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Transcription of the *ibpB* Heat-Shock Gene Is under Control of σ^{32}- and σ^{54}-Promoters, a Third Regulon of Heat-Shock Response

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The expression of the *ibpAibpB* heat-shock operon of *Escherichia coli* was found previously not to conform to the known pattern of expression of the σ^{32}-regulated operons because the *rpoH* gene mutation inactivating the σ^{32} protein did not abolish the *ibp* induction. We show here that this effect can depend partly on the σ^{54}-promoter that is inducible by heat shock, located upstream of the *ibpB*, the distal gene of the operon. It may also depend on a metabolic signal, postulated by others, and possibly required for the expression of the *ibpAB* genes. Thus, the *ibpB* gene can be translated from the transcript covering the whole operon starting from the σ^{32}-promoter and from the *ibpB* gene transcript starting from the σ^{54}-promoter. These results indicate that the *ibpB* gene is a second member of the σ^{54}-heat-shock regulon in *E. coli* besides *pspA-E* operon. Thus, heat-shock response involves three regulons controlled by σ^{32}, σ^{24}, and σ^{54} RNA polymerase subunits. © 2001 Academic Press

***Key Words:* ibpAB operon transcription; σ^{32}- and σ^{54}-promoters; σ^{54}-regulon of the heat-shock; *rpoH15*, Δ*rpoH*, *rpoN208 E. coli* mutants.**

IbpA and IbpB (1) are 16-kDa heat-shock proteins (small heat-shock proteins, sHSPs) whose activity is ATP independent (2, 3). They associate with endogenous proteins of *Escherichia coli* aggregated intracellularly by heat shock (4). Details of their action emerged recently. IbpB (and other sHsps), acting as large oligomers (5, 6) prevents irreversible aggregation of model proteins *in vitro,* and facilitates their specific transfer to the DnaK/DnaJ/GrpE system for the ATP dependent refolding (6). Thus, the mechanism of the reactivation of denatured proteins (7–10) by the HSP system was supplemented by recognition of its preliminary step (3) involving the IbpB protein. IbpAB proteins appear to be dispensable for bacteria cultivated under laboratory conditions (11); however, their evolutionary conservation and abundant production after heat shock seem to point to their importance under natural conditions where nutritional or environmental stresses are to be expected. Recently it has been found that the IbpA and IbpB proteins are involved in protection from superoxide stress (12).

The *ibpA* and *ibpB* genes form an operon that is inducible by heat shock (1, 13, 14). The presence of the σ^{32}-promoter upstream of the *ibpA* gene has been documented (1, 14). However, we found later that in *E. coli rpoH165* (4), and also in *rpoH15* (as we show here), the expression of *ibpAB* gene after heat shock rose to a higher level, in spite of the σ^{32} protein (the *rpoH* gene product; 15) defect. This indicated a more complicated regulation of the *ibpAB* operon expression than sole dependence on σ^{32}.

The IbpA and IbpB proteins have the same mobility in SDS electrophoresis and are indistinguishable by polyclonal antibodies because they share 48% of identical amino acid sequences (14), though the antibodies raised against the two proteins seem to react more strongly with the IbpA protein (unpublished observation of E. Laskowska). Therefore it was not possible to tell whether only one or both genes was/were upregulated in the *rpoH* mutant. Analysis of the *ibpAB* operon sequence (PC/Gene program) revealed, besides the σ^{32}-promoter upstream of the *ibpA* gene (1, 13, 14), a putative, internal σ^{54}-promoter, with potential enhancer and integration host factor (IHF) binding site upstream of the *ibpB* gene (Fig. 1). Therefore, experiments were undertaken in order to assess activity of the putative σ^{54}-promoter.

MATERIALS AND METHODS

Strains and culture conditions. *E. coli* W3350(wt); LS555*rpoH15* (16); KY1620*rpoH30:*Kan (Δ*rpoH*) (17) and ET8045*rpoN208:*Tn10

