Multiple layers of control govern expression of the
Escherichia coli ibpAB heat-shock operon

Lena C. Gaubig, Torsten Waldminghaus† and Franz Narberhaus

Microbial Biology, Ruhr University Bochum, Germany

The Escherichia coli ibpAB operon encodes two small heat-shock proteins, the inclusion-body-binding proteins IbpA and IbpB. Here, we report that expression of ibpAB is a complex process involving at least four different layers of control, namely transcriptional control, RNA processing, translation control and protein stability. As a typical member of the heat-shock regulon, transcription of the ibpAB operon is controlled by the alternative sigma factor σ^{32} (RpoH). Heat-induced transcription of the bicistronic operon is followed by RNase E-mediated processing events, resulting in monocistronic ibpA and ibpB transcripts and short 3′-terminal ibpB fragments. Translation of ibpA is controlled by an RNA thermometer in its 5′ untranslated region, forming a secondary structure that blocks entry of the ribosome at low temperatures. A similar structure upstream of ibpB is functional in vitro but not in vivo, suggesting downregulation of ibpB expression in the presence of IbpA. The recently reported degradation of IbpA and IbpB by the Lon protease and differential regulation of IbpA and IbpB levels in E. coli are discussed.

INTRODUCTION

Bacteria are continuously exposed to changes in environmental conditions. To respond to stress conditions in a timely fashion, they have developed numerous protective strategies that are tightly regulated. Global stress responses are primarily regulated at the level of transcription initiation (Campbell et al., 2008). The induction of stress genes in many bacteria is orchestrated by alternative sigma factors, subunits of the RNA polymerase that direct the holoenzyme to specific promoter sequences. In addition to the housekeeping sigma factor σ^{70}, the Escherichia coli genome encodes six alternative sigma factors. The most prominent examples are σ^{5} (RpoS), responsible for induction of the general stress response, and σ^{32} (RpoH), responsible for initiating heat-shock gene expression (Gross et al., 1998; Guisbert et al., 2008; Hengge-Aronis, 2002).

mRNA degradation and maturation also play important roles in the regulation of gene expression. The inherent stability of an mRNA molecule determines how long it is available as template for translation (Rauhut & Klug, 1999). The half-life of many mRNAs varies with environmental changes; this is consistent with mRNA stability playing a role in the control of differential gene expression (Newbury et al., 1987).

Regulatory RNAs have recently been recognized as important post-transcriptional gene control elements, often being involved in the coordination of stress responses (Narberhaus & Vogel, 2009; Waters & Storz, 2009). Small regulatory RNAs and riboswitches, functioning as trans- and cis-acting regulators, respectively, influence gene expression using various strategies, in most cases depending on RNA–RNA interactions. Riboswitches are structured RNA elements in the 5′ untranslated region (5′ UTR) of certain transcripts. Conformational changes upon the binding of a cellular metabolite alter gene expression, which can be controlled at the level of transcription elongation, translation initiation or RNA processing (Winkler & Breaker, 2005). Likewise, RNA thermometers are structured mRNA regions in the 5′ UTR of temperature-controlled bacterial genes (Narberhaus et al., 2006). They supervise translation initiation by folding into a structure that blocks the Shine–Dalgarno (SD) sequence at low temperatures. A temperature upshift induces melting of the structure, giving access to the ribosome and permitting translation of heat-shock or virulence genes (Narberhaus, 2010).

