Published in final edited form as:

Chromosoma. 2017 February ; 126(1): 45–58. doi:10.1007/s00412-016-0600-y.

Slx4 Scaffolding in Homologous Recombination and Checkpoint Control: Lessons From Yeast

José R. Cussiol¹, Diego Dibitetto², Achille Pellicoli², and Marcus B. Smolka^{1,*}

¹Department of Molecular Biology and Genetics, Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY, 14853, U.S.A

²Department of Biosciences, University of Milan, 20133, Milano, Italy

Abstract

Homologous recombination-mediated DNA repair is essential for maintaining genome integrity. It is a multi-step process that involves resection of DNA ends, strand invasion, DNA synthesis and/or DNA end ligation and, finally, the processing of recombination intermediates such as Holliday junctions or other joint molecules. Over the last 15 years, it has been established that the Slx4 protein plays key roles in the processing of recombination intermediates, functioning as a scaffold to coordinate the action of structure-specific endonucleases. Recent work in budding yeast has uncovered unexpected roles for Slx4 in the initial step of DNA-end resection and in the modulation of DNA damage checkpoint signaling. Here we review these latest findings and discuss the emerging role of yeast Slx4 as an important coordinator of DNA damage signaling responses and a regulator of multiple steps in homologous recombination-mediated repair.

Keywords

Slx4; Dpb11; Homologous Recombination; Checkpoint; Resection

Introduction

Homologous recombination (HR) is essential for the proper repair of double strand breaks (DSBs) and several types of replication-associated structures, including collapsed replication forks and ssDNA gaps (Heyer 2015). Not surprisingly, mutations in genes involved in HR are often linked to human diseases, especially cancer predisposition syndromes (Aparicio et al. 2014; Digweed and Sperling 2004; Luo et al. 2000; Moynahan and Jasin 2010). On the other hand, recombination has also the potential to result in genomic instability, such as increased levels of gross chromosomal rearrangements, due to recombination between non-allelic sequences (Carr and Lambert 2013; Kolodner et al. 2002).

*Correspondence: mbs266@cornell.edu.

Conflict of Interest

The authors declare that they have no conflict of interest.

Compliance with Ethical Standards

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

HR is a multi-step process that is initiated by 5' to 3' exonucleolytic degradation (DNA-end resection) of the DNA lesion, which provides a 3' ssDNA overhang for the strand exchange protein Rad51 to guide strand invasion and search for homology [for recent reviews please see (Cejka 2015; Heyer 2015; Huertas 2010; Symington 2014)]. Successful homology search and subsequent DNA synthesis leads to the formation of recombination intermediates such as Holliday Junctions (HJs), that physically link sister chromatids. Disentanglement of these joint DNA molecules (JMs) marks the completion of HR-mediated repair, which needs to occur before chromosomal segregation. This crucial step of JM processing is achieved through either topological dissolution mediated by the Sgs1-Top3-Rmi1 complex (BLM-TOP3 α -RMI1-RMI2 in mammals) (Cejka et al. 2012) or via nucleolytic resolution mediated by structure-specific endonucleases such as Slx1-Slx4, Mus81-Mms4, and Yen1 (SLX1-SLX4; MUS81-EME1 and GEN1 in mammals respectively) (Wyatt and West 2014). How cells ensure the proper spatio-temporal coordination of these distinct steps in HR-mediated repair, especially in the context of DNA lesions occurring during DNA replication, remains a major question.

Here we review the roles of the Slx4 scaffold in the regulation of HR-mediated repair in the model organism *Saccharomyces cerevisiae*. While Slx4 is known to play roles in the processing of recombination intermediates by coordinating the action of structure-specific endonucleases, recent studies in budding yeast have revealed additional key roles for Slx4 in other aspects of the DNA damage response and HR repair, including the control of DNA damage checkpoint signaling (DDC) and DNA-end resection, the first step of HR (Fig. 1). Here, we discuss how these emerging roles for yeast Slx4 are now positioning this scaffold protein as a central regulator of multiple steps in HR-repair and DNA damage signaling responses.

Slx4 as a scaffold for coordination of structure-specific endonucleases

Slx4 was initially identified in budding yeast in a screen for genes required for cell viability in the absence of the DNA helicase Sgs1 (Mullen et al. 2001). The screen also identified additional “*SLX*” (synthetic lethal of unknown, “x”, function) genes that included the structure-specific nucleases Slx1 and Mus81-Mms4 (Slx3-Slx2). Sgs1 is required for JM processing via dissolution, which in mitotic cells is heavily favored over resolution as the latter can lead to deleterious crossover events (Wu and Hickson 2003). In the absence of Sgs1 the roles of Slx1 and Mus81-Mms4 become critical, especially for the processing of stalled forks generated at hard-to-replicate regions, such as the rDNA locus (Ii et al. 2007; Kaliraman and Brill 2002). Slx4 physically interacts with multiple structure-specific nucleases and is believed to serve as a platform for the coordination of their action (Flott et al. 2007; Fricke and Brill 2003) (Fig. 2). While Slx4 orthologues are found from yeast to higher eukaryotes (Andersen et al. 2009; Fekairi et al. 2009; Munoz et al. 2009; Saito et al. 2009; Svendsen et al. 2009), they share low amino acid identity with the exception of a conserved C-terminus region consisting of a SAP motif (for SAF-A/B, Acinus and PIAS) that is present in many DNA repair proteins (Aravind and Koonin 2000), and a HtH domain (Helix-turn-Helix). Strikingly, the ability of Slx4 to physically interact with structure-specific nucleases is largely conserved from yeast to humans (Fig. 2). Early studies in yeast have revealed that Slx4 physically interacts with Slx1 and with the Rad1-Rad10 complex

[ERCC4 (XPF)–ERCC1 in mammals], another structure-specific nuclease that is not an “*SLX*” gene (Flott et al. 2007). Subsequent reports in mammals have identified similar interactions, in addition to an interaction with the MUS81-EME1 nuclease (yeast Mus81-Mms4) (Fekairi et al. 2009; Svendsen et al. 2009). Recently, yeast Slx4 was found to also form a complex with Mus81-Mms4 (Gritenaite et al. 2014).

Slx1 is a structure-specific endonuclease that cleaves a wide range of DNA substrates *in vitro*, but with a preference for 5′-flaps structures in yeast (Coulon et al. 2004; Fricke and Brill 2003; Munoz-Galvan et al. 2012). The interaction of Slx4 with Slx1 is mediated by Slx4’s HtH domain (Fig. 2) and a recent structural study in *Candida glabrata* showed that the interaction is responsible for dissociating an inactive Slx1 homodimer to promote its endonuclease activity (Gaur et al. 2015). The importance of Slx1-Slx4, especially in the absence of Sgs1, is likely associated with the fact that replication forks stall with high frequency at the rDNA locus due to the presence of a polar replication fork barrier (RFB) (Brewer and Fangman 1988; Linskens and Huberman 1988). As a consequence, the processing of replication intermediates seems required for proper recovery and restart of these stalled forks (Coulon et al. 2004; Fricke and Brill 2003; Kaliraman and Brill 2002). The role of Slx4 in controlling the action of Slx1 is likely what accounts for the synthetic lethality observed upon deletion of *SLX4* and *SGS1*.

Less is understood about the interaction of Slx4 with Rad1-Rad10. Rad1-Rad10 is required for repair of UV lesions by nucleotide excision repair (NER) and is involved in the repair of DSBs by single strand annealing (SSA) (Ciccica et al. 2008). It has been proposed that the interaction of Slx4 and Rad1-Rad10 is important for SSA repair, as cells lacking *SLX4* are deficient for SSA, but not for NER (Flott et al. 2007; Li et al. 2008). However, it remains unclear exactly how Slx4 interacts with Rad1-Rad10 and if the interaction plays a role in the regulation of Rad1-Rad10 action. A more recent study suggested a role of the Saw1 scaffold in bridging the Slx4 and Rad1-Rad10 interaction and coordinating their functions (Sarangi et al. 2014). Because human SLX4 binds to XPF-ERCC1 through a long N-terminal region that is absent in yeast Slx4 (Fig. 2), it was proposed that Saw1 may serve the function of the human N-terminal region. Finally, yeast Slx4 was also shown to indirectly interact with the Mus81-Mms4 nuclease via a phosphorylation mechanism that also involves additional protein scaffolds (Gritenaite et al. 2014). Human SLX4 interacts with MUS81-EME1 through its SAP domain (Fig. 2), and this interaction seems to be crucial for the processing of HJs formed during sister chromatid exchange (Wyatt et al. 2013). However, the functional relevance of the ability of Slx4 to form a complex with Mus81-Mms4 in budding yeast has yet to be established. While it is tempting to extrapolate the identified physical interaction into a role for Slx4 in controlling Mus81-Mms4-mediated resolution, recent findings pointing to roles for Slx4 in the regulation of DNA damage checkpoint signaling and DNA-end resection (see below) reveal a more complex scenario by which Slx4 may be coordinating HR-mediated repair. For more detailed information regarding the roles of Slx4 in the coordination of structure-specific endonucleases, especially in mammals, we refer to recent excellent reviews (Matos and West 2014; Nowotny and Gaur 2016; Rass 2013; Wyatt and West 2014).

