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Integration host factor (IHF) represses a *Chlamydomonas* chloroplast promoter in *E.coli*

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ABSTRACT

We show that in E. coli, a Chlamydomonas chloroplast promoter, P_A, is repressed by Integration Host Factor (IHF). The himA42 mutation, altering the α-subunit of E. coli IHF, leads to over-accumulation of P_A transcripts in vivo. This effect requires upstream chloroplast DNA sequences. DNAase I and methylation protection experiments show that IHF binds in vitro to a site within P_A and band-retardation shows that IHF inhibits formation of P_A-E. coli RNA polymerase open complexes. We interpret these results, together with our previous deletion analyses, to mean that in E. coli, repression of P_A by IHF minimally requires both binding of IHF to a site overlapping P_A and binding of one or more additional proteins, perhaps including IHF itself, to sequences upstream of P_A.

INTRODUCTION

Interaction of RNA polymerase with promoter regions and clearing from these regions can be modulated by DNA structure and topology and by repressors or activators whose target sites overlap. Proteins binding to DNA at a distance may also affect initiation of transcription. It is now becoming apparent that all these regulatory mechanisms operate in both prokaryotes and eukaryotes (for reviews see refs. 1-3).

Chloroplast promoters share several features with bacterial promoters: similar consensus sequences in the so-called "-35 region" and in the "-10 region", separated by approximately 17 bp. The similarity of chloroplast and E. coli promoters is thought to reflect their function, since at least some chloroplast promoters are recognized by E. coli RNA polymerase in vivo and in vitro (for reviews see refs. 4 and 5). Little is known, however, about proteins that modulate chloroplast transcription. We are searching for such regulatory proteins by analyzing the regulation of a Chlamydomonas chloroplast promoter that we call P_A. We have shown that regulation of P_A in E. coli involves upstream chloroplast DNA sequences, suggesting that P_A might be regulated by trans-acting transcriptional factors acting at a

distance. We also noted the presence of several chloroplast DNA sequences resembling the consensus E. coli IHF binding sequence, one of these overlapping P_A (6).

IHF is a member of a family of small, basic, DNA binding proteins which are ubiquitous in prokaryotes (7,8). In E. coli, the protein is a heterodimer consisting of one α-subunit and one β-subunit (9). It specifically binds to the consensus sequence YAA...RTTGAT(A,T) (10,11) and affects phage λ site-specific recombination (12), specificity of transposition of IS1 (13), inversion of a DNA segment that controls phase variation in E. coli (14), replication of certain plasmids (15,16), transcription from certain promoters (17-22), and possibly translation of certain mRNAs (23).

Since IHF appears to be one factor which mediates the response of certain promoters to supercoiling (N.P. Higgins, personal communication), and since supercoiling modulates P_A both in E. coli and in chloroplasts (6), we tested a possible role of IHF in regulating P_A using three approaches: (1) by determining the effects of a himA mutation altering the α-subunit of IHF (24) on P_A activity in vivo using a primer extension assay; (2) by protecting specific sequences in the P_A region in vitro with IHF from nuclease digestion and methylation; and (3) by determining the effects of IHF on open complex formation between RNA polymerase and P_A in vitro using a gel-retardation assay. Together, our results indicate that IHF represses P_A by binding to P_A and inhibiting open complex formation.

MATERIALS AND METHODS

Bacteria

E. coli strain UT481 (met$^-$, thy$^-$, Δ(pro lac), r$^-$m$^-$, supD, F'::Tn10, traD36, proAB, lacI^q, lacZ, ΔM15) was from C. Lark and strains N99 (Str^r, sup°, galK$^-$, F$^-$) and HN414 (Str^r, sup°, galK$^-$, F$^-$, himA42) were from N.P. Higgins. Growth conditions and isolation of RNA for primer extension experiments have been described (6).

Plasmids

Construction of plasmids pRT11, pRT11del4, pRT11del30, pRT11del32, and pRT11del3 has been described (6). Plasmids pRT11 and pRT11del30 were used to transform E. coli strains N99 and HN414 by standard techniques (25).

