Genetic Suppressor Screen of Separase Mutants Identifies Cohesin Subunits

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The following is a transcript of the audio portion included with the poster.

Introduction

Greetings, I'm Dillon Sloan, the technician for the Bembenek and Csankovszki labs in the MCDB department at the University of Michigan. Today I'll be talking to you about our studies of the protease separase in *C. elegans*, focused on elucidating a role of separase in vesicular trafficking during anaphase.

It has long been appreciated that separase plays a role in chromosome segregation during anaphase onset. The current model holds that during anaphase onset, separase is activated to proteolytically cleave the cohesin ring, a protein complex responsible for holding sister chromatids and homologs together during meiosis and/or mitosis. During meiosis, cohesin is removed in a step-wise manner to first allow for the segregation of homologous chromsomes in meiosis I, followed by the removal of the remaining cohesin in meiosis II to allow for the segregation of sister chromatids.

Alongside the role of separase in chromosome segregation, our lab has also demonstrated a role for separase in vesicular trafficking during meiosis and mitosis of *C. elegans* embryos. During anaphase I, separase localizes to specialized vesicles called cortical granules. Separase promotes exocytosis of cargo, which modifies the extracellular matrix of the embryo to form an eggshell and provides an osmotic and mechanical barrier for the developing embryo. In the introduction panel of my poster, note the immunofluorescent image of a meiosis I embryo during anaphase, with separase labeled in green and DNA in blue. At the left, by the arrowhead, you can see separase colocalizing with DNA, as well as localizing to the spindle microtubules between the segregating homologous chromosomes. At the right of the image, by the arrow, you can see separase localizing to several ring-shaped cortical granules. An additional note, we have also demonstrated a role for separase in vesicular trafficking during cytokinesis after mitosis, indicating the impact of its dual function extends beyond meiosis in *C. elegans*.

To identify regulators of separase during vesicular trafficking, we used a hypomorphic, partial separation of function, temperature sensitive allele of separase termed sep-1(e2406) (note the diagram in the Introduction panel). sep-1(e2406) is a point mutant in a well-conserved motif in the N-terminal region of separase, resulting in a Cysteine to Tyrosine amino acid change. At restrictive temperature, this allele of separase has minimal problems with chromosome segregation, but fails to localize to or exocytose cortical granules.

Identifying Suppressors of sep-1(e2406)
We, thus, undertook a massive genetic screen intended to identify genetic regulators of separase during this process. A schematic of this screen can be seen in

the left of the panel titled "Identifying suppressors of sep-1(e2406)". Briefly, worms were treated with ENU, a chemical mutagenic agent, and allowed to grow at permissive temperature for two generations. After this, worms were shifted to the restrictive temperature and suppressors were isolated, characterized, and identified.

Nearly a million haploid genomes were screened by this approach, yielding 68 suppressor mutations. 14 of these were intragenic suppressors located exclusively in the N-terminus of separase. The remaining extragenic suppressor mutations included 47 mutations throughout the coding sequence of pph-5, a phosphatase, including several premature stop codons. Pph-5 had previously been identified in a similar screen. We also identified 1 mutation in hsp-90, a known pph-5 chaperone. Beyond this, 6 suppressing mutations were identified that were not found in any of the known candidate suppressor genes.

Identifying Suppressors in Cohesin

To identify these genes, we used whole genome sequencing and single nucleotide polymorphism (SNP) mapping of ancestral point mutations, as depicted in the the panel titled "Identifying suppressors in Cohesin". Briefly, this approach involved crossing these suppressor mutants with an alternate ancestral strain of *C. elegans* with a known profile of SNPs (the "Hawaiian" strain, per the diagram). We pooled the surviving F2's with different recombination profiles containing the suppressor, and sent them for whole genome sequencing. This produced a plot of ancestral SNP's , as in the example at the top right of the panel. The density of these SNPs should suddenly decrease around the site of the recombined suppressor mutation, as can be seen in the example plot. From the unknown mutants, we identified four containing mutations in cohesin related genes within the suppressed region of interest.

Cohesin is a heteromeric protein complex composed of two SMC subunits, SMC-1 (known as HIM-1 in the worm) and SMC-3, as well as a kleisin subunit which is directly cleaved by separase. These SMC proteins contain ATPase head domains on either end of the protein that meet by folding at a "hinge" domain. Between the hinge domain and the ATPase head domains are coiled coil regions. There are also a number of accessory proteins required for cohesin function, including PDS-5 (known as EVL-14 in the worm), which is implicated in the maintenance of sister chromatid cohesion. PDS-5 contains multiple HEAT repeats.

Each of the cohesin mutants identified were missense mutations. Two of these mutants were identified in him-1, another in smc-3, and one more, from a previous study, was identified in evl-14. The two him-1 mutations are located either within a coiled-coil region of the protein, or right beside the hinge domain. The smc-3 mutation is located in one of the coiled coil regions, and the evl-14 is located between the HEAT repeats and an intrinsically disordered region of the protein.

Cohesin mutant, but not RNAi, suppresses sep-1(e2406) at 20°C

We have begun to verify and characterize these mutants, as shown in the panel titled "Cohesin mutant, but not RNAi, suppresses sep-1(e2406) at 20°C". We used cohesin RNAi as an attempt to verify and characterize these genes as suppressors, as well as CRISPR to begin to re-create the suppressor mutations. Using RNAi at two different doses, we were not able to see suppression of sep-1(e2406) with either him-1 RNAi or evl-14 RNAi, nor with kleisin RNAi. In contrast, depletion of the cleaved kleisin subunit suppresses a lethality in lines expressing a dominant negative protease dead separase. However, we were able to re-create the him-1 suppressor via CRISPR, which resulted in penetrant suppression of the e2406 phenotype by hatching assays, verifying him-1 as a suppressing mutation. In further support of this, RNAi of him-1 in the him-1 candidate mutant background abolishes suppression (data not shown).

These data suggest that these alleles do not merely suppress separase by strict loss of cohesin function, but might affect interactions between separase and cohesin.

Discussion and Future Directions

In conclusion, we have identified potential suppressing mutations in three cohesin genes, and have verified one of them. Based on our preliminary evidence, we also suggest that the mechanism of suppression for these mutants is not simple loss of function, but through another mechanism such as affecting the interaction between separase and cohesin or behaving as hypomorphic alleles. Future efforts will begin to elucidate these possibilities and characterize the mechaism of suppression for all known suppressors. Of particular interest for us will be understanding how these mutants affect chromosome segregation and whether they might also affect vesicle trafficking.

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Thank you for your time.