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Modulating Mek1 kinase alters outcomes of meiotic recombination and the stringency of the recombination checkpoint response

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Summary

Background—During meiosis, recombination between homologous chromosomes promotes their proper segregation. In budding yeast, programmed double-strand breaks (DSBs) promote recombination between homologs versus sister chromatids by dimerizing and activating Mek1, a chromosome axis-associated kinase. Mek1 is also a proposed effector kinase in the recombination checkpoint that arrests exit from pachytene in response to aberrant DNA/axis structures. Elucidating a role for Mek1 in the recombination checkpoint has been difficult since in *mek1* loss-of-function mutants DSBs are rapidly repaired using a sister chromatid thereby bypassing formation of checkpoint-activating lesions. Here we tested the hypothesis that a *MEK1* gain-of-function allele would enhance interhomolog bias and the recombination checkpoint response.

Results—When Mek1 activation was artificially maintained through GST-mediated dimerization, there was an enhanced skew toward interhomolog recombination and reduction of intersister events including multi-chromatid joint molecules. Increased interhomolog events were specifically repaired as noncrossovers rather than crossovers. Ectopic Mek1 dimerization was also sufficient to impose interhomolog bias in the absence of recombination checkpoint functions, thereby uncoupling these two processes. Finally, the stringency of the recombination checkpoint was enhanced in weak meiotic recombination mutants by blocking prophase exit in a subset of cells where arrest is not absolute.

Conclusions—We propose that Mek1 plays dual roles during meiotic prophase I by phosphorylating targets directly involved in the recombination checkpoint as well as targets involved in sister chromatid recombination. We discuss how regulation of pachytene exit by Mek1 or similar kinases could influence checkpoint stringency, which may differ among species and between sexes.

Keywords

meiosis; recombination checkpoint; homologous chromosome; sister chromatid

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Introduction

Meiosis is a specialized process of cell division whereby one round of DNA replication is followed by two rounds of chromosome segregation to produce gametes for sexual reproduction. During meiotic prophase I, programmed recombination and synapsis facilitate the formation of interhomolog crossovers that promote proper disjunction of homologous chromosomes at the first meiotic division (MI; [1-3]). Recombination and synapsis culminate in the late prophase stage known as pachytene, which is defined cytologically by the side-by-side alignment of condensed homologous chromosomes connected by the synaptonemal complex (SC) [4,5]. In budding yeast the structural axis of each homolog is formed by Red1, Hop1 and Mek1 and the central region of the synaptonemal complex is made of Zip1 [6-11]. Notably, pachytene is the last stage of meiotic prophase before cells become committed to undergo MI [12,13].

Meiotic recombination is a step-wise process initiated by Spo11-induced double-strand breaks (DSBs) and implemented using DNA repair factors within the context of meiotic chromosome architecture [14-16]. A subset of DSBs is repaired by a designated interhomolog crossover pathway that transits through single-end invasion (SEI) and double-Holliday Junction (dHJ) intermediates [17-21]. Remaining breaks are repaired using a sister chromatid as a template for repair or by interhomolog repair resulting in noncrossovers (i.e. events detected as a gene conversion without exchange of flanking markers) that arise from a synthesis-dependent strand annealing mechanism [18,22-24]. Finally, there is a minor class of crossovers that are dependent on Mus81/Mms4 [25]. Physical associations between sister chromatids can be detected as well. At least a subset of intersister recombination events involves a dHJ intermediate [17]; multi-chromatid joint molecules, (mcJMs) arise during normal meiosis when two ends of a DSB independently invade different chromatids and/or sequentially invade multiple templates, however they can lead to nonregulated crossing over and missegregation if not processed [26].

The presence of unrepaired DSBs and/or incomplete synapsis activates a recombination checkpoint response that leads to inhibition of Ndt80, a transcription factor required for the exit from pachytene [27,28]. Cells arrest at a pachytene-like stage with compacted chromatin [29]. Repair of DSBs leads to the activation of Ndt80, which then allows for the resolution of dHJs to form crossover products, and finally the completion of MI [18,20]. Mek1 is a proposed downstream effector kinase of the Mec1/Tell (ATR/ATM) signaling that is activated in response to Spo11-induced DSBs [30,31]. Activation of Mek1 requires the C-terminal domain of Hop1 which itself is a target of Mec1/Tel1 [31,32]. Unlike other checkpoint mutations that allow division in the presence of unrepaired DSBs [33-41], the *mek*1Δ mutation acts as a bypass suppressor of the prophase I arrest phenotype conferred by *dmc1*Δ by allowing repair of Spo11 1-induced breaks using the sister chromatid as a substrate [42]. For this reason, the role of Mek1 as a *bona fide* checkpoint protein has comes into question. The role for Mek1 in modulating interhomolog bias is well substantiated; in wild-type cells, activation of Mek1 leads to the phosphorylation and inactivation of Rad54, which is required for sister chromatid recombination [43]

Here we describe a semi-dominant, gain-of-function allele, *MEK1-GST* (similar to that described by Niu et al., 2005 [32]) that confers phenotypes not previously described: i) A net gain of interhomolog events that is coupled to a net loss of intersister events, including intersister-dHJ and multi-chromatid joint molecules; ii) increased levels of interhomolog-noncrossover recombination products that are not associated with either increased DSBs or a change in interhomolog-crossover products; iii) a hyper-barrier to intersister repair; and iv) increased stringency of the output of the recombination checkpoint pathway. Our data support a model in which Mek1 plays dual roles during meiosis I prophase: one is to

promote interhomolog bias and second is to act as a checkpoint effector that controls exit from pachytene.

Results

MEK1-GST is a gain-of function-allele

A conserved sequence at the C-terminus of the Mek1 is implicated in the homodimerization/activation of Mek1 [44]. Here we analyzed the effect of artificial dimerization at the C-terminus of Mek1 in meiotic recombination and checkpoint control via a fused GST moiety similar to that described for an N-terminal *GST-MEK1* fusion created by Niu et al., 2005 [32]. As a control, we created an allele *MEK1-GST(nd)* that is mutated at two residues to prevent GST dimerization. In this case, the dimerization of Mek1-GST(nd) is presumably under the control of Hop1, just as for wild-type Mek1 [32].

