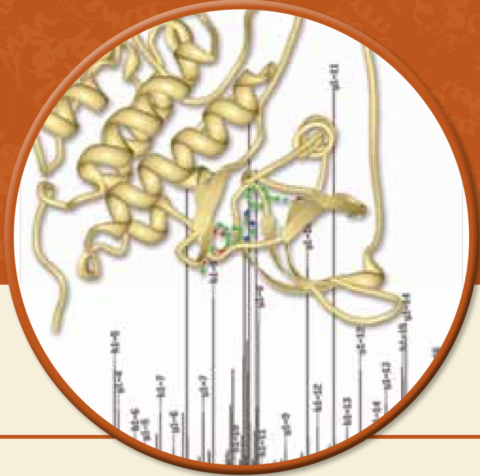

Quantitative Profiling of the Acetylome in Mouse Tissue using Lysine Acetylation Reagents in Immunoaffinity LC-MS/MS

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Introduction

Lysine acetylation of histone proteins was originally associated with the regulatory implication of gene regulation. Rapid progress in the identification of novel proteins subject to lysine acetylation has extended the regulatory scope of this reversible post-translational modification to cancer, muscle contraction, cell cycle machinery and enzymatic metabolism.

Immuno-affinity purification of peptides containing lysine acetylation followed by LC-MS/MS analysis, AcetylScan®, provides a specific and sensitive way to identify and quantify modification sites across samples. The results of such an experiment largely depends on the specificity, affinity, and sequence bias of the lysine acetylation antibody used.

Here we provide a comprehensive analysis of two popular acetylated-lysine (AcK) antibodies from Cell Signaling Technology (CST) and ImmuneChem Pharmaceuticals (ICP) by comparing the number of the MS2 identifications, enrichment specificity, and intensity of enriched peptides at MS1 level.

Based on the broader range of affinity of the AcK antibody from CST, we then performed a large-scale quantitative profiling of lysine acetylation sites from nine mouse tissues including testis, spleen, lung, brain, embryo, heart, liver, brown adipose (BAT), and gastrocnemius muscle (gastroc). In this analysis we identified over 6,000 AcK sites and examined the relative abundance for each site across the nine tissues by label-free quantification using Skyline software.

Methods

Tissues and Western Blots

All tissue except mouse embryos were obtained from mature BALB/c mice. Mouse embryos were harvested at day 16 from BALB/c mice. Equal amounts of lysate from each tissue was loaded into SDS-PAGE and blotted for lysine acetylation using Acetylated-Lysine (Ac-K[2]-100) Rabbit mAb #9814 (Cell Signaling Technology, Danvers, MA).

AcK Antibody Comparison

Lyophilized tryptic peptides from mouse liver were used for the comparison of Acetylated-Lysine (Ac-K[2]-100) Rabbit mAb #9814 from CST and Ack antibody ICP0388 from ICP. Enrichment of AcK peptides was achieved by immunoprecipitation using individual antibody (200 ug) from 10 mg of peptide. Enriched peptides were analyzed by LC-MS/MS over a linear gradient of 90 min. MS/MS spectra were collected in data-dependent mode with the Orbitrap-Elite® mass spectrometer. Raw data was searched against the NCBI *mus musculus* database and the identified AcK peptides were filtered using a 5% FDR. Quantitative analysis of each identified peptide was achieved by using an in-house developed label free algorithm according to ref. 1 and Skyline software.

Quantitative Profiling of AcK Sites across Mouse Tissues

Enrichment of AcK peptides from each tissue was performed using Acetylated-Lysine (Ac-K[2]-100) Rabbit mAb #9814 (Cell Signaling Technology, Danvers, MA), and was subject to LC-MS/MS analysis by Orbitrap-Elite® over a gradient of 90 min in analytical duplicates. Filtered pepXML files containing AcK peptides only and raw data were processed by Skyline v1.4 according to ref. 2 and online tutorial. Quantitative data was evaluated and clustered in Spotfire DecisionSite® v 9.1.2. Protein-protein interactions from the String Database were limited to the experimental and database interaction categories with a minimum score of 0.700.

