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Genetics

Identification of a cold shock transcriptional enhancer of the *Escherichia coli* gene encoding nucleoid protein H-NS

(cold shock regulon/*hns*/histone-like protein/bacterial nucleoid)

ANNA LA TEANA, ANNA BRANDI, MAURIZIO FALCONI, ROBERTO SPURIO, CYNTHIA L. PON, AND CLAUDIO O. GUALERZI

Laboratory of Genetics, Department of Biology, University of Camerino, 62032 Camerino (MC), Italy

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ABSTRACT **The *hns* (27 min) gene encoding the 15.4-kDa nucleoid protein H-NS was shown to belong to the cold shock regulon of *Escherichia coli*, its expression being enhanced 3- to 4-fold during the growth lag that follows a shift from 37°C to 10°C. A 110-base-pair (bp) DNA fragment containing the promoter of *hns* fused to a promoterless *cat* gene (*hns–cat* fusion) conferred a similar cold shock response to the expression of chloramphenicol acetyltransferase (CAT) activity *in vivo* and in coupled transcription–translation systems prepared with extracts of cold-shocked cells. Extracts of the same cells produce a specific gel shift of the 110-bp DNA fragment and this fragment, immobilized on a solid support, specifically retains a single 7-kDa protein present only in cold-shocked cells that was found to be identical to F10.6 (CS7.4), the product of *cspA*. This purified protein, which is homologous to human DNA-binding protein YB-1, recognizes some feature of the 110-bp promoter region of *hns* and acts as a cold shock transcriptional activator of this gene since it stimulates the expression of CAT activity and of *cat* transcription in *in vitro* systems programmed with plasmid DNA carrying the *hns–cat* fusion.**

Several bacterial proteins with DNA-binding property have been implicated in condensation of the chromosome and in organization of the prokaryotic nucleoid. The most abundant and best characterized of these are HU (NS) and H-NS (H1a) proteins (for reviews, see refs. 1–4). H-NS (136 residues) is a neutral, heat-stable, dimeric protein (5) that displays high affinity for curved DNA (6) and has been localized primarily in the nucleoid by immunoelectron microscopy (7). H-NS is encoded by *hns*, a gene that has been cloned and characterized in *Escherichia coli* (8) as well as in other Enterobacteriaceae (9) and that has been ultimately mapped at 27 min on the *E. coli* chromosome (4). Mutations in *hns* were found to increase bacteriophage Mu-specific transcription and to increase dramatically the mini-Mu transposition rate (10). Several mutations causing a number of apparently unrelated phenotypes have been found to be allelic with *hns*. These are *bglY*, which activates expression of the cryptic *bgl* operon (11) and causes large chromosomal deletions (12); *pilG*, which greatly increases the site-specific DNA inversion responsible for fimbrial phase variation (13); *drdX*, which induces expression of the pilus adhesin (*pap*) genes at low temperature in uropathogenic strains (14); *cur-1*, causing a conditional uracil requirement (15); *osmZ*, altering the osmoregulated expression of *proU* operon (16, 17); and *virR*, which affects the temperature-regulated expression of plasmid-borne virulence genes in *Shigella flexneri* (18).

A common basis for these pleiotropic effects could be an altered compaction and fluidity of the genome (12) leading to (or coupled with) a transcriptional derepression of some genes.

An important role of H-NS in controlling the compaction of the nucleoid is also suggested by the observation that the nucleoids undergo a dramatic condensation in cells hyperproducing this protein (19).

In the present paper, we report that H-NS is one of the 13 proteins accumulating during the cold shock response in *E. coli* (20) and we demonstrate that a small DNA fragment derived from the proximal region of its gene and the product of the *cspA* gene (21), the F10.6 (20) 7.4-kDa cold shock protein, are involved in a selective cold shock enhancement of *hns* transcription.

