A Mechanism for Tn5 Inhibition
CARBOXYL-TERMINAL DIMERIZATION

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Tn5 is unique among prokaryotic transposable elements in that it encodes a special inhibitor protein identical to the Tn5 transposase except lacking a short NH2-termin al DNA binding sequence. This protein regulates transposition through nonproductive protein-protein interactions with transposase. We have studied the mechanism of Tn5 inhibition in vitro and find that a heterodimeric complex between the inhibitor and transposase is critical for inhibition, probably via a DNA-bound form of transposase. Two dimerization domains are known in the inhibitor/transposase shared sequence, and we show that the COOH-terminal domain is necessary for inhibition, correlating with the ability of the inhibitor protein to homodimerize via this domain. This regulatory complex may provide clues to the structure of functional synaptic complexes. Additionally, we find that NH2- and COOH-terminal regions of transposase or inhibitor are in functional contact. The NH2 terminus appears to occlude transposase homodimerization (hypothesically mediated by the COOH terminus), an effect that might contribute to productive transposition. Conversely, a deletion of the COOH terminus uncovers a secondary DNA binding region in the inhibitor protein which is probably located near the NH2 terminus.

Transposable DNA elements occur widely in nature and have important roles in evolution, chromosome mutagensis, and gene transfer. Common to all transposable elements is a need to fix transposition at low levels to avoid excessive mutagenesis of the host genome. Transposition is a multistep process involving nucleoprotein intermediates which is catalyzed by an element-encoded transposase (Tnp). The first step is the specific binding of Tnp to short transposon DNA end sequences that are inverted repeats. Synapsis follows such that Tnp-Tnp interactions bridge the ends. Subsequently, the DNA cutting and joining reactions of transposition occur (for a review, see Ref. 1). In the case of transposon Tn5, Tnp completely releases the element from the donor DNA molecule before inserting it into a target DNA site (2).

Tn5 is a 5.8-kilobase composite transposon found in Gram-negative bacteria which consists of two insertion sequences, IS50R and IS50L (for reviews, see Refs. 3 and 4). The insertion sequences, 1.5 kilobases in length, are very nearly identical and are arranged in inverted orientation flanking a central region containing antibiotic resistance genes. The outermost ends of the Tn5 transposon overall are 19-bp inverted repeats called outside ends (OE). IS50R encodes a 53-kDa Tnp protein, 476 amino acids in length, and a 48-kDa inhibitor protein (Inh), 421 amino acids in length.

Inh is encoded in the same reading frame as Tnp but is transcribed from a separate promoter and is translated from a distinct initiation codon such that Inh lacks the first 55 amino acids of Tnp. Inh, apparently, does not bind to OE DNA, as does Tnp (5), but it can participate in a three-molecule complex with an OE-bound Tnp monomer (5, 6). Whereas Tnp acts in cis (i.e. acts on local DNA sequences relative to its own gene), Inh is a trans-acting factor that functions post-translationally to form nonproductive complexes with Tnp (5). Tnp has also been shown to function as a self-inhibitor in trans (7, 8). Tnp and Inh are folded similarly in terms of gross secondary and tertiary structure (9), and thus the convention for numbering the primary amino acid sequence of Inh is based on the Tnp primary sequence (i.e. the initiating methionine of Inh is referred to as residue 56). Tnp and Inh share two distinct dimerization domains (9, 10), one of which is located at the COOH terminus and the other near amino acids 114–314.

Correlating with the fact that Inh-DNA binding has not been detected, the NH2 terminus of Tnp has been implicated in OE DNA recognition (9–11). A series of NH2-terminal point mutations at residues 41, 47, and 54 in Tnp has been shown to enhance OE DNA binding affinity or specificity (11). Also, OE DNA can protect the first 113 amino acids of Tnp against proteolysis (9). Furthermore, the deletion of a very short part of the NH2 terminus disrupts Tnp-DNA binding (10). Additionally, a predicted helix-turn-helix DNA binding motif is present in the NH2-terminal region at residues 35–54.

