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The meiotic-specific Mek1 kinase in budding yeast regulates interhomolog recombination and coordinates meiotic progression with double strand break repair

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Abstract

Recombination, along with sister chromatid cohesion, is used during meiosis to physically connect homologous chromosomes so that they can be segregated properly at the first meiotic division. Recombination is initiated by the introduction of programmed double strand breaks (DSBs) into the genome, a subset of which are processed into crossovers. In budding yeast, the regulation of meiotic DSB repair is controlled by a meiosis-specific kinase called Mek1. Mek1 kinase activity promotes recombination between homologs, rather than sister chromatids, as well as the processing of recombination intermediates along a pathway that results in synapsis of homologous chromosomes and the distribution of crossovers throughout the genome. In addition, Mek1 kinase activity provides a readout for the number of DSBs in the cell as part of the meiotic recombination checkpoint. This checkpoint delays entry into the first meiotic division until DSBs have been repaired by inhibiting the activity of the meiosis-specific transcription factor Ndt80, a site-specific DNA binding protein that activates transcription of over 300 target genes. Recent work has shown that Mek1 binds to Ndt80 and phosphorylates it on multiple sites, including the DNA binding domain, thereby preventing Ndt80 from activating transcription. As DSBs are repaired, Mek1 is removed from chromosomes and its activity decreases. Loss of the inhibitory Mek1 phosphates and phosphorylation of Ndt80 by the meiosis-specific kinase, Ime2, promote Ndt80 activity such that Ndt80 transcribes its own gene in a positive feedback loop, as well as genes required for the completion of recombination and entry into the meiotic divisions. Mek1 is therefore the key regulator of meiotic recombination in yeast.

Keywords

yeast; meiosis; recombination; Mek1; Ndt80; checkpoint; double strand break repair

Meiosis is a fundamental biological process necessary for sexual reproduction. This unique cell division reduces the chromosome number of a cell in a highly specific way to make gametes that contain a single copy of each homolog. Fusion of two haploid gametes then restores the diploid state, keeping the chromosome number constant from generation to generation. The reduction in chromosome number during meiosis results when duplicated

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chromosomes undergo two consecutive rounds of chromosome segregation without an intervening S phase. For many organisms such as budding yeast and humans, which have 16 and 23 pairs of homologous chromosomes, respectively, sorting chromosomes into gametes is a daunting task. To determine which chromosomes are homologs, recombination is used to generate crossovers between the non-sister chromatids of homologous chromosomes. These crossovers, in combination with sister chromatid cohesion, physically connect homologs, allowing them to align and segregate properly at the first meiotic division (Petronczki *et al.* 2003).

Recombination is initiated by double strand breaks (DSBs) that are deliberately created in preferred regions of the genome called “hotspots” by a highly conserved protein called Spo11 (Figure 1A) (Keeney *et al.* 2014). The 5’ ends of the DSBs are resected and the 3’ single strand tails are bound by the mitotic recombinase, Rad51, and a meiosis-specific recombinase, Dmc1 (Hunter 2007; Brown *et al.* 2015). In combination with accessory factors, the resulting nucleoprotein filament mediates “strand invasion”, which involves searching for the homologous sequence on a non-sister chromatid, locally denaturing the duplex and annealing to the complementary strand to create a displacement (D)-loop. Further processing of this intermediate results in a double Holliday junction that can be resolved to form either a crossover or noncrossover (see below).

Because it is critical for the survival of a species that every pair of homologs gets at least one crossover, many safeguards have evolved to ensure this outcome. First, more DSBs are introduced into the genome during meiosis than the number of crossovers required (for example, budding yeast undergoes ~160 DSBs but has only 16 pairs of homologs while mice have 250–300 DSBs for 20 pairs of homologs) (Pan *et al.* 2011; Keeney *et al.* 2014). Second, multiple mechanisms act to promote recombination between homologs, as opposed to sister chromatids. Third, there is a specialized recombination pathway for generating crossovers that are distributed throughout the genome. Finally, DSBs that do not become crossovers are repaired either as non-crossovers or by sister chromatid recombination before chromosomes segregate at the first meiotic division to prevent broken chromosomes from making aneuploid gametes. In budding yeast, the regulation of recombination and its coordination with meiotic progression are under the control of the meiosis-specific kinase, Mek1 (also known as Mre4) (Rockmill AND Roeder 1991; Leem AND Ogawa 1992; Wu *et al.* 2010; Hollingsworth 2016; Chen *et al.* 2018).

Mek1 regulates interhomolog bias as well as recombination pathway choice

When a 3’ end is searching for homology, there are three possible templates: the sister chromatid or the two chromatids of the homolog. Because only interhomolog crossovers connect chromosomes, strand invasion during meiosis is biased to occur between the homologs (Lao AND Hunter 2010). The key player in regulating interhomolog bias is Mek1 (Figure 1B). In the absence of Mek1 kinase activity, DSBs are repaired using the sister chromatid (Niu *et al.* 2005; Kim *et al.* 2010). While this repair produces intact chromosomes, the homologs are not connected, and random chromosome segregation results in high levels of aneuploidy and inviable spores (Rockmill and Roeder 1991; Leem and Ogawa 1992). One way that Mek1 promotes interhomolog recombination is by inhibition of

