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Report

# Rec25 and Rec27, Novel Linear-Element Components, Link Cohesin to Meiotic DNA Breakage and Recombination

**Luther Davis,[1,3] Ana E. Rozalén,[2] Sergio Moreno,[2] Gerald R. Smith,[1,*] and Cristina Martín-Castellanos[2,*]**
[1]Division of Basic Sciences
Fred Hutchinson Cancer Research Center
Seattle, Washington 98109
[2]Instituto de Biología Molecular y Celular del Cáncer
CSIC/Universidad de Salamanca
Campus Miguel de Unamuno
37007 Salamanca
Spain

## Summary

**Meiosis is a specialized nuclear division by which sexually reproducing diploid organisms generate haploid gametes. Recombination between homologous chromosomes facilitates accurate meiotic chromosome segregation [1] and is initiated by DNA double-strand breaks (DSBs) made by the conserved topoisomerase-like protein Spo11 (Rec12 in fission yeast) [2–4], but DSBs are not evenly distributed across the genome [5]. In *Schizosaccharomyces pombe*, proteinaceous structures known as linear elements (LinEs) are formed during meiotic prophase [6]. The meiosis-specific cohesin subunits Rec8 and Rec11 are essential for DSB formation in some regions of the genome [7], as well as for formation of LinEs or the related synaptonemal complex (SC) in other eukaryotes [8–12]. Proteins required for DSB formation decorate LinEs [13], and mutants lacking Rec10, a major component of LinEs, are completely defective for recombination [7, 11]. Although recombination may occur in the context of LinEs, it is not well understood how Rec10 is loaded onto chromosomes. We describe two novel components of LinEs in fission yeast, Rec25 and Rec27. Comparisons of *rec25Δ*, *rec27Δ*, and *rec10Δ* mutants suggest multiple pathways to load Rec10. In the major pathway, Rec10 is loaded, together with Rec25 and Rec27, in a Rec8-dependent manner with subsequent region-specific effects on recombination.**

## Results and Discussion

### Rec25 Promotes Recombination in a Region-Specific Manner and Acts in the Same Pathway as Rec8

Rec25 and Rec27 are small proteins (17 and 16 kDa, respectively) that are important, but not absolutely essential, for meiotic recombination [14]. Deletions of *rec25* and *rec27* have similar phenotypes – aberrant asci with abnormal spore number and morphology – likely resulting from reduced meiotic recombination and chromosome missegregation. Genetic analysis of single and double mutants showed that Rec25 and Rec27 act at the same or closely related steps of meiotic recombination (see the Supplemental Results and Table S1 available online). Although meiotic DSBs are not detectable in the chromosomal regions tested in *rec25Δ* and *rec27Δ* mutants [14], recombination is not reduced to the same level as in other *rec* mutants (e.g., *rec12* [3]). This partial recombination-deficient phenotype is reminiscent of *rec8* and *rec11* deletions and several non-null alleles of *rec10* in which recombination is reduced in a region-specific manner [7, 15, 16].

To determine whether Rec25 also promotes recombination in a region-specific manner, we first measured intragenic recombination at *ura1* on chromosome I (ChrI), which is reduced only modestly by *rec8Δ*, and at *ade6* on chromosome III (ChrIII), where *rec8Δ* strongly impairs recombination [7]. Recombination at *ura1* was reduced by a factor of only 1.6 in a *rec25Δ* mutant ($p < 0.005$ by t test; Table 1), whereas *ade6* recombination was reduced by factors between 10 and 140, depending on the alleles crossed (Table 1 and Tables S1–S3). We next measured intergenic recombination at several intervals throughout the genome. Recombination in the *rec25Δ* mutant was reduced by factors ranging from 2.5 in the *ura1–mes1* interval on ChrI to 30 in the *ade6–arg1* interval on ChrIII (Table 2). These data indicate that Rec25, like Rec8, promotes recombination in a region-specific manner.

