

Studies on a "Jumping Gene Machine":

Higher-Order Nucleoprotein Complexes in Mu DNA Transposition

Abstract

Studies in my lab have focused on DNA transposition in the bacterial virus, Mu. In vitro studies have shown that Mu DNA transposition is a three-step process involving DNA breakage, strand transfer and DNA replication. In the first step a nick is introduced at each end of the transposon. The liberated 3'-OH groups subsequently attack a target DNA molecule resulting in strand transfer. The transposon DNA, now covalently linked to the target, is finally replicated to generate the transposition end-product, referred to as a cointegrate. The DNA cleavage and strand transfer reactions are mediated by a "jumping gene machine" or transpososomes, which we discovered in 1987. They are assembled by bringing together three different DNA regions via a process involving multiple protein-DNA and protein-protein interactions. The action of four different proteins is required in addition to protein-induced DNA bending or wrapping to overcome the intrinsic stiffness of DNA, which would ordinarily prohibit the assembly of such a structure. Transpososome assembly is a gradual process involving multiple steps with an inherent flexibility whereby alternate pathways can be used in the assembly process, biasing the reaction towards completion under different conditions.

Introduction

DNA transposition is an important and ubiquitous process which can have a profound effect upon cells and even entire organisms. The study of DNA transposition at the molecular level (for recent reviews see Hallet & Sherratt, 1997; Mahillon & Chandler, 1998; Mizuuchi, 1997; Plasterk, 1998; Roth & Craig, 1998) has been led by work on phage Mu since the report of the first *in vitro* transposition reaction by Mizuuchi (1983). Figure 1 shows the essential features of this reaction (Lavoie

& Chaconas, 1995; Mizuuchi, 1992a; Mizuuchi, 1992b). DNA cleavage occurs on the bottom strand at the Mu left end and the top strand at the Mu right end. The 3'-OH termini which result are subsequently involved in a nucleophilic attack on a DNA target molecule resulting in a transesterification to generate a q structure intermediate (Craigie & Mizuuchi, 1985; Miller & Chaconas, 1986). Processing of the q structure by DNA replication of the central stem, which contains the Mu DNA, results in the liberation of a cointegrate structure, the transposition end-product.

Reaction components

DNA sites

The three regions of Mu DNA which are required for transposition are shown in Figure 2. All three regions contain binding sites for the Mu A protein. The left-most 200 bp of Mu DNA has the sites L1, L2 and L3 while the right end contains the R1, R2 and R3 sites (Craigie *et al.*, 1984). The transpositional enhancer (Leung *et al.*, 1989; Mizuuchi & Mizuuchi, 1989; Surette *et al.*, 1989), which increases the initial rate of the *in vitro* reaction by a factor of 100-fold or more and is required for synapsis of the two Mu ends in their proper orientation also contains binding sites for the Mu A protein. Enhancer binding by the transposase occurs through a region of the protein which is distinct from that used for end binding (Leung *et al.*, 1989; Mizuuchi & Mizuuchi, 1989). The Mu A binding sites in the enhancer region occur in the O1, O2 and O3 regions which also function to control expression of the Mu early operon. Between O1 and O2 is a binding site for the E. coli IHF protein (see below).

Proteins

There are four proteins involved in the donor cleavage and strand transfer steps of Mu transposition (Lavoie & Chaconas, 1995). The Mu A protein or transposase is a 663 amino acid protein, which in addition to binding the Mu ends and enhancer,

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Figure 1. Mu DNA transposition. *In vitro* studies have used mini-Mu donor molecules, in which over 30 kilobases of Mu DNA carrying phage morphogenesis genes has been deleted. L, R and E represent the Mu left end, right end and transpositional enhancer, respectively.

