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Transcription, topoisomerases and recombination

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Abstract. Transcription, DNA topoisomerases and genetic recombination are interrelated for several structural reasons. Transcription can affect DNA topology, resulting in effects on recombination. It can also affect the chromatin structure in which the DNA resides. Topoisomerases can affect DNA and/or chromatin structure influencing the recombination potential at a given site. Here we briefly review the extent to which homologous direct repeat recombination and site-specific recombination in eukaryotes are affected by transcription and topoisomerases. In some cases, transcription or the absence of topoisomerases have little or no effect on recombination. In others, they are important components in the recombinational process. The common denominator of any effects of transcription and topoisomerases on recombination is their shared role in altering DNA topology.

Key words. Transcription; topoisomerases; direct repeat recombination; yeast repetitive DNA; immunoglobulin class switching; V(D)J recombination.

Introduction

The process of DNA replication and RNA transcription leads to topological changes in chromosomal DNA. The topological stress created by these processes are relieved by DNA topoisomerases[6,7,16,22,30,47,56,58,71]. The failure to deal effectively with the topological alterations associated with DNA metabolism in the cell can lead to DNA damage that results in genetic recombination[11,19,62,70,72]. Alternatively, topological changes can increase the accessibility of DNA to the enzymes involved in recombination. In any case, the alterations themselves are epigenetic in nature since they occur as a consequence of altered DNA topology and not permanent changes in DNA sequence. Therefore, for some biological processes, the topological state of the DNA may be used to control genetic recombination.

Early studies in prokaryotic systems investigated the link between transcription and recombination[34–36]. In some cases, transcription stimulated recombination while in others, it inhibited recombination[12–15,20,21,23,27,28,37,52,64]. Although some of these studies suggest that transcription can stimulate recombination in some assays, others did not. Likewise, studies examining the effects of mutated bacterial topoisomerases on recombination have reported increased recombination[62,72] or decreased recombination[19]. Thus no consistent relationships have emerged between transcription, topoisomerases and recombination in prokaryotes.

Yeast has been used as a major system to study both the loss of topoisomerase function as well as the effect of transcription on recombination. Another eukaryotic system that has been extensively studied for a link between transcription, topology and recombination is immunoglobulin rearrangements in mammalian cells. Results from the study of yeast recombination and immunoglobulin rearrangements form the major portion of this review.

Loss or alteration of topoisomerase function in *Saccharomyces cerevisiae* is associated with increased recombination

Three kinds of assays have been used to study the effect of various topoisomerase mutations on genetic recombination in yeast and these are outlined in figure 1. In the first assay, recombination between multiple tandem repeats is measured as the frequency of marker loss that occurs as a result of recombination between nearby repeats (fig. 1A). At least 4 kinds of events can be envisaged to account for marker loss in multiple tandem repeats and these are illustrated in figure 2. Recombination can also be measured between short (330 bp) dispersed repeated sequences such as delta sequences, the long terminal repeats of the yeast retrotransposon *Ty1*[61] (fig. 1B). The last type of recombination assay involves events between similar sequences on the same or different chromosomes. Examples of two such recombination assays are mating type interconversion[29] (fig. 1C) and recombination between similar but not identical sequences (termed homeologous recombination)[3] (fig. 1D).

Using the assays described above, mutations in the various topoisomerase genes were examined for their effect on recombination. A null mutation in *top1* causes a 25-fold increase in the mitotic frequency of rDNA marker loss[44] (Gangloff and Rothstein, unpubl. observ.). However, the same mutation does not change the

