ClpX and MuB interact with overlapping regions of Mu transposase: implications for control of the transposition pathway

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Transposition of phage Mu is catalyzed by an extremely stable transposase–DNA complex. Once recombination is complete, the *Escherichia coli* ClpX protein, a member of the Clp/Hsp100 chaperone family, initiates disassembly of the complex for phage DNA replication to commence. To understand how the transition between recombination and replication is controlled, we investigated how transposase–DNA complexes are recognized by ClpX. We find that a 10-amino-acid peptide from the carboxy-terminal domain of transposase is required for its recognition by ClpX. This short, positively charged peptide is also sufficient to convert a heterologous protein into a ClpX substrate. The region of transposase that interacts with the transposition activator, MuB protein, is also defined further and found to overlap with that recognized by ClpX. As a consequence, MuB inhibits disassembly of several transposase–DNA complexes that are intermediates in recombination. This ability of MuB to block access to transposase suggests a mechanism for restricting ClpX-mediated remodeling to the proper stage during replicative transposition. We propose that overlap of sequences involved in subunit interactions and those that target a protein for remodeling or destruction may be a useful design for proteins that function in pathways where remodeling or degradation must be regulated.

[Key Words: Phage Mu; transposition; Clp; Hsp100; transposase]

Received March 11, 1997; revised version accepted May 5, 1997.

The regulated assembly of stable protein–nucleic acid complexes controls many genetic processes including transcription, DNA replication, RNA processing, and genetic recombination. These protein–nucleic acid complexes must be dynamic structures that change subunit composition, protein–DNA (or RNA) contacts and protein–protein contacts during a reaction cycle. Energy-dependent chaperones and proteases participate the remodeling of some protein–DNA complexes. For example, the DnaK/DnaJ/GrpE chaperone machine triggers initiation of phage λ DNA replication (Alfano and McMacken 1989; for review, see Echols 1990) and ATP-dependent chromatin remodeling is an important element of transcription initiation in eukaryotes (Tsukiyama and Wu 1995; Tsukiyama et al. 1995). Elucidating how these remodeling factors recognize their substrates and how their activity is restricted to the proper stage in a reaction pathway—or an appropriate time in a cell or developmental cycle—is necessary for the complete understanding of biological control.

Replication of phage Mu requires that its recombina-

tion machinery undergo protein-catalyzed remodeling (Nakai and Kravits 1995; Levchenko et al. 1995; Kravits et al. 1996). Mu uses multiple rounds of replicative transposition to amplify its DNA during lytic growth. The series of events during replicative transposition is as follows. (1) Mu transposase (MuA), a monomer in solution, assembles into a stable tetramer on binding to specific sequences at each end of the phage genome. (2) This transposase tetramer pairs the two ends of the Mu DNA, cleaves these ends, and joins the cleaved ends, by a reaction called strand transfer, to a new DNA site (Craigie and Mizuuchi 1987; Surette et al. 1987, 1991; Lavoie et al. 1991; Mizuuchi et al. 1992; for review, see Mizuuchi 1992). A second phage-encoded transposition protein, MuB, by forming a protein–protein complex with transposase, stimulates assembly of the tetrameric transposase (Mizuuchi et al. 1995), cleavage of the Mu DNA ends (Surette and Chaconas 1991), and strand transfer of these ends into the target site (Maxwell et al. 1987; Baker et al. 1991; Surette and Chaconas 1991). MuB also dramatically influences target-site choice by causing distant segments of DNA to be used preferentially to those located near transposase binding sites (Adzuma and Mizuuchi 1988). (3) Once strand transfer is complete, the *Escherichia coli* replication machinery initiates Mu DNA replication at the recombination site

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(Toussaint and Faelen 1974; Mizuuchi 1983; Toussaint and Resibois 1983; Resibois et al. 1984; Ross et al. 1986; Krukltis and Nakai 1994).

E. coli ClpX protein has an essential protein-remodeling role during the transition between the recombination and replication stages of Mu transposition (Levchenko et al. 1995; Krukltis et al. 1996). Although several factors are probably involved in this transition, recognition of the transposase by ClpX appears to be the first step. Following strand transfer, the transposase–DNA complex is extremely stable in vitro (Surette et al. 1987). ClpX catalyzes the ATP-dependant destabilization of this transposase complex (Levchenko et al. 1995; Krukltis et al. 1996). This chaperone-like activity of ClpX is required for initiation of replication on Mu DNA in vitro (Levchenko et al. 1995; Krukltis et al. 1996) and in vivo (Mhammedi-Alaoui et al. 1994).

E. coli ClpX is a member of a family of ATPases (the Clp/Hsp100 family) present in prokaryotes and eukaryotes. Clp/Hsp100 proteins have been implicated in a wide variety of biological processes including tolerance to heat stress, protein turnover, DNA replication, control of gene expression, and the inheritance of prion-like factors (Tobias et al. 1991; Mhammedi-Alaoui et al. 1994; Parsell et al. 1994; Chernoff et al. 1995; Vogel et al. 1995; Schweder et al. 1996, for review, see Schirmer et al. 1996). Studies of *Saccharomyces cerevisiae* Hsp104 and *E. coli* ClpX and ClpA reveal that the proteins share a ring-like subunit architecture and the ability to use the energy of ATP to disassemble multimeric or aggregated forms of certain proteins (Parsell et al. 1991, 1994; Wickner et al. 1994; Kessel et al. 1995; Levchenko et al. 1995; Wawrzynow et al. 1995; for review, see Schirmer et al. 1996). The ability of Clp/Hsp100 proteins to disassemble protein complexes distinguishes them from the best-studied Hsp chaperone families (Hsp60 and Hsp70), which are relatively inefficient in resolubilizing aggregates once they have formed (for review, see Schirmer et al. 1996).

ClpX and ClpA also participate in energy-dependent proteolysis (Hwang et al. 1988; Katayama et al. 1988; Gottesman et al. 1993; Wojtkowiak et al. 1993; Lehnher and Yarmolinsky 1995; Laachouch et al. 1996; Schweder et al. 1996; for reviews, see Gottesman and Maurizi 1992; Gottesman et al. 1995; Schirmer et al. 1996). Both proteins form complexes with the ClpP serine protease. The large, double-ring protein structure of the ClpAP complex (ClpA₆, ClpP₁₄) resembles the 26S proteasome (Kessel et al. 1995). Current data support the hypothesis that ClpA or ClpX recognize a substrate protein and use their chaperone-like activities to present this protein to ClpP in the conformation necessary for degradation.

Although ClpX and ClpA are homologous, and have very similar activities, they have different substrate specificity. For example, ClpX acts on the phage λ initiator protein (Wawrzynow et al. 1995) and Mu transposase (Levchenko et al. 1995) but not on the P1 plasmid initiator protein, which is a substrate for ClpA (Wickner et al. 1994). Similarly, neither ClpA nor ClpY(HslU) [another *E. coli* family member 50% identical in sequence

of ClpX (Missiakas et al. 1996)] is able to substitute for ClpX during Mu transposition (I. Levchenko, Y. Kim, and T.A. Baker, unpubl.). Based on this type of analysis, the different Clp/Hsp100 proteins are thought to have relatively restricted substrate specificity. What features of a protein determine that it will be recognized by a Clp/Hsp100 protein and how substrate recognition by the different family members varies is not yet understood.

In this study, we investigate how ClpX recognizes Mu transposase. We find that a 10-amino-acid peptide from the carboxyl terminus of transposase is required for it to be recognized by ClpX. This peptide is also necessary and sufficient to convert a protein that is not normally recognized by ClpX into an efficient substrate. The region of transposase that interacts with the MuB activator protein was also further defined and found to overlap with the ClpX recognition signal. As a consequence of this overlapping recognition, MuB inhibits disassembly of the transposase–DNA complex. This ability of MuB to restrict access to the transposase suggests a mechanism by which ClpX-mediated remodeling is restricted to the proper stage during replicative transposition.

