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Linear Element-Independent Meiotic Recombination in *Schizosaccharomyces pombe*

Jennifer L. Wells,* David W. Pryce,* Anna Estreicher,† Josef Loidl† and Ramsay J. McFarlane*,[1]

**North West Cancer Research Fund Institute, University of Wales, Bangor, LL57 2UW, United Kingdom and* †*Department of Chromosome Biology, University of Vienna, A-1030, Vienna, Austria*

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ABSTRACT

Most organisms form protein-rich, linear, ladder-like structures associated with chromosomes during early meiosis, the synaptonemal complex. In *Schizosaccharomyces pombe*, linear elements (LinEs) are thread-like, proteinacious chromosome-associated structures that form during early meiosis. LinEs are related to axial elements, the synaptonemal complex precursors of other organisms. Previous studies have led to the suggestion that axial structures are essential to mediate meiotic recombination. Rec10 protein is a major component of *S. pombe* LinEs and is required for their development. In this report we study recombination in a number of *rec10* mutants, one of which (*rec10-155*) does not form LinEs, but is predicted to encode a truncated Rec10 protein. This mutant has levels of crossing over and gene conversion substantially higher than a *rec10* null mutant (*rec10-175*) and forms cytologically detectable Rad51 foci indicative of meiotic recombination intermediates. These data demonstrate that while Rec10 is required for meiotic recombination, substantial meiotic recombination can occur in *rec10* mutants that do not form LinEs, indicating that LinEs *per se* are not essential for all meiotic recombination.

SEXUAL reproduction in most eukaryotes involves haploid gamete cells fusing to form a diploid cell, which, in metazoans, is the progenitor for the multiple cells found in the different tissues of the body and the germ line. The generation of the haploid gametes is dependent upon a specialized cell division known as meiosis, in which a diploid cell undergoes a single genome replication event followed by two successive rounds of chromosome segregation. The first meiotic chromosome segregation event involves a complex series of interactions between homologous chromosomes, culminating in a physical conjoining via the formation of genetic recombination intermediates that ensure correct segregation of homologs at meiosis I.

In most organisms, proteinacious structures, known as axial elements (AEs), form on chromosomes during meiotic prophase. AEs are the precursors to a synaptic structure, the synaptonemal complex (SC), which forms between homologs. The function of the SC remains poorly understood (Zickler and Kleckner 1999; Bishop and Zickler 2004). The fission yeast, *Schizosaccharomyces pombe*, is a member of a unique group of organisms that do not form fully mature SCs, although *S. pombe* does form cytologically distinct linear elements (LinEs) that exhibit similarities to AEs (Bähler *et al.* 1993; Kohli and Bähler 1994; Lorenz *et al.* 2004). The LinE structures of *S. pombe* and the AEs of *Saccharomyces cerevisiae* have conserved common features and the key structural components in the two organisms, Rec10 (*S. pombe*) and Red1 (*S. cerevisiae*), exhibit some amino acid conservation (Lorenz *et al.* 2004). This structural conservation and the absence of a visible SC in *S. pombe* strongly suggest that AEs serve some function other than providing a precursory platform for SC formation; they may function by contributing to the establishment of chromosomal mechanical forces of compaction, which have been proposed to regulate meiotic recombination (Blat *et al.* 2002; Kleckner *et al.* 2004). Furthermore, AEs (and possibly LinEs) appear to play a critical role in directing interhomolog recombination, precluding intersister recombination events (Schwacha and Kleckner 1997; Thompson and Stahl 1999), possibly by providing a platform for Hop1 protein-mediated dimerization of the Mek1 kinase, which, like Red1 and Hop1, is required to promote interhomolog recombination (Niu *et al.* 2005).

Although LinE formation is *rec10*$^{+}$ dependent (Molnar *et al.* 2003; Lorenz *et al.* 2004), the precise function(s) of Rec10 and LinEs remains unclear. *rec10* mutants have been isolated in a genetic screen for mutants that are defective in meiotic recombination, indicating that Rec10 is required for this process (Ponticelli and Smith 1989; De Veaux *et al.* 1992). However, until now the question of whether or not LinEs are essential for meiotic recombination remained unanswered (Loidl 2006). Further detailed analysis of one particular *rec10* mutant, *rec10-109*, indicated that

[1]*Corresponding author:* North West Cancer Research Fund Institute, University of Wales, Memorial Bldg., Deiniol Rd., Bangor, Gwynedd, LL57 2UW, United Kingdom. E-mail: ramsay@sbs.bangor.ac.uk

Rec10 functions to regulate recombination in a region-specific fashion (De Veaux and Smith 1994; Krawchuk *et al.* 1999). This is consistent with the observation that *S. cerevisae* Red1 null mutants are defective for recombination at some, but not all loci (Rockmill and Roeder 1990). The regional specificity of the *rec10-109* mutant shows that Rec10 regulates recombination in the middle region of chromosomes, a pattern similar to that observed for the meiosis-specific cohesins, Rec8 and Rec11, although residual LinEs have been observed in this mutant, suggesting that it is not totally defective in LinE function (De Veaux and Smith 1994; Krawchuk *et al.* 1999; Parisi *et al.* 1999; Lorenz *et al.* 2004). The similar regional pattern of recombination in the *rec10-109* and cohesion mutants indicate that there is an intimate functional association between meiotic cohesins and LinEs. However, recent studies demonstrate that Rec10 is required to regulate recombination more widely throughout the genome, indicating that the middle region regulation pattern exposed by the study of *rec10-109* is allele specific (Ellermeier and Smith 2005; this study).