Rad51 (Figure 1B). In vegetative cells, Rad51 is used to repair DSBs and this repair is biased to occur between sister chromatids (Kadyk and Hartwell 1992; Bzymek *et al.* 2010). In meiotic cells, the Rad51 protein (but not its activity) is required for interhomolog bias, while Dmc1 is responsible for most of the DSB repair that occurs (Schwacha and Kleckner 1997; Lao *et al.* 2008; Cloud *et al.* 2012). Rad54 is an accessory factor that binds to Rad51 and promotes its strand invasion activity (Petukhova *et al.* 1999). Mek1 inhibits the Rad51-Rad54 interaction in two different ways: (1) it directly phosphorylates threonine (T) 132 on Rad54, thereby reducing the affinity of Rad54 for Rad51 (Niu *et al.* 2009) and (2) it phosphorylates and stabilizes Hed1, a meiosis-specific protein that binds to Rad51, thereby excluding Rad54 (Tsubouchi and ROEDER 2006; Busygina *et al.* 2008; Callender *et al.* 2016). Removing these constraints on Rad51 activity only reduces interhomolog bias by two-fold, as Dmc1 itself can inhibit Rad51 (Hong *et al.* 2013; Lao *et al.* 2013; Liu *et al.* 2014). However, when the *hed1*Δ *RAD54-T132A* mutations are combined with a hypomorphic allele of *DMC1* that is delayed in filament formation, but otherwise behaves like wild type, interhomolog bias is reduced 8-fold and spore viability is reduced (Liu *et al.* 2014). These results argue that Mek1 inhibition helps prevent Rad51 from competing with Dmc1 to repair DSBs. It should be noted that the presence of *DMC1* is not sufficient to ensure interhomolog recombination, as inactivation of Mek1 results in intersister recombination even in *DMC1* cells (Goldfarb and Lichten 2010; Kim *et al.* 2010). Therefore, in addition to the inhibition of Rad51 and the presence of Dmc1, there must be additional mechanisms controlled by Mek1 that promote interhomolog recombination. For example, it has been proposed that Mek1 antagonizes sister chromatid cohesion locally around DSBs to make it easier to invade a homolog, although how this occurs is unknown (Kim *et al.* 2010).

After interhomolog strand invasion, there are different ways in which the resulting D-loop can be processed (Figure 1). One pathway, called synthesis dependent strand annealing (SDSA), occurs when the extended invading strand is disassembled from the D-loop by the STR protein complex containing **S**gs1 (an ortholog of Bloom helicase in humans), **T**opoisomerase III and **R**mi1 (Allers and Lichten 2001; McMahill *et al.* 2007; Oh *et al.* 2007; De Muyt *et al.* 2012; Kaur *et al.* 2015; Tang *et al.* 2015). The extended strand then anneals to the 3’ end on the other side of the break, resulting in a noncrossover. The major pathway for crossover formation in budding yeast occurs via a group of functionally distinct proteins known collectively as the ZMM proteins (Börner *et al.* 2004; Lynn *et al.* 2007). The ZMM pathway of recombination leads to the stable association of homologous chromosomes by formation of a meiosis-specific chromosome structure called the synaptonemal complex (see below) (Börner *et al.* 2004). Crossovers generated by this pathway are distributed throughout the genome by a mysterious process known as “interference” (Kohl and Sekelsky 2013). D-loop intermediates processed using the ZMM pathway are protected from disassembly by STR (De Muyt *et al.* 2012) (Figure 1). Instead D-loops are stabilized to form “single end invasion” (SEI) intermediates (Hunter and Kleckner 2001). Extension of the invading strand by DNA synthesis displaces the complementary strand of the invaded duplex, which then anneals to the other side of the break to form a double Holliday junction (Schwacha and Kleckner 1995). Resolution of

ZMM-mediated double Holliday junctions is biased to generate crossovers (Allers and Lichten 2001; Börner *et al.* 2004).

A key member of the ZMM group is Zip1, which is the transverse filament protein that connects condensed pairs of sister chromatids together to form the synaptonemal complex (Sym *et al.* 1993; Dong and Roeder 2000; Börner *et al.* 2004). In addition to "zippering up the chromosomes", Zip1 also plays an early role in the ZMM pathway that is regulated by Mek1 (Figure 1C)(Chen *et al.* 2015). Mek1 is required for phosphorylation of a conserved region in the C-terminus of Zip1 by the Cdc7-Dbf4 (DDK) cell cycle kinase (Chen *et al.* 2015). Phosphorylation of this region can be prevented using the *zip1–4A* mutant which substitutes alanines for the serines that are phosphorylated. Phenotypic characterization of *zip1–4A* has revealed that phosphorylation of the Zip1 C-terminus is necessary for ZMM-mediated crossovers and synapsis (Chen *et al.* 2015). The double Holliday junctions undergo unbiased resolution by structure-selective endonucleases (SSNs) such as Mus81-Mms4 to give both crossovers and non-crossovers (De muyt *et al.* 2012; Chen *et al.* 2015). Finally, Mek1 indirectly controls the timing of Holliday junction resolution by regulating the activity of the meiosis-specific transcription factor, Ndt80 (Figure 1D)(see below). *NDT80* is required to transcribe the polo-like kinase *CDC5*, which then triggers Holliday junction resolution (Sourirajan and Lichten 2008; Matos *et al.* 2011). Mek1 therefore regulates all the steps of recombination in yeast.

Mek1 controls the meiotic recombination checkpoint to provide time for interhomolog recombination

In vegetative cells, the unexpected appearance of a DSB triggers the "DNA damage checkpoint" (Harrison and Haber 2006). This checkpoint delays cell cycle progression to provide time for DSBs to be repaired and works using the Tel1 and Mec1 kinases (ATM and ATR in mammals, respectively). These kinases are recruited to DSBs where they phosphorylate an adaptor protein called Rad9. An FHA-domain containing effector kinase, Rad53, binds to phosphorylated Rad9 where it autoactivates and phosphorylates target proteins that prevent entry into mitosis.