Results

The carboxy-terminal domain of Mu transposase converts Arc repressor into a substrate for ClpXP

During our initial characterization of the interaction of ClpX with the Mu transposase, we found that the carboxyl terminus domain of transposase was required for ClpX to disassemble transposase–DNA complexes that had completed strand transfer (strand-transfer complexes or STCs). Complexes containing versions of transposase lacking either the last 4 or 8 amino acids were disassembled much less efficiently than those made with the full-length protein. Deletion of the last 48 amino acids (domain IIIB) completely abolished disassembly (Levchenko et al. 1995). The same three carboxy-terminal deletions rendered transposase resistant to degradation by ClpXP (Levchenko et al. 1995).

To investigate whether domain IIIB of transposase contains information sufficient to target a protein to ClpX, the last 41 amino acids of transposase were fused to the carboxyl terminus of the phage P22 Arc repressor (Fig. 1a). The resulting fusion protein (called Arc-41—the fusion proteins are named by the number of residues of transposase they carry, counting from the carboxyl terminus) was assayed for its ability to interact with ClpX by measuring its sensitivity to degradation by ClpXP. Although Arc itself was not a substrate for ClpXP (see below), the Arc–transposase fusion protein was efficiently degraded in vitro in the presence of ClpX, ClpP, and ATP (Fig. 1b). Protease sensitivity was not attributable to misfolding of the fusion protein as Arc-41 formed dimers and bound DNA as efficiently as Arc repressor (data not shown). Therefore, the 41 carboxy-terminal amino acids of transposase are sufficient to target Arc to ClpXP, and therefore contain information critical for substrate recognition by ClpX.

Figure 1. Domain IIIb of MuA is sufficient to target Arc for degradation by ClpXP in vitro. (a) Diagram of the Arc-MuA fusion proteins. Numbers on the left represent the numbers of amino acids of the domain IIIb of MuA fused to the carboxyl terminus of Arc repressor; numbers on the right represent the amino-acid position in full-length MuA fused with the domain IIIb. The six-histidine tag is labeled (H₆). (b) Degradation of Arc-41 by ClpXP. Ten pmoles of the fusion proteins were incubated with 0.68 pmoles of ClpP, and 3 or 6 pmoles of ClpX for 60 min at 30°C. Reaction components are indicated at the top of each lane. Products were analyzed by Western analysis using anti-Arc antibody and ¹²⁵I-labeled protein A. (c) Degradation of Arc-41 or Arc-41Δ8 proteins by ClpXP in vitro. Reaction conditions were as in b, except 0, 1.5, 3, or 6 pmoles of ClpX were added as indicated at the top of the lanes.

To determine the minimal region of domain IIIb required to target Arc to ClpXP, a series of truncations of this domain were fused to Arc (Fig. 1a). As was observed with transposase, degradation of the fusion proteins required residues very near the carboxyl terminus. Arc-41Δ8, which is identical to Arc-41 except for deletion of the last eight amino acids of transposase sequence, was resistant to the protease (Fig. 1c). In contrast, most of the amino-terminal portion of domain IIIb was not essential for ClpX recognition. All five fusion proteins missing information from the amino-terminal region of domain IIIb were degraded with a similar efficiency as intact transposase; degradation of the two shortest fusions, Arc-19 and Arc-10 is shown (Fig. 2a). The kinetics of

Arc-41 degradation were very similar to those of these shorter fusions, with ~60% of the protein degraded in 30 min.

Analysis of these smaller fusion proteins was done by labeling them with ³⁵S, rather than by the Western blots shown above. Release of acid-soluble radioactivity (Fig. 2a), and electrophoresis followed by autoradiography (Fig. 2b), both revealed processive degradation. Degradation was efficient (35%–80% complete in 30 min.), no significant accumulation of intermediates was detected, and the Arc as well as the transposase portion of the fusion proteins were destroyed. Degradation of even the smallest fusion protein, Arc-10, required both ClpX and ATP (data not shown). We conclude that the last 10 carboxy-terminal residues of transposase are sufficient to convert Arc into a substrate for ClpXP; these residues therefore contain a substrate recognition signal for ClpX.

ClpX and MuB interact with overlapping regions of transposase

Domain IIIb of transposase interacts with MuB protein

Figure 2. The 10 last amino acids of transposase target Arc for degradation by ClpXP. (a) Kinetics of the degradation of the ³⁵S-labeled MuA, Arc-19, Arc-10, and Arc proteins by ClpXP. The average from two experiments is shown. Ten picomoles of ³⁵S-labeled proteins were incubated with 1.1 pmoles of ClpP and 1.2 pmoles of ClpX for 60 min at 30°C. Degradation efficiency was estimated by counting TCA-soluble radioactivity. MuA (○), Arc-19 (▲), Arc-10 (◆). Error bars are large for the Arc-10 protein because this substrate has a tendency to stick to plastic. (b) Degradation of Arc and Arc-19 proteins by ClpXP in vitro. Conditions of the reaction are as for a except 0.68 pmoles of ClpP and 3 or 6 pmoles of ClpX were used. Reaction components are indicated at the top of the lanes. Products of the reactions were analyzed by scanning dried gels with a PhosphorImager.

(Baker et al. 1991; Leung and Harshey 1991; Wu and Chaconas 1994). Previous studies have demonstrated that the final 36 residues of transposase interact with MuB and that deletion of as few as four residues from the carboxy-terminal end of the protein disrupts this interaction (Wu and Chaconas 1994). To elucidate the relationship between the region of transposase that interacts with MuB and that needed by ClpX, we investigated transposase–MuB interactions using the Arc–MuA fusion proteins.

The interaction of the Arc–MuA fusion proteins with MuB was measured by their interference in the target DNA delivery process during an *in vitro* transposition reaction. In the presence of MuB, strand transfer occurs principally between two plasmids—a donor DNA, which carries the MuA-binding sites needed for transposase assembly, and a target DNA, which is bound by MuB (Fig. 3a, left). The resulting recombined DNAs are referred to as intermolecular strand-transfer products. When MuB is absent, or its ability to deliver the target DNA is disrupted, transposase uses target sites exclusively within the donor DNA plasmid, thereby generating intramolecular strand-transfer products. Excess transposase inhibits intermolecular strand transfer by the mechanism outlined in Figure 3a (right) (Baker et al. 1991; Wu and Chaconas 1994). The Arc–transposase fusion protein (by analogy to transposase) interacts with a MuB–DNA complex and stimulates the ATPase activity of MuB, resulting in an increased disassociation of MuB from the DNA. The resulting “free” MuB stimulates intramolecular strand transfer by interacting with the transposase–donor DNA complex without bringing a target DNA. Therefore, the ability of a transposase derivative to inhibit intermolecular strand transfer is a sensitive assay for its ability to interact with MuB.

The Arc-41, Arc-36, and Arc-30 fusion proteins inhibited intermolecular strand transfer, indicating that they effectively interact with MuB (Fig. 3b). The minimal inhibitory protein concentration was the same for each of these fusion proteins (data not shown). In contrast, Arc-24 was only slightly inhibitory and the Arc-19 and Arc41Δ8 fusion proteins were inactive (Fig. 3b). These data indicate that the 30 last residues constitute the minimal region of transposase capable of the functional interaction with MuB that disrupts target delivery. These results are in accord with those of Wu and Chaconas (1994). However, the Arc–MuA fusions were ~50-fold more active than isolated domain IIIB peptides used in their study. This difference could be attributable to improved stability of the Arc–MuA fusions, or their ability to form dimers and interact with DNA. Moreover, in this MuB interaction assay, the Arc fusion proteins have a similar specific activity to intact transposase (data not shown, but see Baker et al. 1991), indicating that they carry the principle determinants involved in this MuA–MuB interaction.

