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Genetics

Synaptonemal complex (SC) component Zip1 plays a role in meiotic recombination independent of SC polymerization along the chromosomes

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ABSTRACT **Zip1 is a yeast synaptonemal complex (SC) central region component and is required for normal meiotic recombination and crossover interference. Physical analysis of meiotic recombination in a *zip1* mutant reveals the following: Crossovers appear later than normal and at a reduced level. Noncrossover recombinants, in contrast, seem to appear in two phases: (*i*) a normal number appear with normal timing and (*ii*) then additional products appear late, at the same time as crossovers. Also, Holliday junctions are present at unusually late times, presumably as precursors to late-appearing products. Red1 is an axial structure component required for formation of cytologically discernible axial elements and SC and maximal levels of recombination. In a *red1* mutant, crossovers and noncrossovers occur at coordinately reduced levels but with normal timing. If Zip1 affected recombination exclusively via SC polymerization, a *zip1* mutation should confer no recombination defect in a *red1* strain background. But a *red1 zip1* double mutant exhibits the sum of the two single mutant phenotypes, including the specific deficit of crossovers seen in a *zip1* strain. We infer that Zip1 plays at least one role in recombination that does not involve SC polymerization along the chromosomes. Perhaps some Zip1 molecules act first in or around the sites of recombinational interactions to influence the recombination process and thence nucleate SC formation. We propose that a Zip1-dependent, pre-SC transition early in the recombination reaction is an essential component of meiotic crossover control. A molecular basis for crossover/noncrossover differentiation is also suggested.**

In meiosis, crossovers ensure the disjunction of homologs at the first division. The number and distribution of crossovers are tightly controlled (1–6). One manifestation of control is crossover interference: the presence of a crossover at one position along a chromosome reduces the probability that a crossover will also be found nearby. Crossover interference may act upon an array of undifferentiated recombinational interactions causing certain ones to mature into crossovers and others to mature into noncrossovers (e.g., refs. 3 and 4).

In yeast, meiotic recombination initiates via meiosis-specific double strand breaks (DSBs) (7, 8), which occur prior to bulk polymerization of the synaptonemal complex between the structural axes of paired homologs (9). Resected DSBs then invade an intact duplex to form double Holliday junctions; invasion and ensuing steps are approximately concomitant with initiation and progression of SC polymerization, respectively (10). Double Holliday junctions persist throughout much of the period when SC is full-length ("pachytene"). Mature crossover and noncrossover products form an hour or so after Holliday junctions appear, at about the time that SC disappears (9), but not dependent upon SC disassembly (11, 12). Crossover and noncrossover products likely both arise via double Holliday junctions (13), though noncrossovers could arise in another way.

Experimental data suggest that crossover interference may be imposed early in the recombination reaction. Crossover/noncrossover differentiation apparently occurs no later than the time at which SC is forming (for review, see ref. 7): pachytene recombination nodules often exhibit an "interference distribution" by zygotene. Also, several organisms exhibit a 1:1 correspondence between SC initiation sites and crossovers, which implies that interference is not imposed after SC is formed. If the same is true in yeast, the timing of DNA events relative to cytological events is such that crossover interference would be imposed no later than the time at which double Holliday junctions are forming and long before the time of Holliday junction resolution (7, 10).

The current experiments were provoked by a model that explains diverse aspects of crossover distribution including interference (ref. 7; N.K., J. W. Hutchinson, and G. H. Jones, unpublished work; outlined here as introductory context). In this model, all undifferentiated recombinational interactions are placed under "stress"; in addition, each interaction has an intrinsic "sensitivity to stress." The combination of (stress × sensitivity) "activates" all recombination complexes, though to different extents at different individual complexes. Eventually, along a given bivalent, the recombination complex with the highest activation level "goes critical" and is thereby "committed" to (ultimately) becoming a crossover. Moreover, as a consequence of such an event, stress is rapidly relieved in the immediate vicinity of the committed interaction, thus disfavoring occurrence of additional crossovers nearby. The severity of crossover interference decreases with distance. Thus, stress should be reduced to zero at the point of the committed interaction and then return progressively to the starting level with increasing distance away. Additional recombination complexes may subsequently go critical; if so, the numbers and positions of such events are influenced by previous crossover commitment events on the same bivalent. Most recombinational interactions do not "go critical," either because they undergo relief of stress and/or because they never achieve a high enough activation level to begin with; these interactions all become noncrossovers as the unique default.

A specific mechanism for imposition and relief of stress is also proposed: At the time that stress is imposed, each pair of sister chromatids is organized into a linear array of loops connected at their bases by a continuous structural axis. The two axes of each homolog pair are coaligned via multiple interstitial pairing interactions. Undifferentiated recombinational interactions are present at these interstitial positions, lying between the two axes

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Abbreviations: SC, synaptonemal complex; DSBs, double strand breaks.

