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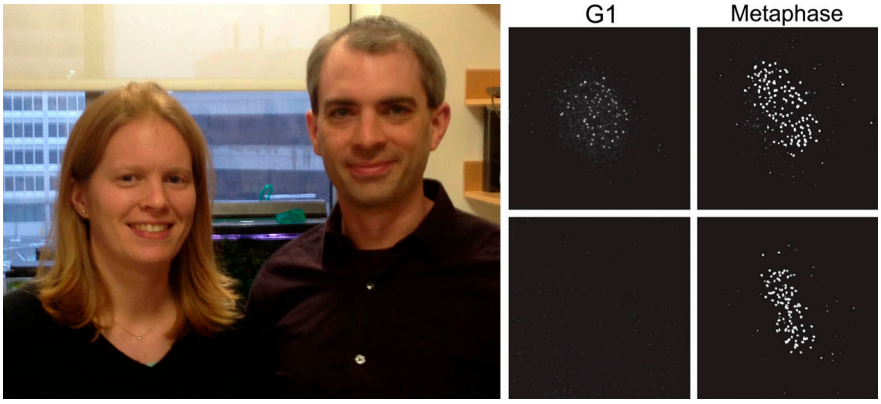
Rise and fall of the kinetochore

Study pinpoints two ways that cells control dynamics of kinetochore proteins.

Focal Point



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Karen Gascoigne (left) and Iain Cheeseman investigated four hypotheses to explain the regulation of kinetochore assembly and disassembly. They tracked kinetochore proteins such as CENP-C and Ndc80 before, during, and after mitosis. As these nuclear shapshots suggest, CENP-C (top row) remains in the nucleus and associates with kinetochores throughout the cell cycle. In contrast, Ndc80 (bottom row) cozies up to the kinetochores only during mitosis.

Like Las Vegas, kinetochores have a few full-time residents and plenty of visitors. Gascoigne and Cheeseman identify two mechanisms that enable cells to specify when some of these visiting kinetochore proteins arrive and when they depart (1).

Around 100 proteins spend time at the kinetochores. Members of the constitutive associated centromere network (CCAN), including CENP-C and CENP-T, stay attached to chromosomes permanently (2) and help draw in other proteins that remain at the kinetochores only during mitosis or that drop by throughout the cell cycle (3, 4). Among these part-timers are the proteins in the Ndc80 complex, part of the KMN network that links the kinetochore to microtubules during mitosis. A cell's task is to attract these transient proteins to the kinetochores at the right time and send them away afterward. The question researchers haven't answered, says senior author Iain Cheeseman, is "How do you make such a stable molecular machine during mitosis and then tear it apart as you exit from mitosis?" Gascoigne and Cheeseman recently proposed four possible mechanisms that could allow cells to adjust the kinetochore's protein lineup (5).

In their new study, Gascoigne and Cheeseman evaluated these mechanisms by tracking the comings and goings of 10 inner and outer kinetochore proteins from 30 minutes before mitosis until 30 minutes after. The results for some members of the CCAN were surprising. Although CENP-C, CENP-T, and CENP-H abide on centromeres throughout the cell cycle, their kinetochore levels shot up 50% after the nuclear envelope dissolved and fell back again after the beginning of anaphase. Why cells need extra copies of these proteins during mitosis isn't clear, but the new arrivals might help stabilize the kinetochores.

> **"How do you make such a stable molecular machine during mitosis and then tear it apart as you exit from mitosis?"**

Two of Gascoigne and Cheeseman's proposed mechanisms didn't jibe with their results: cytoskeletal changes and destruction of transient proteins after they've completed their duties. For example, removing unneeded kinetochore proteins would involve tagging them with ubiquitin and dispatching them to the proteasome for degradation. However, Gascoigne and Cheeseman found that preventing ubiquitylation of Ndc80 didn't hinder its disappearance from kinetochores during anaphase.

Two of the researchers' proposed explanations did pass muster. One mechanism involves limiting proteins' access to the nucleus. The Ndc80 complex, for example, is normally locked out of the nucleus until mitosis. But when the scientists outfitted the Ndc80 protein with a nuclear localization sequence that allowed it to enter at any time, it prematurely attached to the chromosomes, suggesting that its cellular position dictates when it can merge with kinetochores.

Adding and removing phosphates from proteins also helps cells determine kinetochore membership, Gascoigne and Cheeseman discovered. CDK, the master regulator of the cell cycle, instigates these changes. The researchers found that, if they inhibited CDK, Ndc80 and Mis12 rapidly dropped off kinetochores. CDK has an indirect effect on Ndc80, phosphorylating CENP-T proteins that then act as binding sites for Ndc80 molecules and attract them to the kinetochores.

The scientists also tested what happened if they prevented kinetochore disassembly. They equipped cells with a CENP-T version that behaves like it is permanently phosphorylated and then forced Ndc80 to remain in the nucleus. Not only did kinetochore disassembly slow dramatically, but the chromosomes failed to segregate properly in subsequent rounds of division.

Taken together, the team's findings suggest that kinetochore composition depends on proteins' cellular location and phosphorylation status, which is ultimately under the control of CDK. "What's surprising about the kinetochore is how dynamic it is during the cell cycle," says Cheeseman. "This study has told us at least a part of the puzzle of how it assembles and disassembles." Researchers now need to fill in the other steps that result in proteins joining or leaving the kinetochores.

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5. Gascoigne, K.E., and I.M. Cheeseman. 2011. *Curr. Opin. Cell Biol.* 23:102–108.