We analyzed Mek1-GST and Mek1-GST(nd) protein at various time points during meiotic progression in a synchronized cell culture. Both fusion proteins appeared at about the same time (3 hours after transfer of cells to sporulation medium) by immuno-blot using an antibody to GST (Figure 1A). Mek1-GST protein levels were greater and persisted longer compared to Mek1-GST(nd). Similar results were found using two different antibodies to GST including one that recognizes an epitope outside of the dimerization domain (data not shown). The Clb5 cyclin, which is normally degraded prior to the meiosis division (MI), also persisted in the *MEK1-GST* background. MI timing was delayed in *MEK1-GST* compared to wild type *MEK1* and *MEK1-GST(nd)* strains (Figure 1B and 1C). The MI delay was semi-dominant since *MEK1-GST/MEK1* heterozygous strain exhibited an intermediate delay. The *MEK1-GST* induced delay requires *SPO11* and *RED1*, suggesting that Mek1-GST kinase activities require an intact chromosome axis structure (Figure S1A). Spore viability of *MEK1-GST* and *MEK1-GST(nd)* strains was similar to wild type (Table S1). Taken together, *MEK1-GST* exhibits phenotypes consistent with it being a semi-dominant gain-of-function allele of *MEK1*.

The *MEK1-GST* allele behaves similarly to *GST-MEK1* described by Niu et al., 2005 and Niu et al., 2007: Both alleles give nearly wild-type levels of sporulation and spore viability (Table S1) [32,44]. Moreover, phosphorylation of the T327 residue for both GST-Mek1 and Mek1-GST is dependent on genes required for DSB formation including *RED1* and *SPO11* (Figure 1D). Notably, we are not able to distinguish whether the phenotypes associated with *MEK1-GST* are due to an increase in the persistence of a signal that is lower or more transient in wild-type cells or if it creates a toxic gain-of-function effect. We favor the former since *MEK1-GST* gives wild-type levels of spore viability (96.1% versus 96.5%; Table S1).

Repair of DSBs is delayed in *MEK1-GST*

To explore the effect of the *MEK1-GST* allele on modifying outcomes of meiotic recombination, we analyzed the physical intermediates and products of recombination at the *HIS4LEU2* hot spot locus (Figure 2A; [19]). We found that steady-state levels of DSBs and slower migrating joint molecule species were greater in the *MEK1-GST* strain compared to *MEK1-GST(nd)* (Figure 2B). Consistent with this finding was the observation that crossover formation, one outcome of DSB repair, was delayed in *MEK1-GST* compared with *MEK1-GST(nd)* (Figure 2C). We next wanted whether or not the increase in steady-state levels of DSBs corresponded to an increase in their formation. We used a *sae2*Δ mutant background, in which DSBs are not turned over since their resection and repair is blocked. We found that DSB levels were similar in *MEK1-GST sae2*Δ and *sae2*Δ mutants suggesting that the higher levels of breaks are due to their slower turnover (Figure S2A, S2B). We also measured break

levels in a *dmc1* mutant background, in which breaks form and are resected but are unable to use a homolog as a substrate for repair [1-3]. DSB levels in *MEK1-GST dmc1*Δ strains were slightly reduced compared to the *dmc1*Δ single mutant, perhaps due to the inability to detect hyper-resected products by this assay (Figure S2A). Overall these data show that while DSBs form at wild type levels in *MEK1-GST*, their turnover is delayed.

MEK1-GST gives a net gain of interhomolog events that is coupled with a net loss of intersister events compared to WT

Since loss of *MEK1* function is associated with loss of interhomolog bias, we reasoned that the gain of function mutation, *MEK1-GST* might exhibit delayed turnover of DSBs due to a prolonged period of interhomolog recombination. Since crossover levels in *MEK1-GST* are not increased compared to wild type, we tested the possibility that excess interhomolog events are repaired as noncrossovers. For this test we used a strain carrying a *HIS4LEU2* allele variant in which both crossover and noncrossover levels could be measured in the same population of cells [26,45]. Interestingly, we found that noncrossover levels in *MEK1-GST* were two-fold greater than in wild type, while crossover levels were unchanged (Figure 3A-D).

We reasoned that the increase in noncrossover products could arise from breaks that would otherwise have been repaired using a sister chromatid as a template. We tested whether or not intersister events, including both intersister- dHJ and multi-chromatid joint molecules (mcJM), were reduced in *MEK1-GST* compared to wild type by using two-dimensional gel electrophoresis (Figure 4A) [26, 46]. This analysis was carried out in an *ndt80*Δ mutant background, which arrests prior to the exit from pachytene and prevents turnover of the majority of joint-molecule species to final products. In this way, the absolute levels of these intermediates can be directly compared [18, 26, 47]. Indeed, we observed that intermediates of DNA repair events involving sister chromatids were reduced in *MEK1-GST* compared to wild type, with a greater loss of mcJMs compared to IS-dHJ. By contrast, the total levels of intermediates that give rise to interhomolog crossover products, including SEIs and dHJs were the same in *MEK1-GST* compared to wild type (Figure 4B, 4C, 4D). Together, these results suggest that *MEK1-GST* promotes interhomolog recombination events at the expense of intersister recombination. Moreover, the increased level of interhomolog interactions is specifically directed to noncrossover products, suggesting that double-strand breaks are designated for repair as crossovers prior to the activation of *MEK1-GST* by ectopic dimerization. These data also indicate that the increased steady-state levels of JM seen by 1-D Southern blot analysis (Figure 2A) is due to slow turnover of JM into downstream products.

The MI delay conferred by *rad17*Δ or *pch2*Δ single mutants is exacerbated when MEK1-GST is present

Both Rad17, a component of mitotic DNA damage checkpoint, and Pch2, an AAA-ATPase like protein, are involved in checkpoint surveillance during meiosis [33-35,39,40]. Single mutants of *rad17*Δ and *pch2*Δ exhibit an MI delay, presumably because each mutant generates a lesion that activates the other's checkpoint function [39]. We tested the epistatic relationship between the delay conferred by *MEK1-GST* and that of either *rad17*Δ or *pch2*Δ. We found that *MEK1-GST rad17*Δ and *MEK1 pch2*Δ strains were further delayed compared to any of the three single mutants (Figure S1B). Spore viability of *MEK1-GST rad17*Δ was increased compared to *rad17*Δ (63% versus 34%) as were crossover levels measured at a late time point, albeit not to wild-type levels (Table S1,Figure S1C). Spore viability of the *MEK1-GST pch2*Δ double mutant remained high, just as in the *pch2*Δ single mutant (Table S1) even though fewer cells of the *MEK1-GST pch2*Δ genotype sporulated. Crossover levels were substantially reduced but this is likely due to the severe MI delay observed for the

double mutant (Figure S1C). T327 phosphorylation of Mek1-GST occurs in both *rad17*Δ and *pch2*Δ single mutant strains (Figure 1D). The MI delays conferred by either *rad17*Δ or *pch2*Δ are bypassed by *mek1*Δ (Figure S1D, S1E). Since *rad17*Δ and *pch2*Δ function in different processes associated with recombination as well as checkpoint signaling, interpretation of the double-mutant phenotypes must be made cautiously [33,34,39,40,48-51]. Minimally, these data indicate that the delay conferred by *MEK1-GST* is not simply due to the activation of either the Rad17 or Pch2 dependent checkpoint pathways. Below we address the interaction of *MEK1-GST with* the *rad17*Δ *pch2*Δ double mutant where both recombination checkpoint functions are absent.

