*Proc. Natl. Acad. Sci. USA*
Vol. 88, pp. 9031–9035, October 1991
Biochemistry

# DNase protection analysis of the stable synaptic complexes involved in Mu transposition

**(MuA protein/cleaved donor complex/strand-transfer complex/bacteriophage Mu)**

MICHIYO MIZUUCHI, TANIA A. BAKER, AND KIYOSHI MIZUUCHI*

Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD 20892

*Communicated by Howard A. Nash, July 25, 1991 (received for review May 28, 1991)*

ABSTRACT **Several critical steps in phage Mu transposition involve specialized protein–DNA complexes. Cleavage of Mu donor DNA by MuA protein leads to the formation of the stable cleaved donor complex (CDC), in which the two Mu DNA ends are held together by MuA. In the subsequent strand-transfer reaction the CDC attacks a target DNA to generate the strand-transfer complex, in which the donor and the target DNAs are covalently joined. We have carried out DNase I protection experiments on these protein–DNA complexes and found that only three MuA binding sites (L1, R1, and R2 of the six total) at the two Mu ends are stably bound by MuA to maintain the paired Mu end structure. The protection extends beyond the ends of the Mu sequence for different lengths (7–20 nucleotides) depending on the strand and the type of complex. After formation of the CDC, the other MuA binding sites (L2, L3, and R3) and internal activation sequence become dispensable for the subsequent strand-transfer reaction.**

Bacteriophage Mu is a highly efficient transposable element (1). Following induction of a Mu prophage the viral DNA is amplified about 100-fold by replicative transposition. Two phage-encoded proteins are directly involved. The MuA protein, the transposase, pairs the ends of the genome and promotes all the strand cleavage and joining reactions. The MuB protein stimulates the strand-transfer activity of MuA while bound to a target DNA. The host proteins HU and IHF also function early in transposition.

To initiate transposition, MuA binds specifically to each end of the Mu donor DNA (2). In the presence of HU and IHF the ends of the supercoiled Mu genome are paired in a synaptic protein–DNA complex. This complex also involves an internal activating sequence (IAS) and is called a plectosome because its formation depends on the topology of the donor DNA (3, 4). IHF and HU appear to assist in synapsis by influencing DNA conformation (4–6). IHF binds near the middle of the IAS and probably helps the MuA–IAS interaction but is not required *in vitro* when highly supercoiled donor DNA is a substrate (6). While there is substantial indirect evidence for this synaptic complex between the Mu ends and the IAS, it may be transient and has not been isolated as a stable intermediate.

After successful formation of the plectosome, MuA cleaves the donor DNA to expose the 3′-OH ends of the Mu sequence (7). Cleavage generates the stable cleaved donor complex (CDC, also called a type I complex), in which the paired ends of the Mu DNA are held tightly by MuA (7, 8). This MuA–DNA complex can then join the 3′ ends of the Mu DNA to the target site in a concerted reaction (called strand transfer). Sequences bound by MuB are preferred targets for attack by the CDC. Strand transfer generates the strand-transfer complex (STC, also called a type II complex), in which the donor and target DNA are covalently joined and the proteins remain tightly bound (8).

The Mu end sequences involved in transposition are long and asymmetric. Nuclease footprinting reveals three 22- to 30-base-pair (bp) MuA binding sites referred to as L1, L2, and L3 and R1, R2, and R3, at the "left" (L) and "right" (R) ends (2). The binding sites closest to the ends of the genome, L1 and R1, are inverted with respect to each other and start 6 bp from the donor cleavage site. The other four sites are arranged very differently. On the L end, L2 and L3 are contiguous but separated from L1 by about 80 bp; R1, R2, and R3 are all contiguous. Recent results indicate that one site out of the group R3, L2, and L3 can be deleted without drastic consequence to transposition, although initially only R3 was found to be nonessential (ref. 9 and references therein). The IAS, a different type of MuA binding site, is located about 950 bp from the L end and is recognized by a sequence-specific DNA-binding domain of MuA distinct from the part of the protein that recognizes the end sequences (4, 5).

To dissect the roles of these multiple MuA binding sites, we analyzed the structures of the CDC and the STC by DNase I protection analysis.

## MATERIALS AND METHODS

**Proteins.** Restriction enzymes were from New England Biolabs or United States Biochemical, DNase I was from Miles, and polynucleotide kinase was from Phamacia LKB. MuA (10), MuB (11), and *Escherichia coli* protein HU (12) were purified as previously described. IHF was a gift from H. Nash (National Institutes of Health).

