Published in final edited form as:

Science. 2017 January 27; 355(6323): 408–411. doi:10.1126/science.aaf4778.

Control of Meiotic Pairing and Recombination by Chromosomally Tethered 26S Proteasome

Jasvinder S. Ahuja¹, Rima Sandhu¹, Rana Mainpal³, Crystal Lawson⁴, Hanna Henley¹, Patricia A. Hunt⁴, Judith L. Yanowitz³, and G. Valentin Börner^{1,2,*}

¹Center for Gene Regulation in Health and Disease and Department of Biological, Geological and Environmental Sciences, Cleveland State University, Cleveland, Ohio, USA

²Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, Ohio, USA

³Magee-Womens Research Institute, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

⁴School of Molecular Biosciences, Center for Reproductive Biology, Washington State University, Pullman, Washington, USA

Abstract

During meiosis, paired homologous chromosomes (homologues) become linked via the synaptonemal complex (SC) and crossovers. Crossovers mediate homologue segregation and arise from self-inflicted double-strand breaks (DSBs). Here, we identify functions in homologue juxtaposition and crossing over of the proteasome, the multi-subunit protease that degrades proteins in the nucleus and cytoplasm. Without proteasome function, homologues fail to pair and instead remain associated with non-homologous chromosomes. While dispensable for non-crossover formation, a functional proteasome is required for a coordinated transition that entails SC assembly between longitudinally organized chromosome axes and stable strand exchange of crossover-designated DSBs. Remarkably, proteolytic core and regulatory proteasome particles are recruited to chromosomes by Zip3, the orthologue of mammalian E3 ligase RNF212, and SC protein Zip1 as part of an evolutionarily conserved program.

Global homologue juxtaposition and local recombination during meiosis are temporally and spatially coordinated via multiple interdependencies. Pairing between homologue arms occurs at substantial levels prior to DSB formation and is further stabilized by assembly of the SC central element between proteinaceous chromosome axes (1). Centromeres, by contrast, are initially coupled with one or several non-homologous partners, as best documented in yeast and plants (2,3). As SC assembly reaches completion, centromere coupling is replaced by homologous centromere pairing (2,4). Recombination involves

*Correspondence to: g.boerner@csuohio.edu.

Supplementary Materials

Materials and Methods

Supplementary Text

Figs. S1 – S13

Tables S1 – S2

Supplementary References (26 – 40)

processing of Spo11-induced DSBs into crossovers (COs) and noncrossovers (NCOs), i.e. interhomologue recombination products with or without exchange of flanking chromosome arms (1). In several organisms, recombination mediates SC assembly (1) and release of non-homologous centromere coupling (2–4). The ZMM group of proteins, including yeast central element protein Zip1 and E3 ligase Zip3, coordinately control synapsis and CO-specific strand exchange (5,6). Prior to their role in synapsis, SC proteins including Zip1 provide coupling between non-homologous centromeres (2,3).

Meiotic progression in *zmm* deletion mutants in budding yeast is temperature sensitive (ts) (5). A screen for ts mutants (7) identified a transposon insertion in *PRE9* which encodes the universally conserved $\alpha 3$ subunit of the proteasome's fourteen component core particle (CP; 8). Although constitutively expressed like other CP components, $\alpha 3^{\text{Pre9}}$ is non-essential (9, 10). The *pre9* proteasome is modestly impaired for assembly and proteolytic capacity (9, 11). *pre9* diploids grow normally at higher temperatures, yet undergo prophase I arrest when shifted to 30°C following completion of premeiotic replication. This phenotype is also observed in WT exposed to the proteasome inhibitor MG132 (Fig. 1Ai–iii and fig. S1b; 12). Remarkably, abrogation of DSBs restores meiotic progression in *pre9* (Fig. 1Aiv). Thus, at restrictive temperature, the *pre9* -proteasome can promote meiotic divisions, but it fails to resolve Spo11-induced events.

To delineate proteasome functions during meiosis, we first examined homologue pairing. At times when WT exhibits maximum pairing as indicated by predominance of fused homozygous GFP signals, pairing in *pre9* along chromosome arms and at centromeres is dramatically reduced (Fig. 1B,C; fig. S1d). Nonetheless, in both *pre9* and WT, detection of ~16 signals of kinetochore protein Mtw1 indicates pair-wise centromere association (Fig. 1B,D; 2,4). We conclude that in *pre9*, unlike WT, centromeres are frequently coupled with non-homologous partners.