Although Spo11-generated DSBs are programmed to occur during meiosis, they are just as lethal as a break in a mitotic cell if left unrepaired. Meiotic DSB repair is therefore highly regulated using meiosis-specific modifications of the DNA damage checkpoint (Lydall *et al.* 1996; Subramanian and Hochwagen 2014). The meiotic recombination checkpoint delays meiotic prophase to provide time for interhomolog recombination to occur. Given its role in regulating partner and recombination pathway choice, it is not surprising that the effector kinase regulating the meiotic recombination checkpoint is Mek1 (Xu *et al.* 1997; Perez-Hidalgo *et al.* 2003). Similar to Rad53, Mek1 also contains an FHA domain. *MEK1*'s role in this checkpoint was first established using the *rad50S* mutant which makes DSBs whose ends are not resected and therefore cannot be repaired (Alani *et al.* 1990). The unrepaired DSBs trigger the checkpoint, causing a delay in meiotic progression. Deletion of *MEK1* eliminates the *rad50S* progression delay, indicating it is required for the meiotic recombination checkpoint (Xu *et al.* 1997).

Mek1 activation occurs in the context of a meiosis-specific structure formed along sister chromatids called the axial element

One of the key differences between the DNA damage and meiotic recombination checkpoints is that the latter occurs in the context of a meiosis-specific structure called the axial element (Figure 2). Axial elements are generated when condensation of sister chromatids forms chromatin loops that are tethered along a protein core (Blat and Kleckner 1999). In yeast, axial elements contain the meiosis-specific Hop1 and Red1 proteins, as well as cohesin complexes containing the meiosis-specific kleisin subunit, Rec8 (Hollingsworth *et al.* 1990; Smith and Roeder 1997; Blat and Kleckner 1999; Klein *et al.* 1999; Lorenz *et al.* 2004; Panizza *et al.* 2011; Brown *et al.* 2018). Hop1 contains an evolutionarily conserved sequence called the HORMA domain that mediates both Hop1 homoligomerization and heteroligomerization with Red1 (Aravind and Koonin 1998; De los Santos AND Hollingsworth 1999; Woltering *et al.* 2000; West *et al.* 2018).

After recruitment of Tel1 and Mec1 to Spo11 breaks, these checkpoint kinases phosphorylate Hop1 as the adaptor protein instead of Rad9 (Carballo *et al.* 2008). Mek1 binds to phosphorylated Hop1 via its FHA domain where it activates itself in *trans* (Niu *et al.* 2007; Carballo *et al.* 2008). The C-terminus of Hop1 is required for Mek1 activation by promoting Mek1 oligomerization (Niu *et al.* 2005). This function of Hop1 can be bypassed by ectopic dimerization of Mek1 using GST (Niu *et al.* 2005). Ectopic dimerization does not bypass the need for Mek1 to bind to phospho-Hop1 via its FHA domain, but it does enhance Mek1 activity (Niu *et al.* 2005; Wu *et al.* 2010). Activation of Mek1 also requires *RED1*, as well as the Hop1-Red1 interaction (Niu *et al.* 2007). These requirements for Mek1 activation explain why *hop1* and *red1* mutants resemble *mek1*Δ in being defective in interhomolog bias, resulting in inviable spores (Hollingsworth and Byers 1989; Rockmill and Roeder 1990; Schwacha and Kleckner 1994; Kim *et al.* 2010).

How is it that DSBs, which occur in the chromatin loops, activate a kinase that is localized to the chromosome axis? Several discoveries led to the hypothesis that hotspot sequences within chromatin loops are tethered to the axial elements where DSBs are then generated (Figure 2). First, an axis protein called Mer2, which is required for making DSBs, is phosphorylated by DDK as the replication fork passes during premeiotic S-phase (Cool and Malone 1992; Rockmill *et al.* 1995; Sasanuma *et al.* 2008; Wan *et al.* 2008; Murakami and Keeney 2014). Phosphorylated Mer2 recruits Mei4 and Rec114 which brings Spo11 to the axes (Li *et al.* 2006; Sasanuma *et al.* 2008). In addition, Mer2 binds to Spp1 which in turn interacts with trimethylated histones adjacent to DSB hotspots, thereby recruiting hotspot sequences to the axes to be cleaved by Spo11 (Acquaviva *et al.* 2013; Sommermeyer *et al.* 2013; Adam *et al.* 2018). DSBs are therefore generated on the axial elements. Chromatin-immunoprecipitation experiments using an antibody specific for phosphorylated Histone H3 threonine 11, a substrate of Mek1, has shown that Mek1 kinase activity is highest on chromosome axes and correlates with the presence of Hop1 and Red1. Interestingly, Mek1 phosphorylation of histone H3 can spread for several kilobases around a DSB (Kniewel *et al.* 2017).

