Cell, Vol. 83, 375–385, November 3, 1995, Copyright © 1995 by Cell Press

Assembly of Phage Mu Transpososomes: Cooperative Transitions Assisted by Protein and DNA Scaffolds

Michiyo Mizuuchi,* Tania A. Baker,† and Kiyoshi Mizuuchi*
*Laboratory of Molecular Biology
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Bethesda, Maryland 20892
†Howard Hughes Medical Institute and Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Summary

Transposition of phage Mu takes place within higher order protein–DNA complexes called transpososomes. These complexes contain the two Mu genome ends synapsed by a tetramer of Mu transposase (MuA). Transpososome assembly is tightly controlled by multiple protein and DNA sequence cofactors. We find that assembly can occur through two distinct pathways. One previously described pathway depends on an enhancer-like sequence element, the internal activation sequence (IAS). The second pathway depends on a MuB protein–target DNA complex. For both pathways, all four MuA monomers in the tetramer need to interact with an assembly-assisting element, either the IAS or MuB. However, once assembled, not all MuA monomers within the transpososome need to interact with MuB to capture MuB-bound target DNA. The multiple layers of control likely are used in vivo to ensure efficient rounds of DNA replication when needed, while minimizing unwanted transposition products.

Introduction

Formation of higher order protein–DNA complexes is often a critical step in the initiation of transcription, recombination, and replication. The assembly of these complexes is frequently a target of regulation. The protein–DNA complexes involved in transpositional and site-specific recombination are among the best characterized and thus are excellent systems for elucidating general principles governing complex assembly, disassembly, and organization.

The higher order protein–DNA complexes that mediate transposition of the phage Mu genome are called transpososomes (Surette et al., 1987; Craigie and Mizuuchi, 1987; Mizuuchi et al., 1992). These transpososomes contain the two ends of the Mu genome synapsed by a tetramer of the Mu transposase (MuA) (Lavoie et al., 1991; Baker and Mizuuchi, 1992; Mizuuchi et al., 1992). Assembly of the transpososome is a prerequisite for the chemical steps of transposition that move Mu to new locations in the host genome. These steps are single strand cleavage at the two ends of the Mu genome, called donor cleavage, and the insertion of the cleaved Mu ends into a target DNA, called DNA strand transfer. Three forms of the Mu transpososomes are distinguished based on the chemical steps their DNA components have undergone. The stable synaptic complex (SSC or type 0 complex) contains uncleaved donor DNA; the cleaved donor complex (CDC or type I complex) contains donor DNA that has undergone cleavage at the Mu ends; and the strand transfer complex (STC or type II complex) contains the covalently joined donor and target DNAs (Figure 1A).

The key component of the transpososome is the MuA protein. The 663 residue MuA protein can be divided into three domains by partial proteolysis (Nakayama et al., 1987), and each domain can be further divided functionally or structurally into subdomains (Figure 1B). The N- and C-terminal regions of the protein interact with cofactors that regulate transposition (see below), while the internal regions are involved in catalysis of DNA cleavage and strand transfer. The core domain (domain II) of MuA contributes essential functions to both chemical reactions. The crystal structure of this domain has been solved (Rice and Mizuuchi, 1995); the major part of this structure is remarkably similar to that of the catalytic core domain of HIV integrase (Dyda et al., 1994), as well as RNase H (Katayanagi et al., 1990; Yang et al., 1990; Davies et al., 1991) and the Holliday junction resolving enzyme RuvC (Ariyoshi et al., 1994). MuA and HIV integrase catalyze similar reactions, endonucleolytic cleavage at the 3′ end of the donor DNA and subsequent strand transfer (reviewed by Mizuuchi, 1992a, 1992b). A set of acidic amino acid residues in MuA (D269, D336, and E392) align with the D,D-35-E motif present in retroviral integrases and many transposases, and mutations at these amino acids abolish both the donor DNA cleavage and strand transfer activities of MuA (Baker and Luo, 1994; Kim et al., 1995; E. Krementsova and T. A. B., unpublished data). Thus, MuA belongs to a protein family, members of which probably catalyze breakage and joining of DNA and RNA phosphodiesters in a similar manner.

This catalytic domain of MuA becomes chemically active only when properly assembled into a tetramer on the ends of the Mu genome. Both assembly of the transpososome and subsequent reaction steps promoted by the MuA tetramer are influenced by several DNA sites and proteins that can be considered cofactors in the overall reaction (reviewed by Mizuuchi, 1992b; Haniford and Chaconas, 1992). Among these cofactors are the DNA sites at each end of the Mu genome. Each end carries three MuA-binding sites with a 22 bp consensus sequence; the left-end sites are designated L1, L2 and L3, while those on the right end are called R1, R2, and R3 (Craigie et al., 1984). While all six sites appear to participate in transpososome assembly (Allison and Chaconas, 1992), only three sites, L1, R1, and R2, are stably bound by the MuA tetramer within a transpososome (Lavoie et al., 1991; Mizuuchi et al., 1991, 1992). One of the sequence-specific DNA-

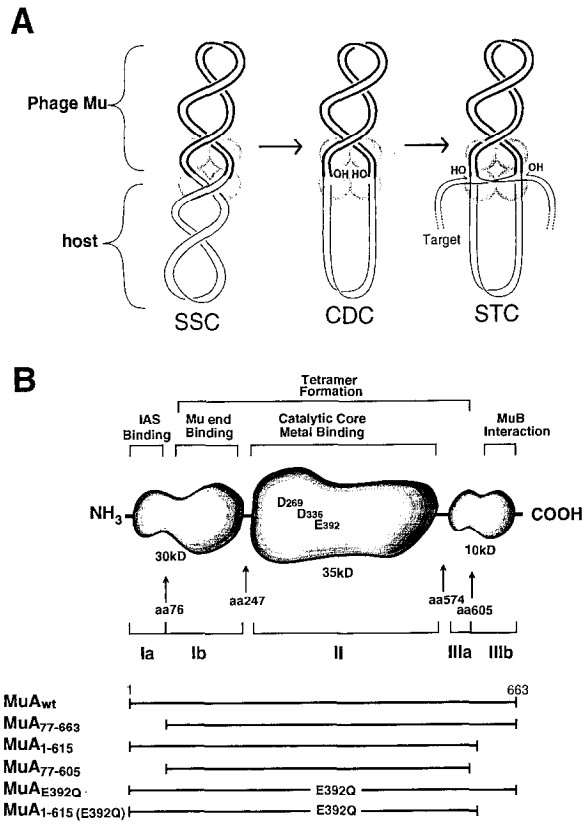

Figure 1. Mu Transpososomes and the Domain Structure of MuA

(A) Three types of Mu transpososomes in the Mu transposition pathway. See the text for details.

(B) The domain structure of MuA protein and the MuA derivatives used in this study. Parts of the MuA protein contained in each derivative are indicated by the horizontal lines. See the text for details. wt, wild type.

binding subdomains of MuA (domain IB) binds to these 22 bp sequences (Leung et al., 1989; Zou et al., 1991).

Efficient assembly of a transpososome also requires an enhancer-like sequence element on the Mu donor DNA, the internal activation sequence (IAS), as another DNA cofactor (Mizuuchi and Mizuuchi, 1989; Leung et al., 1989; Surette et al., 1989). Approximately 100 bp long, the IAS overlaps with the Mu operator sequence and is composed of three components: two clusters of MuA-binding sequences separated by a binding site for integration host factor (IHF), a sequence-specific DNA-bending protein (Mizuuchi and Mizuuchi, 1989; Surette et al., 1989). The N-terminal subdomain (domain IA) forms a winged helix-turn-helix DNA-binding structure (Clubb et al., 1994) and binds to the repeated consensus sequences within the IAS, which also binds Mu repressor (Craigie et al., 1984; Leung et al., 1989; Mizuuchi and Mizuuchi, 1989). MuA protein lacking this subdomain, MuA(77–663), cannot utilize the IAS.