Tnp regions constituting a conserved catalytic domain have been predicted and verified within proteolysis-resistant segments spanning amino acids 93–217 (9) and 312–365 (12). Part of the catalytic domain, therefore, overlaps with the more NH2-terminal of the two known dimerization regions. In vitro, Tnp is the only protein necessary for the catalysis of transposition in the presence of divalent metal ions (2).

Many transposition systems are regulated negatively by repression of transcription, regulation of translation, innate Tnp instability or inactivity, or by accessory protein competition for DNA binding; however, transposase inhibition by nonproduc-
tive multimerization may be unique to Tn5, especially in prokaryotic systems. Here, the molecular mechanism of Tn5 inhibition is explored. We demonstrate a critical importance of the COOH terminus of Tnp/Inh for inhibition in vitro. Because the two proteins are nearly identical, the structure of the inactive complex bound to DNA could provide clues to the nature of functional transposition complexes. An alternative hypothesis separates the inhibitory and synaptic functions of the Tnp protein into distinct regions, inhibition at the COOH terminus and synapsis at an unknown location. Furthermore, we find that the NH2- and COOH-terminal regions of either Tnp or Inh are in close contact or proximity, and we discuss possible functional consequences of this structural arrangement.

**EXPERIMENTAL PROCEDURES**

**Purification of Inh, Tnp, and TnpEK54/LP372**—The overexpression and purification of Inh and Tnp in *Escherichia coli* have been described previously (9). TnpEK54/LP372 was purified similarly except Heparin HyperD (BioSepra) was used during the heparin chromatography step. Based on Coomassie-stained SDS-polyacrylamide gels, Inh was >95% pure, Tnp was >90% pure, and TnpEK54/LP372 was >95% pure. The proteins were stored in 0.4 M NaCl TEG (20 mM Tris-HCl, pH 7.9, 1 mM EDTA, 0.1% Triton X-100, 70 °C).

**Purification of His6 Fusion Proteins**—Fusion protein vectors encoding a 41-amino acid leader sequence with a His6 tag and a protein kinase recognition site were constructed for Inh, InhEQ451, InhAD446, InhA430 (a COOH-terminal deletion), and Tnp using tailed EcoRI-containing primers with Pfu polymerase (Stratagene) polymerase chain reactions (9). The template for the InhEQ451 construct was pRZ622 (13). For InhA466, the A466D mutation was introduced as a mismatch in one of the primers. All oligonucleotides were synthesized by Research Genetics. The polymerase chain reaction products were digested with EcoRI and were ligated into the EcoRI site in pET33b (+) (Novagen). The fusion proteins were overexpressed in *E. coli* and were purified as described previously (9). His6-Inh, InhEQ451, and InhAD446 protein samples were >95% pure based on Coomassie-stained SDS-polyacrylamide gels. His6-InhA430 was partially purified. The proteins were stored in 0.4 M NaCl HEG (20 mM HEPES, pH 7.2, 1 mM EDTA, 10% glycerol) at 70 °C.

**In Vitro Inhibition**—The in vitro assay for Tn5 transposition has been described (2); however, we used modified OE with mutations at positions 10, 11, 12, and 15 which are hyperactive (14) in conjunction with a modified P1 DNA substrate used for detection of DNA transfer events. This 4.4-kilobase DNA substrate contains 1.3 kilobases of “transposon” DNA consisting of the kanamycin resistance gene from Tn903 flanked by the modified OE 19-bp ends. A 430-bp OE-containing DNA fragment (10) and 50 μg/ml bovine serum albumin, 0.5 mM MgCl2, 0.1 M TEG, 40 μg/ml RNase A, and 100 μg/ml tRNA, 50 μM NaCl, 34 mM pGRST2 supercoiled plasmid DNA, 0.2 μM TnpEK54/LP372, and various concentrations of Inh, His6-Inh, or His6-InhEQ451, 0.1 mg/ml each, or 1 μl of a partially purified His6-InhA430 preparation (0.1 mg/ml in fusion protein) or mock preparation was incubated in 20-μl reactions containing 0.7 μg/ml of a 32P-labeled, 60-bp, OE-containing DNA fragment (10) and 5 μg/ml Tnp or TnpEK54/LP372 in 10 mM HEPES, pH 7.2, 200 mM NaCl, 100 mM potassium glutamate, 0.5 mM EDTA, 5% glycerol at 30 °C for 30 min (final ratio is 1:1 Inh:Tnp). 4 μl of loading dye was added, and the samples were electrophoresed at 4 °C with 8% native PAGE in 1× TBE electrophoresis buffer. The gels were analyzed by autoradiography.

