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γδ transposase and integration host factor bind cooperatively at both ends of γδ

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γδ, a prokaryotic transposon, encodes a transposase that is essential for its transposition. We show here, by DNase I protection experiments, that purified γδ transposase binds at the transposon's inverted repeats (IRs). Immediately adjacent to each transposase binding site (and within γδ DNA) we have identified a binding site for an additional protein factor, the *Escherichia coli*-encoded integration host factor (IHF). The binding of transposase and IHF to these adjacent sites is mutually cooperative. An IHF binding-site was also found in the original target DNA, just outside one of the ends of γδ. The affinity of IHF for this flanking site is reduced by transposase. These results demonstrate that γδ transposase binds at the IRs of γδ, and suggest that IHF may be involved in forming a transposase–DNA complex and/or influencing the target site selection during the transposition of γδ.

Key words: transposon/DNase I protection/protein–DNA interaction/negative cooperativity

Introduction

The ends of transposable DNA elements contain information that allow for their precise translocation within the genome of a cell (for reviews, see Grindley and Reed, 1985; Heffron, 1983). Ends of bacterial transposons generally terminate with short inverted repeats (IRs), and it is these IRs that define the transposing segment of DNA. γδ, a member of the Tn*3* family of prokaryotic transposons, has ends that terminate with a perfect inverted repetition of 35 base pairs (bps) (Reed *et al.*, 1979).

The *tnpA* gene of γδ encodes a protein, transposase, which is absolutely required for the transposition of γδ *in vivo* (Kitts *et al.*, 1982). We have purified the transposase of γδ to near homogeneity using both DNA binding activity and SDS–PAGE to follow the protein through its purification (manuscript in preparation). Positive identification of the protein as the product of the γδ *tnpA* gene was made by immunoblotting with a specific antibody. Here we show that γδ transposase binds specifically to the terminal IRs of γδ. We also identified an *Escherichia coli* protein, integration host factor (IHF), that binds at both ends of γδ, adjacent to each IR, and we have investigated the effects of transposase and IHF on each others' binding.

Results

γδ transposase binds at the IRs of γδ

We have used the DNase I footprinting procedure (Galas and Schmitz, 1978) to characterize the interaction of transposase with γδ DNA. Transposase protected a region of DNA covering the terminal 35 bp IR at both γ and δ ends (Figure 1A and 1B, compare final two lanes). Comparison of the transposase titrations shown in Figure 2A and 2B (lanes without IHF) shows that the transposase has a higher affinity for the δ end than for the γ end (~5- to 10-fold), suggesting that transposase may recognize more than just the 35 bp terminal inverted repeat. The binding of transposase occurs in a simple buffered system and needs no cofactors (see Materials and methods for binding conditions); in particular we found that additional ATP was neither required for, nor stimulated, binding (data not shown) as was reported earlier for the related transposase of Tn*3* (Wishart *et al.*, 1985). The protected region ran from the ends of the transposon at about position +40 (at both ends) and extended (although rather weakly) about 15 to 20 bp into the adjacent target DNA (see Figure 3). Within the transposase-protected region were several positions at which cleavage by DNase I was retained or was enhanced (see Figures 1 and 3). The enhanced cleavages resulted from the DNase I digestion since no cuts occurred when DNA was incubated with transposase in the absence of added nuclease (data not shown).

IHF binds adjacent to the IRs, within γδ

In the course of our purification of γδ transposase we discovered a second protein factor that bound to both ends of the transposon. The DNA sequence of the binding site of this factor (identified by methylation-interference experiments) suggested it was the *E.coli* host protein IHF. This was confirmed by DNase I footprinting experiments using purified IHF obtained from H.Nash (N.I.H., Bethesda, Maryland). DNase I footprinting indicated that there were IHF binding sites immediately adjacent to both IRs of γδ (labelled γ1 and δ1; see Figure 2A and 2B, compare final two lanes). Additional IHF binding sites were also seen, one within γδ (labelled δ2; Figure 2B), the other in the target DNA [the chloramphenicol resistance gene in pACYC184 (Alton and Vapnek, 1979; Chang and Cohen, 1978)] close to the γ end (labelled cat1; Figure 2A). The γ1, δ1 and cat1 sites all had similar DNase I protection patterns. The protected region is extensive considering the small size of the monomer IHF [21 805 kd (Flamm and Weisberg, 1985; Mechulam *et al.*, 1985; Miller, 1984)] and is asymmetric relative to the position of the conserved DNA sequence element as has been shown in the studies of IHF binding to the bacteriophage λ attachment site (Craig and Nash, 1984). The γ1 and δ1 IHF sites are positioned identically relative to the terminal IRs, with sequences related to the IHF binding site consensus sequence (Leong *et al.*, 1985) from +43 to +55 (see Figure 3). The γ1 site matches the consensus perfectly (Figure 4) and shows the strongest binding to IHF. The cat1 site is weaker than γ1, but is a