Results

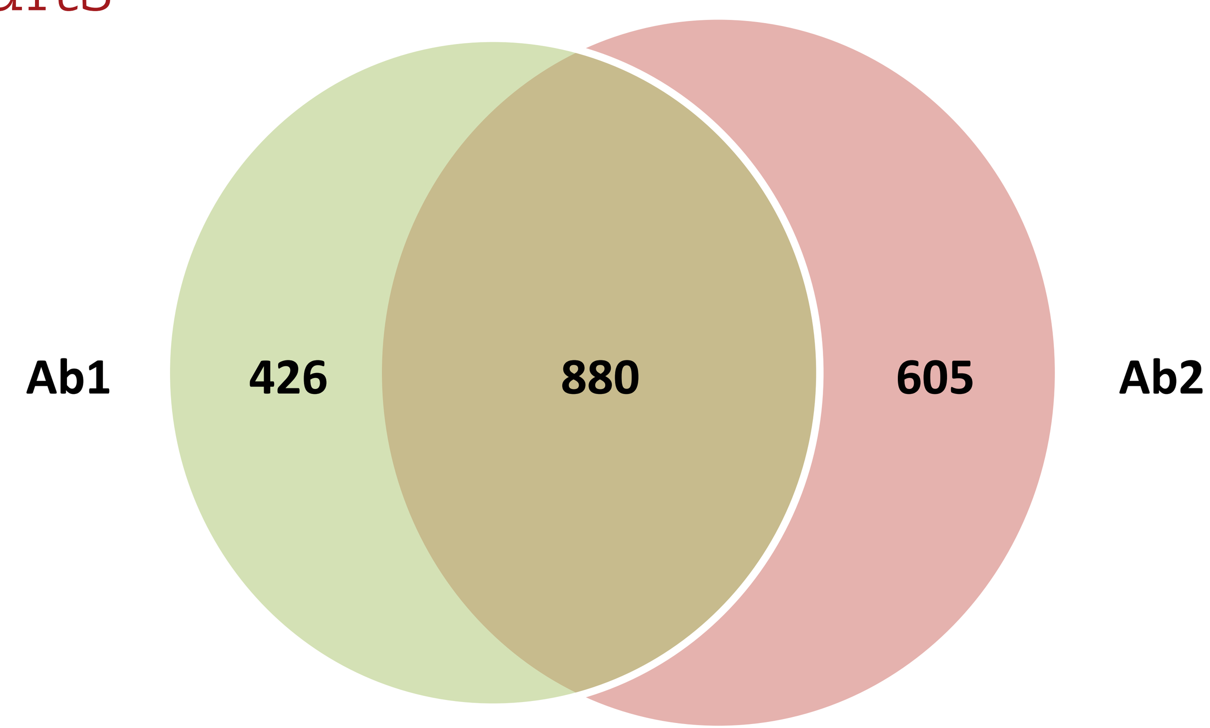
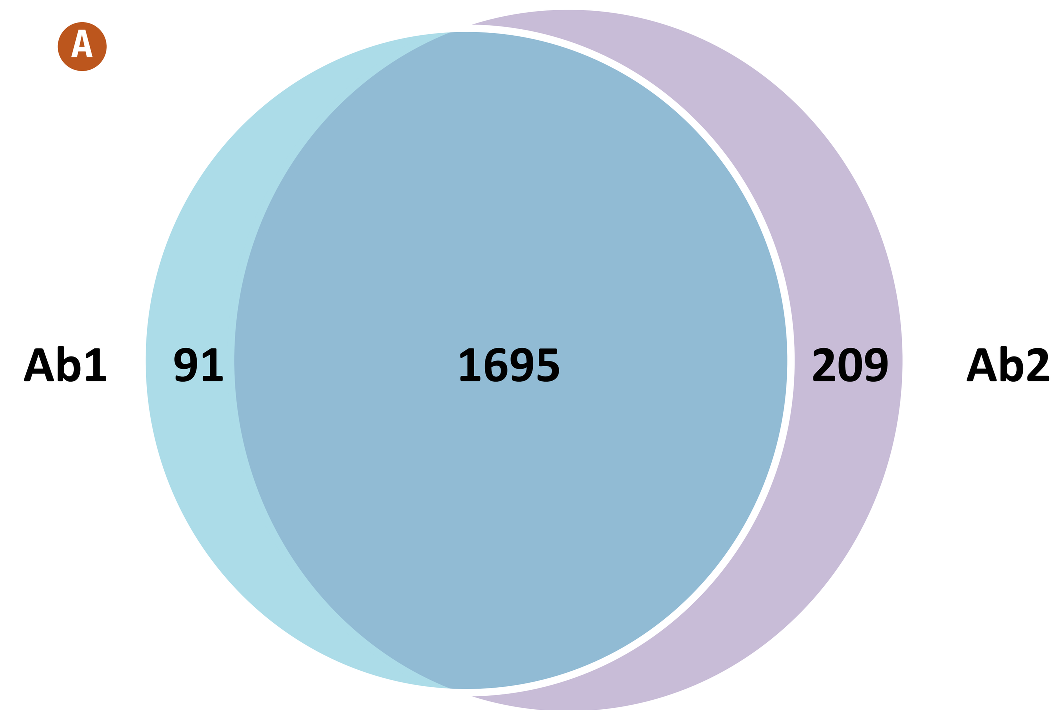


