Symposia

S10-1

Regulation of mesodermal cell behavior in zebrafish embryos by the tumor suppressor Gravin: implications for development and cancer

David Kimelman, Douglas C. Weiser, and Ujjwal J. Pyati
Department of Biochemistry, University of Washington, Seattle, WA 98195-7130, USA

Convergent extension of the mesoderm is the major driving force of vertebrate gastrulation. During this process, mesodermal cells move toward the future dorsal side of the embryo, then radically change behavior as they initiate extension of the body axis. How cells make this transition in behavior is unknown. We have identified the scaffolding protein and tumor suppressor Gravin as a key regulator of this process in zebrafish embryos. We show that Gravin is required for the conversion of mesodermal cells from a highly migratory behavior to the radially intercalative behavior required for body axis extension. In the absence of Gravin, paraxial mesodermal cells fail to shut down the protrusive activity mediated by the Rho/ROCK/Mycosin II pathway, resulting in embryos with severe extension defects. We propose that Gravin functions as an essential scaffold for regulatory proteins that suppress the migratory behavior of the mesoderm during gastrulation, and suggest that this function also explains how Gravin inhibits invasive behaviors in metastatic cells.

S10-2

Rheumatoid arthritis models generated by using transgenic techniques

Yoichiro Iwakura
Center for Experimental Medicine, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108-8639, Japan

Rheumatoid arthritis (RA) is a systemic, chronic inflammatory disorder that mainly affects joints. The disease is autoimmune in nature, and high levels of proinflammatory cytokines are detected in the affected joints. We have generated two different RA models; human T-cell leukemia virus type I (HTLV-I) transgenic (Tg) mice and IL-1 receptor antagonist (Ra) deficient (KO) mice. Both of these models develop arthritis spontaneously, and the histopathology showed marked synovial and periarticular inflammation with articular erosion caused by invasion of granulation tissues, closely resembling that of RA in humans. The disease was not developed in nude or SCID mice and elevated levels of autoantibodies were detected in these mice, suggesting development of autoimmunity. High levels of mRNAs for proinflammatory cytokines, such as IL-1α, IL-6, IL-17 and TNF were detected in the joints of these models, suggesting involvement of these cytokines in the pathogenesis of the disease. The development of arthritis was markedly suppressed in either IL-6 or IL-1 KO mice in HTLV-I Tg model, while TNF-deficiency did not affect the pathology. In contrast, the development of arthritis was completely suppressed in TNF-deficient IL-1Ra KO mice, while IL-6 deficiency did not affect the pathology at all. Thus, the roles of cytokines in these two models are completely different. Interestingly, the development of arthritis in both models was markedly suppressed in IL-17 KO mice. We found that IL-17 was responsible for the priming of T cells and collagen-specific IgG2a production. These observations suggest that IL-17 plays a crucial role in the development of arthritis in the downstream of IL-1 by activating autoantigen-specific cellular and humoral immune responses.

S10-3

Rapsyn stability control by the CUL-3-containing E3 ligase complex and its implication

Seunghee Nam, Kyongwoo Min, Jung Hwa Lee, Jongbok Yoon1, Hyunsook Lee, and Junho Lee
Research Center for Functional Cellomics, School of Biological Sciences, Seoul National University, Seoul, Republic of Korea 151-742, and Ulsan Network Research Center, Department of Biochemistry, Yonsei University, 134 Shinchon, Seoul 120-749, Republic of Korea

Failure of abundance control of the synaptic proteins can be a critical factor for neuronal dysfunction disorders. Here, we show that rapsyn (RPV-1), a postsynaptic protein at the neuromuscular junction required for nicotinic acetylcholine receptor clustering - is degraded by the ubiquitination pathway in Caenorhabditis elegans. UBC-1, UBC-12, NEDD-8, and RBX-1 were required for rapsyn degradation. We identified cullin (CUL)-3 as a component of the E3 ligase and KEL-8 as the substrate adaptor. In vitro assay confirmed rapsyn ubiquilination by the CUL-3/KEL-8-containing E3 ligase. The proteins involved in rapsyn degradation and their interaction are conserved in mammals, and human rapsyn can rescue the nematode mutant phenotypes. Both knockdown of kel-8 and overexpression of rapsyn phosphorylated a loss-of-function mutation of rpy-1, suggesting that rapsyn abundance control is important for proper receptor function. Consistent with this, knockdown of KHL-8, the mammalian KEL-8 homolog, inhibited rapsyn ubiquilination and AChR clustering in mammalian cells. Our results raise the possibility that the human homologs of the genes identified in this study may link rapsyn abundance control to congenital myasthenic syndromes.