MEK1-GST promotes interhomolog bias in the absence of both *RAD17*- and *PCH2*-dependent checkpoint functions

We showed previsously that the *rad17*Δ *pch2*Δ double mutant progresses rapidly through MI, produces reduced levels of crossover products and gives < 1% spore viability. The double mutant is also defective in checkpoint signaling since MI divisions still occur in mutant strain backgrounds that accumulate unrepaired DSBs [39]. We reasoned that if the *MEK1-GST* imposed delay was due the activation of the recombination checkpoint then the *MEK1-GST rad17*Δ *pch2*Δ triple mutant would divide with the same kinetics as the *rad17*Δ *pch2*Δ double mutant. To our surprise, the *MEK1-GST rad17 pch2*Δ triple mutant gave a delayed MI phenotype compared to *rad17*Δ *pch2*Δ (Figure 5A) and spore viability was increased from < 1% to 47% (Table S1). This suppression of spore inviability in the *rad17*Δ *pch2*Δ was semi-dominant since spore viability in *MEK1-GST/MEK1* heterozygous background was ~29% (Table S1). Physical analysis of DSB repair in the *MEK1-GST rad17*Δ *pch2*Δ triple mutant indicated that DSB turnover was slower, yet crossover products were elevated (Figure 5B, 5C and 5D). These results indicate that *MEK1-GST* can uphold interhomolog bias even in the absence of the recombination checkpoint.

We ruled out possibility that another DNA damage checkpoint was activated in the *MEK1-GST rad17*Δ *pch2* Δ triple mutant. First, we found that the addition of the *dmc1*Δ mutation to the triple mutant background did not lead to MI arrest, as would be expected if another checkpoint function were activated. In fact, *dmc 1*Δ *MEK1-GST rad17 Apch2*Δ MI kinetics were indistinguishable from the *MEK1-GST rad17*Δ *pch2*Δ triple mutant (Figure 6A, 6B). Second, we showed that these cells divided even though DSBs were not fully repaired as indicated by physical analysis at the *HIS4LEU2* hot spot (Figure 6C). In addition, DAPI stained chromosomes in the quadruple mutant background were fragmented (data not shown), consistent with the presence of unrepaired breaks.

MEK1-GST increases the stringency of the meiotic recombination checkpoint response

The delay phenotype conferred by *MEK1-GST* suggests that it may be influencing the regulation of MI division, independent of upstream signaling functions. We next wanted to see what effect *MEK1-GST* would have in mutants that exhibit checkpoint mediated delay, but eventually go on to divide. As shown previously, deletion of *ZIP1*, *NDJ1* and *CSM4* results in checkpoint-mediated MI delay yet the majority of cells ultimately divide, albeit with low spore viability (Figure 7 A, 7B and 7C; Table S1) [52-54]. Interestingly, the *MEK1-GST zip1*Δ double mutant exhibited near complete MI arrest, unlike the single mutants (Figure 7A). For the *MEK1-GST ndj1*Δ and *MEK1-GST csm4*Δ double mutants, MI division timing was further delayed compared to either single mutant and a greater fraction of cells failed to undergo the MI division, however crossover levels were not affected (Figure 7B and 7C; Figure S3A and S3B).

We next asked if the effect of *MEK1-GST* on MI delay/arrest in these double mutant situations was due to a synergistic defect causing an accumulation of recombination

intermediates that in turn led to a persistent checkpoint response. We measured DSB formation and repair at the *HIS4LEU2* locus in *zip1*Δ and *MEK1-GST zip 1*Δ mutants and found that DSBs were efficiently repaired in both situations (Figure 7D). We ruled out the possibility that the failure to detect breaks is due to hyper-resection of 5′ ends by analyzing full-length chromosome fragments using clamped homogenous electric field (CHEF) gel analysis. There was no smear that would indicate unrepaired, hyper-resected DSBS in *MEK1-GST zip1*Δ (Figure S3C). arrest conferred by *MEK1-GST* is not due to the failure to repair DSBs, as far as our limits of detection can discern. Together, these results suggest that once Mek1-GST is dimerized/activated, it is less able than wild type to deactivate and shut off an inhibitory affect on MI division, even when checkpoint-activating lesions have been repaired.

Strikingly, compared to each single mutant, *MEK1-GST* rescued the spore inviability patterns conferred by *ndj1* Δ (75% versus 90%) and *csm4*Δ (64% versus 87%; Table S1). The *zip1* Δ *MEK1-GST double* mutant gave no spore products for analysis. For *ndj1* Δ and *csm4*Δ mutants, we classified tetrads into four categories according to the number of viable spores per tetrad (Figure 7E). While all aberrant classes were suppressed to some extent in the presence of the *MEK1-GST* allele, 2:2 and 0:4 classes were significantly reduced in the *ndj1*Δ and *csm4*Δ backgrounds. Combined, these results suggest that *MEK1-GST* selectively suppresses MI division in *ndj1*Δ or *csm4*Δ cells that were unsuccessful in the maturation of chromosome structures that facilitate meiosis I disjunction.

Discussion

Our findings provide insight into the roles of the chromosome axis associated protein Mek1 in meiotic recombination and in regulating the exit from meiosis I in budding yeast. We show evidence that Mek1 kinase has multiple targets that include proteins involved in suppressing sister chromatid recombination as well as those that function to regulate the exit from pachytene. We found that ectopic Mek1 dimerization impacted events of meiosis I prophase in two ways: (i) by enhancing interhomolog bias, perhaps through the prolonged or premature activation of a Mek1 target substrate such as Rad54 [43] and (ii) by inhibiting the re-entry into the cell cycle following checkpoint-mediated arrest in certain mutant situations, perhaps through the persistent phosphorylation of a protein that regulates the exit from pachytene.

