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A Sequence-Specific DNA-Binding Factor (VF1) from *Anabaena* sp. Strain PCC 7120 Vegetative Cells Binds to Three Adjacent Sites in the *xisA* Upstream Region

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A DNA-binding factor (VF1) partially purified from *Anabaena* sp. strain PCC 7120 vegetative cell extracts by heparin-Sepharose chromatography was found to have affinity for the *xisA* upstream region. The *xisA* gene is required for excision of an 11-kilobase element from the *nifD* gene during heterocyst differentiation. Previous studies of the *xisA* upstream sequences demonstrated that deletion of this region is required for the expression of *xisA* from heterologous promoters in vegetative cells. Mobility shift assays with a labeled 250-base-pair fragment containing the binding sites revealed three distinct DNA-protein complexes. Competition experiments showed that VF1 also bound to the upstream sequences of the *rbcL* and *glnA* genes, but the *rbcL* and *glnA* fragments showed only single complexes in mobility shift assays. The upstream region of the *nifH* gene formed a weak complex with VF1. DNase footprinting and deletion analysis of the *xisA* binding site mapped the binding to a 66-base-pair region containing three repeats of the consensus recognition sequence ACATT.

Anabaena sp. strain PCC 7120 is a filamentous cyanobacterium capable of fixing atmospheric dinitrogen in terminally differentiated cells called heterocysts (23). Heterocysts form only during conditions of combined nitrogen deprivation. Differentiation of vegetative cells into heterocysts is accompanied by a globally coordinated regulation of gene expression (8). For example, nitrogen fixation (*nif*) genes are expressed only in heterocysts and genes encoding photosynthetic proteins, such as ribulose-1,5-bisphosphate carboxylase (*rbcLS*) are expressed only in vegetative cells (23). Some genes, such as *glnA*, which encodes glutamine synthetase, are expressed in both cell types (21). An important feature of induced or developmentally expressed genes is their regulation by *trans*-acting DNA-binding proteins. These proteins mediate regulation of transcription by acting as repressors and activators near the promoter (19) or as enhancers (18, 22). Target genes for transcription factors usually have specific DNA-binding sites in their upstream region.

The genome of *Anabaena* sp. strain PCC 7120 undergoes at least two specific developmentally regulated DNA rearrangements during heterocyst differentiation (9). One rearrangement involves the nitrogen fixation genes *nifH*, *nifD*, and *nifK*. During heterocyst differentiation, an 11-kilobase DNA element is excised from the coding region of the *nifD* gene by site-specific recombination between 11-base-pair (bp) directly repeated sequences present at the ends of the element (10). The excision results in formation of the complete *nifD* coding sequence and allows expression of the three genes as an operon transcribed from a single promoter (12). This results in a contiguous *nifHDK* operon necessary for synthesis of nitrogenase mRNA in heterocysts.

The gene *xisA*, located at one end of the 11-kilobase element, is required for the excision of the element cloned into *Escherichia coli* plasmids and for excision during heterocyst differentiation (11, 15). The presence of the *xisA* gene is sufficient to cause rearrangement of a substrate plasmid in *E. coli* (6). It is likely that the *xisA* gene is developmentally regulated because its activity, seen as the excision of the *nifD* element, is expressed only during heterocyst differentiation (10), but nothing is known about the mechanism of this regulation.

Expression of the *xisA* gene from a heterologous promoter in *Anabaena* sp. strain PCC 7120 vegetative cells results in the excision of the 11-kilobase *nifD* element (6). A regulatory region upstream of the *xisA* gene prevented expression of functional XisA in vegetative cells. When this region was deleted, *xisA* was expressed in vegetative cells and caused the excision of the *nifD* element from the chromosome (6).

The mechanism of *xisA* regulation during heterocyst development is not known. In this study, we report that a DNA-binding factor (VF1) partially purified from *Anabaena* sp. strain PCC 7120 vegetative cells has specific affinity for three adjacent sites in the upstream region of the *xisA* gene and that the binding site is in close proximity to sequences involved in suppression of *xisA* expression in vegetative cells. We also show that fractions containing VF1 bind to the upstream regions of the *rbcL*, *glnA*, and *nifH* genes.

MATERIALS AND METHODS

Cyanobacterial and bacterial strains and culture conditions. *Anabaena* sp. strain PCC 7120 was grown in 100-ml BG-11 liquid cultures (2). Large-scale 2-liter cultures were grown in the liquid medium of Allen and Arnon (1) diluted eightfold with the following modifications: the K_2HPO_4 concentration was doubled to 0.5 mM; the nickel, chromium, tungsten, and titanium salts were omitted; and the medium was supplemented with MOPS (3-[*N*-morpholino]propanesulfonic acid) (5 mM) (pH 8.0) and NH_4NO_3 (2.5 mM). Cells were grown at 30°C with illumination at approximately 100 microeinsteins s^{-1} m^{-2} and bubbled with 1% CO_2 in air.

E. coli strains were maintained in LB liquid or on LB agar medium (Lennox L; GIBCO Laboratories, Life Technologies, Inc., Grand Island, N.Y.). For plasmid preparation,

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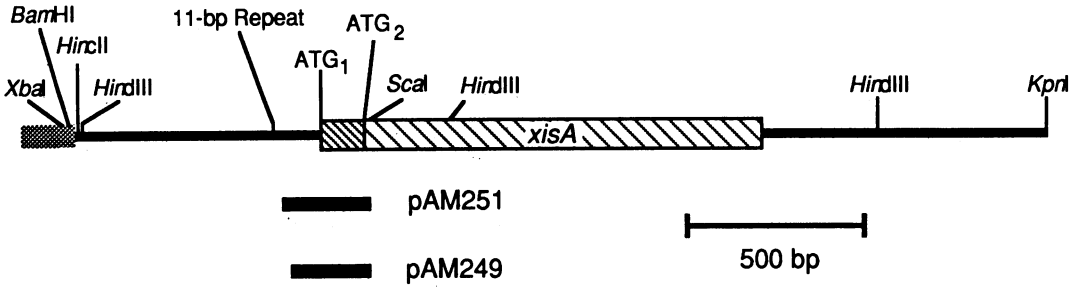


