Wrestling with Chromosomes: The Roles of SUMO During Meiosis

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Abstract

Meiosis is a specialized form of cell division required for the formation of haploid gametes and therefore is essential for successful sexual reproduction. Various steps are exquisitely coordinated to ensure accurate chromosome segregation during meiosis, thereby promoting the formation of haploid gametes from diploid cells. Recent studies are demonstrating that an important form of regulation during meiosis is exerted by the post-translational protein modification known as sumoylation. Here, we review and discuss the various critical steps of meiosis in which SUMO-mediated regulation has been implicated thus far. These include the maintenance of meiotic centromeric heterochromatin, meiotic DNA double-strand break repair and homologous recombination, centromeric coupling, and the assembly of a proteinaceous scaffold between homologous chromosomes known as the synaptonemal complex.

11.1 Introduction

Sexually reproducing organisms depend on the formation of haploid gametes (eggs and sperm) for successful propagation of their species. This requires a specialized cell division process known as meiosis through which chromosome number is reduced by half, generating haploid gametes that upon fertilization will reconstitute a diploid state. The precise reduction in chromosome number is accomplished by following a

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single round of DNA replication with two consecutive rounds of chromosome segregation (meiosis I and II). Homologous chromosomes segregate away from each other in the first (reductional) division, whereas sister chromatids segregate from each other in the second (equational) division. To accurately accomplish a reductional division, chromosomes undergo a series of well-orchestrated steps which are unique to meiosis I. These include homologous chromosome pairing, the formation of a "zipper-like" structure (the synaptonemal complex or SC) between aligned homologs, and the completion of meiotic recombination leading to physical attachments (chiasmata) between homologs. All of these events play a critical role in ensuring the proper alignment of homologous chromosomes at the metaphase I plate, and their subsequent orderly segregation to opposite ends of the spindle upon onset of meiosis I. Significantly, errors in any of these steps lead to chromosome nondisjunction and the formation of aneuploid gametes with tremendously deleterious consequences. Aneuploidy accounts for 30% of miscarriages in humans and is a contributing factor to infertility and birth defects such as Down syndrome (Hassold and Hunt 2001).

Given the importance of achieving accurate chromosome segregation during meiosis, it is not surprising that this is a tightly regulated process. This chapter highlights new findings implicating sumoylation as a key post-translational modification underlying the specificity of several important meiotic events ranging from the sorting of homology, to meiotic double-strand break (DSB) repair and SC morphogenesis.

11.2 Sumoylation

Sumoylation is a post-translational protein modification analogous to ubiquitination, where the SUMO (small ubiquitin-related modifier) protein is covalently linked to lysine residues present in a sumoylation consensus sequence on its target proteins. These target proteins include histones, transcription factors, DNA repair factors and proteins involved in multiple other cellular functions

(reviewed in Gill 2004; Hay 2005; Johnson 2004). Similarly to ubiquitination, sumoylation proceeds via a stepwise transfer of SUMO to its substrate by an E1 activating enzyme, an E2 conjugating enzyme and sometimes an E3 ligase enzyme. In yeast, mature SUMO (cleaved from a precursor form by SUMO-specific proteases or SENPs), forms a thioester bond with the heterodimeric E1 enzyme, Aos1/Uba2, and is then transferred to the E2 conjugating enzyme Ubc9 (Table 11.1). Unlike the ubiquitination pathway, which requires an E3 ligase to proceed, SUMO-conjugated Ubc9 is competent to sumoylate targets *in vitro* (Bencsath et al. 2002). However, several SUMO E3 ligases have been identified that promote sumoylation *in vivo*, suggesting that the E3 ligases may be important for regulating sumoylation in the cellular context (Gill 2004). Unlike ubiquitination, which is frequently associated with proteasomal degradation of its targets, the biological function of sumoylation is less clear. Sumoylation has been linked to transcriptional repression and protein localization, and not surprisingly, appears to affect protein:protein interactions (Gill 2004; Hay 2005). Some insight into the biological roles of sumoylation can be gained from both the phenotypes of sumoylation-deficient model organisms and the recent influx of large-scale proteomic screens that have identified many SUMO-modified substrates. Taken together, both these types of studies indicate a conserved and important role for sumoylation during meiosis, as we will explore below.