Numerous cellular processes, including mRNA translation and protein folding, are sensitive to temperature up- and downshifts (Klinkert & Narberhaus, 2009; Schumann, 2009). Therefore, bacteria precisely monitor changes in their ambient temperature and react accordingly with heat-shock or cold-shock responses. The E. coli heat-shock response is well characterized (Guisbert et al., 2008). A combination of transcriptional and post-transcriptional mechanisms culminate in the synthesis of σ^{32} under heat-stress conditions. The sigma factor initiates transcription of heat-shock genes, most of which encode molecular chaperones or proteases.
The *ibpAB* operon belongs to the $\sigma^{32}$ regulon. The open reading frames are separated by 111 bp and are the most dramatically induced heat-shock genes in *E. coli* (Richmond et al., 1999). The encoded small heat-shock proteins IbpA and IbpB (inclusion body-associated protein A and B) have been found associated with recombinant proteins in inclusion bodies (Allen et al., 1992). They also recognize endogenous *E. coli* proteins that aggregate in response to heat shock (Laskowska et al., 1996). IbpA and IbpB proteins share 57.5% sequence identity and exhibit chaperone activity under *in vitro* conditions (Kitagawa et al., 2000, 2002). IbpB was shown to stabilize stress-denatured proteins for subsequent refolding by a multichaperone network (Veinger et al., 1998). IbpA cooperates with IbpB in this process (Matuszewski et al., 2005). Regulation of the *ibpAB* operon is complex and not yet fully understood. As well as the transcriptional control of *ibpAB*, translational control of *ibpA* by a RNA thermometer in its upstream region has also been reported (Waldminghaus et al., 2009). In this study, we closely examined the regulation of the *ibpAB* operon and discovered additional layers of control.

**METHODS**

**Bacterial strains, plasmids, oligonucleotides and growth conditions.** All strains, plasmids and oligonucleotides used in this study are listed in Table 1. *E. coli* cells were grown at 30 or 37 °C in Luria-Bertani (LB) medium supplemented with ampicillin (Amp, 200 μg ml$^{-1}$) and/or kanamycin (Km, 50 μg ml$^{-1}$), as needed.

**Plasmid construction.** Recombinant DNA work was performed according to standard protocols (Sambrook et al., 1989). Site-directed mutagenesis to generate pBO668 was performed according to the instruction manual of the QuikChange mutagenesis kit (Stratagene). The correct nucleotide sequence of all plasmids was confirmed by automated sequencing. Detailed information on plasmid construction and characteristics is shown in Table 1.

**Construction of an *ibpAB* mutation in *E. coli* C600.** *E. coli* C600 ΔibpAB was constructed by P1 transduction according to standard protocols (Sambrook et al., 1989). *E. coli* JGT14 served as donor (Shearstone & Baneyx, 1999).

**RNA isolation and Northern blot analysis.** Cells were grown at 30 °C to exponential phase and an aliquot of the culture was heat-shocked at 42 °C for 1 min. To measure RNA stability, 250 μg rifampicin ml$^{-1}$ was added 1 min after heat shock. Samples were then taken at 0, 3, 6, 10 and 15 min after the addition of rifampicin. Isolation of total RNA and Northern blotting were performed as described previously (Waldminghaus et al., 2005). Digoxigenin-labelled RNA probes were produced according to the instruction manual (Roche).

**5' Rapid amplification of cDNA ends (RACE).** 5' RACE experiments were conducted as described previously (Willkomm et al., 2005) with minor modifications. DNase treatment was performed with 2 U DNase I (Promega) and 40 U RiboLock (Fermentas). Reverse transcription was carried out with SuperScript III (Invitrogen), according to the manufacturer's instructions. After PCR amplification with the adaptor primer and a gene-specific primer (primers ECibpBPev for the 5' end of the monocistronic *ibpB* transcript and 5RACE_**ibpB**.k for the 5' end of short *ibpB* fragments; Table 1), prominent bands were excised from 2% agarose gels, eluted and blunt-end cloned into pUC18 restricted with *SmaI*. The inserts of several plasmids from each experiment were sequenced.

**In vitro transcription of RNA.** RNA for toeprint analyses and structure probing was synthesized *in vitro* by run-off transcription with T7 RNA polymerase, from linearized plasmids or PCR-generated DNA templates (Table 1).