Crossover/noncrossover designation may be imposed prior to or concomitant with Mek1 dimerization

An outstanding problem in the field of meiosis has been to define the point among the myriad chromosomal processes when DSBs are designated for crossover repair versus noncrossover repair. Crossover designation is thought to occur at early stages of meiosis I prophase prior to formation of SEI joint molecule formation [17-21], however there is evidence that single-stranded 3′ ends of DSBs may sample a number of substrates, including sister chromatids, prior to being committed for repair as a crossover [26]. It is conceivable then that crossover designation occurs prior to or concomitant with Mek1 dimerization, after which point the remaining breaks would be repaired as either noncrossovers or using sister chromatids, with the former being favored due to Mek1 dimerization and/or activation. This model would predict that artificially prolonging the period of interhomolog bias or premature activation of Mek1 might result in an increase in noncrossover products and a decrease in the levels of intersister joint molecules, with no change in crossover levels; the *MEK1-GST* allele confers this same constellation of phenotypes.

Interestingly, the loss of intersister interactions observed in the presence of Mek1-GST comes largely from a pool of mcJM species, which are aberrant products involving three or

four chromatids that occur in an unchallenged meiosis [26]. Our findings using *MEK1-GST* suggest that Mek1 antagonizes mcJM formation, as is also the case for Sgs1 [26].

Sheridan and Bishop have postulated that disassembly of axial constraints at the end of prophase could activate the “clean-up” of residual DSBs via intersister recombination [55]. This constraint might be mechanistically tied to the active state of a Mek1 target substrate and the slow turnover of DSBs that we observe. If this constraint were prolonged due to an artificially maintained active state of Mek1, more breaks may end up being repaired via interhomolog noncrossover recombination rather than using a sister chromatid.

Our finding that *MEK1-GST* preserves crossover levels, even when total interhomolog events are increased is reminiscent of “crossover homeostasis”. Crossover homeostasis has been observed in situations when the number of crossovers per chromosome remains constant even when the total number of breaks is reduced, as in a *spo11* hypomorph [45,56]. Our results indicate that the converse is also true; when the total number of interhomolog events per chromosome is increased, as in the case of *MEK1-GST*, crossover levels remain unchanged. This phenomenon indicates a possible late role for Mek1-GST impacting the output of the crossover/noncrossover decision. In either case, the dimerization/activation of Mek1 may serve as a regulatory landmark to couple these two processes.

Dual roles for Mek1 in interhomolog bias and recombination checkpoint signaling

Findings to date suggest that early stages of meiotic recombination checkpoint function and interhomolog bias are inextricably linked. Both checkpoint signaling and partner choice require the activation of the ATM/ATR signaling pathways [31,33]. It is conceivable that Mek1 acts solely to ensure that the interhomolog bias is upheld, primarily through its role in phosphorylating Rad54 or other like targets [43]. On the other hand, Mek1 is similar to the Rad53 DNA damage checkpoint kinase that acts downstream of ATM/ATR in vegetative cells and regulates a wide range of targets in response to DNA damage [57]. While Rad53 is phosphorylated in response to unrepaired DSBs during MI, it does not affect cell cycle arrest until after the MI division [42,58]. Thus Mek1 is an attractive candidate for maintaining recombination-induced arrest during and after the formation of interhomolog connections.

It would seem reasonable to consider that by extending the period of interhomolog bias, there would be slower turnover of DSBs, which in turn would activate a checkpoint-mediated delay from pachytene exit. We were surprised, however, to find that *MEK1-GST* conferred an MI delay even when the Rad17 and Pch2-dependent recombination checkpoint functions were absent. We were able to rule out the possibility that *MEK1-GST* activated an alternative DNA damage pathway (e.g. Rad9/Rad53) in this situation since deletion of *DMC1* in this strain background did not induce an additional MI delay or arrest phenotype. These data suggest that Mek1 dimerization can influence the regulation of prophase exit even in the absence of surveillance/signaling functions of Rad17 and Pch2. The increased period of interhomolog bias thus can account for the delay observed in the MI division and the turnover of DSBs in *MEK1-GST* cells. We suspect that Mek1-GST exhibits at least some transient association/activation within the chromosome loop-axis structure that has experienced a Spo11-induced DSB, independent of Hop1 phosphorylation by ATM/ATR (Carballo et al; Nui et al). This could be true for wild type Mek1 as well.

Checkpoint stringency can be modulated by controlling exit from pachytene arrest

Deletion of a subset of meiotic genes leads to a checkpoint induced MI delay, followed by a division that gives rise to inviable spore products. We found that *MEK1-GST* exacerbates the delay in which *ndj1*Δ and *csm4*Δ□ mutants progress through MI and even blocks MI division in a subset of cells. The observed increase in spore viability for the double mutants

suggest that those cells arrested in the presence of *MEK1-GST* may represent the same pool of cells that would have otherwise gone on to form inviable spore products.

What mechanism prevents MI division in a subset of cells in *ndj1*Δ/*csm4*Δ mutants, and complete arrest in *zip1*Δ *when MEK1-GST* is present? Conversely, why do *zip1*Δ/*ndj1*Δ/*csm4*Δ single mutants (with wild-type *MEK1*) proceed through MI division, only to give rise to inviable spore products? To accommodate the delay/arrest phenotype conferred by the *zip1*Δ/*ndj1*Δ/*csm4*Δ mutants in combination with *MEK1-GST*, we propose that prolonged dimerization/activation of *MEK1-GST* inhibits the re-entry into the cell cycle following checkpoint-mediated arrest in these mutant backgrounds, perhaps through the persistent phosphorylation of a protein that regulates the exit from pachytene. Based on these data we suggest that Mek1 phosphorylates at least one target that is directly involved in the meiotic recombination checkpoint in a challenged meiosis in addition to its targets that are directly involved in interhomolog bias.

One possibility is that *MEK1-GST* inhibits the process of checkpoint recovery. Checkpoint recovery occurs when signaling through the checkpoint response has ended and cells re-enter the cell cycle [59]. To date, checkpoint recovery has been described only in nonmeiotic cells of yeast, so this would provide the first observation of the phenomenon in meiosis. Another possibility is that *MEK1-GST* mediated arrest in the *zip1*Δ/*ndj1*Δ/*csm4*Δ mutant situations is due to inhibition of the checkpoint adaptation response where divisions occur even though lesions are not repaired [38]. This seems less likely since unrepaired DSBs do not accumulate in the *MEK1-GST zip1*Δ mutant, as would be expected if adaptation were inhibited. On the other hand, there could be a low level of breaks that were not detected with the assays we used here.