**Plasmids and DNAs.** The structure of the mini-Mu plasmid pMK586 is the same as that of pMK584 (13) except that the DNA flanking the L end between the *Hin*dIII and *Eco*NI sites was replaced with a synthetic 54-mer containing the R-end flanking sequence. Plasmid pMK426 (7) was digested with *Hin*dIII to expose the 3′-OH ends of the Mu R-end sequences and used as the precut donor. Mu end competitor was prepared by annealing equal concentrations of oligodeoxynucleotides MM147 and MM148 (5′-GGGCCACGCGTGTATTGATTCACTTGAAGTACGAAAATGTTTCATTAAAAACACGAAAACCGGG-3′ and 5′-CCCGGTTTTCGTGTTTTTAATGAAACATTTTCGTACTTCAAGTGAATCAATAC-3′, respectively). $\phi$X174 replicative form I (RFI) DNA was from BRL.

**Formation of CDC and STC.** Reaction mixtures (25 $\mu$l) contained 15% (vol/vol) glycerol, 25 mM Tris·HCl (pH 8.0), 156 mM NaCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, bovine serum albumin (25 $\mu$g/ml), pMK586 (50 $\mu$g/ml), MuA (35 $\mu$g/ml), and HU (10 $\mu$g/ml) and were incubated at 30°C for 5 min. IHF (0.4 $\mu$g/ml) was added only for the IAS footprint.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CDC, cleaved donor complex; STC, strand-transfer complex; IAS, internal activating sequence.

*To whom reprint requests should be addressed.

To generate the STC, MuB (30 μg/ml), ATP (2 mM), and φX174 RFI DNA (50 μg/ml) were also included and the MuA and HU concentrations were 17.5 μg/ml and 5 μg/ml, respectively. Incubation was at 30°C for 60 min.

**DNase I Protection Experiments ("Footprinting").** After the reaction to form the CDC or STC, 2.4 μg of Mu end competitor, 2.5 μl of 1 mM $CaCl_2$, 1.6 ng of DNase I, 400 units of *Eco*RI (cuts at positions 82 and −78 around the Mu R end), 144 units of *Ban*III (cuts at position 195 at the Mu L end) and 281 units of *Eco*NI (cuts at position −54 at the Mu L end) were added and incubated at 23°C for 15 min. The DNase I-treated CDC or STC was loaded on a 5% polyacrylamide gel in TAE buffer (13) following addition of EDTA and Ficoll 400 and was electrophoresed for 70 min at 9 V/cm in the cold room. The protein–DNA complexes stayed near the origin of the gel. The DNA at this position was recovered by a "crush and soak" method and loaded on an 8% polyacrylamide sequencing gel. DNA blotting and hybridization were performed as described (14).

**Fractionation of the Trimmed CDC.** For the experiment of Fig. 3, the CDC was made using the plasmid pMK584 under the same conditions as for footprinting. Incubation with MuA and HU was for 10 min at 30°C, followed by the addition of competitor DNA and 1800 units of *Sau*3A1 and incubation at 23°C for 20 min prior to the addition of EDTA to 10 mM and NaCl to 600 mM and size-exclusion column chromatography (see legend).

## RESULTS

**CDC Footprint.** To analyze the footprint of the CDC, a supercoiled Mu donor plasmid was incubated with MuA and HU until about 80% of the donor DNA was cleaved. A competitor DNA containing two strong MuA binding sites (sequences in *Materials and Methods*) was then added to "soak up" the unbound and loosely bound MuA. This amount of the competitor (20-fold excess of MuA binding sites relative to those in the donor DNA and 5-fold molar excess to MuA) inhibited CDC formation when added prior to MuA (see below). After addition of the competitor, DNase I and three restriction enzymes that separate short fragments containing the Mu ends (227 bp for L end; 160 bp for R end) from the IAS and the rest of the donor DNA were added. The Mu end fragments remained held together by MuA as judged by their gel mobility (data not shown). The DNA present within this "trimmed" CDC was purified by nondenaturing polyacrylamide gel electrophoresis and the recovered DNA was separated in a sequencing gel. The DNase pattern was visualized by blotting the DNA onto a nylon membrane and probing with $^{32}P$-labeled oligonucleotides complementary to one of the DNA strands. DNase footprinting of the same mini-Mu plasmid that had been linearized prior to the addition of MuA and HU was included for comparison. The linearized DNA binds MuA but fails to form the CDC due to the lack of superhelicity.

