Review

Checkpoint mechanisms: the puppet masters of meiotic prophase

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The coordinated execution of cell cycle processes during meiosis is essential for the production of viable gametes and fertility. Coordination is particularly important during meiotic prophase, when nuclei undergo a dramatic reorganization that requires the precise choreography of chromosome movements, pairing interactions and DNA double-strand break (DSB) repair. Analysis of the underlying regulatory mechanisms has revealed crucial and widespread roles for DNA-damage checkpoint proteins, not only in cell cycle surveillance, but also in controlling many processes uniquely characteristic of meiosis. The resulting regulatory network uses checkpoint machinery to provide an integral coordinating mechanism during every meiotic division and enables cells to safely maintain an error-prone event such as DSB formation as an essential part of the meiotic program.

The coordination of meiosis

When cells divide, be it by mitosis to create two identical daughter cells, or by meiosis to create gametes with half the genome complement of the mother cell, they follow a highly choreographed program. This program involves the precise duplication of chromosomes and other cellular structures, their packaging and alignment with respect to the division plane and finally, their separation. For successful cell division, these processes must be carefully coordinated with one another. One way to achieve such coordination is through checkpoint mechanisms [1,2], which are signaling pathways that detect ongoing cell cycle events and relay this information to other (metabolically independent) processes, thereby establishing dependencies. When things go awry, checkpoint pathways act as surveillance mechanisms that halt cell cycle progression and activate repair responses. In addition, checkpoint mechanisms are also active during unperturbed cell cycles to couple individual cell cycle processes with one another. This latter function is often essential for cell viability [3,4] and becomes particularly apparent in a complex cell division such as meiosis, when a large number of cell cycle events need to be coordinated.

The production of haploid gametes by meiosis is a cornerstone of sexual reproduction. To create haploid cells from diploid progenitors, cells duplicate their DNA and then undergo two chromosomal divisions: homologous chromosomes segregate first (meiosis I), followed by the sister chromatids (meiosis II). During both divisions, the segregating partners of a chromosome pair must be physically associated with one another to ensure proper chromosome orientation in the division plane. Meiotic sister chromatids are connected through sister-chromatid cohesion, which is laid down concurrently with DNA replication, much as in mitotic cells [5]. By contrast, there are no such pre-existing linkages for homologous chromosomes. The function of the extended G2 phase of meiosis (generally referred to as meiotic prophase) is to create these connections.

To establish connections, meiotic prophase nuclei follow an elaborate program of chromosomal movements and structural rearrangements (Figure 1). At the DNA level, meiotic chromosomes undergo crossover recombination, which creates the physical links between homologous chromosomes on which most organisms rely for accurate meiosis I segregation. Meiotic recombination is initiated soon after DNA replication, via a large number of DNA double-strand breaks (DSBs), which are deliberately introduced by the conserved enzyme Spo11 [6]. Meiotic DSBs are directed to preferentially use the homolog rather than the sister chromatid as a template for crossover repair, ultimately leading to the formation of linkages between homologs, called chiasmata [7]. Meiotic recombination occurs alongside dramatic changes in the global organization of chromosomes within the nucleus: homologs typically begin meiosis unpaired, but by the stage at which recombination intermediates appear (Figure 1), chromosomes have identified and fortified an intimate association with their homologous partners, usually within the context of a highly structured protein lattice that assembles along the lengths of paired chromosomes, called the synaptonemal complex (SC) [8].

How are these major chromosomal processes coordinated during meiotic prophase so that, for example, recombination only occurs after DNA replication, SC assembles subsequent to successful homology verification, and DSB repair is completed before the nuclei divide? Perhaps not surprisingly, given the centrality of DNA breakage and repair during this stage of meiosis, components of the DNA-damage checkpoint machinery have emerged as key regulators of prophase chromosomal events. In this review, we attempt to highlight the functional repertoire of these signaling factors and, in so doing, emphasize both their surveillance and regulatory capacities in establishing order during meiotic prophase.

