RNA thermometers

Franz Narberhaus[1], Torsten Waldminghaus[1] & Saheli Chowdhury[1,2]

[1]Lehrstuhl für Biologie der Mikroorganismen, Ruhr-Universität Bochum, Bochum, Germany and [2]Institut für Mikrobiologie, ETH-Hönggerberg, Zürich, Switzerland

Correspondence: Franz Narberhaus, Lehrstuhl für Biologie der Mikroorganismen, Ruhr-Universität Bochum, NDEF 06/783, D-44780 Bochum, Germany. Tel.: +49 234 322 3100; fax: +49 234 321 4620; e-mail: franz.narberhaus@rub.de

Received 28 February 2005; revised 18 April 2005; accepted 10 May 2005
First published online 7 September 2005

doi:10.1111/j.1574-6976.2005.004.x

Editor: Fritz Unden

Keywords:
riboswitch; RNA sensor; heat shock; chaperones; virulence.

Abstract

Temperature is an important parameter that free-living cells monitor constantly. The expression of heat-shock, cold-shock and some virulence genes is coordinated in response to temperature changes. Apart from protein-mediated transcriptional control mechanisms, translational control by RNA thermometers is a widely used regulatory strategy. RNA thermometers are complex RNA structures that change their conformation in response to temperature. Most, but not all, RNA thermometers are located in the 5′-untranslated region and mask ribosome-binding sites by base pairing at low temperatures. Melting of the structure at increasing temperature permits ribosome access and translation initiation. Different *cis*-acting RNA thermometers and a *trans*-acting thermometer will be presented.

Introduction: RNA sensors

Free-living microorganisms are frequently exposed to changing environmental conditions. Nutrient availability, osmolarity, pH, temperature and many other parameters are being surveyed constantly. To avoid hazardous consequences, bacteria have established an intricate network of protective mechanisms. Often, regulation of environmentally controlled genes is realized at the transcriptional level by the action of regulatory proteins. However, several post-transcriptional, RNA-based strategies have been discovered recently. It is becoming increasingly clear that certain messenger RNAs (mRNAs) are not simply a substrate for ribosomes but contain control elements that modulate their own expression in a condition-dependent fashion. Structural changes in such sensory RNAs are induced by specific environmental changes. Two principally different classes can be distinguished: *cis*-acting RNA elements that bear their regulatory potential embedded within the mRNA sequence and *trans*-acting small, noncoding RNAs that function by base-pairing with complementary mRNA sequences encoded elsewhere in the genome.

Unlike classical attenuators which adjust the RNA structure of a leader sequence, according to the position of the translating ribosome (Landick *et al*., 1996; Henkin & Yanofsky, 2002), *cis*-acting RNAs change their conformation in response to physical or chemical signals. So-called riboswitches monitor the metabolic state of a cell by binding to metabolites with high specificity and affinity. They are located in the 5′-UTR of genes coding for the biosynthesis, uptake or degradation of small metabolites and provide feedback control to these pathways. Their complex architecture is comprised of a receptor (aptamer) region characterized by a consensus sequence specifying the substrate molecule and an output (expression platform) region. Binding of a small molecule throws a conformational switch which alters gene expression by one of three possible mechanisms: premature transcription termination (Mironov *et al.,* 2002; Winkler *et al*., 2002a; Epshtein *et al*., 2003; Grundy *et al*., 2003; Mandal *et al*., 2003; McDaniel *et al*., 2003; Sudarsan *et al*., 2003; Winkler *et al*., 2003), translation initiation (Nou & Kadner, 2000; Nahvi *et al*., 2002; Winkler *et al*., 2002a, b) or mRNA processing (Winkler *et al*., 2004) (Fig. 1a). Most riboswitches turn off expression in the bound state. However, a few 'on'-switches have also been found (Mandal & Breaker, 2004a; Mandal *et al*., 2004). The presence of potential riboswitches in eukaryotes suggest a wide distribution of this presumably ancient mode of control (Kaempfer, 2003; Kubodera *et al.,* 2003; Sudarsan *et al*., 2003).

