tion and recrystallization. Many inclusions of coesite and diamond that are shielded in a strong mineral host have recently been reported.

Intergranular coesite crystals sited along grain boundaries are much rarer but have been found in the Sulu UHP eclogites of east-central China (7). These coesite grains exhibit variable degrees of conversion to quartz aggregates. Metagabbro and metagranitic rocks with preserved relict igneous minerals and textures occur in the same Sulu locality; they represent early stages in the progressive conversion of metagabbro to coesite-bearing eclogite. These features suggest that reaction rates are slow; in experiments, the presence of only ~450 ppm of intergranular fluid is sufficient to transform coesite completely to quartz during exhumation at temperatures greater than 300°C (8). The total absence of fluids during subduction to mantle depths (~10 million years) and exhumation to crustal levels (~10 million years) may be the key for such sluggish prograde and retrograde reactions (6). This suggestion is consistent with the occurrence of similar intergranular coesite grains from a mantle-derived eclogitic xenolith in a South African kimberlite pipe (9). It is also compatible with inferences drawn from Sulu UHP rocks regarding the lack of fluid attending recrystallization, as reflected by the lowest $^{18}O/^{16}O$ ratios ever recorded for silicates (10); isotopic equilibrium between minerals and host rocks suggests that these units acquired compositions with low oxygen isotope ratios during meteoric water-rock interactions before UHP metamorphism, then were isolated from fluid interaction during their descent to and return from mantle depths.

Recent research on regional metamorphic rocks containing coesite and diamond has produced a dramatic restructuring of our understanding of continental collision processes. The extremely rare occurrence of intragranular coesite suggests that the lack of fluid during rapid exhumation of UHP rocks may have played a more important role than the fact that the inclusion was contained in a small "pressure vessel." Geologists need to intensify their search for such tiny inclusions in order to uncover more completely the extent and magnitude of profound subduction and exhumation of segments of the continental crust.

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BIOCHEMISTRY

# The Cis-Trans Paradox of Integrase

Makkuni Jayaram

*Successful in vitro analyses marked the beginning of the end of recombination as a geneticist's playground. I can already hear the biochemists circling in the night.*

—F. STAHL (1, p. 1356)

*I smiled because I am a biochemist. Now I see crystallographers and other physical chemists stacking up in the sky.*

—N. COZZARELLI (2, p. 13)

Recombination is the ubiquitous process whereby organisms reshuffle their genetic information. This genetic exchange occurs between DNA molecules from the two parents or between two DNA segments within the same molecule. Such recombination may be general, occurring between two DNA substrates with extensive homology, or site-specific, occurring between two specific, relatively short DNA targets. The "conservative" site-specific recombinases of the integrase (Int) family (from phage λ) execute recombination with a remarkable degree of mechanistic parsimony (3). They break the DNA chains at two specific positions in each recombination partner and rejoin the breaks across partners by using chemical steps that do not require phosphodiester hydrolysis or the degradation or synthesis of DNA. Rather, the DNA exchange is a two-step process involving pairwise single-strand breakage and joining. The chemistry of the reaction is discharged by one or two recombinase enzymes with architectural help, in certain instances, from accessory protein factors. Recent findings that members of the Int family may follow disparate paths to arrive at a common recombination chemistry have been disconcerting. Now in this issue on page 126, Kwon *et al.* (4) present the structure of the λ Int catalytic domain, which promises reasonable solutions to some of the mechanistic concerns.

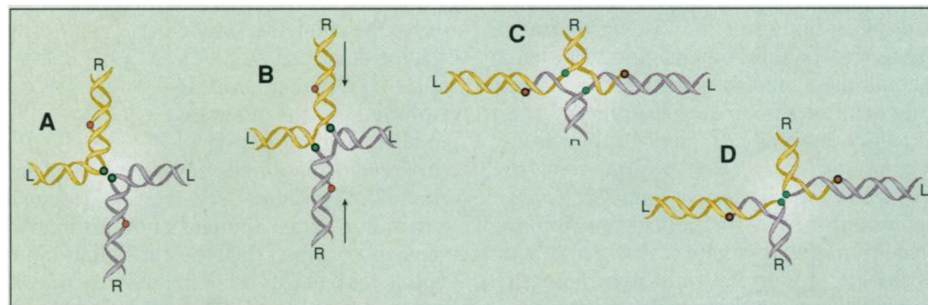


**Fig. 1. "A reversible coalescence of two circles..."** *(5,* p. 136*).* One pair of strands exchanges to form a Holliday junction (A→B); this intermediate isomerizes (B→C), and the reciprocal recombinants form (C→D). Green, the two scissile phosphodiesters that participate in strand exchange; red, the inactive phosphodiesters. The scheme is an elaboration of the original proposal by Campbell (5). L and R refer to left and right arms flanking the strand-exchange region.

