Transposition

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INTRODUCTION

What Is Transposition?

Transposition is a DNA recombination reaction that results in the translocation of a discrete DNA segment called a transposable element or transposon from a donor site to one of many nonhomologous target sites. Transposition can also promote other kinds of DNA rearrangements including deletions, inversions, and replicon fusions. Such rearrangements of DNA can have profound effects. It can result in the stable acquisition of new genetic information, for example, the integration of viral DNA into a host chromosome. The acquisition of a novel DNA segment or other transposon-mediated DNA rearrangements can also have considerable impact on the host genetic information. Element integration, deletion, and inversion can all result in alterations in the host DNA, which can lead to subsequent changes in gene expression. Because some mobile elements encode outwardly firing promoters, element insertion can also activate downstream genes. Transposable elements can also facilitate DNA rearrangements by being substrates for other types of recombination reactions. Homologous recombination between elements at different genomic sites can lead to deletions, inversions, and replicon fusions. Thus, transposons serve as potent agents of genetic change, and their actions contribute substantially to genetic diversity.

Because transposons encode the machinery to execute such rearrangements, i.e., specific recombinases and the DNA sites upon which they act, transposons actually encode the capacity to generate genetic change. These mobile DNA segments also often encode other determinants such as viral genes, antibiotic resistance genes, catabolic genes, and genes for virulence factors. The survival of a transposable element as a discrete entity depends on a delicate balance between the necessity for the element to overreplicate its host and the potentially deleterious impact of DNA rearrangements. There are multiple strategies for element overreplication, including direct copying of the entire element by semiconservative replication during the act of element translocation and also tricking the host into generating transposon copies through recombinational DNA repair and through the use of particular target sites.

Transposable elements are diverse and widespread, having been found in virtually every organism which has been examined. They were originally identified genetically as mobile controlling elements in maize by the pioneering studies of Barbara McClintock on unstable mutations in the 1950s (67, 131) and acquired physical definition upon their identification and characterization as the cause of polar insertion mutations in bacteria in the late 1960s and early 1970s (97, 184). Bacteria, in particular *Escherichia coli*, have provided an important arena for the study of transposition. The powerful genetic, molecular, and biochemical tools available for bacteria have facilitated dissection of both the mechanism and regulation of transposition at the molecular level. Although many of the basic features of transposition were first elucidated in bacteria, it is now clear that transposition is fundamentally similar in all organisms. The study of transposition in bacteria has also contributed greatly to the analysis and understanding of many features of the bacterial lifestyle. One critical contribution of the study of transposition has been the development of powerful genetic tools which exploit and use transposable elements (77, 102, 188; chapter 140, this volume).

Overview of Transposition

Transposition is a type of specialized recombination, i.e., recombination which is specific for and restricted to the sites of action of specialized recombinases. The specialized transposition recombinase is called a transposase. The transposase is usually encoded by the mobile element itself and specifically recognizes and acts on special cognate sites at the ends of the element and promotes their joining to a new target DNA. The transposase thus mediates specific recognition of the transposable element and also executes the DNA strand breakage and joining reactions which underlie element translocation. Transposases often act in concert with both element-encoded and host-encoded accessory proteins. The *cis*-acting recombination sequences lie at the ends of the element, generally arranged as inverted repeats; indeed, these recombination sites delineate the element. Transposition does not require sequence homology between the recombining DNAs and is independent of the host's homologous recombination system, although homologous recombination may act upon the DNA products of transposition.

At the heart of all transposition reactions is the insertion of a discrete DNA segment, i.e., the transposon, into a nonhomologous target site. Transposons insert such that the newly inserted element is flanked by short direct repeats of a sequence that existed only once in the target DNA (Fig. 1). Since the element translocates between many different nonhomologous sites, these duplications are not constant in sequence. These target duplications are, however, constant in length, this length being characteristic for each element. The detailed structures of trans- position products can vary considerably, depending on the relative locations of substrate transposon ends and the target site to which they will join, and on the particular type of transposition reaction. Also, transposition products may be subsequently processed by other recombination systems which will alter their structure.

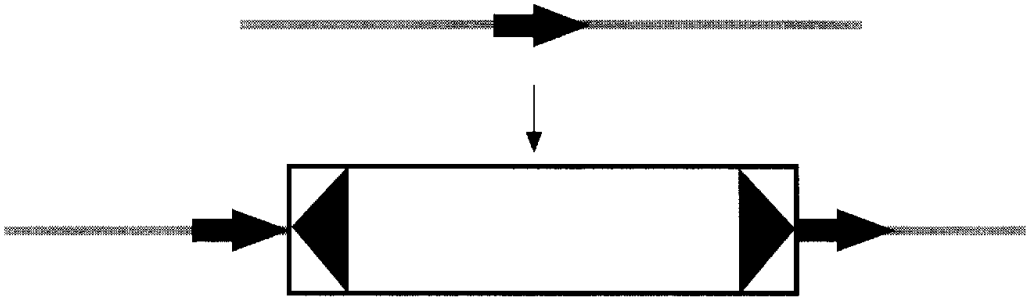

FIGURE 1 Transposon insertion is accompanied by a duplication of target sequences. Insertion of a transposon into a target DNA results in the duplication of a short DNA segment (black arrow) at the insertion site. These duplications flank the newly inserted transposon (white rectangle); the length of the target duplication is characteristic of each element. The *cis*-acting recombination sequences at the transposon ends (triangles) are arranged as inverted repeats.

There also exist some distinctive mobile elements, most notably IS*91* and Tn*916*, whose translocation occurs without the duplication of target sequences. These elements are considered separately below.

Two Types of Transposition Reactions

The transposition mechanisms of bacterial elements can be broadly divided into two types of reactions: (i) nonreplicative or "cut-and-paste" transposition and (ii) replicative transposition (Fig. 2). In cut-and-paste

transposition, the transposon is excised from the donor DNA and inserted into the target DNA to form a simple insertion. The role of replication in this reaction is limited to repair of the new joints between the transposon and the target DNA. This type of transposition also results in a gapped donor DNA which may be processed in a variety of ways. Depending on how the gapped donor is processed, cut-and-paste transposition can be a biologically replicative reaction, i.e., result in an increase in transposon copy number. In replicative transposition, the transposon is directly copied by DNA replication during element translocation to generate a structure called a cointegrate which contains two copies of the transposon joining the donor and target backbones. This cointegrate may be subsequently processed by other types of recombination (i.e., resolved) to yield a simple insertion and another molecule genetically identical to the donor prior to transposition. Although cut-and-paste transposition and replicative transposition may appear quite distinct, they are fundamentally related on many levels, both in chemical terms and also in how such reactions affect the host. Thus, many regulatory concerns affect both classes of reaction.

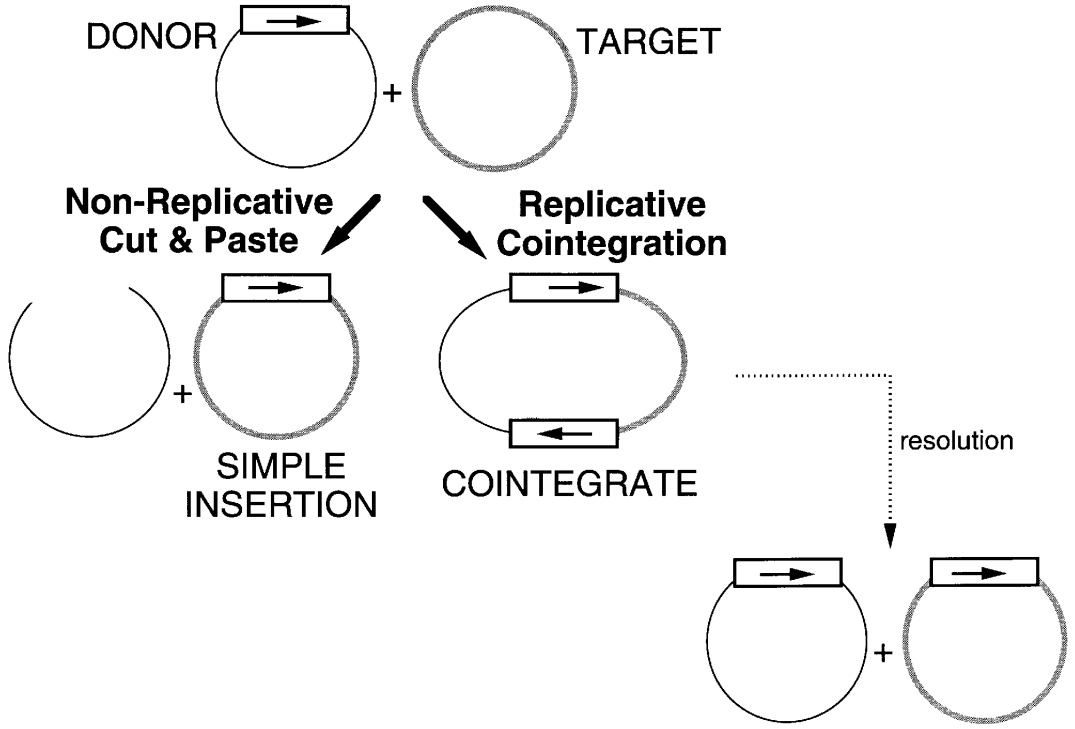

FIGURE 2 Different transposition reactions generate different products. The intermolecular translocation of a transposon from a donor replicon to a target replicon can yield different products, depending on the type of transportation reaction. In nonreplicative (cut-and-paste) transposition, the transposon is excised from the donor backbone and inserted into the target DNA to yield a simple insertion; another product of this reaction is a gapped donor backbone. In replicative transposition, the donor and target are fused through a process that generates another copy of the transposon to yield a cointegrate containing the donor backbone and the target DNA linked by two copies of the transposon. The cointegrate may be resolved either by homologous recombination between the transposon copies or by an element-encoded site-specific recombination system to yield a simple insertion and a molecule which appears genetically identical to the donor.

Differences between Transposition and Site-Specific Recombination

A defining feature of transpositional recombination is that DNA replication is involved. This replication may be limited to repair of the joints between a newly inserted element and its target DNA or may be profoundly involved, actually copying the entire element during the act of transposition. The involvement of replication in transposition distinguishes this recombination reaction from another type of specialized recombination, site-specific recombination. Well-studied site-specific recombination reactions include the integration/excision cycle of bacteriophage lambda, the resolution of plasmid multimers to monomers and DNA inversions (chapter 125, this volume). Whereas transposition involves DNA replication, site-specific recombination involves precise breakage and rejoining in limited regions of homology shared between specialized recombination sites and does not involve replication. Although elements that insert into a target DNA via transposition and those that insert via site-specific recombination (such as bacteriophage lambda) are both flanked by direct repeats of target sequences, the origins of these repeats are quite different. In transposition, target sequence duplications arise from DNA replication. A single copy of this sequence, i.e., the target DNA at the site of element insertion, was present in the reaction substrates; the two copies of this sequence in the products result from replication. By contrast, in site-specific recombination the substrates together contain two copies of the sequences that will appear in the products as target duplications; one copy of the repeat sequence exists in each substrate, and recombination occurs by breaking and joining within this sequence in the absence of replication.

Another distinguishing feature of transposition is that it is nonreciprocal. An example of the nonreciprocal nature of transposition is that the movement of an element from a donor site to an insertion site is not accompanied by the restoration of the donor site to its original, pretransposon state. By contrast, the movement of an element by site-specific recombination regenerates the site from which the element moved. The nonreciprocal nature of transposition is due to the obligatory involvement of replication in this type of recombination whereas site-specific recombination, a reciprocal form of recombination, occurs by breakage and joining without nucleotide loss or gain.

Although transposition and site-specific recombination are distinguished in many of their biological outcomes and do occur by chemically distinct mechanisms, they are also related at many levels. Many host-encoded proteins can act as accessory factors in both types of recombination, and both types of reactions often involve elaborate, multicomponent protein-nucleic acid complexes. Reflecting the potentially lethal nature of any DNA breakage and joining reaction, both of these types of recombination reactions are usually highly regulated.

How To Measure Transposition

Many assays have been developed to detect transposition events (77, 101, 188). One common assay is to monitor the translocation of a transposon-encoded marker, for example an antibiotic resistance determinant, from one replicon to another. The donor and target replicons must be separated to detect both simple insertions and cointegrations. The donor and target replicons can be separated, for example, by transfer of a conjugable target plasmid to a new host or by using a replication-conditional vehicle as a donor, such as a plasmid which is temperature sensitive for replication or an infecting phage which is unable to replicate. When cointegration is being measured, the transfer of a donor backbone marker is also evaluated.

Another powerful way to detect transposition events is to make the expression of a reporter gene within the transposon dependent on its translocation to a new site. For example, a promoterless *lac* gene may be placed within a transposon such that its expression is dependent on an external promoter. Thus, translocation of the element from a donor site lacking an external promoter to a new site where external transcription impinges on the element can be evaluated by monitoring *lac* expression. Changes in gene expression from sequences that flank the donor site are generally used to evaluate transposon-mediated rearrangements such as deletions and inversions.

The transposition frequencies of elements vary widely, generally from 10^{-6} to 10^{-3} per element per generation. The translocation frequency for an element can be highly influenced by the particular donor site being used and, in some cases, by the target being evaluated. Transposition is generally infrequent, probably because of its capacity for deleterious effects on the host, for example, insertion into and inactivation of an essential host gene. For most transposable elements, the transposition frequency is sufficiently low such that genetic selection is required to detect events. A notable exception to the general rule of low-frequency transposition is bacteriophage Mu, which can transpose at very high frequency: Mu uses transposition to replicate its DNA during lytic phage growth. During its 1-h growth cycle, transposition generates more than 100 viral copies from a single infecting Mu phage DNA. This high frequency of Mu transposition has been exploited to investigate transposition biochemistry: the first soluble in vitro transposition system was developed by using Mu in pioneering work by Mizuuchi (136, 137). Another exception to the usual requirement for selection to detect transposition events is Tn*7*, which transposes at high frequency to a single specific chromosomal site. In this case, transposition can be readily detected by direct examination of chromosomal DNA (122). In some other cases, for example when the element has been specifically engineered to express transposase at a very high level, transposon-mediated rearrangements can be detected by simply examining cellular DNA (145, 161).

