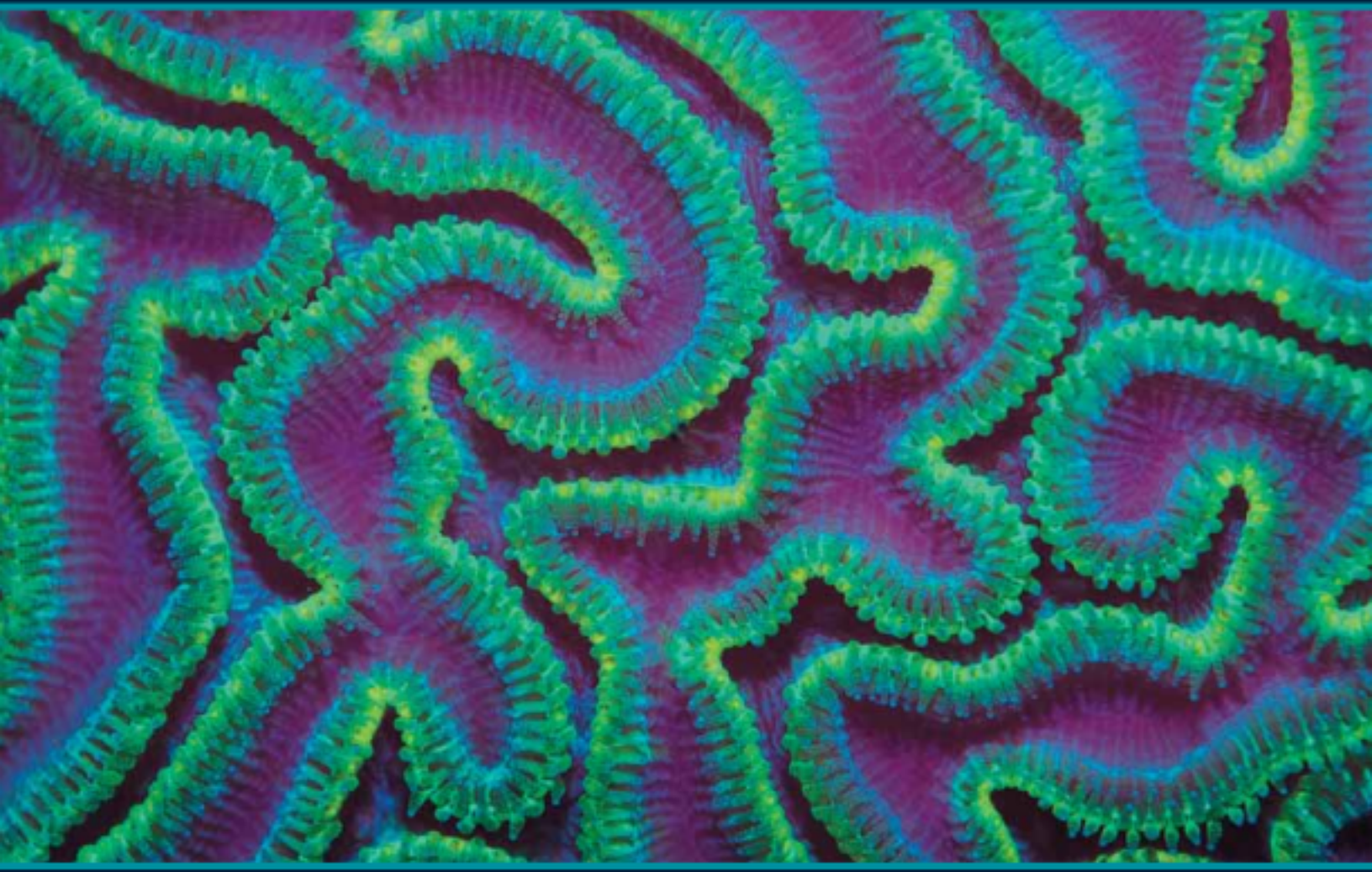
ANTIBODIES AND RELATED REAGENTS FOR SIGNAL TRANSDUCTION RESEARCH



2013/2014 Pathway Reference Guide

Cell Signaling TECHNOLOGY®

XP® Monoclonal Antibodies

one antibody, multiple applications™

XP® monoclonal antibodies are a line of high quality rabbit monoclonal antibodies exclusively available from Cell Signaling Technology (CST). Any product labeled with XP has been carefully selected based on superior performance in the most relevant research applications.

XP monoclonal antibodies are generated using XMT® technology, a proprietary monoclonal technology developed at CST. This technology provides access to a broad range of antibodies unattainable with traditional monoclonal technologies, allowing more comprehensive screening and the identification of XP monoclonal antibodies.

eXceptional specificity

As with all CST™ antibodies, the antibody is specific to your target of interest, saving you valuable time and resources.

+ eXceptional sensitivity

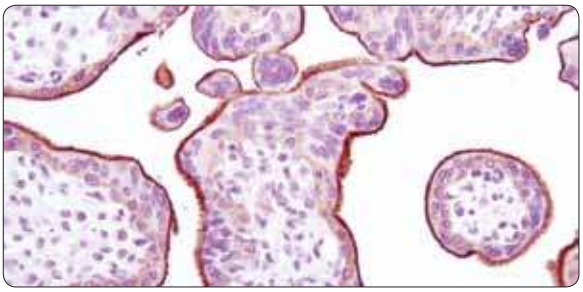
The antibody will provide a stronger signal for your target protein in cells and tissues, allowing you to monitor expression of low levels of endogenous proteins, saving you valuable materials.

+ eXceptional stability and reproducibility

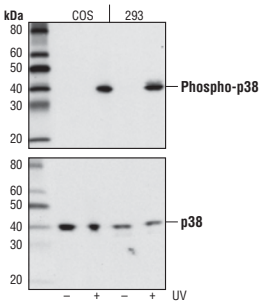
XMT technology combined with our stringent quality control ensures maximum lot-to-lot consistency and the most reproducible results.

= eXceptional Performance™

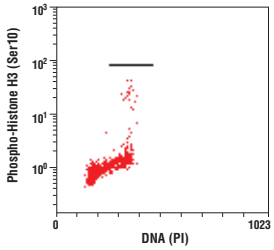
XMT technology coupled with our extensive antibody validation and stringent quality control delivers XP monoclonal antibodies with eXceptional Performance in the widest range of research applications.



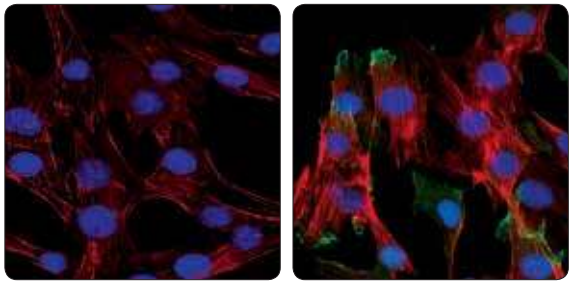
EGF Receptor (D38B1) XP® Rabbit mAb #4267: IHC analysis of paraffin-embedded human placenta using #4267.



Phospho-p38 MAPK (Thr180/Tyr182) (D3F9) XP® Rabbit mAb #4511: Western blot analysis of extracts from COS and 293 cells, untreated or UV-treated, using #4511 (upper) or p38 MAPK Antibody #9212 (lower).



Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb #3377: Flow cytometric analysis of Jurkat cells using #3377 versus propidium iodide (DNA content). The boxed population indicates Phospho-Histone H3 (Ser10) positive cells.



Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060: Confocal IF analysis of C2C12 cells treated with LY294002 #9901 (left) or insulin (right), using #4060 (green). Actin filaments were labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

Visit our website for more experimental details, additional information, and a complete list of available XP Monoclonal Antibodies.

Our Commitment to You...

As a company driven by science, our goal is to accelerate biomedical research by developing a "research tool box" enabling researchers to monitor and measure protein activity. We strive to meet contemporary and future research challenges by creating the highest quality, most specific and thoroughly validated antibodies and related reagents. As a committed member of the research community, we practice responsible and sustainable business methods and invest heavily in research and development. Moreover, we encourage thoughtful use of our limited resources by highlighting environmental issues in our catalog and promoting conservation and recycling.

Highest Quality Products: As scientists we understand your needs—our entire focus is your experimental success. The combination of experienced PhD level scientists overseeing target selection and antibody development processes together with novel antibody production technologies results in the highest possible product quality.

Extensive Product Validation: All product development and production is followed by rigorous in-house testing on a wide range of assay applications. This ensures optimal and reliable performance in critical applications employed in biomedical research.

Highest Quality Support: Technical support is provided by the same scientists who produce the products and know them best.

Research: Research at Cell Signaling Technology (CST) has led to the development of PTMScan™ methodology, a novel affinity/mass spectrometry method for the identification of post-translational modification sites (*Nature Biotech.* 23, 94–101). The combination of PTMScan™ technology with motif antibody development at CST has enabled us to expand discovery efforts from tyrosine to serine/threonine phosphorylation and other modifications such as acetylation and methylation.

PhosphoSitePlus® Bioinformatics Resource: Together with the NIH, we continue to expand PhosphoSitePlus®, available at www.phosphosite.org. PhosphoSitePlus® is the world's most comprehensive resource for the study of *in vivo* phosphorylation events. PhosphoSitePlus® contains thousands of previously unpublished phosphorylation sites discovered at CST, now made freely available to all customers. We encourage you to review this new information and request reagent development.

New Antibody Technology: Over the years, we have invested in the development of new antibody production technologies. XMT™ technology, a new exceptional monoclonal method from CST, allows the production of XP® monoclonal antibodies with exceptional performance.

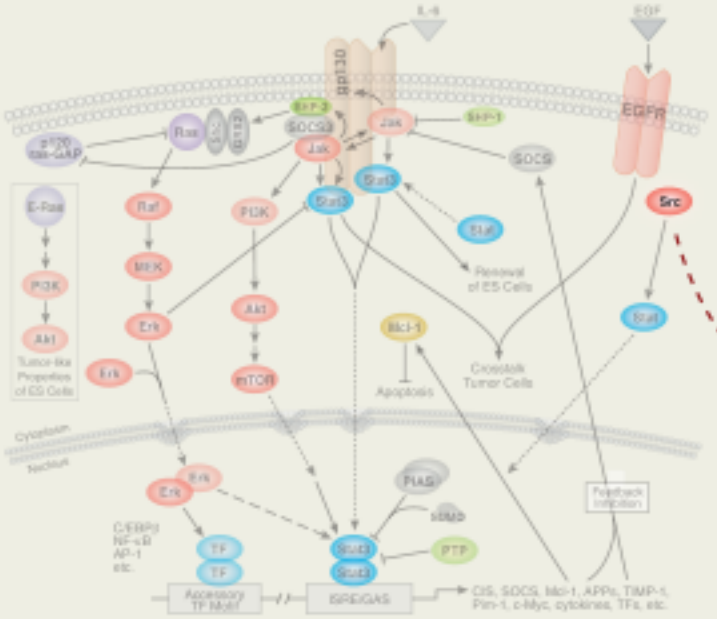
Please visit our website for frequent new product additions. With over 1,300 new products developed at CST during the past two years, the best way to keep up with our rapid rate of new product introduction is to check our website. The website also contains an online version of our unique signal transduction reference material, as well as a special section on our commitment to the environment.

Cover: Fluorescent photo taken to reveal the natural blue light fluorescence of the grooved brain coral (*Diploria labyrinthiformis*) at night with polyps extended to feed, Caribbean Sea. Coral reefs are biologically diverse and productive ecosystems, occupying just 0.1 percent of the oceans by volume while providing a home for about one third of all marine species. For more information about the Mesoamerican reef: **www.eco.cellsignal.com**

Table of Contents

PhosphoSitePlus®

PhosphoSitePlus® (PSP) is an open on-line systems biology resource devoted to commonly studied protein post-translational modifications (PTMs), including phosphorylation, acetylation, ubiquitination, and methylation. PSP contains only experimentally determined sites, not predicted sites, and presents criteria that assist users in evaluating the reliability of site assignments.



Cell Signaling Technology online reference pathway diagrams are now linked to PhosphoSitePlus®.
Click on the proteins in each pathway to navigate to protein pages on the PSP website. Visit these pathways at http://www.cellsignal.com/reference/pathway/index.html.

PhosphoSitePlus®

Comprehensive online protein modification resource provided by Cell Signaling Technology with grant support from the NIH

PSP is a new version of PhosphoSite® that continues to provide broad coverage of current literature and to publish many first reports of protein modifications including phosphorylation, methylation, and acetylation. Previously unpublished modification sites contained in PSP were discovered by Cell Signaling Technology scientists and scientists at other research institutions.

PSP integrates encyclopedic information on experimentally determined protein modification sites, upstream and downstream regulation of these modifications, and powerful analytical tools for investigating the structural and biological significance of protein modifications. Many cutting-edge features make it the premiere resource in protein modification research available today: expansive and continuously curated content; molecular rendering to visualize the location of modification sites; on-the-fly generation of kinase substrate sequence logos; browsing of high-throughput content by disease, cell line, and tissue; new search interfaces that retrieve modification sites and proteins by subcellular locations, sequence and motifs, domains, responsiveness to treatments, disease, tissue, and cell type.

www.phosphosite.org

Home Page

Starting point for querying PhosphoSitePlus®
Users can choose from two types of Simple Searches, three Advanced Searches, and three Browsing Interfaces.

Simple Search:

In addition to the protein search, which will lead to a Protein Page, the substrate search will return a list of experimentally verified *in vivo* and *in vitro* sites on specific proteins. The preferred substrate sequences can be summarized and viewed as a Sequence Logo.

Advanced Search:

Three types of advanced searches give the user the power to explore what is known about proteins and sites that are post-translationally modified. The **Protein, Sequence, or Reference Search** retrieves a list of proteins based on eleven different categories of information. The **Site Search** retrieves a list of modified sites (with surrounding amino-acid sequence) and proteins that can be restricted to eight different categories of information. Users can search for all proteins with modification sites that contain a degenerate motif. **Comparative Site Searches** adds Boolean logic to site searches, giving the user the ability to focus on sites observed under very specific conditions.

Search for Modified Proteins by:

- Name or Accession Number
- Protein Type or Domain
- Subcellular Localization
- Molecular Weight Range
- Sequence/Motif

Search for Sequences by:

- Sequence/Motif
- List of Peptides
- Protein Domain

Search for Sites by:

- Defined Sequences or Motifs
- Observed in Disease State
- Regulation by Treatments
- Protein Type, Domain, Subcellular Location
- Tissue, Cell Line, Cell Type

Browsing Interfaces:

- Specific Diseases
- Specific Cell Lines
- Specific Tissues

What's New

Statistics

Supplements
Downloads
Logo Generator

Substrate Search

Protein Search

PhosphoSitePlus®

Protein Page

Provides detailed information on the parent protein and its modified sites.

Overview Section:

- Brief description of protein function
- Protein type and subcellular localization
- Associated GO terms
- Protein accession number and link to parent database
- Alternative names and gene symbol
- Molecular weight, isoelectric point, and pI calculator
- Associated molecular structures and viewer
- CST protein-centric products

Resources linked from PhosphoSitePlus:

- Major Sequence Repositories
- Scansite: prediction of upstream kinases and downstream interactions
- KinBase: the Kinase Database at Salk
- Pfam: domain structure information
- OMIM: associated diseases

Biological Regulation Overview:

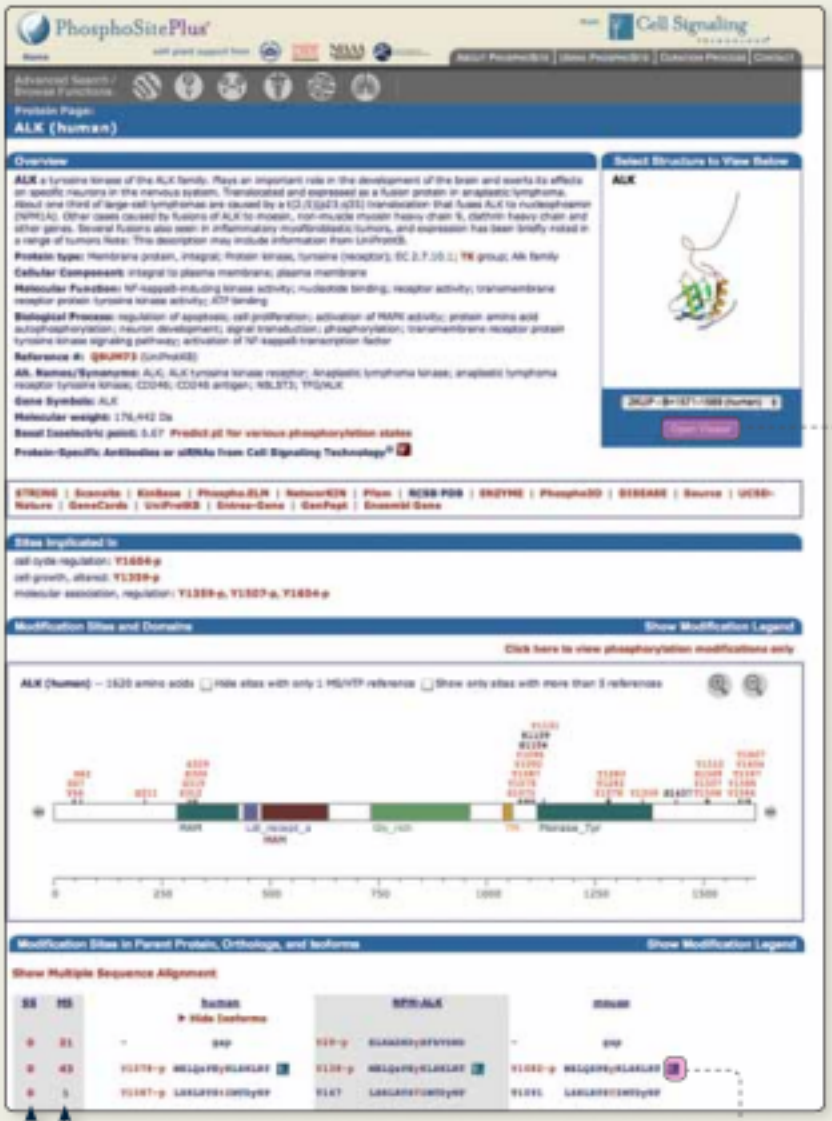
- Lists sites known to regulate biological and molecular processes, including:
  - Enzymatic activation
  - Transcriptional regulation
  - Cell motility regulation
  - Autophagy regulation
  - Conformational changes
- Links to associated Modification Site Pages

Sites and Domains Section:

- Zoomable linear diagram of protein
- Domains and modification sites mapped
- Domain names linked to Pfam
- Filter out sites with > 4 MS/MS sites
- Show only sites with > 2 LTP sites

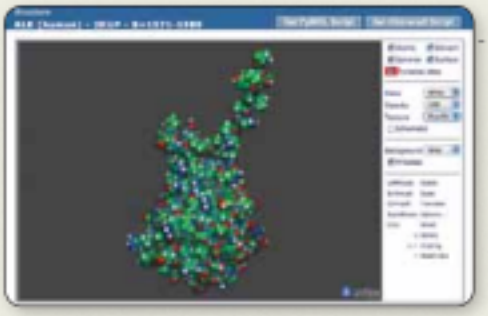
Sites, Sequences, Species, and References Section:

- Modification sites and surrounding sequences (+/- 7 AA) presented
- Sites mapped to other species and isoforms
- Red residues are known modifications
- Red residue numbers are hyper-linked to associated references
- CST logo links to Cell Signaling Technology (CST) product pages



Separate lists for sites determined using low-throughput and high-throughput MS/MS analyses.

Link to CST™ product pages.



Structure Viewer:

- Highlight modified residues

Our Commitment to the Environment

Observing and understanding the array of interactions that shape the living planet on a global level is just as fascinating to us as the complex molecular interactions involved in cellular signaling. The fundamental inter-relationship between ourselves and the biosphere inspires us to draw attention to the magnificence of nature and some of the organizations that struggle to protect it. This year we dedicate pages in our catalog, our annual nature calendar, and CST's nature conservancy website to raising awareness about the Mesoamerican Reef. In addition, CST offers support to four organizations committed to protecting and conserving our marine resources.

For more information about the Mesoamerican reef: **www.eco.cellsignal.com**

EcoLogic Development Fund empowers rural and indigenous peoples to restore and protect tropical ecosystems in Central America and Mexico. We collaborate with local communities to put in place strategies and solutions to increase their self-sufficiency, environmental health, and adaptability in response to climate change in ways that also encourage the long-term survival of the biodiversity around them.

Southern Environmental Association (SEA) is a Belizean non-governmental organization working towards improving stewardship and the environmental integrity of key marine areas in southern Belize through effective, collaborative protected areas management, community involvement, and strategic partnerships for the benefit of all stakeholders. SEA co-manages three important marine protected areas in Southern Belize: Gladden Spit and Silk Cayes Marine Reserve, the Sapodilla Cayes Marine Reserve and the Laughing Bird Caye National Park. Both Laughing Bird Caye National Park and Sapodilla Cayes Marine Reserve form a part of the Belize Barrier Reef Reserve System declared by UNESCO in 1996 as a world heritage site. seabelize.org

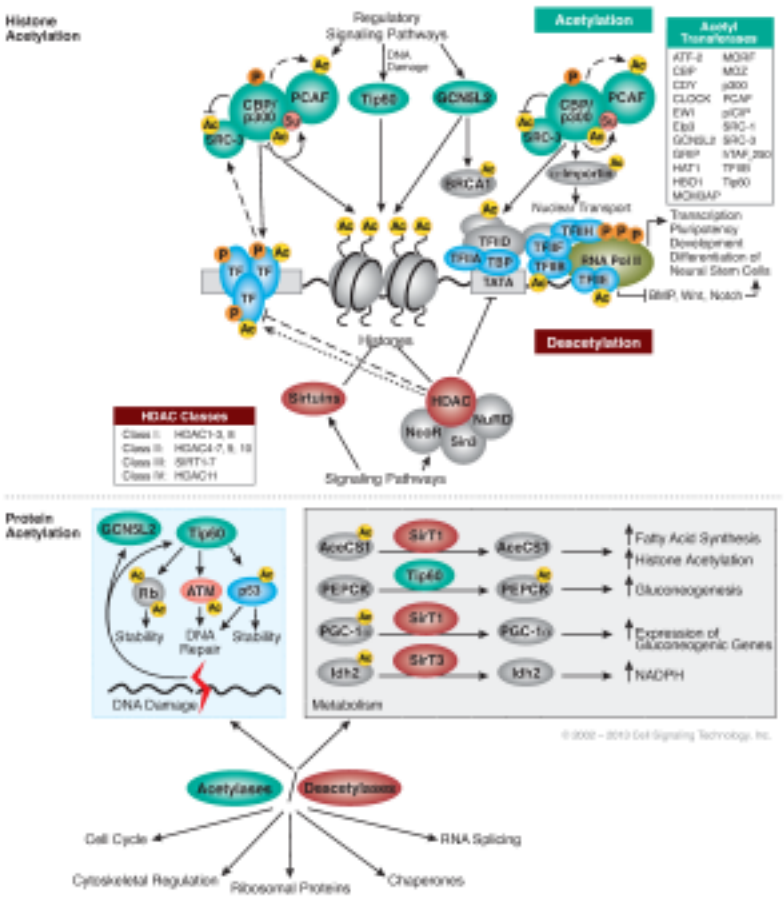
Toledo Institute for Development and Environment (TIDE) is a Belizean non-governmental organization that fosters community participation in resource management and sustainable use of ecosystems within the Maya Mountain Marine Corridor of southern Belize for the benefit of present and future generations. www.tidebelize.org

Large Pelagic Research Center (LPRC) is modeled after the Pelagic Fisheries Research Group (PFRP) in the Pacific. LPRC began as a Center to stimulate and conduct research on key species of interest to commercial and recreational fisheries and marine ecosystems in the Atlantic Ocean. The Center, established in 2003 at the University of New Hampshire, functioned as an academic research group and as a coordinator and source of extramural funding for other large pelagic species research. In 2010, LPRC joined the Department of Environmental Conservation at the University of Massachusetts-Amherst and the Graduate School of Marine Science. We are located in Gloucester, MA and, as part of the Massachusetts Marine Fisheries Institute, are working to revitalize the UMass Marine Station at Gloucester's Hodgkins Cove. www.tunalab.org

Signaling Pathways

These diagrams have been assembled by Cell Signaling Technology (CST) scientists and outside experts to provide succinct and current overviews of selected signal transduction pathways. Knowledge about each signaling pathway has been synthesized and integrated into understandable paradigms of cellular communication.

Please click the literature icon at www.cellsignal.com to obtain copies of our latest posters.

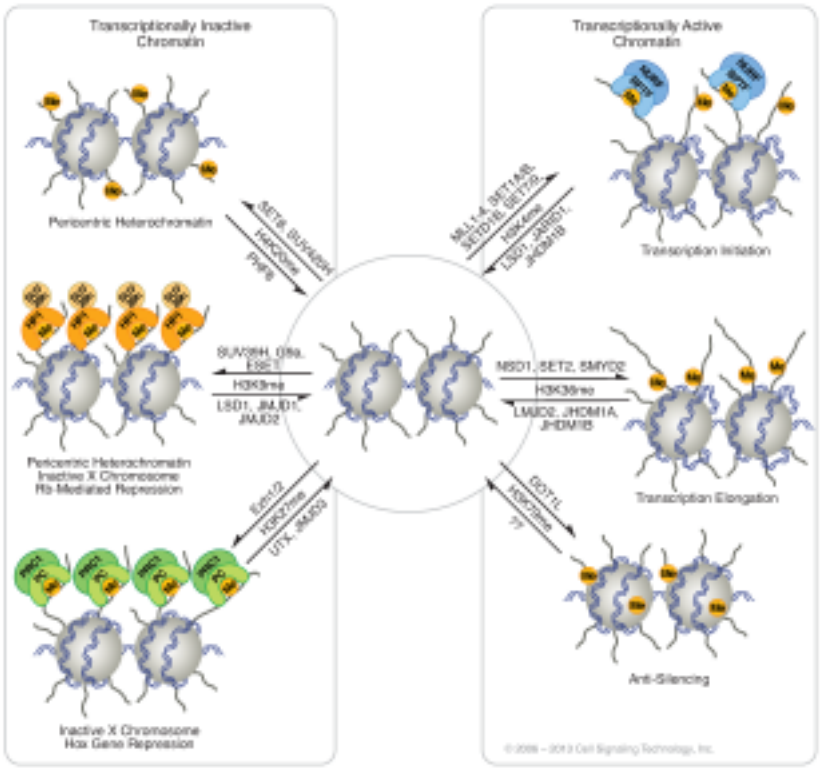


Histone Acetylation

Lysine acetylation is a reversible post-translational modification that plays a crucial role in regulating protein function, chromatin structure, and gene expression. Many transcriptional coactivators possess intrinsic acetylase activity, while transcriptional corepressors are associated with deacetylase activity. Acetylation complexes (such as CBP/p300 and PCAF) or deacetylation complexes (such as Sin3, NuRD, NcoR, and SMRT) are recruited to DNA-bound transcription factors (TFs) in response to signaling pathways. Histone hyperacetylation by histone acetyltransferases (HATs) is associated with transcriptional activation, whereas histone deacetylation by histone deacetylases (HDACs) is associated with transcriptional repression. Histone acetylation stimulates transcription by remodeling higher order chromatin structure, weakening histone-DNA interactions, and providing binding sites for transcriptional activation complexes containing proteins that possess bromodomains, which bind acetylated lysine. Histone deacetylation represses transcription through an inverse mechanism involving the assembly of compact higher order chromatin and the exclusion of bromodomain-containing transcription activation complexes. Histone hypoacetylation is a hallmark of silent heterochromatin. Site-specific acetylation of a growing number of non-histone proteins has been shown to regulate their activity, localization, specific interactions, and stability/degradation. Remarkably, recent advances in mass spectrometry technologies allowed high resolution mapping of most of the acetylation sites in all the proteome. These studies demonstrated that the "acetylome" encompasses nearly ~3600 acetylation sites in roughly ~1750 proteins, suggesting that this modification is one of the most abundant chemical modifications in nature. Indeed, it appears that this mark can influence the activity of proteins in diverse biological processes, including chromatin remodeling, cell cycle, splicing, nuclear transport, mitochondrial biology, and actin nucleation. At an organismal level, acetylation plays an important role in immunity, circadian rhythmicity, and memory formation. Protein acetylation is becoming a favorable target in drug design for numerous disease conditions.

Select Reviews: Allfrey, B.N., Arnold, K.M., and Dasu, J.M. (2011) *Chem. Rev. Chem.* 12, 290–298. | Choudhary, C., Kumar, C., Gnad, F., Nielsen, M.L., Rehman, M., Walther, T.C., Olsen, J.V., and Mann, M. (2009) *Science* 325, 834–840. | Dali-Youcef, N., Lagouge, M., Froelich, S., Koehl, C., Schoonjans, K., and Auwerx, J. (2007) *Ann. Med.* 39, 335–345. | Finkel, T., Deng, C.H., and Mostoslavsky, R. (2009) *Nature* 460, 587–591. | Haberland, M., Montgomery, R.L., and Olson, E.N. (2009) *Nat. Rev. Genet.* 10, 32–42. | Peng, L. and Seto, E. (2011) *Handbook Exp. Pharmac.* 206, 39–56. | Spange, S., Wagner, T., Heinzel, T., and Krämer, O.H. (2009) *Int. J. Biochem. Cell Biol.* 41, 185–198. | Yang, X.J. and Seto, E. (2007) *Oncogene* 26, 5310–5318. | Yang, X.J. and Seto, E. (2008) *Mol. Cell* 31, 449–461.

We would like to thank Prof. Raul Mostoslavsky, Harvard Medical School, Boston, MA, for contributing to this diagram.



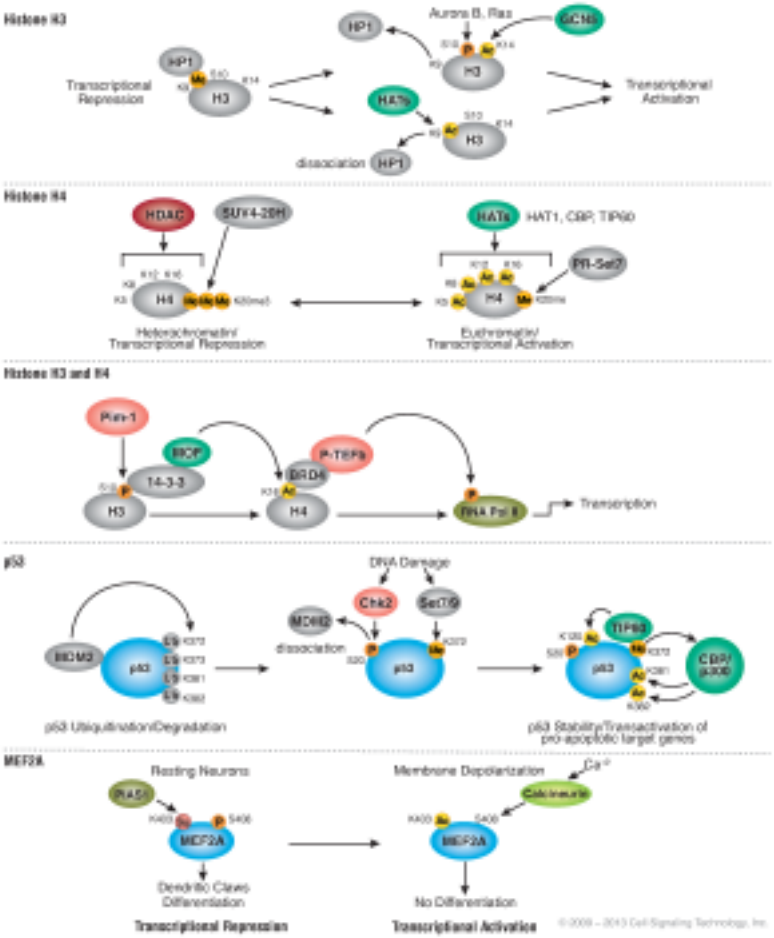
Histone Lysine Methylation

The nucleosome, made up of four histone proteins (H2A, H2B, H3, and H4), is the primary building block of chromatin. Originally thought to function as a static scaffold for DNA packaging, histones have more recently been shown to be dynamic proteins, undergoing multiple types of post-translational modifications. One such modification, methylation of lysine residues, is a major determinant for formation of active and inactive regions of the genome. A set of histone lysine methyltransferases have been identified, all but one of which contain a conserved catalytic SET domain originally identified in the Drosophila Su(var)3-9, Enhancer of zeste, and Trithorax proteins. Lysine methylation has been implicated in both transcriptional activation (H3 Lys4, 36, 79) and silencing (H3 Lys9, 27, H4 Lys20).

Unlike acetylation, methylation does not alter the charge of lysine residues and is unlikely to directly modulate nucleosomal interactions required for chromatin folding. Lysine methylation coordinates the recruitment of chromatin modifying enzymes. Chromodomains (HP1, PRC1), PHD fingers (BPTF, ING2), Tudor domains (53BP1), and WD-40 domains (WDR5) are among a growing list of methyl-lysine binding modules found in histone acetyltransferases, deacetylases, methylases, and ATP-dependent chromatin remodeling enzymes. Lysine methylation provides a binding surface for these enzymes, which then regulate chromatin condensation and nucleosome mobility in order to maintain local regions of active or inactive chromatin. In addition, lysine methylation can block binding of proteins that interact with unmethylated histones or directly inhibit catalysis of other regulatory modifications on neighboring residues. The presence of methyl-lysine binding modules in the DNA repair protein 53BP1 suggests roles for lysine methylation in other cellular processes.

Histone methylation is crucial for proper programming of the genome during development, and misregulation of the methylation machinery can lead to diseased states such as cancer. Until recently, methylation was believed to be an irreversible, stable epigenetic mark that is propagated through multiple cell divisions, maintaining a gene in an active or inactive state. While there is no argument that methylation is a stable mark, recent identification of histone demethylases such as LSD1/KDF2, JMJD1, JMJD2, and JHDM1 has shown that methylation is reversible and provides a rationale for how genomes might be reprogrammed during differentiation of individual cell lineages.

For selected reviews see www.cellsignal.com



Examples of Crosstalk Between Post-translational Modifications

Post-translational modifications (PTMs) have recently emerged as major regulators of protein function. Originally described in histones, these various chemical modifications (methylation, acetylation, phosphorylation, sumoylation, and more) have now been identified in nonhistone proteins as well. Early work defined a putative role for each of these modifications; for instance, acetylation correlates with activation and methylation with repression. However, more recent studies indicate that some of these modifications could trigger either activation or silencing in a context-dependent manner. For instance, methylation of histone H3 Lys9 correlates with repression, while methylation of H3 Lys4 correlates with activation. Furthermore, each of these moieties can be either mono-, di-, or tri-methylated, and depending on the degree of methylation, the biological output will be completely different. We now know that these PTMs are strictly established and maintained by a set of "writers" (histone methyltransferases, acetyltransferases, etc.) and "erasers" (histone demethylases, deacetylases, etc.) that define the different modifications found in our cells. Until recently, PTMs were considered independently, under the assumption that their functions would not be related to one another. It is now clear that PTMs work in concert, and the crosstalk between different modifications determines the final biological read out. In this context, some modifications can influence others, and it appears that specific combinations of these modifications can form a dynamic code. We provide a few examples of this type of crosstalk here. As shown, PTMs can be recognized by "readers" in cis, with a single protein using two different domains to recognize two specific modifications, as well as in trans, where modifications in one histone molecule could be recognized by a particular "reader" to modify another histone, in turn recruiting further readers in a step-wise manner. Further, in some cases, these modifications are themselves recognized by writers and erasers that could then modify neighbor moieties, in this way adjusting the code. Although there are now many examples of these functional networks, it is likely that we have just begun to scratch the surface. Better antibodies and novel technologies will help to complete this crosstalk puzzle, for which the specific fine-tuning appears critical to determine life as we know it.

Select Reviews: Berger, S.L. (2007) *Nature* 447, 407–412. | Gardner, K., Allis, D.A., and Strahl, B.D. (2011) *J. Mol. Biol.* 409, 36–46. | Lee, J.S., Smith, E., and Shilatifard, A. (2010) *Cell* 142, 682–685. | Musselman, K.A. and Kutateladze, T.G. (2011) *Nucl. Acids. Res.* 39, 9061–9071. | Yang, X.J. and Seto, E. (2008) *Mol. Cell* 31, 449–461.

We would like to thank Prof. Ravi Mostoslavsky, Harvard Medical School, Boston, MA, for contributing to this diagram.

Kinase | Transcription Factor | Enzyme | Ribosomal subunit | Acetylase | Methyl-arginine | Methyl-lysine Binding Protein | Nucleosome Core | Methylation / Demethylation
Phosphatase | Receptor | GTPase | | Deacetylase | Methyl-lysine | | Histone Tail | Double Stranded DNA

Histone Modification Table

The nucleosome, made up of four core histone proteins (H2A, H2B, H3, and H4), and linker histone H1 are the primary building blocks of chromatin. Originally thought to function as a static scaffold for DNA packaging, histones have more recently been shown to be dynamic proteins, undergoing multiple types of post-translational modifications that regulate chromatin condensation and DNA accessibility. For example, acetylation of lysine residues has long been associated with histone deposition and transcriptional activation, and more recently found to be associated with DNA repair. Phosphorylation of serine and threonine residues facilitates chromatin condensation during mitosis and transcriptional activation of immediate-early genes. Methylation of lysine and arginine residues function as a major determinant for formation of transcriptionally active and inactive regions of chromatin and is crucial for proper programming of the genome during development. This table provides a referenced list of many known histone modifications, the associated modifying enzymes, and proposed functions.

Acetylation

Histone	Site	Histone-modifying Enzymes	Proposed Function	Ref. #
H2A	Lys4 (S. cerevisiae)	Esa1	transcriptional activation	(1)
	Lys5 (mammals)	Tip60, p300/CBP	transcriptional activation	(2,3)
	Lys7 (S. cerevisiae)	Hat1	unknown	(4)
		Esa1	transcriptional activation	(1)
H2B	Lys5	p300, ATF2	transcriptional activation	(3,5)
	Lys11 (S. cerevisiae)	Gcn5	transcriptional activation	(6)
	Lys12 (mammals)	p300/CBP, ATF2	transcriptional activation	(3,5)
	Lys15 (mammals)	p300/CBP, ATF2	transcriptional activation	(3,5)
	Lys16 (S. cerevisiae)	Gcn5, Esa1	transcriptional activation	(6)
	Lys20	p300	transcriptional activation	(3)
H3	Lys4 (S. cerevisiae)	Esa1	transcriptional activation	(1)
		Hpa2	unknown	(7)
	Lys9	unknown	histone deposition	(8)
		Gcn5, SRC-1	transcriptional activation	(9,10)
	Lys14	unknown	histone deposition	(8)
		Gcn5, PCAF	transcriptional activation	(3,11)
		Esal, Tip60	transcriptional activation	(1,2)
			DNA repair	(11,12)
		SRC-1	transcriptional activation	(10)
		Elp3	transcriptional activation (elongation)	(13)
		Hpa2	unknown	(7)
		hTFIIIC90	RNA polymerase III transcription	(14)
		TAF1	RNA polymerase II transcription	(15)
		Sas2	euchromatin	(16)
		Sas3	transcriptional activation (elongation)	(17)
		p300	transcriptional activation	(3)
	Lys18	Gcn5	transcriptional activation, DNA repair	(9)
		p300/CBP	DNA replication, transcriptional activation	(3,18)
	Lys23	unknown	histone deposition	(8)
		Gcn5	transcriptional activation, DNA repair	(9)
		Sas3	transcriptional activation (elongation)	(17)
		p300/CBP	transcriptional activation	(3,18)
	Lys27	Gcn5	transcriptional activation	(6)
	Lys36	Gcn5	transcriptional activation	(82)
	Lys56 (S. cerevisiae)	Spt10	transcriptional activation	(19)
			DNA repair	(20)
H4	Lys5	Hat1	histone deposition	(21)
		Esal, Tip60	transcriptional activation	(1,2)
			DNA repair	(11,12)
		ATF2	transcriptional activation	(5)
		Hpa2	unknown	(7)
		p300	transcriptional activation	(3)
	Lys8	Gcn5, PCAF	transcriptional activation	(3,22)
		Esal, Tip60	transcriptional activation	(1,2)
			DNA repair	(11,12)
		ATF2	transcriptional activation	(5)
		Elp3	transcriptional activation (elongation)	(13)
		p300	transcriptional activation	(3)
	Lys12	Hat1	histone deposition	(21)
			telomeric silencing	(23)
		Esal, Tip60	transcriptional activation	(1,2)
			DNA repair	(11,12)
		Hpa2	unknown	(7)
		p300	transcriptional activation	(3)
	Lys16	Gcn5	transcriptional activation	(22)
		MOF (D. melanogaster)	transcriptional activation	(24)
		Esal, Tip60	transcriptional activation	(1,2)
			DNA repair	(11,12)
		ATF2	transcriptional activation	(5)
		Sas2	euchromatin	(2,6)
	Lys91 (S. cerevisiae)	Hat1/Hat2	chromatin assembly	(25)

Methylation

Histone	Site	Histone-modifying Enzymes	Proposed Function	Ref. #
H1	Lys26	Ezh2	transcriptional silencing	(48,49)
H2A	Arg3	PRMT 1/6, PRMT 5/7	transcriptional activation, transcriptional repression	(83)
H3	Arg2	PMRT6	transcriptional repression	(83)
	Arg8	PRMT5	transcriptional repression	(31)
	Arg17	CARM1	transcriptional activation	(18)
	Arg26	CARM1	transcriptional activation	(83)
	Lys4	Set1 (S. cerevisiae)	permissive euchromatin (di-Me)	(26)
		Set7/9 (vertebrates)	transcriptional activation (tri-Me)	(27)
		MLL, ALL-1	transcriptional activation	(28,29)
		Ash1 (D. melanogaster)	transcriptional activation	(30)
	Lys9	Suv39h,Clr4	transcriptional silencing (tri-Me)	(32,33)
		G9a	transcriptional repression genomic imprinting	(34)
		SETDB1	transcriptional repression (tri-Me)	(35)
		Dim-5 (N.crassa), Kryptonite (A. thaliana)	DNA methylation (tri-Me)	(36,37)
		Ash1 (D. melanogaster)	transcriptional activation	(30)
	Lys27	Ezh2	transcriptional silencing	(38)
			X inactivation (tri-Me)	
		G9a	transcriptional silencing	(34)
	Lys36	Set2	transcriptional activation (elongation)	(39)
	Lys79	Dot1	euchromatin	(40)
			transcriptional activation (elongation)	(41)
			checkpoint response	(42)
H4	Arg3	PRMT1/6	transcriptional activation	(43)
		PRMT5/7	transcriptional repression	(31)
	Lys20	PR-Set7	transcriptional silencing (mono-Me)	(44)
		Suv4-20h	heterochromatin (tri-Me)	(45)
		Ash1 (D. melanogaster)	transcriptional activation	(30)
		Set9 (S. pombe)	checkpoint response	(46)
	Lys59	unknown	transcriptional silencing	(47)

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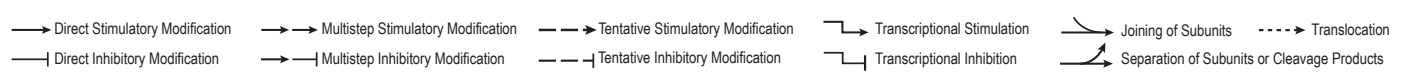

Phosphorylation

Histone	Site	Histone-modifying Enzymes	Proposed Function	Ref. #
H1	Ser27	unknown	transcriptional activation, chromatin decondensation	(48,49)
H2A	Ser1	unknown	mitosis, chromatin assembly	(50)
		MSK1	transcriptional repression	(51)
	Ser122 (S. cerevisiae)	unknown	DNA repair	(53)
	Ser129 (S. cerevisiae)	Mec1, Tel1	DNA repair	(54,55)
	Ser139 (mammalian H2A.X)	ATR, ATM, DNA-PK	DNA repair	(56-58)
	Thr119 (D. melanogaster)	MHK1	mitosis	(52)
H2B	Ser10 (S. cerevisiae)	Ste20	apoptosis	(59)
	Ser14 (vertebrates)	Mst1	apoptosis	(60)
		unknown	DNA repair	(61)
	Ser33 (D. melanogaster)	TAF1	transcriptional activation	(62)
	Ser36	AMPK	transcriptional activation	(84)
H3	Ser10	Aurora-B kinase	mitosis, meiosis	(64,65)
		MSK1, MSK2	immediate-early gene activation	(66)
		IKK-α	transcriptional activation	(67)
		Snf1	transcriptional activation	(68)
	Ser28 (mammals)	Aurora-B kinase	mitosis	(70)
		MSK1, MSK2	immediate-early activation	(66,71)
	Thr3	Haspin/Gsg2	mitosis	(63)
	Thr6	PKCβI		(85)
	Thr11 (mammals)	DAPk/Zip	mitosis	(69)
	Tyr41	JAK2	transcriptional activation	(86)
	Tyr45	PKCδ	apoptosis	(87)
H4	Ser1	unknown	mitosis, chromatin assembly	(50)
		CK2	DNA repair	(72)

Ubiquitylation

Histone	Site	Histone-modifying Enzymes	Proposed Function	Ref. #
H2A	Lys119 (mammals)	Ring2	spermatogenesis	(73)
H2B	Lys120 (mammals)	Ubch6	meiosis	(74)
	Lys123 (S. cerevisiae)	Rad6	transcriptional activation euchromatin	(75)

Sumoylation

Histone	Site	Histone-modifying Enzymes	Proposed Function	Ref. #
H2A	Lys126 (S. cerevisiae)	Ubc9	transcriptional repression	(76)
H2B	Lys6 or Lys7 (S. cerevisiae)	Ubc9	transcriptional repression	(76)
H4	N-terminal tail (S. cerevisiae)	Ubc9	transcriptional repression	(77)

Biotinylation

Histone	Site	Histone-modifying Enzymes	Proposed Function	Ref. #
H2A	Lys9	biotinidase	coloncer	(78)
	Lys12	biotinidase	coloncer	(78)
H3	Lys4	biotinidase	gene expression	(79)
	Lys9	biotinidase	gene expression	(79)
	Lys18	biotinidase	gene expression	(79)
H4	Lys12	biotinidase	DNA damage response	(80,81)

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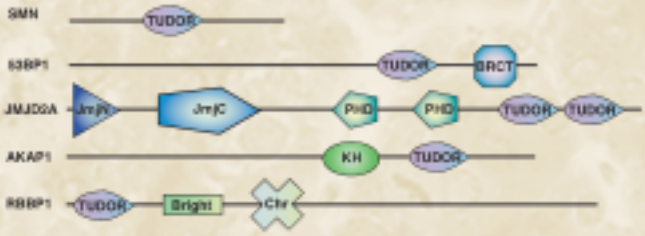

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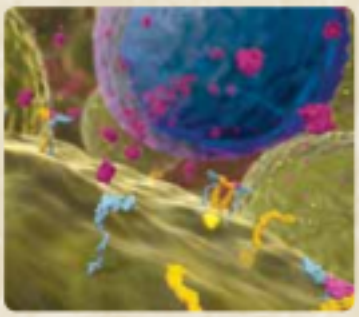
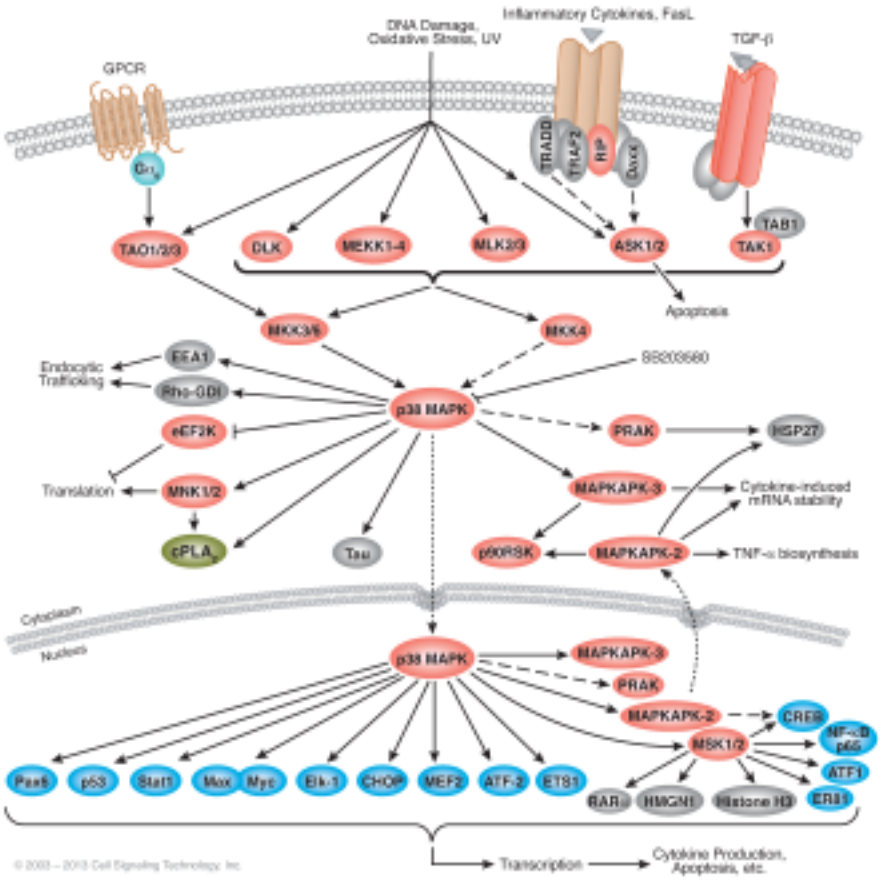


Kinase | Transcription Factor | Caspase | Enzyme | GAP/GEF | G-protein | Acetylase
Phosphatase | pro-apoptotic | Receptor | pro-survival | GTPase | Ribosomal subunit | Deacetylase

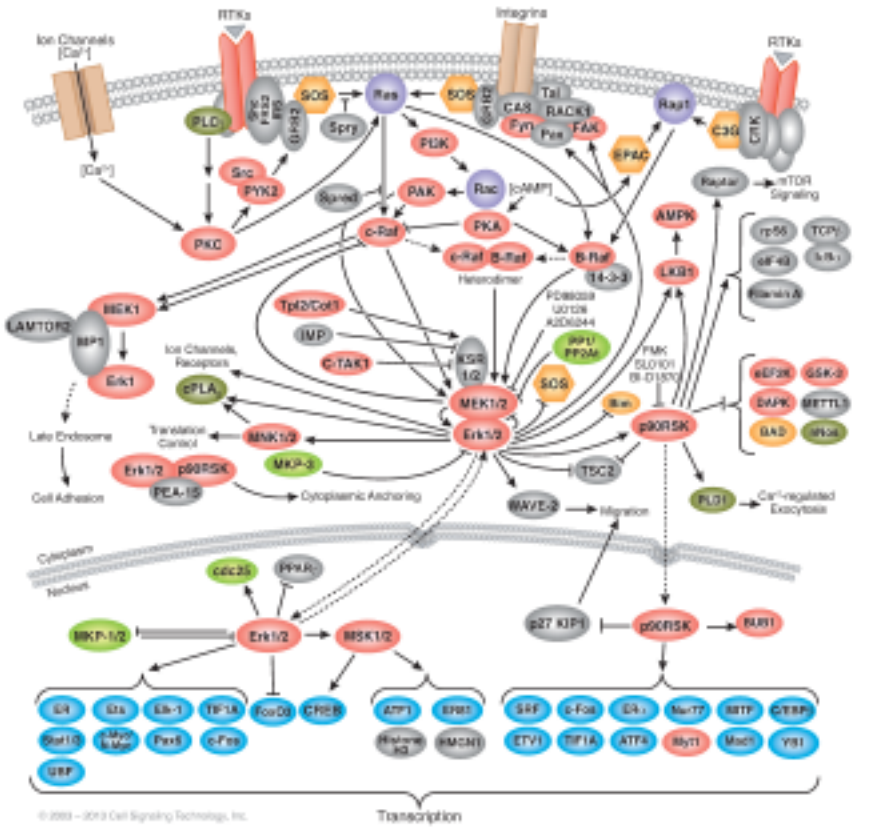


Signaling Pathways Activating p38 MAP Kinase

p38 MAPKs (α, β, γ, and δ) are members of the MAPK family that are activated by a variety of environmental stresses and inflammatory cytokines. As with other MAPK cascades, the membrane-proximal component is a MAPKKK, typically a MEKK or a mixed lineage kinase (MLK). The MAPKKK phosphorylates and activates MKK3/6, the p38 MAPK kinases. MKK3/6 can also be activated directly by ASK1, which is stimulated by apoptotic stimuli. p38 MAPK is involved in regulation of HSP27, MAPKAPK-2 (MK2), MAPKAPK-3 (MK3), and several transcription factors including ATF-2, Stat1, the Max/Myc complex, MEF-2, Elk-1, and indirectly CREB via activation of MSK1.