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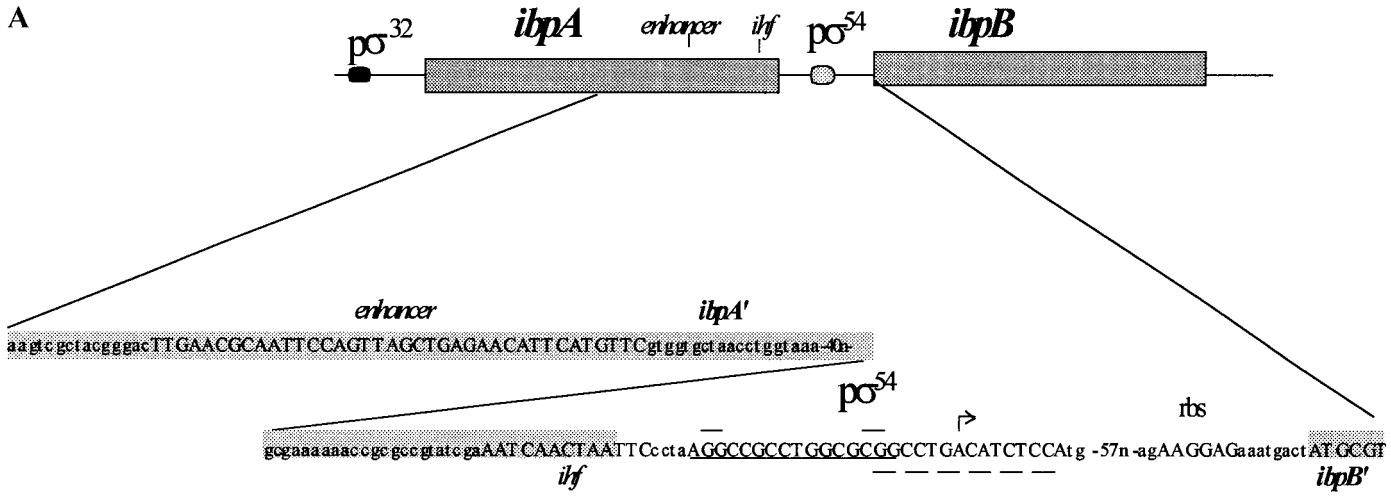

B

ihf consensus WATCAANNNNTTR
ibpB putative ihf site AATCAACTAATTC
W = A/T
N= any nt.
R = G/A

C

psp enhancer TGAAATTCGCCCACTTGTTAGTGTAATTC
ibpB putative enhancer TGAACGCAATTCCAGTTAGCTGAGAACATTC

FIG. 1. The map of the *ibpAB* operon. (A) The upper part shows proposed organization of the *ibpAB* operon. The enlargement presents the sequences surrounding the σ^{54}-promoter with the putative enhancer and IHF binding site (*ihf*). The enhancer is the binding site of an activator necessary for the ATP requiring open complex formation by σ^{54}–RNA polymerase bound to the promoter (unlike the σ^{32}–RNA polymerase complex which reisomerises without such a factor). The IHF is the DNA binding protein, facilitating the contact of activator with the σ^{54}–RNA polymerase bound to the promoter. (B) Alignment of the putative IHF-recognition site upstream the *ibpB* promoter with the 13 bp. consensus sequence (30). (C) Comparison of the PspF (activator of the *pspA* heat shock operon) binding region and putative enhancer sequence upstream *ibpB* gene.

(18) were grown with aeration, in P minimal medium (19): 100 mM Tris, pH 7.4, 20 mM NH_4Cl, 2 mM $MgSO_4$, 0.05 mM $FeCl_3$, 5 mM KCl, 1 mM $CaCl_2$, 1 μg/ml vitamin B_1, 0.3% glucose, 0.3% Casamino acids, at 30°C to A_{575} = 0.3, then submitted to heat shock by transfer to 45°C for 15 min. The P medium, devoid of phosphate was used through all experiments to ensure comparable growth conditions for experiments on transcription start point determination (labeling with ^{32}P) and Western and Northern blottings. Plasmid pTH7 containing *rpoN* gene cloned downstream of the tac promoter was previously described (20). For induction isopropyl-1-thio-β-D-galactopyranoside was added to 1 mM concentration for the last 30 min of growth.

Antibodies. Polyclonal antibodies were used. Anti-IbpA/B serum was prepared as described (4). Anti-DnaJ serum was a gift from K. Krzewski, and anti-DnaK was from J. Osipiuk (Gdańsk).

Polyacrylamide gel electrophoresis (PAGE) PAGE was performed on 0.1% SDS–15% gel with (*N*-tris)[hydroxymethyl]-methylglycine (Tricine) (21) at 25–35 mA, for 20 h. Only in this buffer IbpA and IbpB proteins separate from an outer membrane protein of similar molecular weight.

Western blot (immunoblot) analysis. Electrotransfer of electrophoretically separated proteins to nitrocellulose and immunodetection was carried out as described (22). Anti-IbpA/B, anti-DnaK and anti-DnaJ sera were used as the first and goat anti-rabbit immunoglobulins conjugated with horseradish peroxidase as the second antibodies. 4-Chloro-1-naphthol and H_2O_2 served as the chromogenic substrates for the peroxidase. Densitometry was performed by scanning electropherograms by the UVP (Ultra-Violet Products) EASY system (Cambridge, UK). Results of estimation of intensity of spots on electropherogram are presented in arbitrary units (a.u.) corresponding to percentage of intensity to the most intensive spot on the same electropherogram considered as 100%.