Other Resources

This review will focus on transposable elements in bacteria, providing an overview of both mechanistic and biological aspects of recombination and concentrating on the more extensively studied elements. Other recent reviews by Mizuuchi (138) and Haniford and Chaconas (81) provide more detailed views of transposition biochemistry of bacterial elements and also elements from other organisms. A review by Kleckner (100) provides more extensive discussion of the control of transposition. A review by Plasterk of a recent meeting (157) highlights current issues in both the control and mechanism of transposition in prokaryotes and eukaryotes. There are also several recent collections of detailed and comprehensive reviews on selected transposons in a recent volume of *Current Topics in Microbiology and Immunology* (174) and in two recent monographs, *Mobile DNA* (24) and *Genetic Recombination* (110). *Mobile DNA*, *Ge*netic Recombination, and a recent issue of *Trends in Genetics* (volume 8, no. 12, 1992) also include reviews of a wide variety of other mobile elements and recombination systems.

CLASSES OF TRANSPOSABLE ELEMENTS

Transposable elements which generate a sequence duplication at the target site are common, represent many different classes of elements, and have been studied extensively. These elements may be grouped by several different criteria: their overall structure, their transposition mechanisms, or sequence similarities among their transposases. A grouping of some particularly well-studied elements, based on their structures, is presented in Fig. 3. It should be noted that elements with very similar structures may transpose by substantially different mechanisms and, conversely, that structurally distinct elements may use fundamentally similar transposition mechanisms.

Insertion Sequences and Composite IS Transposons

Insertion sequence (IS) elements are small and compact mobile elements, ranging from about 0.75 to 2.0 kb. They encode only transposition functions, i.e., transposases and the *cis*-acting terminal sites required for recombination. Many different elements have been identified in the chromosomes and plasmids of *E. coli* and other bacteria (55; chapter 111, this volume). Particularly well-studied elements include IS*1*, IS*3*, IS*10*, IS*50*, and IS*903*. Some elements of this class (for example, IS*10* and IS*50*) translocate by a cut-and-paste mechanism, others appear to use replicative transposition to yield cointegrates (IS*3*), and others

appear to yield both simple insertions and cointegrates (IS*1*, IS*903*). It should be noted, however, that not all elements which are called ISs actually make target site duplications, for example, IS*91* (see below).

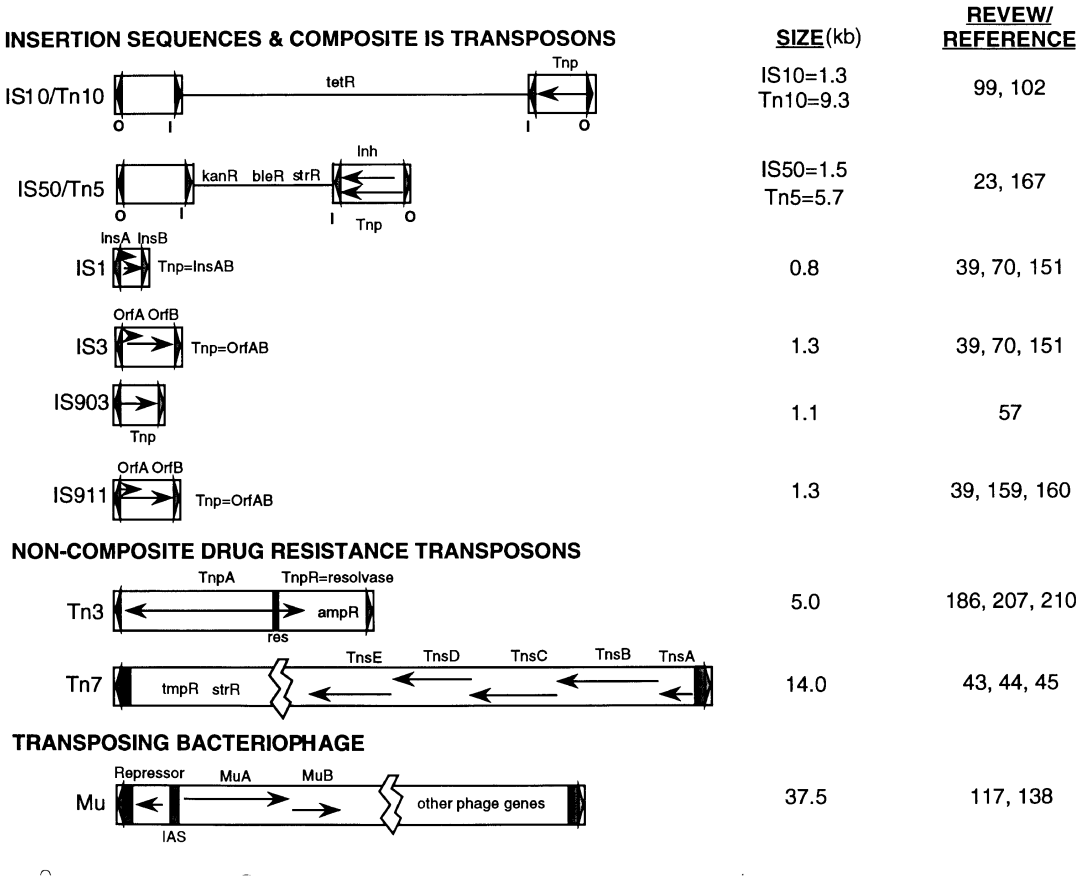

FIGURE 3 There are many different types of bacterial transposons. The most extensively studied elements in bacteria are schematically shown, and references to current reviews and recent literature are given. The recombination sequences at the ends of the elements, generally arranged as inverted repeats, are indicated; in compound transposons containing IS elements, outside (O) and inside (I) ends are also indicated. The terminal recombination sequences are not to scale with respect to the element, nor are differences between elements presented except that the larger terminal regions of Mu and Tn7 reflect the larger size of the recombination sequences of these elements; the internal enhancer of Mu recombination (IAS) is also shown. Also indicated are element-encoded transposition proteins: Tnp or Tns, transposition protein; Inh, transposition inhibitor. Antibiotic resistance genes are as follows: bleR, bleomycin resistance; kanR, kanamycin resistance; strR, streptomycin resistance; tetR, tetracycline resistance; tmpR, trimethoprim resistance.

Composite transposons contain two IS elements bounding, as either direct or inverted repeats, a DNA segment which encodes auxiliary determinants such as antibiotic resistances and virulence factors. The flanking IS components of these composite elements provide the recombination machinery, i.e., transposase and *cis*-acting sites at the termini of the ISs, which mediate translocation. The outside ends of the IS segments collaborate to translocate the entire composite segment; translocation of individual IS

elements is mediated by one inside and one outside end of the IS. Because these composite elements may actually have four reactive termini (two outside and two inside ends), other complex rearrangements can also be observed (see below). Well-studied elements of this class include Tn*5*, which contains IS*50*, Tn*9*, which contains IS*1*, and Tn*10*, which contains IS*10*.

Noncomposite Drug Resistance Transposons

Many transposable elements do not contain smaller component modules which are individually mobile. These elements encode transposition functions; i.e., they encode a transposase (and their termini are the recombination substrates) and other determinants but lack terminal IS elements. Determinates encoded by these elements include antibiotic resistance genes, virulence factors, and catabolic genes.

These elements are structurally very diverse. One well-studied group of elements is the Tn*3* family. These elements use two distinct recombination systems to generate simple insertions. In the first step, transposition generates a replicative cointegrate, and in the second step, an element-encoded resolvase promotes a site-specific recombination reaction between the transposon copies to generate a simple insertion (Fig. 2). Another element of this class is Tn*7*, an elaborate transposon encoding multiple antibiotic resistance determinates and also multiple transposition genes.

Bacteriophage Mu

Bacteriophage Mu is a temperate phage which uses transposition to integrate into the chromosome during the establishment of lysogeny and to replicate its DNA during lytic growth. Chromosomal integration occurs via a nonreplicative cut-and-paste insertion of the transposon into the chromosomal target. Lytic phage DNA replication occurs through multiple rounds of replicative transposition and is highly efficient, generating about 100 progeny phage particles per cell in less than 1 h.

Comparison with Eukaryotic Mobile Elements

Bacterial mobile elements which have been characterized to date contain only DNA. This distinguishes them from a number of eukaryotic transposable elements such as retroviruses (198) and retrotransposons (29), whose transposition life cycle includes an RNA intermediate (30). Transposition of these retroelements begins by transcription of an integrated copy of the element to generate an RNA copy. This RNA intermediate is then turned into double-stranded DNA by the action of an element-encoded reverse transcriptase, a very specialized form of DNA polymerase capable of using an RNA substrate. This double-stranded DNA is then integrated into the host genome. The integration mechanism of the DNA forms of retroviruses and retrotransposons and the movement of DNA elements of eukaryotes and bacteria share many features. They have similar mechanisms of DNA breakage and joining and even, in some cases, shared amino acid motifs between the transposases which execute these reactions (see below). Probably most eukaryotic DNA elements transpose through a cut-and-paste mechanism; no eukaryotic element has yet been identified which appears to be actually copied by DNA replication during its translocation.

Some bacteria, including certain *E. coli* isolates, contain “retrons,” which contain an enzyme related to eukaryotic reverse transcriptase and produce an unusual hybrid nucleic acid containing both RNA and DNA (90). It remains to be determined if retrons are actually mobile elements.

MOLECULAR VIEW OF TRANSPOSITION

Overview: Transposition Involves Specific DNA Breakage and Joining Reactions and DNA Replication

There is a fundamental chemical similarity in the transposition mechanisms of mobile elements of bacteria and eukaryotes which generate target site duplications (138, 139, 159). This similarity has been revealed by biochemical dissection of recombination systems of bacteria, including bacteriophage Mu (137), Tn*10* (20, 21, 146), and Tn*7* (9), and of eukaryotes, including mammalian retroviruses (31, 69), *Saccharomyces cerevisiae* Ty1 (61), and *Drosophila* P element (98). First, specific DNA cleavages expose the 3′ OH ends of the transposon (Fig. 4). In a second step, the 3′ OH transposon ends join to staggered positions in the target DNA such that the 3′ transposon ends are linked to 5′ ends of target DNA with concomitant exposure of new 3′ OH positions in the target DNA. These newly exposed 3′ target ends then serve as primers for host replication such that the sequences complementary to the overhanging target sequences which flank each newly inserted transposon end are regenerated by DNA synthesis. In some cases, this synthesis extends through the transposable element. Thus, the target sequence duplications characteristic of transposition result from DNA repair. During recombination, the transposon-specific breakage and joining steps occur without the involvement of ATP or any other high-energy cofactors in the chemical steps, indicating that transpositional recombination must occur through a phosphoryl transfer mechanism rather than by breakage and ligation. The 5′ transposon ends are handled differently by different elements; their treatment has profound effects on the final nature of the recombination products.

DNA Breakage and Joining Steps in Transposition

The critical initiating chemical steps in transposition are the DNA breakage reactions at each transposon end which expose terminal 3′ OH groups and the joining of these terminal residues to each strand of the target DNA; in some reactions, breakage of the 5′ transposon ends also occurs (Fig. 4). The breakages at the transposon ends can also be considered site-specific endonuclease reactions. In all transposition reactions which have been analyzed biochemically, these breakage reactions are promoted by the element-encoded transposase, positioned at the cleavage sites by its specific DNA-binding activity and also requiring particular sequences at the cleavage points; these reactions also require divalent metals. The cleavage at the 3′ ends of bacterial transposons which separates them from the flanking duplex DNA is equivalent to the processing at the 3′ ends of the double-stranded DNA intermediates during retrotransposition of retroviruses and retrotransposons (30, 198). Mechanistic analysis of the cleavages at retroviral 3′ ends and, by analogy, the 3′ ends of bacterial transposons suggests that this reaction occurs by a one-step mechanism in which water acts as the nucleophile to cleave the phosphodiester bond at the 3′ transposon terminus (62, 199). The critical role of transposase in this reaction may be to activate this position for cleavage. There is no evidence for a covalent protein-DNA linkage in transposon end cleavage.

For only some elements are the 5′ ends of the transposon also cleaved. In IS*10*, this cleavage occurs cleanly at the junction of the 5′ transposon ends and the flanking DNA; IS*10* thus makes flush cuts at the transposon ends (21). In Tn*7*, these cleavages are staggered: the 3′ ends are cut precisely at the transposon-donor junctions, but cleavage on the other strand actually occurs 3 nucleotides within the flanking donor DNA (9). These several nucleotides from donor sequences which flank the 5′ Tn*7* ends are removed during host repair of the element-target junctions; the 5′ ends of integrated retroviral DNAs are apparently processed similarly by the host. How are the active sites which execute the cleavages at the 3′ and 5′ transposon ends related? In Tn*7*, different proteins and hence distinct active sites execute the cleavages at the 3′ and 5′ ends (P. Gary, M. Bievy, K. Bainton, and N. L. Craig, *J. Mol. Biol.*, in press; E. May, R. Sarnovsky, and N. L. Craig, unpublished observations). In IS*10*, both breakage events are mediated by the single transposase protein and may even depend on the same active site (102). Whether or

not the 5′ ends are processed is a key step which has a profound impact on the reaction outcome (see also below).