The lack of a cytologically distinct SC in *S. pombe* makes it an exceptionally amenable system in which to reveal functions of SC components in addition to their role in the immediate juxtapositioning of homologous chromosomes. In this report we address one of the central questions relating to LinE function. We demonstrate that while loss of LinEs correlates with a reduction in recombination throughout the genome, substantial crossing over remains in the absence of LinEs, indicating that LinEs are not essential for all recombination. This also demonstrates that Rec10 has functions distinct from its role in LinE formation. Finally, we demonstrate that LinE-defective cells have a regional bias in the relative reduction of gene conversion events and that at some loci gene conversions are more severely affected than crossovers in the associated intervals. This feature of Rec10 is discussed in parallel with its role in crossing over.

MATERIALS AND METHODS

***S. pombe* strains:** A list of strains employed in this study and their genotypes is provided in Table 1. Culture media and strain storage were as described by Moreno *et al.* (1991). Construction of plasmid pYL167 was previously described by Lin and Smith (1995) and it was transformed into *S. pombe* as described by Moreno *et al.* (1991).

Meiotic crosses: Cultures were grown in yeast extract liquid (YEL), supplemented with 100 mg/ml adenine, to a density of $\sim 2.5 \times 10^7$ cells/ml. A total of 600 μl of each strain to be mated were added to a sterile microfuge tube, pulse centrifuged, and aspirated. Cell pellets were washed with 1 ml sterile dH_2O and finally resuspended in 20 μl dH_2O. Suspensions were spotted onto fully supplemented synthetic sporulation media (SPA) plates and incubated at 30° for 3–4 days. After incubation, sporulating cells were scrapped into a microfuge tube containing 1 ml of 0.6% β-glucuronidase (Sigma, St. Louis)/dH_2O solution and incubated for 16 hr at 25°. After incubation spores were harvested and resuspended in 30% ethanol and incubated at room temperature for no longer than 5 min. Suspensions were then centrifuged and aspirated dry and cell pellets were resuspended in 1 ml sterile dH_2O.

Determination of recombination frequencies: Intragenic recombination frequencies were determined as previously described (Pryce *et al.* 2005). For *ura1* and *lys7* intragenic recombination uracil- and lysine-deficient media were employed, respectively.

To determine intergenic recombination frequencies using prototrophic markers, serial dilutions of spore suspensions were plated onto yeast extract agar (YEA) plates to a colony density of ~50–100 colonies per plate. These were then replica plated onto nitrogen base agar (NBA) plates with and without appropriate supplements to permit the counting of double auxotrophs and prototrophs. The intergenic recombination frequency is the summed values of double prototrophs and double auxotrophs as a percentage of viable spores. To determine the intergenic recombination frequency between *ade6* and *tps16*, serial dilutions of spore suspensions were plated onto YEA plates to a density of ~50–100 colonies per plate and incubated at 25°. Plates were then replica plated onto fresh YEA plates and incubated at 37° (the *tps16-23* restrictive temperature) and recombinants were scored.

Recombination frequencies were used to determine the genetic distance (centimorgans) by employing Haldane's mapping function [genetic distance (centimorgans) $= -50 \ln(1 - 2R)$, where R = the total fraction of recombinant spores among all spores analyzed].

Microscopical preparation and staining: Meiotic time courses and nuclear spreads were carried out as previously described (Lorenz *et al.* 2004). In short, cells from sporulating cultures were freed of cell walls by enzymatic treatment, applied to a microscopic slide, opened up with a detergent, and fixed with paraformaldehyde. Primary antibodies [1:50 mouse monoclonal antibody against recombinant Rad51 protein (NeoMarkers, Fremont, CA) and 1:400 polyclonal rabbit antibody directed against amino acids 670–684 of Rec10] were applied together under a coverslip for overnight at room temperature. Secondary FITC-conjugated anti-mouse and Cy3-conjugated anti-rabbit antibodies were applied for ~4 hr at room temperature. Finally, the slides were mounted in Vectashield antifading agent (Vector Laboratories, Burlingame, CA) supplemented with 1 μg/ml 4′, 6-diamidino-2-phenylindole (DAPI) as a DNA-specific counterstain. Fluorescent signals were detected with a Zeiss Axioskop 2 Plus epifluorescence microscope equipped with an Axiocam HR camera. Black and white images were assigned false color and merged with Axiovision 3.1 software.

RESULTS

Rec10 is required for regulation of recombination throughout the genome: Rec10-controlled recombination in the middle regions of *S. pombe* chromosomes has been demonstrated by genetic analysis using *rec10-109*, a mutant allele generated by chemical mutagenesis (De Veaux and Smith 1994; Krawchuk *et al.* 1999). However, LinEs form in the *rec10-109* mutant during meiosis at the appropriate time, albeit with an altered morphological profile, and may retain some function for activation of recombination (Lorenz *et al.* 2004). Another allele of *rec10*, *rec10-144*, has recently been characterized, which resides in a separate complementation group to *rec10-109* and is a chemically induced missense