Synthetic primers. The primers A1 (−570) 5′-CGAATTCACATCGTAGCCGATGG-3′ and A2 (+1108) 5′CTAAGCTTGAGCATGGAGATGTCAG-3′ flanking the *ibpA* gene (from Isogen, Holland); and B3, (−1157) 5′-AGTCTGCAGTGCGGTACTTACTCGC-3′ and B4, (+1625) 5′-TGGATCGAATAGCTAGTTAGCT-3′ flanking the *ibpB* gene (from DNA-Gdańsk, Poland) and B2, (+1239) 5′-ACCGATCCATTGACGCAT-3′, for primer extension (from ARK Scientific, Germany) were used. The primers contained in addition restriction sites for *Eco*RI, *Hin*dIII, *Pst*I and *Bam*H1 enzymes (underlined), to serve also for cloning of the genes (to be published elsewhere). The sites marked by vertical bars correspond to the beginnings or the ends of the natural gene sequences. The signs "+" and "−" denote oligonucleotides complementary to the coding and noncoding strands respectively, the nucleotide numbers (in parentheses) are those given to *ibpAB* nucleotides in GenBank (1) (Accession No. M94104).

DNA amplification by PCR. DNA fragments encompassing the *ibpA* or *ibpB* or *ibpAB* genes originated from DNA of λ_{566} Kohara clone (23) and were amplified by PCR, using the A1–A2 or B3–B4 or A1–B4 primers according to the Instruction Manual of Invitrogen.

Digoxigenin-labeled probes for ibpA and ibpB genes. The PCR fragments of *ibpA* and *ibpB* genes (3 μg of each) were labeled with digoxigenin-11-dUTP by random priming using the Boehringer (Mannheim) DIG DNA Labeling and Detection Kit and instruction.

The labeled DNA fragments were precipitated and dissolved in 50 μl of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0) and stored at −20°C. Estimation of the yield of the labeling (according to the instruction) showed concentration of the labeled probes close to 20 μg/ml. The probes were used for hybridizations.

Southern hybridization. Southern hybridization was carried out as described (24) and used for testing specificity of the *ibpA* and *ibpB* probes. 120 ng of PCR fragments of DNA of *ibpA* (560 bp), *ibpB* (490 bp) or *ibpAibpB* (1100 bp) genes were resolved in 1.4% agarose gel and transferred to a nylon membrane. The PCR fragments were hybridized with either *ibpA* or *ibpB* probe in SDS-containing buffer (according to the Boehringer instructions).

RNA preparation. *E. coli* wt, *rpoN208*, *rpoN208*/*rpoN*$^+$, *rpoH15* and Δ*rpoH* cultures were grown in P medium at 30°C (28°C for Δ*rpoH*) to A_{575} = 0.4, and each of them was divided into two parts. One was further grown at 30°C, the second was submitted to heat shock (45°C, 15 min). The procedure for total RNA preparation described earlier (19, 25) was applied. Purified RNA was dissolved in TE buffer (1 mM EDTA, 10 mM Tris, pH 7.5) and its purity was checked by electrophoresis in denaturing, agarose gel. The concentration was measured as A_{260} (1 A_{260} unit equals 40 μg/ml of RNA). The RNA preparations were stored at −20°C.

Northern (RNA) hybridization. Total RNA samples (60 μg) were submitted to electrophoresis in 1.4% formaldehyde agarose gel, transferred to positively charged nitrocellulose membrane and bound to the membrane by baking for 30 min at 120°C. The *ibpA* and *ibpB* probes and Boehringer (Mannheim) kit were used for hybridization carried out according to the manufacturer's recommendation.

Primer extension experiment. To locate the transcriptional start site of the *ibpB* gene the B2 synthetic oligonucleotide was used as primer for reverse transcription. The reaction was carried out according to the recommendation of the producer of the M-MuLV (Moloney Murine Leukemia Virus) reverse transcriptase kit (Fermentas, Vilnius). ^{32}P-end-labeled primer (2 pmol) and 5 μg of RNA preparation from *E. coli.* wt, *rpoH15*, *rpoN208*, *rpoN208*/*rpoN*$^+$ were dissolved to a final volume 12 μl and incubated for 10 min at 70°C, then cooled in ice and 5× buffer for the reverse transcriptase and the mixture of dNTP to final concentration 1 mM were added. The reaction mixture was incubated for 2 min at 42°C, then the reverse transcriptase (40 u) was added and again incubated for 50 min at 42°C. The reactions were terminated by 15 min heating at 70°C and 2 μl samples of the products were mixed with an equal volume of loading solution (80% formamide, 1 mM EDTA, 0.1% bromophenol blue, 0.1% xylene cyanol FF) heated for 2 min at 80°C and electrophoretically separated on 6% sequencing gel. The sequencing was performed by Sanger method using the Pharmacia Biotech. (Uppsala) T7 sequencing kit and the manufacturer's instruction.