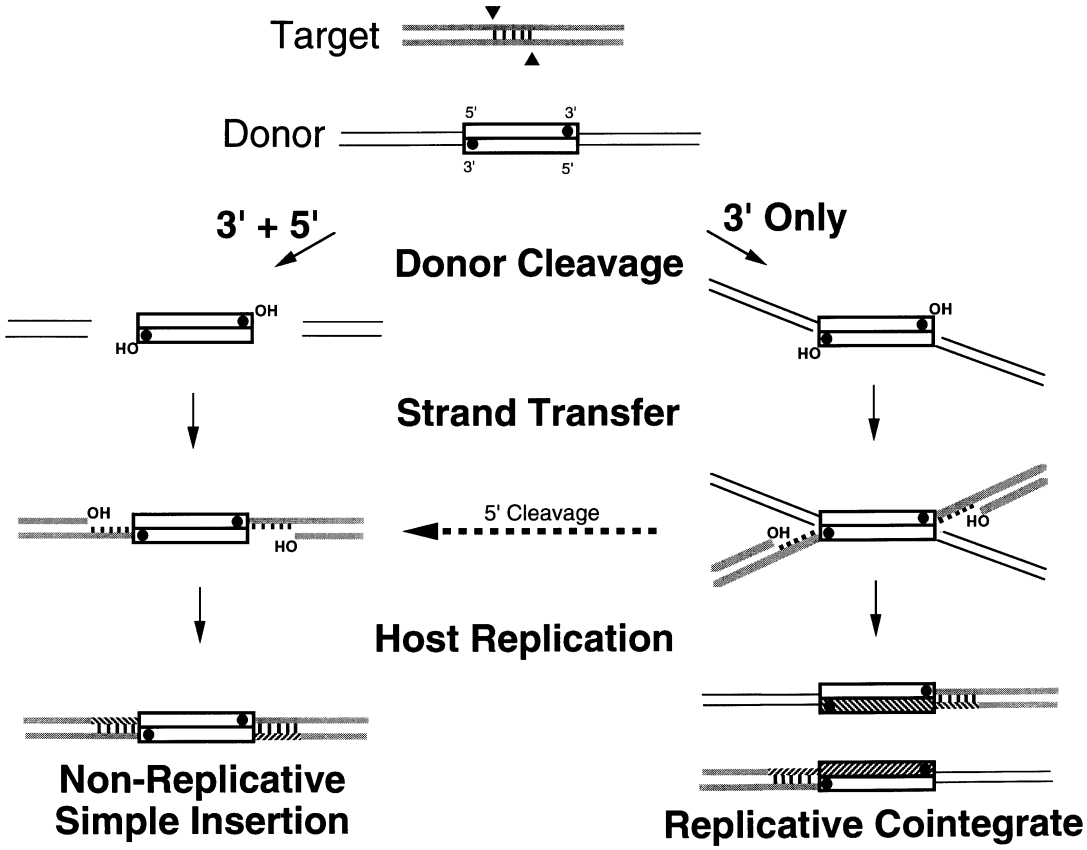

FIGURE 4 Chemical steps in nonreplicative and replicative transposition. The top two lines show the substrates of transposition: the target site, with the base pairs that will be duplicated upon insertion (vertical lines) and the positions at which the 3' transposon ends will be joined (triangles), and the donor site, with the flanking donor DNA and the transposon with the chemical polarities of the transposon strands indicated and the 3' transposon ends marketed by solid circles. The timing of the cleavages at the transposon ends can vary and may occur (i) only at the 3' ends, (ii) first at the 3' ends and then at the 5' ends, or (iii) simultaneously at both the 3' and 5' ends. Following cleavage to expose the 3' transposon ends, these ends are covalently joined to the target DNA in the strand transfer step, the positions of joining being staggered on the top and bottom strands of the target DNA. In some cases, cleavage at the 5' ends may follow the strand transfer step. The strand transfer products are substrates for the host replication machinery, which can act on 3' OH target ends flanking the transposon, which were exposed through the act of joining the transposon ends to the target DNA. When the strand transfer product contains only the transposon and the target DNA, replication is limited to repairing the gaps that flank the 5' ends of the newly inserted transposon. These gaps reflect the joining of the transposon ends to staggered positions on the target DNA; repair of these gaps results in the target site duplication flanking newly inserted transposons. When the strand transfer product contains the transposon and flanking donor DNA linked to the target DNA, semiconservative DNA replication across the element results in the cointegrate form, which contains two copies of the transposon linking the flanking donor backbone and the target DNAs. Newly replicated DNA is indicated by hatching.

Without breakage of the 5′ ends, as in replicative Mu transposition during lytic phage growth, the cleaved intermediate in recombination that precedes target joining has nicks at each end of the element (49, 189). By contrast, in IS*10* (21, 80, 82) and Tn*7* (9, 10) transposition, in which both transposon strands are broken at each end, the key cleaved intermediate is an excised transposon which is completely broken away from the flanking donor DNA.

In the target joining or strand transfer step, the 3′ transposon ends are joined to the target DNA at positions staggered by a few nucleotides. The length of this stagger is characteristic of each element and actually determines the target site duplication length. This joint molecule, sometimes called a Shapiro intermediate, contains a single copy of the transposon covalently linked to both flanking donor sequences and the target DNA. This structure was proposed to be the key intermediate in recombination in several early transposition models (8, 185). It is important to note that each transposon end joins to one strand of the target DNA. It is only the concerted action of the two ends of an element on one target DNA that gives the appearance of recombination occurring through a double-strand break at the target DNA; no such double-strand target breaks have been observed in any system.

In some cases, for example, in nonreplicative Mu transposition during integration, the cleavages at the 3′ and 5′ ends may be temporally distinct, with cleavage of the 3′ ends and their joining to the target DNA actually preceding cleavage of the 5′ ends (Fig. 4) (47).

The strand transfer reaction mechanism of bacteriophage Mu has been studied in detail chemically (140). This reaction can be best described as a concerted one-step transesterification reaction in which the exposed 3′ end of the transposon acts as the nucleophile which attacks a phosphodiester bond in the target DNA. A similar mechanism of strand transfer is observed in retroviral integration (62). This transfer reaction joins the transposon end to the target DNA, accompanied by the generation of a free 3′ end in the target DNA. In other words, in the strand transfer step, a phosphodiester bond in the backbone of the target strand is transformed into a phosphodiester bond between a transposon end and one segment of the target DNA, the other target segment being released as a free end. The fact that under certain conditions an integrated transposon end can be readily released with concomitant rejoining of the target DNA emphasizes that these reactions are isoenergetic and involve phosphoryl exchanges rather than energy-requiring ligation steps (41).

The phosphoryl transfer mechanisms of transposition are in some ways similar to the nucleic acid transactions in RNA splicing (37, 94, 138, 139). This contrasts with site-specific recombination reactions, such as the integration and excision of bacteriophage lambda, which use covalent protein-DNA joints to conserve the high energy of the phosphodiester bond during strand exchange (chapter 125, this volume). Whereas such site-specific recombination reactions generate product molecules which are intact DNA duplexes, transposition products contain broken strands reflecting the multiple steps of strand hydrolysis and strand transfer; DNA repair reactions are required to produce intact DNA products during transposition.

Transposition thus requires two key events involving the 3′ ends of the transposon: specific breakage and strand transfer. The observation that single-amino-acid changes in transposases (13, 102, 139; R. Sarnovsky and N. L. Craig, unpublished observations) can specifically block both of these catalytic steps suggests that these steps probably depend on the same (or at least related) active sites.

DNA Replication Has Different Roles in Nonreplicative and Replicative Transposition

The transposase-executed breakage and joining steps are followed by DNA replication executed by the host machinery, but the extent of replication differs in different types of transposition. In nonreplicative transposition, replication is limited to the repair of new joints between the mobile element and the target DNA, whereas in replicative transposition, a copy of the entire transposon is generated by DNA replication concomitant with element insertion into the new target site (Fig. 4).

In nonreplicative or cut-and-paste transposition, the element is cut away completely from the donor backbone by double-strand breaks at both the 3′ and 5′ transposon ends and the 3′ ends of the transposon are joined to the target DNA. Replication can then initiate from the newly exposed 3′ target ends which result from element insertion. In cut-and-paste reactions, replication is limited to these new joints and basically serves to repair the small gaps resulting from the joining of transposon ends to staggered positions on the target DNA. Elements known to move in this fashion are Tn*10* (16, 20) and Tn*7* (9). With these elements, both transposon strands are cut before target joining, so that a key recombination intermediate is an excised transposon. However, not all nonreplicative recombination reactions involve an excised transposon. In nonreplicative integrative Mu recombination (83), transposition begins with cleavage of only the 3′ ends. These 3′ ends are then joined to the target DNA to generate a product in which the transposon, the donor backbone, and the target DNA are covalently joined; cleavage of the 5′ transposon ends can then follow the target joining step (47, 49, 137, 189). Thus, although the final products of strand transfer, i.e., the transposon inserted in the target DNA and dissociated from the donor backbone, are the same for Tn*10*, Tn*7*, and Mu integration, the temporal orders of their breakage and joining reactions are different. As described below, the Mu strand transfer intermediate can actually yield both nonreplicative and replicative transposition products.

In replicative transposition, recombination begins with the cleavage of the 3′ ends of the element and their transfer to the target DNA without cleavage of the 5′ ends, such that the donor DNA including the transposon and the target DNA are linked (47). As in cut-and-paste transposition, replication then initiates from the newly exposed 3′ ends of target DNA. However, in replicative transposition, replication from the flanking target primer proceeds across the entire transposable element, thereby generating two copies of the transposon by semiconservative DNA replication and also repairing the gaps that flank the newly inserted transposon ends. Because the 5′ transposon ends are still linked to the donor DNA and the 3′ ends are linked to the target DNA, the two transposon copies fuse the donor and target backbones into a single structure called a cointegrate. This mechanism, originally proposed by Shapiro (185) and Arthur and Sherratt (8), has been experimentally observed for the replicative transposition of phage Mu during its lytic growth cycle. The same strategy is also likely to be used by other replicative elements such as Tn*3*.

Thus, the initial events of nonreplicative and replicative transposition are related by the processing of 3′ transposon ends but differ in their handling of 5′ transposon ends. In replicative transposition, the lack of 5′ end cleavage prior to strand transfer and the resulting presence of the 5′ donor strand in the initial strand transfer product are likely to play a key role in the assembly of the replication machinery at the flanking 3′ target ends which will actually proceed to replicate the entire element in semiconservative fashion. It is important to recall, however, that while formation of a strand transfer product containing uncleaved 5′ transposon ends is apparently essential to replicative transposition, this intermediate can also proceed to nonreplicative transposition if breakage of these strands occurs after strand transfer (152). This strategy provides a mechanism by which a single mobile element, for example, bacteriophage Mu, can generate both nonreplicative and replicative transposition products (8, 47, 152, 185). In Mu, both recombination pathways initiate with cleavage of the 3′ transposon ends and their transfer to the target DNA to form a strand transfer intermediate in which the 5′ transposon ends are still attached to the donor DNA (47). Cut-and-paste, nonreplicative transposition ensues if the 5′ transposon ends are broken away from the donor, whereas replicative transposition ensues if the 5′ ends remain attached to the donor. Elements such as Tn*10* (21, 80, 82) and Tn*7* (9, 10) are apparently directed to nonreplicative transposition by the efficient cleavage of their 5′ ends. The decision to cleave the 5′ strand is likely to be a key regulatory step in transposition control. It will be interesting to determine if other elements suggested to yield both nonreplicative and replicative transposition products use a strategy similar to that used by Mu, in which a common intermediate can proceed to these alternative outcomes.

Cointegrate Resolution

A cointegrate may be further processed by other recombination systems (Fig. 2). Reciprocal recombination between the directly repeated transposon copies regenerates a donor replicon which appears genetically identical

to the donor substrate before transposition and generates a target replicon containing a simple insertion of the transposon. Some elements, for example, Tn*3* and its relatives, encode a site-specific recombination system that promotes cointegrate resolution; recombination occurs between specific sites within the transposon called *res* sites and is directed by a recombinase called resolvase (8, 186). This step can be exceedingly efficient, such that virtually no cointegrates are detected but only the substrates and resolution products are present. Other transposons lack a resolvase system; in these cases, cointegrate resolution occurs through the host-encoded homologous recombination system, which is generally less efficient.

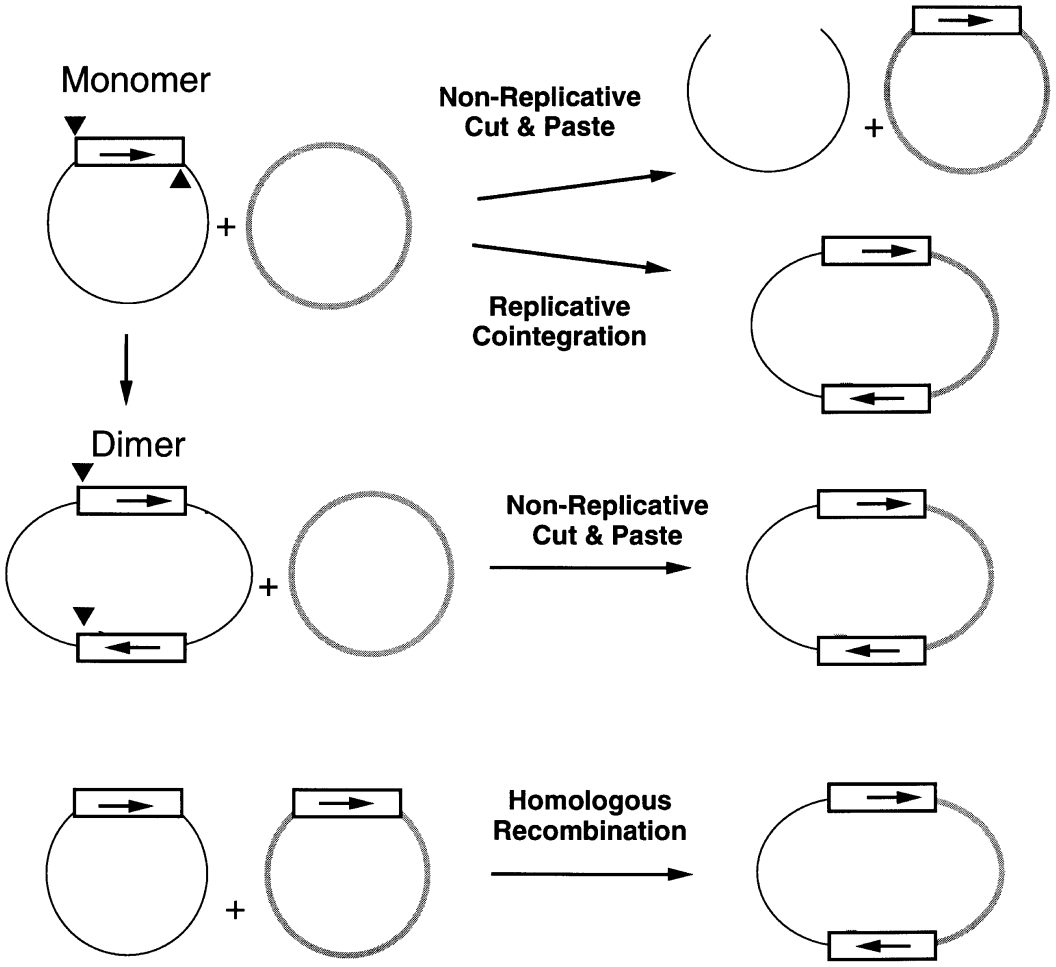

FIGURE 5 A cointegrate is not proof of replicative transposition. A cointegrate can be produced by a variety of DNA transactions. Therefore, observation of a cointegrate does not provide definitive proof of replicative transposition. The top panel shows the outcomes of nonreplicative cut-and-paste transposition and replicative transposition reactions in which the donor substrate is a monomer plasmid containing one copy of the transposon; the transposon ends participating in recombination are indicated by triangles. The middle panel shows that a cointegrate can result from nonreplicative transposition of a dimer substrate containing two transposon copies when recombination involves one end from one element copy and the other end from the other element copy; the participating ends are marked by triangles. The button panel shows that homologous recombination between transposons in a donor molecule and in a simple insertion can result in a cointegrate.