Slx4 interactions with multi-BRCT domain proteins point to functions beyond nuclease scaffolding

Recent reports show that Slx4 controls important aspects of the DNA damage response independently of structure-specific endonucleases. Yeast cells lacking *SLX4* are particularly sensitive to methyl methanesulfonate (MMS) (Chang et al. 2002; Fricke and Brill 2003), an alkylating agent that methylates DNA, leading to replication blocks that are frequently bypassed by replication forks via recombination-mediated template-switching events (Branzei and Foiani 2010). Notably, upon MMS treatment, cells lacking *SLX4* display hyper-activation of the DNA damage checkpoint kinase Rad53 (CHK1/CHK2 in mammals) (Flott and Rouse 2005; Ohouo et al. 2013). However, deletion of any of the nucleases known to interact with Slx4 does not lead to hyperactivation of the checkpoint (Cussiol et al. 2015; Ohouo et al. 2013). Based on these observations, it has been postulated that Slx4 must modulate the DNA damage response independently of structure-specific endonucleases (Ohouo et al. 2013). As discussed later, it is now clear that physical interactions of Slx4 with Rtt107 and Dpb11 (PTIP and TopBP1 in mammals respectively), two scaffolding proteins containing multiple BRCA1 C-terminus (BRCT) domains, are important for the regulation of DNA damage checkpoint signaling and DNA-end resection.

A constitutive interaction with the Rtt107 scaffold

Slx4 was found to interact with the multi-BRCT domain protein Rtt107 over ten years ago (Roberts et al. 2006; Zappulla et al. 2006). However, until recently, the role of this interaction has remained obscure. Rtt107 (Regulator of Ty1 transposition) was initially identified in screens for mutants with increased Ty transposon mobility or DNA repair defects (Chang et al. 2002; Hanway et al. 2002; Scholes et al. 2001). Structurally, Rtt107 contains 6 BRCT domains with four tandem BRCT domains at the N-terminus and two at the C-terminus (Bork et al. 1997; Chin et al. 2006; Zappulla et al. 2006) (Fig. 3a). It is believed that only the C-terminal pair of BRCT (5/6) possesses a phospho-binding pocket capable of interacting with phosphorylated proteins (Li et al. 2012). Slx4 constitutively interacts with Rtt107 through its N-terminal BRCT domains and the interaction is not modulated by DNA damage (Roberts et al. 2006). In addition to Rtt107 being the most stably associated Slx4 binding partner, pieces of evidence pointed to the importance of the joint action of the Slx4-Rtt107 complex for the response to replication stress: i) Cells lacking Rtt107 or Slx4 have similar enhanced sensitivity to MMS and have difficulty recovering from MMS treatment (Roberts et al. 2006); ii) Rtt107 and Slx4 are heavily phosphorylated by the DNA damage signaling kinase Mec1 (ATR in mammals), especially in response to MMS (Flott and Rouse 2005; Rouse 2004); iii) Robust phosphorylation of Slx4 by Mec1 requires the presence of Rtt107, and vice-versa (Ohouo et al. 2010; Roberts et al. 2006). Of note, Rtt107 is not required for survival in the absence of Sgs1, indicating an Slx1-independent role for the Slx4-Rtt107 complex (Zappulla et al. 2006). It has been proposed that the Slx4-Rtt107 complex is somehow involved in the recovery of stalled forks during replication stress and that Rtt107 works as a scaffold to recruit Slx4 to stalled replication forks (Roberts et al. 2008). This notion was further substantiated by the finding that Rtt107 is recruited to chromatin following replication stress via recognition of γ H2A

(histone H2A phosphorylated at serine 129) (Balint et al. 2015; Li et al. 2012; Williams et al. 2010), which is generated by the DNA damage signaling kinases Mec1 and Tel1 (ATR and ATM in mammals). Structural and biochemical work have convincingly shown that such recruitment of Rtt107 is mediated by its C-terminal pair of BRCT domains (5/6) (Li et al. 2012; Williams et al. 2010). It is important to note that Rtt107 has Slx4-independent roles as *rtt107 slx4* cells are more sensitive to MMS than the single mutants and *rtt107* cells show enhanced sensitivity to hydroxyurea (HU) while *slx4* cells do not (Roberts et al. 2006). The more central role of Rtt107 is related to its interactions with other genome maintenance factors. In addition to interacting with Slx4, Rtt107 also forms complexes with the Rtt101 ubiquitin ligase complex and the cohesin-like/sumo-ligase Smc5/6 complex (Ohouo et al. 2010; Roberts et al. 2008). These three distinct interactions occur in a mutually exclusive manner (Hang et al. 2015), suggesting a scenario in which Rtt107 functions as a central and multifunctional scaffold for the coordination of DNA damage and replication stress responses.

A phosphorylation-regulated interaction with the Dpb11 scaffold

Mass spectrometry analysis revealed that Slx4 also interacts with Dpb11 (Ohouo et al. 2010), an evolutionarily conserved multi-BRCT domain protein (orthologue of mammalian TopBP1) that plays essential roles in replication initiation and checkpoint activation (Garcia et al. 2005; Mordes et al. 2008; Navadgi-Patil and Burgers 2008; Tanaka et al. 2007; Zegerman and Diffley 2007). While Slx4 directly interacts with Dpb11, Rtt107 was shown to be important to stabilize the interaction (Ohouo et al. 2010). Importantly, the Slx4-Dpb11 interaction is mediated by BRCT domains in Dpb11, mainly BRCT-1/2, and by multiple phosphorylation sites in Slx4 (Fig. 3b) (Ohouo et al. 2010; Ohouo et al. 2013). The interaction is cell cycle regulated and highly dependent on a canonical CDK site on Slx4 (serine 486) (Gritenaite et al. 2014; Ohouo et al. 2013). Furthermore, consistent with the interaction being induced by replication stress or DNA damage, mutation of seven canonical Mec1 sites (7MUT) in Slx4 strongly reduces its interaction with Dpb11 (Ohouo et al. 2010; Ohouo et al. 2013). While yeast cells expressing the *slx4-7MUT* or the *slx4-S486A* alleles are also sensitive to MMS, they are not synthetic lethal with *SGS1* deletion (Ohouo et al. 2010; Ohouo et al. 2013). Together, these findings revealed that a phosphorylation-regulated Rtt107-Slx4-Dpb11 complex plays a key role in the response to replication stress (Fig. 3).

Precise mechanistic understanding of the roles of the Rtt107-Slx4-Dpb11 complex emerged in recent years and points to the control of DNA damage checkpoint signaling and DNA-end resection. Upon DNA damage or replication stress, Dpb11 is recruited to DNA breaks and other replication-induced lesions such as ssDNA gaps via its interaction with Ddc1 (RAD9 in mammals), a subunit of the 9-1-1 checkpoint clamp. Recruitment of Dpb11 to 9-1-1 requires phosphorylation of Ddc1 by Mec1 at threonine 602 that is recognized by BRCT-3/4 of Dpb11 (Fig. 3a). The phospho-dependent Dpb11-Ddc1 interaction is conserved in mammals (TopBP1-RAD9) and in both yeast and mammals this interaction plays an important role in activation of the DNA damage checkpoint (Delacroix et al. 2007; Navadgi-Patil and Burgers 2009). In budding yeast, both Ddc1 and Dpb11 possess a Mec1 activation domain (MAD), an unstructured region at the C-terminus of these proteins that is required for activation of the Mec1 kinase (Navadgi-Patil and Burgers 2009). In addition to its role in

Mec1 activation, Dpb11 also binds to the checkpoint mediator Rad9 (53BP1 in mammals) in a cell cycle-dependent manner (Pfander and Diffley 2011), which likely helps stabilize Rad9 at DNA lesions. Recruitment of Rad9 by Dpb11 enables Mec1 to extensively phosphorylate Rad9, creating docking phospho-sites for the recruitment and activation of the downstream DDC kinase Rad53 [for details on Rad53 activation see (Pellicioli and Foiani 2005)]. Therefore, Dpb11 is believed to form a complex with Mec1, 9-1-1, Rad9 and Rad53 at sites of lesions, functioning as a pro-checkpoint factor (Fig. 4a). As an intrinsic part of the DNA damage checkpoint response, Rad53 signaling controls a range of functional outputs including cell cycle arrest, inhibition of DNA replication initiation and inhibition of the Exo1 nuclease involved in DNA end resection [for reviews on DNA damage checkpoint, please refer to (Branzei and Foiani 2006; Hustedt et al. 2013; Labib and De Piccoli 2011)]. Because the interactions of Dpb11 with Slx4 and Rad9 are largely dependent on BRCT-1/2 of Dpb11, and therefore mutually exclusive, our group proposed a model in which Slx4 binding to Dpb11 counteracts the engagement of Rad9 at DNA lesions, and thereby dampens checkpoint signaling (Figure 4) (Cussiol et al. 2015; Ohouo et al. 2013). Noteworthy, a recent work provided evidence that the Slx4-Dpb11 interaction is mediated by BRCT-3/4 (Gritenaite et al. 2014), in contrast to our results showing that Slx4 binds preferentially to Dpb11 BRCT-1/2 (Fig. 3c). While we do not exclude the possibility that Slx4 adopts different modes of interaction with Dpb11, as we also observe some level of interaction of BRCT-3/4 with Slx4, more recent experiments demonstrated that mutations in BRCT-1/2 of Dpb11 dramatically reduce its interaction with Slx4, while mutations in BRCT-3/4 have relatively less impact on the interaction (Cussiol et al. 2015). To understand the Slx4-Dpb11 interaction in greater detail, it will be important study the interaction *in vitro* using synthetic phosphopeptides that mimic Slx4 phosphorylated surfaces recognized by BRCT domains of Dpb11. Such reconstitution would enable structural and more quantitative analyses. A possible scenario we envision is that the Slx4-Dpb11 interaction undergoes multiple transitions. For example, the interaction may be initiated by recognition of phosphorylated serine 486 in Slx4 via BRCT-1/2, and formation of a transient “bridge” to 9-1-1 that disengages Rad9, as depicted in Fig. 3c and 4a. This “bridging” configuration would allow extensive phosphorylation of Slx4 by Mec1, creating additional phosphosites that are then recognized by BRCT-3/4. It is tempting to speculate that the ability of Slx4 to bind to multiple BRCT domains in Dpb11 may contribute to strengthening the interaction, so Slx4 may more efficiently sequester Dpb11 and prevent it from re-engaging with Rad9. While this transition would free up 9-1-1 to engage with another Dpb11 molecule, the abundance of Dpb11 in the cell is quite low [~200 copies per cell (Ghaemmamghami et al. 2003; Mantiero et al. 2011)], suggesting that it should become limiting.