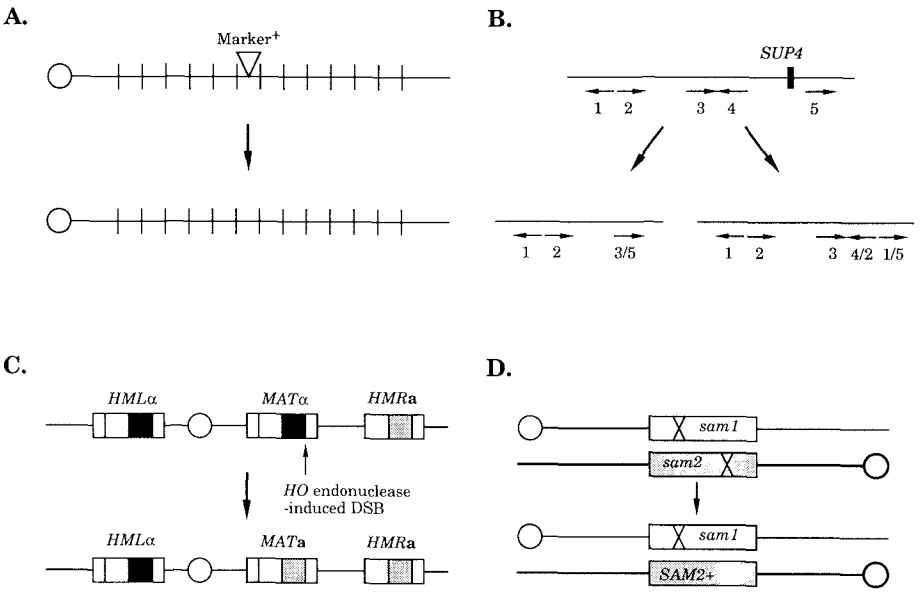

Figure 1. Four assays used to examine the effect of loss or alteration of topoisomerase function on mitotic recombination in *S. cerevisiae*. (A) Marker loss in a multiple tandem repeat such as rDNA ($\approx$200 repeats) or *CUP1* ($\approx$10 repeats) can be measured by inserting a marker into one of the repeats (designated by the inverted triangle). Recombination between repeats leads to marker loss (see fig. 2). (B) the SUP4 region on chromosome X is surrounded by short repeated delta sequences (numbered 1 through 5). The two major classes of deletions that occur via delta-delta recombination are shown. (C) The organization of mating type information in *S. cerevisiae* is shown. *HMLα* and *MATα* contain identical information depicted as 4 blocks (W, X, Y and Z). The filled in block represents Y sequences, which are mating type dependent. *HMR*a does not contain W and contains only part of the Z region. Mating type interconversion is mediated via a site-specific double-strand break (DSB) initiated by the *HO* endonuclease at the Y–Z junction of only the active *MAT* locus (see text for details). (D) The isozymes for S-adenosylmethionine synthetase, *SAM1* and *SAM2* map on chromosomes XII and IV respectively. The nucleotide sequences are 83% identical. Mutations that inactivate each gene were constructed resulting in an S-adenosylmethionine auxotroph. Recombinants that restore function to one or the other of the genes are due to gene conversion only since reciprocal recombinants generate dicentric and acentric chromosomes that are lethal in haploids.

frequency of deletion between the delta sequences at *SUP4* (Gangloff and Rothstein, unpubl. observ.) and the *CUP1* tandem repeats[11]. Similarly, *top1* null mutations do not affect mating type interconversion or *SAM* homologous recombination[2,11]. On the other hand, certain missense mutations of *top1* not only increase recombination but also result in lethality in the absence of the double-strand break repair gene *RAD52*[43,44]. The phenotypic death in a *top1 rad52* strain is best explained by the hypothesis that the topoisomerase mutation leads to a lesion that must be repaired via a double-strand break repair pathway.

Recombination has also been measured in the presence of drugs that interact specifically with topoisomerase I. Yeast cells can be treated with camptothecin, a drug that binds and traps eukaryotic type I topoisomerases with DNA[43,48,49]. Wild type cells are relatively resistant to this drug; however, *rad52* mutants, which are deficient in double-strand break repair, die in the presence of the drug[44,55]. This result suggests that, similarly to the *top1* missense mutation described above, recombination intermediates that require double-strand breaks for repair are generated in camptothecin-treated yeast.

Mutations in *top2* have also been assayed for several of the recombination events mentioned above[11]. Since *TOP2* is an essential gene, the assays are performed at a semi-permissive temperature for cell growth. Similar to that found for *top1*, mutations in *top2* cause strains to exhibit elevated recombination at rDNA. However *top2* mutations do not affect recombination at *CUP1*, mating type or the *SAM* genes[2,11]. Interestingly, when a *top1* mutation is combined with a *top2–4* mutation, more than half of the rDNA exists as extrachromosomal rings, even at the permissive temperature[40].

A second type I topoisomerase, encoded by the *TOP3* gene, exists in yeast and exhibits homology with bacterial type I topoisomerases[70]. Mutations in *TOP3* were first identified by the phenotype of increased recombination between delta sequences surrounding *SUP4*[70]. Mutations in *TOP3* also cause a dramatic increase in rDNA recombination: more than seventy-fold compared to wild type strains (Gangloff and Rothstein, unpubl. observ.). In addition, recombination between the *SAM* homologues is also elevated six-fold in *top3* mutant cells compared to wild type[2].