Primers

The T7 promoter primer was from Promega Biotec. Primer #62 and the 5' end labeling of this primer have been described (6).

Primer extension

Extension of the T7 promoter primer employing total E. coli RNA as template has been described (6).

Preparation of end-labeled chloroplast DNA fragments

In all cases, the DNA fragment to be labeled was contained in the Sma I site in the polylinker of pGEM 1. To label the top strand (Fig. 4), ~10 pmoles of plasmid was cut with Hind III (which cuts once in the polylinker to the left of the Sma I site), dephosphorylated with calf intestinal phosphatase and then 5'-end-labeled with ^{32}P γ-ATP using polynucleotide kinase. A second restriction digestion with Eco RI (which cuts once in the polylinker to the right of the Sma I site) was performed and the released chloroplast DNA fragment, containing some vector sequences at its ends, was then purified by agarose gel electrophoresis. Following electroelution, several phenol/chloroform/isoamyl alcohol extractions and ethanol precipitation, the end-labeled fragments were dissolved at a concentration of 20-60 µg/ml in TE buffer containing 100 mM NaCl. To label the bottom strand, the above protocol was modified such that the first restriction digestion used Eco RI and the second digestion used Hind III.

DNAase I footprinting

The protocol of Craig and Nash (10) was used except that the DNAase I reactions were stopped by the addition of 100 µl of ice cold phenol/chloroform/isoamyl alcohol. The amounts of labeled DNA and IHF used in the various experiments are indicated in the figure legends. A + G and T + C sequencing reactions were carried out as described by Maxam and Gilbert (26). Dried DNA pellets from the footprinting and sequencing reactions were resuspended in loading buffer (90% formamide, 100 mM Tris-borate [pH 8.3], 2.5 mM EDTA, 0.1% xylene cyanol and 0.1% bromphenol blue), boiled for 2 min, quick chilled on ice and loaded onto standard urea-polyacrylamide sequencing gels.

Methylation experiments

Supercoiled plasmid pRT11 was methylated in vitro, in the presence and absence of IHF, with dimethylsulfate (DMS) (Aldrich Chemical Company). Methylation reactions, DNA extraction and restriction (with Hind III and Eco RI), and strand scission reactions were performed as described by Richet et al. (27).

Electroblotting and hybridization

The products of the in vitro methylation experiments were electrophoresed under denaturing conditions as described above and then electroblotted onto Genescreen (New England Nuclear). Electroblotting was

done in 0.05x TBE in a Bio Rad Trans-Blot Cell employing a standard power supply (Model 320-G Dan-Kar Corp.). The polyacrylamide gel was appropriately trimmed before being placed into the Trans-Blot Cell. Electrophoresis was for 30 min at 120 V (max. current 200 mA). The resultant blot was subsequently processed essentially as described by Richet et al. (27) with the following modifications: (1) hybridization was at 42°C and employed ~25 pmoles of end-labeled primer #62 and; (2) the blots were washed eight times at 42°C with 500 ml of washing buffer (5 min per wash).

Formation and separation of P_A-RNA polymerase open complexes (28)

Binding reactions (215 µl at 37°C) contained 52 mM Tris-HCl (pH 7.4), 70 mM KCl, 7 mM $MgCl_2$, 3.0 mM $CaCl_2$, 1.1 mM EDTA, 1.0 mM β-mercaptoethanol, 10% (v/v) glycerol and 200 µg/ml BSA. A ^{32}P-end-labeled 117 bp chloroplast DNA fragment containing P_A (obtained from plasmid pRT11del30 and labeled at the Eco RI site as described above) was present at ~0.54 nM. Reactions were started by adding E. coli RNA polymerase to ~1.4 nM. When IHF was included (~10-40 nM final concentration), it was preincubated with the DNA for 5 min at 37°C prior to adding the RNA polymerase. At various times after polymerase addition, 23 µl aliquots were removed and added to tubes containing 5 µl heparin (250 µg/ml) at 37°C. Incubation was continued for 3 min at which time 2.5 µl loading dye (same buffer as above containing 5% Ficoll and a trace of bromophenol blue) were added and the samples applied to a 4% polyacrylamide gel already running at 80 V. After all samples were loaded the voltage was increased to 140 V. Electrophoresis was at 37°C in 1X TBE buffer for ~2.5 hrs.