Recruitment of Slx4 to DNA lesions

How Slx4 is recruited to sites of DNA lesions is a pivotal question for understanding its function as a bona fide scaffold in the control of DNA damage responses. Efficient recruitment of Slx4 to DNA lesions requires interactions with both Rtt107 and Dpb11 (Fig. 3c) (Cussiol et al. 2015; Dibitetto et al. 2016). Recent work demonstrated that recognition of γ H2A and phospho-Ddc1^{T602} via BRCT-5/6 of Rtt107 and BRCT-3/4 of Dpb11, respectively, is required for dampening of Rad53 signaling (Cussiol et al. 2015). Based on

the available data, Dpb11 seems particularly important for the stabilization of Slx4 precisely at the 5' recessed end of ssDNA::dsDNA junctions formed at DSBs or ssDNA gaps (we note that the specific recruitment to these junctions may not parallel exactly recruitment to the long track of γ H2A). Slx4 is also important to efficiently stabilize Dpb11. A "two-site docking" mechanism was proposed, in which the Rtt107-Slx4-Dpb11 complex recognizes phosphorylated 9-1-1 loaded at these ssDNA::dsDNA junctions, as well as a nearby γ H2A-containing nucleosome (Fig. 3c) (Cussiol et al. 2015). Notably, the proposed model predicts that Slx4 can be recruited to any DNA lesion having a ssDNA::dsDNA junction with a 5' recessed end in which the following events occur: 9-1-1 loading; Mec1 recruitment/activation; and phosphorylation of nearby nucleosomes by Mec1. Consistent with this model, Slx4 is efficiently recruited and phosphorylated upon treatment with MMS, a genotoxin that leads to extensive accumulation of ssDNA gaps behind a moving replication fork and, therefore, multiple ssDNA::dsDNA junctions (Balint et al. 2015; Cussiol et al. 2015; Flott et al. 2007). In addition, resection of double strand breaks also generates ssDNA::dsDNA junctions that should support Slx4 recruitment. Indeed, ChIP approaches were successfully used to detect recruitment of Slx4 to an HO-inducible DSB (Dibitetto et al. 2016; Leung et al. 2016; Toh et al. 2010). Congruent with these findings, functional studies also reveal important roles for Slx4 in the response to DSBs (Dibitetto et al. 2016).

Slx4 recruitment to DNA lesions counteracts Rad53 signaling

A major step toward understanding the roles for Slx4 that are independent of its nuclease scaffolding functions derived from the observation that cells lacking Slx4 show Rad53 hyperactivation upon MMS treatment (Ohouo et al. 2013). Because Rad53 hyperactivation was not observed in cells lacking the nucleases known to interact with Slx4, we hypothesized that Slx4 is a negative regulator of DDC signaling. Importantly, *slx4* cells did not display enhanced phosphorylation of direct targets of the upstream kinase Mec1, supporting the notion that the increased Rad53 activation in these cells is not due to the higher accumulation of damaged and unrepaired DNA (Ohouo et al. 2013). Compelling genetic evidence of a role for Slx4 in DDC dampening came from the finding that a hypomorphic allele of Rad53 (*rad53-R605A*) is able to rescue the MMS sensitivity of *slx4* cells (Jablonowski et al. 2015; Ohouo et al. 2013). Together, these observations indicate that *slx4* cells are sensitive to MMS due to the inability of properly terminating Rad53 signaling. Because activated Rad53 represses DNA replication and cell cycle progression, it is imperative for the cells to terminate Rad53 signaling in order to resume cell growth. Mechanistically, Slx4 was found to dampen Rad53 signaling by competing with Rad9 for Dpb11 binding (Ohouo et al. 2013) (Fig. 4a). As described above, Slx4 and Rad9 both bind to Dpb11 through a similar mode of interaction that involves BRCT-1/2 of Dpb11 (Ohouo et al. 2013). In addition, the Slx4-Rtt107 complex, as well as Rad9, are recruited to chromatin via a BRCT-mediated interaction with γ H2A (Balint et al. 2015; Hammet et al. 2007; Li et al. 2012; Toh et al. 2006; Williams et al. 2010). Therefore, Slx4-Rtt107 and Rad9 are recruited to DNA lesions by interacting with the same docking phosphorylation sites, implying that their recruitment is mutually exclusive. These findings led to the model where Slx4 down-regulates Rad53 signaling via a competition-based mechanism that balances the engagement of the Rad9 adaptor at DNA lesions, a process we named DAMP (Dampens

checkpoint Adaptor Mediated Phospho-signaling) (Ohouo et al. 2013) (Fig. 4a). The checkpoint dampening function of Slx4 is not restricted to MMS-induced replication stress, and a recent report showed that *slx4* cells do not properly down-regulate Rad53 and do not efficiently adapt to one irreparable DSB (Dibitetto et al. 2016). Of importance, these issues were observed in *rtt107* cells, but not in *slx1 rad1 mus81* cells, which adapt normally to one irreparable DSB, further supporting that the Slx4-Rtt107 complex dampens the checkpoint independently of these nucleases (Dibitetto et al. 2016). In addition, consistent with the competition-based model, the checkpoint adaptation defect of *slx4* cells is correlated with higher recruitment of Rad9 to regions flanking the DSB (Dibitetto et al. 2016). In principle, the competition mechanism does not require dephosphorylation of Rad53 or Rad9, so Slx4-mediated dampening of Rad53 signaling may be considered a phosphatase-independent mechanism for down-regulating the checkpoint. Of note, Slx4-mediated dampening is thought to counteract *de novo* Rad53 activation, but not de-activate already activated Rad53. This latter function is mostly performed by the Pph3, Ptc2 and Ptc3 phosphatases (Jablonowski et al. 2015; Kim et al. 2011a; O'Neill et al. 2007; Szyjka et al. 2008).

Slx4 promotes DNA-end resection

Why would a bona-fide HR factor such as Slx4 control Rad53 signaling? The emerging scenario in yeast is that in addition to arresting the cell cycle and inhibiting DNA replication, Rad53 activity also exerts an inhibitory effect on distinct steps of HR. Therefore, down-regulation of DDC would be critical to promote HR. Recent work revealed that the ability of Slx4 to bind to Dpb11 and counteract Rad9 recruitment is also important to promote DNA-end resection (Dibitetto et al. 2016). This first step in HR is best understood in the context of DSBs and requires the coordinated action of the MRX complex (MRN in mammals) and the Exo1 and Dna2 nucleases (EXO1 and DNA2 in mammals). Resection at a DSB starts through an endonucleolytic nick by the action of the MRX subunit Mre11 (MRE11 in mammals), activated by Sae2 (CtIP in mammals) close to the DSB end (Cannavo and Cejka 2014). This action triggers a short 3'-5' nucleolytic processing toward DNA ends, and allows the more extensive 5'-3' degradation mediated by Exo1 and Dna2, in cooperation with the RecQ helicase Sgs1 (Mimitou and Symington 2008; Symington and Gautier 2011; Zhu et al. 2008). Importantly, Rad9 has been shown to block resection (Clerici et al. 2014; Ferrari et al. 2015; Lazzaro et al. 2008), a function that is evolutionary conserved and well documented in the mammalian orthologue 53BP1 (Bunting et al. 2010; Chapman et al. 2013; Di Virgilio et al. 2013; Escribano-Diaz et al. 2013; Zimmermann et al. 2013). In budding yeast, cells lacking *RAD9* resect DSBs faster and more extensively (Chen et al. 2012; Clerici et al. 2014; Lazzaro et al. 2008), and while it remains unknown how Rad9-mediated resection block is achieved, it likely involves multiple mechanisms. One mechanism apparently relies on Rad9-mediated activation of Rad53, which promotes Rad53-dependent phosphorylation and inhibition of Exo1 (Cotta-Ramusino et al. 2005; Jia et al. 2004; Kaochar et al. 2010; Morin et al. 2008; Segurado and Diffley 2008). This Rad53-mediated inhibition of resection is possibly complemented by the ability of Rad9 to oligomerize and form a physical block surrounding the DSB. It is tempting to speculate that the interaction of Rad9 with Dpb11 is important not only to promote Rad53 activation, but

also to stabilize Rad9 at the ssDNA::dsDNA junction to efficiently mask the 5' recessed end, therefore preventing access of Exo1 and Dna2-Sgs1. Congruent with this hypothesis, recent findings have shown that Rad9 reduces the recruitment of Exo1 and Dna2-Sgs1 to DSBs (Bonetti et al. 2015; Ngo and Lydall 2015).