The disparity in the effects observed in the absence of the three topoisomerases suggests that these proteins share overlapping functions involved in suppressing recombination. Biochemical studies have revealed that these three enzymes are capable of relaxing negatively supercoiled DNA[41,71]. However, unlike Top1 and Top2, Top3 can not relax positively supercoiled DNA[41]. This

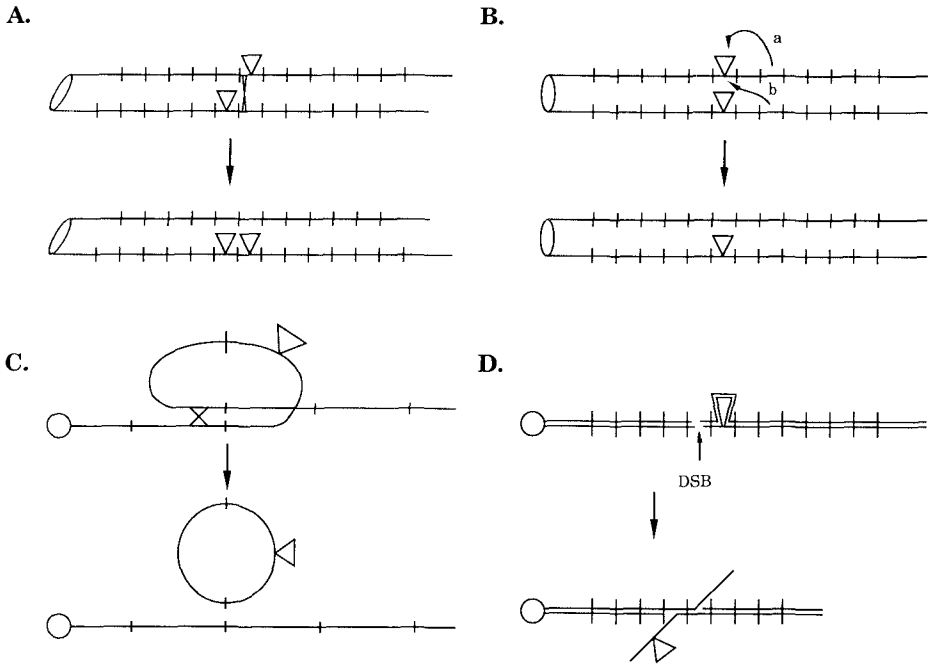

Figure 2. Potential recombination mechanisms for marker loss in a multiple tandem array. (A) Unequal sister chromatid exchange occurs when misaligned repeats recombine reciprocally. If this event occurs between the inserted marker (as depicted in the figure), two non-identical sisters are generated in the following mitosis: one exhibiting marker loss and the other containing two copies of the insert separated by the number of repeats that were misaligned. (B) Gene conversion, that is the non-reciprocal transfer of information from one gene to another, can take place in a multiple tandem array at either G1 or G2. The direction of transfer of information in a G1 event is depicted by the arrow labelled a in the figure. A G2 event is indicated by the arrow labelled b. G2 events can also take place by transfer of information from the same chromatid (a). (C) Ring formation can occur when adjacent repeats pair and reciprocally recombine. The size of the ring varies as a function of the number of copies of the repeat between the paired substrates. In the example illustrated, the crossover takes place with an extra repeat between the paired substrates giving rise to a dimer ring. If the event includes the inserted marker, then the marker is incorporated onto the ring. (D) A double-strand break in a multiple tandem array can be repaired using a sinlge-strand annealing mechanism. Nucleases attack the exposed ends, likely degrading from the 5′ end. When homologous regions between adjacent repeats are revealed, the single strands can anneal to create a repairable structure that leads to an intact array. If the break occurs near the repeat containing the inserted marker, the marker may be lost during the repair of the break as illustrated in the figure. Note that in this panel, each strand of the duplex is shown.

asymmetry may explain why the absence of Top3 increases recombination at multiple loci in the yeast genome. The requirement for all three topoisomerases at rDNA may be a consequence of the intrinsic structure of the nucleolus and the need for relaxing the heavily transcribed rDNA repeats[60]. Further characterization of the type of recombinants stimulated in the absence of the topoisomerases (e.g. unequal sister chromatid exchange or ring formation) may help elucidate the mechanisms of recombination suppression exhibited by the wild-type proteins.