S10-4

The hereditary spastic paraplegia gene, atlantin, regulates microtubules and synaptic development in Drosophila

Mihye Lee, Min-Jung Lee, Yoon-Jung Kim, Sung Jun Jung1, and Seungbook Lee
Department of Cell and Developmental Biology, School of Dentistry, Seoul National University, Seoul 110-740, and Department of Physiology, College of Medicine, Kangwon National University, Chunchon 200-710, Republic of Korea

The pure hereditary spastic paraplegias (HSPs) are inherited human diseases characterized by progressive spasticity and weakness of the lower extremities. Mutations in the atlantin (also, SPPGAA) gene are responsible for 10% of pure autosomal dominant HSP. Although all is known to encode a member of the dynamin family of GTPases, its cellular and developmental roles remain unknown. We show that all is required for normal synaptic development and function at the Drosophila larval neuromuscular junction (NMJ). All is enriched in the body-wall muscles. Removal of all leads to increase in the number of NAI synaptic boutons and depolarization of the resting membrane potential in the body-wall muscle cells. These phenotypes are rescued by targeted expression of all in the muscle, but not by its neuronal expression, suggesting this HSP protein may primarily function in the post-synaptic muscle compartment. In all mutant muscle cells, microtubule bundles are numerous in vivo wild-type, while all overexpression disrupts the microtubule network in the muscle. Interestingly, in all mutant muscle cells, we observed reduced synaptic levels of Dlg, the fly homolog of PSD-95 that is known to be essential for presynaptic assembly. We are currently investigating the cellular mechanisms of how All dependent regulation of microtubules is involved in the regulation of the membrane potential in the muscle.

S10-5

THO complex is required for the environmental stress responses in Drosophila

Bongki Cho, Hye Eun Kim, Sungjin Moon, and Yun Doo Chung
Department of Life Science, University of Seoul, Seoul 130-743, Republic of Korea

Cotranscriptional recruitment of RNA processing and nuclear export factors onto nascent RNA facilitates efficient eukaryotic gene expression. Recent studies showed that a highly conserved multi-subunit protein complex called ‘THO complex’ plays a key role in recruiting these proteins to the mRNA in yeast and metazoans. In yeast cells, the majority of mRNAs requires the THO complex for their transcription and nuclear export, however, unexpectedly only subset of mRNAs appears to be dependent on the THO complex in metazoan cells. Hence, in multicellular higher eukaryotes, the THO complex seems to be required for the expression of certain sets of genes in certain types of cells during development or in response to certain stimuli. Since most studies reported so far have been done in single cell level, the cellular functions of the THO complex in multicellular organisms remains unclear. Here we show that Drosophila THO complex is required for the regulation of life span and stress responses. Mutant flies lacking the components of the THO complex showed severely reduced life span. They also were very sensitive to the various environmental stresses such as heat-shock, oxidative stress and starvation. These data suggested that the THO complex is involved in the expression of stress responsive genes.

S10-6

Leol, a Pafl complex component, controls the endocardial cushion differentiation through the regulation of notch1b expression in zebrafish heart

Jun-Dae Kim, Hyung-Seok Kim, Hang-Suk Chun, Myoung-Jin Kim, Young-Seop Kim, Hye-Kyung Park, Sang-Youb Yeo, and Tae-Lin Huh
Department of Genetic Engineering, College of Natural Sciences, Kyungpook National University, Daegu 701-701, Republic of Korea

METHODS. We have screened a zebrafish dalmurikr947 (dal) mutant showing the developmental defects of heart, jaw, and pigment, through a large-scale ENu mutagenesis. RESULTS. dalumiir (dal) mutants display the abnormal phenotypes in heart looping process at 48 hpf, whereas the early development of heart is not defected. To identify the causative gene to the dal phenotypes, we performed genetic linkage mapping using SSLP markers. As a result, dal mutation is linked to LG18 and mutated gene is characterized to leol, which encodes a RNA polymerase-associated protein and it is conserved from yeast to human. Ecotropic expression of leol as injection of BAC clone, cDNA or synthetic mRNA rescues abnormal phenotypes of mutant and injection of BAC clone, cDNA or synthetic mRNA rescues abnormal phenotypes of mutant and injection of leo1 BAC into the dalmurikr947 (dal) mutant defects. We has investigated the heart development-related gene expression to identify a cause of abnormal heart looping in mutant. The cmlc2 and bmp4, which are expressed in myocardium, is not altered, but notch1b, which is expressed in endocardium, is downregulated in mutant embryos, while it is upregulated in the dalmurikr947 (dal) mutant. To identify a cause of abnormal heart looping in mutant, we performed lacZ reporter construct injection in the dalmurikr947 (dal) mutant. The reporter construct is not expressed in myocardium, but is strongly expressed in endocardium in the wild type embryos, while it is strongly expressed in the dalmurikr947 (dal) mutant. CONCLUSIONS. These results indicated that dal mutation is mutated gene to the leol gene, the component of Pafl complexes, and it plays an important role in endocardial cushion differentiation through the regulation of notch1b expression. The detailed mechanism by which leol regulates several development processes as a component of Pafl complex remains to be elucidated. This work was supported by a grant to

Structural Biology