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as closely held extensions of axis-associated chromatin fibers (7). Tension is imposed along each homolog by differential compaction of the axis-associated chromatin against resistance imposed by the underlying axial structure. Since the axes are (by hypothesis) elastic, this tension is felt as stress by the individual recombinational interactions, i.e., as "pulling" via the DNA/chromatin "arms" that connect them to the axis. Relief of stress occurs by dissociation of chromatin fibers from the structural axes at the point of the committed interaction; stress is zero at that point, but, owing to axis elasticity, relief of stress is also transmitted outward from that point, in both directions, decreasing in magnitude with distance.

An intriguing feature of this specific mechanism is that crossover control does not involve the SC. Here, the two homolog axes communicate exclusively via their shared recombinational interactions. If this feature is correct, crossover control could well be imposed before SC formation. This would in turn imply that crossover control is imposed at or before the DSB stage or during the early stages of double Holliday junction formation.

Crossover control, i.e., differentiation of recombination intermediates into crossovers or noncrossovers, is conceptually distinct from maturation of recombinational interactions. But, given the timing of events in yeast, the proposed mechanism also implies that crossover control is likely imposed long before the final maturation of recombination intermediates into crossover and noncrossover products. This model therefore implies that the process responsible for differentiating recombinational interactions into crossovers and noncrossovers is also responsible for ensuring that those interactions ultimately mature into the appropriate designated products.

In fact, if crossover interference is indeed imposed relatively early in the recombination reaction (above) there would be a need for specific features that "enforce" the interference decision after its made irrespective of any particular model. More specifically, crossover/noncrossover differentiation should ultimately yield two types of strand exchange intermediates which only eventually yield the two types of recombinant products. [Presumptively, two types of double Holliday junctions form which differ in chromosomal or protein/DNA context (e.g., ref. 14) or, perhaps less likely, detailed chemical structure (13).] Postinterference "enforcement features" have been considered previously (e.g., refs. 15–17).

These considerations make three predictions regarding the effects of a mutation that eliminates crossover control:

(*i*) If crossovers must undergo a critical early transition while noncrossovers need not, then a defect in the crossover control process might affect formation of crossovers differentially as compared with formation of noncrossovers.

(*ii*) If imposition of interference and maturation of crossover-type recombinants require a common early event, and if this event is independent of (e.g., precedes) SC formation, then a mutant defective in the relevant process should be defective in crossover formation even in a situation where SC polymerization along the chromosomes cannot occur.

(*iii*) If crossover control is imposed early during recombination and also is important for recombinant maturation, then a defect in crossover control should result in a defect in the formation and/or resolution of at least some double Holliday junctions.

We have also considered the formal notion that the recombination reaction might have an intrinsic binary tendency to occur in two ways which correspond in some way to the "crossover" and "noncrossover" modes and that the crossover control process simply places the choice between these two modes under regulatory control. A defect in crossover control might then cause recombinational interactions to fall randomly into the two modes. If resultant "noncrossover-type" intermediates mature appropriately into noncrossovers, while resultant "crossover-type" intermediates now mature randomly into both crossovers and noncrossovers, a two-fold deficit in the number crossovers would result. Such a deficit is observed by genetic analysis of recombination in several yeast recombination mutants (8, 18–21).

To investigate these ideas, we have analyzed meiotic recombination in a mutant defective in crossover interference (*zip1*; ref. 22). A *zip1* mutant exhibits a deficit of crossovers by physical assay, but this defect was interpreted as an indirect consequence of cell cycle regulatory arrest (23). We have since excluded this explanation and infer that Zip1 affects recombination directly (ref. 11; see *Materials and Methods*). Viable progeny of *zip1* meiosis exhibit a 1.5- to 3-fold deficit of crossovers but normal or elevated levels of "heteroallelic recombination" (ref. 22; A. Storlazzi, unpublished observations; see below), which occurs via "gene conversion" and is thus approximately reflective of total recombinational interactions. This genetic phenotype could be explained by many different mechanisms. Zip1 is also an SC component, a feature proposed to be responsible for its role in interference (22). A *zip1* mutant makes normal axial elements joined by interstitial connectors but lacks SC central regions (22).

Here we analyze recombination in a *zip1* mutant using physical assays (10, 13, 24, 25). Results suggest that Zip1 plays a role in recombination that does not involve SC polymerization along the chromosomes. We suggest that Zip1 acts at the transition from DSBs to double Holliday junctions. Zip1 molecules located in or around the sites of recombinational interactions might influence the recombination process and thence nucleate SC formation at some or all such sites. Implications are considered in the context of models for crossover control.

MATERIALS AND METHODS

Strains. Strains are isogenic SK1 diploids (26) homozygous for *ho::LYS2 lys2 ura3 leu2::hisG* and *arg4-nsp/arg4-bgl* except that NKY2548 is *arg4-bgl/ARG4. HIS4LEU2* genotypes are in Fig. 1*A* (NKY1551, 2515 and 2548 and 2570) and *B* (NKY2571 and 2598). NKY2572 and NKY2598 are *trp1::hisG/trp1::hisG*; NKY2515 is (*trp1::hisG/TRP1*); other strains are *TRP1/TRP1*. Markers are described previously except for *zip1::LEU2* and *red1::LEU2*, which were introduced by transformation from corresponding plasmids [pOL178 = pMB97 (23) and pOL140 = B72 (28)].