Next, we measured intergenic recombination in a *rec8Δ* single mutant and in a *rec8Δ rec25Δ* double mutant. We chose the *ura1–arg3* and *arg3–lys7* intervals on ChrI because we expected these intervals to have enough residual recombination in a *rec8Δ* mutant that a further reduction by *rec25Δ* would be easily detectable [7]. Recombination in the *ura1–arg3* interval was reduced by factors of 3.1 in *rec25Δ*, 7.9 in *rec8Δ*, and 6.4 in the double mutant. Similarly, in the *arg3–lys7* interval, recombination was reduced by factors of 4.6 in *rec25Δ*, 21 in *rec8Δ*, and 15 in the double mutant (Table 2). In both intervals, there was no significant difference between the recombination measured in the *rec8Δ* mutant and *rec8Δ rec25Δ* double mutant (*ura1–arg3*, $p > 0.2$; *arg3–lys7*, $p > 0.1$). These data indicate that Rec25 and Rec8 act in the same pathway, consistent with their similar regional specificity.

### Rec25 and Rec27 Are Required for Homologous Chromosome Pairing

Rec8 is required for linear element (LinE) formation [8, 11] and mutants defective in LinEs are impaired in chromosome pairing [17], although a direct connection between LinEs and chromosome pairing has not been established [18]. We therefore assessed chromosome pairing in *rec25Δ* and *rec27Δ* mutants. Pairing was addressed at the *ade3* locus in *mei4Δ* strains to arrest cells at the end of prophase [19]. In *mei4Δ* control cells, chromosomes were paired at *ade3* in ~25% of prophase nuclei, in the range measured at several other positions, as reported by others [17], whereas in *mei4Δ rec25Δ* and *mei4Δ rec27Δ* mutants the percentage dropped to ~15%, the same level of pairing observed in *mei4Δ rec10Δ* mutant cells (Figure 1). Therefore, chromosome pairing was reduced by the same extent (~40%) in *rec25Δ*, *rec27Δ*, and *rec10Δ* mutants. These results indicate that Rec25 and Rec27 are required for efficient homologous chromosome pairing, as previously suggested for Rec10 by Molnar et al. [17].

There were no significant differences between the pairing observed in control cells and *rec10Δ*, *rec25Δ*, or *rec27Δ*

*Correspondence: gsmith@fhcrc.org (G.R.S.), cmartin@usal.es (C.M.-C.)
[3]Present address: Department of Immunology, University of Washington, Seattle, Washington 98195

Table 1. Region-Specific Activation of Intragenic Recombination by Rec25 and Rec8

| Relevant Genotype | Intragenic Recombination (Ade$^+$ or Ura$^+$ per $10^4$ Viable Spores) | |
|---|---|---|
| | *ade6-M26* × *ade6-52*, ChrIII | *ura1-61* × *ura1-171*, ChrI |
| *rec*$^+$ | 27 ± 2 | 0.62 ± 0.04 |
| *rec25Δ* | 0.20 ± 0.02 (135) | 0.38 ± 0.03 (1.6) |
| *rec8Δ*[a] | (760) | (5.9) |

Recombinant frequencies are the mean ± SEM for at least six experiments. The numbers in parentheses are n-fold reduction relative to wild type.
[a] From [7].

mutants at another chromosomal position, *cut3* in the left arm of ChrII (our unpublished data). This result may reflect different chromosomal regions using diverse strategies for homologous pairing [18, 20].

## Rec25 and Rec27 Are Required for Localization of Rec10 into LinEs

Next, we explored LinE formation in *rec25Δ* and *rec27Δ* mutants. For this purpose we used Rec10 as a marker [11]. LinE formation was analyzed by Rec10 immunostaining of chromosome spreads prepared at different times during prophase in *pat1-114* synchronous meiosis. LinE formation was strongly impaired in *rec8Δ* mutants 3.5 hr after meiotic induction – only a few nuclei contained LinEs (Rec10 structures), and these were abnormally short – whereas normal LinEs were formed in *rec12Δ* mutants (Figure 2), as previously reported [11]. No signal was detected for Rec10 in *rec25Δ* and *rec27Δ* mutant nuclei (Figure 2). These results indicate that Rec25 and Rec27 are required for Rec10 localization and likely for LinE formation. Rec10 is a major component of LinEs [11], and in *rec10* mutants no LinEs are detectable by light or electron microscopy [11, 17]. We, therefore, infer that there are no LinEs in *rec25Δ* or *rec27Δ* mutants, although this inference remains to be tested by electron microscopy, the method by which LinEs were defined [21].