With the linearized mini-Mu DNA, regions of protection corresponding to L1, L2, and L3 were observed as expected (Fig. 1*A*, lane f). However, when the competitor DNA was added after the incubation with MuA and HU, no protection was seen (lane g). Similarly, R1, R2, and R3 were all protected and this protection was sensitive to the competitor DNA (Fig. 1*B*, lanes f and g). These data show that the MuA bound to the linear DNA is free to partition to the competitor. The omission of HU protein, which is required for CDC formation, did not affect the protection pattern (data not shown).

The footprint of the CDC differed from that on the linear mini-Mu DNA in several ways. In the absence of the competitor, in addition to the protection of L1, L2, and L3, about 10 bp outside the Mu L end, including the cleavage site, were

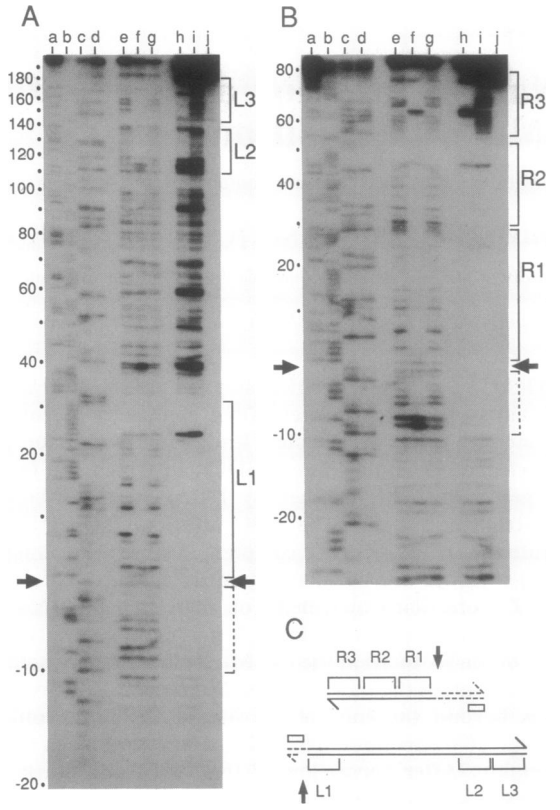


FIG. 1. DNase I footprint of MuA on the CDC. The nuclease protection patterns of the Mu L end (*A*) and R end (*B*) on the strand that is not cut by MuA are shown. The locations of MuA binding sites, MuA cleavage sites (arrows), and the probe used (small boxes; 20 nucleotides long, labeled by T4 polynucleotide kinase and [γ-$^{32}P$]ATP, NEN) within the restriction fragments that contain Mu R and L ends are indicated (*C*). The distances from the probed ends to the start of the Mu sequence are 54 and 78 nucleotides for the L and R end, respectively. Lanes a–d, chemically cleaved sequence markers (G, A+G, T+C, and C, respectively). Lanes e–g, the donor plasmid pMK586 was linearized by *Bam*HI and purified by phenol treatment and ethanol precipitation prior to footprinting without MuA (lanes e), with MuA (lanes f), and with MuA and the Mu end competitor DNA (lanes g). Lanes h and i, the CDC was prepared as described in *Materials and Methods* and footprinted without (lanes h), or with (lanes i) the Mu end competitor DNA. Lanes j, same as lanes h, but without DNase I treatment.

protected (Fig. 1*A*, lane h). When the competitor was added after CDC formation, L1 and the short sequence outside the MuL end remained inaccessible to the nuclease, but L2 and L3 were no longer protected (Fig. 1*A*, lane i). Sequences between L1 and L2 exhibited a periodically modulated DNase sensitivity when these sites were occupied by MuA, suggesting one-sided accessibility of this segment to the nuclease (lane h). This DNase sensitivity pattern persisted, although at a lesser extent, even in the presence of the competitor DNA (lane i), perhaps reflecting limited association of L2 and L3 with the protein–DNA complex even in the presence of the competitor. On the R end, all three sites were protected by MuA in the absence of the competitor (Fig. 1*B*, lane h) as were about 10 bp outside of the Mu end. In the presence of the competitor, R1 and R2, as well as the sequence covering the cleavage site, remained protected, but R3 became nuclease-sensitive (lane i). For both the L end and the R end, the nuclease protection pattern was confirmed by probing from inside the Mu sequences toward the flanking DNA (data not shown). These footprint data indicate that in the CDC, MuA is tightly bound to three sites within the Mu ends, L1, R1, and R2, and about 10 bp outside of both the L and R ends.