MATERIALS AND METHODS

Preparation of the S30 Extracts and Coupled Transcription–Translation Systems. *E. coli* MRE600 cells were grown in 11 liters of LB at 37°C to an A_{450} of 1.4. The cells contained in 3 liters of the culture were then immediately harvested while four 2-liter portions of the culture were incubated at 10°C for 1, 2, 3, and 4 h before harvesting. The cells were washed twice in 10 mM Tris acetate buffer (pH 8) containing 14 mM magnesium acetate, 60 mM potassium acetate, and 1 mM dithiothreitol (DTT) and frozen at −80°C. The cells (≈3 g) were ground in the presence of 4.5 g of alumina and extracted with 2.7 ml of the same buffer. After removal of the alumina by centrifugation at 12,000 rpm for 15 min, the S30 fractions were obtained by centrifugation for 60 min at 15,000 rpm in the Sorvall SA600 rotor. After 90 min preincubation under the conditions described (22), the S30 fractions were divided into small aliquots and stored at −80°C.

In vitro coupled transcription–translation was performed in 100-μl reaction mixtures as described (22). Unless otherwise specified, each mixture contained 0.7 μg of plasmid DNA [purified using Qiagen columns (Diagen, Düsseldorf, F.R.G.)] and 15 μl of the indicated S30 fractions and was incubated for 30 min at 37°C.

Preparation of ^{35}S-Labeled Postribosomal Supernatants. *E. coli* MRE600 cells were grown in M9 medium at 37°C to an A_{500} of ≈0.65. The culture was divided into two 10-ml aliquots; the first was kept at 37°C and received 100 μCi of [^{35}S]methionine (1 Ci = 37 GBq) for 30 min before harvesting, and the second was shifted to 10°C and kept for 1 h at this temperature before receiving 100 μCi of [^{35}S]methionine. Cells were harvested after an additional 30 min incubation at 10°C. The cells from the two aliquots were collected by centrifugation, and the resulting pellets were stored at −80°C. To prepare the extracts, each pellet was suspended in 1 ml of 50 mM Tris·HCl (pH 7.7), incubated 15 min on ice with 160 μg of lysozyme, and sonicated. After addition of 210 μl of

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Abbreviations: DTT, dithiothreitol; CAT, chloramphenicol acetyltransferase.

solution I (20 mM Tris·HCl, pH 7.7/5 mM $MgCl_2$) containing 4 M NaCl (final concentration, 0.8 M) the extracts were centrifuged 1 h at 11°C in the Airfuge (Beckman) to yield postribosomal supernatants (S100), which were exhaustively dialyzed against solution I containing 50 mM NaCl before being passed through DNA cellulose (Sigma) columns to remove most of their DNA-binding proteins.

DNA Coupling to Magnetic Particles. Approximately 50 μg of the 110-base-pair (bp) DNA fragment derived from the upstream region of *hns* (see legend of Fig. 2) was coupled to 20 mg of amino-derivatized magnetic particles (MagniProbe Universal DNA cross-linking kit) according to the procedure given by the supplier (DuPont).

Purification of F10.6 (CS7.4) Cold Shock Protein. A high-salt postribosomal wash fraction (23) derived from 50 g of *E. coli* MRE600 cells subjected to 2 h of cold shock was dialyzed against 20 mM Tris·HCl (pH 7.9) containing 10 mM NH_4Cl, 0.1 mM EDTA, 0.5 mM DTT, and 10% (vol/vol) glycerol. The sample was applied onto a DEAE-cellulose column equilibrated with the same buffer. Protein CS7.4 was eluted within the first fractions of a linear (10–100 mM) gradient of NH_4Cl and freed of contaminating DNA-binding proteins by passage through a DNA cellulose column on which this protein, unlike HU (NS), H-NS, and other DNA-binding proteins, is not retained (5). After concentration by ultrafiltration using the Amicon YM1 membrane, CS7.4 was further purified by gel filtration on Sephadex G-50 (superfine). The fractions containing electrophoretically homogeneous F10.6 (CS7.4) protein were pooled, concentrated as described above, dialyzed, and stored at −80°C.

