index of primary productivity with caution, although on a regional scale the two components are recognized as coherent (*31–34*). In an investigation of the productivity index in the Southern California Bight, Smith and Baker (*35*) found

$$\log P_T = 1.254 + 0.728 \log C_K$$

where P_T is the productivity (in milligrams of carbon per square meter per day) as measured on the ship. This equation presents a possible methodology for deducing regional productivity. We must emphasize that the satellite only detects upwelling spectral radiation from a depth of approximately 1 optical attenuation length, K. Inferences beyond this $1/K$ depth (in meters) to the 1 percent light level ($4.6/K$) depth are based upon regressions (*5, 36*) derived from ship vertical-profile data. Use of the above regression, although inexact, does permit a comparison of productivity in these regions. The last two columns of Table 1 give P_T integrated over the bloom period per square meter per day and the areally integrated result, respectively. The area shoreward of the 200-m isobath between Cape Hatteras and 70°W constitutes the shelf region, and the area between the mean location of the northern edge of the Gulf Stream (*37*) and the 200-m isobath constitutes the slope region for the calculation in Table 1. The calculated productivity integrated per meter square is comparable for the slope and the shelf, whereas the values for the ring and Gulf Stream are less by a factor of 2. These data are in agreement with the available ^{14}C determinations of primary productivity (*38*). The total productivity in clear waters can be within a factor of 2 of that observed in the much more eutrophic waters because of the vertical weighting by euphotic depth, which is part of this calculation. If the P_T-C_K relationship were exact, then, based on the estimated upper limit to the error in C_K, the P_T estimates should be within 15 percent for the shelf and slope and within 30 percent for the clearer waters.

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A Large Deletion Within the T-Cell Receptor Beta-Chain Gene Complex in New Zealand White Mice

Abstract. *The T-cell receptor β-chain gene complex contains a duplication of D_β, J_β, and C_β gene segments in mice and man. When DNA from many inbred strains of mice was screened an unusual allele of the beta locus was identified in New Zealand White (NZW) mice. This allele is distinguished by the deletion of an 8.8-kilobase segment of DNA containing $C_{\beta 1}$, $D_{\beta 2}$ and the $J_{\beta 2}$ cluster. Despite the fact that all NZW T-cell receptors must be derived from a single set of β-chain gene segments, this strain has functional T cells and is phenotypically normal. This deletion of T-cell receptor β-chain segments occurs in a strain known to contribute to lupus-like autoimmune disease.*

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New Zealand White (NZW) mice have generated interest because of their role in the autoimmunity of New Zealand Black (NZB) × NZW F_1 hybrids. Although NZB mice develop an autoimmune hemolytic anemia as the major feature of their autoimmune disease, NZB × NZW F_1 mice exhibit (i) marked antibody production to nuclear antigens, (ii) high serum levels of antibodies to double-stranded DNA, and (iii) a fatal immune-complex glomerulonephritis (*1*). These features are similar to those observed in human systemic lupus erythematosus (SLE). Despite their genetic contribution to NZB × NZW F_1 autoimmunity, NZW mice are phenotypically normal and they have normal life spans (*2*). The basis of their genetic contribution to the F_1 disease has not been determined (*3*).

T-lymphocyte antigen receptors restricted to the major histocompatibility complex (MHC) are disulfide-linked het-

erodimers composed of α and β chains (*4*). Recently, complementary DNA (cDNA) clones for the α and β chains have been isolated, and like immunoglobulin genes, their structure reveals the presence of variable (V), joining (J) and constant (C) region gene segments (*5*). The β-chain cDNA's contain diversity (D) gene segments as well. The β-chain genes are located on chromosome 6 in the mouse and on chromosome 7 in man, and the genomic organization of the β locus has been well characterized (*6*). The β-gene complex contains two highly similar C region genes ($C_{\beta 1}$ and $C_{\beta 2}$), each with a cluster of J_β gene segments and a single D_β gene segment (as shown below). The duplication of D_β, J_β, and C_β gene segments is present in all mouse strains studied to date and in man, although the significance of this genetic redundancy is still unclear.

In screening DNA from NZW mice, we have identified an unusual allele of the β locus. A large segment of DNA encoding several β-chain gene segments has been deleted from the NZW genome, generating a β-gene complex that contains a single D_β gene segment, a single cluster of J_β gene segments and a single C_β gene segment.