Enzymes and other reagents

Pure IHF and RNA polymerase were generous gifts of H. Nash and D. Burgess, respectively. Eco RI was from Boehringer Mannheim and Hind III was purified by M. Gruidl in this laboratory. AMV reverse transcriptase was from Life Sciences. T4 polynucleotide kinase was from U.S. Biochemicals. Calf intestinal alkaline phosphatase was from Boehringer Mannheim. Formic acid was from Aldrich Chemical Company. Hydrazine was from Kodak. Piperidine was from Fisher Scientific Company. Deoxynucleotide triphosphates were from P.L. Biochemicals and [γ-^{32}P]ATP (~4500 Ci/mmole) was from New England Nuclear.

RESULTS

The himA42 mutation increases transcription from P_A

HimA42 is a missense mutation in the gene for the α-subunit of E. coli IHF (24). To test the effect of this mutation on P_A activity, two E. coli

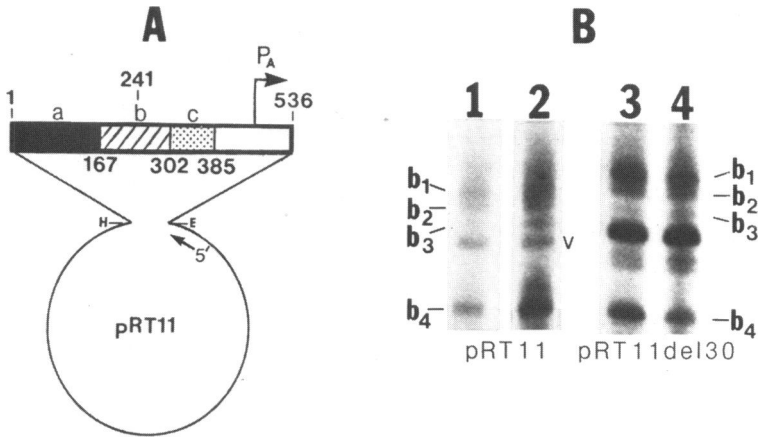

Figure 1. Primer extension analysis of RNA isolated from E. coli bearing plasmids pRT11 and pRT11del30. Panel A: A 536 bp chloroplast DNA fragment containing P_A was cloned into the Sma I site of vector pGEM 1 to generate plasmid pRT11. Flanking Hind III and Eco RI sites are indicated by H and E, respectively. P_A is oriented such that transcripts originating from it are complementary to the T7 promoter primer (counterclockwise arrow). Region a is not crucial for normal regulation of P_A whereas regions b and c each contain different silencing elements which reduce P_A activity in supercoiled plasmids (6). The sequence extending from bp 241 - 536 is shown in Figure 4. The numbered positions beneath the chloroplast DNA insert indicate the right borders of various deletions described in Thompson and Mosig (6). Sequences from bp 1 - 385 are deleted in plasmid pRT11del30. Panel B: Primer extension products. b_1-b_4 correspond to the 5' ends of transcripts initiated at P_A. The small v to the right of lane 2 indicates a product observed with the pGEM 1 vector alone (6). Lanes 1 and 2: 50 µg RNA from N99 IHF^+ cells and HN414 (himA42) cells, respectively, containing pRT11. Lanes 3 and 4: 5 µg RNA from N99 and HN414 cells, respectively, containing pRT11del30.

strains, HN414 (himA42) and its isogenic IHF^+ parent N99, were transformed with plasmid pRT11 or plasmid pRT11del30, both containing the chloroplast promoter P_A (Fig. 1A). In the latter plasmid, chloroplast DNA upstream of P_A from bp 1 - 385 of the sequence of Thompson and Mosig (6) is deleted. Transcription from P_A was measured by primer extension employing total RNA from plasmid-bearing E. coli as template and the T7 promoter primer as primer (Fig. 1A). Analogous primer extension experiments have previously shown (6) that in UT481 bacteria these plasmids give rise to at least four transcripts with 5' ends mapping immediately downstream of P_A (called b_1-b_4).

