expression of voltage-gated calcium channel alpha1 subunit genes. Embryos in which the Notch pathway has been inactivated have shown increased expression of these calcium channel subunits in ectopic areas, while embryos in which Notch signaling has been over-expressed may have differential expression of these genes in several areas of the brain.

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Program/Abstract # 327
Requirements for SAO-1 protein during C. elegans development
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sao-1 (suppressor of aph-one) is a novel C. elegans gene that we isolated as a suppressor of a Notch-signaling defective aph-1 mutant. Our genetic analysis of a soa-1 hypomorphic mutant, a soa-1 null mutant, and soa-1 RNAi, suggests that normally SAO-1 function negatively impacts the efficiency of Notch signaling in the C. elegans embryo and germline. The strongest clue to SAO-1 molecular function comes from its ability to interact with the E3 ubiquitin ligase protein SEL-10, which is thought to negatively regulate Notch and presenilin proteins by facilitating their ubiquitylation. This association has led us to consider a model in which sao-1 works with SEL-10 to achieve protein turnover of one or more components of the Notch signaling pathway. Here we address whether sao-1 mutations have phenotypes other than Notch signaling suppression. We have analyzed a null soa-1 mutation, and found that animals are viable and fertile without sao-1 function, but display a variety of incompletely penetrant phenotypes, such as reduced brood size, decreased embryonic viability, high incidence of males, and increased retention of eggs. We are analyzing these phenotypes to determine whether the sensitivity to soa-1 function is associated with Notch signaling or SEL-10 activity, or perhaps reveals additional roles for SAO-1.

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Program/Abstract # 328
Functional distinctions between HOP-1 and SEL-12 presenilins in C. elegans embryos
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C. elegans has two presenilin genes, sel-12 and hop-1, that can participate in Notch signaling events throughout development. The SEL-12 protein is more closely related to the Drosophila and mammalian presenilins proteins than is HOP-1. Nonetheless, SEL-12 and HOP-1 appear functionally redundant: hop-1 and sel-12 mutants are fully viable and fertile, whereas hop-1 sel-12 double mutants display severe phenotypes that are characteristic of Notch signalizing mutants. The same phenotypes are seen upon removal of any one of the other three gamma-secretase components (APH-1, APH-2, and PEN-2). Recently we have discovered a surprising difference between hop-1 and sel-12 activity by examining maternal presenilin function in worms that carry a leaky aph-1 mutation (zu147). This mutation reduces aph-1 mRNA levels and results in a truncated version of APH-1 that lacks its C-terminal 33 amino acids. In this background, hop-1 and sel-12 show opposite phenotypes: a sel-12 mutation enhances the aph-1(zu147) mutant phenotype, whereas a hop-1 mutation suppresses it. These results suggest that hop-1 gene activity normally has a negative effect in the aph-1(zu147) mutant background, and may thus highlight a functional difference between gamma-secretase complexes that contain SEL-12 versus those that contain HOP-1. We are currently testing the idea that HOP-1-containing gamma secretase complexes show a unique dependence on either the C terminus of APH-1, or on high levels of APH-1.

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Program/Abstract # 329
Suppression of C. elegans aph-1 mutants by increasing mRNA levels
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APH-1 is one of four essential components of the gamma-secretase complex that cleaves the Notch receptor in response to ligand activation. APH-1 and its three partners (APH-2/Nicastrin, PEN-2, and Presenilin) are dependent on one another for stability and proper cell trafficking during complex assembly. Thus it is expected that mutations that alter expression levels of any one component may have significant effects on overall gamma secretase function. In C. elegans, decreased aph-1 activity leads to Notch mutant phenotypes and to mislocalization of gamma-secretase proteins in the early embryo. We analyzed four different aph-1 mutations that show a range of severity in Notch signaling defects. Using quantitative PCR, we demonstrate that three of these four mutations result in decreased aph-1 mRNA levels. In each case, the mutant aph-1 mRNA levels can be increased by inactivating nonsense-mediated mRNA decay. For two of the three mutants, increasing mRNA is sufficient to suppress the mutant phenotype, suggesting that the original mutant mRNA is capable of encoding functional APH-1 product. In the case of an aph-1 nonsense mutant that is predicted to cause a 33 amino acid truncation, this result suggests that the C terminus of APH-1 is not essential for function; we will discuss the implications of this result. In a second case, we show that a start codon mutation in aph-1 can be suppressed by increasing mRNA levels, suggesting that functional product can be generated from this mutant mRNA. We have extrapolated this finding to other start codon mutations, and suggest that such mutations may generally be suppressible by increasing mRNA levels.

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Program/Abstract # 330
FGF signaling is required for parapineal formation
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The zebrafish epithalamus is a region of the forebrain consisting of medially located pineal organ, a parapineal, and two bilaterally opposed habenular nuclei. The parapineal emerges from the left, anterior part of the pineal anlage beginning at approximately 30 h post fertilization (hpf) and migrates leftward to lay adjacent to the left habenula. Parapineal emergence is concomitant with initiation of asymmetry in the habenulae, left habenular neurons do not properly innervate their targets in the midbrain. Despite its importance in establishing epithalamic asymmetries, little is currently known about what factors control parapineal development. One candidate is Fibroblast growth factor 8. fgf8 is expressed within the developing epithalamus beginning at approximately 20 hpf and persisting past 36 hpf, overlapping spatially and temporally with the appearance of the nascent parapineal. In addition, fgf receptor 4 is expressed in migrating parapineal cells. fgf8 mutants display a reduction in the number of parapineal cells. Treatment of embryos with SU5402, a small molecule inhibitor of Fgf receptors, from 24 to 30 hpf also leads to a decrease in parapineal cell number. In addition,