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QUARTERLY

Binding of *Escherichia coli* integration host factor (IHF) to the origin segment of p15A plasmid*

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The integration host factor (IHF) is a sequence-specific, histone-like, multi-functional DNA-binding and -bending protein of *Escherichia coli*. Characterization and functional analysis of this protein has been carried out mainly in bacteriophage λ and other mobile genetic elements.

In this paper we report data concerning the binding of IHF protein to the plasmid *ori*p15A region. IHF binds to the single site of the DNA fragment containing the *ori*p15A, as shown by the gel mobility shift assays and footprinting experiment. On the basis of the *ihf* consensus sequences published, we have been able to identify one sequence of putative *ihf* site into the *ori*p15A sequence with two mismatches in relation to the consensus sequence of Kur *et al.*, 1989, *Gene* 81, 1-15. One *ihf* binding site was also found in the *ori*ColE1 region sequence with three mismatches in relation to this consensus sequence.

The IHF protein of *Escherichia coli* is a histone-like, heterodimeric protein consisting of the products of the *himA* gene (11.2 kDa) [1] and *himD/hip* gene (10.5 kDa) [2]. It was first identified genetically by its function in the integration of bacteriophage λ both *in vivo* and *in vitro* [3]. The IHF protein participates in several regulatory processes, including modulation of transcription, phage packaging, plasmid replication and transfer, bacterial phase variation (for review see [4]). Several related consensus sequences have been proposed from footprinting analyses [5–9]. According to Kur *et al.* [5], IHF specifically binds to the A+T-rich *ihf* consensus sequence $W_6N_7W_4CARNWN_2TTR$. Binding sites, however, can vary significantly from the consensus, and several studies have shown that the context can also play a significant role in binding efficiency [7, 9, 10].

It is known from published data that both *him*A and *hip/him*D mutants fail to maintain plasmid pSC101 [11] and a truncated form of plasmid R6K with only one (the γ) of three overlapping origins of replication [12]; they do not support growth of filamentous phage f1, either [13].

Plasmid p15A was first detected as one of three plasmids in *E. coli* strain $15T^-$ [14]. The plasmid is a circular DNA molecule about 2.2 kb in size [15] and thus one of the smallest

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[1]Abbreviations: bp, base pair(s); IHF, integration host factor; *ihf*, IHF-binding site on DNA; N, any nucleoside; R, A or G; W, A or T.

naturally occurring plasmids known. It has been used to construct a cloning vector, pACYC184, which is useful because it is readily maintained by bacteria carrying also a ColE1 derivative [15]. DNA synthesis on p15A begins within a region whose nucleotide sequence is almost identical with that of the region containing the ColE1 origin [16]. The initiation of p15A replication and its regulation occur through largely the same mechanisms as those of ColE1 [17].

We analyzed, by computer search, the sequence of the *ori*p15A region for the presence of homology to the *ihf* consensus sequence reported by Kur *et al.* [5]. One consensus-like sequences was found in the *ori*p15A DNA region. It overlaps the –10 region of the promoter for synthesis of a primer precursor transcript (RNAII).

The purpose of the present work was (*i*) to determine experimentally and map the *ihf* site in the *ori*p15A segment of DNA, (*ii*) to correlate these experimental data with the predictive value of the consensus sequence published and (iii) to compare IHF-binding to the origin segments of p15A and ColE1.

MATERIALS AND METHODS

IHF was purified by the Biotechnology Center, University of Wisconsin (U.S.A.), according to the protocol of Nash *et al.* [18].

Restriction enzymes were obtained from New England Biolabs and were used according to the manufacturer's recommendations.

DNA manipulations were performed as described by Maniatis *et al.* [19].

The DNA-protein complexes were studied by the gel retardation technique [20], as modified by Kur *et al.* [5]. The DNA fragments and their complexes with IHF were separated on 6% polyacrylamide gel at 6 V/cm and 4°C. After electrophoresis, the gels were stained with ethidium bromide and photographed.

Nucleotides were sequenced as described by Maxam & Gilbert [21]. Footprinting experiments with hydroxyl radicals were carried out as described by Tullius & Dombroski [22] and Kur *et al.* [5].