From plasmid pRT11, the himA42 mutation clearly increased the abundance of all P_A transcripts (Fig. 1B, lanes 1 and 2). This increase was not due to differences in copy number or superhelical density of the plasmid in the

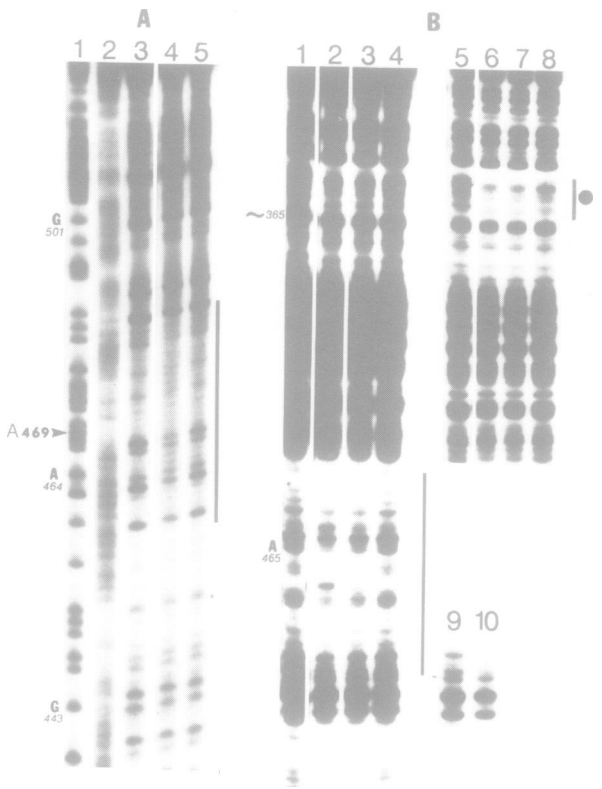

Figure 2. Protection from DNAase I of chloroplast DNA overlapping P_A by IHF. The unlabeled vertical bars indicate the protection of the P_A region. The vertical bar marked with a dot denotes a second IHF binding site located about 100 bp upstream of P_A. The numbered nucleotides correspond to positions in the DNA sequence shown in Figure 4. A_{469} is protected by IHF from methylation (Fig. 3). Panel A: chloroplast DNA (~0.18 pmoles/reaction) extending from position 430 to 536 (Figs. 1A and 4) labeled on the top strand. Lane 1, G + A sequencing reactions; lane 2, T + C sequencing reactions; lane 3, 0 µg/ml IHF; lane 4, 0.25 µg/ml IHF; lane 5, 0.08 µg/ml IHF. Panel B: chloroplast DNA (~0.04 pmoles/reaction) extending from bp 1 - 536 (Figs. 1A and 4) labeled on the bottom strand. Lane 1, 0 µg/ml IHF; lane 2, 0.25 µg/ml IHF; lane 3, 0.08 µg/ml IHF; lane 4, 0.02 µg/ml IHF; lanes 5, 6, 7, and 8 same as lanes 1, 2, 3, and 4, respectively, except that the autoradiogram was exposed less; lanes 9 and 10, same as lanes 1 and 2, respectively, only the autoradiogram was exposed less. The sequencing ladders used to map the DNase I digestion products are not shown.

two bacterial strains (not shown). In contrast to the results with pRT11, the himA42 mutation did not increase the abundance of P_A transcripts from plasmid pRT11del30 (Fig. 1B, lanes 3 and 4). We have previously shown that