TABLE 1

Strains used in this study

Strain[a]	Genotype	Source
BP11	*h⁻ ade6-M26*	McFarlane collection
BP85	*h⁻ lys7-1*	McFarlane collection
BP86	*h⁺ lys7-2*	McFarlane collection
BP265	*h⁻ lys4-95*	McFarlane collection
BP372	*h⁻ ade6-M26 rec10-144*	This study
GP746 (BP404)	*h⁻ ura1-61*	G. R. Smith
GP2020 (BP409)	*h⁻ ade6-704*	G. R. Smith
BP581	*h⁻ ade6-M26 rec10-155:LEU2⁺ leu1-32*	This study
BP670	*h⁺ ade6-M26 his4-239*	This study
BP691	*h⁻ ade6-L52 lys4-95*	This study
BP692	*h⁻ ade6-L52 lys4-95 rec10-144*	This study
BP693	*h⁺ ade6-M26 his4-239 rec10-144*	This study
BP771	*h⁺ ade6-M26 leu2-120*	This study
BP772	*h⁻ ade6-L52 lys7-1*	This study
BP805	*h⁺ ade6-M26 leu2-120 rec10-144*	This study
BP823	*h⁺ ade6-L52 tps16-23*	This study
BP856	*h⁺ ade6-L52 tps16-23 rec10-144*	This study
BP862	*h⁺ ade6-L52 tps16-23 rec10-155:LEU2⁺ leu1-32*	This study
BP863	*h⁻ ade6-L52 lys7-1 rec10-144*	This study
BP874	*h⁺ ade6-M26 lys7-2 rec10-144*	This study
BP880	*h⁻ ade6-M26 arg3-124 leu1-32 rec10-155::LEU2⁺*	This study
BP888	*h⁻ ade6-M26 arg3-124*	This study
BP890	*h⁺/h⁻ ade6-M210/ade6-M216 rec10-155::LEU2⁺/rec10-155::LEU2⁺ leu1-32/leu1-32*	This study
BP892	*h⁺ ade6-L52 pro2-1 leu1-32 rec10-155::LEU2⁺*	This study
BP896	*h⁺ ade6-L52 pro2-1*	This study
BP904	*h⁺ his4-239 leu1-32 rec10-155::LEU2⁺*	This study
BP905	*h⁺ ade6-M26 lys7-2*	This study
BP915	*h⁻ lys4-95 leu1-32 rec10-155::LEU2⁺*	This study
BP926	*h⁺ his4-239*	This study
BP968	*h⁺ pro1-1 leu1-32*	This study
BP970	*h⁻ arg4-55 leu1-32 rec10-155::LEU2⁺*	This study
BP971	*h⁺ ade8-106 rec10-155::LEU2⁺*	This study
BP972	*h⁻ arg4-55*	This study
BP974	*h⁺ ade8-106*	This study
BP981	*h⁺ pro1-1 leu1-32 rec10-155::LEU2⁺*	This study
BP1012	*h⁺ lys7-2 rec10-155::LEU2⁺ leu1-32*	This study
BP1013	*h⁻ lys7-1 rec10-155::LEU2⁺ leu1-32*	This study
BP1027	*h⁺/h⁻ ade-M210/ade6-M216*	This study
BP1045	*h⁻ ura1-61 rec10-175::kanMX6*	This study
BP1046	*h⁺ ura1-171 rec10-175::kanMX6*	This study
BP1047	*h⁺ ura1-171*	This study
BP1048	*h⁻ ura1-171*	This study
BP1049	*h⁺ ura1-61*	This study
BP1100	*h⁺ ura1-61 leu1-32 rec10-155::LEU2⁺*	This study
BP1102	*h⁻ ura1-171 leu1-32 rec10-155::LEU2⁺*	This study
BP1148	*h⁺ rad32Δ::ura4⁺ ura4-D18 leu1-32*	This study
BP1149	*h⁻ rad32Δ::ura4⁺ ura4-D18 leu1-32*	This study
BP1150	*h⁺ rad32Δ::ura4⁺ ura4-D18 leu1-32 rec10-155::LEU2⁺ ade6-704*	This study
BP1151	*h⁻ rad32Δ::ura4⁺ ura4-D18 leu1-32 rec10-155::LEU2⁺ ade6-704*	This study
BP1152	*h⁺ ade6-704*	This study
BP1154	*h⁺ leu1-32 rec10-155::LEU2⁺ ade6-704*	This study
BP1155	*h⁻ leu1-32 rec10-155::LEU2⁺ ade6-704*	This study
BP1156	*h⁻ rec10-175::kanMX6 ura4-D18 ade6-704*	This study
BP1157	*h⁺ rec10-175::kanMX6 ura4-D18 ade6-704*	This study
BP1172	*h⁺/h⁻ ade-M210/ade6-M216 rec10-175::kanMX6/rec10-175::kan MX6*	This study
BP1182	*h⁺ leu1-32 ura1-61 rec10-175::kanMX6* (pYL167)	This study
BP1183	*h⁺ leu1-32 ura1-61 rec10-175::kanMX6* (pSP1)	This study
BP1184	*h⁻ leu1-32 ura1-171 rec10-175::kanMX6* (pYL167)	This study

(*continued*)

TABLE 1

(Continued)

Strain[a]	Genotype	Source
BP1185	*h⁻ leu1-32 ura1-171 rec10-175::kanMX6* (pSP1)	This study
BP1191	*h⁻ rec10-175::kanMX6 rad32Δ::ura4⁺ ura4-D18 ade6-704*	This study
BP1192	*h⁺ rec10-175::kanMX6 rad32Δ::ura4⁺ ura4-D18 ade6-704*	This study
BP1193	*h⁺ leu1-32 lys4-95 ura4-D18 rec10-175::kanMX6* (pYL167)	This study
BP1194	*h⁺ leu1-32 lys4-95 ura4-D18 rec10-175::kanMX6* (pSP1)	This study
BP1195	*h⁻ leu1-32 his4-95 ura4-D18 rec10-175::kanMX6* (pYL167)	This study
BP1196	*h⁻ leu1-32 his4-95 ura4-D18 rec10-175::kanMX6* (pSP1)	This study