Given the roles of Rad9 in blocking resection, it is logical that the ability of Slx4 to counteract Rad9 recruitment to DNA lesions would help avert the block, therefore promoting resection. Indeed, a recent paper by our groups provided experimental evidence that the Slx4-Rtt107 complex favors resection of DSBs (Dibitetto et al. 2016). Taking advantage of a system for induction of a single irreparable DSB at a specific DNA locus by the HO endonuclease it is possible to track the amount of ssDNA that is formed once the DSB is resected. Cells lacking *SLX4* or *RTT107* show a significant decrease in ssDNA accumulation at distal regions from a DSB together with a severe defect to dampen Rad53 signaling (Dibitetto et al. 2016). These observations may suggest that Slx4-Rtt107 is particularly important for long-range resection. Consistent with that, Slx4 was not detected close to DSB ends (Toh et al. 2010), however it is recruited at a few kilobases from the break (Dibitetto et al. 2016; Leung et al. 2016). In agreement with the notion that Slx4-Rtt107 competes with Rad9 for recruitment to DNA lesions (Fig. 4a), decreased resection observed in cells lacking Slx4 or Rtt107 is correlated with increased binding of Rad9 to DSBs (Dibitetto et al. 2016). Moreover, cells expressing the *slx4-S486A* allele phenocopy the resection defects of *slx4* and *rtt107* cells underlining the critical role of the Slx4-Dpb11 interaction. Collectively, these observations corroborate the proposed model for Slx4 recruitment (Fig. 3c), and reveal its role in promoting resection by counteracting Rad9 recruitment and Rad53 signaling (Fig. 4a). While the role of resection in the repair of DSBs by HR is well established, less is known about the importance of resection for the repair of ssDNA gaps by HR. Evidence suggest that expansion of the ssDNA gaps by the action of exonucleases, especially Exo1, would be important to facilitate topological DNA transactions mediated by recombination factors (Giannattasio et al. 2010; Karras et al. 2013; Ma et al. 2013). In agreement with that, Exo1 overexpression can partially rescue the MMS sensitivity of *slx4* cells (unpublished observations).

Checkpoint dampening via Slx4 helps promote Mus81-Mms4 function

In addition to promoting DNA-end resection, the ability of Slx4 to dampen the DDC seems to positively influence other steps in HR. This appears to be the case for Mus81-Mms4-mediated resolution, which is inhibited by DDC signaling. In fission yeast, Cds1 (Rad53 homolog) binds and phosphorylates Mus81 leading to its dissociation from chromatin, thus preventing the processing of stalled forks (Kai et al. 2005). So far a similar mechanism has not been characterized in budding yeast, but there are circumstantial evidence that DDC signaling through Rad53 inhibits Mus81-Mms4 function indirectly through inhibition of Cdc5 activity (Sanchez et al. 1999; Szakal and Branzei 2013; Zhang et al. 2009). Mus81-Mms4 activity is restricted to late G2/M, before chromosome segregation, when Cdc28 (CDK) and Cdc5 phosphorylate the regulatory subunit Mms4 (Gallo-Fernandez et al. 2012; Matos et al. 2013). This mechanism for temporal restriction is important to prevent premature activation of Mus81 during replication, which could lead to crossover-associated chromosome translocations as well as premature processing of joint molecules (JMs) formed

between sister chromatids (Szakal and Branzei 2013). On the other hand, inhibition of Mus81-Mms4 during G2/M would prevent the efficient processing of JMs that escape Sgs1-Top3-Rmi1-mediated dissolution leading to segregation of joint chromosomes and, consequently, chromosome breaks. Consistent with the notion that DDC hypersignaling plays an inhibitory role on Mus81-Mms4 activity, cells lacking the Rad53 phosphatase Pph3 also display MMS sensitivity that seems correlated with the inability to resolve JMs (Jablonowski et al. 2015). These results point to checkpoint dampening as an important mechanism by which Slx4 favors Mus81-Mms4 action, although it remains plausible that this is also achieved via a scaffolding function. Slx4 indirectly interacts with Mus81-Mms4, in a complex that also comprises of Dpb11 and Rtt107 (Gritenaite et al. 2014). Because the SLX4 interaction with MUS81 in human cells has been shown to be important for Holliday Junction resolution (Castor et al. 2013), it is tempting to speculate that the interaction of Slx4 with Mus81-Mms4 in budding yeast is also functionally relevant. However, there are important differences in the architecture of these complexes in yeast compared to humans (Fig. 3) and currently, a separation-of-function mutant that specifically impairs the interaction of Slx4-Dpb11 with Mms4-Mus81 has not been generated. Therefore, the functional relevance of the interaction is yet to be determined. Overall, while yeast Slx4 is likely to play some scaffolding function for Mus81-Mms4 action, lines of evidence point to the control of DDC signaling as being a key mechanism by which Slx4 promotes Mus81-Mms4-mediated resolution.

Slx4 uncouples Mec1 signaling from Rad53 activation

The canonical DDC response involves activation of the apical kinase Mec1 followed by activation of the downstream kinase Rad53, which then mediates several of the established checkpoint outputs (Fig. 4b). As discussed above, Rad53 signaling negatively affects HR, implying that Rad53 needs to be counteracted for pro-HR events such as resection to occur. Consistent with this notion, fork-restart after MMS-induced stalling requires Rad53 deactivation (O'Neill et al. 2007; Szyjka et al. 2008). Interestingly, Mec1 directly phosphorylates many proteins independently of Rad53 (Bastos de Oliveira et al. 2015; Smolka et al. 2007) and, in contrast to Rad53 signaling, this direct mode of Mec1 signaling apparently exerts multiple pro-HR effects. For example, it has been proposed that Mec1 phosphorylation of Rad51 promotes its ATP hydrolysis and DNA binding activity (Flott et al. 2011). In addition, phosphorylation of the Sae2 endonuclease by Mec1 seems to be important to promote DNA-end resection and repair by HR (Chen et al. 2015; Clerici et al. 2006; Puddu et al. 2015). Finally, Rtt107 phosphorylation by Mec1 has been reported to be important for proper sister chromatid recombination (Ullal et al. 2011). Therefore, the ability of Slx4 to specifically counteract Rad53 activation while maintaining active Mec1 signaling represents a clever mechanism for HR regulation (Fig. 4b). The role of Slx4 in uncoupling Mec1 signaling from Rad53 activation was first noticed using quantitative phosphoproteomic analysis (Ohouo et al. 2013), and more recently the Brown lab showed that Slx4 in fact contributes to amplify Mec1 signaling, possibly by stabilizing Dpb11 (a Mec1 activator itself) at the DNA lesion (Figs 3c and 5) (Balint et al. 2015). In addition, Rad9 displacement and Rad53 down-regulation promotes resection, which in turn recruits more Mec1 due to increased ssDNA exposure. Taken together, these findings point to an

intricate regulatory mechanism by which Slx4 controls a range of signaling events with the ultimate goal of facilitating HR (see model in Fig. 5).

An integrated model for HR-control via Slx4

In summary, recent reports in budding yeast have revealed unexpected new roles for Slx4 in the control of HR, suggesting a complex mode of action for this scaffold at the intersection of HR and checkpoint signaling (Fig. 6). Importantly, it seems now clear that yeast Slx4 operates in HR control not only by serving as a scaffold for structure-specific endonucleases, but also by interacting with the BRCT-domain scaffolds Dpb11 and Rtt107 to counteract Rad9 function and regulate DDC signaling. This latter role is critical for Slx4 to function as a pro-HR factor promoting DNA-end resection and JM resolution. Figure 6 depicts our current integrated view for how Slx4 functions in the regulation of HR, differentiating the roles that are mediated via physical interaction with structure-specific nucleases and the roles promoted via counteraction of Rad9 and DDC. It remains unclear the precise contribution of the interactions between Slx4 and Mus81-Mms4 and between Slx4 and Rad1-Rad10 for JM resolution and SSA, respectively.

Does mammalian SLX4 also play pro-HR roles independently of its interaction with structure-specific nucleases?

It is currently unclear whether the roles of yeast Slx4 in regulating DNA-end resection and checkpoint signaling are conserved in mammals. In humans, mutations in the *SLX4* gene are found in Fanconi anemia (FA) patients, an autosomal recessive disease associated with congenital disorders, bone marrow failure and predisposition to cancers (Kim et al. 2011b). The FA pathway plays established roles in protecting chromosome integrity during S-phase, especially when replication forks stall at DNA interstrand cross-links (ICLs). Furthermore, mammalian SLX4 is also important for the repair of other types of lesions induced by camptothecin and PARP inhibitors (Kim 2014). It will be interesting to test whether in mammals SLX4 functions as a pro-resection factor to initiate HR-mediated repair of distinct DNA lesions. As such, it would be important to explore whether human SLX4 retained across evolution the ability to compete with 53BP1 for binding to TopBP1 (human ortholog of Dpb11) to avert a resection block. Consistent with this hypothesis, human SLX4 was shown to interact with TopBP1 in osteosarcoma tumor cells (Gritenaite et al. 2014) and be targeted by the ATR/ATM kinases (Matsuoka et al. 2007). While there are no evidence pointing to the relevance of ATR/ATM phosphorylation in promoting binding to TopBP1, it is tempting to speculate that, like in yeast, ATR/ATM signaling may somehow facilitate SLX4 recruitment to DNA lesions. However, we do not exclude the possibility that in mammals, other protein scaffolds besides SLX4 are in fact functioning as yeast Slx4 in the coordination of ATR signaling, checkpoint responses and HR-mediated repair. Coordination of these processes in mammals is likely to be extremely complex, so understanding the exact mechanisms by which this coordination is achieved will be an enormous challenge. Because DDC and HR dysfunctions are closely linked to many human cancers, we anticipate that a better understanding of the mechanisms interfacing DDC signaling and HR control in humans will provide important new directions to understand tumorigenesis and to develop

treatment. Interestingly, a recent report pointing to roles for mammalian SLX4 in interfacing with sumoylation-mediated pathways may open new avenues to understand how SLX4 regulates DNA damage responses independently of nuclease scaffolding (Guervilly et al. 2015).