Select Reviews: Coulthard, L.R., White, D.E., Jones, D.L., McDermott, M.F., and Burchill, S.A. (2009) *Trends Mol. Med.* 15, 369–379. | Cuadrado, A. and Nebreda, A.R. (2010) *Biochem. J.* 429, 403–417. | del Barco Barrantes, I. and Nebreda, A.R. (2012) *Biochem. Soc. Trans.* 40, 79–84. | Huang, G., Shi, L.Z., and Chi, H. (2009) *Cytokine* 48, 161–169. | Kostenko, S., Dumitriu, G., Lagsgid, K.J., and Moens, U. (2011) *World J. Biol. Chem.* 2, 73–89. | Shiryaev, A. and Moens, U. (2010) *Cell. Signal.* 22, 1185–1192.

We would like to thank Prof. John Blenis, Harvard Medical School, Boston, MA, for contributing to this diagram.



MAPK/Erk in Growth and Differentiation

The MAPK/Erk signaling cascade is activated by a wide variety of receptors involved in growth and differentiation including receptor tyrosine kinases (RTKs), integrins, and ion channels. The specific components of the cascade vary greatly among different stimuli, but the architecture of the pathway usually includes a set of adaptors (Shc, GRB2, Crk, etc.) linking the receptor to a guanine nucleotide exchange factor (SOS, C3G, etc.) transducing the signal to small GTP-binding proteins (Ras, Rap1), which in turn activate the core unit of the cascade composed of a MAPKKK (Raf), a MAPKK (MEK1/2), and MAPK (Erk). An activated Erk dimer can regulate targets in the cytosol and also translocate to the nucleus where it phosphorylates a variety of transcription factors regulating gene expression.

Select Reviews: Anjum, R. and Blenis, J. (2008) *Nat. Rev. Mol. Cell Biol.* 9, 747–758. | De Luca, A., Maiello, M.R., D'Alessio, A., Pergameno, M., and Normanno, N. (2012) *Expert Opin. Ther.* 2, 17–27. | Keyse, S.M. (2008) *Cancer Metastasis Rev.* 27, 253–261. | Kim, E.K. and Choi, E.J. (2010) *Biochim. Biophys. Acta* 1802, 396–405. | Mendoza, M.C., Er, E.E., and Blenis, J. (2011) *Trends Biochem. Sci.* 36, 320–328. | Romeo, Y., Zhang, X., and Roux, P.P. (2012) *Biochem. J.* 441, 553–569. | Roskoski, R. Jr. (2012) *Biochem. Biophys. Res. Commun.* 417, 5–10. | Tidyman, W.E. and Rauen, K.A. (2009) *Curr. Opin. Genet. Dev.* 19, 230–236.

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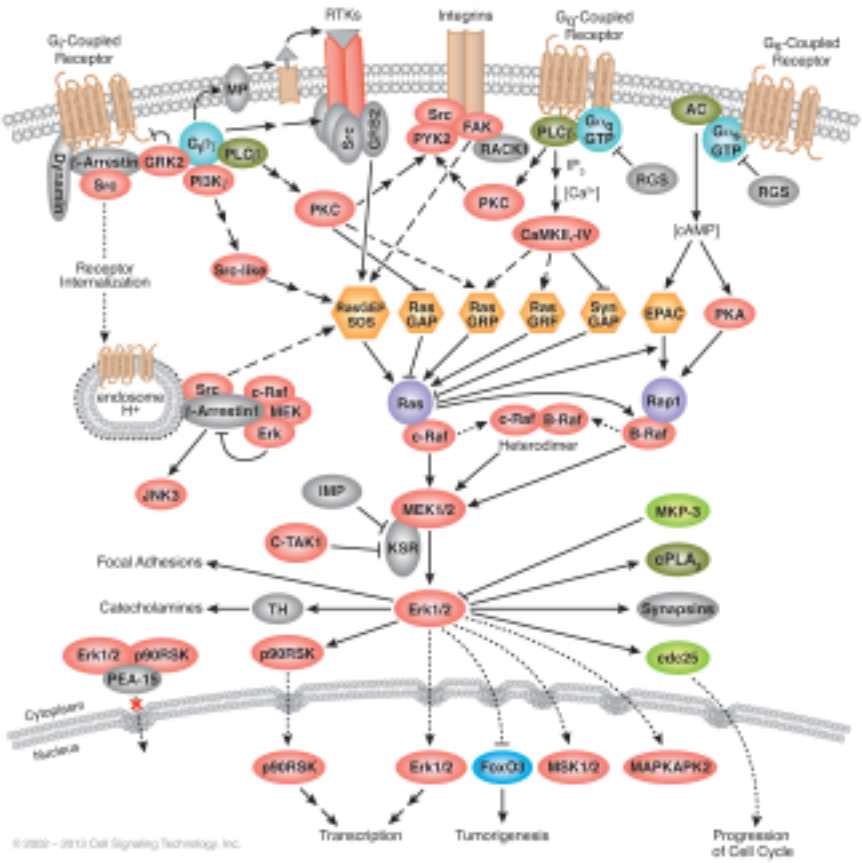

G Protein-coupled Receptors Signaling to MAPK/Erk

G protein-coupled receptors (GPCRs) are activated by a wide variety of external stimuli. Upon receptor activation, the G protein exchanges GDP for GTP, causing the dissociation of the GTP-bound α and βγ subunits and triggering diverse signaling cascades. Receptors coupled to different heterotrimeric G protein subtypes can utilize different scaffolds to activate the small G protein/ MAPK cascade, employing at least three different classes of Tyr kinases. Src family kinases are recruited following activation of PI3Kγ by βγ subunits. They are also recruited by receptor internalization, crossactivation of receptor Tyr kinases, or by signaling through an integrin scaffold involving Pyk2 and/or FAK. GPCRs can also employ PLCβ to mediate activation of PKC and CaMKII, which can have either stimulatory or inhibitory consequences for the downstream MAPK pathway.

Select Reviews: Aoki, Y., Nihori, T., Narumi, Y., Kure, S., and Matsubara, Y. (2008) *Hum. Mutat.* 29, 992–1006. | Caunt, C.J., Finch, A.R., Sedgley, K.R., and McArdle, C.A. (2006) *Trends Endocrinol. Metab.* 17, 276–283. | Goldsmith, Z.G. and Dhanasekaran, D.N. (2007) *Oncogene* 26, 3122–3142. | Kim, E.K. and Choi, E.J. (2010) *Biochim. Biophys. Acta.* 1802, 396–405. | McKay, M.M. and Morrison, D.K. (2007) *Oncogene* 26, 3113–3121.

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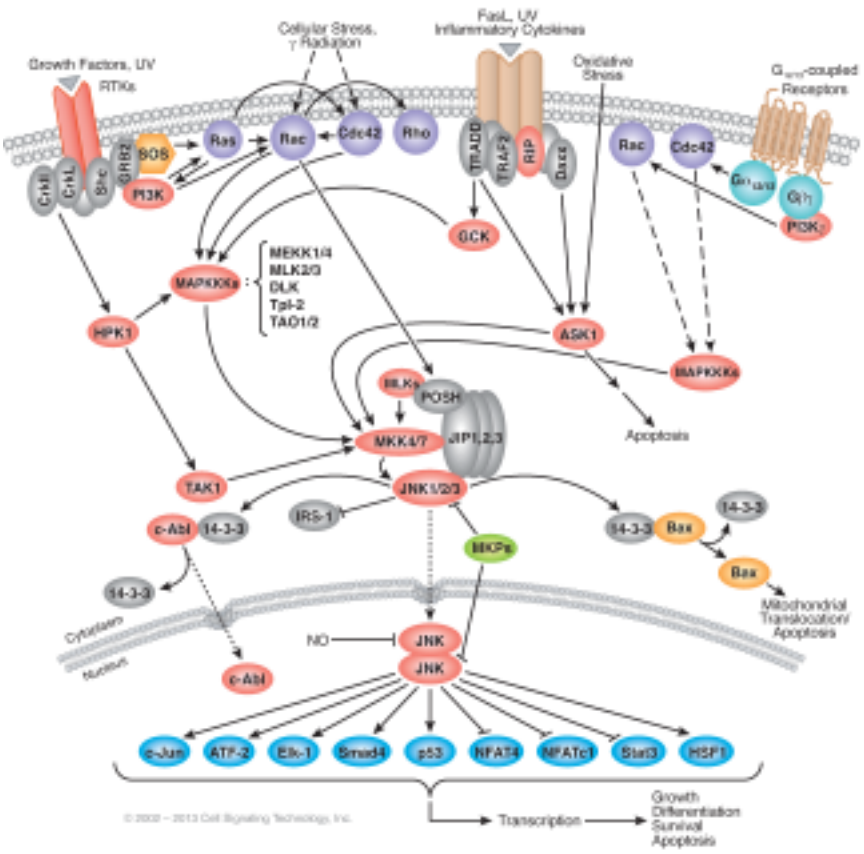


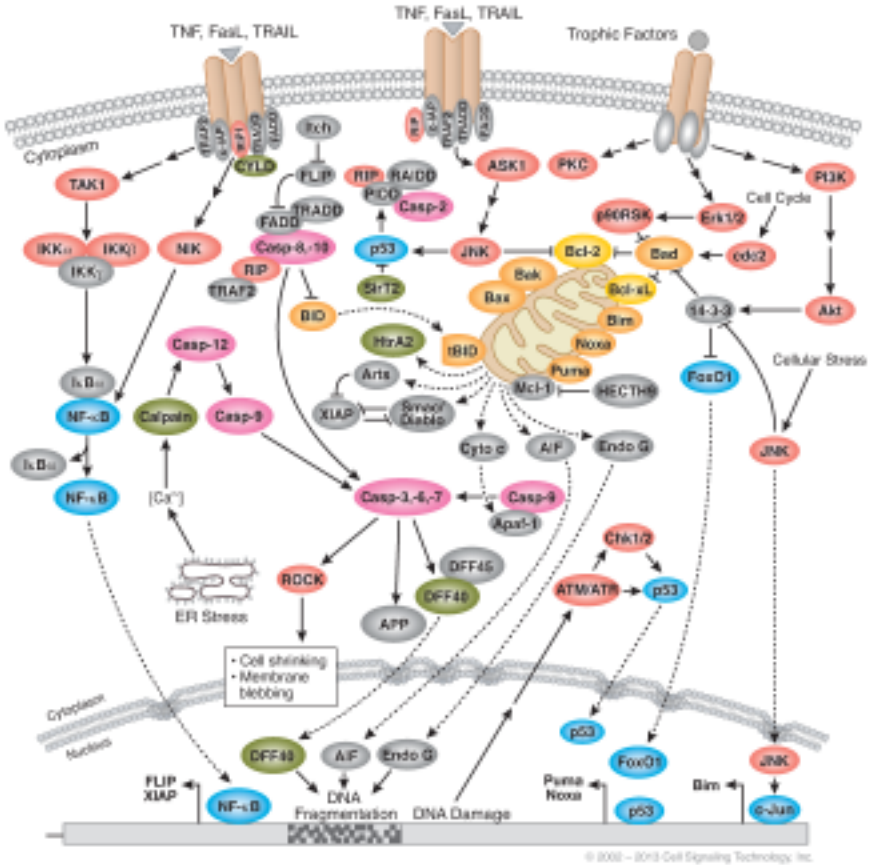
SAPK/JNK Signaling Cascades

Stress-activated protein kinases (SAPK)/Jun amino-terminal kinases (JNK) are members of the MAPK family and are activated by a variety of environmental stresses, inflammatory cytokines, growth factors, and GPCR agonists. Stress signals are delivered to this cascade by small GTPases of the Rho family (Rac, Rho, cdc42). As with the other MAPKs, the membrane proximal kinase is a MAPKKK, typically MEKK1–4, or a member of the mixed lineage kinases (MLK) that phosphorylates and activates MKK4 (SEK) or MKK7, the SAPK/JNK kinases. Alternatively, MKK4/7 can be activated by a member of the germinal center kinase (GCK) family in a GTPase-independent manner. SAPK/JNK translocates to the nucleus where it can regulate the activity of multiple transcription factors.

Select Reviews: Bogoyevitch, M.A., Ngoei, K.R., Zhao, T.T., Yeap, Y.Y., and Ng, D.C. (2010) *Biochim. Biophys. Acta* 1804, 463–475. | Chen F. (2012) *Cancer Res.* 72, 379–386. | Davies, C. and Tournier, C. (2012) *Biochem. Soc. Trans.* 40, 85–89. | Engström, W., Ward, A., and Moorwood, K. (2010) *Cell Prolif.* 43, 56–66. | Haeussgen, W., Herdegen, T., and Waetzig, V. (2011) *Eur. J. Cell Biol.* 90, 536–544. | Verma G., Datta M. (2012) *J. Cell Physiol.* 227, 1791–1795.

We would like to thank Prof. John Blenis, Harvard Medical School, Boston, MA, for reviewing this diagram.

Kinase | Transcription Factor | Caspase | Enzyme | GAP/GEF | G-protein | Acetylase
Phosphatase | pro-apoptotic | Receptor | pro-survival | GTPase | Ribosomal subunit | Deacetylase

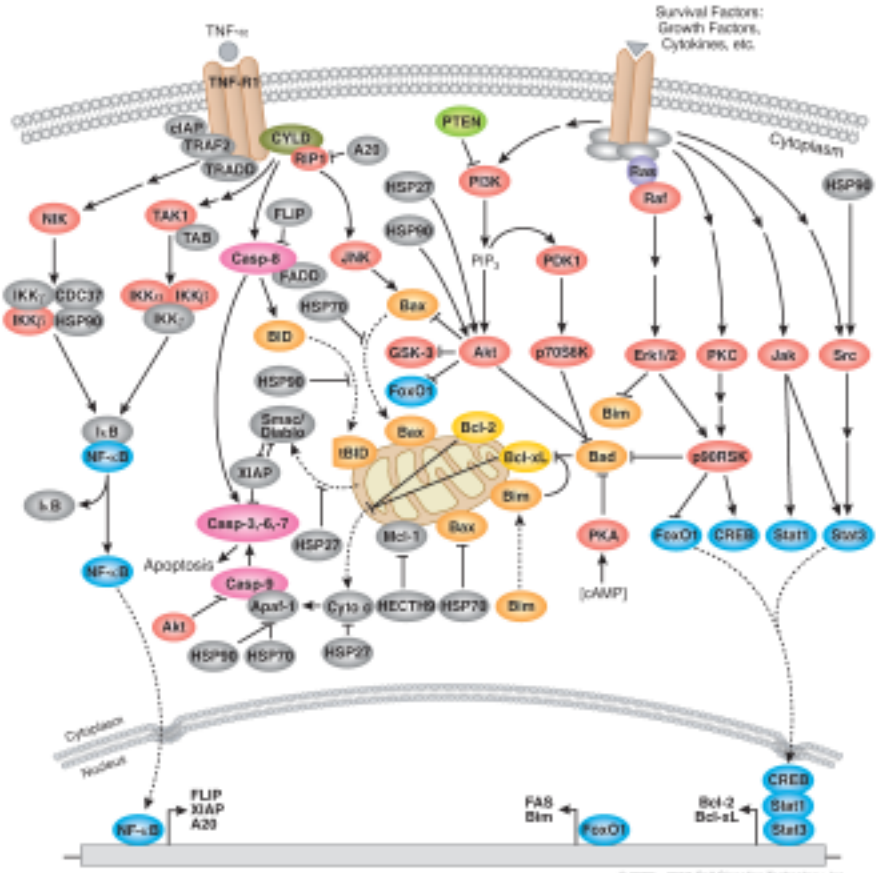


Regulation of Apoptosis Overview

Apoptosis is a regulated cellular suicide mechanism characterized by nuclear condensation, cell shrinkage, membrane blebbing, and DNA fragmentation. Caspases, a family of cysteine proteases, are the central regulators of apoptosis. Initiator caspases (including caspase-2, -8, -9, -10, -11, and -12) are closely coupled to pro-apoptotic signals. Once activated, these caspases cleave and activate downstream effector caspases (including caspase-3, -6, and -7), which in turn execute apoptosis by cleaving cellular proteins following specific Asp residues. Activation of Fas and TNFR by FasL and TNF, respectively, leads to the activation of caspase-8 and -10. DNA damage induces the expression of PIDD, which binds to RAIDD and caspase-2 and leads to the activation of caspase-2. Cytochrome c released from damaged mitochondria is coupled to the activation of caspase-9. XIAP inhibits caspase-3, -7, and -9. Mitochondria release multiple pro-apoptotic molecules, such as Smac/ Diablo, AIF, HtrA2, and Endo G, in addition to cytochrome c. Smac/Diablo binds to XIAP, preventing it from inhibiting caspases. Caspase-11 is induced and activated by pathological pro-inflammatory and pro-apoptotic stimuli and leads to the activation of caspase-1, thereby promoting inflammatory response and apoptosis by directly processing caspase-3. Caspase-12 and caspase-7 are activated under ER stress conditions. Anti-apoptotic ligands, including growth factors and cytokines, activate Akt and p90RSK. Akt inhibits Bad by direct phosphorylation and prevents the expression of Bim by phosphorylating and inhibiting the Forkhead family of transcription factors (FoxO). FoxO promotes apoptosis by upregulating pro-apoptotic molecules such as FasL and Bim.

Select Reviews: Degterev, A. and Yuan J. (2008) *Nat. Rev. Mol. Cell Bio.* 9, 378–390. | Fuchs, Y. and Steller H. (2011) *Cell* 147, 742–758. | Indran, I.R., Tufo, G., Pervaiz, S., and Brenner C. (2011) *Biochim. Biophys. Acta.* 1807, 735–745. | Kaufmann, T., Strasser, A., and Jost, P.J. (2012) *Cell Death Differ.* 19, 42–50. | Kurokawa, M. and Kornbluth, S. (2009) *Cell* 138, 838–854. | Pradelli, L.A., Bénéteau, M., and Ricci, J.E. (2010) *Cell. Mol. Life Sci.* 67, 1589–1597. | Van Herreweghe, F., Festjens, N., Declercq, W., and Vandenabeele, P. (2010) *Cell. Mol. Life Sci.* 67, 1567–1579.

We would like to thank Prof. Junying Yuan, Harvard Medical School, Boston, MA, for reviewing this diagram.



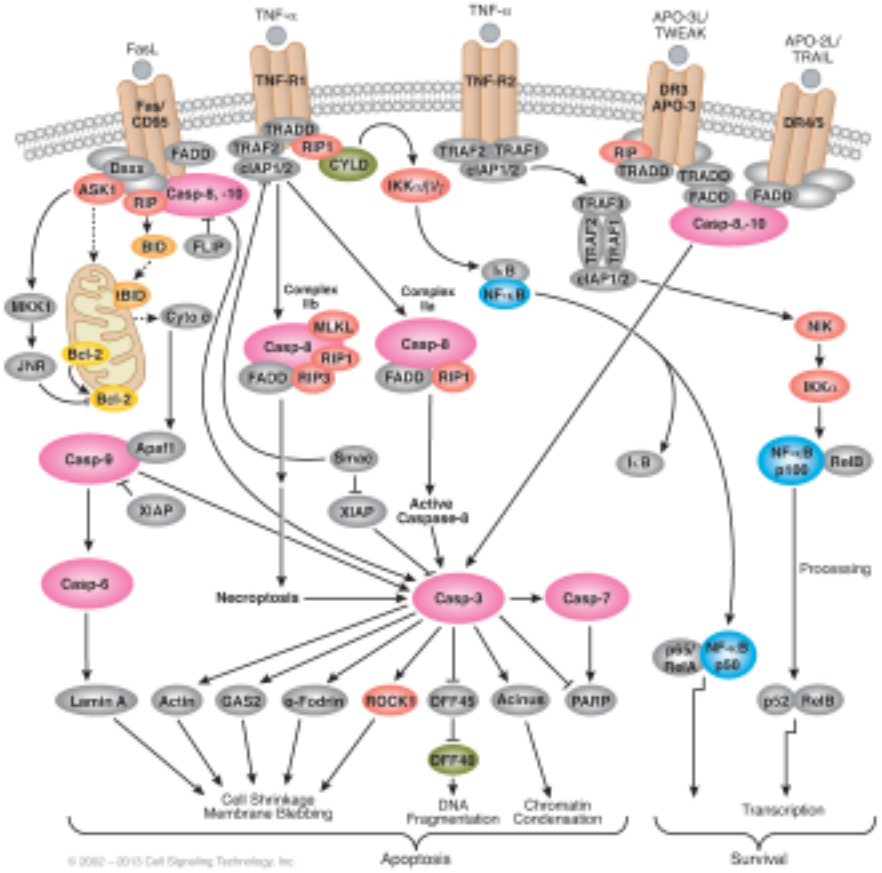
Inhibition of Apoptosis

Cell survival requires the active inhibition of apoptosis, which is accomplished by inhibiting the expression of pro-apoptotic factors as well as promoting the expression of anti-apoptotic factors. The PI3K pathway, activated by many survival factors, leads to the activation of Akt, an important player in survival signaling. PTEN negatively regulates the PI3K/Akt pathway. Activated Akt phosphorylates and inhibits the pro-apoptotic Bcl-2 family members Bad, Bax, caspase-9, GSK-3, and FoxO1. Many growth factors and cytokines induce anti-apoptotic Bcl-2 family members. The Jaks and Src phosphorylate and activate Stat3, which in turn induces the expression of Bcl-xL and Bcl-2. Erk1/2 and PKC activate p90RSK, which activates CREB and induces the expression of Bcl-xL and Bcl-2. These Bcl-2 family members protect the integrity of mitochondria, preventing cytochrome c release and the subsequent activation of caspase-9. TNF-α may activate both pro-apoptotic and anti-apoptotic pathways; TNF-α can induce apoptosis by activating caspase-8 and -10, but can also inhibit apoptosis via NF-κB, which induces the expression of anti-apoptotic genes such as Bcl-2. cIAP1/2 inhibit TNF-α signaling by binding to TRAF2. FLIP inhibits the activation of caspase-8.

Select Reviews: Brunelli, G., Salmanidis, M., and Ekert, P.G. (2010) *Cell. Mol. Life Sci.* 67, 1619–1630. | Fuchs, Y. and Steller H. (2011) *Cell* 147, 742–758. | Fulda, S. and Vucic, D. (2012) *Nat. Rev. Drug Discov.* 11, 109–124. | Kaufmann, T., Strasser, A., and Jost, P.J. (2012) *Cell Death Differ.* 19, 42–50. | Lopez, J. and Meier, P. (2010) *Curr. Opin. Cell Biol.* 22, 872–881. | Rong, Y. and Distelhorst, C.W. (2008) *Annu. Rev. Physiol.* 70, 73–91. | Srinivasula, S.M. and Ashwell, J.D. (2008) *Mol. Cell* 30, 123–135. | Zheng, X., Tang, N., Hadden, T.J., and Rishi, A.K. (2011) *Biochim. Biophys. Acta.* 1813, 1978–1986.

We would like to thank Prof. Junying Yuan, Harvard Medical School, Boston, MA, for reviewing this diagram.

Death Receptor Signaling

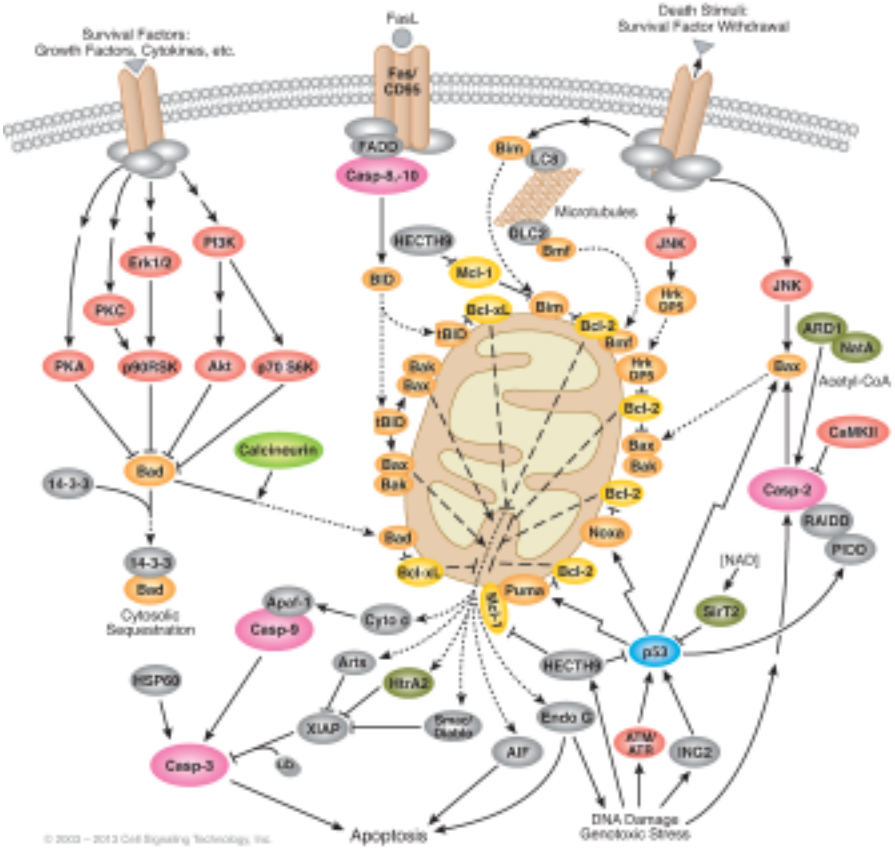


Apoptosis can be induced through the activation of death receptors including Fas, TNFαR, DR3, DR4, and DR5 by their respective ligands. Death receptor ligands characteristically initiate signaling via receptor oligomerization, which in turn results in the recruitment of specialized adaptor proteins and activation of caspase cascades. Binding of FasL induces Fas trimerization, which recruits initiator caspase-8 via the adaptor protein FADD. Caspase-8 then oligomerizes and is activated via autocatalysis. Activated caspase-8 stimulates apoptosis via two parallel cascades: it can directly cleave and activate caspase-3, or alternatively, it can cleave Bid, a pro-apoptotic Bcl-2 family protein. Truncated Bid (tBid) translocates to mitochondria, inducing cytochrome c release, which sequentially activates caspase-9 and -3. TNF-α and DR-3L can deliver pro- or anti-apoptotic signals. TNFαR and DR3 promote apoptosis via the adaptor proteins TRADD/FADD and the activation of caspase-8. Interaction of TNF-α with TNFαR may activate the NF-κB pathway via NIK/IKK. The activation of NF-κB induces the expression of pro-survival genes including Bcl-2 and FLIP, the latter can directly inhibit the activation of caspase-8. FasL and TNF-α may also activate JNK via ASK1/MKK7. Activation of JNK may lead to the inhibition of Bcl-2 by phosphorylation. In the absence of caspase activation, stimulation of death receptors can lead to the activation of an alternative programmed cell death pathway termed necroptosis by forming complex IIb.

Select Reviews: Declercq, W., Vanden Berghe, T., and Vandenabeele, P. (2009) *Cell* 138, 229–232. | Fuchs, Y. and Steller H. (2011) *Cell* 147, 742–758. | Kantari, C. and Walczak, H. (2011) *Biochim. Biophys. Acta.* 1813, 558–563. | Kaufmann, T., Strasser, A., and Jost, P.J. (2012) *Cell Death Differ.* 19, 42–50. | Lavrik, I.N. and Krammer, P.H. (2012) *Cell Death Differ.* 19, 36–41. | Van Herreweghe, F., Festjens, N., Declercq, W., and Vandenabeele, P. (2010) *Cell. Mol. Life Sci.* 67, 1567–79. | Wajant, H. and Scheurich, P. (2011) *FEBS J.* 278, 862–876.

We would like to thank Prof. Junying Yuan, Harvard Medical School, Boston, MA, for reviewing this diagram.

Mitochondrial Control of Apoptosis

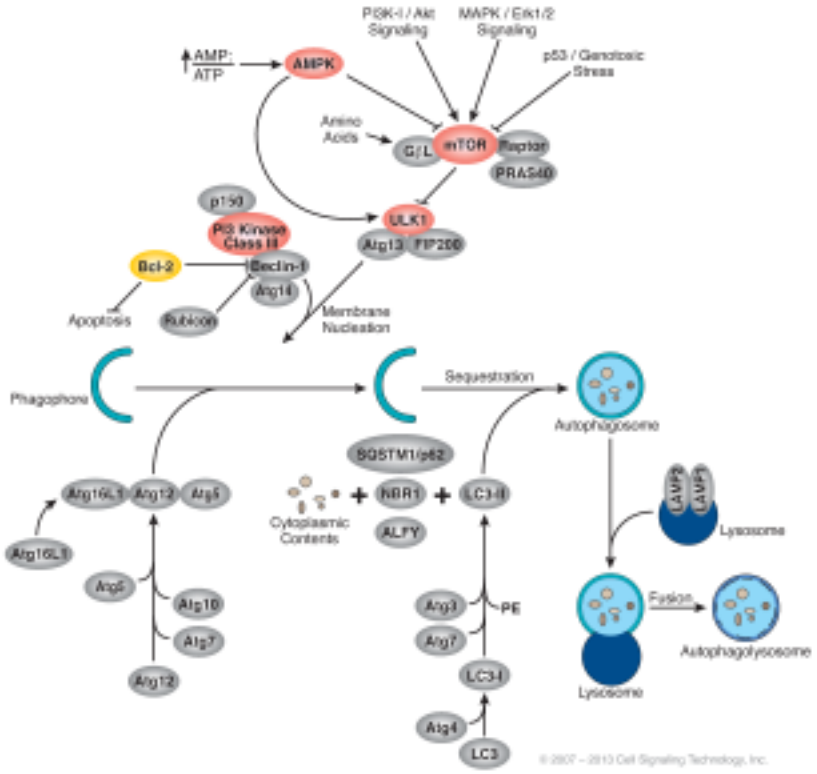


The Bcl-2 family of proteins regulate apoptosis by controlling mitochondrial permeability. The anti-apoptotic proteins Bcl-2 and Bcl-xL reside in the outer mitochondrial wall and inhibit cytochrome c release. The proapoptotic Bcl-2 proteins Bad, Bid, Bax, and Bim may reside in the cytosol but translocate to mitochondria following death signaling, where they promote the release of cytochrome c. Bad translocates to mitochondria and forms a pro-apoptotic complex with Bcl-xL. This translocation is inhibited by survival factors that induce the phosphorylation of Bad, leading to its cytosolic sequestration. Cytosolic Bid is cleaved by caspase-8 following signaling through Fas; its active fragment (tBid) translocates to mitochondria. Bax and Bim translocate to mitochondria in response to death stimuli, including survival factor withdrawal. Activated following DNA damage, p53 induces the transcription of Bax, Noxa, and PUMA. Upon release from mitochondria, cytochrome c binds to Apaf-1 and forms an activation complex with caspase-9. Although the mechanism(s) regulating mitochondrial permeability and the release of cytochrome c during apoptosis are not fully understood, Bcl-xL, Bcl-2, and Bax may influence the voltage-dependent anion channel (VDAC), which may play a role in regulating cytochrome c release. Mule/ARF-BP1 is a DNA damage-activated E3 ubiquitin ligase for p53, and Mcl-1, an anti-apoptotic member of Bcl-2.

Select Reviews: Brenner, D. and Mak, T.W. (2009) *Curr. Opin. Cell Biol.* 21, 871–877. | Chalah, A., Khosravi-Far, R. (2008) *Adv. Exp. Med. Biol.* 615, 25–45. | Lindsay, J., Esposti, M.D., and Gilmore, A.P. (2011) *Biochim. Biophys. Acta.* 1813, 532–539. | Ola, M.S., Nawaz, M., and Ahsan, H. (2011) *Mol. Cell. Biochem.* 351, 41–58. | Pradelli, L.A., Bénéteau, M., and Ricci, J.E. (2010) *Cell. Mol. Life Sci.* 67, 1589–1597. | Rong, Y. and Distelhorst, C.W. (2008) *Annu. Rev. Physiol.* 70, 73–91. | Speidel, D. (2010) *Trends Cell Biol.* 20, 14–24. | Swan, D.F., Norris, K.L., and Youle, R.J. (2008) *Genes Dev.* 22, 1577–1590.

We would like to thank Prof. Junying Yuan, Harvard Medical School, Boston, MA, for reviewing this diagram.

Kinase | Transcription Factor | Caspase | Enzyme | GAP/GEF | G-protein | Acetylase
Phosphatase | pro-apoptotic | Receptor | pro-survival | GTPase | Ribosomal subunit | Deacetylase

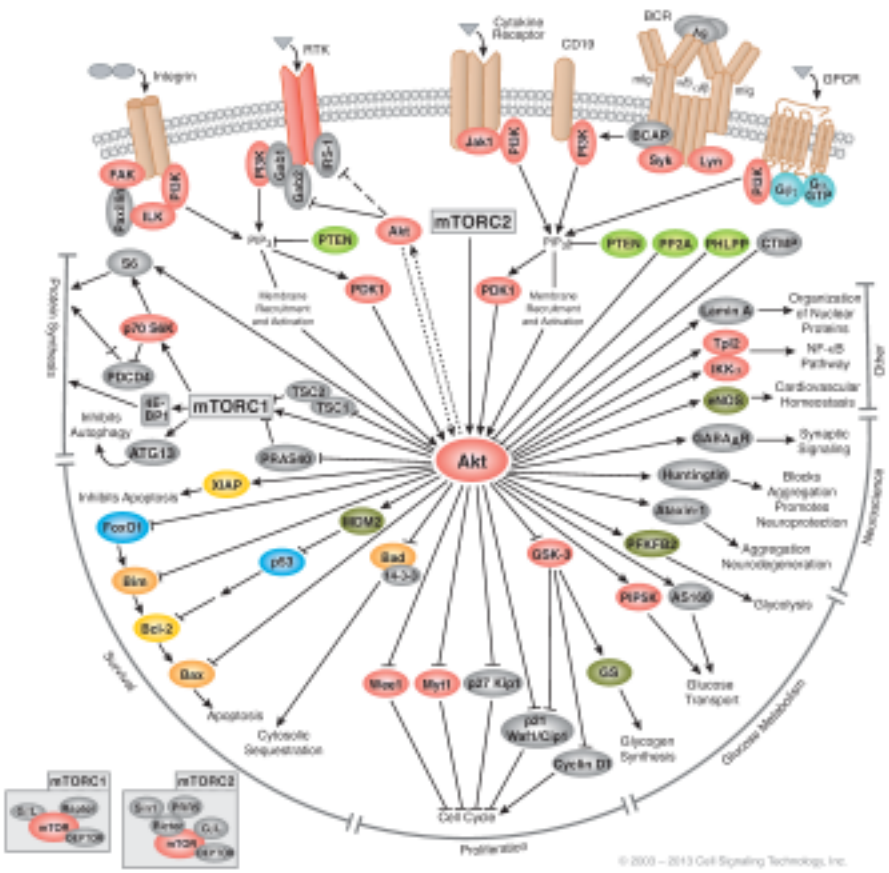


Autophagy Signaling

Macroautophagy, often referred to as autophagy, is a catabolic process that results in the autophagosomic-lysosomal degradation of bulk cytoplasmic contents, abnormal protein aggregates, and excess or damaged organelles. Autophagy is generally activated by conditions of nutrient deprivation but has also been associated with physiological as well as pathological processes such as development, differentiation, neurodegenerative diseases, stress, infection, and cancer. The kinase mTOR is a critical regulator of autophagy induction, with activated mTOR (Akt and MAPK signaling) suppressing autophagy, and negative regulation of mTOR (AMPK and p53 signaling) promoting it. Three related serine/threonine kinases, UNC-51-like kinase -1, -2, and -3 (ULK1, ULK2, ULK3), which play a similar role as the yeast Atg1, act downstream of the mTOR complex. ULK1 and ULK2 form a large complex with the mammalian homolog of an autophagy-related (Atg) gene product (mAtg13) and the scaffold protein FIP200 (an ortholog of yeast Atg17). Class III PI3K complex, containing hVps34, Beclin-1 (a mammalian homolog of yeast Atg6), p150 (a mammalian homolog of yeast Vps15), and Atg14-like protein (Atg14L or Barkor) or ultraviolet irradiation resistance-associated gene (UVRAG), is required for the induction of autophagy. The Atg genes control the autophagosome formation through Atg12-Atg5 and LC3-II (Atg8-II) complexes. Atg12 is conjugated to Atg5 in a ubiquitin-like reaction that requires Atg7 and Atg10 (E1 and E2-like enzymes, respectively). The Atg12–Atg5 conjugate then interacts noncovalently with Atg16 to form a large complex. LC3/Atg8 is cleaved at its C-terminus by Atg4 protease to generate the cytosolic LC3-I. LC3-I is conjugated to phosphatidylethanolamine (PE) also in a ubiquitin-like reaction that requires Atg7 and Atg3 (E1 and E2-like enzymes, respectively). The lipidated form of LC3, known as LC3-II, is attached to the autophagosome membrane. Autophagy and apoptosis are connected both positively and negatively, and extensive crosstalk exists between the two. During nutrient deficiency, autophagy functions as a pro-survival mechanism; however, excessive autophagy may lead to cell death, a process morphologically distinct from apoptosis. Several pro-apoptotic signals, such as TNF, TRAIL, and FADD, also induce autophagy. Additionally, Bcl-2 inhibits Beclin-1-dependent autophagy, thereby functioning both as a pro-survival and as an anti-autophagic regulator.

Select Reviews: Alers, S., Löffler, A.S., Wesselborg, S., and Stork, B. (2012) *Mol. Cell Biol.* 32, 2–11. | Codogno, P., Mehrpour, M., and Proikas-Cezanne, T. (2011) *Nat. Rev. Mol. Cell Biol.* 13, 7–12. | Levine, B. and Kroemer, G. (2008) *Cell* 132, 27–42. | Mizushima, N. and Komatsu, M. (2011) *Cell* 147, 728–741. | Mizushima, N., Levine, B., Cuervo, A.M., and Klionsky, D.J. (2008) *Nature* 451, 1069–1075. | Yang, Z. and Klionsky, D.J. (2010) *Curr. Opin. Cell Biol.* 22, 124–131.

We would like to thank Prof. Bingren Hu, University of Maryland School of Medicine, Baltimore, MD, for reviewing this diagram.



PI3 Kinase/Akt Signaling

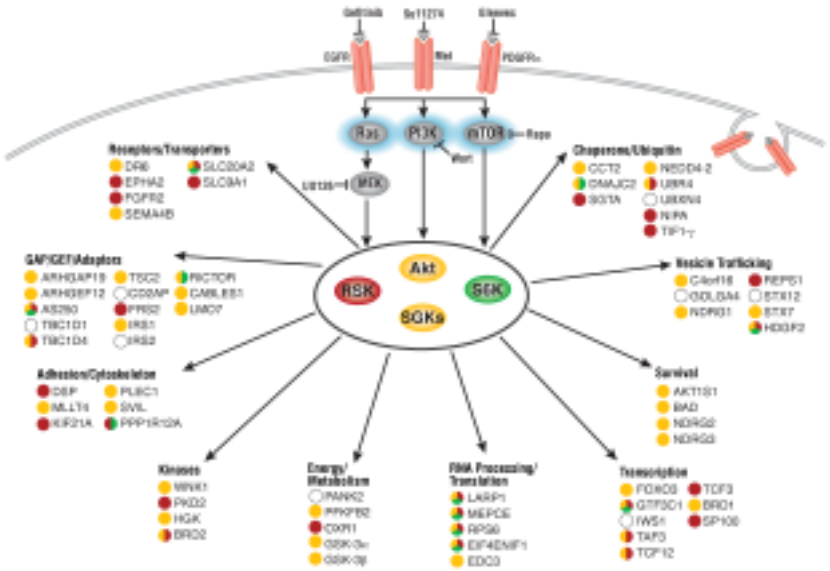
Since its initial discovery as a proto-oncogene, the serine/threonine kinase Akt (also known as protein kinase B or PKB) has become a major focus of attention because of its critical regulatory role in diverse cellular processes, including cancer progression and insulin metabolism. The Akt cascade is activated by receptor tyrosine kinases, integrins, B and T cell receptors, cytokine receptors, G-protein-coupled receptors and other stimuli that induce the production of phosphatidylinositol 3,4,5 triphosphates (PtdIns(3,4,5)P3) by phosphoinositide 3-kinase (PI3K). These lipids serve as plasma membrane docking sites for proteins that harbor pleckstrin-homology (PH) domains, including Akt and its upstream activator PDK1. The tumor suppressor PTEN is recognized as a major inhibitor of Akt and is frequently lost in human tumors. Recently, there has been increased focus on phosphatases that can inactivate Akt, including PHLLP.