Operational definitions

Throughout this review, we use the term 'checkpoint mechanism' in its broadest sense, that is, a signaling mechanism that establishes dependencies between cell cycle events

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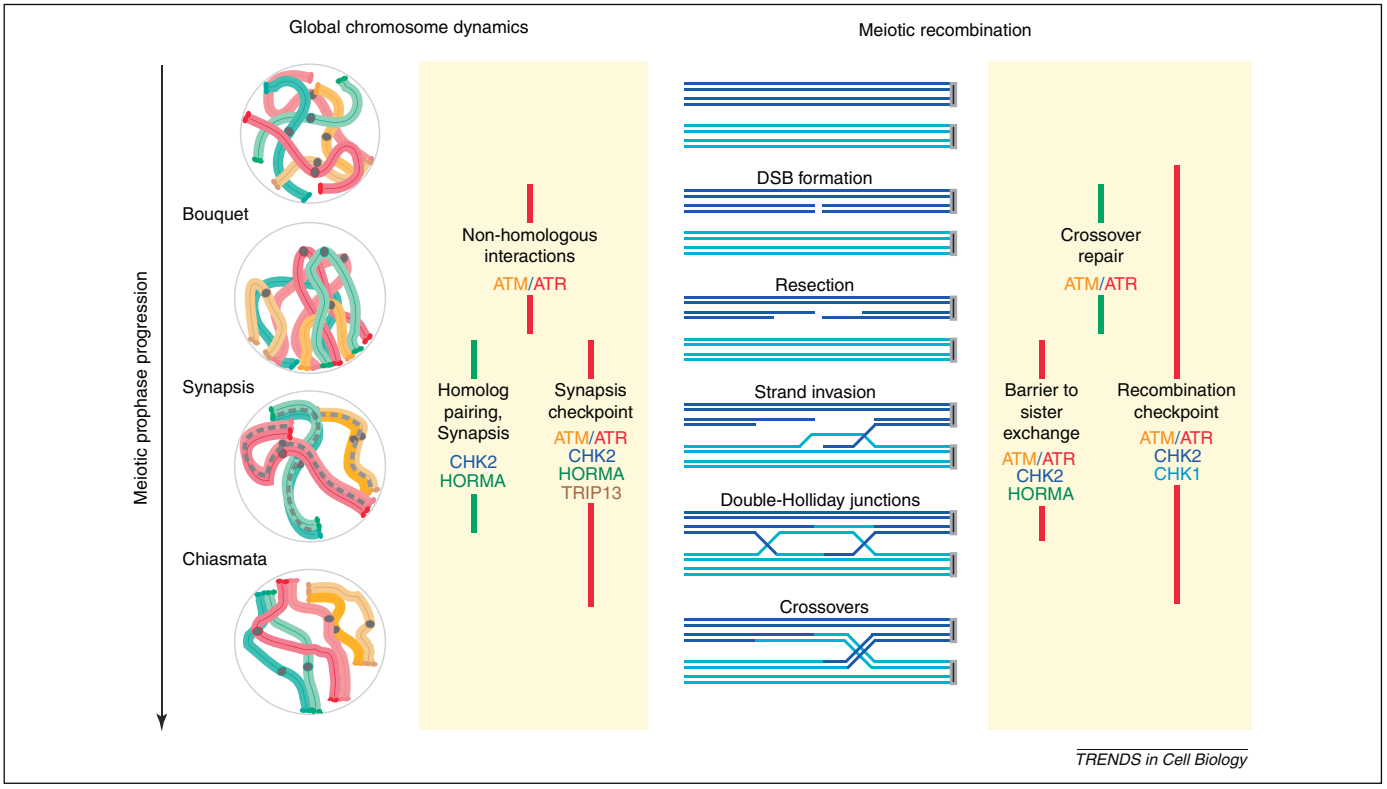

Figure 1. Checkpoint proteins mediate meiotic prophase chromosome dynamics.
Schematic of (left) the major chromosome reorganization and (right) the crossover-recombination events that occur within the meiotic prophase nucleus. The bar to the right of each cartoon depicts processes that represent either positive (green bar) or negative (red bar) regulation imposed by checkpoint proteins. The checkpoint factors that generally regulate these transitions are also indicated.

[1,2]. By this definition, the widely studied checkpoint responses to terminal chromosome damage (i.e. meiotic arrest and apoptosis) are a specialized function of the prophase checkpoint network. We reserve the use of 'recombination checkpoint' and 'synapsis checkpoint' to specifically refer to these terminal responses, in keeping with existing literature [9,10]. Conversely, we refer to the 'prophase checkpoint network' or the 'meiotic checkpoint machinery' when highlighting the coordination roles of meiotic checkpoints. Finally, because DNA-repair processes and chromosome metabolism occur at particular stages during meiotic prophase, they are considered cell cycle events by the above definition. To improve the clarity of interspecies comparisons, we refer to shared checkpoint factors by their closest human orthologs, and where necessary, indicate species-specific names in parentheses (orthology relationships are also indicated in Figure 2 and Table 1).