Acknowledgments

This work was supported by a grant to M.B.S. from the National Institutes of Health (R01-GM097272) and to A.P. from Associazione Italiana per la Ricerca sul Cancro (AIRC IG-15488). We thank all the Smolka lab members for comments and suggestions.

References

- Andersen SL, Bergstralh DT, Kohl KP, LaRocque JR, Moore CB, Sekelsky J. Drosophila MUS312 and the vertebrate ortholog BTBD12 interact with DNA structure-specific endonucleases in DNA repair and recombination. *Molecular cell*. 2009; 35:128–135. DOI: 10.1016/j.molcel.2009.06.019 [PubMed: 19595722]
- Aparicio T, Baer R, Gautier J. DNA double-strand break repair pathway choice and cancer. *DNA repair*. 2014; 19:169–175. DOI: 10.1016/j.dnarep.2014.03.014 [PubMed: 24746645]
- Aravind L, Koonin EV. SAP - a putative DNA-binding motif involved in chromosomal organization. *Trends in biochemical sciences*. 2000; 25:112–114. [PubMed: 10694879]
- Balint A, et al. Assembly of Slx4 signaling complexes behind DNA replication forks. *The EMBO journal*. 2015; 34:2182–2197. DOI: 10.15252/embj.201591190 [PubMed: 26113155]
- Bastos de Oliveira FM, et al. Phosphoproteomics reveals distinct modes of Mec1/ATR signaling during DNA replication. *Molecular cell*. 2015; 57:1124–1132. DOI: 10.1016/j.molcel.2015.01.043 [PubMed: 25752575]
- Bonetti D, Villa M, Gobbin E, Cassani C, Tedeschi G, Longhese MP. Escape of Sgs1 from Rad9 inhibition reduces the requirement for Sae2 and functional MRX in DNA end resection. *EMBO reports*. 2015; 16:351–361. DOI: 10.15252/embr.201439764 [PubMed: 25637499]
- Bork P, Hofmann K, Bucher P, Neuwald AF, Altschul SF, Koonin EV. A superfamily of conserved domains in DNA damage-responsive cell cycle checkpoint proteins. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 1997; 11:68–76. [PubMed: 9034168]
- Branzei D, Foiani M. The Rad53 signal transduction pathway: Replication fork stabilization, DNA repair, and adaptation. *Experimental cell research*. 2006; 312:2654–2659. DOI: 10.1016/j.yexcr.2006.06.012 [PubMed: 16859682]
- Branzei D, Foiani M. Maintaining genome stability at the replication fork. *Nature reviews Molecular cell biology*. 2010; 11:208–219. DOI: 10.1038/nrm2852 [PubMed: 20177396]
- Brewer BJ, Fangman WL. A replication fork barrier at the 3' end of yeast ribosomal RNA genes. *Cell*. 1988; 55:637–643. [PubMed: 3052854]
- Bunting SF, et al. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. *Cell*. 2010; 141:243–254. DOI: 10.1016/j.cell.2010.03.012 [PubMed: 20362325]
- Cannavo E, Cejka P. Sae2 promotes dsDNA endonuclease activity within Mre11-Rad50-Xrs2 to resect DNA breaks. *Nature*. 2014; 514:122–125. DOI: 10.1038/nature13771 [PubMed: 25231868]
- Carr AM, Lambert S. Replication stress-induced genome instability: the dark side of replication maintenance by homologous recombination. *J Mol Biol*. 2013; 425:4733–4744. DOI: 10.1016/j.jmb.2013.04.023 [PubMed: 23643490]
- Castor D, et al. Cooperative control of holliday junction resolution and DNA repair by the SLX1 and MUS81-EME1 nucleases. *Molecular cell*. 2013; 52:221–233. DOI: 10.1016/j.molcel.2013.08.036 [PubMed: 24076219]

- Cejka P. DNA End Resection: Nucleases Team Up with the Right Partners to Initiate Homologous Recombination. *The Journal of biological chemistry*. 2015; 290:22931–22938. DOI: 10.1074/jbc.R115.675942 [PubMed: 26231213]
- Cejka P, Plank JL, Dombrowski CC, Kowalczykowski SC. Decatenation of DNA by the *S. cerevisiae* Sgs1-Top3-Rmi1 and RPA complex: a mechanism for disentangling chromosomes. *Molecular cell*. 2012; 47:886–896. DOI: 10.1016/j.molcel.2012.06.032 [PubMed: 22885009]
- Chang M, Bellaoui M, Boone C, Brown GW. A genome-wide screen for methyl methanesulfonate-sensitive mutants reveals genes required for S phase progression in the presence of DNA damage. *Proceedings of the National Academy of Sciences of the United States of America*. 2002; 99:16934–16939. DOI: 10.1073/pnas.262669299 [PubMed: 12482937]
- Chapman JR, et al. RIF1 is essential for 53BP1-dependent nonhomologous end joining and suppression of DNA double-strand break resection. *Molecular cell*. 2013; 49:858–871. DOI: 10.1016/j.molcel.2013.01.002 [PubMed: 23333305]
- Chen H, et al. Sae2 promotes DNA damage resistance by removing the Mre11-Rad50-Xrs2 complex from DNA and attenuating Rad53 signaling. *Proceedings of the National Academy of Sciences of the United States of America*. 2015; 112:E1880–1887. DOI: 10.1073/pnas.1503331112 [PubMed: 25831494]
- Chen X, et al. The Fun30 nucleosome remodeller promotes resection of DNA double-strand break ends. *Nature*. 2012; 489:576–580. DOI: 10.1038/nature11355 [PubMed: 22960743]
- Chin JK, Bashkurov VI, Heyer WD, Romesberg FE. Esc4/Rtt107 and the control of recombination during replication. *DNA repair*. 2006; 5:618–628. DOI: 10.1016/j.dnarep.2006.02.005 [PubMed: 16569515]
- Ciccio A, McDonald N, West SC. Structural and functional relationships of the XPF/MUS81 family of proteins. *Annual review of biochemistry*. 2008; 77:259–287. DOI: 10.1146/annurev.biochem.77.070306.102408
- Clerici M, Mantiero D, Lucchini G, Longhese MP. The *Saccharomyces cerevisiae* Sae2 protein negatively regulates DNA damage checkpoint signalling. *EMBO reports*. 2006; 7:212–218. DOI: 10.1038/sj.embor.7400593 [PubMed: 16374511]
- Clerici M, Trovesi C, Galbiati A, Lucchini G, Longhese MP. Mec1/ATR regulates the generation of single-stranded DNA that attenuates Tel1/ATM signaling at DNA ends. *The EMBO journal*. 2014; 33:198–216. DOI: 10.1002/embj.201386041 [PubMed: 24357557]
- Cotta-Ramusino C, Fachinetti D, Lucca C, Doksani Y, Lopes M, Sogo J, Foiani M. Exo1 processes stalled replication forks and counteracts fork reversal in checkpoint-defective cells. *Molecular cell*. 2005; 17:153–159. DOI: 10.1016/j.molcel.2004.11.032 [PubMed: 15629726]
- Coulon S, Gaillard PH, Chahwan C, McDonald WH, Yates JR 3rd, Russell P. Slx1–Slx4 are subunits of a structure-specific endonuclease that maintains ribosomal DNA in fission yeast. *Molecular biology of the cell*. 2004; 15:71–80. DOI: 10.1091/mbc.E03-08-0586 [PubMed: 14528010]
- Cussiol JR, Jablonowski CM, Yimit A, Brown GW, Smolka MB. Dampening DNA damage checkpoint signalling via coordinated BRCT domain interactions. *The EMBO journal*. 2015; 34:1704–1717. DOI: 10.15252/embj.201490834 [PubMed: 25896509]
- Delacroix S, Wagner JM, Kobayashi M, Yamamoto K, Karnitz LM. The Rad9-Hus1-Rad1 (9-1-1) clamp activates checkpoint signaling via TopBP1. *Genes & development*. 2007; 21:1472–1477. DOI: 10.1101/gad.1547007 [PubMed: 17575048]
- Di Virgilio M, et al. Rif1 prevents resection of DNA breaks and promotes immunoglobulin class switching. *Science*. 2013; 339:711–715. DOI: 10.1126/science.1230624 [PubMed: 23306439]
- Dibitetto D, et al. Slx4 and Rtt107 control checkpoint signalling and DNA resection at double-strand breaks. *Nucleic acids research*. 2016; 44:669–682. DOI: 10.1093/nar/gkv1080 [PubMed: 26490958]
- Digweed M, Sperling K. Nijmegen breakage syndrome: clinical manifestation of defective response to DNA double-strand breaks. *DNA repair*. 2004; 3:1207–1217. DOI: 10.1016/j.dnarep.2004.03.004 [PubMed: 15279809]
- Escribano-Diaz C, et al. A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. *Molecular cell*. 2013; 49:872–883. DOI: 10.1016/j.molcel.2013.01.001 [PubMed: 23333306]

