Stem Cell & Development Pathways

from Cell Signaling Technology

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As a committed member of the research community, we practice responsible and sustainable business methods and invest heavily in research and development. We also encourage thoughtful use of our limited natural resources by highlighting environmental issues in our catalog and by promoting conservation and recycling.

All pathways were created by research scientists at Cell Signaling Technology and reviewed by leading scientists in the field. Visit www.cellsignal.com for additional reference materials and comprehensive validation data for over 4,000 antibodies and related reagents.

ESC Pluripotency and Differentiation

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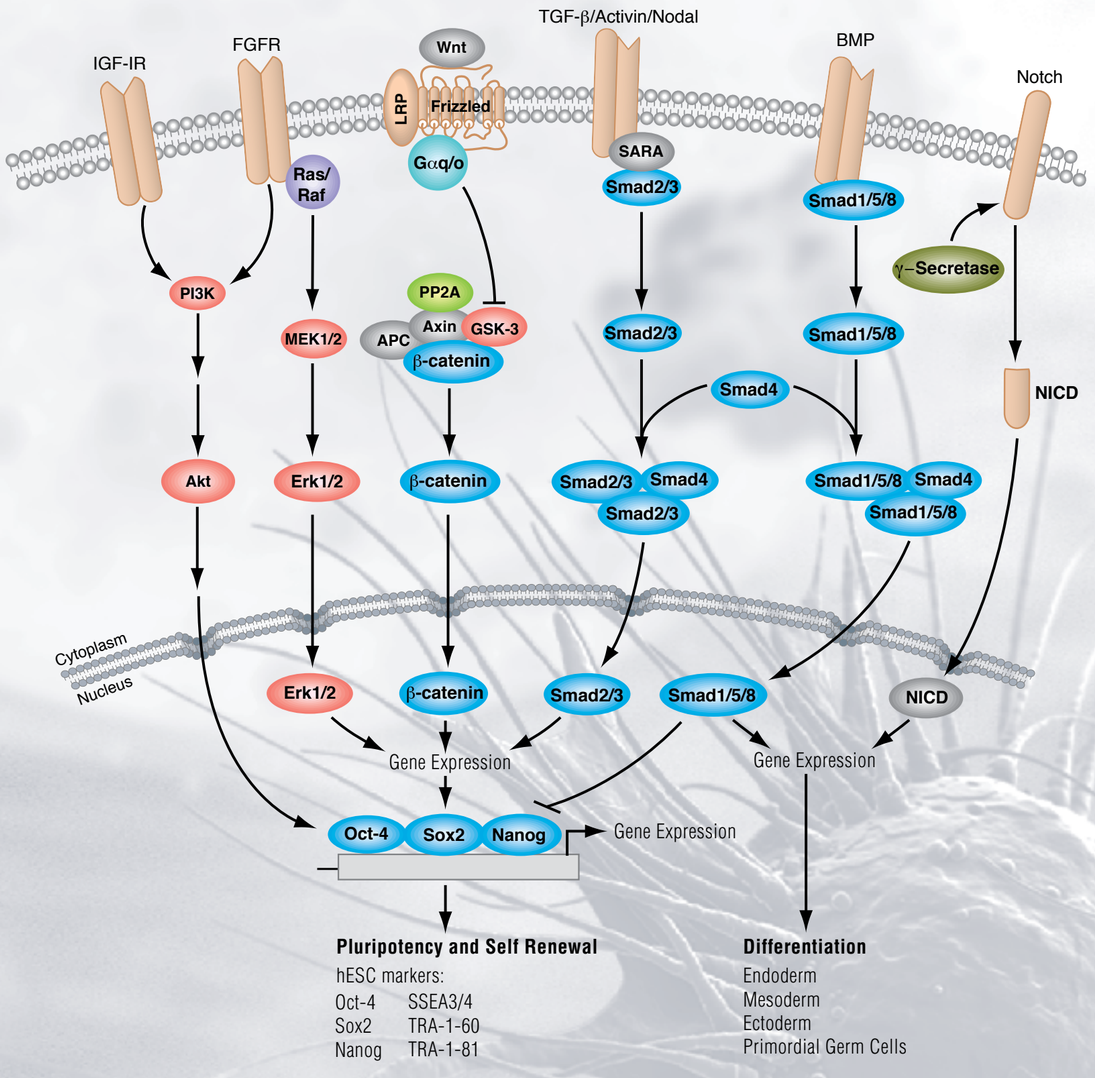