11.2.1 Sumoylation in Meiosis: A Phenotypic Survey

Meiosis involves numerous and tightly coordinated chromosomal processes that must be temporally regulated. Therefore, it is not surprising that a link between a dynamic post-translation modification such as SUMO and meiotic processes has been observed from budding yeast to humans (Tables 11.1 and 11.2). In *Saccharomyces cerevisiae*, sumoylation intersects with at least two proteins required for SC formation. Zip1 is a

Table 11.1 SUMO pathway enzymes and meiotic phenotypes

Species	Sumo Pathway Component	Gene Name	Meiotic Expression and/or *Relevant Phenotype(s)*
S. cerevisiae	SUMO	*SMT3*	
	E1	*AOS1*	
		UBA2	
	E2	*UBC9*	localizes to SC; *perturbed replication fork repair*
	E3	*SIZ1*	*siz1siz2 mutant has mild sporulation defect*
		SIZ2	
		MMS21	*perturbed replication fork repair*
		ZIP3	SC component; *inefficient SC formation; reduced and delayed crossovers*
	De-conjugating	*ULP1*	*synthetic lethal with recombination protein Srs2*
		ULP2/SMT4	increased upon sporulation; *cell cycle arrest at meiotic prophase*
S. pombe	SUMO	*pmt3*	
	E1	*fub2*	
	E2	*hus5*	*aberrant asci; reduced spore viability*
	E3	*pli1*	*aberrant asci; reduced spore viability; reduced crossovers*
		nse1	
	De-conjugating	*ulp1*	
D. melanogaster	SUMO	*smt3*	embryonic germline
	E1	*Aos1*	embryonic germline
		Uba2	embryonic germline
	E2	*lesswright*	embryonic germline; *suppresses mild chromosomal non-disjunction*
	E3	*tonally*	down-regulated in female germline post-mating
		Su(var)2–10	oogenesis
	De-conjugating	*Ulp1*	
C. elegans	SUMO	*smo-1*	high expression in somatic gonad; *sterile, abnormal germline; genetic interaction with zhp-3*
	E1	*aos-1*	
		uba-2	
	E2	*ubc-9*	
	E3	*zhp-3*	SC localization; marker of crossover events; *sterile, high incidence of male progeny (suggesting chromosomal non-disjunction)*
		gei-17	*genetic interaction with mus-101 (required for DNA replication and DNA damage response)*
	De-conjugating	*ulp-1*	*sterile progeny*
		ulp-2	*reduced brood size, sterile, sterile progeny*
Mouse/Human	SUMO	*SUMO-1*	SC and constitutive heterochromatin during spermatogenesis
		SUMO-2/3	constitutive heterochromatin during spermatogenesis
		SUMO-4	
	E1	*SAE1*	
		SAE2	
	E2	*UBE2I*	
	E3	*PIAS family*	PIASx/PIAS1 upregulated during spermatogenesis
		RanBP2	
		Pc2	
		HDAC4	
	De-conjugating	*SENP family*	

Information on the sumoylation pathway members and their expression (normal text) and reported meiotic phenotypes (*italicized*) is gathered from the following online resources, reviews and primary sources. For *S. cerevisiae*: the Saccharomyces Genome Database (SGD), (Cheng et al. 2007; de Carvalho and Colaiácovo 2006; Soustelle et al. 2004; Agarwal and Roeder 2000); for *S. pombe*: (Watts et al. 2007); for *D. melanogaster*: (Talamillo et al. 2008); for *C. elegans*: Wormbase, (Bhalla et al. 2008; Holway et al. 2005; Jones et al. 2002); and for mammalian: (Brown et al. 2008; Yan et al. 2003)

Table 11.2 Budding yeast sumoylated proteins and their roles in meiosis

Name	Known or predicted role in meiosis
DSBR	
Ecm11	Crossover recombination
Mlh3	DNA mismatch repair and meiotic crossover recombination
Rad52	DSB repair during vegetative growth and meiosis
Sgs1	Prevents aberrant crossing over during meiosis
Srs2	Required for proper timing of commitment to meiotic recombination and the transition from Meiosis I to Meiosis II
Top2	Localizes to axial cores in meiosis; meiotic crossover recombination
Structural and chromosome segregation	
Ndc1	Required for nuclear pore complex assembly and spindle pole body duplication; required for chromosome segregation in Meiosis II
Red1	SC axial element component; involved in chromosome segregation during Meiosis I
Slk19	Kinetochore-associated protein required for normal segregation of chromosomes in meiosis and mitosis
Smc4	Structural Maintenance of Chromosomes (SMC) condensin protein
Smc5	SMC condensin protein
Transcriptional	
Sth1	Required for expression of early meiotic genes
Ume1	Negative regulator of meiosis; represses meiotic gene expression during mitotic growth

