Genetic Suppressor Screen of Separase Mutants Identifies Cohesin Subunits

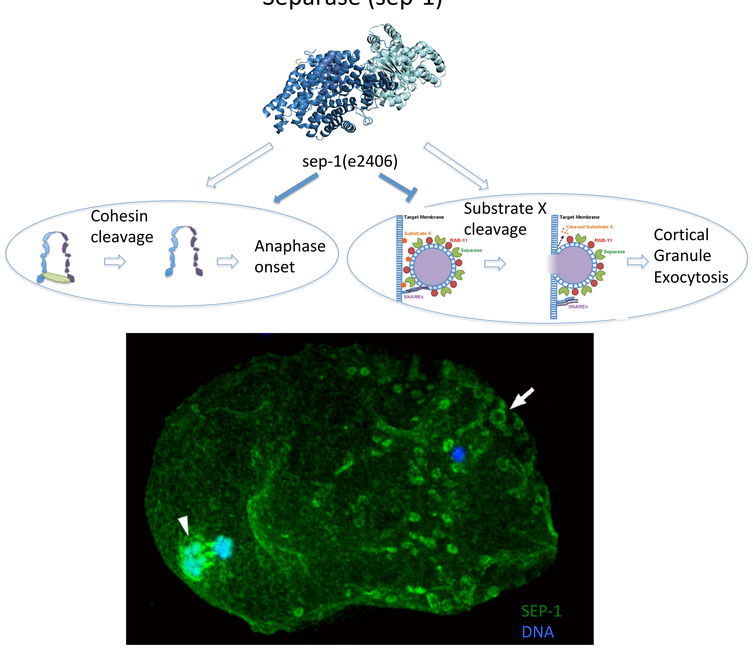
Michael Melesse[2], Dillon E. Sloan[1], Amy Fabritius[3], Harold Smith[3], Andy Golden[3], Györgyi Csankovszki[1], and Joshua N. Bembenek[1]

[1]MCDB, University of Michigan, Ann Arbor, MI; [2]BCMB, University of Tennessee, Knoxville; [3]NIDDK, NIH, Bethesda, MD

Abstract

Separase is a well-conserved protease best known for its function in promoting anaphase onset by cleaving cohesin. However, other roles for separase have been implicated in cytokinesis and vesicular trafficking in different model organisms. In *C. elegans*, it was demonstrated that separase has a role in the formation of the eggshell during cortical granule exocytosis just after anaphase onset of meiosis I, a role independent of chromosome segregation which requires its proteolytic activity. To elucidate the mechanism of separase activity during cortical granule exocytosis, we conducted an ENU mutagenesis screen for suppressors of a temperature sensitive, partial separation-of-function allele of separase that covered nearly a million haploid *C. elegans* genomes. At the restrictive temperature, this allele has minimal issues in chromosome segregation but fails to localize to or exocytose cortical granules. In our screen, we identified 68 suppressor mutations of this allele in 7 different genes, including 14 intragenic suppressors, 47 mutations in *pph-5*, and 7 mutations in previously unidentified genes including *hsp-90* (which regulates *pph-5*), and 3 cohesin genes not directly cleaved by separase. Interestingly, while the mutations in these cohesin genes suppress the lethality associated with this allele, RNAi depletion at varying levels does not. This may indicate that the mechanism of suppression is not simply due to a loss of cohesin function. Currently, our work is aimed at varifying these suppressors by CRISPR and investigating the mechanism of suppression by observing the cellular phenotypes of these cohesin mutants.

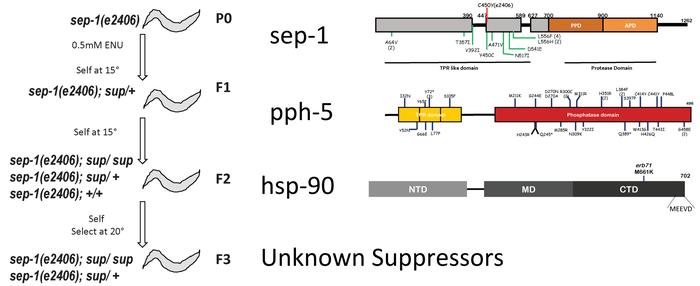
Introduction



Top − Separase cleaves cohesin to promote chromosome segregation and cleaves an unknown target to promote exocytosis. Note that the *sep-1(e2406)* allele, an N-terminal point mutant outside the C-terminal catalytic domain, has been demonstrated to be a partial separation of function allele, which affects cortical granule exocytosis more severely than chromosome segregation. (Separase Structure: Boland et al., 2017)

Bottom − Immunofluoresent image of a *C. elegans* embryo in mid-anaphase I of meiosis. Green is a separase antibody, blue is DAPI-stained DNA. Note SEP-1 localization at cortical granules (arrow), and chromsomes (arrowhead).