**Primer extension inhibition (toeprinting).** Toeprinting experiments were carried out using 30S ribosomal subunits, target mRNA and tRNA$^{Met}$ basically according to Hartz et al. (1988). The 5'-3'$^{-}$-labelled *ibpA*- and *ibpB*-specific oligonucleotides *ibpACDSrv* and *T7ibpBrv* (Table 1), complementary to nucleotides +66 to +43 and +69 to +50 of the *ibpA* and *ibpB* coding sequences, respectively, were used as primers for cDNA synthesis. A 0.08 pmol aliquot of *ibpA* or *ibpB* mRNA annealed to the appropriate oligonucleotide was incubated for 10 min at 30 or 42 °C together with 16 pmol uncharged TRNA$^{Met}$ (Sigma–Aldrich). 30S subunits (6 pmol) or Watanabe buffer (60 mM HEPES/KOH; 10.5 mM Mg(COO)$_2$; 690 mM NH$_4$COO; 12 mM β-mercaptoethanol; 10 mM spermidine; 0.25 mM spermine; negative control) were added, and incubated for another 10 min at 30 or 42 °C. Then 2 μl MMLV-Mix (VD + Mg$^{2+}$ buffer, RSA, dNTPs and MMLV reverse transcriptase; USB) was added. cDNA synthesis was performed at the respective temperature. Reactions were stopped after 10 min by adding formamide loading dye and aliquots were separated on a denaturing 8% polyacrylamide gel.

**In vitro RNA structure probing.** RNAs were 5' end-labelled as described previously (Brantl & Wagner, 1994). Digestions were carried out with RNase T1 (Ambion). In a total volume of 4 μl, RNA corresponding to 30000 c.p.m. was mixed with 1 μl 5× TMN buffer (100 mM Tris/acetate; 10 mM MgCl$_2$; 500 mM NaCl; pH 7.5) and 0.4 μg RNA. One microlitre of RNase T1 was added after 5 min of incubation at the respective temperature. After an additional incubation of 5 min, the reaction was stopped by adding 5 μl formamide loading dye. Samples were denatured at 95 °C for 5 min and an aliquot of each digest was loaded on an 8% polyacrylamide gel. Alkaline ladders were generated as described by Brantl & Wagner (1994).

**β-Galactosidase assay.** β-Galactosidase activity of *E. coli* strains carrying *ibpAB* fusions was measured as described by Miller (1972) with some modifications. For cell disruption, 0.5 μg lysosome μl$^{-1}$ and 25 μg Z-buffer (with 14 μl β-mercaptoethanol μl$^{-1}$) were added to 400 μl of cell suspension and incubated at 55 °C for 15 min. The enzymatic reaction was started at 55 °C by adding ONPG (2 mg ml$^{-1}$ in 1× Z-buffer with 2.7 μl β-mercaptoethanol μl$^{-1}$). Standard deviations (SD) were calculated from at least three independent experiments.

**Computer-based methods.** Sequences for computer-based analysis were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/Genbank/). RNA secondary structure was predicted by using the mfold server (http://frontend.bioinfo.rpi.edu/applications/mfold/cgi-bin/rna-form1.cgi) running version 3.2 (Zuker, 2003). The AIDA image analyser software v. 4.03 was used for toeprint and structure-probing gel analysis.