Measurement of *cat* Gene Transcription. *Quantitation by hybridization on nitrocellulose discs.* An *Eco*RI/*Hin*dIII fragment [1.15 kilobases (kb)] derived from pBR328 (24) comprising the *cat* gene lacking the first 100 nucleotides was purified by electroelution from an agarose gel. Aliquots (≈2 μg of DNA) of this fragment were loaded onto nitrocellulose disks presaturated with 20× standard saline citrate (SSC). The filters were placed on Whatman 3MM paper saturated with 1.5 M NaCl/0.5 M NaOH and allowed to wet completely (5 min); they were then transferred twice to paper saturated with 1.5 M NaCl/0.5 M Tris·HCl, pH 7.4. The filters were finally air-dried and baked for 2 h at 80°C under vacuum. Prehybridization (6 h at 37°C) and hybridization (14 h at 37°C) were carried out in 1× Denhardt's solution/5× SSC/0.1% SDS/1 mM EDTA/40% (vol/vol) formamide containing thermally denatured herring sperm DNA (0.1 mg/ml). The filters were washed for 15 min at 37°C in 40% formamide/2× SSC/0.1% SDS, followed by two 30-min washes at 50°C in 0.5× SSC/0.1% SDS, and finally two 15-min washes at room temperature in 0.5× SSC. The amount of RNA transcript hybridized on each filter was determined by scintillation counting.

The transcription reaction mixture (50 μl) contained 40 mM Tris·HCl (pH 7.4), 120 mM KCl, 5 mM NaCl, 6 mM $MgCl_2$, 0.6 mM ATP, 0.6 mM GTP, 0.6 mM CTP, 0.1 mM UTP, 2 mM spermidine, 10 mM DTT, 0.125 μg of bovine serum albumin per μl, 5 units of RNasin, 1 unit of *E. coli* RNA polymerase (Boehringer Mannheim), 4–5 μCi of [α-^{32}P]UTP (3000 Ci/mmol), 0.5–1 μg of plasmid DNA. After incubation at 37°C for 40 min, 100 μl of water was added and the reaction mixtures were extracted with phenol and chloroform and subjected to ethanol precipitation. Each pellet was dissolved in 50 μl of water and added to 1.5 ml of hybridization solution.

Quantitation by Northern blotting. The conditions for transcription and subsequent quantitative Northern blot analysis were essentially those described by Ross *et al.* (25) using the above-mentioned *Eco*RI/*Hin*dIII fragment labeled by random primer as hybridization probe.

RESULTS

In a preliminary attempt to define the type of regulation governing the expression of the *hns* gene, it was found, in collaboration with F. Neidhardt (University of Michigan, Ann Arbor), that H-NS is likely identical to a protein having the coordinates F14.7 in two-dimensional O'Farrell electrophoresis (20); since the rate of synthesis of this polypeptide increased 3- to 5-fold during the lag that follows the decrease of the growth temperature from 37°C to 10°C (20), this finding suggested that H-NS might be one of the 13 proteins induced by cold shock.

Quantitative Northern and Western blots show that there is an ≈4-fold increase in the cellular levels of both H-NS mRNA and H-NS protein within 2–4 h after the temperature shift to 10°C (Fig. 1*A*). These results conclusively demonstrate that H-NS is one of the *E. coli* proteins involved in the cold shock response.

In a subsequent experiment (Fig. 1*B*), it was found that a 110-bp DNA fragment derived from the promoter region of *hns* and including its promoter (Fig. 2), when fused to a promoterless *cat* gene, confers cold shock enhancement (≈3-fold within 3 h) of the chloramphenicol acetyltransferase

