

# Checkpoints: Chromosome pairing takes an unexpected twist

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**When meiotic cells complete S phase, homologous chromosomes pair, synapse and undergo recombination. A checkpoint protein is somehow required for meiotic chromosome pairing in *C. elegans*, thus providing a direct link between S phase and the rest of the meiotic program.**

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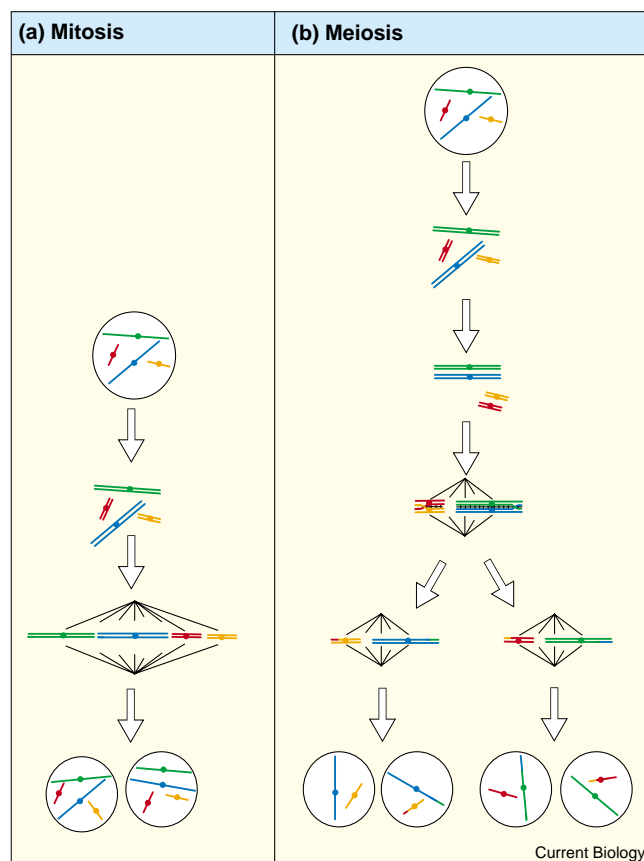
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Chromosome segregation in mitosis is a relatively simple affair, in that two sister chromatids which have recently replicated, and are attached to one another by cohesins, align on the metaphase spindle before being partitioned to either side of a dividing cell (Figure 1a). Chromosome segregation during meiosis is more complicated, in that there is a single round of S phase, followed by two rounds of nuclear and cellular division (Figure 1b). For the first nuclear division, homologous chromosomes must find each other and pair in order for a reductional division to occur. Following chromosome pairing, chromosomes synapse and undergo recombination, which enables both genetic exchange and physical linkage of two homologous chromosomes during nuclear division at metaphase I.

The question of how two homologous chromosomes find each other has been ringing in the ears of cytologists for more than a century. In some organisms, such as the fruit fly *Drosophila melanogaster*, homologous chromosomes are observed in close proximity to each other, even in mitotic cells [1]. In other species, homologous chromosomes are normally dispersed and align only in prophase — following meiotic S phase — accompanied by a spatial nuclear reorganization. Pairing is usually followed by the formation of a proteinaceous scaffold, the synaptonemal complex, which establishes an intimate connection along the lengths of paired homologous chromosomes referred to as synapsis [2].

In some organisms, such as the budding yeast *Saccharomyces cerevisiae* and the mouse, homologue pairing and recombination appear to occur at the same time during meiosis. As some mutants that exhibit reduced recombination also show synapsis defects, these two processes may be linked, or recombination may affect the stability of the synaptonemal complex [3,4]. Strikingly, not all organisms require homologous recombination in order for chromosome