There are three highly related isoforms of Akt (Akt1, Akt2, and Akt3) and these represent the major signaling arm of PI3K. For example, Akt is important for insulin signaling and glucose metabolism, with genetic studies in mice revealing a central role for Akt2 in these processes. In addition, germline mutations of Akt have been identified in pathological conditions of cancer and insulin metabolism.

Akt regulates cell growth through its effects on the TSC1/TSC2 complex and mTOR pathways, as well as cell cycle and cell proliferation through its direct action on the CDK inhibitors p21 and p27, and its indirect effect on the levels of cyclin D1 and p53. Akt is a major mediator of cell survival through direct inhibition of pro-apoptotic signals such as the pro-apoptotic regulator Bad and the FoxO and Myc family of transcription factors. T lymphocyte trafficking to lymphoid tissues is controlled by the expression of adhesion factors downstream of Akt. In addition, Akt has been shown to regulate proteins involved in neuronal function including GABA receptor, sirtuin-1, and huntingtin proteins. Akt has been demonstrated to interact with Smad molecules to regulate TGF-β signaling. Finally, lamin A phosphorylation by Akt could play a role in the structural organization of nuclear proteins. These findings make Akt/PKB an important therapeutic target for the treatment of cancer, diabetes, laminopathies, stroke, and neurodegenerative disease.

For selected reviews see www.cellsignal.com

We would like to thank Prof. Michael Scheid, York University of Toronto, Ontario, for reviewing this diagram.



Akt Substrates Table

MAPK, mTOR, and the PI3K/Akt pathways are key signaling pathways activated downstream of oncogenic receptor tyrosine kinases (RTKs). All of these pathways activate AGC kinase family members, including Akt, RSK, and p70 S6 kinases, whose protein substrates are phosphorylated at the RxRxxS/T motif.

In a phosphoproteomic study co-authored by scientists in the Cell Signaling Technology (CST) Site Discovery Group (Sci. Signal. (2010) 24;3(136):ra84), over 300 novel downstream substrates for these AGC family kinases were identified. The experimental approach involved the use of PhosphoScan®, CST's proprietary methodology for antibody-based peptide enrichment combined with tandem mass spectrometry for quantitative profiling of post-translational modifications. A key step was the development of a RxRxxS/T motif antibody, which was then used as an affinity reagent to selectively immunoprecipitate phosphorylated substrates of Akt, RSK, and p70 S6 kinases. The antibody was employed in PhosphoScan in three different cancer cell lines, dependent on either EGFR, c-Met, or PDGFR, allowing mapping of the signaling network downstream of these RTKs. Substrates included proteins involved in many cellular functions, including scaffolding, protein stability, metabolism, trafficking, and motility (see figure).

For more information, visit PhosphoSitePlus®, CST's manually curated post-translational modification resource available at www.phosphosite.org, where all information on the observed substrate phosphorylation has been made available.

● = wortmannin + RTK inhibitor ● = rapamycin + RTK inhibitor
● = MEK inhibitor + RTK inhibitor ○ = RTK inhibitor only

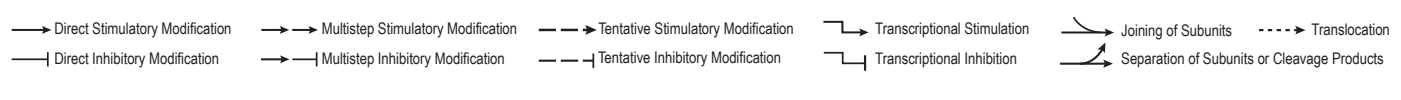
Akt Substrates Table

Substrate	Isoform	Organism	Site	Human Site	Sequence (+/-7)	PMID	Substrate Function and Effect of Phosphorylation
14-3-3 ζ	Akt1	human	S58	S58	VAGARRSsWRVVssI	11956222	a key regulatory protein in signal transduction, checkpoint control, apoptotic, and nutrient-sensing pathways; effect of phosphorylation is unknown
acinus	Akt1	human	S1180	S1180	GPRsRsRsRDRRRKE	18559500, 16177823	induces chromatin condensation during apoptosis; phosphorylation inhibits this process
	Akt1	rat	S1329	S1331	HSRSRSRsTPVRDRG	16177823	induces chromatin condensation during apoptosis; phosphorylation inhibits this process
ACLY	Akt1	mouse	S455	S455	PAPSRtAeFsESRAD	16007201	catalyzes the formation of acetyl-CoA and oxaloacetate (OAA) in the cytosol; phosphorylation enhances the catalytic activity of the enzyme
ADRB2	Akt1	human	S346	S346	LLCLRRssLKAyGNG	11809767	a receptor that binds epinephrine and norepinephrine, acting as a neuromodulator in the central nervous system and as a hormone in the vascular system; phosphorylation in response to insulin stimulation leads to sequestration of ADBR2
Akt1	Akt1	human	S246, T72	S246, T72	LSRERMFsEDRARFY, TERPRPMtRIRCLQ	16549426	activated by insulin and various growth and survival factors to function in a wortmannin-sensitive PO kinase-involved pathway controlling survival and apoptosis; autophosphorylation activates the kinase
	Akt1	mouse	S473	S473	RPHFPQFsYSAsGtA	11570877, 10722653	activated by insulin and various growth and survival factors to function in a wortmannin-sensitive PO kinase-involved pathway controlling survival and apoptosis; autophosphorylation activates the kinase
AMPKA1	Akt1	rat	S485	S485	ATPQRSGdSNVRSC	16340011	heterotrimeric complex that plays a key role in the regulation of energy homeostasis; phosphorylation regulates AMPK activity
AMPKA2	Akt1	rat	S491	S491	STPQRSDsAAGLHRP	16340011	heterotrimeric complex that plays a key role in the regulation of energy homeostasis; phosphorylation regulates AMPK activity
APS	Akt1	rat	S588	S598	SARSRSNsTEHLLEA	16141217	an adaptor protein recruited to the insulin receptor to signal insulin-stimulated glucose transport; phosphorylation promotes membrane localization
AR	Akt1	human	S213, S791	S213, S791	SGRAREAsGAPTsSK, CVRMRHLsQEFGWLQ	11404460, 14555644, 17470458, 11156376	nuclear receptor; phosphorylation suppresses AR activation, expression of AR target genes, and AR-mediated apoptosis
arfaptin 2	Akt1	human	S260	S260	GTRGRLEsADATFQA	15809304	ADP ribosylation factor-interacting protein, implicated as a factor in Huntington's disease; phosphorylation promotes neuronal cell survival and neuroprotection
ARHGAP22	Akt1	human	S16	S16	ARRARSKsLVMGEQS	21969604	a Rho GTPase activator that inhibits Rac1; phosphorylation allows 14-3-3 binding and regulation of cell motility
AS160	Akt1	human	T642	T642	QFRRRAHFsHPPss	16880201, 11994271, 16935857	insulin stimulated Rab GTPase-activating protein, structurally and functionally similar to TBC1D1; phosphorylation results in increased Glut4 translocation
ASK1	Akt1, Akt2	human	S83	S83	ATRGRGssVGGGSRR	11154276, 15782121, 15911620, 14500571, 12697749	MAPKKK, induces apoptosis via JNK pathway; phosphorylation inhibits activity and promotes survival
ataxin-1	Akt1	human	S775	S775	ATRKRRWsAPESRKL	17540006, 12757707	14-3-3 binds to and stabilizes ataxin-1, which forms polyglutamine aggregates and neurodegeneration; phosphorylation promotes 14-3-3 binding
B-Raf	Akt1, Akt3	human	S365, S429	S365, S429	GQRDRssAPNVHIN, PQREPKsasSsEDRN	10869358, 18451171	signaling intermediate in Erk1/2 pathway; phosphorylation causes inhibition
BAD	Akt1	human	S99	S99	PFRGRsRsAPPNLWA	11020382, 10558890, 19667065	pro-apoptotic protein; phosphorylation inhibits function and promotes survival
	Akt1	mouse	S112, S155	S75, S118	ETRsRHsayPAGEE, GRELRRMsDEFEGSF	9381178, 11723239, 10883986, 15123689, 10949026	pro-apoptotic protein; phosphorylation inhibits function and promotes survival
Bcl-10	Akt1	human	S218, S231	S218, S231	EEGTCANsSEMFLPL, PLRSRNVsRQ_____	16260327	a CARD (caspase recruitment domain) containing protein shown to induce apoptosis and activate NF-κB; phosphorylation induces nuclear translocation
Bcl-xL	Akt1	rat	S106	S106	LRYRRAFsDLTSQLH	18951975	prevents apoptosis through binding to apoptotic proteins; phosphorylation promotes VDAC binding
Bex1	Akt1	rat	S105	S102	KLRERQLsHSLRAVS	16408402	a neurotrophin and nerve growth factor signaling adaptor molecule involved in promoting cell cycle progression; phosphorylation prevents degradation by the proteasome
Bim	Akt1	human	S87	S87	FIFMRRssLLSRSss	16262323	pro-apoptotic protein; phosphorylation promotes 14-3-3 binding/inactivation and cell survival

Kinase · Transcription Factor · Caspase · Enzyme · GAP/GEF · G-protein · Acetylase
Phosphatase · pro-apoptotic · Receptor · pro-survival · GTPase · Ribosomal subunit · Deacetylase

Akt Substrates Table

Substrate	Isoform	Organism	Site	Human Site	Sequence (+/-7)	PMID	Substrate Function and Effect of Phosphorylation
BRCA1	Akt1	human	S694, T509	S694, T509	QTSKRHDsDTFPELK, LKRKRRPtsGLHPED	20085797, 10542266	breast cancer susceptibility gene product, tumor suppressor; phosphorylation alters function, perhaps by preventing nuclear localization
BRF1	Akt1	human	S92, S203	S92, S203	RFRDRsFsEGGERLL, PRLQHsFsFAGFPSA	17030608, 15538381	a CCCH zinc-finger protein that binds to AU-rich elements (ARE) found in the 3'-untranslated regions of mRNAs and promotes de-adenylation and rapid degradation by the exosome; phosphorylation results in binding by 14-3-3 protein and inactivation of BRF1
CACNB2	Akt1	rat	S625	S630	KQRSRHKsKDRYCDK	15311280	voltage-dependent calcium channel; phosphorylation regulates channel trafficking to plasma membrane
CaRHSP1	Akt1	human	S52	S52	tRRtRtFsAtVRASQ	15910284	RNA binding protein; phosphorylation effect currently unknown
Casp9	Akt1	human	S196	S196	KLRRRFssLHFMVEV	9812896	protease, initiates apoptosis; phosphorylation inhibits protease activity
CBP	Akt1	mouse	T1872	T1871	LMRRRMAtMNTRNVP	17166829	acetylates histone and non-histone proteins; phosphorylation increases activity
CBY1	Akt1, Akt2	human	S20	S20	TPPRKSAsLSNLHsL	18573912	an inhibitor of the Wnt signaling pathway; phosphorylation allows 14-3-3 binding and β-catenin sequestration in the cytoplasm
CCT2	Akt1	human	S260	S260	GsRVRVDstAkVAEI	19332537	member of the protein chaperone complex; effect of phosphorylation currently unknown
CD34	Akt2	mouse	S343	S346	yssGPGAsPETQGKA	21499536	a type I transmembrane glycophosphoprotein expressed by hematopoietic stem/progenitor cells, vascular endothelium and some fibroblasts as a negative regulator of cell adhesion; effect of phosphorylation currently unknown
Cdc25B	Akt1	mouse	S351	S353	VQSKRRKsVtPLEEQ	17554083	protein phosphatase responsible for cdc2 activation; phosphorylation promotes activation of M-phase promoting factor
CDK2	Akt1	human	T39	T39	LkKIRLDtETEGVPs	18354084	cyclin-dependent kinase functioning in S-phase; phosphorylation increases cyclin A binding
CELF1	Akt1	human	S28	S28	GQVPRTWsEKDLREL	18570922	RNA-binding protein; phosphorylation enhances interaction with cyclin D1 mRNA
CENTB1	Akt1	human	S554	S554	SIRPRPGsLRSKPEP	16256741	GTPase-activating protein (GAP) for ARF proteins; phosphorylation prevents recycling of b1-integrin containing endosomes and cell migration
CENTG1	Akt1	human	S985	S985	THLSRVRsLDLDDWP	19176382	a GTPase activating protein for ARF1 and ARF5; phosphorylation enhances CENTG1 GTP binding and NF-κB activity
CFLAR	Akt1	human	S273	S273	LLRDTFTsLGYEVQK	19339247	a regulator of apoptosis; phosphorylation targets CFLAR for degradation
Chk1	Akt1	human	S280	S280	AKRPRVtsGGVsEsP	15107605, 12062056	DNA damage effector that regulates G2/M transition during DNA damage; phosphorylation inhibits function by preventing phosphorylation by ATM/ATR
CK1-D	Akt1	rat	S370	S370	MERERKVsMRLHRGA	17594292	kinase and core component of circadian clock; phosphorylation inhibits kinase activity
CLK2	Akt1	human	S34, T127	S34, T127	HKRRRSRsWSSSSDR, RRRRRSRtFSRSSSQ	20682768	a dual specificity serine/threonine and tyrosine kinase; phosphorylation increases cell survival after ionizing radiation
Cot	Akt1	human	S400	S400	EDQPRCQsLDSALLE	12138205	oncogene; phosphorylation induces NF-κB-dependent transcription
CREB	Akt1	rat	S133	S133	EILsRRPsYRkILND	9829964	bZIP transcription factor that activates target genes through cAMP response elements; activated by phosphorylation
CTNNB1	Akt1, Akt2	human	S552	S552	QDtQRRtsMGGtQQQ	17287208	Wnt signaling pathway protein; phosphorylation causes nuclear localization
CTNND2	Akt1	mouse	T454	T457	tGTFRtstAPssPGV	17993462	transcriptional activator, plays a role in adhesion molecule regulation; phosphorylation promotes binding to p190RhoGEF, dendritic morphogenesis
Cx43	Akt1	rat	S369	S369	RPssRAssRAssRPR	18163231	gap junction protein; phosphorylation allows 14-3-3 binding
	Akt1, Akt3	rat	S373	S373	RAssRAssRPRPDDL	17008717, 18163231	gap junction protein; phosphorylation allows 14-3-3 binding
DLC1	Akt1	rat	S330	S766	VTRTRSLsTCNKRVG	16338927	tumor suppressor and insulin stimulated phosphoprotein, may play role in Glut4 translocation; phosphorylation may inhibit its GAP activity
DNAJC5	Akt1	rat	S10	S10	DQRQRsLsTSGESLY	16243840	exocytosis; phosphorylation regulates the kinetics of late stage exocytosis
DNMT1	Akt1	human	S143	S143	RtPRRsksDGEAkPE	21151116	a maintenance methyltransferase, transferring proper methylation patterns to newly synthesized DNA during replication; phosphorylation increases DNMT1 stability and prevents methylation
EDC3	Akt1, Akt2	human	S161	S161	sFRRRHNsWssSsRH	20051463	involved in removal of the mRNA 5' cap structure; phosphorylation induces 14-3-3 protein interaction and promotes ED3 mediated post-transcriptional regulation through mRNA
EDG-1	Akt1	human	T236	T236	RTRSRRLtFRKNISK	11583630	G protein-coupled receptor; phosphorylation activates signaling to promote cell migration
eIF4B	Akt1	mouse	S422	S422	RERsRtGsEssQtGA	18836482	necessary for binding of mRNA to ribosomes; phosphorylation increases transcriptional activity
ENaC-α	Akt1	rat	S621	S594	RFRSRYWsPGRGARG	21220922	an amiloride sensitive epithelial sodium channel that mediates sodium reabsorption; phosphorylation increases ENaC specific activity
eNOS	Akt1	human	S615, S1177	S615, S1177	SYKIRFNsISCSDPL, TsRIRtQsFsLQERQ	12511559, 12446767, 10376603, 18622039, 12171920	enzyme that catalyzes the production of nitric oxide (NO); phosphorylation results in enzyme activation, NO production, and cardiovascular homeostasis (vasodilation, vascular remodeling, angiogenesis)
EphA2	Akt1	human	S897	S897	RVsIRLPstsGsEGV	19573808	receptor tyrosine kinase that binds to a GPI-anchored ephrin A ligand for regulation of cell adhesion, cell migration, axon guidance, and homeostasis; phosphorylation regulates EphA2 induced cell migration and invasion
ER-α	Akt1, Akt2	human	S167	S167	GGRERLAsTNDKGSM	11139588, 16113102, 11507039	nuclear receptor and transcription factor; phosphorylation activates the receptor and increases gene expression, causing mammary and uterine cell proliferation
	Akt1	human	S305	S305	IkRSkkNsLALSLtA	20101208	nuclear receptor and transcription factor; phosphorylation activates the receptor and increases gene expression, causing mammary and uterine cell proliferation
ER-β	Akt1	mouse	S236	D236	VRRQRSAsEQVHCLN	17166829	nuclear receptor and transcription factor; phosphorylation prevents cofactor binding and decreases activity
EZH2	Akt1	human	S21	S21	CWRKRVKsEYMRLRQ	16224021	methyltransferase; phosphorylation decreases histone H3 methylation of Lys27 and increases gene expression

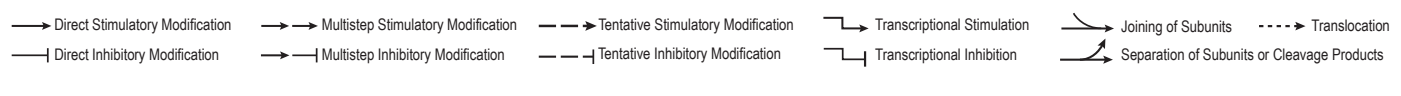


Akt Substrates Table

Substrate	Isoform	Organism	Site	Human Site	Sequence (+/-7)	PMID	Substrate Function and Effect of Phosphorylation
ezrin	Akt2	human	T567	T567	QGRDKYKtLRQIRQG	15531580	plasma membrane/cytoskeletal linker protein; phosphorylation promotes actin binding and cytoskeletal organization
FANCA	Akt1	human	S1149	S1149	CLRSRDPsLMVDFIL	11855836	ATPase involved in DNA repair; phosphorylation is negatively regulated by Akt
FLEG1	Akt1	human	S486	S486	GLEtRRLsLPSsKAK	17256767	a chaperone protein involved in directing specific histones to the centromere; phosphorylation allows binding to 14-3-3
FLNC	Akt1, Akt2	human	S2233	S2233	LGRERLGsFGsItRQ	15461588	muscle-specific filamin functioning in muscle cells; phosphorylation effect currently unknown
FOXA2	Akt1	human	T156	T156	KTYRRSYtHAKPPYS	14500912	transcription factor involved in embryonic development and differentiation; phosphorylation results in nuclear exclusion and inhibition of FoxA2-dependent transcriptional activity
FOXG1	Akt1	human	T279	T279	KLRRRSttsRAKLAF	17435750	transcriptional repression factor involved in brain development; phosphorylation promotes nuclear export
FOXO1A	Akt1	human	S256, S319, T24	S256, S319, T24	sPrRrAAsMDNNSkF, TFRPRtssNAsTIsG, LPRPRSCtWPLPRPE	15668399, 10358075, 11237865, 16076959, 11311120	transcription factor involved in cell cycle arrest, apoptosis, and glucose metabolism; phosphorylation causes export from the nucleus and inhibits activity
FOXO3A	Akt1	human	S253, T32	S253, T32	APRRRAVsMDNSNKY, QSRPRsCtWPLQRPE	10910908, 10995739, 10102273, 11154281	transcription factor involved in cell cycle arrest and apoptosis; phosphorylation causes export from the nucleus and inhibits activity
FOXO4	Akt1	human	S197, S262, T32	S197, S262, T32	APRRRAAsMDSSSKL, TFRPRSssNASSVST, QSRPRsCtWPLPRPE	11313479, 11313479, 10217147, 16272144	transcription factor involved in cell cycle arrest, apoptosis, and insulin signaling; phosphorylation causes export from the nucleus and inhibits activity
Gab2	Akt1	human	S159	S159	LLRERKSsAPSHsSQ	11782427	docking/scaffolding protein, proto-oncogene, RTK signaling intermediate; phosphorylation inhibits activity
GABRB2	Akt1	rat	S434	S434	SRLRRRAsQLKITIP	12818177	receptor that mediates fast inhibitory synaptic transmission in the brain; phosphorylation increases the number of receptors on the cell surface
GAPDH	Akt2	human	T237	T237	GMAFRVPtANVSVVD	21979951	catalyzes the phosphorylation of glyceraldehyde-3-phosphate during glycolysis; phosphorylation decreases nuclear translocation and GAPDH induced apoptosis
GATA1	Akt1	human	S310	S310	QTRNRKAsGkGkkkR	16107690	transcription factor; phosphorylation increases activity and promotes blood cell differentiation
GATA2	Akt1	human	S401	S401	QTRNRKMsNKSKKSK	15837948	transcription factor; phosphorylation inhibits activity to promote adipogenesis and reduce inflammation
girdin	Akt1	human	S1417	S1417	INRERQKsLtLTPTR	16139227	actin binding protein; phosphorylation promotes cell migration
GOLGA3	Akt1	mouse	S174, S385	S174, S389	VKRHRERsSQPAtKM, EVRsRRDsICsSVSM	17888676	golgi auto-antigen; phosphorylation results in reduced apoptosis
Grb10	Akt1	mouse	S455	S428	NAPMRsVsENsLVAM	15722337	an adaptor protein that interacts with many receptor tyrosine kinases as well as downstream signal molecules; phosphorylation allows binding to 14-3-3
GSK3α	Akt1	human	S21	S21	SGRARtssFAEPGGG	11340086, 11563975, 11577096	serine/threonine protein kinase that phosphorylates and inactivates glycogen synthase; phosphorylation inhibits activity
GSK3β	Akt1	human	S9	S9	SGRPRttsFAESCKP	12900420, 15457186, 11563975, 11340086, 11577096, 8985174	serine/threonine protein kinase that phosphorylates and inactivates glycogen synthase; phosphorylation inhibits activity
H2B	Akt1	human	S37	S37	RKRsRkEsyslyVyk	8985174	core component of the nucleosome; phosphorylation effect currently unknown
H3	Akt1	mouse	S10	S10	tKQTARksTGGkAPR	12529330	core component of the nucleosome; phosphorylation is correlated with chromosome condensation during mitosis and meiosis
HMOX1	Akt1	human	S188	S188	LYRSRMNsLEMtPAV	15581622	heme oxygenase involved in stress response; phosphorylation regulates binding affinity
hnRNP A1	Akt1	human	S199	S199	sQrGrsGsGNFGGGr	18562319	involved in pre-mRNA packaging into hnRNP particles and transport of poly(A) mRNA from cytoplasm to nucleus; phosphorylation regulates role in cyclin D1 and c-Myc IRES activity
hnRNP E1	Akt1, Akt2	mouse	S43	S43	VKRIREEsGARINIS	20154680	binds to single-stranded nucleic acid; phosphorylation results in disruption of BAT element binding and translational activation of Dab2 and ILEI mRNA
HSP27	Akt1	human	S82	S82	RALsRQLssGVSEIR	12740362	heat shock protein that confers cellular resistance to stress and adverse environmental change; phosphorylation alters tertiary structure, modulates actin polymerization, and reorganization
HTRA2	Akt1, Akt2	human	S212	S212	RVRVRLLsGDTYEAV	17311912	protease released during apoptosis; phosphorylation inhibits activity and attenuates its pro-apoptotic function
Huntingtin	Akt1	human	S421	S421	GGRsRsGsIVELIAG	12062094, 14725621, 15843398, 16452687	Huntington's disease; Akt phosphorylation blocks nuclear aggregation and provides neuroprotection
IKK-α	Akt1, Akt2	human	T23	T23	EMRERLGtGGFGNVC	18515365, 12048203, 10485710, 19609947	NF-κB signaling intermediate; phosphorylation activates NF-kB and immune/stress response
IP3R1	Akt1	rat	S2682	S2690	FPRMRAMsLVSSDSE	16332683	Ca^{2+} release and signaling; phosphorylation induces resistance to apoptosis, possibly through caspase-3 inactivation
IRAK1	Akt1	human	T100	T100	LRARDIItAWHPPAP	11976320	a serine/threonine-specific IL-1 receptor-associated kinase involved in Toll signaling; phosphorylation inhibits IRAK mediated NK-κB activation
IRS1	Akt1	human	S629	S629	VPSGRKGsGDyMPMs	17640984	insulin receptor signaling intermediate; phosphorylation inhibits function
	Akt1	rat	S522	S527	RFRKRTHsAGTSPTI	17579213	insulin receptor signaling intermediate; phosphorylation inhibits function
KHSRP	Akt1, Akt2	human	S193	S193	GLPERSVsLTGAPES	17177604	recruits degradation machinery, activates mRNA turnover, regulates splicing; phosphorylation inhibits RNA turnover by degradation
Kv11.1 iso5	Akt1	human	T897	T897	SFRRRtDtDtEQPGE	18791070	pore-forming subunit of voltage-gated potassium channels, essential for rhythmic excitability of cardiac muscle and endocrine cells; phosphorylation inhibits channels
lamin A/C	Akt1	rat	S301, S404	S301, S404	RSRGRASsHSSQSQG	18808171	component of nuclear lamina; phosphorylation regulates function of nuclear lamina
LTB4R2	Akt1	human	T355	T355	GGRsREGtMELRTTP	22044535	a low-affinity leukotriene receptor involved in chemotaxis; phosphorylation regulates activation of chemotactic responses

Akt Substrates Table

Substrate	Isoform	Organism	Site	Human Site	Sequence (+/-7)	PMID	Substrate Function and Effect of Phosphorylation
Mad1	Akt1	human	S145	S145	lERlRMDslGSTVSS	18451027, 19526459	component of spindle-assembly checkpoint; phosphorylation results in ubiquitination and degradation through 26S proteasome pathway
MDM2	Akt1	human	S166, S186, S188	S166, S186, S188	SsRRRAlsEtEENsD, RQRKRHKsDslsLsF, RKRHKsDslsLsFDE	11715018, 15169778, 11504915, 11850850, 11923280, 15527798, 11960368	ubiquitin ligase involved in p53 degradation; phosphorylation results in translocation to the nucleus and inhibition of p53
MDM4	Akt1	human	S367	S367	PDCRRtlsAPVVRPK	18356162	RING-finger domain protein involved in p53 degradation and apoptosis; phosphorylation stabilizes MDM4 and MDM2
METTL1	Akt1	human	S27	S27	yYRQrAHsNPMADHT	15861136	catalyzes the formation of m7G46 in tRNA; phosphorylation results in inactivation
MKK4	Akt1	human	S80	S80	lERLRtHslEsSGKL	15911620, 11707464	signaling intermediate of the JNK/SAPK pathway involved in stress/inflammation; phosphorylation inhibits activity
MLK3	Akt1	human	S674	S674	PGRERGEsPTtPPTP	12458207	JNK-mediated neuronal cell death; phosphorylation inhibits activity
MST1	Akt1	human	T120	T120	lIRLRNktLTEDEIA	19940129	pro-apoptotic kinase; phosphorylation inhibits kinase activity and nuclear translocation resulting in inhibition of pro-apoptotic signaling
MST2	Akt1	human	T117, T384	T117, T384	lIRLRNktLIEDEIA, GTMKRNAtsPQVQRP	20231902, 20086174	upstream activator of the MAPK pathway that regulates apoptosis, morphogenesis, and cytoskeletal rearrangements; phosphorylation inhibits pro-apoptotic activity
mTOR	Akt1	human	T2446, S2448	T2446, S2448	RsRtRtDsysAGQsV	15208671, 10910062, 10567225	protein synthesis and cell growth; phosphorylation increases activity
MYO5A	Akt2	mouse	S1650	S1652	GLRKRtsslADEGty	17515613	actin-based motor protein with a role in cytoplasmic vesicle transport and anchorage; phosphorylation promotes insulin-mediated Glut4 vesicle translocation
Myt1	Akt1	starfish	S75	S83	ESRPRAVsFRQSEPS	11802161	Wee1 family member and cell cycle regulator; phosphorylation downregulates Myt1 and initiates M-phase
NDRG2	Akt1	human	S332, T348	S332, T348	LsRsRtAsLtsAAsV, GNRsRsRtLsQssEs	15461589	insulin-stimulated phosphoprotein; phosphorylation promotes insulin signaling
NFAT90	Akt1	human	S647	S647	rGrGRGGslRGRGRG	18097023, 20870937	translation inhibitory protein; phosphorylation required for nuclear export
NHE1	Akt1	human	S648, S703, S796	S648, S703, S796	KTRQRLRsyNRHTLV, MsRARlGsDPLAyEP, QRlQRCLsDPGPHPE	18757828, 20026127	sodium/hydrogen exchanger involved in pH regulation and signal transduction; phosphorylation inhibits activity
NMDAR2C	Akt1	mouse	S1084	S1081	GPRPRHAsLPSSVAE	19477150	Glutamate receptor channel subunit; phosphorylation promotes binding to 14-3-3e and leads to increased surface expression of cerebellar NMDA receptors
	Akt1	rat	S1083	S1081	GPRPRHAsLPSSVAE	19477150	Glutamate receptor channel subunit; phosphorylation promotes binding to 14-3-3e and leads to increased surface expression of cerebellar NMDA receptors
NuaK1	Akt1	human	S600	S600	PARQRlRsCVSAENF	15060171, 12409306	AMPK family member activated under glucose starvation that mediates cell survival; phosphorylation increases kinase activity
Nur77	Akt1	human	S351	S351	GRRGRLPsKPKQPPD	16434970, 11274386	a nuclear receptor and transcription factor regulating T cell apoptosis; phosphorylation inhibits transcriptional activity
p21Cip1	Akt1	human	S146, T145	S146, T145	GRkRRQtsMTDFYHs, QGRkRRQtsMTDFYH	17855660, 11231573, 11756412, 15173090, 11463845, 116982699	regulates cell cycle and cell survival; phosphorylation increases protein stability
p27Kip1	Akt1	human	S10, T157, T198	S10, T157, T198	NVRVsNGsPsLErMD, GlRkrPAtDDSSTQN, PGLRRRQt_____	18710949, 12042314, 12244302	a cyclin-dependent kinase inhibitor that enforces the G1 cell cycle restriction point; phosphorylation promotes 14-3-3 binding and cytoplasmic localization
p300	Akt1	human	S1834	S1834	MLRRRMAsMQRTGVV	16024795, 11116148	transcriptional co-activator; phosphorylation can either activate or suppress transcriptional activity depending on cell type and physiological stimuli
p47phox	Akt1	human	S304, S328	S304, S328	GAPPRRsslRNAHSl, QDAYRRNsVRFLQQR	12734380	a component of the phagocytic NADPH oxidase multiprotein enzyme that catalyzes the reduction of oxygen to superoxide in response to pathogenic invasion; phosphorylation regulates p47hox respiratory burst activity
PAK1	Akt1	mouse	S21	S21	APPMRNTsTMIGAGS	14585966	a p21-activated kinase engaged in cytoskeletal reorganization, MAPK signaling, apoptotic signaling, control of phagocyte NADPH oxidase, and growth factor-induced neurite outgrowth; phosphorylation at Ser21 regulates binding with the adaptor protein Nck
palladin	Akt1	human	S1118	S1118	VRRPRsRsRDsGDEN	20471940	actin-bundling protein; phosphorylation promotes F-actin bundling and inhibits cell migration
PAR-4	Akt1	rat	S249	N257	SRHNRDTsAPANFAS	16209943	a pro-apoptotic factor that activates the Fas-FADD-caspase-8 pathway as well as inhibits the NF-κB pro-survival pathway; phosphorylation prevents nuclear translocation, promoting cell survival
PDCD4	Akt1	human	S67, S457	S67, S457	kRRLRKNssRDsGRG, RGRKRFVsEGDGGRL	16357133	tumor suppressor protein that is strongly induced during apoptosis; phosphorylation inhibits tumor suppressor function
PDE3A	Akt1	mouse	S290, S291, S292	S290, S291, S292	GWKRRRRsssVVAGE, WKRRRRsssVVAGEM, KRRRRsssVVAGEMS	17124499	regulates levels of cAMP and cGMP, insulin-dependent oocyte maturation; phosphorylation increases activity
PDE3B	Akt1	mouse	S273	S295	VlRPRRRssCVsLGE	10454575	regulates levels of cAMP and cGMP, activated by insulin to regulate lipolysis; phosphorylation increases activity
PEA-15	Akt1	human	S116	S116	KDIIRQPsEEEIIKL	12808093	a phosphoprotein shown to coordinate cell growth, death, and glucose utilization; phosphorylation mediates binding to FADD or Erk and further regulates the Erk and apoptosis signaling pathways
peripherin	Akt1	mouse	S66	S59	SSSARLGsFRAPRAG	17569669	neuronal intermediate filament protein; phosphorylation promotes motor nerve regeneration
PFKFB2	Akt1	human	S466, S483	S466, S483	PVRMRRNsFtPLSSS, IRRPRNysVGSRPLK	12853467	glycolytic enzyme, insulin-mediated glucose metabolism; phosphorylation increases activity
PFKFB3	Akt1	human	S461	S461	NPLMRRNsVtPLAsP	15896703	synthesis and degradation of fructose 2,6-bisphosphate; phosphorylation decreases sensitivity to inhibition



Akt Substrates Table

Substrate	Isoform	Organism	Site	Human Site	Sequence (+/-7)	PMID	Substrate Function and Effect of Phosphorylation
PGC-1 α	Akt1, Akt2	mouse	S570	S571	RMRSRsRsFsRHRSC	17554339	regulates gluconeogenesis and fatty acid oxidation; phosphorylation inhibits function
PIP5K	Akt1	human	S307	S307	PARNRsAsItNLsLD	15546921	a protein/ lipid kinase involved in membrane trafficking; phosphorylated in response to insulin
	Akt1	mouse	S105	S105	EELHRRSsVLENTLP	20513353	a protein/ lipid kinase involved in membrane trafficking; phosphorylated in response to insulin
PLB	Akt1	rat	S16	S16	RSAIRRAstIEMPQQ	18838385	a major phosphoprotein calcium regulation component of the sarcoplasmic reticulum; phosphorylation causes release of inhibition and increases calcium uptake by the sarcoplasmic reticulum
PLCG1	Akt1	human	S1248	S1248	HGRAREGsFEsRyQQ	16525023	catalyzes PI 4,5 bisphosphate to IP_3 and DAG, increases intracellular Ca^{2+} levels; phosphorylation increases activity and enhances EGF-stimulated cell motility
PPP1CA	Akt1	human	T320	T320	NPGGRPItPPRNSAK	14633703	a serine/threonine phosphatase involved in cell cycle regulation; phosphorylation inhibits activity
PRAS40	Akt1	human	T246	T246	LPRPRLNtsDFQKLK	12524439, 17277771, 18372248	binds to and inhibits mTOR; phosphorylation causes 14-3-3 binding/inhibition and results in increased protein synthesis
PRPF19	Akt1	human	T193	T193	ERKKRGKtVPEELVK	20629186	a member of the splicesome that also functions in DNA double strand break repair; phosphorylation allows 14-3-3 binding
PRPK	Akt1	human	S250	S250	RLRGRKRsMVG____	17712528	p53 binding protein and kinase; phosphorylation causes activation and results in p53 phosphorylation
PTP1B	Akt1	human	S50	S50	RNRyRDVsPFDHsRI	11579209	protein tyrosine phosphatase that dephosphorylates the insulin receptor; phosphorylation inhibits activity
QIK	Akt2	mouse	S358	S358	DGRQRRPstIAEQTV	17805301	AMPK related protein; phosphorylation leads to kinase activation and promotes ubiquitination/degradation of TORC2
Rac1	Akt1	human	S71	S71	yDRLRPLsYPQTDVF	10617634	Rho-GTPase, actin cytoskeletal organization; phosphorylation inhibits GTP-binding activity
Raf1	Akt1	mouse	S259	S259	SQRQRStsTPNVHMV	12087097, 12087097	signaling intermediate in Erk1/2 pathway; phosphorylation inhibits activity
	Akt1	rat	S259	S259	SQRQRSTsTPNVHMV	11443134	signaling intermediate in Erk1/2 pathway; phosphorylation inhibits activity
RANBP3	Akt1	human	S126	S126	VKRERtssLtQFPPs	18280241	RAN binding protein 3 functions in nuclear transport; phosphorylation mediates Ran binding and regulates nuclear transport
RARA	Akt1	human	S96	S96	FVCQDKSsGYHYGVS	16417524	nuclear receptor for retinoic acid that acts as a direct regulator of gene expression, phosphorylation of the DNA binding domain inhibits RARA activity
RGC32	Akt1	human	S65	S65	ERMKRRSsAsVSDSS	19162005	a regulator of cell cycle-specific kinases in response to DNA damage; phosphorylation leads to activation and regulation of growth factors
RNF11	Akt1	human	T135	T135	DWLMRSFtCPSCMEP	16123141	a member of a ubiquitin editing complex that modulates transient inflammatory signaling; phosphorylation allows 14-3-3 binding
Ron	Akt1	human	S1394	S1394	VRRPRPLsEPPRPT_	12919677, 14505491	receptor tyrosine kinase for macrophage stimulating protein (MSP), cell adhesion, proliferation and migration; phosphorylation causes 14-3-3 binding
RPS3	Akt1	human	T70	T70	GrrIrELtAVVQkRF	20605787	a member of the 40S ribosomal subunit that also induces neuronal apoptosis and acts as an endonuclease; phosphorylation inhibits proapoptotic function, increases nuclear import/accumulation, and increases DNA repair
S6	Akt1	mouse	S236	S236	AKRRRLssLRAstsK	12151408	S6 ribosomal protein; phosphorylation activates the protein and promotes protein synthesis
	Akt1, Akt2	rat	S235, S236	S235, S236	IAKRRRLssLRAsts, AKRRRLssLRAstsK	15358595	S6 ribosomal protein; phosphorylation activates the protein and promotes protein synthesis
SFRS5	Akt2	rat	S86	S86	GRGRGRYsDRFSSRR	15684423	a member of the splicesome involved in constitutive and alternative splicing; phosphorylation activates alternative splicing exon inclusion
SH3BP4	Akt1	mouse	S245	S246	FRSKRSysLsELsVL	19122209	controls selective internalization of the transferrin receptor through endocytosis; phosphorylation promotes 14-3-3 binding at the plasma membrane
SH3RF1	Akt1, Akt2	human	S304	S304	KNTKKRHsFtsLTMA	17535800	scaffolding protein that binds to activated Rac and promotes apoptosis via JNK activation; phosphorylation reduces ability to bind Rac, promoting apoptosis
SKI	Akt1	human	T458	T458	QPRKRKLtVDTPGAP	19875456	negative regulator of TGF-β signaling by binding to Smads; phosphorylation causes its destabilization and reduces SKI-mediated inhibition of expression of Smad7
SOX2	Akt1	mouse	T118	T116	kYRPRRktkTLMkKD	20945330	a transcription factor required for early embryogenesis and embryonic stem cell pluripotency; phosphorylation stabilizes SOX2, increasing transcriptional activity
SRPK2	Akt1	human	T492	T492	PSHDRSRtVsAsstG	19592491	a protein kinase targeting the serine/arginine family of splicing factors; phosphorylation causes nuclear translocation and upregulation of targets regulating cell cycle progression and apoptosis
SSB	Akt1	mouse	T301	T302	LLRNKKVtWKVLEGH	18836485	RNA binding protein, plays a role in processing of RNA polymerase III transcripts; phosphorylation promotes export to cytoplasm where it binds polysomes and regulates expression of a specific set of mRNAs
STXBP4	Akt2	mouse	S99	S99	RAKLRsEsPWEIAFI	15753124	inhibits formation and translocation of intracellular vesicles; insulin-stimulated phosphorylation of STXBP4 releases inhibition
SYTL1	Akt1	human	S241	S241	RMLSSSSsVSSLNSS	15998322	a secretory factor family member that is involved in granule exocytosis; phosphorylation regulates SYTL1 subnuclear localization
TAL1	Akt1	human	T90	T90	EARHRVPttELCRPP	15930267, 19406989	transcription factor; phosphorylation inhibits transcriptional repressor activity and regulates intracellular localization
TBC1D1	Akt1	human	T596	T596	AFRRRANtLsHFPIE	17995453	Rab GTPase-activating protein involved in insulin-stimulated Glut4 trafficking; phosphorylation promotes glucose transport
TERT	Akt1	human	S227, S824	S227, S824	GARRRGGsASRSLPL, AVRIRGKsYVQCQGI	10224060	telomerase reverse transcriptase, chromosome length maintenance; phosphorylation enhances telomerase activity

Kinase
Phosphatase
Transcription Factor
pro-apoptotic
Caspase
Receptor
Enzyme
pro-survival
GAP/GEF
GTPase
G-protein
Ribosomal subunit
Acetylase
Deacetylase

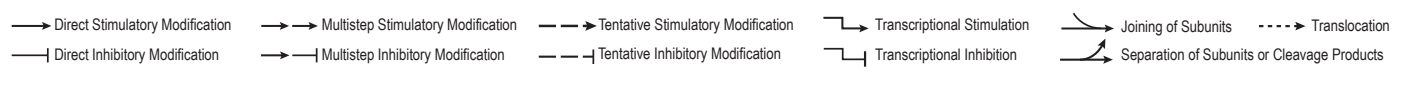
Akt Substrates Table

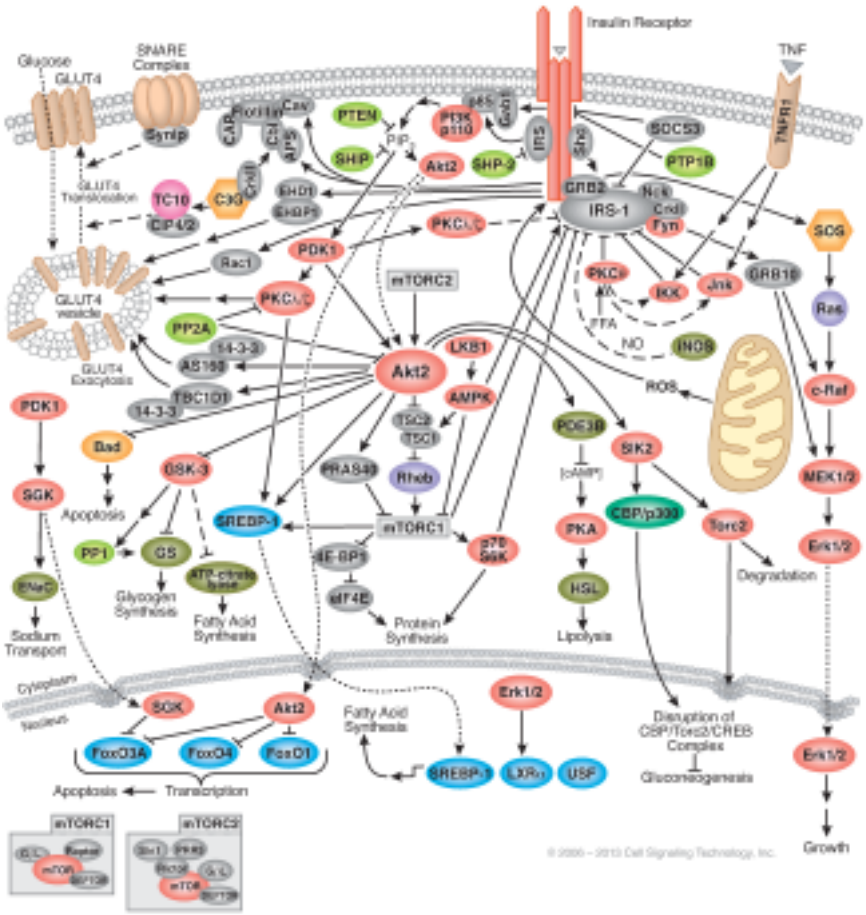
Substrate	Isoform	Organism	Site	Human Site	Sequence (+/-7)	PMID	Substrate Function and Effect of Phosphorylation
THOC4	Akt1	human	S34, T219	S34, T219	RGRGRAGsQGGrGGG, GGGtrRGtRGGARGR	18562279	an RNA binding and export protein that also acts as a chaperone for dimerization of transcription factors; phosphorylation regulates THOC4 subnuclear localization and activates mRNA export and cell proliferation
TOPBP1	Akt1	human	S1159	S1159	EERARLAsNLQWPSC	19477925	induces a large increase in the kinase activity of ATR; phosphorylation prevents the enhanced association of ATR with TopBP1 after DNA damage
TRF1	Akt1	human	T273	T273	SKRTRTItSQDKPSG	19160102	controls telomere structure; phosphorylation decreases telomere length
TSC2	Akt1	human	S939, S981, T1462	S939, S981, T1462	sFRARstsLNERPKs, AFRCRSIsVSEHVVR, GLRPRGytIsDSAPs	15342917, 12150915, 16636147	tumor suppressor that inhibits mTOR; phosphorylation inhibits function and allows protein synthesis to occur
	Akt1	rat	S1130, S1132	S1130, S1132	GARDRVRsMsGGHGL, RDRVRsMsGGHGLRV	12172553	tumor suppressor that inhibits mTOR; phosphorylation inhibits function and allows protein synthesis to occur
TTC3	Akt1	human	S378	S378	AYTPRsLsAPIFTTS	20059950	E3 ligase to Akt; phosphorylation promotes TTC3 function, such as ability to ubiquitinylate and destabilize Akt
TWIST1	Akt1	human	S42, S123	S42, S123	GGRKRRSsRRSAGGG, RERQRTQsLNEAFAA	20400976	a regulatory basic helix-loop-helix anti-apoptotic transcription factor; phosphorylation activates TWIST1, causing inhibition of p53 and promotion of cell survival
USP8	Akt1	mouse	T907	T945	TCRRRSRtFEAFMYL	17210635	deubiquitinating enzyme that plays a role in growth factor receptor trafficking and degradation; phosphorylation increases protein stability
VCP	Akt1	human	S352, S746, S748	S352, S746, S748	AAtNRPNsIDPALRR, AMRFARRsVsDNDIR, RFARRsVsDNDIRky	16551632, 16027165	ATPase and molecular chaperone; phosphorylation may impair its pro-apoptotic effects and promote cell survival
Vimentin	Akt1	human	S39	S39	ttsTrtysLGsALRP	20856200	a cytoskeletal intermediate filament protein; phosphorylation induces cellular motility and invasion by protection from proteolysis
Wee1	Akt1	human	S642	S642	KKMNRsVsLTly___	15964826	a protein kinase that inhibits cell cycle progression by phosphorylation inhibition of cdc2 kinase; phosphorylation promotes a change in Wee1 localization from nuclear to cytoplasmic and is associated with G2/M arrest
WNK1	Akt1	human	T60	T60	EYRRRRHtMDKDSRG	14611643, 16081417	regulates ion channels; phosphorylation of WNK1 causes SGK1 activation and regulation of sodium ion transport
XIAP	Akt1, Akt2	human	S87	S87	VGRHRKVsPNCRFIN	14645242, 17537996	inhibitor of apoptosis; phosphorylation prevents ubiquitination/degradation and causes increased cell survival
YAP1	Akt1	human	S127	S127	PQHVRAHssPAsLQL	12535517	a transcriptional co-activator of PEBP2 and other transcription factors; phosphorylation suppresses p73-mediated apoptosis
YB-1	Akt1	human	S102	S102	NPRKyLRsVGDGEtV	22417301	a transcription/translation factor involved in mRNA stability and expression; phosphorylation induces activation and translocation to the nucleus
zyxin	Akt1	human	S142	S142	PQPREKVssIDLEId	17572661	a focal adhesion molecule that moves between the cytoplasm and nucleus; phosphorylation promotes an association with acinus and anti-apoptotic activity

Akt Binding Partners

The Akt Binding Partners Table outlines Akt binding proteins, along with the effect of this interaction on Akt activity and corresponding references.

