In Brief

Flexible SMC Proteins

Electron microscopy performed by Melby et al. (page 1595) provides new clues as to how structural maintenance of chromosomes (SMC) proteins might help in two enormous tasks: chromosome condensation and sister chromatid cohesion.

The primary structure of the SMCs provides a problem. The two motifs that constitute the putative ATP-binding site are located at opposite ends of the molecule, separated by a long coiled coil, an intervening stretch, and another coiled coil. If the coiled coil is aligned, these motifs could come together in three-dimensional space in one of two ways: the intervening sequence could act as a hinge, allowing the two ends of the molecule to fold back onto each other, or the SMC dimer could form in an antiparallel fashion. Melby et al. find that both of these possibilities occur.

Functional studies of SMCs have focused largely on yeast and *Xenopus* proteins, but Melby et al. look at MukB from *Escherichia coli* and BsSMC from *Bacillus subtilus*. As in a previous study, MukB appeared primarily as a large and small globule separated by a thin rod. In the earlier interpretation, the rod was identified as a single parallel coiled coil. But lighter shadowing and differing sample preparation allow Melby et al. to observe rarer forms of the protein, in which the rod is split in two, or even opened into a V shape. This suggests that the rod is made from two coiled coils, folded back on each other.

A fibronectin domain added to one end of a modified protein appears at both ends of the V-shaped dimer, indicating that each half of the V is an antiparallel coiled coil, ~300 amino acids in length. This far exceeds the previous record for antiparallel coiled coils of 35–45 amino acids. It is unclear how these long, antiparallel coiled coils are stabilized.

The new data suggest a symmetrical structure. "All the previous thinking has been that this is a polar molecule with DNA binding at one end and a motor at the other," says senior author Harold Erickson. "Now both ends have complete and identical functional domains. If this thing is moving along DNA, both ends could be translocating the DNA simultaneously."

Without any proof of motor activity or data on SMC conformation and flexibility in vivo, mechanistic theories are still pure speculation. A *Xenopus* complex that includes SMC proteins can introduce supercoils into DNA; this could be achieved by two SMC-based motors moving along one groove of the DNA in opposite directions, or by SMC arms wrapping DNA around a core particle.

As for sister chromatid cohesion, the extended form of an SMC could reach for up to 100 nm, with an active binding site at each end. "These molecules might act as a bridge," says Erickson. "It might really be making use of the length."

Sorting by a Phosphatase

The furin protease processes a wide variety of substrates—everything from serum proteins, growth factors, cell surface receptors, and extracellular matrix proteins to bacterial toxins and viral coat proteins. Shuttling of furin from the TGN via endosomes to the cell surface and back again allows it to access all these substrates and cleave them in optimal conditions.

On page 1399 and in a recent paper in *Cell* (Wan, L., S.S. Molloy, L. Thomas, G. Liu, Y. Xiang, S.L. Rybak, and G. Thomas. 1998. *Cell.* 94:205–216), Gary Thomas' group shows that phosphorylation of furin directs it in two local cycling loops. Although budding from either the TGN or the cell surface depends on a tyrosine-based motif for recruitment into clathrin-coated pits, retrieval to either the TGN (in the *Cell* paper) or the cell surface (in this issue) requires phosphorylation of an acidic cluster by casein kinase II. Both retrieval events also require phosphofurin acidic cluster sorting protein (PACS-1), which binds the acidic cluster in vitro. Acidic clusters are present on a number of other proteins, including the cation-independent mannose-6-phosphate receptor, which sorts proteins to lysosomes.

The current study shows that an isoform of protein phosphatase 2A is needed for furin to get out of the cell surface–endosome loop and to progress to the TGN. Whether the same or another phosphatase switches furin from the post-TGN–TGN loop towards the cell surface is not yet known.

MAP Kinase at the Kinetochore

Mitogen-activated protein kinase (MAP kinase) is most often associated with signal transduction in response to extracellular signals. Shapiro et al. (page 1533) and Zecevic et al. (page 1547) both show that activated MAP kinase is present at the kinetochore in mitosis. Staining is evident from either prophase or early prometaphase, dropping through metaphase, until it is absent by mid-anaphase. From this common start the results of the two groups diverge into the areas of chromosome movement and checkpoint control.

