V(D)J recombination plays a prominent role in the formation of the antigen receptor repertoires of B and T lymphocytes. It is also likely to be involved in the formation of chromosomal translocations, some of which may result from interchromosomal recombination. We have investigated the potential of the V(D)J recombination machinery to perform intermolecular recombination between two plasmids, either unlinked or linked by catenation. In either case, recombination occurs in trans to yield signal and coding joints, and the results do not support the existence of a mechanistic block to the formation of coding joints in trans. Instead, we observe that linearization of the substrate, which does not alter the cis or trans status of the recombination signals, causes a specific and dramatic reduction in coding joint formation. This unexpected result leads us to propose a “release and recapture” model for V(D)J recombination in which coding ends are frequently released from the postcleavage complex and the efficiency of coding joint formation is influenced by the efficiency with which such ends are recaptured by the complex.

This implies the existence of mechanisms, operative during recombination of chromosomal substrates, that act to prevent coding end release or to facilitate coding end recapture.

V(D)J recombination is the process of assembly of T-cell receptor and immunoglobulin genes from V, J, and sometimes D gene segments (1). Lymphoid-specific proteins RAG1 (recombination activating gene 1) and RAG2 (2, 3) and multiple ubiquitously expressed protein factors are essential for V(D)J recombination. RAG1 and RAG2 bind to and cleave at specific recombination signal sequences (RSSs)1 generating blunt signal ends and covalently sealed, hairpin coding ends (4–7). RSSs consist of conserved heptamer and nonamer sequences separated by a nonconserved spacer 12 or 23 base pairs long (12- and 23-RSS). V(D)J recombination occurs efficiently in vitro only between RSSs with different spacer lengths, a restriction known as the 12/23 rule (8).

Recent biochemical studies indicate that V(D)J recombination proceeds through a series of protein-DNA complexes. Prior to cleavage, the RAG1 and RAG2 proteins form stable complexes with individual and synapsed pairs of RSSs (9–17). After cleavage, the RAG proteins remain tightly bound to a synapsed pair of signal ends (15, 18) and also appear to interact with the two hairpin coding ends, albeit with lower affinity (15). Therefore, the immediate product of cleavage is thought to be a “cleaved signal complex” containing the four free ends (15).

Usually, RSSs involved in V(D)J recombination are located on the same chromosome, i.e. in cis. However, V(D)J recombination has been implicated in the formation of chromosomal translocations leading to lymphoid malignancies (19–23). In certain cases, such translocations could be the result of V(D)J recombination occurring in trans, using RSSs on different chromosomes. There have been attempts to address this issue. The first such study attempted, and failed, to detect V(D)J recombination in trans between two plasmids in a transient transfection assay (24). However, the assay used may not have been sufficiently sensitive to detect the expected level of trans recombination (see “Discussion”). More recently, it was shown that at relatively high concentrations, oligonucleotides separately containing 12- and 23-RSSs can synapse and undergo coupled cleavage in vitro (15). Furthermore, interchromosomal V(D)J recombination has been detected in Abelson virus-transformed pre-B cells (25). Finally, it was reported that 12- and 23-RSSs on separate transiently transfected plasmids could mediate relatively efficient synapsis, coupled cleavage, and signal joint formation. This study also reported a specific and dramatic defect in coding joint formation if the two RSSs were in trans, which led to the proposal that mechanisms exist that selectively disfavor or inhibit coding joint formation in trans (26).

In contrast, we find that both coding and signal joint formation can occur relatively efficiently in trans. Our results do not support the existence of the mechanistic block to trans coding joints formation. Instead, a series of experiments with circular, catenated, or linear substrates suggest a model in which the efficiency of coding joint formation is strongly influenced by the efficiency with which coding ends can be recaptured after being released from the post-cleavage complex. Our data suggest that in the transient transfection assay, the vast majority of coding joints form after at least one cycle of coding end release and recapture.

EXPERIMENTAL PROCEDURES

Cell Lines—The F2A1 cell line, expressing heat shock-inducible murine RAG1 (amino acids 264–1008) and RAG2 (amino acids 1–387), each containing a C-terminal extension of nine histidines and three copies of the c-myc epitope tag, has been described previously (27, 28). Dr3 cells express heat shock-inducible full-length murine RAG1 and RAG2 (29), whereas M12 is the parental cell line of F2A1 and Dr3 and does not express RAG1 or RAG2. Chinese hamster ovary (CHO) cells were grown in α-modified Eagle’s medium supplemented with 2 mM l-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 4 μg/ml each of adenosine, deoxyadenosine, and thymidine, and 10% fetal bovine serum.