The nuclease protection experiments were also carried out on the strand cut by MuA during CDC formation. Essentially the same protection pattern was observed (data not shown). In the gel-purified CDC preparation, while ≈90% of this strand was cut at the Mu ends, ≈10% remained uncut. This uncut DNA is unlikely to represent contaminating unreacted donor, because the protected region extended into the outside sequence of the uncleaved molecules. More likely, the CDC preparations contained a small fraction of stable protein–DNA complex in which at least one of the Mu ends had not been cleaved by MuA. The outside region on the strands cleaved by MuA was accessible to DNase I for 2 or 3 nucleotides immediately adjacent to the cleavage site but was protected further out up to 7 nucleotides from the cleavage site (data not shown).

The IAS is thought to be involved in Mu transposition during formation of an early synaptic protein–DNA complex in which two Mu ends are brought together. To test whether the IAS is tightly bound by MuA in the CDC, we isolated a "trimmed" CDC by using different restriction enzymes so that the IAS segment would not be cut away from the L end. Footprinting was done in the absence or presence of an IAS competitor DNA, using a probe that corresponded to a sequence near the IAS. Weak protection of the IAS by MuA became stronger when IHF was included in the reaction mixture. However, the DNase protection by MuA disappeared regardless of the presence or absence of IHF when the competitor DNA was added (data not shown). Thus, the IAS is not a stable component of the CDC and probably functions only prior to CDC formation (see below).

**Strand-Transfer Reaction with the CDC.** To test whether the CDC used for the footprint was active for strand transfer, reactions were carried out in two stages in the presence and absence of the Mu end competitor. The donor DNA was incubated with MuA and HU for 5 min to make the CDC. Target DNA prebound by MuB was then added to the CDC and the efficiency of strand transfer was determined by the appearance of the strand-transfer product in an agarose gel (Fig. 2).

Strand transfer was inhibited when the Mu end competitor DNA was added before MuA (Fig. 2, lanes c and e). However, when the competitor was added after formation of the CDC, strand transfer occurred at the same efficiency as in its absence (lanes d and f). Thus, the stable complex with MuA bound to the three sites at the Mu ends (L1, R1, and R2) and extending into the flanking DNA retains not only the structural integrity but also the function of the CDC.

Strand transfer by the CDC does not require stable interaction of MuA with L2, L3, and R3. However, these sites could participate in strand transfer, since MuA may still transiently associate with them even in the presence of the competitor. To test this possibility for the L-end sites, we digested the CDC with *Sau*3A1 to separate L2 and L3 from the Mu end–MuA complex. *Sau*3A1 cleaves the donor plasmid into several fragments, the largest of which (830 bp) contains the complete R end and the second largest (764 bp) the L1 sequence. L2, L3, and the IAS are removed from L1 onto separate fragments by this digestion. The trimmed CDC was purified to remove excess MuA and HU by size-exclusion column chromatography in the presence of the competitor DNA (Fig. 3). The column buffer contained 600 mM NaCl to further ensure dissociation of loosely bound MuA and HU (15). R3 remained attached to the R-end

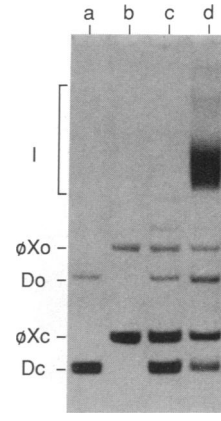


FIG. 2. Strand-transfer activity of the CDC. Strand transfer was carried out essentially as described in *Materials and Methods* except that the reaction was done in two stages as described in the text. Lane a, the donor mini-Mu plasmid pMK586; lane b, $\phi$X174 RF DNA; lanes c and d, Mu end competitor DNA (60 $\mu$g/ml) added before or after formation of the CDC, respectively; lanes e and f, same as lanes c and d except that more (300 $\mu$g/ml) of the competitor DNA was used; lane g, standard strand-transfer reaction without competitor. Electrophoresis was carried out as described (4). Positions of mini-Mu donor DNA [closed circular (Dc) and open circular (Do)] and $\phi$X174 RF DNA [closed circular ($\phi$Xc) and open circular ($\phi$Xo)], and Mu DNA strand-transfer product (I) are indicated.