The elegant simplicity of the Campbell model for λ integrative recombination (5) (see Fig. 1), which unites two DNA circles into one, inspired the design of genetic and biochemical strategies to grapple with the mechanistic problems of the reaction. At least four of the Int family recombinases (λ Int, *Escherichia coli* XerC/XerD, Cre from phage P1, and Flp from the 2-μm yeast plasmid) use fundamentally the same mecha-

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nism, but they do so without sacrificing their individuality. Against the large global sequence diversity among the members of the Int family, local similarities are discernible. The family insignia is an invariant catalytic tetrad of two arginines, a histidine, and a tyrosine (*4, 6, 7*) ($Arg^{212}$, $His^{308}$, $Arg^{311}$, and $Tyr^{342}$ in λ Int). The active-site tyrosine provides the cleavage nucleophile. During the act of strand cutting, it is covalently linked to the 3′-phosphate, exposing the 5′-hydroxyl group at the breakpoint. This 5′-hydroxyl group then provides the nucleophile for strand joining in the recombinant configuration by acting on the 3′-phosphotyrosine target formed within the partner substrate. Reaction at one end of the strand-exchange region (the initiation points) results in the formation of a Holliday junction intermediate, which can be resolved into reciprocal recombinants by repeating the reaction at the other end (the termination points). Analogy to the pancreatic ribonuclease or the *Staphylococcus* nuclease mechanisms (*8*) suggests that the Int family Arg-His-Arg triad contributes to the charge relay or stabilization (or both) during acid-base catalysis of the cleavage and joining steps.

One round of Int recombination between two double-stranded DNA partners requires a total of four strand breakage/union events mediated by four recombinase monomers bound adjacent to the four scissile phosphodiester bonds. It therefore seems reasonable to imagine that each recombinase subunit is responsible for the chemical events at the phosphate closest to it (action in cis). Implicit in this view of recombination is the notion that the recombinase monomer is a catalytically competent entity. The first jolt to this thinking came from the observation that a monomer of the Flp protein has only a partial active site and is hence chemically inactive. The assembly of a full active site requires the donation of the catalytic tyrosine from one Flp protomer to the Arg-His-Arg cleft of a second Flp protomer bound across from it on the other side of the strand-exchange region. This way of assembling the active site directly leads to DNA cleavage in trans (*9–11*). This provocative observation with Flp prompted similar analysis among other Int family members. But all experiments with the XerC/XerD system support the cis-cleavage mode (*12*), whereas most of the experiments with λ Int favor cis cleavage (*13*), although one set suggests trans cleavage (*14*).

Are two types of cleavage performed by members of the same recombinase family? This notion was not merely irksome, but challenged the purported kinship between Int and Flp. Although some of us feared that the parting of the ways between Flp and Int was inevitable (*15*), others were not prepared yet to break up the family (*16*). The presence of the invariant catalytic tetrad in the same sequence order in nearly 70 recombinases, and the similarity in the organization of the recombination target sites, made it painful to reject a common ancestor for these proteins. Perhaps the flexibility of the peptide arm that harbors tyrosine, depending on the constraints imposed by protein-protein and protein-DNA interactions, could accommodate either cis or trans cleavage (*17*). However, in the absence of a three-dimensional structure of an Int family recombinase, this rationalization of cleavage duality was merely wishful thinking.

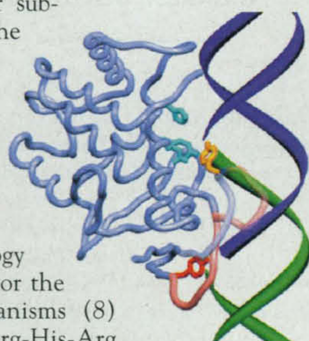

**Fig. 2. Cis and trans mechanisms.** The structure of the catalytic domain of λ Int (*4*), left, and a theoretical model for the equivalent Flp domain (*18*), bottom. Flexibility in the λ Int can account for active-site assembly in cis ($Tyr^{342}$ in yellow) or trans ($Tyr^{342}$in red). The Flp model (*18*) accommodates a trans active site.