**RESULTS**

**Four transcript species derive from the *ibpAB* operon**

To analyse transcriptional and post-transcriptional events involved in *ibpAB* regulation, we synthesized RNA probes against the individual genes (Fig. 1a). The *ibpA* probe reacted with both *ibpA* and *ibpB* whereas the *ibpB* probe...
was highly specific to the *ibpB* transcript (data not shown). Several transcripts of the *ibpAB* operon were detected by Northern blot analysis (Fig. 1b, c). Due to the σ32 dependency of the operon, previously described by Allen et al. (1992), the *ibpAB* transcripts only appeared after heat shock from 30 to 42 °C and were not detectable in an *rpoH* mutant (data not shown). Only 2 min after heat shock at 42 °C, the bicistronic transcript was readily detectable by the *ibpA* (Fig. 1b) and *ibpB* (Fig. 1c) probes. The massive induction was transient, reached a maximum within a few minutes and declined to almost pre-shock levels after another 5–10 minutes. The concomitant appearance of a band between 500 and 600 nt suggested that the bicistronic message was processed into monocistronic *ibpA* and *ibpB* transcripts. Much shorter fragments of approx. 120 nt were detected between 8 and 15 min after heat-shock with the...
shocked. The O2 sample was taken after 30 min from the culture reported previously (Chuang et al., 2006). To determine the 5' ends of the monocistronic ibpA transcript, we measured the stability of the bicistronic mRNA in a temperature-sensitive RNase E mutant and an RNase III-negative strain. RNase E is essential in E. coli. A shift from 30 to 42 °C induced transcription of the ibpAB operon and inactivated RNase E in the mutant strain. Decay of the bicistronic transcript after addition of rifampicin in the presence of RNase E and its stability in the absence of the RNase showed that degradation of the ibpAB transcript depends on RNase E (Fig. 3a). Furthermore, RNase E is responsible for the processing of the bicistronic ibpAB RNA, as the monocistronic ibpB transcript and the short ibpB products were detectable in the wild type (WT), whereas they did not appear in the RNase E mutant strain. Note that the monocistronic transcript in the RNase E mutant at time point zero was generated before the temperature-sensitive enzyme was inactivated. If at all, RNase III contributes only marginally to the processing and degradation of the ibpAB transcript because it had comparable half-lives in the wild-type and the RNase III mutant (Fig. 3b).

**RNases involved in ibpAB processing**

To reveal the identity of the RNase(s) responsible for the processing of the ibpAB transcript, we measured the stability of the bicistronic mRNA in a temperature-sensitive RNase E mutant and an RNase III-negative background. RNase E is essential in E. coli. A shift from 30 to 42 °C induced transcription of the ibpAB operon and inactivated RNase E in the mutant strain. Decay of the bicistronic transcript after addition of rifampicin in the presence of RNase E and its stability in the absence of the RNase showed that degradation of the ibpAB transcript depends on RNase E (Fig. 3a). Furthermore, RNase E is responsible for the processing of the bicistronic ibpAB RNA, as the monocistronic ibpB transcript and the short ibpB products were detectable in the wild type (WT), whereas they did not appear in the RNase E mutant strain. Note that the monocistronic transcript in the RNase E mutant at time point zero was generated before the temperature-sensitive enzyme was inactivated. If at all, RNase III contributes only marginally to the processing and degradation of the ibpAB transcript because it had comparable half-lives in the wild-type and the RNase III mutant (Fig. 3b).

**Heat-stimulated ribosome binding to the ibpB transcript in vitro**

A potential secondary repression of heat-shock gene expression (ROSE)-like RNA structure in the 5' UTR of ibpB suggested that translation might be controlled by an RNA thermometer as is the case for ibpA (Waldminghaus et al., 2009). The putative ibpB thermometer consists of four hairpins in the intergenic region between ibpA and ibpB (see Fig. 5a). The SD sequence is located in hairpin IV and is imperfectly paired with the anti-SD sequence, which exhibits the conserved U(U/C)GCU motif typical of ROSE-like elements (Waldminghaus et al., 2005). To evaluate the identity of the RNase(s) responsible for the processing of the ibpAB transcript, we measured the stability of the bicistronic mRNA in a temperature-sensitive RNase E mutant and an RNase III-negative strain. RNase E is essential in E. coli. A shift from 30 to 42 °C induced transcription of the ibpAB operon and inactivated RNase E in the mutant strain. Decay of the bicistronic transcript after addition of rifampicin in the presence of RNase E and its stability in the absence of the RNase showed that degradation of the ibpAB transcript depends on RNase E (Fig. 3a). Furthermore, RNase E is responsible for the processing of the bicistronic ibpAB RNA, as the monocistronic ibpB transcript and the short ibpB products were detectable in the wild type (WT), whereas they did not appear in the RNase E mutant strain. Note that the monocistronic transcript in the RNase E mutant at time point zero was generated before the temperature-sensitive enzyme was inactivated. If at all, RNase III contributes only marginally to the processing and degradation of the ibpAB transcript because it had comparable half-lives in the wild-type and the RNase III mutant (Fig. 3b).