Binding Partner	Effect of Binding	Effect on Akt Activity	References
α-Actinin 4	Essential role in Akt translocation and activation	Positive	Ding, Z. et al. (2006) *Proc. Natl. Acad. Sci. USA* 103, 15014–15019.
Androgen Receptor (AR)	Forms complex with Akt and Mdm2 which results in AR degradation	N/A	Lin, H.K. et al. (2002) *EMBO J.* 21, 4037–4048.
APE	Associates with the kinase domain of Akt	Positive	Anai, M. et al. (2005) *J. Biol. Chem.* 280, 18525–18535.
APPL1	Associates with the kinase domain of Akt	Positive	Mitsuuchi, Y. et al. (1999) *Oncogene* 18, 4891–4898.
Brk	Binds to Akt and limits its activity	Negative	Zhang, P. et al. (2005) *J. Biol. Chem.* 280, 1982–1991.
cdc25A	Forms complex with Akt and Raf1 to promote cell survival	Positive	Fuhrmann, G. et al. (2001) *Oncogene* 20, 4542–4553.
cdc37	Binds to Akt and prevents its degradation	Positive	Miyata, Y. et al. (2004) *Mol. Cell. Biol.* 24, 4065-4074.
CTMP	Binds to the hydrophobic motif of Akt and prevents Akt activation	Negative	Maira, S.M. et al. (2001) *Science* 294, 374–380.
eNOS	Phosphorylation of eNOS at Ser113 and Ser614 disrupts binding to Akt	N/A	Bauer, P.M. et al. (2003) *J. Biol. Chem.* 278, 14841–14849.
Ft1	Binds to Akt and increases kinase activity	Positive	Remy, I. et al. (2004) *Mol. Cell. Biol.* 24, 1493–1504.
GRB10	Binds to the PH domain of Akt and potentiates its activation	Positive	Jahn, T. et al. (2002) *Mol. Cell. Biol.* 22, 979–991.
HSP27	Formation of Akt/HSP27 complex necessary for Akt activation in neutrophils	Positive	Rane, M.J. et al. (2003) *J. Biol. Chem.* 278, 27828–27835.
ILK	Phosphorylation of ILK is required for association with Akt and phosphorylation of Ser473	Positive	Persad, S. et al. (2001) *J. Biol. Chem.* 276, 27462–27469.
IRAK2	Associates with Akt and promotes NF-κB activity	N/A	Cenni, V. et al. (2003) *Biochem J.* 376, 303–311.
JIP1	Interaction with PH domain of Akt1 inhibits JNK activation	N/A	Kim, A.H. et al. (2002) *Neuron* 35, 697–709.
p21 Cip1	Binds to Akt2 and causes accumulation of p21 Cip1 in the nucleus and cell cyle exit	N/A	Héron-Milhavet, L. et al. (2006) *Mol. Cell Biol.* 26, 8267–8280.
Periplakin	Binds to the PH domain of Akt and regulates intracellular localization	N/A	van den Heuvel, A.P. et al. (2002) *J. Cell. Sci.* 115, 3957–3966.
PIKE-A	Binds to Akt and stimulates kinase activity	Positive	Ahn, J.Y. et al. (2004) *J Biol. Chem.* 279, 16441–16451.
PP2C A	Binds to and dephosphorylates Akt	Negative	Pim, D. et al. (2005) *Oncogene* 24, 7830–7838.
POSH	Binds to Akt2 and downregulates MLK3-JNK activation	N/A	Figueroa, C. et al. (2003) *J. Biol. Chem.* 278, 47922–47927.
Prohibitin 2	Binds to the C-terminus of Akt	N/A	Sun, L. et al. (2004) *J. Cell. Sci.* 117, 3021–3029.
Raf1	Akt binds to and phosphorylates Raf1, resulting in decreased Raf1 activity	N/A	Reusch, H.P. et al. (2001) *J Biol. Chem.* 276, 33630–33637.
Smad3	Insulin-induced Akt and Smad3 association blocks Smad3 phosphorylation and nuclear translocation; TGF-β blocks PKB/Smad3 association	N/A	Conery, A.R. et al. (2004) *Nat. Cell Biol.* 6, 366–372. Remy, I. et al. (2004) *Nat. Cell Biol.* 6, 358–365.
TCL1	Binds to the PH domain of Akt, forming oligomers, leading to increased Akt activity	Positive	Laine, J. et al. (2000) *Mol. Cell* 6, 395–407. Pekarsky, Y. et al. (2000) *Proc. Natl. Acad. Sci. USA* 97, 3028–3033.
TRB3	Insulin-mediated association with Akt blocks Akt activation	Negative	Du, K. et al. (2003) *Science* 300, 1574–1577.



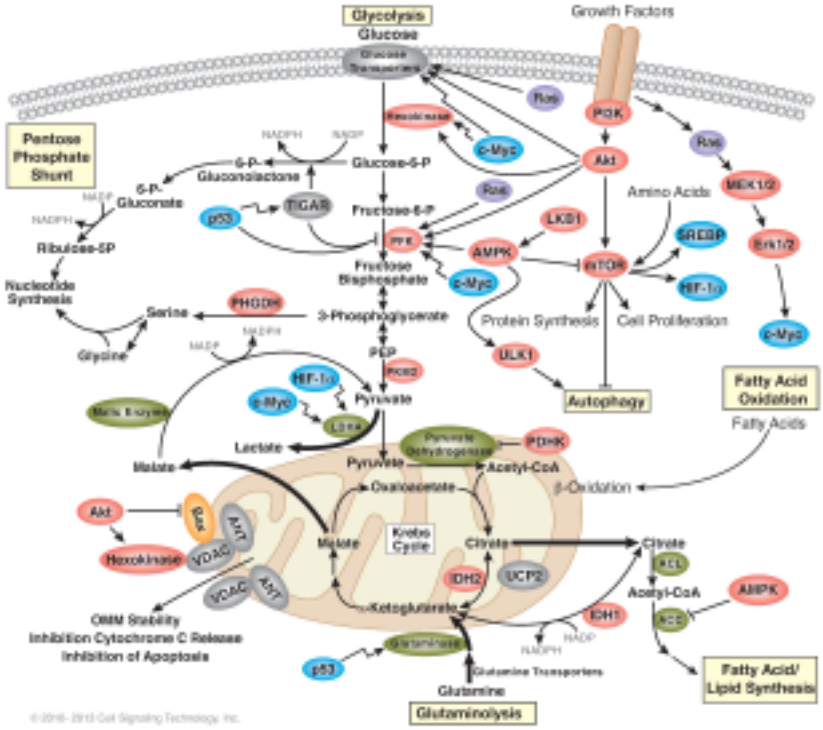


Insulin Receptor Signaling

Insulin is the major hormone controlling critical energy functions such as glucose and lipid metabolism. Insulin activates the insulin receptor tyrosine kinase (IR), which phosphorylates and recruits different substrate adaptors such as the IRS family of proteins. Tyrosine phosphorylated IRS then displays binding sites for numerous signaling partners. Among them, PI3K has a major role in insulin function, mainly via the activation of the Akt/PKB and the PKCζ cascades. Activated Akt induces glycogen synthesis through inhibition of GSK-3; protein synthesis via mTOR and downstream elements; and cell survival through inhibition of several pro-apoptotic agents (Bad, Forkhead family transcription factors, GSK-3). Insulin stimulates glucose uptake in muscle and adipocytes via translocation of GLUT4 vesicles to the plasma membrane. GLUT4 translocation involves the PI3K/Akt pathway and IR-mediated phosphorylation of CAP, and formation of the CAP/Cbl/CrkII complex. Insulin signaling also has growth and mitogenic effects, which are mostly mediated by the Akt cascade as well as by activation of the Ras/MAPK pathway. In addition, insulin signaling inhibits gluconeogenesis in the liver, through disruption of CREB/CBP/Torc2 binding. Insulin signaling also promotes fatty acid synthesis through activation of SREBP-1C, USF1, and LXR. A negative feedback signal emanating from Akt/PKB, PKCζ, p70 S6K, and the MAPK cascades results in serine phosphorylation and inactivation of IRS signaling.

Select Reviews: Alvarejos, J.Y. and Montminy, M. (2011) *Nat. Rev. Mol. Cell Biol.* 12, 141–151. | Cheng, Z., Tseng, Y., and White, M.F. (2010) *Trends Endocrinol. Metab.* 21, 589–598. | Fritsche, L., Weigert, C., Häring, H.U., and Lehmann, R. (2008) *Curr. Med. Chem.* 15, 1316–1329. | Rowland, A.F., Fazakerley, D.J., and James, D.E. (2011) *Traffic* 12, 672–681. | Siddle, K. (2011) *J. Mol. Endocrinol.* 47, 1–10. | Wong, R.H. and Sul, H.S. (2010) *Curr. Opin. Pharmacol.* 10, 684–691.

We would like to thank Ding An and Prof. Laurie Goodyear, Joslin Diabetes Center, Harvard Medical School, for reviewing this diagram.



Warburg Effect

Most cells use glucose as a fuel source. Glucose is metabolized by glycolysis in a multi-step set of reactions resulting in the creation of pyruvate. In typical cells, much of this pyruvate enters the mitochondria where it is oxidized by the Krebs Cycle to generate ATP to meet the cell's energy demands. However, in cancer cells or other highly proliferative cell types, much of the pyruvate from glycolysis is directed away from the mitochondria to create lactate through the action of the enzyme lactate dehydrogenase (LDH). In many normal cells, lactate production is typically restricted to anaerobic conditions when oxygen levels are low, however, cancer cells preferentially channel glucose towards lactate production even when oxygen is plentiful, a process termed "aerobic glycolysis" or the Warburg Effect.

Cancer cells frequently use glutamine as another fuel source, which enters the mitochondria and can be used to replenish Krebs Cycle intermediates or can be used to produce more pyruvate through the action of malic enzyme. Highly proliferative cells need to produce excess lipid, nucleotide, and amino acids for the creation of new biomass. Excess glucose is diverted through the pentose phosphate shunt (PPS) to create nucleotides. Fatty acids are critical for new membrane production and are synthesized from citrate in the cytosol through the action of ATP-citrate lyase (ACL) to generate acetyl-CoA. This process requires NADPH reducing equivalents, which can be generated through the actions of malic enzyme, IDH1, and also from multiple steps within the PPS pathway. Serine and glycine are critical for biosynthesis of nucleic acids and lipids as well as proteins.

Several signaling pathways contribute to the Warburg Effect. Growth factor stimulation results in signaling through RTKs to activate PI3K/Akt and Ras. Akt promotes glucose transporter activity and stimulates glycolysis through activation of several glycolytic enzymes including hexokinase and phosphofructokinase (PFK). Akt phosphorylation of apoptotic proteins such as Bax makes cancer cells resistant to apoptosis and helps stabilize the outer mitochondrial membrane (OMM) by promoting attachment of mitochondrial hexokinase (mtHK) to the VDAC channel complex. RTK signaling to c-Myc results in transcriptional activation of numerous genes involved in glycolysis and lactate production. The p53 oncogene transactivates TP-53-induced Glycolysis and Apoptosis Regulator (TIGAR) and results in increased NADPH production by PPS.

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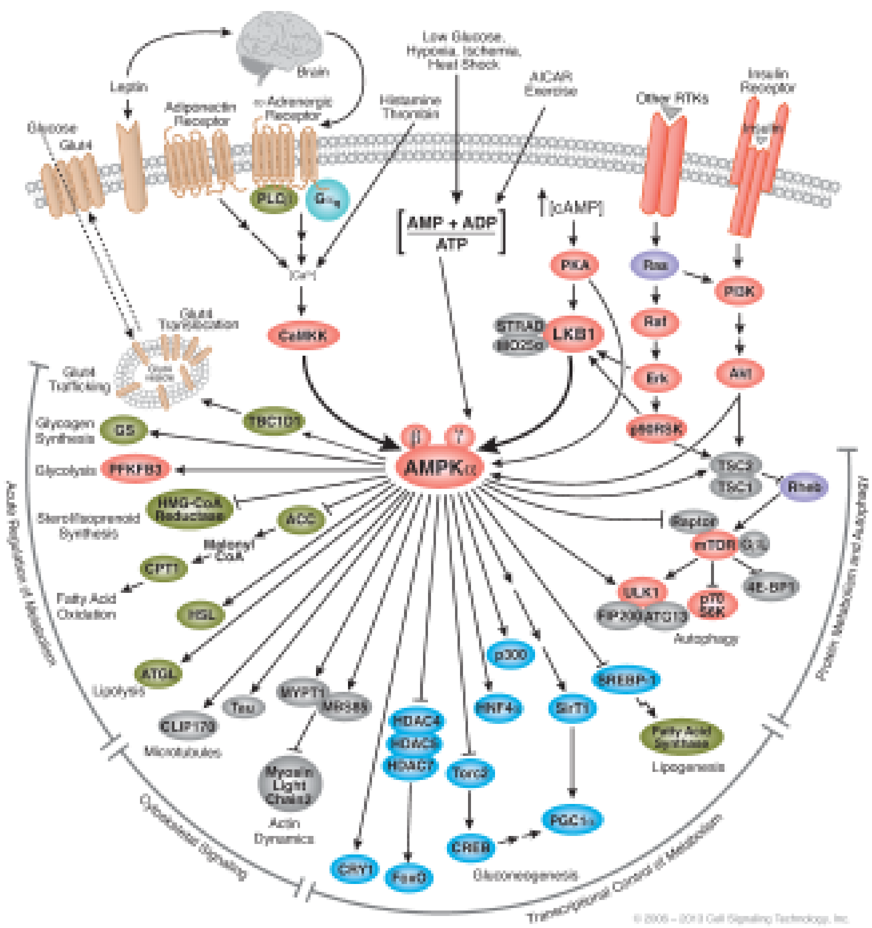
We would like to thank Prof. Matthew G. Vander Heiden, Massachusetts Institute of Technology, Cambridge, MA for reviewing this diagram.

Kinase | Transcription Factor | Caspase | Enzyme | GAP/GEF | G-protein | Acetylase
Phosphatase | pro-apoptotic | Receptor | pro-survival | GTPase | Ribosomal subunit | Deacetylase



AMPK Signaling

AMP-activated protein kinase (AMPK) plays a key role as a master regulator of cellular energy homeostasis. The kinase is activated in response to stresses that deplete cellular ATP supplies such as low glucose, hypoxia, ischemia, and heat shock. It exists as a heterotrimeric complex composed of a catalytic α subunit and regulatory β and γ subunits. Binding of AMP to the γ subunit allosterically activates the complex, making it a more attractive substrate for phosphorylation on Thr172 in the activation loop of the α subunit by its major upstream AMPK kinase, LKB1. AMPK can also be directly phosphorylated on Thr172 by CAMKK2 in response to changes in intracellular calcium as occurs following stimulation by metabolic hormones including adiponectin and leptin.

As a cellular energy sensor responding to low ATP levels, AMPK activation positively regulates signaling pathways that replenish cellular ATP supplies, including fatty acid oxidation and autophagy. AMPK negatively regulates ATP-consuming biosynthetic processes including gluconeogenesis, lipid and protein synthesis. AMPK accomplishes this through direct phosphorylation of a number of enzymes directly involved in these processes as well as through transcriptional control of metabolism by phosphorylating transcription factors, co-activators, and co-repressors.

Due to its role as a central regulator of both lipid and glucose metabolism, AMPK is considered to be a potential therapeutic target for the treatment of type II diabetes mellitus, obesity, and cancer. AMPK has also been implicated in a number of species as a critical modulator of aging through its interactions with mTOR and sirtuins.

Select Reviews: Hardie, D.G., Ross, F.A., and Hawley, S.A. (2012) *Nat. Rev. Mol. Cell Biol.* 13, 251–262. | Mihaylova, M.M. and Shaw, R.J. (2011) *Nat. Cell Biol.* 13, 1016–1023. | Hardie, D.G. (2011) *Genes Dev.* 25, 1895–1908. | Carling, D., Mayer, F.V., Sanders, M.J., and Gamblin, S.J. (2011) *Nat. Chem. Biol.* 7, 512–518. | Canto, C. and Auwerx, J. (2010) *J. Cell. Mol. Life Sci.* 67, 3407–3423. | Steinberg, G.R. and Kemp, B.E. (2009) *Physiol. Rev.* 89, 1025–1078. | Zhang, B.B., Zhou, G., and Li, C. (2009) *Cell Metab.* 9, 407–416.

We would like to thank Prof. Reuben Shaw, The Salk Institute for Biological Studies, La Jolla, CA, for reviewing this diagram.

AMPK Substrate Table

The AMPK Substrate Table provides a list of substrates for AMPK, along with corresponding phosphorylation sites and references. This table was generated using PhosphoSitePlus®, Cell Signaling Technology's protein modification resource. See page 4 for more information on PhosphoSitePlus®.

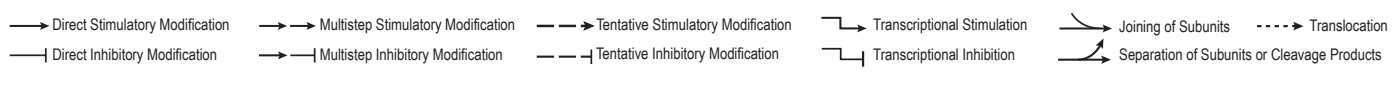
Substrate	AMPKA Isoform	Organism	Site	Human Site	Sequence (+/-7)	PMID	Substrate Function and Effect of Phosphorylation
ACC1	AMPKA1 AMPKA2	**human**	S80	**S80**	LHIRssMsGLHLVKQ	**17276402** **19176702** **18303014** **15371448**	Acetyl-CoA carboxylase (ACC) catalyzes the carboxylation of acetyl-CoA to malonyl-CoA in the biosynthesis and oxidation of fatty acids. Phosphorylation by AMPK inhibits the enzymatic activity of ACC.
	AMPKA2	**mouse**	S79	**S80**	FHMRSSMsGLHLVKQ	**15866171**	Acetyl-CoA carboxylase (ACC) catalyzes the carboxylation of acetyl-CoA to malonyl-CoA in the biosynthesis and oxidation of fatty acids. Phosphorylation by AMPK inhibits the enzymatic activity of ACC.
	AMPKA1 AMPKA2	**rat**	S79 S1200 S1215	**S80** **S1201** **S1216**	FHMRSsMsGLHLVKQ IPTLNRMsFASNLNH YGMTHVAsVSDVLLD	**12015362** **7915280** **1688796** **2900138** **1967580**	Acetyl-CoA carboxylase (ACC) catalyzes the carboxylation of acetyl-CoA to malonyl-CoA in the biosynthesis and oxidation of fatty acids. Phosphorylation by AMPK inhibits the enzymatic activity of ACC.
ACC2	AMPKA1	**human**	S222	**S222**	PTMRPSMsGLHLVKR	**17276402**	Acetyl-CoA carboxylase (ACC) catalyzes the carboxylation of acetyl-CoA to malonyl-CoA in the biosynthesis and oxidation of fatty acids. Phosphorylation by AMPK inhibits the enzymatic activity of ACC.
AMPKA1	AMPKA1	**human**	S360 S486 S494 S496 T183 T388	**S360** **S486** **S494** **S496** **T183** **T388**	LATsPPDsFLDDHHL DEIEAksGTAPQR GtAPQRsGsVsNYR APQRsGsVsNYRSC SDGEFLRtsCGsPNy EIPRARHLLDELMPQ	**19376078**	AMPK is a heterotrimeric complex composed of a catalytic a subunit and regulatory b and g subunits, each of which is encoded by two or three distinct genes (α1, 2; β1, 2; γ1, 2, 3). The kinase is activated by an elevated AMP/ATP ratio due to cellular and environmental stress, such as heat shock, hypoxia, and ischemia. Accumulating evidence indicates that AMPK not only regulates the metabolism of fatty acids and glycogen, but also modulates protein synthesis and cell growth through EF2 and TSC2/mTOR pathways, as well as blood flow via eNOS/nNOS. AMPKA1 phosphorylation is required for AMPK activation.
	AMPKA1	**rat**	S496 T183 T269	**S496** **T183** **T269**	APQRsGsVsNYRSC SDGEFLRtsCGsPNy VDPMKRAtIKDIREH	**17728241** **17023420** **16340011** **9305909**	AMPK is a heterotrimeric complex composed of a catalytic a subunit and regulatory b and g subunits, each of which is encoded by two or three distinct genes (α1, 2; β1, 2; γ1, 2, 3). The kinase is activated by an elevated AMP/ATP ratio due to cellular and environmental stress, such as heat shock, hypoxia, and ischemia. Accumulating evidence indicates that AMPK not only regulates the metabolism of fatty acids and glycogen, but also modulates protein synthesis and cell growth through EF2 and TSC2/mTOR pathways, as well as blood flow via eNOS/nNOS. AMPKA1 phosphorylation is required for AMPK activation.

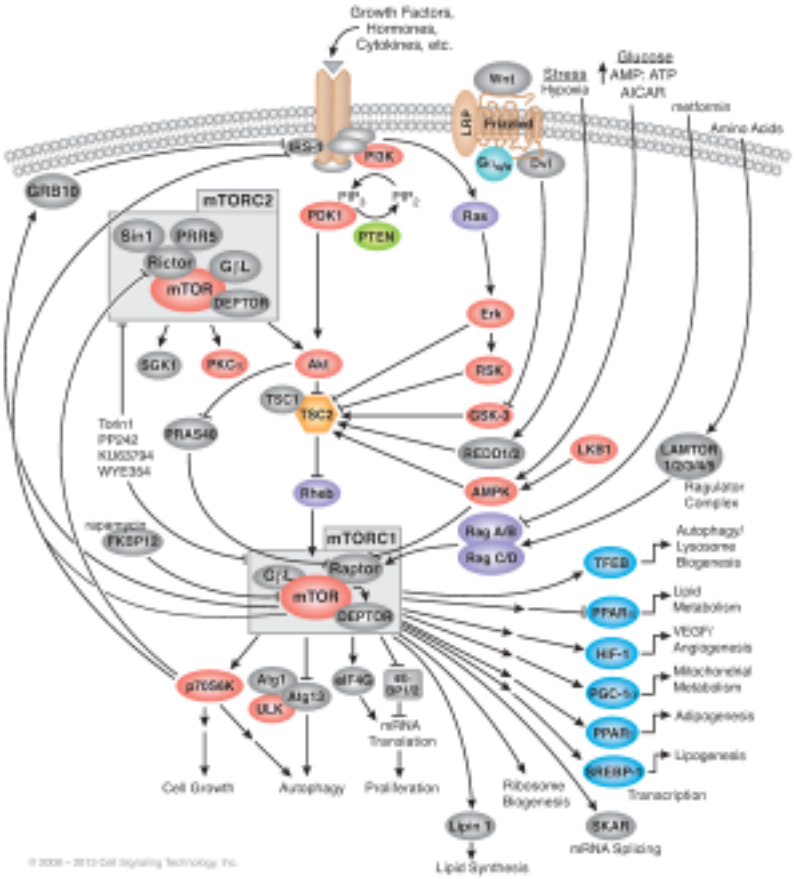
Direct Stimulatory Modification
Multistep Stimulatory Modification
Tentative Stimulatory Modification
Transcriptional Stimulation
Joining of Subunits
Translocation
Direct Inhibitory Modification
Multistep Inhibitory Modification
Tentative Inhibitory Modification
Transcriptional Inhibition
Separation of Subunits or Cleavage Products

Substrate	AMPKA Isoform	Organism	Site	Human Site	Sequence (+/-7)	PMID	Substrate Function and Effect of Phosphorylation
AMPKB1	AMPKA1	**human**	S24 S108 S174 S177 T80 T158	**S24** **S108** **S174** **S177** **T80** **T158**	HKtPRRDssGGTKDG sKLPLTRsHNNFVAI MVDSQKCsDVsELss SQKCsDVsELsssPP APAQARPtVFRWTGG NIIQVKKtDFEVFDA	**19376078**	AMPK is a heterotrimeric complex composed of a catalytic α subunit and regulatory b and g subunits, each of which is encoded by two or three distinct genes (α1, 2; β1, 2; γ1, 2, 3). The kinase is activated by an elevated AMP/ATP ratio due to cellular and environmental stress, such as heat shock, hypoxia, and ischemia. Accumulating evidence indicates that AMPK not only regulates the metabolism of fatty acids and glycogen, but also modulates protein synthesis and cell growth through EF2 and TSC2/mTOR pathways, as well as blood flow via eNOS/nNOS. The β1 subunit is post-translationally modified by multi-site phosphorylation to regulate AMPK activation and localization.
	AMPKA1	**rat**	S24 S25 S96 S101 S108 S182	**S24** **S25** **S96** **S101** **S108** **S182**	HKTPRRDssGGTKDG KTPRRDssGGTKDGD KEVYLSGsFNNWsKL SGsFNNWsKLPLTRs sKLPLTRsQNNFVAI DVSELSSsPPGPYHQ	**9305909** **12764152** **9305909**	AMPK is a heterotrimeric complex composed of a catalytic α subunit and regulatory b and g subunits, each of which is encoded by two or three distinct genes (α1, 2; β1, 2; γ1, 2, 3). The kinase is activated by an elevated AMP/ATP ratio due to cellular and environmental stress, such as heat shock, hypoxia, and ischemia. Accumulating evidence indicates that AMPK not only regulates the metabolism of fatty acids and glycogen, but also modulates protein synthesis and cell growth through EF2 and TSC2/mTOR pathways, as well as blood flow via eNOS/nNOS. The β1 subunit is post-translationally modified by multi-site phosphorylation to regulate AMPK activation and localization.
AS160	AMPKA2	**mouse**	S711	**S704**	PSLHTSFsAPSFTAP	**19923418**	AS160 is a Rab GTPase-activating protein that regulates insulin-stimulated Glut4 trafficking. Phosphorylation of AS160 by AMPK is involved in the regulation of contraction-stimulated Glut4 translocation.
CFTR	AMPKA1	**human**	S737 S768	**S737** **S768**	EPLERRLsLVPDSEQ LQARRRQsVLNLMTH	**19095655** **19419994**	CFTR is a plasma membrane cyclic AMP activated chloride channel that is expressed in the epithelial cells of the lung and several other organs. CFTR channels are kept closed by AMPK mediated phosphorylation in non-stimulated epithelium.
ChREBP	AMPKA1	**rat**	S568	**S556**	TLLRPPEsPDAVPEI	**11724780**	Carbohydrate-responsive element-binding protein (ChREBP) is a transcriptional repressor that regulates cellular energy homeostasis. AMPK phosphorylation inhibits the ability of CHREBP to bind DNA.
CK1-E	AMPKA1	**human**	S389	**S389**	RGAPANVsssDLtGR	**17525164**	CK1-E (Casein Kinase I epsilon) is a member of a family of protein kinases implicated in multiple processes including DNA repair, cell morphology, and Wnt signaling. Multiple inhibitory autophosphorylation sites have been identified near the C-terminus of CK1-E, including an AMPK site that results in increased CK1-E activity.
CRY1	AMPKA1	**mouse**	S71	**S71**	ANLRKLNsRLFVIRG	**19833968**	CRY1 is a member of the DNA photolyase class-1 family that acts as a regulator of the circadian clock. CRY1 is regulated in rhythmic fashion by AMPK phosphorylation-induced degradation.
eEF2K	AMPKA1	**human**	S398	**S398**	DSLPSsPsSATPHSQ	**14709557**	Eukaryotic elongation factor 2 kinase (eEF2k) phosphorylates and inactivates eEF2, resulting in the inhibition of peptide-chain elongation. AMPK phosphorylation of eEF2K increases its ability to phosphorylate eEF2.
eNOS	AMPKA1	**cow**	S1179	**S1177**	TSRIRtQsFSLQERH	**12107173**	Endothelial nitric-oxide synthase (eNOS) catalyzes the production of nitric oxide (NO), a key regulator of blood pressure, vascular remodeling, and angiogenesis. eNOS is activated by AMPK phosphorylating Ser1177 in response to various stimuli.
	AMPKA1	**human**	S633 S1177	**S633,** **S1177**	WRRKRKEssNTDSAG TsRIRtQsFsLQERQ	**12791703** **17276402** **20479254**	Endothelial nitric-oxide synthase (eNOS) catalyzes the production of nitric oxide (NO), a key regulator of blood pressure, vascular remodeling, and angiogenesis. eNOS is activated by AMPK phosphorylating Ser1177 in response to various stimuli.
	AMPKA1	**rat**	T494 S1176	**T495, S1177**	TGITRKKtFKEVANA TSRIRTQsFsLQERQ	**10025949**	Endothelial nitric-oxide synthase (eNOS) catalyzes the production of nitric oxide (NO), a key regulator of blood pressure, vascular remodeling, and angiogenesis. eNOS is activated by AMPK phosphorylating Ser1177 in response to various stimuli.
GABBR1	AMPKA1	**rat**	S948	**S918**	ELRHQLQsRQQLRSR	**17224405**	The metabotropic GABA(B) receptor is coupled to G proteins that modulate slow inhibitory synaptic transmission. Functional GABA(B) receptors form heterodimers of GABA(B)R1 and GABA(B)R2 where GABA(B)R1 binds the GABA ligand and GABA(B)R2 is the primary G protein contact site. AMPK mediated phosphorylation of GABA receptors increases activity as part of a neuroprotective mechanism.
GABBR2	AMPKA1	**rat**	S783	**S784**	VTSVNQAsTSRLEGL	**17224405**	The metabotropic GABA(B) receptor is coupled to G proteins that modulate slow inhibitory synaptic transmission. Functional GABA(B) receptors form heterodimers of GABA(B)R1 and GABA(B)R2 where GABA(B)R1 binds the GABA ligand and GABA(B)R2 is the primary G protein contact site. AMPK mediated phosphorylation of GABA receptors increases activity as part of a neuroprotective mechanism.
GBF1	AMPKA1	**human**	T1337	**T1337**	GKIHRsAtDADVVNs	**18063581**	Golgi-specific brefeldin A resistance factor 1 promotes guanine nucleotide exchange in the Golgi apparatus. GBF1 phosphorylation by AMPK occurs in response to low glucose, resulting in Golgi disassembly and lowered intracellular levels of ATP.
GFAT	AMPKA1	**human**	S261	**S261**	CNLsRVDsttCLFPV	**17941647** **19170765**	GFAT, glutamine:fructose-6-phosphate aminotransferase 1, is the rate-limiting enzyme of the hexosamine biosynthesis pathway generating the building blocks for protein and lipid glycosylation. GFAT activity is regulated by AMPK phosphorylation.
GYS1	AMPKA1	**rabbit**	S8	**S8**	MPLSRTLsVSsLPGL	**2567185**	Glycogen synthase 1 (GYS1) is a key enzyme in the regulation of glycogen synthesis in muscle. AMPK mediated phosphorylation leads to inactivation of GYS1.
H2B	AMPKA1	**human**	S37	**S37**	RKRsRkEsyslyVyk	**20647423**	The nucleosome, made up of four core histone proteins (H2A, H2B, H3, and H4), is the primary building block of chromatin. In response to metabolic stress, AMPK is recruited to responsive genes and phosphorylates histone H2B at S37, activating transcription.
HAS2	AMPKA2	**human**	T110	**T110**	LQSVKRLtYPGIKVV	**21228273**	Hyaluronan synthase 2 (HAS2) regulates the synthesis of hyaluronan (HA), an extracellular matrix protein involved in cell motility, proliferation, tumorigenesis, and inflammation. HAS2 phosphorylation by AMPK results in a loss of HAS2 enzymatic activity and impaired HA regulated functions.
HDAC5	AMPKA1, AMPKA2	**human**	S259, S498	**S259** **S498**	FPLRKTAsEPNLKVR RPLSRtQssPLPQsP	**18184930**	Histone deacetylase 5 (HDAC5) acts as a repressor of transcription by removing histone tail acetylations, promoting a closed chromatin configuration. AMPK mediated phosphorylation inhibits the repression activity of HDAC5.
HNF4	AMPKA1	**human**	S313	**S313**	GkIkRLRsQVQVsLE	**12740371**	Hepatocyte nuclear factor 4α (HNF4α) is a transcription factor that belongs to the steroid hormone receptor superfamily and regulates lipid homeostasis in the liver. AMPK phosphorylation of HNF4α inhibits dimer formation and DNA binding, resulting in increased protein degradation.
HSL	AMPKA1	**human**	S855	**S855**	EPMRRsVsEAALAQP	**16188906,** **2537200**	HSL (hormone-sensitive lipase) catalyzes the hydrolysis of triacylglycerol, the rate-limiting step in lipolysis. AMPK phosphorylation of HSL reduces HSL phosphorylation by PKA and inhibits HSL activity.
IKK	AMPKA2	**human**	S177 S181	**S177** **S181**	AKELDQGsLCtsFVG DQGsLCtsFVGTLQy	**21673972**	The NF-κB/Rel transcription factors are present in the cytosol in an inactive state, in a complex with the inhibitory IκB proteins. IκB kinase (IKK) complex containing the IKKβ catalytic subunit targets IκB for proteasomal degradation. Activation of IKK depends upon AMPK phosphorylation of the activation loop of IKKβ.
IRS1	AMPKA1	**mouse**	S789	**S794**	QHLRLSSsSGRLRYT	**11598104**	Insulin receptor substrate 1 (IRS1) is one of the major substrates of the insulin receptor kinase. Insulin signaling pathway activity is increased by AMPK phosphorylation of IRS1.
KCNMA1	AMPKA1	**mouse**	S722	**S722**	GRSERDCsCMSGRVR	**21209098**	Calcium-activated potassium channel subunit α-1 (KCNMA1) is a K+ channel activated by membrane depolarization, increased cytosolic Ca2+, and cytosolic Mg2+. KCNMA1 regulates several membrane polarization activities, as well as acting as an oxygen mediator under hypoxic conditions. KCNMA1 is inhibited by AMPK phosphorylation in cell types that do not monitor oxygen levels.
Kir6.2	AMPKA1	**rat**	S385	**S385**	AKPKFSIsPDSLS__	**19357830**	ATP-sensitive inward rectifier potassium channel 11 (Kir6.2) is a G protein mediated receptor that allows K+ to flow into the cell. The Kir6.2 channel is closed by AMPK mediated phosphorylation to allow insulin secretion in pancreatic beta cells.
KLC1	AMPKA1	**human**	S521	**S521**	ENMEkRRsREsLNVD	**20074060**	Kinesin light chain 1 (KLC1), also known as KNS2, is a motor protein that associates with microtubule components of the cytoskeleton. The intracellular trafficking of organelles may be regulated by AMPK mediated phosphorylation of KLC1.
KLC2	AMPKA1	**human**	S545 S582	**S545** **S582**	GSLRRsGsFGKLRDA PRMKRAssLNFLNKs	**21725060**	Kinesin light chain 2 (KLC2) is a motor protein that associates with microtubule components of the cytoskeleton. The intracellular trafficking of organelles may be regulated by AMPK mediated phosphorylation of KLC2.

Kinase, Transcription Factor, Caspase, Enzyme, GAP/GEF, G-protein, Acetylase
Phosphatase, pro-apoptotic, Receptor, pro-survival, GTPase, Ribosomal subunit, Deacetylase

Substrate	AMPKA Isoform	Organism	Site	Human Site	Sequence (+/-7)	PMID	Substrate Function and Effect of Phosphorylation
KPNA2	AMPKA1	**human**	S105	**S105**	QAARKLLsREkQPPI	**15342649**	Importin subunit a-2 (KPNA2) is an adaptor subunit of the Importin nuclear protein import receptor. KPNA2 phosphorylation by AMPK is required for Importin nuclear import mediation activity.
Kv2.1	AMPKA1 AMPKA2	**rat**	S444 S541	**S444, S541**	ERAKRNGsIVsMNMK SKMAKTQsQPILNTK	**22006306**	Potassium voltage-gated channel subfamily B member 1 (Kv2.1) mediates voltage dependent flow of K+ across membranes. Kv2.1 mediated action potential frequency is modulated under stress conditions via phosphorylation by AMPK.
mTOR	AMPKA1	**mouse**	T2446	**T2446**	NKRsRtRtDsysAGQ	**14970221**	The mammalian target of rapamycin (mTOR) is a Ser/Thr protein kinase that functions as an ATP and amino acid sensor to balance nutrient availability and cell growth. AMPK phosphorylates mTOR in response to nutrient deprivation and inhibits mTOR response to growth factor phosphorylation.
NKCC2	AMPKA1	**human**	S130	**S130**	GPKVNRPsLLEIHEQ	**19176702**	NKCC2 is an electroneutral cation chloride-coupled efflux cotransporter that regulates cell volume and maintains cellular homeostasis in response to osmotic and oxidative stress. NKCC2 chloride efflux activity is inhibited by AMPK phosphorylation, thereby increasing intracellular chloride concentration in the kidney.
	AMPKA1	**mouse**	S126	**S130**	GPKVNRPsLLEIHEQ	**17341212**	NKCC2 is an electroneutral cation chloride-coupled efflux cotransporter that regulates cell volume and maintains cellular homeostasis in response to osmotic and oxidative stress. NKCC2 chloride efflux activity is inhibited by AMPK phosphorylation, thereby increasing intracellular chloride concentration in the kidney.
p27Kip1	AMPKA1	**human**	T198	**T198**	PGLRRRQt_____	**17237771**	p27 Kip1 is a member of the Cip/Kip family of cyclin-dependent kinase inhibitors that enforces the G1 restriction point via its inhibitory binding to CDK2/cyclin E and other CDK/cyclin complexes. p27Kip1 stability is increased by AMPK mediated phosphorylation, resulting in increased survival under stress conditions.
p27Kip1	AMPKA1	**mouse**	S83 T170 T197	**S83** **T170** **T198**	WQEVERGsLPEFyYR QNKRANRtEENVSDG KPGLRRQt_____	**17237771** **18701472** **20146253**	p27 Kip1 is a member of the Cip/Kip family of cyclin-dependent kinase inhibitors that enforces the G1 restriction point via its inhibitory binding to CDK2/cyclin E and other CDK/cyclin complexes. p27Kip1 stability is increased by AMPK mediated phosphorylation, resulting in increased survival under stress conditions.
p300	AMPKA1	**human**	S89	**S89**	SELLRSGsSPNLNMG	**11518699**	The transcriptional coactivator p300 associates with transcriptional regulators and signaling molecules, integrating multiple signal transduction pathways with the transcriptional machinery. AMPK mediated phosphorylation represses p300 activity by disrupting the association of p300 with nuclear receptors.
p53	AMPKA1	**human**	S20 T18	**S20** **T18**	PLsQEtFsDLWKLLP EPPLsQEtFsDLWKL	**17339337**	The p53 tumor suppressor protein plays a major role in cellular response to DNA damage and other genomic aberrations. Activation of p53 can lead to either cell cycle arrest and DNA repair or apoptosis. DNA damage induces phosphorylation of p53 and leads to a reduced interaction between p53 and its negative regulator, the oncoprotein MDM2.
	AMPKA2	**mouse**	S15	**S15**	IsLELPLsQEtFsGL	**15866171**	The p53 tumor suppressor protein plays a major role in cellular response to DNA damage and other genomic aberrations. Activation of p53 can lead to either cell cycle arrest and DNA repair or apoptosis. DNA damage induces phosphorylation of p53 and leads to a reduced interaction between p53 and its negative regulator, the oncoprotein MDM2.
PFKFB2	AMPKA1	**human**	S466	**S466**	PVRMRRNsFtPLSSS	**12853467**	Phosphofructokinase (PFK) catalyzes the phosphorylation of fructose-6-phosphate in glycolysis. PFKFB2 initiated glycolysis is activated by AMPK phosphorylation.
PFKFB3	AMPKA1	**human**	S461	**S461**	NPLMRRNsVtPLAsP	**12065600,** **15896703**	Phosphofructokinase (PFK) catalyzes the phosphorylation of fructose-6-phosphate in glycolysis. PFKFB2 initiated glycolysis is activated by AMPK phosphorylation.
PGC-1	AMPKA2	**mouse**	T177 S538	**T178** **S539**	NHTHRIRtNPAIVkT SLFDVSPsCSSFNSP	**17609368**	PGC-1α interacts with a diverse array of transcription factors to regulate adaptive thermogenesis, energy metabolism, glucose uptake, gluconeogenesis, insulin secretion, and mitochondrial biogenesis. PGC-1α activity in skeletal muscle is induced by AMPK-mediated phosphorylation.
PLD1	AMPKA2	**human**	S505	**S505**	GSVKRVTsGPsLGSL	**20231899**	Phosphatidylcholine-specific phospholipase D (PLD) hydrolyzes phosphatidylcholine (PC) to produce choline and phosphatidic acid (PA). PA is the precursor of the second messenger, diacylglycerol (DAG). PLD1 is activated by AMPK phosphorylation, leading to an increase in glucose uptake in muscle under stress deprivation conditions.
PPP1R3C	AMPKA1	**human**	S33 S293	**S33** **S293**	MRLCLAHsPPVKSFL LESTIFGsPRLASGL	**19171932**	Protein phosphatase 1 is a serine/threonine phosphatase holoenzyme composed of a catalytic subunit and an inhibitory regulatory subunit. PPP1R3C is a regulatory subunit that confers specificity for increasing glycogen synthesis. AMPK targets PPP1R3C for phosphorylation and proteasomal degradation, which inhibits glycogen synthesis.
PPP2R5C	AMPKA1	**human**	S298 S336	**S298** **S336**	KYWPKTHsPKEVMFL RQLAKCVsSPHFQVA	**19366811**	Protein phosphatase 2 is a tripartite serine/threonine phosphatase holoenzyme composed of a catalytic subunit, a structural subunit and a regulatory subunit. AMPK phosphorylates the regulatory subunit PPP2R5C, which results in dephosphorylation of the catalytic subunit and increased PP2A activity.
Raf1	AMPKA1	**human**	S259 S621	**S259** **S621**	sQRQRststPNVHMV PKINRsAsEPsLHRA	**9091312**	Raf-1 (c-Raf) is recruited by GTP-bound Ras to activate the MEK-MAP kinase pathway. Inhibitory 14-3-3 protein binding sites on c-Raf can be phosphorylated by AMPK.
raptor	AMPKA1	**human**	S792	**S792**	DKMRRASsYSsLNsL	**18439900**	The regulatory associated protein of mTOR (Raptor) was identified as an mTOR binding partner that mediates mTOR signaling to downstream targets. AMPK phosphorylation of raptor is essential for inhibition of the raptor-containing mTOR complex 1 (mTORC1) and induces cell cycle arrest when cells are stressed for energy.
Rb	AMPKA1	**mouse**	S804	**S811**	IYIsPLKsPyKIsEG	**19217427**	The retinoblastoma tumor suppressor protein, Rb, regulates cell proliferation by controlling progression through the restriction point within the G1-phase of the cell cycle. AMPK regulation of brain development is achieved by modulating control of the cell cycle via phosphorylation of Rb.
smMLCK	AMPKA1	**chicken**	S1749	**S1760**	RAIGRLSsMAMISGM	**18426792**	Smooth muscle myosin light chain kinase (smMLCK) is activated by high Ca2+ induced calcium/calmodulin. Smooth muscle contraction is activated smMLCK mediated phosphorylation of myosin light chains. Smooth muscle contraction is attenuated by AMPK phosphorylation and inactivation of smMLCK.
TBC1D1	AMPKA1	**human**	S237 T596	**S237** **T596**	RPMRKSFsQPGLRsL AFRRRANtLsHFPIE	**17995453**	TBC1D1 is a Rab GTPase activating protein involved in vesicle trafficking in response to insulin. AMPK acts in association with insulin and growth factor signaling to activate TBC1D1 mediated vesicle regulation.
TIF-IA	AMPKA1	**human**	S635	**S635**	DTHFRsPsSSVGsPP	**19815529**	RNA polymerase I-specific transcription initiation factor RRN3 (TIF-IA) is required for RNA polymerase I initiation. Transcription of rRNA is inhibited during times of stress by AMPK phosphorylation inhibition of TIF-IA.
TORC2	AMPKA1	**mouse**	S171	**S171**	SALNRtssDsALHTs	**16148943**	Torc2 (transducer of regulated CREB activity 2) functions as a CREB co-activator and is implicated in mediating the effects of hormone and glucose sensing pathways. Torc2 is phosphorylated by AMPK at Ser171 and becomes sequestered in the cytoplasm, inhibiting hepatic gluconeogenesis.
TSC2	AMPKA1	**rat**	S1389 T1271	**S1387,** **T1271**	QPLsKSSsSPELQTL PTLPRSNtVASFSSL	**16959574** **14651849**	Tuberin is a product of the TSC2 tumor suppressor gene and an important regulator of cell proliferation and tumor development. AMPK phosphorylates tuberin during periods of energy starvation, enhancing tuberin activity and resulting in increased translation.
ULK1	AMPKA1	**human**	S638	**S638**	FDFPKtPssQNLLAL	**21383122**	ULK1 is a serine/threonine kinase involved in axon growth, endocytosis of critical growth factors such as NGF, and can act as a convergence point for multiple signals that control autophagy. AMPK, activated during low nutrient conditions, directly phosphorylates ULK1 at multiple sites to promote autophagy.
	AMPKA1	**mouse**	S467 S555	**S467, S556**	sAIRRsGsttPLGFG GLGCRLHsAPNLSDF	**21205641**	ULK1 is a serine/threonine kinase involved in axon growth, endocytosis of critical growth factors such as NGF, and can act as a convergence point for multiple signals that control autophagy. AMPK, activated during low nutrient conditions, directly phosphorylates ULK1 at multiple sites to promote autophagy.
VASP	AMPKA1	**human**	T278	**T278**	LARRRKAtQVGEktP	**17082196**	VASP belongs to the Ena/VASP family of adaptor proteins linking the cytoskeletal system to the signal transduction pathways and that it functions in cytoskeletal organization, fibroblast migration, platelet activation, and axon guidance. AMPK phosphorylation of VASP leads to specific cytoskeletal rearrangements.
ZNF692	AMPKA1	**human**	S470	**S470**	VAAHRSKsHPALLLA	**17097062**	ZNF692, also known as AREBP, is a zinc finger transcription factor involved in the expression of gluconeogenesis genes. ZNF692 DNA binding ability is abrogated by AMPK mediated phosphorylation during times of metabolic stress.





mTOR Signaling

The mammalian target of rapamycin (mTOR) is an atypical serine/threonine kinase that is present in two distinct complexes. mTOR complex 1 (mTORC1) is composed of mTOR, Raptor, GβL (mLST8), and Deptor and is partially inhibited by rapamycin. mTORC1 integrates multiple signals reflecting the availability of growth factors, nutrients, or energy to promote either cellular growth when conditions are favorable or catabolic processes during stress or when conditions are unfavorable. Growth factors and hormones (e.g. insulin) signal to mTORC1 via Akt, which inactivates TSC2 to prevent inhibition of mTORC1. Alternatively, low ATP levels lead to the AMPK-dependent activation of TSC2 and phosphorylation of raptor to reduce mTORC1 signaling. Amino acid availability is signaled to mTORC1 via a pathway involving the Rag and Regulator (LAMTOR1-3) proteins. Active mTORC1 has a number of downstream biological effects including translation of mRNA via the phosphorylation of downstream targets (4E-BP1 and p70 S6 Kinase), suppression of autophagy (Atg13, ULK1), ribosome biogenesis, and activation of transcription leading to mitochondrial metabolism or adipogenesis. The mTOR complex 2 (mTORC2) is composed of mTOR, Rictor, GβL, Sin1, PRR5/Protor-1, and Deptor and promotes cellular survival by activating Akt. mTORC2 also regulates cytoskeletal dynamics by activating PKCα and regulates ion transport and growth via SGK1 phosphorylation. Aberrant mTOR signaling is involved in many disease states including cancer, cardiovascular disease, and metabolic disorders.

