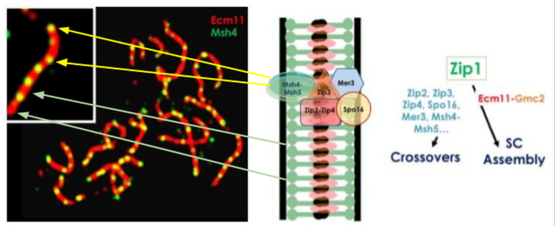
Investigating meiotic protein-protein interactions of the Zip1 synaptonemal complex transverse filament protein via insertion of TurboID biotinylase

Bernard S. R. Chedekel, Amy J. MacQueen
Wesleyan University

Zip1 plays roles in both homologous recombination and synaptonemal complex (SC) formation in meiosis

- Meiosis is an intricate and essential cell division process that allows sexually reproducing organisms to create gametes with half as many chromosomes as the parent, which is required for these organisms to produce viable offspring.
- Chromosome mis-segregation leads to aneuploidy, in which gamete nuclei contain an improper number of chromosomes; aneuploid gametes typically produce inviable offspring.
- The Zip1 coiled-coil protein in *Saccharomyces cerevisiae* (budding yeast) is necessary for assembly of the synaptonemal complex (SC) which bridges between homologous chromosomes in prophase I of meiosis and is unique among SC component proteins in that it is also required for mutS-gamma-mediated homologous recombination of genetic material between chromosomes, which has been shown to prevent chromosome mis-segregation and aneuploidy.[1]
- However, the specific mechanisms by which Zip1 regulates and mediates meiotic chromosome assortment remain largely unclear. Recent developments in proximity labeling techniques hold promise as a means of better understanding the direct interactions of proteins such as Zip1.

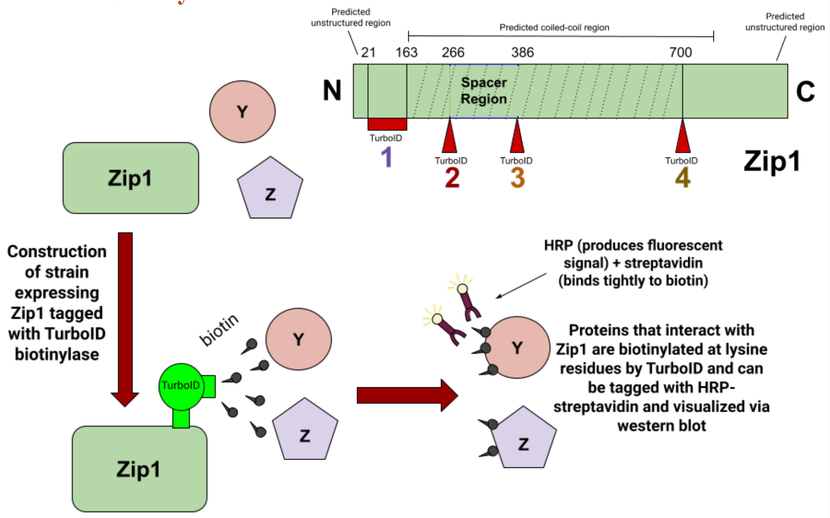
Recombination and SC-associated proteins localize near each other along chromosome pairs, but the mechanism of their interaction is unknown



Question: Which proteins does Zip1 interact with?

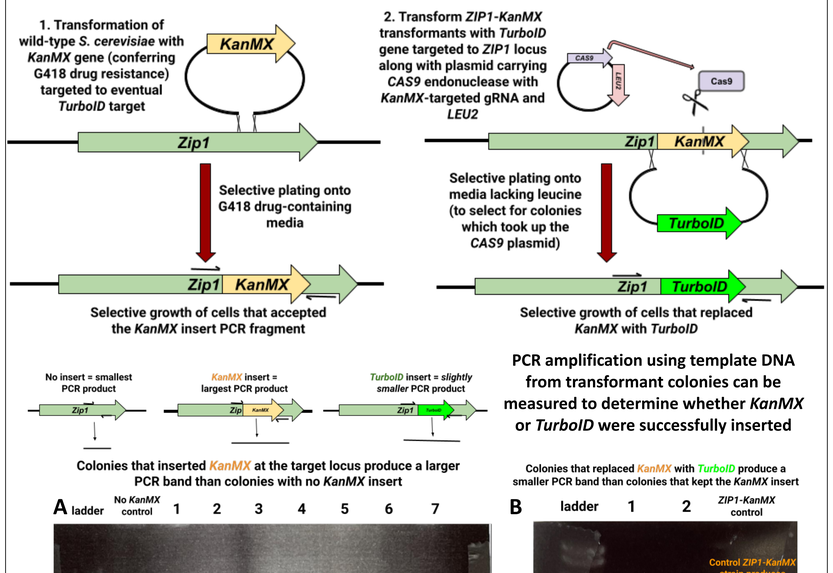
Zip1 proteins naturally assembly into homodimers and are believed to form the transverse filaments of the zipper-like synaptonemal complex (SC) which assembles between homologous chromosomes in meiosis I. Zip1 has been demonstrated to play regulatory roles in both SC formation and homologous recombination; however, the direct protein-protein interactions of Zip1 with proteins involved in SC assembly and homologous recombination have not been experimentally confirmed.

