

## Meiotic roles of Mec1, a budding yeast homolog of mammalian ATR/ATM

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### Abstract

Budding yeast Mec1, a homolog of mammalian ATR/ATM, is an essential chromosome-based signal transduction protein. Mec1 is a key checkpoint regulator and plays a critical role in the maintenance of genome stability. Mec1 is also required for meiosis; loss of Mec1 functions leads to a number of meiotic defects including reduction in recombination, loss of inter-homolog bias, loss of crossover control, and failure in meiotic progression. Here we review currently available data on meiotic defects associated with loss of Mec1 functions and discuss the possibility that Mec1 may participate as a fundamentally positive player in coordinating and promoting basic meiotic chromosomal processes during normal meiosis.

### Introduction

Mec1 belongs to the evolutionarily conserved ATM/ATR family of signal transduction proteins whose members include mammalian ATR and ATM, *Drosophila* Mei-41, fission yeast rad3+ and tel1+, and budding yeast Mec1 and Tel1. They are chromosome-associated proteins, best known for their roles in the DNA damage response where they sense the presence of damage and transmit appropriate signals that trigger cell cycle arrest. Additionally, the ATM/ATR family of proteins play essential roles in the repair of DNA damage by regulating the activity of proteins that participate in the repair process. As expected, inactivation of these proteins confers sensitivity to a wide array of genotoxic agents (Zhou & Elledge 2000).

Interestingly, however, loss of Mec1, ATR, or ATM functions also leads to profound defects during the unchallenged life of the cell, indicating that their functions are required even in the absence of any

exogenously induced damage (Kato & Ogawa 1994, Xu *et al.* 1996). Because the nature of defects observed during unchallenged life is similar to those observed following exposure to DNA damaging agents (e.g. chromosome breakage, genome instability, and inviability), their normal functions were proposed to be analogous to those in damage response – i.e. in processing potentially lethal lesions that arise spontaneously during normal cellular life (Zhou & Elledge 2000). However, because this view could not adequately account for a number of *mec1*, *ATM*<sup>-/-</sup>, and *ATR*<sup>-/-</sup> phenotypes, an alternative model was proposed; in this model the primary function of ATM/ATR/Mec1 during unchallenged life is in coordinating and promoting basic chromosomal events, with the observed mutant phenotypes arising from defects in these fundamental processes regardless of any failure to respond to incidental lesions (Cha & Kleckner 2002).

The ATM/ATR family proteins also play essential roles during meiosis. In budding yeast, loss of Mec1

functions leads to defects in meiotic recombination (Kato & Ogawa 1994, Grushcow *et al.* 1999), chromosome morphogenesis (Xu *et al.* 1996, Grushcow *et al.* 1999), meiotic progression (Kato & Ogawa 1994) and checkpoint arrest (Lydall *et al.* 1996). Inactivation of Mei-41 in fruit flies leads to aberrant crossover distribution (Carpenter 1979). Spermatogenesis in *ATM*<sup>-/-</sup> mice is characterized by chromosome fragmentation and apoptosis (Barlow *et al.* 1996, Xu *et al.* 1996). Because (a) ATM/ATR family proteins are best known for their checkpoint functions and (b) hundreds of programmed double-strand breaks (DSB) are introduced into the genome as a part of the meiotic program, it has been proposed that the essential meiotic role of ATM/ATR family proteins is to monitor the repair process and to delay the onset of meiosis I until the repair is complete (Hochwagen & Amon 2006). However, as is the case for Mec1's essential role during the mitotic cell cycle, it is possible that Mec1 could play a more fundamental role during meiosis. Here we review the nature of the meiotic defects observed in *mec1* mutants and discuss how they could be accounted for in terms of failure in coordinating and promoting basic chromosomal events.

### **Mec1: the functional homolog of mammalian ATM and ATR**

Inactivation of the mammalian ATM or ATR confers phenotypes that are distinguishable from one another at both the cellular and organism level. For example, whereas ATR inactivation in mice is embryonic lethal (Brown & Baltimore 2000), *ATM*<sup>-/-</sup> mice develop pathological conditions during later stages in life (Barlow *et al.* 1996, Xu *et al.* 1996). At the cellular level, *ATR*<sup>-/-</sup> and *ATM*<sup>-/-</sup> cells show sensitivity to different genotoxic agents; *ATR*<sup>-/-</sup> cells are more sensitive to those inducing stalled replication forks or intra-strand crosslinks whereas *ATM*<sup>-/-</sup> cells are more sensitive to those that induce DSB (McGowan & Russell 2004). The ATM and ATR pathways do not appear to function completely independently of each other, and crosstalk between the two has been observed under certain conditions. For example, in facilitating a G2/M checkpoint arrest in response to DSB, ATM mediates the initial processing of the break-ends to generate replication protein A(RPA)-coated single-stranded DNA

(ssDNA), which is required for ATR recruitment and the subsequent phosphorylation and activation of Chk1 (Jazayeri *et al.* 2006, Cuadrado *et al.* 2006).