Both Simple Insertions and Cointegrates Can Result from Both Nonreplicative and Replicative Transposition

It is important to appreciate that a simple insertion transposition product can arise either directly through a nonreplicative mechanism or indirectly through the two steps of cointegrate formation and resolution (Fig. 2). Thus, it cannot be determined by inspection of the transposition products whether element translocation proceeded via a nonreplicative cut-and-paste mechanism or through a replicative reaction. Moreover, nonreplicative transposition reactions can also yield cointegrate structures. Consider, for example, the outcome of cut-and-paste transposition reactions with a dimer plasmid as a donor substrate: reactions involving one end from each element copy will generate a cointegrate structure identical to that produced through a replicative reaction with a monomer substrate (Fig. 5) (22, 23, 175). The utilization of transposon ends on different DNA molecules may also result in cointegrate formation (102, 121). Another difficulty in determining the mechanism of transposition by inspection is that transposition products are generally examined many generations after the actual breakage and joining of DNA strands, so that there are multiple opportunities for other processing events. Another source of cointegrates which do not arise from replicative transposition is homologous recombination between a transposon in a donor plasmid and a simple insertion in a target plasmid (Fig. 5). Clear demonstration that transposition could occur by both replicative and nonreplicative pathways required the establishment of clever biological tests and in vitro systems (56).

Transposition Often Appears Replicative; or,What Happens to the Donor Site?

Transposition often appears replicative: a transposon copy persists at the donor site, and a new insertion at a different site is observed. Indeed, transposon survival requires that the element overreplicate its host, that is, increase in copy number compared with the host. However, only some—certainly not all—transposition reactions involve actual copying of the element by DNA replication during the act of element translocation. Reactions that proceed nonreplicatively through a cut-and-paste mechanism can also appear replicative because of certain features of the cellular conditions under which they occurred (see also below). This appearance of replicative transposition long confounded researchers in this field (56). At issue here is the fate of the broken donor backbone DNA from which transposons have excised during transposition. Possible fates of broken donor molecules include loss of the entire donor molecule from the cell, repair of the break, and rejoining of the broken flanking sequences.

Loss of the Broken Donor Backbone. No experiments to date have critically addressed whether the broken donor molecule may be lost from the cell. Bacterial replicons are usually present in multiple copies. This is true even of replicons such as the chromosome, which are spoken of as single copy but are effectively multicopy under standard growth conditions because of the initiation of new rounds of DNA synthesis prior to segregation. The loss of a single broken donor, especially in light of the low frequency of most transposition events, is unlikely to be deleterious or even noticeable. Moreover, certain mobile elements encode regulatory circuits which ensure that translocation will occur only when the donor replicon is newly replicated, i.e., is present in multiple copies. Thus, if another copy of the donor replicon is maintained, transposition will appear to be replicative even if the replicon from which the element transposes is lost (Fig. 6). Indeed, inspection of the genomes of cells that have undergone transposition events reveals a copy of the transposon at the original donor site and a copy at the new site of insertion (19).

Recombinational Repair of the Broken Donor Backbone. The broken donor backbone may be repaired by recombinational repair when the cell contains a second copy of the transposon donor site (Fig. 7). This reaction effectively restores the broken donor molecule to its state prior to transposition; thus, the donor site genetically appears unchanged by transposition. When the cell contains both a donor molecule from which the transposon has excised to leave a double-strand gap and an unbroken sister molecule, as would

occur, for example, if transposition took place immediately after passage of a replication fork, the unbroken donor site can serve as a homologous template for repair of the gapped site from which the transposon has excised. Such restoration of the donor site dependent on a homologous template has been observed with Tn*7* in *E. coli* (78) and with several eukaryotic transposons including P elements in *Drosophila melanogaster* (63) and Tc1 in nematodes (158).

A consequence of using double-strand gap repair to restore the gap from which the transposon has excised is to make transposition effectively replicative. Although the element is not copied by DNA replication as it translocates to its target site (i.e., the element actually moves by cut-and-paste transposition), a new copy of the transposon is generated at the donor site by replication during repair of the broken donor. While the element-encoded transposition functions play a clear role in the first step, i.e., transposon translocation, it remains to be determined if they play any role in the second step of double-strand gap repair. It may be that the double-strand break itself is sufficient to recruit the DNA repair machinery so that the repair phase is dependent only on the cellular homologous recombination repair machinery.

Rejoining of the Broken Donor Backbone. Another possible fate of the broken donor backbone is that it is rejoined. Several outcomes of rejoining can be imagined. Precise excision may occur, i.e., restoration of the donor site to its pretransposon state resulting from loss of both the transposon and one copy of the target duplication. Alternatively, imprecise excision may occur, i.e., loss of the transposon and rejoining without perfect restoration, an event often detectable in bacteria as relief of polarity on downstream genes because of the polar nature of many transposon insertions. However, little rejoining of the flanking donor sequences is observed following bacterial transposition (19, 23, 99). Such excision events often accompany transposition in other organisms (173), but they are rare in *E. coli.* Although precise excision has been observed in *E. coli,* it occurs at a much lower frequency than does transposition. Precise excision can also occur in the absence of transposition, i.e., in the absence of transposase, and thus is thought to usually reflect aberrant replication events promoted by inverted repeats at the ends of most transposable elements (60, 68).

Transposons Can Promote Deletions and Inversions

Although transposition, i.e., formation of a simple insertion or a cointegrate, is most often thought of as an intermolecular event, transposons can mediate a variety of intramolecular rearrangements, including deletions and inversions, which affect DNA adjacent to the transposon in the donor site. Some of these rearrangements are simply the intramolecular versions of complete intermolecular events, whereas others may reflect aborted transposition reactions.

Both deletions and inversions can be obtained through replicative transposition when the target site lies on the same molecule as the transposon ends, i.e., intramolecular transposition. The relative orientations of the target site with respect to the transposon ends determine whether a deletion or inversion is obtained. The actual steps involved in such intramolecular reactions are identical to those in the intermolecular reaction, only the connection of the target site to transposon ends is changed. Intramolecular rearrangements have been reported with Mu (154) and Tn*3* (186), elements known to move replicatively in intermolecular reactions. Intramolecular deletion reactions mediated by replicative transposition events are a powerful tool for DNA manipulation, for example, for making nested deletions for DNA sequencing (chapter 140, this volume).

With a cut-and-paste mechanism, repair of the donor DNA is essential to the recovery of an intramolecular simple insertion. Adjacent deletions and inversions have been observed with nonreplicative composite IS elements such as Tn*10* (23, 99). These reactions reflect recombination involving the inside ends of the IS sequences rather than the outside ends, which mediate translocation of the intact element (Fig. 8). The use of the inside ends of a composite transposon actually represents intratransposon insertion. Such reactions are of particular interest because they result in the formation of new composite transposable elements (102,186a).

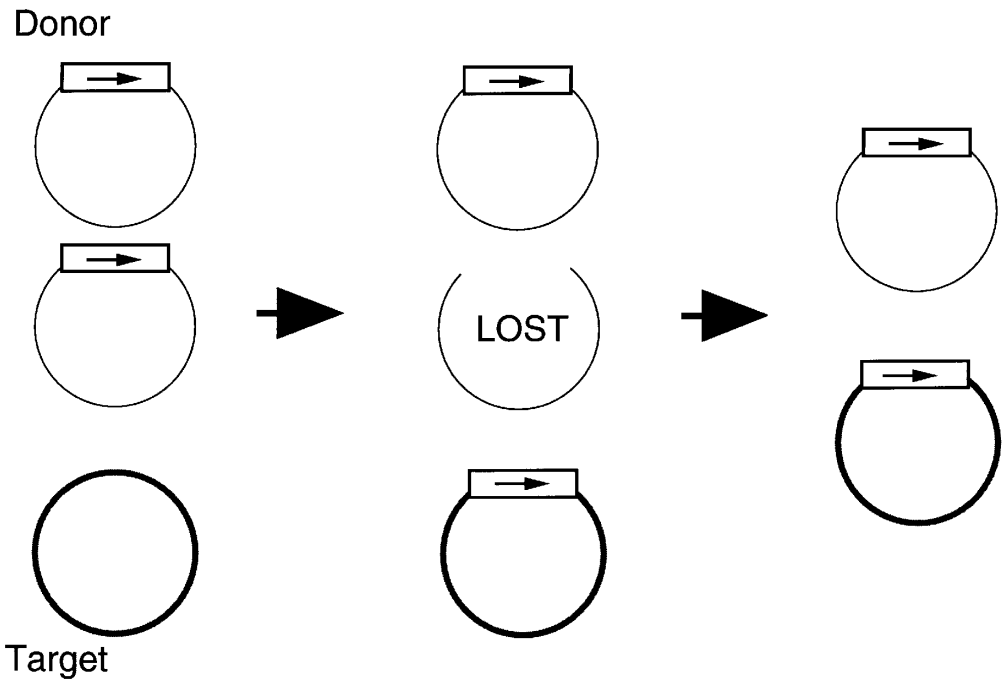

FIGURE 6 Nonreplicative, cut-and-paste transposition can appear replicative. Although cut-and-paste transposition does not involve copying of the element during its translocation, transposition can appear replicative if transposition occurs when multiple donors are present in the cell. Prior to transposition, the element is present in the donor site, and after transposition, the transposon is present at both the donor site and the target site. If transposition occurs when multiple copies of the donor site are present in a cell (thin line), an intact copy of the genome is still present, even if the broken donor site is lost, facilitating cell survival. Another strategy for making cut-and-paste transposition effectively replicative is shown in Fig. 7.

Intratransposon events can also be observed with single IS elements. With IS*10*, such intratransposon products are observed at high frequency when transposase is highly overexpressed in vivo (20, 145) or as a major product of transposition in vitro (21, 80, 146). Although it was long thought that the ability to make adjacent deletions and inversions was a property of replicative transposition or reflected the concerted action of multiple transposon copies, it is now known that adjacent deletions can also be promoted by single-copy nonreplicative elements such as IS*10* (171) and IS*50* (95, 196). These products may reflect aborted transposition products involving breakage and ligation of only one end of an element or, perhaps, the ends of two elements on two sister chromosomes (102, 121, 171).

Another class of intramolecular events which has been observed with the IS*3* family members IS*911* (161) and IS*3* (181) are excised transposon circles in which the two element ends are covalently joined and closely juxtaposed, often separated by just a few nucleotides. These species are not thought to be standard transposition intermediates but, rather, to reflect abnormal reactions resulting from high-level transposase expression. Because such circle formation reactions have thus far been studied most extensively in vivo, it remains to be established which steps are catalyzed by transposase and which are not.

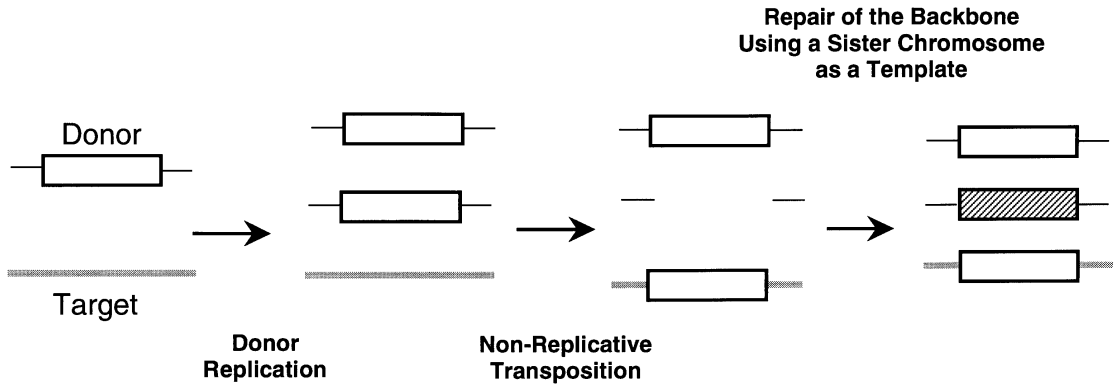

FIGURE 7 Repair of the broken donor backbone. If nonreplicative, cut-and-paste transposition occurs when two copies of the donor site are present, the gapped donor backbone DNA resulting from transposition may use the intact donor site as a template for repair to regenerate an intact donor site (hatched transposon). This repair process can also contribute to making nonreplicative transposition appear replicative: the transposition substrates contain two transposon copies, where the repaired products contain three copies.

Transposons Provide Substrates for Homologous Recombination

Transposition results in the presence of blocks of homologous sequences, i.e., the transposon, at dispersed positions in the genome. These homologous segments provide substrates for the host's general recombination machinery. Homologous recombination between transposon copies in plasmids and chromosomes can result in plasmid integration. For example, homologous recombination between IS sequences in the chromosome and the F plasmid underlies Hfr formation (chapters 126 and 129, this volume). Homologous recombination between directly repeated elements in the same molecule can result in deletions; recombination between inverted elements can result in chromosomal inversions. These changes emphasize the potential of transposable elements for remodeling of the genome. Although rearrangements promoted by these elements can generate new genetic contexts and potential for alternative gene expression, it is also clear that such rearrangements may be hazardous, if not lethal. The potential of transposable elements to effect genetic variation both directly through transposition and indirectly through the action of other systems on transposons is probably the reason that transposition has evolved as a generally low-frequency event.