Plasmids—To construct pAT2, the 6-resolvase site was amplified by PCR from pNG367 (30) (a gift from Dr. Nigel Grindley) with 5′-ccg agg...
In vitro V(D)J Recombination

tgt tta gta cca gca aag agt gga gta cta ctc ggt gat ctt ctt cat aat tca aat g and 5'-cca cca gga tgt cgg aat tat aat taa tta te primers for five cycles and then with the latter primer and 5'-cca cca aga tgt cgg agg gat gtt gta gca ctc for 25 additional cycles and subcloned into the BamHI and BgII sites of pH290 (31). The second y-J-resolution site was amplified from pNG367 using 5'-cca cca gga gct tgt cgg aat tat aat tta te and 5'-cca cca aga tgt cgg agg gat gtt gta gca ctc gtt ctt ctt cat aat tca aat g primers and subcloned into the BamHI and BgII sites of pTR2, thus deleting the 12- RSS. To delete the 23-RSS and produce pAT7, pAT2 was digested with SstI and recircularized.

In Vitro Cleavage Assay—Plasmid substrates (50 ng/μl) were treated with 1/10 volume of y-J-resolvase (a gift from Dr. Nigel Grindley) in 20 mM Tris-HCl, pH 8.3, and 10 mM MgCl2 for 1 h at 37 °C and then for 10 min at 70 °C. The y-J-resolvase stock solution was 10 μg/ml in 10 mM Tris-HCl, pH 7.5, 1 mM NaCl, 0.1 mM EDTA, 0.5 mM dithiothreitol, and 50% glycerol. Samples not requiring catenation were treated in an identical manner, except that y-J-resolvase was substituted with its storage buffer. Samples were then digested with XcmI or ApelIII in an appropriate buffer (New England Biolabs) for 1 h at 37 °C and 10 min at 70 °C. The y-J-resolvase stock solution was 10 μg/ml in 10 mM Tris-HCl, pH 7.5, 1 mM NaCl, 0.1 mM EDTA, 0.5 mM dithiothreitol, and 50% glycerol. Samples not requiring catenation were treated in an identical manner except that restriction endonuclease was substituted with 50% glycerol. To perform RAG-mediated cleavage, DNA samples (200 ng, 45 fmol) were incubated with 80 ng of RAG1 and 80 ng of RAG2 purified from F2A1 cells, and 0.3 ng/μl of murine HMG2 (high mobility group 2; expressed and purified from E. coli) (28) in 20 mM HEPES-NaOH, pH 7.5, 10 mM magnesium acetate, 50 mM sodium acetate, 10 μM ZnSO4, 2 mM dithiothreitol, 5% glycerol, and 0.1 mg/ml acetylated bovine serum albumin (total volume, 100 μl) for 3 h at 37 °C. In the negative control, RAG1 and RAG2 were substituted with 50% glycerol. After cleavage, Tris-HCl, pH 8.3, was then added to 75 mM, EDTA to 7.5 mM, SDS to 0.15%, and proteinase K to 0.2 mg/ml, and samples were incubated for 1 h at 55 °C. 10 μg of yeast tRNA was added as a carrier, NaCl was added to 200 mM, and the samples were ethanol precipitated. They were resuspended in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) containing 10 μg/ml of RNaseA, and samples that had not been treated with a restriction endonuclease were treated with it at this point. Half of the DNA was resolved on a 0.8% agarose gel, transferred to a GeneScreen Plus membrane (NEN Life Science Products), and hybridized to 32P-labeled AT2-2 probe. AT2-2 was excised from pAT2 with Apel and EcoO109I.

In Vivo Recombination—In experiments studying the effect of catenation, substrates were prepared as described above except that larger scale recombination reactions with y-J-resolvase were used and no RAG treatment was performed. In the experiments studying the effect of relaxation and linearization, the substrates (pAT2, pAT6, or pAT7) were treated or not treated with restriction endonuclease at this point, they were treated in an identical manner except that restriction endonuclease was substituted with 50% glycerol. After cleavage, Tris-HCl, pH 8.3, was then added to 75 mM, EDTA to 7.5 mM, SDS to 0.15%, and proteinase K to 0.2 mg/ml, and samples were incubated for 1 h at 55 °C. 10 μg of yeast tRNA was added as a carrier, NaCl was added to 200 mM, and the samples were ethanol precipitated. They were resuspended in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) containing 10 μg/ml of RNaseA, and samples that had not been treated with a restriction endonuclease were treated with it at this point. Half of the DNA was resolved on a 0.8% agarose gel, transferred to a GeneScreen Plus membrane (NEN Life Science Products), and hybridized to 32P-labeled AT2-2 probe. AT2-2 was excised from pAT2 with Apel and EcoO109I.