FIG. 1. Map of *Anabaena* sp. strain PCC 7120 *xisA* gene and flanking sequences. The *Hin*cII-*Kpn*I fragment from pAn207.62 (15) was used to generate 5′ deletions of the *xisA* upstream sequences. The location of the 11-bp repeat involved in site-specific recombination is shown upstream of the *xisA* gene. The first and second translational start sites (ATG_1 and ATG_2) of the *xisA* gene are shown. DNA fragments isolated from deletion plasmids pAM251 (247 bp) and pAM249 (218 bp) used in mobility shift assays are shown below the map. DNA fragments were isolated as *Xba*I or *Bam*HI-*Sca*I fragments; the *Xba*I and *Bam*HI sites are present in the vector multiple-cloning site of the deletion plasmids shown in the grey region at the extreme left.

strains were grown in 0.5× TB liquid medium, a variation of Terrific Broth (20) containing (per liter) 100 ml of KH_2PO_4 (0.17 M)–K_2HPO_4 (0.72 M), 6 g of peptone, 12 g of yeast extract, and 2 ml of glycerol. Media were supplemented with appropriate antibiotics according to standard procedures (16). Plasmids were maintained in *E. coli* DH5α (Bethesda Research Laboratories, Life Technologies, Inc., Gaithersburg, Md.).

***xisA* upstream region deletions.** The *Hin*cII-*Kpn*I fragment from pAn207.62 containing the entire *xisA* open reading frame including flanking 5′ sequences (15) was cloned into the *Pst*I site of pBluescript KS+ (Stratagene, La Jolla, Calif.). Deletions of the upstream region were made by exonuclease III digestion according to the instructions of the vendor (Erase-a-Base kit; Promega, Madison, Wis.).

DNA sequencing. Sequencing from double-stranded DNA was performed with a Sequenase kit (United States Biochemical Corp., Cleveland, Ohio) and [α-^{32}P]dATP. Plasmid DNA was prepared by an alkaline lysis-polyethylene glycol precipitation procedure (13).

Partial purification of vegetative cell DNA-binding factor (VF1). *Anabaena* sp. strain PCC 7120 filaments from a 5-day-grown, 2-liter culture were harvested by centrifugation at 15,000 × *g* for 5 min. The pelleted cells (in approximately 10 ml of medium) were suspended with 15 ml of homogenization medium (50 mM Tris chloride [pH 7.5], 1 mM EDTA, 2 mM dithiothreitol, 0.5% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 10% glycerol) and homogenized with an equal volume of glass beads (0.10-mm diameter) for 2 min at 0 to 4°C in a homogenizing mill (Braun). The cell lysate was centrifuged at 31,000 × *g* for 10 min and 142,000 × *g* for 60 min. The supernatant was fractionated as described before (7) by loading onto a 3-ml heparin-Sepharose CL6B (Pharmacia LKB Biotechnology Inc., Piscataway, N.J.) column, equilibrated with 0.1 M ammonium sulfate in buffer A (50 mM Tris chloride [pH 7.5], 0.1 mM EDTA, 0.1% Triton X-100, 2 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 10% glycerol). After loading, the column was washed with 200 to 250 ml of 0.1 M ammonium sulfate in buffer A and eluted with a 44-ml linear gradient of 0.1 to 1.0 M ammonium sulfate in buffer A. Fractions, 2 ml, were collected and dialyzed against 2.5 liters of 50 mM Tris chloride (pH 7.5)–0.1 mM EDTA–0.5 mM phenylmethylsulfonyl fluoride–10% glycerol. Samples, 5 μl, of each column fraction were assayed for DNA-binding activity in mobility shift assays. All steps after homogenization were carried out at 0 to 4°C. The dye binding assay of Bradford (5) was used to assay protein concentration.

Mobility shift assay. Mobility shift assays were performed as described by Ausubel et al. (3) and included the following modifications. A 5-μl sample of heparin-Sepharose-fractionated protein was equilibrated in binding buffer (4 mM Tris chloride [pH 8.0], 12 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid] [pH 7.9], 12% glycerol, 60 mM KCl, 0.5 mM EDTA, 1 mM dithiothreitol, 5 mM $MgCl_2$) containing 1.0 μg of poly(dI-dC) (Pharmacia LKB Biotechnology Inc.) and 0.2 to 0.5 ng of labeled DNA fragment with a final volume of 20 μl. After 15 min at 22 to 24°C, samples were loaded onto 15-cm 5% polyacrylamide gels (30:1 acrylamide/bisacrylamide, 50 mM Tris chloride, 380 mM glycine, 2 mM EDTA, 2.5% glycerol). Electrophoresis was performed at 4°C in a Tris-glycine running buffer (3). Samples were electrophoresed for 2.5 h at 30 mA. Gels were then dried and exposed to X-ray film.