[a] Full genealogies are available upon request.

mutation (Pryce *et al.* 2005). We noted that crossovers in the *leu2–lys7* interval (see Figure 1 for location) on chromosome I were reduced in *rec10-144* homozygous zygotic crosses, relative to the *rec10⁺* control (Figure 2). Recombination in this interval was previously reported to be unaltered in *rec10-109* homozygous crosses (De Veaux and Smith 1994). Our data using *rec10-144* indicate that Rec10 is influencing recombination in intervals outside the previously reported middle regions (De Veaux and Smith 1994). We examined this further by looking at another interval, *his4–lys4*, on chromosome II (Figure 1), for which recombination was also previously reported to be *rec10* independent (De Veaux and Smith 1994). Crossover levels in the *his4–lys4* interval were also reduced relative to the *rec10⁺* control in the *rec10-144* mutant, indicative of a more extensive, genomewide requirement for Rec10 function (Figure 2). Recombination in the *ade6–tps16* interval, situated close to the centromere of chromosome III, was reduced in the *rec10-144* mutant to a level similar to that previously reported for the *rec10-109* mutant (Figure 2) (De Veaux and Smith 1994).

Although the *rec10-144* mutant exhibits defects in meiotic recombination at noncentral intervals (see above), this mutant forms LinEs, albeit with a morphological profile very different from the wild type, so may retain some LinE functions (Pryce *et al.* 2005). To explore the question of whether or not LinEs are required for recombination we used an insertion inactivation mutant of *rec10*, *rec10-155* (Lin and Smith 1995), which does not form any detectable LinEs by electron microscopy (Molnar *et al.* 2003) or immunocytochemistry (Lorenz *et al.* 2006). The *rec10-155* allele is predicted to encode a truncated Rec10 protein that has lost the C-terminal 103 amino acids containing the homology to the Red1 helical region, proposed to be required for Red1 homo-oligomerization (Hollingsworth and Ponte 1997; Woltering *et al.* 2000; Lorenz *et al.* 2006). We measured crossover frequencies in a number of intervals in the *rec10-155* mutant, some of which appear to retain *rec⁺* levels of recombination in the *rec10-109* mutant (De Veaux and Smith 1994). In all cases crossing over was reduced (Figure 2), demonstrating that Rec10 is required to control crossing over more extensively throughout the genome than previously suggested (De Veaux and Smith 1994; Krawchuk *et al.* 1999).

Recent work has indicated that gene conversions can occur via a crossover-independent pathway (reviewed in Bishop and Zickler 2004; Heyer 2004; Hollingsworth and Brill 2004). In the *rec10-109* mutant, gene conversion levels at the *lys7* and *ura1* loci have been reported to be indistinguishable from *rec10⁺* levels, although data from only one experiment were reported (De Veaux and Smith 1994). To address whether the requirement for Rec10 throughout the genome is specific for crossovers, we also tested gene conversion

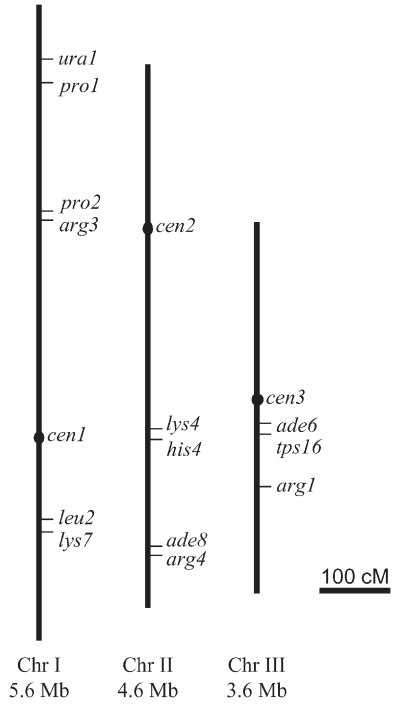

Figure 1.—Schematic genetic map of the *S. pombe* genome showing the approximate positions of the markers employed in this study. *S. pombe* has three chromosomes varying in size (chromosome I, 5.6 Mb; chromosome II, 4.6 Mb; chromosome III, 3.5 Mb). The positions of the three centromeres are marked (*cen1*, *cen2*, *cen3*). The bar is 100 cM based on 0.16 cM/kb (Young *et al.* 2002).