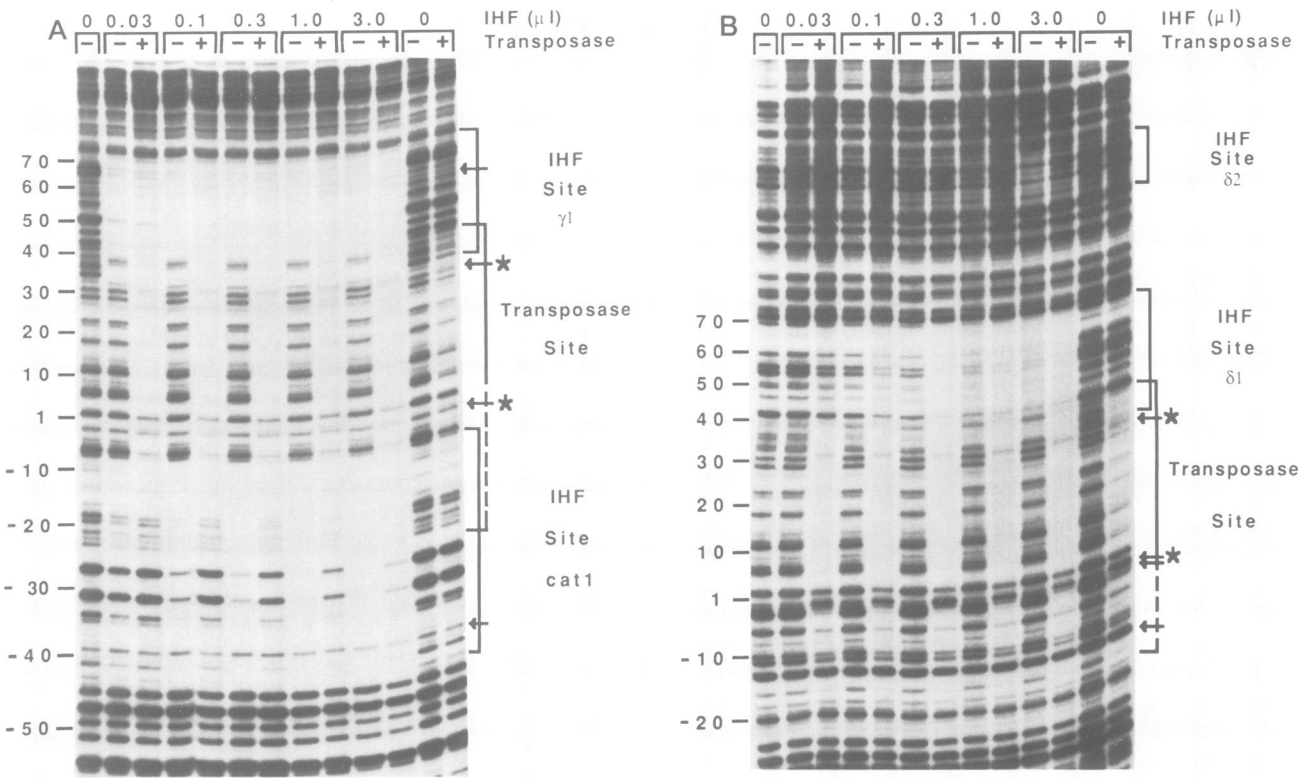

Fig. 1. Effects of transposase on the binding of IHF to the ends of γδ assayed by DNase I footprinting. (**A**) the γ end. (**B**) the δ end. IHF was added to DNA binding mixtures containing either no transposase (−) or an amount of transposase (+) to give protection at the IR. A comparison between transposase (−) and (+) lanes throughout increasing amounts of IHF shows negative cooperativity of IHF binding to the DNA flanking the γ end (cat1) and positive cooperativity of IHF binding to the DNA adjacent to the IR at the δ end (δ1). Autoradiographs of gels show top strand information for γ and δ ends (see Figure 3 for DNA sequence). Positive base numbers indicate γδ sequences and negative numbers indicate flanking DNA sequences; numbering starts from each end of the transposon. Brackets indicate region protected by each protein in individual footprint studies. Dashed portion of bracket for the footprint of transposase indicates weak protection. Arrows indicate DNase I cleavages remaining (a star designates DNase I enhancement) within the protected region in the footprints of transposase and IHF.

little stronger than δ1 (compare Figure 1A and 1B); neither cat1 nor δ1 have a perfect match to the consensus sequence (Figure 4). The δ2 site is the weakest of the four sites (Figure 1B), yet it contains a perfect match to the IHF binding consensus sequence (Figure 4). It would seem, therefore, that the conserved sequence is not the sole determinant of IHF binding strengths.

Transposase and IHF bind cooperatively to the ends of γδ

The proximity of the binding sites of γδ transposase and IHF suggested that these proteins may interact in some way at the ends of the transposon. We tested to see if IHF influenced the binding of transposase (Figure 2A and 2B). Transposase was added to a DNA mixture containing either no IHF, or an amount of IHF sufficient for protection of the IHF binding sites of γ1 and δ1. This was done over a range of transposase concentrations. The results show that IHF stimulated the binding of transposase ~30- to 100-fold at the γ end and ~10- to 30-fold at the δ end.