RESULTS

The production of the IbpAB proteins in wild type *E. coli* and in mutant bearing *rpoH15* (a missense mutation which inactivates the σ^{32} protein; 16), Δ*rpoH30* (insertional mutation; 17) and *rpoN208* (insertional mutation inactivating σ^{54} protein; 18) was estimated by densitometry of electropherograms (Fig. 2) of total bacterial protein after immunoblotting. The *dnaKJ* heat-shock operon served as an internal control of the expression pattern (26). According to expectation, the DnaKJ proteins were synthesized in wild type and *rpoN208* cells at 30°C, and their synthesis increased after transfer to 45°C, however in *rpoN208* cells their levels were not as high as in wild type. In the *rpoH15* and Δ*rpoH30* cells only a small amount of these proteins was detectable as was described earlier (17). These results agree with published data and show that the *rpoH15* mutation has not reverted and is not suppressed.

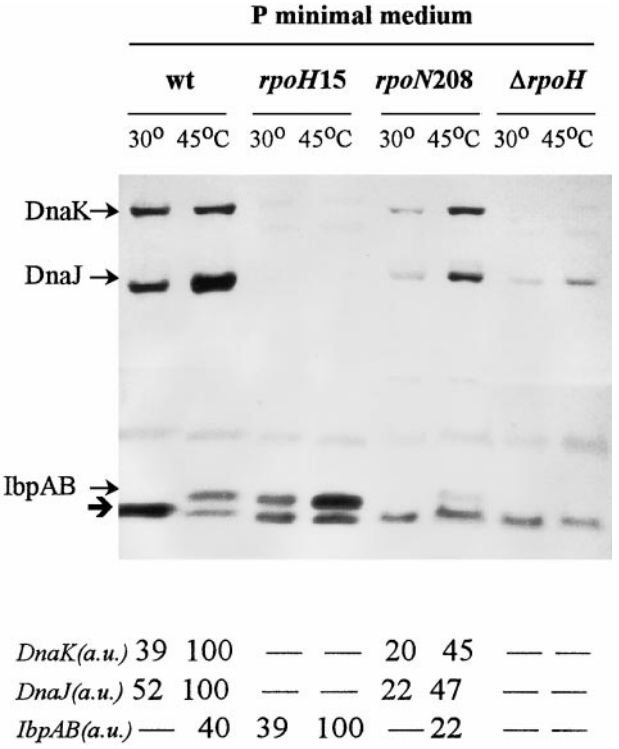

FIG. 2. Comparison by Western blotting of expression of the two heat-shock operons, *dnaKJ* and *ibpAB*. *E. coli* LS556 (wt), LS555*rpoH15*, ET8045*rpoN208*::Tn10, and KY1620Δ*rpoH*30::Kan cultures were grown in P minimal medium at 30°C (28°C for Δ*rpoH30*) and submitted to heat shock at 45°C. Samples (1 ml, 2.4 × 10^8 cells) were taken before and 15 min after the heat shock. The bacteria were sedimented and dissolved in Laemmli lysis buffer (50 μl) for protein separation by PAGE and transfer to nitrocellulose. DnaJ, DnaK, IbpA, and IbpB proteins were detected by immunoblotting with anti-DnaK, anti-DnaJ, and anti-IbpA/B sera followed by goat anti-rabbit immunoglobulins conjugated with horseradish peroxidase. The protein level was estimated by densitometry and expressed in arbitrary units (au). The arrowhead points to the ~16 kDa unidentified outer membrane protein reacting with the anti-*ibpAB* serum.

In contrast to DnaKJ, the IbpAB proteins were not detectable in wild type and *rpoN208* cells grown at 30°C in P minimal medium (Fig. 2), but they were produced after heat shock, though the *rpoN208* mutation reduced their level. Interestingly, IbpAB proteins were found in *rpoH15* cells grown at 30°C (39 densitometric arbitrary units, a.u), and induction of their synthesis (up to 100 a.u.) was observed after the temperature shift. However, the Δ*rpoH30* mutation abolished the *ibpAB* operon expression. We presumed that in wild type and *rpoN208* strains both products of the *ibpAB* operon were present (forming the single 16 kDa spot) due to transcription initiated from the σ^{32}-