## Rec25 and Rec27 Colocalize with Rec10 during LinE Formation

Possible explanations for the apparent loss of LinEs in the mutants are that Rec25 and Rec27, like Rec10, are LinE components; that they help to load Rec10 onto the LinEs; or both. To address these possibilities, we tagged Rec25 and Rec27 with GFP and determined their location during meiosis. Rec25-GFP and Rec27-GFP retain the majority of their function, as shown by the production of normal asci and high levels of recombination (our unpublished data and Table S4), in stark contrast to *rec25Δ* and *rec27Δ* mutants (Tables 1 and 2 and Tables S1–S3; [14]).

*rec25* and *rec27* transcripts are present only in early meiosis [22]. As expected, Rec25-GFP and Rec27-GFP proteins were observed from 1.5–2 hr to 4 hr after meiotic induction in live *pat1-114* cells (our unpublished data), a period that includes DNA replication and recombination [3, 14, 23]. Each protein first displayed a diffuse, transient nuclear signal that evolved into a dotted pattern (our unpublished data; see also Figures S3 and S4). A similar dotted pattern was also observed for Rec10-GFP early in meiosis (our unpublished data). Chromosome spreads taken during prophase and stained with anti-GFP antibodies showed that the Rec25 and Rec27 proteins indeed bind to chromosomes with a pattern similar to that of Rec10 (LinEs); that is, a small number of dots appeared early in prophase and developed into linear structures as prophase progressed (Figure 3 and Figure S1).

Next, we addressed whether Rec25-GFP and Rec27-GFP proteins colocalized with Rec10. Double staining with anti-GFP and anti-Rec10 antibodies of chromosome spreads showed that both Rec25-GFP and Rec10, and Rec27-GFP and Rec10, colocalized (Figure 3 and Figure S1). The colocalization was nearly complete from the beginning to the end of prophase, with nuclear signals positive for both Rec10 and GFP or for neither. The proportion of nuclei with signals increased with time, from a few nuclei with a dotted signal at the beginning of prophase to more nuclei with linear structures later in prophase. The structures observed in our synchronous meiosis appear less developed than the structures reported in *pat1*$^+$ meiosis [11, 21]. In summary these experiments showed that Rec25-GFP and Rec27-GFP always colocalize with Rec10 during LinE formation and, therefore, that Rec25 and Rec27 are components of LinEs. Indeed, genetic analysis showed that Rec10 and Rec25 act together to promote the majority of recombination (Supplemental Results and Table S3).

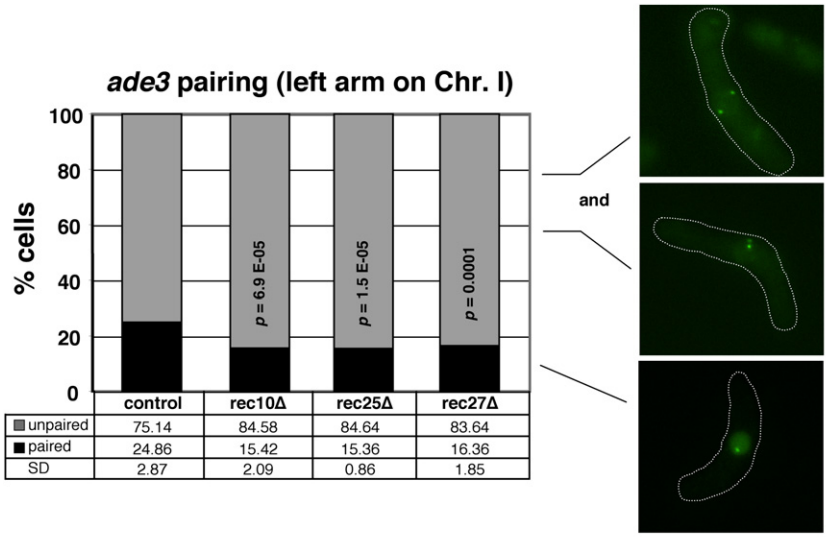
## Normal Loading of a Putative Rec10-Rec25-Rec27 Complex onto Chromosomes Requires Rec8