Histone Methylation

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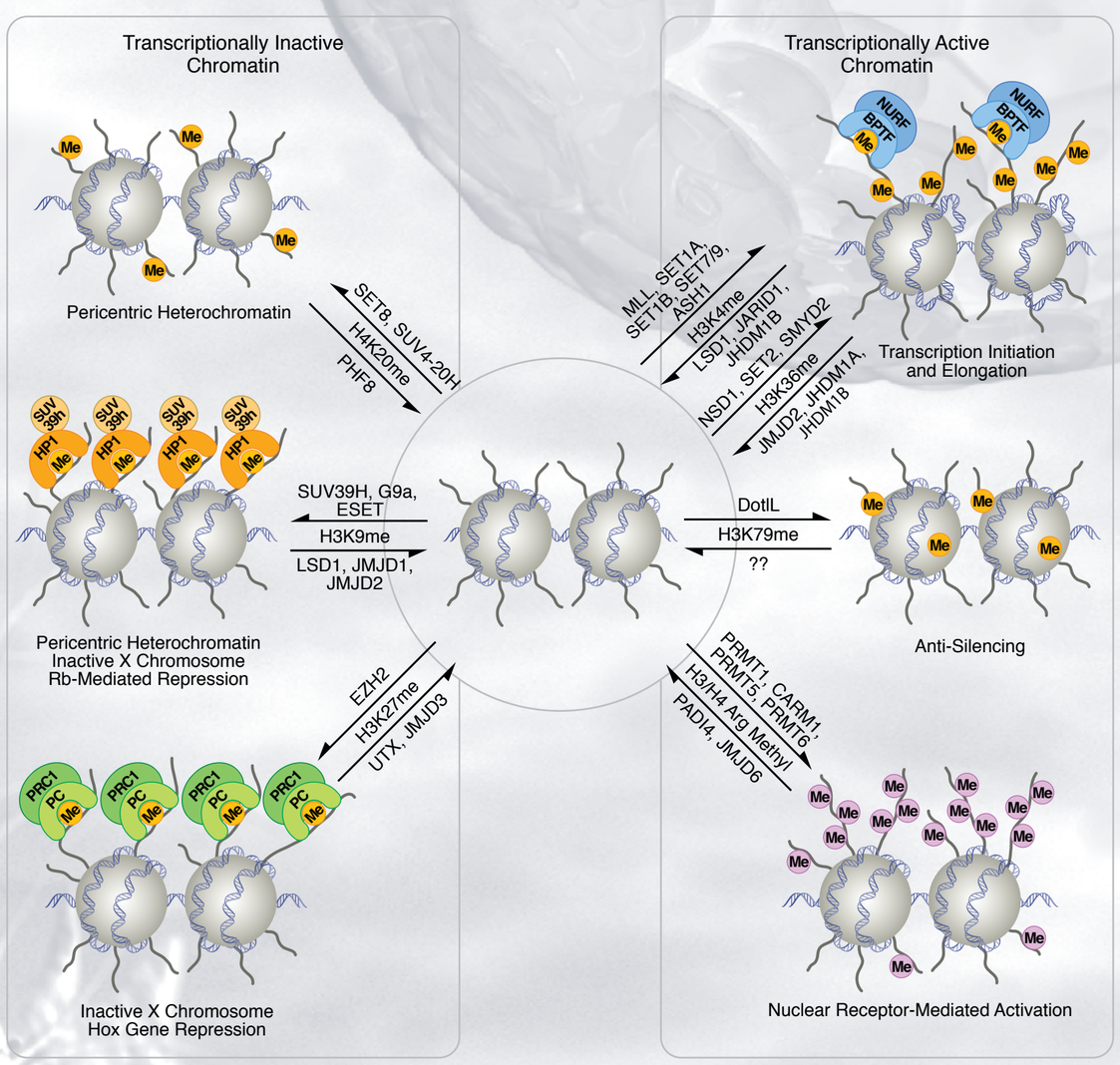