**RESULTS**

*In Vitro Inhibition*—An in vitro assay for Tn5 transposition has been developed which utilizes two point mutations in Tnp, E54K and L372P, to promote both general and trans activities of the otherwise inactive transposase in vitro (2), and we tested Inh for inhibitory activity in this system. The results of an in vitro transposition assay performed in the presence of various concentrations of Inh are shown in Fig. 1. We show that the addition of increasing amounts of Inh produces increasing inhibition. As the molar ratio of Inh:Tnp approaches 1, transposition, as measured by donor backbone excision, is near zero. Moreover, the inhibitory effect is strong; 50% inhibition is achieved with a molar ratio of approximately 1.3 to 1:4, Inh: Tnp. We know that the inhibition is not the result of nonspecific effects because a mutant Inh protein (InhAD466), added in equal concentrations relative to Inh, was found to have no inhibitory effect (this result is discussed below). If we assume that functional synapsis is required for cleavage and that synapsis is the result of one monomer of Tnp binding to each OE followed by Tnp dimerization, then the inhibition experiment suggests inhibition before synapsis because one molecule

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4 Constructed by M. D. Weinreich.
is capable of inhibiting multiple molecules of Tnp. For example, one Inh monomer should be sufficient to prevent the formation of one synapse if it can heterodimerize with one Tnp monomer that is bound to one transposon end, even if the other end is bound by another Tnp monomer. This hypothesis assumes that the nonproductive complex is relatively strong, and it does not rule out Inh-Tnp interactions prior to Tnp-OE binding which might contribute to inhibition.

**Tnp or Inh Gel Filtration and Chemical Cross-linking**—The potential for homodimerization of Inh or Tnp was addressed by both gel filtration and covalent protein-protein cross-linking to probe the role of multimerization in transposition or inhibition. The results of gel filtration experiments conducted under various solution conditions are presented in Table I. We have found that in the presence of glycerol (with buffer containing 0.1 M NaCl) Inh can homodimerize, as has been shown previously (5). However, in the absence of glycerol (with buffer containing 0.1 M NaCl) Inh is predominantly monomeric. At higher salt concentrations (with buffer containing 0.4 M NaCl and glycerol) Inh homodimerizes, whereas Tnp is predominantly monomeric. Glutaraldehyde cross-linking was used to confirm the homodimerization of Inh. Fig. 2 demonstrates that in the presence of 0.01% glutaraldehyde (with buffer containing 0.4 M NaCl and glycerol) His<sub>6</sub>-Inh cross-links into homodimers, whereas in 0.1% glutaraldehyde, His<sub>6</sub>-Inh cross-links into a mixture of homodimers and higher order multimers (Fig. 2A, lanes 3 and 5). Next, we tested His<sub>6</sub>-Tnp in a glutaraldehyde cross-linking experiment and found that, in contrast to His<sub>6</sub>-Inh, His<sub>6</sub>-Tnp did not cross-link into dimers and remained relatively monomeric while exhibiting some aggregation, as deduced by the appearance of His<sub>6</sub>-Tnp in the wells of the polyacrylamide gel (Fig. 2B). The relative instability of Tnp and its propensity for aggregation have been observed by us and by others and correlates with a tendency toward precipitation at lower salt concentrations. In summary, both the gel filtration