It is curious to consider why an intact checkpoint would not function to maximize spore viability in a “challenged meiosis”. It is perhaps advantageous for yeast to progress quickly through sporulation since the resultant spore stage is resistant to myriad environmental insults. Sexually dimorphic levels of checkpoint stringency observed in animals [60] may be determined in part through the modulation of a similar checkpoint-related kinase. To date, Mek1orthologs have only been found in fungi. In *S. pombe*, the Mek1 ortholog phosphorylates Cdc25 phosphatase and causes arrest prior to MI [61]. Since Cdc25 is also a target of Chk1/Chk2 kinases in mitotic checkpoint pathways in metazoans [62], Chk1/Chk2 might play a role similar to Mek1 to uphold checkpoint stringency during meiosis in higher eukaryotes.

Experimental Procedures

Standard methods were used to construct yeast strains, synchronize meiotic cells and prepare DNA and proteins samples for analysis by Southern and western blotting, respectively. Detailed methods are described in supplementary materials.

Highlights

1. *MEK1* gain-of-function mutation enhances meiotic interhomolog recombination
2. Meiotic crossover designation occurs prior to or concomitant with Mek1 dimerization
3. Mek1 plays dual roles in interhomolog bias and the recombination checkpoint
4. Checkpoint stringency can be enhanced by regulating the exit from pachytene

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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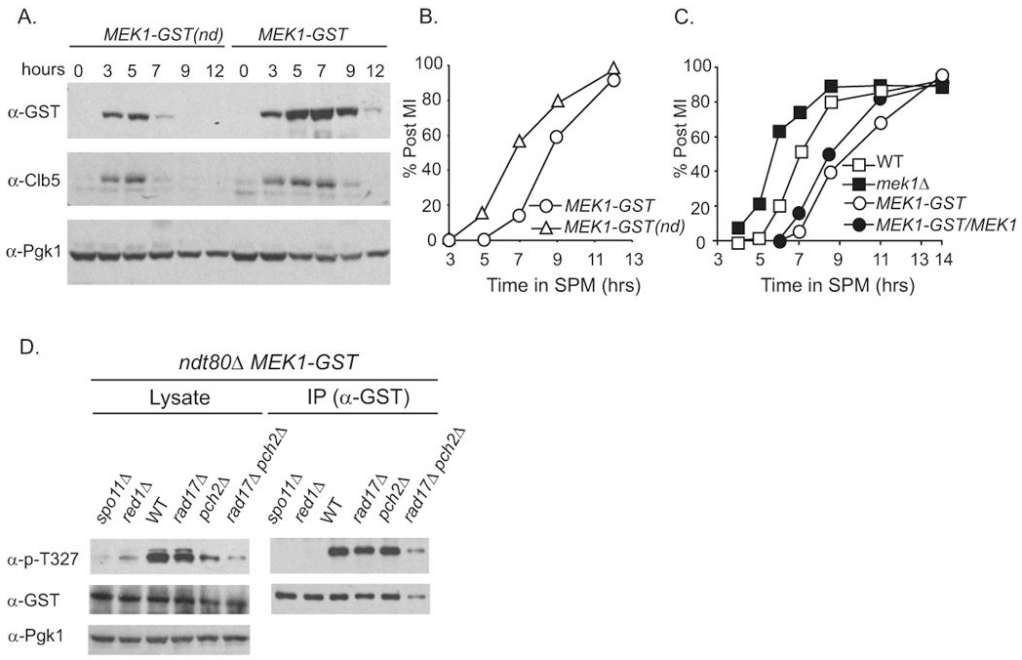

Figure 1. *MEK1-GST*is a gain of function allele

(A) Synchronous meiotic cultures of *MEK1-GST* and *MEK1-GST(nd)* were analyzed by immuno-blot at the indicated times using antibodies to GST (top), Clb5 (middle), and Pgk1 (bottom). (B) Percentage of cells in the culture at each time point that have undergone the first meiotic division as indicated by DAPI staining. (C) Same analysis as in B except for the indicated strains. (D) Western blot of cell lysates or Mek1-GST following immunoprecipitation with antibodies to p-T327, GST or Pgk1 for the indicated strains 4 hours after transfer to SPM. The levels of T-327 detection for immunoprecipitated Mek1-GST normalized to immunoprecipitated -GST for each mutant was compared to Mek1-GST alone. These values are as follows for two independent experiments (the first value corresponds to the figure shown in panel D. The second value shown is from cells collected at 4.5 hours after transfer to SPM from an independent time course): *MEK1-GST spo11*Δ 0.02, not determined; *MEK1-GST red1*Δ < 0.01, not determined; *MEK1-GST*, 1.00, 1.00; *MEK1-GST rad17*Δ, 0.94, 1.22; *MEK1-GST pch2*Δ, 0.88, 1.3 *MEK1-GST rad17*Δ *pch2*Δ 0.60, 0.78. See also Figure S1

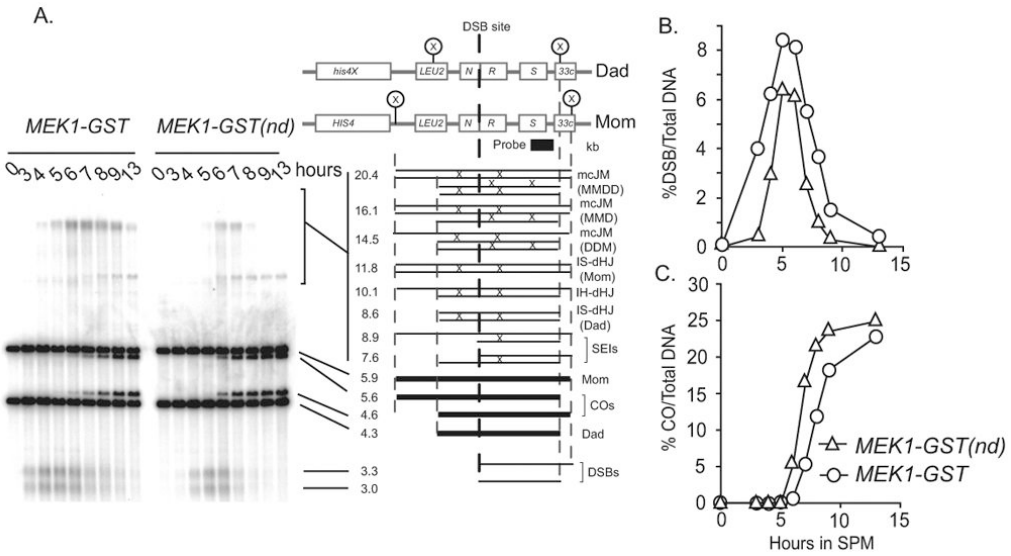

Figure 2. Meiotic recombination analysis at the *HIS4::LEU2* hotspot in *MEK1-GST* compared to *MEK1-GST(nd)*

(A) Physical analysis of recombination by Southern blot to measure the formation and turnover of recombination intermediates at the indicated time points following transfer of synchronized cells to SPM in *MEK1-GST* and *MEK1-GST(nd)* strains. DNA samples in this experiment are psoralen-crosslinked. The physical structure and molecular weights corresponding to each band of a XhoI digest of the *HIS4::LEU2-(NBam)/his4-X::LEU2-(NBam)-URA3* recombination hot spot in the Southern blot is diagramed on the right [19]. The slowly migrating species is labeled with an asterisk. (B and C) Quantification of the formation and turnover of DSB products (%DSBs/Total DNA) and crossover products (%COs/Total DNA) from the Southern blot in part A, respectively. See also Figure S2.