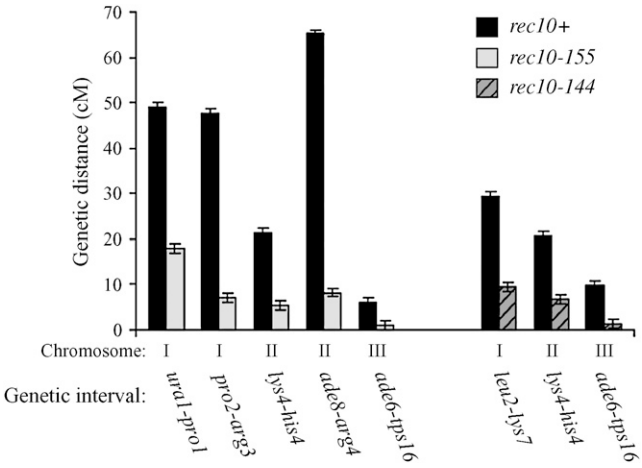

FIGURE 2.—Intergenic meiotic recombination is reduced in the *rec10-144* and *rec10-155* mutants. Intergenic meiotic recombination was measured in *rec10*$^+$, *rec10-155*, and *rec10-144* meioses. Different intervals were measured on each chromosome (see Figure 1 for positions), some of which exhibit little or no reduction in the previously studied *rec10-109* allele (DE VEAUX and SMITH 1994). Recombination levels were reduced at each interval in both mutants, indicating a role for Rec10 throughout the genome. Bars represent 95% confidence intervals. Pairwise comparison of *rec10*$^+$ *vs. rec10-144* or *rec10-155* using Student's *t*-test gave a *P*-value of <0.01 in all cases; $n \geq 4$ in all cases.

frequencies at the *lys7* and *ura1* loci. *lys7* gene conversion levels were greatly reduced in two-factor crosses of *rec10-144* and *rec10-155* mutants. Gene conversion at *ura1* was reduced marginally in the *rec10-155* mutant (Table 2). We also measured conversion frequency at the *ade6* locus in the *rec10-155* mutant and found this to be consistent with the previously reported reduction at this centromere-proximal locus (LIN and SMITH 1995). These results indicate that Rec10 plays a role in regulating both crossing over and gene conversion throughout the genome and not only in restricted regions, as previously reported. This finding corroborates a similar finding made recently by ELLERMEIER and SMITH (2005), who employed a *rec10*Δ mutant. Moreover, there is a differential requirement for a function of Rec10 for controlling gene conversions; this function is lost in the *rec10-155* mutant (see DISCUSSION).

Significant levels of genetic recombination occur in the absence of LinEs: While we demonstrated that Rec10 is required for recombination at all loci tested, we observed only relatively minor reductions in intergenic recombination. In addition, intragenic recombination at the *ura1* locus was reduced only slightly in the *rec10-155* mutant. Recently ELLERMEIER and SMITH (2005) observed much greater levels of reduction in inter- and intragenic recombination at all loci/intervals tested with a *rec10* null mutant (*rec10-175*; ≥40-fold in all cases). This includes loci at which we observed only relatively minor reductions. For example, the *rec10-155* mutation results in a 2-fold reduction in intergenic recombination at the *ura1–pro1* interval on chromosome I, while the *rec10-175* null mutation gives a 42-fold reduction at this interval (ELLERMEIER and SMITH 2005). We confirmed the differences between *rec10-155* and *rec10-175* by measuring *pro1–ura1* intergenic recombination and *ura1* intragenic recombination for both mutants under identical conditions (Table 2; data not shown). Table 3 shows the relative reductions in recombination observed between

TABLE 2

Intragenic gene conversion frequencies for two-factor *rec10-155* and *rec10-144* homozygous crosses

rec10 allele	Plasmid	Locus	Mean recombination frequency[a]	Fold reduction[b]
rec10$^+$	—	*ade6*[c]	297 ± 114	—
rec10-155	—	*ade6*	1.2 ± 0.3	247
rec10$^+$	—	*lys7*[d]	14 ± 10	—
rec10-144	—	*lys7*	0.3 ± 0.2	47
rec10-155	—	*lys7*	0.4 ± 0.4	35
rec10$^+$	—	*ura1*[e]	212 ± 51	—
rec10-155	—	*ura1*	35 ± 5	6
rec10-175	—	*ura1*	3.0 ± 0.5	70[f]
rec10-175	pSP1[g]	*ura1*	1.8 ± 1.2	118
rec10-175	pYL167[g]	*ura1*	24 ± 10	9

[a] Number of prototrophs per 10^6 viable spores ± 95% confidence interval; $n \geq 4$ in all cases.
[b] Student's *t*-test *P*-values from pairwise comparisons of *rec10*$^+$ *vs. rec10*$^-$ gave values of $P \leq 0.01$ in all cases.
[c] Alleles used: *ade6-M375* × *ade6-52*.
[d] Alleles used: *lys7-1* × *lys7-2*.
[e] Alleles used: *ura1-61* × *ura1-171*.
[f] This was the lowest fold reduction obtained in this study for *ura1* intragenic recombination. Other mean data values resulted in fold reductions as high as 155-fold.
[g] pYL167 is the plasmid carrying the *rec10-155* allele (LIN and SMITH 1995). pSP1 is a vector control containing no *rec10* cloned DNA.

TABLE 3

Comparison of levels of reduction for three *rec10* mutants

		Fold reduction in recombination frequency[a]						
		Intergenic						Intragenic:
rec10 allele	LinE status	*pro1–ura1*	*pro2–arg3*	*his4–lys4*	*ade8–arg4*	*ade6–tps16*	*ade6–arg1*	*ura1*
rec10-109[b]	Partial	1.0	1.6	0.8	NA	14.6	NA	0.7
rec10-155[c]	None	2.1	4.7	3.4	6.2	5.6	NA	6.1
rec10-175[d]	None	42.0	NA	64.0	NA	NA	>96.0	70.7

[a] Relative to *rec10*$^+$: These reductions are derived from relative reductions in recombination frequency and not genetic distances (in the case of this study the recombination frequencies were the same values employed to calculate genetic distances; see Figure 1).
[b] Derived from De Veaux and Smith (1994).
[c] Derived from this study.
[d] Derived from this study and Ellermeier and Smith (2005).

rec10-155 and *rec10-175* mutants (data for the *rec10-109* region-specific allele are also shown for comparison). These differences indicate that the truncated Rec10-155 protein retains the ability to mediate high levels of crossing over and region-specific gene conversion, while failing to form LinEs.