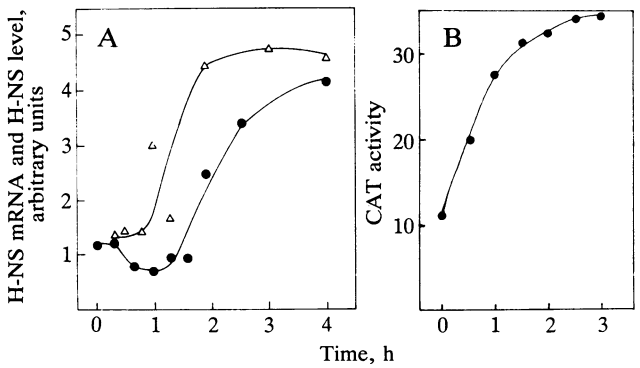

FIG. 1. *hns* belongs to the cold shock regulon and its promoter region shows cold shock inducibility. (*A*) Level of hns mRNA (△) and H-NS (●) during cold shock response. *E. coli* MRE600 cells were grown in LB at 37°C to an A_{600} of 0.8 (≈4 × 10^8 viable cells per ml) and then shifted to 10°C. Samples were withdrawn for RNA and protein extraction at various times between 0 and 4 h after the temperature shift; during this time, there was virtually no increase in the number of viable cells. The RNA was extracted from cells derived from 10 ml of each culture, purified by CsCl centrifugation (9, 26), incubated with DNase I (RNase-free), and subjected to phenol extraction and ethanol precipitation. Ten-microgram aliquots of RNA were subjected to formaldehyde/agarose gel electrophoresis (27). The gels were washed 30 min in 20× SSC and 10 min in 10× SSC and blotted onto Hybond N (Amersham). Hybridization conditions were as described. An ≈450-bp *Eco*RI fragment spanning from the *Eco*RI site upstream of *hns* to the polylinker of M13mp19 and comprising nearly the full length of the gene (8) was ^{32}P-labeled with a random primer kit (Amersham) and used as probe. Cell extracts derived from the same cultures were subjected to PAGE (28) and Western blotting using the apparatus, the detection kit (horseradish peroxidase conjugate immunoblot assay kit with horseradish peroxidase color development reagent containing 4-chloro-1-naphthol), and the experimental procedure from Bio-Rad. Northern and Western blots were quantified by densitometric scanning of the autoradiograms and of the photographic negatives, respectively. (*B*) Cold shock stimulation of *cat* expression *in vivo* from the *hns–cat* fusion. *E. coli* DH5 cells containing pKK110 were subjected to cold shock as described above, withdrawing samples at the indicated times. The amount of cell extract used in the CAT assay was selected on the basis of the results of preliminary experiments in which the linearity of the dose–activity response was ascertained. The CAT activity is expressed as percentage of modified chloramphenicol (mono- and diacetylated) with respect to the total amount of [^{14}C]chloramphenicol (i.e., mono- and diacetylated plus nonacetylated) resolved by silica gel thin-layer chromatography (29).

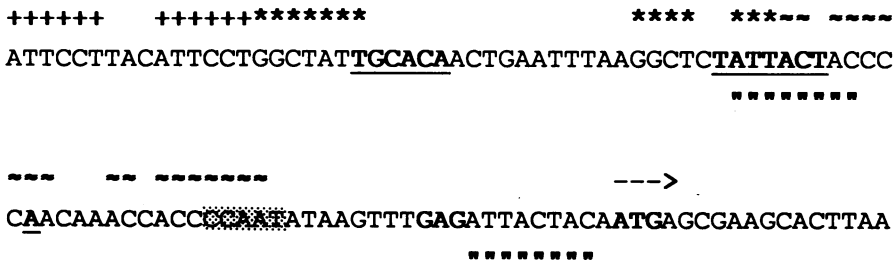

FIG. 2. Sequence of the *Eco*RI/*Fok* I 110-bp DNA fragment from the proximal region of *hns* used to construct pKK110 carrying the *hns–cat* fusion. The *Eco*RI/*Fok* I fragment includes the −35 and −10 promoter elements and the *in vivo* identified transcriptional start point of *hns* (8) (underlined boldface letters), the Shine–Dalgarno sequence (boldface letters), the initiation triplet (boldface letters with arrow), and the first few nucleotides of the coding region of the gene. Four different direct repeats are also indicated by different symbols either above or below the relevant sequence. The CCAAT sequence (see text) is indicated by the shaded area. To construct pKK110, the DNA fragment, after filling in the ends, was ligated into the *Sma* I site of the polylinker of pKK232-8 (Pharmacia), upstream of the promoterless *cat* gene.

