fluorescent proteins (GPI-FPs) in T-cell lines; GPI-anchored proteins are generally thought to be targeted and concentrated in lipid rafts. The FRET signal that they observed was density-dependent, cholesterol-independent and similar to that observed for proteins previously shown to be non-raft associated. These findings suggest that GPI-anchored proteins are not clustered in resting T-cell lines, or put another way, that these cells do not have rafts. Although these conclusions seem reasonable, considering the detection limits of the methodology employed it remains possible that resting T cells contain raft domains that elude detection by conventional FRET technologies. Support for this view comes from recent studies, for example, Mayor and colleagues[6] use FRET in combination with other physical techniques and theoretical modeling[6,7]. These studies suggest that rafts in resting cells are tiny (<10 nm in diameter) and may contain so few GPI-anchored proteins that it may be unlikely that any one raft would contain both the transfected FRET donor and acceptor GPI-FP.

Glebov and Nichols also examined the situation in activated T cells. In the activated state, the TCR and a variety of T-cell signalling components are thought to cluster at the immunological synapse, a highly dynamic structure that is involved in T-cell signalling and activation[8]. In some experiments[9,10], but not in all[11,12], the raft CTB-binding glycosphingolipid GM1 has been shown to concentrate at the synapse. However, it is unclear whether the apparent recruitment of TCR into rafts and the formation of the immunological synapse reflect two temporally correlated, but functionally independent, events[8]. Glebov and Nichols show that although TCR activation resulted in increased fluorescence of both the GPI-FP and GM1 at the site of TCR polarization, FRET analysis does not reveal any clustering of either GPI-FP or GM1. These results lead the authors to conclude that the observed increase in fluorescence intensity could simply reflect changes in membrane shape. Their evidence is convincing that the immunological synapse does not contain a concentration of rafts that bear GPI-linked proteins, in contrast to previous suggestions[9,10]. However, these data do not necessarily preclude a role for rafts in TCR signalling. Again, if the rafts into which TCR partitions initially are indeed small, then few TCR molecules would be predicted to partition into any given GPI-FP-containing raft, and thus TCR cross-linking would have no effect on GPI-FP clustering.

The study of Glebov and Nichols, together with other recent studies, poses a challenge to the traditional view of rafts as relatively large and stable entities. Indeed, these data are important for setting limits on the physical, and potentially functional, definition of rafts in T cells. The question of whether the TCR partitions initially into small dynamic rafts and subsequently induces their clustering to promote signalling will require advanced techniques, such as those used by Mayor[6] and Kusumi[7], to investigate directly the relationships of the TCR with lipid domains and the signalling components that are thought to be compartmentalized in rafts. Clearly, learning what lipid rafts are and whether they are involved in TCR signalling will require a lot more FRETing. □

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Correcting SYNful attachments

Sue Biggins

The remarkable fidelity of chromosome segregation during mitosis and meiosis is critical for preventing birth defects and diseases. The Aurora protein kinases prevent defects in segregation by correcting mistakes in chromosome attachment to the spindle before cells divide.

How do cells get the right number of chromosomes? Every cell division relies on chromosome duplication and segregation occurring with complete fidelity. Ensuring accurate segregation is essential to prevent birth defects and aneuploidy, a hallmark of all tumours. Although many types of DNA repair mechanisms have been described, little is known about the mechanisms that fix errors which would otherwise result in chromosome mis-segregation. On page 232 of this issue, Lampson *et al.* elucidate one of the steps in correcting chromosome attachment defects by showing that the Aurora protein kinases destabilize inappropriately attached microtubules[1].