- Fekairi S, et al. Human SLX4 is a Holliday junction resolvase subunit that binds multiple DNA repair/recombination endonucleases. *Cell*. 2009; 138:78–89. DOI: 10.1016/j.cell.2009.06.029 [PubMed: 19596236]
- Ferrari M, et al. Functional interplay between the 53BP1-ortholog Rad9 and the Mre11 complex regulates resection, end-tethering and repair of a double-strand break. *PLoS genetics*. 2015; 11:e1004928.doi: 10.1371/journal.pgen.1004928 [PubMed: 25569305]
- Flott S, et al. Phosphorylation of Slx4 by Mec1 and Tel1 regulates the single-strand annealing mode of DNA repair in budding yeast. *Molecular and cellular biology*. 2007; 27:6433–6445. DOI: 10.1128/ MCB.00135-07 [PubMed: 17636031]
- Flott S, Kwon Y, Pigli YZ, Rice PA, Sung P, Jackson SP. Regulation of Rad51 function by phosphorylation. *EMBO reports*. 2011; 12:833–839. DOI: 10.1038/embor.2011.127 [PubMed: 21738226]
- Flott S, Rouse J. Slx4 becomes phosphorylated after DNA damage in a Mec1/Tel1-dependent manner and is required for repair of DNA alkylation damage. *The Biochemical journal*. 2005; 391:325–333. DOI: 10.1042/BJ20050768 [PubMed: 15975089]
- Fricke WM, Brill SJ. Slx1-Slx4 is a second structure-specific endonuclease functionally redundant with Sgs1-Top3. *Genes & development*. 2003; 17:1768–1778. DOI: 10.1101/gad.1105203 [PubMed: 12832395]
- Gallo-Fernandez M, Saugar I, Ortiz-Bazan MA, Vazquez MV, Tercero JA. Cell cycle-dependent regulation of the nuclease activity of Mus81-Eme1/Mms4. *Nucleic acids research*. 2012; 40:8325–8335. DOI: 10.1093/nar/gks599 [PubMed: 22730299]
- Garcia V, Furuya K, Carr AM. Identification and functional analysis of TopBP1 and its homologs. *DNA repair*. 2005; 4:1227–1239. DOI: 10.1016/j.dnarep.2005.04.001 [PubMed: 15897014]
- Gaur V, et al. Structural and Mechanistic Analysis of the Slx1-Slx4 Endonuclease. *Cell reports*. 2015; doi: 10.1016/j.celrep.2015.02.019
- Ghaemmaghami S, et al. Global analysis of protein expression in yeast. *Nature*. 2003; 425:737–741. DOI: 10.1038/nature02046 [PubMed: 14562106]
- Giannattasio M, et al. Exo1 competes with repair synthesis, converts NER intermediates to long ssDNA gaps, and promotes checkpoint activation. *Molecular cell*. 2010; 40:50–62. DOI: 10.1016/j.molcel.2010.09.004 [PubMed: 20932474]
- Gritenaite D, et al. A cell cycle-regulated Slx4-Dpb11 complex promotes the resolution of DNA repair intermediates linked to stalled replication. *Genes & development*. 2014; 28:1604–1619. DOI: 10.1101/gad.240515.114 [PubMed: 25030699]
- Guervilly JH, et al. The SLX4 complex is a SUMO E3 ligase that impacts on replication stress outcome and genome stability. *Molecular cell*. 2015; 57:123–137. DOI: 10.1016/j.molcel.2014.11.014 [PubMed: 25533188]
- Hammet A, Magill C, Heierhorst J, Jackson SP. Rad9 BRCT domain interaction with phosphorylated H2AX regulates the G1 checkpoint in budding yeast. *EMBO reports*. 2007; 8:851–857. DOI: 10.1038/sj.embor.7401036 [PubMed: 17721446]
- Hang LE, et al. Rtt107 Is a Multi-functional Scaffold Supporting Replication Progression with Partner SUMO and Ubiquitin Ligases. *Molecular cell*. 2015; 60:268–279. DOI: 10.1016/j.molcel.2015.08.023 [PubMed: 26439300]
- Hanway D, Chin JK, Xia G, Oshiro G, Winzeler EA, Romesberg FE. Previously uncharacterized genes in the UV- and MMS-induced DNA damage response in yeast. *Proceedings of the National Academy of Sciences of the United States of America*. 2002; 99:10605–10610. DOI: 10.1073/pnas.152264899 [PubMed: 12149442]
- Heyer WD. Regulation of recombination and genomic maintenance. *Cold Spring Harbor perspectives in biology*. 2015; 7:a016501.doi: 10.1101/cshperspect.a016501 [PubMed: 26238353]
- Huertas P. DNA resection in eukaryotes: deciding how to fix the break. *Nature structural & molecular biology*. 2010; 17:11–16. DOI: 10.1038/nsmb.1710
- Hustedt N, Gasser SM, Shimada K. Replication checkpoint: tuning and coordination of replication forks in s phase. *Genes*. 2013; 4:388–434. DOI: 10.3390/genes4030388 [PubMed: 24705211]

- Li M, Li T, Brill SJ. Mus81 functions in the quality control of replication forks at the rDNA and is involved in the maintenance of rDNA repeat number in *Saccharomyces cerevisiae*. *Mutation research*. 2007; 625:1–19. DOI: 10.1016/j.mrfmmm.2007.04.007 [PubMed: 17555773]
- Jablonowski CM, et al. Termination of Replication Stress Signaling via Concerted Action of the Slx4 Scaffold and the PP4 Phosphatase. *Genetics*. 2015; 201:937–949. DOI: 10.1534/genetics.115.181479 [PubMed: 26362319]
- Jia X, Weinert T, Lydall D. Mec1 and Rad53 inhibit formation of single-stranded DNA at telomeres of *Saccharomyces cerevisiae* cdc13-1 mutants. *Genetics*. 2004; 166:753–764. [PubMed: 15020465]
- Kai M, Boddy MN, Russell P, Wang TS. Replication checkpoint kinase Cds1 regulates Mus81 to preserve genome integrity during replication stress. *Genes & development*. 2005; 19:919–932. DOI: 10.1101/gad.1304305 [PubMed: 15805465]
- Kaliraman V, Brill SJ. Role of SGS1 and SLX4 in maintaining rDNA structure in *Saccharomyces cerevisiae*. *Current genetics*. 2002; 41:389–400. DOI: 10.1007/s00294-002-0319-6 [PubMed: 12228808]
- Kaochar S, Shanks L, Weinert T. Checkpoint genes and Exo1 regulate nearby inverted repeat fusions that form dicentric chromosomes in *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:21605–21610. DOI: 10.1073/pnas.1001938107 [PubMed: 21098663]
- Karras GI, Fumasoni M, Sienski G, Vanoli F, Branzani D, Jentsch S. Noncanonical role of the 9-1-1 clamp in the error-free DNA damage tolerance pathway. *Molecular cell*. 2013; 49:536–546. DOI: 10.1016/j.molcel.2012.11.016 [PubMed: 23260657]
- Kim JA, Hicks WM, Li J, Tay SY, Haber JE. Protein phosphatases pph3, ptc2, and ptc3 play redundant roles in DNA double-strand break repair by homologous recombination. *Molecular and cellular biology*. 2011a; 31:507–516. DOI: 10.1128/MCB.01168-10 [PubMed: 21135129]
- Kim Y. Nuclease delivery: versatile functions of SLX4/FANCP in genome maintenance. *Molecules and cells*. 2014; 37:569–574. DOI: 10.14348/molcells.2014.0118 [PubMed: 24938228]
- Kim Y, Lach FP, Desetty R, Hanenberg H, Auerbach AD, Smogorzewska A. Mutations of the SLX4 gene in Fanconi anemia. *Nature genetics*. 2011b; 43:142–146. DOI: 10.1038/ng.750 [PubMed: 21240275]
- Kolodner RD, Putnam CD, Myung K. Maintenance of genome stability in *Saccharomyces cerevisiae*. *Science*. 2002; 297:552–557. 297/5581/552 [pii]. DOI: 10.1126/science.1075277 [PubMed: 12142524]
- Labib K, De Piccoli G. Surviving chromosome replication: the many roles of the S-phase checkpoint pathway. *Philos Trans R Soc Lond B Biol Sci*. 2011; 366:3554–3561. 366/1584/3554 [pii]. DOI: 10.1098/rstb.2011.0071 [PubMed: 22084382]
- Lazzaro F, et al. Histone methyltransferase Dot1 and Rad9 inhibit single-stranded DNA accumulation at DSBs and uncapped telomeres. *The EMBO journal*. 2008; 27:1502–1512. DOI: 10.1038/emboj.2008.81 [PubMed: 18418382]
- Leung GP, Brown JA, Glover JN, Kobor MS. Rtt107 BRCT domains act as a targeting module in the DNA damage response. *DNA repair*. 2016; 37:22–32. DOI: 10.1016/j.dnarep.2015.10.007 [PubMed: 26641499]
- Li F, Dong J, Pan X, Oum JH, Boeke JD, Lee SE. Microarray-based genetic screen defines SAW1, a gene required for Rad1/Rad10-dependent processing of recombination intermediates. *Molecular cell*. 2008; 30:325–335. DOI: 10.1016/j.molcel.2008.02.028 [PubMed: 18471978]
- Li X, Liu K, Li F, Wang J, Huang H, Wu J, Shi Y. Structure of C-terminal tandem BRCT repeats of Rtt107 protein reveals critical role in interaction with phosphorylated histone H2A during DNA damage repair. *The Journal of biological chemistry*. 2012; 287:9137–9146. DOI: 10.1074/jbc.M111.311860 [PubMed: 22262834]
- Linskens MH, Huberman JA. Organization of replication of ribosomal DNA in *Saccharomyces cerevisiae*. *Molecular and cellular biology*. 1988; 8:4927–4935. [PubMed: 3062373]
- Luo G, et al. Cancer predisposition caused by elevated mitotic recombination in Bloom mice. *Nature genetics*. 2000; 26:424–429. DOI: 10.1038/82548 [PubMed: 11101838]
- Ma W, Westmoreland JW, Resnick MA. Homologous recombination rescues ssDNA gaps generated by nucleotide excision repair and reduced translesion DNA synthesis in yeast G2 cells. *Proceedings of*