Effects of transcription on recombination

Studies in *S. cerevisiae*

One of the best characterized recombination events in yeast is mating type interconversion (fig. 1C). Regulated expression of the *HO* endonuclease results in a double-strand break at the active locus, while two other loci on chromosome III, which share the identical nucleotide sequence recognized by the *HO* endonuclease, remain uncut. These two loci are the silent cassettes *HML* and *HMR*, which contain *MAT*α and *MAT*a silent mating type information respectively[29]. Chromatin studies on nuclease sensitivity of all three sites revealed that only the active mating type locus is accessible in wild type switching yeast cells[54]. To determine whether the act of transcription per se is necessary for the accessibility, Haber's lab investigated a TATA box deletion that results in transcriptional inactivation of the active *MAT* locus. They found that recombination (switching) still occurred efficiently[26]. This substantiates the hypothesis that transcription is not always a prerequisite for recombination.

However, several studies indicate that transcription can affect recombination. Keil and Roeder demonstrated a true link between transcription and recombination when they cloned the yeast rDNA promoter in front of a non-tandem duplication of the *his4* region (fig. 3Ai). This sequence, named *HOT1*, is comprised of sequences necessary for transcription of the polI-transcribed rDNA[39]. The authors found that only mitotic recombination is stimulated in an orientation-specific fashion that reflects the direction of transcription. Transcription terminators placed downstream of the *HOT1* sequence block the increase in recombination[68]. This experiment

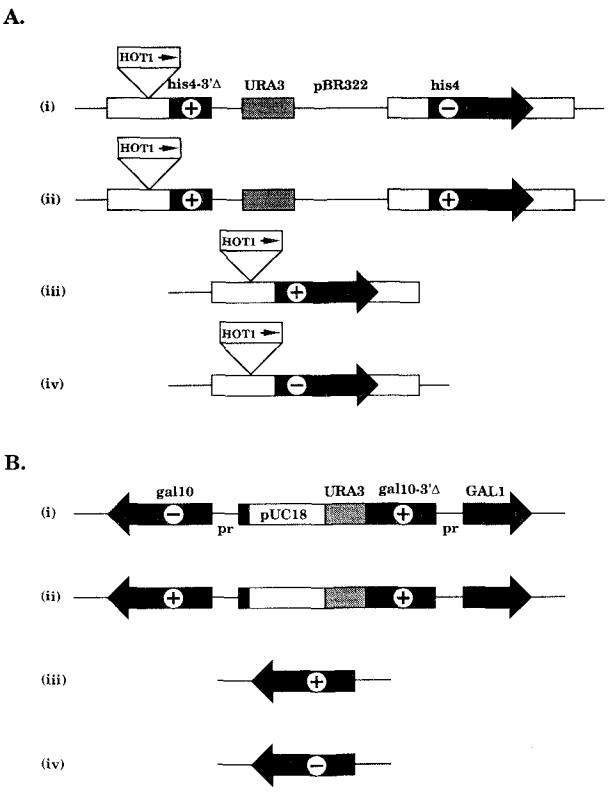

Figure 3. Two constructs for assaying the effects of transcription and recombination. (A) The duplication[68] used by Roeder's lab to assay *HOT1* stimulation of recombination contains a 3′ truncation of the wild type copy of *HIS4* separated from a full-length mutated copy of *his4* by plasmid DNA and the yeast *URA3* gene (i). The direction of transcription of *HOT1* is also shown. Recombinants can be selected for either histidine prototrophy on medium lacking histidine or for loss of the *URA3* gene on 5-fluoro-orotic acid-containing medium[4]. The three kinds of recombinants that can be detected are: (ii) *HIS* + *URA* +, (iii) *HIS* + *ura* −, and (iv) *his* − *ura* −. (B) The *GAL10* duplication used by Thomas and Rothstein[66] to measure the effect of transcription on recombination. As in figure 3A, a 3′ truncation and a full-length copy was separated by plasmid sequence and the yeast *URA3* gene (i). The *GAL10* promoter (pr) is bidirectional since it also regulates the *GAL1* gene. Therefore, this construct results in a promoter capable of transcribing adjacent pUC18 sequences after activation. Recombinants can be selected for either galactose utilization on medium containing galactose or for loss of the *URA3* gene on 5-fluoro-orotic acid-containing medium. The three kinds of recombinants that can be detected are: (ii) *GAL* + *URA* +, (iii) *GAL* + *ura* −, and (iv) *gal* − *ura* −.

demonstrated that, indeed, the act of transcription was necessary for the stimulation of recombination. Further studies showed that an active *HOT1* sequence could increase the conversion tract length and stimulate recombination between homologous chromosomes[39,68,69]. Recent genetic evidence indicates that *HOT1* may also be involved in recombination at its native chromosomal location[46].