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Chromosome segregation appears to be a simple, well-understood process. Large protein–DNA structures called kinetochores bind to a mitotic spindle composed of microtubules nucleated from the spindle poles. Once all of the chromosomes are properly aligned on the metaphase plate, anaphase ensues, pulling chromosomes to opposite poles. However, chromosome segregation is actually much more complicated than the textbook view (for review, see ref. 2). After DNA replication, sister chromatid pairs are held together by cohesion. The sister kinetochores contain multiple microtubule-binding sites that must all make attachments to microtubules from opposite poles. Microtubules are nucleated from spindle poles and exhibit dynamic instability, a property where growing and shrinking polymers co-exist[3]. Microtubules thus find kinetochores by a 'search and capture' mechanism that makes it unlikely for sister kinetochores to be simultaneously captured by microtubules from opposite poles. Instead, one of the two sister kinetochores is first captured by a microtubule, resulting in polewards movement of the chromatid pair (Fig. 1a). Over time, this kinetochore makes additional microtubule

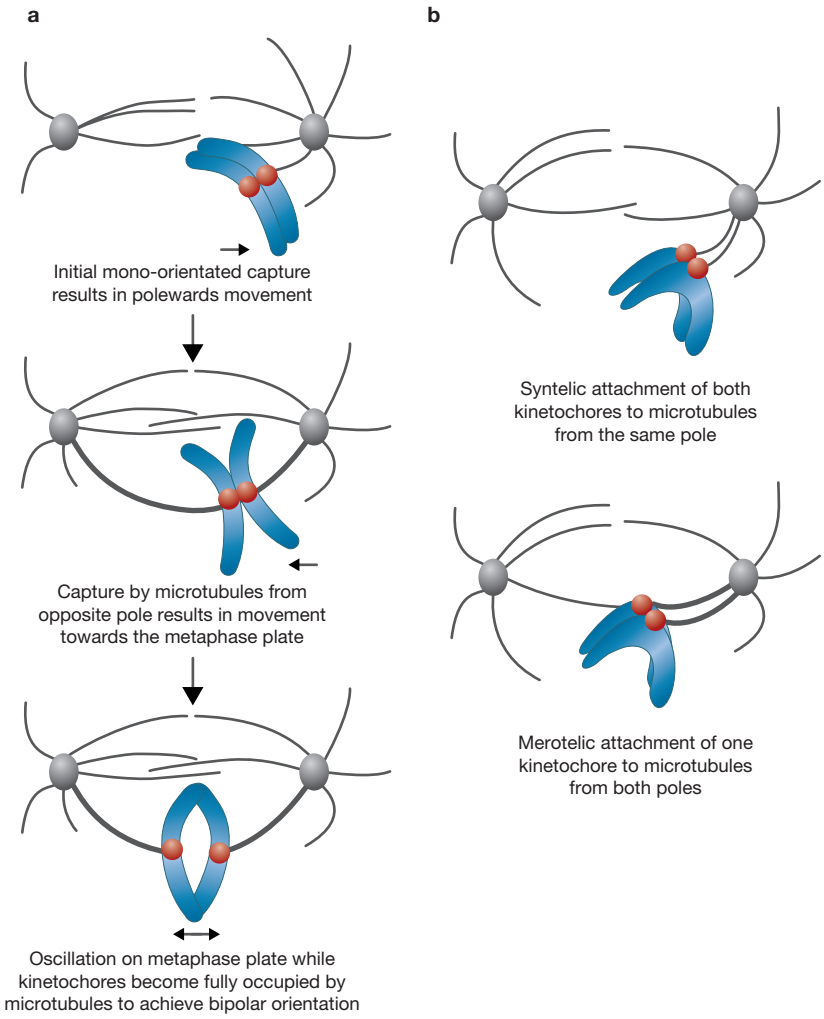

Figure 1 The process of chromosome segregation. (**a**) Paired sister chromatids make bi-orientated attachments to microtubules from opposite poles. (**b**) Sister chromatid pairs can make inappropriate microtubule connections, such as syntelic or merotelic attachments.

attachments to generate a kinetochore–microtubule fibre (K-fibre), whereas its sister kinetochore binds to a microtubule from the opposite pole. This results in movement of the chromatids towards the metaphase plate, where they oscillate until all of the microtubule binding sites become occupied in the bi-oriented fashion that ensures accurate segregation at anaphase. An amazing feature of the kinetochore that allows dynamic movement is its ability to remain bound to the microtubules as subunits are added and removed. This property is caused, at least in part, by motor proteins that connect microtubules to kinetochores. Once a stable bipolar attachment is made, the pulling forces of the microtubules generate physical tension that draws the sister chromatids away from each other.

