D123 Redundant and distinct interactions of apm-1 and unc-101, subunits of AP-1 complex, with various cellular proteins in the Nematode Caenorhabditis elegans

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Clathrin-coated vesicles transport many cellular proteins, and clathrin-associated protein complexes (AP complexes) play an important role in this transport. Out of the 4 subunits of the AP complex, the medium chains (subunits) are associated with the sorting of the cargo proteins. There are two 1 subunits in *C. elegans* similar to mammals. The expression pattern and functions of the two 1 chains, *unc-101* and *apm-1* mostly overlap, but show some differences during various developmental stages of *C. elegans*. We thought that *unc-101* and *apm-1*, being subunits of AP-1 complex, would have redundant and distinct functions in selecting cargo proteins in the cell. Therefore, yeast two-hybrid screening was carried out to find proteins interacting with *unc-101* and *apm-1*. From one million transformants of each screening using either *unc-101* or *apm-1* as bait, most fish proteins interact with *unc-101* and *apm-1* simultaneously and several proteins interact specifically.

D124 Microarray Analysis of Ethanol-Treated Caenorhabditis elegans

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Analysis of changes in gene expression on ethanol treatment can provide insights on the targets and mechanisms of ethanol. We used cDNA microarray analysis to identify genes in *C. elegans* that are either up- or down-regulated by exposure to 7 vol% ethanol. Several gene families, including heat shock protein families and gene families of known or unknown function, showed a change in gene expression to ethanol. Northern results of about 100 genes that were either up- or down-regulated by a 6 hour exposure to ethanol showed 95% consistency with microarray results. Next, by analyzing microarray data corresponding to varied lengths of exposure to ethanol, we were able to classify effected genes largely into 4 classes. Confirmation of these results has been carried out by Northern analysis, and expression studies, promoter analysis and functional studies are currently being carried out.

D125 Transcriptional Regulation and Post-Transcriptional Expression of ADD1/SREBP1c by Insulin

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The ADD1/SREBP1c, a member of the basic helix-loop-helix (bHLH) family of transcription factors, plays a key roles in both adipocyte differentiation and fatty acid metabolism in adipose(fat) and liver. Previously, we have demonstrated that insulin treatment activates the transcription of ADD1/SREBP1c from fat and liver tissue. These findings led us to investigate the promoter region of the ADD1/SREBP1c gene. We isolated the 5'-UTR(about 2.7kb)of the ADD1/ SREBP1c gene by screening a mouse 129 genomic DNA library. And we constructed luciferase reporter containing ADD1/SREBP1c promoter region. Luciferase reporter assays showed that ADD1/SREBP1c promoter is trans-activated by either ADD1/SREBP1c or LXR. In addition, this report activity was further increased (about 1.5 fold) by insulin treatment in the presence of ADD1/

SREBP1c, but not by LXR. These results suggest that insulin appears to contribute to post transcriptional modification of ADD1/SREBP1c and sequentially further activates ADD1/SREBP1c gene expression via auto- regulatory mechanism.

D126 Rapamycin Represses Differentiation by Inhibiting the Expression of the Adipogenic Marker

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Insulin is a potent adipogenic hormone that triggers induction of a series of transcription factors governing differentiation of preadipocytes into mature adipocytes. However, the exact links between the insulin signaling cascade and the intrinsic cascade of adipogenesis have been incompletely understood. The immunosuppressant drug rapamycin has been reported to inhibit adipocyte differentiation of 3T3-L1 and F442A cells. In addition, rapamycin treatment blocked further adipogenesis after some degree of differentiation has been preceded. These observations were examined by morphological changes and by northern blotting with adipocyte marker genes including aP2, FAS, ADD1/SREBP1c, PPAR, C/EBP and adipsin. These results suggest that rapamycin represses adipocyte differentiation by inhibiting the expression of adipogenic marker. The mTOR is a downstream molecule of insulin signaling pathway and potently inhibited by rapamycin. Insulin-induced phosphorylation of mTOR has been shown to activate two translational components, 4E-BP1 and p70 ribosomal protein S6 kinase(p70S6K). Therefore, we are currently investigating the involvement of p70S6K in adipocyte differentiation.

D127 Activity of PKC is required for chondrocyte maturation

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In order to investigate the role of PKC in chondrocyte maturation, chondrocytes of various differentiation stages such as resting, proliferating, maturating, and hypertrophic chondrocytes from day 17 chick embryo sterna were cultured. Phorbol myristate 13-acetate (PMA) were treated to the cultures and maturation of chondrocyte were examined by measuring activity of alkaline phosphatase. Only maturating chondrocytes showed marked increase in alkaline phosphatase activity while other cells weakly responded to PMA treatment. PMA exerts its effect on the ALP activity by activating PKC as evidenced by modulating PKC activity with PKC activator or inhibitor. Our results suggest that activity of PKC is required for chondrocyte maturation.

D128 Nek2 Localizes to Multiple Sites in Mitotic Cells, Suggesting Its Involvement in Multiple Cellular Functions During the Cell Cycle

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Nek2 is a mammalian protein kinase that is structurally homologous to NIMA, a mitotic regulator in *Aspergillus nidulans*. To understand the possible cellular processes in which Nek2 participates during the cell cycle, we investigated the expression and subcellular localization of Nek2 in mitotic cells. Nek2 protein levels were observed to be regulated in a cell cycle stage-specific manner in cultured cells. Nek2 proteins were localized in both the nucleus and