The budding yeast genome has two ATM/ATR family members, Mec1 and Tel1. In general, Mec1 is considered to be the counterpart of ATR, and Tel1 that of ATM. These correspondences are partly based on structural considerations (e.g. Perry & Kleckner 2003) as well as on the observation that certain functions carried out by ATR are carried out by Mec1 (e.g. responding to stalled replication forks and UV damage) whereas certain other functions are primarily carried out by ATM and Tel1 (McGowan & Russell 2004). Like mammalian ATR, Mec1 is an essential protein (Kato & Ogawa 1994). Various hypomorphic alleles of *mec1* confer nearly all cellular phenotypes of *ATR*<sup>-/-</sup> mutants consistent with the view that Mec1 and ATR are involved in the same cellular processes. Interestingly, *mec1* mutants also exhibit defects that are primarily associated with the loss of ATM function (e.g. responding to DSB; Melo *et al.* 2001); thus in yeast, Mec1 is also carrying out (at least some of) ATM functions. Indeed, although inactivation of Tel1 confers some phenotypes such as telomere shortening and a relatively small increase in gross chromosomal recombination (Greenwell *et al.* 1995), *tell1Δ* mutants, by large, proliferate with growth kinetics that are indistinguishable from WT (Sanchez *et al.* 1996). *tell1Δ* strains also exhibit WT resistance to different types of DNA-damaging agents (e.g. Morrow *et al.* 1995), indicating that the cell's repair and checkpoint functions are largely intact. Taken together, these observations suggest that budding yeast Mec1 is primarily responsible for carrying out the functions of both ATR and ATM, and that Tel1 plays a limited role. However, we cannot rule out the possibility that Mec1 and Tel1 each have a specialized set of functions, but that Mec1's roles are more differentiated in that Tel1 is unable to substitute for Mec1, whereas Mec1 can complement the functions of Tel1 effectively.

Mec1 and Tel1 show the same target specificity (below). When tested *in vitro* they phosphorylate many of the same proteins, including Dun1, Xrs2, and RPA (Mallory *et al.* 2003). Further, in all cases examined, inactivation of both Mec1 and Tel1 is required to completely abolish damage-dependent phosphorylation of their targets (e.g. Flott & Rouse 2005, Cartagena-Lirola *et al.* 2006), suggesting a

certain degree of functional overlap and/or redundancy. Nevertheless, the fact that loss of Mec1 alone is sufficient to confer lethality demonstrates that Tel1, when expressed at the physiological level, is unable to carry out the essential functions of Mec1. Since *tell1Δ* strains proceed normally through meiosis to generate viable spores (J. Carballo, N. Hashash & R. Cha unpublished), for the purpose of discussing normal meiotic functions, Mec1 will be considered to be the functional homolog of both ATM and ATR.

### Targets of Mec1

The ATM/ATR family of proteins are serine/threonine kinases. They preferentially phosphorylate their substrates on serine or threonine residues that precede glutamine residues, so-called SQ/TQ or S/TQ motifs. Many known targets of the kinases contain SQ/TQ cluster domains or, in short, 'SCD' (Traven & Heierhorst 2005).

One consequence of phosphorylation is regulation of the interaction of modified proteins with other proteins. Some protein-protein interactions depend on SCD phosphorylation at specific sites, whereas others occur when a certain threshold level of phosphorylated SQ/TQ motifs is achieved. In all cases examined, the phospho-threonines, rather than the phospho-serines, of phosphorylated SCD function as binding sites for the forkhead-associated (FHA) domains of other proteins. A certain level of promiscuity exists in phosphorylated SCD- and FHA domain-mediated interactions – e.g. the Rad53 (Chk2 family of kinases) SCD can interact with the FHA domain of Dun1 and Rad9, as well as the FHA domain of other Rad53 (Traven & Heierhorst 2005).

Mec1 phosphorylates proteins involved in a wide array of cellular and chromosomal processes. Targets of Mec1 include proteins required for checkpoint arrest (Rad9, Schwartz *et al.* 2002, Ma *et al.* 2006), DNA replication (RPA, Bartrand *et al.* 2005), fork stability (Mrc1, Osborn & Elledge 2003), DNA repair/recombination (Sae2, Flott & Rouse 2005, Cartagena-Lirola *et al.* 2006), and chromosome structure (H2A, Downs *et al.* 2000). Additionally, *in-vitro* protein CHIP analysis identified Ax12, Hog1, and Ptk2 as high-to-moderately specific substrates of Mec1, and histone H1, Hgl1, Mek1, Rad9, Red1, Swi6, and Tub4 as low specificity substrates (Zhu *et al.* 2000). The nature of the mammalian ATM and

ATR targets also reflects their functions. The targets identified to date include proteins involved in checkpoint regulation (Rad9, CHEK2), DNA repair/recombination (BRCA1, Mre11A, BLM [budding yeast Sgs1]), and chromosome structure ( $\gamma$ H2AX, Smc1) (Kim *et al.* 1999, Traven & Heierhorst 2005).

