Chromosome Segregation: Monopolin Attracts Condensin

To segregate chromosomes properly, the cell must prevent merotely, an error that occurs when a single kinetochore is attached to microtubules emanating from both spindle poles. Recent evidence suggests that cooperation between Pcs1/Mde4 and condensin complexes plays an important role in preventing merotely.

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Accurate segregation of the genetic material during cell division requires that sister kinetochores attach to microtubules emanating from opposite spindle poles. Merotelic kinetochore orientation is an error in which a single kinetochore is attached to microtubules emanating from both spindle poles [1,2]. If a merotelically attached kinetochore remains uncorrected, it causes the chromatid to lag on the anaphase spindle, hindering its poleward segregation (Figure 1). It is important to understand how cells prevent and correct merotelic kinetochore attachments because merotely represents a major mechanism of aneuploidy in mitotic cells and is the primary mechanism of chromosomal instability in cancer cells [3–8]. Several proteins have been implicated in correcting or preventing merotelic attachments, including condensin and the fission yeast Pcs1/Mde4 complex, a homolog of the budding yeast monopolin complex [9–13]. Two recent studies provide important insights into how Pcs1/Mde4 and condensin prevent merotelic kinetochore attachments [14,15].

Previous studies suggested that both the Csm1/Lrs4 monopolin subcomplex in the budding yeast Saccharomyces cerevisiae and its counterpart Pcs1/Mde4 in the fission yeast Schizosaccharomyces pombe act at kinetochores as molecular clamps which lock together microtubule attachment sites. While the Pcs1/Mde4 complex clamps together microtubule attachment sites on a single kinetochore in order to prevent merotelic attachments, the Csm1/Lrs4 complex clamps together microtubule binding sites from sister kinetochores during meiosis I in order to establish mono-orientation (attachment of sister kinetochores to microtubules emanating from the same pole) [11,16]. Although this model was consistent with the experimental data and nicely explained the mutant phenotype observed in cells lacking Csm1/Lrs4 or Pcs1/Mde4, it was rather speculative. A strong argument in favour of the ‘clamp’ model came only recently from the structural analysis of the Csm1/Lrs4 complex. Corbett et al. [15] showed that the Csm1/Lrs4 complex has a distinctive V-shaped structure, with two pairs of kinetochore-binding domains that are likely to act as molecular clamps for microtubule attachment sites.

Figure 1. Chromosome segregation during mitosis.

(A) In order to segregate chromosomes properly, sister kinetochores must attach to microtubules emanating from opposite spindle poles (amphitelic kinetochore attachment). (B) Merotelic kinetochore attachment is an error in which a single kinetochore is attached to microtubules emanating from both spindle poles. During anaphase, a merotelically attached chromatid lags behind and its timely segregation is hindered.

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domains positioned about 10 nm apart. Thus, a plausible model for Csm1/Lrs4-mediated mono-orientation of sister kinetochores is that these two pairs of kinetochore-binding domains bind across sister kinetochores, bringing them so close together that they effectively prevent bi-orientation (attachment of sister kinetochores to microtubules emanating from the opposite poles). Moreover, Corbett et al. [15] showed that the S. pombe Pcs1/Mde4 complex has the same general architecture as Csm1/Lrs4, suggesting that both Csm1/Lrs4 and its S. pombe counterpart Pcs1/Lrs4 may function as molecular clamps or crosslinkers at kinetochores.

Although the clamp model is now supported by structure–function analyses, it has been challenged by a recent study from Tada et al. [14], who showed that the role of Pcs1 and Mde4 is to recruit condensin to kinetochores and proposed that condensin at kinetochores clamps together microtubule attachment sites. Chromatin immunoprecipitation experiments clearly showed that kinetochore condensin localization is diminished in pcs1Δ cells and, notably, artificial targeting of condensin to kinetochores largely suppressed the growth defect and halved the incidence of lagging chromosomes in pcs1Δ cells [14]. These observations are consistent with previous studies showing that both Pcs1/Mde4 and condensin are important for preventing merotelic attachments [10,11,16] and that in budding yeast, monopolin proteins Csm1 and Lrs4 are required for recruitment of condensin to ribosomal DNA [17]. However, other studies showed that condensin associates with kinetochores independently of Csm1 and Lrs4 [18] and that condensin is not an obligate component of a system preventing merotelic attachments in vertebrate kinetochores [19]. These apparent discrepancies in the literature further underscore the importance of the Tada et al. [14] study. Crucially, the work of Tada et al. raises the following key question. Does the Pcs1/Mde4 complex act as a microtubule site clamp (Figure 2A), or does it prevent merotelic attachments solely by recruiting condensin to kinetochores (Figure 2B)? Although Tada et al. nicely showed that Pcs1 and Mde4 act as a condensin recruiter at kinetochores and that this is an important mechanism for preventing merotelic attachments [14], further experiments are needed to establish whether condensin recruitment is the only role of the Pcs1/Mde4 complex in preventing merotely, or whether Pcs1/Mde4 also functions as a microtubule site clamp, as suggested by previous studies (Figure 2) [11,15,16]. Elegant experiments in which kinetochore condensin was specifically inactivated by proteolytic cleavage showed that this disturbed the structure of centromeric chromatin, and frequent separation of core and pericentromeric domains was observed [14]. It is likely that this defect contributes to the high incidence of merotelic attachments observed in condensin mutant cells; therefore, it will be important to determine whether the absence of Pcs1 or Mde4 leads to a similar phenotype. Moreover, the distinct V-shape structure of the monopolin complex makes important predictions about its putative clamping function [15]. Using this structure as a guide, mutations that prevent the clamping ability should be designed and tested in vivo. Finally, in order to extend the current studies to other organisms, it will be important to identify counterparts of the fission yeast Pcs1/Mde4 complex in higher eukaryotes. Although structural and sequence analyses showed that the Pcs1/Mde4 complex shares similar features with the conserved kinetochore complex Spc24/Spc25 [12,15], it is not known whether in higher eukaryotes the Spc24/Spc25 complex took over the function of the Pcs1/Mde4 or whether there are true homologs of Pcs1/Mde4 which have not been identified yet.