The proteins are subdivided into functional categories of DSBR (Double-strand break repair), Structural and Chromosome Segregation, and Transcriptional based on published literature. Identification of sumoylation and description of meiotic roles are consolidated from the Saccharomyces Genome Database (SGD) and the following primary sources: (Branzei et al. 2006; Cheng et al. 2006; Denison et al. 2005; Hannich et al. 2005; Panse et al. 2004; Sacher et al. 2006; Zavec et al. 2008)

structural component of the SC which may recognize SUMO-conjugated proteins on the chromosomal axes, and Zip3 is a SUMO E3 ligase which appears to regulate Zip1 polymerization (Cheng et al. 2006). Conversely, mutations in the *S. cerevisiae* SUMO deconjugating enzyme *ulp2/smt4* lead to arrest in meiotic prophase

(Li and Hochstrasser 2000), further linking control of sumoylation to meiosis. In *Schizosaccharomyces pombe*, mutation of the SUMO E3 ligase *pli1* leads to reduced spore viability and aberrant asci, a phenotype resulting from defective meiotic recombination (Watts et al. 2007). Mutations in *lesswright*, the *Drosophila* homolog of the E2 enzyme Ubc9, were found to suppress a mild meiotic nondisjunction phenotype, implicating sumoylation in the regulation of accurate meiotic chromosome segregation (Apionishev et al. 2001). Meanwhile, the *C. elegans* genome contains a single SUMO homolog, *smo-1*, and *smo-1* mutants display a pleiotropic phenotype including highly aberrant germlines (Broday et al. 2004). A partially rescued *zhp-3* (the Zip3 ortholog) mutation appears to phenocopy *smo-1* mutations (Bhalla et al. 2008), suggesting a potential conservation of the SUMO and SC connection first reported in budding yeast. In rodents and humans, SUMO shows a stage-specific and chromosomal-specific localization during spermatogenesis (Brown et al. 2008; Metzler-Guillemain et al. 2008; Rogers et al. 2004; Vigodner et al. 2006; Vigodner and Morris 2005), as we describe further below. Moreover, infertile men show a decrease in SUMO in the Sertoli cells, implicating sumoylation in human infertility (Vigodner et al. 2006).

11.2.2 Targets of Sumoylation in Meiosis

Large-scale proteomic studies to identify sumoylated targets have been predominantly done in *S. cerevisiae* thus far. These studies have identified several sumoylated proteins with roles in meiosis, underscoring the breadth of regulatory control exerted by this mode of post-translational modification during this cell division program (Table 11.2). These sumoylated targets can be separated into several groups, including DNA repair proteins and proteins involved in the structural organization of chromosomes during meiosis.

The function of sumoylation has been studied further for at least two proteins with roles in meiotic DSB repair in *S. cerevisiae*. First, the homologous recombination protein Rad52 has been reported to be sumoylated upon an accumulation of meiotic DSBs, in a manner that interferes with its proteasomal degradation and results in its stabilization (Sacher et al. 2006). Second, the budding yeast protein Ecm11, with important functions in DNA replication and meiotic crossover recombination, is sumoylated during meiosis and not mitosis. Mutation of the Ecm11 sumoylation site phenocopies the sporulation defect of the *ecm11* mutant, suggesting sumoylation is required for the meiotic function of this protein (Zavec et al. 2008). Taken together, these studies thus far implicate sumoylation as essential for promoting the stability and function of at least two proteins with known roles in meiotic DSB repair.

Another set of important processes during meiosis involve the assembly and disassembly of the SC, as well as the subsequent chromosomal segregation events that depend on proper SC formation earlier in prophase. Several key players in these processes are sumoylated: one notable example being the axial element component Red1 (Cheng et al. 2006). This sumoylation appears to serve as a recognition site for the SC component Zip1, and has therefore been suggested to play a role in SC assembly (Cheng et al. 2006). Several proteins involved later in chromosome segregation, such as the integral membrane protein Ndc1 and the Separase-binding protein Slk19, are sumoylated (Table 11.2). Although it is currently unknown how sumoylation affects their function, these proteins are involved in the regulation of proper chromosome redistribution and therefore suggest at least a potential role for sumoylation in this process.