Mammalian ATM is also a phospho-protein being phosphorylated at Ser1981 in response to DSB, stalled forks, and general changes in chromosome structure (Bakkenist & Kastan 2003, Stiff *et al.* 2006). Although initially characterized as an auto-phosphorylation event (Bakkenist & Kastan 2003), ATM phosphorylation at Ser1981 can also occur in ATR-dependent (and ATM-independent) manner (Stiff *et al.* 2006). In general, phosphorylation of ATM is thought to be essential for its activation at the cellular level. Currently there is no evidence for a similar requirement for phosphorylation of ATR, Mec1, or Tel1 for their activation. In this regard it is noteworthy that ATM contains the highest number of SQ/TQ motifs, many of which occur in clusters. The numbers of SQ/TQ motifs present over the entire length of each protein are: ATM (28/3066), ATR (16/2635), Mec1 (9/2324), and Tel1 (12/2787).

All *mec1* mutations that abolish its kinase activity show a *mec1-null* phenotype (Paciotti *et al.* 2001). Similarly, over-expression of an *ATR-kd* (kinase dead) allele confers many defects associated with ATR inactivation (Cliby *et al.* 1998). These observations indicate that kinase activity is essential for their functions.

### Roles of Mec1 during meiosis

Meiosis is a specialized cell division program during which a single round of genome duplication is followed by two successive rounds of chromosome segregation, resulting in the halving of the genome. The meiosis-specific part of the program is the period leading up to the first chromosome segregation during which homologs rather than sister chromatids segregate. In most organisms, including budding yeast and mammals, accurate disjunction of homologs requires the generation of hundreds of DSB catalyzed by Spo11 and the repair of these breaks using one homolog to create crossovers between each pair of homologs; such crossovers provide the necessary physical connection between each homolog pair at the time of chromosome segregation. Analogous

to the mitotic cell cycle program, the meiotic program has the regulatory network poised to trigger arrest in response to exogenously induced DNA damage as well as aberrations in the processing of Spo11-induced DSB. Based on the nature of defects responsible for triggering checkpoint response and the specific stage at which cells remain arrested, programmed arrests or delays during meiosis are referred to as ‘pachytene-’, ‘*rad50S*-’, ‘recombination-’, or ‘damage-’ checkpoint arrests (Hochwagen & Amon 2006). In all cases examined, cells delay or prevent the onset of meiosis I and remain in prophase I; for simplicity we will refer all conditions that lead to programmed arrest/delay prior to meiosis I as triggering ‘prophase checkpoint’ when discussing meiotic checkpoint roles of Mec1/Tel1.

*MEC1* (mitosis entry checkpoint gene 1), also known as *ESR1* (essential for recombination), was cloned by two independent research groups based on its roles in preventing mitosis in the presence of unrepaired DNA damage (Weinert *et al.* 1994), and as a gene required for conferring MMS resistance (Kato & Ogawa 1994). Kato & Ogawa examined the meiotic roles of *ESR1/MEC1* and observed an induction of its expression during meiosis, reaching maximum levels (about 20 times the mitotic level) in pachytene. The *esr1-1* allele isolated also exhibited defects in meiotic recombination and progression. Subsequent studies characterizing various *mecl*-null strains (e.g. *mecl1Δ*, *mecl1-kd*, or *mecl1-1* [different from *esr1-1*] strains maintained viable by *sml1*, a suppressor mutation) confirmed their meiotic roles, as viability of the resulting spores is reduced to 30–50% compared with > 90% for *MEC1* (e.g. Lydall *et al.* 1996, J. Carballo, N. Hashash & R. Cha unpublished).

Since Mec1 is a central player in mediating checkpoint responses, the observed spore inviability in *mecl* mutants could stem from the inability to mount an appropriate ‘checkpoint’ response following the induction of programmed meiotic DSB. On the other hand, Mec1’s primary role during the unchallenged life of the cell could be coordination of fundamental chromosomal processes (Cha & Kleckner 2002), and *mecl* spore inviability could have stemmed from failure to regulate essential meiotic events. Below, we present a case for the latter possibility by reviewing the available data from the viewpoint that Mec1 may be an essential component of basic meiotic chromosomal processes.