The protein cofactors for transposition include the host-encoded DNA-bending proteins HU and IHF and the Mu-encoded target DNA–binding protein MuB. HU and IHF play important roles in SSC assembly: IHF by binding to its site in the IAS and effecting a DNA bend (Surette et al., 1989; Surette and Chaconas, 1989) and HU by interacting with the left end, probably between the L1 and L2 sites (Lavoie and Chaconas, 1993, 1994). MuB participates at several steps in the transposition pathway. Efficient strand transfer requires activation of the CDC by interaction of the MuA tetramer with MuB (Baker et al., 1991). MuB is especially important for delivering a nonimmune target DNA site to the CDC by binding tightly in a sequence-independent manner to DNA in the presence of ATP. A DNA molecule is an "immune" target if it carries MuA-binding sites and "nonimmune" if it lacks such sites. These descriptions reflect the fact that a Mu donor DNA plasmid (or other plasmid carrying MuA-binding sites) only rarely acquires an additional insertion of Mu DNA by transposition. The poor use of these DNAs as transposition target is due to preferential dissociation of MuB from DNA molecules that are bound by MuA (Adzuma and Mizuuchi, 1988). As a result of this "clearing" of MuB from the DNA near MuA-binding sites, stable oligomers of MuB can only accumulate on nonimmune DNAs. These MuB-bound DNA sites are preferentially used as target, so self-destruction of the Mu genome by transposition is avoided (see Mizuuchi, 1992b). In the absence of MuB (or ATP), the intermolecular strand transfer reaction is very inefficient (Maxwell et al., 1987; Adzuma and Mizuuchi, 1988). The C-terminal subdomain of MuA (domain IIIB, amino acids 606–663) is required for interaction with MuB (Baker et al., 1991; Leung and Harshey, 1991; Wu and Chaconas, 1994). MuA protein that lacks this subdomain does not recognize MuB and thus cannot carry out efficient intermolecular strand transfer.

Once assembled into a stable tetramer, the four MuA monomers within the complex promote a single round of the cleavage and strand transfer reactions without dissociating or exchanging with free monomers. In this study, we focus on the question of which monomers interact with two of the reaction cofactors, the IAS and MuB protein, during assembly of the transpososome and within the stable tetramer. The results provide insight into how the cofactors function. During assembly, both the IAS and the MuB–target DNA complex appear to contact all four of the monomers that will end up in the tetramer, thus providing a scaffolding for assembly of the complex. In contrast, for MuB to stimulate strand transfer it need only contact a subset of the monomers in the tetramer, but in doing so appears to activate catalysis by the whole complex.

Results

To probe the mechanism of action of the DNA and protein cofactors involved in Mu transposition, we used mutant versions of MuA defective in interacting with the particular cofactor of interest (Figure 1B). These proteins were mixed with wild-type MuA or MuA derivatives carrying mutations that render the protein defective in cleavage and strand transfer reactions. The activities expressed by the resulting transpososomes, as well as the amounts of the different forms of MuA incorporated into them, provided insight into how the cofactors influence the assembly and

activity of the complexes. One protein in each reaction mixture was labeled with ^{35}S so that its presence in the stable protein–DNA complexes could be detected and quantitated by autoradiography with storage phosphor plates after separation of the SSC, CDC, and STC by gel electrophoresis. By carrying out parallel reactions with the same protein concentrations but with the ^{35}S label in a different partner, we could calculate the ratio of each MuA derivative in the different complexes. The presence of a mutant protein in one of the stable complexes indicates that the mutant was present in the tetramer that formed the complex; thus, the mixed tetramer must be functional for all the steps required to generate the observed complex.

Every MuA Monomer in the Tetramer Must Interact with the IAS for Efficient IAS-Assisted SSC Assembly

Under our standard reaction conditions, which include MuB (0.6 μM) and target DNA (10 μg/ml), both the IAS and the N-terminal IAS-binding domain of MuA are critical for efficient assembly of the SSC (Mizuuchi and Mizuuchi, 1989; see Figures 2A and 2B). We were interested in finding out how many of the monomers that constitute the MuA tetramer interact with the IAS during assembly.

To address this question, we performed reactions using wild-type MuA and MuA(77–663) and assayed for the presence of each protein in the stable protein–DNA complexes by autoradiography after electrophoresis on a native agarose gel (Figure 2). As expected, little formation of any transpososome occurred with MuA(77–663) alone, even after 3 hr (Figure 2A). In contrast, wild-type MuA alone efficiently formed all three types of transpososome, with strand transfer complexes accumulating within 5 min (Figure 2B). When equal concentrations of wild-type MuA and MuA(77–663) were mixed, only a small amount of MuA(77–663) was incorporated into the complexes (Figure 2C). The ratio of the two proteins in each of the complexes was estimated by comparing the lanes in Figures 2C and 2D. Approximately 95% of the MuA in each of the types of stable complexes was wild-type MuA, with MuA(77–663) contributing only about 5%. Therefore, most tetramers formed under these conditions have four wild-type monomers, indicating that during assembly the deletion protein is specifically excluded. These data suggest that all four of the MuA monomers that become incorporated into the stable tetramer interact with the IAS during assembly.

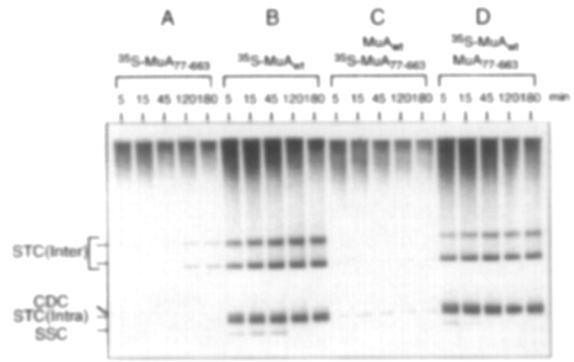

Figure 2. Transpososome Assembly in the Presence of MuA(77–663)

All four MuA monomers must be able to interact with the IAS for an efficient MuA tetramer assembly by the IAS-assisted pathway. Autoradiogram after nondenaturing agarose gel electrophoresis of the complexes formed by wild-type (wt) MuA and MuA(77–663). We used 50 nM each of the two MuA derivatives separately or in combination. One of the MuA derivatives in each reaction was labeled by ^{35}S. pBR322 was used as the target DNA. The reactions were incubated at 30°C for the times indicated. The MuA derivatives included in the reactions are shown above each lane. STC(intra), intramolecular strand transfer complex. The STC(inter) migrated as two distinct bands under the conditions used. The slower migrating band contained the complex with its target DNA segment relaxed, while in the faster migrating complex the target DNA segment was held supercoiled by the bound MuA tetramer (data not shown).

MuB–Target DNA Complex Can Assist Transpososome Assembly by MuA(77-663)

Although virtually inactive under standard conditions, MuA(77–663) was partially activated by higher concentrations of MuB protein. As shown above, little donor cleavage or strand transfer by MuA(77–663) was detectable under standard reaction conditions. However, when the concentration of MuB was increased, intermolecular strand transfer became efficient; this increased efficiency was clear at 1.2 μM MuB, and maximum activity was observed at 2.4 μM (higher concentrations did not improve the reaction efficiency further; data not shown). Reactions with wild-type MuA were not dramatically influenced by these changes in MuB concentration (Figure 3A).

The stimulation of MuA(77–663) by MuB depended on the presence of nonimmune intermolecular target DNA. In the absence of this type of target DNA, higher concentrations of MuB did not rescue the defect of MuA(77–663); no product could be detected irrespective of the concentration of MuB (data not shown). The specific requirement for a nonimmune target DNA indicates that a stable MuB–DNA complex is needed to rescue the defect of MuA(77–663), as neither free MuB (not stably bound to DNA) nor MuB that forms a transient complex with the donor DNA can function in this capacity. Since MuA(77–663) is defective in transposition under standard reaction conditions because it fails to assemble, it is logical to conclude that high concentrations of MuB–target DNA complex activate MuA(77–663) by allowing it to assemble into an active transpososome. Thus, the MuB–target DNA complex can substitute functionally for the IAS in assembly of the MuA tetramer.

This MuB–target DNA-dependent assembly pathway was most apparent with MuA derivatives that lack the IAS-binding domain. When reactions were done with wild-type MuA or MuA(77–663) using a donor DNA lacking the IAS (to prevent IAS-assisted assembly by the wild-type protein), high concentrations of MuB were not as effective at stimulating strand transfer by wild-type MuA as by MuA(77–663) (Figure 3B). Therefore, it appears that the IAS-binding domain, in the absence of the IAS, inhibits the MuB–target DNA complex-assisted pathway of transpososome assembly (see below).