Figure 1: Overlap of AcK sites identified by LC-MS/MS from mouse liver tryptic peptides. CST AcK Ab2 identified slightly more unique AcK sites than ICP Ab1 (1485 versus 1306), while about 46% of the identified AcK sites were common to both antibodies.



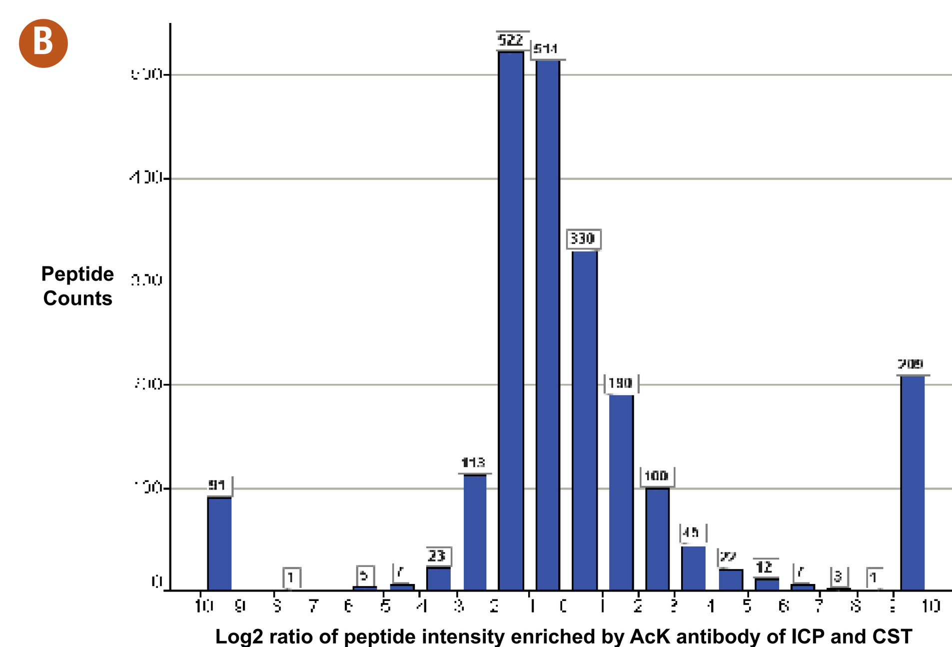


Figure 2: Comparison of MS1 intensity of enriched AcK peptides. (A) A total number of 1,995 unique AcK peptides were obtained from the composite analysis of both CST Ab2 and ICP Ab1. A quantitative comparison of the AcK peptides were obtained by label-free methods using Skyline software. A total of 209 and 91 peptides were uniquely enriched by CST Ab2 and ICP Ab1, respectively. **(B)** Distribution of the Log2 ratio of MS1 intensity of AcK peptides identified by CST Ab2 and ICP Ab1. CST AcK Ab2 has affinity for a broad range of AcK peptides.