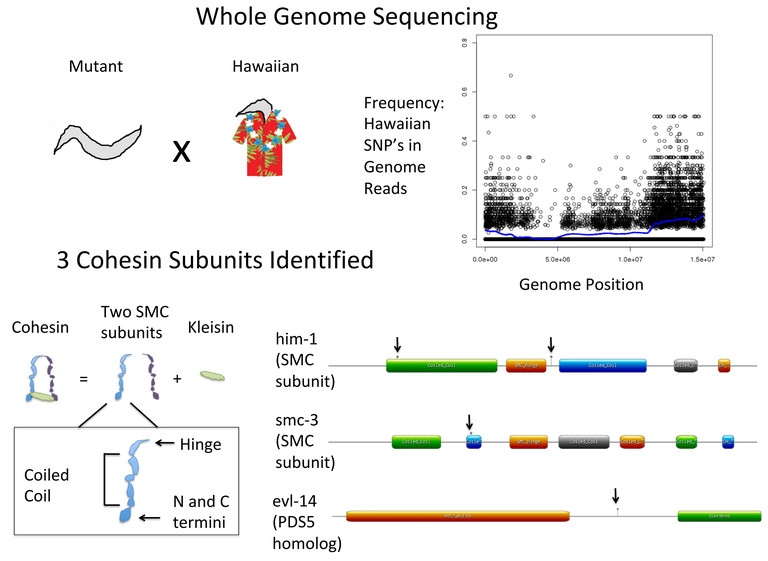
Identifying Suppressors of sep-1(e2406)



Left− Diagram depicting the isolation of suppressors for sep-1(e2406). Hermaphrodites were treated with a chemical mutagen and allowed to self for two generations at the permissive temperature. Worms were then shifted to the restrictive temperature, and suppressor lines were isolated.

Right − Suppressor lines were lysed and PCR'd to identify mutations in sep-1, pph-5, and hsp-90. We isolated 14 intragenic sep-1 suppressors, 47 pph-5 suppressors, 1 hsp-90 suppressor, and 6 suppressors that did not show mutations in sep-1, pph-5, or hsp-90. (Melesse et al., 2018)

Identifying Suppressors in Cohesin



Top Panel - Unknown suppressors were crossed for multiple generations with an alternate ancestral strain (Hawaiian) containing a known SNP profile. Suppressed cross progeny were sequenced to create an SNP profile. Regions with fewer percent Hawaiian reads indicate recombinant regions containing unique SNPs.

Bottom Panel - These regions were analyzed for each suppressor strain, and found to include four mutations in three cohesin genes, including two SMC subunits (him-1 and smc-3) and a PDS5 homolog (evl-14).

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

	Hatching (%)			
	15°C		20°C	
RNAi	**N2**	***sep-1(e2406)***	**N2**	***sep-1(e2406)***
Empty Vector	96.9 (n=96)	81.6 (n=38)	91.3 (n=321)	7.0 (n=57)
him-1	95.8 (n=84)	92.6 (n=27)	96.7 (n=120)	1.1 (n=89)
him-1 (Diluted)	83.1 (n=72)	56.1 (n=57)	94.7 (n=152)	0.0 (n=111)
evl-14	92.6 (n=68)	71 .0 (n=31)	93.0 (n=158)	0.0 (n=5)
evl-14 (Diluted)	92.3 (n=78)	87.8 (n=41)	92.4 (n=158)	0.0 (n=5)
scc-1	94.5 (n=73)	82.9 (n=35)	94.3 (n=123)	0.0 (n=0)
scc-1 (Diluted)	95.8 (n=71)	89.5 (n=38)	61.4 (n=272)	0.0 (n=1)
Mutants				
him-1 mutant1 (ENU)	-	-	-	29 (n=1977)
him-1 mutant1 (CRISPR)	-	22 (n=194)	-	75 (n=640)
him-1 mutant2 (ENU)				46 (n=1691)
smc-3 mutant (ENU)				37 (n=2397)

Hatching data. The top half shows hatching data for RNAi-treated worms, and the bottom half shows hatching data in non-RNAi treated mutant lines.. Diluted RNAi cultures were diluted 1:1 with the control empty vector. Note that at the restrictive temperature, hatching is not recovered in RNAi treated worms, but in the mutant lines.

Discussion and Future Directions

- We identified suppressors of a temperature sensitive separase in 3 cohesin genes.
- The mechanism of suppression for these cohesin mutants may not be explained by strict loss of function.
- Do the cohesin mutants rescue chromosome segregation defects?
- Do the cohesin mutants have any effect on cortical granule exocytosis defects?

Acknowledgements

Many thanks to members of the Bembenek, Golden, and Csankovszki labs for providing helpful feedback and resources.

Also thanks to our funding source, the NIH.