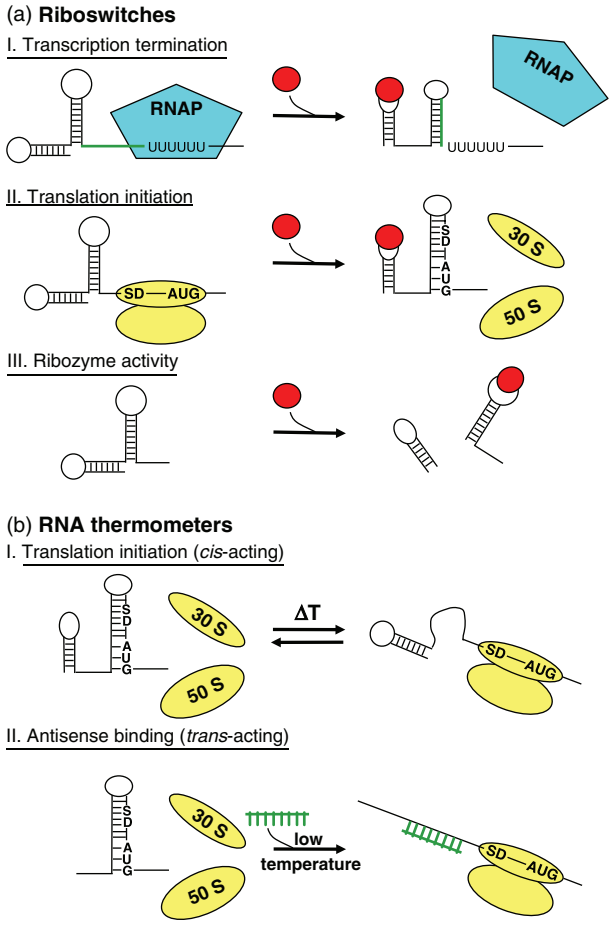

Fig. 1. Schematic illustration of regulatory principles of riboswitches (a) and RNA thermometers (b). The processes that are controlled are indicated in each case. Red circles represent metabolites. RNAP, RNA polymerase; SD, Shine–Dalgarno sequence; 30S and 50S, ribosomal subunits, Δ*T*, temperature change. AUG and UUUUUU; ribonucleotide sequences.

Because numerous excellent reviews have been published recently on this fascinating subject (Narberhaus, 2002; Lai, 2003; Winkler & Breaker, 2003; Grundy & Henkin, 2004; Mandal & Breaker, 2004b; Nudler & Mironov, 2004; Soukup & Soukup, 2004; Vitreschak *et al.*, 2004), the present review will focus on a closely related type of sensory mRNAs, the RNA thermometers. In contrast to highly specific metabolite-binding riboswitches, they respond to a fairly global physical signal, i.e. the intracellular temperature, which is an important parameter under constant vigilance. It is a well-known characteristic of structured nucleic acid molecules that they melt as the temperature increases. Hence, it is easily conceivable that temperature shifts are able to modulate the conformation of regulatory RNAs. A collection of structurally and functionally diverse RNA thermosensors that control a variety of cellular processes will be presented in this review. All currently known *cis*- and *trans*-acting molecular thermometers bring about translational control by sequestration of the ribosome-binding region (Fig. 1b).

Control of various cellular processes by *cis*-acting RNA thermometers

Considering possible temperature-controlled genes, several processes come to mind. The most obvious are the heat- and cold-shock responses, i.e. the expression of genes that protect a living cell from deleterious consequences of sudden temperature up- or downshifts. Virulence gene expression is a possibility if one considers that many pathogenic micro-organisms spend most of their life outside the comfort of a warm-blooded host. All these processes are, at least in part, temperature-regulated and RNA thermometers play an important role. Much less intuitively, it is possible that regulatory circuits during infection of *Escherichia coli* with phage λ or of the starvation response are also modulated by RNA thermometers. However, the first RNA thermometer was discovered some 15 years ago in the λ cIII gene.

Lysis-lysogeny decision of phage λ

Pioneering studies showed that alternative mRNA structures determine the translation rate of the λ cIII gene in a temperature-dependent manner (Altuvia *et al.*, 1989). The cIII gene product plays a role in the decision of whether phage λ enters the lytic or lysogenic cycle. It serves as an alternative substrate for the FtsH protease, which degrades the cII protein, a central regulator in the lysogenic pathway (Herman *et al.*, 1997; Shotland *et al.*, 1997). High concentrations of the cIII protein thus result in the stabilization of cII, which in turn promotes lysogeny. Several point mutations affecting translation of cIII were mapped in a region covering approximately 85 nucleotides upstream of the AUG start codon and 45 nucleotides in the coding region (Altuvia & Oppenheim, 1986; Kornitzer *et al.*, 1989). Two alternative mRNA structures were predicted and verified experimentally by structure probing *in vitro* and *in vivo* (Altuvia *et al.*, 1989, 1991). One structure sequesters part of the Shine–Dalgarno (SD) sequence and the AUG start codon, whereas the other structure leaves the translation initiation region accessible to the ribosome (Fig. 2). Single-point mutations are able to lock the RNA into either one or the other conformation. The equilibrium between both structures is temperature-dependent. High temperatures (45 °C) favour the energetically more stable conformation in which the ribosome-binding site is occluded. Blocked translation of cIII results in decreased cII concentration and initiates the lytic pathway. Physiological temperatures (37 °C) shift the equilibrium towards the structure in which the ribosome binding is available and cIII protein is produced.

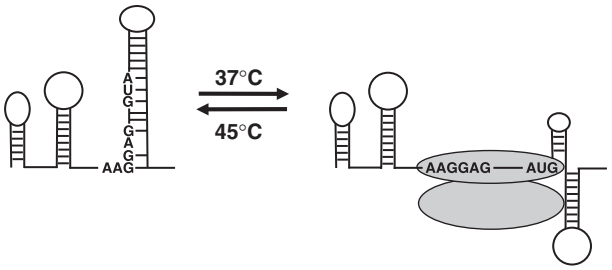

Fig. 2. Temperature-dependent sequestration of the λ cIII ribosome-binding site. Grey ovals, ribosome; AAGGAG, Shine–Dalgarno (SD) sequence; AUG, start codon.