Thomas and Rothstein examined the effect of polII transcription on recombination by measuring direct repeat recombination between a non-tandem duplication of a regulatable gene, *GAL10*[66] (fig. 3B). Two kinds of recombination events can be measured in the presence or absence of transcription: direct-repeat recombination resulting in loss of intervening vector sequences (popout, fig. 3Biii and iv) or recombination between the repeats leading to replacement of a mutant copy of *GAL10* (gene conversion, fig. 3Bii). Transcription between the direct repeats increased popout recombination approximately 15-fold compared to the absence of transcription. The frequency of gene conversion events was unaffected by transcription. Furthermore, the frequency of recombination correlated with the amount of transcription in the *GAL10* repeats as well as transcription that entered the plasmid sequence itself from the adjacent bi-directional *GAL1–10* promoter. These results suggest that the transcribed sequences become more accessible to the recombination machinery leading to the stimulation in direct repeat recombination.

Transcription and antigen receptor gene rearrangements

There are six loci in vertebrate genomes at which DNA rearrangements are known to occur. These are the immunoglobulin (Ig) heavy chain, kappa and lambda loci and the T-cell receptor (TCR) beta, gamma and alpha/delta loci reviewed in Lieber[45]. V(D)J recombination occurs at all six loci. This is the process by which the exon encoding the antigen binding pocket, the variable domain exon, is created (fig. 4). This is a site-specific recombination reaction in that the cut sites appear to occur precisely adjacent to a heptamer/nonamer signal sequence. The Ig heavy chain locus undergoes a second type of reaction called class switch recombination (fig. 5)[24]. This is the process by which

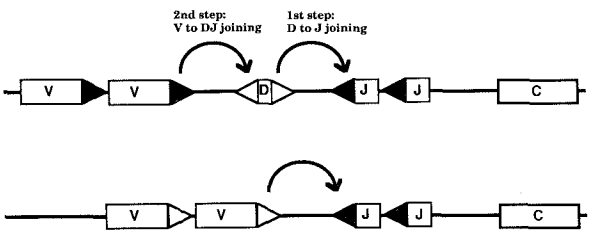

Figure 4. Architecture of the immunoglobulin heavy chain locus and the process of V(D)J recombination. The assembly of the variable domain of immunoglobulin and T-cell receptor genes is from component sub-exon segments called V, D or J segments. Simplified versions of the immunoglobulin heavy (top) and light (bottom) chain loci are illustrated here. At the Ig heavy chain locus, there are up to several hundred V (variable) segments, over 13 D (diversity) segments and 4 J (joining) segments. For simplicity, this diagram shows far fewer segments. Once a D joins to a J, and a V joins to a DJ, a complete VDJ variable domain exon exists. The signals that the recombinase sees are adjacent to each V, D or J segment and consist of a heptamer (CACAGTG) and a nonamer (ACAAAAACC) separated by either 12 or 23 nonconserved base pairs. The recombinase acts on a pair of signals, one with a 12-base pair spacer (open triangle) and the other with a 23-base pair spacer (closed triangle). The reaction depicted here is a deletional one in which the intervening DNA is looped out of the chromosome and discarded. Inversional reactions also occur at some antigen receptor loci. C represents the several constant domain exons that encode the constant portion of the heavy and light chains.