RESULTS

Cleavage in Trans in Vitro—We have examined the ability of RAG1 and RAG2 proteins to cleave large plasmid-based substrates with 12- and 23-signals in cis and in trans in the presence of HMG2 in vitro. Placing the RAG sites in trans may affect the reaction in two ways: first, the local concentration of one RAG in the vicinity of another RAG decreases; and second, the different topological location of the RAGs may affect the reaction via some unknown mechanism. To distinguish between these two possibilities, we constructed catenated substrates in which RAG sites were located on two different circles that were not covalently attached but were topologically connected. In this way, the local concentrations of RAGs in the vicinity of each other remained high as in the case of the cis configuration, and only the topological effect, if any, could be investigated.

To create such a substrate, we introduced two y-J-resolvase sites (30) into a plasmid containing two RAG sites to generate pAT2 (Fig. 1). Control substrates, identical to pAT2 but lacking either the 12-RSS (pAT6) or the 23-RSS (pAT7) were also constructed. Treatment of supercoiled pAT2 with y-J-resolvase yielded two catenated circles (labeled A and B in Fig. 1), each containing one RAG, whereas pAT6 yielded the same products but with the 12-RSS missing from circle A. Although the y-J-resolvase-mediated reaction did not go to completion, the RAG-generated cleavage products from pAT2 and the catenated circles could easily be distinguished by Southern blots using pAT2 and digoxigenin-labeled XcmI (Fig. 1). Supercoiled pAT2 yielded a 6.8-kb band when hybridized to XcmI (band 1 in Fig. 2), a 3.6-kb band when cleaved by the RAG proteins at only the 23-RSS (band 3), and a 3.1-kb band when cleaved at both RAG sites. pAT6 yielded bands at both 12-RSS or only at the 12-RSS (band 4). The catenated circle B (to which AT2-2 hybridizes) gives rise to a 4.2-kb band when linearized with XcmI (band 2 in Fig. 2) and a 0.9-kb band when cleaved at the 23-RSS (band 5).
plasmid pAT2 contains 12- and 23-RSSs (agarose gel, transferred, and hybridized with probe AT2-2. Treated with various combinations of gds, markers are shown in kb.

Treatment of pAT2 with y-resolvase generates catenated circles, each containing one RSS. Subsequent V(D)J recombination results in coding and signal joints that can be detected by PCR, with arrows indicating the approximate position of PCR primers and dotted lines indicating the locations of probes used for Southern hybridizations. Note that the primer pairs used to detect joints formed with the catenated substrate do not amplify products after V(D)J recombination of pAT2. The distance between the two RSSs in pAT2 is 405 base pairs, and the diagrams are not drawn to scale. The approximate locations of the unique XcmI and XcmI restriction enzyme sites are indicated.

We conclude that RSSs can undergo productive synapsis and coupled cleavage in trans. A similar conclusion was reached using oligonucleotide substrates at a concentration of 2 nM (15). It is clear that with plasmid DNA substrates at the concentration used in our experiments (0.45 nM), the two RSSs must either be in cis or constrained by catenation to undergo detectable synapsis.

V(D)J Recombination of Catenated Substrates in Vivo—To determine whether V(D)J recombination could occur in vivo between RSSs located in trans, we transfected cells expressing RAG1 and RAG2 with various plasmid substrates and measured the amounts of coding and signal joints formed by PCR. In most experiments, substrates were transfected by electroporation into the F2A1 cell line, which expresses truncated forms of RAG1 (amino acids 264–1008) and RAG2 (amino acids 1–387). Cells were heat shocked 6–10 h before electroporation to induce expression of RAG1 and RAG2, although omitting the heat shock produced equivalent results because of leaky expression of RAG1 and RAG2 in F2A1 (data not shown). Transfection efficiency was estimated by transfection with pCMVβ and staining for β-galactosidase as described under “Experimental Procedures.” Although substrates contained polyoma virus sequences necessary for replication, extrachromosomal DNA was harvested 14–16 h post-transfection, before detectable replication occurs (33). The relatively early time of harvest also reduced the possible effects on detection of recombination caused by unequal stability of various recombination products.

