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Meiosis: Polo, FEAR and the Art of Dividing Reductionally

Dispatch

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Recent studies on the regulation of meiosis have uncovered new roles for old acquaintances: the polo-like kinase Cdc5 has been found to dictate proper kinetochore orientation during meiosis I, while the FEAR pathway is essential for some, but not all, aspects of meiosis I exit.

A key question in the cell-cycle field is how processes that seem independent become coordinated. Many cell-cycle processes occur in parallel, like instruments in an orchestra, each playing their individual parts that are brought together by the conductor, creating a harmonious melody. The conductor can be viewed as a master regulator: at his signal the symphony commences; after that, one can only hope that the tuba will be able to keep up… Recent studies on meiotic regulation in budding yeast have uncovered master regulators and parallel processes that bring about the successful generation of haploid gametes. One master regulator is the polo-like kinase Cdc5, for which new functions have been identified [1,2]. Our 'tuba' is the FEAR pathway, recently discovered as a regulator of mitotic exit. But whereas mitotic cells can survive without FEAR, in meiosis inactivation of the FEAR pathway results in an aberrant cell division, with some processes coming to a halt, while others continue unabated [3,4].

The Key to a Successful Meiosis

Successful meiosis is contingent upon the segregation of homologous chromosomes in the first (reductional) meiotic division, and the segregation of sister chromatids during the second (equational) meiotic division (Figure 1; reviewed in [5]). This necessitates a change in kinetochore-spindle association between meiosis I and meiosis II: in meiosis I, sister kinetochores must associate with the same spindle pole, an arrangement known as co-orientation, whereas during meiosis II, sister kinetochores must associate with opposite spindle poles, known as bi-orientation. Co-orientation is imposed by the monopolin complex, which binds kinetochores in meiosis I and prevents the bi-orientation of sister kinetochores (reviewed in [6]).

Segregation of homologous chromosomes depends on their ability to align via recombination intermediates known as chiasmata. The cohesin complex, which associates the two sister chromatids, stabilizes the chiasmata between the homologs [7] and allows the cells to keep the sisters together until meiosis II. In meiosis I, cohesion is dissolved along the chromatid arms but importantly not at the centromeres, allowing chiasmata to resolve and the homologs to segregate while maintaining contact between the sisters. The remaining cohesion is dissolved during meiosis II (Figure 1). In yeast, this two-phase removal of cohesin requires a meiosis specific cohesin subunit, Rec8; substituting Rec8 with its mitotic counterpart, Scc1/Mcd1, results in dissolution of both arm and centromere cohesion in meiosis I [7,8]. In both meiosis and mitosis, cohesion is dissolved through the proteolytic cleavage of Rec8 and Scc1/Mcd1, respectively, by the protease separase [7].

Budding yeast mutants that undergo an aberrant meiosis have been known for well over a decade. Relevant to our discussion are those that appear to undergo a single division, with a significant fraction of the chromosomes dividing equationally. Recent studies, made possible by experimental advances, show that not all equational divisions are, in fact, equal.

Determining Co-orientation

Budding yeast *cdc5* mutant strains undergo an aberrant meiosis, with some chromosomes segregating reductionally while others ssegregate equationally [9]. These cells divide only once, after a very long delay in metaphase I, well beyond the time it would have taken a wild-type cell to complete the whole meiotic process [1,2]. In mitosis, Cdc5 promotes cohesion dissolution by stimulating the proteolytic cleavage of Scc1/Mcd1 [10]. The prolonged metaphase I might thus have been a result of persistent sister chromatid cohesion resulting from the inability to cleave Rec8 and resolve chiasmata. Had that been the case, abolishing recombination altogether, by removing the endonuclease that initiates recombination, Spo11 [11], should have allowed anaphase I to take place. In *spo11* mutants, sister chromatids still co-orient in metaphase I and chromosomes segregate despite the lack of alignment; however, *cdc5 spo11* mutants show no sign of anaphase [2].

In *cdc5 spo11* mutants the homologous chromosomes are apart, so one is left to conclude that the failure of this double mutant to segregate chromosomes occurs either as a result of defective kinetochore–microtubule attachments, or because sister kinetochores do not co-orient but rather bi-orient, segregation being prevented by the presence of sister chromatid cohesion. Chromatid cohesion *per se* is not required for co-orientation [12]. When, in addition to the absence of chiasmata, both arm and centromeric cohesion were dissolved in anaphase I — as in a *cdc5 spo11* double mutant expressing Scc1/Mcd1 instead of Rec8 — equational chromosome segregation took place [2]. What could be the cause of this equational division? In meiosis I, equational division is normally prevented by the monopolin complex (Figure 1) [6]. In *cdc5* mutants, metaphase I kinetochores are devoid of monopolin, indicating that Cdc5 is responsible for kinetochore co-orientation in meiosis I [1,2]. These studies also showed that Cdc5 is needed for exit from meiosis I and for efficient resolution of chiasmata [1].