Preparation of DNA fragments for mobility shift assays. Plasmids pAM251, pAM249, and pAM254 contain the *xisA* gene with different deletions of the upstream sequences. They correspond to plasmids pAM265, pAM264, and pAM268, respectively (6), except that the vector is pBluescript KS+ rather than pRL191*tac*. DNA fragments used for *xisA* upstream-region fragments were released from pAM251 and pAM249 by digestion with either *Xba*I or *Bam*HI and *Sca*I (Fig. 1). The *Xba*I and *Bam*HI sites are in the plasmid vector, and their position relative to the *Sca*I site varies depending on the extent of the deletion (6). A 195-bp DNA fragment containing the *Anabaena* sp. strain PCC 7120 *nifH* promoter region (21) (nucleotides −260 to −70) was subcloned into the *Eco*RV/*Hin*dIII site of pBluescript KS+ and released by digestion with *Xba*I and *Hin*dIII, producing a 233-bp fragment. A 270-bp *Hin*cII/*Xba*I fragment of plasmid pCP106 (P. Lammers, unpublished results) containing the *glnA* promoter region (nucleotides −220 to +29) of *Anabaena* sp. strain PCC 7120 was subcloned into the *Xba*I/*Sma*I site of pBluescript KS+ and released by digestion with *Xba*I and *Hin*dIII, producing a 292-bp fragment. A 397-bp *Hpa*I fragment from pAn602 (17) containing the *rbcL* promoter (nucleotides −602 to −205) of *Anabaena* sp. strain PCC 7120 was subcloned into the *Eco*RV site of pBluescript KS+ and released by digestion with *Xba*I and *Hin*dIII, producing a 439-bp fragment. The above fragments were labeled with either [α-^{32}P]dCTP and the Klenow fragment of DNA polymerase I or [γ-^{32}P]ATP and T4 polynucleotide kinase, using standard procedures (3).

DNase I footprinting. The 5′ ends of fragments used for DNase I footprinting were labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase to a specific activity of 8.9×10^7

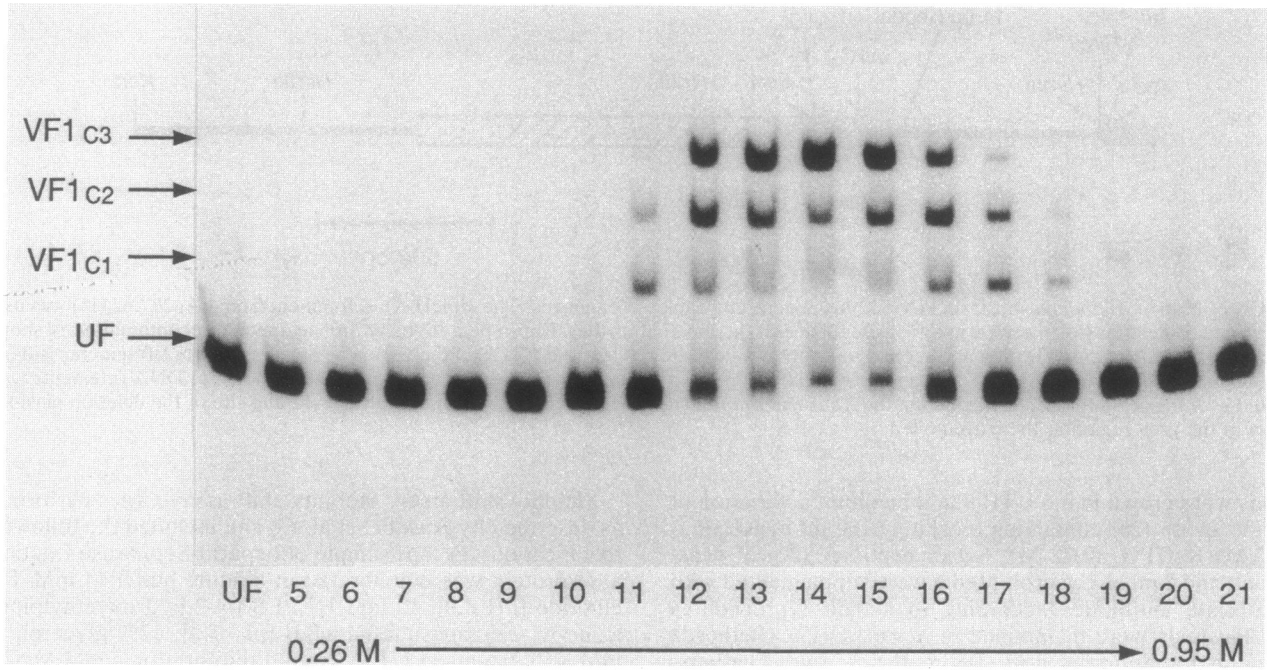


FIG. 2. Mobility shift assays of *Anabaena* sp. strain PCC 7120 vegetative cell lysate fractionated by heparin-Sepharose chromatography. Samples (5 μl) from column fractions 5 to 21 collected during elution with a linear 0.1 to 1.0 M ammonium sulfate gradient were equilibrated with 15,000 dpm of labeled pAM251 *Bam*HI-*Sca*I fragment and analyzed by a mobility shift assay. Positions of high, intermediate, and low relative molecular mass VF1 complexes and the unbound fragment are designated $VF1_{C3}$, $VF1_{C2}$, $VF1_{C1}$, and UF, respectively.

dpm/μg. The 3′ end of fragments were labeled with [α-^{32}P] dCTP and the Klenow fragment of DNA polymerase I to a specific activity of 3.2×10^7 dpm/μg. Approximately 25,000 dpm of either labeled fragment was incubated with various amounts of a heparin-Sepharose fraction containing the vegetative cell DNA-binding factor (VF1) in a 20-μl reaction volume containing 50 mM Tris chloride (pH 7.5), 5 mM $MgCl_2$, 6% glycerol, and 1 μg of poly(dI-dC). After a 20-min incubation at 22 to 24°C, 1 μl of DNase I (23 U/ml; Sigma Chemical Co.) was added. DNase I digestions were terminated after 2 min with 25 μl of stop solution containing 0.3 M sodium acetate (pH 5.2), 25 μg of tRNA, and 80 mM EDTA. The DNA was precipitated with 125 μl of ethanol, and the dry pellet taken up with 10 μl of 1:3-diluted sucrose-urea loading dye (300 mg of sucrose, 840 mg of urea, 40 μl of 0.5 M EDTA, 100 μl of 0.1% bromophenol blue, 100 μl of 0.1% xylene cyanol) (14). The samples were heated to 90°C for 3 min and loaded onto 10% sequencing gels.