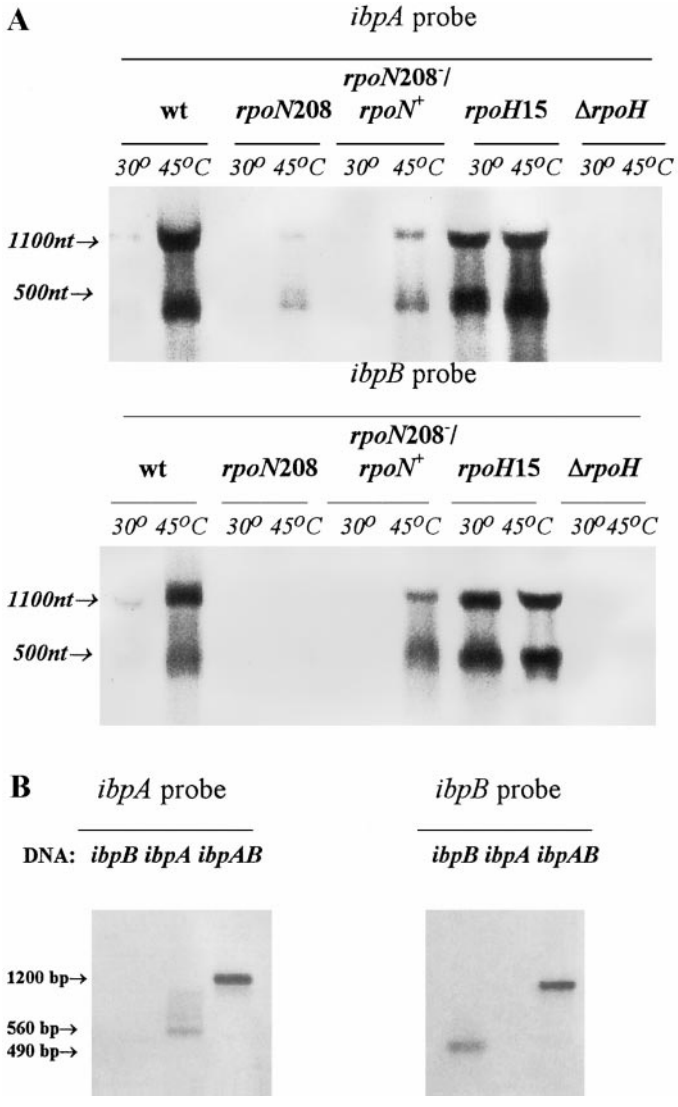

FIG. 3. Northern analysis of the *ibpAB* operon transcripts. (A) The digoxigenin-labeled, specific *ibpA* and *ibpB* probes were used for detection of ~1100 nt transcripts of the *ibpAB* operon and ~500 nt transcripts of *ibpA* or *ibpB* genes in total bacterial RNA. (B) The specificity of *ibpA* and *ibpB* probes: the PCR fragments corresponding to the *ibpA* and *ibpB* genes were submitted to agarose gel electrophoresis, transferred to nylon membrane, and hybridized with the digoxigenin-labeled *ibpA* or *ibpB* probes. For immunodetection, anti-digoxigenin Fab fragments, conjugated to alkaline phosphatase, were used and visualized with chromogenic substrate as recommended by Boehringer (Mannheim), the producer of the DIG DNA labeling and detection kit.

promoter. The 16-kDa product of the *ibpB* gene could be produced in the *rpoH15* mutant if the gene was transcribed from the putative σ^{54}-promoter.

Therefore, the transcription of the *ibpB* gene from the putative σ^{54}-promoter was examined by the use of specific *ibpA* and *ibpB* molecular probes. The test for probe specificity (Fig. 3B) showed that each of them recognized only sequences of their cognate genes, with no cross-reactions: the *ibpA* probe reacted with the A1–B4 and A1–A2 PCR products (1200 and 560 nt) covering the *ibpAB* or *ibpA* genes respectively. The *ibpB* probe reacted with the A1–B4 and B3–B4 PCR products corresponding to the *ibpAB* and *ibpB* genes (1200 and 490 nt, respectively).

The probes were used to search for transcripts of the whole *ibpAB* operon or *ibpA*, or *ibpB* genes in the total cellular RNA isolated from the wild type, *rpoN208*, *rpoN208*/*rpoN*$^+$, *rpoH15* and Δ*rpoH30* cells grown in P minimal medium, at 30°C or submitted to heat shock (Fig. 3A). Three kinds of transcripts were detected: the long one (~1100 nt) which hybridized with both *ibpA* and *ibpB* probes and thus covered both *ibp* genes, and two short transcripts (~500 nt), recognized by the *ibpA* or by the *ibpB* probes.