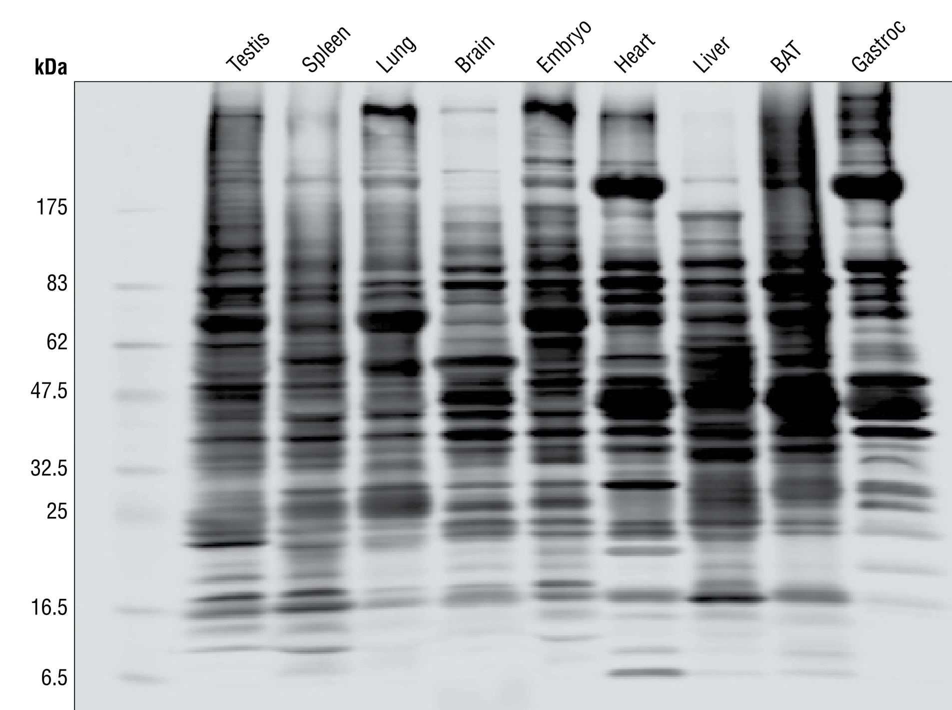
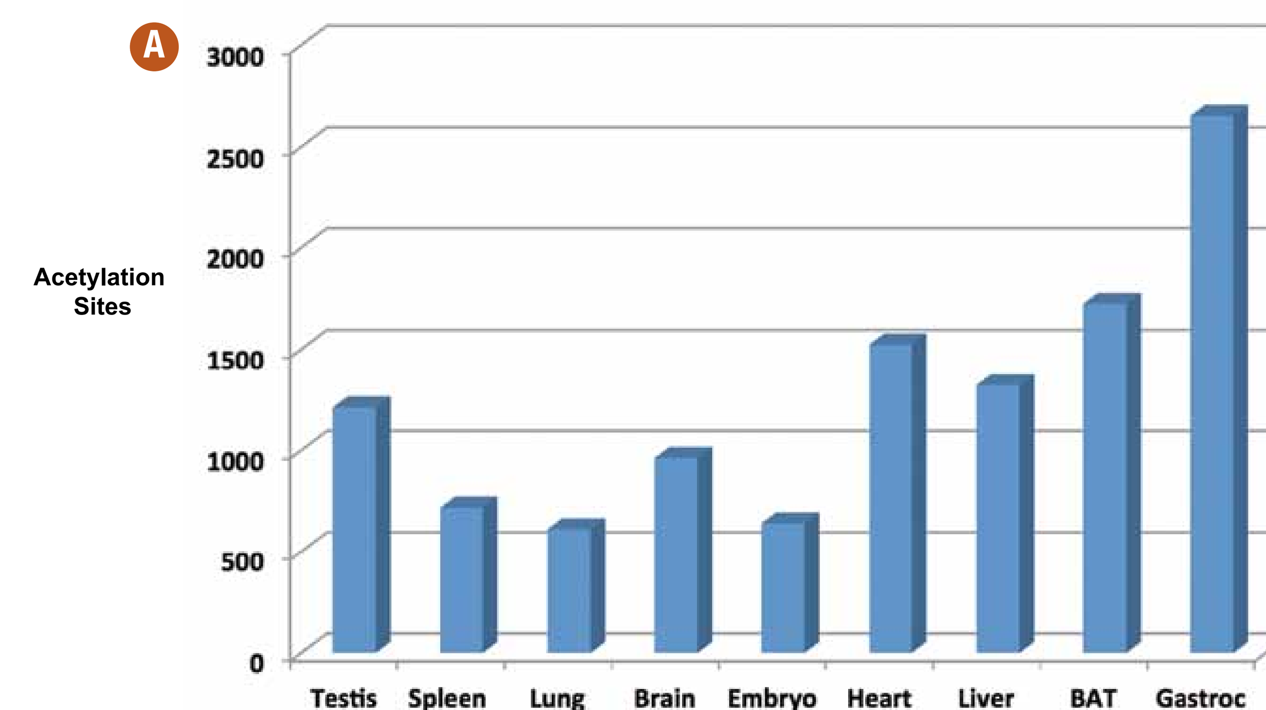