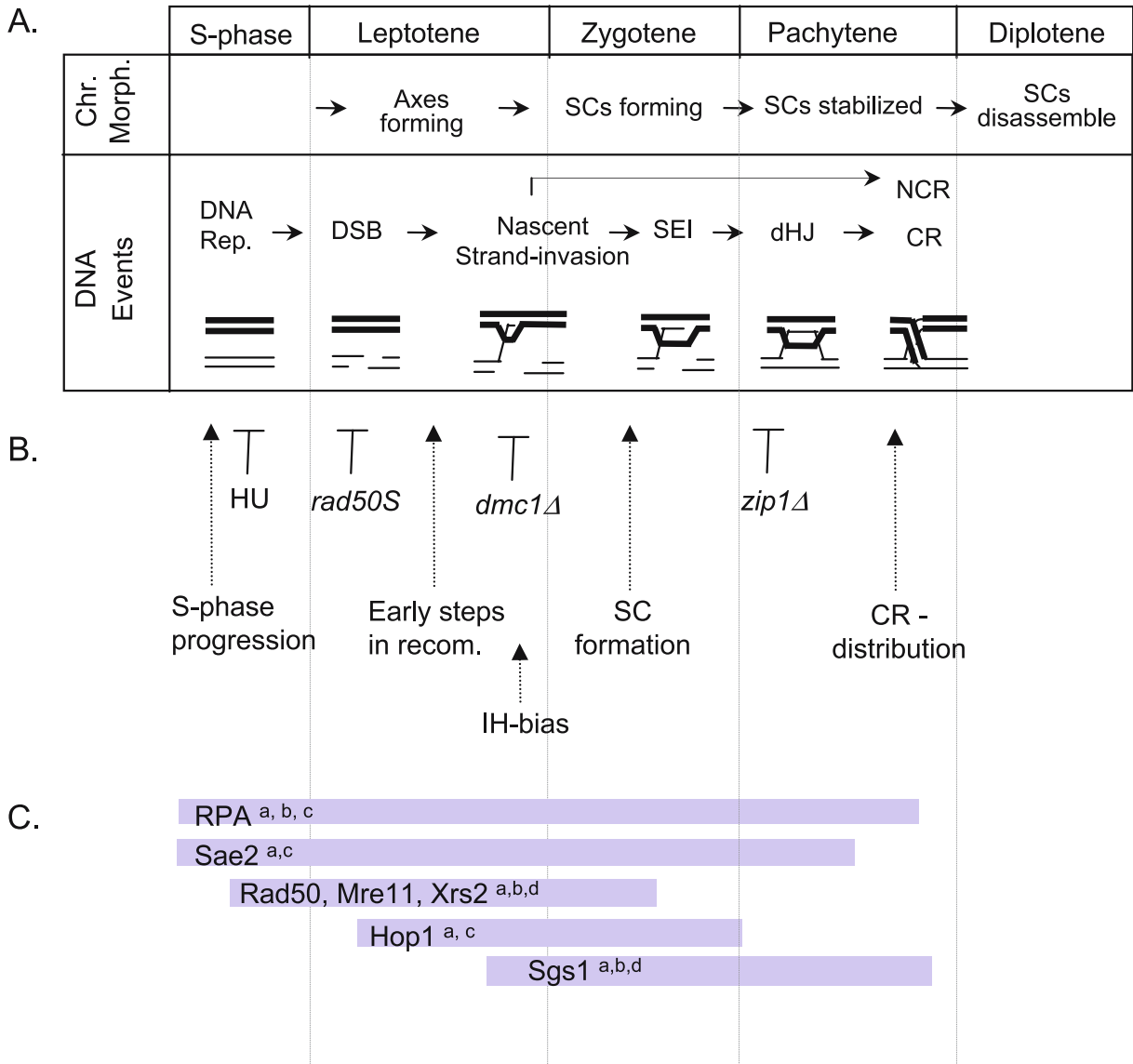
The checkpoint role of Mec1 is also discussed, but only briefly, as an in-depth review on the matter has recently been published (Hochwagen & Amon 2006).

#### *Mec1 is an essential checkpoint regulator during challenged meiosis*

During mitotic cell cycle progression, Mec1 mediates G1/S and G2/M checkpoint arrests in response to DNA-damaging agents (Weinert *et al.* 1994, Siede *et al.* 1996). It is also required for S-phase arrest triggered by accidentally stalled replication forks (Sogo *et al.* 2002). Similarly Mec1 is required for the prophase arrest induced by mutations (e.g. *rad50S*, *dmc1Δ*, *zip1Δ*) that affect the processing of Spo11-catalyzed DSB (Lydall *et al.* 1996) as well as for hydroxyurea (HU)-induced meiotic S-phase checkpoint arrest (Borde *et al.* 2000). In all cases examined (e.g. HU, *rad50S*, *dmc1Δ*, or *zip1Δ*), inactivation of Mec1 leads to efficient progression through meiotic divisions and generation of inviable spores (Hochwagen & Amon 2006; Figure 1).

The nature of DNA aberrations triggering S-phase arrest in HU-treated cells is likely to be the same during both mitotic and meiotic S-phases – i.e. the persistence of RPA-coated single-stranded (ss) DNA, which results from the uncoupling of leading and lagging strand DNA synthesis on accidentally stalled replication forks (Sogo *et al.* 2002). The signal sensed by Mec1 in *dmc1Δ* mutants is also likely to be similar to that sensed during the mitotic G2/M checkpoint triggered by the presence of unrepaired DSB – i.e. the persistence of RPA-coated ssDNA following resection of the 5’ end of DSB (Pelliccioli *et al.* 2001, Lisby *et al.* 2004). Prophase arrest in *zip1Δ* mutants is interesting in that the arrest occurs despite the fact that DSB disappear with an apparently normal kinetics (e.g. Borner *et al.* 2004). The nature of aberration responsible for triggering the checkpoint following Zip1 activation is currently unclear.