The unusual organization of the β-chain locus in NZW mice was first noted while screening NZW, NZB, BALB/c, and SJL DNA for β-chain gene polymorphism. A Southern blot of liver DNA from these four different mouse strains was probed with a ^{32}P-labeled β-chain cDNA fragment (*7*) that detects both $C_{\beta 1}$ and $C_{\beta 2}$ germline genes (Fig. 1A). The NZW DNA contains a single fragment that hybridizes to the C_β probe, regardless of the enzyme used to digest the DNA. Since it is highly unlikely that five different restriction enzymes would generate two C_β restriction fragments of similar length in NZW DNA, these results indicate that NZW mice carry a single C_β gene. As expected, two restriction fragments that hybridize to the C_β probe are present in BALB/c, NZB, and SJL DNA. There are actually two C_β restriction fragments (6.0 and 6.2 kb) in the Pvu II digests of NZB and BALB/c DNA which were not resolved in this particular experiment. The C_β fragments in NZB DNA are indistinguishable from those seen in BALB/c DNA, and several restriction enzymes reveal C_β fragments that are polymorphic in SJL DNA (*8*).

To determine which Cβ gene is present in NZW DNA, a fragment that distinguishes $C_{\beta 2}$ from $C_{\beta 1}$ was prepared from the 3′ untranslated region of a $C_{\beta 2}$ cDNA clone. Fragments carrying $C_{\beta 2}$ are identified in NZB, BALB/c, and SJL DNA, but fragments containing $C_{\beta 1}$ genes are not detected with this probe (Fig. 1B). NZW DNA also contains a single fragment hybridizing with the $C_{\beta 2}$-specific probe. Similar experiments with a $C_{\beta 1}$-specific probe (*7*) reveal $C_{\beta 1}$ restriction fragments in BALB/c, NZB, and SJL but not in NZW DNA (*9*). Taken together these data indicate that the NZW β-chain gene complex contains $C_{\beta 2}$ but not $C_{\beta 1}$.

We also considered the possibility that other β-chain gene segments are absent from NZW mice. Therefore, a 1.8-kb genomic Pst 1 fragment containing $D_{\beta 1}$ and flanking sequences (*7*) was used to probe liver DNA from NZW, NZB, BALB/c, and SJL mice (Fig. 2A). The NZW mice contained the $D_{\beta 1}$ gene segment on restriction fragments identical to those seen in BALB/c and NZB DNA. Since these restriction fragments contain part or all of the $J_{\beta 1}$ cluster, the data imply that $J_{\beta 1}$ genes are present in the germline of NZW mice. Liver DNA from these strains was also probed with a 2.4-kb genomic fragment containing $D_{\beta 2}$ and flanking sequences (*7*) (Fig. 2B). While restriction fragments hybridizing to $D_{\beta 2}$ are present in BALB/c and SJL mice, this 2.4-kb fragment does not hybridize to NZW DNA, indicating that the genomic fragment containing the $D_{\beta 2}$ gene segment is not present in the NZW genome. Examination of mouse DNA for the presence of $J_{\beta 2}$ sequences (*7*) revealed that the $J_{\beta 2}$ cluster is present in BALB/c, NZB, and SJL, but that it is absent from NZW DNA (*9*).

A ^{32}P-labeled variable region fragment isolated from a β-chain cDNA (*7*) was used to probe genomic DNA from several mouse strains. This particular V_β gene is present in NZW DNA and is indistinguishable from the V_β in BALB/c and NZB mice (*9*). NZW mice were also examined for polymorphism at the α-chain locus (*7*). A Southern analysis of various DNA's revealed no obvious polymorphic differences at the α locus among NZW, NZB, and BALB/c mice. Although the examination of T-cell receptor genes in NZW mice has not been exhaustive, the most striking differences seem to be confined to the segment of the β-chain locus containing D_β, J_β, and C_β genes.