Pairing defects, including persistence of non-homologous centromere coupling, are frequently associated with compromised DSB formation (1,2). Physical analysis shows indeed that both *pre9* and MG132 exposure decrease DSB levels by ~35% (Fig. 2A and fig. S2). DSB reductions in a *spo11* hypomorph (13) by >50%, however, do not result in homologous centromere pairing, consistent with distinct proteasome roles in homologue pairing and DSB formation (Fig. 1Ci; Fig. 2A).

Processing of DSBs is also similarly affected by *pre9* and MG132 exposure as indicated by analysis at the *HIS4::LEU2* recombination hotspot (fig. S3). Increased DSB steady-state levels observed under both conditions (Fig. 2B,C and fig. S4a,b) are likely due to delayed DSB processing since proteasome defects do not increase DSB formation (above). Furthermore, proteasome defects reduce COs to < 50% of WT levels, whereas NCOs form normally (Fig. 2B,C and fig. S4). Thus, a structurally and functionally intact proteasome is required for DSB processing into COs, but not into NCOs. Efficient NCO formation in *pre9* further intimates that homologue arms can undergo allelic recombination despite compromised pairing.

Crossover formation involves two successive strand invasions of 5' resected DSBs, generating first interhomologue single end invasions (IH-SEIs) and then double Holliday junctions (IH-dHJs) (1). Three findings in *pre9* show that a structurally intact proteasome mediates the CO-specific transition from DSBs to IH-SEIs: (i) DSBs persist at late time points (Fig. 2C), (ii) both IH-SEIs and IH-dHJs are reduced and delayed, whereas (iii) intersister joint molecules (IS-JMs) appear with normal timing (Fig. 2D,E and fig. S5a,b). By inference, in *pre9* as in *zip1* (5) fewer IH-JM form, but they accumulate to substantial levels at late times due to their increased lifespan. Recombination defects in MG132-treated cultures are qualitatively similar, but less severe (supporting online material (SOM) text and fig. S5a,b). We conclude that during meiosis, the proteasome mediates DSB processing specifically along the CO pathway.

At 23°C, COs are only modestly reduced in *pre9* [fig. S6 and SOM text]. By implication, high levels of chromosome missegregation observed in *pre9* under permissive conditions are likely due to recombination-independent proteasome effects.

A role of proteasome function in synapsis was examined by monitoring chromosomal localization of Zip1, Zip3 and axis protein Red1. During WT leptotema, Zip1 colocalizes with 10–20 Zip3 foci at centromeres (Class I, Fig. 3A,B; fig. S7a–c; 14), followed by appearance of zygotene nuclei exhibiting Zip1 foci and lines along with ~40 Zip3 foci (Class II). Pachytene entry is indicated by (semi-)continuous Zip1 lines that contain >35 Zip3 foci (Class III). Red1 also localizes in multiple foci with little linear organization at earlier stages, but becomes organized as semi-continuous lines during pachynema (Fig. 3C) (15).

Proteasome defects result in a dramatic reduction and/or delay in synapsis. Class III nuclei reach only ~one fifth (*pre9*) or half (MG132) of WT peak levels, whereas Class I and/or Class II nuclei remain abundant (Fig. 3A,B; fig. S7d). Moreover in *pre9*, (i) Zip1 foci in Class I nuclei remain associated with centromeres even at late times, a configuration normally limited to pre-DSB nuclei (fig. S7a,b); (ii) Zip1 polymerization occurs despite lack of homologous centromere pairing (fig. S7e,f); and (iii) Red1 fails to assume its longitudinally-organized pachytene morphology, despite chromatin association at normal levels (Fig. 3C,D and fig. S7g). Zip3 foci along chromosome arms, by contrast, are detected at normal numbers in *pre9* or MG132 (fig. S7c) (t = 6h; Fig. 3A, E), although this analysis does not indicate to what extent Zip3 associates with its normal targets (16). We conclude that the proteasome mediates a coordinated transition involving homologue pairing, synapsis of longitudinally organized chromosome axes, and CO-specific strand exchange. Continued Zip3 recruitment and NCO formation in *pre9* and MG132 further argue for specific proteasome requirements in meiotic processes rather than generic cytotoxicity of proteasome inhibition.