RESULTS

Partial purification of *xisA* binding factor (VF1). Our identification of a regulatory region upstream of the *xisA* gene (6) prompted experiments designed to detect *trans*-acting DNA-binding factors in *Anabaena* sp. strain PCC 7120 vegetative cells. We initially used a fragment from pAM249 (shown in Fig. 1) because this was the smallest *xisA* fragment that did not allow expression of the *xisA* gene when conjugated to vegetative cells of *Anabaena* sp. strain PCC 7120 (6). Crude extracts of vegetative cells appeared to contain binding activity for a 230-bp *Xba*I-*Sca*I fragment from pAM249 that contains the *xisA* upstream region. These crude extracts also contained nonspecific DNA-binding activity. Partial purification of a vegetative cell DNA-binding factor (VF1) was accomplished by fractionation of a crude lysate by heparin-Sepharose column chromatography (7). Samples from a linear 0.1 to 1.0 M ammonium sulfate gradient were assayed with a mobility shift gel for binding activity by equilibration with a labeled 230-bp *Xba*I-*Sca*I fragment from pAM249. Two retarded bands were detected with heparin-Sepharose fractions containing VF1. Subsequent DNase I footprint analysis of the pAM249 fragment revealed that the pAM249 probe did not contain the complete binding site. A fragment derived from the next largest plasmid in our series of deletions, the 247-bp *Bam*HI-*Sca*I pAM251 fragment, was found to contain the complete VF1 binding site (see below and Fig. 5 and 6). The pAM251 fragment was used to analyze the heparin-Sepharose column fractions with a mobility shift assay (Fig. 2). Peak fractions of VF1 high ($VF1_{C3}$), intermediate ($VF1_{C2}$), and low ($VF1_{C1}$) relative molecular mass complexes were found to coelute between 0.55 and 0.71 M ammonium sulfate (Fig. 2, fractions 12 to 16).

Effect of VF1 concentration on DNA-protein complex formation. Increasing amounts of a heparin-Sepharose fraction containing VF1 resulted in a shift from the predominance of the $VF1_{C1}$ complex to the predominance of the $VF1_{C3}$ complex in a mobility shift assay with the *xisA* upstream fragment (Fig. 3). The heparin-Sepharose elution profile and the effect of VF1 concentration on the relative levels of $VF1_{C1}$, $VF1_{C2}$, and $VF1_{C3}$ suggest that with increasing concentration first one, then two, and finally three VF1 proteins bind the DNA fragment.

VF1 binding to *glnA*, *rbcL*, and *nifH* promoter regions. Restriction fragments from *Anabaena* sp. strain PCC 7120 *glnA*, *rbcL*, and *nifH* promoter regions were used in a mobility shift assay with the VF1-containing heparin-Sepharose fractions (Fig. 4). Relatively stable single DNA-protein complexes were observed for *glnA* and *rbcL* promoter fragments. The *nifH* promoter fragment formed a weak

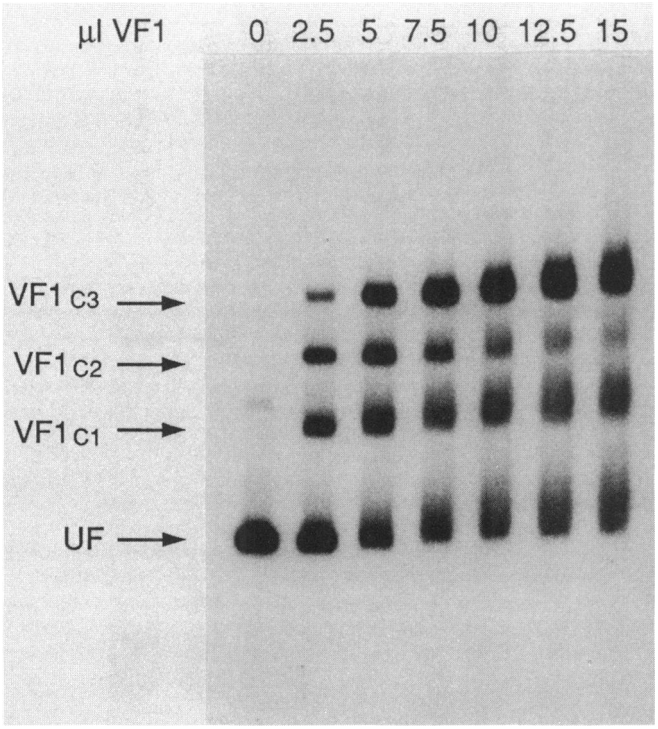


FIG. 3. Effect of increasing amounts of VF1 on high ($VF1_{C3}$), intermediate ($VF1_{C2}$), and low ($VF1_{C1}$) relative molecular mass complex stoichiometry. Mobility shift assays were carried out with the indicated volumes of a VF1-containing heparin-Sepharose fraction (0.1 µg of protein μl^{-1}) and 15,000 dpm of labeled *Bam*HI-*Sca*I pAM251 fragment. UF, Unbound fragment.

single DNA-protein complex. Detection of this less stable interaction was favored by preincubation at 37°C and by running the mobility shift assay gels for a shorter time. The single DNA-protein complexes formed with the *glnA*, *rbcL*, and *nifH* promoter regions contrast with the three VF1 complexes formed with the *xisA* upstream region. Competition experiments with unlabeled *glnA* and *rbcL* fragments demonstrated that these fragments compete with the labeled *xisA* pAM251 fragment for binding to VF1. A 50-fold excess of an unlabeled *glnA* fragment or a 100-fold excess of an unlabeled *xisA* fragment was required to compete effectively with the labeled *xisA* fragment for binding to VF1. A 200-fold excess of unlabeled *rbcL* fragment was required to effectively compete with the labeled *xisA* fragment for binding to VF1, while a 200-fold excess of a *nifH* fragment only partially competed with the labeled *xisA* fragment for binding. In reciprocal experiments, a 100-fold excess of unlabeled *xisA* pAM251 fragment was able to reduce VF1 binding to a labeled *glnA* fragment significantly, while a 50- and 25-fold excess of unlabeled *xisA* fragment completely blocked binding of VF1 to labeled *rbcL* and *nifH* fragments, respectively. These results suggest that VF1 binds strongly to the *glnA* fragment, moderately to the *xisA* and *rbcL* fragments, and weakly to the *nifH* fragment. A 175-bp *Pvu*I-*Xba*I fragment from the vector pBluescript KS+ and a 103-bp *Xba*I-*Sca*I fragment from the *xisA* deletion plasmid pAM254 did not show VF1 binding, and they did not compete with VF1 binding to the pAM249 fragment in mobility shift assays.