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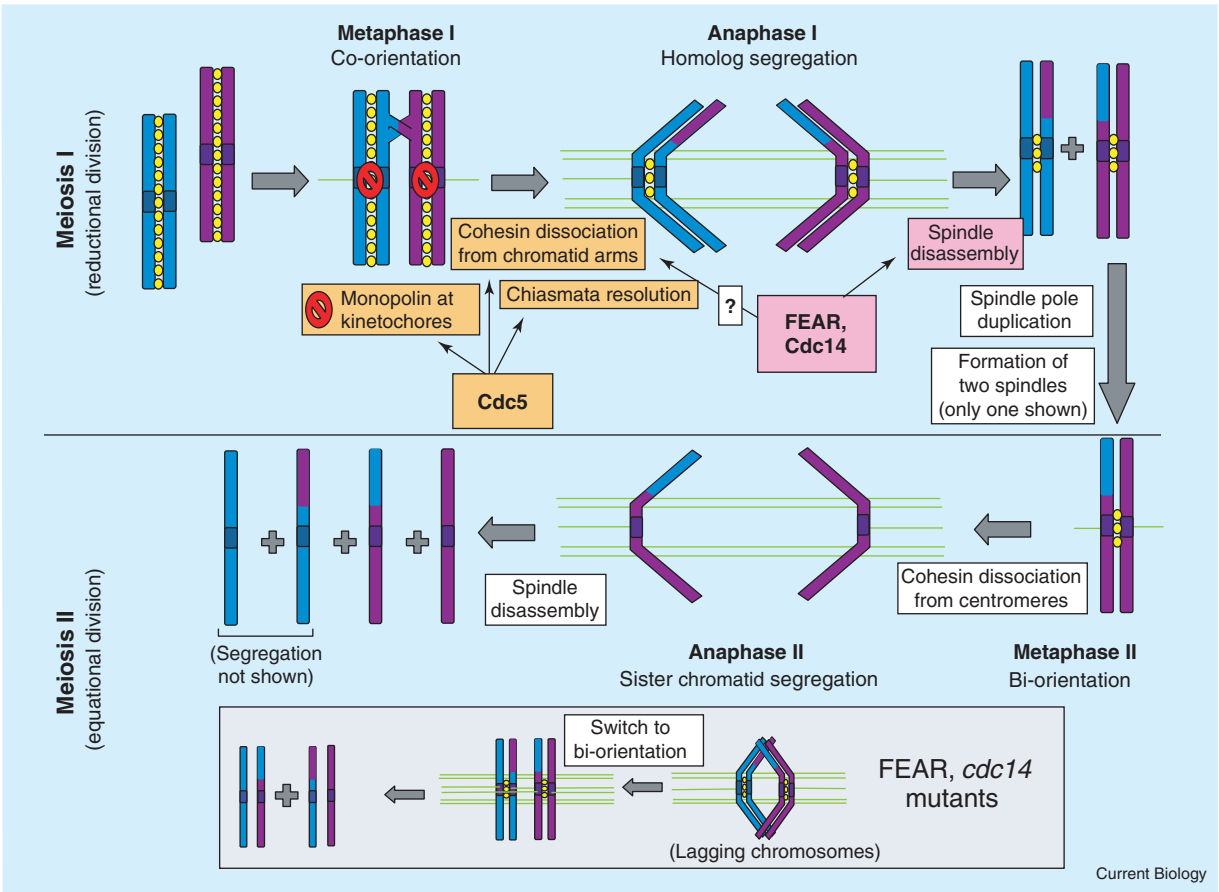

Figure 1: Kinetochore orientation and chromosome segregation in meiosis.

During meiosis, immediately following DNA replication, sister chromatids are associated along their entire length via the cohesin complex (yellow). Prior to metaphase I, chiasmata are formed between homologous chromosomes (blue and purple) and the Cdc5-dependent association of monopolin (red) with kinetochores dictates the co-orientation of the sister kinetochores, such that they associate with spindle microtubules (green) emanating from the same spindle pole. Whether one or both sister kinetochores are active in microtubule binding is not known. Cdc5 and the FEAR pathway control several aspects of anaphase I (see text for details). After meiosis I, wild-type cells go on to meiosis II (bottom panel, only one of the homologs is shown). FEAR and *cdc14* mutants fail to disassemble the anaphase I spindle, but they nonetheless continue on to an equational division.

Thus, as in mitosis, Cdc5 appears to have multiple roles in meiosis: it promotes co-orientation, cohesin cleavage, chiasmata resolution and meiosis I exit. Cdc5 homologs are also essential for meiosis in other organisms [13,14]. In yeast, the absence of Cdc5 significantly delays meiotic progression, suggesting that Cdc5 may act as a master regulator.