The use of the *ibpA* probe revealed that there was no *ibpAB* transcript in the wild type cells grown at 30°C, but the long transcript and the short *ibpA* transcript became abundant after heat shock. There was no transcript at all in *rpoN208* cells grown at 30°C, but after heat shock the *ibpA* probe detected a trace amount of the long transcript and a very small amount of the *ibpA* transcript. Complementation of the *rpoN208* mutation by expression of the *rpoN*$^+$ gene from pTH7 plasmid reverted the effect; after heat shock both transcripts were produced. The long transcript and the short, *ibpA* transcript, were abundant in the *rpoH15* cells grown at 30°C, and their amount increased after heat shock. There was no transcript at all in the Δ*rpoH30* cells.

The presence of the *ibpA* short transcript was not expected, but it might be explained by release of the transcript at the stem and loop structure present in the intergenic space, facilitated by steric hindrance created by promoter-bound σ^{54}-RNA polymerase. The stem and loop structure is not followed by the string of U's that typifies rho-independent terminators.

The *ibpB* probe revealed a trace of the *ibpAB* (long) transcript in wild type cells grown at 30°C. After heat shock both *ibpAB* and *ibpB* (short) transcripts were abundantly produced in the wild type and the *rpoH15* cells. Transcripts were not detected in the *rpoN208* cells by the *ibpB* probe, either before or after heat shock. However, complementation of the mutation by the *rpoN* gene product resulted in restoration of the transcription after heat shock. Again no transcript was detected in the Δ*rpoH30* cells. The absence of the *ibpB* transcript in *rpoN208* cells and the positive result of the complementation confirmed the expected dependence of this transcription on σ^{54}. But it was surprising that transcription from the σ^{32}-promoter was defective in the *rpoN208* strain grown in the P minimal medium as indicated by the absence or very low level of the long *ibpAB* transcript.

In summary, transcription from σ^{32}-promoter resulted in the long transcript (1100 nt) covering the *ibpAB* operon. However, transcription termination occurred sometimes to produce the *ibpA* transcript (~500

nt). The *ibpB* transcripts as separate units were produced in the wild type (45°C) and the *rpoH15* strains (30 and 45°C) but not in the *rpoN208* strains, unless the $rpoN^+$ gene product complemented the mutation. These results pointed to the activity of the σ^{54}-promoter.

The primer extension technique was used to map the transcription start point (tsp) of the *ibpB* gene at the putative σ^{54}-promoter (Fig. 4A). The experiment has shown that transcription of *ibpB* started at A_{1111} (numbered according to the Gene Bank; Accession No. M94 104) 84 nt upstream from the translational start. The transcript starting at this point was found in the wild type strain only after heat shock demonstrating that the σ^{54}-promoter was heat-inducible. The transcript was also present in the *rpoH15* strain grown at 30°C and was produced at higher levels after heat shock. However, no *ibpB* transcript was found in the *rpoN208* mutant. Figure 4B shows that the complementation by the $rpoN^+$ gene product restored the transcription of the *ibpB* gene. Thus the role of the σ^{54}-promoter in the heat-shock inducible expression of *ibpB* gene has been demonstrated.

DISCUSSION

The presence of active σ^{54}-promoter located internally in the *ibpAB* operon, upstream of the *ibpB* gene was documented. The evidence for this conclusion is (i) identification of canonical DNA sequence of the σ^{54}-promoter with putative enhancer and IHF binding sites by PC-Gene program (Fig. 1), (ii) Northern hybridizations of total RNA of *E. coli* wild type, and *rpoN208*, *rpoN208*/$rpoN^+$, *rpoH15* and Δ*rpoH30* with *ibpA* and *ibpB* specific probes which showed that the *ibpB* gene could be transcribed as a separate unit in wild type and *rpoH15* strains, but that the *rpoN208* mutation abolished its transcription (Fig. 3A), (iii) the transcription in *rpoN208* cells was restored by the $rpoN^+$ gene product delivered from pTH7 plasmid, (iv) the primer extension experiment that established the transcriptional start point of the *ibpB* gene at A_{1111}, 6 nt downstream of the pσ^{54} (Fig. 4A), (v) confirmation of the previous findings by showing that no transcriptional start from this promoter was observed in the *rpoN208* mutant (Figs. 4A and 4B), and that the transcription was restored by complementation of the mutation.

The promoter region contains two overlapping sequences (Fig. 1, underlined by continuous and broken lines correspondingly) recognized by the PC Gene program as potential σ^{54}-promoters. Both differ from most σ^{54}-promoters characterized by conserved $_{5'}$GG-10nt-GC$_{3'}$ sequence (27, 28). The distal one contains CG at its conserved 3′ side instead of GC, the proximal to the transcriptional start contains CC. According to Barrios *et al.* (29) GC at −13, −12 positions are conserved in 96%. All changes in the conserved sequence may be regarded as down mutations reducing the binding of σ^{54}. The position of the transcriptional start, which is separated by 6 nt from CG, indicates that rather the distal promoter than the proximal one is active under conditions of the experiment.