To explore the recombination proficiency of the *rec10-155* allele further we introduced a plasmid (pYL167; Lin and Smith 1995) encoding the truncated *rec10-155* allele into the *rec10-175* strain. pYL167 elevates recombination in the *rec10-175* null mutant to levels comparable to those in the *rec10-155* mutant (Table 2; data not shown).

Further evidence for the occurrence of recombination in the *rec10-155* mutant: It has been proposed that—in accordance with evidence from other organisms—recombination in *S. pombe* is initiated by the formation of DNA double-strand breaks (DSBs) (Cervantes *et al.* 2000). Such lesions in the DNA, which appear as fragmented DNA in physical assays, are not detected in the *rec10-175* mutant (Ellermeier and Smith 2005). Moreover, while Rad51 associates with meiotic recombination-initiating lesions in *S. pombe* (Grishchuk *et al.* 2004), no Rad51 foci are observed in *rec10-175* cells traversing meiosis (Lorenz *et al.* 2006). The failure to observe either DNA fragmentation or Rad51 foci indicates that initiation of meiotic recombination does not take place in the *rec10-175* mutant (Ellermeier and Smith 2005; Lorenz *et al.* 2006). This is consistent with the recombination analysis of the *rec10-175* mutant in which genetic recombination is at a level similar to that of mutants defective in the initiation of meiotic recombination (Ellermeier and Smith 2005). Since our data indicated that recombination is being initiated to significant levels in the absence of LinEs (see above), we predicted that Rad51 foci should be measurable at a significant level in the absence of LinEs in the *rec10-155* mutant. To test this we induced homozygous *rec10-155* h^+/h^- diploid cells to traverse meiosis by transferring them to sporulation medium and employed immunocytochemistry to determine whether Rad51 foci could be observed. Rad51 foci form in the *rec10-155* mutant with a high frequency (Figure 3), confirming that Rad51-dependent recombination is occurring. Higher-resolution temporal comparison of the *rec10-155* mutant

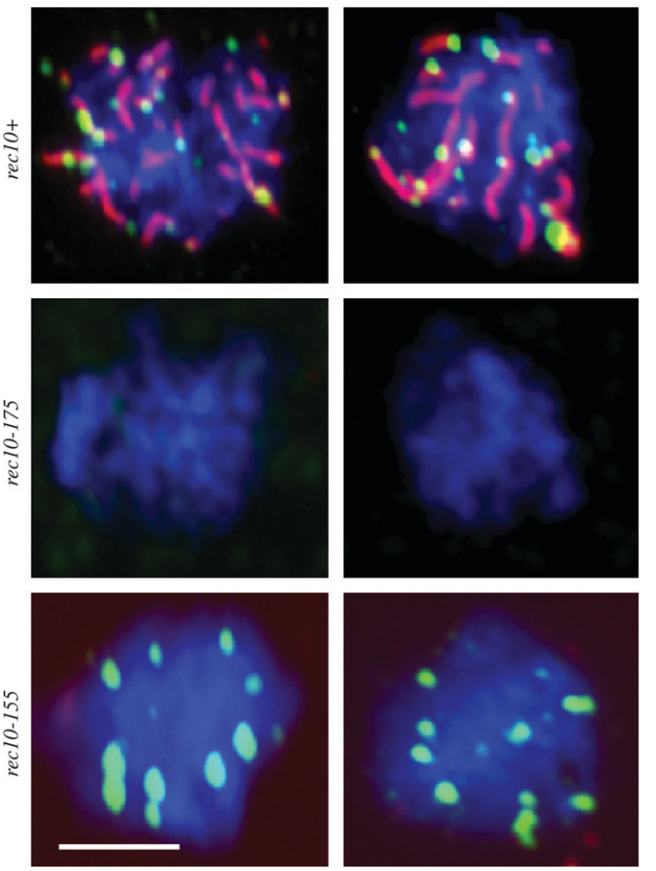

Figure 3.—Rad51 foci are present in meiotic cells devoid of LinEs. Examples of meiotic nuclei from diploid *rec10*$^+$ (LinEs and Rad51 foci), *rec10-175* (no LinEs and no Rad51 foci), and *rec10-155* (no LinEs and Rad51 present) are shown. *rec10-155* mutants do not form any LinEs detectable by electron microscopic analysis of silver-stained nuclear spreads (Molnar *et al.* 2003), so the loss of staining is not likely to be due to epitope loss. Blue is DAPI, Red is Rec10 (LinEs), and green is Rad51. Bar, 5 μm.

TABLE 4

***rad32*Δ reduced spore viability is not rescued by the *rec10-155* mutation**

Genotype	Spore viability (%)[a]	
	rad32$^+$	*rad32*Δ
rec10$^+$	76.5 ± 9.34	0.7 ± 0.3[b]
rec10-155	29.1 ± 9.2	1.0 ± 0.4
rec10-175	37.5 ± 10.4	14.4 ± 3.0

[a] $n \geq 4$ in all cases; values are ± SD.
[b] Similar to values previously reported for the *rad32*Δ mutant (Tavassoli *et al.* 1995).

and the wild type is not possible due to the fact that *rec10-155* diploids traverse meiosis more rapidly (data not shown) and LinEs are missing as specific meiotic landmarks. However, the number of Rad51 foci detected in the *rec10-155* mutant was similar to the number observed in *rec10*$^+$ meioses, albeit with a different temporal profile (data not shown).