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bicistronic ibpAB region ranging from the ibpA 5′ UTR to 69 nt into the coding region of ibpB or a shorter fragment containing only the ibpB 5′ UTR up to 69 nt into the coding region of ibpB. Temperature-dependent binding of the 30S ribosomes to the ibpA SD sequence has already been demonstrated by using a short fragment containing only the ibpA 5′ UTR (Waldminghaus et al., 2009). Inefficient binding of the 30S ribosome to the ibpA SD sequence at 30 °C and enhanced formation of the RNA–ribosome complex at 42 °C was also observed with the bicistronic ibpAB fragment (Fig. 4b). Binding of the ribosome to the ibpB leader region was equally temperature-sensitive. The absence of a prominent toeprint signal at 30 °C despite the presence of 30S ribosomes suggests that access of the ribosome to the ibpB SD sequence is blocked by the predicted RNA thermometer structure in
both the long and the short transcript (Fig. 4c and d, respectively). Prematurely terminated reverse transcripts, at 42 °C and at the typical toeprint position around 15 nt downstream from the AUG start codon, showed that melting of the structured RNA under heat-shock conditions permits translation initiation of ibpB in vitro.

**Structural features of the ibpB 5’ UTR**

To gain structural insights into the putative ibpB thermometer and two ibpB 5’ UTR variants, structure-probing experiments were carried out with RNase T1 (cleaves 3′ of single-stranded guanines) over a temperature range from 20 to 50 °C (Fig. 5). To reduce complexity, constructs analogous to the previously studied ibpA thermometer, containing the predicted hairpins II, III and IV of the ibpB 5’ UTR, were chosen (Fig. 5a). The overall cleavage pattern of the ibpB WT UTR supports the predicted structure (Fig. 5b). Cleavage at positions G50, G51 and G52 at low temperatures suggests that the top loop is wider as predicted. The structure of hairpin IV was temperature-responsive. G101, G102 and G104 were barely cleaved at low temperatures but showed increased accessibility to RNase T1 at temperatures above 40 °C, suggesting liberation of the SD sequence according to the melting model. Probing of the C91A and ΔG90 variants (Fig. 5c, d) provided structural evidence for their derepressed (‘Derep’) and repressed (‘Rep’) phenotypes, respectively, in reporter-gene assays (data not shown). In both RNAs, hairpins II and III behaved like the corresponding hairpins of the WT RNA. Hairpin IV of the ‘Derep’ RNA was more sensitive to RNase T1 cleavage compared to that of the WT (Fig. 5c); prominent cleavage of the guanines in the SD region already occurred at low temperatures. In the ‘Rep’ RNA, the SD sequence did not become accessible in the physiological temperature range (Fig. 5d).

**IbpA-mediated regulation of ibpB expression**

To further analyse the putative RNA thermometer of the ibpB 5’ UTR we constructed translational ibpB and ibpAB reporter-gene fusions (Fig. 6a) by using our previously described bgab-reporter system taking advantage of the thermostable β-galactosidase BgaB from Bacillus steaorthemophilus (Waldminghaus et al., 2007). At 30 °C, expression of the ibpB fusion measured in E. coli C600 was about 110 MU and increased only slightly to 160 MU at 42 °C (Fig. 6b). Expression of the ibpAB fusion was comparable, whereas the ibpA fusion was heat-inducible, as reported previously (Waldminghaus et al., 2009). Hence, in
Fig. 5. Structure probing of the \textit{ibpB} 5' UTR. (a) Secondary structure prediction of the \textit{ibpB} 5' UTR made with the mfold program (Zuker, 2003). Nucleotide numbers were arbitrarily chosen starting from the second A of the UAA stop codon of \textit{ibpA}. The sequence shown in black represents the \textit{in vitro}-synthesized RNA used for structure probing. The SD sequence and AUG start codon of \textit{ibpB} are indicated as well as single-point mutations of repressed ('Rep') and derepressed ('Derep') variants. (b–d) Structure probing of the \textit{ibpB} WT 5' UTR (b), the predicted derepressed (c) and the predicted repressed (d) variants. L, alkaline ladder; K, water-treated control RNA.
contrast to the in vitro results, which showed temperature-dependent melting of, and ribosome binding to, the ibpB RNA, the in vivo data argue against the temperature-regulated translation of ibpB.