Recovered DNA was quantitated by UV absorbance and by PCR with primers complementary to a nonrearranging portion of the plasmid (polyoma backbone). Results obtained by the two methods were in good agreement, and the amount of input DNA in PCR reactions was calculated based on its UV absorbance. However, we were not able to determine the proportion of recovered DNA that had actually entered the cell (substantial amounts of substrate adhere to the outside of the cells). We note that experiments were repeated multiple times, and good reproducibility in the results was observed between similar samples within a given transfection experiment. Furthermore, although variations in the efficiency of transfection or DNA
recovery will affect the absolute number of signal and coding joints obtained, they should not affect the ratio of coding to signal joints because both are measured in the same DNA preparation. Hence this CJ/SJ ratio can be compared between different samples and different transfections using only the assumption that the relative detection efficiency of coding and signal joints was the same for all samples for the given PCR primers. As negative controls, we mock transfected F2A1 (omitting the electric pulse) or transfected substrates into M12, the parental line of F2A1 that does not express RAG1 or RAG2.

In the first group of experiments, we transfected catenated substrates identical to those used in the *in vitro* cleavage experiments (that is, a mixture of catenated and uncatenated plasmids, because the γδ resolvase reaction does not go to completion). We designed PCR primers (AT2a-f and Inne1 for coding joints, and AT2c-r and AT2b-r for signal joints; Fig. 1) that detected the recombination products of the catenated substrate but not those of the original noncatenated plasmid pAT2 (Fig. 1). PCR products were resolved on 5% native acrylamide gels, transferred to a membrane, and hybridized to AT2-5 or AT2-4 radiolabeled oligonucleotides specific for coding or signal joints, respectively. AT2-5 annealed to coding sequences on each side of and five base pairs away from the position of a precise coding joint and contained two inosine residues at the position spanning the joint, thereby reducing effects of coding joint diversity on the intensity of the hybridization signal. Blots were quantitated on a PhosphorImager.

We observed formation of both coding and signal joints using the catenated substrate with 12- and the 23-RSSs in *trans* (Fig. 3, lanes 3–6). Formation of these joints was dependent on the presence of RSSs on both catenated circles (data not shown). The catenated substrate was also digested with a restriction endonuclease that cleaved circle A, and in a separate reaction, with one that cleaved circle B, and the products of digestion were mixed together. This procedure yielded a sample containing the same circles as the catenated substrate but that were not catenated. Transfection of this sample also yielded coding and signal joints (Fig. 3, lanes 7–10). Together, the results demonstrate that coding and signal joints can be detected in *trans* and that this does not depend on catenation of the two recombining partners. We did not attempt to calculate a relative CJ/SJ ratio for these two samples. This experimental system was not explored further because it did not allow for a ready comparison of joining efficiencies in *cis* versus *trans* (different PCR primers were required for detection of joints with the two different configurations of RSSs).

*Cis versus Trans and the Effect of Substrate Topological State on Recombination in Vivo*—To determine the relative efficiencies of coding and signal joint formation in *cis* and *trans*, we repeated the transfection assay described in the previous section but compared pAT2 (RSSs in *cis*) with a mixture of pAT6 and pAT7 (RSSs in *trans*). In this situation, the same pairs of PCR primers (Stop2 and Inne1 for coding joints and Mark1 and AT2c-r for signal joints; Fig. 1) could be used to amplify joints for both the *cis* and *trans* configurations. This strategy is essentially identical to that employed in a recent analysis of the same issue (26). We also explored the effect of substrate topology by transfecting supercoiled, relaxed circular, and linearized forms of the substrates. Relaxed circular and linear plasmids were generated by treatment of supercoiled plasmids with topoisomerase I and NcoI, respectively. The results of agarose gel electrophoresis of the various topological forms of the substrates are shown in Fig. 4.

The initial experiment revealed that relaxation of supercoiling had little if any effect on the relative efficiencies of coding and signal joint formation (Fig. 5A, compare lane 2 with lane 3 and lane 5 with lanes 6 and 7). In contrast, linearization led to a dramatic reduction in coding joint formation relative to signal joint formation (compare lane 2 with lane 4 and lane 5 with lanes 8 and 9), and linearization of both pAT6 and pAT7 had a greater effect in this regard than linearization of either one alone (compare lane 10 with lanes 8 and 9).