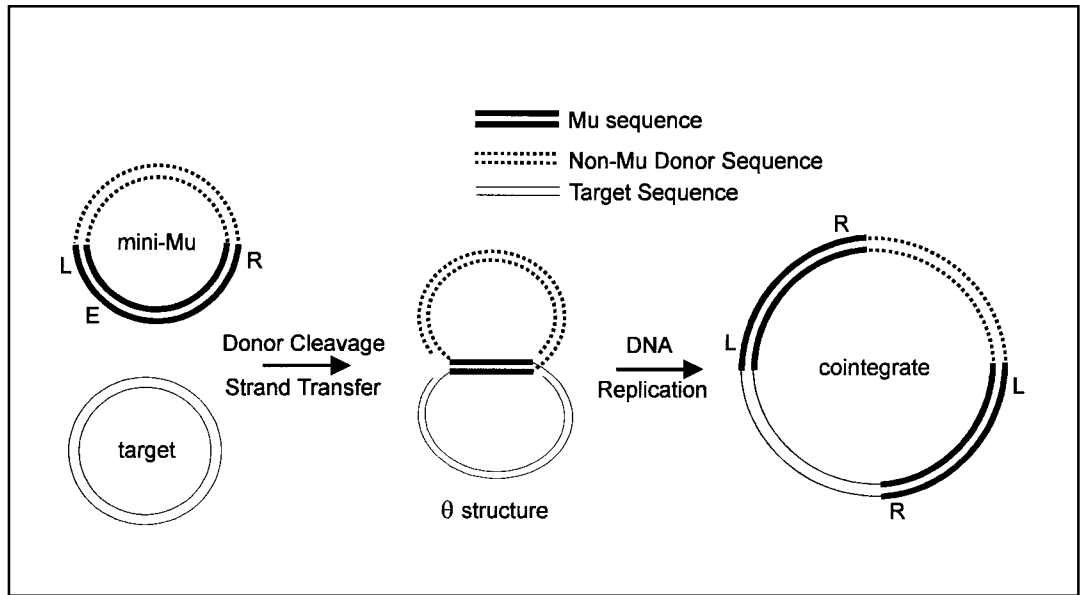
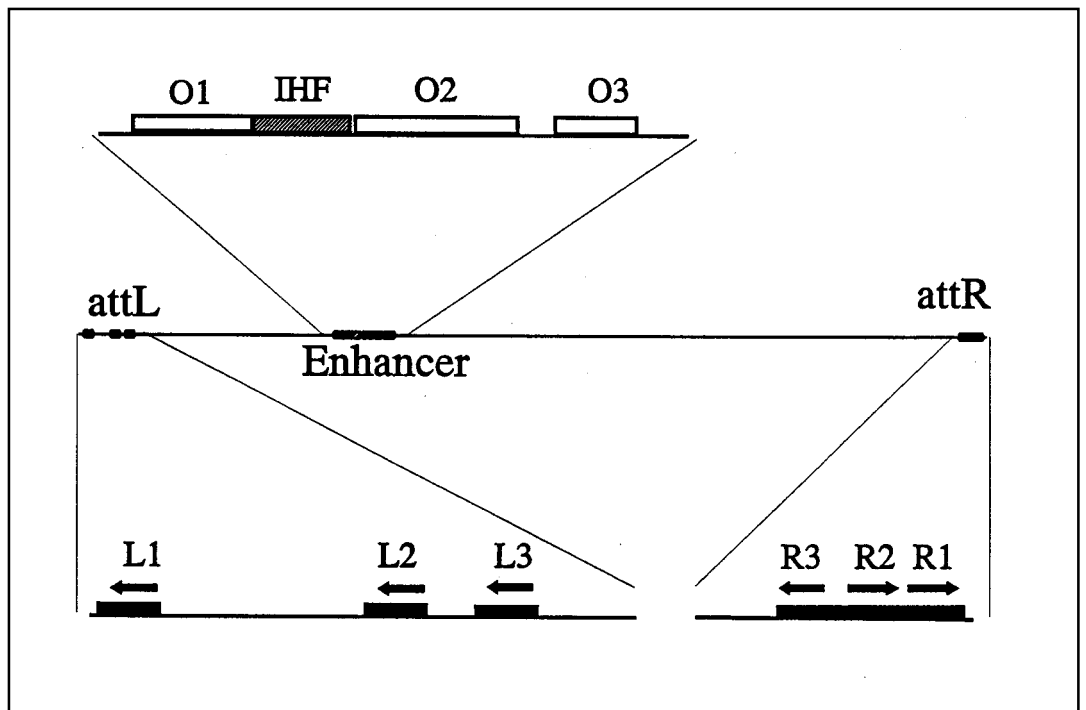


Figure 2. Required regions of Mu DNA for the strand transfer reaction. The substructure of the three required regions are shown in the blowups (from Allison & Chaconas, 1992, used with permission).