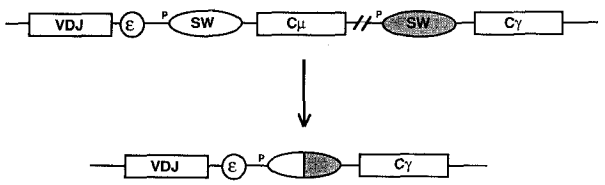

Figure 5. Class switch recombination. After the assembly of a VDJ variable domain exon, the constant domain exons can be switched by an entirely different recombination process called class switch recombination. In this diagram, ε represents the intron enhancer; Cμ represents the mu constant domain exons; Cγ represents the gamma constant domain exons. There are several other constant domain exon alternatives not shown for simplicity (such as Cα, Cε and other Cγ exons). The open and shaded ovals represent the Sμ and Sγ repetitive class switch regions, which are 2 to 10 kb in length. A class switch recombination reaction involves cuts in these two respective regions and deletion of the intervening DNA. The mechanism of this reaction is uncertain. P indicates the site of promoters that send 'sterile' transcripts (to the right) through the switch regions upon B lymphocyte stimulation. These transcripts are termed 'sterile' because they do not appear to encode protein.

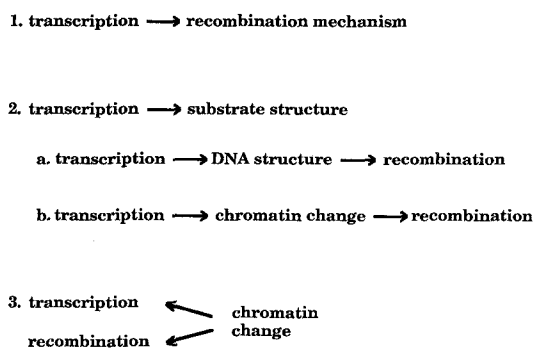

Figure 6. Possible relationships between transcription and recombination. The ways in which transcription and recombination might be related are schematized. In (1), RNA polymerase is part of the recombination machinery. In (2), transcription alters the substrate structure in an essential way. In (2a), this is at the DNA level. In (2b), this is at the chromatin level. In (3), there is no causal connection between transcription and recombination; they simply happen at about the same time (temporally related, but not causally related).

the mu constant domains are deleted so that the downstream gamma, alpha or epsilon constant domains can be utilized in order to synthesize IgG, IgA and IgE instead of IgM. Class switch recombination is regionally-specific insofar as the recombination sites occur anywhere within 2 to 10 kb switch regions. There is no indication that V(D)J recombination and class switch recombination share any enzymatic components or that the reactions are similar in any way.

V(D)J recombination

The signals for V(D)J recombination are present at all six antigen receptor loci. Yet these loci do not all recombine simultaneously. Ig loci recombine in B lymphoid cells but much less so and to only a limited extent in T lymphoid cells. TCR loci recombine in T lymphoid cells, but again, only to a limited extent in B lymphoid cells. Within cells differentiating along the B cell developmental pathway, the kappa and lambda loci usually recombine after the heavy chain locus. V_H to D_H recombination occurs only after a DJ_H recombination event has occurred. Because not all loci are available for V(D)J recombination simultaneously, the concept of accessibility emerged as a restatement of this fact[1]. That is, loci must be made accessible; otherwise, they remain inaccessible to the recombinase.

The molecular nature of accessible and inaccessible DNA has been very difficult to define. An early and dominating hypothesis in the V(D)J recombination field for how the transition from the inaccessible to the accessible state is achieved proposed that transcription was the cause of this transition[1]. The notion that transcription makes an antigen receptor locus accessible was based on the fact that transcription from promoters that are upstream of V, D or J segments can be detected at roughly the time when recombination occurs at the corresponding segments. This temporal association between transcription and V(D)J recombination can be due to any of three major relationships between these two processes (fig. 6). Firstly, transcription might be part of the V(D)J recombination mechanism. For example, the RNA polymerase might be a component of the recombination complex. Secondly, transcription might alter substrate structure at the DNA or the chromatin level in a way that is necessary for the substrate to acquire its appropriate configuration. Thirdly, transcription and recombination might only be temporally related. That is, a chromatin accessibility change not caused by transcription may occur, thereby allowing nuclear proteins for both processes to gain access to the DNA.