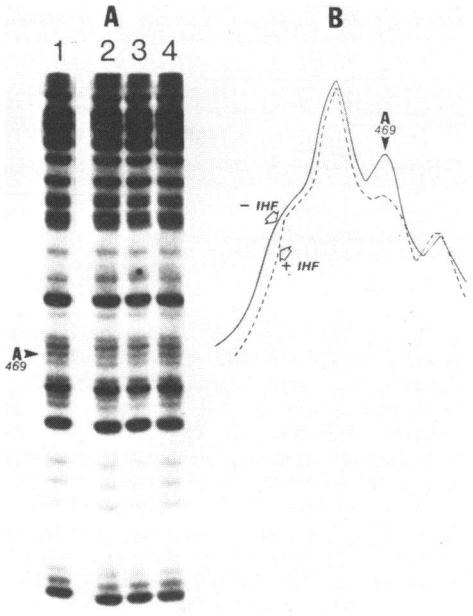

Figure 3. Methylation of supercoiled plasmid pRT11 in vitro in the presence and absence of IHF. The protocol used detects cleavages at purines on the top strand of the chloroplast DNA in the immediate vicinity upstream of primer #62 (Fig. 4). Details are in Materials and Methods. Numbered nucleotides refer to positions in Figure 4. Panel A: Lane 1, 0 µg/ml IHF; lane 2, 7.5 µg/ml IHF; lane 3, 2.5 µg/ml IHF; lane 4, 0.8 µg/ml IHF. Methylation of A_{469} is reduced by IHF. Panel B: densitometric scan of lanes 1 and 2 from Panel A.

at least two upstream DNA elements which are deleted in this plasmid silence P_A (6) (Fig. 1A). Consistent with these results, P_A transcripts made from pRT11del30 were much more abundant than P_A transcripts from pRT11 (Fig. 1B, compare lanes 1 and 3 and note the different amounts of RNA used). The himA42 mutation thus appears to increase the abundance of P_A transcripts only in the presence of these silencing sequences upstream of P_A.

Our results with plasmid pRT11del30 also exclude the possibility that the himA mutation increases transcript stability. We conclude that the mutation increases transcription from P_A.

In vitro, IHF binds to a site overlapping P_A

We have used protection against DNAase I attack and against methylation to probe for specific interactions between IHF and the chloroplast DNA encompassing P_A. The results show that IHF binds, among others, to a site which physically overlaps P_A. The experiments shown in Figure 2 demonstrate

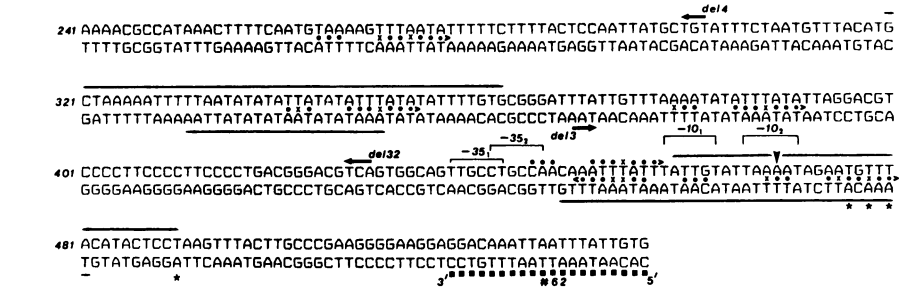

Figure 4. DNA sequence of the last 295 bp of the chloroplast DNA insert of plasmid pRT11 showing P_A and both IHF binding sites. Numbering of the sequence is as in Figure 1A. Bars indicate regions of strong protection from DNAase I. On the upper strand of the stronger IHF site there is a gap in the DNAase I protection from A_{326} to T_{332}. We have marked this region as protected because bonds further upstream are clearly protected. The -35 and -10 regions of P_A are indicated by brackets and the 5' ends of transcripts b_1-b_4 are indicated by asterisks. Several IHF-like consensus binding sequences, YAA...RTTGAT(A,T), are indicated (dots represent a match with the consensus, Xs a mismatch). The polarity of these sequences is indicated by an arrowhead. The end points of various deletions are indicated by horizontal arrows labeled del4, del32 (tail denotes the right border of the deletion) and del3 (tail denotes the left deletion end point). DNA fragments containing these deletions were used in the DNAase I footprinting experiments thus allowing for accurate mapping (i.e., ±1 bp) of the DNAase I cutting sites. Primer #62, used in the methylation protection experiments, is indicated by the labeled squares. A_{469} (arrowhead) is weakly protected from methylation in vitro by IHF.

the protection against DNAase I provided by IHF to the top (A) and bottom (B) strands of this site. Protection against methylation at this site by IHF is demonstrated in Figure 3. The results of both approaches are summarized in Figure 4 and are discussed below.