Time Courses. Cell cultures were taken through synchronous meiosis by protocols 1 (27) or 2 (10); DNA was extracted

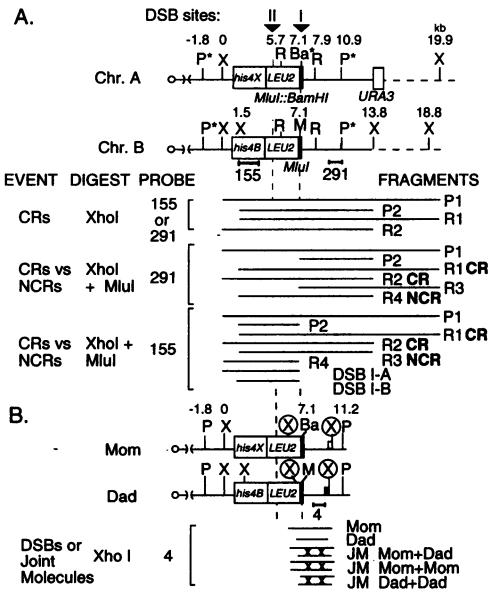

FIG. 1. Physical assays. (*A*) Analysis of crossovers and noncrossovers (25, 27). (*B*) Analysis of Holliday junction formation (13). P, parental fragment(s); R, recombinant fragment(s); Ba, *Bam*HI; Bg, *Bgl*II; *, only relevant sites indicated; M, *Mlu*I; P, *Pst*I; X, *Xho*I (in *B*, relevant sites circled).

and analyzed by protocols A (27) or B (10). Probes are 291 and 155 (27) or 4 (10). For meiosis I ± meiosis II and spores, cell aliquots were pelleted in a microfuge, resuspended in 40% ethanol and 0.1 M sorbitol, stored at 4°C, and analyzed (29).

Cell Cycle Arrest. A *red1 zip1* mutant exhibits no delay or arrest in meiosis I or meiosis II but still exhibits the characteristic *zip1* crossover defect in both BR and SK1 strains (ref. 11; see below). Thus, the *zip1* crossover defect (11, 22, 23) does not result from cell cycle regulatory arrest in any strain background.

RESULTS

Temporal Analysis of Crossovers and Noncrossovers in a *zip1* Mutant. Formation of crossover and noncrossover recombination products is monitored physically at the *HIS4LEU2* recombination hot spot in appropriately marked strains (ref. 25; Fig. 1*A*). Particular restriction fragments obtained by double digestion with *Xho*I and *Mlu*I are known from tetrad analysis to arise specifically via either crossover or noncrossover recombination events. Crossovers can also be examined in *Xho*I single digests (ref. 24; Fig. 1*A*). Wild-type and *zip1* cultures were carried through synchronous meiosis in parallel and analyzed (Fig. 2).

Crossovers. In wild type, crossovers are maximal at t = 7–10 hr with an apparent decrease at later time points due to inefficient DNA recovery from spores (9). In *zip1*, crossovers occur at a reduced level at all time points and reach 25% the maximal wild-type level at late times. Crossovers also appear