Wnt/β-Catenin Signaling

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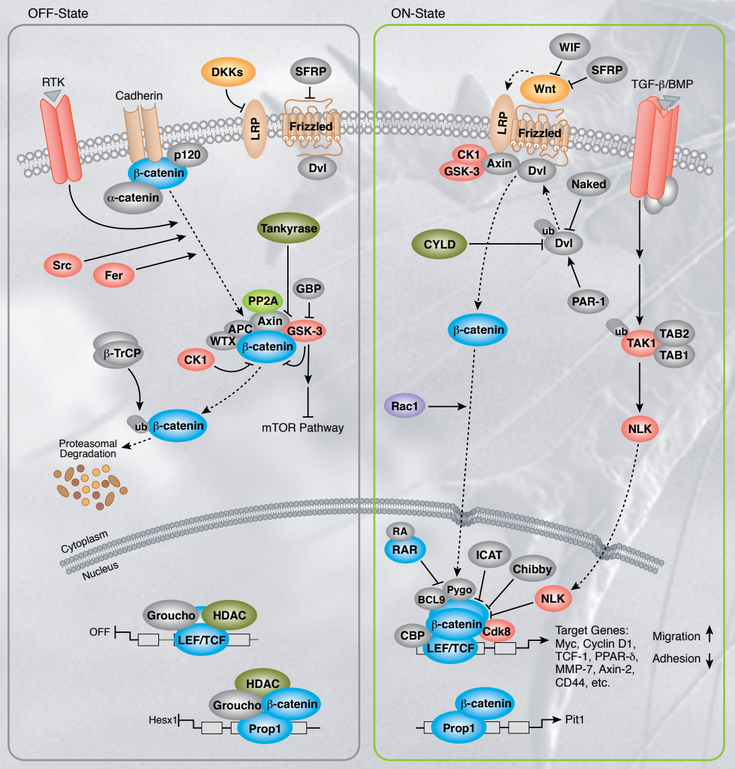

Notch Signaling

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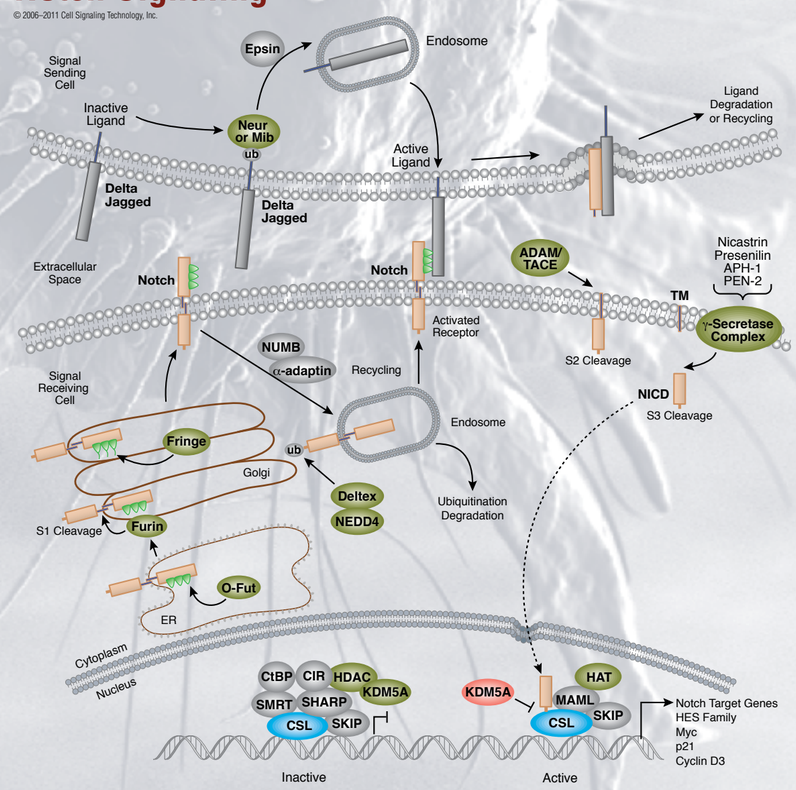