carries the donor cleavage and strand transfer activities. The Mu B protein is a 312 amino acid protein which functions in ATP-dependent capture of target DNA and acts as an allosteric effector of Mu A. The host accessory factors HU and IHF are both small, basic architectural proteins, or DNA flexers, which mediate DNA bending. HU is essential for the reaction (Craigie *et al.*, 1985). It is a general DNA binding protein which we have recently found to bind specifically in a supercoiling-dependent fashion to the L1-L2 spacer (Kobryn *et al.*, 1999; Lavoie *et al.*, 1996; Lavoie & Chaconas, 1993). HU is believed to facilitate the interaction of Mu A bound at the L1 and L2 sites by looping out the DNA between the sites. IHF is essential for the reaction with DNA substrates containing *in vivo* levels of supercoiling (Surette & Chaconas, 1989) and functions by binding and introducing a sharp DNA bend in the enhancer (Surette *et al.*, 1989). This DNA bending is required to facilitate interactions between Mu A monomers bound on the left and right sides of the enhancer or between Mu A monomers bound at the enhancer and the Mu ends.

Transpososomes

The Mu DNA strand transfer reaction is a complex process involving multiple proteins, multiple DNA sites and a complex circuit of cooperative protein-protein and protein-DNA interactions.

It also involves DNA bending or wrapping and interaction of proteins bound at distant sites on the DNA. The reaction is mediated by a series of non-covalent protein-DNA complexes or transpososomes, whose stability increases as the reaction proceeds (see also (Chaconas *et al.*, 1996)). Such higher-order structures are common in high-precision DNA transactions (Echols, 1990) and transpososomes have now been observed in several other transposition systems.

Since our initial report of the Type 1 and Type 2 complexes (Surette *et al.*, 1987) a number of other transpososomes in the Mu reaction have been identified as shown in Figure 3.

LER

The earliest characterized intermediate on the reaction pathway is the LER, a 3-site synaptic complex containing the left (L) and right (R) Mu ends and the transpositional enhancer (E) (Watson & Chaconas, 1996). This complex forms on a supercoiled donor DNA molecule in the presence of Mu A, HU, IHF and Mg^{2+} . The LER is unstable and can only be detected following treatment with a protein cross-linker. The active site of the transposase has not yet engaged the terminal nucleotides where cleavage will occur. The LER is the only complex containing the enhancer, which is required for transpososome assembly but not for donor cleavage (Surette & Chaconas, 1992;