Remarkably, *pre9* affects Zip1 localization even in absence of DSBs. In *spo11*, Zip1 association with chromatin is limited to centromeres, whereas bulk Zip1 aggregates into polycomplexes (PCs) not associated with chromatin (Fig. 3F) (2). A less specific Zip1 association with chromatin in *pre9 spo11* is indicated by prevalence of nuclei with a multitude (>20) of Zip1 foci [speckled], whereas PCs are reduced, despite normal Zip1

abundance (Fig. 3F, G and fig. S7h). Thus, the proteasome controls association of Zip1 with chromatin independently of its effects on recombination.

How does the proteasome control chromosomal events during meiosis? In the 26S proteasome, the barrel-shaped 20S CP is flanked by one or two 19S regulatory particles (RP; 8). The RP recognizes polyubiquitinated substrates and feeds them into the CP's proteolytic tunnel. Meiosis-specific recruitment of fully assembled 26S proteasome to chromatin is indicated by localization analysis of fully-functional, constitutive CP^{20S} protein $\alpha 5$ ($\alpha 5^{\text{Pup}2}$ -GFP, Fig. 4A,D and fig. S8; 17) and RP protein Rpn12 (GFP-Rpn12 or HA-Rpn12; Fig. 4B, E; fig. S9; 18). Low numbers of CP^{20S} and RP^{19S} foci in pre-meiotic cells (t = 0h) indicate proteasome occupancy of many genome regions in vegetative cells (Fig. 4A,B) (19). Proteasome foci increase during pre-meiotic S-phase and/or leptotema (t = 2h), with an intermittent dip or steadily, reaching peak levels of 29.2 ($\pm$ 14.4) CP^{20S} and 32.6 ($\pm$ 16.6) RP^{19S} foci, respectively. Proteasome levels peak one hour after pachynema (Fig. 4D, E), in nuclei exhibiting fragmented Zip1 staining as typically observed at pachytene exit. Thus, RP^{19S} and/or CP^{20S} recruitment to chromatin occurs in two waves, the first during leptotema and the second during pachytene exit. Both CP^{20S} and RP^{19S} tend to localize to chromatin regions devoid of Zip1 (Fig. 4A, B), consistent with a proteasome role in displacing Zip1 from the corresponding chromosome regions.

CP^{20S} recruitment to chromatin is controlled by a distinct subset of meiosis-specific factors. High levels of CP^{20S} in a strain expressing catalytically inactive *spo11-yf*(13) suggests that meiotic DSBs are dispensable at least for early CP^{20S} recruitment whereas $\alpha 5^{\text{Pup}2}$ foci are dramatically reduced at all times in both *zip1* and *zip3* (Fig. 4C, D; fig. S10). PCs in *zip3* and *spo11-yf* also frequently contain at least one CP^{20S} focus (>90%; Fig. 4C), indicating a potential Zip1 role in CP^{20S} recruitment. A role in CP^{20S} recruitment of synapsis itself, however, is unlikely since *spo11-yf* and *zip3* are similarly defective for SC central element assembly (2, 5). More likely, Zip1 and Zip3 control CP^{20S} recruitment via their spatial and/or functional association with coupled centromeres (2, 14, 16).

A conserved meiotic proteasome function was garnered from examination of the *C. elegans* germline. Depletion of the $\alpha 3^{\text{Pre}9}$ orthologue PAS-3 disrupts both early and late SC morphogenesis, as suggested by increased abundance in zygotene nuclei of PCs containing both axis protein HTP-3 and central element protein SYP-1 (Fig. 4F, fig. S12) (20, 21). Moreover, during pachynema, SYP-1 undergoes premature and excessive polarization (fig. S12a, iii), a distribution normally limited to diplonema (21). Consistent with a function along meiotic chromosomes, the CP^{20S} extensively associates with SYP-1-decorated, pachytene chromosome squashes (Fig. 4G).

Meiosis-specific proteasome association with chromosome axes is also detected in mouse spermatocytes where CP^{20S} signal overlaps with SYCP3-stained chromosome axes (2) at all meiotic stages except leptotema (Fig. 4H), while staining is absent in non-meiotic testicular cells (fig. S13c).