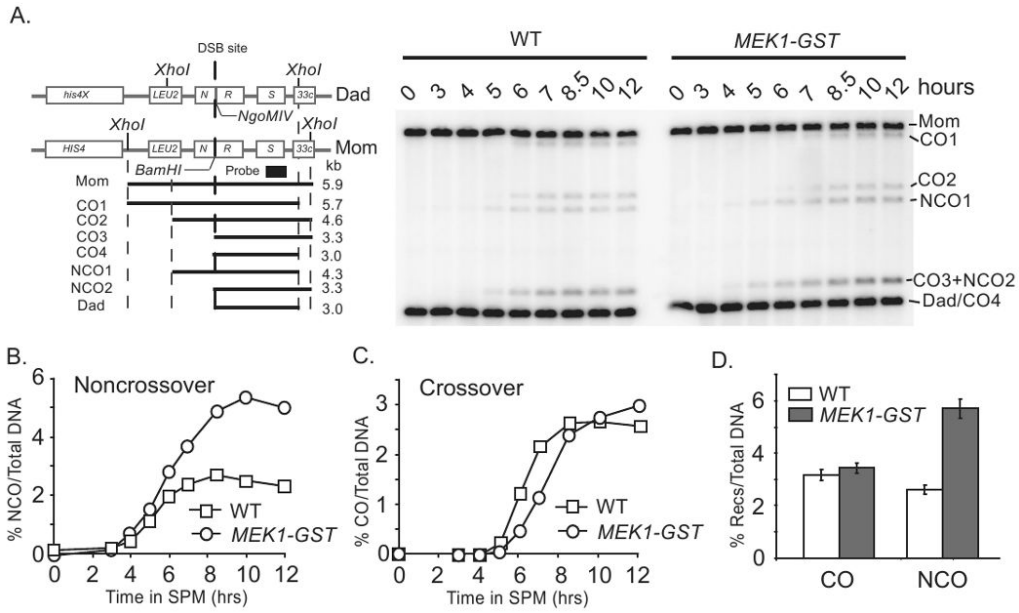

Figure 3. Noncrossover recombination products are increased in *MEK1-GST* compared to WT while crossover levels are unchanged

(A) Schematic *of the HIS4::LEU2-(BamHI)/his4-X::LEU2-(NgoMIV)* hotspot (left) and the timing of noncrossover and crossover formation in WT and *MEK1-GST*. DNA was isolated from synchronous meiotic cultures and digested with XhoI and NgoMIV prior to Southern analysis. At this hot spot, the DSB can occur at either the BamHI chromosome or the NgoMIV chromosome (the chance is equal). By digesting with XhoI and NgoMIV, there are four species of crossover products. CO4 is the same size as the DAD band (3.0kb). Further details of this hotspot are described in Martini et al. 2006 and Oh et al., 2007 [26,45]. (B and C) Quantification of noncrossover and crossover levels at indicated times, respectively. (D) Quantification of crossover (CO) and noncrossover (NCO) levels at t = 12 hours after transfer to SPM. For each strain, four independent meiosis cultures from the same time course were analyzed.

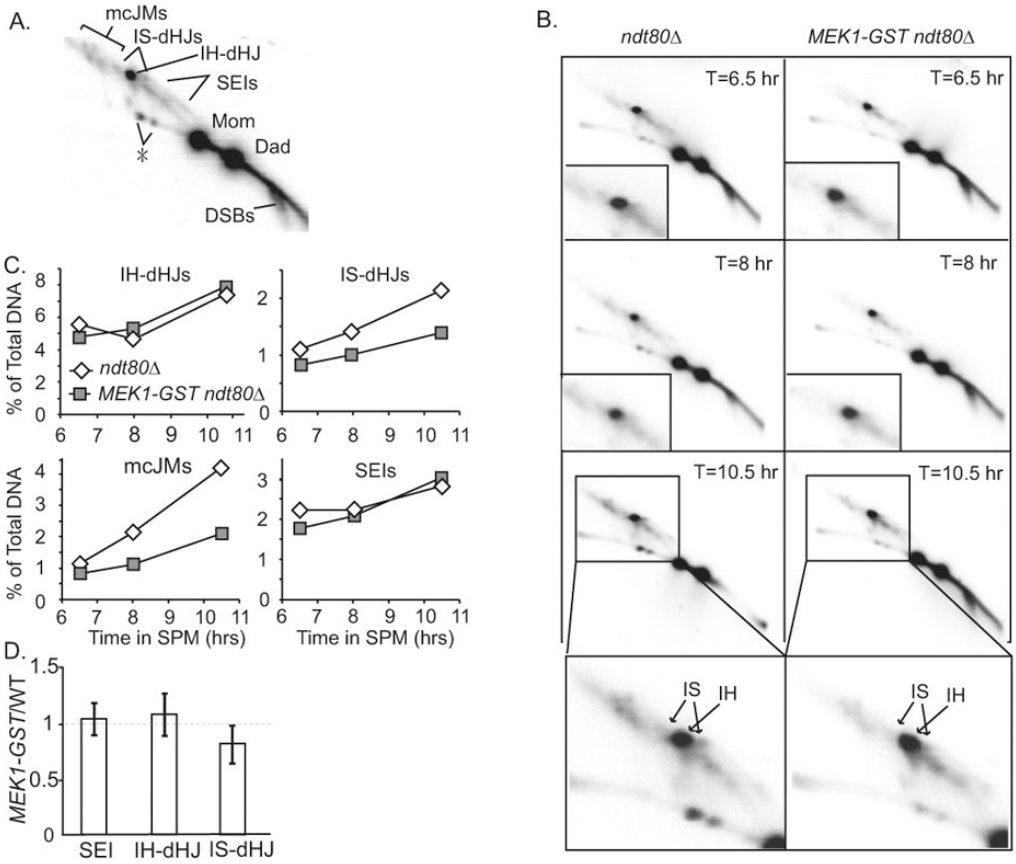

Figure 4. The levels of intersister-dHJs and mc-JMs are decreased in *MEK1-GST* compared to WT