MAP Kinase and CENP-E

The kinesin motor centromere-binding protein E (CENP-E) is found at kinetochores, has possible MAP kinase phosphorylation sites, and is phosphorylated in mitosis. Zecevic et al. therefore test CENP-E in in vitro reactions and find that the MAP kinase extracellular signal-regulated kinase 2 (ERK2) can phosphorylate CENP-E. (The two classical MAP kinases, ERK1 and ERK2, are not distinguished by the antibodies used for kinetochore localiza-

tion.) ERK2 and CENP-E also coimmunoprecipitate, with activated ERK2 preferentially associating with CENP-E in mitosis, but not in interphase.

One effect of the ERK2 phosphorylations is known because Cdc2 phosphorylates the same residues and reduces microtubule binding to the CENP-E COOH terminus. The NH_2-terminal motor domain can also bind microtubules, but the effect of kinases on this activity has not been investigated.

What all of this means for mitosis is not clear. "Our understanding of how MAP kinase controls mitosis is limited by our knowledge of how CENP-E controls mitosis, both in terms of its interaction with kinetochores and its interaction with microtubules," says senior author Michael Weber. CENP-E is needed for alignment of chromosomes on the metaphase plate. But the effect of expressing mutant versions of CENP-E that lack the MAP kinase phosphorylation sites is not known, so whether MAP kinase regulates chromosome alignment is anyone's guess. The situation is further complicated by uncertainty over the directionality of the CENP-E motor. The native CENP-E exhibits only minus end–directed activity, but this could be due to a different, copurified motor. Alternatively, the plus end–directed recombinant motor domain may lack a crucial domain specifying directionality.

Later in mitosis, CENP-E is likely to lose any MAP kinase phosphorylation and regain microtubule-binding activity at the COOH terminus. By then most CENP-E has left the kinetochore, and its two microtubule-binding sites could potentially bridge the antiparallel microtubules in the midbody of the spindle.

MAP Kinase and 3F3/2

Shapiro et al. note that the temporal pattern of MAP kinase staining is reminiscent of 3F3/2 staining. The 3F3/2 antibody was first raised against thiophosphorylated frog extracts. It stains kinetochores that either have not attached to the spindle or are not experiencing tension from their spindle attachment. Originally this was thought to be part of a signal to move chromosomes closer to the metaphase plate, where a bipolar attachment (and therefore tension) could be established. But when antibody microinjection kept 3F3/2 staining high on both kinetochores, chromosomes still moved normally to the metaphase plate. The cells then arrested in metaphase. The antibody may be sterically blocking anaphase induction or locking on a checkpoint function. The 3F3/2 checkpoint hypothesis states that the appearance of 3F3/2 is a signal telling the cell to stop before dividing because a chromosome is not yet aligned.

Shapiro et al. find that kinetochore staining of activated MAP kinase and 3F3/2 are both enhanced in three conditions: when the spindle is dissolved with nocodazole, when chromosomes are displaced near one pole, and when chromosomes are isolated in vitro. The 3F3/2 staining of isolated chromosomes is reduced by phosphatase treatment but then reaccumulates upon addition of MgATP. This recovery is greatly enhanced by the addition of ERK2 or the MAP kinase kinase MKK1, which is specific for the ERKs.

Based on this evidence, Shapiro et al. suggest that the kinetochore-bound ERKs are, directly or indirectly, responsible for phosphorylating the 3F3/2 antigen(s). Earlier studies in *Xenopus* implicated ERK2 in the checkpoint; a recent report implicates p38, a relative of MAP kinase. Shapiro et al. look for but do not find activated p38 at the kinetochore. The resolution of who controls the checkpoint may have to wait for more specific phosphatases and kinase inhibitors or the isolation of the protein(s) recognized by the 3F3/2 antibody.

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