Rec10, Rec25, and Rec27 colocalize in LinEs, suggesting they may act as a complex. If so their localization might be interdependent. To test this possibility, we determined the genetic requirements for the loading of these proteins onto chromosomes. As noted above (Figure 2), Rec10 was not loaded into LinEs in *rec25Δ* or *rec27Δ* mutants. The loading of Rec25-GFP was assessed in *rec10Δ* and *rec27Δ* mutants analyzing chromosome spreads from synchronous meioses. Rec25-GFP signal was not observed on chromosomes in *rec10Δ* or *rec27Δ* mutants (Figure 4A). Similar results were

Table 2. Rec25 Promotes Intergenic Recombination in a Region-Specific Manner and Acts in the Same Pathway as Rec8

| Relevant Genotype | Intergenic Recombination (cM) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *lys3–ura1*, ChrI | *ura1–met5*, ChrI | *ura1–mes1*, ChrI | *mes1–arg3*, ChrI | *ura1–arg3*, ChrI | *arg3–lys7*, ChrI | *leu1–his5*[a], ChrII | *ade6–arg1*, ChrIII |
| *rec*$^+$ | 20 | 43 | 91 | 117 | 245 | 355 | 39 | 92 |
| *rec25Δ* | 1 (20) | 8 (5.4) | 37 (2.5) | 41 (2.8) | 79 (3.1) | 78 (4.6) | 3 (13) | 3 (30) |
| *rec8Δ* | 2.5 (10)[b] | ND | ND | ND | 31 (7.9) | 17 (21) | ND | 0.8 (90)[b] |
| *rec8Δ rec25Δ* | ND | ND | ND | ND | 38 (6.4) | 23 (15) | ND | ND |

Two or more independent crosses were performed for each interval. Each genetic distance is based on the cumulative number of spore colonies, with more than 300 colonies in each case. Recombinant frequencies were converted to genetic distance with Haldane's formula, cM = $-50\,ln\,(1 - 2R)$, where R is the recombinant frequency. The numbers in parentheses are n-fold reduction relative to wild type.
[a] From [14].
[b] From [7].




| | control | rec10Δ | rec25Δ | rec27Δ |
|---|---|---|---|---|
| unpaired | 75.14 | 84.58 | 84.64 | 83.64 |
| paired | 24.86 | 15.42 | 15.36 | 16.36 |
| SD | 2.87 | 2.09 | 0.86 | 1.85 |

Figure 1. Rec25 and Rec27 Are Required for Efficient Homologous Chromosome Pairing

$h^{90}$ *ade3*$^+$*::lacO his7*$^+$*::GFP-LacI-NLS mei4Δ* cells with the indicated *rec* deletions (strains S1899, S1901, S1903, and S1904) were grown on sporulation medium (MEA) at 25°C for more than 29 hr to ensure that cells were blocked in meiotic prophase and examined under a fluorescence microscope. The percentage of cells with unpaired chromosomes (gray bar) and paired chromosomes (black bar) is shown. Previously described criteria for pairing were used [17]. Homologous chromosomes were scored as paired when their GFP signals touched each other or only a single signal was visible, or as unpaired when two independent GFP signals were observed even if they were in close proximity. The data are the mean of six independent experiments in which 200–300 asci were counted for each genotype in each experiment. Standard deviation (SD) and p values based on Student's t test are shown.

found with Rec27-GFP; signal was not detected on chromosomes in *rec10Δ* or *rec25Δ* mutants (Figure S2). These results show that loading of these three components of LinEs onto chromosomes is mutually interdependent and suggest that they are not loaded in a stepwise manner to form LinEs. Among the three LinE components only Rec10 has a putative nuclear localization signal (NLS), so the interdependency of the loadings could be explained if the complex is formed in the cytoplasm before it reaches the nucleus. The NLS is maintained in the C terminally truncated Rec10-155 protein (Supplemental Data), which does not form LinEs although it promotes a low but significant level of recombination independent of Rec25 (Table S3) [16]. Thus, this mutant protein likely still reaches the nucleus and the missing part of the protein may be required for formation of a complex with Rec25 and Rec27. In agreement with this hypothesis, Rec10 and Rec25 strongly interact in two-hybrid assays in budding yeast cells (M. Spirek and J. Loidl, personal communication).