(CAT) activity expressed by cells harboring this construct (pKK110). In contrast, the CAT activity expressed by cells harboring pBR328, which carries *cat* preceded by its own promoter (24), did not vary appreciably under comparable conditions (data not shown). Cold shock enhancement of CAT activity using the *hns–cat* fusion was seen also *in vitro*. Thus, cell-free transcription–translation systems programmed with pKK110 (Fig. 3 *A*, *C*, and *D*) but not with pBR328 (Fig. 3*B*) always expressed significantly higher (2- to 4-fold) CAT activity when the cell extracts from control cells (i.e., grown at 37°C) were replaced by extracts from cold-shocked cells.

This difference did not depend on the amount of cell extract used (data not shown), on the amount of DNA offered (Fig. 3*A*), or on the time allowed for CAT synthesis (Fig. 3*C*). Furthermore, the amount of CAT synthesized *in vitro* depended on the length of cold exposure to which the cells used for the preparation of the cell-free systems had been subjected; maximum activity was reproducibly obtained with extracts of cells cold shocked for 2–3 h (Fig. 3*D*). Thus, both extent and time course of the cold shock stimulation of *hns* expression *in vivo* (Fig. 1*A*) are roughly paralleled by the cold shock enhancement of CAT activity from *hns–cat* fusion *in vivo* (Fig. 1*B*) and *in vitro* (Fig. 3*D*). Considering that *hns–cat* fusion contains two translational stop codons and two Shine–Dalgarno sequences upstream of the *cat* coding sequence, the observed cold shock enhancement of CAT expression can almost certainly be attributed to transcriptional (rather than translational) activation.

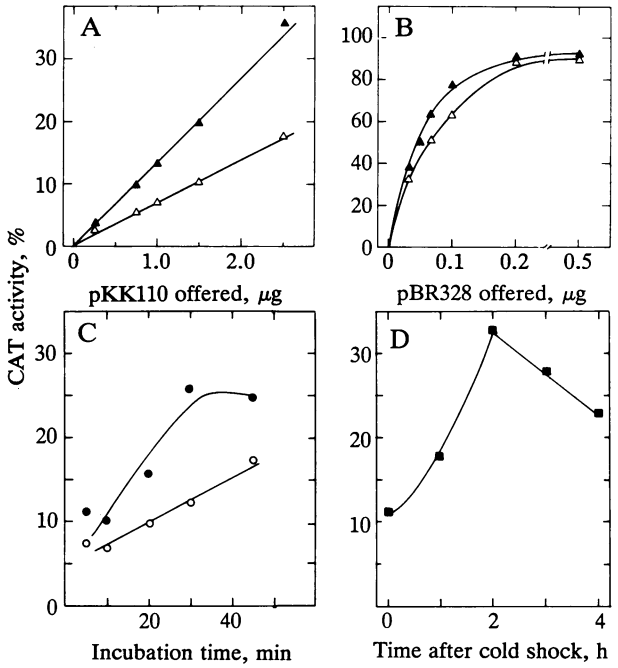

FIG. 3. CAT activity expressed *in vitro* in coupled transcription–translation systems from normal and cold-shocked cells. CAT activity produced as a function of an increasing amount of template (pKK110) containing the *hns–cat* fusion (*A*), an increasing amount of template (pBR328) not containing the fusion (*B*), the time of incubation (*C*), and the stage of cold shock at which cells were harvested to prepare the cell-free extracts (*D*). The transcription–translation systems were prepared as described using S30 fractions derived from control cells growing at 37°C (△ and ○) and from cells subjected to cold shock for 1 h (●), 2 h (▲), or the times indicated on the abscissa (■).