Curiously, Zip1 physically interacts with Psy2, a component of the phosphatase complex known as HTP-C (histone H2A phosphatase complex) (Ito *et al.* 2001). HTP-C regulates recovery from DNA damage checkpoint arrest by dephosphorylating H2A, a key target of Mec1/Tel1 (Keogh *et al.* 2005). Similarly, dephosphorylation of  $\gamma$ H2AX by protein phosphatase 2 (PP2) is required for recovery from damage checkpoint arrest in mammals (Chowdhury *et al.* 2005). Involvement of PP1



**Figure 1.** Meiotic roles of Mec1. **A:** DNA and chromosomal events during meiotic prophase I. Chr.Morph: chromosome morphology, CR: crossover, NCR; non-crossover, SEI: single-end invasion, dHJ: double Holliday junction, SC: synaptonemal complex. Schematically depicted in the ‘DNA event’ are the recombination intermediates involved in the formation of CR. Commitment to the CR or NCR pathways takes place after the nascent strand invasion step, and the majority of NCR arise independently of dHJ (e.g. Borner *et al.* 2004). **B:** Mec1 is a key checkpoint protein during challenged meiosis. It is essentially required for arrests induced by HU, *rad50S*, *dmc1Δ*, and *zip1Δ* (reviewed in Hochwagen & Amon 2006). During normal meiosis, Mec1 is likely to play a critical role in promoting or regulating the key meiotic chromosomal processes. The fundamentally positive nature of Mec1 function(s) during unchallenged meiosis is based on the observations that inactivation of Mec1 leads to a block in meiotic progression rather than faster transit (see text). Molecular mechanisms underlying inter-homolog bias, SC development, and crossover distribution are still unclear; the positions of arrows along the meiotic time-line simply denotes Mec1’s involvement in these processes rather than marking the precise execution point. **C:** Confirmed (or likely) meiotic targets of Mec1. The nature of evidence in each case is as indicated (**a:** genetics data in yeast, **b:** genetics data in mammals, **c:** biochemical data in yeast, **d:** biochemical data in mammals). The width of rectangle denotes either the period during which the function of each protein is required and/or the period during which phosphorylation of each protein is observed.

(protein phosphatase I) in *dmc1Δ* checkpoint adaptation during yeast meiosis has also been reported (Hochwagen *et al.* 2005). Taken together, these observations raise an intriguing possibility that Zip1 (and/or the status of synaptonemal complex [SC]) may regulate checkpoint recovery during yeast meiosis. The notion that SC polymerization plays a role in sensing interchromosomal entanglements and triggering an appropriate response has been presented previously (Kleckner *et al.* 1991, Bishop *et al.* 1992).

Essential components of the Mec1/Tel1 damage checkpoint pathway include Rad24, the budding yeast '9-1-1 complex' (Rad17/Mec3/Ddc1), Rad9, and Rad53. Rad24 and Rad17/Mec3/Ddc1 are considered to be acting upstream of Mec1, whereas Rad9 and Rad53 function as downstream effectors (Zhou & Elledge 2000). Available evidence suggests that the upstream components are required for all Mec1/Tel1-mediated meiotic checkpoint responses; the requirement for Rad9, on the other hand, appears to depend on the nature of defect(s) responsible for triggering the arrest (Hochwagen & Amon 2006). The potential involvement of Rad53 in meiotic checkpoint regulation has not been directly examined; however, it is likely that at least some of its functions are carried out by Mek1, the meiosis-specific paralog of Rad53 (Usui *et al.* 2001, Hochwagen & Amon 2006).

Dissecting the checkpoint roles of Rad24, Rad17, Mec3, and Ddc1 has been complicated by the fact that these proteins are required not only for checkpoint response signaling but also in the process of recombination or SC formation (Shinohara *et al.* 2003, Wu & Burgess 2006). For instance, elimination of Rad17 leads to defective processing of meiotic DSB, which in itself triggers a checkpoint response (Wu & Burgess 2006). It is noteworthy that meiotic phenotypes conferred by *mec1*, *rad24*, and *rad17* are not identical (Lydall *et al.* 1996, Wu & Burgess 2006) raising the possibility that these proteins carry out functions that might be independent of one another during meiosis.

#### *Mec1 functions as a positive regulator of normal meiotic progression*

Meiotic prophase (i.e. the period between the completion of the genome duplication and the first meiotic division) in budding yeast (SK1 background) is about 2 h long. This meiotic prophase duration of 2 h

coincides with the time it takes to process the Spo11-induced breaks into the final crossover recombination products (e.g. Padmore *et al.* 1991), raising the possibility that the status of ongoing meiotic recombination is monitored, and that the onset of meiosis I is coupled to the completion of DSB repair. Given the essential nature of Mec1 in triggering prophase arrest in *dmc1Δ* and *zip1Δ*, it is reasonable to propose that Mec1 plays similar roles during normal meiosis in monitoring the processing of Spo11-catalyzed DSB and that defects in this process lead to the observed reduction in spore viability.