Conversely, we tested to see if transposase affected the binding of IHF (Figure 1). IHF was added to a DNA mixture containing either no transposase, or an amount of transposase sufficient to protect either IR. This was done over a range of IHF concentrations. Transposase stimulated the binding of IHF >10-fold at the δ end (Figure 1B). Therefore, the binding of transposase and IHF at the ends of γδ seems to be mutually cooperative.

The footprint of IHF and transposase together is very similar to the sum of their individual footprints. DNase I cleavages, present or enhanced within the footprint of IHF, but protected by transposase, are protected in the footprint of IHF and transposase (Figure 3). The weak protection of DNA afforded by transposase at the flanking DNA (bases 1 to −17 at the δ end) is similar in the footprint of transposase with IHF (Figures 1B, 2B and 3).

Transposase inhibits IHF binding at flanking DNA

The presence of an IHF binding site in the target DNA close to the site of γδ insertion was intriguing. In the experiment described above and shown in Figure 1A it can be seen that transposase inhibited the binding of IHF at this flanking DNA site ~10-fold (Figure 1A) in contrast to the mutual, positive cooperative binding seen at the ends of γδ.

Discussion

Terminal inverted repeats are a striking characteristic of bacterial transposons. For many transposons, these IRs are the only DNA sequences required in *cis* for transposition (Grindley and Reed, 1985; Shapiro, 1983), and it has been assumed that these sequences are recognition sites for transposase, the transposon-encoded protein essential for transposition. As expected, we find that the purified γδ transposase binds specifically to the transposon's ends.