Our findings support the following model: Prior to the DSB-induced homology search, non-homologous interactions can become stabilized by promiscuous association with

chromosomes of SC proteins including yeast Zip1 [Fig 4I, panel i]. Following its recruitment, the proteasome displaces SC proteins, restricting the latter to centromeres (panel ii). At this stage, the proteasome may also ensure high DSB levels via its role in axis morphogenesis (1) and/or via removal of proteins that normally render DSB formation by Spo11 reversible (1, 22). Non-homologous centromere coupling could provide a kinetic barrier against interstitial non-allelic pairing (3). This pairing block is then destabilized by proteasome-mediated removal of SC proteins (panel iii).. Once integrated into the meiotic program, chromosomally-tethered proteasome may have acquired lineage-specific functions and localization patterns. Our model postulates independent proteasome functions in axis morphogenesis, homologue pairing and DSB formation that in turn control SC assembly and CO formation. The proteasome may also have distinct roles in the two latter processes. Notably, not all proteasome effects can be attributed to its roles in Zip1 recruitment or DSB formation (SOM text).

Interactions between the proteasome and polyubiquitinated substrates are assumed to be stochastic (8). Our findings suggest that the proteasome is targeted to chromosomal sites by appropriately modified substrates, analogous to its association e.g., with the endoplasmatic reticulum or with sites of DNA damage (8,17,19,23). Notably, meiotic recombination sites in many organisms are occupied by RING finger E3 ubiquitin and/or SUMO ligases (6). Moreover, the ubiquitin-SUMO-proteasome system controls CO formation and positioning (7, 24). Our work identifies chromosomal tethering of 26S proteasome during meiosis as an evolutionarily conserved mechanism for controlling protein dynamics at distinct chromosomal sites thus ensuring homologue juxtaposition and CO exchange.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank V. Matthews for assistance; A. Severson, A. Tartakoff, A. Almasan, B. Li, M. Lichten, R. Pezza and R. Kondratov for advice; N. Hunter for communicating unpublished results; and A. Amon, S. Ben-Aroya, D. Dawson, S. Keeney, A. Peyroche, A. Villeneuve, and M. Zetka for strains and antibodies. Research was supported by NIH grants R15GM099056 (G.V.B.), R01GM104007 (J.L.Y.) and R01HD083177 (P.A.H.). Additional funding from CSU's Faculty Research Development Program and John Vitullo's Bridge Funding Program (to G.V.B.).

References

- 1Zickler D, Kleckner N. Recombination, Pairing, and Synapsis of Homologs during Meiosis. Cold Spring Harb. Perspect. Biol. 2015; 18:7. pii: a016626.
- 2Tsubouchi T, Roeder GS. A synaptonemal complex protein promotes homology-independent centromere coupling. Science. 2005; 308:870–873. [PubMed: 15879219]
- 3Obeso D, Pezza RJ, Dawson D. Couples, pairs, and clusters: mechanisms and implications of centromere associations in meiosis. Chromosoma. 2014; 123:43–55. [PubMed: 24126501]
- 4Obeso D, Dawson DS. Temporal characterization of homology-independent centromere coupling in meiotic prophase. PLoS One. 2010; 5:e10336. [PubMed: 20428251]
- 5Börner GV, Kleckner N, Hunter N. Crossover/ noncrossover differentiation, synaptonemal complex formation, and regulatory surveillance at the leptotene/zygotene transition of meiosis. Cell. 2004; 117:29–45. [PubMed: 15066280]