Approach: proximity labeling of protein-interacting partners using TurboID biotinylase



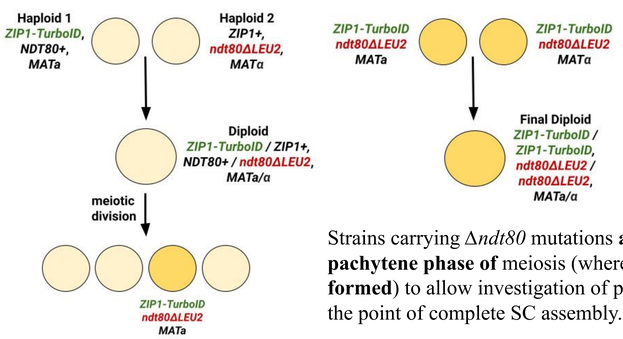
Using CRISPR-Cas9 to create strains expressing custom Zip1-TurboID fusion proteins

Insertion of the *TurboID* biotinylase gene into the Zip1 open reading frame



Colony PCR identification of successful *ZIP1-KanMX* and *ZIP1-TurboID* transformants. Agarose gels of colony PCR products amplified off potential transformant colonies that inserted *KanMX* at Zip1 amino acid 700 (gel A) or replaced *KanMX* with *TurboID* after the Zip1 spacer region (gel B). Both gels also contain PCR products amplified at the same genetic locus on untransformed colonies, which are expected to produce band sizes corresponding to the size of unsuccessful transformants that did not insert the PCR product. Gel A identified two PCR fragments with a size of approximately 1.7 kilobases, which correspond to colonies with a successful *KanMX* insertion at amino acid 700 on Zip1. Gel B identified two PCR fragments with a size of approximately 1.8 kilobases, which are the expected fragment size of PCR products amplified off successful Zip1-TurboID transformants, while the Zip1-*KanMX* control produced a fragment of the size expected of unsuccessful Zip1-TurboID transformants that still contain *KanMX*.

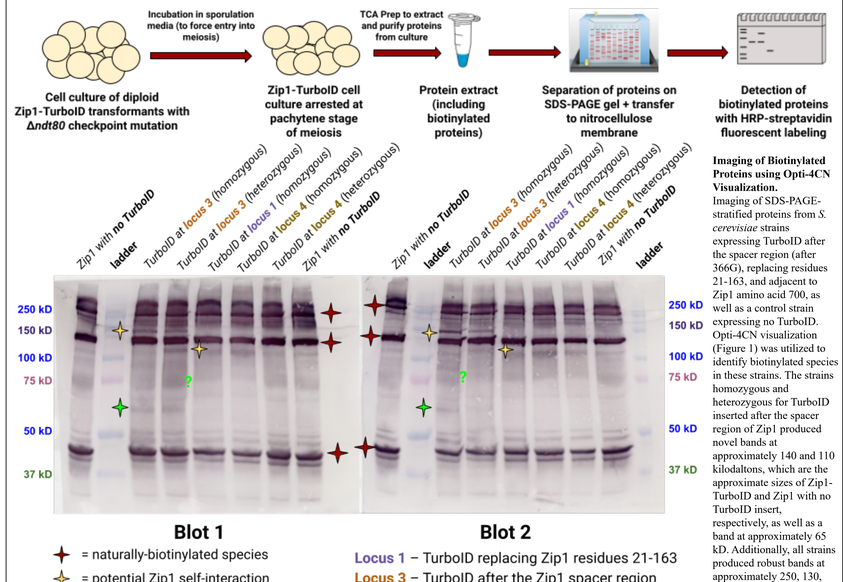
Haploid Zip1-TurboID transformants are used to construct diploid *ZIP1-TurboID Δndt80* western blot strains



Strains carrying Δ*ndt80* mutations **arrest at the pachytene phase of** meiosis (where **SC is fully formed**) to allow investigation of protein activity at the point of complete SC assembly.