Taken together, the analysis of mutant phenotypes and sumoylated substrates hints at interesting functions for sumoylation in meiosis and further highlights the importance of studies in various organisms to determine its degree of conservation.

11.3 Centromeric Heterochromatin and Sumoylation

Centromeric function is important for both mitosis and meiosis, and sumoylation seems to play a particularly important role in the establishment and/or maintenance of heterochromatin at the centromere from yeast to mammals. Both Smt3 (the *S. cerevisiae* SUMO-1 homolog) and Smt4 (a SUMO de-conjugating enzyme) were originally identified as suppressors of mutations in the centromere binding protein Mif2/Cenp-C (Meluh and Koshland 1995), supporting an important functional connection between sumoylation and centromeres. In *S. pombe*, deletion of *pli1*, which encodes for a SUMO E3 ligase, results in a mild dysfunction of the kinetochore and/or centromere (Xhemalce et al. 2004). Moreover, de-silencing of a reporter gene located in the centromeric region in *pli1* mutants suggests a defect in heterochromatin maintenance in this region (Xhemalce et al. 2004). In *Drosophila*, SUMO is seen localizing to heterochromatic sites (Lehembre et al. 2000), and in *S. pombe*, sumoylation has more recently been shown to play a role in heterochromatin maintenance at the centromere and other heterochromatic regions of the genome (Shin et al. 2005).

Heterochromatin can either be transiently induced ("facultative") or be permanent ("constitutive"), and sumoylation has been implicated in both types of heterochromatin. One classic example of meiotic facultative heterochromatin that has been linked with sumoylation is the sex body or XY body, formed by the mammalian sex chromosomes during pachytene spermatogenesis (Rogers et al. 2004; Vigodner et al. 2006; Vigodner and Morris 2005). However, a recent study suggests that at least in humans, the observation of XY body sumoylation (which would be considered facultative heterochromatin) may actually be the result of a large region of constitutive heterochromatin on the Y chromosome (Metzler-Guillemain et al. 2008) as opposed to an XY body-specific process. This is in agree-

ment with the observation of other large SUMO-1 signals on chromosomes 1, 9 and 16 (Brown et al. 2008; Metzler-Guillemain et al. 2008), which also contain large regions of constitutive heterochromatin. These data, along with the frequent observation of SUMO-1 at mammalian centromeres (known sites of constitutive heterochromatin) during meiosis (Brown et al. 2008; La Salle et al. 2008; Metzler-Guillemain et al. 2008; Vigodner et al. 2006), suggests that sumoylation may in fact be more specific to constitutive heterochromatin. However, numerous studies link sumoylation to transcriptional repression (reviewed in Gill 2004), suggesting that sumoylation may contribute to facultative heterochromatin in non-meiotic situations. Further studies are therefore required to determine the extent to which sumoylation plays a role, if any, in facultative heterochromatin during meiosis.
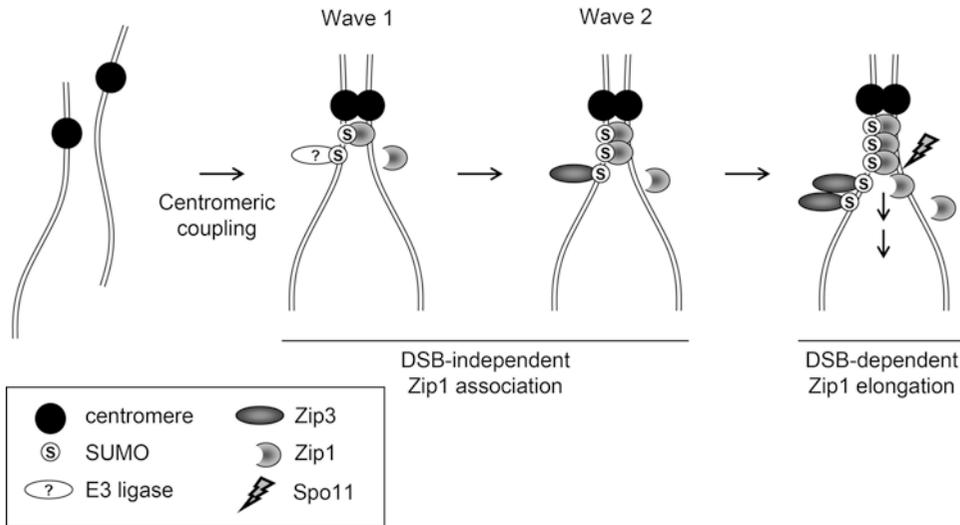
11.4 Centromeric Coupling