Furthermore, since both *hns* and the *hns–cat* fusion are efficiently expressed at 37°C, albeit to a lower level than during cold shock, one could postulate the existence of a somewhat "leaky" and metabolically unstable transcriptional repressor of *hns* present only at 37°C or, alternatively, of a cold shock transcriptional activator. Indeed, comparison of the electrophoretic patterns obtained with extracts of control and cold-shocked cells provided potential support for either type of control by showing that during cold shock the amount of some proteins is drastically reduced while other proteins are increased in amount or appear *de novo*. Some indication against the existence of a labile inhibitor came, however, from the finding that preincubation of the control and cold-shocked cell extracts did not reduce the difference in their activity in CAT synthesis (data not shown). On the other hand, a strong clue for the presence of a cold shock transcriptional activator came from a gel-shift assay in which the electrophoretic mobility of the 110-bp DNA fragment was selectively retarded upon incubation with a postribosomal supernatant (previously deprived of the main DNA-binding proteins) derived from cold-shocked cells (Fig. 4, even-numbered lanes). To identify the protein(s) of the cold shock extracts that interacts with the 110-bp DNA fragment, this fragment was immobilized on a solid support and used to "fish" for the protein from an S100 fraction of ^{35}S-labeled cells. While no specific protein was found by this method when the extract from control cells was used (Fig. 5*A*), a specific small (≈7 kDa) protein was retained up to a high ionic strength (≥200 mM NaCl) when the extract was prepared from cold-shocked cells (Fig. 5*B*). The specificity of the interaction between this protein and the 110-bp fragment is also documented by the fact that the S100 fraction from which the protein was isolated had been deprived of all major DNA-binding proteins by prior passage through a DNA cellulose column. Furthermore, the protein isolated by this affinity purification corresponded to one present primarily, if not exclusively, in cold-shocked cells (e.g., compare lanes 1 in Fig. 5 *A* and *B*). After purification to electrophoretic homogeneity (Fig. 6*A*, lane 2) and determination of its N-terminal amino acid sequence (20 residues), this protein was found to be identical to CS7.4, the product of *cspA* (21),

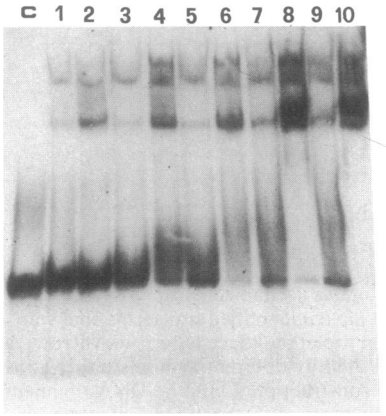

FIG. 4. Electrophoretic mobility shift of the *hns* 110-bp promoter region by cell extracts from control and cold-shocked cells. Each reaction mixture (15 μl) contained 26 mM Tris·HCl (pH 7.7), 60 mM KCl, 40 mM NH_4Cl, 0.1 mM NaEDTA, 1 mM DTT, 10% glycerol, 0.15 μg of bovine serum albumin, 0.6 μg of poly d(IC), and 5′ ^{32}P-end-labeled 110-bp DNA fragment. As specified below, each reaction mixture also contained different amounts of postribosomal supernatant (S100) fraction passed through DNA cellulose columns. After 30 min incubation at 10°C, the samples were subjected to electrophoresis on 10% acrylamide in 90 mM Tris/64.6 mM boric acid/2.5 mM EDTA, pH 8.3, followed by autoradiography. The sample loaded in lane C contained no S100 fraction. The mixtures loaded in the other wells contained S100 fractions derived either from control cells (odd-numbered lanes) or from cells subjected to 3 h of cold shock (even-numbered lanes). The total amounts of protein [determined by the Coomassie blue colorimetric assay (30)] present in each mixture were 2.2 μg (lanes 1 and 2), 4.4 μg (lanes 3 and 4), 6.5 μg (lanes 5 and 6), 8.6 μg (lanes 7 and 8), and 10.8 μg (lanes 9 and 10).

which, in turn, corresponds to protein F10.6 originally described by Jones *et al.* (20). Addition of this purified protein to a coupled transcription–translation system prepared from control cell extract and programmed with pKK110 resulted in a clear increase of CAT synthesis (Fig. 6*B*). Likewise, this protein induced an obvious increase of *cat* gene transcription in a purified *in vitro* system programmed with the *hns–cat* fusion (Fig. 6 *C* and *D*). The transcriptional enhancement was observed with two different detection methods and was seen at both 37°C and 10°C (Fig. 6*C*) but could not be detected when pBR328 instead of pKK110 was used as template (Fig. 6*D*). Similar results were also obtained using a sample of purified CS7.4 protein kindly provided by J. Goldstein and M. Inouye (University of Medicine and Dentistry of New Jersey/Robert W. Johnson Medical School at Rutgers, Piscataway, NJ).