Mek1 activity provides a readout for the presence of DSBs

For the meiotic recombination checkpoint to monitor DSB repair, it must be able to detect the presence and amount of DSBs in the cell. Mek1 activity correlates with both DSB levels and the rate of meiotic progression, suggesting it is the sensor for the checkpoint (Prugar *et al.* 2017). In DSB-defective mutants (as well as *mek1*Δ, *hop1*Δ and *red1*Δ), Mek1 is inactive and meiotic progression occurs faster than in wild type due to the absence of the checkpoint (Malone *et al.* 2004; Niu *et al.* 2007). In *dmc1*Δ strains, DSBs are formed but unrepaired due to the inhibition of Rad51 and the cells arrest in meiotic prophase with high levels of DSBs (Bishop *et al.* 1992; Hunter and Kleckner 2001; Niu *et al.* 2005). Mek1 kinase activity (detected using an antibody specific for the Mek1-phosphorylated form of Hed1) persists at a high level in *dmc1*Δ strains as well (Callender *et al.* 2016; Prugar *et al.* 2017). In contrast, deletion of *NDT80*, which is needed to exit from meiotic prophase, arrests cells at the pachytene stage when synapsis is complete (Xu *et al.* 1995; Winter 2012). By this stage most DSBs have been processed either into noncrossovers or double Holliday junctions awaiting resolution (Allers and Lichten 2001). Furthermore, synapsis downregulates, but does not abolish, Spo11 activity, resulting in a low number of DSBs (1–10/cell) (Thacker *et al.* 2014; Subramanian *et al.* 2016) (Figure 2). In an *ndt80*Δ diploid, Hed1 phosphorylation is reduced, but not eliminated, indicating a decrease in Mek1 activity that is consistent with the removal of Mek1 from chromosomes that occurs as homologs synapse (Subramanian *et al.* 2016; Prugar *et al.* 2017). Another example of the correlation between DSB levels and the activity of the meiotic recombination checkpoint is the meiotic progression delay observed for the *zip1*Δ and *zip1–4A* mutants. Both of these mutants are defective in synapsis and therefore in turning down Spo11 activity, resulting in increased numbers of DSBs compared to wild type (Sym *et al.* 1993; Thacker *et al.* 2014; Chen *et al.* 2015). However, for reasons that are unclear, the *zip1–4A* diploid exhibits significantly higher levels of DSBs compared to the *zip1*Δ (Chen *et al.* 2015). This mutant also enters into the meiotic divisions more slowly than *zip1*Δ. Taken together, these results indicate that meiotic progression in yeast is regulated using Mek1 kinase activity as a readout for the number of DSBs. Furthermore, they argue against the presence of a synapsis checkpoint in yeast. The *spo11*Δ, *zip1*Δ and *zip1–4A* mutants are all defective in synapsis but exhibit differences in meiotic progression that mirror instead the number of DSBs.

The target of the meiotic recombination checkpoint is the meiosis-specific transcription factor, Ndt80

Ndt80 is a transcriptional activator that can be divided in three domains: an N-terminal DNA binding domain, a C-terminal activation domain, and the region in the middle (Lamoureux *et al.* 2002; Montano *et al.* 2002; Sopko *et al.* 2002). Ndt80 binds to a specific DNA sequence called the middle sporulation element (MSE) in the promoters of >300 target genes to activate their transcription (Hepworth *et al.* 1995; Ozsarac *et al.* 1997; Chu and Herskowitz 1998). One of these genes is *CDC5*, which, together with DDK, is sufficient to trigger Holliday junction resolution and degradation of Red1, resulting in the destruction of the SC (Sourirajan and Lichten 2008; Okaz *et al.* 2012; Argunhan *et al.* 2017; Prugar *et al.* 2017; Tsubouchi *et al.* 2018). Another Ndt80 target is the *CLB1* cyclin, which together with Cdc28

forms the cyclin-dependent kinase (CDK) needed for Meiosis I spindle formation and meiotic progression (Shuster and Byers 1989; Benjamin *et al.* 2003). When cells enter meiosis, a meiosis-specific E3 ligase degrades mitotic regulators like Cdc5 and Clb1, thereby making meiotic progression dependent upon their transcription by Ndt80 (OKAZ *et al.* 2012). Compelling evidence showing that Ndt80 is the target of the meiotic recombination checkpoint was the discovery of a 57 amino acid domain within the middle region of the protein that is required for the meiotic recombination checkpoint delay/arrest in mutants such as *zip1*Δ and *dmc1*Δ (WANG *et al.* 2011). Deletion of this "bypass checkpoint" (*bc*) domain creates a version of Ndt80 (*NDT80-bc*) which functions as a transcriptional activator but no longer responds to the meiotic recombination checkpoint (Wang *et al.* 2011). Mek1 signals the presence of DSBs and Ndt80 receives the signal—the question is how?

Mek1 coordinates exit from meiotic prophase with DSB repair by directly inhibiting the transcriptional activity of Ndt80

The answer to this question was recently revealed by the discovery that Mek1 binds and negatively regulates the Ndt80 transcription factor in response to the level of DSBs in the cell (Chen *et al.* 2018). The transcriptional regulation of the *NDT80* gene itself is key to understanding how it functions in the checkpoint (WINTER 2012). In vegetative cells, transcription of early meiotic genes such as *SPO11*, *RED1*, *HOP1*, *MEK1* and *DMC1* is repressed by the Ume6 complex (Strich *et al.* 1994; Goldmark *et al.* 2000).

When diploid cells are transferred to sporulation (Spo) medium which lacks nitrogen and uses a non-fermentable carbon source such as acetate, the Ume6 repressor is replaced by the transcriptional activator Ime1 and early gene expression proceeds (Figure 3a)(Pak and Segall 2002; Mallory *et al.* 2007). The *NDT80* promoter is also repressed by Ume6, but has an additional repressor bound at MSE sites within the promoter called Sum1 (Xie *et al.* 1999). In meiotic cells, Sum1 must be removed before Ime1 can activate transcription of the *NDT80* gene (Pak and Segall 2002). This removal requires phosphorylation by Ime2, which is encoded by an early gene (Figure 3b). The need to transcribe and translate *IME2*, as well as the fact that phosphorylation of Sum1 by DDK and CDK is also necessary to eliminate repression, means that transcription of *NDT80* is delayed relative to the other early genes (Figure 3c) (Ahmed *et al.* 2009; Shin *et al.* 2010; Lo *et al.* 2012). This delay provides time for making axial elements, generating DSBs and activating Mek1 (Figure 3d) (Chen *et al.* 2018).