Select Reviews: Dowling, R.J., Topisirovic, I., Fonseca, B.D., and Sonenberg, N. (2010) *Biochim. Biophys. Acta.* 1804, 433–439. | Dunlop, E.A., and Tee, A.R. (2009) *Cell. Signal.* 21, 827–835. | Hoeffer, C.A., and Klann, E. (2010) *Trends Neurosci.* 33, 67–75. | Laplante, M., and Sabatini, D.M. (2009) *J. Cell. Sci.* 122, 3589–3594. | Neufeld, T.P. (2010) *Curr. Opin. Cell Biol.* 22, 157–168. | Zoncu, R., Efeyan, A., and Sabatini, D.M. (2011) *Nat. Rev. Mol. Cell Biol.* 12, 21–35.

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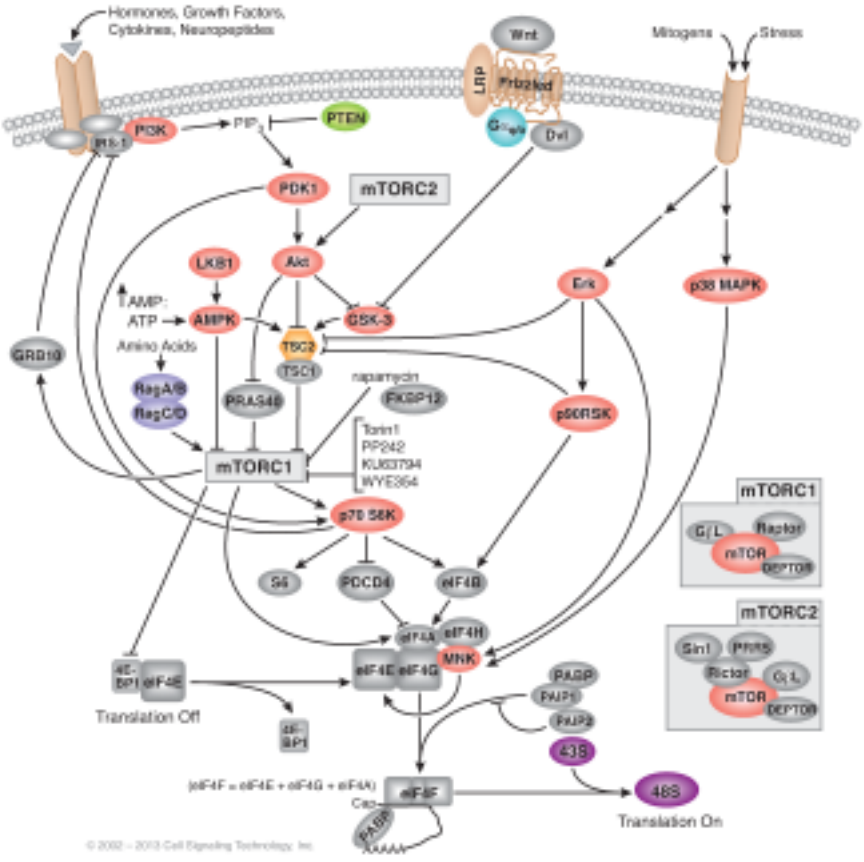

Translational Control: eIF4E and p70 S6K

eIF4E and S6 kinase (S6K) play critical roles in translational regulation. eIF4E binds the 5′ cap-structure of cytoplasmic mRNA and nucleates the eIF4F pre-initiation complex, which also includes eIF4A, a helicase that unwinds complex secondary structure in the mRNA leader sequence, and eIF4G, a large scaffolding protein that coordinates delivery of the mRNA to eIF3 and circularizes the mRNA through an association with poly(A) binding protein (PABP). Several stimuli, including growth factors, cytokines, and nutrient availability, regulate both eIF4F and S6K through mTORC1 (mTOR Complex 1: mTOR, GβL, Raptor, and Deptor). mTORC1 directly phosphorylates the translational inhibitory eIF4E-binding proteins (4E-BPs), which, when hypo-phosphorylated, prevent the interaction between eIF4E and eIF4G. mTORC1 also directly phosphorylates S6K, which has many targets in the translational machinery: S6 small ribosomal subunit; eIF4B, an activator of the eIF4A helicase; PDCD4, an inhibitor of eIF4A that is inhibited by phosphorylation; and SKAR, an mRNA splicing factor. Activation of S6K can also lead to suppression of insulin signaling through a negative feedback loop that destabilizes IRS1.

Select Reviews: Dowling, R.J., Topisirovic, I., Fonseca, B.D., and Sonenberg, N. (2010) *Biochim. Biophys. Acta.* 1804, 433–439. | Graff, J.R., Konicek, B.W., Carter, J.H., and Marcusson, E.G. (2008) *Cancer Res.* 68, 631–634. | Holcik, M. and Sonenberg, N. (2005) *Nat. Rev. Mol. Cell Biol.* 6, 318–327. | Huang, J. and Manning, B.D. (2008) *Biochem. J.* 412, 179–190. | Ruvinsky, I. and Meyuhas, O. (2006) *Trends Biochem. Sci.* 31, 342–348. | Sonenberg, N. and Hinnebusch, A.G. (2009) *Cell* 136, 731–745.

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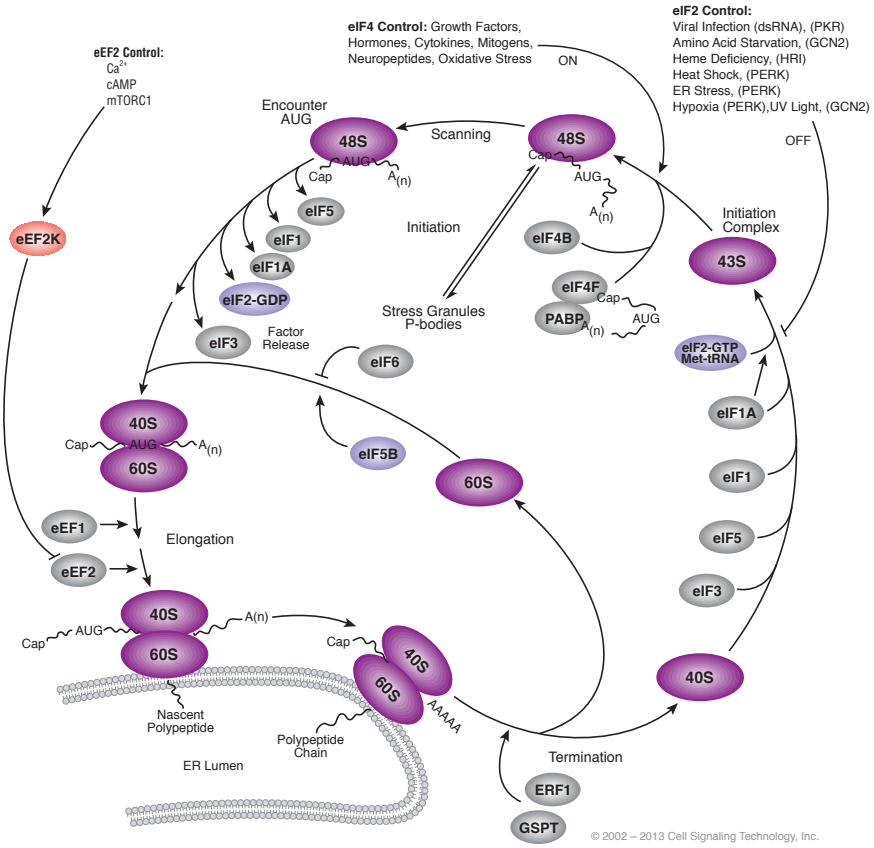
Kinase | Transcription Factor | Caspase | Enzyme | GAP/GEF | G-protein | Acetylase
Phosphatase | pro-apoptotic | Receptor | pro-survival | GTPase | Ribosomal subunit | Deacetylase



Translational Control: Overview

The synthesis of new protein is a highly regulated process that allows rapid cellular responses to diverse stimuli post-transcriptionally. Eukaryotic translation initiation factors (eIFs) catalyze the assembly of a functional ribosomal complex, which includes the 40S subunit, mRNA, and the initiator Met-tRNA, and finally the 60S subunit before the first peptide bond is formed. Most regulatory stimuli, such as growth factors and stress, control rate-limiting steps of the initiation process by either stimulating or inhibiting specific eIFs. Elevated levels of Ca^{2+} or cAMP can also attenuate translation by blocking the action of eukaryotic elongation factor 2 (eEF2).

Select Reviews: Gebauer, F. and Hentze, M.W. (2004) *Nat. Rev. Mol. Cell Biol.* 5, 827–835. | Sonenberg, N. and Hinnebusch, A.G. (2009) *Cell* 136, 731–745. | Hinnebusch, A.G. (2011) *Microbiol. Mol. Biol. Rev.* 75, 434–467. | Spirin, A.S. (2009) *Biochemistry* 48, 10688–10692. | Steitz, T.A. (2008) *Nat. Rev. Mol. Cell Biol.* 9, 242–253.

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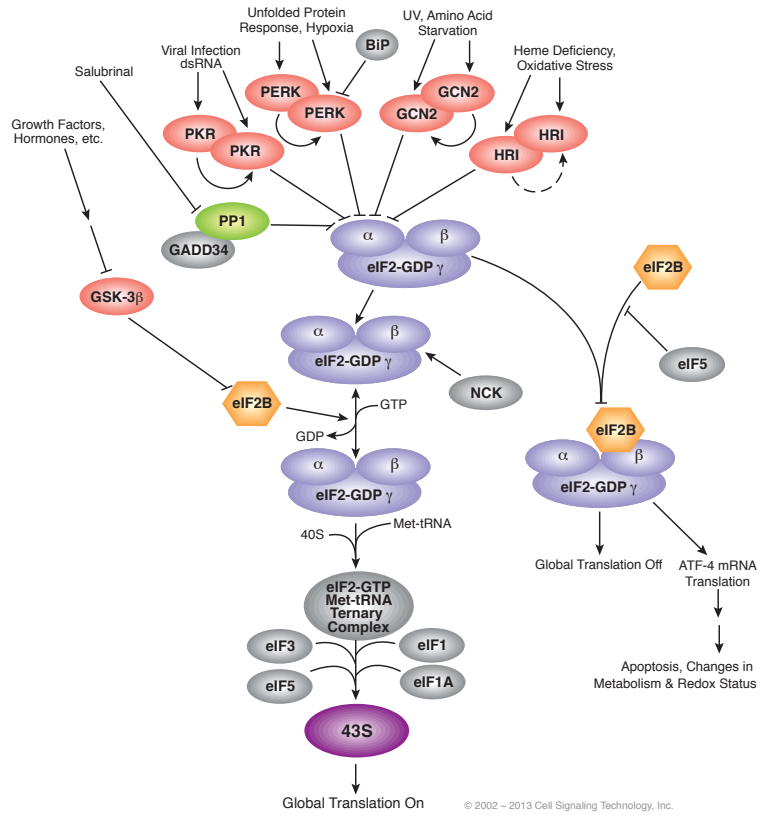

Translational Control: Regulation of eIF2

The eIF2 initiation complex integrates a diverse array of stress-related signals to regulate both global and specific mRNA translation. Under permissive conditions, eIF2 binds GTP and Met-tRNA to form the ternary complex (TC), which then associates with the 40S ribosomal subunit, eIF1, eIF1A, eIF5, and eIF3 to form the 43S pre-initiation complex (PIC). The 43S PIC scans the mRNA UTR for an AUG start codon. Upon AUG recognition, eIF2 hydrolyzes GTP to GDP and dissociates from the mRNA, permitting the binding of the 60S ribosomal subunit and elongation of the polypeptide chain. eIF2 is unable to participate in another round of initiation until GDP is exchanged for GTP, a reaction catalyzed by the guanine nucleotide exchange factor (GEF) eIF2B. This step is tightly regulated, and phosphorylation of eIF2α by a diverse family of four stress-activated kinases—PKR (dsRNA), PERK (ER stress), GCN2 (amino acid starvation), and HRI (heme deficiency)—prevents nucleotide exchange. An increase in eIF2α-GDP limits the availability of the ternary complex and causes a decrease in global protein synthesis while enhancing the translation of specific stress-related mRNA transcripts, such as the transcription factor ATF-4.

Select Reviews: Hinnebusch, A.G. (2011) *Microbiol. Mol. Biol. Rev.* 75, 434–467. | Raven, J.F. and Koromilas, A.E. (2008) *Cell Cycle* 7, 1146–1150. | Schmitt, E., Naveau, M., and Mechulam, Y. (2010) *FEBS Lett.* 584, 405–412. | Wek, R.C., Jiang, H.Y., and Anthony, T.G. (2006) *Biochem. Soc. Trans.* 34, 7–11.

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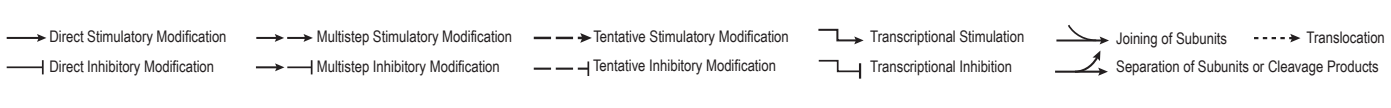

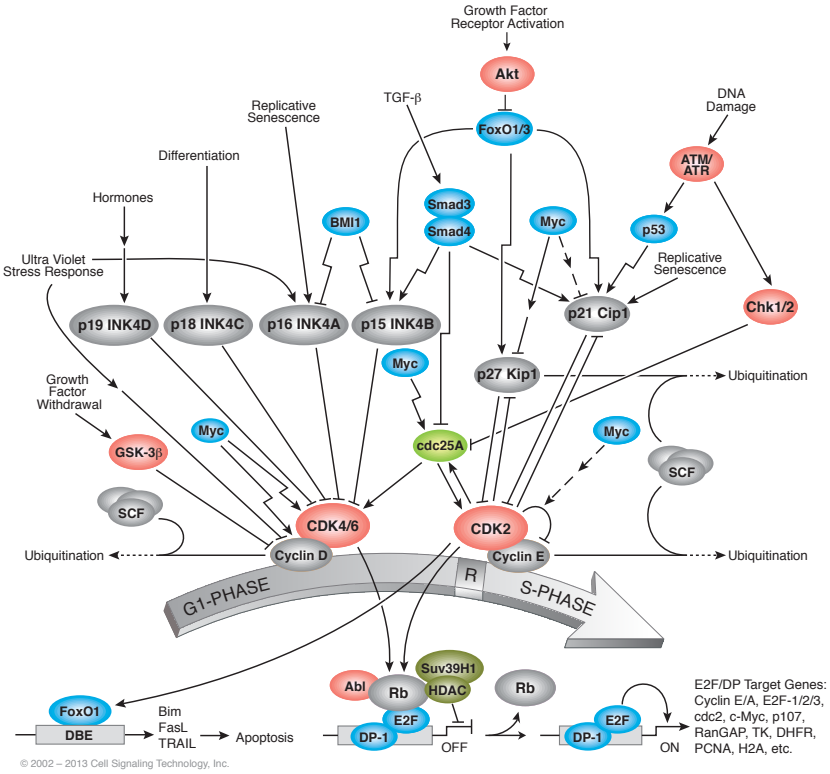

Cell Cycle Control: G1/S Checkpoint

The primary G1/S cell cycle checkpoint controls the commitment of eukaryotic cells to transition through the G1 phase to enter into the DNA synthesis S phase. Two cell cycle kinase complexes, CDK4/6-Cyclin D and CDK2-Cyclin E, work in concert to relieve inhibition of a dynamic transcription complex that contains the retinoblastoma protein (Rb) and E2F. In G1-phase uncommitted cells, hypo-phosphorylated Rb binds to the E2F-DP1 transcription factors forming an inhibitory complex with HDAC to repress key downstream transcription events. Commitment to enter S-phase occurs through sequential phosphorylation of Rb by Cyclin D-CDK4/6 and Cyclin E-CDK2 that dissociates the HDAC-repressor complex, permitting transcription of genes required for DNA replication. In the presence of growth factors, Akt can phosphorylate FoxO1/3, which inhibits their function by nuclear export, thereby allowing cell survival and proliferation. Importantly, a multitude of different stimuli exert checkpoint control, including TGF-β, DNA damage, replicative senescence, and growth factor withdrawal. These stimuli act though transcription factors to induce specific members of the INK4 or Kip/Cip families of cyclin dependent kinase inhibitors (CKIs). Notably, the oncogenic polycomb protein Bmi1 acts as a negative regulator of INK4A/B expression in stem cells and human cancer. In addition to regulating CKIs, TGF-β also inhibits cdc25A transcription, a phosphatase directly required for CDK activation. At a critical convergence point with the DNA-damage checkpoint, cdc25A is ubiquitinated and targeted for degradation via the SCF ubiquitin ligase complex downstream of the ATM/ATR/Chk-pathway. However, timely degradation of cdc25A in mitosis (M-phase) via the APC ubiquitin ligase complex allows progression through mitosis. Furthermore, growth factor withdrawal activates GSK-3β to phosphorylate Cyclin D, which leads to its rapid ubiquitination and proteasomal degradation. Collectively, ubiquitin/proteasome-dependent degradation and nuclear export are mechanisms commonly used to effectively reduce the concentration of cell cycle control proteins. Importantly, Cyclin D1/CKD4/6 complexes are explored as therapeutic targets for cancer treatment as researchers have found this checkpoint to be invariantly deregulated in human tumors.

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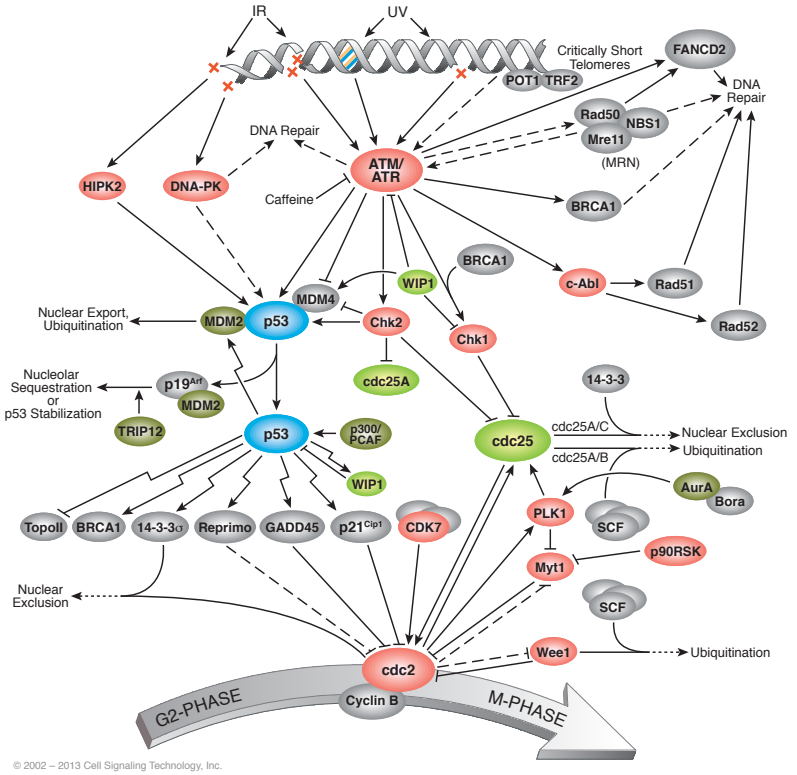

Cell Cycle Control: G2/M DNA Damage Checkpoint

The G2/M DNA damage checkpoint serves to prevent the cell from entering mitosis (M-phase) with genomic DNA damage. Specifically, the activity of the Cyclin B-cdc2 (CDK1) complex is pivotal in regulating the G2-phase transition wherein cdc2 is maintained in an inactive state by the tyrosine kinases Wee1 and Myt1. It is thought that coordinated action of the kinase Aurora A and the cofactor Bora activate PLK1 as cells approach the M-phase, which in turn activates the phosphatase cdc25 and downstream cdc2 activity, hence establishing a feedback amplification loop that efficiently drives the cell into mitosis. Importantly, DNA damage cues activate the sensory DNA-PK/ATM/ATR kinases, which relay two parallel cascades that ultimately serve to inactivate the Cyclin B-cdc2 complex. The first cascade rapidly inhibits progression into mitosis: the Chk kinases phosphorylate and inactivate cdc25, which prevents activation of cdc2. The slower second parallel cascade involves phosphorylation of p53 and allows for its dissociation from MDM2 and MDM4 (MdmX), which activates DNA binding and transcriptional regulatory activity, respectively. The transcriptional ability of p53 is further augmented through acetylation by the co-activator complex p300/PCAF. The second cascade constitutes the p53 downstream-regulated genes including: 14-3-3, which binds to the phosphorylated Cyclin B-cdc2 complex and exports it from the nucleus; GADD45, which binds to and dissociates the Cyclin B-cdc2 complex; and p21 Cip1, an inhibitor of a subset of the cyclin-dependent kinases including cdc2. Recent data suggest an important role for the p53-regulated WIP1 phosphatase that acts as a critical dampener of DNA damage signaling in cancer. In human cancer, researchers have found p53 to be commonly mutated, indicating that this checkpoint is a critical barrier to tumor formation. In addition, sporadic and familial mutations in the DNA-repair proteins such as the BRCA-family, ATM, and the Fanconi Anemia proteins further highlight this as a key tumor suppressor checkpoint.

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Jak/Stat Utilization

The Jak/Stat Utilization Table tabulates the combinatorial use of tyrosine kinases and Stat proteins in cytokine/growth factor signaling.

Ligand	Receptor	Jak-Kinase	Other Tyrosine Kinases	Stat Family Members
IL-6	IL-6Rα+gp130	Jak1,2, Tyk2	Hck	Stat1, Stat3
IL-11	IL-11R+gp130	Jak1,2, Tyk2	Src, Yes	Stat3
CNTF, CT-1, LIF, OSM	CNTFR+gp130, CT-1R+gp130, LIFR+gp130, OSMR+gp130	Jak1,2, Tyk2	Src family	Predominant: Stat3 Secondary: Stat1,5
G-CSF	G-CSFR	Jak2, Tyk2	Lyn	Stat3
IL-12 (p40+p35)	IL-12Rβ1+IL-12Rβ2	Jak2, Tyk2	Lck	Stat4
Leptin	LeptinR	Jak2	not determined	Stat3,5,6
IL-3	IL-3Rα+βc	Jak2	Fyn, Hck, Lyn	Stat3,5,6
IL-5	IL-5R+βc	Jak2	Btk	Stat3,5,6
GM-CSF	GM-CSFR+βc	Jak2	Hck, Lyn	Stat3,5
Angiotensin	GPCR	Jak2, Tyk2		Stat1,2,3
Serotonin	GPCR	Jak2		Stat3
α-Thrombin	GPCR	Jak2		Stat1,3
Chemokines	CXCR4	Jak2,3		
IL-2	IL-2Rα+IL-2Rβ+γc	Jak1,2,3	Fyn, Hck, Lck, Syk, Tec	Stat3,5
IL-4	IL-4Rα+γcR or IL-4Rα+ IL-13Rα1	Jak1,3	Lck, Tec	Stat6
IL-7	IL-7R+γc	Jak1,3	Lyn	Stat3,5
IL-9	IL-9R+γc	Jak1,3	not determined	Stat1,3,5
IL-13	IL-13Rα1+ IL-4Rα	Jak1,2, Tyk2	Ctk	Stat6
IL-15	IL-15Rα+IL-2Rβ+γc	Jak1,3	Lck	Stat3,5
IL-19	IL-20Rα+IL-20Rβ	Jak1, ?		Stat3
IL-20	IL-20Rα+IL-20Rβ, IL-22R+IL-20Rβ	Jak1, ?		Stat3
IL-21	IL-21R+γc	Jak1,3		Stat1,3,5
IL-22	IL-22R+IL-10Rβ	Jak1, Tyk2		Stat1,3,5
IL-23 (p40+p19)	IL-12Rβ1+IL-23R	Jak2	Tyk2	Stat4
IL-24	same as IL-20	Jak1, ?		Stat3
IL-26	IL-20Rα+IL-10Rβ	Jak1, Tyk2		Stat3
IL-27 (EBI3+p28)	gp130+WSX1	Jak1,2, Tyk2		Stat1,2,3,4,5
IL-28A, IL-28B, IL-29	IL-28R+IL-10Rβ	Jak1, Tyk2		Stat1,2,3,4,5
IL-31	IL-31Rα+OSMR	Jak1,2, Tyk2		Stat1,3,5
IL-35 (p35+EBI3)	gp130+WSX1	Jak1,2, Tyk2		Stat1,3,5
GH	GHR	Jak2	Src family	Stat3,5 (mainly Stat5a)
Tpo	TpoR (c-Mpl)	Jak2, Tyk2	Lyn	Stat1,3,5
Epo, Pro	EpoR, ProlactinR	Jak2	Src Family	Stat5 (mainly Stat5a)
Interferon (IFNα/β)	IFNAR1+IFNAR2	Jak1, Tyk2	Lck	Predominant: Stat1,2 Secondary: Stat3,4,5
IFN-γ	IFN-γR1+IFN-γR2	Jak1, Jak2	Hck, Lyn	Stat1
IL-10	IL-10Rα+ IL-10Rβ	Jak1, Tyk2	not determined	Stat1,3,5
TLSP	TLSPR and IL-7R	Jak1, possibly Jak2	not determined	Stat3,5
EGF	EGFR	Jak1	EGFR, Src	Stat1,3,5
PDGF	PDGFR	Jak1,2	PDGFR, Src	Stat1,3,5

Jak and Cytokine Receptor

The Jak and Cytokine Receptor Table lists mutations found in various cancers, along with the corresponding references.

Jak Mutants	Cytokine Receptor	Disease	References
Jak2 V617F	EpoR, TpoR (MPL), G-CSFR	Myeloproliferative neoplasms (MNPs), PV, ET, PMF	1–5
Jak2 K539L / exon 12 mutants	EpoR	MNP: PV	6
Jak2 T875N	undetermined	AML (AMKL)	7
Jak3 A572V	undetermined	AML (AMKL) (cell lines)	8
Jak1 V658F / Jak1 A634D / R879H / R724S	IL2R, IL9R, other undetermined	T-ALL	9,10
Jak1 R683G/S Jak2 ΔIREED	TLSPR	Pediatric and Down syndrome ALL	11–15

Receptor Mutants	Cytokine Receptor	Disease	References
TpoR W515L/K/A	Jak2	MPNs: ET, PMF	16–18
TpoR S505N			19
TpoR S487A			20
TLSPR F232S / TLSPR translocations	Jak2 R683 mutants	Pediatric and Down syndrome ALL	13, 21–23

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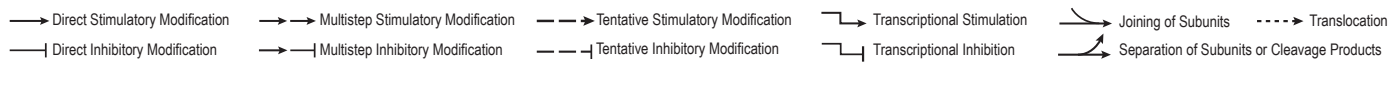
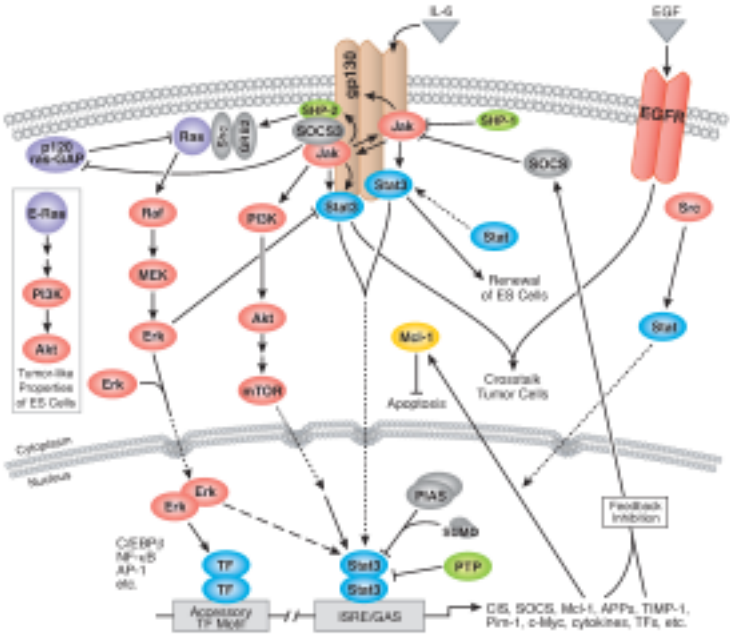


Jak/Stat Signaling: IL-6 Receptor Family



Jaks and Stats are critical components of many cytokine receptor systems, regulating growth, survival, differentiation, and pathogen resistance. An example of these pathways is shown for the IL-6 (or gp130) family of receptors, which coregulate B cell differentiation, plasmacytogenesis, and the acute phase reaction. Cytokine binding induces receptor dimerization, activating the associated Jaks, which phosphorylate themselves and the receptor. The phosphorylated sites on the receptor and Jaks serve as docking sites for the SH2-containing Stats, such as Stat3, and for SH2-containing proteins and adaptors that link the receptor to MAP kinase, PI3K/Akt, and other cellular pathways.

Phosphorylated Stats dimerize and translocate into the nucleus to regulate target gene transcription. Members of the suppressor of cytokine signaling (SOCS) family dampen receptor signaling via homologous or heterologous feedback regulation. Jaks or Stats can also participate in signaling through other receptor classes, as outlined in the Jak/Stat Utilization Table. Researchers have found Stat3 and Stat5 to be constitutively activated by tyrosine kinases other than Jaks in several solid tumors.

The Jak/Stat pathway mediates the effects of cytokines, like erythropoietin, thrombopoietin, and G-CSF, which are protein drugs for the treatment of anemia, thrombocytopenia, and neutropenia, respectively. The pathway also mediates signaling by interferons, which are used as antiviral and antiproliferative agents.

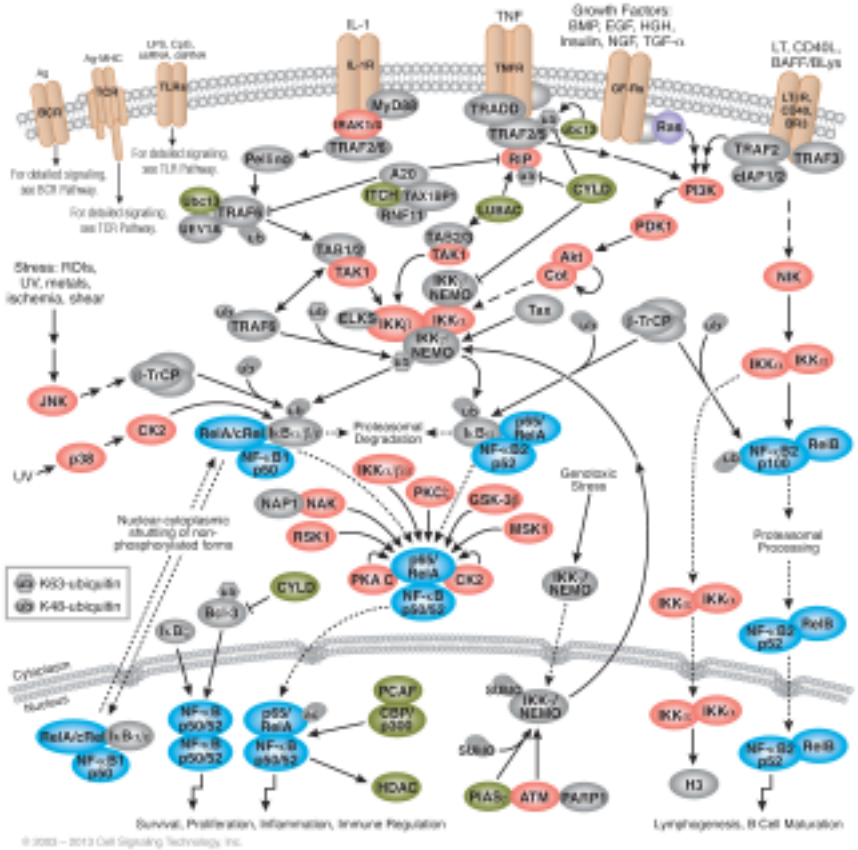
Researchers have found that dysregulated cytokine signaling contributes to cancer. Aberrant IL-6 signaling contributes to the pathogenesis of autoimmune diseases, inflammation, and cancers such as prostate cancer and multiple myeloma. Jak inhibitors currently are being tested in models of multiple myeloma. Stat3 can act as an oncogene and is constitutively active in many tumors. Crosstalk between cytokine signaling and EGFR family members is seen in some cancer cells.

Activating Jak mutations are major molecular events in human hematological malignancies. Researchers have found a unique somatic mutation in the Jak2 pseudokinase domain (V617F) that commonly occurs in polycythemia vera, essential thrombocythemia, and idiopathic myelofibrosis. This mutation results in the pathologic activation Jak2, associated with receptors for erythropoietin, thrombopoietin, and G-CSF, which control erythroid, megakaryocytic, and granulocytic proliferation and differentiation. Researchers have also found somatic acquired gain-of-function mutations in Jak1 have been discovered in adult T cell acute lymphoblastic leukemia. Somatic activating mutations in Jak1, Jak2, and Jak3 have been also identified in pediatric acute lymphoblastic leukemia (ALL). Furthermore, Jak2 mutations have been detected around pseudokinase domain R683 (R683G or duoNREED) in Down syndrome childhood B-ALL and pediatric B-ALL.

For selected reviews see www.cellsignal.com

We would like to thank Prof. Stefan Constantinescu, Ludwig Institute for Cancer Research, Brussels, Belgium for contributing to this diagram

Kinase · Phosphatase · Transcription Factor · pro-apoptotic · Caspase · Receptor · Enzyme · pro-survival · GAP/GEF · GTPase · G-protein · Ribosomal subunit · Acetylase · Deacetylase

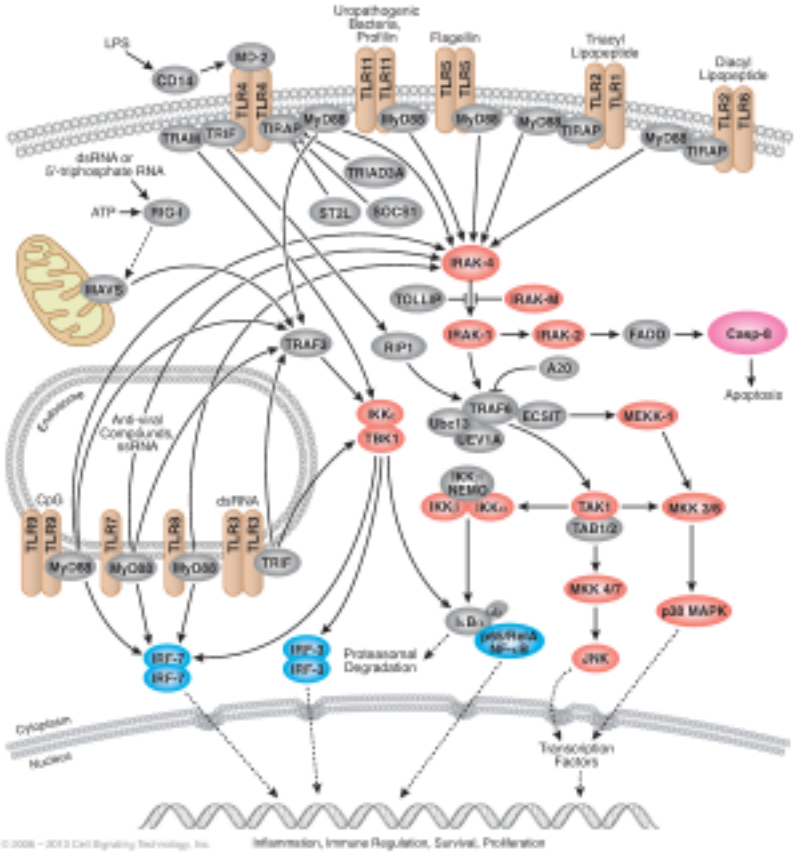


NF-κB Signaling

Nuclear factor κB (NF-κB)/Rel proteins include NF-κB2 p52/p100, NF-κB1 p50/p105, c-Rel, RelA/p65, and RelB. These proteins function as dimeric transcription factors that control genes regulating a broad range of biological processes including innate and adaptive immunity, inflammation, stress responses, B cell development, and lymphoid organogenesis. In the classical (or canonical) pathway, NF-κB/Rel proteins are bound and inhibited by IκB proteins. Proinflammatory cytokines, LPS, growth factors, and antigen receptors activate an IKK complex (IKKβ, IKKα, and NEMO), which phosphorylates IκB proteins. Phosphorylation of IκB leads to its ubiquitination and proteasomal degradation, freeing NF-κB/Rel complexes. Active NF-κB/Rel complexes are further activated by phosphorylation and translocate to the nucleus where, either alone or in combination with other transcription factor families including AP-1, Ets, and Stat, they induce target gene expression. In the alternative (or noncanonical) NF-κB pathway, NF-κB2 p100/RelB complexes are inactive in the cytoplasm. Signaling through a subset of receptors, including LTβR, CD40, and BR3, activates the kinase NIK, which in turn activates IKKα complexes that phosphorylate C-terminal residues in NF-κB2 p100. Phosphorylation of NF-κB2 p100 leads to its ubiquitination and proteasomal processing to NF-κB2 p52, creating transcriptionally competent NF-κB p52/RelB complexes that translocate to the nucleus and induce target gene expression. Only a subset of NF-κB agonists and target genes are shown here.

Select Reviews: Gilmore, T.D. (2006) Rel/NF-κB *Transcription Factors* www.nf-kb.org. | Hayden, M.S. and Ghosh, S. (2012) *Genes Dev.* 26, 203–234. | Perkins, N.D. (2012) *Nat. Rev. Cancer.* 12, 121–132. | Razani, B., Reichardt, A.D., and Cheng, G. (2011) *Immunol Rev.* 244, 44–54. | Vanhaesebroeck, K., Carpentier, I., and Beyaert, R. (2011) *Cytokine Growth Factor Rev.* 22, 277–286.

We would like to thank Prof. Thomas D. Gilmore, Boston University, Boston, MA, for contributing to this diagram.



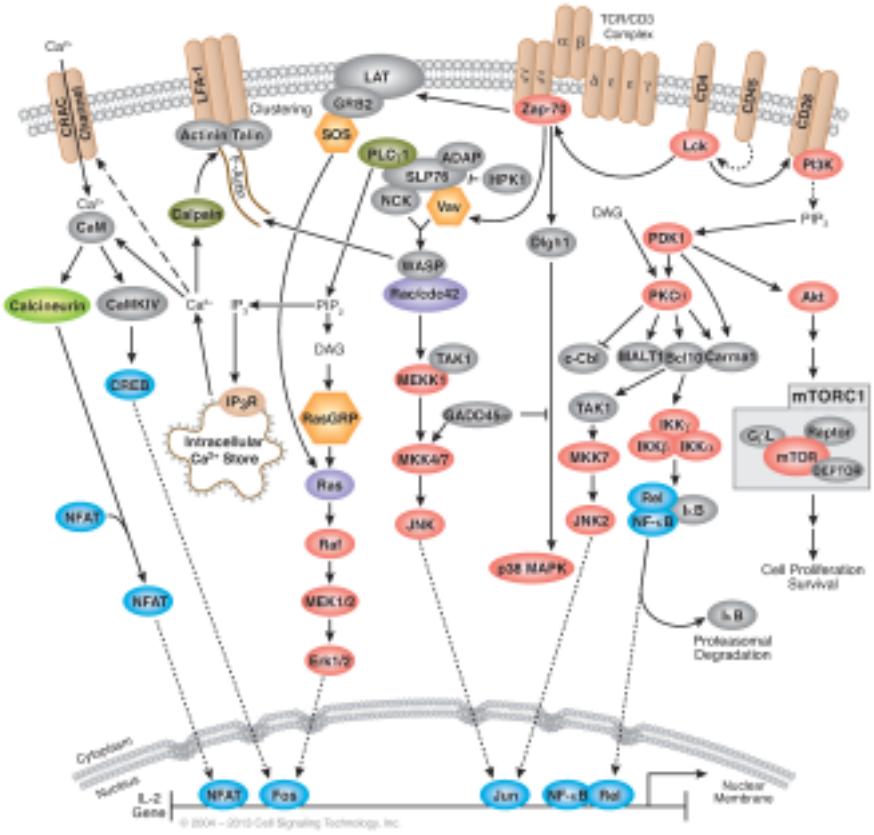
Toll-like Receptor Signaling

Toll-like receptors (TLRs) recognize distinct pathogen-associated molecular patterns and play a critical role in innate immune responses. They participate in the first line of defense against invading pathogens and play a significant role in inflammation, immune cell regulation, survival, and proliferation. To date, 11 members of the TLR family have been identified, of which TLR1, TLR2, TLR4, TLR5, TLR6, and TLR11 are located on the cell surface and TLR3, TLR7, TLR8, and TLR9 are localized to the endosomal/lysosomal compartment. The activation of the TLR signaling pathway originates from the cytoplasmic Toll/IL-1 receptor (TIR) domain that associates with a TIR domain-containing adaptor, MyD88. Upon stimulation with ligands, MyD88 recruits IL-1 receptor-associated kinase-4 (IRAK-4) to TLRs through interaction of the death domains of both molecules. IRAK-1 is activated by phosphorylation and associates with TRAF6, thereby activating the IKK complex and leading to activation of MAP kinases (JNK, p38 MAPK) and NF-κB. Tollip and IRAK-M interact with IRAK-1 and negatively regulate the TLR-mediated signaling pathways. Additional modes of regulation for these pathways include TRIF-dependent induction of TRAF6 signaling by RIP1 and negative regulation of TRAP-mediated downstream signaling by ST2L, TRIAD3A, and SOCS1. Activation of MyD88-independent pathways occurs via TRIF and TRAF3, leading to recruitment of IKKε/TBK1, phosphorylation of IRF3, and expression of interferon-β. TIR domain-containing adaptors such as TIRAP, TRIF, and TRAM regulate TLR-mediated signaling pathways by providing specificity for individual TLR signaling cascades. TRAF3 plays a critical role in the regulation of both MyD88-dependent and TRIF-dependent signaling via TRAF3 degradation, which activates MyD88-dependent signaling and suppresses TRIF-dependent signaling (and vice versa).

Select Reviews: Barton, G.M. and Kagan, J.C. (2009) *Nat. Rev. Immunol.* 9, 535–542. | Blasius, A.L. and Beutler, B. (2010) *Immunity* 32, 305–315. | Li, X., Jiang, S., and Tapping, R.I. (2010) *Cytokine* 49, 1–9. | McGettrick, A.F. and O'Neill, L.A. (2010) *Curr. Opin. Immunol.* 22, 20–27. | Miggin, S.M. and O'Neill, L.A. (2006) *J. Leukoc. Biol.* 80, 220–226. | Passer, C. and Medzhitov, R. (2005) *Adv. Exp. Med. Biol.* 560, 11–18. | Kawai, T. and Akira, S. (2010) *Nature Immunol.* 11, 373–384.

We would like to thank Dr. Pranoti Mandrekar, University of Massachusetts Medical School, Worcester, MA, for contributing to this diagram.