Interestingly, the switch region is preceded by two additional stem-loop structures which are identical in either conformation (Fig. 2). The integrity of the second stem loop is required for efficient ribosome binding (Altuvia *et al.*, 1991). It has been proposed that this region is involved in tertiary RNA–RNA interactions (Altuvia *et al.*, 1989, 1991). It has also been shown that RNaseIII binds to this stem loop and it is assumed that binding (but not RNA cleavage) introduces a structural transition that stimulates translation (Altuvia *et al.*, 1987). Based on this finding, the cIII mRNA might be considered a protein-dependent riboswitch. However, the temperature-induced structural alterations shown *in vitro* (Altuvia *et al.*, 1989) demonstrate that it is a *bona fide* RNA thermometer.

What might be the physiological reason for temperature control of the cIII gene? The lysis-lysogeny decision of phage λ is regulated by a complex network of transcriptional and post-transcriptional mechanisms in response to multiple environmental signals, among them nutritional status and ambient temperature. The phage tends to proliferate effectively when the host cells are healthy and it is more likely to integrate into the chromosome when growth conditions are poor. However, under life-threatening conditions (e.g. ultraviolet (UV) damage, severe heat shock) it might be beneficial for the phage to escape from the host. The amount of cII protein is critical to the fate of the phage. Enhanced degradation of cII by the heat-inducible FtsH protease in combination with reduced production of the competitor cIII will stimulate the lytic pathway under severe heat stress conditions.

Heat-shock gene expression

A sudden temperature upshift poses a serious threat to the integrity of almost all cellular macromolecules. The structure of membrane lipids, DNA, RNA and proteins is altered as the temperature rises. The expression of heat-shock proteins is a universal response found in all living cells. The induced chaperones or proteases constitute a protein quality-control system that either rescues misfolded proteins or promotes their degradation (Gross, 1996; Yura *et al.*, 2000). The cellular concentration of heat-shock proteins is regulated primarily at the level of transcription and involves positive control by alternative sigma factors or negative control by repressor proteins (Narberhaus, 1999; Yura *et al.*, 2000; Servant & Mazodier, 2001; Schumann, 2003). An additional level of control is often brought about by built-in feedback loops. A subset of heat-induced chaperones or proteases is commonly recruited to control the activity or stability of the regulatory factors involved. For example, the activity and stability of the *E. coli* heat-shock σ factor σ^{32} (RpoH) is tightly controlled by the DnaK chaperone and the FtsH protease (Straus *et al.*, 1990; Herman *et al.*, 1995; Tomoyasu *et al.*, 1995). While this mechanism acts on the already established cellular pool of the transcription factor, an RNA thermometer in the *rpoH* gene ensures that translation efficiency of the master regulator of the heat-shock response correlates with the growth temperature.

The *E. coli rpoH* gene

Expression of the *rpoH* gene in *E. coli* is modulated by four different promoters which respond to different environmental signals (Yura *et al.*, 2000) and by two intragenic segments (regions A and B) in the coding sequence (Fig. 3a). As *rpoH* transcription is only moderately altered in response to heat stress, translational control was responsible for accumulation of the sigma factor at high temperatures (Tilly

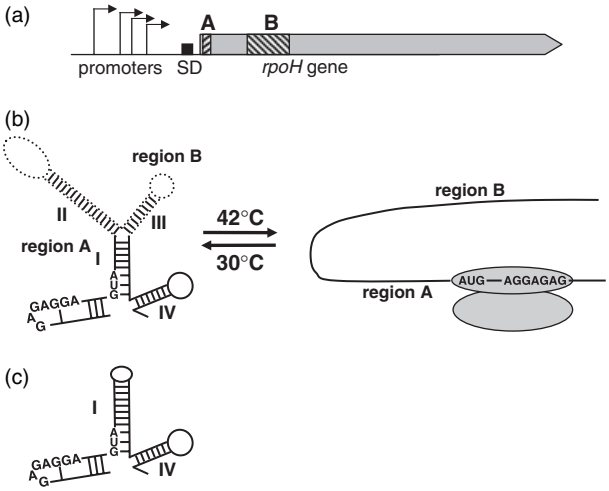

Fig. 3. Regulatory elements of the *Escherichia coli rpoH* gene (a) and schematic drawing of the secondary structures involved in translational control (b, c). The entire *rpoH* gene is comprised of 852 nucleotides and regions A and B extend from 6 to 20 and 112 to 208, respectively. The actual secondary structure of the region represented by dotted lines is more extensive than shown in (b). The minimal, functional thermoregulator is illustrated in (c). AAGGAG, Shine–Dalgarno (SD) sequence; AUG, start codon.