The establishment of stable pairing between homologous chromosomes is a critical step for successful meiosis I progression. Before homologous chromosomes can synapse and progress through meiosis, they must first search for homology and pair, and recent studies in yeast have uncovered an important link between centromeric sumoylation and these early pairing events. Pairing and synapsis of homologous chromosomes during meiosis in *S. cerevisiae* relies on both recombination-dependent and -independent mechanisms. Once homologous chromosomes are paired, several proteins, including Zip1, Zip2 and Zip3, form the Synapsis Initiation Complex (SIC) at sites called Axial Associations (AA) where the chromosomes are in close contact (Rockmill et al. 1995). Synapsis (polymerization of the SC between paired and aligned homologous chromosomes) is believed to then proceed from these sites. In many organisms, synapsis is dependent upon DSB formation and subsequent recombination (reviewed in Page and Hawley 2004). This and other lines of evidence have suggested that the SICs form at the sites

of crossover recombination (reviewed in Henderson and Keeney 2005).

However, several recent studies have implicated sumoylation in a recombination-independent form of early chromosomal pairing termed "centromeric coupling" (Cheng et al. 2006; Hooker and Roeder 2006; Tsubouchi et al. 2008; Tsubouchi and Roeder 2005) (Fig. 11.1). In a *spo11* mutant that lacks DSB formation and fails to synapse, the SC component Zip1 and the SUMO E3 ligase Zip3 do not polymerize along chromosomes, but instead, form foci at (or near) the centromeres (Tsubouchi et al. 2008; Tsubouchi and Roeder 2005). Moreover, the number of observable centromere-associated foci is approximately half that of the number of chromosomes, suggesting that even in the absence of recombination and synapsis, the chromosomes are pairing at or near the centromeres (Tsubouchi and Roeder 2005). This "centromeric coupling" occurs even in the absence of bouquet formation, a process of telomere clustering that is important for efficient homolog pairing (Trelles-Sticken et al. 2000). Interestingly, the earliest centromeric coupling is not between homologous chromosomes, although over time the proportion of paired homologs increases (Tsubouchi and Roeder 2005). In a *spo11 zip1* double mutant the number of centromere foci double, indicating that Zip1 is required for centromeric coupling (Tsubouchi and Roeder 2005). In a wild-type background, AAs can be found at the centromeres, and Zip1 and Zip3 linear staining appears to initiate from the centromeres, further supporting a model where SC formation during early stages of meiosis initiates from the sites of centromeric coupling (Tsubouchi et al. 2008; Tsubouchi and Roeder 2005).

Taken together, the authors propose a model in which homologous pairing is modulated not just by the previously-studied bouquet formation and recombination, but also by Zip1-dependent centromeric coupling. Thus, centromeric coupling and/or bouquet formation may serve to sequentially match together different chromosomes until homology is determined (Tsubouchi and Roeder 2005). They propose that Spo11 then initiates recombination via the production of DSBs,

Fig. 11.1 *A model for the roles of sumoylation in meiotic chromosome dynamics: Centromeric coupling and SC assembly.* In budding yeast, Zip1, a structural component of the synaptonemal complex, is required for centromeric coupling early in meiotic prophase I. Once homologous chromosomes are coupled, synapsis ensues. Two distinct waves of sumoylation are believed to participate in these processes. **Wave 1** involves centromeric (or pericentromeric) sumoylation and the recognition of SUMO-conjugated products at the centromeres by Zip1 in a Zip3-independent fashion. Thus, centromeric sumoylation may be the result of the activity of an as yet unidentified E3 ligase. **Wave 2** involves the Zip3 SUMO E3 ligase and results in the formation of short Zip1 stretches. Initiation of synapsis is not DSB–dependent, however, DSB formation via Spo11 function is required for the Zip3-dependent Zip1 elongation resulting in a fully-formed SC. Therefore, it appears that sumoylation is important both in the early stages of chromosome pairing/homology sorting and later on in the assembly of the mature SC