On the top strand (Fig. 2A), IHF protected a region of ~32 nucleotides overlapping P_A, from A_{459} to T_{490} (Fig. 4). On the bottom strand (Fig. 2B), it protected a region of ~34 nucleotides, from G_{448} to T_{481}. There are several IHF-like consensus binding sequences in the immediate vicinity of these protected regions (Fig. 4). Like other IHF binding sites (10), this site contains DNAase I sensitive and hypersensitive phosphodiester bonds within the protected region (Fig. 2). Based on the degree of protection as a function of IHF concentration (Fig. 2 and not shown), we estimate that ~0.1-0.3 µg/ml of IHF renders this IHF site 50% resistant to DNAase I. This estimate is similar to the value of 0.4 µg/ml IHF reported by Krause and Higgins (21) to render the IHF site in the phage Mu operator 50% resistant

to DNAase I. Binding of IHF to this site is known to modulate Mu transcription (21).

DNAase I footprinting also revealed another IHF binding site about 100 bp upstream of P_A (Fig. 2B, lanes 5-8). This upstream site had a greater affinity for IHF than the site overlapping P_A (Fig. 2B, compare lanes 4 and 8). Precise mapping of the upstream site (not shown) showed that it is contained within silencing element c (Fig. 1A) in a stretch of DNA consisting almost entirely of AT base-pairs (Fig. 4). There was no detectable difference in the amount of IHF required to footprint the site at P_A when this upstream IHF site was deleted (not shown).

Additional footprinting experiments (not shown) revealed no other preferred IHF binding sites on the chloroplast DNA of plasmid pRT11 at least up to bp 62 (Fig. 1A), despite the fact that several other sequences within the region resemble IHF consensus target binding sequences (Fig. 4 and Fig. 1 in ref. 6). Other investigators have observed that at least some sequences resembling the IHF consensus binding sequence do not bind IHF *in vitro* (13).

Protection from methylation showed that IHF makes at least one tight contact with the DNA containing P_A. Supercoiled plasmid pRT11 was methylated *in vitro* with DMS in the presence or absence of IHF. After cutting with the appropriate restriction enzymes, strand cleavage at methylated purines (G>A) was performed using standard techniques (26). The resulting DNA fragments were separated by denaturing polyacrylamide gel electrophoresis, electroblotted to Genescreen and probed with ^{32}P-end-labeled primer #62 (Fig. 4). IHF clearly protected A_{469}, within the core of the IHF binding site which overlaps P_A (Fig. 4), from methylation (Fig. 3).

IHF inhibits formation of P_A-RNA polymerase open complexes

The above results suggested that binding of IHF to P_A might directly inhibit transcription initiation from this promoter. Therefore, we have examined *in vitro* if IHF inhibits formation of P_A-RNA polymerase open complexes using the gel-retardation assay described by Straney and Crothers (28). A ^{32}P-end-labeled chloroplast DNA fragment containing P_A (but lacking the upstream IHF site), was incubated with purified *E. coli* RNA polymerase in the presence or absence of IHF. Protection of this fragment by RNA polymerase from DNAase I digestion indicated that the polymerase bound specifically to P_A (not shown). At various times after addition of RNA polymerase, heparin was added to prevent further open complex formation and to dissociate RNA polymerase from closed complexes at P_A and from