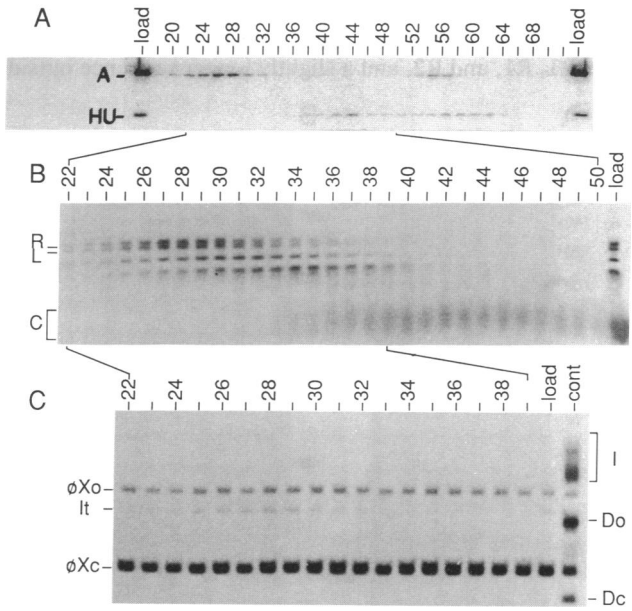


FIG. 3. Trimmed CDC retains strand-transfer activity. The CDC was prepared with the donor plasmid pMK584. Two hundred microliters of the CDC digested by *Sau*3A1 was applied to a 4-ml Sephacryl S-400 superfine (Pharmacia) column equilibrated with 25 mM Tris·HCl, pH 8/600 mM NaCl/10 mM EDTA/1 mM dithiothreitol/15% glycerol and developed with the same buffer at 20 $\mu$l/min. Fractions of 80 $\mu$l were collected and 10 $\mu$l samples of each were subjected to electrophoresis in 10% polyacrylamide gel (Tris/glycine, NOVEX) and Western blotting with MuA and HU antibodies to visualize the protein profile (*A*) and in 1.8% agarose gel (in 89 mM Tris/89 mM boric acid/2.5 mM EDTA) after addition of SDS containing a dye mixture to visualize the DNA pattern (*B*). The strand-transfer activity of the fractions (*C*) was assayed by mixing 6.25-$\mu$l samples with 19-$\mu$l mixtures containing $\phi$X174 DNA (10 $\mu$g/ml), MuB (9 $\mu$g/ml), and ATP (2 mM) in the standard reaction buffer lacking NaCl. Samples were incubated for 20 min at 30°C prior to termination of the reaction and analysis by electrophoresis. Fraction numbers are shown above each lane. The sample prior to the column fractionation (load) was also assayed. The control sample (cont) in *C* shows the strand-transfer activity of the CDC prior to the *Sau*3AI digestion. Positions marked are A, MuA protein; HU, HU protein; R, R-end *Sau*3AI fragment; L, L-end *Sau*3AI fragment; C, Mu end competitor DNA; $\phi$Xo, open circular $\phi$X174 DNA; $\phi$Xc, closed circular $\phi$X174 DNA; Do, open circular pMK584; Dc, closed circular pMK584; It, strand-transfer intermediate with the trimmed CDC; and I, strand-transfer intermediate with undigested CDC.

fragment but the presence of high salt and the competitor DNA made associations between free MuA and this site unlikely; a transient interaction between R3 and the MuA in the complex cannot be ruled out.

The strand-transfer activity in each column fraction was assayed in the presence of MuB, ATP, and $\phi$X174 target DNA. The MuA and HU content of each fraction was determined by Western immunoblot analysis. The peak of strand-transfer activity corresponded to the position of the MuA–Mu ends complex, whereas the competitor DNA and free MuA were well separated. HU was exclusively in the included fractions away from the CDC. We conclude that the protein–DNA complex in which only L1, R1, and R2 are bound by MuA is active for strand transfer.

**STC Footprint.** Nuclease protection experiments similar to those of the CDC were done on the STC. The method was identical, except strand transfer was carried out under the standard conditions with $\phi$X174 target DNA and MuB, and the competitor was added after strand transfer but prior to DNase I digestion. The restriction enzymes used do not digest the $\phi$X174 DNA. About 70% of the donor DNA formed the STC under these conditions.