The deletion analysis described above indicates that the last 30 residues of transposase interact with MuB, the last 10 interact with ClpX, and both proteins require the last 8 residues. This arrangement of the protein in-

Figure 3. The carboxy-terminal 30 amino acids of MuA are sufficient to interact with MuB. (a) Diagram of the Mu transposition reaction *in vitro* with (right) and without (left) a fusion protein that interacts with MuB. (b) Reactions showing inhibition of the intermolecular strand-transfer reaction by Arc–MuA proteins were performed with 10 μ g/ml of mini-Mu donor DNA, 10 μ g/ml of ϕ X174 DNA, 1.3 pmoles of MuA, 5 pmoles of MuB, 3 pmoles of HU, and 5 or 10 pmoles of the fusion proteins. Reactions were incubated at 30°C for 30 min, stopped by the addition of SDS and EDTA (final concentrations: 0.5% and 10 mM, respectively), and analyzed by agarose gel electrophoresis. Supercoiled and relaxed donor DNA (Dc, Do) and target DNA (Tc, To) are indicated. Intermolecular strand-transfer products (INTER STP) are detected as a series of topoisomers as indicated by the bracket. The INTRA products are not resolved under this electrophoresis condition.

teraction determinants was further investigated by engineering amino acid substitutions into the Arc–MuA fusion proteins (Fig. 4). The goals of this mutagenesis were to (1) confirm existence of overlapping signals and (2) isolate mutant proteins defective in interacting with MuB but recognized by ClpX. This series of mutants were called Bid (MuB interaction defective). As expected from the deletion analysis, most substitution mutations between 30 and 10 amino acids from the carboxyl terminus (Fig. 4, Bid1, Bid5, Bid6, and Bid8) had less than a twofold affect on the ability of these proteins to be recognized by ClpX. Only one mutation within this region (Bid7, Y639 $\rightarrow$ A) decreased degradation by ClpXP more than twofold. Of these proteins, the Bid1 mutant had the desired characteristic of being impaired in MuB interac-

MuB Interaction		ClpX Interaction		protein	MuB Interaction	ClpX Interaction
30	24	19	10	8		
---ERDDEYETERDEYLNHSLDILEQNRKKAI				WT	+++ ^d	+++
A-AAA-A-A-----				Bid1a	+/-	+++
-DD-----				Bid2b	+/-	+++
		-MMMMAI		Bid3b	+	++
		-DDDDAI		Bid4a	-	+/-
-A--A-A-A-----				Bid5c	++	++
A-AA-----				Bid6c	+++	+++
--A-----				Bid7c	++	+/-
--AA-----				Bid8c	+++	++

^a Assayed in Arc-30 and in full length transposase.
^b Assayed in full length transposase only.
^c Assayed in Arc-30 only.

^d +++ > 75% of wt
 ++ 50-75 %
 + 25-50 %
 +/- 5-25 %
 - < 5 %

Figure 4. Substitution mutagenesis of the MuB and ClpX recognition signals. The sequence of the carboxy-terminal 30 amino acids of MuA (Harshey et. al. 1985) is shown, and substitutions are shown below the wild-type sequence. The ability of Arc-Bid fusions to interact with MuB was estimated by quantitation of intermolecular strand-transfer products generated as described in Fig. 3; the ability of MuA-Bid proteins to interact with MuB was estimated by quantitation of intermolecular strand-transfer products generated in the standard transposition reaction as described in the legend to Fig. 5. The ability of MuA-Bid proteins to interact with ClpX was estimated by quantitation of intramolecular STC as described in the legend to Fig. 5. The Arc-MuA fusions were assayed for ClpX interaction by degradation as described in the legend to Fig. 1.

tion, but well recognized by ClpX. In contrast, replacement of the positively charged residues between 6 and 3 residues from the carboxyl terminus with aspartates (Bid4) caused a severe defect (<15% residual activity) in the ability of the protein to interact with both ClpX and MuB, confirming the conclusion of the deletion studies that this region interacts with both proteins.

Two of the Bid alleles (Bid1 and Bid4) were reconstructed into full-length transposase. Two additional mutant transposase proteins were generated (Bid2, NH₆₄₉ → DD, and Bid3, RRKK₆₆₁ → MMMM). In the absence of MuB, each of the four MuA-Bid proteins catalyzed *in vitro* transposition, through the strand-transfer stage, with an efficiency comparable with wild-type transposase. Therefore, the Bid mutations do not disrupt transposase assembly, DNA cleavage, or strand transfer. In contrast, in the presence of MuB protein, each Bid protein was defective in intermolecular strand transfer compared with wild-type transposase (between 2 and 30-fold less active) (Fig. 5a). These data establish that residues 634–642, 648–649, and 658–661 of transposase are important for its interaction with MuB.

Recognition of the MuA-Bid proteins by ClpX was assayed by measuring disassembly of intramolecular STCs containing the mutant proteins (Fig. 5b; data for Bid1 is in Fig. 6). In agreement with the behavior of the fusion proteins carrying the same sequences, transposase complexes containing either the MuA-Bid1 or the MuA-Bid2 mutant proteins were disassembled efficiently by ClpX. Therefore, these mutations, which change residues >10 amino acids from the carboxy-terminal end, do not substantially disrupt ClpX recognition. In contrast, substitution of the RRKK sequence 6 amino acids from the carboxyl terminus with aspartates (MuA-Bid4) almost completely prevented disassembly; 86% of the STCs present in the absence of ClpX, remained intact at the highest ClpX concentration (calculated based on the presence of 57% of donor DNA in the STC after ClpX treatment, compared with 66% in the absence of ClpX). The methionine substitution of the same residues (MuA-Bid3) inhibited disassembly less severely; 40% of complexes present in the absence of resisted disassembly at the highest level of ClpX (Fig. 5b). The MuA-Bid1 and MuA-Bid4 proteins were also assayed for degradation by ClpX. In

Table 1. Disassembly and degradation are influenced in parallel by changes in the carboxy-terminal domain of transposase

Allele	Disassembly (% of wt)	MuA degradation (% of wt)	Arc-MuA degradation (% of wt)	Arc fusion protein
Wild type	100	100	100	Arc41
Δ4	28	26	N.D.	—
Δ8	28	12	<2	Arc41Δ8
Δ48	2	<2	<2	Arc
Bid1	90	94	69	Arc30-Bid1
Bid4	14	15	14	Arc30-Bid4

Disassembly of STCs formed by MuAwt, MuA-Bid1, and MuA-Bid4 by ClpX is shown in Figs. 5 and 6. The efficiency of disassembly was estimated as described in Materials and Methods and the legends to Figs. 5 and 6. Disassembly of STCs formed with MuAwt, MuAΔ4, MuAΔ8, and MuAΔ48 have been published (Levchenko et al. 1995). Efficiency of the reaction was estimated by scanning negatives of the EtBr-stained gels and calculating the percent of the total donor DNA in STC. Degradation of the MuA and Arc-MuA fusions by ClpXP are described (see Figs. 1 and 2). Degradation of MuAΔ4 and MuAΔ48 were published (Levchenko et al. 1995). Degradation of MuAΔ8, MuA-Bid1, MuA-Bid4, Arc30-Bid1, and Arc-Bid4 and the calculation of the reaction efficiency are as described in Materials and Methods.

Figure 5. MuB and ClpX recognize overlapping signals in domain IIIb of MuA. (a) Mu transposition with MuA-Bid mutants. Reactions were performed as described in the legend to Fig. 3, except Arc-MuA fusion proteins were omitted. The amounts of MuB added were 5, 10, and 20 pmoles, except in the reactions with MuA-Bid4 where MuB was included at 10, 20, and 40 pmoles. Reactions were incubated at 30°C for 20 min and were analyzed by agarose gel electrophoresis. Supercoiled and relaxed donor DNA (Dc, Do) and target DNA (Tc, To) are indicated by dashes. Topoisomers of intermolecular STP (INTER-STP) and intramolecular STP (INTRA-STP) are indicated by brackets and an arrow. Numbers below each lane indicate the percent of donor DNA in the intermolecular STP. (b) MuA release from STC by ClpX with MuA-Bid mutants. Intramolecular STC were assembled with 10 µg/ml of pSG-1, 1.3 pmoles of MuA and 3 pmoles of HU. Reactions were incubated at 30°C for 60 min before the addition of ClpX. ClpX levels were 0, 1.7 and 2.5 pmoles. Reactions were incubated at 30°C for 60 min and analyzed by agarose gel electrophoresis. STC and free DNA are indicated by dashes. Numbers below each lane indicate percent of donor DNA in intramolecular STC.

agreement with the effect of these substitutions on disassembly, MuA-Bid4 was much more resistant to degradation than either MuA-Bid1 or wild-type transposase (Table 1).