Detecting DSBs

In most organisms, the initial detection of DSBs relies on two related and highly conserved kinases, ATM (ataxia telangiectasia mutated) and ATR (ATM and Rad3-related) [11,12]. Unprocessed DSBs are first sensed by ATM, which binds to DNA ends with the help of the MRE11/RAD50/NBS1 (MRN) complex. This leads to further processing of break ends and the recruitment and activation of ATR (Figure 2). ATR is recruited to DSBs by the presence of replication protein A (RPA)-coated single-stranded DNA (ssDNA) through its activator ATR-interacting protein (ATRIP) [11,12]. Full activation of ATR requires the co-recruitment of the 9-1-1 complex (RAD9/RAD1/HUS1), which is independently loaded at ssDNA-duplex junctions. Once active, ATM and ATR phosphorylate a large set of substrates, including the checkpoint mediator kinases CHK1 and CHK2, to activate the DNA-damage response (Figure 2).

The role of ATM and ATR in the DNA-damage response appears largely similar between mitosis and meiosis [13,14]. However, it is noteworthy that, at least in budding yeast, meiotic cells might have the capacity to distinguish between Spo11-induced DSBs versus exogenous (Spo11-independent) DSBs, and initiate somewhat distinct checkpoint-signaling pathways. For example, the budding yeast CHK2 protein, Rad53, accumulates in a phosphorylated form in response to exogenous DSBs during meiosis, but not after Spo11-induced DSBs [15]. Furthermore, as outlined in more detail below, Spo11-induced DSBs not only initiate crossover recombination, but are also required for SC assembly between homologous chromosomes (synapsis) [8,16]. However, although exogenous DSBs can restore crossover recombination to yeast *spo11* mutants, they fail to trigger chromosome synapsis [17,18] (MacQueen and Rockmill, unpublished). These observations suggest that components of the DNA-damage checkpoint mechanism have been specifically adapted to meiosis, and therefore these surveillance mechanisms now regulate meiotic targets in response to meiotic triggers. An intriguing possibility is that Spo11, which becomes covalently linked to the ends of meiotic DSBs [6], directly modulates the

Table 1. Meiotic checkpoint proteins and their homologs[a]

Human	*S. cerevisiae*	*C. elegans*	*S. pombe*	*Drosophila*	Mouse	Function
Checkpoint-signaling proteins						
ATR	**Mec1**	**ATL-1**	**Rad3p**	**Mei-41**	Atr	PI3[b] kinase-like kinase
ATM	**Tel1**	ATM-1	Tel1p	Atm	**Atm**	PI3 kinase-like kinase
RAD9	**Ddc1**	**HPR-9**	Rad9p	Rad9	Rad9	PCNA-like clamp (9-1-1 complex)
RAD1	**Rad17**	**MRT-2**	Rad1p	Rad1	Rad1	PCNA[c]-like clamp (9-1-1 complex)
HUS1	**Mec3**	**HUS-1**	Hus1p	**Hus1**	Hus1	PCNA-like clamp (9-1-1 complex)
CHK1	(Chk1)	**CHK-1**	Chk1p	(Grp)	Chk1	Protein kinase
CHK2	Rad53, **Mek1**	**CHK-2**	**Cds1p**, **Mek1p**	**Mnk**	Chk2	Kinase with FHA[d] domain
HORMAD1, HORMAD2	**Hop1**	**HTP-1**, **HTP-3**	Hop1	-	Hormad1, Hormad2	Chromosomal HORMA-domain proteins
several	**Sir2**	**SIR-2**	Sir2p	**Sir2**	several	NAD-dependent deacetylase
TRIP13	**Pch2**	**PCH-2**	-	**Pch2**	(Trip13)	AAA$^+$-ATPase[e]
Signaling targets						
H2AX	**H2A**	-	H2A	**H2Av**	**H2AX**	Histone 2A variant
SUN1, SUN2	Mps3	**SUN-1**	Sad1p	-	Sun1, Sun2	Nuclear envelope proteins
SYCP1	**Zip1**	SYP-1	-	C(3)G	Sycp1	Central SC protein
CtIP	**Sae2**	COM-1	Ctp1p		CtIP	Nuclease component
p53	-	**CED-1**	-	**p53**	(p53)	Transcription factor

[a]Protein names in bold indicate checkpoint proteins whose role in meiotic coordination has been demonstrated experimentally, and names in parentheses indicate proteins for which experiments did not identify a role in meiotic coordination.

