fertilization. Thus, Rsk seems to be an essential mediator or itself of CSF. To evaluate it in other frog oocytes, we have cloned and characterized Rsk cDNA in *Rana dybowskii* oocytes. The cloned Rana Rsk cDNA is about 2950 bp of nucleotides, which is consisting of a complete single open-reading frame with ATG codon and polyadenylation signal. The deduced amino acid sequence of Rana Rsk is 733 amino acids with 83 kDa. Rana Rsk shows a high homology (about 90 %) Xenopus Rsks. It also well conserved the two kinase domains with the specific phosphorylation sites, which is essential for activation of Rsk. Interestingly, we have cloned two type of Rsk variants, which may be splicing variants. Northern analysis is exhibited that Rana Rsk mRNA is strongly expressed in ovary tissue but weakly in other tissues. Rana Rsk protein is expressed with pTYB1 vector and purified with IMPACT-CN system. The purified Rana Rsk is cross-reacted with Xenopus $p90^{Rsk}$ antiserum. Therefore, the cloned Rana Rsk will be very useful for study of CSF during meiotic maturation in seasonal breeding animals.

D121

Functional Analysis of Rapsyn in *C.elegans*

Seung Hee Nam* and Junho Lee
Department of Biology, Yonsei University, Seoul

Nicotinic acetylcholine receptors (nAChRs) are clustered at high density in the postsynsptic membrane of neuromuscular junction. Rapsyn (achR Associated protein at SYNapse) is a peripheral membrane protein that is required for the AChR clustering at the neuromuscular junction. Rapsyn is conserved in many species including C. *elegans*. To understand rapsyn-mediated AChR clustering at the neuromuscular junction, we have examined various constructs of rapsyn fused to the green fluorescent protein in transgenic animals. In wild-type *C. elegans*, rapsyn was expressed specifically in muscle synapses and neurons. When a putative dominant negative mutant rapsyn gene, in which the histidine residue of the zinc finger motif was substituted by the amino acid glutamine, was examined, we found that its expression in muscle cells was altered and that the transgenic animals showed an uncoordinated phenotype, suggesting that the zinc finger motif of rapsyn is essential for AChR clustering. Examination of the transgenic animals bearing either wild type rapsyn or dominant negative mutant rapsyn in the genetic background of *unc-29* (AChR mutant), and *ric-4* (md1088) showed that both functional presynaptic activities and functional postsynaptic receptors are required for normal localization and function of rapsyn. To investigate the function of rapsyn, we analyzed rapsyn deletion mutant. Rapsyn deletion mutant animals grow slowly and their brood size is smaller than that of wild type. In order to investigate the interaction of rapsyn and AChR, we are examining co-localization of AchR and wild-type or mutant rapsyn proteins by using LacZ and GFP reporters. From these various assays, we will be able to elucidate the functions of rapsyn in C. *elegans*.

D122

Biological Functions of Transcriptional Mediators in the Nematode *Caenorhabditis elegans*

Jae Young Kwon* and Junho Lee
Department of Biology, Yonsei University, Seoul

Mediator is required for transcriptional regulation of most genes in yeast. Mammalian Mediator homologs function as transcriptional coactivators in vitro, however, their physiological role in gene-specific

transcription is not yet known. To determine the role of Mediator in development, we analyzed the phenotypes caused by RNA interference. RNA interference assays showed that Med-6, Med-7, and Med-10 are required for the expression of developmentally regulated genes, but are dispensable for expression of ubiquitously expressed genes · tested in this study. Therefore, the gene-specific function of Mediator as an integrator of transcriptional regulatory signals is evolutionarily conserved and is essential for *C. elegans* development. We have characterized the biological functions of the *C. elegans* gene *med-6*, which is the homolog of the yeast mediator med-6. We first identified a genetic mutation in the *med-6* gene by comparing genetic and physical maps and determining the molecular lesion. Next, we demonstrated that *med-6* plays an important role in development by regulating the transcription of genes in several evolutionarily conserved signaling pathways. One pathway with which we found *med-6* to be associated is the Ras pathway, as is assayed in the hermaphrodite vulval development system. We also found that med-6 is involved in a transcription factor cascade and the Wnt pathway which work together to mediate male ray development. Thus, *med-6* mediates regulated transcription of genes in various metazoan developmental signaling pathways. Since Med-6 is universally conserved including in yeast, and the mediator-related proteins that function in vulval and male ray development are metazoan-specific, our results imply the role of *med-6* as a point of convergence where signals transmitted through metazoan-specific mediator-related proteins meet. In addition, RNAi experiments in *rde-1* background showed that maternal and zygotic *med-6* activities have distinct roles in development.

D123

Ethanol Sensitivity Genes in the Nematode *Caenorhabditis elegans*

Min Gi Hong*, Jae Young Kwon, MinSung Choi and Junho Lee

Department of Biology, Yonsei University, Seoul

The mechanisms and sites of action of volatile anesthetics and ethanol are not fully understood. In the hope of understanding the mechanisms of ethanol, we first identified genes that control sensitivity to ethanol and anesthetics in the invertebrate system Caenorhabditis elegans. We identified 24 mutations that confer ethanol resistance either by EMS mutagenesis or transposon insertion mutagenesis. The genes are being cloned by positional cloning and analyses on the mutations are under way. In the next experiments, we used the cDNA microarray to identify genes that are either up-regulated or down-regulated by exposure of the animals to 7 % ethanol at various times. Several gene families including heat-shock protein family, glutamate receptor family, and gene families with unknown function, were up-regulated by ethanol after 6 hours. Also, there are gene families down-regulated. We are now examining these candidate ethanol-affected genes by northen analysis and in situ hybridization analysis. To establish an experimental system by which one can study Fetal Alcohol Syndrome using the nematode, we examined the effect of ethanol on embryogenesis. After incubating adult hermaphrodites in 7% EtOH for 12 hours, we observed egg-laying defects and abnormal embryogenesis. Based on this preliminary data, we will investigate and characterize the ethanol sensitivity genes involved in embryogenesis. In summary, we identified ethanol resistance genes by EMS or transposon mutagenesis; we identified genes whose transcription levels are altered by ethanol in the microarray analysis; and we established an experimental model system to study Fetal Alcohol Syndrome.