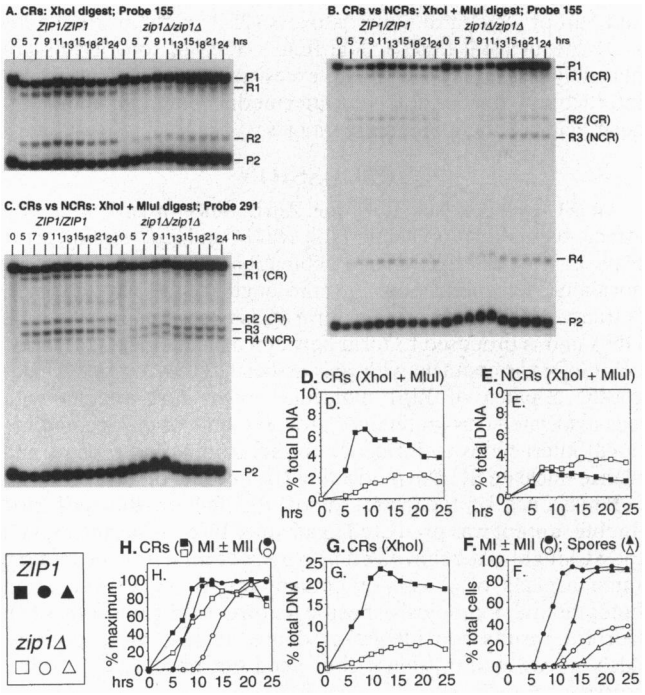

FIG. 2. Recombination, meiosis I, and spore formation in a *zip1D* mutant. Wild-type and *zip1* cultures were taken through synchronous meiosis in parallel (protocol 1); DNA was extracted at the indicated times after transfer of cells to sporulation medium (protocol A) and analyzed as in Fig. 1*A*. (*A*) *Xho*I digestion, probe 155. (*B* and *C*) *Xho*I + *Mlu*I digestion, probes 291 and 155. (*D–G*) Quantitation of events. (*D–G*) Crossovers and noncrossovers: levels of *Xho*I + *Mlu*I fragments R2 and R3 and *Xho*I fragment R2, respectively (probe 155). Each value is level of the relevant fragment as a percent of total DNA in the corresponding lane. Because each fragment represents only a subset of crossover or noncrossover products, measured product levels do not reflect total (relative or absolute) crossover or noncrossover levels. (*G*) Meiosis I ± meiosis II = % of cells that have undergone telophase I (± telophase II). Spores = % of cells containing one or more regular, refractile body. (*H*) Crossover formation (from *G*) and meiosis I (from *F*) plotted with the maximum absolute level observed during the time course defined as 100%.

≈3 hr later than normal and are maximal at t = ≈15 hr, with a small decrease at very late times reflecting delayed and deficient spore formation (see below). In viable progeny of *zip1* meiosis as assayed genetically at the same locus in closely related strains, crossing over also occurs at ≈25% the wild-type level (*his4X LEU2-MluI URA3/HIS4 LEU2-MluI*; K. Haack, unpublished work).

Noncrossovers. In wild type, noncrossovers appear with the same kinetics as crossovers, reaching a maximal level at t = 7–10 hr, with an apparent decline at late times. In *zip1*, the noncrossover-specific fragment appears with kinetics and at levels identical to wild type at these times, plateauing at t = 7–10 hr. Then, between t = 11 and 15 hr, the level of fragment rises again and plateaus after an additional increase of 25% beyond the previous plateau level.

Notably, the second increase in noncrossover products is contemporaneous with the appearance of crossover products. Furthermore, the absolute numbers of crossovers and the number of presumptive late-appearing noncrossovers are comparable. At this locus, in wild-type meiosis, about half of products are crossovers and half are noncrossovers as determined by genetic analysis (A. Storlazzi, L. Cao, and N.K., unpublished work); *zip1* crossovers occur at 25% the normal level of crossovers and the additional noncrossovers occur at 25% the normal level of noncrossovers.

These findings are suggestive that, in a *zip1* mutant, recombinants mature in two waves: a first wave that yields normal levels of noncrossovers with normal timing and a second wave that yields equal numbers of crossovers and noncrossovers with delayed timing. This interpretation assumes that the noncrossover-specific fragments are diagnostic of mature noncrossover products in this mutant case as in wild type (25). This assumption is supported circumstantially by the close correspondence between wild-type and *zip1* strains at early times, which would otherwise be attributed to chance. Also, even if the early "wave" did represent forms other than mature recombinants, the final occurrence of excess noncrossovers in a number that corresponds to a reduced number of crossovers would still suggest the existence of two types of intermediates as described above.

Cell cycle progression. In *zip1*, telophase I, telophase II, and spore formation all occur later than in wild type (11, 22, 23; Fig. 2). Here, 70% of *zip1* cells never undergo meiosis I, whereas 30% do so with a delay of 6 hr. Crossover and noncrossover recombinants both appear several hours before the delayed telophase I.

A *zip1* Mutation Confers the Same Specific Defect in Crossover Formation in the Presence or Absence of RED1 Function. To evaluate the importance of the SC for Zip1's role in recombination, the effects of a *zip1* mutation were examined in a *red1* mutant strain background. In a *red1* single mutant, recombination occurs at ≥25% the wild-type level but no SC is detectable cytologically (30, 31). Moreover, a *red1* mutant also lacks discernible axial elements, the silver-staining features that form the lateral elements of the SC, and so might also be deficient in even the potential for SC central region formation. We reasoned that a mutation which affects recombination independent of the SC might have the same effect in both a *red1* mutant strain background and an otherwise wild-type strain background. This experiment could seem peculiar, however, for two reasons: (*i*) Zip1 protein is itself a component of SC central regions (23) and the *zip1* crossover/interference defect has been attributed to the absence of SC polymerization along the chromosomes (22). If Zip1 affected recombination only via SC formation, a *zip1* mutation should have no phenotype in a *red1* strain background where SC is not detected. (*ii*) Our model envisions that information is transmitted along the structural axes of the homolog. But a *red1* mutant lacks normal axial structure. Thus, the *red1* mutation might potentially affect the activation step of recombination in a complex way. However, the ideas that triggered our model

included the notion that meiotic interhomolog interactions in general, and meiotic crossover control in particular, might have arisen from interactions between sister chromatids in mitotically cycling cells (7). If so, fundamental components of the crossover interference process might be retained even in the absence of meiosis-specific chromosome structures. Furthermore, since *red1* mutant chromosomes compact effectively during midprophase (31), they must retain some type of continuous structural axis even though the axial element feature is not discernible.