Hedgehog Signaling in Vertebrates

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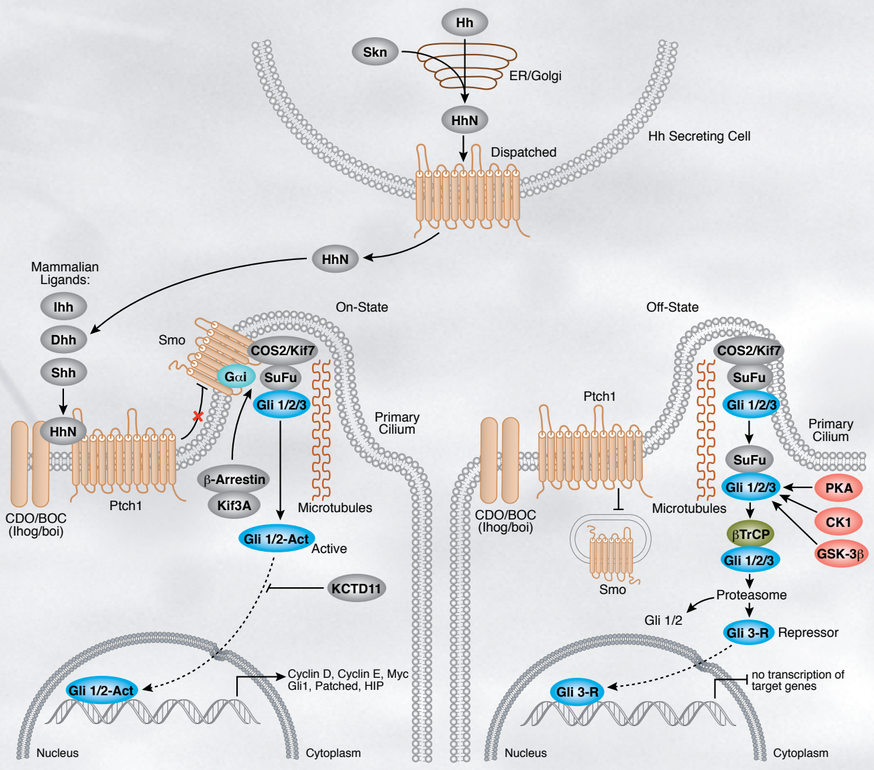

Direct Stimulatory Modification
Direct Inhibitory Modification
Multistep Stimulatory Modification
Multistep Inhibitory Modification
Tentative Stimulatory Modification
Tentative Inhibitory Modification
Transcriptional Stimulation
Transcriptional Inhibition
Separation of Subunits or Cleavage Products
Joining of Subunits
Translocation
Kinase
Phosphatase
Transcription Factor
Enzyme
GTPase
G-protein
Receptor

ESC Pluripotency and Differentiation

Pathway Description: Two distinguishing characteristics of embryonic stem cells (ESCs) are pluripotency and their ability to self renew. These traits, which allow ESCs to grow into any cell type in the body and to divide continuously in the undifferentiated state, are regulated by a number of cell signaling pathways. In human 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 through activation of β-catenin. 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.

Histone Methylation

Pathway Description: 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. Two such modifications, methylation of arginine and lysine residues are major determinants for formation of active and inactive regions of the genome. Arginine methylation of histones H3 (Arg2, 17, 26) and H4 (Arg3) promotes transcriptional activation and is mediated by a family of protein arginine methyltransferases (PRMTs), including the co-activators PRMT1 and CARM1 (PRMT4). In contrast, a more diverse set of histone lysine methyltransferases has 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 arginine and lysine residues and is unlikely to directly modulate nucleosomal interactions required for chromatin folding. While the mechanisms by which arginine methylation regulates transcription are unknown, 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 disease 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/AOF2, JMJD1, JMJD2, and JHDM1 has shown that methylation is reversible and provides a rational for how genomes might be reprogrammed during differentiation of individual cell lineages.

