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# Transpososome Dynamics and Regulation in Tn10 Transposition

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**ABSTRACT** Tn10 is a bacterial transposon that transposes through a non-replicative mechanism. This mode of DNA transposition is widely used in bacteria and is also used by "DNA-based" transposons in eukaryotes. Tn10 has served as a paradigm for this mode of transposition and continues to provide novel insights into how steps in transposition reactions occur and how these steps are regulated. A common feature of transposition reactions is that they require the formation of a higher order protein-DNA complex called a transpososome. A major objective in the last few years has been to better understand the dynamics of transpososome assembly and progression through the course of transposition reactions. This problem is particularly interesting in the Tn10 system because two important host proteins, IHF and H-NS, have been implicated in regulating transpososome assembly and/or function. Interestingly, H-NS is an integral part of stress response pathways in bacteria, and its function is known to be sensitive to changes in environmental conditions. Consequently, H-NS may provide a means of allowing Tn10 to responed to changing environmental conditions. The current review focuses on the roles of both IHF and H-NS on Tn10 transposition.

**KEYWORDS** Transposon, IHF, H-NS, Transposase, IS10



## TRANSPOSOSOME FORMATION IN DNA TRANSPOSITION REACTIONS

Transposons are mobile DNA elements that are highly abundant in most organisms. Transposons mediate a wide range of DNA rearrangements, which can be harmful to their hosts, and consequently host organisms have developed a range of defenses that limit the frequency of transposition events. DNA methylation and RNA interference are two systems that may have evolved to reduce the expression of transposon-encoded proteins that are necessary for transposition events (Yoder *et al.*, 1997; Plasterk, 2002). More generally, limiting the expression of transposon-encoded proteins represents a major mechanism for down-regulating DNA transposition. However, regulation of DNA transposition also occurs after transposition proteins are synthesized. The formation of the transpososome, a higher-order protein-DNA complex in which transposon-encoded proteins bind to and synapse

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the two ends of the transposon, represents another key control point in transposition reactions. Evidence from several bacterial systems, including bacteriophage Mu, Tn5, and Tn7, now suggests that transpososome assembly is required to assemble active site components (Aldaz *et al.*, 1996; Savilahti and Mizuuchi, 1996; Watson and Chaconas, 1996; Yang *et al.*, 1996; Davies *et al.*, 2000; Skelding *et al.*, 2002). Thus, it is generally thought that transpososome assembly is a key prerequisite for the chemical steps in transposition reactions.

Importantly, host proteins can contribute significantly to transpososome assembly, and this represents another level at which the host can influence the transposition frequency (Nagy and Chandler, 2004). Tn10 is one such system where a host protein, in this case integration host factor (IHF), has been shown to play an important role in transpososome assembly (Sakai *et al.*, 1995). IHF and the functionally related protein HU also play important roles in the assembly of the Mu transpososome (Craigie and Mizuuchi, 1987; Surette *et al.*, 1987; Surette *et al.*, 1989). More recently work in the Tn10 system has revealed that host factors, including IHF and H-NS (histone-like nucleoid structuring protein), also contribute to target site selection (Chalmers *et al.*, 1998; Wardle *et al.*, 2005). IHF promotes intramolecular transposition events (where the transposon inserts into itself), whereas H-NS promotes intermolecular transposition (where the transposon inserts into a target site that is separate from the transposon). This represents a new way in which host proteins can regulate transposition because intramolecular events are self-destructive and therefore reduce the transposition frequency. Preventing intramolecular transposition events can be particularly important for the continued survival of transposons that are already subject to strong negative regulation at the level of transposase expression, such as Tn10 and Tn5 (reviewed in Kleckner, 1989; and Berg, 1989). The major focus of the current review will be on the mechanisms by which host factors regulate Tn10 transposition subsequent to the expression of the transposase protein.

## BASIC FEATURES OF THE TN10/IS10 SYSTEM

Tn10 is a composite bacterial transposon that encodes resistance to the antibiotic tetracycline (Figure 1). The ends of Tn10 are inverted repeats of the insertion sequence IS10. IS10-Right encodes a functional transposase protein, whereas IS10-Left is a degenerate copy of IS10-Right and does not encode a transposase protein (Foster *et al.*, 1981). The two termini of IS10-Right and IS10-Left are referred to as the outside and inside ends (OE and IE, respectively), as defined by their positions relative to the tetracycline resistance determinants of Tn10. The OE and IE contains the primary specificity determinants for transposase binding, which is present within the 23 base pairs (bp) (nearly perfect) terminal inverted repeats (TIR) (Way and Kleckner 1984). However, an important feature that distinguishes OE from IE is that only the OE includes a binding site for the host protein IHF.

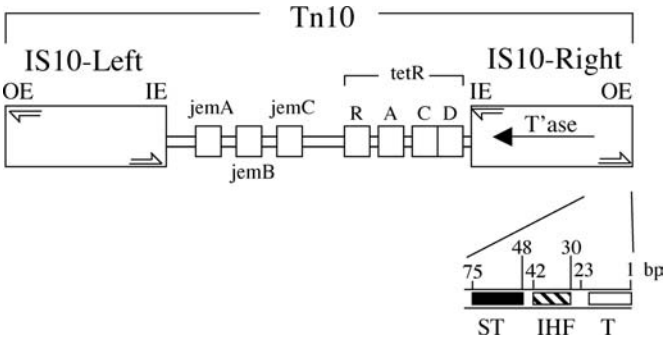


**FIGURE 1** **Structure of Tn10. Tn10 is 9147 basepairs in length. IS10-Right encodes a transposase protein and the non-IS10 portion includes seven open reading frames, four of which are determinants for tetracycline resistance, and three of which (JemA through JemC) have no defined function. Half arrows indicate 23 bp terminal inverted repeats (TIR) in the outside and inside ends (OE and IE, respectively). The specificity determinants for transposase binding are within the TIR. Transposase contacts the OE in the terminal (T—open rectangle) and subterminal (ST—*black rectangle*) regions. The IHF binding site (*striped rectangle*) is also shown. The first basepair of IS10-Right is defined as basepair 1 of the transposon.**

Tn10 and IS10 transpose by a non-replicative mechanism (Bender and Kleckner 1986; Haniford *et al.*, 1991)–hereafter Tn10 will be used unless an event/phenomenon specific to IS10 is being discussed. Tn10 is first excised from a donor molecule and then the excised transposon is inserted into a new site (Figure 2). The Tn10 transposase protein mediates the pairing of transposon ends prior to the initiation of the chemical steps in transposition (Sakai *et al.*, 1995). This ensures that the two ends are positioned to integrate into a common target site, a requirement for a successful transposition event. As will be described below, many of the new insights into the mechanism and regulation of Tn10 transposition are centered around the conformational changes that the transpososome goes through during the course of the transposition reaction.