If Mec1 functions as a negative regulator of meiotic progression delaying the onset of meiosis I until the completion of DSB repair, inactivation of Mec1 should lead to a faster transit through meiosis, similar to that observed in *rec-* mutants (above); *mec1-1 sml1* strains, however, proceed through meiosis with WT kinetics with regard to meiotic divisions (e.g. Lydall *et al.* 1996, Borde *et al.* 2000). Although this 'unexpected' observation could be due to Tel1 compensating for Mec1's normal function, the existence of *mec1* alleles that confer a meiotic arrest phenotype, even in the presence of a fully functional *TELI*, argues against this notion. For example, the original mutant isolated by Kato & Ogawa, *esr1-1*, exhibits a progression defect; following transfer into sporulation medium, *esr1-1* cells undergo meiotic DNA replication but only 2% of the cells proceed to form spores (Kato & Ogawa 1994). We have also independently isolated alleles of *mec1* that fail to form spores (R. Cha unpublished). Finally, sporulation efficiency in *mec1Δ tel1Δ sml1Δ* mutants is also reduced below 20% (J. Carballo, N. Hashash & R. Cha unpublished). This apparent failure to progress through meiosis associated with loss of Mec1 functions suggests Mec1 has a positive, rather than negative, role in promoting meiosis. Presented below are essential meiotic DNA and chromosomal processes and the potential involvement of Mec1 in each.

#### *Meiotic S-phase progression*

Meiotic S-phase is several times longer than the mitotic counterpart in all organisms examined to date. In budding yeast (SK1 background), mitotic- and meiotic-S phases are about 20 min and 60–80 min, respectively (Williamson *et al.* 1983, Cha *et al.* 2000). The lengthening of S-phase during meiosis is

proposed to reflect the extra time needed for developing additional chromosomal features that are later utilized for meiosis-specific chromosomal metabolism (Holm 1977). Consistent with this notion, the lengthening of meiotic S-phase is dependent on the establishment of DSB-independent homolog pairing mediated by Spo11 (Cha *et al.* 2000).

Mec1 is essentially required for replication fork progression through genetically specified replication slow zones (RSZ) during unchallenged mitotic S-phase (Cha & Kleckner 2002). This essential role of Mec1 is bypassed by mutations that result in higher intracellular dNTP levels (Zhao *et al.* 1998, Cha & Kleckner 2002), suggesting that Mec1 may regulate fork progression by coupling fork processivity to the availability of dNTP in the cell. With regard to a possible physiological relevance for such programmed fork slowing and the subsequent Mec1-regulated progression, it was proposed that Mec1 might be playing a role in coupling ongoing DNA replication to the replication-dependent chromosome feature development – e.g. sister chromatid cohesion (Uhlmann & Nasmyth 1998), installation of nucleosomes (Shibahara & Stillman 1999), and meiosis-specific chromosome modification (Wu & Lichten 1994, Ohta *et al.* 1998, Cha *et al.* 2000). More specifically, Mec1 would monitor the installation of key features in newly replicated regions, and (a) promote progression of nearby replication forks, and (b) activate late firing origins when these events have been completed.

A number of observations are consistent with the notion that Mec1 is a central regulator of the basic S-phase program. These include: (a) Rad53, a key downstream target of Mec1, interacts physically with Asf1 – Asf1 is a component of the replication-dependent chromatin remodelling complex, which is needed for proper completion of DNA synthesis during normal S phase (Le *et al.* 1997); (b) Mec1/Rad53 regulates the timing of late origins activation during normal S-phase (Shirahige *et al.* 1998); (c) Mec1 regulates fork progression through RSZ (Cha & Kleckner 2002); (d) Mre11, a known genetic interactor of Mec1/Tel1, and a confirmed target of ATM in mammals, promotes S-phase-specific chromatin remodeling during meiosis (Ohta *et al.* 1998, Kim *et al.* 1999, Nakada *et al.* 2004); and (e) the mammalian sister chromatid cohesin molecule Smc1 is a target of ATM/ATR (Kim & Kastan 2002, Yazdi *et al.* 2002).

We have isolated a *mec1* allele that confers defects in meiotic S-phase progression (J. Cauwood & R. Cha unpublished). Further, *mec1Δ tel1Δ sml1Δ* cells are unable to progress through meiotic S-phase (J. Carballo & R. Cha unpublished). Since these mutants can proliferate, they presumably undergo relatively normal mitotic DNA replication. Thus, the meiotic S-phase problem in *mec1* mutants is unlikely to stem from a failure in regulating dNTP pools, responding to spontaneously stalled replication forks, or from other ‘usual’ challenges associated with the genome duplication in general. Given that additional meiosis-specific chromosomal features are laid down during meiotic S-phase (Wu & Lichten 1994, Ohta *et al.* 1998, Murakami *et al.* 2003) and that they are required for the timely transit through S-phase, a differential requirement for Mec1 in meiotic S-phase progression may reflect its involvement in these processes. It will be interesting to examine potential genetic interactions between *mec1* and meiotic chromosomal proteins known to affect meiotic S-phase progression – i.e. Spo11, Rec8, and Mre11 – in regard to meiotic S-phase progression and establishment of replication-dependent chromosome features.