Direct Stimulatory Modification
Direct Inhibitory Modification
Multistep Stimulatory Modification
Multistep Inhibitory Modification
Tentative Stimulatory Modification
Tentative Inhibitory Modification
Transcriptional Stimulation
Transcriptional Inhibition
Joining of Subunits
Separation of Subunits or Cleavage Products
Translocation

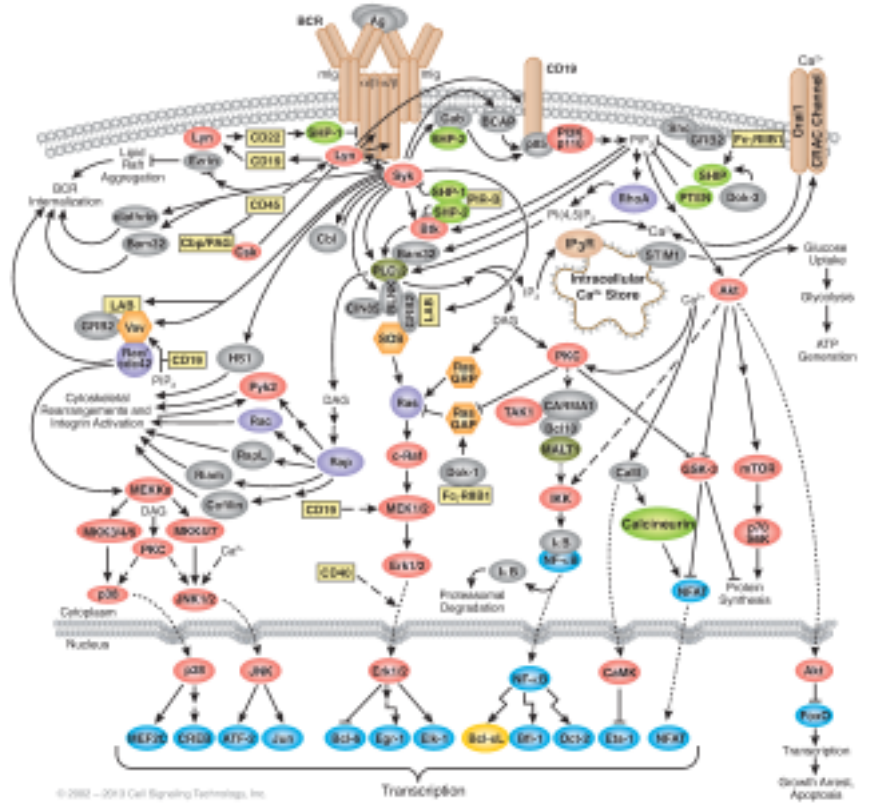


T Cell Receptor Signaling

T Cell Receptor (TCR) activation promotes a number of signaling cascades that ultimately determine cell fate through regulating cytokine production, cell survival, proliferation, and differentiation. An early event in TCR activation is phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) on the cytosolic side of the TCR/CD3 complex by lymphocyte protein tyrosine kinase (Lck). The CD45 receptor tyrosine phosphatase modulates the phosphorylation and activation of Lck and other Src family tyrosine kinases. ζ-chain associated protein kinase (Zap-70) is recruited to the TCR/CD3 complex where it becomes activated, promoting recruitment and phosphorylation of downstream adaptor or scaffold proteins. Phosphorylation of SLP-76 by Zap-70 promotes recruitment of Vav (a guanine nucleotide exchange factor), the adaptor proteins NCK and GADS, and an inducible T cell kinase (Itk). Phosphorylation of phospholipase C γ1 (PLCγ1) by Itk results in the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) to produce the second messengers diacylglycerol (DAG) and inositol trisphosphate (IP_3). DAG activates PKCθ and the MAPK/Erk pathways, both promoting transcription factor NF-κB activation. IP_3 triggers the release of Ca^{2+} from the ER, which promotes entry of extracellular Ca^{2+} into cells through calcium release-activated Ca^{2+} (CRAC) channels. Calcium-bound calmodulin (Ca^{2+}/CaM) activates the phosphatase calcineurin, which promotes IL-2 gene transcription through the transcription factor NFAT. Feedback regulation at several points within these pathways allows for different outcomes, depending on the cell type and environment. The incorporation of signals from additional cell surface receptors (such as CD28 or LFA-1) further regulates cellular response.

Select Reviews: Burbach, B.J., Medeiros, R.B., Mueller, K.L., and Shimizu, Y. (2007) *Immunol. Rev.* 218, 65–81. | Cheng, J., Montecalvo, A., and Kane, L.P. (2011) *Immunol. Res.* 50, 113–117. | Cronin, S.J. and Penninger, J.M. (2007) *Immunol. Rev.* 220, 151–168. | Marsland, B.J. and Kopf, M. (2008) *Trends Immunol.* 29, 179–185. | Oh, O. and August, A. (2007) *Sci. STKE* pe39. | Thome, M. (2008) *Nat. Rev. Immunol.* 8, 495–500.

We would like to thank Prof. Sankar Ghosh, Columbia University, New York, NY for contributing to this diagram.



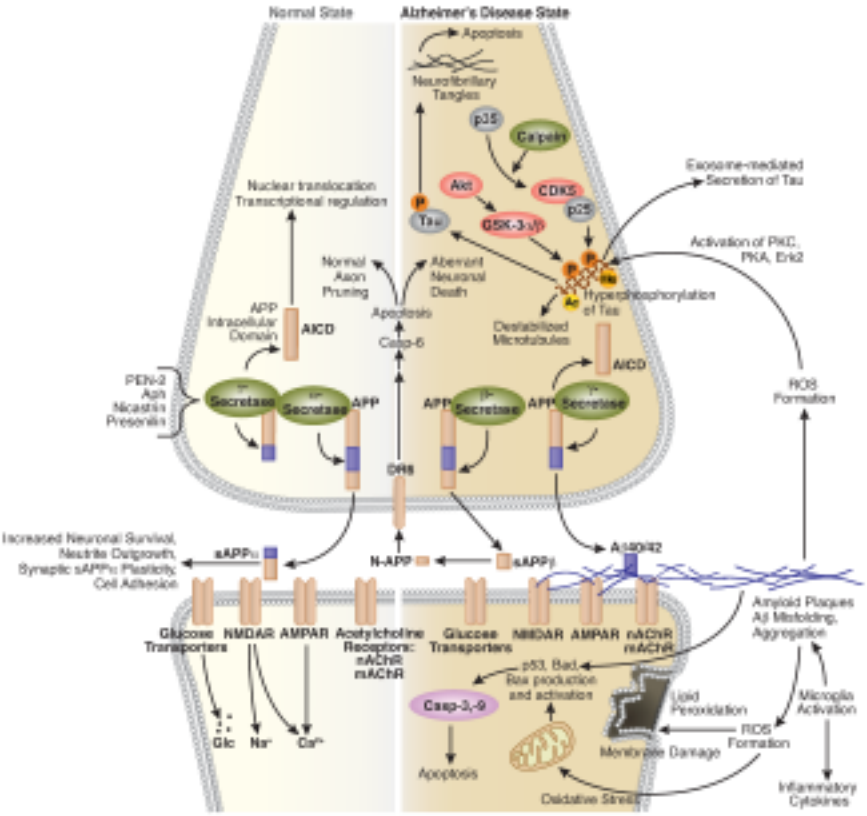
B Cell Receptor Signaling

The B cell antigen receptor (BCR) is composed of membrane immunoglobulin (mIg) molecules and associated Igα/Igβ (CD79a/CD79b) heterodimers (Igβ). The mIg subunits bind antigen, resulting in receptor aggregation, while the αβ subunits transduce signals to the cell interior. BCR aggregation rapidly activates the Src family kinases Lyn, Blk, and Fyn as well as the Syk and Btk tyrosine kinases. This initiates the formation of a 'signalosome' composed of the BCR, the aforementioned tyrosine kinases, adaptor proteins such as CD19 and BLNK, and signaling enzymes such as PLCγ2, PI3K, and Vav. Signals emanating from the signalosome activate multiple signaling cascades that involve kinases, GTPases, and transcription factors. This results in changes in cell metabolism, gene expression, and cytoskeletal organization. The complexity of BCR signaling permits many distinct outcomes, including survival, tolerance (anergy) or apoptosis, proliferation, and differentiation into antibody-producing cells or memory B cells. The outcome of the response is determined by the maturation state of the cell, the nature of the antigen, the magnitude and duration of BCR signaling, and signals from other receptors such as CD40, the IL-21 receptor, and BAFF-R. Many other transmembrane proteins, some of which are receptors, modulate specific elements of BCR signaling. A few of these, including CD45, CD19, CD22, PIR-B, and FcγRIIB1 (CD32), are indicated here in yellow. The magnitude and duration of BCR signaling are limited by negative feedback loops including those involving the Lyn/CD22/SHP-1 pathway, the Csk/Cbp pathway, SHIP, Cbl, Dok-1, Dok-3, FcγRIIB1, PIR-B, and internalization of the BCR. *In vivo*, B cells are often activated by antigen-presenting cells that capture antigens and display them on their cell surface. Activation of B cells by such membrane-associated antigens requires BCR-induced cytoskeletal reorganization. Please refer to the diagrams for the PI3K/Akt signaling pathway, the NF-κB signaling pathway, and the regulation of actin dynamics for more details about these pathways.

Select Reviews: Dal Porto, J.M., Gauld, S.B., Merrell, K.T., Mills, D., Pugh-Bernard, A.E., and Cambier, J. (2004) *Mol. Immunol.* 41, 599–613. | Goodnow, C.C., Vinuesa, C.G., Randall, K.L., Mackay, F., and Brink, R. (2010) *Nature Immunol.* 11, 681–688. | Harwood, N.E. and Batista, F.D. (2008) *Immunity* 29, 609–619. | Harwood, N.E. and Batista, F.D. (2010) *Annu. Rev. Immunol.* 28, 185–210. | Kurosaki, T., Shinohara, H., and Baba, Y. (2010) *Annu. Rev. Immunol.* 28, 21–55.

We would like to thank Prof. Michael R. Gold, University of British Columbia, Vancouver, British Columbia for contributing to this diagram.

Kinase | Transcription Factor | Caspase | Enzyme | GAP/GEF | G-protein | Acetylase
Phosphatase | pro-apoptotic | Receptor | pro-survival | GTPase | Ribosomal subunit | Deacetylase

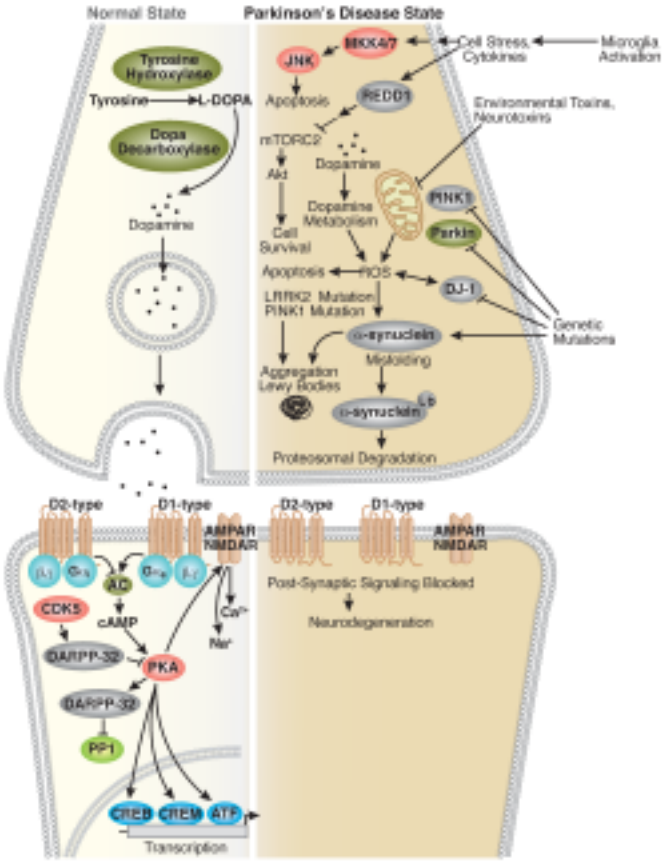


Amyloid Plaque and Neurofibrillary Tangle Formation in Alzheimer's Disease

Alzheimer's disease is one of the most common neurodegenerative diseases worldwide. Clinically, it is characterized by the presence of extracellular amyloid plaques and intracellular neurofibrillary tangles, resulting in neuronal dysfunction and cell death. Central to this disease is the differential processing of the integral membrane protein APP (Amyloid Precursor Protein) in the normal versus disease state. In the normal state, APP is initially cleaved by α-secretase to generate sAPPα and a C83 carboxy-terminal fragment. The presence of sAPPα is associated with normal synaptic signaling and results in synaptic plasticity, learning and memory, emotional behaviors, and neuronal survival. In the disease state, APP is cleaved sequentially by β-secretase and γ-secretase to release an extracellular fragment called Aβ40/42. This neurotoxic fragment frequently aggregates and results in Aβ40/42 oligomerization and plaque formation. Aβ40/42 aggregation results in blocked ion channels, disruption of calcium homeostasis, mitochondrial oxidative stress, impaired energy metabolism and abnormal glucose regulation, and ultimately neuronal cell death. Alzheimer's disease is also characterized by the presence of neurofibrillary tangles. These tangles are the result of hyperphosphorylation of the microtubule-associated protein Tau. GSK-3β and CDK5 are the kinases primarily responsible for phosphorylation of Tau, although other kinases such as PKC, PKA, and ERK2 are also involved. Hyperphosphorylation of Tau results in the dissociation of Tau from the microtubule, leading to microtubule destabilization and oligomerization of the Tau protein within the cell. Neurofibrillary tangles form as a result of Tau oligomerization and lead to apoptosis of the neuron.

Select Reviews: Rossi-Wetzel, E., Schwarzenbacher, R., and Lipton, S.A. (2004) *Nat. Med.* 10, 2–9. | Chen, J.X. and Yan, S.S. (2010) *J. Alzheimers Dis.* 2, S569–S578. | Claeysen, S., Cochet, M., Donneger, R., Dumuis, A., Bockaert, J., and Giannoni, P. (2012) *Cell. Signal.* 24, 1831-1840. | Marcus, J.N. and Schachter, J. (2011) *J. Neurogenet.* 25, 127–133. | Müller, W.E., Eckert, A., Kurz, C., Eckert, G.P., and Leuner, K. (2010) *Mol. Neurobiol.* 41, 159–171. | Nizzari, M., Thellung, S., Corsaro, A., Villa, V., Pagano, A., Porcile, C., Russo, C., and Florio T. (2012) *J. Toxicol.* 2012, 187297. | Thinakaran, G. and Koo, E.H. (2008) *J. Biol. Chem.* 283, 29615–29619.

We would like to thank Prof. Christopher Pfeil, University of Colorado Denver, and Prof. Jeff Kuret, The Ohio State University, Columbus, OH for contributing to this diagram.



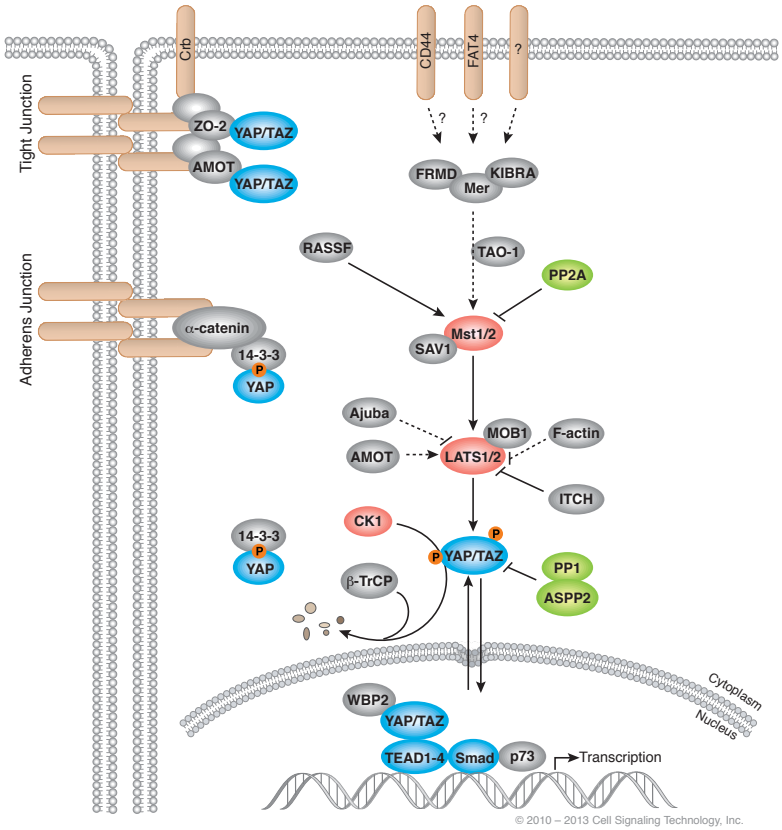
Dopamine Signaling in Parkinson's Disease

Parkinson's disease is the second most prevalent neurodegenerative disorder. Clinically, this disease is characterized by bradykinesia, resting tremors, and rigidity due to loss of dopaminergic neurons within the substantia nigra section of the ventral midbrain. In the normal state, release of the neurotransmitter dopamine in the presynaptic neuron results in signaling in the postsynaptic neuron through D1- and D2-type dopamine receptors. D1 receptors signal through G proteins to activate adenylate cyclase, causing cAMP formation and activation of PKA. D2-type receptors block this signaling by inhibiting adenylate cyclase. Parkinson's disease can occur through both genetic mutation (familial) and exposure to environmental and neurotoxins (sporadic). Recessively inherited loss-of-function mutations in parkin, DJ-1, and PINK1 cause mitochondrial dysfunction and accumulation of reactive oxidative species (ROS), whereas dominantly inherited missense mutations in α-synuclein and LRRK2 may affect protein degradation pathways, leading to protein aggregation and accumulation of Lewy bodies. Mitochondrial dysfunction and protein aggregation in dopaminergic neurons may be responsible for their premature degeneration. Another common feature of the mutations in α-synuclein, parkin, DJ-1, PINK1, and LRRK2 is the impairment in dopamine release and dopaminergic neurotransmission, which may be an early pathogenic precursor prior to death of dopaminergic neurons. Exposure to environmental and neurotoxins can also cause mitochondrial functional impairment and release of ROS, leading to a number of cellular responses including apoptosis and disruption of protein degradation pathways. There is also an inflammatory component to this disease, resulting from activation of microglia that cause the release of inflammatory cytokines and cell stress. This microglia activation causes apoptosis via the JNK pathway and by blocking the Akt signaling pathway via REDD1.

Select Reviews: Dauer, W. and Przedborski, S. (2003) *Neuron* 39, 889–909. | Ginsalt, J.A. and Greengard, P. (2004) *Arch. Neurol.* 61, 641–644. | Patten, D.A., Germain, M., Kelly, M.A., and Slack, R.S. (2010) *J. Alzheimers Dis.* 20 Suppl 2, S357–367. | Inoai, Y. and Lu, B. (2011) *Curr. Opin. Neurobiol.* 21, 935–941. | Springer, W. and Kahle, P.J. (2011) *Autophagy* 7, 266–278.

We would like to thank Prof. Jie Shen, Harvard Medical School, Boston, MA, for contributing to this diagram.

Hippo Signaling

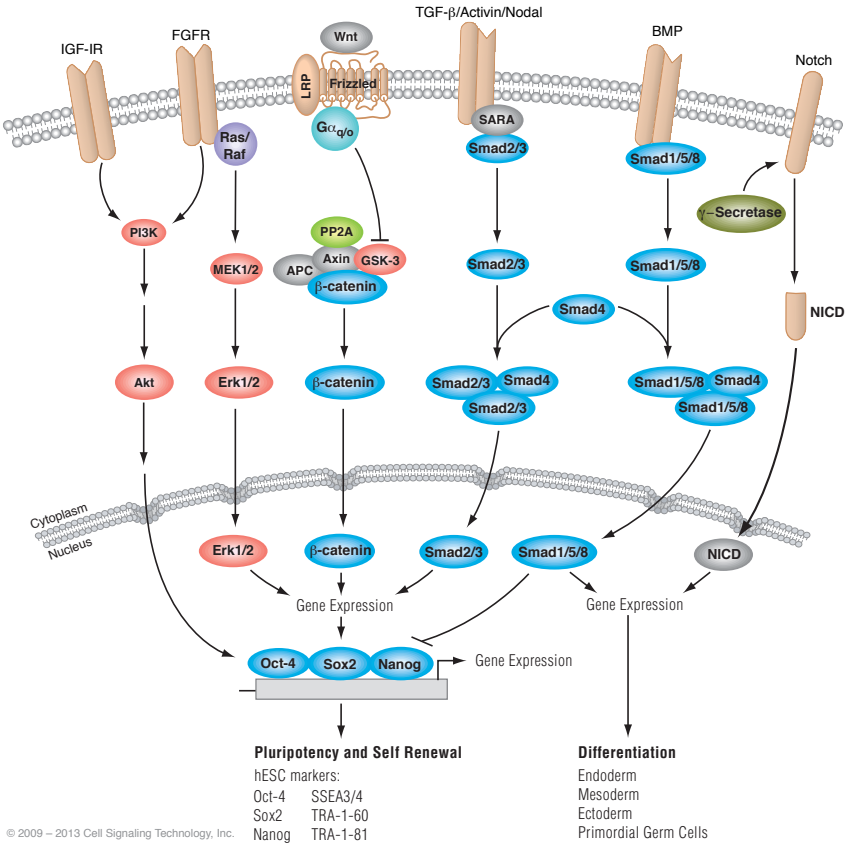


Hippo signaling is an evolutionarily conserved pathway that controls organ size by regulating cell proliferation, apoptosis, and stem cell self renewal. In addition, dysregulation of the Hippo pathway contributes to cancer development. Core to the Hippo pathway is a kinase cascade, wherein Mst1/2 (ortholog of Drosophila Hippo) kinases and Sav1 form a complex to phosphorylate and activate LATS1/2. LATS1/2 kinases in turn phosphorylate and inhibit the transcription co-activators YAP and TAZ, two major downstream effectors of the Hippo pathway. When dephosphorylated, YAP/TAZ translocate into the nucleus and interact with TEAD1-4 and other transcription factors to induce expression of genes that promote cell proliferation and inhibit apoptosis. The Hippo pathway is involved in cell contact inhibition, and its activity is regulated at multiple levels: Mst1/2 and LATS1/2 are regulated by upstream molecules such as Merlin, KIBRA, RASSFs, and Ajuba; 14-3-3, α-catenin, AMOT, and ZO-2 retain YAP/TAZ in the cytoplasm, adherens junctions, or tight junctions by binding; Mst1/2 and YAP/TAZ phosphorylation and activity are modulated by phosphatases; Lats1/2 and YAP/TAZ stability are regulated by protein ubiquitination; and LATS1/2 activity is also regulated by the cytoskeleton. Despite extensive study of the Hippo pathway in the past decade, the exact nature of extracellular signals and membrane receptors regulating the Hippo pathway remains elusive.

Select Reviews: Badouel, C. and McNeill, H. (2011) SnapShot: The hippo signaling pathway. *Cell* 145, 481–484. | Genevet, A. and Tapon, N. (2011) *Biochem. J.* 436, 213–224. | Pan, D. (2010) *Dev. Cell* 19, 491-505. | Sudol, M. and Harvey, K.F. (2010) *Trends Biochem. Sci.* 35, 627–633. | Zhao, B., Li, L., Lei, Q., and Guan, K.L. (2010) *Genes Dev.* 24, 862–874. | Zhao, B., Tumaneng, K., and Guan, K.L. (2011) *Nat. Cell Biol.* 13, 877–883.

We would like to thank Prof. Kun-Liang Guan, University of California, San Diego, for contributing to this diagram.

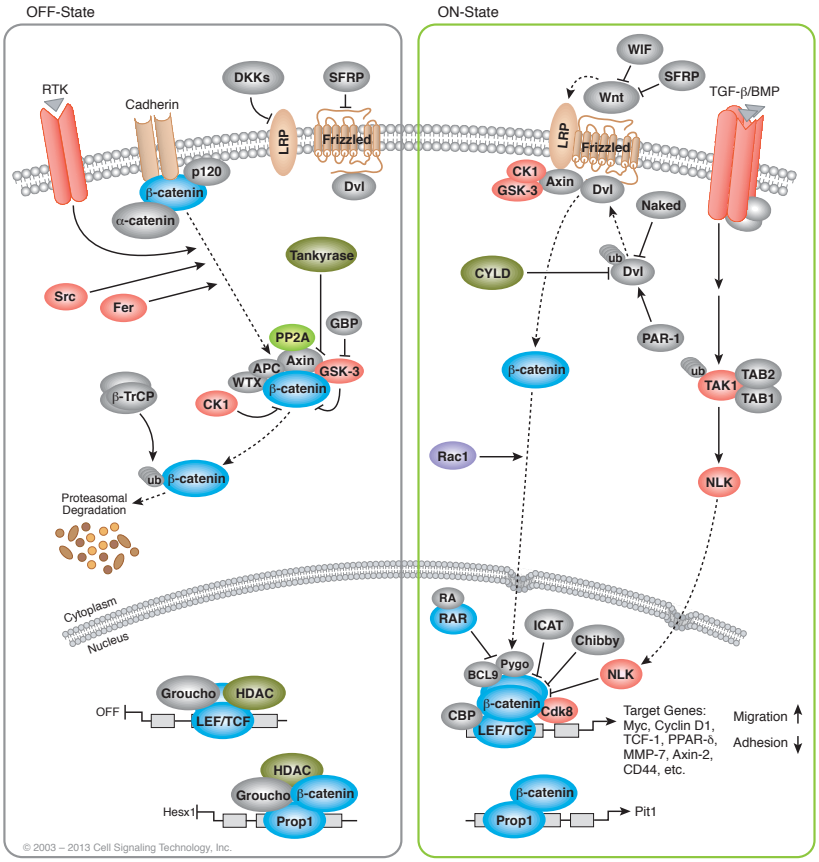
ESC Pluripotency and Differentiation



ESCs (hESCs), the predominant signaling pathways involved in pluripotency and self renewal are TGF-β, which signals through Smad2/3/4, and FGFR, which activates the MAPK and Akt pathways. The Wnt pathway also promotes pluripotency, although this may occur through a non-canonical mechanism involving a balance between the transcriptional activator TCF1 and the repressor TCF3. Signaling through these pathways results in the expression and activation of three key transcription factors: Oct-4, Sox2, and Nanog. These transcription factors activate gene expression of ESC-specific genes, regulate their own expression, and also serve as hESCs markers. Other markers used to identify hESCs are the cell surface glycolipid SSEA3/4, and glycoproteins TRA-1-60 and TRA-1-81. Loss of pluripotency results in differentiation into primordial germ cells or one of the three primary germ layers: endoderm, mesoderm, or ectoderm. One of the primary signaling pathways responsible for this process is the BMP pathway, which uses Smad/1/5/8 to promote differentiation by both inhibiting expression of Nanog, as well as activating the expression of differentiation-specific genes. Notch also plays a role in this process through the notch intracellular domain (NICD). As differentiation continues, cells from each primary germ layer further differentiate along lineage-specific pathways.

Select Reviews: Bilic, J. and Izpisua Belmonte, J.C. (2012) *Stem Cells* 30, 33–41. | Boiani, M. and Schöler, H.R. (2005) *Nat. Rev. Mol. Cell Biol.* 6, 872–884. | Guenther, M.G. (2011) *Epigenomics* 3, 323–343. | Heng, J.C., Orlov, Y.L., and Ng, H.H. (2010) *Cold Spring Harb. Symp. Quant. Biol.* 75, 237–244. | Pan, G. and Thomson, J.A. (2007) *Cell Res.* 17, 42–49. | Pei, D. (2009) *J. Biol. Chem.* 284, 3365–3369. | Welham, M.J., Kingham, E., Sanchez-Ripoll, Y., Kumpfmueller, B., Storm, M., and Bone, H. (2011) *Biochem. Soc. Trans.* 39, 674–678.

Kinase | Transcription Factor | Caspase | Enzyme | GAP/GEF | G-protein | Acetylase
Phosphatase | pro-apoptotic | Receptor | pro-survival | GTPase | Ribosomal subunit | Deacetylase

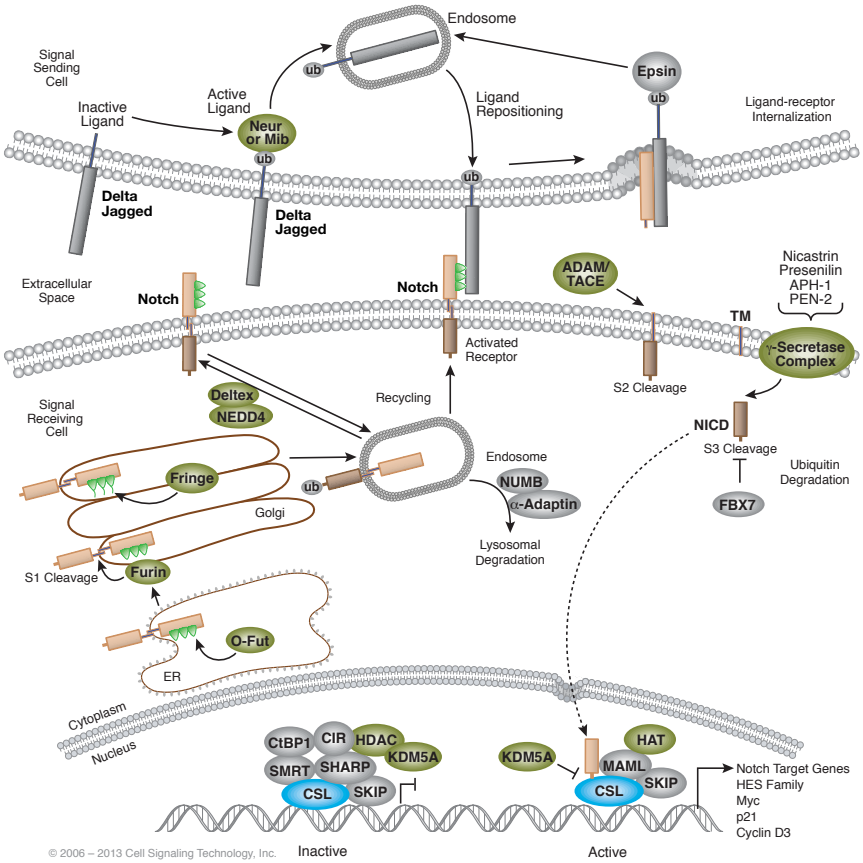


Wnt/β-Catenin Signaling

The conserved Wnt/β-Catenin pathway regulates stem cell pluripotency and cell fate decisions during development. This developmental cascade integrates signals from other pathways, including retinoic acid, FGF, TGF-β, and BMP, within different cell types and tissues. The Wnt ligand is a secreted glycoprotein that binds to Frizzled receptors, which triggers displacement of the multifunctional kinase GSK-3β from a regulatory APC/Axin/GSK-3β-complex. In the absence of Wnt-signal (Off-state), β-catenin, an integral E-cadherin cell-cell adhesion adaptor protein and transcriptional co-regulator, is targeted by coordinated phosphorylation by CK1 and the APC/Axin/GSK-3β-complex leading to its ubiquitination and proteasomal degradation through the β-TrCP/SKP pathway. In the presence of Wnt ligand (On-state), the co-receptor LRP5/6 is brought in complex with Wnt-bound Frizzled. This leads to activation of Dishevelled (Dvl) by sequential phosphorylation, poly-ubiquitination, and polymerization, which displaces GSK-3β from APC/Axin through an unclear mechanism that may involve substrate trapping and/or endosome sequestration. The transcriptional effects of Wnt ligand is mediated via Rac1-dependent nuclear translocation of β-catenin and the subsequent recruitment of LEF/ TCF DNA-binding factors as co-activators for transcription, acting partly by displacing Groucho-HDAC co-repressors. Additionally, β-catenin has also been shown to cooperate with the homeodomain factor Prop1 in context-dependent activation as well as repression complexes. Importantly, researchers have found β-catenin point mutations in human tumors that prevent GSK-3β phosphorylation and thus lead to its aberrant accumulation. E-cadherin, APC, and Axin mutations have also been documented in tumor samples, underscoring the deregulation of this pathway in cancer. Furthermore, GSK-3β is involved in glycogen metabolism and other signaling pathways, which has made its inhibition relevant to diabetes and neurodegenerative disorders.

Select Reviews: Angers, S. and Moon, R.T. (2009) *Nat. Rev. Mol. Cell Biol.* 10, 468–477. | Clevers, H. and Nusse, R. (2012) *Cell* 149, 1192-1205. | Fearon, E.R. (2009) *Cancer Cell* 16, 366–368. | MacDonald, B.T., Tamai, K., and He, X. (2009) *Dev. Cell* 17, 9–26. | Metcalfe, C. and Bienz, M. (2011) *J. Cell Sci.* 124, 3537–3544. | Mosimann, C., Hausmann, G., and Basler, K. (2009) *Nat. Rev. Mol. Cell Biol.* 10, 276–286. | Nusse, R. (2010) The Wnt Homepage. http://www.stanford.edu/group/nusselab/cgi-bin/wnt/. | Petersen, C.P. and Reddien, P.W. (2009) *Cell* 139, 1056–1068. | Sokol, S.Y. (2011) *Development* 138, 4341–4350. | van Amerongen, R. and Nusse, R. (2009) *Development* 136, 3205–3214.

We would like to thank Dr. Hans Widlund, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, for contributing to this diagram.

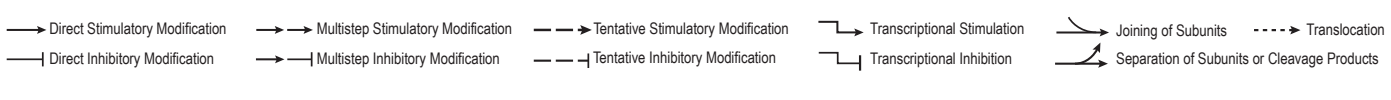


Notch Signaling

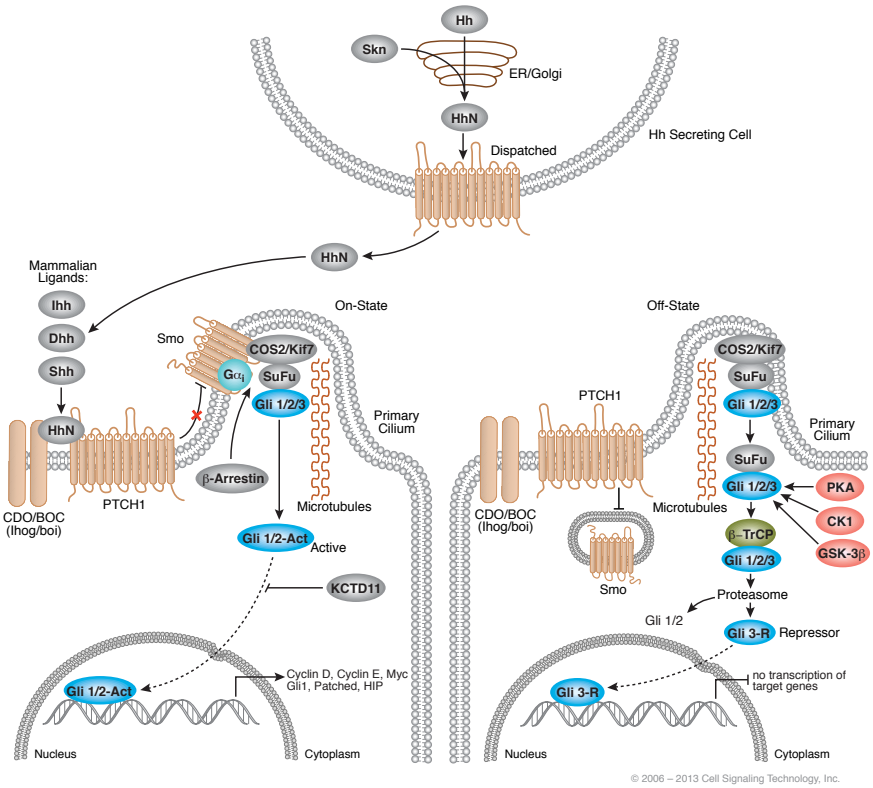
Notch signaling is an evolutionarily conserved pathway in multicellular organisms that regulates cell-fate determination during development and maintains adult tissue homeostasis. The Notch pathway mediates juxtacrine cellular signaling wherein both the signal sending and receiving cells are affected through ligand-receptor crosstalk by which an array of cell fate decisions in neuronal, cardiac, immune, and endocrine development are regulated. Notch receptors are single-pass transmembrane proteins composed of functional extracellular (NECD), transmembrane (TM), and intracellular (NICD) domains. Notch receptors are processed in the ER and Golgi within the signal-receiving cell through cleavage and glycosylation, generating a Ca^{2+}-stabilized heterodimer composed of NECD noncovalently attached to the TM-NICD inserted in the membrane (S1 cleavage). The processed receptor is then endosome-transported to the plasma membrane to enable ligand binding in a manner regulated by Deltex and inhibited by NUMB. In mammalian signal-sending cells, members of the Delta-like (DLL1, DLL3, DLL4) and the Jagged (JAG1, JAG2) families serve as ligands for Notch signaling receptors. Upon ligand binding, the NECD is cleaved away (S2 cleavage) from the TM-NICD domain by TACE (TNF-α ADAM metalloprotease converting enzyme). The NECD remains bound to the ligand and this complex undergoes endocytosis/recycling within the signal-sending cell in a manner dependent on ubiquitination by Mib. In the signal-receiving cell, γ-secretase (also involved in Alzheimer's disease) releases the NICD from the TM (S3 cleavage), which allows for nuclear translocation where it associates with the CSL (CBF1/Su(H)/Lag-1) transcription factor complex, resulting in subsequent activation of the canonical Notch target genes: Myc, p21, and the HES-family members. The Notch signaling pathway has spurred interest for pharmacological intervention due to its connection to human disease. Importantly, researchers have found Notch receptor activating mutations leading to nuclear accumulation of NICD are common in adult T cell acute lymphoblastic leukemia and lymphoma. In addition, loss-of-function Notch receptor and ligand mutations are implicated in several disorders, including Alagille syndrome and CADASIL, an autosomal dominant form of cerebral arteriopathy.

Select Reviews: Ables, J.L., Breunig, J.J., Eisch, A.J., and Rakic, P. (2010) *Nat. Rev. Neurosci.* 12, 269–283. | Andersson, E.R., Sandberg, R., and Lendahl, U. (2011) *Development* 138, 3593–3612. | Aster, J.C., Blacklow, S.C., and Pear, W.S. (2010) *J. Pathol.* 223, 262–273. | Bai, G. and Pfaff, S.L. (2011) *Neuron* 72, 9–21. | de la Pompa, J.L. and Epstein, J.A. (2012) *Dev. Cell* 22, 244–254. | Ranganathan, P., Weaver, K.L., and Capobianco, A.J. (2011) *Nat. Rev. Cancer* 11, 338–351. | Weinmaster, G. and Fischer, J.A. (2011) *Dev. Cell* 21, 134–144. | Yuan, J.S., Kousis, P.C., Suliman, S., Visan, I., and Guidos, C.J. (2010) *Annu. Rev. Immunol.* 28, 343–365.

We would like to thank Dr. Hans Widlund, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, for contributing to this diagram.



Hedgehog Signaling

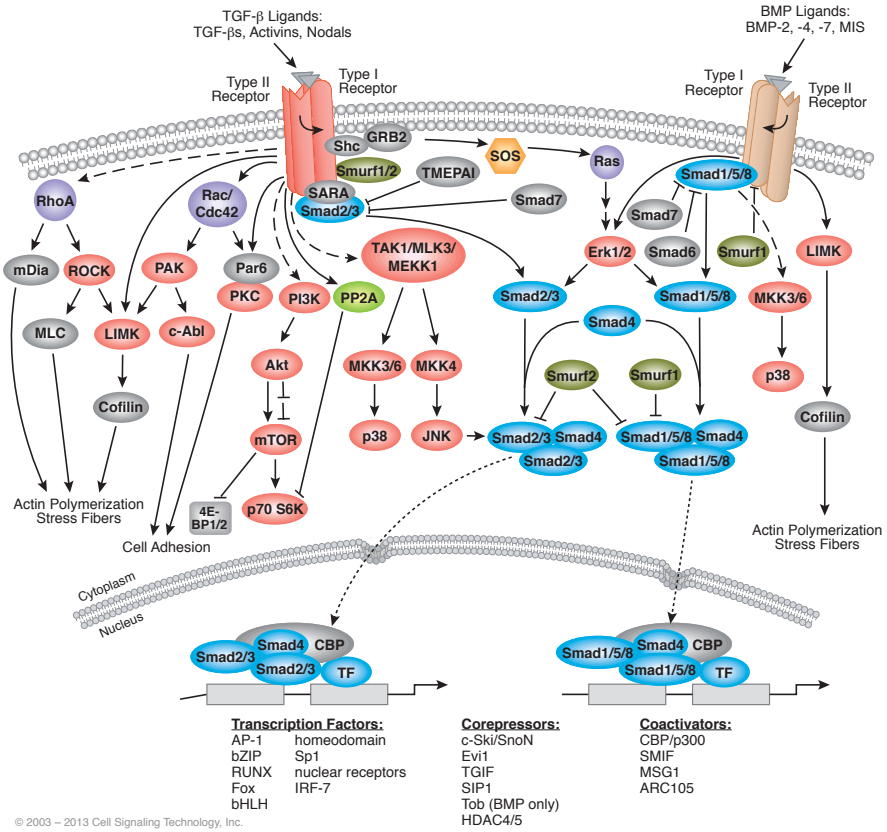


The evolutionarily conserved Hedgehog pathway plays a critical role in a time and position-dependent fashion during development by regulating patterning and maintenance of proliferative niches. Proper secretion and gradient diffusion of the vertebrate Hedgehog-family ligands, including Sonic, Desert, and Indian Hedgehog, all require autoprocessive cleavage as well as cholesterol and palmitate lipid modifications. In the receiving cell in the absence of Hedgehog ligand (off-state), the Patched receptor is associated with Smoothened, a G-coupled transmembrane protein, and prevents its membrane incorporation from endosomes. Further, the Hedgehog Off-state allows SuFu and COS2 (Kif7 in vertebrates) to sequester the microtubule-bound pool of the Gli transcription factor within the primary cilium, thereby allowing its phosphorylation by PKA, CK1, and GSK-3. This results in β-TrCP-mediated degradation of Gli activators (Gli1 and Gli2 in mammals) or the generation of repressor-Gli (Gli3 or truncated-Ci in Drosophila) in the conserved pathway that collectively leads to repression of Hedgehog target genes. In the on-state, Hedgehog binding sequesters the co-receptor Ihog to Patched that permits β-arrestin to freely facilitate incorporation of Smoothened to the primary cilium membrane. In the primary cilium, Smoothened's associated G protein activity relieves Gli from microtubule association, enables nuclear translocation, and activation of Hedgehog/Gli target genes including Cyclin D, Cyclin E, Myc, and Patched. Consequently, the conserved action of Hedgehog ligands is to switch the Gli factors from transcriptional repressors into activators and allow for well-coordinated bursts of transcriptional events. Loss-of-function Patched mutations are associated with Gorlin syndrome and predisposes to basal cell carcinomas, medulloblastomas, and rhabdomyosarcomas. In addition, researchers have found activating mutations of Smoothened in basal cell carcinomas and rare SuFu mutations in medulloblastomas, underscoring the involvement of this developmental pathway in cancer; consequently, significant interest is focused on targeting this pathway for therapeutic purposes.

Select Reviews: Beachy, P.A., Hymowitz, S.G., Lazarus, R.A., Leahy, D.J., and Siebold, C. (2010) *Genes Dev.* 24, 2001–2012. | Eaton, S. (2008) *Nat. Rev. Mol. Cell Biol.* 9, 437–445. | Hui, C.C. and Angers, S. (2011) *Annu. Rev. Cell Dev. Biol.* 27, 513–537. | Ingham, P.W., Nakano, Y., and Seger, C. (2011) *Nat. Rev. Genet.* 12, 393–406. | Ng, J.M. and Curran, T. (2011) *Nat. Rev. Cancer* 11, 493–501. | Oh, E.C. and Katsanis, N. (2012) *Development* 139, 443–448. | Theunissen, J.W. and de Sauvage, F.J. (2009) *Cancer Res.* 69, 6007–6010. | Wilson, C.W. and Chuang, P.T. (2010) *Development* 137, 2079–2094.

We would like to thank Dr. Hans Widlund, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, for contributing to this diagram.

TGF-β Signaling



Transcription Factors:
AP-1 homeodomain
bZIP Sp1
RUNX nuclear receptors
Fox IRF-7
bHLH

Corepressors:
c-Ski/SnoN
Evi1
TGIF
SIP1
Tob (BMP only)
HDAC4/5

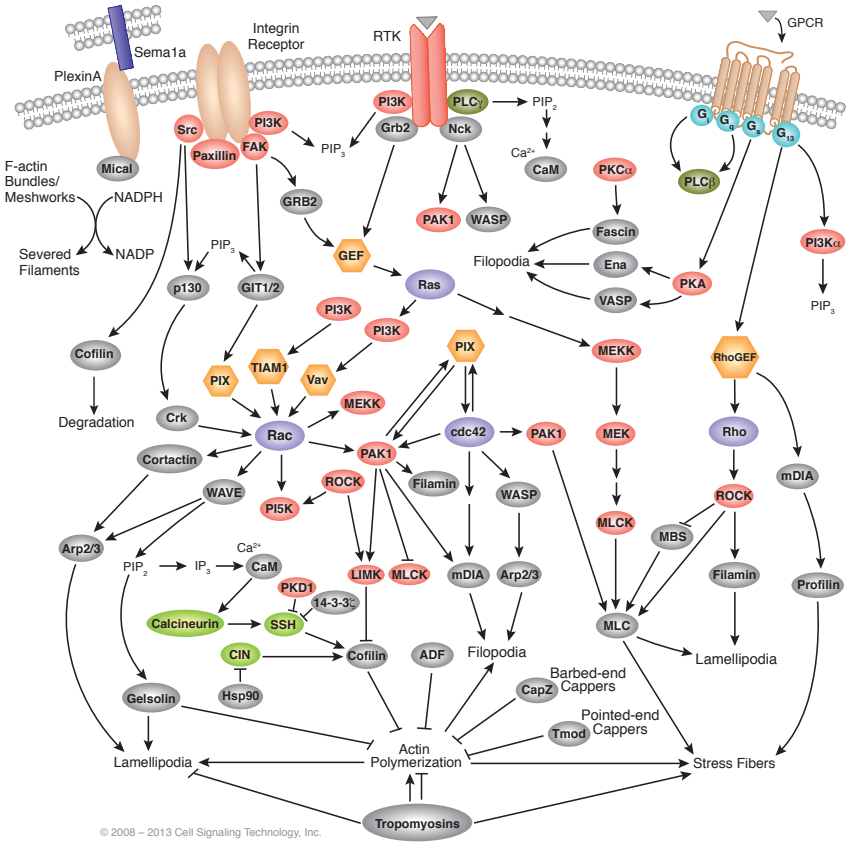
Coactivators:
CBP/p300
SMIF
MSG1
ARC105

The regulation of cell growth, differentiation, and development in a wide range of biological systems. In general, signaling is initiated with ligand-induced oligomerization of serine/ threonine receptor kinases and phosphorylation of the cytoplasmic signaling molecules Smad2 and Smad3 for the TGF-β/activin pathway, or Smad1/5/8 for the bone morphogenetic protein (BMP) pathway. Carboxy-terminal phosphorylation of Smads by activated receptors results in their partnering with the common signaling transducer Smad4, and translocation to the nucleus. Activated Smads regulate diverse biological effects by partnering with transcription factors resulting in cell-state specific modulation of transcription. The activin and BMP pathways are themselves attenuated by MAPK signaling at a number of levels, while the expression of inhibitory Smads (I-Smads) 6 and 7 is induced by both activin/TGF-β and BMP signaling as part of a negative feedback loop. In certain contexts, TGF-β signaling can also affect Smad-independent pathways, including Erk, SAPK/JNK, and p38 MAPK pathways. Activation of Smad-independent pathways through TGF-β signaling is also common. Rho GTPase (RhoA) activates downstream target proteins, such as mDia and ROCK, to prompt rearrangement of the cytoskeletal elements associated with cell spreading, cell growth regulation, and cytokinesis. Cdc42/Rac regulates cell adhesion through downstream effector kinases PAK, PKC, and c-Abl following TGF-β activation.