Recent data on the relationship between transcription and V(D)J recombination permits us to rule out some of these possibilities. Integrated constructs that recapitulate the V(D)J recombination process suggested that removal of a eukaryotic promoter from the construct does not affect the recombination efficiency[17]. In addition, in the genome, there are circumstances where transcription can not be demonstrated through V, D or J segments that recombine well. Studies with integrated substrates and genomic analysis are complicated by the fact that the recombination rate is very difficult to measure. Extrachromosomal substrates provide a much more reliable way to measure recombination rates. Moreover, transcription can be controlled more easily without concerns of transcription from the surrounding DNA. In a study in which transcription was varied over a 1000-fold range on extrachromosomal substrates, there was no effect on the rate of V(D)J recombination[33]. In all studies of transcription, one is confronted with the issue of how low a transcriptional rate is still sufficient to activate the process. Nevertheless, the lack

of any dependence of V(D)J recombination on transcriptional variation over three orders of magnitude indicates that transcription is unlikely to be related to the recombination machinery or involved in any alterations of substrate structure at the DNA level. In addition, it is unlikely that transcription alters substrate structure in any necessary way at the simplest levels of chromatin structure (10 nm filament)[33]. Even the higher order chromatin structure effects (30 nm fiber) appear unlikely based on the observations with integrated substrates[17].

More recently, gene replacement analysis has also begun to support the view that transcription is inadequate as a means of activating a locus for V(D)J recombination. In one study, the heavy chain locus was altered by deletion of the J_H segments. This prevented any D to J_H joining. Despite the fact that the upstream V_H segments were intact and transcriptionally active, there was no V_H to D_H joining[10].

Given that neither the process of transcription nor the presence of promoters appear to play causal roles in making a locus accessible for V(D)J recombination, what *cis*-acting elements cause the conversion from the inaccessible to the accessible state? Recent data indicates that enhancers can determine chromatin accessibility for transcription and V(D)J recombination. In one study, the Ig heavy chain intron enhancer conferred position-independent transcriptional accessibility for an adjacent T7 promoter[38]. In another study, the V(D)J recombination of TCR Vβ-Dβ-Jβ constructs was determined by the type of enhancer in the construct. A TCR alpha locus enhancer resulted in the construct rearranging with a T cell developmental profile known to occur for the TCR alpha locus. A TCR beta locus enhancer resulted in the construct rearranging with a developmental profile of the endogenous TCR beta locus[9].

What structural features determine the inaccessible state for V(D)J recombination? Recent data indicate that CpG methylation is a key inhibitor of V(D)J recombination. These data come from both transgenic studies which correlated recombination with CpG methylation and from studies with extrachromosomal substrates[18,32]. However, is the effect of CpG methylation directly inhibitory to recombinase binding at the signals or does it create an inaccessible chromatin domain? Studies with extrachromosomal substrates indicate that the CpG methylation ushers the DNA into an inaccessible chromatin configuration only after the DNA has undergone replication[32]. That is, DNA that is methylated but has not replicated does not acquire the inaccessible chromatin configuration. Hence, the inhibitory effect of CpG methylation occurs by directing the DNA into an inaccessible chromatin state. Demethylation may then be required to achieve an accessible chromatin state. Methylation analysis of the endogenous antigen receptor loci is consistent with this. It is not clear how demethylation would be achieved.

In summary, there is no indication that transcription affects V(D)J recombination or that it targets a locus for this site-specific recombination reaction. Rather, both transcription and recombination occur at approximately the same time, but in a causally unlinked manner. Both processes are likely to occur after a chromatin change opens a previously inaccessible locus and makes it accessible.

Class switch recombination

In contrast to the site-specific nature of the V(D)J recombination reaction, class switch recombination is regionally-specific[24]. Like V(D)J recombination, there has long been observed a temporal association between transcription of the switch regions and their recombination. Upstream of each switch region is a promoter, which becomes active in response to cytokines and mitogenic stimuli that are known to cause the heavy chain isotype to switch from mu (in IgM) to gamma, alpha, or epsilon[50,65]. These transcripts are called sterile transcripts because they are not known to encode for a protein.

Recent information supports a much more important role for transcription in class switch recombination than has emerged for V(D)J recombination. Removal of the promoter in gene replacement studies ablates switch recombination[25]. Replacement of the inducible promoter with a constitutive promoter obviates the need for cytokine or mitogenic stimulation; hence, rearrangement occurs regardless of cytokine stimulation[73]. These studies indicate that the association between class switch recombination and transcription hardly is a purely temporal one.

The mechanistic basis for the association between class switch recombination and transcription is still not certain. The possibilities range from a mechanistic role to an alteration of substrate structure at the DNA or chromatin level (fig. 6). For example, the process of transcription may alter the DNA or chromatin substrate in a way that is necessary for recombination. Or the movement of RNA polymerase through the region may be sufficient, regardless of any change in DNA or chromatin. Or the RNA transcript may play a role in the reaction mechanism or in stabilizing some non-B DNA conformation.