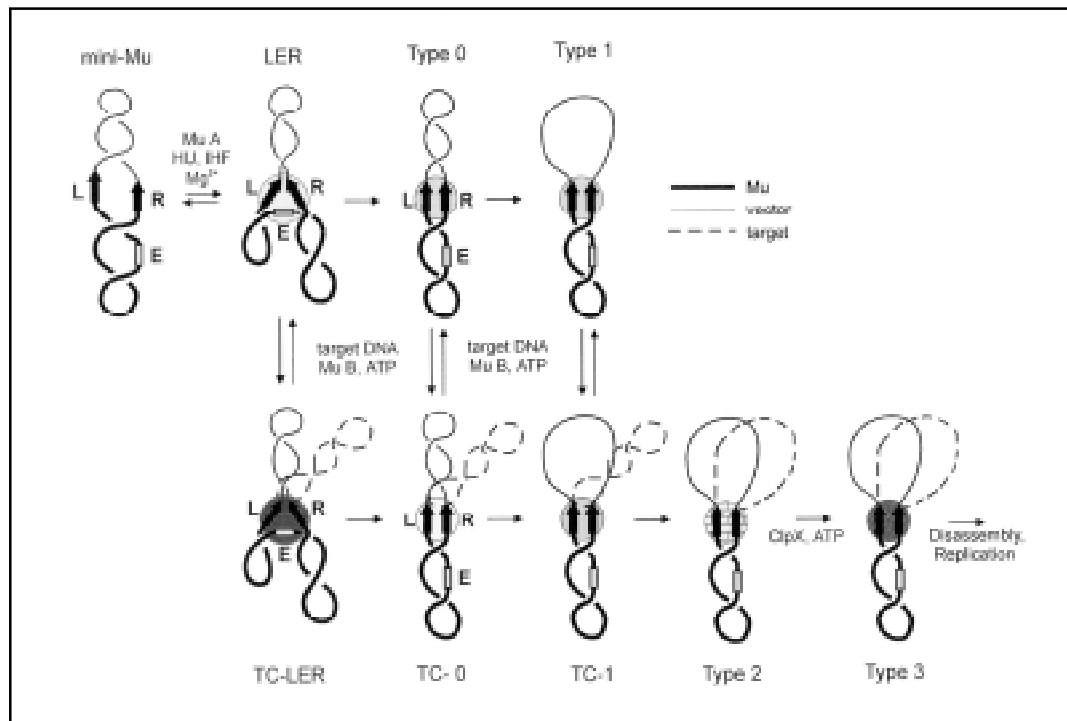


Figure 3. Transpososomes (non-covalent protein-DNA reaction intermediates) which mediate the Mu DNA transposition reaction.

Mizuuchi *et al.*, 1992). Earlier two-site complexes have also been identified, but proof of their role as reaction intermediates has not yet been possible.

Type 0- The LER is rapidly converted into the Type 0, or Stable Synaptic Complex (Mizuuchi *et al.*, 1992). This complex accumulates when Ca^{2+} is substituted for Mg^{2+} in the reaction buffer. There are a number of important developments during the LER to Type 0 transition. The enhancer is dislodged and conformational changes occur in the transposase, resulting in the formation of a stable Mu A tetramer. Moreover, the terminal nucleotide at each end is engaged by the active site. The Mu A tetramer formed at the Type 0 stage is the structural and functional core of all subsequent transposition complexes (Lavoie *et al.*, 1991).

Type 1- In the presence of Mg^{2+} , the first chemical step, concerted cleavage of the two Mu ends occurs. The Type 0 is thereby converted into the Type 1 transpososome or Cleaved Donor Complex, which displays an increase in stability (Surette & Chaconas, 1987; Craigie & Mizuuchi, 1987). The introduction of nicks at the Mu ends results in the loss of supercoils in the vector DNA but the presence of the complex inside the Mu ends blocks the release of DNA supercoils in the Mu domain; the strength of the non-covalent protein-DNA interaction is great enough to withstand the free energy of supercoiling stored in the Mu DNA.

Target capture (TC) complexes

The addition of Mu B, target DNA and ATP to the LER, the Type 0 or the Type 1 results in formation of the target capture complexes TC-LER, TC-0 and TC-1, respectively (Naigamwalla & Chaconas, 1997). Non-covalent recruitment of target DNA to the transpososome can occur at any of these stages, even before donor cleavage has occurred. The Mu transposition system is very flexible in this respect compared to other characterized transposons. In Tn10 target capture can only occur after donor cleavage (Sakai, 1997) while in Tn7 target capture must occur as part of the transpososome assembly process before donor cleavage can occur (Bainton *et al.*, 1993). The flexibility in the Mu target capture step may help to bias the reaction towards completion, especially when an immune target is encountered. Mu integration into itself or other Mu DNA molecules is prohibited by a process known as transposition immunity (Adzuma & Mizuuchi, 1988; Adzuma & Mizuuchi, 1989). When a Mu target is captured, Mu A induces ATP hydrolysis and target release by Mu B. The ability to capture target DNA at several

steps along the reaction pathway allows for the recruitment of a suitable target even if an immune target has already been disengaged from the transpososome.

Type 2- The second chemical step, a transesterification, occurs in the TC-1 and results in the Type 2 or Strand Transfer complex, where the nicks at the two Mu ends have been transferred to the target DNA resulting in the Type 2 transpososome (Surette & Chaconas, 1987; Craigie & Mizuuchi, 1987). This structure contains the q structure depicted in Figure 1 and shows the greatest stability of all the Mu transposition complexes. The complex is so stable that it needs to be actively disassembled for replication to proceed.

Type 3- Interaction of the Type 2 transpososome with ClpX in the presence of ATP destabilizes the complex resulting in the formation of the Type 3 transpososome (Kruklytis *et al.*, 1996; Levchenko *et al.*, 1995). This structure is then dismantled to allow replication to occur (Jones, 1997).

Future directions

Studies are now in progress to identify earlier intermediates in transpososome assembly as well as to define the structure/function relationships of the transpososomes and the individual transposition proteins.

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