Exiting Meiosis I: All Together Now

The phenotype of a single meiotic division of mixed equational and reductional segregation is also exhibited by *spo12*, *slk19* and *cdc14* mutant strains [9,15–17]. Spo12 and Slk19, along with separase and Cdc5, are part of the FEAR pathway which regulates the exit from mitosis, although mitotic cells lacking Spo12 or Slk19 exhibit only minor defects [18]. The FEAR pathway — for Cdc fourteen early anaphase release — controls the release of the Cdc14 phosphatase from the nucleolus, which in turn down regulates cyclin B activity, an essential step in mitotic exit. The FEAR pathway is not needed in mitosis because of the existence of an essential pathway, the mitotic exit network (MEN), which also promotes the nucleolar release of Cdc14 (reviewed in [18]). Mitotic exit requires a drastic reduction in cyclin B activity, whereas during the exit from meiosis I, moderate cyclin B levels need to be maintained to prevent re-replication ([19] and references therein), and for which the activity of the FEAR pathway may be necessary and sufficient. But if the FEAR pathway were essential for meiotic exit, why would *slk19* and *spo12* mutants go on to divide, let alone equationally?

New studies from the Amon [3] and Nasmyth [4] labs show that *slk19*, *spo12* and *cdc14* mutants fail to disassemble the anaphase I spindle, and instead of having two clearly separated DNA masses, these mutants have a significant amount of DNA stretched along the entire spindle. At least part of this lagging DNA can be accounted for by the inability of the nucleolus, and the rDNA chromosomal region it contains, to divide in *slk19*, *spo12* and *cdc14* mutants [4]. Why this is the case, and whether other chromosome regions suffer from the same problem, are not known. It seemed that the defect was due, at least in part, to high cyclin B levels, consistent with the mitotic role of the FEAR pathway; however,

unlike mitosis, where high cyclin B levels block exit, in meiosis I, despite high cyclin B levels and the lack of spindle disassembly, these cells divided and in a significant number of cases an equational division was observed [3,4].

Could this be similar to the *cdc5* mutant, where co-orientation of sister kinetochores in meiosis I fails to take place? This seems unlikely; both groups noted that, in the *slk19*, *spo12* and *cdc14* mutants, sister kinetochores do initially co-orient, and they show no defect in monopolin localization. But although anaphase I spindle disassembly did not occur, other aspects of meiotic progression did — such as spindle pole duplication and the biphasic dissociation of Rec8 from chromosomes (Figure 1). Careful analysis revealed that the timing of the equational division in these mutants coincides with the time at which wild type cells would have undergone meiosis II. On the basis of this and other findings, both groups [3,4] conclude that, despite the presence of an anaphase I spindle, some kinetochores switched at the scheduled time from co-oriented to bi-oriented spindle attachments; those that switched underwent an equational division while those that did not segregated reductionally [3,4] (Figure 1, inset).

Why only some chromosomes divide equationally in these mutants remains a mystery. One possibility is that centromeres/kinetochores differ in their microtubule binding, some being more prone to switch than others. Alternatively, the chromosomes that lag behind around the spindle midzone, possibly as a result of defects in removing Rec8 [3], may be more prone to segregate equationally as they are more likely to capture microtubules emanating from opposite poles, whereas those that reached the poles during meiosis I would segregate reductionally. How the FEAR pathway may promote certain aspects of meiotic exit is not known; for reasons that are not understood, the removal for Spo11, thereby suppressing recombination, rescues some of the *spo12* and *slk19* defects. It is especially intriguing that, for meiosis I exit, the activity of separase is needed, but its protease activity is not [4].

Parallel Processes and Master Regulators

Recent studies of Cdc5 and FEAR pathway involvement in meiotic progression have changed the way we interpret a single meiotic division: indeed, not all equational divisions are alike. For the most part, the absence of Cdc5 delays all aspects of meiosis examined; and while it is not clear how *cdc5* mutants eventually manage to divide, their ability to divide equationally was gained during meiosis I. In the case of *slk19*, *spo12* and *cdc14* mutants, equational segregation occurs as a result of the uncoupling of meiotic processes, with some chromosomes segregating equationally via a meiosis II mechanism.

The uncoupling of meiotic events is clearly an undesirable outcome, resulting in germ line defects. Cells do have checks and balances along the way that allow them to monitor proper meiotic progression and stop the process when defects arise. The targets of such surveillance mechanisms are likely to be master regulators, the inactivation of which blocks or delays all aspects of meiotic progression. A case in point is the meiotic pachytene checkpoint, which exists across species, and that stops meiotic progression in response to an unrepaired double-strand DNA break. This is accomplished by inhibiting the activation of the Cdc28 cyclin-dependent kinase and the Ndt80 transcription factor, both of which can be considered master regulators (reviewed in [20]). Whether there is a meiotic checkpoint that uses Cdc5 as its target remains to be determined.

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