Quantitative protein analysis to map inner kinetochore assembly

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Introduction

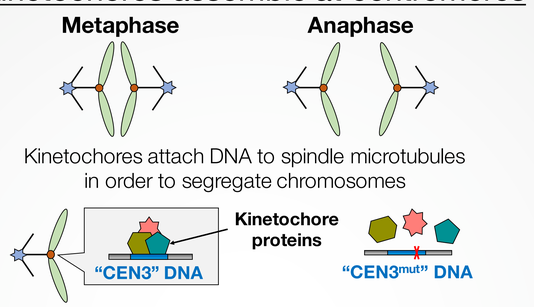
A dividing cell must ensure that its sister chromatids properly segregate into its daughter cells to avoid aneuploidy. A key component in mediating chromosomal segregation is the kinetochore, a highly conserved protein complex that physically connects spindle microtubules to the chromosome.

Kinetochore protein recruitment during assembly has remained difficult to study *in vivo*, so we instead use a **cell-free method for kinetochore assembly**. We currently analyze these assemblies by Western blot, but this technique is limited in that it can only identify a small number of proteins out of over sixty kinetochore components.

To overcome these limitations, we have developed a **quantitative mass spectrometry approach**, in which kinetochore proteins assembled *in vitro* are labeled with a molecular tag. Independent kinetochore assemblies can be labeled with unique tags and combined for direct and quantitative comparison by mass spectrometry.

We demonstrate that this technique can not only identify all known kinetochore proteins, but can also quantify the relative amount of each kinetochore protein across multiple assemblies. Furthermore, our new approach has allowed us to investigate subtle effects on kinetochore assembly when performed with various **inner kinetochore** mutants. This novel approach will allow us to gain a more comprehensive understanding of protein-protein interactions during kinetochore assembly and has the potential to reveal novel proteins important in this process.

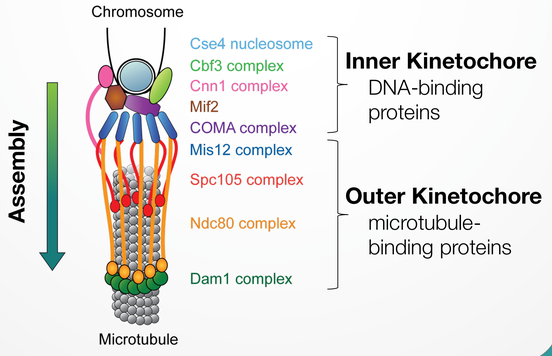
Kinetochores assemble at centromeres



Kinetochores attach DNA to spindle microtubules in order to segregate chromosomes

Mutations in the centromere prevent kinetochore assembly

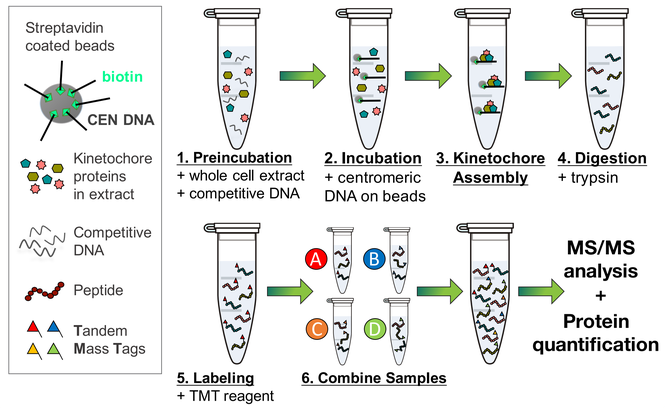
Kinetochore Structure



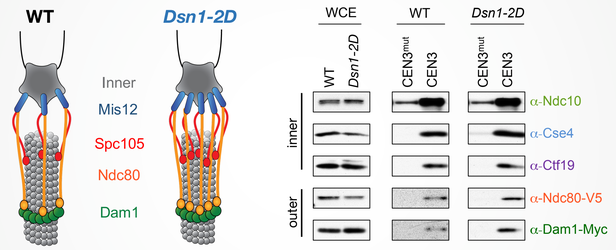
Goals

1. **Develop a quantitative, high-throughput approach to analyze kinetochore assembly.**
2. **Characterize how inner kinetochore proteins interact and recruit each other during assembly.**

Method for kinetochore assembly and quantitative mass spectrometry (MS)