The protection by MuA of the L and R ends in the STC (Fig. 4) was basically the same as that of the CDC. Three sites, L1, R1, and R2, and a slightly longer sequence outside of both Mu ends were protected by MuA in the presence of the competitor. A probe different from that used for Fig. 4 was used to demonstrate that in the STC preparation, the majority of the cut strand on both the L and R ends in the CDC has been covalently joined to the target DNA as expected. This target DNA strand showed extended protection of about 20 nucleotides adjacent to the Mu end and hypersensitivity to DNase I slightly further out (data not shown; see Fig. 5 for a similar pattern). The donor flanking DNA on the cut strand showed a short region of protection similar to that seen in the CDC.

Previous work (7) has shown that a pair of R ends that have been precut by a restriction enzyme to expose the exact 3′ ends of the Mu sequence can complete strand transfer when presented with MuA and a MuB-coated target DNA. HU is not required for this reaction (7). The nuclease protection by MuA of this two-R-end STC was analyzed under the same conditions as used for the normal STC. The strand probed was that which is covalently joined to the target. In the footprint both the R1 and R2 sites were fully protected by MuA, as was the region outside of the Mu ends (Fig. 5, lanes i and j). These results differ from that seen with the natural asymmetric pair of ends in that four MuA binding sites (two R1 and two R2) instead of three (L1, R1, and R2) were stably protected in the complex. Since the CDC containing the natural Mu ends has been suggested to contain a tetramer of MuA (15), one monomer in this complex should have an unoccupied DNA binding site. Perhaps, due to the proximity of the additional copy of R2 within the two-R-end complex, this fourth monomer left a visible footprint.

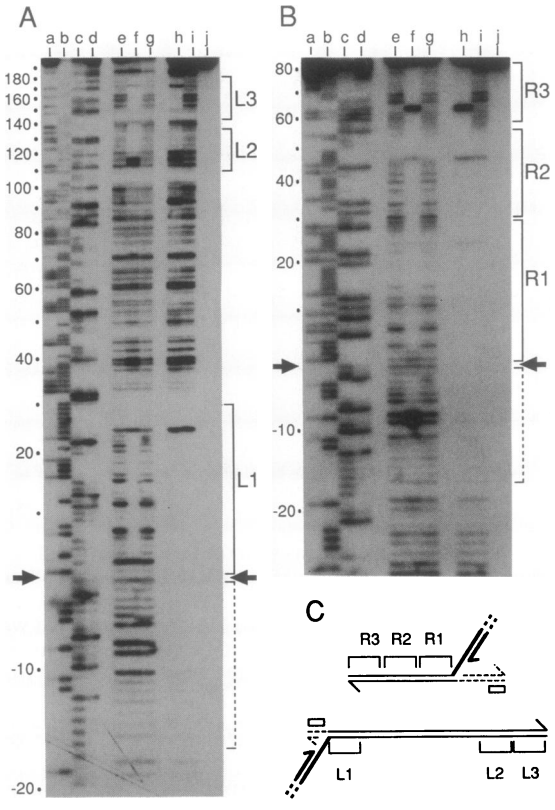


FIG. 4. DNase I footprint of MuA on the STC. The nuclease protection patterns of the Mu L end (*A*) and R end (*B*) on the strand that is not joined to the target DNA are shown. The location of MuA binding sites, junction (arrows) with the target DNA (thick lines) that is not cut by the restriction enzymes used, and the $^{32}$P-labeled probes (small boxes) within restriction fragments that contain the R and L ends of the Mu sequence are indicated (*C*). Lanes a–d, sequence markers (G, A+G, T+C, C, respectively). Lanes e–g, the donor plasmid pMK586 was linearized prior to footprinting without MuA (lanes e), with MuA (lanes f), and with MuA and the Mu end competitor DNA (lanes g). Lanes h and i, the STC was prepared as described in *Materials and Methods* and footprinted without (lanes h) or with (lanes i) the Mu end competitor. Lanes j, same as lanes h, but without DNase I treatment.