In vegetative yeast cells, a single DSB is sufficient to arrest cells, but in meiosis, a threshold number of DSBs is required to trigger the meiotic recombination checkpoint (Callender and Hollingsworth 2010; Gray *et al.* 2013). A threshold amount of DSBs is also necessary to activate sufficient Mek1 to impose interhomolog bias, as evidenced by the fact that early in meiotic prophase DSBs are repaired preferentially using sister chromatids (JOSHI *et al.* 2015). By the time Ime1-activated *NDT80* transcription occurs, sufficient Mek1 has been activated (indicated as $Mek1^{H}$ in Figure 3) to inhibit the low level of Ndt80 protein that

results from Ime1 ($Ndt80^{Ime1}$) (Figure 3e). In addition, $Mek1^{H}$ inhibits Rad51 activity and promotes interhomolog bias (Figure 3f).

Recent work has shown that the mechanism by which Mek1 inhibits Ndt80 is direct (Chen *et al.* 2018). Mek1 binds to a conserved five amino acid sequence (RPSKR) within the Ndt80 *bc* domain and phosphorylates the transcription factor at multiple sites within the DNA binding domain and the middle region (Chen *et al.* 2018). The consensus phosphorylation site of Mek1 is RXXT/S and Ndt80 contains 10 such sites (Mok *et al.* 2010; Suhandynata *et al.* 2016). Preventing phosphorylation using alanine substitutions at these sites, as well as a few non-consensus sites (*ndt80–10AMS*), results in partial bypass of the *dmc1*Δ arrest (Chen *et al.* 2018). Nearly complete checkpoint bypass occurs when the RPSKR sequence is deleted from the *bc* domain in *NDT80*, suggesting that binding of Mek1 to Ndt80 allows phosphorylation of non-consensus sites in the *ndt80–10AMS* mutant.

Four of the Mek1 consensus sites in the DNA binding domain are located immediately adjacent to the DNA sugar-phosphate backbone when bound to an MS (Lamoureux *et al.* 2002; Montano *et al.* 2002; Chen *et al.* 2018). A compelling hypothesis therefore is that negative charges on the phosphates prevent DNA binding by repelling the negatively charged DNA sugar-phosphate backbone. Consistent with this idea, substitution of the DNA binding domain consensus sites with negatively charged aspartic acid creates a constitutively inactive form of Ndt80 in both *dmc1*Δ and *DMC1* diploids (CHEN *et al.* 2018). The *ndt80–6D* mutant enters meiosis but arrests in meiotic prophase and is unable to activate transcription of itself, *CDC5* or *CLB1* (Chen *et al.* 2018). The amino acid asparagine has a nearly identical side chain as aspartic acid but is not negatively charged. The *NDT80–6N* mutant sporulates efficiently, indicating that it is the negative charge which is inhibiting the activity of *ndt80–6D*. Negative charges disrupt both specific and non-specific DNA binding by recombinant Ndt80 DNA binding domain *in vitro* (Chen *et al.* 2018). Finally, Mek1 directly phosphorylates at least one of the DNA binding domain consensus sites *in vitro*.

As DSBs are repaired by the ZMM pathway resulting in synapsis, Mek1 is removed from chromosomes and Mek1 kinase activity goes down (indicated as $Mek1^{L}$ in Figure 3)(Figure 3g) (Subramanian *et al.* 2016; Prugar *et al.* 2017). The combination of Ime2 phosphorylation and reduced level of Mek1 phosphorylation activates Ndt80 (Figure 3h), which binds to MSEs in its own promoter to activate transcription of the *NDT80* gene in a positive feedback loop (indicated $Ndt80^{Ndt80}$ in Figure 3i). Ndt80 also promotes transcription of *CDC5*, which, in addition to triggering Holliday junction resolution, works in conjunction with DDK to promote degradation of Red1 (Figure 3j), resulting in the complete inactivation of Mek1 (Figure 3k). Rad51 is then able to repair any remaining DSBs (Figure 3l) while Ndt80-activated transcription of *CLB1* allows entry into Meiosis I (Figure 3m) (Argunhan *et al.* 2017; Prugar *et al.* 2017)..

Ndt80 activity is controlled by an antagonistic interaction between Mek1 and Ime2

It was known for over 20 years that *MEK1* is required for the meiotic recombination checkpoint and that the target is Ndt80 (Xu *et al.* 1997; Chu and Herskowitz 1998). Yet the

simple model that Mek1 negatively regulates Ndt80 by phosphorylating the transcription factor was ignored until recently. A major reason is because the inhibitory phosphorylation by Mek1 is obscured by phosphorylation from a second kinase, Ime2. During wild-type meiosis, Ndt80 exhibits a robust mobility shift that is dependent upon phosphorylation by Ime2 (Sopko *et al.* 2002; Benjamin *et al.* 2003; Shubassi *et al.* 2003). In checkpoint arrested cells, hyperphosphorylation of Ndt80 is lost, and only a low level of Ime1-dependent Ndt80 protein is observed (Tung *et al.* 2000; Shubassi *et al.* 2003; Chen *et al.* 2018). Ndt80 hyperphosphorylation is restored in the absence of Mek1 kinase activity (Tung *et al.* 2000; Chen *et al.* 2018). These observations were interpreted to mean that (1) Ndt80 is not a substrate of Mek1 and (2) Ime2 phosphorylation activates Ndt80 and the checkpoint works by preventing this activating phosphorylation (Tung *et al.* 2000). One problem with this simple interpretation, however, is that there is no checkpoint in the absence of *MEK1*, and so Ndt80 in active and phosphorylated by Ime2. Therefore, whether Ndt80 is phosphorylated by Mek1 cannot be determined simply by looking at Ndt80 mobility in the presence or absence of Mek1 activity.