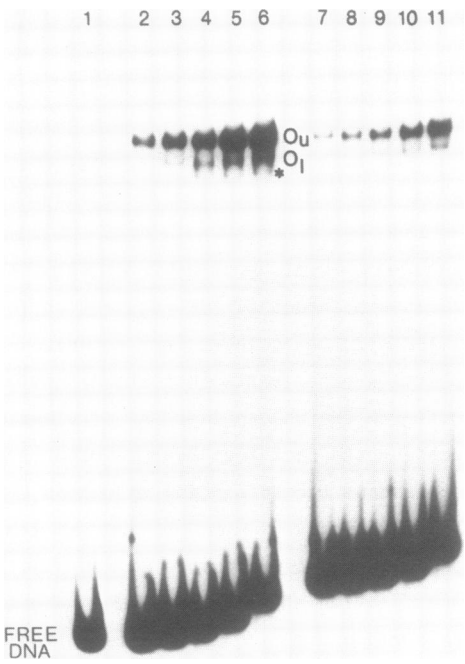

Figure 5. IHF inhibits formation of P_A-RNA polymerase open complexes. Binding reactions were at 37°C and contained ~60 ng/ml (0.54 nM) ^{32}P-end-labeled chloroplast DNA (171 bp from bp 386 - 534 in Figure 4 plus some vector sequences) containing P_A, ~0.69 µg/ml (1.4 nM) E. coli RNA polymerase (lanes 2-11) and where indicated ~0.4 µg/ml (20 nM) pure IHF (lanes 7-11) (see Materials and Methods for further details). The IHF was added 5 min prior to the RNA polymerase. Reactions were terminated with heparin (50 µg/ml final concentration for 3 min at 37°C), loading dye was added and the samples were electrophoresed in a non-denaturing 4% polyacrylamide gel at 37°C. O_u (open upper) and O_l (open lower) correspond to the two types of open complexes described by Straney and Crothers (28). A minor complex of unknown origin is marked with an asterisk. Lane 1: no proteins added; lanes 2 and 7, 1 min; lanes 3 and 8, 2 min; lanes 4 and 9, 4 min; lanes 5 and 10, 8 min; lanes 6 and 11, 16 min.

The absence of a retarded band resulting from IHF binding (lanes 7-11) might be due to the instability of the P_A-IHF complex at 37°C (especially in the presence of heparin). In addition, IHF may be unable to stably bend the DNA at this temperature. At 4°C a retarded band resulting from IHF binding was observed (not shown).

non-specific complexes. The heparin resistant open complexes were then separated from the protein-free DNA by electrophoresis in non-denaturing polyacrylamide gels.

Addition of RNA polymerase alone to DNA yielded a major retarded band, open complex upper (O_u), and a minor retarded band of slightly greater

mobility, open complex lower (O_1) (Fig. 5) (28). An additional minor complex of unknown origin was also seen in some experiments in the absence of IHF (Fig. 5, lanes 4-6). IHF clearly inhibited formation of all of these complexes (Fig. 5, lanes 7-11). Our results do not distinguish whether IHF inhibits closed complex formation at P_A or isomerization of the closed to the open complex.

The amount of IHF required to inhibit formation of P_A-RNA polymerase open complexes was several fold higher than that required to footprint P_A. For example, in the experiment shown in Figure 5 the molar ratio of IHF (~20 nM) to DNA (~0.54 nM) was ~40. Lower IHF concentrations did not detectably inhibit open complex formation (not shown). In contrast, molar ratios of IHF to DNA of less than 10 clearly were sufficient to footprint P_A (Fig. 2). Most likely, temperature contributed to this apparent difference; P_A-RNA polymerase open complexes had to be formed at 37°C because none were detected at 25°C, the temperature used in the footprinting experiments. Using protection against DNAase I, we have observed that binding of IHF to P_A is reduced at 37°C as compared to 25°C. Prentki *et al.* (29) have previously reported that binding of IHF to at least some sites becomes weaker as the temperature is increased.

DISCUSSION

We have presented three lines of evidence that in *E. coli*, *Chlamydomonas* chloroplast promoter P_A is repressed by IHF. Firstly, *in vivo* the *himA42* mutation, altering the α-subunit of IHF, results in the over-accumulation of P_A transcripts assayed by primer extension (Fig. 1). Secondly, *in vitro* DNAase I (Fig. 2) and methylation protection (Fig. 3) experiments show that IHF binds to a site which physically overlaps P_A (Fig. 4). This site contains sequences which resemble the consensus IHF-binding sequence. Thirdly, *in vitro*, IHF inhibits formation of P_A-*E. coli* RNA polymerase open complexes assayed by gel-retardation (Fig. 5). We interpret our results, taken together, to mean that repression of P_A by IHF requires, at least in part, binding of IHF to a site which physically overlaps P_A. This repression may be at the level of P_A-RNA polymerase closed complex formation (i.e., IHF hinders RNA polymerase binding to P_A) and/or at the level of open complex formation (i.e., binding of IHF to P_A alters the isomerization of RNA polymerase bound to P_A). The *himA42* mutation, by functionally altering IHF, allows constitutive transcription from P_A. Since inhibition by IHF involves sequences upstream of P_A (see below), the IHF

mutation is unlikely to act at the level of RNA stability. We have also excluded the possibilities that it changes the copy number or the superhelical density of the P_A containing plasmids.