[b]Phosphoinositide 3-kinase

[c]Proliferating cell nuclear antigen,

[d]Forkhead-associated domain,

[e]ATPases associated with diverse cellular activities ATPase

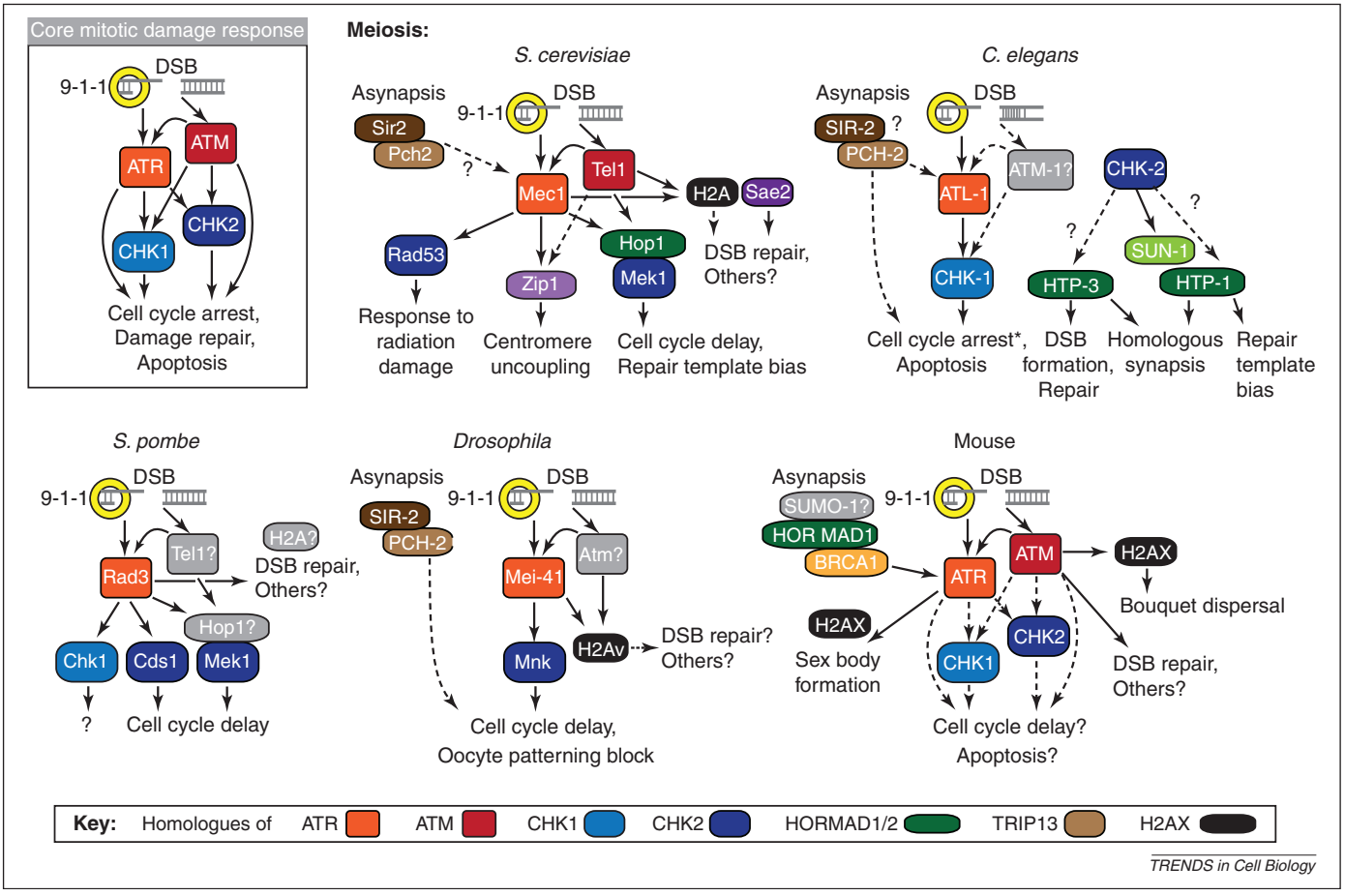

Figure 2. The role of DNA-damage checkpoint proteins in integrating meiotic prophase events is evolutionarily conserved.
Cartoons depict signaling pathways, most triggered by DSBs, during meiotic prophase. Color refers to orthologous proteins. Solid arrows represent a relationship that is well supported by data in the literature; dotted arrows reflect a speculative interaction, which might be supported by some published data but has not been rigorously proven. *Meiotic nuclei in the proliferative zone of the germline undergo arrest in response to unrepaired breaks, whereas unrepaired breaks trigger apoptosis during meiotic prophase.

response by the DNA-damage checkpoint machinery in meiosis.