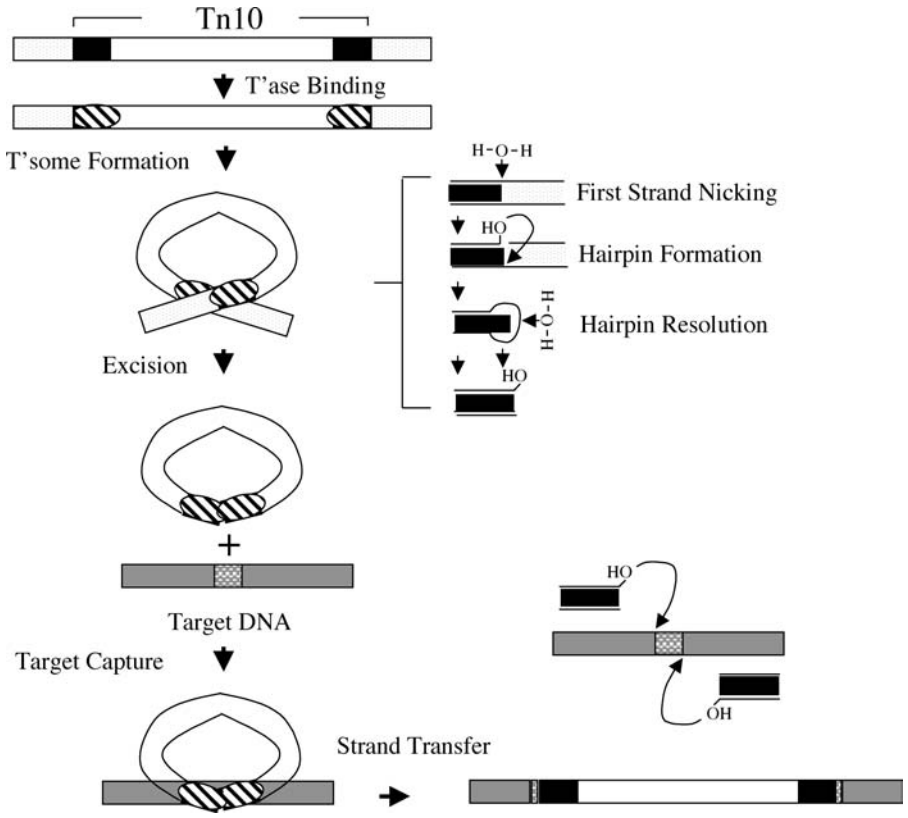


**FIGURE 2** **Mechanism of Tn10 transposition. Tn10 is shown imbedded within donor DNA. Transposase (*striped oval*) is shown bound to OE sequences (*black rectangles*). After mediating transpososome formation, transposase catalyzes the chemical steps in excision. Once the flanking donor DNA is removed from both transposon ends the transpososome binds a target DNA (*gray rectangle*) and catalyzes the chemical steps in the strand transfer reaction. Host repair functions then fill in the 9 bp gaps created by the strand transfer reaction, leading to the duplication of the original short target site (*checkered rectangle*).**

Once the transpososome is formed, and a suitable divalent metal cation is present (either $Mg^{2+}$ or $Mn^{2+}$), the chemical steps in Tn10 transposition occur. Transposon excision involves three chemical steps at each transposon end, first strand nicking, hairpin formation and hairpin resolution (Kennedy *et al.*, 1998). First strand nicking and hairpin formation result in a double strand break precisely at the transposon-donor junction, thereby cleanly separating the transposon from the flanking donor sequences. Hairpin resolution is necessary to re-expose a 3′ OH at each transposon terminus, as the second phase of the reaction involves the transfer, by direct transesterification (Kennedy *et al.*, 2000), of the 3′ OH termini to phosphate groups separated by 9 bp, and on opposite strands of a target site (Halling and Kleckner, 1982). This latter phase is called strand transfer.

Target site selection in Tn10 transposition is highly DNA sequence specific (Halling and Kleckner, 1982). A functionally significant interaction between the transpososome and a target DNA requires prior separation of the transposon from flanking DNA at both ends. The fully excised transpososome initially binds weakly to a target DNA and then, upon encountering a target DNA with a preferred DNA sequence, a more stable target capture complex is formed (Junop and Haniford, 1997; Sakai and Kleckner, 1997). Part of what constitutes an optimal sequence for Tn10 insertion is that the target site has to be able to accommodate DNA bends precisely at the sites of strand transfer chemistry. Target DNA bending permits the formation of a more extensive interface between the target site and the transpososome and in addition is likely important for activating the scissile phosphates for the strand transfer chemistry (Pribil and Haniford, 2000; Pribil and Haniford, 2003; Pribil *et al.*, 2004). Importantly, Tn10 does not exhibit transposition immunity, a phenomenon wherein the presence of a transposon within a DNA molecule prevents further transposition events close to, or within, the existing copy of the transposon. Consequently, Tn10 is able to insert into itself, as well as other DNA molecules (Morisato and Kleckner, 1987;

Benjamin and Kleckner, 1989). The decision to transpose *via* the inter- *versus* intramolecular pathway represents a major regulatory point in Tn10 transposition and host proteins including IHF and H-NS appear to influence the distribution of these events (Signon and Kleckner, 1995; Chalmers *et al.*, 1998; Wardle *et al.*, 2005).

There are two general classes of intramolecular Tn10 transposition products. An inversion circle (IC) is formed when inter-strand strand transfer events take place, whereas when intra-strand strand transfer takes place, a pair of deletion circles (DC) are formed (see Figure 1 of Benjamin and Kleckner, 1989). Both types of products can be either topologically simple or complex (Figure 3) (Chalmers *et al.*, 1998). If DNA supercoils, which are retained in the excision complex, are trapped between the transposon ends and the insertion site, topologically complex products are formed. In the case of the IC, these trapped supercoils produce knots (and thus a knotted IC, or KIC for short). In the case of DCs these trapped supercoils produce interlinks between the DCs. If the transposon ends are constrained in a way that forces insertion close to an end, the chance of trapping a supercoil in the strand transfer product is low and an unkotted IC (UKIC) or unlinked DCs are produced. Thus, the distribution of topologically complex and simple intramolecular transposition products provides information regarding the structure of the fully excised transpososome. A mechanism for constraining the transposon ends within the transpososome in a manner that favors the production of the UKIC is discussed below.

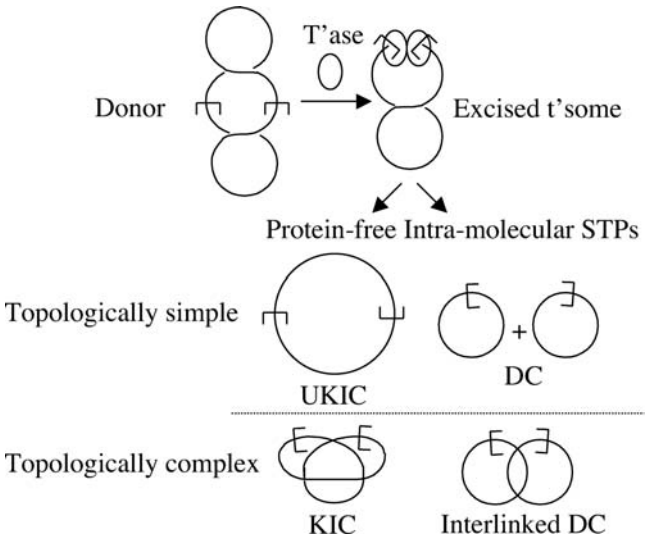


**FIGURE 3** **Intramolecular transposition products. Brackets represent transposon ends and other short forms are defined in the text.**

## TRANSPOSOSOME STRUCTURE AND DYNAMICS—EARLY DEVELOPMENTS

### Utilization of Short OE Fragments to Form Tn10 Transpososomes

Tn10 transpososomes were initially identified in the lysates of cells over-producing transposase in the presence of a Tn10 derivative contained on a multicopy plasmid. Both excision and intramolecular strand transfer complexes were characterized. The former was shown to have retained DNA supercoils. The supercoils were released upon treatment with a protein denaturant, indicating that the two transposon ends are held tightly together through protein-DNA (and presumably protein-protein) interactions without being covalently linked (Haniford *et al.*, 1991). However, an important limitation to studying Tn10 transpososomes generated from plasmid substrates is that it is difficult to distinguish the starting donor DNA from pre-cleavage complexes. Much of what we now know of the structure and dynamics of Tn10 transpososomes has been revealed through the use of an experimental system wherein the ends of the transposon are present on short, linear DNA fragments (Figure 4). In this system the difference in mobility of the initial donor DNA and the transpososome is relatively large, facilitating the separation of these molecules by native gel electrophoresis. In addition, it is possible to separate different forms of the transpososome by this technique (Sakai *et al.*, 1995, 2000).

One notable difference between plasmid and fragment reactions is that IHF is required in the fragment reaction where there is no negative supercoiling of the substrate DNA. In contrast, in the plasmid reaction, IHF is not essential as long as the substrate DNA is negatively supercoiled (Sakai *et al.*, 1995; Chalmers *et al.*, 1998). Since IHF introduces a 180° bend into DNA upon binding (Rice *et al.*, 1996), it is likely that the requirement for DNA supercoiling in the plasmid reaction, in the absence of IHF, is due to DNA bending induced by supercoiling. Thus, studying the dynamics of transpososome formation and the progression of transpososomes through the reaction in the absence of DNA supercoiling does not appear to be a significant limitation.