A restriction map of this unusual β-chain gene complex was constructed with the use of the above data and compared to the restriction map (*6*) of the β-

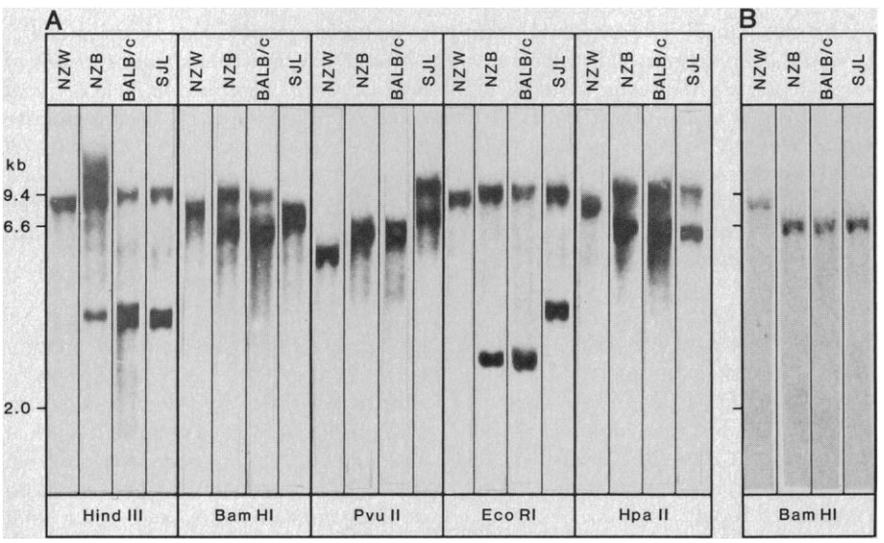

Fig. 1. (A) Southern analysis of liver DNA with a C_β probe. Liver DNA from various strains was isolated as in (*23*) and digested with different restriction enzymes. Digested DNA's were subjected to electrophoresis through 0.7 percent agarose gels, transferred to nitrocellulose filters, and hybridized with a ^{32}P-labeled probe as described (*24*); the acid depurination step was omitted and the gel was irradiated for 5 minutes with shortwave ultraviolet light prior to the denaturation step. Filters were washed several times in 2× SSC (1× SSC is 0.15*M* NaCl, 0.015*M* sodium citrate) at room temperature and once in 0.1× SSC at 55°C for 30 minutes. Hybridizing fragments were detected by autoradiography. The C_β probe, pDOβ2, is derived from a $C_{\beta 2}$ cDNA clone and hybridizes to restriction fragments containing $C_{\beta 1}$ and $C_{\beta 2}$ (probe C in Fig. 3A). The probe is described in (*25*). There are actually two C_β-containing restriction fragments (6.0 and 6.2 kb) in the Pvu II digests of NZB and BALB/c DNA which are not resolved in this particular experiment. Mice are described in (*26*). (B) Southern analysis of liver DNA's with a $C_{\beta 2}$-specific probe. The experiment was carried out according to the legend of Fig. 1A except that the probe consisted of 3′ untranslated exon of the $C_{\beta 2}$ cDNA clone (Fig. 1A) (probe 2 in Fig. 3A). This 260-bp Sau 3A–Eco RI fragment distinguishes $C_{\beta 2}$ from $C_{\beta 1}$ (*7*).

chain allele in B10 mice (Fig. 3A). The B10 restriction map is valid for BALB/c mice in that we have not discovered any polymorphic differences between B10 and BALB/c mice at this locus (*10*). The restriction map for the β-chain allele in NZW mice accounts for (i) the presence of $D_{\beta 1}$ and flanking DNA sequences that are contained on restriction fragments identical in size to those seen in BALB/c DNA; (ii) the absence of DNA that hybridizes to the 3′ untranslated exon of the $C_{\beta 1}$ gene; (iii) the absence of DNA that hybridizes to the $D_{\beta 2}$ gene segment and 2.3 kb of flanking DNA; (iv) the absence of DNA that hybridizes to the $J_{\beta 2}$ cluster; (v) the presence of a single C_β gene that contains the 3′ untranslated exon of $C_{\beta 2}$; and (vi) the sizes of seven different C_β-containing restriction fragments seen in NZW DNA. Thus, the NZW β-chain allele is related to the B10 allele except for the deletion of 8.8 kb of DNA containing $C_{\beta 1}$, $D_{\beta 2}$, and the $J_{\beta 2}$ cluster. The deletion begins 5′ to $C_{\beta 1}$ and ends 5′ to $C_{\beta 2}$, which may have implications regarding the mechanism by which the NZW β-gene complex was generated (see below). In view of the organization of β-chain genes in NZW mice, all T-cell receptor β chains in this strain are derived from a combination of $D_{\beta 1}$, $J_{\beta 1}$, and $C_{\beta 2}$ gene segments.