Western blotting of Zip1-TurboID strains allows identification of Zip1-interacting proteins

Extraction of proteins from cell cultures and stratification on a gel



Imaging of Biotinylated Proteins using Opti-4CN Visualization. Imaging of SDS-PAGE-stratified proteins from *S. cerevisiae* strains expressing TurboID after the spacer region (after 366G), replacing residues 21-163, and adjacent to Zip1 amino acid 700, as well as a control strain expressing no TurboID. Opti-4CN visualization (Figure 1) was utilized to identify biotinylated species in these strains. The strains homozygous and heterozygous for TurboID inserted after the spacer region of Zip1 produced novel bands at approximately 140 and 110 kilodaltons, which are the approximate sizes of Zip1-TurboID and Zip1 with no TurboID insert, respectively, as well as a band at approximately 65 kD. Additionally, all strains produced robust bands at approximately 250, 130, and 40 kD which likely correspond to naturally-biotinylated proteins in a wild-type *S. cerevisiae* cell.

Key Observations

- All strains express robustly-biotinylated proteins at approximately **250**, **130**, and **40 kDa**, which are likely naturally-biotinylated species.
- Strains expressing Zip1-TurboID after the spacer region (locus 3) also produce bands at approximately **140** and **110 kDa**, which are the sizes of Zip1-TurboID and Zip1 with no TurboID.
- Additionally, this strain produces a **new, unknown band** at approximately **65 kDa**, which may be an **interacting partner of Zip1**.
- Note that a lack of exposed lysine residues or a brief period of interaction can cause a Zip1-protein interaction to occur but *not be accounted for* in a western blot analysis.

Conclusions

- TurboID insertion after the spacer region of Zip1 may hold promise for future investigation of Zip1 protein-protein interaction.
- Zip1 may interact with a protein **~65 kilodaltons in size**, which is the band size of meiotic proteins **Fpr3** and **Pch2**. Fpr3 is required for formation of Zip1 polycomplexes (conglomerations of meiotic proteins in cells unable to construct a stable SC),[2] and Pch2 is believed to serve as a checkpoint protein that prevents SC assembly in cells lacking Zip1.[3]
- The spacer region of Zip1, which is part of the Zip1 structured alpha-helical region, may contact its partner Zip1 protein in the homodimer that forms a transverse filament of the SC. Zip1 lacking residues 21-163 cannot assemble a full SC, which may explain the lack of Zip1 and Zip1-TurboID bands in the strain with Zip1-TurboID replacing residues 21-163.

Next Steps

- Repeat the western blot in strains carrying **tagged or removed Fpr3/Pch2** genes to determine whether the 65 kDa band corresponds to one of these proteins.
- Use **anti-Zip1 fluorescent labeling** to confirm that the 110 and 140 kDa bands are Zip1.
- Use fluorescent labeling to image the localization of biotinylated proteins on the chromosome.
- Continue to **construct new strains** expressing TurboID at more loci to gain a more complete understanding of Zip1 interactions with meiotic proteins.

References

1. MacQueen, Amy, et al. "Crossover Recombination AND Synapsis Are Linked by Adjacent Regions within the N Terminus of the ZIP1 Synaptonemal Complex Protein." *PLOS Genetics*, vol. 15, no. 6, 2019, doi:10.1371/journal.pgen.1008201.
2. MacQueen, Amy J, and G Shirleen Roeder. "Fpr3 and Zip3 ensure that initiation of meiotic recombination precedes chromosome synapsis in budding yeast." *Current biology : CB* vol. 19,18 (2009): 1519-26. doi:10.1016/j.cub.2009.08.048
3. Mukherjee, P. (2014). Regulation of the Synaptonemal Complex Protein, Zip1, During Meiosis in Budding Yeast.
4. "Synaptonemal Complex." *Wikipedia*, Wikimedia Foundation, 18 Apr. 2021, en.wikipedia.org/wiki/Synaptonemal_complex.

Acknowledgements

Special thanks to Dr. Amy MacQueen, Karen Voelkel-Meiman, Chandni Ravindan, and Alex Poppel for all your help, guidance, and support during this project.