The *xisA*, *glnA*, *rbcL*, and *nifH* binding activities all coeluted in the same fractions during heparin-Sepharose column chromatography. This result is consistent with the competition studies and suggests that all four upstream regions interact with VF1. Although the VF1-containing heparin-Sepharose fractions are impure, we think that the chromatographic coelution results are significant because these procedures did resolve VF1 from additional DNA-binding factors with properties distinct from VF1.

VF1 binding site localization. Mobility shift assays of fragments deleted for various lengths of the *xisA* upstream region were used to identify the sequences involved in the formation of the three VF1 complexes. The *xisA* 5′ deletion plasmids generated in our previous studies, pAM251, pAM249, and pAM254 (6), contained fragments that produced three, two, and no VF1 complexes, respectively, in mobility shift assays (Fig. 5 and data not shown). To define more precisely the VF1 binding site, 5′ deletions of the *xisA* upstream region were prepared by exonuclease III digestion of pAM249. DNA fragments obtained from these deletion clones were used in a mobility shift assay (Fig. 5). pAM249 deletion Δ4 retained the $VF1_{C1}$ and $VF1_{C2}$ complexes. Deletions Δ12 and Δ13 showed only trace amounts of the $VF1_{C2}$ complex (only seen on longer exposures), but allowed the $VF1_{C1}$ complex to form. Deletion Δ18 showed no $VF1_{C2}$ complex and showed significantly reduced levels of the $VF1_{C1}$ complex. Further deletions, Δ30, Δ31, and Δ34, eliminate formation of the $VF1_{C1}$ complex. These experiments show that three distinct VF1 binding sites are adjacent to one another and that removal of upstream binding sites does not affect the formation of a complex with downstream binding sites significantly.

DNase I protection experiments were used to determine the region of DNA involved in VF1 binding (Fig. 6). Footprints from both *xisA* upstream coding and noncoding strands showed a protected region of approximately 63 (coding) and 66 (noncoding) bp beginning 143 bp upstream from the second translational start site of *xisA* (Fig. 5 and 6). The region shows uniform protection within the footprint with different amounts of VF1. This suggests that the three binding sites have approximately equal affinity for VF1.

DISCUSSION

Sequences shown to inhibit expression of the *Anabaena* sp. strain PCC 7120 *xisA* gene from heterologous promoters (6) specifically bind to a factor (VF1) present in vegetative cell extracts. VF1 binds to three adjacent sites in the *xisA* upstream region. Coelution of binding activity for the three *xisA* complexes as well as the *glnA*, *rbcL*, and *nifH* complexes during heparin-Sepharose column chromatography and the competition studies supports the contention that a single protein gives rise to all observed DNA-protein complexes. We do not know the subunit composition of the VF1 protein. Analysis of binding-site affinities and the possibility of cooperative binding to the *xisA* upstream region will require quantitative studies with purified VF1.

The symmetrical heparin-Sepharose column elution profile and the data shown in Fig. 3 demonstrate that increasing concentrations of VF1 result in a shift to higher-molecular-weight complexes with the *xisA* DNA fragments. This suggests that the same protein is binding to each of the three adjacent sites. Using the method of Bading (4), the protein components of the $VF1_{C1}$, $VF1_{C2}$, and $VF1_{C3}$ complexes were estimated to have relative molecular masses of approximately 45,000, 80,000, and 120,000, respectively. The additive differences in estimated relative molecular mass is consistent with the view that the three complexes are formed