The steps that result in chromosomes lining up on the metaphase plate create the possibility for numerous errors. Although the continual probing of the kinetochore by microtubules ensures they will all eventually be captured, it is difficult to prevent inappropriate microtubule attachments from being made. For instance, microtubules from the same pole can bind to both sister kinetochores, creating a mono-oriented syntelic attachment (Fig. 1b). Merotelic attachments are generated when microtubules from both poles bind to the same kinetochore. Although these defects would be lethal if left uncorrected, they are almost always resolved before chromosome segregation. The inappropriate microtubule attachments are sensed by a signal transduction system called the 'spindle checkpoint', which halts the cell cycle until the defects are corrected (for review, see ref. 4). Although it is known that incorrect microtubule attachments are unstable, the mechanisms that cause instability of incorrect attachments have remained elusive.

The first clue to understanding how errors are corrected comes from recent work on the Aurora protein kinase family (which contains three members, Aurora A–C; for review, see ref. 5). Cells defective in Aurora B function accumulate syntelic attachments. Other studies showed that transiently depolymerizing the microtubules in cells defective in the budding yeast Aurora kinase (Ipl1p) could restore bipolar attachments[6]. This result led to the proposal that Aurora kinases correct syntelic attachments, and recent work in budding yeast has provided additional evidence for this model[7,8]. Lampson *et al.* now extend this work to show that the Aurora kinases destabilize incorrectly attached microtubules[1]. To demonstrate this activity, the authors made clever use of small-molecule inhibitors. First, they used monastrol to prevent pole separation and increase the likelihood that syntelic microtubule attachments were made (Fig. 2). They then removed monastrol as they added a second inhibitor against the Aurora kinases, allowing the cells to accumulate bipolar spindles while maintaining syntelic attachments. To analyse how the inappropriate connections are resolved, the Aurora inhibitor was removed and the microtubules and chromosomes were directly visualized by high-resolution microscopy techniques. The major observation was that chromosomes that had syntelic attachments moved polewards because the attached K-fibres shortened simultaneously. The Aurora-induced polewards movement of the mono-oriented chromosomes is reminiscent of the initial movement of chromosomes towards the pole in prometaphase, and occurred at a comparable rate. This raises the interesting hypothesis that Aurora kinases can 're-set' chromosomes to a state similar to prometaphase, giving them another chance to go through the normal search and capture mechanism.

The work described in this issue provides the first direct demonstration of a mechanism used to correct mal-oriented attachments. It is remarkable that Aurora kinase activity resulted in the shortening of mono-oriented, but not bi-oriented, kinetochore microtubules. This suggests that the Aurora kinases may detect the difference between proper and improper attachments to mediate selective microtubule depolymerization. One hypothesis is that the Aurora B kinase senses the lack of tension on mono-oriented kinetochores, therefore distinguishing the inappropriate attachments from the proper ones. This idea stems from the demonstration that in some organisms Aurora B activity is required to