The region of γδ protected from DNase I cleavage by its

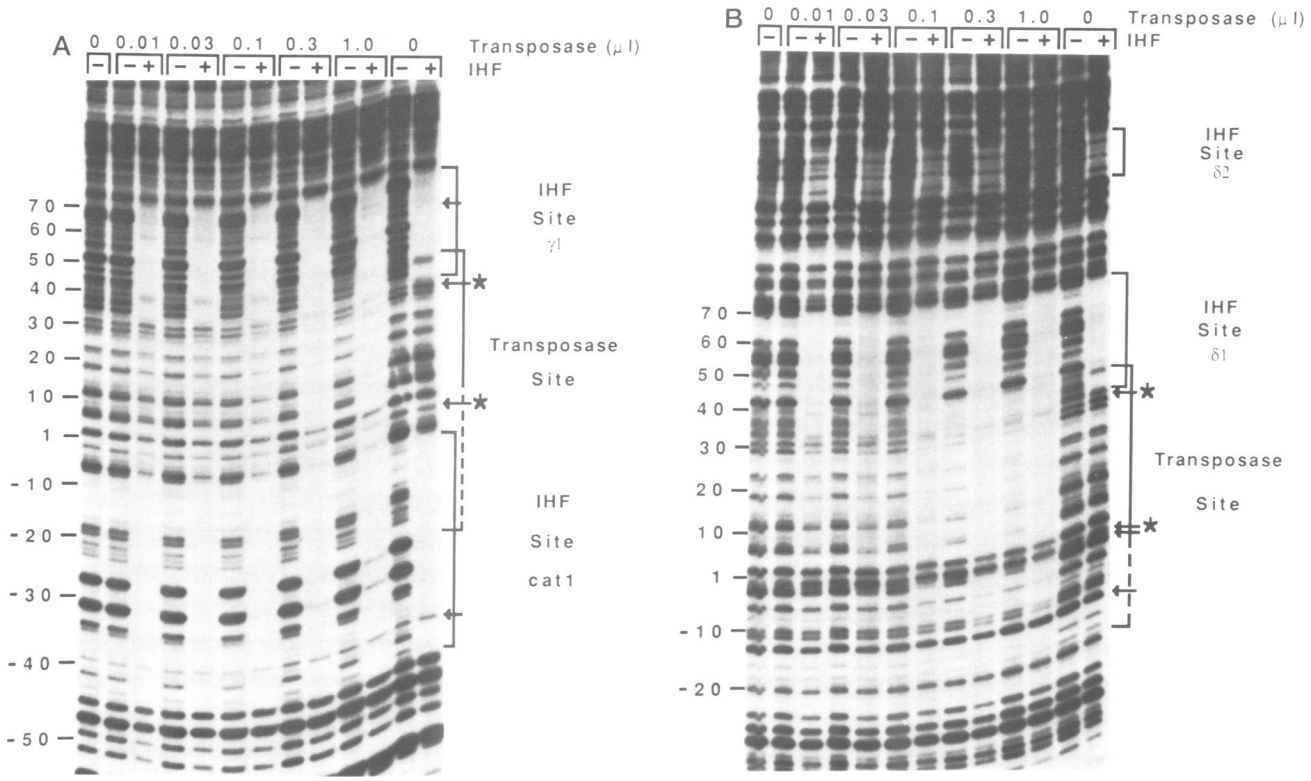

Fig. 2. Effects of IHF on the binding of transposase at its IRs. (**A**) the γ end. (**B**) the δ end. Transposase was added to a DNA binding mixture containing either no IHF (−) or an amount of IHF (+) to give protection at IHF binding sites. A comparison between IHF (−) and (+) lanes throughout increasing amounts of transposase shows that IHF increases the binding of transposase at both ends of γδ. Autoradiographs of gels show top strand information for γ and δ ends (see Figure 3 for DNA sequence). Positive base numbers indicate γδ sequences, and negative numbers indicate flanking DNA sequences; numbering starts from each end of the transposon. Brackets indicate region protected by each protein in individual footprint studies. Dashed portion of bracket for the footprint of transposase indicates weak protection. Arrows indicate DNase I cleavages remaining (a star designates DNase I enhancement) within the protected region in the footprints of transposase and IHF.

transposase is some 40 bp long and includes the 35 bp IRs. The fact that transposase appears to bind more tightly to the δ end that to the γ end suggests that the sequences involved in recognition extend slightly beyond the terminal 35 bp IR (since these are identical at both ends). In this regard, it is of interest that the IRs of the closely related transposon, Tn*3*, are 38 bp long and have the same sequence from positions 36−38 as the higher affinity δ end of γδ. The DNA which flanks the IRs outside the transposon may also contribute to binding since it is weakly protected. A recognition region of about 3.5 helical turns of DNA (nearly 120 Å) without any clear dyad symmetry or repeated elements is extraordinarily long for a single protein and raises interesting problems in protein−DNA recognition.

In the transposase−DNA complex the phosphodiester bonds at both 5′-ends of γδ remain susceptible to DNase I cleavage while the 3′-ends are protected. The accessibility of the 5′-ends to DNase I indicates that transposase does not cover these sites (at least in a complex with linear DNA fragments) and implies that the action of transposase may be directed to the 3′-ends as has been shown with the unrelated transposon, bacteriophage Mu (Mizuuchi, 1984). We saw no evidence for transposase-mediated cleavages at the ends of γδ on the linear DNA fragments used in the footprinting experiments (data not shown).