TRANSPOSITION MACHINERY

Transposition involves three DNA substrates, the two transposon ends and the target site, and recombination proteins, including an element-encoded transposase and, often, host-encoded proteins. Although experimentally demonstrated for only a small number of elements, it is generally assumed that the element-encoded transposase mediates specific recognition of the transposon ends and the DNA breakage and joining steps of recombination and that host-encoded proteins play accessory roles.

DNA Substrates

Transposon Ends. The termini of transposons provide the substrates for transposition, i.e., the *cis*-acting DNA sequences which are recognized and acted upon by the transposition proteins. Two functions are provided by these recombination substrates: recognition sequences for the specific binding of recombination proteins and sites for DNA breakage and joining.

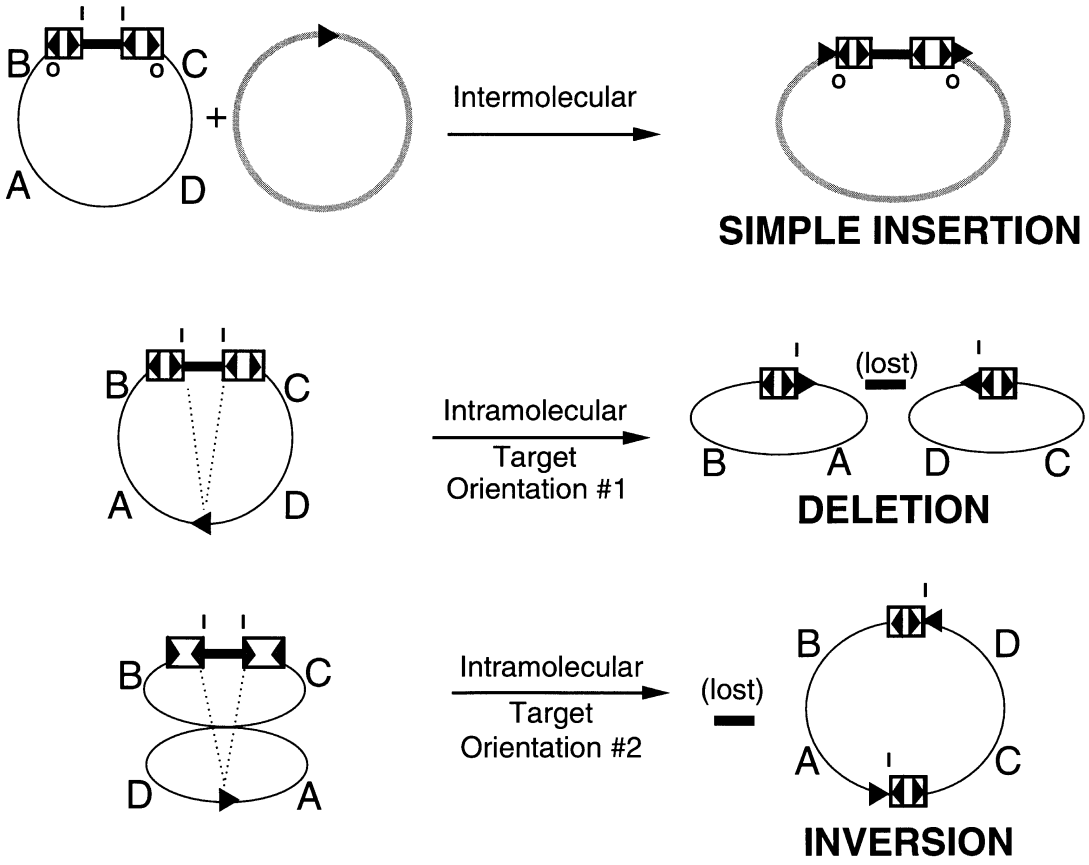

FIGURE 8 Intramolecular nonreplicative transposition. The substrate donor molecule contains a composite IS transposon, which includes four recombination sites: two outside ends at the extreme termini of the composite element (O), and two inside ends (I). Intermolecular nonreplicative transposition of the composite element with the O ends results in a simple insertion. When the inside ends are used in an intramolecular reaction, two different outcomes are possible, depending on the orientation of the target site. With the target site in one configuration, two circles are generated, each containing one transposon end with concomitant loss of the sequences that were between the I ends in the substrate. With the target site in the opposite orientation, inversion of segments on the donor substrate occurs, accompanied by the loss of the sequences between the reactive I ends.

Transposition requires that each element end contain recombination sites which define the DNA segment which will be moved during transposition. The terminal recombination sites are organized as inverted repeats so that the recombination proteins are positioned in symmetrical fashion at each end. Although the ends of an element are generally homologous, there is no evidence for a role for homologous or complementary DNA sequences per se during transposition; rather, their homologous nature simply reflects identical or related protein-binding sites at each end.

The size and complexity of the sequences at transposon ends required for recombination vary. The ends of an element are generally identical or closely related to each other and are organized as inverted repeats, usually between 25 and 50 bp in length. Genetic and biochemical analysis of some elements, for example,

IS*10* (79, 88, 102), IS*50* (95), IS*903* (57, 58), and IS*1* (217), as well as members of the Tn*3* family (89, 210), has revealed that an inner region of these repeats provides primary recognition determinants for binding of the cognate transposase; the outer region including the tip of the transposon is essential for subsequent steps, probably provoking DNA breakage and joining. In some other elements, for example, Mu (4, 50, 118) and Tn*7* (6, 7, 195), the recombination sequences at each end are more elaborate, with multiple, internal transposase-binding sites in addition to a multifunctional transposase binding site at each transposon terminus. These internal sites probably play a role in assembling a protein complex which positions and may even activate transposase at the terminal sites where DNA breakage and joining will occur (14, 138, 139, 192). The ends of bacteriophage Mu are particularly elaborate. In addition to requiring recombination sites at each end of Mu, another internal sequence, the transpositional enhancer or internal activating sequence, is required for efficient recombination (120, 143, 190, 192). This DNA site is usually located about 1 kb from one end of Mu but can activate recombination when located at any position between the left and right ends. The enhancer promotes the assembly of the active recombination complex involving the Mu ends and transposase (14, 138, 139, 192). Notably, this segment can act in *trans* (192) and provides a useful paradigm for a variety of protein-DNA transactions such as transcription and replication, which require multiple DNA sites which may be well separated on a linear DNA sequence.

Host-encoded proteins also interact with transposon ends. In some elements, such as IS*1* (216) and γδ (207), there are specific recognition sites for host factors within the inverted repeats. In other elements, such as IS*10* (88, 102, 169) and IS*50* (206, 214, 215), host-factor-binding sites flank the inverted repeat and are present only at one end. Although the roles of such host-factor-binding sites are not in general well understood, a popular view is that their primary role is in modulating transposase binding and activity, in many cases by influencing the conformation of DNA. For bacteriophage Mu, a key host protein involved in transposition is HU (47), a sequence-nonspecific DNA-binding protein whose interaction with DNA facilitates DNA bending and flexibility (85, 87, 177). Such changes in DNA bendability are critical to assembling the elaborate nucleoprotein complex that executes transposition (114–116, 141). Integration host factor (IHF), another host DNA-bending protein (150), can also be involved in recombination and also probably acts to facilitate DNA conformational changes which allow nucleoprotein complex assembly (14, 138, 139, 190, 192, 193). IHF and HU also play key roles in the regulation and execution of IS*10* transposition (102, 186a).

While the termini of some elements are identical or very nearly so, the ends of some other elements are distinct in both structure and function, although they remain generally related. For example, although both ends of IS*10* contain a single transposase-binding site, one end is highly active in the absence of host factors while the other end requires an adjacent IHF site and IHF for activity (88, 146, 186a). Another example of distinctive end functions occurs with Tn*7* (5–7, 195). The ends of Tn*7* are related by the presence of multiple transposase-binding sites, but the spatial organization of these sites is different at each end. The ends are also functionally distinct, as revealed by the fact that Tn*7* inserts in orientation-dependent fashion into certain target sites (123); moreover, artificial elements containing two Tn*7* right ends are highly active while those containing two left ends cannot translocate (6). The *cis*-acting recombination sites at the ends of Mu are also structurally and functionally distinct (4, 50, 118). It should also be noted that in many elements the terminal recombination sites are often closely apposed to or even interdigitated with sites that regulate gene expression and that both transposases and host-encoded proteins may be involved in both processes (see below).

Our understanding of what roles the host DNA sequences which flank the element in the donor site may play in recombination is limited. These flanking sequences acted as the insertion site in the previous round of transposition. It has often been observed in vivo that the frequency of element transposition from different donor sites is different, but these differences have not been systematically dissected. Such variation could reflect effects either on transposase expression or on the activity of the ends. Recent investigations in vitro of the Mu system have suggested that the flanking sequences can, under some conditions, directly affect the efficiency of DNA breakage at the donor site (212).

In vitro analysis of several transposition reactions has revealed that supercoiling of the donor substrate often plays a critical role in recombination. Supercoiling probably has several roles: promoting communication and synapsis between transposon ends to ensure that ends on the same substrate molecule and in the correct relative orientation are used (14, 48, 80, 142, 192, 203), and facilitating DNA distortions at the ends that may accompany synapsis and/or strand exchange (112, 118, 218).

Insertion Sites. Although a distinctive feature of transposable elements is their ability to insert into many different target sites, they do display a wide spectrum of target site selectivity. Some elements appear to be able to insert at virtually any position in DNA, while others display preferences for particular hot-spot sequences or regions or even the extreme selectivity of inserting into a single site in the bacterial genome. It should be noted that because target site selectivity has been explored mostly in vivo, much remains to be learned about the relative contributions of target DNA sequence, DNA structure (for example, a bend), and other processes (for example, transcription of the target DNA) to the site specificity of element insertion.

Two key steps in target selection are the positioning of the transposase at a particular site on the target DNA and the efficiency of the transposase in the actual steps of joining the transposon ends to the target DNA. These functions may be executed by distinct domains of the transposase or may actually be distributed among several polypeptides. For example, although the actual chemistry of DNA breakage and joining is executed by one protein in Mu (1, 15, 130, 144, 191) or several proteins in Tn*7* (10; Gary et al., in press; May et al., unpublished), the positioning of these transposase proteins on the target DNA is determined by another transposon-encoded protein(s). Another interesting possibility is that the position of a transposase on the target DNA could be determined by interaction with a host protein, although no example of this sort of reaction has yet been described.

The target site selectivity of bacteriophage Mu has been explored both in vivo and in vitro. MuA protein is the transposase which interacts specifically with the element ends and executes DNA breakage and joining, while MuB is a regulatory protein involved in target molecule selection and in the positioning of MuA onto the target DNA (117, 138). In vivo and in vitro, Mu inserts into many different target positions, with a preference for a consensus duplication of N-Py-G/C-Pu-N (NYSRN) (144). As this sequence occurs frequently in DNA, insertions are observed at many different positions. This target sequence selectivity is observed in both the presence and absence of MuB. In the presence of MuB, Mu displays a regional specificity, i.e., clustered insertions in a small segment of the target, while continuing to maintain preferential insertion into NYSRN sites (144). In the presence of MuB, Mu also displays another form of target selection called target immunity; i.e., it avoids insertion into DNAs that already contain a copy of Mu (1, 2; also see below). While Mu insertion in vivo can be observed at many sites, considerable site selectivity can also be detected (36): the element seems to avoid actively transcribed genes (34) and may prefer insertion into the control regions of repressed genes (202). The mechanism(s) of these choices is not understood.

Tn*7* can display extreme target site selectivity. This element inserts into a single site called *att*Tn*7* in the *E. coli* chromosome and also in many other bacterial chromosomes (44, 45, 122, 123). This target site selectivity is imposed by recognition of *att*Tn*7* through the specific binding of the Tn*7*-encoded protein TnsD to *att*Tn*7*. The site-specific insertion results from the interaction of the targeting TnsD protein with the rest of the transposition proteins, TnsA+TnsB+TnsC (10). Thus, the protein responsible for target site selectivity is distinct from the transposase which executes breakage and joining. In the absence of TnsD and in the presence of another Tn*7*-encoded targeting protein, TnsE, Tn*7* inserts into many different sites which are unrelated to *att*Tn*7* in nucleotide sequence. Thus, Tn*7* is distinguished by its ability to use two different classes of target sites in reactions which are mediated by distinct but overlapping sets of transposition proteins.

Tn*10* also displays considerable site selectivity, inserting preferentially both in vivo and in vitro into hot spots that share a particular consensus sequence (18, 80, 99). However, the stringency of this selection is not extreme; therefore, insertion into a wide array of target sites is observed. The isolation of Tn*10* transposase mutants that display altered target site selectivity has begun to identify parts of the protein involved in target site

selection (17, 18). Some other elements, for example, IS*1* (70), can show a preference for particular regions, but the controlling determinants of this selectivity have not been identified.

The target site selectivity of an element can have considerable influence on its suitability for a variety of genetic manipulations. Although γδ and Tn*5* can display some insertion selectivity, the relatively random nature of the insertion distributions they generally display has made them particularly effective tools for genetic engineering (chapter 140, this volume).

Target Immunity. The sequence of a potential insertion site may not be the only determinant of transposon insertion. The ability of some transposable elements to insert into a target DNA is highly influenced by whether that target DNA already contains a copy of the transposon, even at positions distant from the potential point of element insertion. The elements Tn*3* (119), γδ (208), Mu (1, 51, 166), and Tn*7* (6, 10, 84) display this property, termed target or transposition immunity. Their frequency of insertion into a target replicon is much reduced when that replicon already contains a copy of the transposon. The inhibition of transposition is not global, since only insertions into the target replicon already containing the element are affected; insertions into other targets in the same cell are not affected. Of particular interest is the considerable distances over which target immunity can be effective. For example, Tn*7* can provide immunity over distances exceeding 175 kb in the *E. coli* chromosome (R. DeBoy and N. L. Craig, unpublished results). Target immunity is specific for a particular element; that is, the presence of Tn*3* in a target replicon provides immunity only to Tn*3* insertion, not even to other closely related elements such as γδ (186). Immunity is provided by the presence of the ends of the transposon in the target DNA; i.e., the same sequences which promote transposition when present in the donor molecule actually inhibit transposition when present in the target molecule. However, while two ends are required to promote transposition, the presence of a single end is sufficient to provide immunity.