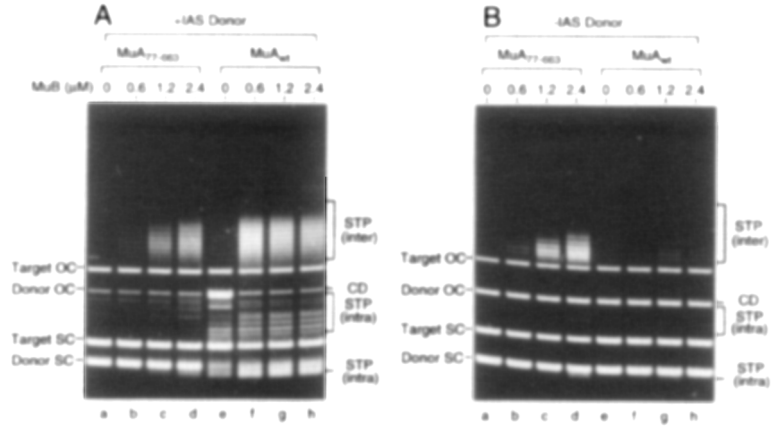

Figure 3. MuB–Target-Assisted Transpososome Assembly with MuA(77–663)

(A) MuB–target DNA complex can assist the transpososome assembly by MuA(77–663). Agarose gel electrophoresis of DNA reaction products after dissociation of proteins by SDS. The reaction was incubated at 30°C for 60 min in the presence of different concentrations of MuB as indicated. The MuA derivatives included in the reactions (50 nM each) are shown above each lane. The donor DNA was pMK586. (B) The IAS-binding domain of MuA inhibits MuB-assisted assembly of the transpososome in the absence of the IAS. Reactions were exactly the same as in (A), except that the donor DNA used, pMK588, did not carry the IAS. Band positions are labeled as follows: Donor SC, supercoiled donor; Donor OC, open circle donor; Target SC, supercoiled ΦX174 RF target; Target OC, open circle ΦX174 RF target; STP(inter), intermolecular strand transfer product; STP(intra), intramolecular strand transfer product; CD, cleaved donor.

How many of the MuA monomers interact with MuB during tetramer assembly by the MuB–target DNA complex-assisted pathway? To answer this question, we took a similar approach to the experiment of Figure 2; this time we made use of a doubly defective protein, MuA(77–605), which cannot interact with either the IAS or MuB. MuA(77–605) was virtually inactive on its own, regardless of high concentrations of MuB; only a small amount of the intramolecular STC was generated even after a 90 min incubation (Figure 4A, lane b). MuA(77–605) is inactive only because of defects in cofactor-mediated complex assembly; it is fully active under conditions that bypass the cofactor requirements for assembly (data not shown). When added to a reaction containing MuA(77–663), an equal concentration of MuA(77–605) was inhibitory (approximately 70% inhibition; compare lanes a and c in Figure 4), and very little MuA(77–605) (less than 5% of the total MuA in the complex) was incorporated into the complex (lane d). Thus, it appears that the four MuA monomers that constitute the MuA tetramer interact with MuB during MuB–target-assisted transpososome assembly.

The cofactor requirements for transpososome assembly are less stringent in the presence of glycerol (Mizuuchi and Mizuuchi, 1989), and the standard reaction conditions used in this study included 15% glycerol because the reaction is more efficient in its presence. When glycerol was omitted from the reactions containing a high concentration of MuB, MuA(77–663) was less active (Figure 4B, lane a), while MuA(77–605) had no detectable cleavage or strand transfer activity (lane b). Further, the demand for all four MuA monomers to interact with MuB for the MuB–target-assisted transpososome assembly was more stringent in the absence of glycerol; no double mutant protein could be detected in the complexes formed in the presence of MuA(77–663) and MuA(77–605) (lane d).

The experiments presented in the last two sections reveal two pathways for assembly of active transpososomes, one that involves interaction of four monomers with the IAS and a second in which four monomers interact with the MuB–target DNA complex. These data contribute to models for the mechanism of cofactor-dependent complex assembly (see Discussion).

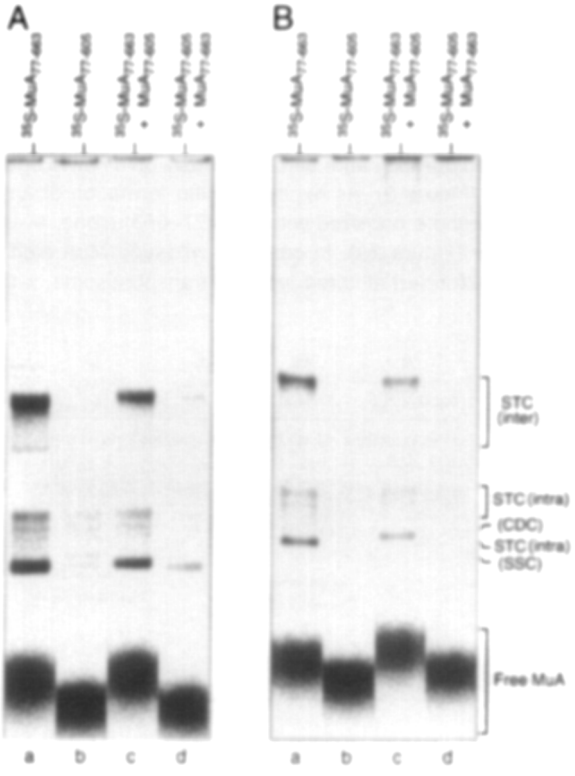

Figure 4. Requirements for the MuB–Target-Assisted Transpososome Assembly

All four MuA monomers must be able to interact with MuB for an efficient assembly of the MuA tetramer by the MuB–target DNA-assisted pathway. Autoradiogram after nondenaturing agarose gel electrophoresis (see Experimental Procedures for details) of the complexes formed by MuA(77–663) and MuA(77–605), separately or in combination. Standard reaction conditions were used, except that it contained 2.3 μM MuB. pBR322 was used as the target DNA, and reactions were incubated for 90 min. Reactions shown in (A) included 15% glycerol, while those in (B) did not contain glycerol. The MuA derivatives included in the reactions (50 nM each) are shown above each lane. Labeling is the same as in Figure 2.

MuB Does Not Have to Interact with All Four MuA Monomers to Assist Intermolecular Strand Transfer

In the last section, a pathway for assembly of an active Mu transpososome that depends on a MuB–target DNA complex was demonstrated. For MuB to promote this assembly reaction, all four monomers in the MuA tetramer apparently need to interact with MuB. The more commonly appreciated role of MuB in transposition is its delivery of intermolecular target DNA to an assembled transpososome and stimulation of strand transfer by the MuA tetramer. The following experiments address how many monomers in the tetramer need to contact MuB for this stimulation of intermolecular strand transfer. These experiments utilized another deletion protein, MuA(1–615), which is unable to interact with MuB, but can assemble transpososomes and carry out donor DNA cleavage and intramolecular strand transfer as efficiently as the full-length MuA in the absence of MuB (Baker et al., 1991).

To address the question of how many monomers need to interact with MuB during strand transfer, we mixed equal concentrations of wild-type MuA and MuA(1–615) in a reaction containing MuB and pBR322 as target DNA (Figure 5). The donor DNA carried the IAS, and both proteins had the IAS-binding domain, so under these conditions assembly could take place efficiently by the IAS-dependent pathway. The presence of equimolar MuA(1–615) inhibited intermolecular strand transfer by wild-type MuA; the level of intermolecular STC was about 30% of that made by wild-type MuA alone (compare Figure 5A with the sum of Figures 5C and 5D). However, MuA(1–615) was stably incorporated into the STC containing intermolecular target DNA (STC(inter); Figure 5D), indicating that not all monomers in the tetramer need to contact MuB during MuB-dependent intermolecular strand transfer. The contributions of wild-type MuA and MuA(1–615) in the intermolecular STC were approximately 75% and 25%, respectively. Values obtained from independent experiments ranged between 65% and 80% for wild-type MuA and between 35% and 20% for MuA(1–615) in the complex.