In our footprinting experiments we found that specific DNA binding by the γδ transposase was independent of (and not enhanced by) ATP. This observation conflicted with the report of Wishart *et al.* (1985) that binding of the related Tn*3* transposase to its cognate IRs was ATP-dependent. During the preparation of this paper a report appeared in which the Tn*3* transposase was shown to be an ATP-independent DNA binding protein (Ichikawa *et al.*, 1987). This confirms our results and shows that the γδ and Tn*3* transposases in this respect are similar to the transposases from two other unrelated bacterial transposons, Mu (Mizuuchi and Craigie, 1986) and Tn*10* (Morisato and Kleckner, 1987).

The discovery of binding sites for the *E.coli* protein, IHF, adjacent to the γδ terminal IRs (and at one of the insertion sites) was not anticipated. IHF and the structurally homologous (but non-sequence specific) protein HU belong to a class of histone-like proteins that are implicated in altering the topology of DNA (Drlica and Rouviere-Yaniv, 1987). Both IHF and HU are involved in a variety of specialized recombination processes. IHF is required for integrative recombination of bacteriophage λ (Miller and Friedman, 1977; Williams *et al.*, 1977) and has been shown to facilitate the binding of λ integrase at the phage recombination site, *attP* (Craig and Nash, 1984; Nash and Robertson, 1981). Insertion sequence IS*1* has IHF binding sites at its ends (Gamas *et al.*, 1985, 1987), although the role of IHF in IS*1* transposition is as yet unclear. Both IHF and HU stimulate transposase-mediated circle formation by Tn*10 in vitro* (Morisato and Kleckner, 1987). HU is required for transposition of phage Mu *in vitro* (Craigie *et al.*, 1985), and stimulates site-specific recombination by the DNA-invertase,