To assess the effect of this deletion on T-cell function, T cells from NZW mice were analyzed for the presence of different surface markers and for the ability to function in different assays. The major T-cell subsets are normally represented in NZW spleen. The percentages of Thy-1.2, Lyt-1, Lyt-2, and L3T4 bearing cells are similar to those seen in BALB/c and CBA/J mice (*11*). The in vitro proliferative responses to the T-cell mitogens phytohemagglutinin (PHA) and concanavalin A (ConA) are also similar to those with cells from normal strains. Stimulation indices are greater than 20 for both mitogens (*11*). We also studied the in vivo primary antibody response to injected sheep red blood cells (SRBC), which requires antigen-specific helper T cells (*12*). NZW and BALB/c mice are similar in their total and immunoglobulin G (IgG) antibody responses to SRBC. The kinetics of these antibody responses are also similar in the two strains (*11*). Our data are consistent with those (*13*) demonstrating a normal antibody response to other T cell–dependent antigens such as keyhole-limpet hemocyanin (KLH). In vitro proliferative responses to $H\text{-}2^b$ and $H\text{-}2^d$ allogeneic cells (in the mixed leukocyte reaction) are within the normal range (*11*).

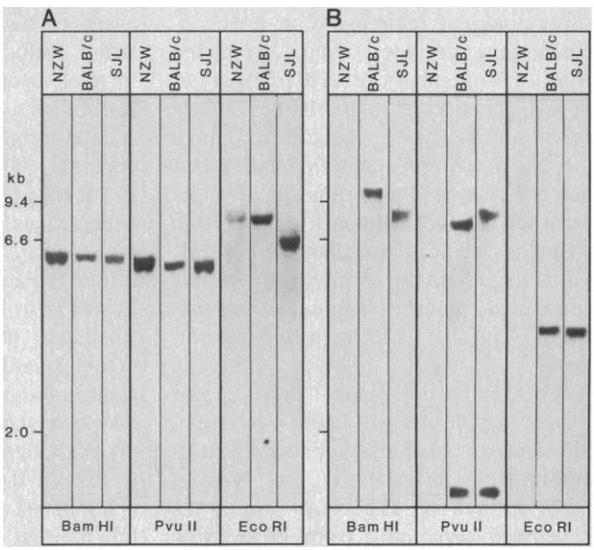

Fig. 2. (A) Southern analysis of liver DNA's with a $D_{\beta 1}$ genomic probe. Southern analysis was performed as described in Fig. 1A. The probe was a 1.8-kb Pst fragment containing $D_{\beta 1}$ and flanking DNA. The probe (probe A in Fig. 3A) is described in (*7*). (B) Southern analysis of liver DNA's with $D_{\beta 2}$ genomic probe. Southern analysis was performed as in the legend to Fig. 1A. The probe was a 2.4-kb Eco RI–Hind III fragment containing $D_{\beta 2}$ and flanking DNA. The probe (probe E in Fig. 3A) is described in (*7*).

This deletion of β-chain gene segments is rare among strains of mice. We and others (*6*) have examined a number of inbred strains including BALB/c, DBA/2, AKR, SJL, SWR, C57BL10, C57BL/6, C57BR, C57LE, MRL, and NZB and have always found the C_β genes to be duplicated. The duplication of β-chain gene segments is present in human DNA as well (*14*), indicating that this arrangement of β-chain genes may be common among vertebrate organisms with T cells. If one considers the $C_{\beta 1}$ and $C_{\beta 2}$ coding sequences, they are 96 percent homologous in mice and 97 percent homologous in man. On the other hand, the 3′ untranslated exons of $C_{\beta 1}$ and $C_{\beta 2}$ are only 45 and 23 percent homologous in mice and man, respectively. Assuming a rate of genetic drift of 1 percent per 2.2×10^6 years, Gascoigne *et al.* (*6*) calculated that these two genes may be the product of a duplication which occurred $\sim 1.2 \times 10^8$ years ago. Despite the age of the duplication and the stringent selection on both genes, there is no correlation of helper or cytotoxic function with expression of $C_{\beta 1}$ or $C_{\beta 2}$ (*15*). NZW mice further illustrate this point since both functional cytotoxic and helper T cells are present in a strain that has deleted $C_{\beta 1}$ (*11, 13, 16, 17*).