DISCUSSION

When the growth temperature of an *E. coli* culture is shifted from 37°C to 10°C, the cells enter a 4- to 5-h-long lag before resuming growth at a much reduced rate. During this lag, the expression of ≈13 genes is increased between 2- and 10-fold, while the expression of *cspA*, the gene encoding protein F10.6 (CS7.4), is either turned on *de novo* or dramatically increased (20, 21).

In the present paper, we show that *hns*, the structural gene for the nucleoid protein H-NS (8), belongs to the cold shock regulon, its expression being increased 3- to 4-fold after a temperature shift to 10°C. We also show that a 110-bp segment of DNA spanning from 55 bp upstream of *hns* to the proximal portion of its coding sequence (Fig. 2) can confer cold shock enhancement to the expression of a promoterless *cat* gene *in vivo* and *in vitro*. In addition to the −35 and −10 promoter elements of *hns*, this fragment is characterized by

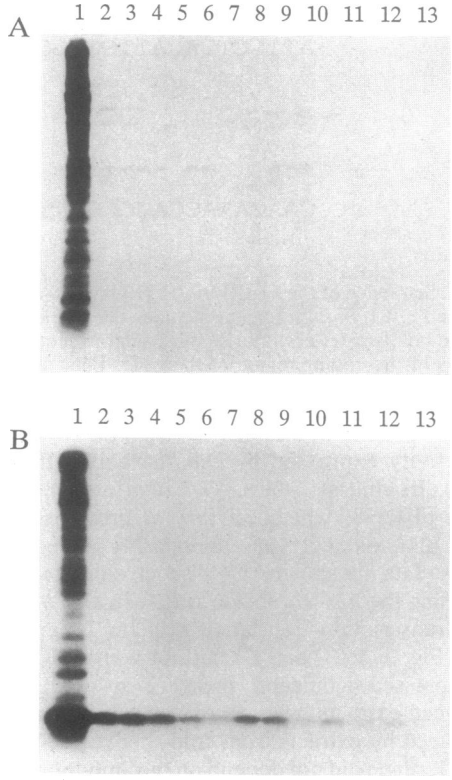

FIG. 5. Fractionation of ^{35}S-labeled proteins by affinity binding to immobilized *hns* 110-bp promoter region. Each incubation mixture contained the 110-bp DNA fragment immobilized on MagniProbe resuspended in 540 μl of 20 mM Tris·HCl, pH 7.7/5 mM $MgCl_2$/20 mM NaCl and 260 μl of ^{35}S-labeled S100 fraction, both prepared as described. After 1 h incubation at 10°C with gentle shaking, the magnetic particles carrying the DNA were sedimented by centrifugation for 20 sec at 3000 rpm and the supernatant was recovered. The magnetic particles were subjected to a series of washes with 800 μl of the buffer described above containing increasing concentrations of NaCl. Aliquots of each supernatant were then analyzed by SDS/18% PAGE and autoradiography. Lanes: 1, the first supernatant (i.e., total unbound proteins); 2–4, three successive washes with 20 mM NaCl; 5–13, washes with 50 mM, 100 mM, 200 mM, 300 mM, 400 mM, 500 mM, 600 mM, 800 mM, and 1.0 M NaCl with S100 fraction from control cells growing at 37°C (*A*) and from cells subjected to 1.5 h of cold shock (*B*).

the unusual presence of four different direct repeats of at least 6 bp and by a CCAAT sequence located at the presumed leading edge of the RNA polymerase binding site (see below).

We have also found that some component(s) present in the S100 fraction of cold-shocked cells can interact with this DNA fragment, specifically affecting its electrophoretic mobility, and that the same DNA fragment can be used in affinity purification of a small (≈7 kDa) protein present only in cold-shocked cells later identified as protein CS7.4. When added to *in vitro* systems, this purified protein was found to act as a transcriptional enhancer specifically recognizing the proximal region of *hns*.