Figure 1

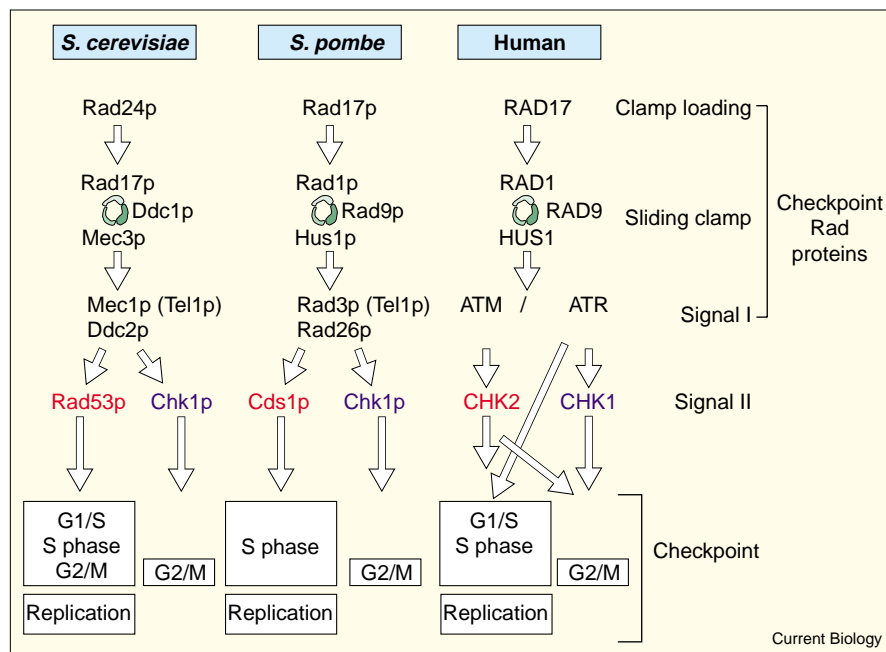


Chromosome alignment and segregation in (a) mitosis and (b) meiosis.

pairing and synapsis to occur. In *Drosophila*, no recombination is detectable in male flies or on chromosome IV in females. Furthermore, the nematode *Caenorhabditis elegans* displays synapsis of meiotic chromosomes that is independent of recombination [5].

*C. elegans* mutants that are defective for chromosome pairing, synapsis or recombination display high levels of chromosome non-disjunction, the result of random chromosome segregation at metaphase I [5–8]. Many of the strong non-disjunction mutants identified to date have defects in proteins required for recombination, rather than for chromosome pairing or for synapsis. Recently, however, MacQueen and Villeneuve [7] identified a *C. elegans* gene that is required both for the initiation of chromosome pairing and for recombination, suggesting that they had found an early player in post-S-phase meiotic chromosome pairing. Much to their surprise, this protein, CHK-2, turned out to be homologous to a DNA damage checkpoint protein

Figure 2



The DNA damage and replication checkpoints in yeast and mammals. The core checkpoint Rad proteins are indicated, but do not include Tel1p in yeast. Phases of the cell cycle known to be controlled by the downstream 'signal II' kinases are indicated, including DNA damage checkpoint responses that occur in G1/S, S and G2/M phases, and the replication checkpoint.

called Rad53p in *S. cerevisiae*, Cds1p in the fission yeast *Schizosaccharomyces pombe* and CHK2 in mammals [9,10].

The DNA damage checkpoint is mediated by a group of proteins that are required to sense DNA damage and that, when activated, induce either cell-cycle arrest and DNA repair, which removes the damage, or programmed cell death, which removes the damaged cell. The latter response is found only in higher eukaryotes, such as worms, flies and mammals. Most checkpoint proteins have been identified through genetic studies of yeast and mammals, and they tend to be conserved, both structurally and functionally, through evolution (Figure 2). A DNA damage checkpoint protein can play roles in one or more of the G1/S, S phase and G2/M checkpoints that respond to DNA damage [11,12]. Some of these proteins are also required for an S phase replication checkpoint that coordinates the end of S phase with the onset of mitosis.

A core group of checkpoint Rad proteins is required for all three DNA damage checkpoints (Figure 2) [11]. These proteins are thought to be involved in sensing DNA damage and generating the initial signal. The proteins Rad17p, Ddc1p and Mec3p in *S. cerevisiae*, and Rad1p, Rad9p and Hus1p in *S. pombe*, may form a complex that resembles the donut-shaped 'sliding clamp' formed by the DNA replication processivity factor PCNA. The complex may be loaded onto DNA damage by Rad24p in *S. cerevisiae* or Rad17p in *S. pombe*, which are similar to the replication clamp-loading protein RFC. Finally, *S. cerevisiae* Mec1p,

*S. pombe* Rad3p and the mammalian proteins ATR and ATM all resemble lipid kinases and may be involved in transmitting the original signal from damaged DNA. It is also possible that these large proteins can sense DNA damage independently of other checkpoint Rad proteins [13]. Downstream of the checkpoint Rad proteins are a pair of protein kinases, Chk1 and Rad53p/Cds1p/CHK2 (Figure 2), which are responsible for activating or inhibiting a number of effectors of the DNA damage response, such as the Wee1, Mik1, Dun1 and Pds1 kinases, the Cdc25 phosphatase and p53 [11].

The CHK-2 protein identified by MacQueen and Villeneuve [7] is homologous to Rad53p/Cds1p/CHK2, a checkpoint protein that functions at several stages of the cell cycle in *S. cerevisiae* and mammals, and in the case of *S. pombe*, at the S phase checkpoint [12,14]. Curiously, the *C. elegans* CHK-2 protein appears to be dispensable for typical DNA damage checkpoint responses to  $\gamma$  irradiation or hydroxyurea, and though CHK-2 is required for the response to meiotic recombination intermediates induced by *rad-51* inhibition, it is unclear whether or not recombination actually initiates in *chk-2* mutants, where homologous chromosomes fail to pair in the first place [7].