In two other promoters which contain IHF target sites, i.e., the phage Mu P_{CM} promoter and the phage λ promoter formed by the cin-1 mutation, initiation of transcription is inhibited by binding of IHF (N.P. Higgins, personal communication and M. Gottesman, A. Oppenheim and G. Griffo, personal communication). Autoregulation of the α-subunit gene (22) and perhaps of the β-subunit gene (7) of IHF is also thought to involve binding of IHF to the respective promoters. Our results with a chloroplast promoter, together with these other observations suggest that inhibition of promoter activity by binding of IHF (or IHF-like proteins) may be a rather general phenomenon.

Our in vitro results suggest that binding of IHF to P_A alone can suffice to repress P_A (Fig. 5). In vivo, however, chloroplast DNA sequences upstream of P_A are important for this repression (Fig. 1B). The relevant upstream chloroplast sequences most likely reside between bp 167 and 385 in Fig. 1A, i.e., DNA upstream of bp 167 is not important for the control of P_A (6) while P_A is derepressed when DNA up to bp 385 is deleted in plasmid pRT11del30 (Fig. 1B). We suggest that in vivo, physical and/or functional interactions between IHF and one or more proteins bound to this upstream region augment repression of P_A by IHF at low intracellular IHF concentrations. This situation would be analogous to the requirement for the enhancer for binding of transcriptional activator NR_1 to the glnAp1 promoter of E. coli; the enhancer is required at low, but not high, concentrations of NR_1 (30). Upstream DNA might in addition silence P_A by mechanisms not involving IHF.

The presence of a strong IHF binding site between bp 167 and 385 suggested that one of the regulatory proteins proposed to bind in this region might be IHF itself. Site-specific deletion of this IHF site alone (i.e., bp 317-362 in Fig. 4), however, did not derepress P_A (not shown) suggesting that one or more factors other than IHF bind to the region between bp 167 and 385. One of these factors could be E. coli FIS protein (Factor for Inversion Stimulation) (31). Purified FIS footprinted a region of DNA between the two IHF binding sites (i.e., bp 346 - 396 in Fig. 4; not shown) and most of this region is deleted in plasmid pRT11del30 (Fig. 4). In other systems, interacting IHF and FIS binding sites are in close proximity (32).

It is possible that the upstream IHF site could still function in the control of P_A at least under certain conditions. For example, the silencer of the yeast mating type locus consists of three short DNA sequences which bind trans-acting factors (33). Each of these elements can be mutated individually without seriously reducing silencer activity. Mutating any two of three elements, however, destroys this activity. By analogy, deletion of the upstream IHF site might reduce repression of P_A only when the function of some other trans-acting factor (FIS?) is simultaneously altered (e.g., by environment or mutation).

The silencing of P_A by proteins bound to upstream sequences could also be influenced by supercoiling of the DNA. Although we have no direct evidence linking the supercoiling control of P_A (6) with IHF, we note that in the divergent promoters of the phage Mu operator region (i.e., P_E and P_{CM}) IHF modulates the relative activities of these promoters in a manner which is dependent upon DNA supercoiling (N.P. Higgins, personal communication).

A significant implication of our results is that in the chloroplast, like in bacteria and nuclei of eukaryotic cells, combinations of trans-acting proteins modulate promoters by binding to cis-acting DNA elements. Our results suggest that one such chloroplast factor is an analog of E. coli IHF. A chloroplast analog of E. coli HU, a protein related to IHF, has already been described (34). We are presently attempting to isolate genes from Chlamydomonas chloroplast and/or nuclear DNA which might code for IHF-like factors and to express these genes in E. coli.

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