The extent and the time course of cold shock activation were found to be similar with *hns in vivo* and with the *hns–cat* fusion *in vivo* and *in vitro*, suggesting that a common mechanism is responsible for these effects. The transcriptional enhancement produced by CS7.4 in the *in vitro* systems directed by the *hns–cat* fusion is also of similar magnitude. Thus, our results suggest that the cold shock activation of *hns* expression is mediated by a transcriptional activator (protein CS7.4) and by a target localized within the 110-bp DNA fragment comprising the promoter of the gene.

The fact that the cold shock activation of *cspA* expression somewhat precedes that of *hns* and of most of the other cold

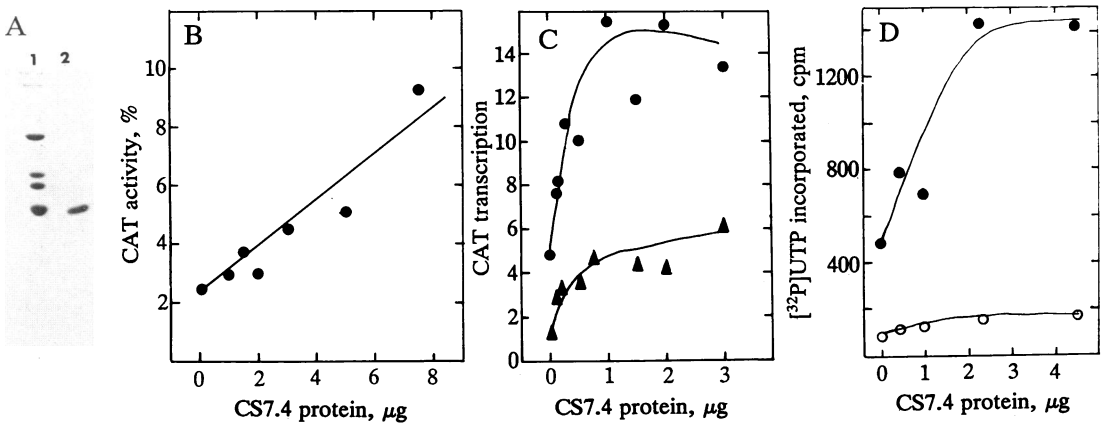

FIG. 6. Purified CS7.4 (F10.6) protein stimulates *in vitro* expression of CAT activity and transcription of *cat* from *hns–cat* fusion. (*A*) Electrophoretic purity of CS7.4 on SDS/polyacrylamide gel containing 15% acrylamide (28). Lanes: 1, size standards—from top to bottom, IF3 (20.5 kDa), H-NS (15.4 kDa), lysozyme (14.3 kDa), HU(NS) (9.5 kDa); 2, 2.5 μg of purified CS7.4. Effect of the indicated amounts of purified CS7.4 on expression of CAT activity in a transcription–translation system prepared from an extract of control cells programmed with pKK110 (*B*), transcription in arbitrary units (assayed by Northern blotting) of *cat* at 37°C (●) and at 10°C (▲) using pKK110 as template (*C*), and transcription (assayed by hybridization) of *cat* at 37°C using pKK110 (●) or pBR328 (○) as template (*D*).

shock genes (20) is fully compatible with this hypothesis. Whether the basis for the activation of other cold shock genes is the same as that of *hns*, however, remains an open question.

The exact mechanism by which CS7.4 activates transcription remains a matter of speculation at this time; nevertheless, it is worth mentioning that the primary structure of this protein is strikingly homologous to a conserved region of a class of eukaryotic transcriptional factors comprising the Y box binding protein YB-1 (31), whose binding to DNA displays an absolute requirement for a CCAAT sequence (32). Thus, if the presence of a CCAAT sequence at +12 of the 110-bp DNA fragment (Fig. 2) is not coincidental, one can imagine that CS7.4 protein may bind just in front of the promoter-bound RNA polymerase somehow enhancing its activity. In addition to investigating this hypothesis, further questions that future work should answer concern the nature of the "constitutive" (i.e., under conditions of nonstress) regulation of *hns* expression and the role of H-NS during cold shock. In fact, since H-NS is the product of a monocistronic transcript (9), it is very unlikely that the stimulation of its production during cold shock is merely coincidental.

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