et al., 1986; Straus *et al.*, 1987). Using a large set of *rpoH–lacZ* fusions it was found that two distinct 5′-proximal regions in the coding sequence of *rpoH* are responsible for translational control (Nagai *et al.*, 1991). Secondary structure formation between these two regions was postulated to repress translation initiation at low temperatures (Fig. 3a). Disruption of that structure by a temperature upshift would liberate the ribosome-binding site. Similar structures were predicted to occur in the *rpoH* genes from other Gram-negative bacteria (Nakahigashi *et al.*, 1995). Experimental evidence for such an RNA structure was generated by deletion analysis, point mutagenesis and structure probing of *E. coli rpoH* (Morita *et al.*, 1999a). The structure in stem I appears to be weak as several nucleotides were modified by chemical probes, although they are predicted to base pair. A certain instability of the RNA structure might be required to permit residual ribosome entry and basal production of the σ factor at low temperatures. Starting from a *lacZ* reporter fusion downstream of box B, a minimal RNA thermometer was created (Morita *et al.*, 1999a). Its secondary structure was trimmed down to the SD region followed by stem loops I and IV (Fig. 3c). In the minimal fusion construct, mismatches had to be introduced into stem I in order to maintain heat inducibility, indicating that imperfect base pairing is required for proper thermosensing. It is notable that stem loops II and III were dispensable only when certain destabilizing mismatches were introduced into the remaining sequence. It thus seems that each individual hairpin plays a distinct role in the complex architecture of the wild-type structure. Stem loop III was suggested to function as a 'wedge' between stems I and II, conferring the instability to the mRNA structure required for melting in the physiological temperature range (Morita *et al.*, 1999a). Whether the region downstream of stem I opens up entirely after heat shock or whether movement of the ribosome during translation disrupts the remaining base pairs is not known.

Further support for the importance of secondary structure formation was obtained by an extensive mutagenesis study (Morita *et al.*, 1999b). Point mutations predicted to loosen the RNA structure increased reporter fusion expression, whereas mutations predicted to increase RNA stability reduced expression. Compensatory mutations recovered almost normal expression demonstrating that the mRNA structure, rather than the primary nucleotide sequence, is critical for thermoregulation. In agreement, circular dichroism (CD) spectroscopy of synthetic *rpoH* RNA fragments revealed a strong negative correlation between the RNA thermostability *in vitro* and corresponding expression levels *in vivo*.

The key prediction of the RNA thermometer model is that ribosomes have access to the *rpoH* transcript only at high temperatures. Indeed, toeprinting experiments (primer extension inhibition assays) showed that high temperatures were required for ribosome loading (Morita *et al.*, 1999b). Full-length reverse transcripts initiated from a primer complementary to the coding region of *rpoH* were obtained at 30 °C. At 42 °C, however, primer extension was blocked by the assembly of a ternary complex between the *rpoH* transcript, 30S ribosome and $tRNA_f^{Met}$. Experiments with RNA sequences carrying mismatches or stabilizing point mutations in the thermometer region were used to establish that ribosome binding is dependent upon the stability of the RNA structure. Evidence for the contribution of additional cellular factors was not found in these *in vitro* assays, suggesting that the RNA structure itself suffices as molecular thermometer.

*R*epression *O*f heat-*S*hock gene *E*xpression (ROSE)

While the *rpoH* thermosensor reaches far into the coding region, most other thermometers are located primarily upstream of the translation start codon. A conserved sequence of about 100 nucleotides in the 5′-UTR of multiple small heat-shock genes in *Bradyrhizobium japonicum* was strongly indicative of a novel regulatory element (Narberhaus *et al.*, 1996, 1998; Münchbach *et al.*, 1999). The expression of a translational *lacZ* fusion to the *hspA* gene downstream of such a sequence was very low at 30 °C, but increased dramatically when large internal regions were deleted. Based on this inhibitory effect, the *cis*-acting regulatory element was designated ROSE for *R*epression *O*f heat-*S*hock gene *E*xpression (Narberhaus *et al.*, 1998). The absence of ROSE-containing transcripts at low temperatures was interpreted initially as evidence for transcriptional control, presumably by a repressor protein binding to this conserved region. However, all attempts to isolate a regulatory factor binding to ROSE-containing DNA failed (Nocker *et al.*, 2001a). The first evidence that the mRNA itself might comprise the regulatory entity originated from computer calculations using the mfold program (Zuker *et al.*, 1999; Zuker, 2003). The $ROSE_1$ RNA from *B. japonicum* was predicted to fold into a complex structure occluding both the SD sequence and AUG start codon (Fig. 4a). ROSE elements from related *Rhizobiaceae* such as *Mesorhizobium loti*, *Rhizobium* sp. NGR 234 and *Agrobacterium tumefaciens* (Fig. 4b) are assumed to fold into very similar structures (Nocker *et al.*, 2001a, b; Balsiger *et al.*, 2004). The regulatory potential of these structures to act as RNA thermometers was examined by mutational studies with $ROSE_1$ preceding the *hspArpoH*$_1$ operon of *B. japonicum* (Fig. 4a). Introduction of mismatches in the paired regions of stem-loop IV increased expression of translational *lacZ* fusions, compensatory mutations restored repression and alterations in unpaired nucleotides were neutral, demonstrating that a structured RNA rather than a particular RNA sequence is