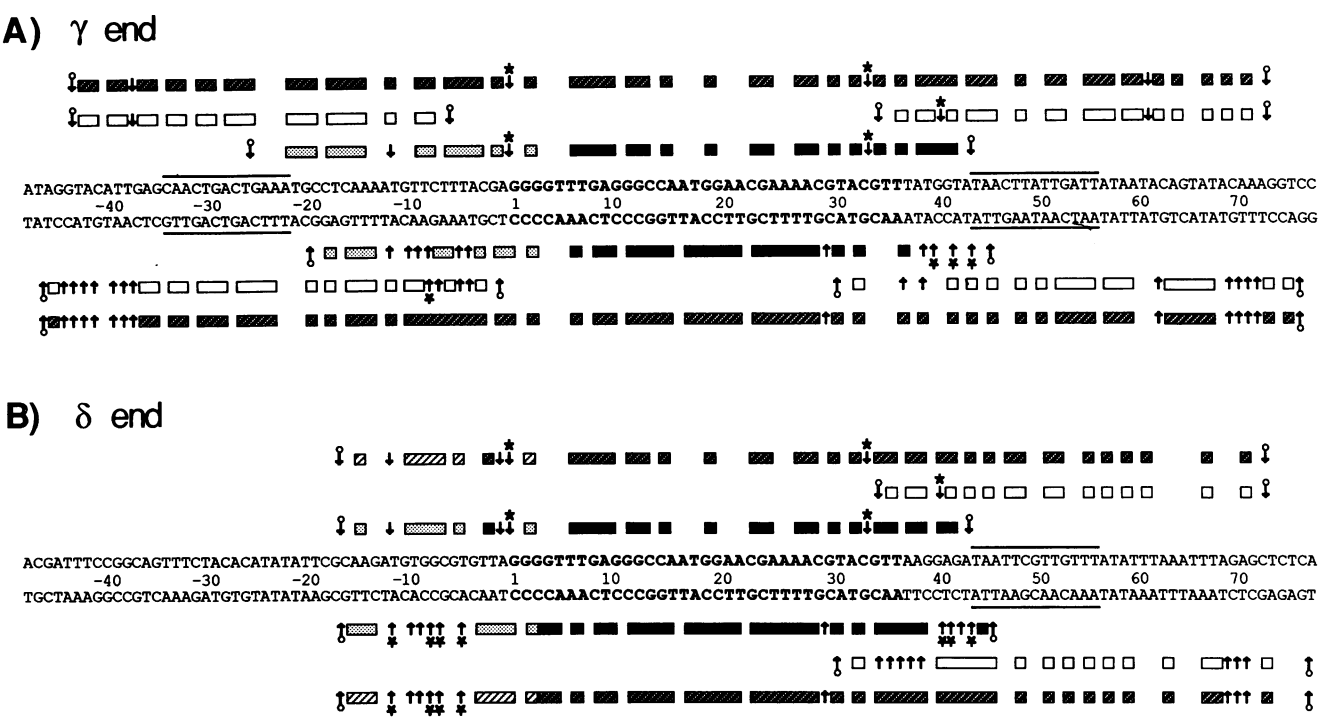

Fig. 3. DNA sites protected from DNase I digestion by γδ transposase and IHF separately and in combination. (**A**) the γ end. (**B**) the δ end. Boxes indicate protection from DNase I as follows: ■ and ▩, strong and weak protection by transposase; □, protection by IHF; ▨ and ▨, strong and weak protection by IHF and transposase combined. Arrows indicate DNase I sites still present within the protected region; asterisks over arrows indicate enhanced DNase I cleavages. Circles over arrows indicate the first unaffected DNase I cleavages outside of the protected region and hence demarcate the DNase I protected region. Numbering begins at the terminal base of each end of γδ with the 35 bp IR in bold; negative numbers indicate flanking DNA sequences. Sequences related to the IHF consensus sequence (see Figure 4) which appear at similar places relative to the known pattern of IHF protections (Craig and Nash, 1984) are indicated by horizontal lines. IHF binding sites at the γ end are cat1 (−35 to −23) and γ1 (43 to 55), and at the δ end are δ1 (43 to 55) and δ2 (124 to 136, sequence shown in Figure 4).

Hin (Johnson *et al.*, 1986). It seems likely that IHF plays some role in γδ transposition, either by facilitating binding of transposase or by altering the activity of the transposase−IR complex. We note, however, that the ends of Tn*3* do not appear [by DNA sequence (Heffron *et al.*, 1979)] to have similarly positioned binding sites for IHF.

A role for IHF in formation of transposase−IR complexes is suggested by recent studies of γδ transposition immunity (Goto *et al.*, 1987). Immunity is a phenomenon exhibited by Tn*3*-family transposons in which a replicon containing a transposon is a very poor target for further insertions of the same element (Robinson *et al.*, 1977). A single copy of the transposon terminus is sufficient to effect immunity (Lee *et al.*, 1983). Goto *et al.* found that a replicon with the 38 bp δ end exhibited strong immunity, whereas the 38 bp γ end (in the identical vector) showed only weak immunity. These results parallel our finding that the δ end binds transposase with higher affinity than the γ end. Strikingly, immunity was substantially increased when the target replicon contained longer terminal segments of γδ (400 bp at the δ end or 200 bp at the γ end). We suggest that this increase in immunity results from the presence of the terminal IHF binding sites which increase the affinity of the ends for transposase.

The presence of an IHF binding site in the target DNA close to the site of γδ insertion was intriguing. Transposase inhibits the binding of IHF at this flanking DNA site (Figure 1A) in contrast to the mutual cooperativity seen at the transposon's ends. Although we have no data that address the role of the target IHF site, its location suggests that it (and IHF) may be involved in target site selection. A similar suggestion has been made by Gamas *et al.* (1987) who found IHF binding sites within the major hot spot for IS*1* insertion in the plasmid pBR322. We are currently exploring the role of IHF in the transposition of γδ.

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cat1      ATAGGTACATTGAGCAACTGACTGAAATGCCTCAAAATGTTCTTTACG
γ1        GTACGTTTATGGTATAACTTATTGATTATAATACAGTATACAAAGGTC
δ1        GTACGTTAAGGAGATAATTCGTTGTTTATATTTAAATTTAGAGCTCTC
δ2        AATGTATAATGGCCCAACATATTGATATGCCCGTGCATCAGGGGAGAT

Consensus           YAANNNNTTGAT[A/T]
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Fig. 4. IHF binding sites aligned. The consensus is taken from Leong *et al.*, 1985.