Wnt/β-Catenin Signaling

Pathway Description: The Wnt/β-Catenin pathway regulates cell fate decisions during development of vertebrates and invertebrates. The Wnt ligand is a secreted glycoprotein that binds to Frizzled receptors, which triggers a cascade resulting in displacement of the multifunctional kinase GSK-3β from the APC/Axin/GSK-3β-complex. In the absence of Wnt signal (Off-state), β-catenin, an integral cell-cell adhesion adaptor protein as well as transcriptional co-regulator, is targeted for degradation by the APC/Axin/GSK-3β-complex. Appropriate phosphorylation of β-catenin by coordinated action of CK1 and GSK-3β leads to its ubiquitination and proteasomal degradation through the β-TrCP/SKP complex. In the presence of Wnt binding (On-state), Dishevelled (Dvl) is activated by phosphorylation and poly-ubiquitination, which in turn recruits GSK-3β away from the degradation complex. This allows for stabilization of β-catenin levels, Rac1-dependent nuclear translocation, and recruitment to the LEF/TCF DNA-binding factors where it acts as an activator for transcription by displacement of Groucho-HDAC co-repressors. Additionally, in complex with the homeodomain factor Prop1, β-catenin has also been shown to act in context-dependent activation as well as repression complexes. Importantly, point-mutations in β-catenin lead to its deregulated stabilization. APC and Axin mutations also have been documented in some tumors, underscoring the deregulation of this pathway in human cancer. During development, the Wnt/β-catenin pathway integrates signals from many other pathways including retinoic acid, FGF, TGF-β, and BMP in many different cell-types and tissues. In addition, GSK-3β is also involved in glycogen metabolism and other key pathways, which has made its inhibition relevant to diabetes and neurodegenerative disorders.

Notch Signaling

Pathway Description: Notch signaling is an evolutionarily conserved pathway in multicellular organisms that regulates cell-fate determination during development and in stem cells. The Notch pathway mediates juxtacrine signaling among adjacent cells by which a diverse array of cell fate decisions in neuronal, cardiac, immune, and endocrine development are regulated. Notch receptors are single-pass trans-membrane proteins composed of functional extracellular (NECD), transmembrane (TM), and intracellular domains. ER and Golgi processing of Notch receptors in the signal-receiving cell results in cleavage and produces a glycosylated, Ca^{2+}-stabilized heterodimer composed of NECD non-covalently attached to the TM-NICD inserted in the membrane (S1 cleavage). This processed receptor is then translocated to the plasma membrane to enable ligand binding. In mammals, members of the Delta-like (DLL1, DLL3, DLL4) and the Jagged (JAG1, JAG2) families, which are located in the signal-sending cell, serve as ligands for Notch signaling receptors. Upon ligand binding, the NECD is cleaved away (S2 cleavage) from the TM-NICD domain by TACE (ADAM metalloprotease TNF-α converting enzyme). The NECD remains bound to the ligand and this complex undergoes endocytosis and recycling/degradation within the signal-sending cell. In the signal-receiving cell, γ-secretase (also involved in Alzheimer's disease) releases the NICD from the TM (S3 cleavage), which translocates to the nucleus where it associates with the CSL (CBF1/Su(H)/Lag-1) family transcription factor complex, resulting in subsequent activation of the canonical Notch target genes Myc, p21 and HES-family members.

Hedgehog Signaling in Vertebrates

Pathway Description: 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 and cholesterol as well as palmitate lipid modifications. In the absence of Hedgehog ligand in the receiving cell (Off-state), the receptor for Hedgehog-family ligands, Patched, is normally bound to and prevents membrane association of Smoothened, a G-coupled transmembrane protein. In the Off-state, SuFu and COS2 (Kif7 in vertebrates) sequester the microtubule-bound pool of the transcription factor Gli in the primary cilium. Gli can be phosphorylated by PKA, CKI, and GSK-3 resulting in β-TrCP-mediated degradation of Gli activators (Gli1 and Gli2 in mammals) or in the conserved pathway generation of repressor-Gli (Gli3 or truncated-Ci in Drosophila), which leads to repression of Hedgehog target genes. In the On-state, Hedgehog binding to Patched enables β-arrestin mediated translocation of Smoothened to the primary cilium where its associated G protein activity inhibits suppressive kinase action on Gli, leaving Gli free to translocate to the nucleus and activate Hedgehog target genes, including Cyclin D, Cyclin E, Myc, and Patched. Consequently, the conserved action of Hedgehog ligands is to switch the Gli-factors from being transcriptional repressors to activators. Loss of function mutations in Patched are associated with Gorlin-syndrome and predisposes to basal cell carcinomas, medulloblastomas, and rhabdomyosarcomas.

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