Recently, (Chen *et al.* 2018) showed that inactive Ndt80 is phosphorylated in *dmc1*Δ-arrested cells independently of *IME2*, raising the possibility that this phosphorylation is due to Mek1. The dogma is that Ime2 phosphorylation of Ndt80 activates the transcription factor, although there are studies which show that Ime2 phosphorylation is not required for Ndt80 to activate transcription (Sopko *et al.* 2002; Benjamin *et al.* 2003). We propose an alternative hypothesis that Ime2 phosphorylation indirectly activates Ndt80 by promoting the removal of the inhibitory Mek1 phosphates. This idea was tested using a *dmc1*Δ *IME2*Δ*C241-as NDT80-IN* diploid. The *NDT80-IN* genotype consists of *NDT80* under the control of the *GAL1* promoter in combination with a *GAL4*-estrogen receptor (*GAL4-ER*) fusion that allows induction of *NDT80* transcription by addition of estradiol to the Spo medium (BENJAMIN *et al.* 2003; CARLILE AND AMON 2008). Using a heterologous promoter allows the production of similar levels of *NDT80* whether or not the checkpoint is active (Chen *et al.* 2018). *IME2*Δ*C241-as* contains a truncation of a C-terminal inhibitory domain, resulting in a constitutively active kinase, as well as an analog-sensitive mutation that allows inactivation of the kinase using 3-MB-PP1 (Benjamin *et al.* 2003; Sari *et al.* 2008; Jin *et al.* 2015). After *NDT80* induction with estradiol, approximately 40% of the *dmc1*Δ *IME2*Δ*C241-as NDT80-IN* cells active Ime2ΔC241 entered the meiotic divisions, in contrast to a *dmc1*Δ *NDT80-IN* diploid which exhibited a nearly complete prophase arrest, as expected if the meiotic recombination checkpoint is working (Figure 4)(Chen *et al.* 2018). One explanation for the partial checkpoint bypass is that the truncated, constitutively active Ime2ΔC241-as protein has enhanced stability compared to wild type and thus may be able to counteract the inhibitory Mek1 phosphates in a way that normal levels of Ime2 cannot. Inhibiting Ime2ΔC241-as with 3MB-PP1 decreases the rate of meiotic progression, confirming that the partial bypass requires Ime2 kinase activity (Figure 4)(Chen *et al.* 2018).

To ask whether the role of Ime2 phosphorylation in Ndt80 activation is direct or indirect, the same experiment was performed in an isogenic diploid homozygous for *mek1*Δ. In this diploid, no inhibitory Mek1 phosphates are present on Ndt80 and, when Ime2ΔC241-as is active (i.e. no inhibitor), meiotic progression occurred with similar kinetics to a *DMC1 IME2*Δ*C241-as NDT80-IN* diploid (Figure 4). If Ime2 phosphorylation directly promotes

the transcriptional activity of Ndt80, then inhibition of Ime2ΔC241-as in the *dmc1*Δ *IME2*Δ*C241-as mek1*Δ *NDT80-IN* diploid should slow down meiotic progression. If, instead, Ime2 helps remove the inhibitory Mek1 phosphates, there should be no difference with or without inhibitor, since no inhibitory phosphates are present due to the *mek1*Δ. The latter result was observed, suggesting that an important role for Ime2 is to counteract Mek1 phosphorylation on Ndt80. One possibility is that Ime2 phosphorylation recruits phosphatases to the transcription factor that remove the Mek1 phosphates. When Mek1 levels are above a threshold level, the necessary inhibitory phosphates are maintained. However, lowering the amount of active Mek1, or increasing the amount of active Ime2, tips the balance so that Ndt80 is able to bind to the MSEs in its own promoter to start a positive feedback loop.

In summary, by using the same kinase, Mek1, to regulate both DSB repair and the checkpoint that monitors that repair, the cell is able to ensure that meiotic progression does not occur until all of the potentially lethal DSBs have been fixed.

Strains

The experiment shown in Figure 3 used the following SK1 isogenic diploids:

yLJ92 (Jin *et al.* 2015):