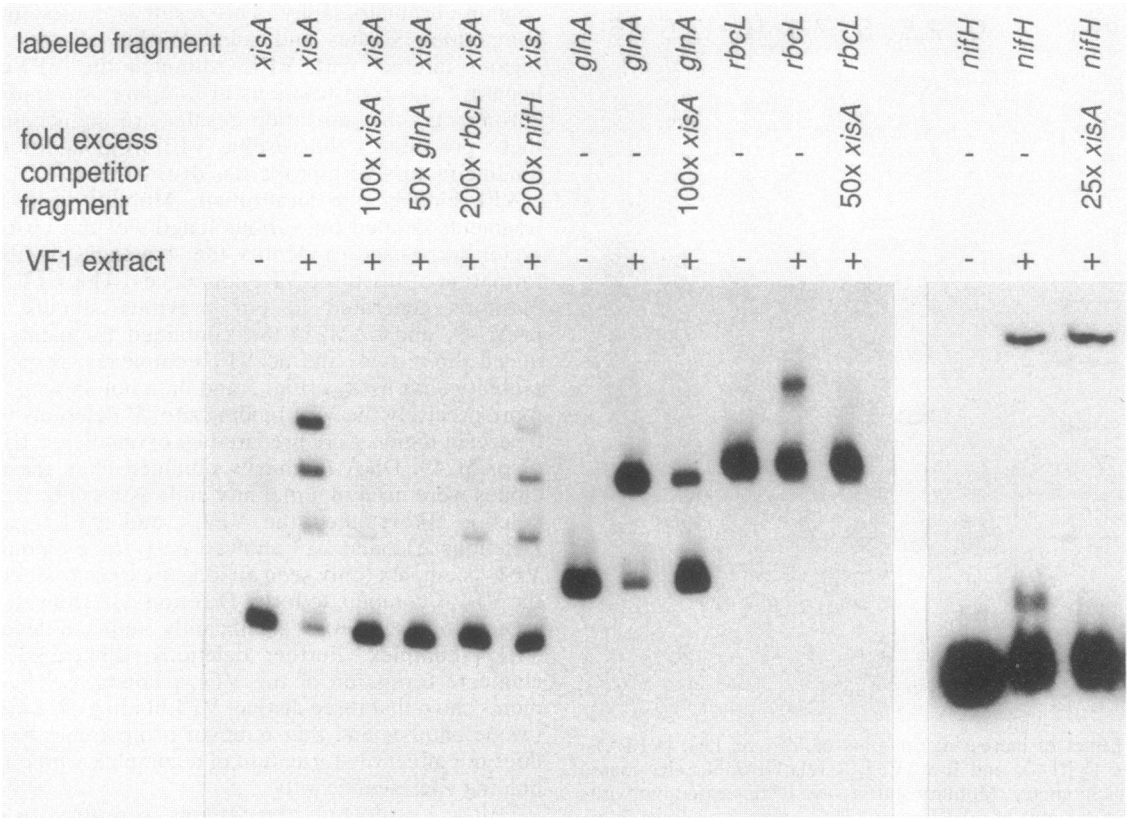


FIG. 4. VF1 binding with *glnA*, *rbcL*, and *nifH* promoter region fragments. Mobility shift assays were carried out with 0.4 μg of protein from a VF1-containing heparin-Sepharose fraction and 6,000 dpm of the indicated labeled DNA fragment with or without unlabeled competitor DNA fragment. The fold excess unlabeled competitor fragment added on a weight basis is indicated above the lanes. Labeled fragments had a specific activity of 1.2×10^7 to 3.0×10^7 dpm μg^{-1}. The *xisA*, *glnA*, and *rbcL* samples were run on a 5% polyacrylamide gel. The mobility shift assay with the labeled *nifH* fragment was preincubated at 37°C and run on a 7-cm gel to avoid dissociation of the complex, since the *nifH*-VF1 complex is relatively unstable. The upper bands produced in the presence of VF1 with the labeled *nifH* fragment mark the position of the sample wells. The position of the unbound fragment for each labeled fragment can be seen in lanes lacking VF1 extract.

from the same species, perhaps binding as monomer-dimer-trimer or dimer-tetramer-hexamer complexes.

DNase I footprinting of *xisA* with VF1 showed the protection of approximately 66 bp beginning 143 bp upstream from the second translational start site (Fig. 5 and 6). Deletion of 16 bp into this region eliminated the formation of the largest ($VF1_{C3}$) of three complexes in mobility shift assays. Further deletion of 18 bp eliminated the $VF1_{C2}$ complex and resulted in the reduction of signal from the $VF1_{C1}$ complex. Finally, deletion of another 12 bp eliminated VF1 binding altogether. These deletions and the uniform protection within the DNase I footprint indicate that the three VF1 binding sites are adjacent to one another; this close spacing could facilitate protein-protein interactions between VF1 proteins. Sequences containing obvious dyad symmetry are absent within this protected area. The DNase I-protected sequences correspond well with the region identified by mobility shift assays of deletion fragments. The VF1 binding site sequence contains three repeated sequences (ACATT) that may be involved in the VF1-DNA interaction (Fig. 5). The ACATT repeats can be aligned to appear in the same portion of three adjacent 18-bp protected regions within the VF1-*xisA* footprint (Fig. 5A). The *xisA* distal repeat (labeled a) is on the opposite strand relative to the other two repeats (labeled b and c), suggesting that VF1 binds to this site in an inverted orientation relative to the two *xisA* proximal sites. If the ambiguous *xisA* proximal border of the footprint is neglected, the three 18-bp sequences contain all but 3 bp of the footprint. An alignment of the three 18-bp sequences shows some similarity between the 5′ ends of each sequence, as well as the perfectly conserved ACATT sequence at the 3′ end of each sequence (Fig. 5C). Since deletions of the less conserved region of the b and c 18-bp sequences is sufficient to eliminate formation of a VF1 complex, we think that the weakly conserved region of each 18-bp sequence is important in the formation of a VF1 complex. We cannot assign any of the 18-bp sequences to the $VF1_{C1}$, $VF1_{C2}$, or $VF1_{C3}$ complexes. It may be that $VF1_{C1}$ and $VF1_{C2}$ contain mixtures of DNA molecules with VF1 bound to any one or two of the three possible binding sites. The DNase footprint at lower VF1 concentrations did not show preferential binding to any of the three binding sites.

The role of VF1 in *xisA* gene expression is unclear. *xisA* activity is developmentally regulated as indicated by excision of the *nifD* element only during heterocyst formation, but *xisA* transcription has not been detected in vitro and the *xisA* promoter has not been identified (6). We have recently demonstrated that *xisA* activity can be detected in *Anabaena* sp. strain PCC 7120 vegetative cells transformed with an expression vector containing *xisA* preceded by the *E. coli tac* promoter (6). Deletion of upstream sequences was re-

