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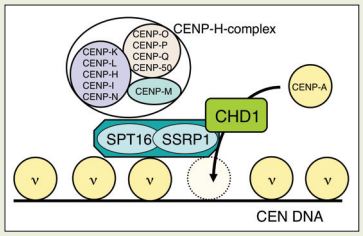


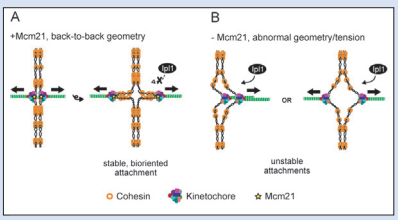
CENP-H–containing Complex Facilitates Centromere Deposition of CENP-A in Cooperation with FACT and CHD1

Masahiro Okada, Katsuya Okawa, Toshiaki Isobe, and Tatsuo Fukagawa

The centromere-specific histone H3 variant CENP-A plays a central role in specifying the locus where the centromere is constructed. Thus defining the mechanisms by which CENP-A is specifically deposited to centromeric chromatin will provide valuable insight into how the centromere is specified. The authors previously identified the CENP-H–containing complex and showed that it is required for targeting nascent CENP-A to centromeres. Here they show that the histone chaperone FACT localizes to centromeres in a manner that is dependent on the CENP-H–containing complex. In knockout cell lines for SSRP1, a subunit of FACT, centromere targeting of newly synthesized CENP-A is severely inhibited. The ATP-dependent chromatin remodeling factor CHD1 also associates with centromeres through direct binding to SSRP1. RNAi knockdown of CHD1 leads to a decrease in the amount of centromere-localized CENP-A. These findings indicate that the CENP-H–containing complex facilitates centromere-specific deposition of newly synthesized CENP-A by recruiting the chromatin-remodeling factors FACT and CHD1 to centromeres.

Pericentromeric Sister Chromatid Cohesion Promotes Kinetochore Biorientation

Tessie M. Ng, William G. Waples, Brigitte D. Lavoie, and Sue Biggins



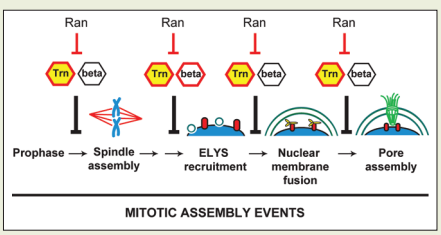
Faithful chromosome segregation requires sister kinetochores to make bioriented attachments to microtubules from opposite poles. An essential regulator of biorientation is the Ipl1/Aurora B kinase, which destabilizes improper microtubule–kinetochore attachments. To identify novel biorientation pathways, the authors isolated mutants sensitive to reduced Ipl1 activity. One of the mutants was in the *MCM21* gene that encodes a kinetochore protein with unknown function. *Mcm21* mutants precociously separate pericentromeres due to a reduction in cohesin binding. The levels of the Scc2 cohesin-loading factor were also reduced at the centromere, providing the first demonstration that the kinetochore recruits Scc2. Furthermore, Mcm21 becomes essential for biorientation when Ipl1 function is reduced. Strikingly, when pericentromeres were artificially tethered, Mcm21 was no longer needed for biorientation despite decreased Ipl1 activity. Taken together, these data are consistent with a specific requirement for cohesin enrichment at pericentromeres to facilitate kinetochore biorientation.

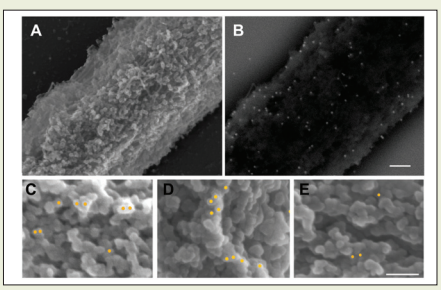
Transportin Regulates Major Mitotic Assembly Events: From Spindle to Nuclear Pore Assembly

Corine K. Lau, Valerie A. Delmar, Rene C. Chan, Quang Phung, Cyril Bernis, Boris Fichtman, Beth A. Rasala, and Douglass J. Forbes

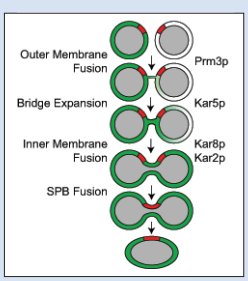
Importin β Regulates the Seeding of Chromatin with Initiation Sites for Nuclear Pore Assembly

Asaf Rotem, Rita Gruber, Hagai Shorer, Lihi Shaulov, Eugenia Klein, and Amnon Harel





Shuttling nuclear transport receptors mediate nucleocytoplasmic traffic through nuclear pore complexes (NPCs). Importin β, the best studied receptor, is also a key regulator of cell cycle events from mitotic spindle assembly to nuclear envelope fusion and NPC assembly. Now, Lau, Delmar *et al.* show that a second import receptor, transportin, regulates the same set of mitotic assembly events, including spindle assembly. Both transportin and importin β are seen to negatively regulate the earliest known step in NPC assembly, the seeding of chromatin with the critical proteins ELYS and the Nup107-160 complex. Indeed, the two import receptors bind directly to the C-terminus of ELYS. Rotem, Gruber, Shorer *et al.* focus on the chromatin seeding step and its regulation by importin β. Importin β is shown to form a high molecular weight complex with both ELYS and Nup107-160, preventing them from binding chromatin. Seeding sites consisting of ELYS and the Nup107-160 complex are formed along the topmost ridges of the chromatin landscape and can be visualized by immunolabeling and high-resolution scanning electron microscopy.



Distinct Roles for Key Karyogamy Proteins during Yeast Nuclear Fusion

Patricia Melloy, Shu Shen, Erin White, and Mark D. Rose

Yeast cells never break down their nuclear envelopes. After cells mate, the two nuclei must move together and fuse (karyogamy) to form the diploid nucleus. Like mitochondria, nuclei are surrounded by two membranes, the inner and outer nuclear envelopes, both of which must fuse correctly. Previously, the authors showed that nuclear envelope fusion occurs in two steps, with outer membrane fusion preceding inner membrane fusion. Using electron tomography and live cell studies, the authors have examined the roles of different karyogamy genes and find that they block at different steps of fusion. Mutation of an outer nuclear envelope protein, Prm3p, blocked prior to initiation of outer membrane fusion. Mutation of an integral membrane protein, Kar5p, blocked dilation of the initial fusion pore. Finally, mutations in the lumenal HSP70 chaperone Kar2p and its co-chaperone Kar8p blocked inner membrane fusion. Thus the proteins mediate different steps in the pathway of nuclear fusion, consistent with their locations. ■