Measurements of the same fusions in an E. coli C600 ΔibpAB strain suggested a solution for this apparent puzzle. In the absence of any IbpA and IbpB protein, the ibpB fusion showed heat induction like the ibpA fusion (Fig. 6b) (Waldminghaus et al., 2009). Interestingly, expression was reduced in the ibpAB fusion, which is able to produce IbpA from the reporter plasmid. The possible influence of the IbpA protein on ibpB expression was determined by testing the introduction of a nonsense mutation at the beginning of the coding sequence of ibpA in the ibpAB fusion so that no IbpA protein could be synthesized from this plasmid. The temperature response of this fusion in the ΔibpAB background was restored, suggesting that IbpA plays a role in controlling ibpB expression.

DISCUSSION

Regulation of the heat-shock response in E. coli is complex and involves several mechanisms, mostly controlling the cellular level of the key regulator σ^{32} in response to the physiological need (Guisbert et al., 2008). Here, we describe novel mechanisms that control expression of the ibpAB operon at regulatory levels downstream of σ^{32}-mediated transcriptional control (Fig. 7).

Transcriptional regulation and RNA processing

Transcription of the ibpAB operon has been reported to depend on the alternative sigma factor σ^{32} (Allen et al., 1992). In certain nutrient-poor media, the distal ibpB gene is transcribed from a σ^{34}-dependent promoter (Kuczyńska-Wisniski et al., 2001). However, this does not play a role under the conditions used in the present study because corresponding 5' ends were not found by 5' RACE experiments. The σ^{32} promoter upstream of ibpA is responsible for the massive, several hundred-fold induction of the ibpAB operon after heat shock (Rasouly et al., 2009; Wade et al., 2006). The requirement for the heat-shock sigma factor was recapitulated in our experiments. Further, we discovered several RNA processing events that might result in differential expression of ibpA and ibpB. Cleavage of the bicistronic ibpAB transcript results in monocistronic ibpA and ibpB transcripts, the latter of which is further processed into short 3' ends. The 5' end of the monocistronic ibpB transcript was mapped within the stop codon of ibpA. There are no apparent promoter sequences upstream of this site. Instead, the sequence around the mapped 5' end (A^QAUUC) closely resembles the consensus sequence of an RNase E cleavage site RAUUW (R=A/U; W=A/U), in which the cleavage usually occurs in the central AUU sequence (Ehretsmann et al., 1992). The g55/zgt RNA from the T4 phage was demonstrated to be cleaved by RNase E in E. coli at exactly the same nucleotide sequence (A^QAUUC) as in the ibpAB transcript (Loayza et al., 1991). Fully
consistent with the putative RNase E cleavage site, the \(ibpB\) transcript was only detectable in the WT strain, whereas it did not appear in the temperature-sensitive RNase E mutant after heat inactivation of the enzyme.

Subsequent decay of the \(ibpB\) RNA in the wild-type strain led to the transient accumulation of short degradation products containing the 3' end of \(ibpB\). The 5' fragment was not detected, suggesting that it is rapidly degraded. In contrast to the well-defined initial cut in the \(ibpAB\) transcript, the \(ibpB\) fragments carry diverse 5' ends. RNase E-dependent cleavage is known to be the rate-limiting step in mRNA degradation. It processes polyribosomal mRNA by cleaving in the translation-initiation region or in the intercistronic regions of polycistronic messages. The mRNA fragments generated are further cleaved at RNase E-sensitive sites located downstream (Carpousis, 2007; Jain, 2002). Several of the mapped 5' ends within the \(ibpB\) mRNA are characterized by AU-rich sequences suggesting that RNase E is also responsible for this process.