Target immunity has been most extensively investigated for bacteriophage Mu, and a molecular view of this process has been provided by in vitro dissection (1, 2, 117, 138). The transposase MuA binds specifically to the ends of Mu; targets bound by MuB, which can activate the breakage and joining activities of MuA, are most efficiently used by MuA. Immunity results from the fact that the presence of MuA bound to a transposon end in a target DNA discourages target binding by MuB. MuB binding to the target DNA is discouraged by the interaction of target-bound MuA with MuB, which promotes MuB dissociation from the target DNA. The DNA-binding activity of MuB is modulated by ATP, and MuA stimulates the dissociation of MuB from DNA by stimulating MuB ATP hydrolysis. The decreased presence of MuB on the immune target in the presence of target-bound MuA thus reduces insertions into that target.

The utility of transposition immunity is probably severalfold: it can prevent multiple insertions into the same target DNA, a potentially hazardous situation because other recombination systems acting on these large resulting regions of homology could destroy the replicon, and it can also discourage intramolecular insertions (28). It is important to note, however, that only some elements display target immunity; such immunity has not been observed with IS elements or their composite relatives. The ability of these elements to carry out intrachromosomal rearrangements that would be discouraged by target immunity probably contributes to the formation of novel transposable elements (102, 186a).

Recombination Proteins

Element-Encoded Transposases. At the heart of a transposition reaction is the transposase, the protein(s) which must specifically recognize the ends of the transposon and execute the DNA breakage and joining reactions. These enzymes must execute breakage at the 3′ ends of the transposon, in some cases also execute breakage at the 5′ ends, and also transfer the 3′ ends to the target DNA to effect target joining. It should be noted that most mobile elements in bacteria are intact; that is, they encode a transposase and have terminal recombination sequences. By contrast, eukaryotes often contain many copies of defective elements

composed of only transposon ends and lacking a transposase; such elements can, however, be mobilized by transposase provided in *trans* by an intact element.

Many transposable elements encode a single transposition protein. For example, IS*10* encodes a single protein which executes all the functions associated with recombination, including specific binding to the transposon ends, interacting with the target DNA, and executing DNA breakage and joining (38, 146). It is of some interest to know how a single protein interacts selectively and distinctly with different DNA sites, i.e., the ends of the transposon and the target DNA, and how the catalytic sites will be related to these specificity determinants.

In some other elements, alternate protein forms generated from the same reading frame may be produced to yield both a transposase and transposition regulators. IS*50* encodes a single protein transposase and an inhibitor of transposition which is a truncated form of the transposase (167). The transposition inhibitor lacks the 55 N-terminal amino acids of the transposase and the DNA-binding activity, but does retain the ability to interact with transposase through oligomerization (53). In some elements, for example, IS*1* (182) and IS*3* (160, 181, 200) family elements, the transposase is actually assembled from two open reading frames by translational frameshifting (39). In these cases, an altered form of transposase which lacks the C-terminal portion added by frameshifting can also act as a regulator of transposition (64, 125, 161, 216).

Although many transposases have been sequenced and considerable biochemical work has been done in a few systems, relatively little is actually known in molecular terms about the structure and activities of these proteins. A major activity of these proteins is to bind to DNA; sequence-specific binding is required at the transposon ends, but much less or at least a different sequence specificity is generally involved at the target DNA. MuA protein actually contains two distinct domains for transposon DNA binding; one mediates binding to the ends of the transposon, and the other mediates binding to the enhancer (120, 143). Amino acid sequence inspection reveals that some transposases have recognizable motifs for DNA binding, such as helix-turn-helix sequences, but little detailed structural information is available for any transposase DNA-binding domain.

Also of considerable interest are the structure and functions of the active sites which execute DNA breakage and joining. The biochemical details of DNA breakage and joining have actually been examined for only a few elements in bacteria, Mu (46, 136), Tn*10* (21, 80, 146), and Tn*7* (10). However, strikingly, all elements examined from both prokaryotes and eukaryotes (138, 139, 159) display the same fundamental biochemistry: recombination requires a divalent metal, and the key reactions are DNA breakage to expose the 3′ ends of the transposon and subsequent transfer of this 3′ end to the target DNA. Although seemingly quite different, these breakage and joining reactions are quite closely related when thought of as transesterification reactions that alter phosphodiester bonds (62, 138–140). In the breakage step, water is used as a nucleophile to promote the hydrolysis of a phosphodiester bond, and in the second step, the 3′ end of the transposon apparently acts as a nucleophile to attack a phosphodiester bond in the target DNA. Sequence comparisons among transposases have identified some conserved amino acid motifs thought to be closely related to the catalytic active sites which execute these reactions. A large number of bacterial transposases, including those of elements of the IS*3* family (66, 111), bacteriophage Mu, and Tn*7* (13, 45, 164, 168), contain the conserved amino acid motif D,D-35-E. This motif has also been identified in other eukaryotic recombinases, such as the integrases of retroviruses and retrotransposons, and, moreover, is thought to be intimately associated with catalysis (66, 110, 159, 199). It has been suggested that this motif is involved in metal binding for catalysis and may thus play a central role in recombination (32, 199). Although it has been suggested that this D,D-35-E motif can be found in virtually all bacterial transposases (168), in only a few cases have the effects of mutations in this motif actually been experimentally examined, i.e., in Mu (13) and in Tn*7* (E. May, R. Sarnovsky, and N. L. Craig, unpublished observations). Mutational analysis of IS*10* transposase has identified a number of positions essential to recombination including conserved acidic amino acids that may be related to the D,D-35-E motif (102). Several families of transposases have been identified by amino acid similarities (Table 1).

As described in more detail below, our molecular understanding of the structure and function of transposase is most advanced with the MuA transposase. Briefly, a key step in transposition is the conversion of MuA from a monomer form, which is active in specific DNA binding, to a tetramer form, which is active in specific binding and strand exchange (14, 142, 192). It seems likely that this tetramer is the fundamental protein unit which executes strand exchange, two protomers probably being most intimately involved in strand breakage at the donor DNA and the other two being involved with strand joining at the target DNA (12). The use of an oligomer at the heart of the recombination machinery provides a mechanism for the close apposition of the substrates during recombination and coordination between DNA breakage and joining events. Dissection of IS*10* transposase suggests that a single monomer of transposase at each end of the element executes all the strand breakage and joining steps (102). A key step in this reaction is the conversion of a transposase-transposon end complex through DNA cleavage at the transposon end to a form which can interact with the target DNA.

TABLE 1 Transposase families based on amino acid sequence homology

Family	Reference(s)
IS*3*, IS*911*, Mu, Tn*7*[a]	13, 45, 66, 111, 164
IS*10*, IS*50*, IS*903*, IS4	102, 103, 128, 194
Tn*3*	186
IS*1*	70

[a]This family also includes retroviruses and retrotransposons.

Element-Encoded Accessory Proteins. In addition to transposase itself, many elements encode other transposition proteins which modulate transposase activity. Tn*7* is a particularly elaborate transposon, encoding five transposition proteins. Overlapping subsets of these Tn*7* proteins direct transposition to two different classes of target sites (172, 201). One of these Tn*7* proteins mediates specific end recognition and, in combination with a second Tn*7*-encoded protein, mediates DNA breakage and joining. The breakage and joining activity of these proteins is controlled by several other transposon-encoded proteins. Two other proteins are alternative targeting proteins, and the last is a connector protein between those which act at the transposon ends and those which dictate the target choice (9, 10, 44, 45). In other elements, variant forms of transposase itself can regulate transposition. Several such negative regulators of transposition were mentioned above, including the IS*50* inhibitor, a truncated form of transposase which lacks DNA-binding activity and reduces transposase activity through the formation of mixed oligomers (53), and the IS*1* inhibitor, which may exclude authentic transposase from the element ends because it retains DNA-binding activity but apparently lacks catalytic activity (125, 216). Another variation of this theme is the Mu repressor, which controls Mu transcription, including transcription of transposition genes, by binding to specific operator sites (154). The Mu repressor can also directly modulate Mu transposition because the operator sites for Mu repressor binding lie within the enhancer which stimulates Mu transposition. Mu repressor has the same DNA-binding specificity as one of the DNA-binding domains of MuA, so that the binding of repressor at its operators can block MuA binding to the enhancer, thereby inhibiting transposition by blocking the assembly of the active MuA complex at the ends of Mu (120, 142, 143). The interaction of Mu repressor with its operators is, like the interaction of MuA with the enhancer, modulated by the host IHF protein (3, 71). Another example of a transposon-encoded regulator that controls the expression of a transposition gene occurs in Tn*3*. The TnpR protein is a sequence-specific DNA-binding protein which can act both as a repressor of transposase transcription and as a recombinase, a resolvase, to promote recombination between the directly repeated transposon copies in a cointegrate (186).

Some other elements encode positive activators of transposition. Although these regulatory proteins are distinct from the transposase which actually recognizes the ends and executes breakage and joining, efficient transposition requires these transposase activators. A benefit of such regulators is that they provide an opportunity for additional circuitry and inputs in the control of transposition. Both Mu and Tn*7* encode

such transposition activators, the MuB protein (117, 138) and the TnsC protein (44, 45), respectively. These proteins regulate transposition though their ability to hydrolyze ATP and are ATP-dependent nonspecific DNA-binding proteins. It is important to note that although both Mu and Tn*7* recombination are ATP dependent, ATP plays a regulatory role and is not directly involved in DNA breakage and joining. Our understanding of MuB is most advanced. This protein plays a key role in juxtaposing the transposon ends bound by transposase to the target DNA: DNA bound by MuB is the preferred insertion site. In addition to promoting the capture of a target DNA by the transposase, MuB activates its breakage and joining activities (15, 191). A key regulatory function of MuB is that its does not bind effectively to, and thus does not promote insertion into, DNAs that already contain Mu, i.e., immune target DNAs (1, 2). MuB is removed from an immune target by MuA binding to the immunity-conferring Mu end in the target DNA; then a MuB-MuA interaction prompts MuB ATP hydrolysis and thus releases MuB from the target DNA. MuB thus acts to channel insertions into intermolecular targets.

TnsC may act in a related fashion to confer immunity on target DNAs which already contain Tn*7* (10, 73). An additional regulatory input in Tn*7* recombination is that the transposase TnsA+TnsB and the transposase activator TnsC require other transposon-encoded regulatory proteins that appear to modulate the activity of TnsC. Two other Tn*7* proteins, TnsD and TnsE, are alternative activators of TnsA+TnsB+TnsC and also mediate specific target site selectivity (44, 45, 172, 201). In the presence of TnsD, Tn*7* is activated to insert into the specific chromosomal site *att*Tn*7;* TnsD binds to *att*Tn*7* and also recruits TnsC to the target site. This target complex appears to position and activate the transposase on the target DNA. TnsE activates insertion into many other non-*att*Tn*7* insertion sites, perhaps similarly recruiting and activating the TnsA+TnsB+TnsC machinery.

Host-Encoded Accessory Proteins. The involvement of host factors in transposition provides a potential route for communication between the transposon and its host bacterium. Host proteins may participate in transposition at many levels: in transposase expression, directly in the transposition reaction itself, and in the bacterial responses to transposition, i.e., the DNA replication-repair events that complete transposition and regenerate intact DNA duplexes. A variety of host proteins have been implicated in each of these steps, and some proteins appear to act at several levels (Table 2). A particularly interesting issue is how transposition might be modulated by certain cellular conditions.

The *E. coli* Dam methylase, which acts on GATC sites, affects the transposition of IS*10* (169), IS*50* (213), and IS*903* at multiple levels (99, 100); the methylation state of GATC sequences in the ends of the elements can affect both transposase expression and the activity of an element end in recombination. In particular, GATC hemimethylation, because it occurs just after a replication fork passes through an element, appears to stimulate transposition by stimulating transposase synthesis and by activating element ends (169). The regulation of transposition by methylation state provides a link between a cellular condition, i.e., the stage of the cell cycle, and recombination. This linkage is a key survival technique for elements such as these which transpose by cut-and-paste mechanisms. Another bacterial replication protein which has been implicated in Tn*5* transposition is DnaA (214), but its role is unknown.

There are several other host proteins whose molecular effects on transposition are at least in part understood but whose physiological role is less well defined, because there is yet no clear linkage of these proteins to a particular cellular condition. IHF and probably HU are host proteins that can affect transposase expression of both Mu (117) and IS*10* (102, 186a) and also participate directly in recombination. Both of these proteins can alter DNA conformation, IHF by promoting DNA bending at specific sequences and HU by promoting DNA flexibility in sequence-independent fashion and thereby facilitating the assembly of protein-DNA complexes. The interaction of IHF with Mu DNA can modulate transposase expression by influencing the interaction of the Mu repressor on its operators. IHF can also influence transposition directly by promoting the assembly of active MuA transposase complexes at the ends of the element though interaction with the transposition enhancer internal activating sequence (190, 193). HU can also directly modulate recombination, apparently by facilitating the assembly of active MuA

complexes at the ends of Mu (14, 142, 192). It is not unreasonable to suspect that changes in DNA conformation facilitated by IHF and HU are similarly involved in IS*10* transposition (102, 186a) and perhaps with other elements as well. Other proteins which affect DNA conformation, FIS and H-NS, have also been implicated in other transposition events (Table 2).

TABLE 2 Host factors implicated in transposition

Protein	Element	Reference
ClpX	Mu	120a, 135, 149a
Dam	IS*10*/Tn*10*	169
	IS*50*/Tn*5*	213
	IS*903*	169
DnaA	Tn*5*	156, 214
FIS	Mu	25
	IS*50*/Tn*5*	206
H-NS	Mu	65
HU	Mu	46, 115, 116
	IS*10*/Tn*10*	102, 146
IHF	Mu	3, 71, 190, 193
	IS*10*/Tn*10*	88, 102, 146, 186a
	γδ	207, 209
	IS*1*	72, 163
	IS*50*/Tn*5*	129
LexA	Tn*5*	107–109, 205
Lon	IS*903*	59
Replication proteins (DnaB, DnaC, DnaG, DnaE, DnaF, gyrase)	Mu	106, 154

The response of the host cell to transposition is also an important facet of transposition. One cellular response which has been observed with some elements is induction of the SOS system, an ensemble of cellular functions expressed in response to DNA damage. Transposition-dependent SOS induction has been observed with Tn*10* (170) and Tn*7* (A. Stellwagen and N. L. Craig, unpublished results), elements known to transpose via a cut-and-paste mechanism that would generate a double-strand-break-inducing signal. The observation that IS*1* transposition can induce SOS suggests that this element may also introduce double-strand breaks into DNA (113). Although the role of the SOS system in transposition is not known, its induction in response to transposition has provided a valuable assay for partial reactions, which has enabled the isolation of interesting transposase mutants (82).