- the National Academy of Sciences of the United States of America. 2013; 110:E2895–2904. DOI: 10.1073/pnas.1301676110 [PubMed: 23858457]
- Mantiero D, Mackenzie A, Donaldson A, Zegerman P. Limiting replication initiation factors execute the temporal programme of origin firing in budding yeast. *The EMBO journal*. 2011; 30:4805–4814. DOI: 10.1038/emboj.2011.404 [PubMed: 22081107]
- Matos J, Blanco MG, West SC. Cell-cycle kinases coordinate the resolution of recombination intermediates with chromosome segregation. *Cell reports*. 2013; 4:76–86. DOI: 10.1016/j.celrep.2013.05.039 [PubMed: 23810555]
- Matos J, West SC. Holliday junction resolution: regulation in space and time. *DNA repair*. 2014; 19:176–181. DOI: 10.1016/j.dnarep.2014.03.013 [PubMed: 24767945]
- Matsuoka S, et al. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science*. 2007; 316:1160–1166. DOI: 10.1126/science.1140321 [PubMed: 17525332]
- Mimitou EP, Symington LS. Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. *Nature*. 2008; 455:770–774. DOI: 10.1038/nature07312 [PubMed: 18806779]
- Mordes DA, Nam EA, Cortez D. Dpb11 activates the Mec1-Ddc2 complex. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105:18730–18734. DOI: 10.1073/pnas.0806621105 [PubMed: 19028869]
- Morin I, Ngo HP, Greenall A, Zubko MK, Morrice N, Lydall D. Checkpoint-dependent phosphorylation of Exo1 modulates the DNA damage response. *The EMBO journal*. 2008; 27:2400–2410. DOI: 10.1038/emboj.2008.171 [PubMed: 18756267]
- Moynahan ME, Jasin M. Mitotic homologous recombination maintains genomic stability and suppresses tumorigenesis. *Nature reviews Molecular cell biology*. 2010; 11:196–207. DOI: 10.1038/nrm2851 [PubMed: 20177395]
- Mullen JR, Kaliraman V, Ibrahim SS, Brill SJ. Requirement for three novel protein complexes in the absence of the Sgs1 DNA helicase in *Saccharomyces cerevisiae*. *Genetics*. 2001; 157:103–118. [PubMed: 11139495]
- Munoz IM, et al. Coordination of structure-specific nucleases by human SLX4/BTBD12 is required for DNA repair. *Molecular cell*. 2009; 35:116–127. DOI: 10.1016/j.molcel.2009.06.020 [PubMed: 19595721]
- Munoz-Galvan S, et al. Distinct roles of Mus81, Yen1, Slx1-Slx4, and Rad1 nucleases in the repair of replication-born double-strand breaks by sister chromatid exchange. *Molecular and cellular biology*. 2012; 32:1592–1603. DOI: 10.1128/MCB.00111-12 [PubMed: 22354996]
- Navadgi-Patil VM, Burgers PM. Yeast DNA replication protein Dpb11 activates the Mec1/ATR checkpoint kinase. *J Biol Chem*. 2008; M807435200 [pii]. doi: 10.1074/jbc.M807435200
- Navadgi-Patil VM, Burgers PM. A tale of two tails: activation of DNA damage checkpoint kinase Mec1/ATR by the 9-1-1 clamp and by Dpb11/TopBP1. *DNA repair*. 2009; 8:996–1003. DOI: 10.1016/j.dnarep.2009.03.011 [PubMed: 19464966]
- Ngo GH, Lydall D. The 9-1-1 checkpoint clamp coordinates resection at DNA double strand breaks. *Nucleic acids research*. 2015; 43:5017–5032. DOI: 10.1093/nar/gkv409 [PubMed: 25925573]
- Nowotny M, Gaur V. Structure and mechanism of nucleases regulated by SLX4. *Current opinion in structural biology*. 2016; 36:97–105. DOI: 10.1016/j.sbi.2016.01.003 [PubMed: 26827285]
- O'Neill BM, Szyjka SJ, Lis ET, Bailey AO, Yates JR 3rd, Aparicio OM, Romesberg FE. Pph3-Psy2 is a phosphatase complex required for Rad53 dephosphorylation and replication fork restart during recovery from DNA damage. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:9290–9295. DOI: 10.1073/pnas.0703252104 [PubMed: 17517611]
- Ohouo PY, Bastos de Oliveira FM, Almeida BS, Smolka MB. DNA damage signaling recruits the Rtt107-Slx4 scaffolds via Dpb11 to mediate replication stress response. *Molecular cell*. 2010; 39:300–306. DOI: 10.1016/j.molcel.2010.06.019 [PubMed: 20670896]
- Ohouo PY, Bastos de Oliveira FM, Liu Y, Ma CJ, Smolka MB. DNA-repair scaffolds dampen checkpoint signalling by counteracting the adaptor Rad9. *Nature*. 2013; 493:120–124. DOI: 10.1038/nature11658 [PubMed: 23160493]
- Pelliccioli A, Foiani M. Signal transduction: how rad53 kinase is activated. *Current biology : CB*. 2005; 15:R769–771. DOI: 10.1016/j.cub.2005.08.057 [PubMed: 16169479]

- Pfander B, Diffley JF. Dpb11 coordinates Mec1 kinase activation with cell cycle-regulated Rad9 recruitment. *The EMBO journal*. 2011; 30:4897–4907. DOI: 10.1038/emboj.2011.345 [PubMed: 21946560]
- Puddu F, et al. Synthetic viability genomic screening defines Sae2 function in DNA repair. *The EMBO journal*. 2015; 34:1509–1522. DOI: 10.15252/emboj.201590973 [PubMed: 25899817]
- Rass U. Resolving branched DNA intermediates with structure-specific nucleases during replication in eukaryotes. *Chromosoma*. 2013; 122:499–515. DOI: 10.1007/s00412-013-0431-z [PubMed: 24008669]
- Roberts TM, et al. Slx4 regulates DNA damage checkpoint-dependent phosphorylation of the BRCT domain protein Rtt107/Esc4. *Molecular biology of the cell*. 2006; 17:539–548. DOI: 10.1091/mbc.E05-08-0785 [PubMed: 16267268]
- Roberts TM, Zaidi IW, Vaisica JA, Peter M, Brown GW. Regulation of rtt107 recruitment to stalled DNA replication forks by the cullin rtt101 and the rtt109 acetyltransferase. *Molecular biology of the cell*. 2008; 19:171–180. DOI: 10.1091/mbc.E07-09-0961 [PubMed: 17978089]
- Rouse J. Esc4p, a new target of Mec1p (ATR), promotes resumption of DNA synthesis after DNA damage. *The EMBO journal*. 2004; 23:1188–1197. DOI: 10.1038/sj.emboj.7600129 [PubMed: 14988729]
- Saito TT, Youds JL, Boulton SJ, Colaiacovo MP. *Caenorhabditis elegans* HIM-18/SLX-4 interacts with SLX-1 and XPF-1 and maintains genomic integrity in the germline by processing recombination intermediates. *PLoS genetics*. 2009; 5:e1000735.doi: 10.1371/journal.pgen.1000735 [PubMed: 19936019]
- Sanchez Y, Bachant J, Wang H, Hu F, Liu D, Tetzlaff M, Elledge SJ. Control of the DNA damage checkpoint by chk1 and rad53 protein kinases through distinct mechanisms. *Science*. 1999; 286:1166–1171. [PubMed: 10550056]
- Sarangi P, et al. A versatile scaffold contributes to damage survival via sumoylation and nuclease interactions. *Cell reports*. 2014; 9:143–152. DOI: 10.1016/j.celrep.2014.08.054 [PubMed: 25263559]
- Scholes DT, Banerjee M, Bowen B, Curcio MJ. Multiple regulators of Ty1 transposition in *Saccharomyces cerevisiae* have conserved roles in genome maintenance. *Genetics*. 2001; 159:1449–1465. [PubMed: 11779788]
- Segurado M, Diffley JF. Separate roles for the DNA damage checkpoint protein kinases in stabilizing DNA replication forks. *Genes & development*. 2008; 22:1816–1827. DOI: 10.1101/gad.477208 [PubMed: 18593882]
- Smolka MB, Albuquerque CP, Chen SH, Zhou H. Proteome-wide identification of in vivo targets of DNA damage checkpoint kinases. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:10364–10369. DOI: 10.1073/pnas.0701622104 [PubMed: 17563356]
- Svendsen JM, Smogorzewska A, Sowa ME, O'Connell BC, Gygi SP, Elledge SJ, Harper JW. Mammalian BTBD12/SLX4 assembles a Holliday junction resolvase and is required for DNA repair. *Cell*. 2009; 138:63–77. DOI: 10.1016/j.cell.2009.06.030 [PubMed: 19596235]
- Symington LS. End resection at double-strand breaks: mechanism and regulation. *Cold Spring Harbor perspectives in biology*. 2014; 6doi: 10.1101/cshperspect.a016436
- Symington LS, Gautier J. Double-strand break end resection and repair pathway choice. *Annual review of genetics*. 2011; 45:247–271. DOI: 10.1146/annurev-genet-110410-132435
- Szakal B, Branzei D. Premature Cdk1/Cdc5/Mus81 pathway activation induces aberrant replication and deleterious crossover. *The EMBO journal*. 2013; 32:1155–1167. DOI: 10.1038/emboj.2013.67 [PubMed: 23531881]
- Szyjka SJ, Aparicio JG, Viggiani CJ, Knott S, Xu W, Tavaré S, Aparicio OM. Rad53 regulates replication fork restart after DNA damage in *Saccharomyces cerevisiae*. *Genes & development*. 2008; 22:1906–1920. DOI: 10.1101/gad.1660408 [PubMed: 18628397]
- Tanaka S, Umemori T, Hirai K, Muramatsu S, Kamimura Y, Araki H. CDK-dependent phosphorylation of Sld2 and Sld3 initiates DNA replication in budding yeast. *Nature*. 2007; 445:328–332. nature05465 [pii]. DOI: 10.1038/nature05465 [PubMed: 17167415]

