Meiosis is a special cell division program that reduces the diploid chromosome set to a haploid set for sexual reproduction. Reductive segregation of homologous chromosomes is helped by physical linkages (crossovers) provided by recombination. A long-standing problem in biology has been to understand how meiotic recombination is controlled, since its initiation by DNA double-strand breaks (DSBs) is a programmed damage that could be lethal and is highly organized temporally and spatially throughout the genome. Much of our current knowledge comes from work in fungi, notably the budding yeast Saccharomyces cerevisiae and the distantly related fission yeast Schizosaccharomyces pombe. As anticipated from well-known recombination hotspots, in both yeasts there are DSB hotspots. A major effort has been to map these hotspots and, more recently, the genome binding positions of the dozen or more proteins involved, to determine what makes particular genome sites hot for DSBs. DSB hotspot location is clearly related to other aspects of genome architecture. Hotspots are preferentially located in large intergenic regions in S. pombe and in promoter regions in S. cerevisiae. Furthermore, chromosome structure—histone modifications, transcription factor binding, meiosis-specific sister chromatid cohesin binding, etc.—influences DSB distribution. However, until recently no single factor seemed responsible for determining most DSB hotspots, and little was known about how the conserved topoisomerase-like protein Spo11 (Rec12 in S. pombe), which possesses the active site for DSB formation, is recruited or activated. We recently reported2 that in fission yeast three proteins—Rec25, Rec27 and Mug20—are DSB hotspot determinants, since they bind with high specificity to hotspots in proportion to the frequency of DNA breakage and are required for most DSBs at hotspots (Fig. 1). These proteins interdependently colocalize in the nucleus (by fluorescence microscopy) and bind the same sites along the genome (by chromatin immunoprecipitation and microarray hybridization; ChIP-chip), suggesting they act as a complex. By ChIP-chip they bind to 86% of all hotspots (97% of the hottest two-thirds) independently of, and presumably before, DSB formation. Furthermore, in the absence of Rec27, DSBs are eliminated or strongly reduced at > 80% of hotspots. It was already known that meiotic chromosome components control recombination. During meiosis, the synaptonemal complex (SC) binds sites along the entire chromosome length, forming a ladderlike structure with emanating loops and holds homologs together. S. pombe lacks an SC but has related structures called linear elements (LinEs) thought to extend across large parts of the chromosomes. Mutants lacking SC or LinE components, or cohesins required for their formation, are impaired in meiotic recombination and DSB formation. In S. cerevisiae, SC axis proteins Hop1 and Red1 and cohesin protein Rec8 have been reported to bind at genome sites anti-correlated with DSB hotspots. In stark contrast, S. pombe LinE components Rec25, Rec27 and Mug20 show a strong positive correlation with hotspots. We revisited the anti-correlation reported in budding yeast and noted that the degree of anti-correlation of Red1 (or Hop1) binding and DSBs is not high: the coefficient of determination $R^2 = 0.068$ for Red1 and 0.04 for Hop1 vs. DSBs. Thus, the binding of Red1 and Hop1 (negatively) accounts for < 10% of the DSB distribution, whereas that of Rec25-Rec27-Mug20 (positively) accounts for > 60%. Therefore, a single hotspot determinant acting genome-wide has been found in S. pombe but remains unknown in S. cerevisiae. In mice, hotspot position is correlated with variants of Prdm9, which methylates histone H3 Lys9; however, only a minority of the testis-specific H3 Lys9 marks are DSB hotspots, and in the absence of Prdm9, hotspots appear at novel positions. Thus, Prdm9 appears to contribute to hotspot positioning but is not required for DSB formation.
Dissimilar controls of meiotic events in budding and fission yeast, and between them and other species, are well-documented and may reflect the rapid evolution of meiotic proteins. However, we have noticed amino-acid sequence similarity between Rec27 in four Schizosaccharomyces species and the SC protein SYP-2 in four Caenorhabditis species; Mug20 and DDL-1 (a SYP-2 interacting protein) also show conserved sequence similarity. Thus, our work has uncovered an unexpected role for chromosomal-axial proteins in hotspot determination that may be conserved in other organisms. Our results also help clarify other aspects of meiotic recombination. Rec12 (wild type or active-site null) binds across the genome more uniformly than Rec25-Rec27-Mug20 or hotspots and, in a few cases, binds at elevated frequency where DSB hotspots are absent. It also binds the same with or without Rec27. Thus, we proposed that Rec25-Rec27-Mug20 activates already-bound Rec12 to make DSBs rather than promoting Rec12’s binding (Fig. 1).

Because Rec25-Rec27-Mug20 is at hotspots, where DSBs are preferentially repaired with the sister chromatid, we further proposed that this complex directs partner choice for DSB repair. In DSBcold regions DSBs appear to be repaired preferentially with the homolog and can give genetic recombinants, unlike repair with the sister. Thus, these proteins may be key factors in keeping crossovers nearly constant across the genome in spite of DSB hotspots, an aspect of crossover control called crossover invariance (Fig. 1), which may be an evolutionarily important factor to promote reassortment of alleles to generate more fit genotypes.
Figure 1. Meiotic DS B hotspot determination in *S. pombe*. Top panel: LinEs form discrete nuclear foci during meiotic prophase; in vivo localization by microscopy of a representative LinE-component tagged with GFP. Middle panel: LinE components bind with exceptional specificity to DS B hotspots. A representative part of the genome showing localization by ChIP-chip of three LinE components and DS Bs. Bottom panel: Rec12, which binds more uniformly along chromosomes, may be locally activated at DS B hotspots by LinEs. In addition, LinEs may bias DS B repair at hotspots with the sister chromatid, contributing to crossover invariance (uniform crossover distribution in spite of DS B hotspots).