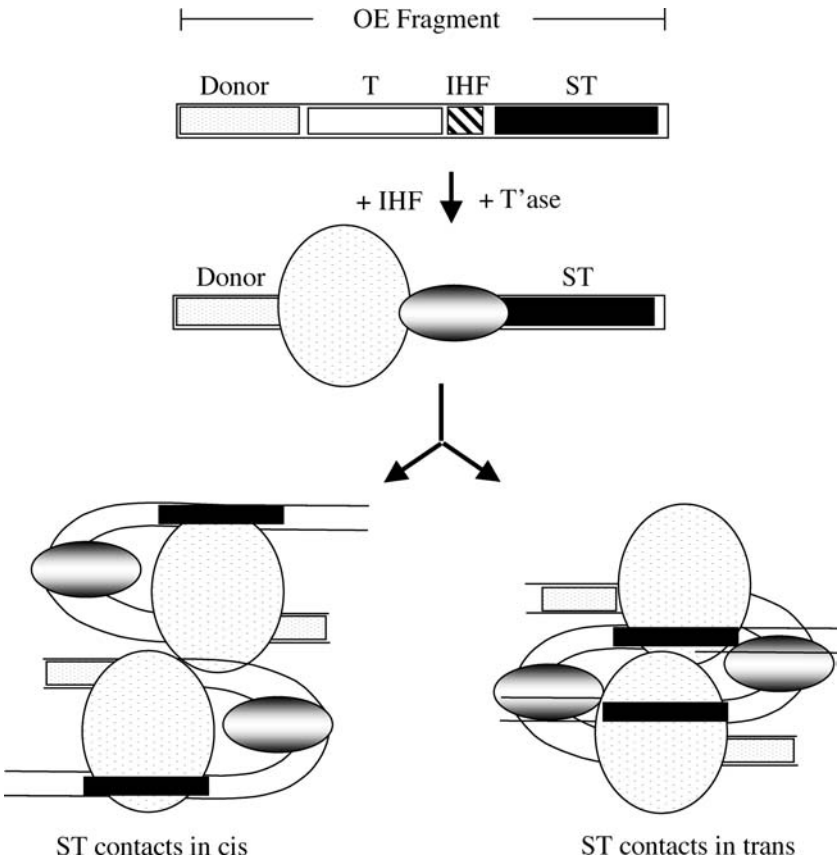


**FIGURE 4** **Models for IHF-directed transpososome formation. Transpososomes can be formed by mixing an OE fragment, transposase and IHF. It is anticipated that end pairing results from the random collision of a pair of "single end" complexes, however, the single end complexes do not appear to be stable entities—they either fall apart or are rapidly converted to transpososomes. Two transposon end complexes are shown coming together in two different orientations. In one orientation (defined as cis) the transposase bound to the terminal binding site of one OE also contacts the subterminal binding site of the same OE. In the trans arrangement the transposase bound to the terminal binding site of one OE makes subterminal contacts with the other OE. Note that the transpososome are drawn as symmetric structures in these diagrams.**



## Initial Transpososome Assembly and Impact of IHF on Transpososome Structure and the Choice Between Intra- and Intermolecular Transposition Pathways

The earliest stages in transpososome assembly have not been directly defined largely because the first intermediate detected is the fully formed transpososome. A transposase-single end complex is either too unstable to be detected or is very quickly converted to a transpososome (Figure 4) (Sakai *et al.*, 1995). The available evidence from protein-protein cross-linking studies suggests that transposase forms dimers, but only in the context of the transpososome (Kennedy, 1999). It is therefore likely that transposase associates with each transposon end as a monomer and that dimerization occurs coincidently with the pairing of two ends. It is not currently known if the ends are brought together in a parallel or anti-parallel arrangement. Tn5 is closely related to Tn10 in several ways and thus the available information on transpososome assembly in this system may be relevant to the Tn10 system. In the Tn5 transpososome the transposon ends are held together in an anti-parallel arrangement and each of the two transposase monomers contacts both ends. In fact, most of the contacts stabilizing the transpososome are derived from protein-DNA interactions, as there is only a very limited interface between the transposase monomers (Davies *et al.*, 2000). As in the Tn10 system, no monomeric transposase-end complex is detected in the presence of full-length transposase, implying that only a subset of the total protein-DNA contacts form upon the initial encounter of the transposase and the end sequence (York and Reznikoff, 1996). Furthermore, dimerization of the transposase does not occur with the full-length protein in the absence of the end sequence (Braam *et al.*, 1999). Both of the above

processes are actually inhibited by intramolecular interactions between N- and C-terminal domains (Braam *et al.*, 1999; Davies *et al.*, 2000). Thus, transpososome formation is thought to proceed via a monomer of transposase binding weakly to each transposon end. This initial interaction would then trigger a conformational change in the transposase that permits dimerization to occur, followed by multiple protein-DNA interactions in trans, with the latter providing the bulk of the stabilizing interactions for the transpososome (Davies *et al.*, 2000).

While the initial formation of the Tn10 transpososome in the linear fragment assay shows an absolute dependence on IHF, once formed, IHF can be removed from the transpososome by addition of competitor DNA or heparin (a polyanion) without causing the transpososome to fall apart. Importantly, the mobility of the resulting transpososome decreases significantly on a native polyacrylamide gel. This observation may seem counter-intuitive, given that the mass of the transpososome decreases with the loss of IHF. However, the decrease in mobility can be explained by the over-riding effect of IHF removal being on the conformation of the DNA rather than the overall mass of the complex. Dissociation of IHF from the transpososome is expected to remove a 180° bend in the OE that forms when IHF binds, and for this reason it has been inferred that the reduced mobility of the transpososome lacking IHF is due to the conversion of the transpososome from a folded to an unfolded form (Figure 5) (Sakai *et al.*, 2000). In support of this idea it has been shown in DNA footprinting experiments that, only when IHF is present, and the transpososome adopts the higher mobility form on a native gel, does transposase makes additional contacts with OE DNA. These contacts, which are non-specific in nature, span a region from approximately bp 48 to 75 and are referred to as subterminal contacts. Together, the IHF induced bend and the resultant formation of subterminal contacts, is expected to generate a microloop in the OE DNA with one anchor point being the transposase-OE contacts in the TIR and another being transposase-OE contacts in the subterminal region (Figures 4 and 5) (Sakai *et al.*, 2000; Crellin and Chalmers, 2001; Sewitz *et al.*, 2003). Operationally, the additional stability provided by the formation of subterminal contacts is analogous to the stabilizing effect of the trans contacts formed in the Tn5 system (see above) (Davies *et al.*, 2000). In fact, the possibility does exist that in the Tn10 system the subterminal contacts could be provided in trans (see below and Figure 4).

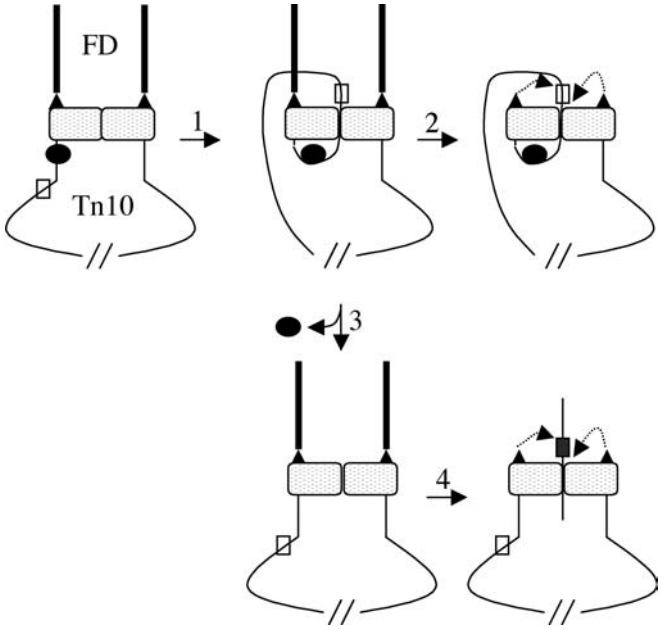


**FIGURE 5** **Model for how IHF-directed transpososome folding prevents target interactions via random collision. Transposase (*stippled oval*) and IHF (*black oval*) are shown bound to the OE of Tn10, producing an unstable form of the transpososome (FD is flanking donor DNA). IHF-mediated bending of the OE DNA stabilizes the transpososome by allowing the formation of additional transposase-DNA contacts (1). The aforementioned bend results in the close alignment of a segment of the bent end (*unfilled box*) and the two OE termini (*arrow heads*). This conformation either persists through the excision reaction (2) or re-forms upon IHF binding to the fully cleaved excision intermediate and results in a high frequency of transposition events into the OE (*i.e.*, an intramolecular transposition event). If the transposon end unfolds due to the release of IHF (3), either before or after excision, then the distal segment of the OE (*unfilled box*) is no longer in position to act as a target site. Consequently, a random collision mode of target interactions (4) is now favored over the highly constrained mode, and as such more intermolecular transposition events occur (shown as insertions into the *gray box*), as well as intramolecular events that generate topologically complex products (not shown).**

It is presumed that OE folding (induced by IHF binding or by negative supercoiling) is important for transpososome assembly because the formation of additional transposase-OE contacts helps to stabilize transposase binding to the OE. If this inference is correct, then the fact that the unfolded transpososome is stable, even though the subterminal contacts have been lost, implies that the process of unfolding the OE alters the contacts between transposase and the OE substrate in a manner that either increases the strength of the transposase-OE substrate interaction or the strength of transposase-transposase interactions. Consistent with the former possibility, DNA footprinting experiments

have revealed some substantial differences in the reactivity of backbone phosphodiester bond linkages to chemical nucleases in the terminal inverted repeat and the flanking donor DNA regions of the fully folded and unfolded transpososomes (Crellin and Chalmers, 2001; Crellin *et al.*, 2004).