In *rec8* and *rec11* mutants, which lack meiosis-specific cohesin subunits [24–26], only a few aberrant LinEs containing Rec10 are formed [8, 11, 17] (see above), indicating that the formation of LinEs depends on meiotic cohesin. We, therefore, determined whether the loading of Rec25-GFP and Rec27-GFP also was dependent on meiotic cohesin and whether the rudimentary structures formed in *rec8Δ* mutants also contained Rec25 and Rec27 proteins. As with Rec10, fewer and shorter structures containing Rec25-GFP or Rec27-GFP were formed in *rec8Δ* cells compared to *rec8*$^+$ cells (Figure 4A and Figure S2). In addition, double staining with anti-Rec10 and anti-GFP antibodies showed that the rudimentary structures observed in *rec8Δ* cells contained both Rec10 and Rec25-GFP (or Rec27-GFP) (our unpublished data). These data indicate that Rec10, Rec25, and Rec27 are loaded onto chromosomes in a Rec8-dependent manner. Because Rec8 is required for normal chromosome compaction during prophase [27], we cannot exclude the possibility that the loading of the LinE components onto chromosomes requires proper chromatin organization more than a direct interaction with Rec8.

The loading of the putative Rec10-Rec25-Rec27 complex also was studied in live cells, instead of nuclear spreads, and similar results were obtained (Supplemental Results and Figures S3 and S4) with the exception that in *rec8Δ* meiosis Rec25-GFP appeared in all nuclei but as short, linear structures instead of dots (Figure S3; see Supplemental Data for discussion). Rec25-GFP and Rec27-GFP proteins were present in all the mutant backgrounds as shown by Western blot (Figure S5), excluding the possibility that the proteins were not expressed.

Our data predict that Rec10, Rec25, and Rec27 are loaded after Rec8 and that the loading of Rec8 onto chromosomes would not be affected by their absence. Therefore, we examined Rec8 loading by using Rec8-GFP in mutants lacking components of the putative complex. Rec8-GFP showed a punctate pattern throughout the entire prophase nucleus 3.5 hr after meiotic induction, as previously described [24]

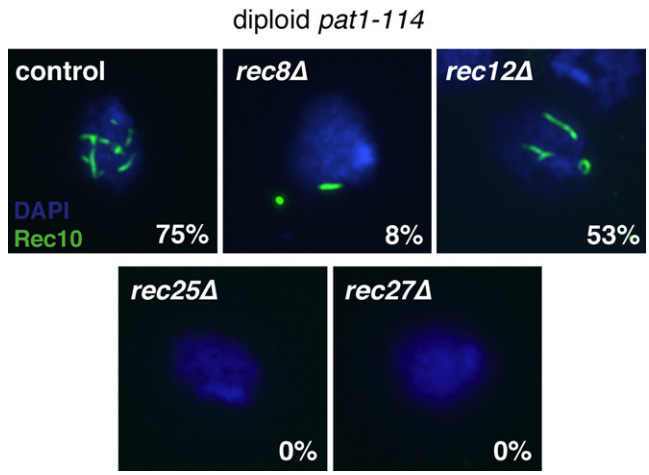



Figure 2. Rec10 Structures Are Not Formed in *rec25Δ* and *rec27Δ* Mutants

Diploid *pat1-114* cells with the indicated deletions (strains S964, S1628, S1624, S1554, and S1572) were induced for meiosis, and cells in prophase were collected for preparation of nuclear spreads. The spreads were stained with DAPI (DNA; blue) and anti-Rec10 antibodies (green), and photographed under a fluorescence microscope. Results at 3.5 hr after meiotic induction are shown. The fraction of nuclei with Rec10 structures (LinEs) is indicated. 100 nuclei were counted in a well-stained area of the preparation, except for *rec25Δ* and *rec27Δ*, in which the entire preparation (more than $5 \times 10^3$ nuclei examined) was screened. Similar results were obtained with 3 hr chromosome spreads and in an independent experiment (our unpublished data).