MATα leu2::hisG ho::LYS2 his3::hisG trp1::hisG::P$_{GAL1}$*-NDT80-TRP1*

MATa leu2::hisG ho::LYS2 his3::hisG trp1::hisG::P$_{GAL1}$*-NDT80-TRP1*

ura3::P$_{CPD1}$*-GAL4(848).ER::URA3 IME2ΔC241-as*

ura3::P$_{CPD1}$*-GAL4(848).ER::URA3 IME2ΔC241-as*

NH2451 (Chen *et al.* 2018): same as yLJ92 only *dmc1-2EΔ::kanMX6*

dmc1-2EΔ::kanMX6

NH2478 (this work): same as yLJ92 only *dmc1-2EΔ::kanMX6 mek1Δ::hphMX4*

dmc1-2EΔ::kanMX6 mek1Δ::hphMX4

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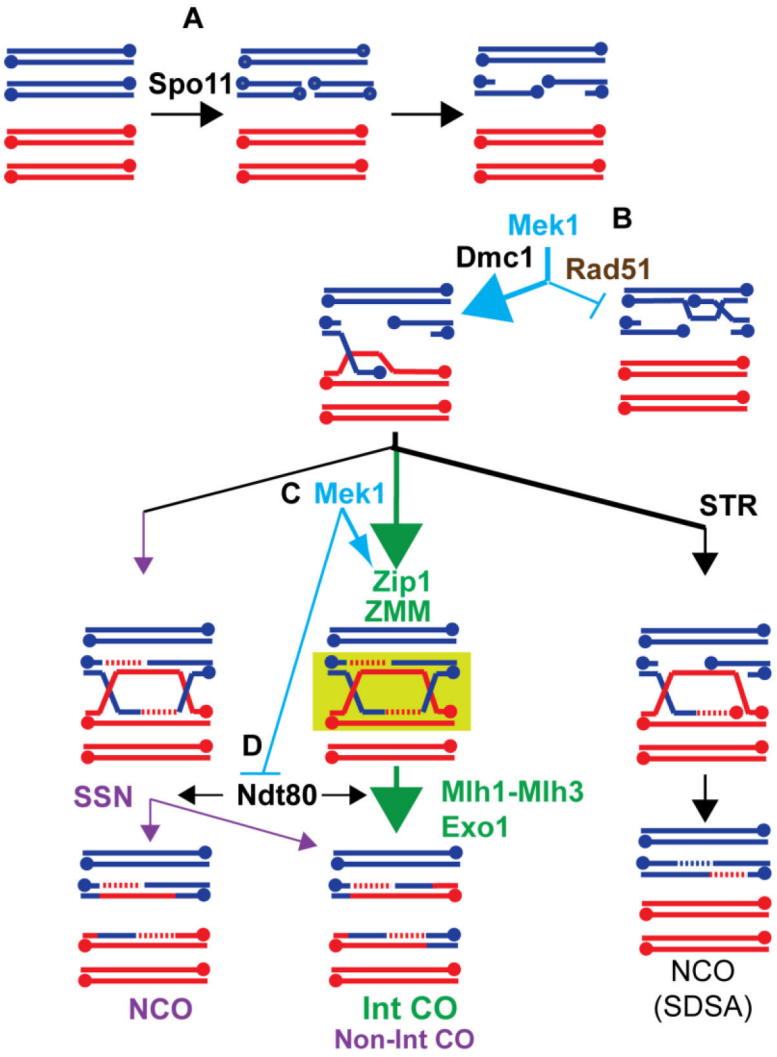

Figure 1. Mek1 regulates multiple steps during meiotic recombination to promote the formation of interhomolog crossovers.

(A) Homologous chromosomes (indicated by red and blue) replicate to make pairs of identical sister chromatids. Spo11 introduces a DSB on one of the four chromatids. The 5' ends of the DSB are then resected to generated 3' single stranded tails (3' ends are indicated by dots). (B) The 3' ends are bound by the Rad51 and Dmc1 recombinases which mediate strand invasion of an homologous duplex. Mek1 activity ensures that the bulk of strand invasion events occur via the homolog using Dmc1, in part by preventing Rad51 from interacting with its accessory factor, Rad54. (C) Mek1 promotes the ZMM pathway of crossover formation by enabling Cdc7-Dbf4 to phosphorylate a conserved region of the C-terminus of the transverse filament protein, Zip1. The green box indicates the protection of the double Holliday junction from disassembly or dissolution, resulting in biased resolution to form interfering crossovers (Int-CO). (D) Mek1 phosphorylates the Ndt80 transcription factor, keeping it inactive while double Holliday junction formation is occurring. Activation of Ndt80 results in the production of the polo-like kinase Cdc5, which triggers Holliday junction resolution.

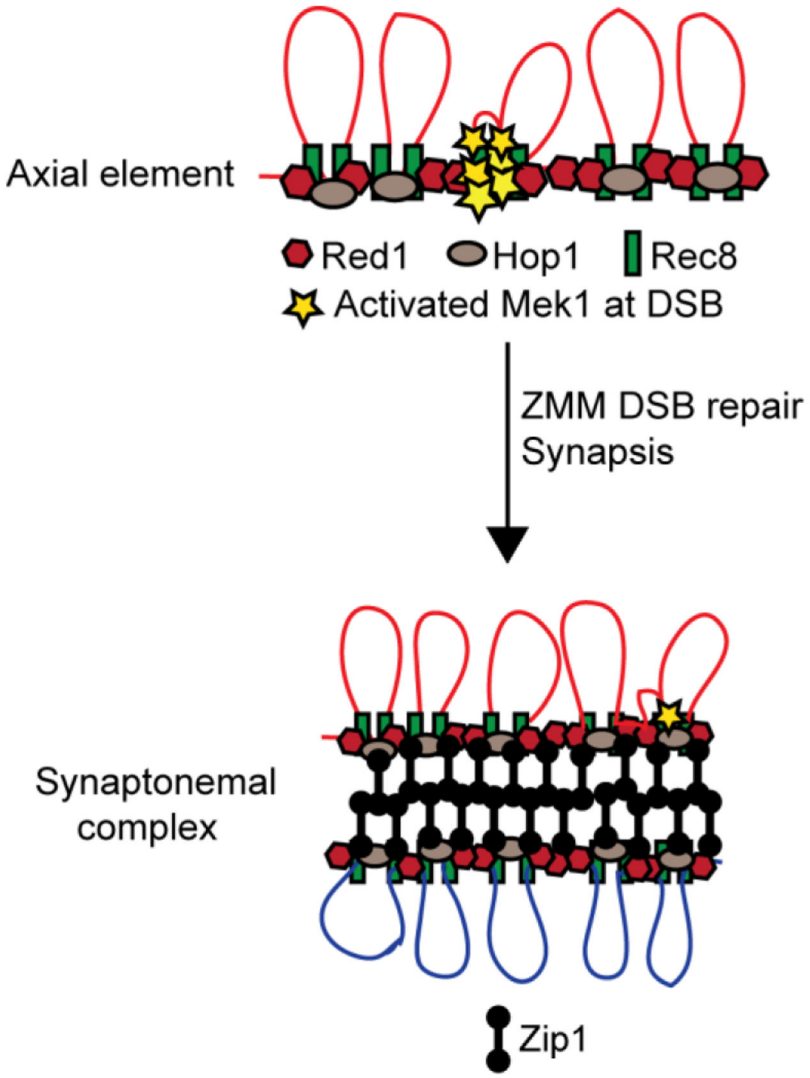

Figure 2. Cartoon of an axial element and synaptonemal complex from budding yeast.