### Table I

**Gel filtration results of Inh or Tnp analyzed under various conditions**

<table>
<thead>
<tr>
<th>Tris-HCl buffer</th>
<th>Major peak</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inh 0.1 M NaCl</td>
<td>52 Monomer</td>
<td></td>
</tr>
<tr>
<td>Inh 0.1 M NaCl, 10% glycerol</td>
<td>109 Dimer</td>
<td></td>
</tr>
<tr>
<td>Inh 0.4 M NaCl, 10% glycerol</td>
<td>110 Dimer</td>
<td></td>
</tr>
<tr>
<td>Tnp 0.4 M NaCl, 10% glycerol</td>
<td>54 Monomer</td>
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**FIG. 1. In vitro inhibition of transposition.** Panel A, transposition reactions were performed using a supercoiled plasmid DNA substrate and TnpEK54/LP372 with added Inh at various molar ratios, Inh:Tnp. After 1 h, the reactions were digested with XhoI to linearize all DNA molecules, and the reactions were analyzed using 1% agarose gel electrophoresis and fluoroimaging of SYBR Green-stained gels, which were quantitated. The full-length linear substrate and excised donor backbone fragment are indicated, although other transposition products are apparent at low molar ratios. Panel B, percent donor backbone DNA, a measure of transposon double-ended cleavage, was calculated, and the results were plotted against the molar ratio, Inh:Tnp, for each lane in panel A. 50% inhibition is taken as 18.5% donor backbone release because the uninhibited reaction reached 37% donor backbone release.

**FIG. 2. Glutaraldehyde protein-protein cross-linking.** Panel A, His<sub>6</sub>-Inh or His<sub>6</sub>-InhEQ451 in 0.4 M NaCl HEPES buffer with 10% glycerol was reacted with 0.01 or 0.1% final concentrations of glutaraldehyde for 5 h at 25 °C. The reactions were analyzed by 12% SDS-PAGE, and the gel was stained with Coomassie Blue. The expected molecular masses of His<sub>6</sub>-Inh or His<sub>6</sub>-InhEQ451 monomers and dimers are 52.0 and 104 kDa, respectively. Panel B, His<sub>6</sub>-Tnp in 0.4 M NaCl HEPES buffer with 10% glycerol was reacted with 0.01% glutaraldehyde for various times, and the reactions were analyzed by 9% SDS-PAGE. The gel was stained with Coomassie Blue. Expected molecular masses are as follows: His<sub>6</sub>-Tnp, 57.8 kDa, monomer, and 115.6 kDa, dimer.
and cross-linking experiments indicate that Inh homodimerizes whereas Tnp is monomeric. Furthermore, the dimerization of Inh is facilitated by glycerol and is relatively insensitive to salt, suggesting that hydrophobic effects may be important at the dimer interface. Finally, because Inh lacks a short part of the Tnp NH2 terminus, we speculate that the NH2-terminal region of Tnp interferes with Inh-like homodimerization.

**GST-Tnp Pull-down Assay with Inh**—Interactions between Inh and Tnp have been reported using a gel shift assay in which Tnp is bound to one molecule of OE DNA (5, 6); here, we have sought any heterodimer interactions that might occur in the absence of DNA. We used a pull-down assay in which GST-Tnp was immobilized onto glutathione beads before incubation with Inh. Then, the beads were washed and analyzed for retained protein. The assay was performed under the same conditions in which Inh is known to dimerize, whereas Tnp is monomeric (0.4 M NaCl with glycerol). Fig. 3A shows an SDS-PAGE analysis of proteins recovered from this assay. We find that Inh is immobilized only in the presence of GST-Tnp, and we conclude that Inh heterodimerizes with Tnp.

Because two modes of heterodimerization have now been observed (free and DNA-bound heterodimers), we repeated the pull-down assays to reveal any differences between dimerization in the presence of OE DNA and dimerization in the absence of DNA. To accomplish this, immobilized GST-Tnp was incubated with or without a 3-fold molar excess of OE DNA before addition of Inh. The beads were washed and analyzed for retained protein. The assay was performed under the same conditions in which OE DNA is known to dimerize, whereas Tnp is monomeric (0.4 M NaCl with glycerol). Fig. 3B shows an SDS-PAGE analysis of proteins recovered from this assay. We find that Inh is immobilized only in the presence of GST-Tnp, and we conclude that Inh heterodimerizes with Tnp.