In summary, mounting evidence suggests that both the Pcs1/Mde4 and condensin complexes are required to prevent merotely. There are two models of how Pcs1 and Mde4 prevent merotelic kinetochore attachments. Whereas the clamp model suggests that Pcs1/Mde4 complex itself acts as a molecular clamp which locks together microtubule attachment sites (Figure 2A), an alternative model suggests that the Pcs1/Mde4 complex prevents merotely indirectly by recruiting condensin to kinetochores and that condensin acts as a molecular clamp which locks together microtubule attachment sites (Figure 2B). These two models are not mutually exclusive and it is possible
that both Pcs1/Mde4’s clamping activity and its role as a condensin recruiter are required to efficiently prevent merotelic kinetochore attachments. Further studies are needed to unveil molecular details of how kinetochore pools of Pcs1/Mde4 and condensin complexes prevent merotely. Given the importance of this process for our understanding of how cells ensure faithful segregation of chromosomes, it is likely that this will continue to be an area of intense research in the future.

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Circadian Rhythms: FLOWERING LOCUS T Extends Opening Hours

Plants are more sensitive to light in the day than at night due to the circadian clock. The protein that acts downstream from the clock to modulate blue light signalling in stomata comes as a surprise; it is FT, which is thought to be the long-distance regulator of flowering.

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In Cambridge University, where we write, Francis Darwin, son to a famous father, studied the daily rhythms of stomatal movements and found stomata opened greater in response to light in the day than at night [1]. We now know that this is an example of rhythmic sensitivity to light due to modulation by the oscillator of the circadian clock, a process known as ‘circadian gating’ [2]. In a recent issue of Current Biology, Kinoshita et al. [3] report mechanisms involving FLOWERING LOCUS T (FT) that might permit circadian and photoperiodic regulation of stomatal sensitivity to blue light.

The stomatal pore provides an interface between the plant and the atmosphere through which CO2 can enter the leaf to act as a substrate in photosynthesis, while at the same time H2O is lost by evapotranspiration. Regulation of guard cell movements by the circadian oscillator conserves water by favouring stomatal opening in the morning, when ambient temperatures are low and promoting stomatal closure long before dusk to prevent water loss in the heat of the afternoon [4,5]. Mutant Arabidopsis lines with a compromised circadian oscillator have increased water loss during the day when compared to wild-type plants [5,6].

Stomatal aperture is ultimately regulated by the guard cell plasma membrane potential. In the morning, blue light and the circadian clock activate an electrogenic proton-pumping ATPase through a 14-3-3 protein-dependent pathway. The resulting H+ efflux hyperpolarises the plasma membrane up to ~250 mV, creating a driving force for the influx of K+ through the KAT1 channel. K+, along with Cl− and malate, accumulate in the vacule, resulting in water influx and an increase in guard cell turgor that opens the stomatal pore. Stomatal closure is brought about by inhibiting the H+-ATPase and by Ca2+ - and OPEN STOMATA 1 kinase-dependent activation of SLOW ANION CHANNEL1 (SLAC1) to promote prolonged Cl− efflux, which depolarises the plasma membrane. At plasma membrane potentials positive of ~120 mV, the GUARD CELL OUTER RECTIFIER K CHANNEL opens, permitting K+ efflux [7]. Loss of K+, Cl− and malate results in water efflux from the guard cell and stomatal closure.

Kinoshita et al. [3] provide evidence for a role in the regulation of stomatal aperture for EARLY FLOWERING 3 (ELF3) and FT, genes that are more