One issue that has not been addressed is whether the subterminal contacts in the folded transpososome are made in cis or trans–the two possibilities are illustrated in Figure 4–cis refers to the situation where one transposase monomer contacts terminal and subterminal binding sites located on the same end, whereas trans refers to the situation where a transposase monomer binds the terminal binding site of one end and the subterminal binding site of the other end. It is worth considering the trans arrangement because this would provide a relatively straightforward explanation as to why transposon arm folding would favor transpososome formation, as opposed to stabilizing a single end complex.

While IHF-mediated folding of the OE has positive effects on transpososome formation, persistence of the folded state actually interferes with intermolecular strand transfer events (Figure 5). An early indication of this came from the finding that it was necessary to unfold the transpososome with heparin treatment in order to get efficient capture of a DNA fragment containing a hotspot for Tn10 insertion (Junop and Haniford, 1997). It was also shown in plasmid reactions that addition of IHF qualitatively changed the distribution of strand transfer products formed. In the absence of IHF, or the presence of low concentrations of IHF, intermolecular and topologically complex intramolecular transposition products (*i.e.*, KIC and catenated DCs) predominated over topologically simple intramolecular transposition products (*i.e.*, UKIC and unlinked DC). In contrast, at higher IHF levels, UKIC was the predominant strand transfer product. This led to the idea that IHF-induced microloop formation in the OE inhibits the binding of a target DNA through a random collision pathway. Furthermore, the finding that the predominant product is the UKIC implies that the position of the OEs is tightly constrained in the folded transpososome so that insertion into sites close to a transposon end are favored (Chalmers *et al.*, 1998; Sakai *et al.*, 2000)–this would account for the fact that the transposition product observed is topologically simple, as only insertion close to an OE would avoid the trapping of supercoils in the transposition product. This phenomenon is referred to as IHF channeling.

## Transposase and IHF Contacts Within the Transpososome and $Me^{2+}$ Induced Transpososome Unfolding

DNA footprinting has been a very important tool in the characterization of Tn10 transpososomes and the conformational changes these transpososomes undergo. The most detailed protein-DNA contact maps for Tn10 transpososomes have been generated by hydroxyl radical and 1,10-phenanthroline-copper (OP-Cu) footprinting (collectively referred to here as chemical nucleases). In the folded transpososome, transposase makes contacts on one face of the DNA spanning approximately 3 turns of the helix (Figure 6). These contacts extend from roughly basepair 19 of the terminal inverted repeat to basepair -8 of the flanking donor DNA (Allingham *et al.*, 2001; Crellin and Chalmers, 2001).

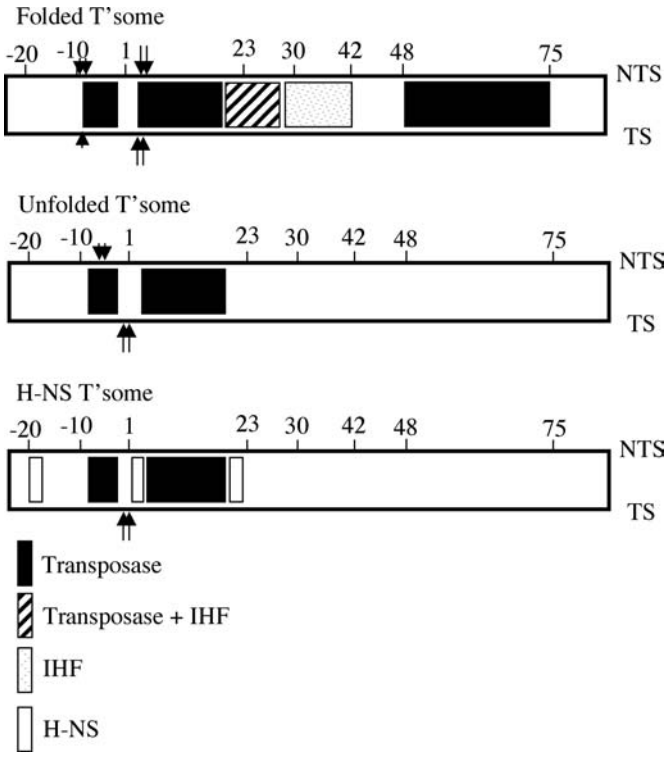


**FIGURE 6** **Summary of chemical nuclease footprinting data. Regions within an OE fragment of different transpososomes showing either reduced (*rectangles*) or enhanced (arrows) chemical nuclease cleavage are indicated. Note that strand specific information is presented only for sites of chemical nuclease hypersensitivity and the size of the arrows defining hypersensitive sites defines the relative degree of hypersensitivity. NTS and TS indicate nontransferred and transferred strands, respectively.**

Interestingly, residues spanning the transposon-donor junction (basepairs 2 to –3) are not protected, although OP-Cu footprinting revealed strong hyper-reactivity to this reagent on both strands in the region spanning basepairs 2 to 6 (Allingham *et al.*, 2001). This latter result indicates that the DNA in the aforementioned region adopts a distorted structure. In the region spanning basepairs 28 to 43, IHF makes contacts on the opposite face of the DNA, relative to transposase, over two consecutive turns of the helix–this region include the consensus site for IHF binding (Crellin and Chalmers, 2001). Both transposase and IHF contribute to protection in the region spanning the IHF and transposase binding sites. The convergence of the IHF and transposase protection patterns raises the possibility that the two proteins may be able to interact within the context of the transpososome and preliminary results from protein-protein crosslinking studies support this possibility (S. Wardle and D. Haniford, unpublished data). Interestingly, the OEs of Tn5 also include overlapping transposase-host factor binding sites, in this case for DnaA (Yin and Reznikoff, 1987). However, here host factor and transposase binding are competitive in nature and it has been suggested that DnaA acts as a positive regulator of Tn5 transposition by helping to disengage the transposase from the final transposition product so as to facilitate gap repair (Reznikoff, 2002).

The addition of a $Ca^{2+}$ to the folded Tn10 transpososome was shown to result in a rather remarkable set of changes in chemical nuclease footprinting patterns. In hydroxyl radical experiments residues +1 and –1 on the 'transferred strand' (*i.e.*, the strand that is joined by transposase to the target DNA) exhibited hypersensitivity to cleavage. In addition, the zone of contact in the flanking donor DNA was reduced (Crellin and Chalmers, 2001). In OP-Cu experiments there was a shift in the residues hypersensitive to cleavage; hypersensitivity at residues 2–6 was dampened and, as in the case of hydroxyl radical footprinting, residues +1 and −1 of the transferred strand became hypersensitive (Allingham *et al.*, 2001). As is discussed in more detail below, the appearance of chemical nuclease hypersensitivity at residues +1 and −1 correlates with unfolding of the transpososome, and the distortion in DNA structure that is responsible for this hypersensitivity has been proposed to facilitate one or more of the chemical steps in the Tn10 excision reaction.

# TRANSPOSOSOME STRUCTURE AND DYNAMICS—RECENT DEVELOPMENTS

## Asymmetry Within the Tn10 Transpososome

While there are two IHF binding sites in the Tn10 transpososome, one contributed by each OE, it appears that transpososome formation requires that IHF be bound to only one end. This inference was initially made based on the observation that an IS10 transpososome, consisting of an OE and an IE (that does not bind IHF), can readily be assembled in the presence of IHF. The dependence of transpososome formation on IHF binding to only one transposon end indicates that transposase binding to a transposon end is not absolutely dependent on that end also binding IHF. Furthermore, this observation raised the possibility that the Tn10 transpososome might not be a symmetric structure. In support of this idea, conditions have been defined where IHF is titrated off one but not both OEs. The difference in binding affinity of IHF for the two OE in the Tn10 transpososome was further underscored by the finding that a concentration of heparin sufficient to strip IHF off of the residual IHF-OE complex present in assembly reactions, was not sufficient to efficiently convert the folded transpososome to the fully unfolded form (Sewitz *et al.*, 2003). Partial unfolding of the transpososome by (for example) heparin treatment is thought to reflect a situation wherein IHF is in equilibrium with the IHF binding site of only one of the two OEs within the transpososome. The OE to which IHF is more tightly bound is referred to as the $\alpha$ arm of transpososome, while the OE to which IHF is less tightly bound is referred to as the $\beta$ arm (Sewitz *et al.*, 2003; Crellin *et al.*, 2004).