further linking the homologs together and promoting SC formation (Tsubouchi and Roeder 2005). Therefore, the SUMO-mediated centromeric coupling observed in yeast, along with the observations by immunofluorescence studies that centromeric regions are sumoylated during meiosis in mouse, rat and human (Brown et al. 2008; Metzler-Guillemain et al. 2008; Vigodner et al. 2006; Vigodner and Morris 2005), suggest that sumoylation may play a conserved role in centromere function as it relates to early chromosome pairing in meiosis.

11.5 SUMO-Mediated Regulation of SC Dynamics

After homologous chromosomes find and pair with one another, they undergo synapsis via assembly of the SC. The establishment of this proteinaceous scaffold is crucial for the stabilization of homologous pairing interactions and the completion of crossover recombination (Page and Hawley 2004). Interestingly, despite the ubiquitous presence of the SC from yeast to humans, and its fundamental importance for reproductive biology, the regulation of the assembly and disassembly of this macromolecular structure remains poorly understood. However, recent studies in several model systems are linking sumoylation with the regulation of SC morphogenesis.

11.5.1 ZIP1 and ZIP3: A SUMO Connection

Analysis of human testes samples has shown that SCP1 and SCP2, structural components of the SC, are sumoylated, and that SUMO-1 localizes to the SC (Brown et al. 2008). Although the co-localization observed in mammals is still controversial (Metzler-Guillemain et al. 2008), recent studies observed a co-localization of the yeast

SUMO homolog Smt3 to the SC in budding yeast (Cheng et al. 2006; Hooker and Roeder 2006). The SIC components Zip1 and Zip3, and the topoisomerase-like enzyme Spo11, involved in generating programmed meiotic DSB breaks, are required for this localization (Cheng et al. 2006; Hooker and Roeder 2006), suggesting that these proteins are involved in SC sumoylation in yeast. In synapsis-defective mutants, both Smt3 and Zip1 co-localize to non-SC aggregates termed polycomplexes (reviewed in de Carvalho and Colaiácovo 2006; Zickler and Kleckner 1999), further supporting their SC-related interaction.

Earlier in prophase, Smt3 is present at the Zip1 foci implicated in centromeric coupling (Cheng et al. 2006; Hooker and Roeder 2006; Tsubouchi and Roeder 2005). In both wild type and *zip3* mutants, these early Zip1 foci disappear by mid-prophase, but in a *zip3* mutant background, an additional mutation of the Smt3 deconjugating enzyme, *ulp2*, leads to prolonged maintenance of these foci on chromosomes (Cheng et al. 2006), suggesting sumoylation may support their stability. In addition, Hooker and Roeder 2006 find that mutations in the yeast SUMO E2 conjugating enzyme, Ubc9, lead to delays in synapsis, further supporting the importance of sumoylation for proper synapsis (Hooker and Roeder 2006).

Zip3 acts as a Smt3 E3 ligase *in vitro* leading Cheng et al. (2006) to conclude that Zip3-dependent sumoylation is necessary for proper SC formation (Cheng et al. 2006). They also describe Zip1 as a Smt3-conjugate binding protein, with both Zip3-independent (to Smt3-Top2 during early prophase) and Zip3-dependent (to Smt3-Red1 during mid-to-late prophase) interactions, implicating sumoylation in both centromeric coupling and subsequent SC formation (Cheng et al. 2006) (Fig. 11.1).

These findings suggest that at least two "waves" of sumoylation may be involved in the association of Zip1 onto chromosomes during meiosis in budding yeast (Fig. 11.1). The first wave results in Zip1 localization to centromeric and pericentromeric regions in early meiotic pro-phase, thereby promoting centromeric coupling and early synapsis, and involves sumoylation mediated by an as of yet unidentified SUMO E3 ligase. The second wave results in the extensive polymerization of Zip1 along the full length of chromosomes, thereby promoting completion of SC assembly, and is Zip3-dependent (Cheng et al. 2006; Tsubouchi et al. 2008; reviewed in de Carvalho and Colaiácovo 2006). Furthermore, taken together these studies suggest that the Zip1 foci implicated in centromeric coupling could also be sites of synapsis initiation (Fig. 11.1).

