Elucidating the Role of Securin in Regulating Separase during Cortical Granule Exocytosis

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Abstract

Meiosis is a tightly regulated series of events leading to the production of haploid gametes. A key player in this process is the cysteine protease separase (SEP-1). Known for its canonical role in chromosome segregation, recent studies suggest that SEP-1 has an additional function in vesicular trafficking during cell division. We hypothesize that cell cycle machinery known to control SEP-1 protease activity for chromosome segregation also controls its localization to the cortex and subsequent exocytic activity. To ensure proper activity of SEP-1, the spindle assembly checkpoint (SAC) monitors for proper spindle attachment to kinetochores and chromosome alignment during the meiotic M phase. After proper attachment and alignment are established, the anaphase promoting complex/cyclosome (APC/C) is activated, resulting in the degradation of SEP-1 inhibitory chaperone securin (IFY-1) and entry into anaphase I. In recent studies, we have observed that SEP-1 localizes to specialized vesicles called cortical granules and regulates their exocytosis during anaphase I. Cortical granule exocytosis is necessary for the process of eggshell formation. Before it appears on cortical granules, SEP-1 localizes to cytosolic filaments near the plasma membrane. We have shown that SEP-1 colocalizes with its inhibitor, IFY-1, on filaments during prometaphase I, and both disassociate from these structures after the onset of anaphase I. Kinetochore proteins also associate with these filaments. Inactivating the kinetochore protein CZW-1, the APC/C and IFY-1 differentially affect SEP-1 localization. Interestingly, inhibition of APC/C activity prevents SEP-1 and IFY-1 from leaving the filaments, and depletion of IFY-1 via RNAi causes premature localization of SEP-1 to cortical granules and blocks their exocytosis. Surprisingly, simultaneous inactivation of the APC/C and IFY-1 prevent SEP-1 from localizing to vesicles. These data suggest the hypothesis that degradation of IFY-1 may regulate the timing of SEP-1 localization to vesicles and exocytic activity at vesicles. To address whether IFY-1 degradation is required for SEP-1 activity at vesicles, we generated a non-degradable IFY-1 (IFY-1^{DM}::GFP). Consistent with enhanced IFY-1 stability, IFY-1^{DM}::GFP is not degraded following anaphase I onset, persisting on chromosomes and in the cytoplasm. As expected, expression of IFY-1^{DM}::GFP causes embryonic lethality, chromosome segregation defects, and blocks cortical granule exocytosis. IFY-1^{DM}::GFP localizes to filaments normally and, in contrast to wild-type, localizes to vesicles with SEP-1 during anaphase I. In the future we will further investigate how key regulatory components of the cell cycle differentially affect SEP-1 localization and function to promote cortical granule exocytosis. Furthermore, we will investigate the IFY-1 degradation-independent mechanism by which APC/C promotes SEP-1 transfer to cortical granules during meiosis I.

Separase Regulation and Dynamics During Meiosis I

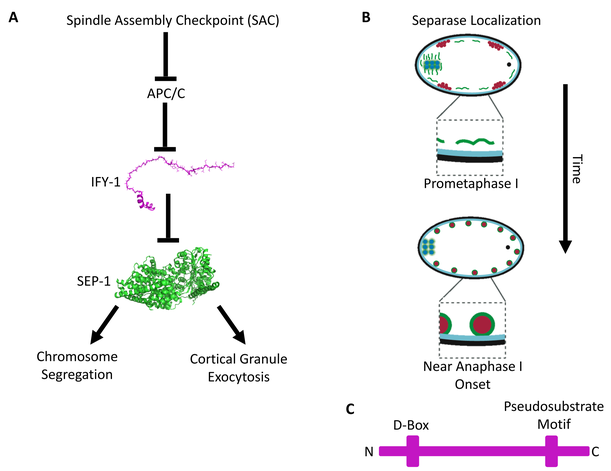
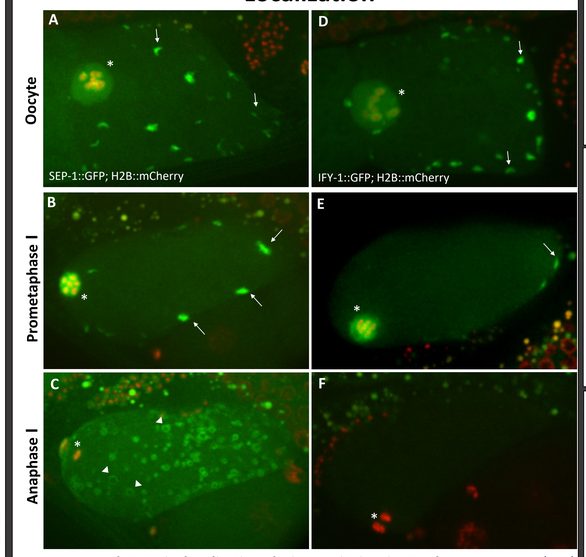