Since $C_{\beta 1}$ and $C_{\beta 2}$ appear to be functionally equivalent, it is possible that the duplication has been maintained in order to double the number of germline D_β and J_β gene segments. This might serve to diversify the T-cell repertoire. If the number of T-cell specificities is generated in a purely combinatorial fashion, then one would expect that deleting one-half of the D_β and one-half of the J_β genes would have a marked effect on the number of T-cell specificities in this strain. The occurrence of this deletion in NZW mice is not lethal, although mortality is a rather insensitive measure of the size of a particular T-cell repertoire, especially in a strain maintained in the laboratory. In addition, T-cell responsiveness to antigens is not obviously depressed in NZW mice although further study of T-cell receptor specificities in NZW mice should clarify the contribution of D_β and J_β gene segments to repertoire diversity. In that NZW mice have a rare H-2 haplotype [$H\text{-}2^z$] (*18*) and in view of the role of self-MHC in T-cell recognition of antigens, this unusual β-chain allele and rare H-2 haplotype may have possibly been co-selected.

The NZW β-chain allele was most likely generated by a meiotic recombination between two homologs of chromosome 6 (Fig. 3B). This kind of event would give rise to one homolog with a deletion (that is, the NZW allele) and another with a duplication of β-chain gene segments. Misalignment of the two chromosome homologs occurred between the coding exons of $C_{\beta 1}$ on one homolog and the coding exons of $C_{\beta 2}$ on the other. This is certainly plausible given the remarkable conservation of nucleotide sequences between $C_{\beta 1}$ and $C_{\beta 2}$ (96 percent homology in mice and 97 percent homology in man). Our data suggest that the actual recombination occurred not within the C_β coding exons but within intervening DNA sequences 5′ to $C_{\beta 1}$ and $C_{\beta 2}$.

One might expect this allele to be relatively frequent in a population since the heterozygote would not be at a serious selective disadvantage. To account for the rarity of this allele, at least among inbred mice and outbred humans, we propose a suppression of recombination

in this locus. Additional evidence for suppression of recombination in the β-chain gene complex comes from the work of Kronenberg *et al.* (*15*). Analysis of 65 $J_{\beta 1}$ clusters in 35 T-cell lines did not reveal any $J_{\beta 1}$ cluster joined to $C_{\beta 2}$ as in the NZW genome. Mitotic recombination which would generate this arrangement of β-gene segments occurs at a frequency of not more than 1.5 percent. To achieve greater confidence in this conclusion, more β-chain gene complexes in T-cell lines will have to be examined.

We initially studied the T-cell receptor β-gene complex in NZW mice because of the known contribution of this strain to autoimmune disease in the NZB × NZW F_1 hybrid. The NZW strain was developed separately from other New Zealand strains and therefore the presence of this allele in the NZW and not in the NZB is not surprising (*19*). The NZB × NZW F_1 hybrid exhibits a markedly different autoimmune process compared to the disease seen in the NZB strain, including high levels of antibodies to nuclear antigens, high levels of IgG antibodies to double-stranded DNA, and a fatal immune-complex glomerulonephritis (*1*). The nature of the NZW genetic contribution remains unclear. It has been suggested that NZW mice are high responders to DNA and contribute this trait to the autoimmune NZB × NZW F_1 hybrid (*20*). Backcross studies are most consistent with the contribution being complex and related to more than one genetic locus, with evidence that some involved genes may be linked to $H\text{-}2^z$ (*3*). Although NZB × NZW mice appear to have a B-cell defect (also present in NZB mice) which is necessary for the full expression of the disease (*3, 21*), there is considerable evidence that T cells are also important for the production of antibody (IgG) to nuclear antigens (*3, 22*). Whether the T-cell component of the F_1 autoimmune disease is related to the deletion present in the NZW β-chain gene complex remains to be determined.