**OE DNA Gel Shift Assay**—To address whether Inh could heterodimerize with TnpEK54/LP372 (the mutant protein used in the *in vitro* transposition/inhibition assay), a gel mobility shift experiment was performed in which Inh was added to Tnp-DNA binding reactions (the Inh was added last, but the order of addition has no effect on complex formation). In such assays, Tnp exhibits little or no monomeric binding, except in cases in which a COOH-terminal truncation of Tnp is present (6, 8, 10). However, the addition of Inh is known to promote strongly the binding of Tnp into a heterodimeric complex that contains one DNA molecule (5, 6). In Fig. 4, the results of a gel shift assay with radiolabeled OE DNA are presented. The heterodimeric complex bound to one molecule of DNA is stimulated in the presence of the Inh fusion protein (compare lanes 2 and 3, Fig. 4). This experiment confirms that Inh heterodimerizes with TnpEK54/LP372 in a DNA-bound complex, and we suspect that this complex may be important for inhibition. The addition of Inh also appears to increase the complexes remaining in the well. This is a variable result that is thought to be an artifact of Tnp aggregation.

**InhE451Q**—A hypertransposing mutation in Tnp and Inh, E451Q, has been identified previously *in vivo* (13) which, because of its location near one of the known dimerization domains, was predicted to function through a reduction in inhibitory regulation of Tn5 transposition (9). We studied this mutation in the context of His<sub>6</sub>-Inh for dimerization and for inhibitory activity. Gel shift analysis with TnpEK54/LP372 (data not shown) revealed that His<sub>6</sub>-InhE451Q could heterodimerize with Tnp-DNA as efficiently as His<sub>6</sub>-Inh. In addition, cross-linking experiments indicated that His<sub>6</sub>-InhE451Q could homodimerize (Fig. 2A, lanes 4 and 6). Furthermore, His<sub>6</sub>-InhE451Q was found to inhibit TnpEK/LP *in vitro* to the same extent as His<sub>6</sub>-Inh (data not shown). Thus, residue 451 is apparently not involved in either Inh-like homodimerization, Inh-Tnp-DNA heterodimerization or inhibition, as thought previously. However, recent structural studies of Inh may explain why E451Q does not disrupt dimerization. These studies reveal a COOH-terminal dimerization domain in which Glu-451 is not part of the dimerization interface itself but resides near the beginning of a long helix whose end forms the basis of the interface (discussed further below). We hypothesize that the E451Q mutation in Tnp influences transposition during some step other than regulatory inhibition, perhaps during transpo-
son DNA end binding or during the capture of target DNA.

**InhAD466—** We wished to mutagenize the COOH-terminal dimerization domain of Inh at the recently discovered dimer interface to test for functional effects. The dimer interface is formed predominantly by the close interaction of two helices in which the β-carbon of alanine 466 faces the interface, adjacent to the corresponding β-carbon of residue 466 in the dimer partner. Thus, the substitution of aspartic acid for alanine at this position should disrupt the close interaction of the dimer helices by steric occlusion and electrostatic repulsion. We introduced the Ala → Asp mutation at position 466 by site-directed mutagenesis and found the overexpressed His6-InhAD466 protein to be stable, as judged by relative expression levels and the absence of detectable degradation fragments during purification (data not shown). His6-InhAD466 was compared with His6-Inh for inhibitory activity and, as shown in an in vitro transposition assay in Fig. 5, was found to be inactive for inhibition at all protein concentrations tested. We note that the His6-Inh protein was slightly less active for inhibition than Inh itself (this may reflect NH2-terminal fusion and COOH-terminal domain interactions; the importance of the COOH terminus for inhibition and evidence for the close proximity of NH2 and COOH termini is discussed below). Furthermore, His6-InhAD466 did not homodimerize when tested in a cross-linking experiment (Fig. 6), nor did it heterodimerize in gel shift assays with DNA-bound Tnp (data not shown). His6-InhAD466 was found to be inactive for inhibitory activity and, as shown in an in vitro transposition assay in Fig. 5, was found to be inactive for inhibition at all protein concentrations tested. We note that the His6-Inh protein was slightly less active for inhibition than Inh itself (this may reflect NH2-terminal fusion and COOH-terminal domain interactions; the importance of the COOH terminus for inhibition and evidence for the close proximity of NH2 and COOH termini is discussed below). Furthermore, His6-InhAD466 did not homodimerize when tested in a cross-linking experiment (Fig. 6), nor did it heterodimerize in gel shift assays with DNA-bound Tnp (data not shown) or TnpEK54/LP372 (Fig. 4, lane 4), in contrast to His6-Inh. We conclude that the A466D mutation disrupts the dimer interface at the COOH terminus of the protein and that this interface is critical for inhibition.