Based on the above deletion and substitution mutagenesis of domain IIIb, in the context of the Arc-MuA fusion proteins and in intact transposase, we conclude that MuB and ClpX use overlapping sequences near the carboxyl terminus of transposase to perform their functions during transposition. MuB-transposase interactions require the last 30 residues of transposase, encompassing the region recognized by ClpX. The impact of these overlapping protein-protein interaction determinants is investigated below.

MuB inhibits disassembly by ClpX and can protect early transposition intermediates

The observation that MuB and ClpX use overlapping regions of transposase to interact with and influence the transposase complex raised the possibility that the two proteins compete for their recognition regions. To investigate this possibility, we tested the impact of MuB on the ability of ClpX to disassemble STCs. STCs were

Figure 6. MuB inhibits ClpX-dependent release of MuA from STCs by interacting with the carboxy-terminal domain of MuA. Intramolecular STC were assembled with ³⁵S-labeled MuA and MuA-Bid1. STCs were then purified from free MuA. Purified STC's (0.3 pmoles of MuA) were incubated with 1.98 pmoles of ClpX and 0, 2.8, 5.6, 8.4, or 11.2 pmoles of MuB at the conditions described in the legend to Fig. 5. Reaction components are as indicated. (a) The protein-DNA complexes and free DNA products were visualized by staining of the agarose gel with ethidium bromide; a photograph of the negative is shown. STC and released DNA are indicated by arrows. (b) Detection of ³⁵S-labeled MuA both free and in complex. ³⁵S-labeled STC and released MuA were visualized by autoradiography of the dried agarose gels from a. STC and released MuA are indicated by arrows. (c) Efficiency of the reactions of STC disassembly for wild-type MuA and MuA-Bid1 was estimated by scanning and quantifying radioactivity remaining in the STC after incubation with ClpX on dried gels on a PhosphorImager. The average from three experiments is shown.

made in the absence of MuB using ^{35}S -labeled transposase to allow the protein present in the complexes to be quantitated. MuB protein was then added to the reaction, and disassembly was initiated by addition of ClpX. This experiment was done in parallel with wild-type transposase and the Bid1 mutant, which has a reduced ability to interact with MuB.

The STCs formed by both wild-type transposase and MuA–Bid1 were efficiently disassembled by ClpX (Fig. 6a shows the DNA, and 6b shows the radiolabeled transposase). Addition of MuB to the reaction containing wild-type transposase clearly inhibited disassembly; in the presence of 5.6 pmoles or more of MuB, 80%–90 % of the transposase in strand-transfer complexes was protected from ClpX (Fig. 6c). In contrast, the MuA–Bid1 complexes were less well-protected by MuB; only 30% of the transposase was protected from disassembly in the presence of 5.6 pmoles of MuB, and ~50% was still disassembled when the MuB level was doubled. Similar results were obtained with a when MuA–Bid2 was used in place of MuA–Bid1 (data not shown). This effect of the Bid mutations on the ability of MuB to inhibit ClpX, indicates that the mechanism of inhibition involves interaction of MuB with the carboxy-terminal domain of transposase. In further support of this conclusion, MuB treated with NEM (*N*-ethyl-maleimide), which impairs its ability to bind DNA but not its ability to activate transposase (Baker et al. 1991; Surette and Chaconas 1991), inhibited disassembly at similar concentrations as did untreated MuB (data not shown). Therefore, we conclude that MuB inhibits disassembly of STCs by forming a protein–protein complex with the carboxyl terminus of transposase; this interaction presumably blocks the access of ClpX.

The ability of MuB to inhibit disassembly of STCs suggested that it may have a role in regulating the transposition pathway. As MuB is involved in several of the early stages of transposition (tetramer assembly, cleavage, and strand transfer), whereas ClpX is essential after strand transfer, this inhibitory activity of MuB could serve to prevent premature disassembly of transposase–DNA complexes. To investigate this possibility, we asked (1) whether ClpX disassembles transposase–DNA complexes that have not yet completed strand transfer and (2) whether MuB protects these early recombination intermediates from ClpX.

The stable synaptic complex is an intermediate in transposition that forms before DNA cleavage (Mizuuchi et al. 1992). This complex, like later complexes, contains a tetramer of transposase bound to the paired ends of the Mu DNA. Transposase carrying the point mutation E392Q, which disables the active site, efficiently forms stable synaptic complexes but does not proceed further with recombination (Baker and Luo 1994). The stable synaptic complexes formed with E392Q were disassembled by ClpX (Fig. 7, lane 9; top panel shows DNA, bottom shows transposase). These data reveal that early transposition complexes are not intrinsically resistant to ClpX in the absence of MuB. MuB inhibited disassembly of these stable synaptic complexes to a similar extent as

Figure 7. MuB inhibits disassembly of SSC by ClpX. Wild-type MuA and E392Q MuA were used to assemble intramolecular STC (MuAwt) and SSC (E392Q). Purified complexes were incubated with 1.73 pmoles of ClpX and 0, 5.6, or 11.2 pmoles of MuB under the conditions described in the legend to Fig. 5. (Top) The protein–DNA complexes and free DNA products were visualized by staining of the agarose gel; a photograph of the negative is shown. (Bottom) Western analysis of the MuA in the STC and SSC with anti-MuA antibody. Supercoiled (Dc) and relaxed (Do) donor DNA, intramolecular STCs (INTRA STC), stable synaptic complexes (SSC) are shown with arrows; released intramolecular strand-transfer DNA products are indicated by (* →).

it protected the STCs (Fig. 7, cf. lanes 5 and 6 with lanes 10 and 11). MuB also inhibited disassembly of cleaved donor complexes, the next stable transposase–DNA complex in the transposition pathway (data not shown). Therefore, transposase is susceptible to ClpX at several stages of transposition and MuB has the capacity to control the access of ClpX to these complexes.

Discussion

Identification of a ClpX recognition signal

We have established that a 10-amino-acid peptide from the carboxy-terminal domain of Mu transposase contains information that is both necessary and sufficient to target a protein as a substrate for the *E. coli* ClpX protein. The ability of this peptide to tag a protein as a ClpX substrate was measured by (1) the sensitivity of the Arc–MuA fusion proteins to degradation by ClpXP; (2) the sensitivity of transposase to degradation by ClpXP; and (3) the ability of transposase–DNA complexes to be disassembled by ClpX (see Table 1 for summary). All three assays were affected similarly by alterations in the targeting peptide, indicating that this sequence is the major determinant governing whether these proteins are recognized as substrates by ClpX. Furthermore, the fact that disassembly by ClpX and degradation by ClpXP were influenced in parallel by changes in the peptide strongly supports the conclusion that, at least for these substrates, the ability of a protein to be recognized by ClpX determines if it will be degraded by ClpXP.

Inspection of the sequences near the carboxyl termini

of the *E. coli* and phage proteins identified as substrates for ClpXP fails to reveal an obvious relationship to the transposase sequence. There are, however, similarities. Positively charged and aliphatic residues are common, whereas negatively charged amino acids are infrequent. Furthermore, in two other cases, sequences near the carboxyl termini are known to be important determinants for protease sensitivity. Mutations in Mu repressor (*vir* mutations) that enhance its susceptibility to degradation by ClpXP are frame shifts that change the amino-acid sequence at the carboxyl terminus (Geuskens et al. 1992; Laachouch et al. 1996). A seven-residue peptide from one of the *vir* repressors (sequence FMNRKVL) converted a "recipient" protein into a ClpXP substrate *in vivo* when fused to its carboxyl terminus (Laachouch et al. 1996). Proteins translated from mRNAs that lack a stop codon have been shown recently to contain a 10-amino-acid sequence (ANDENYALAA) at their carboxy-terminal end, which arises from translation of the *ssrA* RNA (Keiler et al. 1996). The resulting tagged proteins are degraded by both ClpXP and ClpAP (S. Gottesman, E. Roche, Y. Zhou, and R. Sauer, in prep.). The high incidence of alanine in the *ssrA*-tag sequence may explain why alanine substitutions in the transposase recognition signal did not prevent ClpX recognition (I. Levchenko, unpubl.). In fact, substitution of the last residue of the transposase sequence (I $\rightarrow$ A) increased susceptibility of the Arc-MuA fusion protein to ClpXP approximately twofold (data not shown). Randomization of the transposase sequence is in progress to determine what peptides can function for ClpX substrate recognition.