## DISCUSSION

During assembly of the nucleoprotein complexes required for Mu transposition, MuA protein initially binds to all six Mu-end binding sites in a freely reversible manner. The next stage, formation of the plectosome, involves communication between the two Mu ends and the IAS mediated by MuA, HU protein, and the superhelical state of the donor plasmid. Here

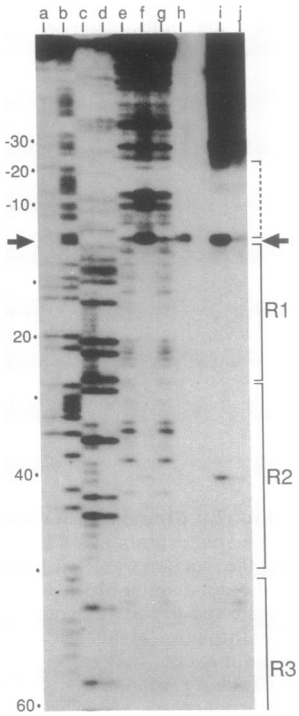


FIG. 5. DNase I footprint of MuA on the STC made by precut donor DNA. The STC involving pMK426 DNA cut by *Hin*dIII was prepared essentially as described for the standard STC, except that HU, which is not required for this reaction, was omitted. The STC was digested by *Eco*RI, which cuts at the end of the R3 site (position 85). The nuclease protection patterns on the strand that is joined to the target DNA are shown. The strand was probed with $^{32}$P-labeled oligonucleotide complementary to this strand at the end of the R3 site and sequenced toward the target DNA sequence. Lanes a–d, sequence markers (G, A+G, T+C, and C, respectively) were made by using the *Eco*RI fragment of pMK586 that carried the same Mu R-end sequence as the precut Mu R-end fragment. Lanes e–g, *Bam*HI-linearized pMK586 was footprinted without MuA (lane e), with MuA (lane f), and with MuA and the Mu end competitor (lane g). Lane h, the STC made by the precut Mu R-end DNA without DNase I treatment. Lanes i and j, the STC made by the precut Mu R-end DNA footprinted without (lane i) or with (lane j) the Mu end competitor.

we demonstrate that the IAS, L2, and L3 are required only prior to CDC formation; other experiments have confirmed earlier results (see Introduction) that these sites are needed to make the CDC (M.M. and K. Adzuma, unpublished observation). Whether the sites nearest to the ends R1, R2, and L1 also participate in this early synapsis has not been determined, as they are essential for later stages.

After donor cleavage, MuA protein becomes essentially irreversibly bound to three sites (L1, R1, and R2) at the ends of the Mu DNA. The DNA sequence at the cleavage site and a short sequence extending further into the flanking DNA are also protected from nuclease digestion. Lavoie *et al.* (9) obtained similar results by using a slightly different method. This outside protection is probably necessary for MuA to cleave the end sequences and must be almost completely sequence-nonspecific, since Mu can transpose into essentially any DNA sequence. MuA binding to these endmost sites and the flanking DNA is sufficient to hold the two Mu ends together. Part of the protection of the outside sequences in CDC and STC may be attributable to the proximity of the DNA strands rather than the bound protein, as observed in the case of protein-free Holiday DNA structure (16). HU protein, while necessary to form the CDC, is not needed for its continued stability (ref. 15 and Fig. 3). In light of its reversible binding, HU is not likely to be responsible for the protection of the flanking DNA sequences. Further, the protection of flanking target DNA in the STC is not due to HU, since the DNase I footprint of the STC made from the precut donor in the absence of HU shows the same extended protection as the normal STC.

The CDC with only irreversibly bound MuA is competent for strand transfer. This reaction covalently joins the donor 3′ ends to a target site to generate the second stable protein–DNA complex, the STC. In the STC, MuA remains bound to the same three Mu end sites (L1, R1, and R2) and the outside sequences at both the L and R ends. Thus, the basic structure of the CDC seems to be largely retained in the STC after strand transfer, despite addition of the target DNA and MuB (15) to the complex.

MuA protein serves multiple functions during the Mu transposition reaction. There are at least two classes of MuA–end site interaction: the reversible binding at R3, L3, and L2 and the irreversible binding at L1, R1, and R2. These data further suggest that at least three monomers [a 1:1 stoichiometry for MuA binding to the end sites has been demonstrated (17), although perhaps four monomers are present in the CDC (9)] are bound to the paired Mu ends in the CDC, and in the absence of competitor DNA, three more MuA molecules are bound. How are the multiple functions of MuA delegated to the different monomers? Since the R1, R2, L1 complex is capable of strand transfer and of interacting with MuB, the MuA monomers bound at these sites are assigned to these functions. Further, considering the proximity to the ends, the R1- and L1-bound subunits are mostly likely to have the Mu-end-cutting as well as the strand-transfer function. In contrast, since the IAS, L2, L3, and R3 sites are involved in formation of the CDC but are no longer needed after it is made, the MuA subunits contacting these sites must help the initial pairing of the Mu DNA ends.