**Translational and post-translational regulation**

Transcriptional induction of the \(ibpAB\) operon and mRNA processing are complemented by further control mechanisms (Fig. 7). Translation of the \(ibpA\) gene is temperature-controlled by an RNA thermometer (Waldminghaus et al., 2009). The 5' UTR of the \(ibpB\) gene also exhibits characteristics of a ROSE-like element (Narberhaus et al., 2006). Toeprinting experiments indeed showed heat-dependent binding of the 30S ribosomal subunit to the \(ibpB\) SD sequence in the monocistronic as well as in the bicistronic context. Structure-probing experiments suggested an opening of the RNA structure in hairpin IV, which harbours the \(ibpB\) SD sequence. These in vitro results demonstrated that the structural properties of the \(ibpB\) 5' UTR allow thermoregulation of \(ibpB\) translation. However, the temperature response in vitro was not reflected by translational fusions in vivo. Heat induction of an \(ibpB\)-\(bgaB\) fusion was only detected when no \(ibpA\) protein was present in the cells. The two homologous small heat-shock genes \(ibpA\) and \(ibpB\) are believed to originate from a duplication event and were initially discovered as inclusion-body-binding proteins during the production of heterologous proteins in *E. coli* (Allen et al., 1992; Vaillancourt, 2003). In other studies it was shown that \(IbpA\) and \(IbpB\) levels increase almost 10-fold during overexpression of recombinant proteins while other heat-shock proteins like DnaK and GroEL increase only 2-fold (Han et al., 2004). This suggests that \(IbpA\) and \(IbpB\) play an important role during recombinant protein production. Nevertheless, their cooperation and exact function in vivo are still not fully understood (Han et al., 2004; Matuszewska et al., 2008). The pool of substrates interacting with \(IbpA\) is much larger than that of \(IbpB\) (Butland et al., 2005). The presence of \(IbpA\) alone during substrate denaturation did not increase the efficiency of reactivation but rather inhibited this process. \(IbpA\)-mediated recruitment of \(IbpB\) reversed this inhibitory effect (Ratajczak et al.,...
2009). Although IbpA and IbpB seem to have distinct activities, they depend on an intricate interplay between the two proteins.

For reasons not yet fully understood, IbpA accumulation is stronger than IbpB accumulation during ibpAB overexpression and in cells exposed to copper (Lethanh et al., 2005; Matuszewska et al., 2008). Our results suggest two possible reasons for differential expression of ibpA and ibpB (Fig. 7). First, processing of the ibpAB transcript between ibpA and ibpB and subsequent cleavage within ibpB might result in preferential accumulation of ibpA transcripts in the cell. In addition, regulation of ibpB expression by IbpA might constitute a second mechanism involved in reducing ibpB expression. At present, we can only speculate on the putative mechanism of IbpA-mediated control. It is conceivable that IbpA interacts with the ibpB mRNA to prevent translation and/or stimulate ribonucleolytic decay. In plants, small heat-shock proteins are associated with mRNAs in heat-shock granules (Nover et al., 1989). Other reports have shown that small heat-shock proteins are able to interact with single-stranded and double-stranded DNA (Singh et al., 1998). Owing to its polydispersity and its tendency to precipitate, purification of IbpA for biochemical characterization is notoriously difficult (Shearstone & Baneyx, 1999). Hence, all our attempts to demonstrate a direct interaction between the ibpB 5′ UTR and IbpA failed. The recently discovered degradation of IbpA and IbpB by the Lon protease provides another explanation for differing cellular levels of the two proteins (Bissonnette et al., 2010). Proteinolyis of IbpB was 15-fold faster than IbpA degradation. Interestingly, decay of IbpA was accelerated 7-fold in the presence of IbpB. Taking these findings together with our results, it is tempting to speculate an antagonistic mechanism, in which the IbpA protein represses its own degradation by inhibiting the translation of ibpB. It is going to be a challenging task to obtain deeper mechanistic insights into the control of the ibpAB operon.

ACKNOWLEDGEMENTS

We thank Birgit Klinkert for the generous gift of 30S ribosome preparations, Ursula Aschke-Sonnenborn for technical assistance, and Gabriele Klug (University of Giessen) for sending RNase mutant strains. We are grateful to the German Research Foundation (DFG, SPP 1258) for financial support.

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Edited by: M. S. Paget