The long-awaited structure of the catalytic domain of λ Int described by Kwon *et al.* (*4*) offers a tantalizing resolution of the cis-trans paradox. The clustering of conserved, functionally important residues around the presumed active site (as deduced from amino acid alignment of 66 Int family members) suggests that the constellation of peptide folds in this Int domainal structure is likely to be representative of the whole family. Within the two Int protomers that form the crystallographic asymmetric unit, the peptide segment immediately preceding $Tyr^{342}$ is disordered in both Int monomers, indicating conformational flexibility. Although $Tyr^{342}$ in one monomer is positioned in trans with respect to the Arg-His-Arg triad, its counterpart is not visible in the electron density of the second monomer. What is especially interesting about the structure is that disruptions in the β6 to β7 loop at the base of the flexible tether harboring $Tyr^{342}$ could trigger its movement toward the triad cluster to establish the cis configuration of the active site. In a model structure of Flp built by using the "genetic algorithm" (see Fig. 2) (*18*), the relative placement of the active site tyrosine with respect to the Arg-His-Arg triad is similar to that seen in λ Int and agrees with DNA cleavage in trans by Flp. Finally, the structure of the catalytic domain of the Int protein of the *Haemophilus* phage solved in the presence of $SO_4^-$ and metal ions (*19*) and the emerging structure of XerD (*20*) display a clustering of the invariant catalytic residues consistent with a cis-cleavage mechanism.

How can these multiple cleavage modes be reconciled with the uniform chemical mechanism within the Int family? In the recombinase tetramer, which is the functionally relevant unit, the question of cis versus trans cleavage would be irrelevant as long as two catalytic tyrosines are properly positioned in relation to the appropriate phosphates during the Holliday-forming and Holliday-resolving steps of recombination. It may be useful to invoke here the "Cheshire cat" conjecture, first proposed for different RNA enzymes that use identical metal-ion catalysis (*21*). If we were to "erase" the complex three-dimensional structure of the recombinase tetramer to leave only the critical active-site residues and the scissile phosphates in view, I suspect that the "catalytic grin" of the Int family may be unmistakably recognized, regardless of the action of tyrosine in cis or trans.

Note, also, that an essential requirement of the two-step strand-transfer mechanics is that the assembly of one pair of active sites be coupled to the disassembly of the second pair. The opposing cleavage mode may then reflect differences in how individual recombinases have evolved to deal with the requisite conformational transitions. The structures of recombinase tetramers, complexed with the DNA substrates (perhaps waiting in the wings), may tell us more about the subtleties of the reaction mechanism when they make their appearance. Although experience with the structures of other enzymes that mediate phosphoryl transfer in nucleic acids (*22*) cautions us against irrational exuberance, the structure of the Int catalytic domain and its implications encourage (guarded) optimism.

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MEDICINE

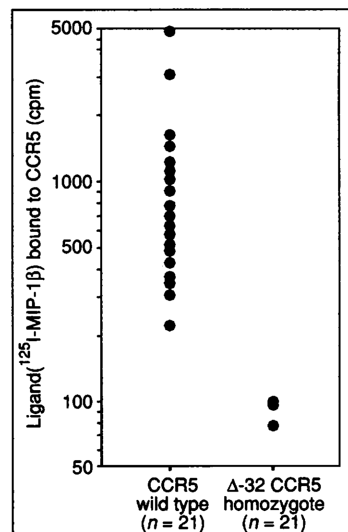
# Coreceptors: Implications for HIV Pathogenesis and Therapy

John P. Moore

Human immunodeficiency virus (HIV) cannot enter human cells unless it first binds to two key molecules on the cell surface. The identity of one of these, CD4, has been known since 1984, but only last year did the decade-long search for the second receptor molecules end (*1*). Identification of these coreceptors—CCR5 and CXCR4—has changed the view in several arenas of acquired immunodeficiency syndrome (AIDS) research.