(A) Position of DNA joint molecules on Southern blot of a 2-D gel from synchronized cultures of *ndt80*Δ after transfer to SPM. The identity of the spot denoted by the asterisk is not known. (B) Southern blots of 2-D gels from a representative time course of *ndt80*Δ and *ndt80* Δ *MEK1-GST*. DNA samples in this experiment are psoralen-crosslinked. The relative area used for quantification of intersister- and interhomolog-dHJs are as indicated in the lower panel. (C) Quantification of JM structures from time course shown in B. (D) Quantification of JMs from two independent time-courses including time points ranging from 6.5 hours to 11.5 hours. Mean +/- SD of the value of the ratio of SEI, interhomolog-dHJ and intersister-dHJ joint molecules in *ndt80*Δ *MEK1-GST* strain compared to *WT*. In *ndt80*Δ *MEK1-GST*, intersister-dHJs are decreased to 82% of WT levels ($p = 0.035$; paired t-test). Interhomolog-dHJ and SEI in *MEK1-GST* are not significantly different from WT ($p = 0.283$ and $p = 0.619$, respectively). The authors were able to visually assign all relevant blots with the correct strains in a blind analysis.

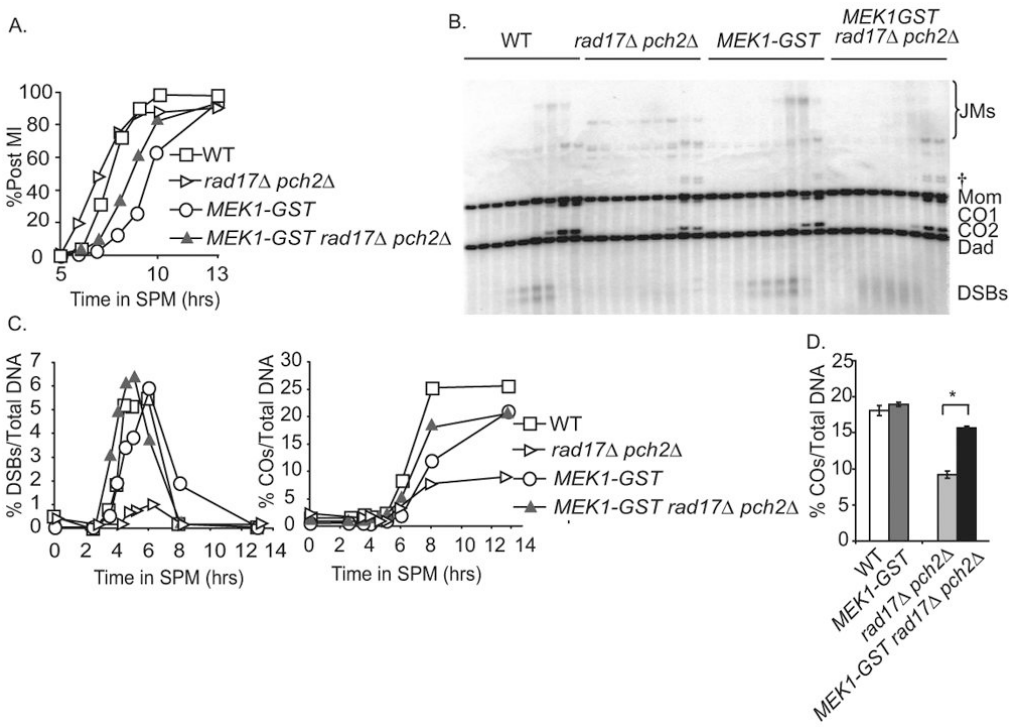

Figure 5. *MEK1-GST*restores interhomolog bias in the absence of*RAD17*and*PCH2*-dependent surveillance/signaling functions

(A) Percentage of cells in a synchronized meiotic cultures that have undergone the first meiotic division at the indicated time points in WT, *rad17*Δ *pch2*Δ, *MEK1-GST*, *MEK1-GST rad17*Δ *pch2*Δ. (B) Southern blot and quantification of recombination intermediates and crossovers at the *HIS4LEU2* hotspot in WT, *rad17*Δ *pch2*Δ, *MEK1-GST* and *MEK1-GST rad17*Δ *pch2*Δ. For each strain, the 0, 2.5, 3.5 4, 4.5, 5, 6, 8 and 13 hour time points were analyzed. The bands immediately above the Mom fragment in the *rad17*Δ *pch2*Δ–containing strains are ectopic recombination species [34]. DNA samples in this experiment are Psoralen-crosslinked. †, DNA fragments arising from ectopic recombination between *HIS4::LEU2* and the linked, endogenous *leu2* locus [34]. (C) Quantification of the formation and turnover of DSB products (%DSBs/Total DNA), crossover products (%COs/Total DNA) and %post MI cells from the Southern blot experiment is shown in part B, (D) Final crossover levels/total DNA in WT and checkpoint mutants used in parts B and C. Mean +/- SD of three independent cultures is shown. The asterisks indicate the differences were statistically significant ($p < 0.05$) by paired t-test. Results presented in (A) to (C) are from the same time course.