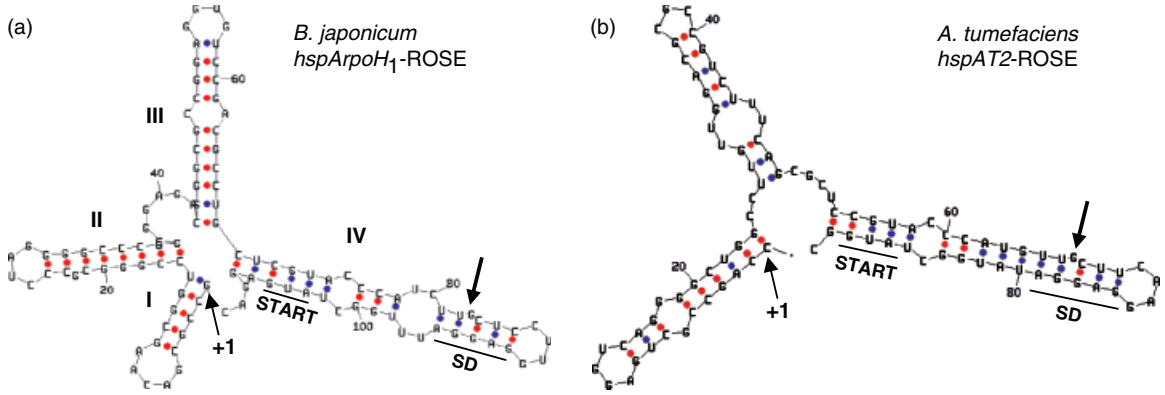

Fig. 4. *R*epression *O*f heat-*S*hock gene *E*xpression (ROSE) elements from *Rhizobiaceae*. The predicted secondary structures of *Bradyrhizobium japonicum* $ROSE_1$ (a) and *Agrobacterium tumefaciens* $ROSE_{AT2}$ (b) is shown. Transcriptional start sites (+1), Shine–Dalgarno sequence (SD) and the translational start site (START) are indicated. A conserved bulged G is marked with an arrow. The four hairpin structures of $ROSE_1$ are labelled I, II, III and IV.

critical for thermosensing (Nocker *et al*., 2001a; Chowdhury *et al*., 2003).

A common feature of all ROSE elements is the presence of a G residue opposite to the SD sequence that appears to be bulged in all the predicted structures (Fig. 4). This nucleotide was found to be functionally important, as its elimination made the thermometer unresponsive to high temperatures (Nocker *et al*., 2001a). Presumably, optimized base pairing with the SD sequence in this mutant prevents opening of the folded structure in the temperature range between 30 and 40 °C. It is currently unknown whether the entire ROSE RNA melts as the temperature increases, or whether local perturbations around the ribosome-binding site are sufficient to initiate translation. Only subtle structural changes in a synthetic ROSE transcript were recorded by UV and CD spectroscopy, suggesting that the latter might be the case (Chowdhury *et al*., 2003). *In vitro*, thermally induced alterations were reversible provided that the temperature was not raised far beyond the physiological temperature range.

Recent nuclear magnetic resonance (NMR) studies with a portion of the fourth stem loop containing the SD sequence showed that opening of the thermometer begins at an internal loop adjacent to the SD sequence, which appears to be the key temperature-sensing element (Chowdhury *et al*., unpublished results). The conserved G residue opposite the SD sequence closes the loop by a weak GG base pair which breaks at higher temperatures, making the ribosome-binding site accessible. Deletion of the G leads to the formation of a more stable hairpin that fails to open up within the physiological temperature range.

Similar to the cIII and *rpoH* thermometers, all ROSE structures consist of more than a single stem loop sequestering the SD sequence and AUG codon. In each case, two or three additional structural elements are present (Figs 2–4). Truncation of stem loops I–III of $ROSE_1$ retaining only hairpin IV did not abolish repression at 30 °C but reduced heat induction *in vivo* (Chowdhury *et al*., 2003). Mutations that destabilized the top of stem loop III resulted in enhanced expression at low temperatures. Compensatory mutations in the same region restored repression (Nocker *et al*., 2001a). Proper temperature sensing might require the entire and intact structure of the ROSE mRNA. The hairpins upstream of the one that sequesters the ribosome-binding site might have several important functions. (i) They might serve as a zipper enforcing correct folding of the thermometer as the RNA is being transcribed. It is noteworthy that the initial nucleotide (+1) of the ROSE transcript is paired (Fig. 4). Folding from the very beginning of the transcript might provide a scaffold preventing the ultimate stem loop engaging in fortuitous interactions when it emerges from the RNA polymerase. The observation that synthetic ROSE RNA did not return to its original conformation after it was heat-denatured at 80 °C supports the importance of cotranscriptional folding (Chowdhury *et al*., 2003). (ii) In analogy to the proposed wedge function of stem III in the *rpoH* thermometer (Morita *et al*., 1999a), it is conceivable that structures I–III in the ROSE-containing transcript might be involved in tertiary contacts with the SD region. With increasing temperature, these tertiary interactions might act in a cooperative fashion once the melting commences at the internal loop and help to open up the entire ribosome-binding site to facilitate 30S binding and translation initiation. (iii) The complex architecture might also play a role in another aspect of ROSE-mediated regulation. *In vivo*, ROSE-containing mRNA is barely detectable at low temperatures, suggesting that the transcript is rapidly degraded when it is in the folded conformation. After a shift to 42 °C, however, the translation-competent transcript is extremely abundant (Narberhaus *et al*., 1996, 1998; Nocker, 2001b). It is possible that the folded RNA displays recognition motifs to cellular RNases, which eliminate the untranslatable

transcript. The presence of an instability element early in the transcript would make economic sense. Stabilization of the RNA at higher temperature could be achieved by melting of recognition motif(s) and/or by shielding of the translated transcript by the ribosome.