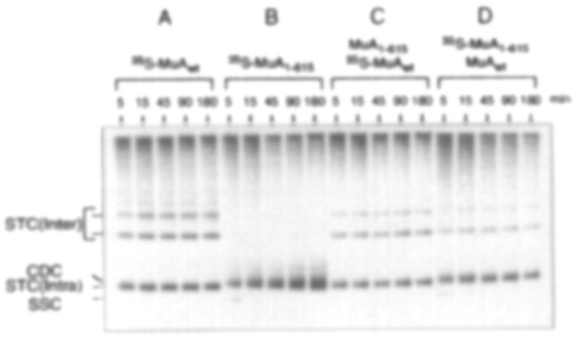

Figure 5. Intermolecular Strand Transfer in the Presence of MuA (1–615)

Not all MuA monomers in the CDC must be able to interact with MuB for MuB-assisted intermolecular strand transfer. Autoradiogram after nondenaturing agarose gel electrophoresis of the complexes formed by wild-type (wt) MuA and MuA(1–615). We used 50 nM each of the two MuA derivatives separately or in combination. One of the MuA derivatives in each reaction was labeled by ^{35}S. pBR322 was used as the target DNA. The reactions were incubated at 30°C for the times indicated. The MuA derivatives included in the reactions are shown above each lane. Labeling is the same as in Figure 2.

The relative contributions of the two proteins in the intermolecular STC remained at approximately 3 to 1 when reactions contained one fifth of our standard concentration of wild-type MuA (10 nM) and 50 nM MuA(1–615) (data not shown). Although calculation of the ratio is not precise owing to errors inherent to the measurements, we conclude that at least one, and possibly two, MuA monomers that are unable to interact with MuB can be present within a MuA tetramer without seriously impeding its capacity for MuB-assisted intermolecular strand transfer.

Which MuA Monomer Interacts with MuB Is Not Critical for MuB-Assisted Intermolecular Strand Transfer

To address whether or not the monomer that catalyzes strand transfer needs to interact with MuB directly, we carried out a complementation experiment using MuA(1–615) and MuA(E392Q). MuA(E392Q) is defective in cleavage and strand transfer, but assembles into the SSC and retains the MuB-binding domain (domain IIIB) (Baker and Luo, 1994; Baker et al., 1994; Kim et al., 1995). If this mutant protein can work with MuA(1–615) to form the intermolecular STC, different monomers within the tetramer must be able to contribute the MuB interaction domain and the critical acidic residues in domain IIA (referred to as active site in the following discussion).

When MuA(1–615) was mixed with an equal concentration of MuA(E392Q), a single discrete intermolecular strand transfer product band was formed (Figure 6A, lane e). This discrete band contained products in which only one of the two Mu DNA ends was covalently joined to the target DNA (data not shown; Baker et al., 1994). It migrates slower in an agarose gel than the intermolecular strand transfer products made by wild-type MuA, which have both cleaved ends of the Mu DNA joined to the target DNA and migrate as a series of topoisomers (Figure 6A, lane f). Roughly equal amounts of each MuA derivative were found in the intermolecular STC, as judged by autoradiography of the complexes containing ^{35}S-labeled proteins (Figure 6B, lanes c and d). Was the Mu end that remained unjoined in the single end strand transfer products cleaved or uncleaved? The single end strand transfer products were isolated from an agarose gel, and the overall extent of cleavage at the two Mu ends was measured. Approximately half of the Mu ends (60% and 40% at the left and right ends, respectively) remained uncleaved (and unjoined to target DNA) (data not shown). Since the 50% of the Mu ends that completed strand transfer must have been cleaved, almost none of the unjoined ends in these complexes could have been cleaved. Therefore, it appears that, although two molecules each of MuA(1–615) and MuA(E392Q) can form a mixed tetramer able to promote MuB-dependent intermolecular strand transfer, only one of the donor ends can be cut and joined to the target DNA by this complex.

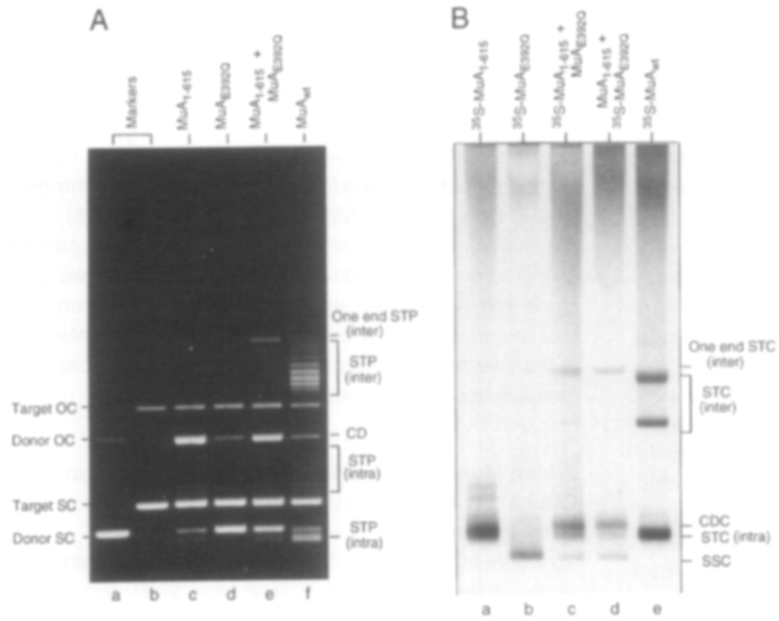

Figure 6. A Mixed Tetramer Containing MuA (1–615) and MuA(E392Q) Generates Single-Ended STC(inter)

(A) Agarose gel electrophoresis of DNA reaction products after dissociation of proteins by SDS. Reactions were incubated at 30°C for 60 min. The MuA derivatives included in the reactions (50 nM each) are shown above each lane. Markers were as follows: lane a, donor pMK586 DNA; lane b, ΦX174 RF target DNA. Labeling is the same as in Figure 3.

(B) Autoradiogram of a nondenaturing agarose gel of the complexes made by MuA(1–615) and MuA(E392Q). pBR322 was used as target DNA, and the reactions were incubated at 30°C for 60 min. We included 50 nM each of the MuA derivatives shown above each lane in the reactions. Labeling is the same as in Figure 2.

Is it obligatory then for the two monomers that donate the domain II for cutting and joining to be activated by the other two monomers contacting MuB by their domain IIIB? If this were the case, a double mutant protein that retains neither the chemical activities nor the MuB interaction domain should not be able to participate in intermolecular strand transfer. We tested whether the double mutant MuA(1–615[E392Q]) protein could participate in intermolecular strand transfer. Generation of single end intermolecular strand transfer product in a reaction containing a mixture of the double mutant and wild-type MuA would indicate that incorporation of this mutant protein into a tetramer does not block productive interaction with MuB.

When the reaction was carried out in the presence of equal concentrations of wild-type MuA and MuA(1–615[E392Q]), both single end intermolecular strand transfer product and complete product accumulated (Figure 7A). The double-ended strand transfer products are expected to result from the MuA tetramers composed of four wild-type monomers. On the other hand, the single-ended strand transfer product should have at least one, possibly two, mutant MuA monomers in the tetramer. Analysis of the gel-purified single-ended strand transfer product as described above indicated that the majority of the ends that did not complete strand transfer in this product had not been cleaved either (data not shown). Thus, most of the single end strand transfer product was generated by a MuA tetramer that incorporated the double mutant pro-

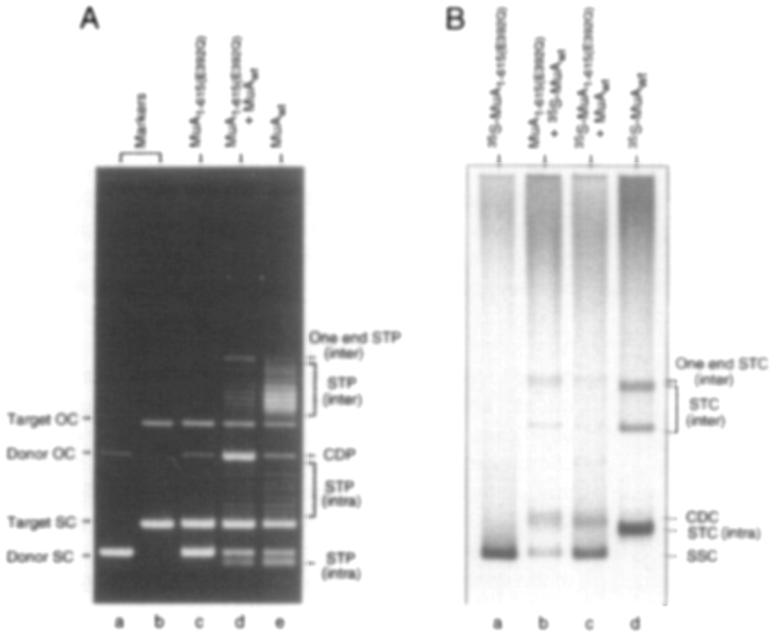

Figure 7. MuA(1–615[E392Q]) Can Form a Single-Ended STC(inter) with Wild-Type MuA