## Unlocking the $\alpha$ Arm of the Transpososome

While both $Ca^{2+}$ and heparin addition are required to detect the fully unfolded transpososome, $Ca^{2+}$ addition on its own has a significant impact on the structure of the transpososome. This was manifested mainly as the appearance of chemical nuclease hypersensitivity at the +1 and −1 positions of the transferred strand upon treatment of the initial transpososome with $Ca^{2+}$

(Figure 6). Given that $Ca^{2+}$ is required to get full transpososome unfolding, the possibility was considered that unlocking of the $\alpha$ arm and the +1/−1 hypersensitivity might be linked. Support for this has come from the observation that the +1/−1 hypersensitivity is fully reversible under conditions where $Ca^{2+}$ is titrated out of the transpososome, but IHF is allowed to rebind. Furthermore, evidence for a direct dependence of the +1/−1 hypersensitivity on transpososome unfolding, as opposed to $Ca^{2+}$ binding per se, has been provided by showing that an OE predisposed to unfolding because of a truncated subterminal binding site, exhibits the +1/−1 hypersensitivity in the absence of $Ca^{2+}$ (Crellin *et al.*, 2004).

Evidence that $Me^{2+}$ exerts its affects on the transposon-donor structure through binding to the active site has been provided. When two of the three key $Me^{2+}$ binding residues in the active site (the DDE motif) were mutated to alanine, $Ca^{2+}$ treatment did not produce the +1/−1 chemical nuclease hypersensitivity. It follows that if the +1/−1 hypersensitivity is produced by unlocking of the $\alpha$ arm, which in turn is initiated by $Me^{2+}$ binding to the active site, the $\alpha$ arm of a DDE mutant should be locked in the folded form. However, this was not observed as a transpososome formed with the DDE mutant unfolded more easily than a transpososome formed with WT transposase (Crellin *et al.*, 2004). A possible explanation for this result is that the loss of negative charge in the active site associated with the D to A substitutions actually mimics the $Me^{2+}$ bound state of the active site, where these charges would normally be neutralized by $Me^{2+}$ binding. However, if this were the case, then it is unclear why +1/−1 hypersensitivity was not observed for the DDE mutant, especially considering the finding that $\alpha$ arm unfolding was capable of inducing +1/−1 hypersensitivity in the absence of $Me^{2+}$ (Crellin *et al.*, 2004). A similar result to that observed with the DDE mutant was observed in reactions with WT transposase and a substrate with a terminal basepair mutation. Transpososome unfolding occurred but no +1/−1 hypersensitivity was observed (Allingham and Haniford, 2002). Thus, it may be that different perturbations in the active site are capable of uncoupling $\alpha$ arm unfolding and the conformational change in the transposon-donor junction that this unfolding is thought to direct. Alternatively, these results could be taken as evidence that the above events are not actually linked.

## A Possible Link Between Transpososome Unfolding and Excision

It is particularly interesting that $Ca^{2+}$ addition causes a deformation in the DNA structure of the transpososome specifically at the site of reaction chemistry (*i.e.*, the transposon donor junction). Since this deformation has been linked with $\alpha$ arm unfolding (although see paragraph above), it has been proposed that this unfolding might regulate one or more of the chemical steps in the transposon excision reaction. Two lines of evidence support this possibility.

First, in reactions performed with an OE substrate containing a truncated subterminal binding site, only one transposon end was cleaved. Characterization of the resulting transpososome revealed the OE linked to the donor DNA had not undergone first strand nicking. In addition, the OE that was separated from the donor DNA was in a hairpin form. It was concluded that the inability to form a properly locked $\alpha$ arm (because of the truncated subterminal region), and therefore to complete at least one cycle of $\alpha$ arm folding and unfolding, is responsible for the defect in excision. Also, the finding that one end was a hairpin and the other contained no strand breaks led to the proposal that hairpin resolution must occur at one end before first strand nicking can occur at the second end (Crellin *et al.*, 2004).

The second line of evidence that transpososome unfolding may be linked to transposon excision came from the analysis of a transposase mutant that is partially defective in transpososome unfolding. An R to A substitution at residue 182 resulted in a transposase that promotes efficient transpososome assembly, however, treatment of the R182A transpososome with both heparin and $Ca^{2+}$ did not efficiently convert this transpososome to the fully unfolded form. Furthermore, upon addition of $Mg^{2+}$, complete separation of transposon from flanking donor DNA was very inefficient. However, in this case biochemical analysis of the $Mg^{2+}$ treated transpososome revealed that both OEs had a single strand nick, but no hairpin. This suggests that the inability of the transpososome to unfold, blocks hairpin formation and not hairpin resolution. Furthermore, the finding that both transposon ends efficiently underwent first strand nicking goes against the idea that it is necessary to complete all of the steps in excision at one end

before first strand nicking at the other end can occur (Humayun *et al.*, 2005).

While the results of the two approaches described above are both in agreement with a coupling between $\alpha$ arm unfolding and excision, it is unclear why different results, with regard to the execution point of the excision defect, were observed. In the case of the R182A mutant, it could be argued that the amino acid substitution causes additional defects, in which case it may just be coincidental that a single substitution affects both transpososome unfolding and hairpin formation. While this possibility cannot be ruled out, R182A was shown to promote efficient excision in a reaction with a plasmid substrate containing two inside ends (Humayun *et al.*, 2005). Thus, the R182A mutation does not confer an intrinsic defect to hairpin formation (at least with inside ends). In the case of the reaction with WT transposase and an OE substrate with a truncated subterminal binding region, it may be that improper assembly of the initial transpososome causes defects partway along the excision pathway. The reason for thinking that transpososome assembly is not "normal" in this case is that subterminal contacts are expected to play a key role in the assembly process and the substrate used does not support a full set of these contacts. It is also worth noting that hairpin formation seems a more likely step than hairpin resoluton to require a significant input of energy because only the former requires major alterations in DNA structure (Kennedy *et al.*, 2000).

It has been proposed that energy released from $\alpha$ arm unfolding is transduced to the transposon-donor junction to license one or more steps in excision. While a theoretical model for this energy transduction has been presented, this model has not been rigorously tested. A key aspect of the model is that the DNA microloop in the $\alpha$ arm is under torsional strain and that it is the release of this strain that provides a source of mechanical energy to drive excision through to completion (Chalmers *et al.*, 1998). Notably, there has been no direct demonstration that the $\alpha$ arm is under torsional strain, although some indirect evidence supporting this possibility has been presented. This has come from the analysis of the transposase mutant R119H. R119H was originally identified in a genetic screen for dominant negative transposase mutants. This mutant was shown to form a transpososome at normal levels, however, upon addition of $Ca^{2+}$ or $Mg^{2+}$, the transpososome dissociated and, in the case of $Mg^{2+}$, no cleaved species were detected. However, if steps in excision were bypassed by using a pre-cleaved OE substrate, the above $Me^{2+}$ sensitivity was not observed and the R119H transpososome was able to efficiently carry out strand transfer (Kennedy and Haniford, 1996).

More recently, it has been shown that the $Me^{2+}$ sensitivity of the R119H mutant can also be suppressed by using an OE fragment lacking most of the subterminal transposase binding site. Suppression of the R119H defect through bypassing excision, and the presumed interference with a normal cycle of $\alpha$ arm folding and unfolding, have led to the suggestion that it is $\alpha$ arm unfolding that actually causes dissociation of the R119H transpososome. The reason R119H is thought to be sensitive to this step is that the mutation either weakens transposase-OE interactions or the dimer interface so that dissipation of mechanical energy through the transpososome would disrupt an already destabilized transpososome (Crellin *et al.*, 2004). While the above experiments with the R119H mutant are extremely interesting, it is somewhat premature to take these results as strong support for the idea that unfolding of the $\alpha$ arm provides a source of mechanical energy that drives transposon excision to completion. First, it has not been directly demonstrated that the R119H mutation weakens either transposase-OE interactions or the dimer interface. Second, analysis of R119H in plasmid reactions revealed that this mutant could undergo full excision (and strand transfer) when $Mn^{2+}$ was used in place of $Mg^{2+}$ (Kennedy and Haniford 1996). It is unclear why this simple $Me^{2+}$ substitution would be sufficient to prevent transpososome dissociation by the above mechanism.