All currently known ROSE elements derive from Rhizobiaceae (Münchbach *et al.*, 1999; Nocker *et al.*, 2001b; Balsiger *et al.*, 2004). A search for ROSE-type sequences in completed bacterial genome sequences using the basic linear alignment sequence tool (BLAST) program (Altschul *et al.*, 1997) did not provide new candidates, either because they do not exist or because the sequence conservation is too low. The latter seems to apply, as a closer bioinformatic inspection of the upstream region of genes coding for small heat-shock proteins revealed a number of ROSE-type sequences (Fig. 5a; T. Waldminghaus *et al.*, unpublished results). Some were found in *Alphaproteobacteria* belonging, or related closely, to *Rhizobiaceae* such as *Brucella mellitensis* and *Caulobacter crescentus*. More surprisingly, good candidates also exist in *Gammaproteobacteria*; for example, upstream of small heat-shock genes in *E. coli*, *Salmonella* or *Vibrio*. The predicted secondary structures of *C. crescentus* and *E. coli* ROSE elements are shown in Fig. 5b and c, respectively.

According to their amino acid sequence, bacterial small heat-shock proteins can be divided into two distinct classes, A and B (Münchbach *et al.*, 1999; Narberhaus, 2002b). Class A proteins are related closely to inclusion body-binding proteins A and B (IbpA and IbpB), the two *E. coli* small heat-shock proteins. Class B proteins comprise a very heterogeneous family. Many rhizobia contain both class A and class B proteins. All ROSE elements found so far are linked to genes coding for class A small heat-shock proteins. Neither Gram-positives nor *Archaea* seem to contain ROSE-like sequences, probably because their small heat-shock genes belong to class B.

The presence of four hairpin structures might be restricted to *Bradyrhizobium* sp. (Fig. 4a). Other ROSE elements seem to consist of three stem loops equivalent to the bradyrhizobial stems II, III and IV (Fig. 5a). It is important to note, however, that these predictions are preliminary, as the transcriptional start sites of most of the candidate thermometers are unknown. Sequence conservation in the first hairpins is only marginal and hence not included in the alignment (Fig. 5a). Stem loop IV, on the other hand, is characterized by several highly conserved residues, including the SD region and the complementary sequence blocking it at low temperatures. It is evident from all the studies conducted so far that the G, appearing as bulged from the predicted secondary structures just opposite to the SD sequence, is universally conserved and its deletion leads to severe compromise of the thermosensitivity of ROSE. Interestingly, exchanging it for an A in $ROSE_1$ did not alter expression (Nocker *et al.*, 2001a).

In summary, the bioinformatic search uncovered many new ROSE-type RNA thermometers in the 5′-UTR of class A small heat-shock genes from *Alpha-* and *Gammaproteobacteria*. They are predicted to fold into complex secondary structures which have an evolutionarily conserved hairpin in common, including the SD sequence. ROSE elements are clearly the most widely distributed RNA thermometers known to date.

Other *cis*-acting heat-shock sensors in prokaryotes

That *rpoH*- and ROSE-type thermometers were found only in *Alpha-* and *Gammaproteobacteria* does not exclude the fact that other organisms make use of RNA thermometers with entirely different sequence and structure. Given that this simple mechanism was predicted to originate from an early RNA world (Narberhaus, 2002a; Lai, 2003; Mandal & Breaker, 2004b; Vitreschak *et al.*, 2004), the presence of RNA thermometers in other bacteria and in *Archaea* is entirely conceivable. In fact, potential RNA thermometers have been described in various organisms. The *C. crescentus dnaKJ* transcript comprises an extended 5′-UTR, in which the SD sequence is trapped by complementary base pairs (Avedissian *et al.*, 1995). Elimination of most of the UTR stimulated expression. Temperature-controlled accessibility of the SD sequence was also postulated for the *Haemophilus ducreyi dnaK* transcript (Parsons *et al.*, 1999). Translation of the small heat-shock gene *hsp18* in the Gram-positive organism *Streptomyces albus* is temperature dependent. Here, too, a predicted secondary structure in the 5′-UTR might interfere with ribosome binding at low temperatures (Servant & Mazodier, 2001). Although a great deal more work is required to demonstrate that these candidates qualify as RNA thermometers, the findings already suggest that many new RNA-based heat-shock sensors remain to be discovered in phylogenetically diverse prokaryotes.

RNA thermometers in eukaryotes?

There is accumulating evidence that mRNA folding also might be an important parameter in translational control of some eukaryotic heat-shock genes. As in prokaryotes, thermal stress in eukaryotes triggers a heat-shock response (Morimoto, 1998). In addition to transcriptional control, expression of heat-shock genes can also be modulated at the level of mRNA processing (Yost & Lindquist, 1991) and by control of translation initiation. Here we discuss some of the diverse modes of translational control adapted by different eukaryotic systems to combat thermal stress.