Figure 3: Western blot showing different lysine acetylation patterns across nine mouse tissues using the CST AcK antibody.



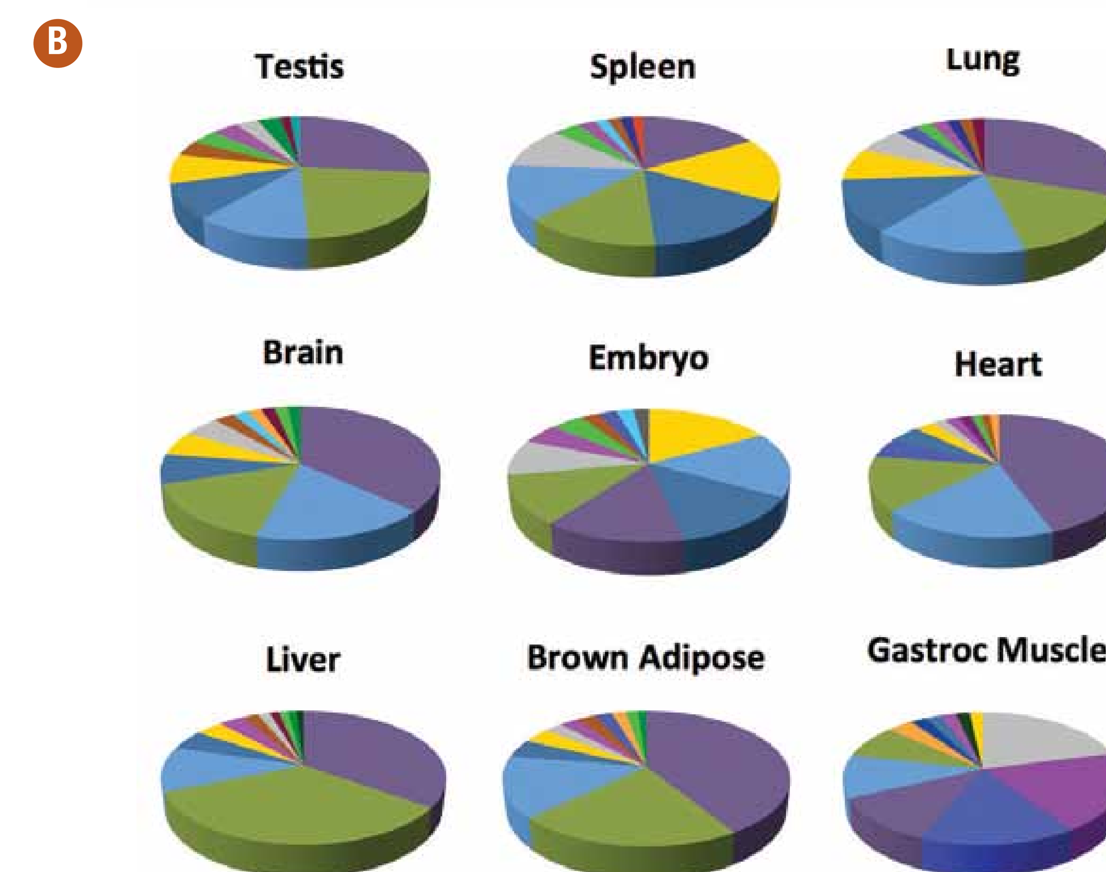


Figure 4: Identification of lysine acetylation sites from mouse tissues. (A) Number of unique AcK sites identified from each tissue. **(B)** Distribution of identified AcK sites containing proteins by their protein types. Interestingly, gastroc muscle and heart showed a different pattern of protein type