S7-3

Metazoan Transcriptional Mediator Complex as the Integrator of Gene-specific Transcriptional Regulation Signals.

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The Mediator complex is generally required for transcriptional regulation in species ranging from yeast to human. Throughout evolution, the functional diversity of the Mediator complex has been enhanced to meet the increasing requirement for sophisticated gene regulation. We took systematic biochemical and genomics approaches to examine various types of Mediator proteins in *Drosophila melanogaster*. Such efforts led to the identification of three distinct forms of Mediator complexes. In exploring their compositional and fun ctional heterogeneity, we found that different cell types may require distinct Mediator complexes, some of which may participate in nuclear processes other than the previously identified functions. On the top of the complex heterogeneity, several Mediator gene s show distinct mutant phenotype thus were suggested to mediate transcriptional regulation of distinct group of genes via activator-specific interactions. However, their physiological relevance and activator-specificities in cell have not been addressed. Using biochemical analysis coupled with RNAi-mutational analysis, we confirmed that physical interaction between specific activator proteins and dTRAP80 is required for transcriptional activation of distinct group of genes in vivo. We extended this stud y to other Mediator genes by generating mutant Mediator cells and examining their defect on transcriptional regulation with the use of microarray analysis.

S7-4

Applications of RNA Interference Technology to Deciphering the Coupling Specificity bewteen Phospholipase C-β Isoforms and G-protein Coupled Receptors

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When activated by G-protein coupled receptor (GPCR), phospholipase C (PLC)-β plays a key role in signal transduction by triggering IP_3-induced Ca^{2+} molilization and DAG-induced PKC activation. Mammals have 4 isoforms of PLC-β, which are known to play distinct roles in organisms. However, the coupling specificity and the underlying mechanism in GPCR-PLC-β signaling have remained elusive so far. Firstly, we applied RNAi techenique to delineate the specific coupling between PLC-β isoform and GPCR. We found that bradykinin-induced IPs generation was selectively affected by PLC-β1 knock-down, whereas LPA-induced one was by PLC-β3 knock-down. Secondly, we aimed to reveal the mechanism, underlying the coupling specificity of PLC-β isoforms. In this study, we identified NHERF2 as the specific binding partner of both PLC-β3 and LPA receptor 2 (LPA2). Moreover, we found that NHERF2 physically links PLC-β3 to LPA2. Using siRNA directed against NHERF2, we showed that the ternary complex formation between LPA2, NHERF2, and PLC-β3 enhances the efficiency of signal propagation and ensures the specificity of the signaling pathway, especially in terms of the activation of the PLC-β isoforms. Taken together, we suggest that PLC-β isoform is specifically coupled to respective GPCRs and the coupling specificity between them can be crucially determined by the selective scaffolds such as NHERF2.