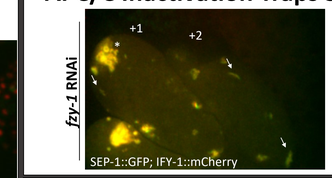
Diagram of Separase regulation and subcellular localization. (**A**) The SAC inhibits the APC/C until proper chromosome alignment in metaphase I. At anaphase I onset, the APC/C degrades IFY-1, which liberates SEP-1 to promote chromosome segregation and cortical granule exocytosis. (**B**) During prometaphase I, SEP-1 (green) localizes to chromosomes and cortical filaments. Shortly before anaphase I onset, SEP-1 localizes to cortical granules (maroon). (**C**) Schematic of IFY-1. IFY-1 has a pseudosubstrate motif which binds SEP-1 protease domain. The APC/C recognizes IFY-1 D-box in order to polyubiquinate IFY-1, targeting it for degradation. **Whether the regulatory pathway outlined in (A) controls SEP-1 localization to and localization at vesicles (B) has not been investigated.**

Securin Is Degraded Before Separase Vesicle Localization



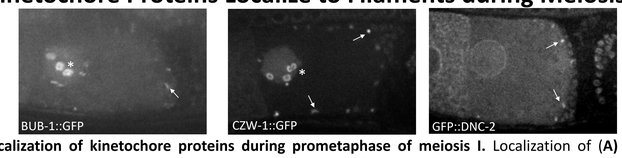
Separase and Securin localization during meiosis I in *C. elegans* oocyte. (**A-C**) SEP-1::GFP and (green) or (**D-F**) IFY-1::GFP (green) localization with H2B::mCherry (red). Both SEP-1 and IFY-1 localize to chromosomes (asterisks) and cortical filaments (arrows) before anaphase I. During anaphase I IFY-1 is degraded and SEP-1 is localized on cortical granules (arrowheads).

APC/C Inactivation Traps Separase & Securin on Filaments



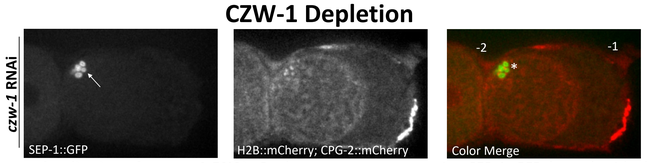
Separase and Securin localization after *fzy-1* RNAi. SEP-1::GFP (green) and IFY-1::mCherry (red) remain localized on chromosomes (asterisk), spindle (caret), and cortical filaments (arrows). This suggest that APC/C activity is required for SEP-1 to localize to cortical granules. Number indicates relative age of embryo.

Kinetochore Proteins Localize to Filaments during Meiosis I



Localization of kinetochore proteins during prometaphase of meiosis I. Localization of (**A**) BUB-1::GFP, (**B**) CZW-1::GFP, and (**C**) GFP::DNC-2. Arrows indicate localization to filaments (arrows) and chromosome localization denoted by asterisks. Numerous kinetochore proteins have been reported to localize to cortical filaments: KNL-1, HIM-10, and NDC-80.

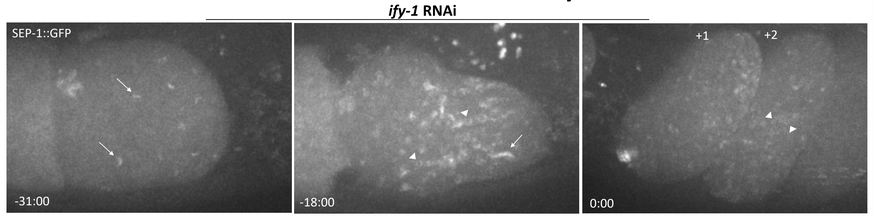
Separase Does not Localize to Filaments or Vesicles after CZW-1 Depletion



Separase localization after *czw-1* RNAi. *C. elegans* oocytes expressing SEP-1::GFP (green) with the cortical granule marker CPG-2::mCherry and H2B::mCherry (red) during prometaphase I. Separase is able to localize to chromosomes (asterisk) but is not observed on filaments or vesicles. Number indicates relative age of embryo.