Placing the RSSs in *trans* resulted in a reduction in both signal and coding joint formation compared with the *cis* configuration (Fig. 5A; note the different exposure times for coding
Joint Detection in cis and trans. This was best visualized and quantitated in the experiment shown in Fig. 5B (compare lanes 2–5 with lanes 11–13), in which the amount of template added to the PCR reactions was varied, and a single exposure time for each assay is shown. PhosphorImager quantitation of these data revealed that placing the RSSs in trans reduced signal and coding joint formation 8- and 40-fold, respectively, compared with the cis configuration. However, as discussed above, it is more meaningful to compare the CJ/SJ ratio for the cis-circular substrate divided by that for the trans-circular substrates, indicating a 5-fold increase in coding joint formation relative to signal joint formation for the former compared with the latter.

Far (143 base pairs) from the coding junction, coding joint detection was identical to that with AT2-5 (data not shown). Most (90%) signal joints formed in the experiments with F2A1 were precise fusions of the two RSSs, as determined by sensitivity of the PCR products to ApaLI digestion (data not shown). This was true regardless of substrate topology. Representative coding joints were cloned and sequenced for supercoiled and linear pAT2 (RSSs in cis) and for supercoiled pAT6 plus supercoiled pAT7 (RSSs in trans). In all cases, coding joints were found to have the expected structure, with deletion of small numbers of nucleotides from one or both coding ends (data not shown). We note that the clones sequenced were chosen at random and were not selected based on hybridization to the AT2-5 probe.

These results lead to two primary conclusions. First, synapsis, cleavage, and signal and coding joint formation can occur with RSSs located in trans. Second, substrate linearization (which does not alter the cis/trans configuration of the RSSs) has a much larger effect on the relative efficiency of coding joint formation than does changing from a cis to a trans configuration. A model to explain these results is presented below.

**DISCUSSION**

V(D)J recombination in trans is an important issue because of its potential involvement in a variety of chromosomal rearrangements leading to the development of neoplasias (19–23, 34). However, it remains difficult to study V(D)J recombination in trans directly using chromosomal substrates, primarily because of the low efficiency of the reaction.

In this paper, we show that the cleavage step of V(D)J recombination can be performed in vitro in the presence of Mg2+ and HMG2 with a substrate containing 12- and 23-RSSs in trans. Cleavage is stimulated by the presence of the appropriate...
brate partner RSS in trans and, based on previous studies, is likely to occur in the context of a synaptic complex containing the two RSSs (15, 35). Efficient cleavage in trans was also obtained using oligonucleotide substrates at RSS concentrations somewhat higher than those used in our experiments (2 nM versus 0.45 nM) (15). Taken together, the results suggest that the reduction of cleavage efficiency we observe for RSSs on unlinked molecules is due to the decrease in RSS local concentration and not to their topological (cis versus trans) location.

Given that cleavage in vitro can occur efficiently in trans, it was possible that V(D)J recombination in vivo might also occur in trans. An early study attempted to recover the dimeric circle that would result from recombination in trans between two extrachromosomal substrates but failed after screening 40,000 recovered plasmids (24). The authors of this study did not draw strong conclusions based on these results, but more recently, the results were cited as evidence against V(D)J recombination in trans (26). This does not appear to be a valid argument. The recombination frequency in the PD31 cell line used was sufficiently low that only 150 recombinants would have been expected for RSSs in cis, and several factors would have reduced that number for the trans configuration: (i) many cells would likely have taken up only one of the two plasmids because of the poor transfectability of this cell line; (ii) even in those cells that obtained both plasmids, our results and those of Han et al. (26) indicate that the events leading to signal joint formation would be reduced in efficiency by an order of magnitude because of the trans configuration; and (iii) the only plasmids that would have been scored as trans-recombinants were those that contained both a signal and a coding joint, and prior evidence indicates that this can be quite inefficient because the efficiency of joint formation after cleavage is not 100% (36, 37). Hence, it is likely that the methods employed in this early study were not sufficiently sensitive to detect trans recombination.