- 6Lake CM, Hawley RS. Becoming a crossover-competent DSB. *Semin. Cell. Dev. Biol.* 2016; 54:117–125. [PubMed: 26806636]
- 7Perry J, Kleckner N, Börner GV. Bioinformatic analyses implicate the collaborating meiotic crossover/chiasma proteins Zip2, Zip3, and Spo22/Zip4 in ubiquitin labeling. *Proc. Natl. Acad. Sci. U. S. A.* 2005; 102:17594–9. [PubMed: 16314568]
- 8Glickman MH, Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol. Rev.* 2002; 82:373–428. [PubMed: 11917093]
- 9Velichutina I, Connerly PL, Arendt CS, Li X, Hochstrasser M. Plasticity in eucaryotic 20S proteasome ring assembly revealed by a subunit deletion in yeast. *EMBO J.* 2004; 23:500–10. [PubMed: 14739934]
- 10Brar GA, et al. High-resolution view of the yeast meiotic program revealed by ribosome profiling. *Science.* 2012; 335:552–557. [PubMed: 22194413]
- 11Wilson MA, Meaux S, van Hoof A. A genomic screen in yeast reveals novel aspects of nonstop mRNA metabolism. *Genetics.* 2007; 177:773–784. [PubMed: 17660569]
- 12Materials and methods are available as supporting material on *Science* Online.
- 13Martini E, Diaz RL, Hunter N, Keeney S. Crossover homeostasis in yeast meiosis. *Cell.* 2006; 126:285–295. [PubMed: 16873061]
- 14Tsubouchi T, MacQueen AJ, Roeder GS. Initiation of meiotic chromosome synapsis at centromeres in budding yeast. *Genes Dev.* 2008; 22:3217–3226. [PubMed: 19056898]
- 15Smith AV, Roeder GS. The yeast Red1 protein localizes to the cores of meiotic chromosomes. *J Cell Biol.* 1997; 136:957–67. [PubMed: 9060462]
- 16Serrentino ME, Chaplais E, Sommermeyer V, Borde V. Differential association of the conserved SUMO ligase Zip3 with meiotic double-strand break sites reveals regional variations in the outcome of meiotic recombination. *PLoS Genet.* 2013; 9:e1003416. [PubMed: 23593021]
- 17Ben-Aroya S, et al. Proteasome nuclear activity affects chromosome stability by controlling the turnover of Mms22, a protein important for DNA repair. *PLoS Genet.* 2010; 6:e1000852. [PubMed: 20174551]
- 18Tomko RJ Jr, Hochstrasser M. Incorporation of the Rpn12 subunit couples completion of proteasome regulatory particle lid assembly to lid-base joining. *Mol. Cell.* 2011; 44:907–917. [PubMed: 22195964]
- 19McCann TS, Tansey WP. Functions of the proteasome on chromatin. *Biomolecules.* 2014; 4:1026–1044. [PubMed: 25422899]
- 20Goodyer W, et al. HTP-3 links DSB formation with homolog pairing and crossing over during *C. elegans* meiosis. *Dev. Cell.* 2008; 14:263–274. [PubMed: 18267094]
- 21Nabeshima K, Villeneuve AM, Colaiácovo MP. Crossing over is coupled to late meiotic prophase bivalent differentiation through asymmetric disassembly of the SC. *J Cell. Biol.* 2005; 168:683–689. [PubMed: 15738262]
- 22Zhang A, et al. A protease pathway for the repair of topoisomerase II-DNA covalent complexes. *J Biol. Chem.* 2006; 281:35997–36003. [PubMed: 16973621]
- 23Krogan NJ, et al. Proteasome involvement in the repair of DNA double-strand breaks. *Mol. Cell.* 2004; 16:1027–1034. [PubMed: 15610744]
- 24Zhang L, et al. Topoisomerase II mediates meiotic crossover interference. *Nature.* 2014; 511:551–556. [PubMed: 25043020]
- 25Brar GA, Hochwagen A, Ee LS, Amon A. The multiple roles of cohesin in meiotic chromosome morphogenesis and pairing. *Mol. Biol. Cell.* 2009; 20:1030–1047. [PubMed: 19073884]
- 26Ross-Macdonald P, et al. Large-scale analysis of the yeast genome by transposon tagging and gene disruption. *Nature.* 1999; 402:413–418. [PubMed: 10586881]
- 27Janke C, et al. A versatile toolbox for PCR-based tagging of yeast genes: new fluorescent proteins, more markers and promoter substitution cassettes. *Yeast.* 2004; 21:947–962. [PubMed: 15334558]
- 28Ahuja JS, Börner GV. Analysis of meiotic recombination intermediates by two-dimensional gel electrophoresis. *Methods Mol. Biol.* 2011; 745:99–116. [PubMed: 21660691]
- 29Collins GA, Gomez TA, Deshaies RJ, Tansey WP. Combined chemical and genetic approach to inhibit proteolysis by the proteasome. *Yeast.* 2010; 27:965–74. [PubMed: 20625982]

