Introduction

The Musacchio lab aims at understanding the molecular components and processes involved in eukaryotic cell division. The distribution of two exact copies of the genome from the mother to the two daughter cells depends on a large number of protein interactions and regulation systems.

For the physical separation of the two sister chromatids, the centromere is a key player. It is a specific chromosomal region that binds both chromatids together and enables kinetochore assembly and microtubule attachment.

In this study we look closer at how centromeric chromatin links to kinetochore assembly and centromere identity. For this, we reconstituted a minimal, simplified kinetochore module containing all known core kinetochore subunits at stoichiometries that resemble those of previously reported intracellular quantifications of entire human kinetochores.
Main Players

CENP-C:
- is the largest CCAN member and provides a blueprint for kinetochore assembly
- contains an N-terminal binding site for the MIS12 complex, two conserved motifs mediating binding to CENP-A nucleosomes, a binding region for the CCAN members CENP-HIKM/-LN and a C-terminal dimerization domain
- is crucial for the assembly of a complete kinetochore

CENP-T:
- forms a link to the outer kinetochore besides CENP-C
- recruits two NDC80 complexes and one KMN network in a CDK1-Cyclin B phosphorylation dependent manner

CENP-A and H3 nucleosomes:
- Nucleosomes are the fundamental subunit of the chromatin
- consist of a segment of DNA that is wrapped around a histone octamer
- CENP-A is a histone H3 variant specifically enriched at the centromere
- CENP-A is recognized by two CCAN members, CENP-N and CENP-C

Aurora B kinase:
- phosphorylates various kinetochore proteins
- enables the binding between the MIS12 complex and CENP-C
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Science Advances, Vol. 7, no. 27, 2021.
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Questions

Can we reconstitute a simplified, but complete kinetochore module that represents the minimal building block of the human kinetochore?

How many nucleosomes are included in such a kinetochore module?

Can we incorporate the outer kinetochore components at the right stoichiometries?

Can we reconstitute full-length CENP-C, a key component for kinetochore assembly, and demonstrate that it is functional?

Which binding sites of CENP-C are crucial for its centromere targeting?

What is the role of CENP-C dimerization?

How is the CENP-T:MIS12 complex interaction regulated?

Can we explain why one MIS12 complex cannot simultaneously bind to CENP-C and CENP-T?
Main methods used

In this study we used:

- **The SpyCatcher/SpyTag system** to obtain a full-length version of human CENP-C by joining together two complementary CENP-C fragments (A).

- **Powerful biophysical methods** like *Analytical Ultracentrifugation* and size-exclusion chromatography to determine the molecular weight and stoichiometries of complexes containing full-length dimeric CENP-C mixed with CENP-A nucleosomes at defined ratios (B).

- **Electroporation** as a tool for the efficient and rapid delivery of recombinant fluorescently tagged proteins into living human cells to test the subcellular localization and the functionality of wild type CENP-C and CENP-T as well as site directed mutants (C).

- **Fluorescence polarization assays** to identify residues of CENP-T that are critical for the interaction with the MIS12 complex (D).

- **Pull-down assays** on amylose resin to reconstitute large megadalton-scale complexes containing all main components of the human kinetochore on solid phase (E).
Results

- We reconstituted a full-length CENP-C and demonstrated that cells with electroporated mCherry-CENP-C were functional at the same level as cells with endogenous CENP-C. (A)

- We discovered that the CENP-HIKM/-LN binding sites are crucial for the centromere recruitment of CENP-C.

- We determined that the dimerization of the Cupin domain stabilizes kinetochore CENP-C, probably through enforcement of multivalent interactions with other CCAN subunits.

- We showed that for the CENP-T:MIS12 complex interaction, CENP-T needs to be phosphorylated by CDK1, and the MIS12 complex needs to be phosphorylated by Aurora B.

- We created a model describing the competitive binding of CENP-C and CENP-T to the MIS12 complex. (B)

For the first time, we reconstituted a complete kinetochore containing all core components incorporated with the expected stoichiometries. Our final model summarizes the obtained results:

The CENP-C dimer binds two CENP-A nucleosomes and two copies of CENP-OPQR (not depicted), CENP-LN and CENP-HIKM, the latter recruits CENP-TWSX which binds two NDC80 complexes and one additional KMN complex (containing KNL1, the MIS12 complex and the NDC80 complex). The CENP-C N-Terminus binds another KMN complex. (C)
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Outlook

By combining biochemical reconstitution and structural biology, we want to gain detailed insights into the organization of centromeric nucleosomes and their interaction with kinetochore proteins.

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