Although the sequence near the carboxyl terminus of some proteins clearly constitute important determinants for substrate recognition by ClpX, other evidence indicates that the key recognition determinants will not always be restricted to this region. For example, an internal segment of the alternative σ factor, σ^S , is implicated as a determinant for degradation, and σ^S is degraded only when the regulatory protein SprE (RssB) is also present (Muffler et al. 1996; Pratt and Silhavy 1996). Further-

more, in several cases the quaternary structure of the protein appears to be important for susceptibility. For example, heterodimers of a UV mutagenesis protein (UmuD'-UmuD) are degraded by ClpXP, whereas other forms of the protein that have the same carboxyl termini appear not to be substrates (Frank et al. 1996). Even with transposase it appears that other regions of the protein can influence the efficiency of ClpX action. For example, deletion of the final 8 residues of the transposase sequence had a larger inhibitory affect on degradation of the Arc-41 fusion protein than it did on transposase (see Table 1). Likewise, substitution mutations outside the final 10 residues (especially Y639A) inhibited ClpXP degradation of the Arc-30 protein, although this region is not essential for degradation by deletion analysis. We also do not yet know how many proteins can be converted into a ClpX substrate by addition of the 10-amino-acid transposase tag. Arc was chosen as a recipient protein for these fusion studies because it has an exposed carboxyl terminus and is a dimer (Raumann et al. 1994), as we reasoned these characteristics may be important to ClpX recognition. Further studies are needed to determine if this is the case.

MuB and ClpX recognize overlapping regions of transposase: implications for control

ClpX and MuB both use sequences within the carboxy-terminal domain of transposase to execute their roles in transposition. The region essential for MuB clearly includes the sequence needed by ClpX. As a consequence, MuB antagonizes disassembly of transposase-DNA complexes by interacting with, and probably occluding, the carboxy-terminal peptide from ClpX. This ability of MuB to prevent ClpX from recognizing transposase suggests a mechanism by which specific transposase-DNA complexes could be substrates for remodeling by ClpX, whereas others would be protected (Fig. 8). MuB stimulates assembly of the active tetramer, cleavage of the Mu DNA ends, and strand transfer, and delivers the target

Figure 8. Proposed model for MuB-mediated protection of the recombination complex from ClpX. (Assembly) MuB recognizes domain IIIb of MuA, participates in assembly of the recombination complex and brings in the target DNA. (Recombination) MuB interacts with MuA in the recombination complex, protecting the transpososome from premature disassembly by ClpX and stimulating cleavage and strand transfer. Efficient complexes involve interaction of all four transposase subunits with MuB. (Disassembly and initiation of replication) MuB leaves transpososome after completion of strand transfer, exposing recognition signal for ClpX, which catalyzes disassembly of the STC during the initiation of DNA replication. ATP hydrolysis by MuB protein is proposed as a likely mechanism triggering the exit of MuB from the STC.

DNA (Maxwell et al. 1987; Adzuma and Mizuuchi 1988; Baker et al. 1991; Surette and Chaconas 1991; Mizuuchi et al. 1995). All of these activities require that MuB interact with the carboxy-terminal domain of transposase (Baker et al. 1991; Wu and Chaconas 1994; Mizuuchi et al. 1995). Furthermore, MuB activation is most efficient when it contacts all four of the subunits in the transposase tetramer, although this is not essential under some circumstances (Mizuuchi et al. 1995; Nakai and Krukltis 1995). Therefore, the most efficient transposition pathway almost certainly involves a MuB-transposase complex throughout recombination, at least until strand transfer is complete.

Based on our current analysis, we would expect these early complexes to be protected from ClpX by virtue of the presence of MuB. In vitro, in the absence of MuB, ClpX disassembles transposase-DNA complexes that have not completed strand transfer. If this also occurs in vivo, ClpX could serve a "proofreading" function, removing transposase subassemblies that fail to successfully recruit MuB. Therefore, this role for MuB in protecting early transposition intermediates from premature remodeling by ClpX would enforce the dependence of transposition on MuB, thereby increasing the probability of efficient transposition into distant target sites. MuB stimulates all types of Mu transposition in vivo, and is required for replicative transposition (Chaconas et al. 1985 and references therein).

The observation that MuB restricts the ability of ClpX to recognize transposase strongly suggests that MuB must release the carboxy-terminal peptide for ClpX to initiate the transition to the replication stages of transposition. How this occurs is not yet understood, however, a role for ATP hydrolysis by MuB is attractive. MuB bound to ATP is optimal for stimulating transposase (Maxwell et al. 1987; Adzuma and Mizuuchi 1988; Baker et al. 1991; Surette and Chaconas 1991). Furthermore, ATP hydrolysis-dependant release of MuB from DNA is stimulated by an interaction with the carboxy-terminal domain of transposase (Baker et al. 1991; Wu and Chaconas 1994). Therefore, transposase-regulated ATP hydrolysis may destabilize interaction of MuB with both the target DNA and the transposase complex. A prediction of this model is that if ATP hydrolysis by MuB were blocked, ClpX-catalyzed disassembly would be prevented. Experiments are in progress to address directly interaction of MuB and ClpX with transposase at different stages of the recombination pathway and the role of ATP in these interactions.

Control of protein remodeling and degradation by regulating substrate recognition

Elucidating how remodeling factors and proteases recognize their substrates and how their activity is restricted to the appropriate stage in a pathway, or time in a developmental cycle, is important to understanding biological control. MuB-mediated sequestration of the region of transposase required for substrate recognition by ClpX provides an example of how access of a chaperone or a

protease to its substrate can be regulated by another protein. It is well known that in protein complexes that consist of multiple subunits, if certain components are synthesized in excess to the level needed to form the complex, these free subunits are often degraded rapidly. There are also several examples of unstable regulatory proteins whose degradation is influenced by a protein-binding partner. For example, degradation of RcsA—a positive regulator of capsular polysaccharide synthesis in *E. coli*—by the Lon protease, is slowed when it forms a complex with a second regulator, RcsB (Stout et al. 1991). Activation of AMP-dependent protein kinase provides another example of protein-binding partners controlling degradation. cAMP causes disassociation of the regulatory subunits from the inactive holoenzyme; on release from the complex, these regulatory proteins are selectively degraded (Greenberg et al. 1987; Hegde et al. 1993). We suggest that a protein-complex architecture that uses overlapping sequences for subunit interactions and for targeting a protein for remodeling or destruction provides a useful design for this type of regulation.

Materials and methods

DNA

Plasmid pET14b-ClpX was constructed by cloning the *NdeI* to *BamHI* fragment of pET3a-ClpX (Levchenko et al. 1995) containing the ClpX-coding sequence into pET14b (Novagen). pET3a-Arc-41, used to overexpress a fusion of Arc repressor from P22 to the 41 carboxy-terminal amino acids of the MuA transposase (domain IIIb; Wu and Chaconas 1994) was constructed as follows. The Arc gene was amplified by PCR from plasmid pSA600 (kindly provided by R. Sauer; Milla et al. 1993). The coding sequence for domain IIIb of MuA with a silent alteration creating an *EagI* site at nucleotide 1864 (primer TB287) of MuA (Harshey et al. 1985) was PCR amplified from plasmid pET3d-MuA (Baker et al. 1993). The two PCR products were digested with *EagI* and ligated. Ligation products were digested with *NdeI* and *ClaI* and cloned into pET3a digested with *NdeI* and *ClaI*. Constructs expressing fusions of amino-terminal truncations or substitutions of domain IIIb of MuA to Arc were constructed analogously. Altered domain IIIb fragments were similarly PCR amplified from pET3a-MuA.