- 30Börner GV, Cha RS. Analysis of Recombination and Chromosome Structure during Yeast Meiosis. Cold Spring Harb. Protoc. 2015; 11 pdb.top077636.
- 31Lai YJ, Lin FM, Chuang MJ, Shen HJ, Wang TF. Genetic requirements and meiotic function of phosphorylation of the yeast axial element protein Red1. Mol. Cell. Biol. 2011; 31:912–923. [PubMed: 21173162]
- 32Brenner S. The genetics of *Caenorhabditis elegans*. Genetics. 1974; 77:71–94. [PubMed: 4366476]
- 33Chan RC, et al. Chromosome cohesion is regulated by a clock gene paralogue TIM-1. Nature. 2003; 423:1002–1009. [PubMed: 12827206]
- 34Mainpal R, Priti A, Subramaniam K. PUF-8 suppresses the somatic transcription factor PAL-1 expression in *C. elegans* germline stem cells. Dev. Biol. 2011; 360:195–207. [PubMed: 21968099]
- 35Timmons L, Court DL, Fire A. Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in *Caenorhabditis elegans*. Gene. 2001; 263:103–112. [PubMed: 11223248]
- 36Vrooman LA, Oatley JM, Griswold JE, Hassold TJ, Hunt PA. Estrogenic exposure alters the spermatogonial stem cells in the developing testis, permanently reducing crossover levels in the adult. PLoS Genet. 2015; 11:e1004949. [PubMed: 25615633]
- 37Gladstone MN, Obeso D, Chuong H, Dawson DS. The synaptonemal complex protein Zip1 promotes bi-orientation of centromeres at meiosis I. PLoS Genet. 2009; 5:e1000771. [PubMed: 20011112]
- 38Nag DK, Scherthan H, Rockmill B, Bhargava J, Roeder GS. Heteroduplex DNA formation and homolog pairing in yeast meiotic mutants. Genetics. 1995; 141:75–86. [PubMed: 8536992]
- 39Pan J, et al. A hierarchical combination of factors shapes the genome-wide topography of yeast meiotic recombination initiation. Cell. 2011; 144:719–731. [PubMed: 21376234]
- 40Wagner CR, Kuervers L, Baillie DL, Yanowitz JL. XND-1 regulates the global recombination landscape in *Caenorhabditis elegans*. Nature. 2010; 467:839–843. [PubMed: 20944745]

Fig. 1. The proteasome controls meiotic progression and homologue pairing

(A) Meiotic progression in WT, *pre9*, *zip1* at (i) 23°C and (ii) 33°C, in presence of MG132 (iii, pink arrow and (iv) in *spo11 pre9* versus *pre9*. n = 2; error bars indicate data range.

(B) Localization of GFP-tagged centromere V (*cenV*, arrows) and total kinetochore signals (*MTWI-13xMyc*) in nuclei with homologous centromere pairing (WT, left) or homology-independent centromere coupling (*pre9*, right). Blue lines (DNA) trace edges of spread nuclei stained with 4',6'-diamidino-2-phenylindole (DAPI). Bar 1 μ m.

(C) Fraction of prophase I-arrested nuclei with one, two, or 0 or > 2 ("other") GFP signals in strains homozygous for *tetO* arrays (i) near *cenV*, on the long arms of (ii) chromosome V, or (iii) chromosome II; or (iv) heterozygous for *tetO* at non-allelic arm loci on chromosomes II and V (25). n = 2; error bars indicate data range (also see fig. S1d).

(D) Number of kinetochore signals in WT (14.1 ± 2.5 SD) and in *pre9* (12.9 ± 3.8 SD). For nucleus numbers, see Table S1.

Fig. 2. The proteasome controls DSB formation and the crossover-specific DSB-to-SEI transition (33°C)

(A) Cumulative DSB signals at five recombination hotspots at $t = 7.5$ h in a *sae2* background. $n = 3$, error bars are SD (also see fig. S2).

(B) 1D gel Southern analyses of DSBs and COs (i, ii), and NCOs (iii) in *pre9*, *zip1*, and WT. Time points are 0, 2.5, 4, 5, 6, 7, 8.5, 10, 11, and 24 h (asterisk in (iii): inverted loading order of 2.5 h and 4 h *zip1* samples).

(C) Quantitation of DSBs, COs and NCOs in *pre9*, *zip1* and WT. Dotted vertical line, $t = 5$ h (also see fig. S4).