**InhAD430—** To study further the effects of mutating the COOH terminus of Inh, a truncation was introduced which deletes all residues COOH-terminal to residue 430. His6-InhAD430 was found to have relatively low expression levels in E. coli compared with His6-Inh. We tested partially purified preparations in DNA binding assays (in the absence of Tnp). Because Inh has not been previously reported to bind to DNA, we were surprised to find that His6-InhAD430 could bind to OE DNA (Fig. 7). Mock preparations did not show OE DNA binding activity (data not shown), nor did His6-Inh (Fig. 7). The shifted complex consists of a bound His6-InhAD430 monomer, as judged by comparison with the monomeric binding of a previously characterized protein, TnpA387 (6, 10) (data not shown). Thus, the COOH-terminal deletion in Inh has apparently exposed a previously unknown DNA binding region.

**DISCUSSION**

**Inh Inhibits Synapsis—** Here we find that purified Inh strongly inhibits Tn5 transposition in vitro such that one molecule of Inh apparently inhibits multiple molecules of TnpEK54/LP372. This effect suggests that inhibition occurs prior to synapsis because the present model of transposition requires two or more functional Tnp molecules for every synapse, and therefore the nonproductive complexation of one Tnp molecule at one transposon end would block synapsis and effectively
inactivate a second Tnp molecule at the other end. Corresponding to our in vitro data, Inh has been demonstrated to inhibit TnpEKLP in vivo whereas InhAD466 (see below) does not.  

**DNA-bound Heterodimerization Is Likely the Predominant Inhibitory Mechanism**—Inh and Tnp heterodimers were detected both free in solution and within a DNA-bound complex, raising the possibility that either of these heterodimeric complexes may be important for inhibition. Because the DNA-bound heterodimer is more abundant than the free heterodimer in immobilization assays we suspect that the DNA-bound complex is highly stable. Correspondingly, we confirmed that Inh heterodimerizes with TnpEK54/LP372 in a gel shift experiment. Although the shift experiment and our in vitro inhibition experiments were performed under different conditions using different OE DNA substrates (short OE-containing fragments and mutated OE-containing DNA supercoiled circles, respectively), we are able to correlate DNA-bound heterodimerization with inhibition. Further evidence that the DNA-bound heterodimer is more stable than the free heterodimer has been found in gel shift experiments in which the DNA-bound heterodimer product was neither reduced nor enhanced by premixing Tnp and Inh. Therefore, it appears that monomers of Tnp bind to DNA, that heterodimerization occurs after DNA binding, and that free heterodimerization has only minimal effects on DNA-bound heterodimer complex formation. In summary, we suspect that DNA-bound heterodimers may be the major mediators of inhibition.

In support of the hypothesis described above, we find that monomers of Tnp are abundant. In gel filtration studies, Tnp was found to be predominantly monomeric, whereas Inh could homodimerize. In addition, no Tnp-Tnp dimers were detected in cross-linking studies although Inh-Inh dimers were found. As discussed above, monomer-DNA binding is probably prerequisite to dimer complex formation with Inh and could also be important for synapsis, even though monomeric, untruncated Tnp apparently binds slowly or with low affinity to OE DNA.