(A) Agarose gel electrophoresis of DNA reaction products after dissociation of proteins by SDS. The reactions were incubated at 30°C for 60 min. Protein concentrations and reaction conditions are the same as in Figure 5. We used 50 nM each of the MuA derivatives shown above each lane in the reactions. Markers were as follows: lane a, donor pMK586; lane b, ΦX174 RF target DNA. Labeling is the same as in Figure 3. wt, wild type.

(B) Autoradiogram of a nondenaturing gel of the complexes formed by wild-type (wt) MuA and MuA(1–615[E392Q]). pBR322 was used as target DNA, and the reactions were incubated at 30°C for 60 min. We used 50 nM each of the MuA derivatives shown above each lane in the reactions. Labeling is the same as in Figure 2.

tein at a position in the tetramer where it prevents donor DNA cleavage.

The ratio of the two proteins in the intermolecular STC was analyzed using ^{35}S-labeled proteins and autoradiography. Unfortunately, the STC(inter) containing the double-ended and single-ended strand transfer product could not be separated well enough to assess their protein compositions separately. Most of the single end STC(inter) migrates slightly slower than the slower migrating band of the normal STC(inter). As can be seen in Figure 7B (compare lanes c and d), approximately 20%–30% of the MuA in the slower migrating band of the STC(inter) was the double mutant protein MuA(1–615[E392Q]). This is a minimum estimate of the contribution by the double mutant protein in the single end STC(inter). A significant fraction of the complex migrating at this gel position is expected to be the complete STC containing only the wild-type protein. Thus, at least some of the single end STC(inter) probably contained two MuA(1–615[E392Q]) monomers.

Taken together, the last two mixing experiments argue that there are no two unique monomer positions within the tetramer that must interact with MuB during MuB-dependent intermolecular strand transfer. Mixtures of MuA(E392Q) and MuA(1–615) gave rise to single end strand transfer products in which only one end was usually cleaved; most tetramers appeared to consist of two of each type of monomers. With this pair, MuA(E392Q) must provide the MuB interaction while MuA(1–615) provides the active site residues from domain II. Similarly, mixtures of wild-type MuA and MuA(1–615[E392Q]) also generated single end STCs with only one end cleaved, and these complexes appeared to contain one or two MuA(1–615[E392Q]) monomers. However, in this second pair, wild-type MuA must be providing both the MuB interaction and the domain II active site residues. Clearly, MuB can deliver a target DNA and activate catalysis of strand transfer by interacting with only a subset of the MuA monomers in the tetramer. Furthermore, it appears that the MuB contact with the monomers promoting the strand transfer reaction can be either direct or indirect. The implications of these data for the mechanism of MuB activation of catalysis are discussed below.

Discussion

Two Pathways for Cofactor-Dependent Transpososome Assembly

Assembly of an active transpososome is a critical step in Mu transposition. The requirements for complex assembly in vitro vary widely depending on the reaction conditions and the substrate DNA. Under certain in vitro conditions, MuA can assemble into an active complex on the Mu end DNA without the participation of cofactors (Savilahti et al., 1995). However, under conditions that we consider to be close to physiological, assembly is very inefficient in the absence of any one of a number of cofactors. The participation of these cofactors allows transposition to be controlled at an early step prior to cleavage of the DNA strands. In a sense, the requirement for the multiple cofactors is analogous to multiple checkpoint controls, all of which must be met prior to initiating a reaction or committing to a developmental pathway. In this study, examining the assembly behavior of wild-type MuA and MuA derivatives defective in cofactor interactions provided several insights into the mechanisms by which transpososomes can be assembled.

One of the critical cofactors for transpososome assembly is an enhancer-like DNA sequence element, the IAS. Here we demonstrated that for efficient IAS-assisted assembly, all four MuA monomers within the resulting transpososome needed to be able to interact with the IAS. Furthermore, in the process of studying IAS-dependent assembly, we discovered that the assembly defect of MuA that is unable to interact with the IAS is rescued by high concentrations of another reaction cofactor, MuB. The requirements for assistance of assembly by MuB are a stable MuB–target DNA complex and the ability of all four monomers that end up in the tetramer to interact with MuB. Both of these requirements are specific for MuB-assisted assembly of the MuA tetramer and are not observed during MuB stimulation of strand transfer.

What the IAS and the MuB–target DNA complex have in common is that they contain DNA segments that can make multivalent contacts with MuA. How might the interaction between the IAS, or the MuB–target DNA complex, and the four MuA monomers assist assembly of the transpososome? In the following model, the general scheme is described for the IAS interaction; however, the MuB–target DNA complex could function in an analogous manner.

The IAS contains two clusters of MuA-binding sequences separated by an IHF-binding site. It is therefore attractive to imagine that each cluster of MuA-binding sequences interacts with one of the two Mu end DNA segments bound by MuA monomers, as discussed by Allison and Chaconas (1992). By providing a bridging platform for close association between the two Mu DNA ends bound by MuA monomers, perhaps at the branch point of the supercoiled donor DNA, the IAS may significantly influence the association equilibrium between the two protein–DNA complexes at the two ends of the Mu genome. We imagine there is a reversible association of the two Mu end–MuA complexes prior to the irreversible conformational change that leads to formation of the stable transpososome. In the simplified scheme shown in Figure 8, we speculate that the dissociation rate constant, k_{-2}, becomes slow in the presence of the IAS ($k_{-2} >> k_{-2}^*$), although the rate constant of the irreversible step, k_3, may become fast as well ($k_3 < k_3^*$).

By this scheme, it is clear that the IAS can promote assembly without becoming a stable component of the product, the SSC (A_4D_2). Indeed, the IAS is not a stable component of the SSC, CDC, or STC and is not required for the chemical steps once the SSC is assembled (Mizuuchi et al., 1992; Surette and Chaconas, 1992). By providing a stable platform on which MuA monomers bound to the two Mu DNA ends can simultaneously interact, the MuB-bound target DNA complex could functionally substitute for the IAS in transpososome assembly. This type of role would explain the requirement for a high concentration of MuB and a nonimmune target DNA, since the stable higher

$$3A + D \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} A_3D$$

$$2A_3D \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} (A_6D_2) \overset{k_3}{\rightarrow} A_4D_2 + 2A$$

$$2A_3D + E \underset{k^*_{-2}}{\overset{k^*_2}{\rightleftharpoons}} (A_6D_2E) \overset{k^*_3}{\rightarrow} A_4D_2 + E + 2A$$

Figure 8. Possible Pathways of Transpososome Assembly

Abbreviations are as follows: A, MuA; D, a Mu end DNA segment; E, the IAS or enhancer. (A_6D_2) and (EA_6D_2) are the hypothetical reversible complexes between the two ends, each bound by three MuA monomers prior to transition to the stable Mu end–bound MuA tetramer, A_4D_2. The above equations are oversimplified in many respects and should not be taken literally. For example, free MuA monomers reversibly associate with A_4D_2, and the IAS is expected to do the same. See the text for details.

order MuB–DNA complex needed for this pathway should only accumulate on non-Mu-containing DNA owing to the target immunity mechanism that continually dissociates MuB from DNA containing MuA-binding sites.