The virology of HIV has now become more understandable. The HIV-1 strains that cause most transmissions of viruses by sexual contact are called M-tropic viruses. These HIV-1 strains (also known as NSI primary viruses) can replicate in primary CD4$^+$ T cells and macrophages and use the β-chemokine receptor CCR5 (and, less often, CCR3) as their coreceptor. The T-tropic viruses (sometimes called SI primary) can also replicate in primary CD4$^+$ T cells but can in addition infect established CD4$^+$ T cell lines in vitro, which they do via the α-chemokine receptor CXCR4 (fusin). Many of these T-tropic strains can use CCR5 in addition to CXCR4, and some can enter macrophages via CCR5, at least under certain in vitro conditions (*1*). Whether other coreceptors contribute to HIV-1 pathogenesis is unresolved, but the existence of another coreceptor for some T-tropic strains can be inferred from in vitro studies. What is occurring in patients? Because M-tropic HIV-1 strains are implicated in about 90% of sexual transmissions of HIV, CCR5 is the predominant coreceptor for the virus in patients; transmission (or systemic establishment) of CXCR4-using (T-tropic) strains is rare (*1, 2*). However, once SI viruses evolve in vivo (or if they are transmitted), they are especially virulent and cause faster disease progression (*1, 3*).

The numbers and identity of coreceptor molecules on target cells, and the ability of HIV-1 strains to likely enter cells via the different coreceptors, seem to be critical determinants of disease progression. These factors are major influences on both host- and virus-dependent aspects of HIV-1 infection. For example, a homozygous defect (Δ32) in CCR5 correlates strongly with resistance to HIV-1 infection in vivo and in vitro. Individuals who are heterozygous for a defective CCR5 allele are at best weakly protected against infection and have only a modestly slowed disease progression (*2*). However, other factors can influence the level of CCR5 expression on activated CD4$^+$ T cells and thereby affect the efficiency of HIV-1 infection in vitro (*4, 5*). For reasons that are not yet clear, the amount of CCR5 expression on the cell surface (as measured by MIP-1β binding) varies by 20-fold on CD4$^+$ T cells from individuals with two wild-type CCR5 alleles (*4*) (see figure). Staining with a CCR5-specific monoclonal antibody indicates a similar large variability (*6*). Such variation may far outweigh any effect of one defective allele for CCR5. The causes of this variation should be the subject of intensive studies, as they point to controllable factors that could increase resistance to disease.




**Binding to a coreceptor.** The binding of the chemokine $^{125}$I-MIP-1β to the coreceptor of activated CD4$^+$ T cells differs markedly in 21 individuals with two wild-type CCR5 alleles and three individuals homozygous for defective CCR5 alleles (*19*).

The state of activation of CD4$^+$ T cells also affects coreceptor expression. Quiescent CD4$^+$ T cells express CCR5 only minimally or not at all, but they do express CXCR4. Activation with interleukin-2 (IL-2) causes strong, sustained up-regulation of CCR5 expression and transient up-regulation of CXCR4 (*5*). Hence, M-tropic strains that only use CCR5 for entry do not fuse efficiently with quiescent T cells, whereas T-tropic primary and lab strains can do so without difficulty (*7*). In assessing antibody effectiveness, assays for virus neutralization that rely on HIV-1 entry into resting cells are badly skewed by these variations in coreceptor expression. Memory T cells (CD45RO$^+$) are susceptible to the effects of β-chemokines that bind CCR5 (*8*) and express much more CCR5 than naive T cells (CD45RA$^+$), but CXCR4 expression appears to be less variable between T cell subsets (*5*). A phenotypic switch (M- to T-tropic) that may be associated with escape from β-chemokines, or reduced production of α-chemokines, can occur during disease progression (*1, 3*). This could render a whole new set of naive CD4$^+$ T cells susceptible to efficient HIV-1 infection (through CXCR4). Another important but as yet uncharted area is the interaction of CD4 with CCR5 and CXCR4. The specifics of this process will be important for understanding HIV-1 infection and perhaps also for more general immunology studies.

How does the new information about coreceptors affect the prospects for a successful vaccine for HIV? It may be possible to create a human CD4$^+$, CCR5$^+$ transgenic mouse or rabbit for vaccine testing. However, the infectious inoculum that would be required in such animals might prove problematically high, because HIV-1 replication in nonhuman cells is not very efficient, even if entry blocks are overcome. Among the existing primate models, CCR5 is an important coreceptor for simian immunodeficiency virus (SIV) stocks used for experimentation, but another (as yet unreported) coreceptor is probably the simian counterpart of CXCR4 (CXCR4 does not usually function with SIV) (*9*). It will be important to completely characterize the coreceptor us-

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