(D) 2D gel Southern analyses of JM intermediates in WT and *pre9*.

(E) Quantitative analysis of JMs in WT, *pre9* and *zip1* (also see fig. S5a,b).

Fig. 3. The proteasome controls axis morphogenesis and synapsis (33°C)

(A) Zip1 and Zip3-GFP localization in WT Class I, II or III nuclear spreads; in *pre9* ; and in presence of MG132 (bottom). Blue dotted lines, see Fig. 1B. Bar 1 μ m.

(B) Zip1 localization in WT and *pre9* cultures. For Zip1 classes, see Fig. 3A.

(C) Red1-staining classes. Bar 1 μ m.

(D) Quantitation of Red1 classes at t = 6 h in *PRE9* and *pre9* in *ndt80* background.

“other” indicates poorly spread nuclei. n = 3; error bars, SD; * p < 0.03, two-tailed t-test.

(E) Zip3-GFP focus numbers in a *pdr5* culture treated with DMSO (mock; 37.4 ± 16.1) or with MG132 (34.8 ± 15.0), in *pre9* (38.3 ± 14.3) or in an untagged WT strain (5.3 ± 3.1) at time of WT pachytene (t = 6h). Errors are SD (also see fig. S7c).

(F) Zip1 localization in *PRE9 spo11* (n = 3) and *pre9 spo11* (n = 3) nuclei. Arrows indicate polycomplexes. Bar 1 μ m.

(G) Quantitation of Zip1 localization in a *spo11* background. # Polycomplexes (PC) were scored independent of patterns of chromatin-associated Zip1. Fractions are averages from two cultures at t = 3.5 h and 5 h or t = 4 h and 6 h, respectively; error bars, SD; * p < 0.03, two-tailed t-test. For nucleus numbers, see Table S1.

Fig. 4. Meiosis-specific recruitment of the 26S proteasome to chromosomes is evolutionarily conserved

(A) Localization of the proteasome CP ($\alpha 5^{\text{Pup2}}$) and Zip1 on WT $G_{0/1}$ -arrested ($t = 0\text{h}$), leptotene ($t = 2\text{h}$), early pachytene ($t = 5\text{h}$) and late pachytene/ diplotene ($t = 6\text{h}$) nuclear spreads. Bar $1\ \mu\text{m}$.

(B) Same as (A), but for proteasome RP component Rpn12-GFP without 2 h sample.

(C) CP ($\alpha 5^{\text{Pup2}}$ -GFP) and Zip1 localization in *spo11-yf* ($t = 5\text{h}$), *zip3* ($t = 6\text{h}$) and *zip1* ($t = 6\text{h}$). Arrows, CP foci associated with PCs. Bar $1\ \mu\text{m}$.

(D) CP focus counts in WT, *spo11-yf*, *zip3* and *zip1*. Pachytene (Zip1 Class III; red), meiotic divisions (blue). §, for focus scoring see 30; fig. S11). Asterisks indicate significant differences versus corresponding WT sample (two-tailed Wilcoxon rank sum test): **, $p < 0.01$; *, $p < 0.05$; n.s., $p > 0.05$.

(E) RP focus counts in WT (also see Fig. 4D).

(F) Requirement for *C. elegans* $\alpha 3^{\text{PAS-3}}$ CP for SC axis and central element assembly.

Region 1 (transition zone) excerpts from (i) WT and (ii) *pas-3(RNAi)* knockdown animals stained with HTP-3 and SYP-1. Arrows indicate HTP-3/SYP-1 coaggregates (also see fig. S12). Bar $4\ \mu\text{m}$.

(G) Squashed pachytene nucleus stained with anti-CP^{20S} and anti-SYP-1 antibodies.

Overlap is similar in 99% of nuclei ($n > 100$) from 20 germ lines (also see fig. S13b). Bar $2\ \mu\text{m}$.

(H) Proteasome and SYCP3 localization along surface-spread mouse spermatocytes at the leptotene (n=5), zygotene (n = 34), pachytene (n = 115), or diplotene stages (n = 47). Bar 5 μ m. Insets show magnified views of boxed areas (also see fig. S13c,d).

(I) Model for proteasome functions in homologue pairing, synapsis (SC) and crossover (CO) formation (see text for details).