Arrest and eliminate

Activation of ATM/ATR by unrepaired (Spo11-induced) DSBs leads to a delay in meiotic progression in many organisms, similar to the effect of the mitotic DNA-damage checkpoint response. This response is called the recombination checkpoint or pachytene checkpoint, and might provide extra time to complete DSB repair before the meiotic divisions. It also provides an opportunity to cull meiotic cells unable to complete DSB repair through apoptosis [9,10,19]. Interestingly, although the recombination checkpoint in most organisms relies on the activity of ATR and 9-1-1 (and to a lesser extent ATM) to sense persistent meiotic DSBs, organisms exhibit different specializations of the downstream recombination checkpoint-signaling pathways. For example, the recombination checkpoint-dependent cell cycle arrest in flies and yeast requires CHK2 (Mnk) or a meiosis-specific CHK2 homolog (Mek1), whereas CHK1 is dispensable (Figure 2) [9,20]. By contrast, CHK1 is necessary in the worm germline for p53 activation and apoptosis in response to the recombination checkpoint, whereas CHK2 has been adapted for other meiotic roles (see below) [21–23].

Perhaps connected to these specializations in signaling is the observation that the downstream effectors of the recombination checkpoint differ substantially between organisms (although they often achieve the same outcome, such as arrest of the meiotic cell cycle and a delay in the meiotic gene expression program). In *Schizosaccharomyces pombe*, the recombination checkpoint prevents cyclin-dependent kinase (CDK) activation and cell cycle progression through nuclear exclusion of the CDK-activating phosphatase Cdc25 [24]. The *Saccharomyces cerevisiae* recombination checkpoint also inhibits CDK, but this occurs predominantly through inhibition of the meiotic transcription factor Ndt80, which controls transcription of B-type cyclins [9]. Indeed, Ndt80 is a key checkpoint target in *S. cerevisiae* because it also controls expression of the majority of genes required for later meiotic development, including Polo-like kinase, another central cell cycle regulator [25]. In *Drosophila*, the recombination checkpoint was recently found to target the nucleosomal histone kinase NHK-1, thereby blocking meiotic prophase exit and nuclear restructuring [26]. Moreover, the *Drosophila* recombination checkpoint acts at least in part post-transcriptionally, by preventing translation of the oocyte-patterning factor Gurken [9,19].

This striking diversity of downstream targets might indicate that the control of meiotic cell cycle progression is not the ancestral function of ATR/ATM activation in meiosis. Indeed, some organisms, most notably the plant *Arabidopsis thaliana*, activate ATR/ATM in response to meiotic DSB formation, but have no apparent cell cycle delay if these DSBs remain unrepaired [27]. Similarly, in fission yeast, only the most severe repair defects elicit a detectable cell cycle delay [28]. Other organisms, including *Caenorhabditis elegans* and mice, exhibit strong sex-specific differences in their recombination checkpoint response [13,23]. Thus, the common denominator of checkpoint regulation during meiosis between organisms is not cell cycle arrest or apoptosis, but the activation of ATM/ATR. As outlined below, the near-universal meiotic activation of ATM/ATR is crucial for meiosis-specific DSB-repair outcomes and to stabilize interactions between homologous chromosomes. As has been previously noted (but perhaps not widely appreciated) [3,14,29], in this regulatory capacity, the DNA-damage checkpoint machinery functions as an integral, organizing component of every meiotic prophase.

The DNA-damage checkpoint machinery regulates meiotic DSB repair

Signaling how meiotic DSBs are to be repaired is a widely conserved meiotic function of DNA-damage checkpoint proteins. As in the mitotic DNA-damage response, this signal only initiates once DSBs have formed [12,14]. In some cases, the role of the meiotic checkpoint machinery appears to be a relatively general activation of DNA-repair functions. For example, mice or plants lacking ATM are strongly defective in meiotic DSB repair [30–32]. Similarly, in *S. cerevisiae*, ATR/ATM (Mec1/Tel1)-dependent phosphorylation of the DSB resection protein CtIP (Sae2) is important to promote homologous recombination [33,34]. In addition, checkpoint proteins often selectively affect specific meiotic DSB-repair processes. For example, in *C. elegans* mutants lacking ATR (ATL-1) or the 9-1-1 complex component HUS1, meiotic DSBs are repaired but chromosomes mis-segregate, suggesting that crossover formation is defective [23,35]. Similar effects occur in flies lacking HUS1 or p53 [36,37]. Crossover formation defects are also observed in mutants unable to remove ATM/ATR-dependent phosphorylation marks. Budding yeast or worms lacking protein phosphatase 4, the phosphatase needed to reverse many ATR-dependent phosphorylation events, exhibit severe crossover repair defects [38,39], suggesting that the dynamic interplay between checkpoint protein-dependent phosphorylation and dephosphorylation is important for crossover formation.