distribution although they shared a similar western blot signal pattern (**Fig. 3**).

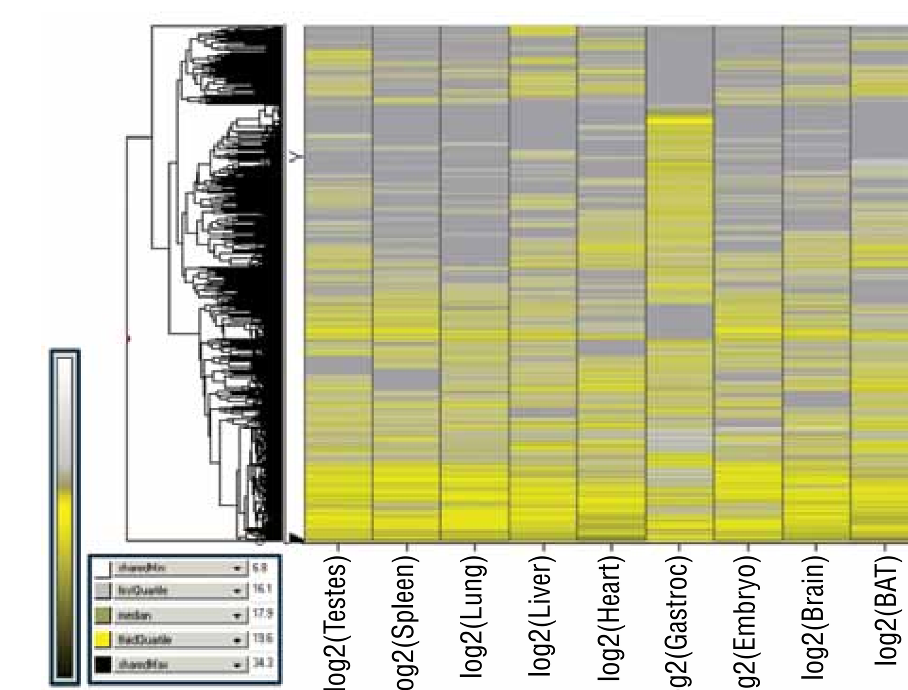


Figure 5: Hierarchical clustering analysis of the abundance, Log2 (Intensity), of AcK sites across nine mouse tissues using Spotfire DecisionSite® software (9.1.2). Hierarchical Clustering was performed using UPGMA clustering method by Euclidean distance. The color-coding was performed as illustrated in the legend located on the bottom left.