Ellermeier and Smith (2005) further demonstrated that recombination-initiating lesions are not generated to any significant level in the *rec10-175* mutant by showing that the *rec10-175* mutation could rescue the low spore production of a *rad32*Δ mutant. Rad32 is required to process recombination-initiating lesions. If such lesions are formed in the absence of Rad32 they cannot be properly processed and there is a low viable spore yield (Tavassoli *et al.* 1995). However, if no initiating lesions form then the viable spore yield is elevated to levels consistent with random segregation of the three *S. pombe* chromosomes, *i.e.*, ~12% (see Ellermeier and Smith 2005 for further details). If recombination-initiating lesions are generated in *rec10-155* cells, it follows that the *rec10-155* mutation should not rescue the poor spore viability of the *rad32*Δ mutant to the same extent as does the *rec10-175* mutation. Table 4 shows that this is indeed the case, indicating that lesions that require Rad32 processing, most likely DSBs, are being formed in the *rec10-155* mutant.

Collectively these data indicate that the Rec10-155 protein is capable of mediating significant levels of recombination in the absence of LinEs.

DISCUSSION

The discovery that Rec10 is a central component of LinEs in *S. pombe*, and shares features in common with *S. cerevisiae* Red1, suggests that LinEs have functional parallels with the SC of other organisms or at least with SC axial precursor structures (Lorenz *et al.* 2004). However, the exact role of these structures remains unclear (Loidl 2006; Wells *et al.* 2006). Whether or not recombination can occur outside the context of LinEs has developed into a central question. Here we provide data that answer this question, but unravel a more complex picture.

Rec10 controls recombination throughout the genome: Previous work using the chemically induced *rec10* mutant, *rec10-109*, demonstrated that recombination in strains carrying this allele was defective only in middle regions of each of the three chromosomes (De Veaux and Smith 1994; Krawchuk *et al.* 1999). Early on during this study we found that loci/intervals that retained *rec10*$^+$ levels of recombination in the *rec10-109* mutant exhibited reductions in both the *rec10-144* and *rec10-155* mutants. In fact, we observed some degree of recombination loss at all loci/intervals tested, implying that Rec10 is needed, to some extent, for the regulation of recombination throughout the genome. This conclusion corroborates that recently made by Ellermeier and Smith (2005), who observed genomewide recombination reductions in a *rec10*Δ null mutant (*rec10-175*). Null mutants of the meiosis-specific cohesion genes, *rec8* and *rec11*, result in region-specific reductions in recombination in the central regions of chromosomes as does *rec10-109* (Parisi *et al.* 1999; Ellermeier and Smith 2005). This suggests that the *rec10-109* mutant is defective in some function of Rec10 that is intimately associated with the meiotic cohesins and, further, would imply Rec10 having more than one functional role (also see below). It is possible that in central chromosomal regions it functions, in part, to modulate meiosis-specific cohesion dynamics. In the more distal regions it might partner with the Rad21–Psc3 cohesin complex, although there is not yet direct experimental evidence to support this.

This and other studies have now found that various *rec10* mutants exhibit different phenotypes, which go some way to demonstrate that Rec10 has more than one function. In the interest of clarity Table 5 lists the mutants that have been characterized in detail to date.

Rec10 has a LinE-independent function in mediating recombination: The *rec10-155* mutant does not form LinEs. This has been determined by immunocytochemistry (Lorenz *et al.* 2004); this is not simply an artifact, due to loss of antibody epitope recognition, as LinEs cannot be detected in this mutant by electron microscopy (Molnar *et al.* 2003). However, we have shown that relative to the *rec10*Δ null mutant (*rec10-175*) there is substantial recombination occurring throughout the genome in the *rec10-155* mutant. Furthermore, a clone carrying the *rec10-155* allele is capable of restoring significant levels of recombination to the *rec10*Δ null mutant. This clearly demonstrates that while Rec10 protein is essential for meiotic recombination, LinEs are not, and at best LinEs only enhance meiotic recombination. Moreover, while the *rec10-155* mutant is defective in recombination, we cannot dismiss the possibility that LinEs have no function in regulating interhomolog meiotic recombination to any degree and that the reduced recombination levels observed in this allele are

TABLE 5

Characteristics of the well-studied mutant alleles of *rec10*

rec10 allele	Mutation	DSB proficient?	Crossover proficiency	Gene conversion proficiency	*ade6–M26* hot spot proficiency	LinE status	Other
rec10-175[a]	Full open reading frame deletion	No	None[b]	None[b]	None[b]	None[c]	—
rec10-109[d]	Two point mutations[a] G526A (V176I) and G533A (G178D)[e]	Levels of DSBs diminished[f]	Regional[g,h]	Regional[g,h]	Partial[g]	Abnormal LinEs formed[c,i]	Complements *rec10-144*[j,k]. Slightly temperature sensitive for recombination at some regions[a]
rec10-144[j,k]	Single point mutation[j] G2180A (G727E)[e]	Not determined	Limited reduction at all intervals tested[l]	Limited reduction at all intervals tested[j,l]	Reduced hot spot activity[j]	Limited abnormal LinEs formed[j,m]	Complements *rec10-109*[j,k]
rec10-155[n]	Insertion inactivation mutant; presumed C-terminal truncation of last 103 amino acids[n]	Not determined	Limited reduction at all intervals tested[l]	Limited reduction at all intervals tested[l,n]	Reduced hot spot activity[o]	None[i,m,p]	—

Other *rec10* mutant alleles have been isolated/generated and sequenced (De Veaux *et al.* 1992; Ellermeier and Smith 2005; our unpublished data), but they have undergone only limited analysis to date and are not listed here.