There are some interesting parallels between Tn10 transposition and V(D)J recombination. Both systems generate a double strand break at their respective recombination sites *via* a hairpin mechanism (McBlane *et al.*, 1995)–Tn5 and Hermes also use a hairpin mechanism for transposon excision (Bhasin *et al.*, 1999; Zhou *et al.*, 2004). V(D)J recombination is facilitated by the binding of HMG (DNA bending) proteins to the recombination sites. HMG binding is thought to promote the pairing of recombination sites and activate the cleavage step (van Gent *et al.*, 1997; Hiom and Gellert, 1998; Kwon *et al.*, 1998). In addition, a significant proportion of RAG-mediated DNA transposition events observed in vitro are intramolecular events, giving rise to UKICs (Agrawal *et al.*, 1998). It is tempting to speculate that release of HMG proteins from the synaptic complex helps

to drive the cleavage reaction in V(D)J recombination to completion and/or influences target site selection in RAG-mediated transposition events.

## Transpososomes Representing Different Excision Intermediates Unfold at Different Rates

The ability of pre-assembled transposition intermediates to undergo the transpososome unfolding transition has been assessed (Liu *et al.*, 2005). A transpososome assembled with an un-cleaved end and a pre-cleaved end was shown to unfold significantly faster than transpososomes made with only un-cleaved ends or only pre-cleaved ends. Furthermore, the faster unfolding rate was only observed when the preformed "single end break complex" was generated by mixing a precleaved OE and an IE with flanking donor DNA. In this arrangement the $\alpha$ arm can only form on the side of the transpososome lacking flanking donor DNA because the IE doesn't bind IHF. This observation suggests that the flanking donor DNA plays an important role in allowing the IHF to dissociate from the $\alpha$ arm of the transpososome. Also, with regard to the idea that transpososome unfolding is coupled to hairpin resolution, it is interesting that the single end break complex would be the easiest transpososome to unfold. If earlier intermediates are resistant to unfolding, even though $Me^{2+}$ is bound to at least one active site, then this would provide a means of coupling the unfolding event directly with the chemical step at which the energy input is required.

One expectation of the above result is that for IS10 transposition to occur efficiently, there should be a bias in which end undergoes the first steps in the excision reaction. That is, cleavage at the OE should be favored over cleavage at the IE, as only this order of events would produce a single end break intermediate that readily unfolds, thereby licensing cleavage at the other end. In support of this idea it was shown that close to 80% of single end cleavage intermediate present in an IS10 cleavage reaction underwent cleavage at the OE. Importantly, a significant amount of fully cleaved intermediate produced in this experiment adopted a folded form (Liu *et al.*, 2005). This indicates that IHF readily re-binds the fully cleaved transpososome, an occurrence that will influence the type of strand transfer products that are formed.

## H-NS Binds Directly to the Transpososome and Promotes Tn10 Transposition by Inducing Transpososome Unfolding

H-NS (histone-like nucleoid structuring protein) is a member of a group of proteins found associated with the *Escherichia coli* nucleoid. Disruption of the H-NS gene in *E. coli* was shown to strongly inhibit Tn10 transposition, suggesting that H-NS is a positive regulator of Tn10 transposition (Swingle *et al.*, 2004). H-NS was previously shown to act as a positive regulator of IS1 transposition by enhancing the translation of the IS1 transposase protein, and thus there is precedent for H-NS up-regulating DNA transposition by increasing the amount of transposase protein made (Rouquette *et al.*, 2004). On the other hand, IHF and FIS are examples of other nucleoid-associated proteins that influence a range of recombination reactions by binding directly to recombination intermediates (Craig and Nash, 1984; Bruist *et al.*, 1987). It is likely that H-NS acts in this latter fashion to promote Tn10 transposition as purified H-NS was shown to bind directly to Tn10 transpososomes. This binding interaction was shown to be highly specific, as concentrations of H-NS sufficient to fully shift the transpososome in the gel mobility shift assay, did not shift either the unbound OE DNA or the IHF-OE complex. Importantly, binding of H-NS to the transpososome was dependent on the OE substrate containing greater than 15 bp of flanking donor DNA, implying that H-NS binds to the transpososome through an interaction with the flanking donor DNA. However, H-NS was also shown to bind a transpososome lacking the flanking donor DNA under conditions where the transpososome was first partially unfolded by heparin treatment. This indicates that at least one additional binding site for H-NS is present within the OE sequence (or core) of the transpososome (Wardle *et al.*, 2005).

Importantly, When H-NS was added to an otherwise complete transposition reaction an increase (at least fivefold) in the level of intermolecular strand transfer was observed. This indicates that the binding of H-NS to the transpososome actually does have a positive impact on the transposition reaction *in vitro*. Interestingly, the increase in intermolecular strand transfer observed upon addition of H-NS did not coincide with either an increase in the efficiency of transpososome assembly or a change in the kinetics of the excision reaction. It

therefore appears that H-NS promotes Tn10 transposition *in vitro* by acting at a step after transposon excision (Wardle *et al.*, 2005).

H-NS binding to the transpososome appears to promote transpososome unfolding and maintain the transpososome in the unfolded state. Such actions would be consistent with the above inference that H-NS promotes transposition by acting subsequent to excision, as intermolecular strand transfer should be promoted by an activity that prevents refolding of the full excision complex (Chalmers *et al.*, 1998). Some of the evidence supporting a role for H-NS in transpososome unfolding includes: (1) Addition of H-NS to the folded transpososome, in the absence of heparin or CaCl2 treatment, resulted in the appearance of chemical nuclease hypersensitivity precisely at residues +1 and −1 of the transferred strand (Wardle *et al.*, 2005). As noted previously the appearance of this pattern of hypersensitivity correlates with unlocking of the $\alpha$ arm of the transpososome and therefore with full transpososome unfolding (Crellin *et al.*, 2004). (2) When equivalent amounts of H-NS were added either to the initial transpososome or a transpososome that was unfolded by CaCl2 and heparin treatment, the extent of the transpososome mobility shifts were equivalent (Wardle *et al.*, 2005). Because the conformation of the transposon arms is the major determinant of transpososome mobility (Sakai *et al.*, 2000), the co-migration of the two H-NS complexes can best be explained by the two treatments generating structurally equivalent complexes (*i.e.*, H-NS bound to an unfolded transpososome). (3) H-NS has also been shown to facilitate unfolding of the R182A transpososome (S. Wardle and D. Haniford, unpublished data).

## Possible Mechanisms for Recruitment of H-NS to the Transpososome and Induction of Transpososome Unfolding

Understanding how H-NS unfolds the Tn10 transpososome requires knowledge of where in the transpososome H-NS binds and the temporal order of the H-NS binding events. As noted above, initiating transpososome unfolding is dependent on the presence of greater than 15 basepairs of flanking donor DNA and accordingly, addition of H-NS to the folded transpososome, generated a region of chemical nuclease protection spanning bp −15 to −21 of the flanking donor (Figure 6). In addition, the region of protection in the TIR caused by transposase binding was extended by about 2 to 3 bp both towards the transposon-donor junction and the IHF site, in the presence of H-NS. This extra protection provides preliminary evidence for at least two additional H-NS binding sites in the core portion of the transpososome (Wardle *et al.*, 2005). Since H-NS does not bind to the core of the transpososome in the absence of flanking donor, but does bind the core upon heparin treatment, it can be inferred that core binding is dependent on transpososome unfolding. Notably, in order to influence the mode of strand transfer used, it is important that H-NS bind to the transpososome core because the flanking donor DNA is removed from the transposon ends prior to target binding (Figure 2). (Sakai and Kleckner, 1997). Thus, it would appear that H-NS binding to the transpososome core is important in maintaining cleaved forms of the transpososome in the unfolded state. This would be consistent with the observation that the fully excised transpososome readily adopts a folded conformation (Liu *et al.*, 2005).