Translation initiation in eukaryotes differs substantially from the prokaryotic process and proceeds by two principal pathways. The first, termed 'cap-dependent translation initiation', involves the interaction of a 5′-cap with the

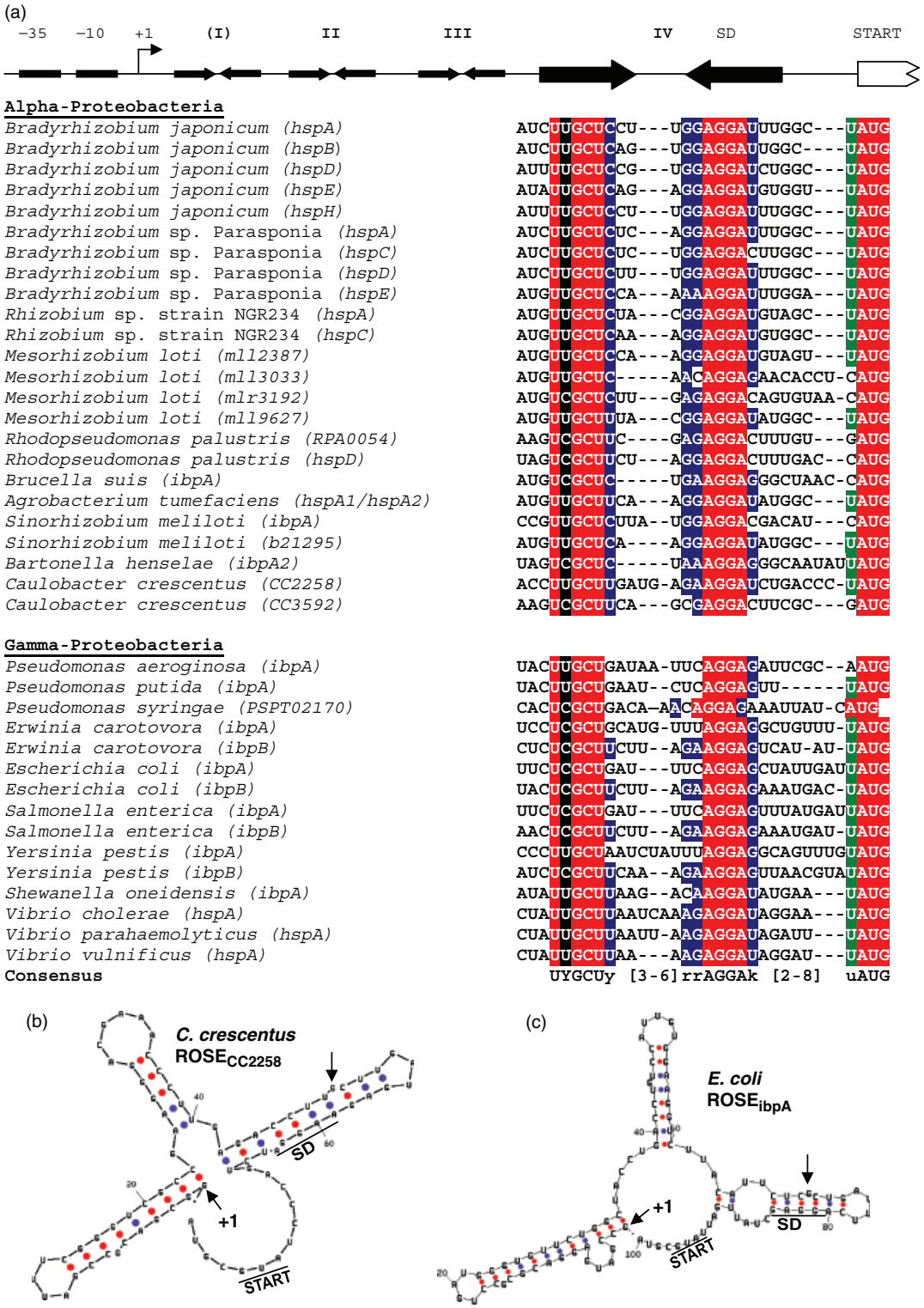

Fig. 5. Predicted *R*epression *O*f heat *S*hock gene *E*xpression (ROSE)-like sequences and structures from Gram-negative bacteria. The principal modules of ROSE-type thermometers with the promoters (−35 and −10), the transcription start site (+1), several hairpin structures (I–IV), the Shine–Dalgarno sequence (SD) and the AUG codon (START) is drawn at the top of panel (a). An alignment of region IV of predicted ROSE elements from *Alpha-* and *Gammaproteobacteria* is shown below. The predicted secondary structures of two candidate RNA thermometers are presented in (b) and (c). Characteristic features such as the predicted transcription start (+1), the SD sequence and a conserved bulged G (arrow) are labelled.

cap-binding protein complex that mediates recruitment of the small ribosomal subunit at the extreme 5′-end of the mRNA. Cap-dependent translation initiation is severely compromised under heat-shock conditions (Lamphear & Panniers, 1991; Cuesta *et al.*, 2000). A second pathway, which is emerging as the potential choice for eukaryotic cells under stress, uses complex secondary structure elements in the mRNA termed 'internal ribosomal entry segments' (IRES). They are present in the mRNAs of several survival factors and act directly by RNA–ribosome contacts or indirectly via initiation factors that can bind both the IRES and the ribosome (Holcik *et al.*, 2000; Von der Haar *et al.*, 2004).