V(D)J recombination between antigen receptor loci located on different chromosomes has been detected in normal lymphoid cells (reviewed in Ref. 1), but the low frequency of these events (on the order of one/10^6 lymphocytes) is consistent with the possibility that they occur subsequent to a chromosomal translocation that puts the two loci in cis (1). Recombination between the murine Ig κ and λ loci has been detected in an Abelson murine leukemia virus transformed pre-B cell line, at a frequency about 1000-fold lower than rearrangements in cis (25). These events were unusual in that they were mediated by two 12-RSSs and generated atypical, imprecise signal joints. Overall, these studies indicate that V(D)J recombination between RSSs located on different chromosomes is a rare event.

Our results demonstrate that extrachromosomal substrates containing RSSs in trans can successfully recombine in vivo to yield both signal and coding joints. This was true for RSSs on catenated, circular substrates and for RSSs on unlinked DNA molecules. We conclude that there is nothing intrinsic to the V(D)J recombination reaction that prevents synopsis, cleavage, and joint formation with RSSs located in trans. This, together with the very low frequency of interchromosomal V(D)J recombination events, suggests that regulatory mechanisms exist to suppress V(D)J recombination between different chromosomes. We infer that these mechanisms do not function, or function inefficiently, with transiently transfected extrachromosomal elements. Although the nature of these mechanisms are unknown, it is reasonable to think that they act to inhibit synopsis of inappropriate RSS pairs, either those on different chromosomes or in different antigen receptor loci (38).

How is it that, with extrachromosomal substrates, RSSs in trans undergo synopsis and cleavage at levels only moderately reduced (less than an order of magnitude) compared with the cis configuration? One possibility, raised by the fact that the 12-RSS and 23-RSS in pAT7 and pAT6 are contained in nearly identical plasmid backbones, is that pairing of homologous sequences between different plasmid molecules increases the effective local concentration of the two RSSs. This notion is consistent with the finding that the human homologous pairing protein HPP-1 is an important component of an in vitro end joining system that preferentially joins homologous ends (39). It will be important to determine whether the efficiency of trans V(D)J recombination is altered when the two RSSs reside in plasmids lacking homology.

Using circular substrates, placing the RSSs in trans resulted in roughly 8- and 40-fold decreases in signal and coding joint formation, respectively, compared with the cis configuration. The decrease in signal joint formation that we observe is in good agreement with the 5–10-fold decrease reported in a recent study by Han et al. (26). This study, which was quite similar to ours in design, also assessed the efficiency of RAG-mediated cleavage of the transfected substrates by measuring the abundance of signal ends. Substrates with RSSs in trans yielded about 10-fold fewer signal ends than did a substrate with RSSs in cis, a decrease that mirrored the drop in signal joints (26). A simple explanation of these results is that RSSs in trans are less likely to synapse and undergo cleavage, resulting in the parallel drop in cleaved products and signal joints.

Surprisingly, the aforementioned study found that intermolecular coding joint formation was at least 1000-fold less efficient than intramolecular coding joint formation, yielding a greater than 100-fold drop in the CJ/SJ ratio (26). In contrast, we find only a modest 5-fold decrease in this ratio. Each study examined lymphoid and nonlymphoid cell lines and truncated and full-length RAG proteins. There are no obvious methodological differences between the two studies apart from the method used to recover transfected plasmid DNA (alkaline lysis followed by Qiagen spin column purification in this study; a nondenaturing Hirt method in the study of Han et al.), and this does not appear to provide a straightforward explanation for the differences in the results.

To explain the dramatic drop in intermolecular coding joints formation in trans, Han et al. postulated the existence of a mechanism capable of discerning whether two RSSs are in cis or in trans at an early step in the reaction and transmitting this information to the postcleavage complex in such a way as to allow signal but not coding joint formation. Because antigen receptor loci are typically quite large, such a mechanism would have to be able to distinguish between synopsis of two RSSs hundreds of kilobases apart on the same chromosome from synopsis of two RSSs on different chromosomes.

Our results lead to a quite different model in which there is no intrinsic difference between intermolecular and intramolecular recombination. Rather, we propose that the specific reduction in coding joint formation we observe when two RSSs are in trans or on linear substrates is explained by inefficient retention of coding ends, but not signal ends, in the postcleavage complex. This is consistent with biochemical experiments demonstrating that signal ends are bound much more tightly than coding ends after cleavage (14, 18). Our model predicts frequent accidental release of a coding end from the postcleavage synaptic complex. In addition, it predicts that this coding end can be recaptured by the synaptic complex and successfully incorporated into a coding joint (Fig. 6). The effects we observe with a trans configuration of RSSs or with linearized substrates can be explained by a decreased efficiency of recapture of the released coding end with these substrates.