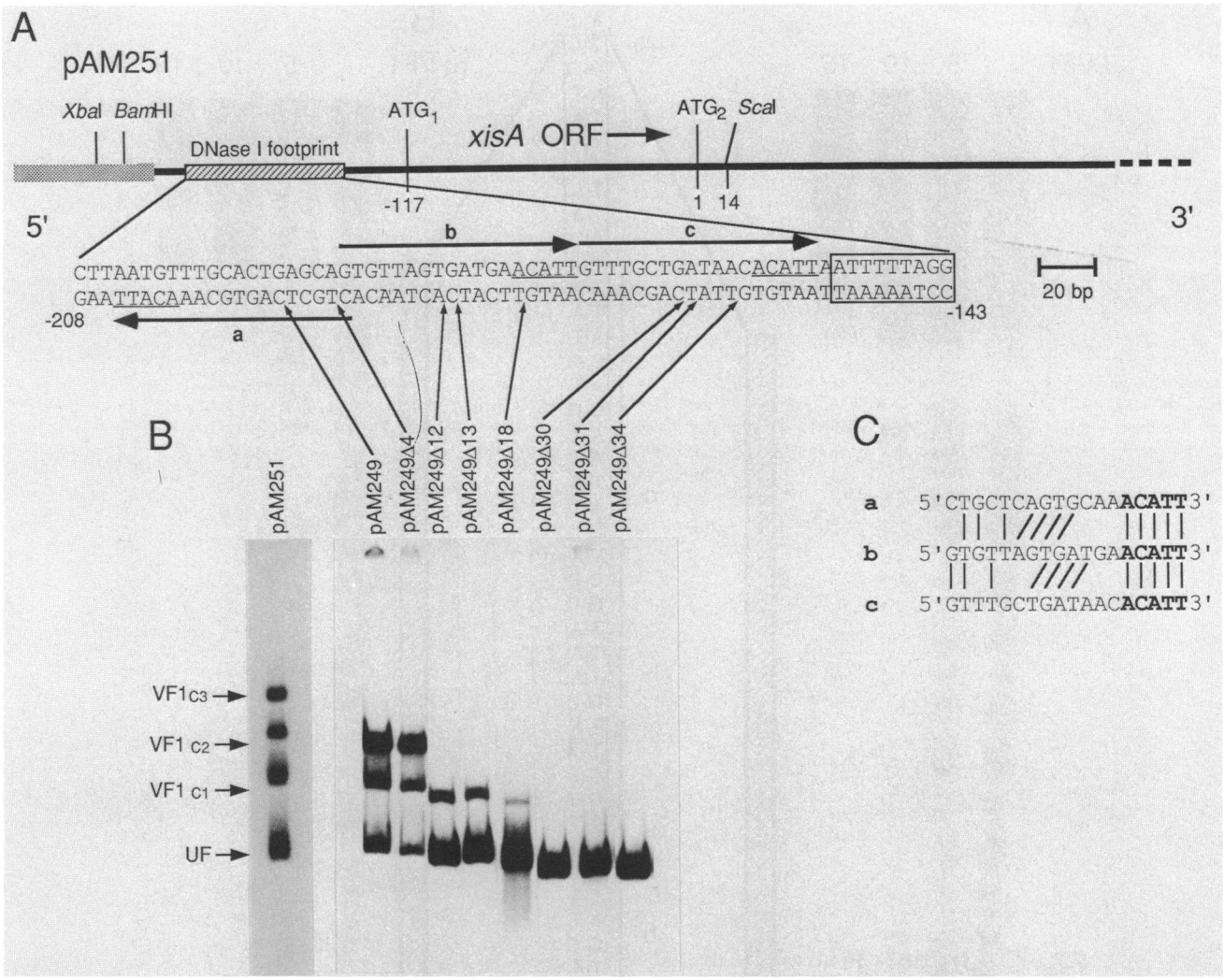
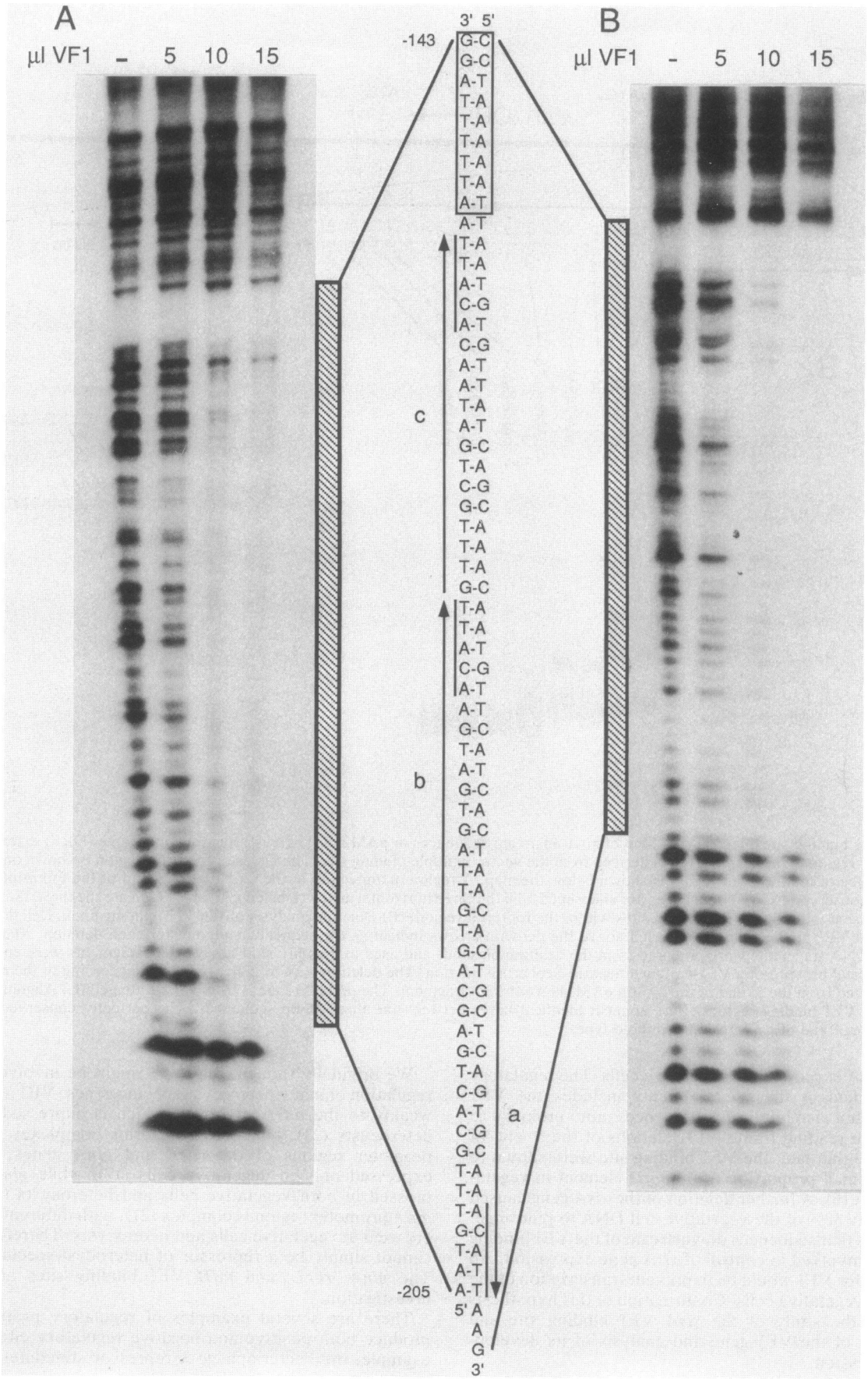