Materials and methods

Materials

The following materials are listed with their suppliers: restriction endonucleases, New England Biolabs and Boehringer Mannheim; polynucleotide kinase, New England Biolabs; Klenow, gift from Cathy Joyce, Yale University; DNase I, Worthington; Proteinase K and poly[d(I-C)], Sigma; and bovine serum albumin, International Biotechnologies, Inc. IHF was a gift from Howard Nash, National Institutes of Health; the protein had been purified through step V as described in Nash and Robertson, 1981.

Origin and preparation of DNA substrates

Labelled DNA fragments containing either the γ or δ end came from one of two different γδ inserts, one on each side of the *Eco*RI site in the chloramphenicol resistance gene of pACYC184 (Alton and Vapnek, 1979; Chang and Cohen, 1978). The γ end was obtained on a 401 bp *Eco*RI−*Pst*I fragment from pLAW61 with the beginning of the γ end 168 bp from the

*Eco*RI site. The δ end was obtained on a 553 bp *Eco*RI−*Bam*HI fragment from pLAW83 with the beginning of the δ end 166 bp from the *Eco*RI site.

Top strand information for both ends (see Figure 3) was obtained by 5′-end-labelling (Maxam and Gilbert, 1977) at the *Eco*RI site and secondarily cleaving within γδ sequences with either *Pst*I for pLAW61 or *Bam*HI for pLAW83. Bottom strand information was obtained using the same DNA fragments that were 3′-end-labelled (Maniatis *et al.*, 1981) at their *Eco*RI sites. All DNA fragments were purified by gel electrophoresis.

DNase I footprinting

Binding reactions (10 μl) contained end-labelled DNA fragments in 10 mM $KHPO_4$ (pH 7.3), 100 mM NaCl, 3 mM $MgCl_2$, 0.1 mM dithiothreitol, 10 μg/ml bovine serum albumin, 10 μg/ml Poly[d(I-C)], 0.5% glycerol, and 0.02% Triton X-100. After addition of protein (see below) the binding reactions were incubated at 23°C. Reactions were shifted to 20°C and equilibrated for 1 min. DNase I was added to 10 μg/ml, and after 1 min the digestion was stopped by the addition of an equal volume of 20 mM EDTA. Proteinase K was added to 1 mg/ml and incubated for 15 min at 20°C. The DNA was then precipitated with ethanol, washed in 70% ethanol and redissolved in loading buffer (98% deionized formamide, 10 mM EDTA, 0.1% xylene cyanol and bromophenol blue). After boiling for 1 min, samples were analyzed by electrophoresis on a 0.4 mm thick 8% polyacrylamide gel in 50 mM Tris-borate (pH 8.3), 1 mM EDTA, 8 M urea. Gel lanes on autoradiographs were scanned by densitometry (Ultroscan XL, LKB) to enable comparison of DNase I cuts.

For the experiments shown in Figure 1, transposase (350 μg/ml final concentration) was added to the (+) reactions and incubated for 1 min at 23°C. IHF (from a 33 μg/ml stock solution) was added to the binding mixture at the indicated amounts to both transposase (−) and (+) reactions and incubated for 5 min at 23°C. For the experiments shown in Figure 2, IHF (8 μg/ml final concentration) was added to the (+) reactions and incubated for 1 min at 23°C. Transposase (from a 3.5 mg/ml stock solution) was added to the binding mixture at the indicated amounts to both IHF (−) and (+) reactions and incubated for 5 min at 23°C. As an additional control, an incubation of each protein at the highest concentration used in footprinting, but without addition of DNase I, was performed to determine if either protein contained an intrinsic DNA 'nicking' activity.

γδ transposase

γδ transposase was purified to near homogeneity as visualized by Coomassie Blue staining of an SDS−polyacrylamide gel, and its identity was confirmed through immunoblotting with a specific antibody. A detailed description of the purification of γδ transposase will be given elsewhere (manuscript in preparation). Briefly, the steps used to purify transposase are as follows: cell lysis, precipitation using polymin P, resuspension in high salt (1.0 M ammonium sulfate), precipitation by dialysis against low salt (25 mM NaCl), resuspension in medium salt (300 mM NaCl), chromatography on Bio-Rex 70 (Bio-Rad), concentration using Centricon-30 (Amicon) and gel filtration using Sephacryl S-300 (Pharmacia).

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