Interestingly, studies of ZHP-3 (the Zip3 homolog) function during meiosis in the nematode *C. elegans* reveal it is required for crossover recombination in a SC-dependent manner (Jantsch et al. 2004). However, in contrast to yeast, SC assembly is not impaired in either *zhp-3* or *smo-1* (the SUMO homolog) mutants (Bhalla et al. 2008; Jantsch et al. 2004). Instead, comparisons between a *zhp-3::gfp* integrated transgene which partially complements a *zhp-3* null mutant, *smo-1* and *smo-1; zhp-3::gfp* double mutants revealed that ZHP-3 coordinates recombination with SC disassembly and bivalent differentiation (Bhalla et al. 2008). Therefore, both Zip3 and ZHP-3 may function to coordinate crossover recombination with SC morphogenesis. However, in *S. cerevisiae*, where DSB-formation is critical to promote synapsis, Zip3 coordinates crossover formation with SC assembly (Agarwal and Roeder 2000). Meanwhile, in *C. elegans*, where synapsis is DSB-independent (Dernburg et al. 1998), ZHP-3 coordinates crossover formation with SC disassembly and biva-lent formation. The role of SUMO in these processes during *C. elegans* meiosis remains to be further examined and its potential role in the formation of functional bivalents (stably attached through chiasmata) needs to be investigated across species. Taken together, these studies further highlight the importance of identifying additional meiotic SUMO targets and pursuing the analysis of their roles in SC assembly and disassembly to understand the crucial regulation of SC dynamics.

11.6 Meiotic DSB Repair/ Recombination

Proper control of DNA double-strand break repair (DSBR) is essential for promoting interhomolog recombination resulting in crossovers and subsequent accurate chromosome segregation. Several proteins with roles in meiotic DSBR are known to be sumoylated, therefore implicating this post-translational modification in the critical regulation of this meiotic process (Table 11.2). Many of the proteins involved in DSBR are highly conserved across species (reviewed in Villeneuve and Hillers 2001), however, their roles in meiotic DSBR have been more extensively investigated in yeast, and therefore, we will primarily focus on the roles of the yeast proteins with links to sumoylation.

In yeast and metazoans, the endonuclease Spo11 creates the DSBs during the early stages of prophase I (Villeneuve and Hillers 2001). The Mre11/Rad50/Xrs2 complex then resects the 5′ ends of the DSBs thereby creating 3′ overhangs, where the ssDNA binding factor RPA binds, allowing Rad51 and Rad52 to participate in the homology search and strand invasion that allows homologous recombination to proceed. The Srs2 helicase opposes this activity by disrupting Rad51 binding and serves an important function in preventing inappropriate recombination events from proceeding (Veaute et al. 2003). The RecQ helicase homolog Sgs1 also acts to prevent inappropriate crossovers, although its mechanism is less well understood (Rockmill et al. 2003). The MutL homologs, Mlh1 and Mlh3, act downstream to promote crossover formation (Hoffmann and Borts 2004). Finally, topoisomerases such as Top2 are proposed to "untangle" recombined chromosomes upon completion of DSBR, thereby allowing for efficient segregation (Hartsuiker et al. 1998).

Several of these proteins are known to be sumoylated: specifically, Rad52, Sgs1, Srs2, Mlh3 and Top2 (Table 11.2). In *S. cerevisiae*, Rad52 is sumoylated on at least two sites upon induction of DSBs (Sacher et al. 2006). While Rad52 mutants that lack the sumoylation sites are still able to complete meiotic DSBR, the sumoylation does appear to stabilize Rad52 and promote its activity (Sacher et al. 2006). More recent studies uncovered a remarkable link between Rad52 sumoylation and relocalization of damage sites to "damage foci" for repair, where repair of ribosomal DNA sites requires Rad52 sumoylation for formation of Mre11 and Rad52-containing extranucleolar foci (Torres-Rosell et al. 2007). Additional studies have implicated the SUMO E3 ligase Slx5/8 in the relocalization of damaged DNA to nuclear pore complexes (Nagai et al. 2008), suggesting that sumoylation plays a role in relocalizing damaged DNA to sites of repair after experimentally-induced damage and perhaps during endogenous meiotic DSBR as well.