RESULTS AND DISCUSSION

Computer search for IHF-binding sites (ihf). We analyzed the sequence of the *ori*p15A region [23] for the presence of homology to the *ihf* consensus sequences [5–7] and the results of this search are presented in Table 1. The analyzed sequence was an *Xmn*I-*Eco*RV restriction fragment (1044 bp) and represents the nucleotides 635 to 1679 *ori*p15A region in pACYC184 plasmid. Only one consensus-like sequences (*ihf*) was found on this DNA segment. This sequence was found to overlap the –10 region (Pribnov box) of *ori*p15A primer promoter in-

Table 1
Homology with the consensus binding sequence

Sequence	*ihf*	Consensus agreement[a]
WWWWWW NNNNNNN WWWW CAR NWNN TTR	Kur *et al.* [5] (consensus)	
TTTTTT CGTTTTC AgAg CAA GAGA TTA	*ori*p 15A	2
gTTTTT TTGTTTG cAAg CAG CAGA TTA	*ori*ColE1	3
YNY AA NNNN TTG ATW	Gardner & Nash [7] (consensus)	
CGT AA TCTC TTG cTc	*ori*p 15A	2
CGT AA TCTG cTG cTT	*ori*ColE1	2
TNY AA NNNR TTG AT	Craig & Nash [6] (consensus)	
cGT AA TCTC TTG cT	*ori*p 15A	2
cGT AA TCTG cTG cT	*ori*ColE1	3

[a] Consensus agreement is calculated in relation to the three published consensus sequences shown in the Table. Lower-case letters represent nucleotides unmatched to the consensus.

N, any nucleoside; R, A or G; W, A or T; Y, T or C.

dicating that there is good reason to expect that IHF might affect plasmid replication by changing RNAII transcription. The sequence homologous to the *ihf* consensus sequence according to Kur *et al.* [5] in relation to the *ori*p15A fragment is shown below:

5'-TTTTTT CGTTTTC AgAg CAA GAGA TTA,

where the underlined nucleotides belong to the –10 promoter sequence, and lower-case letters represent the nucleotides unmatched to the consensus (two mismatches). The *ori*p15A sequence was also analyzed for the presence of homologues to other previously reported consensus sequences (see Table 1).

There is also one sequence homologous to the consensus sequences (Table 1) for the *ori*ColE1 in the analysed *Xmn*I-*Pst*I restriction fragment (1588 bp). It represents the nucleotides 2029 to 3607 *ori*ColE1 region in pBR322 plasmid segment [24]. The sequence homologous to the *ihf* consensus sequence according to Kur *et al.* [5] is shown below:

5'-gTTTTT TTGTTTG cAAg CAG CAGA TTA,

where the underlined nucleotides belong to the –10 promoter sequence, and lower-case letters represent the nucleotides unmatched to the consensus (three mismatches).

Location of the IHF-binding sites using gel mobility shift analysis. To study IHF-DNA interactions *in vitro*, we performed gel mobility shift assays using restriction fragments containing the putative *ihf* sites. In this technique DNA bound to a protein has a lower mobility as compared with the unbound DNA, when electrophoresed in a native polyacrylamide gel. The procedure has been applied successfully in studies of binding and specific complex formation of many proteins. It was also applied to the study of the IHF binding. As evident from Fig. 1A, the *Hin*fI-*Hin*dIII *ori*p15A restriction fragment (nucleotides 1140 to 1523 of plasmid pACYC184) interacts with IHF, as it forms complexes that are retarded. Only one kind of complex is observed, even at higher IHF concentrations, indicating that only one *ihf* binding site is present on this fragment.

We have also investigated IHF binding to the *Alu*I-*Hae*III *ori*ColE1 restriction fragment (nucleotides 3035 to 3410 of plasmid pBR322). IHF binding to this DNA also revealed one retarded band (Fig. 1B).

The affinity of IHF to the *ori*p15A *ihf* site is rather high, as it is very similar to that observed for the H' *ihf* of the λ *att*P (the same relative IHF concentration resulted in a complete shift of the fragment). This is shown in Fig. 1C, where the *Hin*dIII-*Hin*dIII restriction fragment (341 bp) of the λ *att*P, carrying only the H' *ihf* site, was treated exactly in the same way as the *ori*p15A *ihf* fragment. In the case of the *ori*ColE1 *ihf* site (Fig. 1B), we found that it binds IHF less strongly than the *ori*p15A (or H') fragments. As seen in Fig. 1B, migration of the *ori*ColE1 fragment is not affected at 3 ng IHF/μl, whereas the *ori*p15A fragment is partially retarded (Fig. 1A). This difference in binding was expected be-