#### Meiotic recombination

The original *esr1-1* allele confers a reduction in both intra- and inter-genic meiotic recombinational events (Kato & Ogawa 1994). In budding yeast, such overall deficit in meiotic recombination could stem from either the reduction in the amount of breaks generated and/or failure to process DSB into recombination products. For instance, the recombination defect in *hop1Δ*, *red1Δ* or *mek1Δ* is accompanied by a significant reduction in the amount of breaks generated (e.g. Schwacha & Kleckner 1997, Xu *et al.* 1997), whereas that observed in mutations in the *RAD52* epistasis group stems from defects in processing Spo11-catalyzed DSB (e.g. Schwacha & Kleckner 1997). Currently available evidence points to the latter possibility as a mechanism underlying the observed *esr1-1* phenotype; a potential link between Mec1(Tel1) and DSB formation has not yet been reported.

Although the context in which meiotic breaks arise (i.e. programmed vs accidental) and the specific mechanisms involved are different, the basic mechanisms of repairing DSB are conserved during mitotic and meiotic programs (Paques & Haber

1999). In general, all of the players in mitotic DSB repair participate in the repair of meiotic DSB, but with a number of meiosis-specific adaptations that facilitate the eventual formation of crossovers between homologs (e.g. Kleckner 1996).

The repair of Spo11-catalyzed DSB begins with the enzymatic removal of Spo11 from the break ends followed by nucleolytic resection of the 5'-strand to generate 3'-single-stranded tails (Neale *et al.* 2005). These tails are coated first by RPA, followed by Rad51 and Dmc1 (a meiosis-specific RecA protein) and participate in the homology search and strand invasion (Paques & Haber 1999). Many proteins become localized to the site of breaks including Mre11 and the rest of the recombinational repair machinery (e.g. *RAD52* epistasis group) (Lisby *et al.* 2004). A number of chromosome structural and repair proteins become phosphorylated during this process (Downs *et al.* 2004, Cartagena-Lirola *et al.* 2006). Since Mec1 (or ATM/ATR) plays a central role in the localization and phosphorylation of many of these proteins (e.g. RPA, H2A, Mre11, Smc1, and Rad55) the observed reduction in meiotic recombination could easily be due to a failure to promote (early steps of) DSB repair.

#### *Inter-homolog bias*

In contrast to mitotic recombination where DSB are repaired utilizing a sister chromatid, meiotic recombination takes place between homologs. In budding yeast the repair of meiotic DSB occurs with an estimated 5-fold bias toward inter-homolog events (Schwacha & Kleckner 1997). Although the molecular mechanism underlying establishment and enforcement of the inter-homolog bias is unclear, proteins involved in recombination (e.g. Dmc1, Mer3, and Msh5), chromosome structure (e.g. Hop1, Red1, and Zip1), and a regulatory kinase (Mek1) have been shown to be required (Schwacha & Kleckner 1997, Niu *et al.* 2005).

In *mec1-1 sml1* mutants, recombination events between homologs are observed at reduced rates; this reduction is accompanied by an increase in ectopic recombination. The observed loss of inter-homolog bias is not a secondary effect of inappropriate progression (e.g. compromising inter-homolog recombination by premature separation of homologous chromosomes), but is likely to reflect Mec1's involvement in regulating recombination partner

choice (Grushcow *et al.* 1999). Thompson & Stahl also isolated Mec1 as a protein whose inactivation leads to increased recombination between sister chromatids during meiosis, presumably at the expense of inter-homolog events (Thompson & Stahl 1999).

Recently, we identified Hop1 as a meiosis-specific target of Mec1 (J. Carballo, A. Johnston & R. Cha unpublished). Hop1 is an evolutionarily conserved protein required for multiple meiotic processes. One of its functions, together with Red1 and Mek1, is to ensure that meiotic DSB are repaired via the Dmc1-mediated (i.e. inter-homolog) pathway (Schwacha & Kleckner 1997, Niu *et al.* 2005). Dmc1 is an evolutionarily conserved meiosis-specific RecA homolog and is essential for meiotic inter-homolog recombination (Bishop *et al.* 1992, Gasior *et al.* 2001). Thus, characterization of Mec1/Tel1 dependent phosphorylation of Hop1 may provide a plausible mechanism by which Mec1 participates in enforcing inter-homolog bias.

#### *Homolog synapsis*

The synaptonemal complex (SC), a proteinaceous structure, comprising two lateral elements and a central element, mediates the intimate synapsis between the homologs during meiotic prophase I. SC formation begins during the leptotene to zygotene transition, is completed by pachytene, and becomes dissolved by diplotene (Figure 1). The SC is observed in most organisms including budding yeast, plants, worms, and mammals, where intimate homolog synapsis mediated by the SC is essential for normal meiosis (Page & Hawley 2004, Zickler 2006). Status of SC development can be assessed by immunolocalization of either the central or the lateral components of the SC; in budding yeast, Zip1, a component of the central element, is typically utilized.