The alignment of the putative *ihf* site from the *ibpB* regulatory region with the 13 nt consensus sequence of Craig and Nash (30) shows that 1 nt does not conform (Fig. 1). A comparison with the 48 nt extended consensus sequence of *ihf* (31) resulted in the base score 426 for the *ibpB* site, while the maximum site score and baseline score in the *ihf* target file were 682 and 323, respectively. The similarity score for the *ibpB* 48 nt sequence was 29. The authors suggest, as the most concise description of an IHF binding site, the combined use of the 10 base sequence AATCAA*aAgTTA (to indicate the site location) and the similarity score for extended 48 nt site (to indicate similarity to the entire site).

The PC Gene program indicated the putative enhancer site. The site is compared with that binding the PspF activator of the *pspA-E* operon (32, 33), which is the first heat-shock operon of *E. coli* controlled by the σ^{54} protein (Fig. 1). The two sequences contain several identical 4–5 nt stretches.

Taking into account all these properties of the regulatory sequence of the *ibpB* gene one should expect that pσ^{54} is a weak promoter, nevertheless its activity has been demonstrated.

A drastic difference was revealed (by Western and Northern blottings; Figs. 2 and 3) between the expression of the *dnaKJ* and *ibpAB* heat-shock operons in Δ*rpoH30*, *rpoH15* and *rpoN208* strains, despite the presence of the σ^{32}-promoter upstream of both operons. The mutations *rpoH15* or Δ*rpoH30* inhibited the expression of the *dnaKJ* operon, as expected. The Δ*rpoH*30 mutation was equally inhibitory for the *dnaKJ* and *ibpAB* operons (Figs. 2 and 3A). However, a high expression of *ibpAB* was observed in *rpoH15* and in the *rpoH165* strain, as reported previously (4). This observation showed that the defective σ^{32}_{15} or σ^{32}_{165} proteins could somehow satisfy the requirements for transcriptional start from this particular heat-shock promoter, in contrast to those from other σ^{32}-promoters.

Mathews and Neidhardt (34) and Matthews (35), reported another peculiarity of the regulation of the *ibpAB* operon. Increase in the σ^{32}-protein concentration by induction of the cloned *rpoH* gene by IPTG, at 28°C caused induction of most of the heat-shock transcriptional units. The exceptions were the three genes: *lysU* (coding for lysyl-tRNA synthetase), *ibpA* and *ibpB* (35). Moreover, it was found that in *metK* genetic background (reduced *S*-adenosylmethionine synthetase activity and therefore disturbed flux of one-carbon units), the genes *lysU*, *ibpA* and *ibpB* were not inducible by