- Toh GW, et al. Histone H2A phosphorylation and H3 methylation are required for a novel Rad9 DSB repair function following checkpoint activation. *DNA repair*. 2006; 5:693–703. DOI: 10.1016/j.dnarep.2006.03.005 [PubMed: 16650810]
- Toh GW, Sugawara N, Dong J, Toth R, Lee SE, Haber JE, Rouse J. Mec1/Tel1-dependent phosphorylation of Slx4 stimulates Rad1-Rad10-dependent cleavage of non-homologous DNA tails. *DNA repair*. 2010; 9:718–726. DOI: 10.1016/j.dnarep.2010.02.013 [PubMed: 20382573]
- Ullal P, Vilella-Mitjana F, Jarmuz A, Aragon L. Rtt107 phosphorylation promotes localisation to DNA double-stranded breaks (DSBs) and recombinational repair between sister chromatids. *PloS one*. 2011; 6:e20152.doi: 10.1371/journal.pone.0020152 [PubMed: 21647453]
- Williams JS, Williams RS, Dovey CL, Guenther G, Tainer JA, Russell P. gammaH2A binds Brc1 to maintain genome integrity during S-phase. *The EMBO journal*. 2010; 29:1136–1148. DOI: 10.1038/emboj.2009.413 [PubMed: 20094029]
- Wu L, Hickson ID. The Bloom's syndrome helicase suppresses crossing over during homologous recombination. *Nature*. 2003; 426:870–874. DOI: 10.1038/nature02253 [PubMed: 14685245]
- Wyatt HD, Sarbajna S, Matos J, West SC. Coordinated actions of SLX1-SLX4 and MUS81-EME1 for Holliday junction resolution in human cells. *Molecular cell*. 2013; 52:234–247. DOI: 10.1016/j.molcel.2013.08.035 [PubMed: 24076221]
- Wyatt HD, West SC. Holliday junction resolvases. *Cold Spring Harbor perspectives in biology*. 2014; 6:a023192.doi: 10.1101/cshperspect.a023192 [PubMed: 25183833]
- Zappulla DC, Maharaj AS, Connelly JJ, Jockusch RA, Sternglanz R. Rtt107/Esc4 binds silent chromatin and DNA repair proteins using different BRCT motifs. *BMC molecular biology*. 2006; 7:40.doi: 10.1186/1471-2199-7-40 [PubMed: 17094803]
- Zegerman P, Diffley JF. Phosphorylation of Sld2 and Sld3 by cyclin-dependent kinases promotes DNA replication in budding yeast. *Nature*. 2007; 445:281–285. nature05432 [pii]. DOI: 10.1038/nature05432 [PubMed: 17167417]
- Zhang T, Nirantar S, Lim HH, Sinha I, Surana U. DNA damage checkpoint maintains CDH1 in an active state to inhibit anaphase progression. *Developmental cell*. 2009; 17:541–551. DOI: 10.1016/j.devcel.2009.09.006 [PubMed: 19853567]
- Zhu Z, Chung WH, Shim EY, Lee SE, Ira G. Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. *Cell*. 2008; 134:981–994. DOI: 10.1016/j.cell.2008.08.037 [PubMed: 18805091]
- Zimmermann M, Lottersberger F, Buonomo SB, Sfeir A, de Lange T. 53BP1 regulates DSB repair using Rif1 to control 5' end resection. *Science*. 2013; 339:700–704. DOI: 10.1126/science.1231573 [PubMed: 23306437]

Fig. 1.

Simplified depiction of homologous recombination (HR)-mediated repair indicating the steps regulated by Slx4. Repair by HR requires an initial step of resection of 5' DNA ends, which allows strand invasion and homology search, often leading to the formation of DNA-repair intermediates (joint molecules, JMs). These JMs need to be processed via dissolution or resolution pathways before chromosome segregation (see text for more details). Slx4 is important for the regulation of resection and JM resolution. While Slx4 is required for proper single strand annealing (SSA) repair, it is currently unclear if the SSA defects observed in *slx4* cells are mostly a consequence of improper resection control.

Fig. 2.

Domain organization of human and budding yeast Slx4 and modes of interaction with structure-specific endonucleases. Dashed lines indicate indirect interaction or lack of evidence for a direct interaction. UBZ (Ubiquitin-binding zinc-finger domain); BTB (for Broad-complex, Tramtrack, Bric-a-brac domain); MLR (MEI9^{XPF} interaction like region); SAP (for SAF-A/B, Acinus and PIAS motif); HtH (helix-turn-helix motif).

Fig. 3. Modes of interaction of Slx4 with the multi-BRCT domain proteins Dpb11 and Rtt107. (A) While Slx4 interaction with Rtt107 is constitutive and independent of phosphorylation, interaction with Dpb11 requires phosphorylation and is regulated in a cell cycle and DNA damage-dependent manner. (B) Depiction of Slx4 regions and phosphorylation sites important for Rtt107 and Dpb11 binding. (C) Working model for how the Rtt107-Slx4-Dpb11 complex is recruited to DNA lesions. Mec1 phosphorylates the Ddc1 subunit of the 9-1-1 complex (phospho-T602) and histone H2A (phospho-S129) creating a docking sites that are recognized by the BRCT domains of Dpb11 (BRCT-3/4) and Rtt107 (BRCT-5/6), respectively.

Fig. 4.

Slx4 dampens Rad53 signaling by counteracting the Rad9 checkpoint adaptor. (A) Working model for a competition-based mechanism by which Slx4 counteracts the engagement of Rad9 to the 5' recessed end of a ssDNA:dsDNA junction where the 9-1-1 complex is loaded. "P" indicates phosphorylation sites and "m" indicates methylation of histone H3K79 (see text for details). (B) Simplified scheme for the role of Slx4 in modulating DNA damage signaling. In addition to uncoupling Mec1 signaling from Rad53 activation, Slx4 contributes to further potentiate Mec1 signaling, likely through the stabilization of Dpb11. As a result, Mec1 partakes on the regulation of processes such as DNA repair without invoking the canonical checkpoint response.

Fig. 5.

Spatio-temporal regulation of DNA damage signaling via Slx4. 1) DNA damage leads to the exposure of ssDNA that is rapidly coated by the ssDNA binding protein RPA (not depicted in the cartoon). RPA recruits the Mec1 kinase (via Ddc2 interaction) to DNA lesions, while the 9-1-1 complex is loaded at the 5' recessed end of the ssDNA::dsDNA junction. The Ddc1 subunit of the 9-1-1 complex interacts with Mec1 through its Mec1 Activation Domain (MAD), hence stimulating Mec1 kinase activity. 2) Mec1 phosphorylates histone H2A (γ -H2A) and the Ddc1 subunit of the 9-1-1 (phospho-Ddc1) complex. 3) Rad9 and Dpb11 are recruited to the site of lesion via interactions with γ -H2A and phospho-Ddc1, respectively. Once recruited, Dpb11 further stimulates Mec1 kinase activity as it also possesses a MAD. Rad9 becomes extensively phosphorylated by Mec1, which promotes Rad53 recruitment creating the Rad53 activation complex. At this step, resection is not favored due to the inhibition of Exo1 by Rad53 and potentially via Rad9 oligomerization (not depicted in the picture) that could prevent exonucleases from accessing the 5' recessed end. 4) Build up in the γ -H2A platform surrounding the lesion site helps recruit the Slx4-Rtt107 complex nearby the lesion site, leading to Slx4 phosphorylation by Mec1 (not depicted in the picture), which enhances Slx4 interaction with Dpb11 and competes out Rad9 from the site of lesion. As a result, Mec1 signaling is uncoupled from Rad53 signaling (checkpoint dampening). 5) Resection is favored as Rad53 dampening ceases Exo1 inhibition and Rad9 displacement allows accessibility of nucleases to the recessed 5' end. 6) Increased ssDNA exposure allows more Mec1 recruitment, while stabilization of Dpb11 and 9-1-1 further stimulates Mec1 signaling that may help regulate subsequent steps in HR-mediated repair.

Fig. 6.

Integrated view for how Slx4 facilitates HR repair in budding yeast. In addition to functioning as a scaffold for the coordination of structure-specific nucleases, Slx4 plays key roles in the modulation of DNA damage signaling to favor multiple steps in HR-mediated repair. Slx4 positively regulates DNA-end resection by counteracting Rad9 as detailed in Figs. 4 and 5. Moreover, Slx4 dampening of Rad53 signaling favors JM resolution via Mus81-Mms4 by allowing cell cycle progression and timely activation of the cell cycle kinases CDK and Cdc5. Asterisks indicate where the role of the physical interaction with Slx4 has not yet been established.