Introduction & Background

Hi, I'm Chris Turpin, a PhD candidate at the University of Tennessee, under the guidance of Josh Bembenek. I will be presenting the progress we have made in understanding of how the protease, separase, is regulated during vesicle trafficking that occurs during meiotic anaphase I in the *C. elegans* early embryo.

Meiosis is a tightly regulated series of events that leading to the production of gametes. A key player in this process is separase (SEP-1 in worms). During meiosis I, separase is activated during anaphase I to promote homologous chromosome segregation. Interestingly, recent studies have shown that separase promotes vesicle trafficking during anaphase I.

A well documented signaling pathway ensures that separase becomes catalytically active at the right time to ensure faithful segregation of chromosomes during anaphase I. To ensure proper activity of separase, the spindle assembly checkpoint ensures proper attachment of the meiotic spindle to the kinetochores and proper chromosome alignment. Following proper attachment and alignment, the anaphase promoting complex/cyclosome (APC/C) is activated, resulting in the degradation of separase inhibitory chaperone, securin (IFY-1 in worms).

In recent studies, we have observed separase localize to specialized vesicles called cortical granules and regulates their exocytosis during anaphase I. Cortical granules are necessary for eggshell formation in the early worm embryo. These observations raise exciting, novel questions regarding separase during meiosis I: The first being how is separase protease activity regulated during cortical granule exocytosis?; and second how is the localization of separase during meiosis regulated.

We hypothesize that the cell cycle machinery known to regulate separase protease activity during chromosome segregation control its localization to and subsequent catalytic activity at cortical granules during anaphase I.

Securin Is Degraded Before Separase Vesicle Localization

To test this hypothesis, we conducted live cell imaging of embryos expressing fluorescently labeled separase::GFP or securin::GFP with chromosome marker H2B::mCherry.

Interestingly, prior to localizing to cortical granules separase localizes to cytoplasmic filaments near the plasma membrane and spindle, which we call cortical filaments. We observed securin also localizes to cortical filaments. And we noticed that securin is degraded by the time that separase localizes to cortical granules.

Given that securin is a key protein targeted for destruction by the APC/C to allow for separase to become activated during chromosome segregation, we hypothesized that APC/C promotes degradation of securin to allow separase to localize and function on cortical granules.

APC/C Inactivation Traps Separase & Securin on Filaments

To test this hypothesis, we used RNAi to knockdown the APC/C activator Cdc20 (*fzy-1* in worms) in worms expressing separase::GFP and securin::mCherry. Under these conditions, embryos remain arrested in a metaphase-I-like state and never enter anaphase I.

We observed that separase and securin remain trapped on cortical filaments, in addition to being trapped on chromosomes. This suggests that APC/C activity is required for separase to localize to cortical granules.

Kinetochore Proteins Localize to Filaments

Surprisingly, numerous kinetochore proteins, including members of the spindle assembly checkpoint, localize to the cortical filaments with separase and securin, but their function here has not been carefully assessed.

We were able to observe BUB-1:GFP, CZW-1::GFP (which is a member of the RZZ complex), and DNC-2::GFP on these filaments prior to anaphase I.

These observations indicate that the spindle assembly checkpoint and kinetochore proteins may function with the APC/C to regulate separase localization and activity during meiosis I.

To test this hypothesis, we analyzed SEP-1::GFP localization following RNAi of components of the SAC-APC/C pathway.

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

We knocked down the RZZ component CZW-1 by RNAi in worms expressing separase::GFP together with H2B::mCherry, and a cortical granule cargo marker CPG-2::mCherry.

Under this condition separase was able to localize to chromosomes, but we never saw separase localize to filaments or vesicles. Therefore, CZW-1 is required for separase to localize to cortical filaments. In the future we will determine whether filament localization regulates separase transfer onto vesicles.

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

A primary target of the APC/C is securin. Previous studies show that securin-depletion prevents cortical granule exocytosis.

We imaged separase::GFP following secruin RNAi. We observed separase prematurely localizing to cortical granules. These vesicles were not exocytosed, which is in agreement with previously published data.

This supports the hypothesis that securin degradation is required for separase localization to vesicles.

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

Our hypothesis predicts that we would be able to rescue separase localization from filaments to vesicles in our APC/C-inhibited embryos by knocking down securin.

To test this, we analyzed separase::GFP localization following simultaneous inhibition the APC/C and depleted securin. Unexpectedly, separase remains on cortical filaments.

This suggests the APC/C is required for separase to localize to cortical granules, independent of its targeting of securin for destruction.

Non-degradable Securin Is Stable during Meiosis I

To further test our hypothesis**,** we generated a non-degrdable securin::GFP (ify-1(dm)::GFP) and expressed it in embryos with active APC/C. This will allow us to test if APC/C targets a substrate besides securin to allow vesicle localization and to determine how non-degradable securin affects vesicle exocytosis. To do this, we mutated conserved residues in securin's destruction motif (d-box), which prevent its recognition by the APC/C.

This transgene was predicted to be dominant negative. As expected, we observed embryonic lethality. Our lab has developed special techniques to control toxic transgene expression.

We expressed non-degradable securin::gfp with H2B::mcherry. During meiosis I non-degradable securin::gfp localizes to chromosomes and filaments. In contrast to wild-type, our mutant signal remains stable in the cytoplasm during anaphase I.

Non-degradable Securin Causes Chromosome Segregation Defects

In other systems, non-degradable securin causes chromosome segregation defects. Non-degradable securin should inhibit separase protease activity, preventing cohesin cleavage and causing defects in chromosome segregation.

To test this, we imaged wild-type or non-degradable securin::GFP with H2B::mCherry through anaphase I. Consistent with our prediction, we noticed a significant delay in chromosome segregation comparted to wild-type. It also took longer for non degradable securin expressing embryos to finish anaphase I.

Non-degradable Securin Blocks Cortical Granule Exocytosis

To determine if non-degradable securin affects cortical granule exocytosis, we imaged wild-type or non-degradable securin::gfp with the cortical granule cargo marker CPG-2::mCherry through anaphase I.

Given that separase protease activity is required for cortical granule exocytosis, we predicted that non-degradable securin would also block exocytosis. In contrast to wild-type, non-degradable securin blocks cortical granule exocytosis, and the number of vesicles remain constant over time in the embryo.

Non-degradable Securin Ectopically Localizes to Vesicles and Blocks Exocytosis

If non-degradable securin blocks separase protease activity, we would expect vesicle colocalization.

Separase and non-degradable securin colocalize at chromosomes and on cortical filaments in prometaphase I (embryo 1). As predicted non-degradable securin and separase colocalize on vesicles in older embryos (+2and beyond), and their exocytosis is blocked. This indicates that securin inhibits separase activity on vesicles preventing exocytosis. It also indicates that non-degradable securin does not prevent separase from localizing to vesicles.

Conclusion

In summary, separase, securin, and kinetochore proteins localize to cortical filaments during prometaphase I. We suggest these cortical filaments may serve as a signaling hub for separase localization.

The localization of separase to vesicles is regulated by the SAC-APC/C pathway.

Securin controls the timing of separase localization to and function at cortical granules.

Non-degradable securin ectopically localizes with separase on vesicles and blocks their exocytosis.

Our data suggest that another mechanism promotes vesicle localization independent of securin degradation, downstream of APC/C.