[a] Ellermeier and Smith (2005).
[b] None equates to the levels observed for a *rec12* null mutant.
[c] As measured by immunocytochemistry.
[d] Ponticelli and Smith (1989).
[e] Numbered from the start of the open reading frame.
[f] Cervantes *et al.* (2000).
[g] De Veaux and Smith (1994).
[h] Krawchuk *et al.* (1999).
[i] Lorenz *et al.* (2004).
[j] Pryce *et al.* (2005).
[k] De Veaux *et al.* (1992).
[l] This study.
[m] As determined by immunocytochemistry and electron microscopy.
[n] Lin and Smith (1995).
[o] D. W. Pryce and R. J. Mc Farlane (unpublished observation).
[p] Molnar *et al.* (2003).

caused by other functions of Rec10 being impaired due to the truncation of the Rec10 protein and not due to the loss of LinEs *per se*.

The *rec10-155* allele encodes a truncated Rec10 protein that has lost the C-terminal 103-amino-acids (aa) domain (Lin and Smith 1995) that contains homology to the helical region of Red1 (Hollingsworth and Ponte 1997; Lorenz *et al.* 2004). This domain is proposed to be required for Red1 homo-oligomerization (Woltering *et al.* 2000). Its loss in *S. pombe* might explain the failure of this mutant to develop LinEs (Molnar *et al.* 2003; Lorenz *et al.* 2004). Limited *in silico* analysis of the truncated Rec10-155 protein did not identify any obvious features that may provide clues to the function of Rec10 essential for meiotic recombination.

It might be argued that the truncated Rec10-155 protein goes undetected by immunostaining with the antibody that is directed to aa 670–684 of the 791-aa wild-type Rec10 (Lorenz *et al.* 2004). However, *rec10-155* mutants do not form any LinEs detectable by electron microscopic analysis of silver-stained nuclear spreads either (Molnar *et al.* 2003), so the loss of staining is not likely to be due to epitope loss. On the other hand, we cannot dismiss the possibility that tiny Rec10 dots go undetected both by electron microscopy (due to background silver grains) and by immunostaining (due to a weakened affinity).

A disparity between gene conversions and crossovers in the *rec10-155* mutant: An unexpected feature of recombination in the *rec10-155* mutant is the greater reduction in gene conversion relative to the reduction in crossovers at some intervals; for example, at the *ade6* locus gene conversions are reduced 247-fold in the *rec10-155* mutant (Table 1), while crossovers in the associated *ade6–tps16* interval are reduced only ~6-fold (Figure 2). At the present time, there is no clear explanation for this observation. If conversions at *ade6* occurred without high levels of associated crossing over, this observation could be accounted for; *i.e.*, *rec10-155* is defective in the conversions at *ade6* that have little associated crossing over. However, Cromie *et al.* (2005) recently demonstrated that there are high levels of crossovers associated with conversions at *ade6*, dismissing the proposal that there is a greater reduction in conversions at *ade6* than crossovers in the *rec10-155* mutant because there is limited crossing over associated with *ade6* gene conversions. A similar, but less dramatic, observation has also been made for mutants defective in the meiosis-specific cyclin Rem1, where a reduction in gene conversions is observed (~4-fold) at *ade6*, but no reduction in crossing over in the adjacent *ade6–arg1* interval (Malapeira *et al.* 2005).

Another explanation for this observation might be that there is increased marker coconversion in the *rec10-155* mutant in two-factor crosses at some loci (for example, *ade6*), resulting in a reduction in measurable gene conversions. However, there is no other evidence to support mechanistic models based on elevated marker coconversion, such as extended gene conversion tracts in the *rec10-155* mutant resulting in elevated coconversions.

LinEs and crossing over: It has been proposed that axial proteins, such as Red1 in *S. cerevisiae*, function to introduce a compaction stress into the chromosomal structure that facilitates crossing over and might play a role in genetic interference (Blat *et al.* 2002; Kleckner *et al.* 2004), the latter not being apparent in *S. pombe* (Munz 1994). Our data are not inconsistent with LinEs generating a recombinogenic compaction stress that may enhance crossing over to above the level observed in the *rec10-155* mutant.

In conclusion, we demonstrate that Rec10 has more than one distinct function. Rec10 is required for DSB formation (Cervantes *et al.* 2000; Ellermeier and Smith 2005), but we show that with the loss of the C-terminal domain of Rec10 substantial levels of recombination occur despite the fact that LinEs do not form. Some function of Rec10 is differentially required for regulating gene conversions with some loci being more dependent upon this function than others; whether this function is linked to LinE function or not remains unresolved. Finally, we can conclude that LinEs are not absolutely essential for all programmed meiotic recombination. LinEs have been implicated in other processes, such as interhomolog pairing (Bähler *et al.* 1993), but loss of pairing might be an indirect effect of mutating *rec10*. Furthermore, LinEs hold Hop1 in place, which in turn may play a role in recombination partner choice (Loidl 2006). It remains to be determined whether or not LinEs are dispensable for all programmed meiotic recombination.

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