In this light, it is noteworthy that an *S. cerevisiae* meiosis-specific version of CHK-2, Mek1p, is required both for detecting intermediates that result from incomplete meiotic recombination and for synapsis of homologous chromosomes, but not for chromosome pairing [15,16].

Mek1p, along with several core checkpoint proteins such as Rad17p, Rad24p, Ddc1p and Mec1p, is also required to ensure that homologous chromosomes recombine with each other rather than with their sister chromatids [17]. Thus, it seems possible that the CHK-2 protein identified by Villeneuve and MacQueen [7] might be more closely related to Mek1p than to Rad53p. Alternatively, given that orthologues such as *S. cerevisiae* Rad53p, *S. pombe* Cds1p and human CHK2 do not all act at the same time in the cell cycle (Figure 2), this protein family may have a significant degree of evolutionary plasticity, perhaps enough for the *C. elegans* protein to have acquired a role in meiotic chromosome pairing.

How might a *C. elegans* CHK-2 homologue act to bring two homologous chromosomes together? CHK-2 is likely to act by phosphorylating a regulatory protein, similar to the observed phosphorylation of BRCA1 or p53 by mammalian CHK2 in response to DNA damage [18,19]. Given that the *S. pombe* CHK-2 homolog, Cds1p, specifically monitors the S phase replication checkpoint, *C. elegans* CHK-2 might be involved in coupling the end of meiotic S phase with the onset of chromosome pairing. CHK-2 might negatively regulate chromosome pairing until the end of S phase, when DNA replication is completed and the chromosomes are competent to pair. Alternatively, *C. elegans* CHK-2 might be directly involved in organizing the chromatin of meiotic S phase into a form that is capable of pairing. This possibility is supported by the findings that the *S. cerevisiae* homolog, Rad53p, regulates chromatin remodeling in response to DNA damage [20,21], and that pairing and segregation of the fourth chromosome in female flies depend on heterochromatin [22]. Finally, CHK-2 might play an active role helping post-S-phase sister chromatids search for their homologues.

Is the role of CHK-2 in meiotic chromosome pairing generally dependent on DNA damage checkpoint proteins? Mutations in three other *C. elegans* checkpoint genes, at least one of which is conserved, have been shown to be required for canonical DNA damage checkpoint responses such as cell-cycle arrest and apoptosis [23,24]. However, these mutations fail to cause the strong non-disjunction phenotype observed for *chk-2* mutants [23,24]. Furthermore, an RNAi scan of *C. elegans* chromosome I failed to detect a non-disjunction phenotype for conserved DNA damage checkpoint genes such as H26D21.1 (*scDDC1* / *sphus1(+)* / hHUS1), Y48G1BL.2 (*scTEL1* / *sptel1(+)* / hATM) or T05F1.6 (*scRAD9* / *sprhp9p(+)* / h53BP1) [25]. Together, these results suggest that most *C. elegans* DNA damage checkpoint proteins are unlikely to be required for meiotic chromosome pairing or recombination. Thus, in order to regulate meiotic chromosome pairing, the signal that *C. elegans* CHK-2 responds to is either separate from, or redundant with, most upstream checkpoint Rad proteins.

Several observations do suggest, however, that the core checkpoint Rad proteins may be active during meiosis. Most checkpoint proteins display strong germline expression, probably in part to protect this special tissue, but these proteins may also help with the chromosome dynamics that occur in meiosis. In cases where it has been examined, mammalian checkpoint proteins like ATR, CHK1 and RAD1 have been observed coating unsynapsed prophase chromosomes, suggesting a possible role in chromosome pairing [26–28]. In addition, yeast strains defective for some checkpoint Rad proteins undergo ectopic recombination, genetic exchange between non-homologous chromosomes, a phenotype accompanied by defective synapsis [17,29]. We conclude that other members of this conserved checkpoint pathway are likely to act during meiotic prophase, despite being dispensable for the chromosome pairing activity of Chk-2 in *C. elegans*.

Further insight into the mechanism by which CHK-2 enables homologous chromosomes to pair awaits analysis of other *C. elegans* mutants with chromosome pairing defects [6]. Searching for direct targets of *C. elegans* CHK-2 may also prove illuminating. In addition, *C. elegans* genetics is providing advances in defining new DNA damage checkpoint genes, such as *rad-5* (A. Gartner, personal communication). Finally, protein interaction mapping of *C. elegans* DNA damage and repair proteins is underway, and in combination with phenotypic analysis following RNAi gene silencing, this approach has identified several novel DNA damage checkpoint and DNA repair proteins (S. Boulton and M. Vidal, personal communication). Although initiated with the hope of understanding a pathway that is often mutated in cancer cells, studies of the DNA damage checkpoint now hold some promise for a better understanding of meiotic chromosome pairing as well.

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