The altered domain IIIb PCR products were ligated through the *EagI* site to the Arc PCR product described above. Ligated fragments were digested with *NdeI* and *ClaI* and cloned into the pET3a *NdeI*-*ClaI* fragment. To make pET3a-ArcΔ8, the domain IIIb PCR product was obtained from pET3a-MuA with primers TB288: 5'-gggcatgattattccagaatattccagc and TB287 from above. This fragment was ligated through the *EagI* site to Arc and cloned into pET3a as for pET3a-Arc-41. To make pET3a-Arc-Bid4, Arc-30 was PCR amplified with TB309 (an amino-terminal Arc primer) and TB375 (5'-gggcatgattaaatggcatcatcatcatgttctgttcag). This fragment was cloned into pET3a after digestion with *ClaI* and *NdeI*. To make pET3d-MuA-Bid3 and pET3d-MuA-Bid4, an altered domain III fragment with an introduced *EagI* site at nucleotide 1778 was PCR amplified from pET3a-MuA with TB258 (5'-ccccggccggccgtgaatatccgccgccgcaaacaa) and TB389 (5'-gggcatgattaaatggcatcatcatcatgttctgttcag, Bid3) or TB375 (described above for MuA-Bid4); amplified DNA was digested with *EagI* and *ClaI* and cloned into the *EagI*-*ClaI* fragment of pWZ170 (Wu and Chaconas 1995). pET3d-MuA-Bid1 was constructed by PCR amplification from pET3a-

MuA with TB289, (a carboxy-terminal MuA primer), and TB372: 5'-ggaacgggtgaagaatcaggcgctgctgctgc of an altered domain IIIb. The amplified DNA was digested with *HphI* and *Clal*. The 81-bp fragment (nucleotides 1900–1981) was ligated to a 184-bp *EagI*-*HphI* DNA fragment obtained from a PCR amplification of domain III with primers TB258 and TB289 as described above. The ligation products were PCR amplified with TB258 and TB289, digested with *EagI* and *Clal* and cloned in the *EagI*-*Clal* fragment of pWZ170. To make pET3d-MuA-Bid2, a modified domain IIIb fragment was PCR amplified from pET3a-MuA with TB381: 5'-aaactgagcgtgatgaatatctggatgattcgctg and TB289 (described above). This PCR fragment was digested with *DdeI* and *Clal* and the 58-bp fragment was ligated to the 207-bp *EagI* to *DdeI* fragment generated from digestion by *EagI* and *DdeI* of the PCR product generated from pET3a-MuA using primers TB258 and TB289 (described above). The ligation products were cloned into pWZ170 as for MuA-Bid1.

Proteins

Wild-type MuA and MuA-Bid1–4 were purified as described (Baker et al. 1993). HU protein was purified as described (Baker et al. 1994). MuB was subcloned into the pET14b vector (Novagen) which, when expressed, produced a hexahistidine-tagged MuB protein. MuB was overproduced in HMS174(DE3)pLysS (Novagen) and lysed as described previously (Baker et al. 1994). Purification was performed under native conditions. MuB was dialyzed against MuB dilution buffer (25 mM HEPES-KOH, pH 7.6; 1 M NaCl; 0.1 mM EDTA; 1 mM DTT; 20% glycerol).

His-tagged ClpX (H-ClpX) was purified from *E. coli* cells (HMS174 pLysS; Novagen) containing the pET14b-ClpX plasmid using a spermidine heat lysis procedure described previously (Baker et al. 1993) with the exception that the lysis buffer contained 5 mM β -ME instead of DTT and was supplemented with 1 mM imidazole. The lysate was centrifuged at 12,500 rpm for 1 hr at 4°C. Protein from the supernatant was purified under native conditions according to manufacturer's recommendations. Elution fractions containing ClpX, as determined by SDS-PAGE, were pooled and loaded onto a Sephacryl S-300HR 26/60 column equilibrated with 50 mM Tris-HCl, pH 7.5; 100 mM NaCl; 2 mM DTT; and 20% glycerol. Fractions were analyzed by coomassie SDS-PAGE and those containing ClpX (>95% pure) were pooled.

Overexpressed ClpP protein was purified from an *E. coli* strain HMS(DE3)174pLysS (Novagen) containing the plasmid pAED4 (kindly provided by A.L. Horwich, Yale University, New Haven, CT). Purification was as described previously (Thompson and Maurizi 1994) with modifications. Cell lysis was as described for MuA (Baker et al. 1993). Nucleic acids from the soluble cell lysate were removed by polyethylenimine precipitation (final concentration 0.13%). The precipitated material was removed by centrifugation at 30,000 rpm for 30 min at 4°C. After Q-sepharose chromatography, ClpP-containing fractions were applied to a heparin-sepharose column equilibrated with 50 mM Tris-HCl (pH 7.2); 20 mM NaCl; 5 mM MgCl₂; 2 mM DTT; 10% glycerol. Bound proteins were eluted with 20–250 mM NaCl gradient. ClpP-containing fractions, were pooled and loaded onto a Sephacryl S-300HR 26/60 (Pharmacia) column equilibrated with ClpP storage buffer (50 mM Tris-HCl at pH 7.5; 100 mM NaCl; 2 mM DTT; 10% glycerol).

Arc-His6 (Milla et al. 1993) protein or Arc-MuA fusions were purified as described (Milla et al. 1993). After Ni-NTA-agarose purification, Arc-containing fractions were pooled and passed through a PD-10 desalting column equilibrated with Arc storage buffer (50 mM Tris-HCl at pH 7.5; 100 mM NaCl; 1 mM EDTA). Arc was further purified in Arc storage buffer by Mono-S (PC

1.6/5) chromatography and eluted with a 100–1500 mM NaCl gradient. After Mono-S chromatography, Arc was >95% pure as determined by coomassie SDS-PAGE.

MuA, Arc, and Arc-MuA fusions were labeled *in vivo* with ³⁵S [EXPRE³⁵S-labeling mix (Du Pont)] essentially as described previously (Baker et al. 1993).

Transposition and disassembly

Strand-transfer reactions were performed as described previously (Baker et al. 1993). Reaction mixtures (25 μ l) contained 25 mM HEPES-KOH (pH 7.6), 156 mM NaCl, 10 mM MgCl₂, 2 mM ATP, 15% glycerol, 10 μ g/ml of mini-Mu donor DNA (pSG1; Baker and Luo 1994), and 10 μ g/ml of ϕ X174 DNA. The amounts of protein were as follows: HU, 3 pmoles; MuB, 6.5 pmoles; MuA (or MuA-Bid1–4) 1.33 pmoles. Reactions were incubated at 30°C for 20 min. Intramolecular strand-transfer reactions were performed as above except that MuB, ϕ X174 DNA, and ATP were omitted. Reaction products were analyzed by agarose gel electrophoresis. Southern analysis with ³²P-labeled pSG-1 DNA followed by scanning using a PhosphorImager was performed to estimate the efficiency of intermolecular strand transfer (percent of donor DNA in inter-strand transfer product).

MuA release by ClpX was performed as described (Levchenko et al. 1995). Mu strand-transfer reactions were supplemented by 4 mM ATP, 4 mM creatine phosphate, and 0.1 mg/ml creatine kinase; 1.25–2.5 pmoles of heptameric ClpX protein was added to start the reaction. When release was measured with ³⁵S-labeled MuA, INTRA-STCs were purified from free ³⁵S-labeled MuA by addition of NaCl to 400 mM and passing the reaction mix over a S400HR spin column. After incubation at 30°C for 60 min, portions of the samples were analyzed by gel electrophoresis in the presence of BSA (10 μ g/ml) and heparin (80 μ g/ml) (Levchenko et al. 1995). Efficiency of the disassembly reaction was estimated by Southern analysis with unlabeled protein, or by quantitation of the ³⁵S-labeled MuA in the STC after scanning with a PhosphorImager.