While important for assembly, the dependence on the interaction between MuA and the IAS is clearly not absolute; the requirement can be abolished or relaxed in vitro, for example in the presence of dimethyl sulfoxide or glycerol, or by the use of precleaved donor DNA. We have also found that several MuA derivatives with alterations at the essential active site residues exhibit reduced stringency for the assembly cofactor requirements. More specifically, the E392Q mutation partially rescues the defect in assembly of both the domain IA deletion protein and the domain III deletion protein in complementation experiments (D. Pincus, T. A. B., M. M., and K. M., unpublished data). Thus, assembly with an active site mutant, especially in the presence of higher concentrations of glycerol, should be considered partially IAS independent. We therefore avoided using this mutant for the investigation of transpososome assembly. In contrast, we have not found evidence indicating that the active site mutations strongly affect MuB dependence for intermolecular strand transfer.

Yang et al. (1995) have recently reported experiments using MuA derivatives lacking domain IA or containing this region from phage D108-A protein, which recognizes a different sequence at the IAS (Mizuuchi et al., 1986; Kukolj and DuBow, 1991). When these proteins, which cannot interact with the Mu IAS, were mixed with mutants defective in catalysis owing to an alteration at residue E392, partially active complexes containing approximately equal amounts of both proteins were formed. We believe these data, which on the surface contradict our conclusion that all the monomers in the tetramer need to interact with the IAS for efficient assembly, reflect the partially IAS-independent conditions discussed above. Assembly of mixed tetramers containing MuA(E392Q) and MuA(77–663) has also been observed in our laboratories. However, the majority of the resulting complexes appear to be cleaved only at the right end, while the left end is left uncleaved (M. M. and K. M., unpublished data), suggesting that it is more critical for the MuA monomers in the position to cut the left end to be able to interact with the IAS. The mixed tetramers that form under these conditions may be useful in further dissecting the role of individual monomers in catalysis.

Possible Functions of MuB-Dependent Transpososome Assembly

In the MuB–target DNA-assisted pathway of transpososome assembly described in this report, complete STCs are efficiently produced without detectable accumulation of either the SSC or the CDC (data not shown). This is in contrast with the IAS-assisted pathway, in which significant sequential accumulation of the SSC and the CDC is detected prior to accumulation of the STC (data not shown; also see Mizuuchi et al., 1992). Apparently, the presence of the target-bound MuB in association with the transpososome as it is formed accelerates the donor DNA cleavage and strand transfer steps, preventing significant accumulation of reaction intermediates. This pathway is also of interest because it alters the early steps of the in vitro Mu transposition reaction to make it resemble that of transposon Tn7, which does not have an IAS-like element. Efficient assembly of the Tn7 transposition complex requires preassembly of TnsC and TnsD proteins with the target DNA (Bainton et al., 1991, 1993); these target DNA–binding proteins of Tn7, in combination, appear to be functionally equivalent to MuB of phage Mu. Not only strand transfer, but also donor DNA cleavage, does not take place in the absence of a proper Tn7 target DNA.

What might be the physiological significance of the MuB–target-assisted pathway for transpososome assembly? It is possible that for a physiological reaction with wild-type MuA acting on the IAS-containing phage DNA this pathway may be insignificant. It may be simply an evolutionary remnant of a Tn7-like ancestral transposon from which phage Mu evolved. Since phage Mu developed the IAS pathway, which renders transposition sensitive to the presence or absence of the phage repressor (Craigie et al., 1984; Mizuuchi and Mizuuchi, 1989), therefore helping its lifestyle as a temperate bacteriophage, MuB–target-assisted transpososome assembly may have become unimportant. On the other hand, it is attractive to consider that, in vivo, the two pathways cooperate for efficient, physiologically sensible transpososome assembly. This notion is supported by an observation that CDC formation is inefficient in vivo in the absence of MuB (B. S. Chan and G. Chaconas, personal communication). Furthermore, several previous observations have indicated that MuB can participate in transposition prior to strand transfer (Surette et al., 1991; Baker et al., 1991). These earlier roles of MuB have been particularly noticeable when the reaction was partially crippled, for example by mutations in the donor DNA substrate (Surette et al., 1991; Wu and Chaconas, 1992) or in MuA (this study; Z. Wu and G. Chaconas, personal communication).

Obligatory accumulation of the stable higher order MuB–target DNA complex prior to assembly of the trans-

pososome by the MuB-dependent pathway would enhance stringency of target immunity. This way, establishment of a proper distribution of the functional higher order MuB complex among DNA sites must precede the transpososome assembly: the target-free CDC, which is ready for strand transfer as soon as it encounters even MuB-free DNA, albeit with low efficiency, will not accumulate. Indeed, the ratio of STCs containing nonimmune target DNA to those utilizing intramolecular target sites was substantially higher when the reaction was carried out in the absence of glycerol and in the presence of higher concentrations of MuB (M. M. and K. M., unpublished data).

The MuB–target DNA-assisted transpososome assembly was inefficient with wild-type MuA when tested with a donor DNA missing the IAS. This may appear to indicate that this pathway does not operate with the wild-type protein. We believe this is not the case based on other observations mentioned above. We propose that in the absence of the IAS, the domain IA of the monomers bound to the two Mu ends interacts with fortuitous DNA sites that are not closely positioned as are the sites in the IAS. Thus, this domain becomes inhibitory rather than stimulatory for the assembly. Such a dual effect of the domain IA, positive in the presence of the IAS and negative in its absence, would effectively enhance the substrate specificity of the reaction.

MuB Stimulates Strand Transfer by Promoting a Cooperative Structural Transition in the MuA Tetramer

Regardless of whether it is necessary for assembly of the transpososome, MuB is a critical cofactor during strand transfer, delivering intermolecular target DNA and stimulating catalysis by MuA. Mixtures of MuA derivatives, some of which were unable to interact with MuB, were used to address several questions about how this MuA–MuB interaction activates strand transfer. These data reveal that not all monomers in the tetramer need to interact with MuB for MuB-assisted intermolecular strand transfer. Mixed tetramers containing an active site mutant, MuA(E392Q), and MuA(1–615), which cannot interact with MuB, were able to accomplish MuB-dependent single end strand transfer. These complexes almost certainly are composed of two MuA(1–615) monomers and two monomers of MuA(E392Q). Therefore, a transpososome containing two MuB-interacting MuA monomers, while perhaps not as efficient as one with four MuB-interacting monomers, is capable of capturing MuB-bound target DNA. Can a MuA tetramer containing only one monomer that can interact with MuB capture MuB-bound target DNA? We believe the answer is no, or at least not efficiently; otherwise, the intermolecular STC should have contained more than 50% of MuA(1–615) when mixed with MuA(E392Q). The strong inhibition of intermolecular strand transfer when MuA(1–615) was mixed with wild-type MuA, and relatively low occupancy by the mutant protein in the resulting STC(inter), further supports the notion that the larger the number of MuA monomers capable of interacting with MuB, the more efficiently MuB-bound target DNA is captured.

Protein–protein contact between the domain IIIB of MuA and MuB stimulates the rate of strand transfer about 40-fold, and the DNA segment that has been bound by MuB is used as the target (Baker et al., 1991; Adzuma and Mizuuchi, 1988). We asked whether MuB must activate strand transfer by directly contacting the monomer that performs the chemical reaction of strand transfer (*cis* activation) or instead must interact through a specific partner across the tetramer (*trans* activation). The physical arrangements of the monomers within the tetramer that perform the specific reactions is not yet known. However, the activities of the mixed tetramers presented here argue that domain IIIB can function in *trans* to the domain II catalytic residues. The mixing experiments also indicate that the *cis* configuration is probably functional too, indicating that MuA monomers that carry out strand transfer can be activated either way. Thus, it appears that the efficiency of a productive capture of the MuB-bound target DNA by the MuA tetramer depends on the number, rather than the positions, of the MuA monomers within the tetramer that can interact with MuB. The interactions between the MuA and MuB molecules that stimulate strand transfer by the CDC do not appear to operate independently at the individual MuA monomers within the tetramer. Instead, this stimulation must take place cooperatively through all four MuA monomers within the complex.