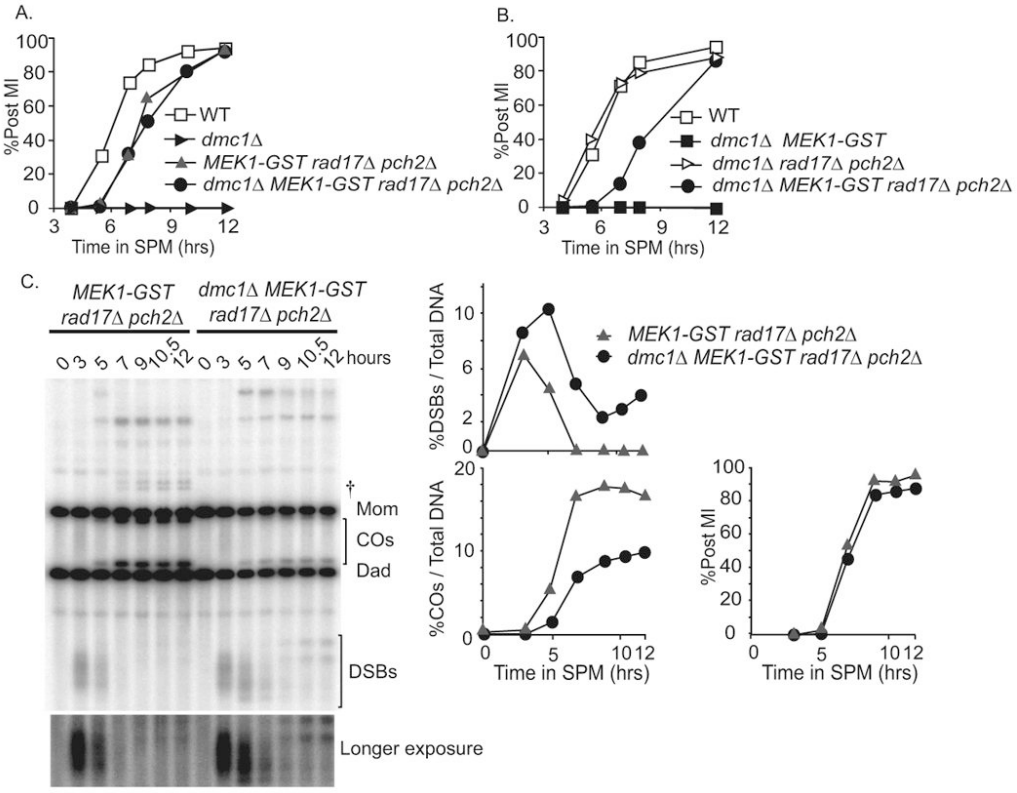

Figure 6. *MEK1-GST*imposes a delay independent of meiotic checkpoint

(A-B) Percentage of cells in synchronized meiotic cultures that have undergone the first meiotic division. Strains used include WT, *MEK1-GST*, *dmc1*Δ, *MEK1-GST rad17*Δ *pch2*Δ, *dmc 1*Δ *MEK1-GST* and *dmc1*Δ *MEK1-GST rad17*Δ *pch2*Δ (C) Southern blot analysis of *MEK1-GST rad17*Δ *pch2*Δ versus *dmc1*Δ *MEK1-GST rad17*Δ *pch2*Δ DSB formation and turnover (%DSBs/Total DNA), CO formation (%COs/Total DNA) and post MI kinetics (%Post MI/Total DNA) are shown. DNA samples in this experiment are not Psoralen-crosslinked. Two slower migrating bands appeared in the quadruple mutant at late time points, after the majority of cells had already undergone MI division. The migration pattern of these bands is reminiscent of hairpin structures that form from hyper-extended 3′ ends of DSBs at *HIS4LEU2* locus in the *rad52* mutants [47]. †, meiosis-specific hybridization bands, probably ectopic recombination resulted from *HIS4::LEU2* recombining with the *leu2* locus [34].

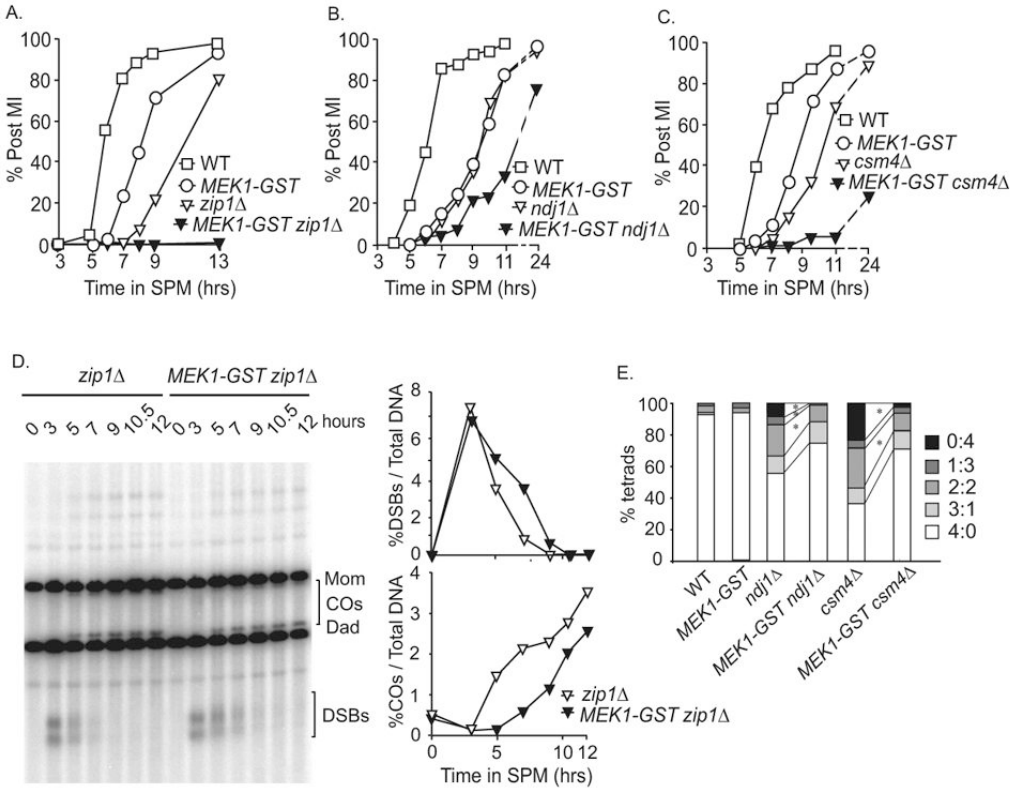

Figure 7. *MEK1-GST* exhibits a hyper-checkpoint function

(A-C) Percentage of cells in a synchronized meiotic cultures that have undergone the first meiotic division at the indicated time points in WT, *MEK1-GST*, *zip1*Δ, *MEK1-GST zip1*Δ, *ndj1*Δ,*MEK1-GST ndj1*Δ, *csm4*Δ, and *MEK1-GST csm4*Δ. (D) Southern blot and quantification of *zip1*Δ compared to *MEK1-GST zip 1*Δ (E) Categories of tetrads from *ndj1*Δ and *csm4*Δ mutants (viable: inviable). A 2:2 pattern of viable to nonviable spores is expected from a MI non-disjunction event. A 3:1 pattern might arise by non-disjunction in MII or premature sister chromatid segregation in MI. The 0:4 and 1:3 classes indicate multiple meiosis I and/or meiosis II non-disjunction events so were not further considered in this analysis. Only four spore tetrads were analyzed. The asterisks indicate the differences were statistically significant ($p < 0.05$) by Fisher's exact test. Note: the strains used in A do not contain the *HIS4LEU2* hotspot and are described in the strain Table S2 See also Figure S3.