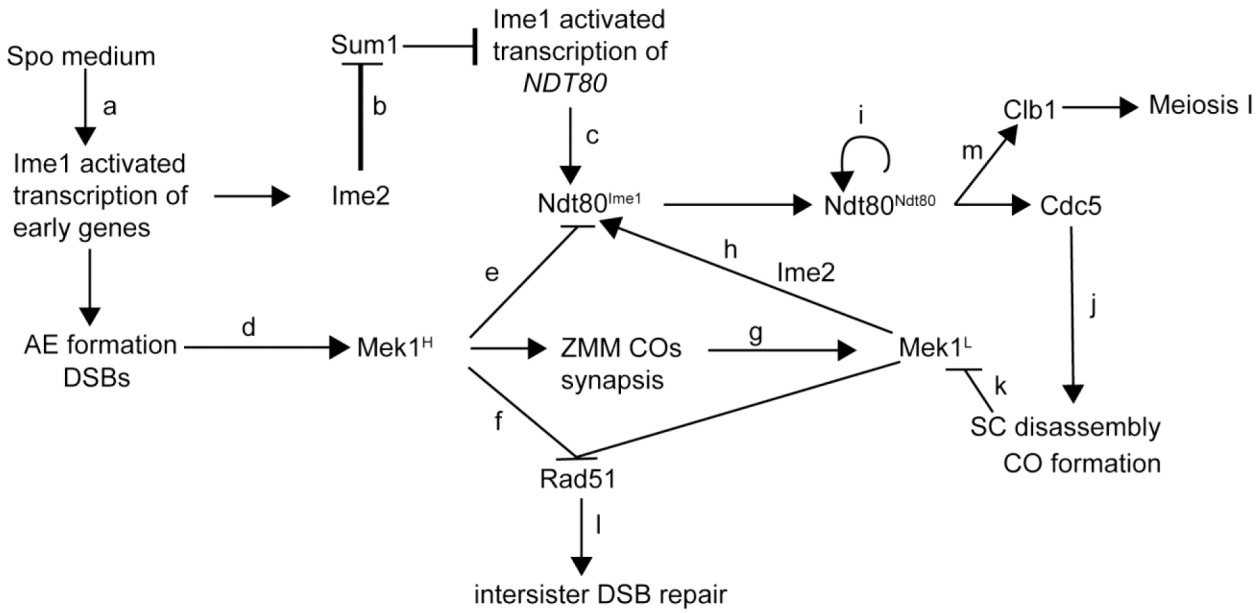

Figure 3. Model for how Mek1 coordinates DSB repair with meiotic progression in budding yeast.

See text for description of the indicated steps.

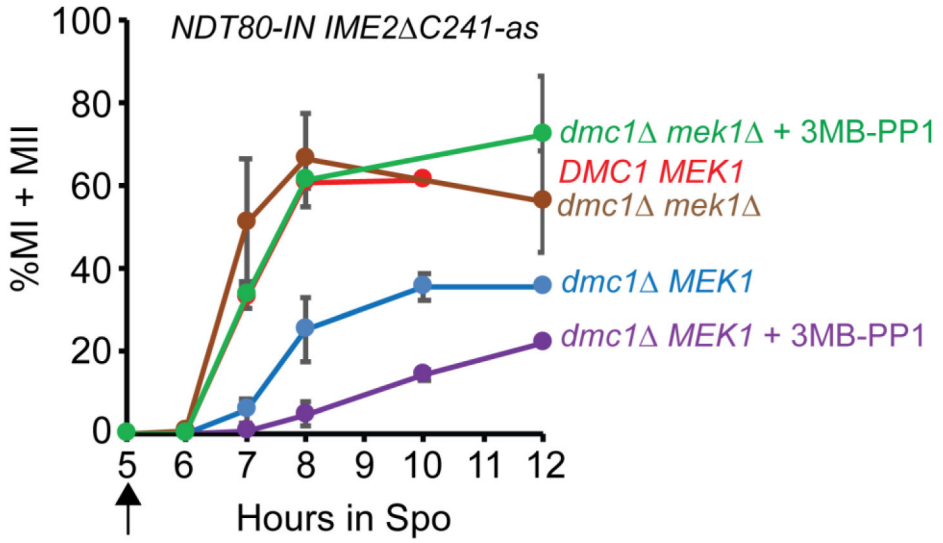

Figure 4. Ime2 is not required to activate Ndt80 in the absence of *MEK1*.

Diploids containing *NDT80-IN IME2ΔC241-as* and either *DMC1 MEK1* (yLJ92), *dmc1Δ MEK1* (NH2451) or *dmc1Δ mek1Δ* (NH2478) were incubated in Spo medium for five hours at which time a final concentration of 1 μM estradiol was added to induce transcription of *NDT80* (indicated by the arrow). For the *dmc1Δ* and *dmc1Δ mek1Δ* diploids, the cultures were split in half and the Ime2ΔC241-as inhibitor, 3MB-PP1, was add to a concentration of 50 μM to one half. Cells were fixed at the indicated timepoints and stained with 4'6-diamidino-2-phenylindole (DAPI) and examined by fluorescent microscopy to count the number of mono-, bi- (MII) and tetra-nucleate (MII) cells. Two hundred cells were counted for each timepoint. Error bars indicate the standard deviation for $n \geq 3$, or the range when $n = 2$. For yLJ92, $n = 1$, NH2451, $n = 4$, NH2478, $n = 2$.