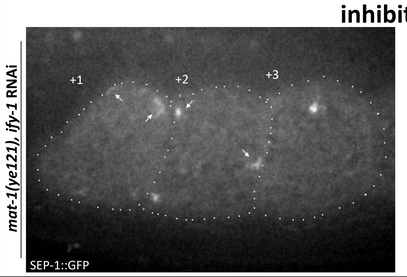
Securin Depletion Causes Precocious Separase Localization to Cortical Granules and Blocks Their Exocytosis

ify-1 RNAi



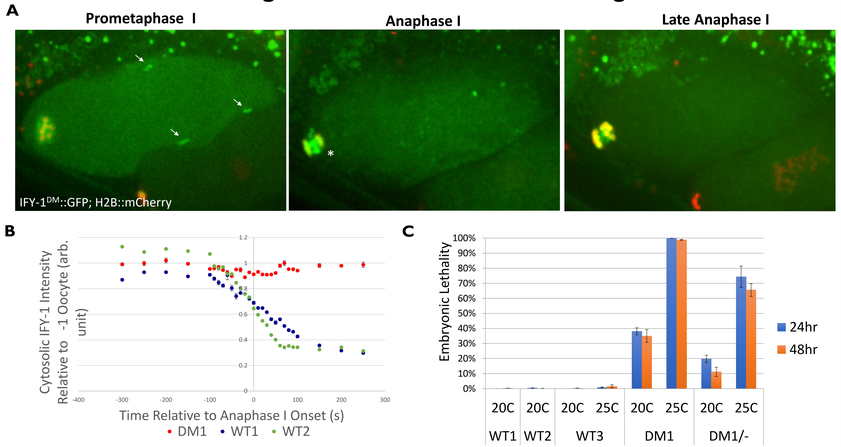
Time series images of *C. elegans* oocytes during meiosis I expressing SEP-1::GFP and depleted of *ify-1* by RNAi. SEP-1 localizes to filaments (arrow, -31 minutes), but prematurely associates with cortical granules (arrowhead, -18 minutes) during ovulation. After premature SEP-1 localization to cortical granules, exocytosis does not occur and separase remains on vesicles for an extended period of time. Time is relative to anaphase I onset (minutes:seconds). Number indicates relative age of embryo.

Securin Depletion Does Not Allow Separase Vesicle Localization in APC/C-inhibited Embryos



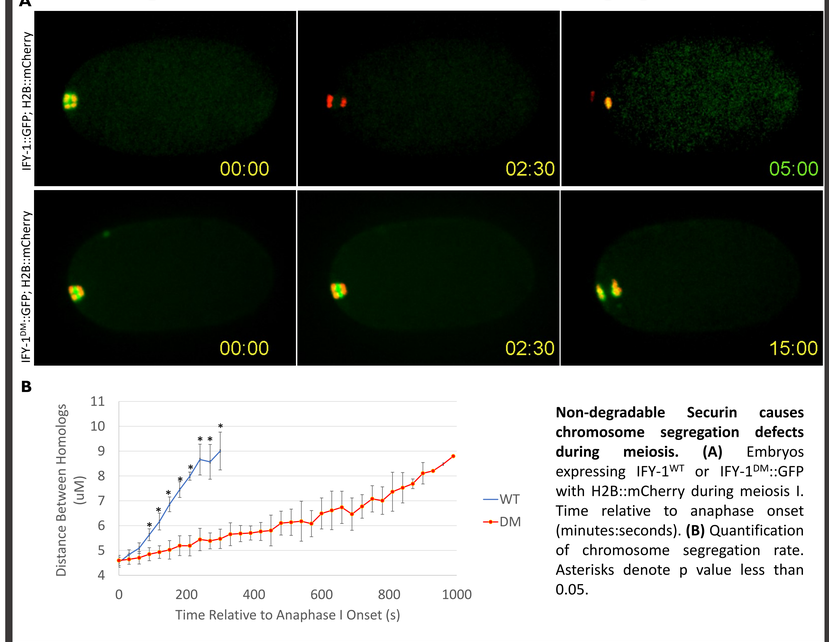
Separase localization following depletion of Securin and inhibition of APC/C. *C. elegans* embryos expressing SEP-1::GFP in a temperature-sensitive APC/C mutant, *mat-1(ye121),* depleted of *ify-1* by RNAi. SEP-1 remains on filaments (arrows) and is not observed on vesicles, suggesting that IFY-1 degradation is not sufficient to allow SEP-1 vesicle localization when APC/C is inhibited. Number indicates relative age of embryo.