The importance of nucleoprotein complexes in initiation of other transposition reactions [e.g., Tn*10* (18) and Tn*7* (19)] and site-specific recombination reactions [e.g., phage λ integration and excision and the "invertase" family (20–23)] as well as in replication and transcription is emerging (24). One message from this work is that some protein binding sites may be required for the proper assembly of a nucleoprotein complex and then become dispensable once the active complex is constructed. In the case of Mu transposition, assembly of the nucleoprotein complex that pairs the ends of Mu is a rate-limiting step (M.M., unpublished work) and subject to regulation (4). Similarly, the proper protein–DNA complex assembly as a prerequisite for the DNA cleavage and joining steps has been demonstrated for both Tn*7* transposition (19) and λ integration (21) and excision (22). The complexity of these assembly stages probably helps prevent abortive events and reactions between improperly positioned sites. Further characterization of the assembly, subunit organization, and structure of these complexes should provide insight into general mechanisms of biological function and regulation.

We are grateful to G. Chaconas, B. Lavoie, and coworkers for communicating their results prior to publication. We thank K. Adzuma, F. Bushman, R. Craigie, A. Engelman, and M. Gellert for careful reading of the manuscript. This work was supported in part by the National Institutes of Health Intramural AIDS Targeted Antiviral Program. T.A.B. is supported by a Helen Hay Whitney Foundation postdoctoral fellowship.

1. Symonds, N., Toussaint, A., van de Putte, P. & Howe, M. M., eds. (1987) *Phage Mu* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
2. Craigie, R., Mizuuchi, M. & Mizuuchi, K. (1984) *Cell* **39**, 387–394.
3. Craigie, R. & Mizuuchi, K. (1986) *Cell* **45**, 793–800.
4. Mizuuchi, M. & Mizuuchi, K. (1989) *Cell* **58**, 399–408.
5. Leung, D. C., Teplow, D. B. & Harshey, R. M. (1989) *Nature (London)* **338**, 656–658.
6. Surette, M. G., Lavoie, B. D. & Chaconas, G. (1989) *EMBO J.* **8**, 3483–3489.
7. Craigie, R. & Mizuuchi, K. (1987) *Cell* **51**, 493–501.
8. Surette, M. G., Buch, S. J. & Chaconas, G. (1987) *Cell* **49**, 253–262.
9. Lavoie, B. D., Chan, B. S., Allison, R. G. & Chaconas, G. (1991) *EMBO J.*, in press.
10. Craigie, R. & Mizuuchi, K. (1985) *J. Biol. Chem.* **260**, 1832–1835.
11. Chaconas, G., Gloor, G. & Miller, J. L. (1985) *J. Biol. Chem.* **260**, 2662–2669.
12. Craigie, R., Arndt-Jovin, D. J. & Mizuuchi, K. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7570–7574.
13. Baker, T. A., Mizuuchi, M. & Mizuuchi, K. (1991) *Cell* **65**, 1003–1013.
14. Richet, E., Abcarian, P. & Nash, H. A. (1986) *Cell* **46**, 1011–1021.
15. Lavoie, B. D. & Chaconas, G. (1990) *J. Biol. Chem.* **265**, 1623–1627.
16. Churchill, M. E. A., Tullius, T. D., Kallenbach, N. R. & Seeman, N. C. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4653–4656.
17. Kuo, C.-F., Zou, A., Jayarm, M., Getzoff, E. & Harshey, R. (1991) *EMBO J.* **10**, 1585–1591.
18. Haniford, D. B., Benjamin, H. W. & Kleckner, N. (1991) *Cell* **64**, 171–179.
19. Bainton, R., Gamas, P. & Craig, N. L. (1991) *Cell* **65**, 805–816.
20. Landy, A. (1989) *Annu. Rev. Biochem.* **58**, 913–949.
21. Richet, E., Abcarian, P. & Nash, H. A. (1988) *Cell* **52**, 9–17.
22. Kim, S., Moitoso de Vargas, L., Nunes-Düby, S. E. & Landy, A. (1990) *Cell* **63**, 773–781.
23. Heichman, K. A. & Johnson, R. C. (1990) *Science* **249**, 511–517.
24. Echols, H. (1990) *J. Biol. Chem.* **265**, 14697–14700.