How does H-NS binding induce transpososome unfolding and maintain the fully cleaved transpososome in the unfolded state? With regard to the latter question, it is interesting that the putative H-NS binding site on the 'IHF side' of the transpososome core includes a portion of the OE where transposase and IHF footprints merge in the folded transpososome. Thus, while there is no clear evidence for H-NS binding directly to the IHF binding site, H-NS appears to bind very close to the IHF site and could therefore alter the structure of this site in a way that reduces the affinity of this site for IHF. This may be a comparable situation to that observed in the transcriptional regulation of the *rrnB* gene where Fis and H-NS binding to closely situated sites in the *rrnB* promoter act antagonistically to influence transcription (Afflerbach *et al.*, 1998). Alternatively, or in addition, H-NS binding immediately adjacent to transposase (on one or both sides) might alter the conformation of transposase, or the TIR portion of the OE, in a way that interferes with IHF binding.

With regard to how H-NS initiates transpososome unfolding, it is interesting that two independent studies have identified an important role for the flanking donor DNA in transpososome unfolding. As noted above, the interaction of H-NS with the flanking donor DNA is critical for H-NS-induced transpososome unfolding (Wardle *et al.*, 2005), and studies with pre-formed

transposition intermediates identified the flanking donor DNA on the $\beta$ arm of the single end break complex as being the key determinant in making this transpososome the easiest to unfold (Liu *et al.*, 2005). A possibility to consider is that the flanking donor DNA of the $\beta$ arm is actually in close proximity to the tightly folded $\alpha$ arm (Figure 4 and 5). H-NS bound to the $\beta$ arm of the flanking donor DNA might interact directly with either IHF or transposase in the $\alpha$ arm and one of these interactions could facilitate the release of IHF from the $\alpha$ arm. Interestingly, preliminary data from protein-protein crosslinking experiments is consistent with H-NS binding to transposase under conditions where IHF has been released from the transpososome (S.Wardle and D. Haniford, unpublished data).

The requirement for H-NS binding to the flanking donor DNA and the core sites of the transpososome are clearly different, implying that there are at least two distinct modes of H-NS binding in the transpososome. In principle, the high selectivity H-NS has for binding the transpososome could result from H-NS recognition of specific DNA structures in the transpososome and/or from H-NS binding to transposase (and possibly IHF). H-NS is known to bind preferentially to curved DNA (Yamada *et al.*, 1991) and H-NS has been shown to interact with other proteins (Dorman, 2004). With regard to the structure-specific binding potential of H-NS, it is interesting that in the folded transpososome there is evidence that transposase binding in the flanking donor DNA deforms the DNA structure in the −10 region (Crellin and Chalmers, 2001). Given that the H-NS binding site is immediately adjacent to this region, it is likely that an altered DNA structure, possibly a bend, induced by transpososome formation, creates an H-NS binding site. While distortions in DNA structure within the transpososome core could also account for H-NS binding in core regions, the overlap of H-NS and transposase footprints in the inverted repeat also raises the possibility that H-NS binding in the core is at least partly mediated by H-NS interactions with transposase. An interestingly possibility to consider is that transpososome unfolding alters the structure of transposase in a way that facilitates H-NS-transposase interactions within the core.

One puzzling observation regarding the ability of H-NS to induce transpososome unfolding in the Tn10 system is that addition of H-NS did not have an obvious positive effect on either the efficiency or kinetics of the excision reaction (Wardle *et al.*, 2005). This could be taken as evidence that either steps in excision are not dependent on transpososome unfolding, or that under the standard reaction conditions used, $Mg^{2+}$ binding to the transpososome is sufficient to induce transpososome unfolding. If the latter is true, then it is likely that the enhancement in intermolecular strand transfer observed in H-NS containing reactions is due entirely to the stabilization of the unfolded form of excision intermediates. It will be interesting to see if, under suboptimal conditions for excision, such as reduced $Me^{2+}$ concentrations (Junop and Haniford, 1996), H-NS is needed to drive the excision reaction through to completion.

## Impact of H-NS on Tn10 Transposition *in vivo*

Is there evidence that H-NS acts as a positive regulator of Tn10 transposition *in vivo* by inhibiting the intramolecular transposition pathway? This has been studied by comparing the transposition product distributions of cells not expressing H-NS and cells expressing either WT or mutant forms of H-NS. In these experiments full excision and intramolecular transposition products were measured. However, for technical reasons, the total number of transposition events, and the number of excision events that failed to go on to strand transfer, were not measured and thus a complete picture of the fate of Tn10 in the different H-NS backgrounds was not obtained. Nevertheless, as described below, some interesting differences in transposition product distributions were observed in these experiments.

Comparison of the product distributions in hns+ versus hns- cells reveals that hns+ cells produce more UKIC than hns- cells (Swingle *et al.*, 2004). This actually goes against the idea that H-NS helps to unfold the transpososome and maintain the fully excised transpososome in the unfolded form. However, higher levels of the excision intermediate were observed in the hns- relative to hns+ cells and therefore the higher level of UKIC in hns+ may simply reflect more efficient conversion of excision to strand transfer products in hns+. The fact that UKIC was formed at all in hns+ might reflect there being insufficient H-NS available to interact with all of the transpososomes formed in this strain.

It is also intriguing that the absence of H-NS results in the accumulation of the excision product. This could reflect a more general role for H-NS in target binding than has previously been appreciated. In this regard it is notable that deletion of the *hns* gene has significant

affects on target site selection in IS903 (Swingle *et al.*, 2004). Alternatively, H-NS might help stabilize the excision intermediate. It is known that, *in vitro*, conversion of the folded form of the fully cleaved transpososome to the unfolded form results in significant destabilization of the transpososome (Junop and Haniford, 1997).

In another experiment *in vivo* product distributions were compared for isogenic strains encoding either WT H-NS or a mutant form of H-NS where cysteine was substituted for arginine at position 12 (*i.e.*, R12C) (Wardle *et al.*, 2005). Notably, the R12 position in H-NS is thought to be important in mediating H-NS interactions with other proteins (Ueguchi *et al.*, 1996; Bloch *et al.*, 2003; Garcia *et al.*, 2006). The R12C mutation resulted in a dramatic change in the Tn10 transposition product distribution, as a significant decrease in UKIC, and a significant increase in KIC and DC, was observed. The increase in KIC and DC provides an indication that the degree to which the conformation of the transposon ends within the transpososome is constrained is significantly relaxed in the H-NS R12C strain. Thus, this experiment provides evidence, albeit indirect, that H-NS influences the structure of the transpososome *in vivo*. Given the evidence that H-NS is in very close proximity to transposase in the transpososome, and the R12 position is important for H-NS interactions with other proteins, it is reasonable to predict that an altered interaction between H-NS and transposase is responsible for the change in the strand transfer product distribution. However, it should be recognized that H-NS is itself an important regulator of gene expression (see below), and therefore there is the concern that any differences in product distribution observed may be due to indirect affects. It will therefore be important to demonstrate that H-NS is actually bound to transpososomes *in vivo*. Given that a methodology for partially purifying transpososomes generated *in vivo* has been developed (Haniford *et al.*, 1991), this objective should be feasible.

## Stress Response, H-NS and Tn10 Transposition

According to the current model, H-NS promotes Tn10 transposition by acting in opposition to IHF to promote transpososome unfolding and/or to maintain the fully cleaved transpososome in the unfolded state. These functions could be particularly important in ensuring the long-term survival of Tn10 in bacterial populations because the overall level of Tn10 and IS10 transposition is quite low (Morisato *et al.*, 1983; Shen *et al.*, 1987), primarily due to a very low level of transposase expression (Raleigh and Kleckner 1986). Thus, at times when a transposition event is initiated it would make sense, with regard to the long-term survival of the transposon, to ensure that the net outcome is an intermolecular, as opposed to a self-destructive, intramolecular transposition event (Figure 7). Furthermore, H-NS is a highly expressed protein in gram-negative bacteria (Dorman, 2004) and therefore it is quite likely that H-NS would be able to bind Tn10 transpososomes and influence the mode of transposition. The relationship between Tn10 and H-NS could also favor the host cell