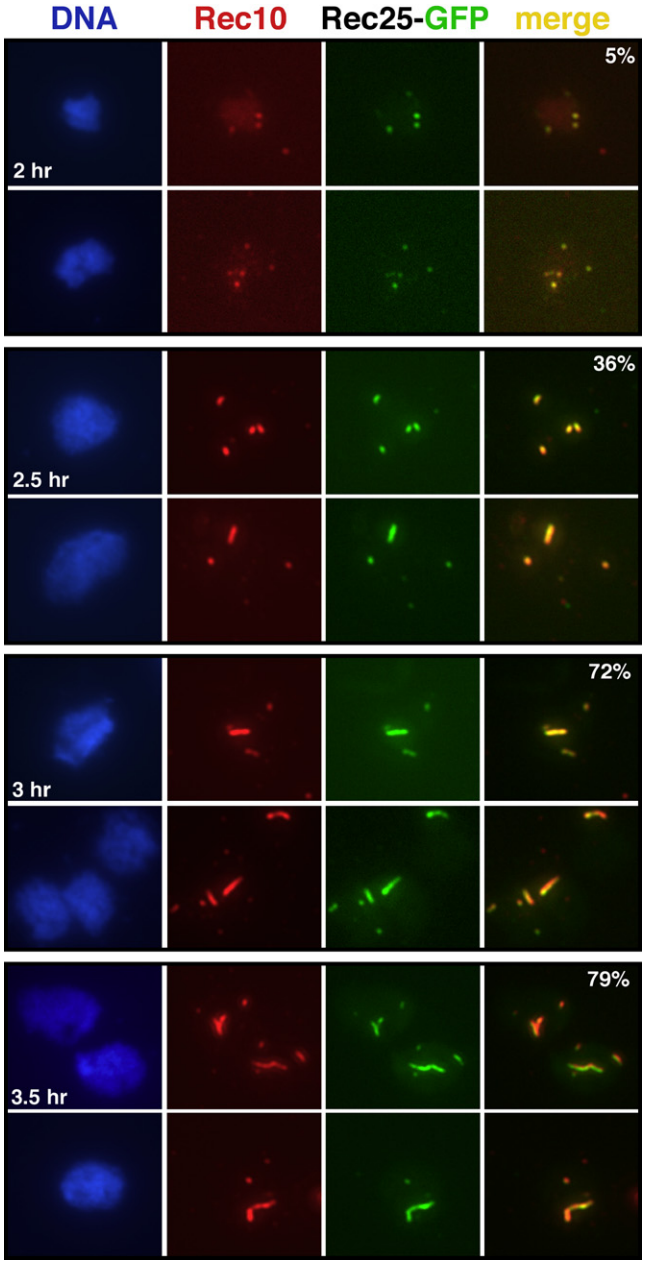



Figure 3. Rec25-GFP and Rec10 Colocalize during LinE Formation

Chromosome spreads of diploid *pat1-114 rec25-GFP* (strain S1702) cells were prepared at the indicated times after meiotic induction, stained with DAPI (DNA; blue), anti-GFP (Rec25; green), and anti-Rec10 (red) antibodies and photographed under a fluorescence microscope. The fraction of nuclei with structures (LinEs or precursors) is indicated. 200 nuclei, 100 in each of two different well-stained areas of the same preparation, were counted. Nuclear signals were positive for both GFP and Rec10 or for neither. Staining was done twice with duplicate spreads of the same meiotic induction.