We applied the *Xho*I and *Xho*I+*Mlu*I cleavage assays to parallel analysis of wild-type (*RED1 ZIP1*), the two single mutant strains (*red1 ZIP1* and *RED1 zip1*), and the *red1 zip1* double mutant (Fig. 3). (*i*) In wild-type strain, crossovers and noncrossovers appear coordinately as usual. (*ii*) A *zip1* single mutant exhibits a deficit of crossover recombinants but not noncrossover recombinants, as above. (*iii*) In the *red1* single mutant, crossovers and noncrossovers again appear coordinately, with the same general kinetics as in wild type, but at reduced levels. Quantitation of *Xho*I crossover products in wild-type and *red1* cultures analyzed in parallel reveals no detectable difference in the time of crossover formation (≤20 min; ref. 11). (*iv*) The pattern of recombination in the *red1 zip1* strain is the simple product of the two single mutant patterns: a specific deficit of crossovers as compared with noncrossovers comparable to that seen in the *zip1* mutant plus a several-fold reduction in the total levels of both products as compared with a *zip1* single mutant, comparable to the coordinate reduction seen in *red1 ZIP1* as compared with wild type. Quantitation of crossovers in the *Xho*I digests of these and other experiments confirms these results (11).

These results strongly suggest that Zip1 plays a role in meiotic recombination which is independent of normal SC polymerization along the chromosomes.

DSBs and Holliday Junction Intermediates in a *zip1* Mutant. Resected DSBs appear and disappear normally in a *zip1* mutant except that a few resected DSBs are still observed at very late times (ref. 11; A. Schwacha, unpublished results; see *Discussion*). Holliday junction kinetics, in contrast, are substantially altered in a *zip1* mutant (Fig. 4).

In two-dimensional gels that separate molecules first according to mass and then according to shape, double Holliday junctions at an appropriately marked *HIS4LEU2* locus form a discrete signal at a specific diagnostic position. Also, interhomolog Holliday junctions are distinguishable from intersister Holliday junctions (Fig. 1; ref. 10).

Holliday junctions appear at similar times in *zip1* and wild-type strains (t = 3 hr). In wild-type strains, double Holliday junctions are maximal around t = 5 hr and diminish thereafter. In *zip1*, Holliday junctions accumulate to higher than wild-type levels at t = 5 hr, are diminished only slightly by t = 10 hr, and are further diminished at later time points (data not shown). *zip1* Holliday junctions might be either double or single junction structures, which run similarly in such gels. Presumptively, Holliday junctions present at the later than normal times in *zip1* mature into the late-appearing recombination products (see above).

Aberrant Holliday junction kinetics could in principle reflect alterations in the number, formation, and/or resolution of these forms. Importantly, however, the kinetics observed are not easily explained by a simple uniform delay in Holliday junction maturation: in this scenario, the level of branched forms should rise to a much higher than normal level and then decrease. Instead, branched forms arise to only slightly higher than normal levels and then decay relatively slowly. These kinetics are, however, fully consistent with the possibility that some Holliday junctions form and mature with normal kinetics while others undergo aberrant Holliday junction formation and maturation.

Interhomolog Holliday junctions and presumptive intersister junctions exhibit similar kinetics in both wild-type and *zip1* strains (Fig. 4*C*). The ratio of interhomolog to apparent intersister forms is reproducibly lower in the *zip1* mutant than in the wild-type case, however, ≈2.3 and ≈4, respectively (Fig. 4*C*; A. Schwacha, unpublished results). This difference could be explained in several ways (see legend to Fig. 4*C*).

The fates of virtually all DSBs in a *zip1* mutant can be accounted for comfortably according to the ideas in the Introduction. The ≈50% of DSBs that would normally appear as noncrossovers still do so; the remaining ≈50% of DSBs, which normally would appear as crossovers, suffer aberrant fates. A few remain at the DSB stage (≈10% of total DSBs). Most of the remainder mature into interhomolog recombination products that are detected as such, half of which are crossovers (25% × the normal 50% = 12.5% of total DSBs) and half of which are noncrossovers (25% × the normal 50% = 12.5% of total DSBs). The final ≈15% of total DSBs are plausibly accounted for by the excess of (pseudo?)-intersister interactions, rare undetected intermediates and/or minor inaccuracies in the above percentages.

DISCUSSION

An SC-Independent Role for Zip1. Since a *zip1* mutation affects recombination similarly in *RED1* and *red1* strains, Zip1 plays at least one role in recombination that does not involve normal SC polymerization along the lengths of the chromosomes. A transverse filament protein of rat SCs is known to interact with DNA and is proposed to form between axial elements of paired homologs via contacts with axis-associated DNA (32). Analogously, a patch of Zip1 molecules might first interact with axis-associated chromatin in or around (some or all) recombinational interactions to influence the recombination process and thence nucleate SC polymerization (from some or all such sites).