Select Reviews: Herpin, A. and Cunningham, C. (2007) *FEBS J.* 274, 2977–2985. | Horbelt, D., Denkis, A., and Knaus, P. (2012) *Int. J. Biochem. Cell Biol.* 44, 469–474. | Ikushima, H. and Miyazono, K. (2010) *Nat. Rev. Cancer* 10, 415–424. | Kitisin, K., Saha, T., Blake, T., Golestaneh, N., Deng, M., Kim, C., Tang, Y., Shetty, K., Mishra, B., and Mishra, L. (2007) *Sci. STKE* cm1. | Meulmeester, E. and ten Dijke, P. (2011) *J. Pathol.* 223, 205–218. | Schmierer, B. and Hill, C.S. (2007) *Nat. Rev. Mol. Cell Biol.* 8, 970–982. | Verheyen, E.M. (2007) *Dev. Cell* 13, 755–756. | Xiao, Y.T., Xiang, L.X., and Shao, J.Z. (2007) *Biochem. Biophys. Res. Commun.* 362, 550–553.

We would like to thank Luuk Hawinkels and Prof. Peter ten Dijke, Leiden University Medical Center, Leiden, The Netherlands, for contributing to this diagram.

Kinase | Transcription Factor | Caspase | Enzyme | GAP/GEF | G-protein | Acetylase
Phosphatase | pro-apoptotic | Receptor | pro-survival | GTPase | Ribosomal subunit | Deacetylase



Regulation of Actin Dynamics

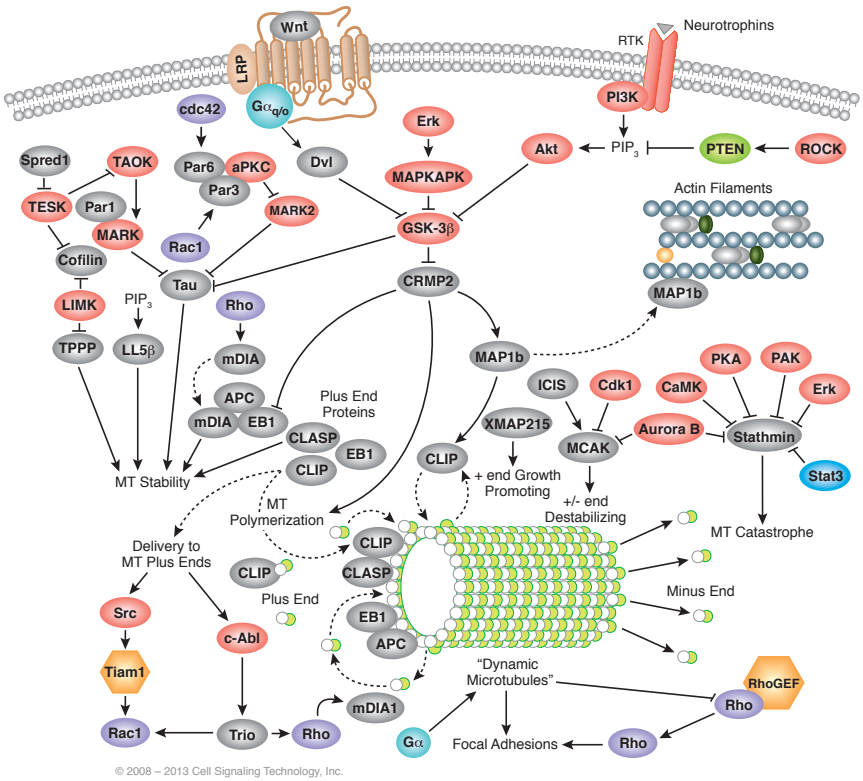
Signaling to the cytoskeleton through G protein-coupled receptors (GPCRs), integrins, receptor tyrosine kinases (RTKs), and numerous other specialized receptors, such as the semaphorin 1a receptor PlexinA, can lead to diverse effects on cell activity, including changes in cell shape, migration, proliferation, and survival. Integrins, in conjuction with other components of focal adhesion complexes, serve as the link between the extracellular matrix and cytoskeleton in many cell types. Integrin activation leads to activation of focal adhesion kinase (FAK) and Src kinase, resulting in phosphorylation of other FA components such as paxillin and the Crk-associated substrate p130 Cas, as well as the recruitment of signaling adapter proteins.

Intracellular regulation of the cell's response to external cues occurs through a large number of signaling cascades that include the Rho family of small GTPases (Rho, Rac, and Cdc42) and their activators, guanine nucleotide exchange factors (GEFs), their downstream protein kinase effectors, including Rho-kinase/ROCK and p21 activated kinase (PAK), as well as through direct binding of the GTPases to several actin regulatory proteins, such as cortactin, mDia, WAVE, and WASP. These cascades converge on proteins that directly regulate the behavior and organization of the actin cytoskeleton, including actin interacting regulatory proteins such as cofilin, Arp2/3 complex, Ena/VASP, forminins, profilin, and gelsolin. Signaling through different pathways can lead to the formation of distinct actin-dependent structures whose coordinated assembly/disassembly is important for directed cell migration and other cellular behaviors. Migration is also regulated by signaling to myosin, which participates in leading edge actin dynamics and enables retraction of the rear of the cells. Tropomyosins stabilize F-actin by preventing binding of severing and dynamizing factors. Some tropomyosins may also enhance filament dynamics. Dynamic actin is required for most cellular actin-dependent processes; inhibiting actin assembly and preventing actin disassembly are equally inhibitory to most behaviors.

Aberrant control of cytoskeletal signaling, which can result in a disconnection between extracellular stimuli and cellular responses, is often seen in immune pathologies, developmental defects, and cancer.

Select Reviews: Bernstein, B.W. and Bamburg, J.R. (2010) *Trends Cell Biol.* 20, 187–195. | Lee, S.H. and Dominguez, R. (2010) *Mol. Cells* 29, 311–325. | Levayer, R. and Lecuit, T. (2012) *Trends Cell Biol.* 22, 61–81. | Poukkula, M., Kremneva, E., Serlachius, M., and Lappalainen, P. (2011) *Cytoskeleton (Hoboken)* 68, 471–490. | Ridley, A.J. (2011) *Cell* 145, 1012–1022. | Rottner, K. and Stradal, T.E. (2011) *Curr. Opin. Cell Biol.* 23, 569–578.

We would like to thank Prof. James Bamburg, Colorado State University, for updates to the Regulation of Actin Dynamics and the Regulation of Microtubule Dynamics pathways.



Regulation of Microtubule Dynamics

Microtubules are required for the establishment of cell polarity, polarized migration of cells, intracellular vesicle transport, and chromosomal segregation in mitosis. Microtubules (MTs) are nonequilibrium polymers of α/β-tubulin heterodimers, in which GTP hydrolysis on the β-tubulin subunit occurs following assembly. Most microtubules are nucleated from organizing centers. The most prevalent microtubule behavior is dynamic instability, a process of slow plus end growth coupled with rapid depolymerization ("catastrophe") and subsequent rescue. Although microtubule minus ends show dynamic instability, albeit at a lower rate than the plus ends, the minus ends are usually capped and anchored at MT organizing centers and thus often do not participate in microtubule dynamics.

Maintaining a balance between dynamically unstable and stable microtubules is regulated in large part by proteins that bind either tubulin dimers or assembled microtubules. Proteins that bind tubulin dimers include stathmin, which sequesters tubulin and enhances MT dynamics by increasing catastrophe frequency, and collapsin response mediator protein (CRMP2), which increases MT growth rate by promoting addition of tubulin dimers onto microtubule plus ends. Other proteins that associate with assembled MTs include those that bundle MTs (e.g. MAP1c), those that stabilize MTs (e.g. tau), and those that maintain MTs in a dynamic state (MAP1b). A major signaling pathway that regulates MT dynamics involves GSK-3β, a kinase typically active under basal growth conditions but locally inactive in response to signals that enhance MT growth and dynamics.

Tubulin undergoes several post-translational modifications such as acetylation, poly-glutamylation, and poly-glycylation, which have been shown to alter the association with certain MT motors as well as other proteins that can affect MT stability and dynamics.

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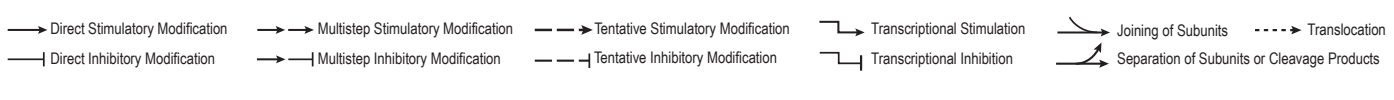
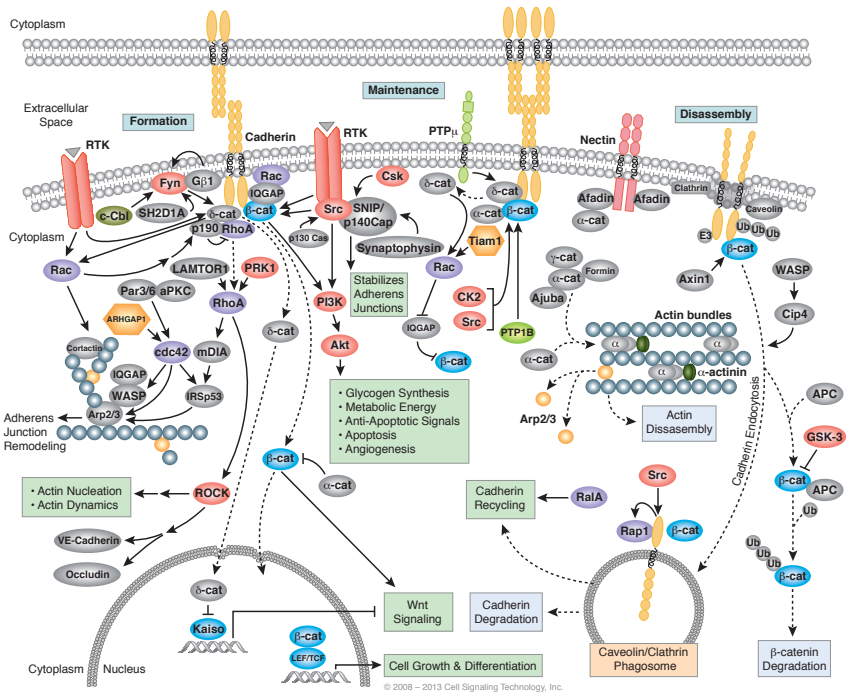


Adherens Junction Dynamics



Adherens junctions are dynamic structures that form, strengthen and spread, degrade, and then re-form as their associated proteins create ephemeral connections with counterparts from adjacent cells. This view updates the traditional model of a stable complex composed of cadherin, β-catenin, and α-catenin bound to the actin cytoskeleton. Although cadherin does exist in a complex with β-catenin and α-catenin, this cadherin-catenin complex does not associate with the actin cytoskeleton. α-catenin does not directly anchor cell adhesion proteins to the actin cytoskeleton but acts as a regulatory protein to control actin filament dynamics.

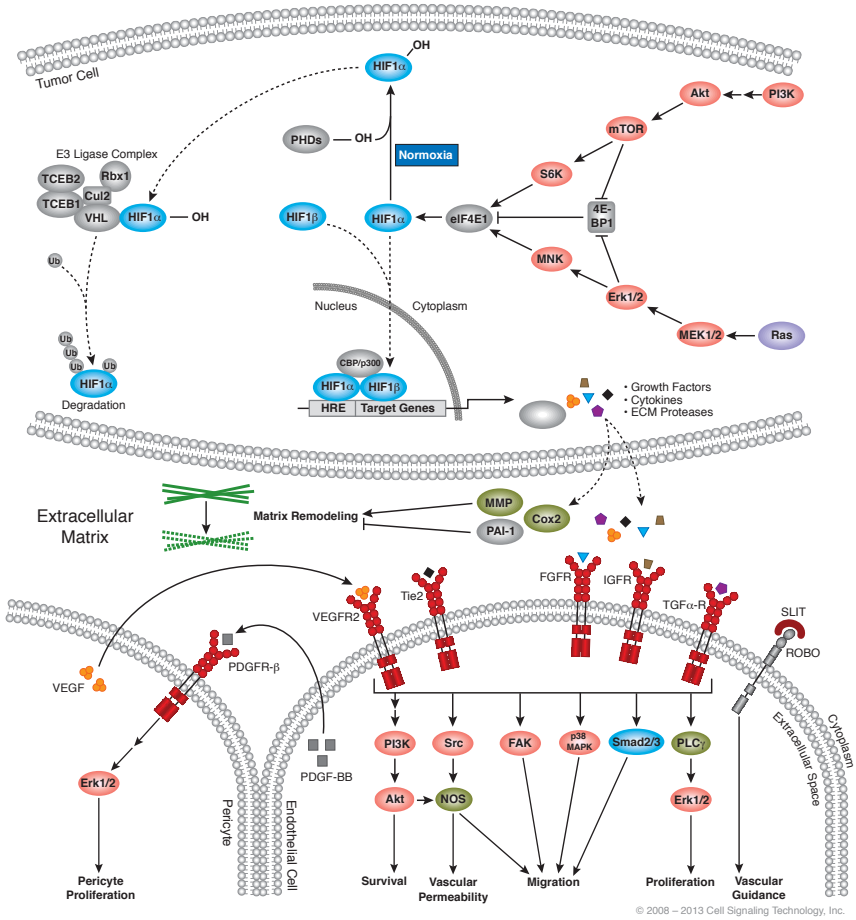
Monomeric α-catenin binds β-catenin at adherens junctions and upon release forms α-catenin dimers that promote actin bundle formation. The transition from branched actin networks to bundled actin filaments correlates with the creation of mature, strong adherens junctions and a decrease in membrane lamellipodia. The connection between cell junctions and the cytoskeleton may be more dynamic than originally considered and may rely on multiple, weak associations between the cadherin-catenin complex and the actin cytoskeleton or rely on other membrane-associated proteins (i.e. nectin and afadin).

As with most dynamic cellular systems, a collection of kinases, phosphatases, and adaptor proteins regulate the activity and localization of a few key effector proteins. p120 catenin (δ-catenin) binds and stabilizes cadherin at the plasma membrane. Membrane-bound and cytosolic tyrosine kinases phosphorylate β-catenin at weak or nascent junctions, while phosphatases remove added phosphates from β-catenin and δ-catenin at established junctions. Rho family GTPases modulate the availability and activation state of catenins and other essential adherens proteins. Together, this collection of structural proteins, enzymes, and adaptor proteins create dynamic cell-cell junctions necessary for temporary associations during morphogenesis and maintains the integrity of complex tissues and structures following development.

Select Reviews: Baum, B. and Georgiou, M. (2011) *J. Cell Biol.* 192, 907–917. | Citi, S., Spadaro, D., Schneider, Y., Stutz, J., and Pulimeno, P. (2011) *Mol. Membr. Biol.* 28, 427–444. | Harris, T.J. and Tepass, U. (2010) *Nat. Rev. Mol. Cell Biol.* 11, 502–514. | Niessen, C.M. and Gottardi, C.J. (2008) *Biochim. Biophys. Acta.* 1778, 562–571. | Pieters, T., van Roy, F., and van Hengel, J. (2012) *Front Biosci.* 17, 1669–1694. | Yonemura, S. (2011) *Curr. Opin. Cell Biol.* 23, 515–522.

We would like to thank Prof. Rakesh Kumar of The George Washington University, Washington, D.C. for reviewing this diagram.

Angiogenesis



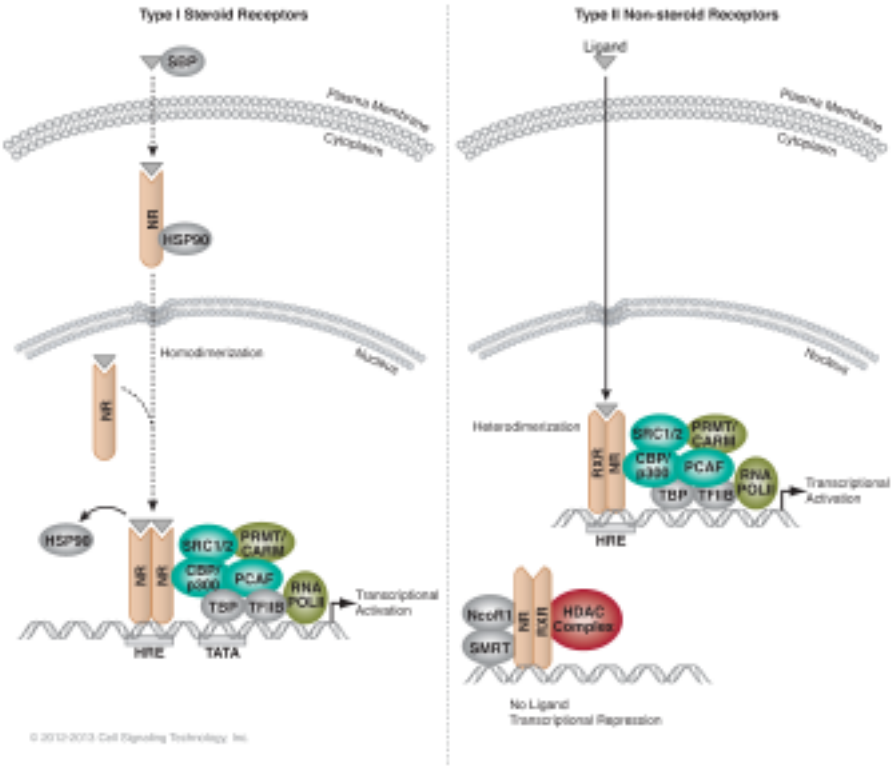
Angiogenesis is the formation of new blood vessels and can be induced by tumor growth, tissue wound, and inflammation. Rapid tumor cell growth creates intracellular hypoxia. Hypoxia-inducible factor (HIF) is a transcription factor that responds to changing intracellular oxygen concentration. Under typical oxygen levels (normoxia), HIF is hydroxylated and acetylated, modifications that target the transcription factor for VHL-mediated ubiquitin degradation. During hypoxia, HIF accumulates and is transported to the nucleus where it induces expression of numerous target gene products. Secreted growth factors (such as VEGF, FGF, and TGF) induce signaling pathways (including PLCγ, PI3K, Src, and Smad signaling) that result in endothelial cell proliferation, increased vascular permeability, and cell migration. In addition to hypoxia, the PI3K and Ras pathways can increase HIF expression by promoting HIF translation.

Pericytes are support cells that provide structural support for newly formed blood vessels, promote endothelial cell survival, guide sprouting vessels, and regulate vasoconstriction and dilation. This is done through a reciprocal signaling mechanism in which PDGF-BB secreted into the matrix by endothelial cells acts as a ligand for PDGF receptor-β located on the pericyte membrane. In return, pericytes produce and secrete VEGF that signals through the endothelial VEGF receptor.

Extracellular matrix proteases and regulators induce tissue matrix remodeling in preparation for migration of endothelial cells from existing vessels to form new tubing. Tissue wounding, ischemia, or inflammation recruit macrophages and bone marrow-derived inflammatory cells (BDMC) to wound areas and secrete a similar panel of proteins to induce angiogenesis.

Select Reviews: Keith, B., Johnson, R.S., and Simon, M.C. (2011) *Nat. Rev. Cancer* 12, 9–22. | Raza, A., Franklin, M.J., and Dudek, A.Z. (2010) *Am. J. Hematol.* 85, 593–598. | Sakurai, T. and Kudo, M. (2011) *Oncology 81 Suppl* 1, 24–29. | Senger, D.R. and Davis, G.E. (2011) *Cold Spring Harb. Perspect. Biol.* 3, a005090. | Tie, J. and Desai, J. (2012) *Crit. Rev. Oncog.* 17, 51–67. | van Hinsbergh, V.W. and Koolwijk, P. (2008) *Cardiovasc. Res.* 78, 203–212.

Kinase | Transcription Factor | Caspase | Enzyme | GAP/GEF | G-protein | Acetylase
Phosphatase | pro-apoptotic | Receptor | pro-survival | GTPase | Ribosomal subunit | Deacetylase



Nuclear Receptor Signaling

The nuclear receptor superfamily are ligand-activated transcription factors that play diverse roles in cell differentiation/ development, proliferation, and metabolism and are associated with numerous pathologies such as cancer, cardiovascular disease, inflammation, and reproductive abnormalities. Members of this family contain an N-terminal transactivation domain, a highly conserved central region zinc-finger DNA binding domain, and a C-terminal ligand-binding domain. Ligand binding to its cognate nuclear receptor results in transactivation of specific genes within a target tissue.

Type I nuclear receptors, also called steroid receptors, include the estrogen receptor, androgen receptor, progesterone receptor, and glucocorticoid receptor. Steroid hormone ligands for this subgroup of receptors travel from their respective endocrine gland through the bloodstream bound to steroid binding globulin. Some type 1 nuclear receptors are activated, in part, upon binding their respective ligand in the cytoplasmic compartment. The ligand-receptor complex enters the nucleus where it homodimerizes, dissociates from HSP90, and binds a hormone response element within the promoter of a target gene. The receptor transactivation domain is responsible for interaction at the promoter with acetyltransferases, co-activators, and the general transcription machinery (TBP, TF1B, RNA polymerase II), thereby resulting in transcriptional activation.

Type II non-steroid nuclear receptors include thyroid hormone receptor, retinoic acid receptor, vitamin D receptor, and PPARγ. Members of this family heterodimerize with the retinoid X receptor (RXR). Prior to ligand binding, receptor heterodimers are located in the nucleus as part of complexes with histone deacetylases (HDACs) and other co-repressors that keep target DNA in a tightly wound conformation, preventing exposure to transactivation factors. Ligand binding results in HDAC dissociation, chromatin derepression, and transcriptional activation.

In addition to ligand binding, nuclear receptor activity can be modulated through the action of numerous growth factor and cytokine signaling cascades that result in receptor phosphorylation or other post-translational modifications, typically within the N-terminal transactivation domain. For example, the estrogen receptor is phosphorylated on multiple serine residues that affect receptor activity. Ser118 may be the substrate of the transcription regulatory kinase CDK7, whereas Ser167 may be phosphorylated by p90RSK and Akt. Phosphorylation of Ser167 may confer resistance to tamoxifen in breast cancer patients.

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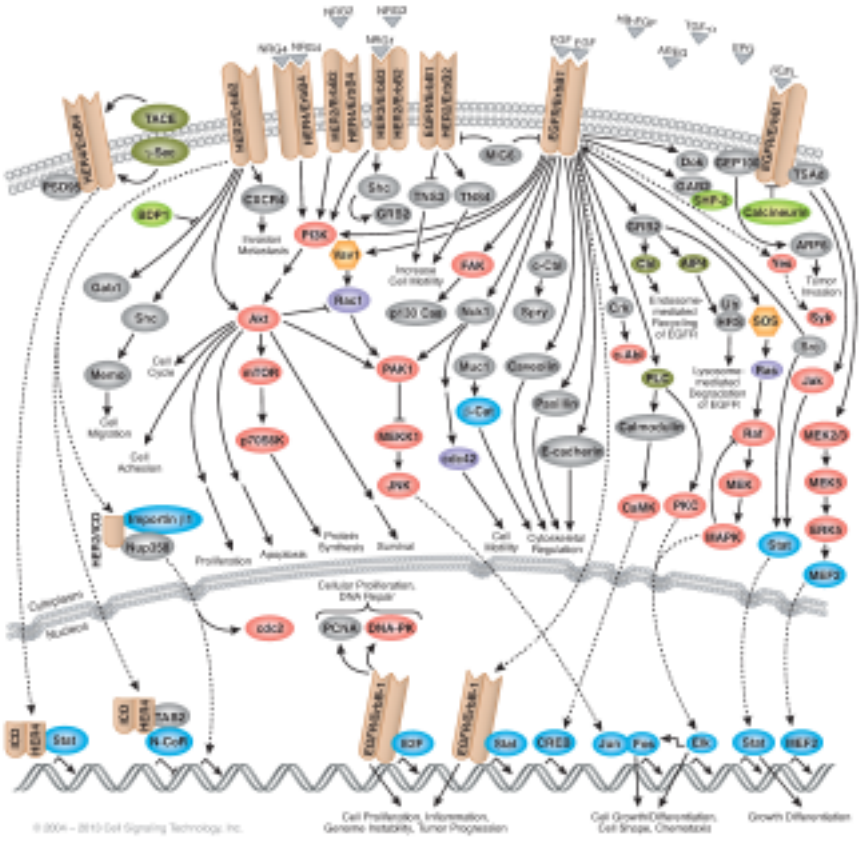

ErbB/HER Signaling

The ErbB receptor tyrosine kinase family consists of four cell surface receptors: ErbB1/EGFR/HER1, ErbB2/HER2, ErbB3/HER3, and ErbB4/HER4. ErbB receptors are typical cell membrane receptor tyrosine kinases that are activated following ligand binding and receptor dimerization. Ligands can either display receptor specificity (i.e. EGF, TGF-α, AR, and Epigen bind EGFR) or bind to one or more related receptors; neuregulins 1-4 bind ErbB3 and ErbB4 while HB-EGF, epiregulin, and β-cellulin activate EGFR and ErbB4. ErbB2 lacks a known ligand, but recent structural studies suggest ErbB2 is probably also regulated by ligand.

The ErbB receptors signal through Akt, MAPK, and many other pathways to regulate cell proliferation, migration, differentiation, apoptosis, and cell motility. ErbB family members are often over-expressed, amplified, or mutated in many forms of cancer, making them important therapeutic targets. Researchers have found EGFR to be amplified in gliomas and NSCLC while ErbB2 amplifications are seen in breast, ovarian, bladder, NSCLC, as well as several other tumor types.

Besides functioning as receptors on the cell surface, ErbB family proteins are also present in the nucleus to act as both kinases and transcriptional regulators. For example, EGFR could be transported into the nucleus where it functions as a tyrosine kinase to phosphorylate and stabilize PCNA. Similarly, membrane-bound ErbB2 interacts with importin β1 and Nup358 and migrates to the nucleus via endocytic vesicles. Inside the nucleus, ErbB2 modulates the transcription of multiple downstream genes including COX-2. In addition, NRG or TPA stimulation promotes ErbB4 cleavage by γ-secretase, releasing an 80 kDa intracellular domain that translocates to the nucleus to induce differentiation or apoptosis. Upon activation and cleavage, ErbB4 can also form a complex with TAB2 and N-CoR to repress gene expression.

Signaling through ErbB networks is modulated through dense positive and negative feedback and feed forward loops, including transcription-independent early loops and late loops mediated by newly synthesized proteins and miRNAs. For example, activated receptors can be switched "off" through dephosphorylation, receptor ubiquitination, or removal of active receptors from the cell surface through endosomal sorting and lysosomal degradation.

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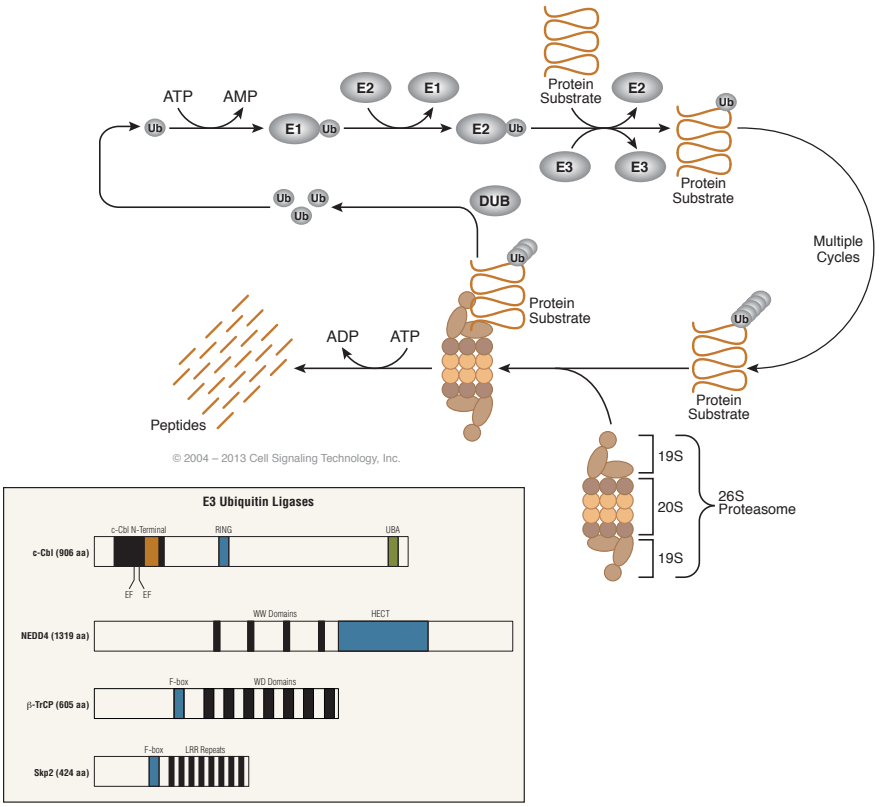
We would like to thank Dr. Jiayan Du, Merrimack Pharmaceuticals Inc., Cambridge, MA, for contributing to this diagram.

Ubiquitin/Proteasome



The ubiquitin proteasome pathway, conserved from yeast to mammals, is required for the targeted degradation of most short-lived proteins in the eukaryotic cell. Targets include cell cycle regulatory proteins, whose timely destruction is vital for controlled cell division, as well as proteins unable to fold properly within the endoplasmic reticulum.

Ubiquitin modification is an ATP-dependent process carried out by three classes of enzymes. A "ubiquitin activating enzyme" (E1) forms a thio-ester bond with ubiquitin, a highly conserved 76-amino acid protein. This reaction allows subsequent binding of ubiquitin to a "ubiquitin conjugating enzyme" (E2), followed by the formation of an isopeptide bond between the carboxy-terminus of ubiquitin and a lysine residue on the substrate protein. The latter reaction requires a "ubiquitin ligase" (E3). E3 ligases can be single- or multi-subunit enzymes. In some cases, the ubiquitin-binding and substrate-binding domains reside on separate polypeptides brought together by adaptor proteins or cullins. Numerous E3 ligases provide specificity in that each can modify only a subset of substrate proteins. Further specificity is achieved by post-translational modification of substrate proteins, including, but not limited to, phosphorylation.

Effects of monoubiquitination include changes in subcellular localization. However, multiple ubiquitination cycles resulting in a polyubiquitin chain are required for targeting a protein to the proteasome for degradation. The multisubunit 26S proteasome recognizes, unfolds, and degrades polyubiquitinated substrates into small peptides. The reaction occurs within the cylindrical core of the proteasome complex, and peptide bond hydrolysis employs a core threonine residue as the catalytic nucleophile.

Recent work has indicated that an additional layer of complexity, in the form of multiubiquitin chain receptors, may lie between the polyubiquitination and degradation steps. These receptors react with a subset of polyubiquitinated substrates, aiding in their recognition by the 26S proteasome, and thereby promoting their degradation.

This pathway is not only important in cellular homeostasis, but also in human disease. Because ubiquitin/proteasome-dependent degradation is often employed in control of the cell division cycle and cell growth, researchers have found that proteasome inhibitors hold some promise of being developed into potential cancer therapeutic agents.

Select Reviews: Budhidarmo, R., Nakatani, Y., and Day, C.L. (2012) *Trends Biochem. Sci.* 37, 58–65. | Burrows, J.F. and Johnston, J.A. (2012) *Front. Biosci.* 17, 1184–200. | Hammond-Martel, I., Yu, H., and Affar el, B. (2012) *Cell Signal.* 24, 410–421. | Schaefer, A., Nethe, M., and Hordijk, P.L. (2012) *Biochem. J.* 442, 13–25. | Weissman, A.M., Shabek, N., and Ciechanover, A. (2011) *Nat. Rev. Mol. Cell Biol.*12, 605–620.

Ubiquitin Ligase Table

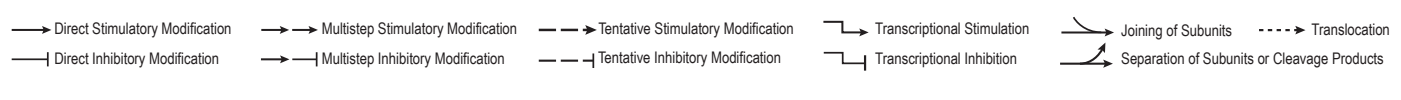
The Ubiquitin Ligase Table provides a list of E3 ubiquitin ligases, along with their substrates (when known), and corresponding references. This table was generated using PhosphoSitePlus®, Cell Signaling Technology's protein modification resource. See page 4 for more information on PhosphoSitePlus®.

We would like to thank Prof. Wenyi Wei, Beth Israel Deaconess Medical Center, Harvard Medical School, for contributing to this table.

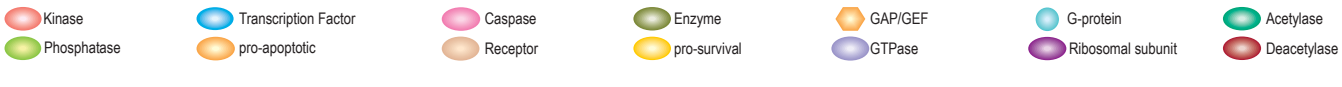
Ligase	Substrate	Function	PMID
AMFR	KAI1	AMFR is also known as gp78. AMFR is an integral ER membrane protein and functions in ER-associated degradation (ERAD). AMFR has been found to promote tumor metastasis through ubiquitination of the metastasis suppressor, KAI1.	18037895
APC/Cdc20	Cyclin B, Securin	The anaphase promoting complex/cyclosome (APC/C) is a multiprotein complex with E3 ligase activity that regulates cell cycle progression through degradation of cyclins and other mitotic proteins. APC is found in a complex with CDC20, CDC27, SPATC1, and TUBG1.	17609108, 12070128
APC/Cdh1	Cdc20, Cyclin B, Cyclin A, Aurora A, Skp2, Claspin	The anaphase promoting complex/cyclosome (APC/C) is a multiprotein complex with E3 ligase activity that regulates cell cycle progression through degradation of cyclins and other mitotic proteins. The APC/C-Cdh1 dimeric complex is activated during anaphase and telophase, and remains active until onset of the next S phase.	10548110, 11562349, 15014503, 19477924
ARIH1	4EHP	ARIH1 is an E3 ubiquitin ligase that may regulate protein translation by targeting eIF4E2 for ubiquitination and degradation by the proteasome.	14623119
BIRC2	Smac, TRAF2	BIRC2 is an apoptotic suppressor that prevents caspase activation by forming a complex with TNF receptor associated factors 1 and 2 (TRAF1 and TRAF2), which is then recruited to the tumor necrosis factor receptor 2 (TNFR2).	12525502, 18434593
BIRC3	Caspase 3 and 7, Smac, TRAF1	BIRC3 is an apoptotic suppressor that prevents caspase activation by forming a complex with TNF receptor associated factors 1 and 2 (TRAF1 and TRAF2), which is then recruited to the tumor necrosis factor receptor 2 (TNFR2).	10862606, 12525502, 15468071
BIRC4	Caspase 3, Smac, MEKK2	BIRC4 is an apoptotic suppressor that prevents caspase activation by forming a complex with TNF receptor associated factors 1 and 2 (TRAF1 and TRAF2), which is then recruited to the tumor necrosis factor receptor 2 (TNFR2). BIRC4 is also known as XIAP.	11447297, 12121969, 18761086
BIRC7	Smac	BIRC7 is an E3 ubiquitin ligase with anti-apoptotic activity. BIRC7 supports cell survival by targeting Smac for ubiquitination and degradation by the proteasome.	16729033
Bmi1	H2A K119	Bmi1 is a component of the polycomb group multiprotein PRC1- like (PcG PRC1) complex. Bmi1 is required for stimulating PcG PRC1 ubiquitin-protein ligase activity.	18650381
BRCA1	ER-α, Rpb8, CtIP, FANCD2	BRCA1 is an E3 ubiquitin ligase that maintains genomic stability by repairing DNA damage. Research studies have shown that mutations of this gene have been linked to breast cancer.	17392432, 17283126, 16818604, 11239454
C6orf157	Cyclin B	C6orf157 is also known as H10BH. C6orf157 is an E3 ubiquitin ligase that has been shown to ubiquitinate cyclin B.	15749827
Cbl		Cbl-b and c-Cbl are members of the Cbl family of adaptor proteins that are highly expressed in hematopoietic cells. Cbl proteins possess E3 ubiquitin ligase activity that downregulates numerous signaling proteins and RTKs in several pathways such as EGFR, T cell and B cell receptors, and integrin receptors. Cbl proteins play an important role in T cell receptor signaling pathways.	18759930, 9797470
CBLL1	CDH1	CBLL1 is also known as Hakai. CBLL1 is an E3 ubiquitin ligase that ubiquitinates the phosphorylated form of E-Cadherin, causing its degradation and loss of cell-cell adhesions.	11836526
CHFR	PLK1, Aurora A	CHFR is an E3 ubiquitin ligase that functions as a mitotic stress checkpoint protein that delays entry into mitosis in response to stress. CHFR has been shown to ubiquitinate and degrade the kinases PLK1 and Aurora A.	14562038, 19326084
CHIP	HSP70/90, iNOS, Runx1, LRRK2	CHIP is an E3 ubiquitin ligase that acts as a co-chaperone protein and interacts with several heat shock proteins, including HSP70 and HSP90, as well as the nonheat shock proteins iNOS, Runx1, and LRRK2.	19913553, 19362296, 19524548, 19536328
Cul3/HIB	Ci/Gli	Cul3/HIB is an E3 ubiquitin ligase complex composed of Cullin3, Hedgehog-induced MATH and BTB domain-containing protein (HIB), and SPOP. Cul3/HIB targets the Hedgehog pathway transcription factor (Ci)/Gli for ubiquitination and degradation by the proteasome.	16740475
Cul3/Keap1	Nrf2	Cul3/Keap1 is part of an E3 ubiquitin ligase complex composed of RBX1, cullin3 and the substrate-recognition component Keap1. Cul3/Keap1 targets Nrf2, a transcription factor that regulates antioxidant genes in response to oxidative stress for ubiquitination and degradation by the proteasome.	12682069

Kinase | Transcription Factor | Caspase | Enzyme | GAP/GEF | G-protein | Acetylase
Phosphatase | pro-apoptotic | Receptor | pro-survival | GTPase | Ribosomal subunit | Deacetylase

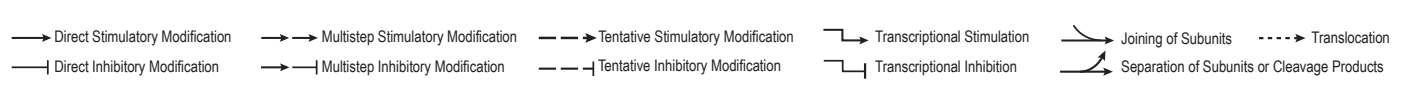
Ligase	Substrate	Function	PMID
Cul3/MEL-26	mei-1	Cul3/MEL-26 is an E3 ubiquitin ligase complex composed of cullin3 and the substrate-recognition component MEL-26. MEL-26 targets mei-1 for ubiquitination and subsequent proteasomal degradation.	13679922
Cul4/Cdt2	Cdt1, p21, Set8	Cul4/Cdt2 is an E3 ubiquitin ligase complex composed of DCX (DDB1-CUL4-X-box) and the substrate recognition component Cul4/Cdt2. Cul4/Cdt2 regulates cell cycle progression into S phase by targeting cdt1 and spd1 for ubiquitination and degradation by the proteasome.	16949367, 18794347, 20932471
Cul4/COP1	c-Jun, p53	Cul4/COP1 is an E3 ubiquitin ligase that mediates ubiquitination and subsequent proteasomal degradation of target proteins. COP1 targets the oncoprotein c-Jun and may target the tumor suppressor p53 for ubiquitination and degradation.	12615916, 16931761, 21572435
Cul4/DDB2	XPC, H3, H4	Cul4/DDB2 is an E3 ubiquitin ligase complex composed of DCX (DDB1-CUL4-ROC1) and the substrate recognition component Cul4/DDB2. Cul4/DDB2 may target histone H2A, histone H3, and histone H4 at sites of UV-induced DNA damage to induce ubiquitination and degradation by the proteasome.	15882621, 16678110
Cul5/SOCS1	Dab1	Cul5/SOCS1 is part of an SCF-like ECS (Elongin BC-CUL2/5-SOCS-box protein) E3 ubiquitin ligase complex. Cul5/SOCS1 targets components of the Jak/Stat pathway as well as Dab1, a regulator of cortical development, for ubiquitination and degradation by the proteasome.	17974915
Cul5/SOCS4	EGFR	Cul5/SOCS4 is part of an SCF-like ECS (Elongin BC-CUL2/5-SOCS-box protein) E3 ubiquitin ligase complex. SOCS4 may target components of cytokine signal transduction pathways, such as EGF receptor (EGFR) for ubiquitination and degradation by the proteasome.	17997974
Cul5/Vif	APOBEC3G	Cul5/Vif is part of an SCF-like ECS (Elongin BC-CUL2/5-SOCS-box protein) E3 ubiquitin ligase complex. Vif targets APOBEC3G and APOBEC3F for ubiquitination and degradation by the proteasome. The interaction of Vif with APOBEC3G also blocks its cytidine deaminase activity in a proteasome-independent manner,	15574592
Cul7/FBXW8	cyclin D1	Cul7/FBXW8 is an SCF-like E3 ubiquitin ligase complex composed of SKP1, CUL7, RBX1, GLMN isoform 1, and the substrate recognition component, FBXW8.	17205132
DZIP3	H2AK119	DZIP3 is an E3 ubiquitin ligase that blocks transcriptional elongation by ubiquinating H2A at lysine 119.	12538761
E6-AP	p53, Dlg	E6-AP is also known as UBE3A. E6-AP is a HECT domain E3 ubiquitin ligase that interacts with Hepatitis C virus (HCV) core protein and targets it for degradation. The HCV core protein is central to packaging viral DNA and other cellular processes. E6-AP also interacts with the E6 protein of the human papillomavirus types 16 and 18, and targets the p53 tumor-suppressor protein for degradation.	17108031
FANCL	FANC D2	FANCL is an ubiquitin ligase protein integral to the DNA repair pathway.	12973351
HACE1		HACE1 is an E3 ubiquitin ligase and tumor suppressor. Research has shown that aberrant methylation of HACE1 is frequently found in Wilms' tumors and colorectal cancer.	17694067
HECTD1		HECTD1 is an ubiquitin E3 ligase required for neural tube closure and normal development of the mesenchyme.	17442300
HECTD2		HECTD2 is a probable E3 ubiquitin ligase and may act as a susceptibility gene for neurodegeneration and prion disease.	19214206
HECTD3		HECTD3 is a probable E3 ubiquitin ligase and may play a role in cytoskeletal regulation, actin remodeling, and vesicle trafficking.	18194665
HECW1	DVL1, mutant SOD1, p53	HECW1 is also known as NEDL1. HECW1 interacts with p53 and the Wnt signaling protein DVL1, and may play a role in p53-mediated cell death in neurons.	14684739, 18223681
HECW2	p73	HECW2 is also known as NEDL2. HECW2 ubiquitinates p73, which is a p53 family member. Ubiquitination of p73 increases protein stability.	12890487
HERC2	RNF8	HERC2 belongs to a family of E3 ubiquitin ligases involved in membrane trafficking events. HERC2 plays a role in the DNA damage response through interaction with RNF8.	20023648
HERC3		HERC3 belongs to a family of E3 ubiquitin ligases involved in membrane trafficking events. HERC3 interacts with hPLIC-1 and hPLIC-2 and localizes to the late endosomes and lysosomes.	18535780
HERC4		HERC4 belongs to a family of E3 ubiquitin ligases involved in membrane trafficking events. HERC4 is highly expressed in testis and may play a role in spermatogenesis.	17967448
HERC5		HERC5 belongs to a family of E3 ubiquitin ligases involved in membrane trafficking events. HERC5 is induced by interferon and other pro-inflammatory cytokines and plays a role in interferon-induced ISG15 conjugation during the innate immune response.	16407192, 16815975
HLTF	PCNA	HLTF is both a helicasee and an E3 ubiquitin ligase. HLTF participates in postreplication repair (PRR) of damaged DNA by polyubiquitination of chromatin-bound PCNA.	18316726
HOIP	PKC	HOIP is the E3 ubiquitin ligase of the LUBEC (linear ubiquitin chain assembly complex) which ubiquitinates signaling proteins, targeting them for proteasomal degradation.	17069764
HUWE1	N-Myc, C-Myc, p53, Mcl-1, TopBP1	HUWE1 is also known as Mule. HUWE1 is a HECT domain E3 ubiquitin ligase that regulates degradation of Mcl-1 and therefore regulates DNA damage-induced apoptosis. HUWE1 also controls neuronal differentiation by destabilizing N-Myc, and regulates p53-dependent and independent tumor suppression via ARF.	15989957
HYD	CHK2	HYD is also known as EDD or UBR5. HYD is a regulator of the DNA damage response and is overexpressed in many forms of cancer.	18073532
IBRDC2	p21, Bax	IBRDC2 is an E3 ubiquitin ligase involved in the regulation of apoptosis. IBRDC2 expression can be induced by p53 and may target apoptosis related proteins p21 and Bax.	12853982
IBRDC3	UCKL-1	IBRDC3 is an E3 ubiquitin ligase involved in the cytolytic activities of hematopoietic natural killer cells and T cells.	16709802
ITCH	MKK4, RIP2, Foxp3	ITCH plays a role in T cell receptor activation and signaling through ubiquitination of multiple proteins including MKK4, RIP2, and Foxp3. Loss of ITCH function leads to an aberrant immune response and T helper cell differentiation.	19737936, 19592251, 20108139
LNX1	NUMB	LNX1 is an E3 ubiquitin ligase that plays a role in cell fate determination during embryogenesis through regulation of NUMB, the negative regulator of Notch signaling.	11782429
LRSAM1	Tsg101	LRSAM is an E3 ubiquitin ligase that mediates intracellular vesicular trafficking by monoubiquitination of TSG101.	15256501
Mahogunin		Mahogunin is an E3 ubiquitin ligase involved in melanocortin signaling. Loss of mahogunin function leads to neurodegeneration and loss of pigmentation, and may be the mechanism of action in prion disease.	19737927, 19524515
MALIN	laforin	Malin, also known as NHLRC1, is an E3 ubiquitin ligase that promotes the ubiquitination and proteasomal degradation of misfolded proteins.	15930137
MARCH-I	HLA-DRβ	MARCH1 is an E3 ubiquitin ligase found on antigen presenting cells (APCs). MARCH1 ubiquitinates MHC class II proteins and downregulates their cell surface expression.	19880452
MARCH-II		MARCH-II is a member of the MARCH family of E3 ubiquitin ligases. It associates with syntaxin6 in the endosomes and helps to regulate vesicle trafficking.	15689499
MARCH-III		MARCH-III is a member of the MARCH family of E3 ubiquitin ligases. MARCH-III associates with syntaxin6 in the endosomes and helps to regulate vesicle trafficking.	16428329
MARCH-IV	MHC class I	MARCH-IV is a member of the MARCH family of E3 ubiquitin ligases. MARCH-IV ubiquitinates MHC class I proteins and downregulates their cell surface expression.	14722266
MARCH-V	DRP1	MARCH-V is a member of the MARCH family of E3 ubiquitin ligases. March-V is located in the mitochondria and aids in the control of mitochondrial morphology.	16936636
MARCH-VI		MARCH-VI is also known as TEB4 and is a member of the MARCH family of E3 ubiquitin ligases. It localizes to the endoplasmic reticulum and participates in ER-associated protein degradation.	16373356
MARCH-VII	gp190	MARCH-VII is also known as axotrophin. MARCH-VII was originally identified as a neural stem cell gene, but has since been shown to play a role in LIF signaling in T lymphocytes through degradation of the LIF receptor subunit, gp190.	19901269
MARCH-VIII	B7-2, MHC class II	MARCH-VIII is also known as c-MIR. MARCH-VIII causes the ubiquitination/degradation of B7-2, which is a co-stimulatory molecule for antigen presentation. MARCH-VIII has also been shown to ubiquitinate MHC class II proteins.	16785530
MARCH-IX	ICAM-1, MHC-I	MARCH-IX is a member of the MARCH family of E3 ubiquitin ligases. MARCH-IX mediates ubiquitination of transmembrane proteins, marking them for endocytosis and sorting to lysosomes via multivesicular bodies.	17174307, 14722266
MARCH-X		MARCH-X is also known as RNF190. MARCH-X is a member of the MARCH family of E3 ubiquitin ligases. MARCH-X may be involved in spermiogenesis.	21937444