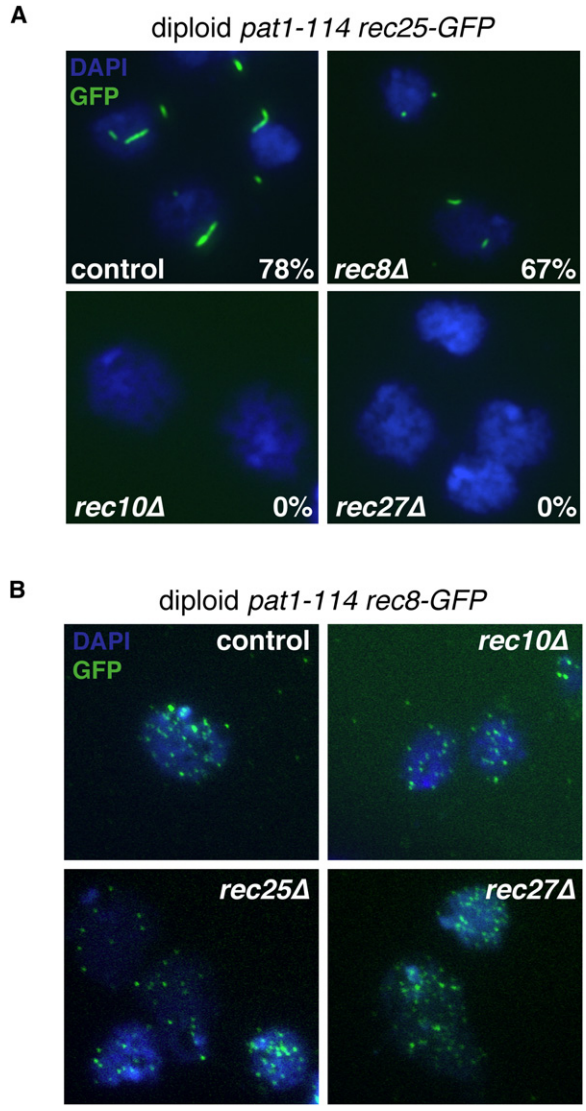



Figure 4. Loading of LinE Components and Cohesins

(A) Rec25-GFP is defectively loaded in *rec8Δ* and not loaded in *rec10Δ* and *rec27Δ* mutants. Chromosome spreads, prepared from strains S1702, S1812, S1816, and S1814 as in Figure 2, were stained with DAPI (DNA; blue) and anti-GFP antibodies (Rec25; green) and photographed under a fluorescence microscope. The fraction of nuclei with Rec25 structures is indicated. Results at 3.5 hr after meiotic induction are shown. 200 nuclei, 100 in each of two different well-stained areas of the same preparation, were counted, except for *rec10Δ* and *rec27Δ*, in which the entire preparation (more than $5 \times 10^3$ nuclei examined) was screened. Similar results were obtained with spreads at 3 hr after meiotic induction (our unpublished data).

(B) Rec8-GFP is normally loaded in *rec10Δ*, *rec25Δ*, and *rec27Δ* mutants. Chromosome spreads, prepared from strains S1855, S1809, S1815, and S1854 as in Figure 2, were stained with DAPI (DNA; blue) and anti-GFP antibodies (Rec8; green), and photographed under a fluorescence microscope. Results at 3.5 hr after meiotic induction are shown. The fluorescent signal was too weak to quantify, but in every genotype positive Rec8-GFP nuclei were found.

(Figure 4B). Supporting our hypothesis, in the three mutant backgrounds (*rec10Δ*, *rec25Δ*, and *rec27Δ*) we observed loading of Rec8-GFP onto chromosomes similar to the loading in the control strain (*rec10*$^+$ *rec25*$^+$ *rec27*$^+$).

## Conclusions

Our data expand on the current model for *S. pombe* meiotic recombination [7]. Although Rec10 is absolutely essential for DSB formation and recombination throughout the genome, Rec8, Rec25, and Rec27 are not. Therefore, a high level of recombination requires all four proteins, but a low level of recombination occurs independently of meiotic cohesins and LinE components. This conclusion agrees with the recent observations that in *rec10-155* mutants no LinEs are observed,

but still there are readily detectable recombination and Rad51 foci (this report and [16]), and that some Rad51 foci are located outside of LinEs in wild-type meiosis [13]. The different modes of Rec10 action may reflect different ways of loading Rec10 onto chromosomes. In addition the new *rec25* and *rec27* mutants described here may provide us with a tool to address the specific role of LinEs, rather than Rec10 per se, in the biology of the meiotic chromosomes. Furthermore, our genetic analysis—by using complete deletions and double mutants—suggests that LinEs are required for most recombination in the same chromosomal regions where Rec8 and Rec11 function to promote recombination. The region-specific effect of mutations altering LinEs on DSB formation and recombination appears to reflect a region-specific function of Rec8 and Rec11, whose molecular basis remains to be elucidated. The recent report of the distribution of meiotic DSBs in *S. pombe* provides us with the framework to address this question [28]. The position of crossovers along chromosomes differentially influences homolog segregation. In yeast, flies, and humans, chromosomes with distally located exchanges appear more likely to nondisjoin than those with more proximally positioned exchanges [29–32]. The regional effect on recombination investigated here may be related to this general observation, and meiotic cohesins and LinEs may be required to promote these "properly" placed crossovers.

### Supplemental Data

Additional results, Experimental Procedures, five figures, and five tables are available at http://www.current-biology.com/cgi/content/full/18/11/849/DC1/.

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