In yeast, one manifestation of aberrant SC development is the appearance of large structures called polycomplexes (PC), which stain brightly with anti-Zip1 antibody. PC appear in addition to or instead of the extended structures typical of normal meiosis, especially in mutants with defects in synapsis. As expected, eliminating genes that encode for the lateral (e.g. Red1 and Hop1) components of the SC leads to the appearance of extensive PC. Additional proteins required for normal SC development include those involved in (a) basic chromosome structure



(e.g. Rec8), (b) the generation of DSB (e.g. Spo11), and (c) the processing of meiotic DSB (e.g. Dmc1, Msh5) (Page & Hawley 2004, Zickler 2006).

Inactivation of Mec1 has been linked to the formation of PC (Grushcow *et al.* 1999). Although this could be an indirect consequence of an earlier problem (e.g. in recombination), it is also possible that Mec1 plays a more direct role in SC development by phosphorylating a relevant chromosome structure protein(s). The observations that (a) Mec1/ATM/ATR interact both genetically and physically with chromosome structure proteins (e.g. Asf1, Smc1, CAF, and H2A; reviewed in Koundrioukoff *et al.* 2004); and (b) ATM can be activated by conditions that alter chromatin structure in the absence of DNA damage (Bakkenist & Kastan 2003) highlight a potential interplay between chromosome structure and ATM/ATR family proteins. Also relevant in this regard is our recent identification of Hop1 as a Mec1 target (J. Carballo, A. Johnson & R. Cha unpublished). As mentioned above, Hop1 is essentially required for SC formation, being a component of the lateral element. An attractive possibility would be that Mec1 participates in SC development by regulating Hop1 activity.

#### Regulation of crossover distribution

In budding yeast, meiotic recombination begins with the formation of approximately 300 Spo11-catalyzed DSB; at the end of the process each pair of homologs ends up with at least one but no more than a few crossovers. A similar numerical relationship between the number of breaks and crossovers exists for meiotic recombination in mammals. In many organisms, including budding yeast, worms, fruit flies, and mammals, the distribution of crossovers in the genome is highly regulated in that: (a) every pair of homologs acquires at least one crossover, and (b) when two or more crossovers occur between the same pair of homologs, adjacent ones are located further apart than expected from a random distribution (e.g. Jones 1987, Carpenter 1988). These two features of crossover distribution are referred to as 'obligatory crossover' and 'crossover interference', respectively. The molecular mechanisms governing the distribution of crossovers are not known. However, mutations that confer defects in chromosome structure development, recombination, and homolog pairing have been shown to weaken crossover interference.

Involvement of Mec1 in crossover distribution has been examined by Bartrand *et al.* (2005) where the authors investigated the consequences of altering Mec1-dependent phosphorylation sites in RPA. The RPA serine-to-aspartate (S122D) mutation significantly altered the pattern of meiotic crossover events for certain genetic intervals, suggesting that Mec1-mediated RPA phosphorylation can influence the mechanism of crossing over during meiosis in a region-specific manner (Bartrand *et al.* 2005). Another yeast gene shown to affect meiotic crossover recombination is Sgs1, a RecQ helicase homolog. Inactivation of Sgs1 leads to a moderate to a dramatic increase in crossover recombination events depending on the strain background, indicating that Sgs1 has potent meiotic anti-crossover activity (Rockmill *et al.* 2003, Jessop *et al.* 2006). Interestingly, the mammalian homolog, BLM, is a confirmed target of ATM (Beamish *et al.* 2002). In yeast, Mec1 and Sgs1 function synergistically in suppressing gross chromosomal aberrations (Cobb *et al.* 2005), and Mec1 is required for the localization of Sgs1 to a damage-induced multi-protein complex (Chiolo *et al.* 2005). Further, a few of the proteins involved in crossover regulation contain an unusually high number of SQ/TQ motifs including Msh5 (8/901) and Exo1 (10/702). It will be interesting to examine whether they are targets of Mec1 and, if so, whether Mec1-dependent phosphorylation regulates crossover distribution. Finally, it is noteworthy that one of the original defects reported for the fly *mei-41* mutant is defects in crossover regulation (Carpenter 1979).

#### Summary

Here, we presented meiotic defects of *mec1* mutants from the perspective that the observed phenotype stems from failure to regulate and promote essential chromosomal events. The complex nature of the *mec1* phenotype suggests that its function(s) is likely to be required for a number of different meiotic processes such as S-phase progression, recombination, inter-homolog bias, and chromosome synapsis (Figure 1). Although a number of Mec1/Tel1 and ATM/ATR targets have been identified, the focus thus far has been on those involved in checkpoint regulation and DNA repair in response to exogenously induced DNA damage. Identification and characterization of meiosis-specific targets of Mec1/

Tell during normal meiotic progression should provide invaluable information regarding Mec1's essential meiotic function(s).

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