Consider first the 5-fold decrease in the CJ/SJ ratio observed for recombination in trans compared with cis. The recapture of
a coding end is influenced by the local concentration of the end in the vicinity of the synaptic complex. In a simple model, this in turn depends on the length of the DNA segment connecting the coding end to the signal end, which is acting to tether the coding end to the synaptic complex. With circular substrates, the cis and trans configurations give rise to structurally very similar putative postcleavage complexes (Fig. 6, A and C), with one important difference: in the cis configuration, the lengths of the “tethers” are 6.4 and 0.4 kb, whereas for the trans configuration, the lengths are both 6.8 kb. Theoretical and empirical considerations (40) indicate that the difference between 0.4 and 6.8 kb for one end can account for the 5-fold decrease in the CJ/SJ ratio during recombination (7, 41–43). Together with our results and the considerations noted above, these findings strongly suggest that mechanisms exist to stabilize the postcleavage complex (either by increasing the release or reducing the recapture of coding ends) leads to reduced coding joint, but not signal joint, formation.

During V(D)J recombination of endogenous antigen receptor genes, one or both of the coding ends is not tethered to a signal end, and yet coding joints appear to form efficiently and quickly. In particular, coding ends are difficult to detect in normal lymphoid precursors and in cell lines actively performing recombination (7, 41–43). Together with our results and the considerations noted above, these findings strongly suggest that mechanisms exist to stabilize the postcleavage complex during recombination of endogenous antigen receptor loci and that these mechanisms do not act or act inefficiently with

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FIG. 6. Release and recapture model for V(D)J recombination. Proteins of the postcleavage complex are indicated by a shaded oval, coding ends are shown as rectangles, and signal ends are shown as triangles. Signal ends are assumed to be held tightly in the complex. In contrast, coding ends are proposed to be readily released, with the efficiency of recapture depending on whether the coding end is tethered to a signal end, and if so, how long the tethering piece of DNA is. If one or both coding ends are released and not recaptured, only signal joint formation can occur. A, in cis with a circular substrate. B, in cis with a linearized substrate. C, in trans with circular substrates. D, in trans with linearized substrates. Note that the postcleavage complexes in A and C differ only in the lengths of the DNA strands linking the coding ends to the signal ends (0.4 and 6.4 kb in A and 6.8 and 6.8 kb in B).

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2 E. A. Agard and S. M. Lewis, submitted for publication.
transiently transfected substrates. These mechanisms may function to prevent the release of coding ends or may act to retain released coding ends in the vicinity of the synaptic complex for rapid recapture. Intriguingly, deletion of the T-cell receptor β enhancer from the mouse germline preferentially interferes with coding joint formation compared with signal joint formation, leading to the suggestion that factors associated with the enhancer may act to stabilize coding ends in the postcleavage complex (44).

Our ability to detect significant amounts of both coding and signal joints in trans further supports the possibility of a direct involvement of V(D)J recombination in the generation of chromosomal translocations. The rate of V(D)J recombination in trans on chromosomal substrates, and especially of the relevant loci, remains to be determined. We expect that it will be significantly lower than on extrachromosomal circles. The rate of loss and recapture of coding ends of chromosomal substrates of loss and recapture of coding ends of chromosomal substrates of V(D)J recombination in the generation of chromosomal translocations. The rate of V(D)J recombination in trans on chromosomal substrates, and especially of the relevant loci, remains to be determined. We expect that it will be significantly lower than on extrachromosomal circles. The rate of loss and recapture of coding ends of chromosomal substrates by the synaptic complex also remains to be investigated.

Acknowledgments—We are grateful to Nigel Grindley for the kind gift of ψφ-resolvase and pNG367. We also thank Quinn Eastman and Isabelle Villey for purified RAG1, RAG2, and HMG2 proteins and for help with the in vitro cleavage assay. We thank Alfred Lee for insightful suggestions concerning coding end release and recapture and Emily Agard and Susanna Lewis for sharing their unpublished results. We are grateful to Susanna Lewis for numerous helpful suggestions on the manuscript and an anonymous reviewer for ideas concerning homologous pairing.

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J. Biol. Chem. 2000, 275:8341-8348.
doi: 10.1074/jbc.275.12.8341

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