Persistence of the *zip1* crossover defect in the *red1 zip1* double mutant was predicted in advance by considering that (*i*) the SC might not be involved in crossover interference and (*ii*) some aspects of crossover control might be derived from fundamental nonmeiosis-specific features of chromosomes (7). Current results provide no evidence against these notions. Also, a *red1* mutant might (but need not necessarily) exhibit crossover interference.

At Which Step in Recombination Does Zip1 Act? We suggest that Zip1 acts relatively early in the recombination reaction, before and/or during Holliday junction formation and that it is required primarily to make qualitatively normal Holliday

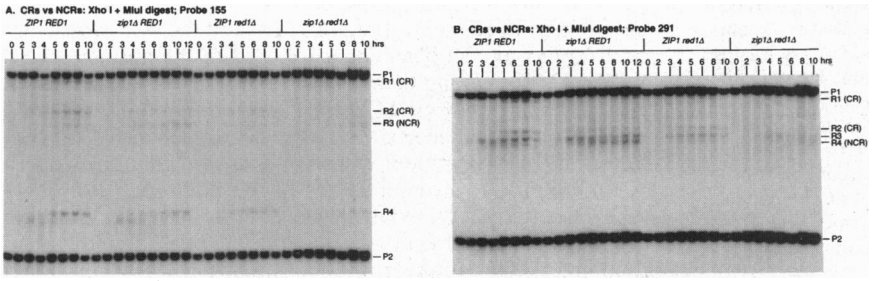

FIG. 3. Effects of *zip1* and/or *red1* mutations. *ZIP1 RED1*, NKY1551; *zip1*::*LEU2 RED1*, NKY2515; *ZIP1 red1*::*LEU2*, NKY2548; *zip1*::*LEU2 red1*::*LEU2*, NKY2570. Cultures analyzed in parallel as in Fig. 2 *B* and *C*. Identical results obtained in a second parallel analysis of the four strains and in other experiments. Crossovers were also analyzed by *Xho*I digestion, with identical results (11).

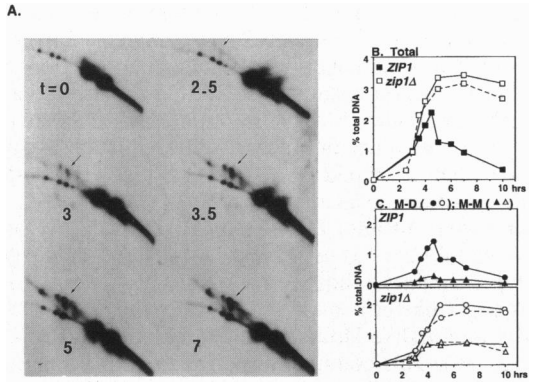

FIG. 4. Holliday junctions in *zip1* strains. (*A*) *zip1*::*LEU2* (NKY2572; Fig. 1*B*) was taken through synchronous meiosis (protocol 2), and *Xho*I-digested DNA (protocol B) was analyzed (ref. 10; probe 4). Arrow indicates interhomolog Holliday junctions; presumptive intersister Holliday junction species occur immediately above and below the arrow. (*B* and *C*) Kinetics of Holliday junction formation in *zip1* and wild-type strains. The dashed *zip1* line is the experiment in *A*; the solid *zip1* line is an independent analysis of the same strain. Wild type, NKY2598. (*B*) Total Holliday junctions; (*C*) interhomolog Holliday junctions and one type of intersister Holliday junction (between "Mom" chromatids; Fig. 1*B*). Two other *zip1* cultures and several other wild-type cultures have given the same results. Altered ratios of interhomolog and intersister forms (see text) could be explained by: (*i*) a differential effect of the *zip1* mutation on interhomolog and intersister Holliday junction lifespans, (*ii*) channeling of some recombination intermediates into *bona fide* (Zip1-dependent) intersister recombination, or (*iii*) formation of Holliday junctions having the genetic composition (and physical properties) of the intersister species from an interhomolog recombination event, by appropriate "gene conversion" of flanking marker(s) (which are located very close to the initiation DSB site in this construct as compared to that used for product analysis above; Fig. 1). Given the sometimes elevated frequency of heteroallelic recombination and heteroduplex DNA in a *zip1* mutant (refs. 22 and 33; A. Storlazzi, unpublished results), we favor the latter view.

junctions. A *zip1* mutant would thus exhibit the observed aberrant Holliday junction kinetics because at least some such intermediates are delayed in their resolution (and perhaps also in their formation). Zip1 is not needed for DSB formation nor to ensure efficient conversion of DSBs to some type of later form. Also, if Zip1 acts early, a *zip1* defect at a single (early) step could account simultaneously for both loss of crossover interference and aberrant product formation.