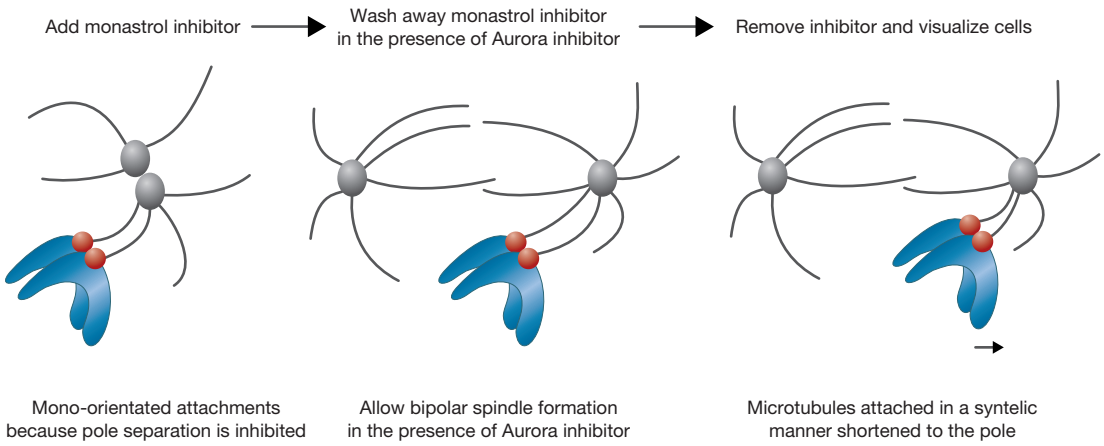

Figure 2 Outline of the Lampson *et al.* experiment. Aurora protein kinase activity results in destabilization of microtubules when chemical inhibitors are removed.

mediate the cell-cycle delay that occurs in response to defects in tension, but not attachment[9–11]. Consistently, tension is known to stabilize microtubule attachments[12]. Current work is therefore aimed at understanding how tension defects might be translated into changes in Aurora B activity. However, it is important to note that the inhibitors used by Lampson *et al.* blocked both Aurora A and B activity, making it unclear which kinase is directly responsible for the correction activity.

Despite the exciting discovery that Aurora kinases can destabilize microtubules, it is still not clear how bi-orientation is eventually achieved. As the chromosomes moved with both shortening sets of K-fibres, it is likely that the kinetochores remained bound to the depolymerizing microtubules. However, electron microscopy will be required to confirm that the full complement of microtubules stays attached to the kinetochore as the microtubules shorten. If the microtubules remain bound to the kinetochores as the authors propose, then the syntelic attachment will be maintained. Microtubule capture from the opposite pole will result in merotelic attachments, creating yet another problem that needs to be fixed. It is likely that additional correction steps must occur that were not revealed in the reported experiments. One possibility is that after depolymerization, the microtubules are released at the pole thus allowing re-orientation. Another idea is that Aurora activity contributes to the steric orientation of the kinetochores, allowing them to re-orientate in a manner that makes it favourable to capture microtubules from the opposite pole. However, as unreplicated dicentric chromosomes still bi-orientate in an Aurora-dependent manner in budding yeast, the orientation of the kinetochores may not be essential to achieve bi-orientation[7]. Alternatively, the syntelic attachments might be weakened during the microtubule shortening process, resulting in their release as the chromosomes subsequently move back towards the metaphase plate. In budding yeast, Aurora activity generates unattached chromosomes in response to defects in bipolar attachment[8]. In addition, the re-orientation of budding yeast monocentric chromosomes that are not under tension requires Aurora activity[7]. Although this might reflect a difference between the organisms, it may also mean that Aurora has a second function that causes complete detachment of inappropriately connected microtubules. A key area of future research will be to identify the additional correction steps that convert syntelic attachments into bi-oriented ones and determine how many of these steps require Aurora activity.

How do Aurora kinases destabilize microtubules? Although they can bind directly to microtubules, they are not known to have depolymerization activity. The most likely possibility is that one or more substrates exist that promote microtubule turnover when phosphorylated. Although many Aurora substrates have been identified, one intriguing newly discovered target is the mitotic centromere-associated kinesin (MCAK), which can depolymerize microtubules[13]. Aurora B phosphorylation of MCAK alters both its localization and activity[14,15] (and R. Ohi and T. Mitchison, personal communication). However, Aurora inhibits MCAK activity *in vitro*, suggesting there are additional controls *in vivo* that create the destabilizing activity assayed by the Lampson *et al.* The use of chemical inhibitors combined with small-interfering RNA (siRNA) approaches should allow future identification of the targets that mediate destabilization. These studies, combined with advances in other systems, should continue to elucidate the mechanisms that correct chromosome attachment errors and thus ensure genomic stability.

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