Non-degradable Securin Is Stable during Meiosis I



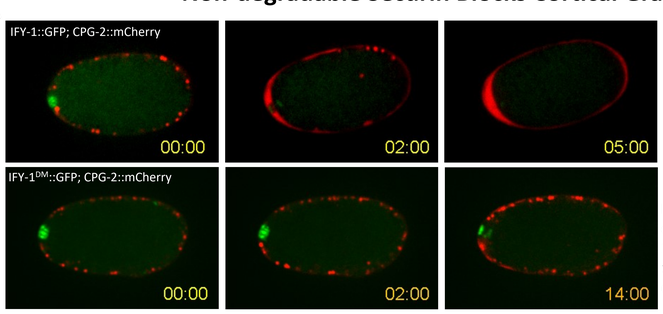
Non-degradable Securin is stably expressed throughout meiosis I and causes embryonic lethality. (**A**) Embryos expressing IFY-1^{DM}::GFP and H2B::mCherry during meiosis I. During prometaphase I, IFY-1^{DM}::GFP temporarily associates with filaments (arrows) until anaphase I, where it also remains on chromosomes (asterisk). (**B**) GFP intensity through anaphase I of IFY-1^{DM}::GFP and IFY-1^{WT}::GFP lines. (**C**) Lethality assays of IFY-1^{WT}::GFP lines compared to homozygous and heterozygous IFY-1^{DM}::GFP lines.

Non-degradable Securin Causes Chromosome Segregation Defects



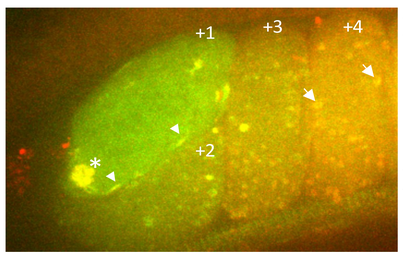
Non-degradable Securin causes chromosome segregation defects during meiosis. (A) Embryos expressing IFY-1^{WT} or IFY-1^{DM}::GFP with H2B::mCherry during meiosis I. Time relative to anaphase onset (minutes:seconds). **(B)** Quantification of chromosome segregation rate. Asterisks denote p value less than 0.05.

Non-degradable Securin Blocks Cortical Granule Exocytosis



Non-degradable Securin causes defects in cortical granule exocytosis in meiosis I. (A) Embryos expressing IFY-1^{WT} or IFY-1^{DM}::GFP with CPG-2::mCherry during meiosis I. Time relative to anaphase onset (minutes:seconds). **(B)** Quantification of exocytosis rate. Asterisks denote p value less than 0.05.

Non-degradable Securin Ectopically Localizes to Vesicles and Blocks Exocytosis



Separase localization in early embryos expressing IFY-1(DM)::GFP. Embryos expressing IFY-1^{DM}::GFP and SEP-1::mCherry. During prometaphase I (+1 embryo), IFY-1^{DM}::GFP and SEP-1 colocalize on filaments (arrows) and chromosomes (asterisks). In older embryos, IFY-1^{DM}::GFP localizes to vesicles (arrowheads) abnormally together with SEP-1, which are not exocytosed. Number indicates relative age of embryo.

Conclusions

- **Kinetochore proteins, Securin, and Separase localize to cortical filaments during prometaphase I. These filaments may be a signaling platform for Separase localization.**
- **The dynamic localization of Separase is regulated by the APC/C pathway.**
- **Securin controls timing of Separase localization to and its function at cortical granules.**
- **Non-degradable Securin ectopically localizes to cortical granules and blocks exocytosis.**