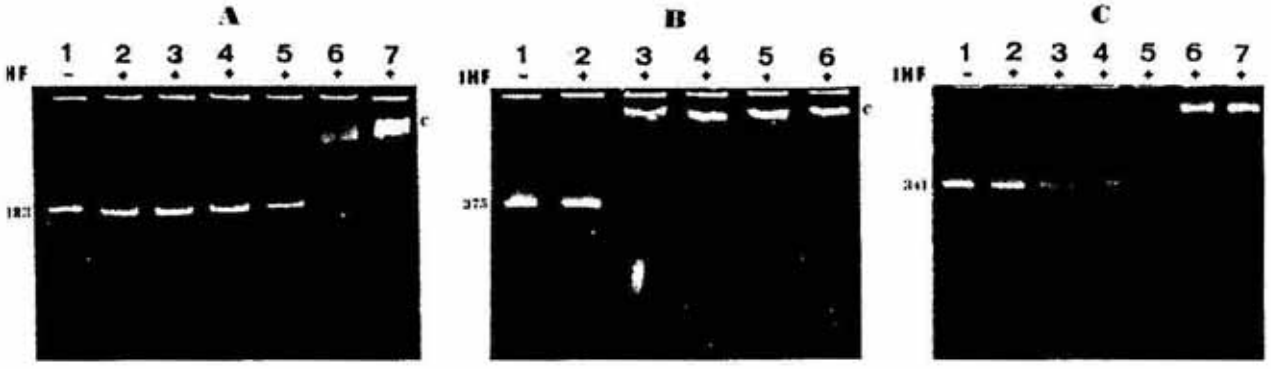

Fig. 1. Gel-mobility shifts induced by IHF binding.
Panel **A**, IHF binding to the *Hin*fI-*Hin*dIII *ori*p15A restriction fragment (383 bp) of pACYC184 plasmid. Panel **B**, IHF binding to the *Alu*I-*Hae*III *ori*ColE1 restriction fragment (375 bp) of pBR322. Panel **C**, IHF binding to the *Hin*dIII-*Hin*dIII restriction fragment (341 bp) containing H' *ihf* of the λ *att*P. The IHF binding was carried out in the presence of unlabeled DNA fragments (1 μg) and increasing concentrations of IHF (0.0, 1.2, 1.8, 2.4, 3.0, 3.6 and 4.2 ng/μl for lanes 1–7, respectively, for panel A and C; and 0.0, 3.0, 4.0, 6.0, 8.0 and 10.0 ng/μl for lanes 1–6, respectively, for panel B). The DNA fragments and their complexes with IHF were separated on 6% polyacrylamide gel at 6 V/cm. The size of fragments is indicated (in bp) on the left margin and the position of their complexes (C) on the right margin.

cause of better homology to the consensus-like *ihf* sequence for *ori*p15A *ihf* site (two mismatches in relation to three mismatches for *ori*-ColE1 *ihf* site). The retarded fragments must contain only one *ihf* site, as only a single complex is observed, even at high IHF concentrations.

Another test for the specificity of the IHF-*ori*p15A interaction is shown in Fig. 2. Calf thymus DNA was used as the nonspecific competitor DNA. About a 25-fold excess of this DNA was used and the totally retarded fragment was still observed even at a very small IHF concentration (0.4 ng/µl).

Location of the IHF-binding sites using footprinting analyses. Footprinting techniques permit not only to demonstrate protein binding to DNA, but also to locate the site(s) of binding. The hydroxyl radicals, generated by reduction of H_2O_2 by Fe^{2+}, are the reagent of choice because they break the DNA backbone at practically every nucleotide; thus every position can be monitored for contact with protein. Our experiments on IHF protection of the *ori*p15A region show three distinct areas of protection (Fig. 3A). The central (main) protected region shows five nucleotide contacts. Moreover, there are upstream and downstream contact points, each at a distance of about one turn of the DNA helix. One of these coincides with the –10 primer promoter sequence (Fig. 3B).

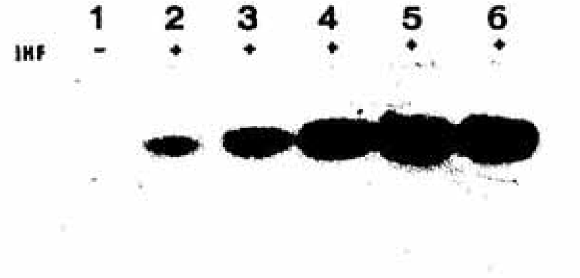

Fig. 2. Gel-mobility shifts induced by IHF binding to the fragment containing the ori of the p15A plasmid.
The *Xba*I-*Dde*I (117 bp, 0.2 µg) ^{32}P-end-labeled fragment of p15A *ori* was used. IHF binding was carried out as described elsewhere [5, 10] in the presence of 50 µg/ml of calf thymus DNA as a nonspecific competitor. IHF concentrations used were 0.1, 0.2, 0.4, 0.6 and 1 ng/µl for lanes 2, 3, 4, 5 and 6, respectively. The DNA fragments and their complexes with IHF were separated on 6% polyacrylamide gel at 6 V/cm and autoradiographed.