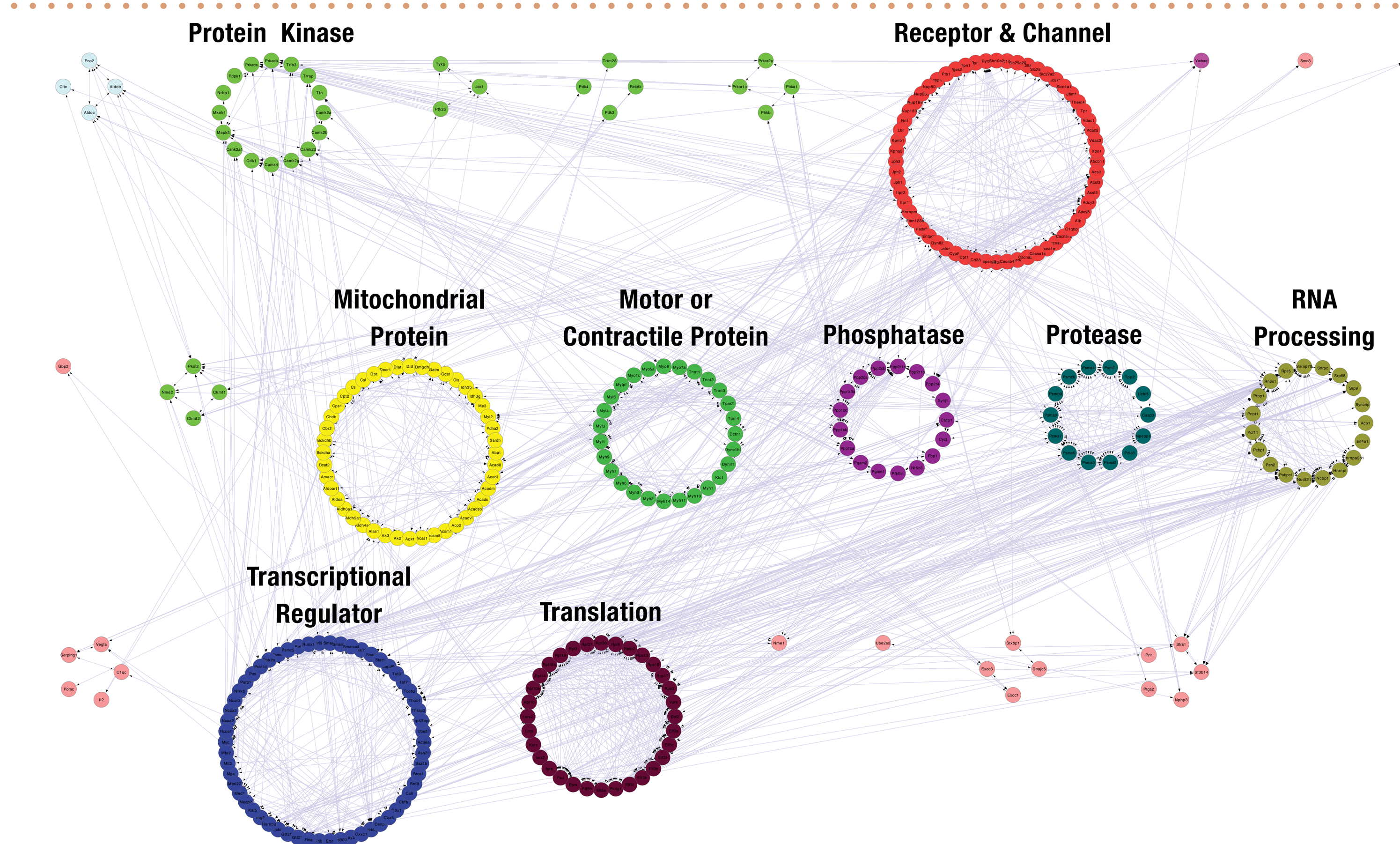


Figure 6: Interaction map of proteins with lysine acetylation. Protein-protein interaction was extracted from the STRING database, which was further processed according to protein type groups by Cytoscape.

Conclusion

A broadly reactive acetylated-lysine antibody has been developed by Cell Signaling Technology for conducting Acetylome profiling studies via LC-MS/MS methods (AcetylScan®).

References

1. Stokes M. et al (2012) *Mol. Cell Proteomics* 11:187–201.

2. Schilling B. et al (2012) *Mol. Cell. Proteomics* 11:202–221.

Visit **http://www.cellsignal.com/services/index.html** for more information regarding AcetylScan®.

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