The meiotic checkpoint machinery probably functions at multiple levels to promote crossover repair. In *S. cerevisiae*, ATR (Mec1)-dependent phosphorylation of RPA (Rfa2) controls crossover distribution [40]. In addition, ATR also controls repair-template choice. The preferential use of a 'non-sister' chromatid belonging to a homologous chromosome (rather than the sister chromatid) as a template for DSB repair is a conserved feature of meiotic recombination. Mutants defective in ATR or 9-1-1 exhibit increased sister repair as well as otherwise aberrant recombination in a number of organisms [37,41–43], suggesting that the meiotic crossover-recombination defects exhibited by checkpoint mutants can often be attributed to a loss of repair bias.

The involvement of checkpoint-signaling components in sister repair bias is best understood in budding yeast, in which it involves the CHK2-like kinase Mek1 [44]. Mek1 is a component of meiotic chromosome axes, and requires the HORMA (Hop1p, Rev7p and Mad2)-domain protein Hop1 for dimerization and activation [20,45,46]. Hop1 function, in turn, depends on phosphorylation by ATM (Tel1) and ATR (Mec1) [47]. Once active, Mek1 autophosphorylates

and promotes homolog bias, partly through inhibitory phosphorylation of the conserved inter-sister repair factor Rad54 [45,48]. Histone H3 threonine 11 was also recently identified as a putative Mek1 target [49]. This latter finding is interesting because altering the chromatin state around a DSB site on the broken chromosome and its closely associated sister chromatid potentially provides a way to exclude the sister chromatid from participating in repair.

HORMA-domain proteins: adaptors for meiotic checkpoint protein signaling

Proteins homologous to the Hop1 family of HORMA-domain proteins have emerged as key regulators of meiotic chromosome behavior in many organisms. These proteins harbor a protein-interaction domain of approximately 200 residues, whose function remains poorly understood [4], and are integral components of meiotic chromosomes. Notably, HORMA proteins often simultaneously govern multiple meiotic processes. For example, Hop1 in budding yeast not only controls repair bias, but also modulates DSB initiation and recombination checkpoint activation [50] and analogous roles have recently been identified for mouse HORMAD1 [51]. Similarly, the combined action of the four *C. elegans* HORMA-domain proteins promotes meiotic DSB formation, interhomolog repair bias, homolog pairing, SC assembly, and meiotic prophase progression [52–54]. These observations suggest that HORMA-domain proteins provide central nodes for the coordination of meiotic processes.

An example of this coordinating function is the *C. elegans* HORMA-domain protein HTP-1, which couples homology verification and SC formation. Unlike in wild-type cells, which initiate chromosome synapsis only after matching homologous chromosomes have been aligned, SC formation occurs between non-homologous chromosomes in *htp-1* mutants, suggesting that HTP-1 triggers a 'wait' signal for synapsis progression until homology has been verified [52,53]. The release of this 'wait' signal appears to depend on signaling by CHK2 kinase: homologous chromosome pairing and SC assembly fail in *chk-2* mutants worms; however, if HTP-1 is also removed, then SC assembles despite an absence of homolog pairing [52]. Interestingly, recent data suggest a role for cytoskeletal-driven motion and force-dependent signaling in homology verification in *C. elegans*, raising intriguing parallels to the spindle-assembly checkpoint, a process that also involves a HORMA-domain protein (Mad2) [4,52,55].

A role in communicating chromosomal pairing behavior or synapsis during meiosis is also suggested by the chromosomal distribution of HORMA domain proteins. Mouse HORMAD1 and HORMAD2 localize specifically to unsynapsed chromosome axes during meiotic prophase [56], and Hop1 marks alternating chromosomal domains with the SC component Zip1 in budding yeast [57]. Similarly, HTP-1 disassembles to form mutually exclusive domains with the worm SC central region component, SYP-1, at the end of meiotic prophase [58]. Because many HORMA factors exhibit genetic or physical interactions with components of the DNA-damage checkpoint machinery [45,46,51,52,54], these observations suggest that HORMA-domain proteins provide a key interface to coordinate checkpoint signaling and chromosome behavior during meiosis.