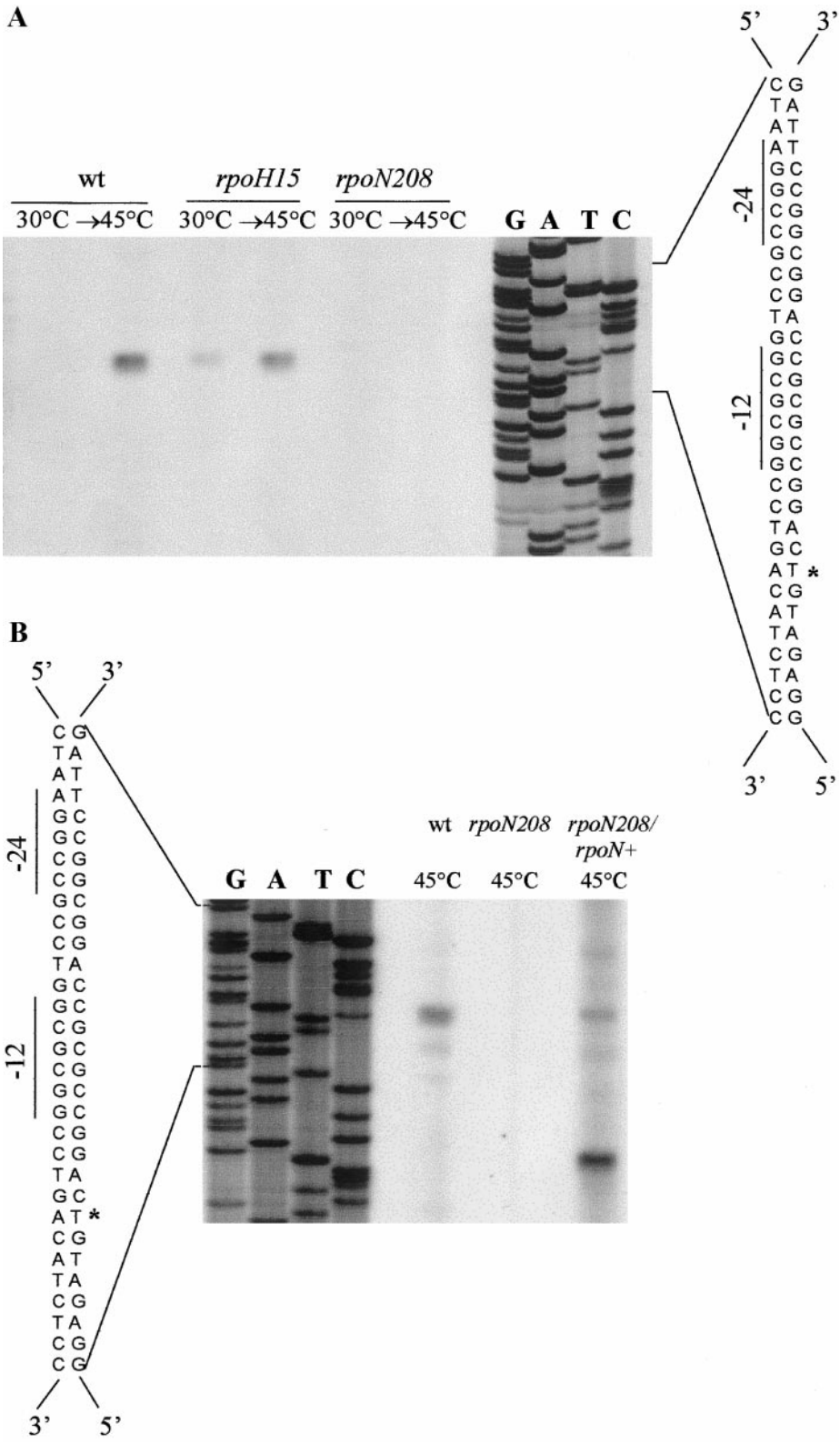

FIG. 4. Transcriptional start site of the *ibpB* gene. RNAs, used as matrices for reverse transcription, were extracted from *E. coli* (A) wt, *rpoH15* and *rpoN208* before (30°C) and 15 min. after heat shock (45°C) or (B) wt, *rpoN208* and *rpoN208*/*rpoN*$^+$ submitted to heat shock (15 min at 45°C). For *rpoN208*/*rpoN*$^+$ cells isopropyl-1-thio-β-D-galactopyranoside was added to 1 mM concentration 30 min before heat shock. To locate the transcriptional start site of the *ibpB* gene the B2 synthetic oligonucleotide was used as primer for reverse transcription. The samples were electrophoretically separated on 6% sequencing gel alongside a dideoxy sequence ladder generated with the same primer. The sequencing was performed by Sanger method using the Pharmacia Biotech (Uppsala) T7 sequencing kit and the manufacturer's instruction. The noncoding strand sequence is shown. T nucleotide on the ladder (marked with a star) corresponds to A transcriptional start of the *ibpB* gene. For scanning the Microtek Scan Wizard (U.S.A.) program was used.

heat shock. Taken together, these features were interpreted as demonstrating a requirement for a metabolic signal generated by one-carbon metabolism, absent when the *rpoH* gene was induced at 28°C, but not yet identified on the molecular level (35). Hence, one could assume that the defective σ^{32}-protein in *rpoH15* and *rpoH165* strains together with the metabolic signal fulfilled the requirements for transcriptional start of the *ibpAB* operon. Sze and Shingler (36) draw attention to the role of global regulator (p)ppGpp dominating the specific regulatory mechanisms involving several σ^{54}-promoters that respond to different stresses. It would be interesting to check its possible control over the *ibpB* σ^{54}-promoter.

The first heat-shock operon under the control of the σ^{54}-promoter, the *psp* (phage-shock-protein) operon of *E. coli* that is induced upon filamentous phage infection and by a variety of stresses, was recently described (32). Thus we have identified the second member of the σ^{54}-heat-shock regulon of *E. coli,* the *ibpB* gene.

The last point for discussion is the absence, or a very strong reduction of the level of the IbpAB proteins (Fig. 2) and their transcripts starting at the pσ^{32} (Fig. 3) in the *rpoN208* mutant cells grown in the P minimal medium (devoid of phosphate). This observation seems to be in agreement with the notion that the sequence about 40 nts 5′ to the *rpoH* gene might represent a σ^{54}-promoter (37). It seems possible, that it could be used for expression of the *rpoH* gene during growth in poor media like the P medium. If so, the *rpoH* gene might be a third member of the σ^{54}-heat-shock regulon.

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