We suggest specifically that Zip1 modulates the recombination process exactly at the transition from resected DSBs to the next chemical step, before, after, or during the point at which resected DSBs have begun to invade an homologous duplex to form a nascent strand exchange intermediate. This scenario would fit with a pre-SC role. Moreover, the presence of a few resected DSBs at late times in *zip1* meiosis hints at a mechanistic "glitch" at this point, with a few recombination complexes failing to make the critical transition at all. Interestingly, a recently described mutant, *mer3*, exhibits an even more dramatic version of this mixed phenotype: many recombinational interactions are blocked at the DSB stage and many others proceed to products but with a deficit of crossovers (T. Nakagawa and H. Ogawa, personal communication).

Action of Zip1 late in recombination, i.e., at the time of Holliday junction resolution (8), is less attractive. *zip1* Holliday junction kinetics do not favor this view. Also, since resolution occurs at the end of pachytene (9) and is independent of SC disassembly (11, 12), this model would imply an SC-independent role for Zip1 at a time when SC is full length, which is not the most obvious possibility. Finally, if crossover control is indeed imposed "early" but the *zip1* defect in recombinant maturation results a failure of Zip1 action "late," it would be necessary to postulate additional role(s) for Zip1 during the (early) interference process, independent of any effects on product maturation.§

Recombinant Formation, Crossover Interference, and the SC. Fig. 5 presents a model that accommodates: (*i*) SC-independent imposition of crossover control via the imposition and relief of stress; (*ii*) two types of recombination modes, a "crossover-type" and a "noncrossover-type"; and (*iii*) an early process which precedes and is prerequisite to crossover/noncrossover differentiation *per se*. During this early process, all intermediates acquire two essential features: susceptibility to the special factors as required for commitment to the crossover mode; and, most importantly, constraints such that, in the absence of such commitment, an intermediate will mature into a noncrossover as the unique default option.

Additionally, to accommodate early imposition of crossover control (and in accord with action of Zip1 at the point of nascent strand invasion), we propose that the crossover and noncrossover modes of recombination reflect two different geometries by which the two ends of a resected DSB can invade an intact duplex, i.e., from the "same" side or from "opposite" sides as defined by the axis of the bivalent. In consequence of these early constraints, two geometrically distinct types of double Holliday junctions form; furthermore, these same early constraints then continue to hold the resultant Holliday junctions arms in the corresponding geometries as needed to ensure their appropriate resolution at a later time.

More specifically, the early events constrain the DSB arms in the "noncrossover" configuration (simultaneously making the intermediate susceptible to crossover designation); then, for the subset of interactions where crossover designation occurs, the designation process alters DSB arm geometry into the alternative "crossover" configuration. Strand exchange then occurs in the two appropriate modes, with those intermediates not designated as crossovers continuing in the noncrossover mode as the default. The Holliday junction arms continue to be held in their respective geometries until they are resolved. Stress, sensitivity to stress, activation, and stress relief are assigned appropriate roles.

In previous models, Holliday junctions undergo isomerization after they are formed. Here, because differentiation occurs at a very early step, the crossover attack mode leads, in essence, to the "preisomerization" of one junction. This feature avoids problems implicit in mechanisms for postformation isomerization, e.g., the steric complications of braided junctions or the physical awkwardness of dramatic arm rotations. Intriguingly, it has recently been found that the directionality of RuvC-promoted Holliday junction resolution is determined as much by the geometry of the emanating arms (via effects on protein binding geometry) as by the strand configuration within the junction itself (34).

In the proposed model, Zip1 acts upon all intermediates at the very beginning of the process, as a component of "sensitivity to stress," and is thus required for the resultant constraining of all intermediates. In the absence of Zip1, developing recombinational interactions fall arbitrarily into proto-crossover and proto-noncrossover modes as the geometry by which a DSB attacks an intact duplex is random; pre-noncrossover intermediates mature relatively normally, in consequence of the fact that maturation from this mode of attack does not normally require special processes; pre-crossover intermediates, lacking their normal special promoting factors, mature aberrantly and also, via randomization at the resolution stage, into both crossovers and noncrossovers. The numbers and kinetics of observed species is compati-

§Inefficient maturation of precrossover intermediates at a point after interference is imposed would not result in a loss of interference, as a random sampling of a nonrandom distribution will still exhibit nonrandomness. Thus, if the occurrence of closely-spaced crossovers has already been precluded, the intermediates already fated to become noncrossovers cannot be recovered back into the population of crossovers as a consequence of a crossover maturation defect.