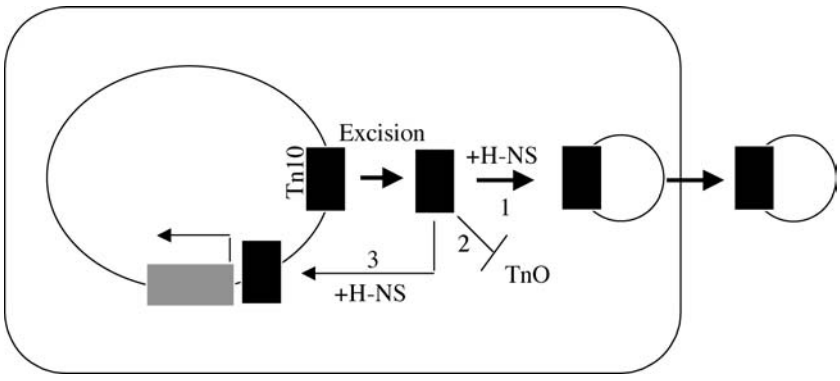


**FIGURE 7** **Model for how H-NS binding to the transpososome can benefit both the transposon and the host. Different outcomes for a Tn10 transposition event from the bacterial chromosome are shown. If H-NS is bound to the excised transpososome (pathway 1 and 3), then an intermolecular transposition event is favored. Alternatively, if H-NS is not bound to the excised transpososome (pathway 2), an intramolecular transposition event (TnO) is favored. In pathway 1, insertion into a plasmid leads to transfer of Tn10 out of the cell, thereby increasing the chance of Tn10 disseminating throughout a population of bacteria. In pathway 3, insertion back into the chromosome is shown turning on the expression of a chromosomal gene (*gray rectangle*). An environmental stress that stimulates either H-NS function or expression would increase the probability of pathways 1 and 3 being used. In pathway 3 the transposition event would benefit the host if the gene being turned-on helps the cell adapt to the environmental stress.**

as genomic rearrangements induced by transposable elements can help the host adapt in suboptimal growth conditions (Reynolds *et al.*, 1981; Schnetz and Rak, 1992; Manna *et al.*, 2001).

H-NS is an important regulator of gene expression. It has been estimated that approximately 5% of *E. coli* genes are regulated by H-NS, and many of these genes play important roles in stress response pathways and virulence (Dorman, 2004). Interestingly, the expression and activity of H-NS are both influenced by environmental factors. For example, H-NS expression is stimulated by cold shock (La Teana *et al.*, 1991) and elevated hydrostatic pressure (Welch *et al.*, 1993) and by entry into stationary phase. (Ali Azam *et al.*, 1999). With regard to the modulation of H-NS function, it is known that the oligomeric state of H-NS, an important factor in H-NS function (Stella *et al.*, 2005), is also affected by temperature and osmolarity (Stella *et al.*, 2006). In addition, the interaction of H-NS with other proteins can alter the function of H-NS, improving its ability to function under suboptimal conditions. For example, H-NS interacts with the Hha protein and the resulting complex represses the *hly* operon at low temperature (Madrid *et al.*, 2002). Thus, it is reasonable to predict that, through H-NS, Tn10 transposition can be coupled to host cell physiology. In fact, some evidence for this already exists, as Tn10 transposition is much more dependent on H-NS when cells are subjected to gradual nutrient starvation relative to growth in exponential phase (Swingle *et al.*, 2004). Interestingly, the expression of IHF increases about 10-fold when cells enter stationary phase (Ditto *et al.*, 1994). Since H-NS and IHF appear to act in opposition, with regard to determining the type of Tn10 transposition product formed (*i.e.*, inter-*versus* intramolecular), the greater dependence of Tn10 transposition on H-NS under conditions of nutritional stress could be linked to the higher concentration of IHF in stationary phase cells. It will be interesting to see if others stresses known to affect H-NS function and/or IHF levels, also affect the transposition frequency of Tn10. Notably, it has been shown that IS903 transposition, which shows a conditional dependence on H-NS for transposition, is modulated by a diverse set of host factors and is stimulated by nutritional stress (Coros *et al.*, 2005; Twiss *et al.*, 2005). Other transposons that are mobilized in response to metabolic stress include IS1, IS5, bacteriophage Mu (Reynolds *et al.*, 1981; Schnetz and Rak, 1992; Manna *et al.*, 2001) and Tn4562 (Ilves *et al.*, 2001).

Is H-NS a component of a signal transduction pathway that links external stimuli and DNA transposition? A definitive answer to this question is not yet available. The sensitivity of H-NS function to environmental factors may be directly related to the impact these factors have on DNA structure. Correlations between H-NS function and the imposition of a stress, such as low temperature, have been linked to alterations in the conformational flexibility of DNA (Madrid *et al.*, 2002). Thus, if H-NS does promote intermolecular Tn10 transposition events under stress conditions, there is the potential for this affect to be mediated entirely through increased binding of H-NS to the transpososome. That is, an environmental factor that facilitates the formation of an appropriate DNA structure in the transpososome might permit higher affinity H-NS binding to that transpososome. Alternatively, additional components might be involved in a more complex pathway. For example, it has been demonstrated that Tn4652 transposition is regulated in response to metabolic stress through a two-component phospho-relay system (Horak *et al.*, 2004). Interestingly, Tn4652 transposition is regulated by IHF. It has been shown that Tn4652 transposition is not observed when a gene encoding one of the two IHF subunits is knocked out. Furthermore, *in vitro* studies have shown that IHF binds to the ends of Tn4652 and that this binding is necessary for transposase binding (Ilves *et al.*, 2004). Thus, in at least one respect, IHF performs the analogous function in both Tn10 and Tn4652 transposition. While it is currently not known what component of the Tn4652 transposition machinery is the target of the phospho-relay system, it will be interesting to see if phospho-relay systems are involved in regulating other transposition reactions and if nucleoid-associated proteins might be targets in these signaling pathways. While currently there is no evidence that H-NS is phosphorylated, it is interesting that H-NS undergoes post-translational modification by short chain poly-(R)-3-hydroxybutyrate, a modification expected to alter the binding properties of H-NS (Reusch *et al.*, 2002).

## SUMMARY AND PROSPECTS FOR THE FUTURE

The efficiency of transpososome formation in Tn10 transposition is greatly increased by IHF binding to the OE of Tn10. After binding the OE, IHF bends the DNA, thereby allowing transposase to contact the OE

on either side of the IHF binding site. This arrangement apparently stabilizes the transposase-OE interaction, thus increasing the probability that a transpososome will form. However, an important consequence of this mechanism of promoting transpososome formation is that the IHF-mediated folding of the OE restricts entry of target DNAs into the transpososome for integration so that intramolecular transposition events are favored. A second host protein, H-NS, can act in opposition to IHF to unfold the transpososome, so that target DNA entry into the transpososome is not restricted and thus intermolecular transposition events can occur. Thus, the involvement of IHF in transpososome formation, which is precipitated by weak binding of transposase to the OE, has introduced an additional layer of regulation into the Tn10 system. Moreover, because both the expression of H-NS and IHF is growth phase regulated, and the function of H-NS is modulated by a variety of environmental stimuli, the regulation of Tn10 transposition, at the level of target interactions, is expected to be influenced by both growth phase and the environment. This would permit Tn10 transposition to be linked to the physiology of the host cell, a situation that could benefit both the transposon and the host (Figure 7).

It will be important in the future to test the hypothesis that Tn10 transposition is modulated by changes in host physiology in an H-NS dependent manner. If this is confirmed, then it will be very interesting to determine if other modulators of H-NS function are involved in this regulation. Also, from a biochemical perspective it will be interesting to work out precisely how H-NS promotes transpososome unfolding and to determine if H-NS influences other aspects of the Tn10 transposition reaction. While H-NS has previously been shown to influence other transposition reactions, it has not been previously shown to do so by directly interacting with a transposition intermediate. It will therefore be important to establish if H-NS is able to influence other transposition reactions through direct binding to transpososomes. There is already preliminary evidence in the Tn5 system to support this possibility (C. Whitfield and D. Haniford, unpublished data).

Finally, there needs to be some clarification regarding the role of transpososome arm unfolding on Tn10 excision. The argument that transpososome arm unfolding is coupled to excision has largely been supported by experiments utilizing OE substrates with truncated subterminal transposase binding sites. It is presumed that such substrates provide enough subterminal DNA to promote transpososome formation but insufficient subterminal DNA to promote the formation of a stably folded, torsionally strained transpososome arm. In order to better define the biochemical properties of transpososomes formed with truncated OE ends, it will be important to establish that transpososomes formed with a full length OE do actually contain a stably folded transpososome arm that is under torsional strain–and thus harbor a store of energy that can be used to drive conformational changes in the transpososome necessary for excision. In this regard it would be useful to ask if OE substrates containing a nick between the anchor points are able to complete excision. Presumably, the $\alpha$ arm of a transposon containing such an OE would not develop torsional strain because of the discontinuity in a DNA strand, and therefore would not be available to drive the excision reaction to completion. It will also be important to determine if other transposons, (presumably) with binding sites for DNA bending proteins in their termini, also undergo the same type of transposon arm folding and unfolding cycle that has been documented for Tn10.

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