A critical feature of cellular metabolism involved in transposition is replication of the DNA products of transposition. In some cases this replication is a “gap-filling” repair reaction limited to the joints between the newly inserted transposon and the target DNA, and in other cases it is semiconservative replication of many kilobases of transposon DNA during replicative transposition. Little is known in molecular terms about these replication processes in transposition. Genetic and biochemical experiments suggest that the PolIII system is involved in Mu replication (106, 154). It will be very interesting to identify the components of these replication machines and to understand how they are directed to the sites of replication at the joints between the transposon and the target DNA.

Nucleoprotein Complexes in Transposition

Like many other complex transactions involving nucleic acids, transposition requires multiple DNA sites which interact with many different, specific proteins. Elegant studies of the Mu (117, 138) and IS*10* (102) transposition systems have revealed that recombination involves the assembly of these components into a variety of stable protein-DNA complexes and that transitions between these complexes are key regulatory points. Studies with Mu have revealed that the multiple DNA sites in such transactions may be seen as effectors of the assembly of particular protein structures and that these protein assemblies may have distinct

chemical activities from the unassembled protein components (11, 14, 117, 138, 142). The strategy of activating the chemical steps of recombination by assembly of a particular nucleoprotein complex can ensure that recombination will be tightly regulated and occur only when a wide variety of conditions are met. Thus, DNA breakage, a potentially lethal event, will occur only under a limited set of conditions. Only a few transposition systems are sufficiently well developed at the biochemical level to allow direct investigation of these nucleoprotein complexes.

Although its nucleoprotein complexes have not yet been examined in detail, Tn*7* provides a striking example of recombination control that is readily explained by the hypothesis that recombination proceeds in an elaborate nucleoprotein complex containing the ends of the transposon, the target DNA, and, for this element, four recombination proteins. Few recombination intermediates or products are detected if any component is omitted (10). The strategy of requiring assembly of such a complex to initiate recombination ensures that recognition and selection of the appropriate DNA substrates occur before DNA breakage begins.

Nucleoprotein complexes involved in transposition and the transitions between them have been investigated most extensively with bacteriophage Mu (11, 117, 138). Elegant biochemical studies from a number of laboratories have elucidated how the individual protein components—the transposase MuA, the regulator MuB, and host factors IHF and HU—are converted into a recombination machine in the presence of the DNA substrates. At the heart of this machine is the MuA transposase, which is converted from a monomer which binds specifically to sites on the Mu ends to a tetramer which is also active in DNA breakage and joining. Assembly of the MuA tetramer also promotes recombination by specifically synapsing the Mu ends and apposing them to the target DNA (14, 118, 142, 192).

Recombination begins with the reversible specific binding of MuA to multiple sites of the ends of the element. When a variety of regulatory conditions are satisfied, synapsis of the ends occurs by the conversion of MuA to a stable tetramer. Requirements for the production of the stable synaptic complex are as follows: (i) the Mu ends must be of the correct sequence and be present in inverted orientation on a supercoiled substrate DNA, and (ii) the internal activating sequence must be available, i.e., not blocked by the Mu repressor. DNA deformations facilitated by the DNA-bending proteins IHF and HU play a key role in facilitating the assembly process. Once formed, this synaptic complex is very stable, and many of the effectors required to promote its assembly (for example, the IAS and particular MuA end binding sites) are dispensable. Divalent metal, an essential recombination cofactor, is required for assembly of the synaptic complex. In the presence of Ca^{2+}, the complex contains intact, i.e., uncut, Mu DNA; in the presence of Mg^{2+}, donor cleavage occurs to expose the 3′ ends of the element. It is likely that MuB, which is an activator of MuA cleavage, can also play a role in this assembly and cleavage process. The cleaved donor complex then executes strand transfer of the transposon ends to the target DNA upon interaction with MuB bound to the target DNA. The distribution of MuB on target DNA is also a key regulatory step, reflected particularly in transposition immunity.

IS*10* nucleoprotein complexes have also been characterized (80, 102). Like Mu recombination, IS*10* recombination involves transitions between several distinct protein-DNA complexes. In the case of IS*10*, transposition begins with synapsis of the transposon ends and involves an excised transposon species, which has been cut out of the donor backbone by double-strand breaks. A transposase-containing cleaved donor complex in which the transposon ends are synapsed and the intervening DNA is in supercoiled form has been detected; the donor backbone is not retained in this complex. After donor cleavage, the synapsed transposon ends engage the target DNA, followed by strand transfer. Interestingly, the target DNA is apparently apposed to the transposon ends only after the cleavage events which disconnect the ends from the donor backbone. Thus, IS*10* transposition, in contrast to Mu and Tn*7* transposition, does not appear to involve evaluation of the target DNA prior to the initiation of recombination. The principal strand transfer product produced in vitro involves a target site inside the transposon.

The strategy of using the assembly of multimeric protein structures to mediate the stable juxtaposition of several DNA segments with concomitant formation and activation of sites for DNA breakage and joining may be a general one in transposition and site-specific recombination (11, 94, 138).

REGULATION OF TRANSPOSITION

How is transposition frequency regulated? What factors influence changes in recombination frequency? Transposable elements must satisfy two major conflicting biological forces: transposition is essential to the survival of the element, but high-frequency transposition is generally detrimental to the host upon which the element depends. Most elements solve this dilemma by transposing at a low basal frequency.

An issue intimately related to the control of transposition is the interplay between the element and its bacterial host. A popular (and appealing) view is that transposition is modulated by the cellular environment, there being certain cellular conditions under which transposition will be favored and other conditions under which it will be disfavored. Although interesting variations in transposition behavior have been observed, there are relatively few well-understood examples of this sort of interplay between an element and its cellular environment. Indeed, there is to date relatively little insight into what might actually define favorable or unfavorable cellular conditions for transposition. The interplay between a transposon and its host and their influence on each other is a particularly interesting and, in many ways, poorly understood topic.

Transposition frequency can be regulated indirectly through the control of transposase level or directly by modulation of the recombination reaction by alterations in transposase activity and the reactivity of the DNA substrates. The regulatory mechanisms used by transposable elements to control transposition are numerous and diverse, acting at virtually every level of gene expression and also using a variety of strategies to directly modulate the recombination reaction. It should be noted, however, that only a few elements have actually been studied in detail. Most of our understanding about transposition control derives from analysis of IS*10*/Tn*10*, IS*50*/Tn*5*, IS*1*/Tn*9*, Mu, and Tn*3*. Some specific regulatory scenarios that have been investigated include maintaining a low basal level of transposition, protecting the transposon from external regulatory influences which reflect its site of insertion, linking transposition to cellular DNA replication, and facilitating the horizontal transmission of elements. The diversity, richness, and complexity of the regulatory circuitry of transposable elements reflect and emphasize their highly evolved nature.

Maintaining a Low Level of Transposition

For many elements, the frequency of transposition is determined by the level of transposase. Thus, a low level of transposase provides for low-frequency transposition.

Modulation of Transcription. Both strong and weak transposase promoters have been observed. The binding of element-encoded proteins to the promoter region may also influence promoter activity. For example, control of Mu transposase transcription by the phage lysis/lysogeny repressor plays a key role in Mu development (154). Another example of transcriptional regulation is in the Tn*3* family, in which transposase transcription is modulated by the specific DNA-binding activity of another element-encoded recombinase, the resolvase (186). In many elements, the recombination sequences at the ends of the element are often closely apposed to, interdigitated with, or even identical to the DNA sites that regulate transposase expression. In IS*1* (125, 216) and Tn*7* (7, 153, 195), transposase expression appears to be autoregulated, since its binding to DNA can affect both recombination and transcription. This sort of regulation may play an especially important role in promoting transposition upon the initial entry of a transposon into a naive cell (see below). Promoter activity may be modulated by the action of host factors on the transposase promoter region. IHF is known to play a key role in regulating several recombination reactions. In Mu (3, 71, 75, 86, 197) and IS*10* (88, 102, 186a), IHF-binding sites are present in transposase

promoter regions and transposition is altered in IHF$^-$ strains. Another interesting host modulator of promoter activity is the Dam methylase. Alteration in the methylation state of some transposase promoters is used to link transposition to the cell cycle (169; also see below). In some cases, transposition is observed to be stimulated by expression of a transposon-encoded determinant. For example, transposition of Tn*501*, which carries mercury resistance determinants, is induced when mercury resistance, i.e., expression of the enzymes which detoxify mercury, is induced by the presence of mercury (186).

Inefficient Translation. The efficiency of transposase translation plays a key role in controlling the level of transposase. Indeed, for many elements, inefficient translation, often because of nonconsensus initiation signals, appears to be the primary method of ensuring low levels of transposase (100). IS*10* mRNA is one of the most poorly translated mRNAs in *E. coli* (165). Translational control may be particularly useful, because it provides an effective buffer against changes in transcription. Some other elements, for example, IS*1* and IS*3* elements, modulate transposase translation not at the level of initiation but, rather, during the elongation phase. The transposases of these elements are assembled by frameshifting during the translation of two adjoining and overlapping open reading frames which lie in two different reading frames (39). One of these open reading frames contains the DNA-binding domain which recognizes the transposon ends while the other probably contains the catalytic domain.

Influence of External Promoters. The elaborate mechanisms by which elements modulate transposase expression would not be sufficient to ensure low levels of transposase if transposase expression could also be directed by transcription from an external promoter, as would occur if the element inserted into an actively transcribed gene. Although many elements actually avoid insertion in actively transcribed genes (34), some elements also directly protect themselves from external promoters. One strategy is to block the entry of external transcripts into the element by transcriptional termination signals within the element (70). The presence of such internal transcription terminators also plays a role in turning off the expressing of genes downstream of a transposon insertion by imposing transcriptional polarity. Another scenario is that transposase bound to the ends of an element may prevent external transcription from entry into the element, thereby also imposing polarity on downstream genes through blocking the progress of RNA polymerase by steric hindrance transposon-bound proteins (183).

Another protective strategy is to ensure that transposase mRNAs which initiate outside the element, i.e., upstream in flanking DNA, are not efficiently translated (99, 100, 167). Such inhibition of readthrough RNAs can be achieved by including the translation initiation codon in an RNA stem-loop which can be formed only when the mRNA initiates upstream of the usual, element-internal promoter. Thus, mRNAs that initiate at the proper element-internal site will not include this translation inhibitor, i.e., will lack RNA of the upstream portion of the stem-loop, and translation will be allowed. RNAs that enter the element from upstream sites will form the stem-loop, thereby occluding the initiation codon and blocking translation (52, 88, 104, 178).

Another possible external influence on the activity of an element inserted into an actively transcribed gene is that transcription across the transposon ends may decrease the activity of the ends, as has been observed with elements such as IS*1* (27, 40, 126, 127), IS*10* (52), and IS*50* (176), perhaps by disrupting transposase binding or by changing DNA supercoiling.

Transposon Tn*7* has a unique relationship with a bacterial host gene. This element inserts site specifically into the *E. coli* chromosome (44, 45, 122), into the transcription terminator of the bacterial *glmS* gene, an essential gene involved in cell wall biosynthesis (74, 76). Although transposon insertion disrupts the terminator, *glmS* mRNAs are apparently blocked from entering Tn*7* by a transcription terminator in the transposon end (74). An interesting question is whether expression of *glmS* affects Tn*7* transposition, either by altering the expression of Tn*7* genes or by influencing the reactivity of this specific target site.

Transposase Stability and Preferential *cis* Action. The level of transposase activity may also be influenced by its stability. Some transposases have been observed to be chemically unstable, with a strong correlation between stability and transposase activity. With IS*903*, a functional reflection of such instability is that this transposase is preferentially *cis* acting. The transposition frequency of an IS*903* element actually synthesizing transposase, i.e., with the transposon ends *cis* or in close proximity to the site of transposase synthesis, is much higher than the transposition frequency of equivalent transposon ends at some other position in the cell (59). These observations imply that this transposase cannot effectively diffuse from its site of synthesis to other cellular positions. Mu transposase has also been observed to be functionally unstable (155).

Although many IS element transposases have been observed to be preferentially *cis* acting, including those of IS*10* (147), IS*1* (127, 163), and IS*50* (92, 96), not all are observed to be chemically unstable, so that there must be other factors which influence transposase activity. The functional stability of transposase may vary because of conformational changes or modification (99, 100). For example, it has been proposed that transposase activity might be influenced by its connection to the translation machinery (54, 93, 167). Another possibility is that a preferentially *cis*-acting transposase is physically sequestered near its point of synthesis, for example, by a high affinity for nonspecific DNA, which precludes effective diffusion within the cell (147). The degree of *cis* action of a protein may also be influenced by its multimerization state, interacting with either itself or other proteins (54, 204a, 204b). The preferential *cis* action of some insertion sequences transposases probably plays a critical role in controlling the copy number of such elements in a cell (see below). However, not all transposases are preferentially *cis* acting. Efficient complementation between separated sources of transposase and their cognate transposon ends can be observed for many elements, including Tn*3*-like transposons and Tn*7*. Thus, different elements have quite different strategies for controlling transposase activity**.**

Alternate Forms of Transposase Can Modulate Transposase Activity. Transposase activity can also be affected by proteins which interact with transposase or which may compete with transposase for DNA-binding sites. For example, a potent inhibitor of Tn*5* transposition is a truncated version of transposase itself (91, 96, 213) and is proposed to modulate several facets of transposition, including recombination, when this element enters a naive cell (see below). This truncated form is derived from a different transcriptional and translational start and lacks about 55 amino acids from the amino terminus of transposase. While the transposase can bind to the ends of Tn*5*, the inhibitor cannot. The transposase and transposase inhibitor interact to form inactive mixed multimers, thereby reducing transposase activity (53). The IS*50* transposase itself can also act as transposition inhibitor (211); this inhibitory activity may be another reflection of the functional instability of transposase that results in preferential *cis* action (167, 204a, 204b).