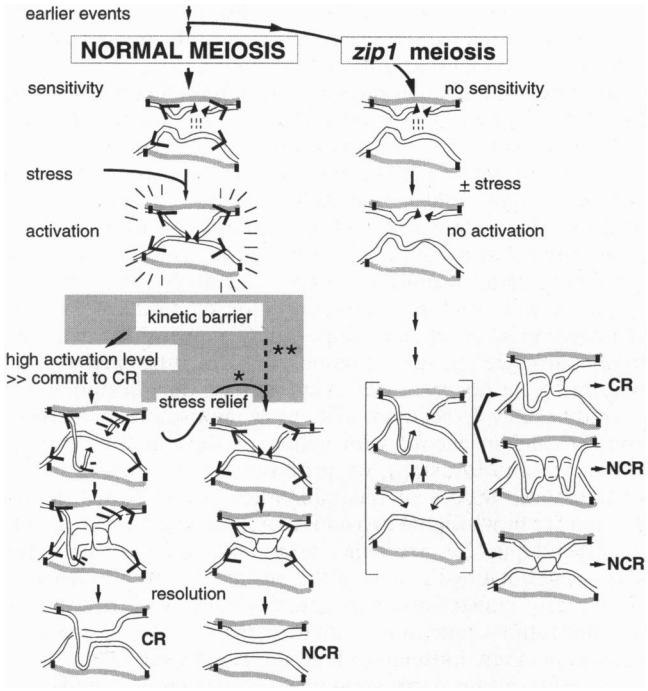

FIG. 5. A model for crossover/noncrossover differentiation. Structural axes of homologs are shown by grey bars; axial associations of DNA segments participating in recombination are shown by black rectangles. Part I: Stress and stress relief. Sensitivity to stress is shown by large "V" shapes; "stress" is not indicated (to be discussed elsewhere). The combined effects of stress and sensitivity to stress "activate" all recombination intermediates (halo of lines). As part of this process, recombination intermediates are placed in a configuration such that, in the absence of any further modulation, they will be resolved into noncrossovers as the automatic default. The level of activation varies amongst different recombinational interactions, e.g., due to differences in sensitivity levels. The interaction with the highest level of activation (i.e., the highest level of stress × sensitivity to stress) eventually "goes critical" and thereby becomes committed to becoming a crossover (curved anchors); concomitantly, stress is rapidly relieved in the immediate vicinity. Additional intermediate(s) on the same bivalent may then go critical if their activation level(s) are high enough. Many intermediates fail to go critical either because they are subjected to stress relief emanating from a nearby intermediate committed to crossing over (*) and/or because they began with an intrinsically low activation level (**); all such intermediates mature as noncrossovers due to the default constraint. As a final feature, all activated interactions are initially impeded from further progress by a kinetic barrier. This barrier provides an opportunity for specific positive activation of certain intermediates into the crossover mode and also implies that interactions which never go critical do, nonetheless, eventually proceed to the next stage while still under the influence of the noncrossover constraint. Part II: Molecular discrimination between crossover and noncrossover modes. The two modes involve two different geometries for attack of DSB ends upon the intact duplex partner (see text). In consequence, two chemically identical but geometrically different types of double Holliday junctions are formed; in effect, the crossover mode has "preisomerized" one junction (see text). Geometric constraints imposed during Holliday junction formation then persist until the time of Holliday junction resolution, at which step both types of forms can be resolved by the same activity: e.g., a nuclease that gives "horizontal" resolution of all junctions as drawn. In a *zip1* mutant (*Right*), recombinational interactions fail to acquire sensitivity to stress with consequences as shown.

bel with this model. No role for Zip1 is invoked at later stages in the process. It is not excluded that Zip1 is also involved in stress relief, however; the earlier defect would make any defect at a later stage invisble in a *zip1* mutant.

The *zip1* mutant phenotypes could be explained by any specific mechanism having the same formal logic as that described in Fig. 5. In particular, communication along the chromosomes could be mediated by polymerization of the SC or some other substance (3, 35). Notably, however, even an SC-based model would now also seem to imply modulation of the recombination reaction prior to the Holliday junction resolution step. Thus, by this model, too, Zip1 might act exclusively at the transition from DSBs to double Holliday junctions, once during early differentiation of intermediates and then a second time, during SC polymerization, to preclude crossover designation.

Other Yeast Mutants That Resemble *zip1*. Four yeast mutants, *mer1MER2++*, *msh4*, *msh5*, and *srs2*, have genetic phenotypes similar to those of *zip1* (8; 18–21); *mer1MER2++* exhibits a *zip1*-like crossover deficit by physical assay (25); and *srs2* exhibits *zip1*-like Holliday junction kinetics (A.S., unpublished results). This commonality of phenotypes suggests the existence of a specific mechanistic breakpoint in the recombination process. Those mutants tested cytologically still make substantially normal SCs, consistent with the idea that absence of SC is not responsible for the *zip1* crossover deficit.

***Drosophila* Precondition Mutants.** *Drosophila* precondition mutants are defective for crossover and crossover interference, suggesting that "aspects of the recombination process . . . not only promote the normal high level of meiotic exchange but also are involved in specifying [crossover control]" (15). *zip1* is suggested to be such a mutant (22) in contrast to the suggestion that its recombination defects result from a cell cycle block (23). The ideas presented here arose from a different perspective but are consonant with earlier considerations of *Drosophila* mutant phenotypes and their implications for models of crossover interference (e.g., refs. 15 and 17).

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