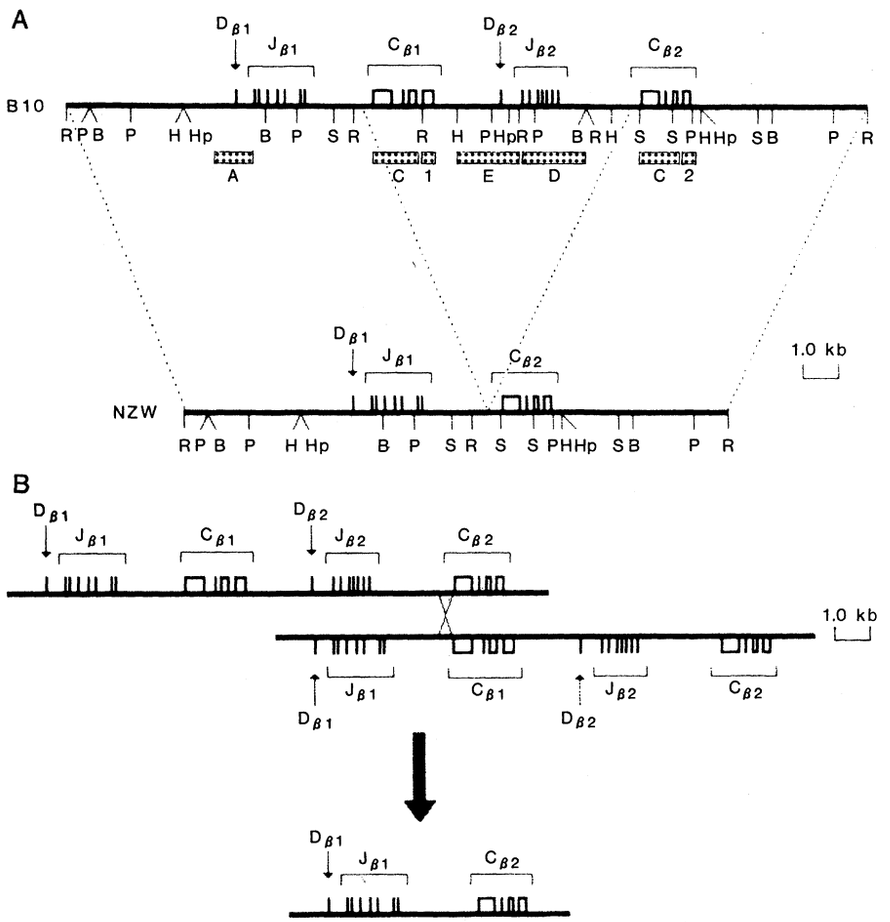

Fig. 3. (A) Restriction maps of two alleles of the β-chain gene complex in mice. The B10 restriction map is described (*6*), and the NZW restriction map is derived from the Southern analyses of NZW DNA. Abbreviations: B, Bam HI; E, Eco RI; H, Hind III; Hp, Hpa I; P, Pvu II; S, Sst I; A, $D_{\beta 1}$ probe; C, C_β probe; 1, $C_{\beta 1}$-specific probe; E, $D_{\beta 2}$ probe; D, $J_{\beta 2}$ probe; 2, $C_{\beta 2}$-specific probe. The probes are described in (*7*). (B) Probable recombination which generated the deletion in the NZW β-chain gene complex. A meiotic recombination between two chromosome 6 homologs may have produced a deletion of several β-chain gene segments, generating the allelic form of the β locus, which is present in NZW mice.

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Discovery of a Predicted DNA Knot Substantiates a Model for Site-Specific Recombination

Abstract. *The mechanism of site-specific genetic recombination mediated by Tn3 resolvase has been investigated by a topological approach. Extrapolation of a detailed model of synapsis and strand exchange predicts the formation of an additional DNA product with a specific knotted structure. Two-dimensional gel electrophoresis of DNA reacted* in vitro *revealed a product, about 0.1 percent of the total, with the appropriate mobility. A technique for determining DNA topology by electron microscopy was improved such that less than a nanogram of DNA was required. The structure of the knot was as predicted, providing strong evidence for the model and showing the power of the topological method.*

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The resolvase proteins encoded by transposons Tn3 and γδ efficiently promote site-specific recombination of supercoiled DNA containing directly repeated resolution (*res*) sites (*1, 2*). Distant sites are first brought together in an ordered fashion; the strands of this synapsed DNA are then broken in four locations, generating eight free ends, each of which undergoes defined reorientation and ligation. Such movements of recombining DNA strands often result in knotted or catenated products from whose structure it is possible to deduce critical features of a reaction pathway (*3, 4*).