**The COOH-terminal Domain of Inh/Tnp Is Critical for Inhibition**—Mutation of the recently discovered dimerization interface in Inh at the COOH terminus by site-directed mutagenesis abolishes not only in vitro inhibition but also homodimerization and OE-bound Tnp-Inh heterodimerization. We conclude that this domain represents the critical dimerization main for inhibition. The point mutation, A660D, is not predicted to expose unfavorable side chains to solvent upon disruption of the dimer interface, and we correspondingly found that the mutant protein was stable during overexpression and purification. Evidence that the COOH-terminal region can be disrupted without disturbing other functional regions in Inh is also found in our studies of InhAD430 because this protein can bind to DNA. A second COOH-terminal mutated protein, InhEQ451, was found to be active for inhibition and could homodimerize and also heterodimerize with OE-bound Tnp. Although residue 451 is located near the COOH-terminal dimerization domain, this result confirms that the critical dimer interface is located at the end of the long COOH-terminal helices of the dimer. In summary, Inh COOH-terminal homodimerization reflects the mechanism of Tn5 inhibition, and this mechanism likely occurs through Inh heterodimerization with Tnp, probably via DNA-bound complexes.

**The NH2 and COOH Terminals of Tnp and of Inh Are in Close Proximity**—The COOH-terminal regulatory dimerization exerted by Inh is a functional consequence of the deficiency of the first 55 amino acids of Tnp, perhaps uncovering the COOH-terminal domain and making it available for dimerization. This hypothesis explains the monomeric nature of Tnp in contrast to Inh and suggests that the NH2- and COOH-terminal regions of Tnp are in close proximity or close contact. In much the same manner, dominant-negative mutants of the maize **Activator** transposase have been found by mutating the NH2-terminal DNA binding region of the protein; these mutants act by nonproductive oligomerization with functional transposase (16). There could be a purpose for the interaction of NH2 and COOH termini in Tn5 Tnp and, perhaps, other transposases, at least in bacterial cells; usually the NH2 terminus is associated with DNA binding, and because it is produced first during translation it might bind to DNA before the inhibitory COOH terminus can be produced. Perhaps such self-regulation contributes to the cis phenotype of transposases in prokaryotic cells, including that of Tn5, where transcription and translation are coupled, and thus the nascent protein is physically close to transposon end sequences. In fact, the COOH terminus of Tn5 Tnp does appear to inhibit DNA binding activity, as discussed below.

Interactions between NH2- and COOH-terminal regions probably also occur in Inh, even though Inh is missing a short part of the corresponding Tnp NH2 terminus. When we removed 46 amino acids from the Inh COOH terminus we uncovered a secondary DNA binding region that we suspect may be located near the NH2 terminus of the protein. In fact, structural studies show that the NH2- and COOH-terminal regions are spatially close in a three-dimensional structure of Inh, and nearby is a disordered region containing a predicted helix-turn-helix, 107–124 amino acids. We can also expect the NH2 terminus of Tnp to be situated near this region, and it contains a second predicted helix-turn-helix DNA binding region, amino acids 35–54. If this arrangement exists, it may resemble the dual helix-turn-helix DNA binding motifs that have been identified in protein-DNA structural studies of the Tc3 transposase (17) and the MuA transposase (18).

Just as a COOH-terminal deletion uncovered a DNA binding function in Inh, such deletions have also been found to stabilize monomeric Tnp binding to OE DNA (5, 7, 9), presumably via the known NH2-terminal DNA binding domain. Thus, the COOH terminus of Tnp partially interferes with DNA binding. Tnp-DNA binding is also stabilized by heterodimerization with Inh (5), which could be caused by removal of a hinderance of the COOH terminus via COOH-terminal heterodimerization. A somewhat similar situation is found in the *Drosophila* transposable P element system in which a KP repressor, an internal deletion of transposase, binds DNA more readily than the transposase and utilizes dimerization for high affinity binding (19).

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