We believe the multiple layers of regulatory checkpoints built into the assembly, activity, and disassembly of Mu transpososome help efficiently produce physiologically sensible types of transposition products with judicious timing. Higher order protein–nucleic acid complexes involved in many other reactions are also likely to be able to accommodate elaborate regulatory mechanisms suitable for their function. Understanding several systems in depth should be generally useful, since it is likely that similar types of macromolecular interactions are used as the building blocks of different regulatory mechanisms. However, the purpose and placement of these regulatory checkpoints would vary among different reactions because of the different physiological demands imposed on them. For example, the role of the IAS in Mu transpositional recombination is distinct from the role of the recombination enhancer in Hin site-specific recombination. The protein–DNA complex containing a pair of hix recombination sites and the Hin recombinase can be formed, but DNA cutting and ligation are slow without the enhancer or the enhancer-binding protein, Fis (Heichman and Johnson, 1990; Johnson and Bruist, 1989). The Fis-bound enhancer appears to play a major role in a conformational step after DNA cutting, leading to ligation of the DNA ends in the recombined configuration instead of the nonrecombined configuration (R. Johnson, personal communication). The difference in the placement of the major control point among different types of recombination reactions probably reflects, in part, the different nature of the chemistry. The reversible cutting and joining reaction of conservative site-specific recombination systems is well suited to have a control point further downstream than transpositional recombination systems, in which the first chemical step is an irreversible endonucleolytic DNA cleavage.

Experimental Procedures

DNA and Reagents

Buffers and standard DNA substrates were as previously described (Baker et al., 1993). The standard mini-Mu donor DNA was pMK586 (Mizuuchi et al., 1992). The mini-Mu DNA without the IAS, pMK588, was constructed from pMK586 by replacing the IAS-containing BamHI–ClaI fragment with a linker DNA. ΦX174 RFI (GIBCO BRL) or pBR322 (GIBCO BRL) was used as target DNA. The competitor DNA, a double-stranded synthetic oligonucleotide containing two MuA-binding sites, has been previously described (Mizuuchi et al., 1991). All restriction endonucleases were obtained from New England BioLabs.

The expression plasmid (pET-MuA149) for MuA(E392Q) has been described in Baker et al. (1994). The expression plasmid for the double mutant, MuA(1–615[E392Q]) (pMK618), was constructed by combining appropriate DNA fragments from plasmids coding for the separate mutant proteins: the 1566 bp NcoI–AseI fragment from pET-MuA149 was ligated with the 279 bp fragment of pMK576 plasmid containing the MuA(1–615) coding sequence digested with BamHI and AseI. Then, the resulting fragment was ligated with the 3.65 kb NcoI–BamHI fragment of pET-3d (Novagen). The expression plasmid for the double deletion protein, MuA(77–605) (pMK599), was constructed, using as the template pMK482 coding for MuA(77–663) by PCR-mediated deletion of the DNA coding for the C-terminal domain and ligating the NdeI–BamHI fragment coding for the deletion protein with the NdeI–BamHI fragment of pET-3c (Novagen).

Proteins

MuB protein was purified as described by Chaconas et al. (1985) with the additional step described by Adzuma and Mizuuchi (1991) to remove aggregated protein. HU protein was a gift from Dr. M. H. Werner (National Institutes of Health).

Wild-type MuA, MuA(1–615), MuA(E392Q), MuA(E392Q[1–615]), MuA(77–663), and MuA(77–605) were purified essentially as described by Baker et al. (1993). MuA derivatives were radiolabeled with [^{35}S]methionine in vivo, as described by Baker et al. (1993) with minor modifications and purified essentially according to Baker et al. (1993). The specific radioactivity of protein preparations was determined by scintillation counting and adjusted by adding the corresponding unlabeled protein so that the specific radioactivities of the pair of labeled proteins used within each experiment matched. [^{35}S]methionine was obtained from DuPont New England Nuclear Research Products, and methionine assay medium was purchased from Difco; 2.5 mCi (1175 Ci/mmole) was used to label 100 ml of cell culture. The protein concentration of unlabeled and ^{35}S-labeled MuA preparations was determined spectrophotometrically with a value of ε_{280} = 1.58 for 1 mg/ml wild-type MuA, MuA(E392Q), and MuA(77–663) and ε_{280} = 1.7 for MuA(1–615), MuA(E392Q[1–615]), and MuA(77–605).

Transposition Reactions

The standard reactions were as previously described (Baker et al., 1993). Reaction mixtures contained 25 mM Tris–HCl (pH 8), 156 mM NaCl, 10 mM $MgCl_2$, 2 mM ATP, 15% glycerol, 10 μg/ml donor DNA (3.4 nM), 10 μg/ml ΦX174 RFI or pBR322 DNA, 20 μg/ml MuB (600 nM), 2.4 μg/ml HU (130 nM), and 3.7 μg/ml MuA (50 nM) unless otherwise noted. The reactions were incubated at 30°C for the time indicated. For nondenaturing electrophoresis of protein–DNA complexes, reactions were stopped by adding the competitor DNA to 20 times molar excess of MuA protein (30 μg/ml) and cooling in ice water or freezing in liquid nitrogen. Ficoll 400 (Pharmacia) was added to 5% (w/v) prior to the electrophoresis. For the analysis of DNA products freed from proteins, reactions were stopped by adding 0.2 vol of stop solution (0.1% bromophenol blue, 2.5% SDS, 50 mM EDTA, and 50% Ficoll 400). Electrophoresis was as described in Baker et al. (1993), except for the experiment shown in Figure 3, in which the electrophoresis buffer contained 80 μg/ml bovine serum albumin (fraction V; Miles) and 10 μg/ml heparin (Sigma). These buffer additives drastically reduced a high background smear due to free MuA(77–605) protein and also the band of MuA(77–605) monomers bound to supercoiled donor DNA during electrophoresis.

Quantitation of Labeled Proteins in Complexes

The quantity of ^{35}S-labeled protein in transpososome bands was determined by drying the agarose gels and exposing them to a Fuji imaging plate. Band intensities were measured by a Fujix BAS 2000 (Fuji Medical Systems).

Acknowledgments

We thank Phoebe Rice, Harri Savilahti, and Bob Craigie for careful reading of the manuscript. This work was in part supported by the National Institutes of Health (NIH) Intramural AIDS Targeted Antiviral Program. The work in the lab of T. A. B. was supported by United States Public Health Service grant GM499224 from the NIH. T. A. B. is a recipient of a National Science Foundation Young Investigator Award.

Received July 25, 1995; revised August 11, 1995.

References

Adzuma, K., and Mizuuchi, K. (1988). Target immunity of Mu transposition reflects a differential distribution of Mu B protein. Cell *53*, 257–266.

Adzuma, K., and Mizuuchi, K. (1991). Steady-state kinetic analysis of ATP hydrolysis by the B protein of bacteriophage Mu. J. Biol. Chem. *266*, 6159–6167.

Allison, R.G., and Chaconas, G. (1992). Role of the A protein-binding sites in the *in vitro* transposition of Mu DNA: a complex circuit of interaction involving the Mu ends and the transpositional enhancer. J. Biol. Chem. *267*, 19963–19970.

Ariyoshi, M., Vassylyev, D.G., Iwasaki, H., Shinagawa, H., and Morikawa, K. (1994). Atomic structure of the RuvC resolvase: a Holliday junction–specific endonuclease from E. coli. Cell *78*, 1063–1072.

Bainton, R., Gamas, P., and Craig, N.L. (1991). Tn7 transposition in vitro proceeds through an excised transposon intermediate generated by staggered breaks in DNA. Cell *65*, 805–816.

Bainton, R.J., Kubo, K.M., Feng, J.-N., and Craig, N.L. (1993). Tn7 transposition: target DNA recognition is mediated by multiple Tn7-encoded proteins in a purified in vitro system. Cell *72*, 931–943.

Baker, T.A., and Luo, L. (1994). Identification of residues in the Mu transposase essential for catalysis. Proc. Natl. Acad. Sci. USA *91*, 6654–6658.

Baker, T.A., and Mizuuchi, K. (1992). DNA-promoted assembly of the active tetramer of the Mu transposase. Genes Dev. *6*, 2221–2232.

Baker, T.A., Mizuuchi, M., and Mizuuchi, K. (1991). MuB protein allosterically activates strand transfer by the transposase of phage Mu. Cell *65*, 1003–1013.

Baker, T.A., Mizuuchi, M., Savilahti, H., and Mizuuchi, K. (1993). Division of labor among monomers within the Mu transposase tetramer. Cell *74*, 723–733.