Another regulatory strategy is involved in IS*1* transposition. Active IS*1* transposase is synthesized by translational frameshifting, which joins an N-terminal DNA-binding domain with a C-terminal catalytic domain (160, 182). The N-terminal DNA-binding domain can act as a negative regulator of transposition, presumably by excluding intact transposase from the ends of the element by competitive binding (64, 125, 161, 216). Thus, transposase activity can be determined not only by the actual local level of active transposase but also by the levels of other regulatory proteins which act directly on the components of the recombination reaction itself.

Linking Transposition to Cellular DNA Replication

Because transposition involves DNA breakage and joining, it is a potentially lethal event. This danger is particularly true of elements which transpose by a cut-and-paste mechanism in which the element is excised from the donor DNA, resulting in a broken molecule: persistence of this break in a single-copy genome will result in cell death. Some elements which transpose via a cut-and-paste mechanism have

reduced the potentially deleterious effects of transposition by linking recombination to cellular DNA replication, such that transposition occurs preferentially just after a replication fork has passed through the element in the donor site (169). Thus, even if the broken donor molecule from which transposition has occurred is not repaired, there is an intact copy of the genome which allows cell survival. Moreover, the intact sister chromosome may serve as a template for the repair of the broken chromosome. Preferential transposition from newly replicated DNA can also probably facilitate the horizontal transmission of transposable elements (see below).

Several elements link their transposition to chromosomal DNA replication through the action of the Dam methylase, whose action allows newly replicated DNA to be distinguished from other DNA. For a brief window after the passage of a replication fork, newly replicated DNA is hemimethylated whereas other cellular DNA is fully methylated. Both *dam* host mutations and hemimethylated DNA stimulate transposition of Tn*10* and IS*903* (99, 169), as well as Tn*5* (213). The role of Dam in transposition was revealed through hunts for host mutants which affect Tn*10* transposition (169). Hemimethylated DNA can apparently modulate transposition at several levels: the Tn*10* transposase promoter is activated by hemimethylation, and a hemimethylated transposon end has increased activity in transposition. There are Dam recognition sites (GATC sequences) at the ends of these elements in regions involved directly in recombination and in transposase expression. By this means, a transposon that is hemimethylated, i.e., has just undergone DNA replication (and thus multiple copies of this donor site exist in the same cell), is the preferential substrate for transposition.

There may be other means for linking transposition to DNA replication. For example, the ends of IS*50* also contain a binding site for DnaA, a key host protein in the control of chromosomal DNA replication; moreover, Tn*5* transposition is altered in *dnaA* mutant hosts (214). However, the molecular basis of a potential role for DnaA in Tn*5* transposition has not been established.

It should also be noted that DNA replication executed by the host machinery plays essential roles in several aspects of transposition. Host-mediated replication is critical in repairing the gaps between the newly inserted transposon and the target DNA, in carrying out semiconservative replication of a transposon to produce a cointegrate from a Shapiro intermediate, and also probably in the repair by homologous recombination of broken donor backbones. However, little is yet known about how the cellular replication apparatus may recognize and be directed to the various DNAs that result from transposition (106, 120a, 149a).

Controlling Transposon Copy Number

Another issue central to the control of transposition is how recombination is affected by the number of copies of that element within the cell. Because the act of transposition can lead to an increase in the number of transposon copies within a cell, the total frequency of transposition events will increase with increasing transposon copy number if each of these copies acts independently. If unchecked, this process could lead to cellular catastrophe, with exponentially increasing numbers of transposition events and transposons. To avoid such catastrophe, many elements have control mechanisms which keep the total number of transposition events small even with increased transposon copy number; in other words, the frequency of transposition of one particular element is sensitive to the total number of transposon copies in the cell (99, 100). Such strategies avoid the escalating cycle of “transposition leads to more transposon copies leads to more transposition. . . .”

One mechanism that contributes to make transposition of an element insensitive to potential stimulation by transposase expressed by another copy of that element is that, as noted above, many transposases are preferentially *cis* acting; that is, they are most effective on the element from which they are synthesized and are not freely diffusible to other transposon copies within the cell. This feature can also contribute to the control of transposon copy number. The effective transposase level available to a single element reflects only the amount of transposase made by that element, i.e., is independent of the presence

of other elements. While this regulatory mechanism does not directly address how to keep the total number of transposition events small, it does mean that transposition frequency will not increase exponentially with increasing copy number.

Additional control mechanisms ensure that for some elements, the overall frequency of transposition of that element remains constant even as the number of copies of the transposon may increase. In other words, as the element number increases, the transposition frequency per element actually decreases. This can be accomplished by coupling a *cis*-acting transposase, i.e., a recombinase that communicates only poorly with other transposon copies, with a *trans*-acting negative regulator of transposition which can act on all copies within a cell (99, 100).

In the case of Tn*10*, the *trans*-acting negative regulator is an antisense RNA complementary to the 5′ end of the transposase mRNA which acts at the level of transposase synthesis (35, 187). By pairing to authentic transposase mRNA, this inhibitory antisense RNA blocks ribosome binding and, hence, translation initiation (124). By contrast to transposase, this antisense inhibitor works effectively in *trans*, so that as the transposon copy number increases, the level of this inhibitor also increases, and the inhibitor can inhibit transposase expression from all transposon copies.

Another strategy to maintain a constant transposition frequency is to generate a *trans*-acting negative regulator that acts directly on the recombination machinery. This strategy is used by Tn*5*. The Tn*5* *trans*-acting negative regulator is a truncated form of transposase which can inhibit transposase by forming mixed oligomers inactive in transposition (53). A related but distinct strategy is displayed by IS*1*. Here, the *trans*-acting negative regulator is also the N-terminal portion of transposase, which is produced in the absence of translational frameshifting, and has DNA end-binding activity but lacks the catalytic sites for strand exchange. This shorter form of transposase may inhibit recombination by blocking the access of authentic transposase to the transposon ends (64, 125, 161, 216).

The strategy of a *trans*-acting transposition inhibitor coupled with a *cis*-acting transposase can effectively reduce transposition activity per element as the element copy number increases.

Encouraging Horizontal Transposon Transmission

A prominent feature of the lifestyle of bacterial transposons is their ready horizontal transmission between bacteria, both within and between bacterial species. This process underlies the rapid dissemination of antibiotic resistance determinants. The usual strategy for such transmission is the translocation of the transposon to plasmids which can, in turn, move into other cells by conjugation. Other vehicles for intercellular transmission include bacteriophages and even DNA via transformation. Some specialized mobile elements actually encode their own conjugation functions (see below).

Elements can facilitate their horizontal transfer by several means. For many elements, plasmids rather than the bacterial chromosome actually appear to be the preferred targets of transmission. For example, Tn*3* readily transposes between plasmids, but no insertions of Tn*3* into the chromosome have been observed (105, 149). The molecular basis of this preference is not understood. The preferential transposition of elements from newly replicated DNA may also facilitate horizontal transposon transmission. During conjugation, a single DNA strand containing the transposon is transferred into the recipient cell. The host replication machinery then converts this single strand into double-stranded DNA. Thus conjugation, like chromosomal DNA replication, provides a brief window in which newly replicated DNA is marked by hemimethylation. The hemimethylated DNA generated during conjugation can act as a preferential substrate for transposition, both by increased transposase synthesis because of a hemimethylated promoter region and from a high activity of a hemimethylated transposon end directly in transposition (99, 169).

As described above, *trans*-acting negative regulators, which are often truncated forms of transposase, can profoundly affect transposition frequency, either through promotion of alterations in transposase expression or by direct modulation of the transposition reaction itself. Upon entry into a naive cell, the

element will begin to express transposase and these regulatory molecules. There may then be an interval when the concentration of the inhibitor relative to transposase is much lower than in a cell in which the transposon has long resided. Such a high-transposase–low-inhibitor window would result in a relatively high frequency of transposition when an element enters a naive cell before the level of the negative regulator rises to again decrease transposition in a cell to a low basal level. Thus, zygotic induction of transposition may occur upon entry of an element into a naive cell. Studies of Tn*5* transposition into naive cells and cells already containing Tn*5* revealed a higher frequency of transposition upon entry into the naive cell and provided an early example of the regulation of transposition by transposon-encoded proteins (26). A variation on this strategy may be used by elements such as Tn*7*, in which the transposase binding sites at the element ends apparently both mediate transposition and autoregulate transposase expression (7, 84, 153, 195). Similar to the effect of the inhibitor of transposase, upon transfer to a naive cell there will be an initial burst of transposase synthesis, which may encourage transposition to the chromosome, thereby facilitating the stable incorporation of the element into the host chromosome and promoting its spread from cell to cell. A related strategy may be involved in Tn*3* transposition, in which an element-encoded repressor regulates transposase transcription (186). These strategies would be particularly important when, for example, because of host range restrictions, stable replication of the incoming conjugable plasmid cannot be maintained.

OTHER TYPES OF MOBILE ELEMENTS

There are several types of bacterial mobile DNA elements that translocate between nonhomologous positions and thus can be broadly termed transposable elements but are distinctive in that they do not make target site duplications upon insertion. Although the translocation mechanisms of these elements are different from the target site duplication pathways described above, many of the same biological issues of control and regulation are similar.

IS*91*

IS*91* has several characteristics which distinguish it from most other insertion sequences: (i) insertion is not accompanied by target site duplication (133); (ii) insertion is target sequence specific, the element always inserting 5′ of either of two sequences (133); and (iii) its transposase is related in sequence to site-specific nicking-closing proteins involved in the initiation of rolling-circle replication mechanisms (134). It is proposed (132) that IS*91* transposition begins with sequence-specific breakage events at one transposon end and at the target DNA, followed by strand transfer of one transposon end to the target DNA, displacement of this target-linked transposon strand from the donor DNA by replication, and, finally, passive replication of the transferred transposon strand as part of the target chromosome. This proposed mechanism is closely related to several other DNA transactions, the conjugal transfer of DNA between bacteria, and formation of the Ti plasmid segment which is transferred into plant cells (204). In these cases, a “daughter” DNA element is generated by sequence-specific nicking and strand displacement by DNA replication, but there is no interaction of the “mobile element” with the target DNA at the initiation of recombination. Rather, the DNA segment to be transferred is generated in the absence of the target DNA, and insertion results from a subsequent encounter with a target DNA. This proposed mechanism of IS*91* transposition emphasizes the close relationships between transposition, replication, and conjugation.

Conjugative Transposons

Conjugative transposons are discrete DNA segments, generally encoding antibiotic resistance determinants, which have the ability to translocate between the chromosomes of different cells without the need for another DNA vehicle such as a plasmid (42, 179, 180). These elements encode functions which mediate their excision

from the donor chromosome, the transfer of this excised species between cells, and the integration of the element into the target chromosome in the recipient cell. Well-studied conjugative transposons include Tn*916* and Tn*1545;* Tn*554* is another element which may translocate in a related fashion. Although these elements were first identified in gram-positive bacteria and appear to be most prevalent in such organisms, they do occur naturally in gram-negative bacteria, and some can transpose in *E. coli.* It should also be noted that gram-positive bacteria also contain many other mobile elements similar to the gram-negative elements discussed above, which do make target site duplications (148).

Distinctive features of the translocation of conjugative transposons include the following: (i) target insertion is not accompanied by target sequence duplication, (ii) nonelement sequences from the donor site can also be joined to the target site, and (iii) the recombinases of these elements are related to the lambdoid phage integrase family (see below). The translocation of these conjugative elements can be described by the following model (33, 162). Excision occurs by staggered breaks at the transposon ends and in flanking DNA, followed by circularization to form intermediates that contain heteroduplex DNA between the juxtaposed transposon ends derived from flanking donor sequences. Following transfer to the recipient, integration occurs via staggered breaks in the circular intermediate and target DNA, followed by joining. These steps result in the insertion of the element into the target DNA, flanked by heteroduplex DNA containing one strand from the target and one strand from sequences that flanked the element in its donor site. This mechanism accounts for the joining of nonelement DNA from the donor site to the target site. Target selection is not random, because preferred insertion sites are observed, but the mechanism of target selection is not understood; target selection does appear to be independent of the host sequences which flank the element in the donor site. Production of the final recombination products, i.e., DNAs lacking heteroduplex, requires a round of chromosomal DNA replication.

The proposed mechanism of movement of conjugative transposons is highly reminiscent of the integration-excision cycle of bacteriophage lambda; indeed, the homologies between the conjugative transposon recombinases and the lambdoid phage integrases suggest that the basic chemical features of these reactions may be similar (chapter 125, this volume). However, these reactions differ substantially in the involvement of DNA homology. In conjugative transposition, there is no requirement for strict homology between the recombination sites; intermediates at several stages involve heteroduplex DNA. By contrast, site-specific recombination reactions such as lambda integration are dependent on homology between the recombining DNAs; such positions of homology include the sites of strand exchange. It should also be appreciated that in the movement of conjugative transposons, like more classical transposition reactions, DNA replication does play a key role; a round of replication is required to produce homoduplexes from the direct products of recombination, which include regions of heteroduplex DNA.

FUTURE DIRECTIONS

The study of bacterial transposition reactions has provided an outline at the molecular level of both the mechanism and regulation of these interesting DNA rearrangements. In addition to revealing the basic features of an important type of recombination, the study of bacterial transposition has contributed in important and fundamental ways to our understanding of protein-DNA interactions and of a variety of bacterial regulatory processes and has also provided powerful genetic tools for bacterial genome manipulation.

What are future studies of these bacterial reactions likely to explore and reveal? It seems likely that, having arrived at a molecular outline of transposition, an important advance will be to provide atomic views of both the protein and DNA components of these reactions. As transposition is a reaction involving changes in DNA, it will be especially critical to have a dynamic view of these components and their interactions. How, for example, is a recombinase converted from a simple DNA-binding protein to a machine that effects the rearrangement of DNA strands in highly coupled and precise fashion? An important first step has recently come from the crytallographic analysis of the catalytic center of the Mu transposase (168a). Dissection of the structure and function of transposition proteins is certain to contribute deeply to our understanding of general protein structure and function. Understanding the superstructure and dynamics of the complex assemblies of proteins and DNAs in

which recombination occurs will also be fundamental. Another fruitful arena will be to further explore the complex relationships between transposable elements and their hosts. A deeper understanding of how the cellular (and also probably the extracellular) environment communicates with the transposon and influences transposition is needed for *E. coli, Salmonella typhimurium* (*Salmonella enterica*), and other bacterial systems.

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