The anti-recombinogenic helicases Sgs1 and Srs2 are both known to be sumoylated (Table 11.2), and Srs2 is also known to interact specifically with sumoylated PCNA earlier in pre-meiotic S phase in order to prevent inappropriate recombination at stalled replication forks (Pfander et al. 2005). The *in vivo* functions of Sgs1 sumoylation are not yet known, however sumoylation of the mammalian Sgs1 homolog BLM is required for DNA damage-induced foci (Eladad et al. 2005). Formation of these foci involves relocalization of sumoylated BLM (Eladad et al. 2005), further supporting a general role for sumoylation in subnuclear relocalization during DSBR. However, these studies have yet to be repeated in the context of meiosis, so future studies are critical to see whether SUMO does in fact play a role in meiotic DSBR-induced relocalization.

Another sumoylated protein that plays an important role in meiosis is the topoisomerase Top2. During mitosis, Top2 is known to be sumoylated, and mutation of the Top2 sumoylation sites contributes to mitotic chromosomal missegregation (Bachant et al. 2002; Takahashi et al. 2006). During meiosis, immuno-fluorescence analysis shows colocalization of Top2 and the yeast SUMO homolog Smt3 (Cheng et al. 2006), suggesting Top2 is sumoylated during meiosis as well. Furthermore, sumoylated Top2 (localized near the centromeres) is believed to interact with the sumo-binding SC component

Zip1 (Cheng et al. 2006), suggesting that sumoylated Top2 may act both early and late in meiosis with functions in SC assembly and chromosome segregation.

Finally, *C. elegans* ZTF-8, a functional analog of mammalian RHINO, which plays roles in both DSBR and DNA damage-induced apoptosis, is a direct target for sumoylation at its consensus CKxE sites *in vivo* (Cotta-Ramusino et al. 2011; Kim and Colaiácovo 2014; Kim and Colaiácovo 2015). Non-sumoylatable transgenic worms mimic the phenotypes observed in the null mutants such as reduced fertility, impaired DNA damage repair, and mislocalization of the 9–1-1 complex component HUS-1, suggesting that sumoylation is indispensable for DSBR and DNA damage-mediated checkpoint activation in the germline. However, while mutants for components acting in the sumoylation pathway fail to properly localize ZTF-8, its localization is not altered in the ZTF-8 non-sumoylatable mutants. These observations suggest that while direct sumoylation of ZTF-8 is required for its roles in DSBR and DNA damage response, it is not required for its localization. Instead, another factor may be a target for sumoylation, and it in turn may be required for proper localization of ZTF-8.

11.7 Conclusions

Sumoylation has been implicated in various ways for several essential events of meiosis, including homologous pairing, synapsis, and DSBR leading to crossover events. The importance of sumoylation in meiosis is highlighted by the meiotic phenotypes of sumoylation pathway mutants across species (Table 11.1). Furthermore, many proteins with known important roles in meiotic processes are known to be sumoylated (Table 11.2), although further studies are needed to determine the precise role or function for the sumoylation undergone by some of these proteins. In yeast, sumoylation is involved in both centromeric coupling and the subsequent polymerization of the SC (Fig. 11.1), and at least one report of SC

sumoylation in human spermatocytes suggests a general conservation of this role (Brown et al. 2008). In contrast, in the nematode *C. elegans*, sumoylation is apparently not required for SC assembly and instead is important for proper SC disassembly (Bhalla et al. 2008). Further work is therefore needed in mammalian and other model systems to determine whether the role of SUMO in centromeric coupling and SC morphogenesis is in fact conserved across species.

The role of sumoylation in meiotic DSBR is supported by mutant phenotypes and the identification of sumoylated DSBR proteins (Tables 11.1 and 11.2), but even more intriguing is the potential connection of sumoylation with DNA damage-induced re-localization and repair (Eladad et al. 2005; Nagai et al. 2008; Torres-Rosell et al. 2007; Kim and Colaiácovo 2014, 2015). Sumoylation has long been implicated in intracellular re-localization (reviewed in Gill 2004), and future studies may specifically implicate this re-localization in meiotic DSBR, potentially uncovering entirely novel mechanisms of DSBR regulation in meiosis. Taken together, the studies reviewed here hint at many possible avenues for research, and future studies will undoubtedly strengthen the connections between sumoylation and meiotic processes.

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