Ligase	Substrate	Function	PMID
MARCH-XI	CD4	MARCH-XI is a member of the MARCH family of E3 ubiquitin ligases. MARCH-IX mediates ubiquitination of CD4, marking it for endocytosis and sorting to lysosomes via multivesicular bodies.	17604280
MDM2	p53	MDM2, an E3 ubiquitin ligase for p53, plays a central role in regulation of the stability of p53. Akt-mediated phosphorylation of MDM2 at Ser166 and Ser186 increases its interaction with p300, allowing MDM2-mediated ubiquitination and degradation of p53.	9153395
MEKK1	c-Jun, Erk	MEKK1 is a well known protein kinase of the STE11 family. MEKK1 phosphorylates and activates MKK4/7, which in turn activates JNK1/2/3. MEKK1 contains a RING finger domain and exhibits E3 ubiquitin ligase activity toward c-Jun and Erk.	12049732, 17101801
MGRN1	Tsg101	MGRN1 is an E3 ubiquitin ligase that mediates intracellular vesicular trafficking by monoubiquitination of TSG101.	17229889
MIB1	Delta, Jagged	Mindbomb homolog 1 (MIB1) is an E3 ligase that facilitates the ubiquitination and subsequent endocytosis of the Notch ligands, Delta and Jagged.	16000382
MIB2	Delta, Jagged	Mind Bomb 2 (MIB2) is an E3 ligase that positively regulates Notch Signaling. MIB2 has been shown to play a role in myotube differentiation and muscle stability. MIB2 ubiquitinates NMDAR subunits to help regulate synaptic plasticity in neurons.	15824097, 18216171, 17962190
MID1	PP2A	Mid1, also known as Midline-1, is an E3 ubiquitin ligase that may target protein phosphatase 2 for ubiquitination and proteasomal degradation.	11685209
MKRN1	hTERT, p53, CDKN1A, FLIP1	MKRN1 is an E3 ubiquitin ligase that regulates both anti- and pro-apoptotic functions.	15805468
MycBP2	Fbxo45, TSC2	MycBP2 is an E3 ubiquitin ligase also known as PAM. MycBP2 associates with Fbxo45 to play a role in neuronal development. MycBP2 also regulates the mTOR pathway through ubiquitination of TSC2.	19398581, 18308511
NEDD4		NEDD4 is an E3 ubiquitin ligase highly expressed in the early mouse embryonic central nervous system. NEDD4 downregulates both neuronal voltage-gated Na+ channels (NaVs) and epithelial Na+ channels (ENaCs) in response to increased intracellular Na+ concentrations.	9618557, 9792722
NEDD4L	Smad2, PTEN	NEDD4L is an E3 ubiquitin ligase highly expressed in the early mouse embryonic central nervous system. NEDD4L has been shown to negatively regulate TGF-β signaling by targeting Smad2 for degradation.	19917253, 17218260
NEURL	Jagged 1, Delta	NEURL is an E3 ubiquitin ligase involved in Notch signaling and neurological determination of cell fate.	17003037, 11696324
OSTM1	Gai3	OSTM1 is an E3 ubiquitin ligase localized to the cell membrane that regulates membrane associated G-proteins by ubiquitination and proteasomal degradation.	12826607
PARC		PARC is a cullin family member that acts as a p53-binding cytoplasmic anchor protein and is part of an atypical cullin-RING- based E3 ubiquitin ligase complex.	12526791
Parkin	Pael-R, CDC-rel, PLC-g1	Parkin is an E3 ubiquitin ligase that has been shown to be a key regulator of the autophagy pathway. Mutations in Parkin can lead to Parkinson's Disease.	20074049, 18671761, 17553932, 16672220
PCGF1	H2A, K119	PCGF1 is a component of the polycomb group multiprotein PRC1- like (PcG PRC1) complex. PCGF1 is required for PcG PRC1 mediated monoubiquitination of H2A Lys119, which is central to the histone code and gene regulation.	18460542
PELI1	TRIP, IRAK	PELI1 is an E3 ubiquitin ligase that plays a role in Toll-like Receptor (TLR3 and TLR4) signaling to NF-κB via the TRIP adaptor protein. PELI1 has also been shown to ubiquitinate IRAK.	19734906, 17675297
PEX10	Pex5	PEX10 is localized to peroxisome membranes and has been associated with several peroxisomal biogenesis disorders.	15283676
PJA1	ELF	PJA1 is also known as PRAJA. PJA1 plays a role in downregulating TGF-β signaling in gastric cancer via ubiquitination of the Smad4 adaptor protein ELF.	16096365
PJA2		PJA2 is an E3 ubiquitin ligase found in neuronal synapses. The exact role and substrates of PJA2 are unclear.	12036302
RAD18	PCNA	RAD18 is an E3 ubiquitin ligase involved in post-replication repair of UV-damaged DNA.	17720710, 18245774
RBCK1	SOCS6, PKC, TAB2/3	RBCK1 is an E3 ligase that acts as an iron sensor by promoting the ubiquitination of oxidized IREB2 in the presence of high iron and oxygen. RBCK1 is a component of the LUBAC (linear ubiquitin chain assembly complex).	17449468, 16643902,
RCHY1	P27, KIP1, P53	RCHY1, also known as Pirh2, is an E3 ubiquitin ligase that contributes to the regulation of the cell cycle. RCHY1 is primarily associated with the ubiquitination and proteasomal degradation of tetrameric p53.	12654245, 18006823, 18344599
RFFL	p53	RFFL is also known as CARP2 and is an E3 ubiquitin ligase that inhibits endosome recycling. RFFL also degrades p53 through stabilization of MDM2.	15229288, 18382127
RFWD2	MTA1, p53, FoxO1	RFWD2 is also known as COP1. RFWD2 is an E3 ubiquitin ligase that ubiquitinates several proteins involved in the DNA damage response and apoptosis including MTA1, p53, and FoxO1.	19805145, 16931761, 18815134
Rictor	SGK1	Rictor interacts with Cullin1-Rbx1 to form an E3 ubiquitin ligase complex and promotes ubiquitination and degradation of SGK1.	20832730
RING1	H2A, K119	RING1, also known as RNF1, is an E3 ubiquitin ligase of the polycomb group multiprotein PRC1-like (PcG PRC1) complex. RING1 is required for PcG PRC1 mediated monoubiquitination of H2A Lys119, which is central to the histone code and gene regulation.	16359901
RNF2	H2A, K119, Geminin	RNF2, also known as Ring2, is an E3 ubiquitin ligase of the polycomb group multiprotein PRC1-like (PcG PRC1) complex. RNF2 is required for PcG PRC mediated monoubiquitination of H2A Lys119, which is central to the histone code and gene regulation.	17157253, 15509584
RNF5	JAMP, paxillin	RNF5 is also known as RMA5. RNF5 plays a role in ER-associated degradation of misfolded proteins and ER stress response through ubiquitination of JAMP. RNF5 also plays a role in cell motility and has been shown to ubiquitinate paxillin.	19269966, 12861019
RNF6	LIM1, Androgen receptor	RNF6 is an E3 ubiquitin ligase involved in the regulation of cell motility and differentiation. RNF6 targets LIMK for ubiquitination and degradation, inhibiting cytoskeleton stability.	16204183
RNF8	H2A, H2AX	RNF8 is a RING domain E3 ubiquitin ligase that plays a role in the repair of damaged chromosomes. RNF8 ubiquitinates Histone H2A and H2A.X at double-strand breaks (DSBs) which recruits 53BP1 and BRCA1 repair proteins.	18001824
RNF11	Smurf2	RNF11 is a required component of a ubiquitin-editing protein complex involved in modifying cellular inflammatory response to LPS and TNF signaling.	14562029
RNF12	CLIM, Ldb1, Ldb2	RNF12, also known as RLIM, is an E3 ubiquitin ligase. RNF12 is involved in telomere regulation and X chromosome inactivation.	12874135, 11882901
RNF19	SOD1	RNF19 is also known as Dorfin. Accumulation and aggregation of mutant SOD1 leads to ALS disease. RNF19 ubiquitinates mutant SOD1 protein, causing a decrease in neurotoxicity.	19610091
RNF20	Histone H2B	RNF20 is also known as BRE1. RNF20 is an E3 ubiquitin ligase that monoubiquitinates Histone H2B. H2B ubiquitination is associated with areas of active transcription.	18832071
RNF34	Caspase-8, -10	RNF34 is also known as RFI. RNF34 inhibits death receptor mediated apoptosis through ubiquitination/degradation of caspase-8 and -10.	16596200
RNF40	Histone H2B	RNF40 is also known as BRE1-B. RNF40 forms a protein complex with RNF20 resulting in the ubiquitination of Histone H2B. H2B ubiquitination is associated with areas of active transcription.	16307923
RNF41	ErbB3, BIRC6, Parkin	RNF41 is an E3 ubiquitin ligase that has been implicated in the regulation of hematopoietic progenitor cell differentiation.	14765125, 12411582, 18541373
RNF111	Smad, SnoN, c-Ski	RNF111 is an E3 ubiquitin ligase that participates in mesoderm patterning by promoting the ubiquitination and proteasomal degradation of downstream Smads.	18451154, 14657019, 17591695
RNF123	CDKN1B	RNF123 is an E3 ubiquitin ligase that functions as part of the KPC complex. RNF123 aids in cell cycle regulation by targeting CDKN1B for ubiquitination and proteasomal degradation during G1.	15531880
RNF125		RNF125 is also known as TRAC-1. RNF125 has been shown to positively regulate T cell activation.	17990982
RNF128		RNF128 is also known as GRAIL. RNF128 promotes T cell anergy and may play a role in actin cytoskeletal organization in T cell/APC interactions.	19833735
RNF135	RIG-1	RNF135 is an E3 ubiquitin ligase involved in viral innate immunity. RNF135 targets the cytoplasmic viral nucleic acid receptor RIG-1 for ubiquitination and degradation by the proteasome.	19017631
RNF138	TCF/LEF	RNF138 is also known as NARF. RNF138 is associated with Nemo-like Kinase (NLK) and suppresses Wnt/β-Catenin signaling through ubiquitination/degradation of TCF/LEF.	16714285
RNF167	TSSC5 (SLC22A18)	RNF167 may act as an E3 ubiquitin ligase involved in the regulation of kidney transporter function.	16314844
RNF168	H2A, H2A.X	RNF168 is an E3 ubiquitin ligase that helps protect genome integrity by working together with RNF8 to ubiquitinate Histone H2A and H2A.X at DNA double-strand breaks (DSB).	19203579



Ligase	Substrate	Function	PMID
RNF180	Zic2	RNF180 is an E3 ubiquitin ligase involved in neurological development. RNF180 targets the ZIC2 transcription factor for polyubiquitination and degradation by the proteasome.	18363970
RNF182	ATP6VOC	RNF 182 is an E3 ubiquitin ligase that targets ATP6V0C, a component of vacuolar ATPase, for polyubiquitination and degradation by the proteasome.	18298843
RNF190		see MARCH-X	
SCF/FBW7	Cyclin-E, c-Myc, c-Jun	SCF/FBW7 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBW7. SCF/FBW7 mediates the ubiquitination of proteins involved in cell cycle progression, signal transduction, and transcription. Target proteins for SCF/FBW7 include the phosphorylated forms of c-Myc, Cyclin E, Notch intracellular domain (NICD), and c-Jun. Research has found that defects in FBXW7 may be a cause of breast cancer.	11533444, 15150404, 16023596
SCF/FBXL3	CRY1, CRY2	SCF/FBXL3 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXL3. SCF/FBXL3 mediates circadian clock function by ubiquitination and subsequent degradation of CRY1 and CRY2.	17463251, 17463252, 17462724
SCF/FBXL5	IRP2	SCF/FBXL5 is an ubiquitin ligase complex also known as SCF (SKP1-cullin-F-box). FBXL5 is an F-box protein that functions as an iron sensor by promoting the ubiquitination and subsequent degradation of IREB2/IRP2 under high iron and oxygen conditions.	19762597, 19762596
SCF/FBXL14	SNAIL1	SCF/FBXL14 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate-recognition component FBXL14. The SCF/FBXL14 complex is thought to contribute to mesoderm formation by ubiquitination and subsequent degradation of SNAI1.	19955572
SCF/FBXL15	Timeless, SMURF1, SMURF2	SCF/FBXL15 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate-recognition component FBXL15. FBXL15 targets negative regulators of the BMP signaling pathway, including SMURF1 and SMURF2, for ubiquitination and subsequent proteasomal degradation. FBXL15 is required for dorsal/ventral pattern formation and bone mass maintenance. SCF/FBXL15 also targets the Drosophila circadian clock protein timeless.	16794082, 21572392
SCF/FBXL20	RIM1	SCF/FBXL20 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate-recognition component FBXL20. FBXL20 is localized to the synapse and its regulation of RIM1by ubiquitination may play a role in neural transmission.	17803915
SCF/FBXO1	CP110	SCF/FBXO1 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate-recognition component FBXO1. FBXO1 targets Cp110 for ubiquitination and subsequent proteasomal degradation during cell cycle G2 phase, thereby inhibiting centrosome reduplication.	20596027
SCF/FBXO2	Pre-integrin β1, CFTR	SCF/FBXO2 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate-recognition component FBXO2. FBXO2 targets misfolded glycoproteins for ubiquitination and proteasomal degradation by recognition of sugar chains in the endoplasmic reticulum-associated degradation (ERAD) pathway.	12140560
SCF/FBXO3	HIPK2, p300	SCF/FBXO3 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate-recognition component FBXO3. FBXO3 targets HIPK2 and p300 for ubiquitination and rapid degradation by the proteasome. The inclusion of PML in a complex with SCF/FBXO3, HIPK2, and p300 delays degradation of HIPK2 and allows synergistic activation of p53/TP53-dependent transactivation.	18809579
SCF/FBXO4	TERF1	SCF/FBXO4 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate-recognition component FBXO4. FBXO4 may play a role in telomere homeostasis by recognition of TERF1 and promotion of its ubiquitination together with UBE2D1.	17081987
SCF/FBXO6	Chk1	SCF/FBXO6 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate-recognition component FBXO6. FBXO6 targets misfolded glycoproteins for ubiquitination and proteasomal degradation by recognition of sugar chains in the endoplasmic reticulum-associated degradation (ERAD) pathway. FBXO6 also targets the kinase Chk1, a cell cycle regulator involved in entry into mitosis.	19716789
SCF/FBXO7	BIRC2, DLGAP5	SCF/FBXO7 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate-recognition component FBXO7. SCF/FBXO7 targets BIRC2 (cIAP1), an inhibitor of apoptosis, and DLGAP5, a cell cycle regulator, for ubiquitination and proteasomal degradation.	16510124
SCF/FBXO031	Cyclin D1	SCF/FBXO31 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate-recognition component FBXO31. FBXO31 is also known as FBXO14. SCF/FBXO31 targets phosphorylated cyclin D1 for ubiquitination and degradation by the proteasome, resulting in G1 cell cycle arrest.	19412162
SCF/FBXO42	p53	SCF/FBXO42 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate-recognition component FBXO42. FBXO42 targets p53/TP53 for ubiquitination and degradation by the proteasome.	19509332
SCF/FBXO45	UNC13A, p73	SCF/FBXO45 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate-recognition component FBXO45. SCF/FBXO45 aids in the regulation of neurotransmission at mature neurons by targeting UNC13A for ubiquitin dependent degradation by the proteasome. FBXO45 also targets the apoptotic protein p73 for ubiquitination and degradation.	19581926
SCF/FBXW5	SASS6	SCF/FBXW5 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXW5. SCF/FBXW5 mediates the ubiquitination of SASS6, preventing centriole duplication.	18381890
SCF/FBXW10	CBX1, CBX5	SCF/FBXW10 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, FBXW10. SCF/FBXW10 has been shown to contribute to gene expression by degradation of heterochromatin components CBX5 and CBX1	20498703
SCF/Skp2	p27, p21, FoxO1	SCF/Skp2 is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, Skp2. SCF/Skp2 mediates the ubiquitination of proteins involved in cell cycle progression (specifically the G1/S transition), signal transduction and transcription. Target proteins for SCF/Skp2 include the phosphorylated forms of p27Kip1, p21Waf1/Cip1, and FoxO1.	15668399, 10559916
SCF/β-TrCP	IκBα, Wee1, Cdc25A, β-Catenin	SCF/β-TrCP is an E3 ubiquitin ligase complex composed of SCF (SKP1-CUL1-F-box protein) and the substrate recognition component, β-TrCP (also known as BTRC). SCF/β-TrCP mediates the ubiquitination of proteins involved in cell cycle progression, signal transduction, and transcription. SCF/β-TrCP also regulates the stability of β-catenin and participates in Wnt signaling.	10230406, 15070733, 14603323, 10339577
SHPRH	PCNA	SHPRH is an E3 ubiquitin ligase that plays a role in DNA replication through ubiquitination of PCNA. PCNA ubiquitination prevents genomic instability from stalled replication forks after DNA damage.	18719106
SIAH1	β-catenin, Bim, TRB3	SIAH1 is an E3 ubiquitin ligase that plays a role in inhibition of Wnt signaling through ubiquitination of β-catenin. SIAH1 has also been shown to promote apoptosis through upregulation of Bim, and to ubiquitinate the signaling adaptor protein TRB3.	16413921, 19775288, 18276110
SIAH2	HIPK2, PHD1/3	SIAH2 is an E3 ubiquitin ligase that plays a role in hypoxia through ubiquitination and degradation of HIPK2. SIAH2 also ubiquitinates PHD1/3, which regulates levels of HIF-1α in response to hypoxia.	19043406, 15210114
SMURF1	Smad1/5, RhoA, MEKK2	SMURF1 is an E3 ubiquitin ligase that interacts with BMP pathway Smad effectors, leading to Smad protein ubiquitination and degradation. Smurf1 negatively regulates osteoblast differentiation and bone formation in vivo.	10458166, 15820682
SMURF2	Smads, Mad2	SMURF2 is an E3 ubiquitin ligase that interacts with Smads from both the BMP and TGF-β pathways. SMURF2 also regulates the mitotic spindle checkpoint through ubiquitination of Mad2.	11158580, 18852296
SYVN1	ERAD, Pael-R, p53,IRE-1	SYVN1 is an E3 ubiquitin ligase involved in the ER-associated degradation (ERAD) pathway. SYVN1 targets misfolded proteins and appropriately folded short-lived proteins for ubiquitination and degredation by the proteasome.	14593114, 17059562, 17170702, 18369366
TOPORS	p53, NKX3.1	TOPORS is an E3 ubiquitin ligase and a SUMO ligase. TOPORS ubiquitinates and sumoylates p53, which regulates p53 stability. TOPORS has also been shown to ubiquitinate the tumor suppressor NKX3.1.	19473992, 18077445
TRAF2	Rip1, other TRAFs	TRAF2 is a weak E3 ubiquitin ligase that acts as a component of several ubiquitination complexes. TRAF2 ligase activity is activated in the presence of cytoplasmic sphingosine-1-phosphate. TRAF2 is a major regulator of the apoptosis and cell survival machinery.	11909853, 15175328
TRAF6	NEMO, Akt1	TRAF6 is an E3 ubiquitin ligase that functions as an adaptor protein in IL-1R, CD40, and TLR signaling. TRAF6 promotes NF-κB signaling through K63 polyubiquitination of IKK, resulting in IKK activation. TRAF6 has also been shown to ubiquitinate Akt1, causing its translocation to the cell membrane.	19713527, 11057907
TRAF7		TRAF7 is an E3 ubiquitin ligase and SUMO ligase that functions as an adaptor protein in TNF Receptor and TLR signaling. TRAF7 has been shown to be capable of self-ubiquitination and plays a role in apoptosis via MEKK3-mediated activation of NF-κB.	15001576
TRIAD3	TLRs, RIP1	Triad 3 is an E3 ubiquitin ligase found in peripheral blood leukocytes of the immune system that regulates antiviral and cytokine induced cellular responses.	15107846, 16968706
TRIM8	SOCS-1	TRIM8 is an E3 ubiquitin ligase that regulates cytokine induced signal transduction by targeting SOCS1 for ubiquitination and degradation by the proteasome.	12163497
TRIM11	Humanin,ARC105,Pax6	TRIM11 is an E3 ubiquitin ligase that may promote the degradation of insoluble ubiquitinated proteins. TRIMM11 may also aid in anti-viral cellular functions.	12670303, 16904669, 18628401



Ligase	Substrate	Function	PMID
TRIM13		TRIM13 is an E3 ubiquitin ligase that targets membrane and secretory proteins for ubiquitination and proteasomal degradation in the endoplasmic reticulum-associated degradation (ERAD) pathway.	17314412
TRIM21	IgG1 HC, IRF3	TRIM21 is an E3 ubiquitin ligase involved in intracellular antibody-mediated degradation of viral components by the proteasome.	18022694, 18641315
TRIM25	RIG-1	TRIM25 is an E3 ubiquitin ligase involved in viral innate immunity. TRIM25 targets the cytoplasmic viral nucleic acid receptor RIG-1 for ubiquitination and degradation by the proteasome.	12075357
TRIM32	actin, piasy	TRIM32 is an E3 ubiquitin ligase involved in viral lysosome related vesicle trafficking. TRIM32 targets DTNBP1 for ubiquitination and degradation by the proteasome. TRIM32 may also mediate the activity of HIV Tat proteins.	14578165, 16243356
TRIM33	Smad4	TRIM33 is an E3 ubiquitin ligase involved in the regulation of the TGF-b/ BMP signaling pathway . TRIM33 targets SMAD4 for ubiquitination, nuclear exclusion, and proteasomal degradation.	15820681
TRIM41	PKC	TRIM41 is an E3 ubiquitin ligase that targets protein kinase C for ubiquitination and proteasomal degradation.	17893151
TRIM54		TRIM54 is an E3 ubiquitin ligase that may target and stabilize microtubules.	15967462
TRIM55		TRIM55 is an E3 ubiquitin ligase that may regulate gene expression and protein turnover in muscle cells	15967462
TRIM63	Troponin I, MyBP-C, MyLC1/2	TRIM63 is also known as Murf-1. TRIM63 is a muscle-specific E3 ubiquitin ligase whose expression is upregulated during muscle atrophy. TRIM63 has been shown to ubiquitinate several important muscle proteins including troponin I, MyBP-C, and MyLC1/2.	19506036
UBE3B		UBE3B is an E3 ubiquitin ligase identified through sequence analysis. The specific substrates and cellular function of UBE3B is currently unknown.	12837265
UBE3C		UBE3C is an E3 ubiquitin ligase also known as KIAA10. UBE3C is highly expressed in muscle and may interact with the transcriptional regulator TIP120B.	12692129
UBR1		UBR1 is an E3 ubiquitin ligase responsible for proteasomal degradation of misfolded cytoplasmic proteins. UBR1 has also been shown to be a ubiquitin ligase of the N-end rule proteolytic pathway, which regulates degradation of short-lived proteins.	19041308, 17962019
UBR2	Histone H2A	UBR2 is an E3 ubiquitin ligase that has been shown to ubiquitinate histone H2A, resulting in transcriptional silencing. UBR2 is also part of the N-end rule proteolytic pathway.	20080676, 19008229
UHRF1	Histone H3	UHRF1 is an epigenetic regulator that is also a putative E3 ubiquitin ligase.	14993289
UHRF2	PCNP	UHRF2 is also known as NIRF. UHRF2 is a nuclear protein that may regulate cell cycle progression through association with Chk2. UHRF2 also ubiquitinates PCNP and has been shown to play a role in degradation of nuclear aggregates containing polyglutamine repeats.	15178429, 14741369, 19218238
VHL	HIF-1α	VHL is the substrate recognition component of the ECV (Elongin B/C, Cullen-2, VHL) E3 ubiquitin ligase complex responsible for degradation of the transcription factor HIF-1α. Ubiquitination and degradation of HIF-1α takes place only during periods of normoxia, but not during hypoxia, thereby playing a central role in the regulation of gene expression by oxygen.	11292862
VPS18	SNK	VPS18 is an E3 ubiquitin ligase that regulates intracellular vesicle trafficking. VPS18 may also regulate the POLO-like kinase SNK during the cell cycle.	16203730
WWP1	ErbB4	WWP1 is an E3 ubiquitin ligase commonly found to be overexpressed in breast cancer. WWP1 has been shown to ubiquitinate and degrade ErbB4. Interestingly, the WWP1 homolog in C. elegans was found to increase life expectancy in response to dietary restriction.	19561640, 19553937
WWP2	oct-4, PTEN	WWP2 is an E3 ubiquitin ligase that has been shown to ubiquitinate/degrade the stem cell pluripotency factor Oct-4. WWP2 also ubiquitinates the transcription factor EGR2 to inhibit activation-induced T cell death.	19274063, 19651900, 21532586
ZNRF1		ZNRF1 is an E3 ubiquitin ligase highly expressed in neuronal cells. ZNRF1 is found in synaptic vesicle membranes and may regulate neuronal transmissions and plasticity.	14561866

Deubiquitinase Table

The Deubiquitinase Table provides a list of deubiquitinases, along with their substrates (when known) and corresponding references. This table was generated using PhosphoSitePlus®, Cell Signaling Technology's protein modification resource. See page 4 for more information on PhosphoSitePlus®.

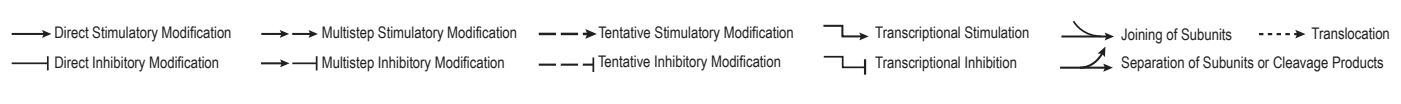
We would like to thank Prof. Wenyi Wei, Beth Israel Deaconess Medical Center, Harvard Medical School, for contributing to this table.

Deubiquitinase Table

Ligase	Substrate	Function	PMID
STAMBP		STAM-binding protein (STAMBP or AMSH) is an endosomal deubiquitination enzyme that preferentially displays ubiquitin isopeptidase activity toward K63-linked chains.	18758443
STAMBPL1		STAM-binding protein-like 1 (STAMBPL1 or AMSHLP) is a deubiquitination enzyme that preferentially displays ubiquitin isopeptidase activity toward K63-linked chains.	18758443
ATXN3	RAD23A, RAD23B	ATXN3 is a transcriptional regulation deubiquitination enzyme that preferentially displays ubiquitin isopeptidase activity toward long, four or greater, ubiquitin chains.	17696782
ATXN3L		ATXN3-like is a deubiquitination enzyme that displays ubiquitin isopeptidase activity toward K48- and K63-linked chains.	21118805
BRCC36	FAM175A/Abraxas	BRCC36 (BRCC3) is a deubiquitination enzyme that preferentially displays ubiquitin isopeptidase activity toward K63-linked chains. BRCC36 targets K63-linked ubiquitin chains on H2A and H2X at the site of DNA double strand breaks as a component of the BRCA complex.	14636569, 16707425
COPS5	TP53, MIF, JUN, UCHL1	COPS5 (CSN5) is the protease subunit of the COP9 signalosome complex (CSN), a key regulator of the ubiquitin conjugation pathway. COPS5 is essential for the CSN isopeptidase activity responsible for deneddylation of cullin-RING E3 ubiquitin ligase complexes.	9535219, 11285227
DUB3	CDC25A	DUB3 is a deubiquitination enzyme that preferentially displays ubiquitin isopeptidase activity toward CDC25A, preventing CDC25A degradation and allowing cell cycle progression.	14699124, 20228808
JOSD1		JOSD1 is a deubiquitination enzyme that displays low ubiquitin isopeptidase activity in vitro.	21118805
JOSD2		JOSD2 is a deubiquitination enzyme that displays ubiquitin isopeptidase activity toward K63-linked chains, and to a lesser extent K48-linked chains.	17696782, 21118805
MPND		MPND is an MPN domain and JAMM motif-containing protein with predicted ubiquitin isopeptidase activity.	
MYSM1	H2A	MYSM1 is a deubiquitinating enzyme that acts as a transcriptional co-activator by directing preferential ubiquitin isopeptidase activity toward monoubiquinated H2A in hyperacetylated nucleosomes.	17707232
OTU1	VCP	OTU1, also known as YOD1, is a deubiquitination enzyme that displays ubiquitin isopeptidase activity toward K48- and K63-linked polyubiquitin or di-ubiquitin chains. OTU1 is a part of the endoplasmic reticulum-associated degradation (ERAD) pathway for misfolded lumenal proteins.	19818707
OTUB1	RNF128	OTUB1 is a deubiquitination enzyme that preferentially displays ubiquitin isopeptidase activity toward polyubiquitinated K48-linked chains. OTUB1 regulates protein turnover by preventing degradation and also plays a unique role in the regulation of T cell anergy.	12704427, 14661020
OTUB2		OTUB2 is a deubiquitination enzyme that displays ubiquitin isopeptidase activity toward K48- and K63-linked chains. OTUB2 regulates protein turnover by preventing degradation.	12704427, 18954305

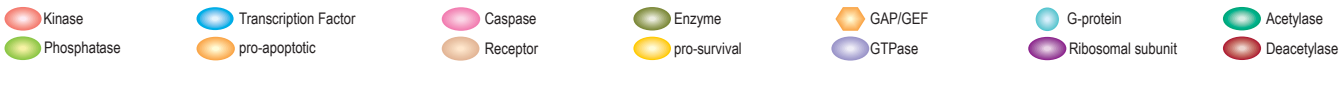
Deubiquitinase Table

Ligase	Substrate	Function	PMID
OTUD1		OTUD1 is a member of the deubiquitinating enzyme ovarian tumor domain (OTU) superfamily.	17991829
OTUD3		OTUD3 is a member of the deubiquitinating enzyme ovarian tumor domain (OTU) superfamily.	17991829
OTUD4		OTUD4 is a member of the deubiquitinating enzyme ovarian tumor domain (OTU) superfamily.	17991829
OTUD5	TRAF3	OTUD5 is a deubiquitination enzyme that displays ubiquitin isopeptidase activity toward K48- and K63-linked chains. OTUD5 negatively regulates type I interferon (IFN) production by deubiquitination of TRAF3.	17991829
OTUD6A		OTUD6A is a member of the deubiquitinating enzyme ovarian tumor domain (OTU) superfamily.	17991829
OTUD6B		OTUD6B is a member of the deubiquitinating enzyme ovarian tumor domain (OTU) superfamily.	17991829
OTUD7A/ Cezanne 2		OTUD7A is a deubiquitination enzyme that displays ubiquitin isopeptidase activity toward K48- and K63-linked chains.	12682062
OTUD7B/ Cezanne	TRAF6	OTUD7B is a deubiquitination enzyme that displays ubiquitin isopeptidase activity toward K48- and K63-linked chains. OTUD7B negatively regulates NF-κB.	11463333
OTUD7C/A20	NAF1, TAX1BP1, TRAF2	OTUD7C is a ubiquitination-editing enzyme that displays ubiquitin isopeptidase activity toward K63-linked chains and ubiquitination of K48-linked chains. OTUD7C is an essential regulator of inflammatory signaling pathways in the lymphoid system.	9882303, 14748687
POH1		POH1 is the metalloprotease deubiquitination enzyme component of the 26S proteasome that displays ubiquitin isopeptidase activity toward K63-linked chains.	9374539, 19214193
PRPF8	SNRP116, WDR57/ SPF38	PRPF8 is a member of the deubiquitinating enzyme metalloprotease JAMM domain superfamily. PRPF8 is known to be a central component of the spliceosome, while PRPF8 ubiquitin isopeptidase activity is controversial.	2139226, 8702566
TRABID	TRAF6, APC	TRABID is a deubiquitination enzyme that preferentially displays ubiquitin isopeptidase activity toward K63-linked chains. TRABID acts as a positive regulator of the Wnt signaling pathway by deubiquitinating APC protein, a Wnt signaling pathway negative regulator.	18281465, 21834987
UCHL1	COPS5	UCHL1 is a member of the ubiquitin C-terminal hydrolase (UCH) deubiquitinase superfamily. UCHL1 functions as a ubiquitin hydrolase involved in the processing of both ubiquitin precursors and ubiquitinated substrates, generating free monomeric Ub.	9790970
UCHL2/BAP1	BRCA1, HCFC1	UCHL1/Bap1 is a member of the ubiquitin C-terminal hydrolase (UCH) deubiquitinase superfamily. UCHL1/ Bap1 is a BRCA1-associated, nuclear localized ubiquitin hydrolase that suppresses cell growth.	9528852
UCHL3	ENAC	UCHL3 is a member of the ubiquitin C-terminal hydrolase (UCH) deubiquitinase superfamily. UCHL3 functions as a ubiquitin hydrolase involved in the processing of both ubiquitin precursors and ubiquitinated substrates, generating free monomeric Ub. UCHL3 shows dual specificity toward both ubiquitin (Ub) and NEDD8, a Ub-like molecule.	2530630
UCHL5		UCHL5 is a member of the ubiquitin C-terminal hydrolase (UCH) deubiquitinase superfamily. UCHL5 is the deubiquitination enzyme component of the 19S regulatory subunit of the 26S proteasome that displays ubiquitin isopeptidase activity toward K48-linked chains.	16906146, 18922472
USP1	FANCD2, PCNA	USP1 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP1 is a negative regulator of DNA repair machinery.	15694335, 16531995
USP2	CCND1	USP2 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP2 is characterized by its C19 peptidase activity, which is involved in ubiquitin recycling and in the disassembly of various forms of polymeric ubiquitin and ubiquitin-like protein complexes.	17290220, 19917254, 19838211
USP3	H2A	USP3 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP3 deubiquitinates monoubiquitinated histone H2A and H2B. USP3 is required for proper progression through S phase and subsequent mitotic entry.	17980597
USP4	ADORA2A, RB1	USP4 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP4 is a proto-oncogene that deubiquitinates target proteins such as the receptor ADORA2A and TRIM21 and plays a role in the regulation of quality control in the ER.	7784062, 16316627
USP5/ISOT	p53, TRIML1	USP5 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP5 preferentially cleaves branched and K48-linked polymers. USP5 binds linear and K63-linked polyubiquitin with a lower affinity. Knock-down of USP5 causes the accumulation of p53/TP53 and an increase in p53/TP53 transcriptional activity.	19098288
USP6		USP6 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP6 exhibits an ATP-dependent C-terminal isopeptidase activity.	20418905
USP7/HAUSP	FOXO4, PTEN p53, MDM2	USP7, also known as herpes virus-associated ubiquitin-specific protease (Hausp), is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP7 deubiquitinates target proteins such as FoxO4, p53/TP53, MDM2, PTEN and DAXX. USP7 is Involved in cell proliferation during early embryonic development.	11923872, 14506283, 15053880
USP8/UBPY	EPS15	USP8 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP8 is an essential growth-regulated enzyme that is indispensible for cell proliferation and survival. USP8 regulates endosomal ubiquitin dynamics, cargo sorting, membrane traffic at early endosomes, and maintenance of EGFR stability.	9628861, 16520378, 17711858
USP9X	SMAD4, MARK4, NUAK1, BIRC5/survivin	USP9X is an X-linked member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP9X hydrolyzes both 'Lys-29'- and 'Lys-33'-linked polyubiquitin chains. USP9X functions to regulate cell-cell contact interactions, TGF-β/BMP signaling, chromosome alignment and segregation, and specifically deubiquitinates monoubiquitinated Smad4.	16322459, 18254724, 19135894
USP9Y	SMAD4	USP9Y is a Y-linked member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily required for sperm production. USP9Y functions to regulate TGF-β/BMP signaling, and specifically deubiquitinates monoubiquitinated Smad4.	19246359
USP10	G3BP, p53/TP53, SNX3	USP10 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP10 functions as an essential regulator of p53/TP53 stability following DNA damage.	11439350, 18632802, 19398555
USP11	BRCA2, CHUK/IKKA, RANBP9/RANBPM	USP11 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP11 aids in the regulation of pathways leading to NF-κB activation and also DNA repair after double-stranded DNA breaks.	15314155, 17897950, 18408009
USP12	WDR48	USP12 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP12 requires interaction with WDR48 for high deubiquitinase activity.	19075014
USP13/ISOT3		USP13 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP13 is part of an autophagy regulatory loop involving the deubiquitination of USP10 that leads to regulation of p53 stability.	9841226
USP14	FANCC, CXCR4, ERN1	USP14 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP14 is one of three proteasome-associated deubiquitinases, along with POH and UCHL5. USP14 is thought to antagonize substrate degradation as a part of the proteasome.	18162577, 19135427, 19106094
USP15	E6	USP15 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP15 preferentially cleaves K48-linked polymers. USP15 deubiquitination protects APC and human papillomavirus type 16 protein E6 target proteins against proteasomal degradation.	16005295, 19576224
USP16	H2A	USP16 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP16 acts as a transcriptional co-activator by specifically targeting H2A for deubiquitination. USP16 deubiquitination of H2A is also required for entry into mitosis.	10077596, 17914355
USP18		USP18 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP18 catalyzes the removal of ISG15, an interferon-regulated ubiquitin-like protein, which maintains the critical cellular balance of ISG15-conjugated proteins important for normal development and brain function.	10777664
USP19	RNF123	USP19 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP19 deubiquitinates target proteins involved in cell proliferation, myogenesis, regulation of hypoxia, and modulation of the ERAD protein degradation pathway.	19465887



Deubiquitinase Table

Ligase	Substrate	Function	PMID
USP20	VHL, DIO2, HIF1A	USP20 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP20 cleaves K48- and K63-linked chains. USP20 deubiquitinates β2-adrenergic receptor (ADRB2) as well as target proteins involved in thyroid hormone regulation and regulation of hypoxia.	12056827, 12865408, 15776016
USP21	H2A	USP21 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP21 is also known as USP23. USP21 acts as a transcriptional co-activator by specifically targeting H2A for deubiquitination. USP21 is capable of removing the ubiquitin-like NEDD8 from NEDD8 conjugates.	10799498
USP22	ATXN7L3	USP22 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP22 deubiquitinates histones H2A and H2B as a component of the histone acetylation (HAT) complex SAGA. USP22 deubiquitinates specific targets required for transcription, nuclear receptor-mediated transactivation, and cell cycle progression.	18206972, 18206973, 18469533
USP23	H2A	See USP21	10799498
USP24		USP24 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. Mutations of the USP24 gene may correlate with risk of Parkinson's disease.	16917932
USP25	ACTA1, MYBPC1	USP25 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP25 cleaves both K48- and K63-linked chains. The USP25 muscle-specific isoform may have a role in the regulation of muscular differentiation and function.	10612803, 11597335, 16501887
USP26	AR	USP26 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP26 regulates the androgen receptor signaling pathway by targeting the androgen receptor for deubiquitination.	20501646
USP27X		USP27X is an X-linked member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily.	12838346
USP28	P53bp1, Chk2, FBW7α, Myc	USP28 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP28 can bind to and deubiquitinate several target proteins in the DNA damage pathway, resulting in their stability, including p53BP1 and Chk2. USP28 also plays an important role in Myc related signaling by binding through FBW7α to Myc.	17558397, 16901780
USP29		USP29 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily.	10958632
USP30		USP30 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP30 may participate in the maintenance of mitochondrial morphology.	18287522
USP31		USP31 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily.	14715245
USP32		USP32 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP32 is highly expressed in breast cancer cell lines and may be involved in tumorigenesis.	12604796, 20549504
USP33	ADRB2	USP33 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP33 is involved in cellular migration, β2-adrenergic receptor/ADRB2 recycling, and G protein-coupled receptor (GPCR) signaling.	12865408
USP34	AXIN1, AXIN2	USP34 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP34 acts as an activator of the Wnt signaling pathway downstream of the β-catenin destruction complex by deubiquitinating and stabilizing AXIN1 and AXIN2.	21383061
USP35		USP35 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily.	14715245
USP36		USP36 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP36 may play a role in the maintenance of stem cells and regulation of cellular differentiation.	22622177
USP37	FZR1/CDH1	USP37 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP37 antagonizes the anaphase-promoting complex (APC/C) during G1/S transition by mediating deubiquitination of cyclin A (CCNA1 and CCNA2), thereby promoting S phase entry.	21596315
USP38		USP38 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP38 is expressed in skeletal muscle and adrenal gland.	19615732
USP39		USP39 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP39 may play a role in mRNA splicing as a competitor of ubiquitin C-terminal hydrolases (UCHs).	11350945
USP40		USP40 may be a nonprotease homologue of the ubiquitin-specific processing protease (USP/USB) superfamily.	16917932
USP41		USP41 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily.	14715245
USP42		USP42 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP42 may play a role in spermatogenesis.	14715245
USP43		USP43 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily.	14715245
USP44	Cdc20	USP44 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP44 regulates the cell cycle by deubiquitination of CDC20, leading to stabilization of the MAD2L1-CDC20-APC/C ternary complex and avoidance of premature anaphase entry.	17443180
USP45		USP45 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily.	14715245
USP46	GAD1/GAD67	USP46 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP46 requires interaction with WDR48 for high deubiquitinase activity. USP46 may act by mediating the deubiquitination of GAD1/GAD67.	19075014
USP47	POLB, CDC25A	USP47 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP47 regulates base excision repair by deubiquitinating monoubiquitinated DNA polymerase β (POLB). USP47 may also regulate cell growth and survival by targeting CDC25A.	19966869
USP48	TRAF2, RELA	USP48 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP48 may be involved in the regulation of NF-κB activation by the TNF receptor superfamily via its interactions with RelA and TRAF2.	16214042
USP49		USP49 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily.	14715245
USP50		USP50 is a nonprotease homologue of the ubiquitin-specific processing protease (USP/USB) superfamily.	14715245
USP51		USP51 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily.	14715245
USP52	PAN3	USP52 is a member of the ubiquitin-specific processing protease (USP/USB) deubiquitinase superfamily. USP52 is a member of the Pan nuclease complex, which regulates mRNA stability.	14583602, 16284618
USP53		USP53 is a nonprotease homologue of the ubiquitin-specific processing protease (USP/USB) superfamily.	14715245
USP54		USP54 is a nonprotease homologue of the ubiquitin-specific processing protease (USP/USB) superfamily.	14715245
USPL1		USPL1 is a nonprotease homologue of the ubiquitin-specific processing protease (USP/USB) superfamily.	
USPL2/CYLD	NF-κB, HDAC6	CYLD deubiquitinase regulates inflammation and cell proliferation by down regulating NF-κB signaling through removal of ubiquitin chains from several NF-κB pathway proteins. CYLD is a negative regulator of proximal events in Wnt/β-catenin signaling and is a critical regulator of natural killer T cell development.	12917689, 12917690
VCPIP1	VCP	VCPIP1 (valosin containing protein p97/p47 complex-interacting protein) is a member of the deubiquitinating enzyme ovarian tumor domain (OTU) superfamily. VCPIP1 is necessary for VCP-mediated reassembly of Golgi stacks after mitosis.	15037600



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