In the absence of a functional experimental assay system for this reaction, it is difficult to distinguish among these possibilities. Although there is no cell-free reconstitution of this reaction, one line of cell-free analysis of the switch region DNA has been intriguing. The Griffin laboratory has done structural analysis of the alpha switch region[59]. This switch region is somewhat unique in that it contains one 30-nucleotide stretch of purines on one strand. Earlier work by their laboratory

showed that this 30-nucleotide region could form DNA triplexes under negative supercoiling conditions. Subsequently, they found that when in vitro transcription is directed in the physiologic orientation through the alpha switch region on a supercoiled plasmid, a change in supercoiling is observed. Moreover, this alteration is resistant to phenol extraction and is RNase A resistant and RNase H sensitive. These investigators proposed that DNA triplex formation occurs between the repeats within the switch regions. The fourth DNA strand not included in the DNA triplex formation would then be stabilized by the RNA strand generated by the process of transcription. This interesting set of observations has not yet been extended to the other switch regions, which do not have such polypurine stretches within them. Nevertheless, it provides the suggestion that transcription might be coupled to class switch recombination by causing an altered or non-B DNA substrate configuration. The nature, stability and role of such a non-B DNA configuration are uncertain.

What is the relationship between topoisomerases and transcription that may affect genetic recombination?

The common thread connecting the absence of topoisomerase function and the act of transcription is that they both cause an alteration in DNA topology. Topoisomerases remove positive and/or negative superhelicity from DNA[41,71]. In the absence of such a function, an imbalance occurs, leading to the potential recombinogenic lesions described below. The act of transcription itself similarly creates positive and negative superhelicity. This idea, the twin domain hypothesis, was first proposed by Liu and Wang[47].

Altered topology may promote genetic recombination by changing the accessibility of DNA sequences. For example, if single-stranded regions result from increased negative supercoiling in DNA, then these single-stranded regions may be more prone to breakage by nucleases. Another possibility is that altered superhelicity may change or alter chromatin structure[53]. For example, the altered DNA may not package histones properly, again resulting in increased accessibility to nuclease attack. Finally, accessibility may be altered by the occurrence of non-B DNA that can directly cause changes in superhelicity. For example, negatively supercoiled DNA can assume Z-DNA conformation or triple helical non-B conformations with greater ease[8,42,67]. These structures can be recognized by proteins that promote recombination or may initiate recombination events themselves.

On the other hand, altered topology may promote recombination by changing the process of recombination itself. For example, during strand uptake, the recA protein itself spools DNA from one molecule to another resulting in a change of DNA topology[31]. If a topoisomerase is removed from such a reaction, it could favor an event that gives rise to enhanced recombination. Additionally, a resolution event may be altered as a result of the change in topology. For example, the generation of rDNA rings in *top1 top2* mutant cells may be directly due to the failure to appropriately regulate the superhelicity of the rDNA leading to an effect on resolution of a Holliday junction intermediate.

There are many cases where a link has been established between transcription and recombination. On close examination of some of these cases, the link appears to be due to accessibility of the recombination machinery to a region rather than to the act of transcription itself. This is true for both V(D)J joining during immunoglobulin rearrangements and mating type interconversion in yeast. In the case of *HOT1* and *GAL10* in yeast, transcription stimulates recombination, but it is not a prerequisite, since basal levels of recombination can occur. Finally, there is one example, immunoglobulin class switching, in which transcription appears to be a prerequisite for rearrangements to take place.

Thus far, we have reviewed solely the relationships between either transcription and recombination, or topoisomerases and recombination. However, at rDNA, one can argue for an association between all three processes. Topoisomerase I has been shown to be involved in the regulation of rRNA transcription[63]. The loss of RNA polymerase I destabilizes the rDNA[57] and leads to a massive loss of repeats (Gangloff and Rothstein, unpubl. observ.; see also ref. 5). The extent of the rearrangements is greater than the effects observed in the absence of any of the topoisomerases, and suggests that transcription, topoisomerases and recombination are very closely linked in rDNA. One possible role for RNA polymerase I may be to attract the topoisomerases, and hence suppress recombination where it is most likely to occur (between repetitive DNA). This type of relationship may also be applicable to mammalian polII transcription where topoisomerase I has been shown to be part of the polII transcription complex and to be responsible for the activation or repression of this process at certain promoters[51].

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