Regulation of global chromosome dynamics

Signaling by the prophase checkpoint network also provides a direct link between DSB formation and chromosome dynamics. This control function is particularly evident in the case of organisms that require meiotic recombination initiation as a prerequisite for homologous chromosome synapsis, such as budding yeast, mammals and plants. It presumably aids the transition from early (often homology-independent) chromosomal interactions to *bona fide* homologous pairing associations. Homology-independent meiotic chromosome interactions are thought to help restrict the search space for homology identification, and include the pairwise association (coupling) of non-homologous centromeres and the clustering of telomeres (the 'bouquet' configuration) [59,60]. In budding yeast, ATR (Mec1)-dependent phosphorylation of centromere-localized Zip1 protein promotes dispersal of coupled centromeres [38], whereas in mice, ATM and its phosphorylation target histone H2AX are required for dispersal of the bouquet [61,62], provided that DSBs have formed [63]. Consistent with a role for checkpoint proteins in shifting these early interactions towards homologous association, *Arabidopsis* mutants lacking both ATM and ATR display increased non-homologous interactions between chromosomes [64], and a similar effect might also occur in yeast [42].

Perhaps the most dramatic example of checkpoint protein-dependent changes in nuclear dynamics occurs in the protist *Tetrahymena thermophila*. In this organism, meiotic prophase nuclei adopt an extremely elongated morphology (~50 times their normal diameter) that is thought to assist homologous chromosome pairing. This nuclear elongation occurs in response to DSB formation, and is crucially dependent on the activity of ATR [65].

DSB-independent signaling by the checkpoint machinery

DSB-activated ATM and/or ATR appear to play less prominent roles in regulating homologous chromosome pairing and/or SC assembly in organisms such as *C. elegans* and *Drosophila*, in which DSB formation, chromosome pairing and synapsis can occur independently of each other [66,67]. Interestingly, DSB-independent signals nevertheless activate checkpoint protein 'modules' that, in turn, play crucial roles in regulating meiotic chromosome dynamics in these organisms. Perhaps the best example of this is the *C. elegans* CHK-2 homolog, CHK-2 [68]. *chk-2* mutants display a remarkable phenotype: germ cells progress through meiosis, but recombination initiation, early meiotic chromosome clustering, chromosome pairing and SC assembly all fail [22], implicating CHK-2 as a 'linchpin' that couples the execution of these meiotic prophase events. The signals activating CHK-2 have yet to be revealed, as its activity in this context is independent of ATM (ATM-1) and ATR (ATL-1), (consistent with the fact that DSBs form downstream of CHK-2 [35,69]. CHK-2 presumably promotes pairing and synapsis through phosphorylating adaptor

proteins involved in the homology-verification step of meiotic prophase. One such CHK-2 target is the nuclear envelope protein, SUN-1; strains carrying alleles of *sun-1* that mimic CHK-2-dependent phosphorylation exhibit non-homologous synapsis, indicating an uncoupling of homologous pairing and SC assembly [69]. The HORMA-domain-containing protein HTP-3 has also been implicated in promoting DSB initiation, homolog pairing and synapsis initiation [54], raising the possibility that HTP-3 is a target of CHK-2 signaling in these pathways.

Interestingly, CHK-2 might also act downstream of DSB initiation in *C. elegans* meiocytes: In a *him-19* mutant, which displays many *chk-2*-like phenotypes (including failure to initiate meiotic DSBs), SUN-1 phosphorylation (which is normally CHK-2-dependent) is restored by ionizing radiation [69]. As a target of DSB signaling, CHK-2 could potentially contribute to coordinating recombination with synapsis progression.

The synapsis checkpoint

Certain defects in chromosome axis formation or aberrant SC assembly can trigger a delay in meiotic progression or apoptosis independently of DSB formation. This 'synapsis checkpoint' has been most clearly elucidated by studies in *C. elegans* and *Drosophila*, in which defects in homologous synapsis can be unambiguously assessed apart from defects in meiotic recombination [70,71]. Interestingly, despite its apparent DSB independence, the *C. elegans* synapsis checkpoint depends on some components of the DNA-damage checkpoint machinery, including the ATR/ATM target CHK1 (CHK-1) [72], and probably also requires ATR, although this has yet to be tested directly. An involvement of ATR/ATM would be remarkable because it would suggest that during meiotic prophase, features of chromosome structure other than DSBs could activate these kinases.