FIG. 5. VF1 binding site analysis. (A) Map of *xisA* upstream sequences in pAM251. The *xisA* open reading frame (ORF) extends to the right. The grey region at the extreme left is derived from the vector multiple-cloning site. The DNase I footprint region is shown on the map, and the sequence of the footprint region is shown below the map. A region of ambiguity at the *xisA* proximal end of the footprint region is boxed. Three conserved ACATT sequences are underlined, and the three horizontal arrows labeled a, b, and c indicate the three 18-bp regions that may represent individual VF1 binding sites within the footprint region. (B) Deletion analysis of the VF1 binding sites. Deletions which extend into the VF1 binding sites are labeled above the gel with arrows indicating the sequence endpoint for each deletion. *Xba*I-*Sca*I or *Bam*HI-*Sca*I DNA fragments were prepared from the deletion plasmids and used in mobility shift assays. The fragments were equilibrated with 0.35 μg (total protein) of a VF1-enriched heparin-Sepharose fraction. The deletions (Δ4 to Δ34) are labeled according to the number of base pairs deleted from the 5′ end of the 205-bp pAM249 *Xba*I-*Sca*I fragment. The pAM251 lane is from a separate gel. (C) Alignment of the three putative VF1 binding regions. Bars connect identical bases between the three 18-bp sequences. The perfectly conserved ACATT sequence at the 3′ end of each region is in bold type.

quired for *xisA* expression in vegetative cells. The regulatory region identified in these experiments includes the VF1 binding site, but also implicates sequences more proximal to the *xisA* open reading frame (6). Deletions of the 5′ end of the *xisA* gene that lack the VF1 binding site were shown to rearrange a small proportion of the *nifD* element in vegetative cell DNA (6). A further deletion of the *xisA* gene caused approximately 50% of the vegetative cell DNA to rearrange. This suggests that sequences downstream of the VF1 binding site are also involved in control of *xisA* gene expression. An obvious role for VF1 would be to prevent transcription of the *xisA* gene in vegetative cells. Confirmation of this hypothesis will require the study of the *xisA* VF1 binding site and identification of the VF1 gene and analysis of its developmental expression.

We originally thought that VF1 might be involved in the regulation of many heterocyst-specific genes. VF1 also binds weakly to the *nifH* promoter, which is expressed only in heterocysts (23), but VF1 also forms complexes with the promoter regions of the *rbcL* and *glnA* genes. *rbcL* is expressed only in vegetative cells (17), while *glnA* is expressed in both vegetative cells and heterocysts (21). The *glnA* promoter region is complex (21), with different promoters used in vegetative cells and heterocysts. Therefore, VF1 cannot simply be a repressor of heterocyst-specific genes. The *glnA*, *rbcL*, and *nifH* VF1 binding sites are under investigation.

There are several examples of regulatory proteins that produce both positive and negative regulatory effects. For example, the bacteriophage λ repressor stimulates its own


A
μl VF1 - 5 10 15
B
μl VF1 - 5 10 15
3' 5'
-143
G-C
G-C
A-T
T-A
T-A
T-A
T-A
T-A
A-T
A-T
T-A
T-A
A-T
C-G
A-T
C-G
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A-T
T-A
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G-C
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G-C
T-A
C-G
A-T
C-G
G-C
T-A
T-A
T-A
G-C
T-A
A-T
A-T
-205
5' A
A
G
3'
a
b
c

FIG. 6. DNase I footprints of the VF1-protected *xisA* upstream region. A 5'-end-labeled, 247-bp pAM251 *Bam*HI-*Sca*I fragment coding strand (A) and a 3'-end-labeled 259-bp pAM251 *Xba*I-*Sca*I fragment noncoding strand (B) were equilibrated with the indicated amount of VF1-containing heparin-Sepharose fraction (0.08 μg of protein μl^{-1}), followed by DNase I treatment and electrophoresis as described in Materials and Methods section. The nucleotide numbers are assigned as in the legend to Fig. 5. The boxed sequence contains a region of ambiguity at the *xisA* proximal border of the footprint, due to the absence of bands in this region for native DNA. The three conserved ACATT sequences are marked with a line, and the three arrows labeled a, b, and c indicate the three 18-bp sequences, as in Fig. 5.

expression and represses expression of the *cro* gene (19). One explanation of our results is that VF1 binds to the *rbcL* and *glnA* genes as a single DNA-protein complex and not as the multimeric complex seen with *xisA* (Fig. 5). Thus, it is possible that VF1 positively regulates transcription in vegetative cells when it is bound as a monomeric complex, as in *rbcL* and *glnA*, but negatively regulates transcription of *xisA* as a multimeric complex. Further insights into the function of VF1 will require identification of the *xisA* promoter sequences and information on the role of VF1 in the regulation of the *rbcL*, *glnA*, and *nifH* genes.

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