Protein degradation by ClpXP

Degradation of MuA and Arc-MuA fusion proteins were performed as follows. Reaction mixtures (30 μ l) contained 25 mM HEPES-KOH (pH 7.6), 12.5 mM KCl, 5 mM MgCl₂, 0.026% NP-40, 15% glycerol, 4 mM ATP, 4 mM creatine phosphate, and 0.1 mg/ml of creatine kinase. Protein levels were as indicated in the figure legends. After incubation at 30°C for 60 min, samples were analyzed by Western blot, using anti-MuA or anti-Arc polyclonal antibodies and ¹²⁵I-labeled protein A (Amersham). Blots were scanned on PhosphorImager. Degradation of ³⁵S-labeled MuA or Arc-MuA proteins was monitored by autoradiography or by scintillation counting of TCA-soluble material as described (Wojtkowiak et al. 1993).

Acknowledgments

We thank Tracy Smith for help with designing the Arc protein fusions, and Stephen Bell, Robert Sauer, Susan Gottesman, Eric Roche, Arie Berggrun, and the members of the laboratory for comments on the manuscript. Robert Sauer, Susan Gottesman, and Eric Roche are also acknowledged for communicating results before publication. This work was supported by the United States Public Health Service grant GM499224 from the National Institutes of Health and by the Howard Hughes Medical Institute. I.L. and T.A.B. are employees of the Howard Hughes

Medical Institute and T.A.B. is a recipient of a National Science Foundation Young Investigator Award.

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References

- Adzuma, K. and K. Mizuuchi. 1988. Target immunity of Mu transposition reflects a differential distribution of MuB protein. *Cell* **53**: 257–266.
- Alfano, C. and R. McMacken. 1989. Heat shock protein-mediated disassembly of nucleoprotein structures is required for the initiation of bacteriophage λ DNA replication. *J. Biol. Chem.* **264**: 10709–10718.
- Baker, T.A. and L. Luo. 1994. Identification of residues in the Mu transposase essential for catalysis. *Proc. Natl. Acad. Sci.* **91**: 6654–6658.
- Baker, T.A., M. Mizuuchi, and K. Mizuuchi. 1991. MuB protein allosterically activates strand transfer by the transposase of phage Mu. *Cell* **65**: 1003–1013.
- Baker, T.A., M. Mizuuchi, H. Savilahti, and K. Mizuuchi. 1993. Division of labor among monomers within the Mu transposase tetramer. *Cell* **74**: 723–733.
- Baker, T.A., E. Kremmentsova, and L. Luo. 1994. Complete transposition requires four active monomers in the Mu transposase tetramer. *Genes & Dev.* **8**: 2416–2428.
- Chaconas, G., E.B. Giddens, J.L. Miller, and G. Gloor. 1985. A truncated form of the bacteriophage MuB protein promotes conservative integration, but not replicative transposition of Mu DNA. *Cell* **41**: 857–865.
- Chernoff, Y.O., S.L. Lindquist, B.I. Ono, S.G. Inge-Vechtomov, and S.W. Liebman. 1995. Role of the chaperone protein Hsp104 in propagation of the yeast prion-like factor [psi⁺]. *Science* **268**: 880–884.
- Craigie, R. and K. Mizuuchi. 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.
- Echols, H. 1990. Nucleoprotein structures initiating DNA replication, transcription, and site-specific recombination. *J. Biol. Chem.* **265**: 14697–14700.
- Frank, E.G., D.G. Ennis, M. Gonzalez, A.S. Levine, and R. Woodgate. 1996. Regulation of SOS mutagenesis by proteolysis. *Proc. Natl. Acad. Sci.* **93**: 10291–10296.
- Geuskens, V., A. Mhammedi-Alaoui, L. Desmet, and A. Toussaint. 1992. Virulence in bacteriophage Mu: A case of trans-dominant proteolysis by the *E. coli* Clp serine protease. *EMBO J.* **11**: 5121–5127.
- Gottesman, S. and M.R. Maurizi. 1992. Regulation by proteolysis: Energy-dependent proteases and their targets. *Microbiol. Rev.* **56**: 592–621.
- Gottesman, S., W.P. Clark, V. Crecy-Lagard, and M.R. Maurizi. 1993. ClpX, an alternative subunit for ATP-dependent Clp protease of *E. coli*. *J. Biol. Chem.* **268**: 22618–22626.
- Gottesman, S., S. Wickner, Y. Jubete, S.K. Singh, M. Kessel, and M. Maurizi. 1995. Selective, energy-dependent proteolysis. In *Escherichia coli. Cold Spring Harbor Symposia on Quantitative Biology*, Vol. LX, pp. 533–548. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Greenberg, S.M., V.F. Castellucci, H. Bayley, and J.H. Schwartz. 1987. A molecular mechanism for long-term sensitization in *Aplysia*. *Nature* **329**: 62–65.
- Harshey, R.M., E.D. Getzoff, D.L. Baldwin, J.L. Miller, and G. Chaconas. 1985. Primary structure of phage mu transposase: Homology to mu repressor. *Proc. Natl. Acad. Sci.* **82**: 7676–7680.
- Hegde, A.N., A.L. Goldberg, and J.H. Schwartz. 1993. Regulatory subunits of cAMP-dependent protein kinases are degraded after conjugation to ubiquitin: A molecular mechanism underlying long-term synaptic plasticity. *Proc. Natl. Acad. Sci.* **90**: 7436–7440.
- Hwang, B.J., K.M. Woo, A.L. Goldberg, and C.H. Chung. 1988. Protease Ti, a new ATP-dependent protease in *Escherichia coli* contains protein-activated ATPase and proteolytic functions in distinct subunits. *J. Biol. Chem.* **263**: 8727–8734.
- Katayama, Y., S. Gottesman, J. Pumphrey, S. Rudikoff, W.P. Clark, and M.R. Maurizi. 1988. The two ATP-component, ATP-dependent Clp protease of *Escherichia coli*. *J. Biol. Chem.* **263**: 15226–15236.
- Keiler, K.C., P.R.H. Waller, and R.T. Sauer. 1996. Role of a peptide tagging system in degradation of protein synthesized from damaged messenger RNA. *Science* **271**: 990–993.
- Kessel, M., M.R. Maurizi, B. Kim, E. Kocsis, B. Trus, S.K. Singh, and A.C. Steven. 1995. Homology in structural organization between *E. coli* ClpAP protease and the eukaryotic 26 S proteasome. *J. Mol. Biol.* **250**: 587–594.
- Krukltis, R. and H. Nakai. 1994. Participation of the bacteriophage Mu A protein and host factors in the initiation of Mu DNA synthesis in vitro. *J. Biol. Chem.* **269**: 16469–16477.
- Krukltis, R., D.J. Welty, and H. Nakai. 1996. ClpX protein of *Escherichia coli* activates bacteriophage Mu transposase in the strand transfer complex for initiation of Mu DNA synthesis. *EMBO J.* **15**: 935–944.
- Laachouch, J.E., L. Desmet, V. Geuskens, R. Grimaud, and A. Toussaint. 1996. Bacteriophage Mu repressor as a target for the *E. coli* ATP-dependent Clp protease. *EMBO J.* **15**: 437–444.
- Lavoie, B.D., B.S. Chan, R.G. Allison, and G. Chaconas. 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.
- Lehnherr, H. and M.B. Yarmolinsky. 1995. Addiction protein Phd of plasmid prophage P1 is a substrate of ClpXP serine protease of *Escherichia coli*. *Proc. Natl. Acad. Sci.* **92**: 3274–3277.
- Leung, P.C. and R.M. Harshey. 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.
- Levchenko, I., L. Luo, and T.A. Baker. 1995. Disassembly of the Mu transposase tetramer by the ClpX chaperone. *Genes & Dev.* **9**: 2399–2408.
- Maxwell, A., R. Craigie, and K. Mizuuchi. 1987. B protein of bacteriophage Mu is an ATPase that preferentially stimulates intermolecular DNA strand transfer. *Proc. Natl. Acad. Sci.* **84**: 699–703.
- Mhammedi-Alaoui, A., M. Pato, M. Gama, and A. Toussaint. 1994. A new component of bacteriophage Mu replicative transposition machinery: The *Escherichia coli* ClpX protein. *Mol. Microbiol.* **11**: 1109–1116.
- Milla, M.E., B.M. Brown, and R.T. Sauer. 1993. P22 Arc repressor: Enhanced expression of unstable mutants by addition of polar C-terminal sequences. *Protein Sci.* **2**: 2198–2205.
- Missiakas, D., F. Schwager, J.-M. Betton, C. Georgopoulos, and S. Raina. 1996. Identification and characterization of HslV and HslU (ClpQ ClpY) proteins involved in overall proteolysis of misfolded proteins in *Escherichia coli*. *EMBO J.* **15**: 6899–6909.
- Mizuuchi, K. 1983. In vitro transposition of bacteriophage Mu:

- A biochemical approach to a novel replication reaction. *Cell* **35**: 785–794.
- . 1992. Transpositional recombination: mechanistic insights from studies of Mu and other elements. *Annu. Rev. Biochem.* **61**: 1011–1051.
- Mizuuchi, M., T.A. Baker, and K. Mizuuchi. 1992. Assembly of the active form of the transposase-Mu DNA complex: A critical control point in Mu transposition. *Cell* **70**: 303–311.
- Mizuuchi, M., T.A. Baker, and K. Mizuuchi. 1995. Assembly of phage Mu transpososomes: Cooperative transitions assisted by protein and DNA scaffolds. *Cell* **83**: 375–385.
- Muffler, A., D. Fischer, S. Altuvia, G. Storz, and R. Hengge-Aronis. 1996. The response regulator RssB controls stability of the σ S subunit of RNA polymerase in *Escherichia coli*. *EMBO J.* **15**: 1333–1339.
- Nakai, H. and R. Krukltis. 1995. Disassembly of the bacteriophage Mu transposase for the initiation of Mu DNA replication. *J. Biol. Chem.* **270**: 19591–19598.
- Parsell, D.A., Y. Sanchez, J.D. Stitzel, and S. Lindquist. 1991. Hsp104 is a highly conserved protein with two essential nucleotide-binding sites. *Nature* **353**: 270–273.
- Parsell, D.A., A.S. Kowal, M.A. Singer, and S. Lindquist. 1994. Protein disaggregation mediated by heat-shock protein Hsp104. *Nature* **372**: 475–477.
- Pratt, L.A. and T.J. Silhavy. 1996. The response regulator SprE controls the stability of RpoS. *Proc. Natl. Acad. Sci.* **93**: 2488–2492.
- Raumann, B.E., B.M. Brown, and R.T. Sauer. 1994. DNA recognition by β -sheets in the Arc repressor-operator crystal structure. *Nature* **367**: 754–757.
- Resibois, A., M. Pato, P. Higgins, and A. Toussaint. 1984. Replication of bacteriophage Mu and its mini-Mu derivatives. In *Proteins involved in DNA replication* (ed. U. Hubscher and S. Spadari), pp. 69–76. Plenum Press, New York, NY.
- Ross, W., S.H. Shore, and M.M. Howe. 1986. Mutants of *Escherichia coli* defective for replicative transposition of Bacteriophage Mu. *J. Bacteriol.* **167**: 905–919.
- Schirmer, E.C., J.R. Glover, M.A. Singer, and S. Lindquist. 1996. Hsp100/Clp proteins: A common mechanism explains diverse functions. *Trends Biochem. Sci.* **21**: 289–296.
- Schweder, T., K.-H. Lee, O. Lomovskaya, and A. Matin. 1996. Regulation of *Escherichia coli* starvation sigma factor (σ^S) by ClpXP protease. *J. Bacteriol.* **178**: 470–476.
- Stout, V., A. Torres-Cabassa, M.R. Maurizi, D. Gutnick, and S. Gottesman. 1991. RcsA, an unstable positive regulator of capsular polysaccharide synthesis. *J. Bacteriol.* **173**: 1738–1747.
- Surette, M.G. and G. Chaconas. 1991. Stimulation of the Mu DNA strand cleavage and intramolecular strand transfer reactions by the MuB protein is dependent of stable binding of the MuB protein to DNA. *J. Biol. Chem.* **266**: 17306–17313.
- Surette, M.G., S.J. Buch, and G. Chaconas. 1987. Transpososomes: Stable protein-DNA complexes involved in the in vitro transposition of bacteriophage Mu DNA. *Cell* **49**: 253–262.
- Surette, M.G., T. Harkness, and G. Chaconas. 1991. Stimulation of the MuA protein-mediated strand cleavage reaction by the MuB protein, and the requirement of DNA nicking for stable type I transpososome formation. In vitro transposition characteristics of mini-Mu plasmids carrying terminal base pair mutations. *J. Biol. Chem.* **266**: 3118–3124.
- Thompson, M.W. and M. Maurizi. 1994. Activity and specificity of *Escherichia coli* ClpAP protease in cleaving model peptide substrates. *J. Biol. Chem.* **269**: 18201–18208.
- Tobias, J.W., T.E. Shrader, G. Rocap, and A. Varshavsky. 1991. The N-end rule in bacteria. *Science* **254**: 1374–1377.
- Toussaint, A. and M. Faellen. 1974. The dependence of temperate phage Mu-1 upon the replication functions of *E. coli* K12. *Mol. Gen. Genet.* **131**: 209–214.
- Toussaint, A. and A. Resibois. 1983. Phage Mu: Transposition as a life-style. In *Mobile genetic elements* (ed. J. Shapiro), pp. 105–158. Academic Press, New York, NY.
- Tsukiyama, T., C. Daniel, J. Tamkun, and C. Wu. 1995. ISWI, a member of the SWI2/SNF2 ATPase family, encodes the 140 kDa subunit of the nucleosome remodeling factor. *Cell* **83**: 1021–1026.
- Tsukiyama, T. and C. Wu. 1995. Purification and properties of an ATP-dependent nucleosome remodeling factor. *Cell* **83**: 1011–1020.
- Vogel, J.L., D.A. Parsell, and S. Lindquist. 1995. Heat-shock proteins Hsp104 and Hsp70 reactivate mRNA splicing after heat inactivation. *Curr. Biol.* **5**: 306–317.
- Wawrzynow, A., D. Wojtkowiak, J. Marzalek, B. Banekki, M. Jonsen, B. Graves, C. Georgopoulos, and M. Zylicz. 1995. The ClpX heat-shock protein of *Escherichia coli*, the ATP-dependent substrate specificity component of the ClpP-ClpX protease, is a novel molecular chaperone. *EMBO J.* **14**: 1867–1877.
- Wickner, S., S. Gottesman, D. Skowrya, J. Hiskins, K. McKenney, and M.R. Maurizi. 1994. A molecular chaperone, ClpA, functions like DnaK and DnaJ. *Proc. Natl. Acad. Sci.* **91**: 12218–12222.
- Wojtkowiak, D., C. Georgopoulos, and M. Zylicz. 1993. Isolation and characterization of ClpX, a new ATP-dependent specificity component of ClpP protease of *E. coli*. *J. Biol. Chem.* **268**: 22609–22617.
- Wu, Z. and G. Chaconas. 1994. Characterization of a region in phage Mu transposase that is involved in interaction with the MuB protein. *J. Biol. Chem.* **269**: 28829–28833.
- Wu, Z. and G. Chaconas. 1995. A novel DNA binding and nuclease activity in domain III of Mu transposase: Evidence for a catalytic region involved in donor cleavage. *EMBO J.* **14**: 3835–3843.

ClpX and MuB interact with overlapping regions of Mu transposase: implications for control of the transposition pathway.

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Genes Dev. 1997, **11**:

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