The nature of the signal sensed by the synapsis checkpoint is still unclear, but probably involves aspects of the meiotic chromosome interface. For example, the *C. elegans* synapsis checkpoint is dependent upon pairing centers, *cis*-acting elements located at a single end of every *C. elegans* chromosome, which mediate initial stable pairing between homologous chromosomes [70,73]. Moreover, both *Drosophila* and worms require the chromosome structure regulator TRIP13 (PCH-2) for synapsis checkpoint activation [70,71]. TRIP13 (PCH2) is also necessary for a cell cycle arrest response in a set of budding yeast mutants that exhibit defects in SC assembly (and recombination), suggesting that the synapsis checkpoint might also be active in this organism [74,75]. TRIP13 is a conserved AAA^{+}-ATPase that regulates HORMAD-protein distribution along meiotic chromosomes in yeast and mice [56,57]. In yeast and flies, the function of TRIP13 (Pch2) is controlled in part by the histone deacetylase SIR2 (although the exact mechanism might vary between organisms) and SIR2 (SIR-2) is also required for meiotic checkpoint regulation in *C. elegans* [23,76]. By modulating chromosome axis structure and HORMAD protein distribution, TRIP13 and SIR2 might facilitate the establishment or competency of a regulatory interface that can link chromosome structural intermediates to checkpoint signaling.

Several pieces of evidence indicate that a synapsis checkpoint might also be active in mice. Like *C. elegans* or flies, mouse *Spo11*$^{-/-}$ mutants, which are unable to properly pair and synapse meiotic chromosomes, mount a DSB-independent apoptotic response [29,77]. In addition, the checkpoint factors BRCA1, ATR and phosphorylated H2AX (γ-H2AX) accumulate on unsynapsed chromosome axes [78,79]. Consistent with a role for chromosome axes in signal generation, checkpoint signaling is impaired in mice lacking the meiotic axis component Sycp3 [78], and HORMAD1, which specifically localizes to unsynapsed chromosome axes, is required for ATM activation [51]. By contrast, to date, no checkpoint role for TRIP13 has been observed in mouse, although such a role might be obscured by the more prominent DNA-repair function of TRIP13 in this organism [80,81].

Sex body formation

A specialized signaling response by the meiotic prophase checkpoint network, triggered by unpaired/unsynapsed chromosomes, might also underlie sex chromosome inactivation in the mouse. During mouse spermatogenesis, the X and Y chromosomes are sequestered from the autosomes, and form the transcriptionally repressed and condensed sex body [82], which requires histone H2AX [81,83] and is characterized by abundant H2AX phosphorylation [82]. Moreover, the formation of robust γ-H2AX domains during spermatogenesis is strongly correlated with the recruitment of ATR to sex chromosomes by the DNA-repair factor BRCA1, suggesting that it is ATR-dependent [81,84,85]. Importantly, checkpoint factors, including ATM, ATR and γ-H2AX, localize to (unsynapsed) meiotic chromatin even in the absence of DSBs. However, in the latter situation, these factors frequently accumulate on autosomes rather than exclusively on the sex chromosomes [84,86].

The primary (DSB-independent) signal that recruits BRCA1 and activates ATR specifically on sex chromosomes remains unclear, although recent work revealed a role for HORMAD1 in this process [51]. Intriguingly, the productive accumulation of BRCA1 at mitotic DSBs depends on the small ubiquitin modifier (SUMO) E3 ligases PIAS1 and PIAS4 [87], and SUMO-1 accumulates on sex chromosomes prior to γ-H2AX [88]. A role for the protein modifier SUMO in ATR activation would be interesting, because SUMO is an abundant component of meiotic chromosome axes in a variety of organisms [89]. In principle, SUMO could regulate ATR as a component of the synapsis checkpoint.

Conclusion

A large body of work accumulated over the past several years indicates that the role of DNA-damage checkpoint proteins in meiotic prophase is substantially more complex than simply providing a mechanism for quality control. Rather, the prophase checkpoint network appears to be employed as a major integrating principle of meiotic prophase, which both responds to errors and drives the coordinated progression of meiotic DSB repair, chromosome dynamics and homologous synapsis. Meiotic chromosome morphogenesis has probably evolved to rely on DNA-damage checkpoint proteins because of their unique capacity to both recognize chromatin structures and interact with cell

cycle machinery. Moreover, some components of the meiotic prophase checkpoint network (in particular HORMA-domain proteins) are in proximity to DSB sites even before DSB formation. This arrangement might provide a fail-safe way for meiotic cells to ensure that whenever DSB formation is initiated, downstream pathways respond in a manner that will both repair the break and regulate prophase progression, while directing key meiotic prophase chromosome events.

Although the study of meiotic checkpoints has benefited dramatically from the use of different experimental systems, only a handful of meiotic checkpoint substrates have been discovered to date. In addition, it will be a major challenge to understand how the DNA-damage machinery integrates a variety of signals (some DSB-independent) to direct the appropriate chromosomal responses. Future progress will rely on even more diverse approaches, including modeling, genomics and live imaging, to fully grasp the checkpoint-dependent nuclear choreography that ensures the faithful transmission of genetic material to the next generation.

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