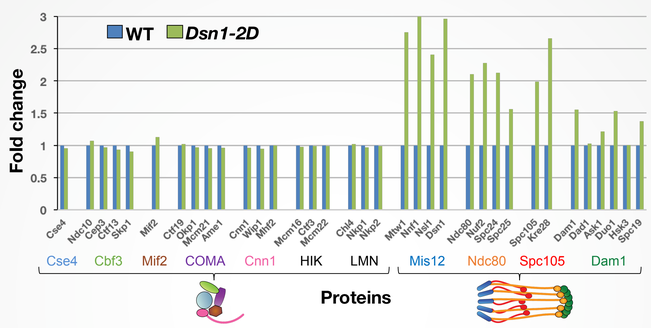


Assay to validate quantitative MS

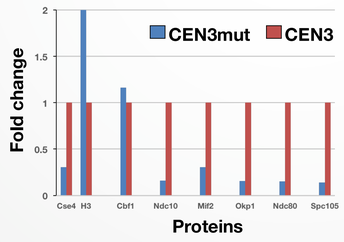


Phosphomimic mutations in **Dsn1** promote the interaction between outer and inner kinetochore proteins

Validating the quantitative MS protocol

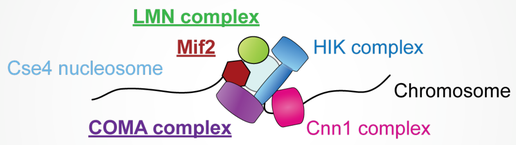


Post-labeling mass spectrometry analysis identifies and quantifies most kinetochore proteins



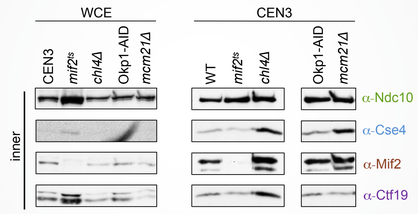
Mutations within the centromere inhibit kinetochore assembly

Inner Kinetochore Assembly



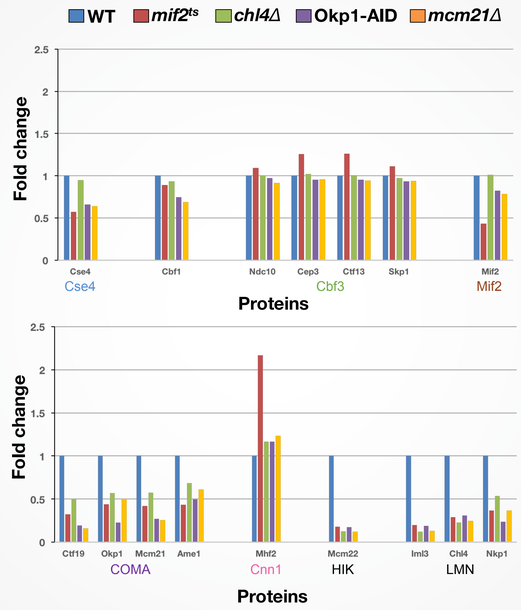
Can *in vitro* assembly in the absence of specific inner kinetochore proteins reveal how these proteins interact?

The effects of inner kinetochore mutants are subtle



Western blot analysis provides limited insight into inner kinetochore interactions

Analyzing the hierarchy of inner kinetochore assembly

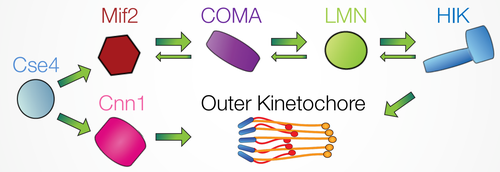


Kinetochore assembly with various inner kinetochore mutant extracts reveals a complex network of interactions

Key Discoveries

1. **Post-labeling mass spectrometry analysis identifies and quantifies virtually all kinetochore proteins.**
2. **Inner kinetochore proteins assemble in a primarily interdependent manner.**

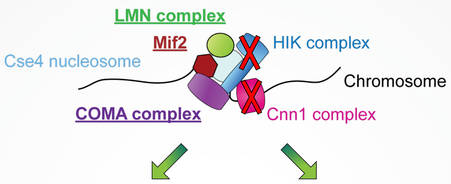
Hierarchical model of inner kinetochore assembly



Mif2 and Cnn1 complexes comprise independent pathways for kinetochore assembly

Future Directions

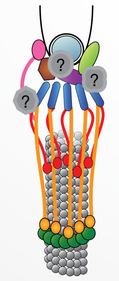
1. Generate additional inner kinetochore mutants



Analyze ***in vitro*** kinetochore assembly

Analyze effects ***in vivo***

2. Identify new kinetochore-associated proteins



Identify and confirm novel proteins enriched on centromeric DNA

Determine how these novel proteins contribute to assembly

Acknowledgements

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