Baker, T.A., Kremenstova, E., and Luo, L. (1994). Complete transposition requires four active monomers in the Mu transposase tetramer. Genes Dev. *8*, 2416–2428.

Chaconas, G., Gloor, G., and Miller, J.L. (1985). Amplification and purification of the bacteriophage Mu encoded B transposition protein. J. Biol. Chem. *260*, 2662–2669.

Clubb, R.T., Omichinski, J.G., Savilahti, H., Mizuuchi, K., Gronenborn, A.M., and Clore, G.M. (1994). A novel class of winged helix-turn-helix protein: the DNA-binding domain of Mu transposase. Structure *2*, 1041–1048.

Craigie, R., and Mizuuchi, K. (1987). Transposition of Mu DNA: joining of Mu to target DNA can be uncoupled from cleavage at the ends of Mu. Cell *51*, 493–501.

Craigie, R., Mizuuchi, M., and Mizuuchi, K. (1984). Site-specific recognition of the bacteriophage Mu ends by the Mu A protein. Cell *39*, 387–394.

Davies, J.F.d., Hostomska, Z., Hostomsky, Z., Jordan, S.R., and Mattews, D.A. (1991). Crystal structure of the ribonuclease H domain of HIV-1 reverse transcriptase. Science *252*, 88–95.

Dyda, F., Hickman, A.B., Jenkins, T.M., Engelman, A., Craigie, R., and Davies, D.R. (1994). Crystal structure of the catalytic domain of HIV-1 integrase: similarity to other polynucleotidyl transferases. Science *266*, 1981–1986.

Haniford, D.B., and Chaconas, G. (1992). Mechanistic aspects of DNA transposition. Curr. Opin. Genet. Dev. *2*, 698–704.

Heichman, K.A., and Johnson, R.C. (1990). The Hin invertasome: protein-mediated joining of distant recombination sites at the enhancer. Science *249*, 511–517.

Johnson, R.C., and Bruist, M.F. (1989). Intermediates in Hin-mediated DNA inversion: a role for Fis and the recombinational enhancer in the strand exchange reaction. EMBO J. *8*, 1581–1590.

Katayanagi, K., Miyagawa, M., Matsushima, M., Ishikawa, M., Kanaya, S., Ikehara, M., Matsuzaki, T., and Morikawa, K. (1990). Three-dimensional structure of ribonuclease H from *E. coli*. Nature *347*, 306–309.

Kim, K., Namgoong, S.-Y., Jayaram, M., and Harshey, R.M. (1995). Step-arrest mutants of phage Mu transposase: implications in DNA-protein assembly, Mu end cleavage, and strand transfer. J. Biol. Chem. *270*, 1472–1479.

Kukolj, G., and DuBow, M.S. (1991). Characterization of the lysogenic repressor (c) from transposable Mu-like bacteriophage D108. Nucl. Acids Res. *19*, 5949–5956.

Lavoie, B.D., and Chaconas, G. (1993). Site-specific HU binding in the Mu transpososome: conversion of a sequence-independent DNA-binding protein into a chemical nuclease. Genes Dev. *7*, 2510–2519.

Lavoie, B.D., and Chaconas, G. (1994). A second high affinity HU binding site in the phage Mu transpososome. J. Biol. Chem. *269*, 15571–15576.

Lavoie, B.D., Chan, B.S., Allison, R.G., and Chaconas, G. (1991). Structural aspects of a higher order nucleoprotein complex: induction of an altered DNA structure at the Mu-host junction of the Mu type 1 transpososome. EMBO J. *10*, 3051–3059.

Leung, P.C., and Harshey, R.M. (1991). Two mutations of phage Mu transposase that affect strand transfer or interaction with B protein lie in distinct polypeptide domains. J. Mol. Biol. *219*, 189–199.

Leung, P.C., Teplow, D.B., and Harshey, R.M. (1989). Interaction of distinct domains in Mu transposase with Mu DNA ends and an internal transpositional enhancer. Nature *338*, 656–658.

Maxwell, A., Craigie, R., and Mizuuchi, K. (1987). B protein of bacteriophage Mu is an ATPase that preferentially stimulates intermolecular DNA strand transfer. Proc. Natl. Acad. Sci. USA *84*, 699–703.

Mizuuchi, K. (1992a). Polynucleotidyl transfer reaction in transpositional DNA recombination. J. Biol. Chem. *267*, 21273–21276.

Mizuuchi, K. (1992b). Transpositional recombination: mechanistic insights from studies of Mu and other elements. Annu. Rev. Biochem. *61*, 1011–1051.

Mizuuchi, M., and Mizuuchi, K. (1989). Efficient Mu transposition requires interaction of transposase with a DNA sequence at the Mu operator: implications for regulation. Cell *58*, 399–408.

Mizuuchi, M., Weisberg, R.A., and Mizuuchi, K. (1986). DNA sequence of the control region of phage D108: the N-terminal amino acid sequences of repressor and transposase are similar both in phage D108 and its relative phage Mu. Nucl. Acids Res. *14*, 3813–3825.

Mizuuchi, M., Baker, T.A., and Mizuuchi, K. (1991). DNase protection analysis of the stable synaptic complexes involved in Mu transposition. Proc. Natl. Acad. Sci. USA *88*, 9031–9035.

Mizuuchi, M., Baker, T.A., and Mizuuchi, K. (1992). Assembly of the active form of the transposase Mu DNA complex: a critical control point in Mu transposition. Cell *70*, 303–311.

Nakayama, C., Teplow, D.B., and Harshey, R.M. (1987). Structural domains in phage Mu transposase: identification of the site-specific DNA-binding domain. Proc. Natl. Acad. Sci. USA *84*, 1809–1813.

Rice, P.A., and Mizuuchi, K. (1995). Structure of the bacteriophage Mu transposase core: a common structural motif for DNA transposition and retroviral integration. Cell *82*, 209–220.

Savilahti, H., Rice, P.A., and Mizuuchi, K. (1995). The phage Mu transpososome core: DNA requirements for assembly and function. EMBO J. *14*, in press.

Surette, M.G., and Chaconas, G. (1989). A protein factor which reduces the negative supercoiling requirement in the Mu DNA strand transfer reaction is *Escherichia coli* integration host factor. J. Biol. Chem. *264*, 3028–3034.

Surette, M.G., and Chaconas, G. (1992). The Mu transpositional enhancer can function in *trans*: requirement of the enhancer for synapsis but not strand cleavage. Cell *68*, 1101–1108.

Surette, M.G., Buch, S.J., and Chaconas, G. (1987). Transpososomes: stable protein–DNA complexes involved in the in vitro transposition of bacteriophage Mu DNA. Cell *49*, 253–262.

Surette, M.G., Lavoie, B.D., and Chaconas, G. (1989). Action at a distance in Mu DNA transposition: an enhancer-like element is the site of action of supercoiling relief activity by integration host factor (IHF). EMBO J. *8*, 3483–3489.

Surette, M.G., Harkness, T., and Chaconas, G. (1991). Stimulation of the Mu A protein-mediated strand cleavage reaction by the Mu B protein, and the requirement of DNA nicking for stable type 1 transpososome formation: *in vitro* transposition characteristics of mini-Mu plasmids carrying terminal base pair mutations. J. Biol. Chem. *266*, 3118–3124.

Wu, Z., and Chaconas, G. (1992). Flanking host sequences can exert an inhibitory effect on the cleavage step of the *in vitro* Mu DNA strand transfer reaction. J. Biol. Chem. *267*, 9552–9558.

Wu, Z., and Chaconas, G. (1994). Characterization of a region in phage Mu transposase that is involved in interaction with MuB protein. J. Biol. Chem. *269*, 28829–28833.

Yang, J.-Y., Kim, K., Jayaram, M., and Harshey, R.M. (1995). A domain sharing model for active site assembly within the MuA tetramer during transposition: the enhancer may specify domain contributions. EMBO J. *14*, 2374–2384.

Yang, W., Hendrickson, W.A., Crouch, R.J., and Satow, Y. (1990). Structure of ribonuclease H phased at 2 Å resolution by MAD analysis of the selenomethionyl protein. Science *249*, 1398–1405.

Zou, A.H., Leung, P.C., and Harshey, R.M. (1991). Transposase contacts with Mu DNA ends. J. Biol. Chem. *266*, 20476–20482.