The fact that the topology of DNA knots or catenanes, unlike that of supercoils, is invariant in both intact and nicked duplex DNA has made electron microscopy particularly useful in analyzing these products of genetic rearrangement. No amount of stretching, twisting, or other physical distortion can alter the stereostructures of knotted or catenated products; the topological signature of the recombination mechanism remains distinct.

The topological concepts used to analyze recombination are those of nodes and domains (*5*). Most easily defined for molecules viewed in plane projection, nodes are simply the crossings of DNA segments. The number, arrangement, and sign of nodes together define topology, whether that of supercoils, catenanes, or knots. Each node is assigned a value of either +1 or −1; by convention, nodes of naturally occurring supercoiled DNA are negative.

For a resolvase substrate, the two regions of DNA between the *res* sites define two domains (Fig. 1). Alterations in DNA topology inherent to recombination have radically different consequences depending on whether the nodes involved are intradomainal (self-intersections of a domain) or interdomainal (intersections of different domains) (*4*). Only interdomainal nodes contribute to knotting or catenation, whereas both types of nodes contribute to changes in superhelicity.

The principal products of resolvase action are singly linked catenanes (*1, 2*). With sensitive detection methods, other products have been found at the level of 1 to 3 percent (*6, 7*). We have recently presented a model that provides a unitary mechanism for formation of all the observed products (*4, 8*). According to this scheme (Fig. 1), resolvase usually acts dispersively, mediating only a single round of strand exchange on binding and then releasing as sole product the singly linked catenane. We postulate, however, that occasionally—about once every 20 productive encounters—resolvase acts processively to promote additional strand exchanges prior to dissociation, generating the more complex products. The rotation of strands about each other during each exchange introduces a single (+) interdomainal node.

The detailed topology of two of the complex products, the four-noded knot and the figure-8 catenane (*6, 7*), but not of the singly linked catenane were available in constructing the model. Implicit in the scheme, however, was the prediction that the two nodes of the singly

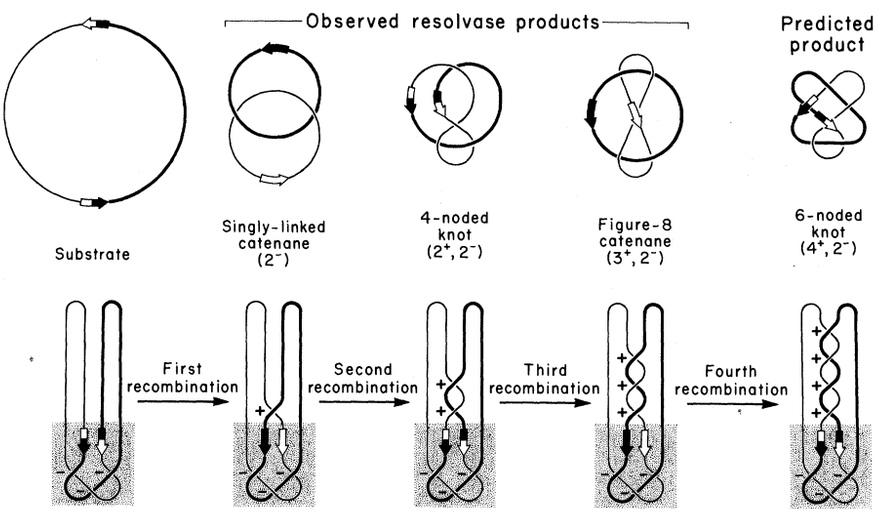

Fig. 1. Scheme for resolvase-mediated recombination. In the upper row, the duplex DNA substrate and products are represented in standard topological form as they might appear after nicking. In the lower row the DNA's are depicted as intact forms bound to resolvase (stippled rectangle) with the two directly repeated *res* sites (thick arrows) dividing the substrate into two domains (thick and thin regions). The substrate at synapsis has three (−) supercoils that entail crossing of the two domains. Successive rounds of recombination, each introducing a single (+) interdomainal node, are drawn in the lower row. Bound resolvase maintains the three synaptic supercoils. After dissociation from resolvase at any stage, product supercoil nodes either cancel with ones of opposite sign or are removed by subsequent nicking. Node composition is indicated in parentheses.