

**789A**

Defects in Nonsense Mediated Decay can partially suppress *sel-12*. Alisson Gontijo, Sylvie Aubert, Ingele Roelens, **Bernard Lakowski**. Nematode Genetics Group, Dept Neuroscience, Pasteur Institute, Paris, France.

In screens for suppressors of the egg-laying defect of mutations in the *C. elegans sel-12* presenilin gene, several suppressors of presenilin (*spr*) genes have been identified. Most *spr* genes encode components of a CoREST-like complex and mutations in these genes suppress *sel-12* by de-repressing the transcription of a second presenilin gene, *hop-1*. However, how the gene *spr-2*, which encodes a Nucleosome Assembly Protein (NAP) orthologous to the human SET/TAF1Beta oncogene, suppresses *sel-12* has remained unclear. To determine if *spr-2* works through the same mechanism as the other *spr* genes we examined the transcript levels of the different presenilin genes in *spr-2* mutants. To our surprise, *spr-2* mutations do not de-repress the transcription of *hop-1*. Furthermore *spr-2* mutations do not strongly affect the modification of histones around the *hop-1* locus. However, *spr-2* mutations appear to reduce Nonsense Mediated Decay (NMD) of *sel-12* transcripts containing early stop mutations, at least in the early larval stages. To determine if defects in NMD can suppress *sel-12* we constructed double mutant strains between *sel-12* and alleles of *smg* genes. The *smg* genes are absolutely required for NMD and all alleles tested can restore *sel-12* transcript levels and partially suppress the egg-laying defect of *sel-12*. This indicates that the proteins encoded by the three non-sense *sel-12* alleles tested, *by125*, *ty11* and *ar171*, retain some residual *sel-12* activity. However, *smg* mutations do not suppress *sel-12* nearly as well as *spr-2* indicating that *spr-2* suppresses *sel-12* through another mechanism. In the process of this work we realized that a new *spr* gene we were pursuing, *spr-8*, mapped close to the gene *smg-6*. *spr-8* has a weak suppressor phenotype and fails to complement *smg-6* for *sel-12* suppression. *spr-8* can suppress the *smg*-suppressible mutation *unc-54(r296)* and *spr-8(pf52)* contains a mutation in *smg-6*. We are presently testing whether *smg* genes can also suppress the phenotype of a deletion and a missense mutation of *sel-12* and whether any additional *smg* mutation uncovered in our screen are *smg* alleles.

**790B**

New Suppressors of *unc-93(e1500sd)* Locomotory Defects Affect Genes Encoding Critical pre-mRNA Splicing Factors. **Long Ma**, Bob Horvitz. HHMI, Dept Biol, MIT, Cambridge, MA.

*sup-9*, *sup-10* and *unc-93* encode components of a presumptive *C. elegans* two-pore domain K<sup>+</sup> channel complex. Rare gain-of-function mutants of each of these three genes have abnormal body muscle contraction and exhibit the "rubberband" phenotype: when prodded on the head, an animal contracts and relaxes along its entire body without moving backwards. Loss-of-function mutants of each of these genes have no obviously abnormal phenotype. The SUP-9 protein is similar to the mammalian Two-pore Acid Sensitive K<sup>+</sup> channels TASK-1 and TASK-3. *sup-10* encodes a novel single transmembrane protein. *unc-93* encodes a multiple transmembrane protein that defines a novel family of proteins conserved from *C. elegans* to mammals. A mammalian UNC-93 homolog, UNC-93b, has been shown recently to play important roles in the innate immune response.

Previous screens for recessive suppressors of the mutant phenotype of *unc-93(e1500sd)* animals were not designed to identify suppressors that cause lethality. To seek essential genes that interact with *unc-93*, we screened ~10,000 EMS-mutagenized F1 *unc-93(e1500sd)* animals clonally for progeny with improved locomotion and identified three new suppressors. *n4562* is a partial suppressor and causes sterility or carries a closely linked mutation that causes sterility. *n4564* and *n4588* are strong recessive suppressors and cause temperature-sensitive (ts) lethality or carry closely linked ts-lethal mutations. We found that *n4588* is a missense mutation in the gene *uaf-1*, which encodes a worm homolog of the splicing factor U2AF65 (U2 snRNP auxiliary factor large subunit). *n4562* is a nonsense mutation in the gene *Y116A8C.32*, which encodes a worm homolog of the splicing factor SF1. U2AF65 and SF1 have been shown to interact with each other to regulate pre-mRNA splicing. Finally, we have identified a missense mutation in the gene *sig-7* in *n4564* mutant animals. *sig-7* encodes a highly conserved protein with a peptidyl-prolyl isomerase domain, an RRM (RNA recognition motif) and an RS (arginine/serine) rich domain.

We found that *uaf-1(n4588) unc-93(e1500)* animals have an alternatively spliced *unc-93* transcript that encodes a truncated UNC-93 protein. This transcript is probably caused by the generation of a cryptic splice acceptor by the *unc-93(e1500)* mutation so that this new splice acceptor, instead of the endogenous splice acceptor, is recognized by the splicing machinery. We hypothesize that *n4588*, *n4562* and *n4564* suppress *unc-93(e1500)* by affecting pre-mRNA splicing of the *unc-93* transcript.

These results suggest that *unc-93(e1500)* can be used as a genetic system for the study of splice-site selection *in vivo*.

**791C**

Regulation of the formation of cytoplasmic processing bodies in *C. elegans*. **Yinyan Sun**, Yuxia Zhang, Peiguo Yang, Wenru Hou, Mengran Wang, Xiangyu Yao, Jinghua Han, Hong Zhang. National Institute of Biological Sciences, Beijing, No. 7, Science Park Road, Zhongguancun Life Science Park, Beijing, P. R. China, 102206.

Gene expression is tightly controlled at both the transcriptional and post-transcriptional levels, establishing the differential gene expression patterns during animal development. Post-transcriptional gene regulation involves miRNA-mediated gene repression and siRNA-mediated RNA degradation. Furthermore, mRNAs with a premature termination codon (PTC) are under the surveillance of the nonsense-mediated decay system (NMD), which serves to protect against the possibility of producing a toxic protein. Defects in each of these processes result in serious diseases: one third of genetic and acquired diseases are attributable to PTCs. Recent results demonstrate that NMD, miRNA-mediated post-transcriptional gene silencing and siRNA interference take place in cytoplasmic processing bodies, called P bodies. The molecular basis for the aggregation of mRNAs into P bodies, as well as their significance in these processes, however, is almost completely unknown. We use *C. elegans* as a model system to study the regulation of P body formation, offering insight into the coordinated regulation of mRNA translation and mRNA decay. As in other systems, the *C. elegans* DCAP-1 is localized in P bodies. Stable transgenic lines carrying *dcap-1::Dsred* were therefore used in a genome-wide RNAi screen to identify mutants with abnormal P body formation. We identified about 500 genes whose loss-of-function affect the P body formation, including changes in the number, size, morphology and distribution. Some genes affect the formation of P bodies only in specific tissues, such as in intestine, uterus, egg and gonad. The physiological function of this altered P body formation in post-transcriptional regulation is being characterized.