It is difficult to assume from this study that the presence of an IHF-binding site in the primer promoter region might contribute significantly to the regulation of transcription and plasmid replication *in vivo*. This problem will be studied further to explain the role of IHF in p15A plasmid replication. Our preliminary results revealed the importance of IHF protein in the effective transformation of plasmid containing *ori*p15A. Two isogenic strains (*him*A *him*D double mutant and its wild-type counterpart) were transformed with pACYC184 (*ori*p15A) or pBR322 (*ori*ColE1) plasmid. Subsequent analysis of the efficiency of the plasmid

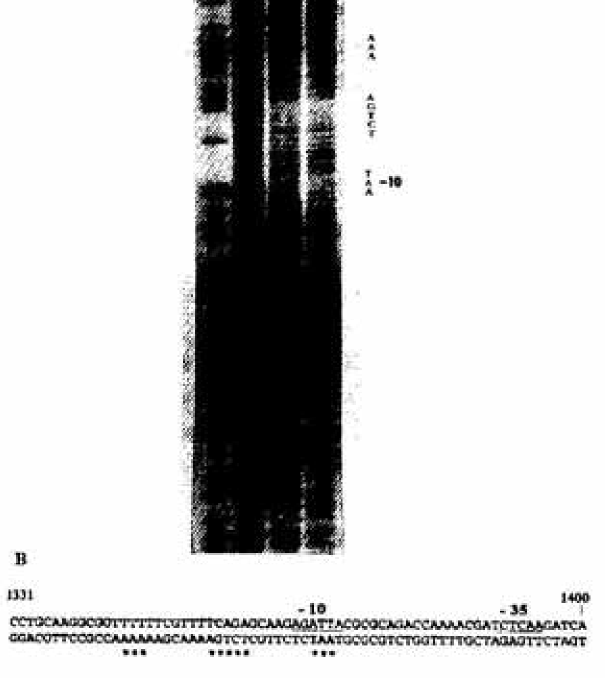

Fig. 3. Protection by IHF of a fragment containing the ori of the p15A plasmid against cleavage by hydroxyl radicals — footprinting (panel A) and nucleotide sequence (panel B).
Panel A: the *Xba*I-*Dde*I (117 bp) ^{32}P-end-labeled fragment of p15A *ori* was used. Lanes 1 and 2, no IHF; lane 3, 0.8 µg IHF/ml; lane 4, 1.6 µg IHF/ml. The footprinting reactions were run (on a denaturing 6% polyacrylamide gel) in parallel with G+A sequencing reaction of the same labeled DNA fragment performed as described by Maxam & Gilbert [21]. The sequence on the right margin shows the nucleotides protected by IHF. Reactions with hydroxyl radicals were carried out as described by Tullius & Dombroski [22] and Kur *et al.* [5]. Panel B: nucleotide sequence of *ori*p15A primer promoter for RNAII transcription. Asterisks indicate the nucleotides protected by IHF against hydroxyl-radical cleavage. Thin lines indicate the –10 and –35 components of promoter.

transformation revealed that there is a substantial reduction in transformation with pACYC184 plasmid in comparison to that obtained with pBR322 (see Table 2). Experiments *in vivo* are continued.

Table 2
Transformation efficiency of plasmids pACYC184 and pBR322 in wild-type and mutant E. coli

Transforming DNA	Numbers of transformants of strain[a]	
	SD 1286 wild type	SD 1287 *him*A and *him*D double mutant
pACYC184	4×10^3	3×10^2 (7.5)[b]
pBR322	6×10^3	2×10^3 (33.3)[b]

[a] Competent cells (4×10^8) were transformed with pACYC184 and pBR322 closed circular plasmid DNA. Data show the numbers of transformants obtained per microgram of DNA.

[b] Relative transformation efficiency is calculated as the number of the mutant transformants divided by the number of wild type transformants, and expressed also as a percentage (in parentheses).

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