Early investigations with Hsp70, an abundant protein under heat-shock conditions in eukaryotes, showed that the 5′-UTR of its mRNA is required for efficient translation in *Drosophila* cells subjected to heat shock (Klemenz *et al.*, 1985; McGarry & Lindquist, 1985). Evidence for specific RNA-binding factors was not found (Hess & Duncan, 1994). It emerged that minimal secondary structure is the key element of preferential translation during heat stress in most invertebrates. Introduction of structural elements into the 5′-UTR of *hsp70* mRNA inhibited translation at higher temperatures without affecting it at unstressed temperatures (Hess & Duncan, 1996). It has been suggested that under thermal stress, the capacity of the translational machinery to unwind the 5′-UTR secondary structure of the mRNA is reduced, which might result from shortage of one or more translation initiation factor activities that are absent under thermal stress in *Drosophila*. Under such circumstances, *hsp70* expression is mediated by the IRES-dependent pathway (Hernandez *et al.*, 2004).

Hsp 90 is an abundant protein in *Drosophila* cells grown at normal temperatures, with its mRNA present at significant levels (Lindquist & Craig, 1988). The 5′-UTR of *hsp90* mRNA is the only Hsp mRNA identified so far in *Drosophila* that possesses significant secondary structural elements typical to non-heat-shock mRNA (Ahmed & Duncan, 2004). Two modes of translation of the *hsp90* mRNA were postulated. Under normal temperature conditions, translation proceeds through the cap-dependent scanning mechanism. This process is relatively inefficient because of secondary structures. During heat shock, translation is assumed to switch to another mechanism that involves a more direct recruitment of the small ribosomal subunit to the AUG initiation codon promoted by mRNA–rRNA base pairing. Deletion of nucleotides close to the AUG severely compromised heat-dependent translation but did not substantially affect translation under non-heat-shock conditions. In analogy to bacterial RNA thermometers, it was postulated that the preferential translation of *hsp90* mRNA under thermal stress might rely on temperature-controlled alterations of the mRNA structure (Ahmed & Duncan, 2004). A related mechanism might be responsible for selective translation of human *hsp70* mRNA during heat stress (Yueh & Schneider, 2000).

The IRES-dependent translation of mRNA has also been shown to be crucial for survival and proliferation of cells under stress such as therapeutic radiation and hypoxia-induced DNA damage. Although it might be a preferred route when the cap-dependant pathway is inactive, there have been few studies on its role in heat-shock gene translation. It was reported to play a role in translation control of the heat-shock response of BiP, a Hsp 70 family member that facilitates protein folding, assembly of nascent proteins and scavenging of misfolded proteins in the endoplasmic reticulum (Kim & Jang, 2002). Enhanced translation of BiP mRNA mediated by its IRES was registered in HeLa cells under heat-shock conditions, suggesting that this mode of translation might be critical to the survival and proliferation of cells under thermal stress.

In trypanosomatids extreme environmental changes, e.g. alterations of temperature and extracellular pH, induce developmental gene expression. Hsp70 plays an important role in cell survival during developmental changes and is regulated exclusively at the post-transcriptional level (Vanhamme & Pays, 1995). Although Hsp70 and Hsp83 are expressed constitutively throughout the life cycle of *Leishmania*, translation of the heat-shock transcripts increases at least 10-fold upon temperature elevation (Shapira *et al.*, 1988). Unlike in other higher eukaryotes, the responsible regulatory element of *Leishmania* and *Trypanosoma brucei* *hsp70* resides in the 3′-UTR (Lee, 1998; Quijada *et al.*, 2000). Deletion analysis confirmed that sequences within the 3′-UTR of *hsp83* mRNA are essential in stabilization and preferential translation of this heat-shock transcript in *Leishmania* (Zilka *et al.*, 2001). The 5′-UTR could not generate this effect alone but had synergistic effects on increasing translation at higher temperatures when combined with the 3′-UTR. This result indicates a possible long-range interaction between the 5′-UTR and the 3′-UTR, which has also been reported in other eukaryotic systems (Jarzembowski *et al.*, 1999).

Virulence gene expression

Bacteria living in the soil, in water or in food usually experience a temperature upshift when swallowed by mammals. The increase in temperature might not be severe enough to induce a heat-shock response, but it comprises an important signal for pathogenic micro-organisms that a warm-blooded host has been entered successfully. As a consequence, many virulence genes are under temperature control (Konkel & Tilly, 2000). The regulatory mechanisms involved are diverse. Several pathogens control transcription of virulence genes by temperature-induced changes in DNA

supercoiling, which modulates the binding of regulatory proteins. Others use protein thermometers, i.e. regulatory proteins that alter their conformation in response to temperature (Hurme & Rhen, 1998). That a thermosensor might also reside in the mRNA of a virulence gene was first postulated in the early 1990s. The cellular level of the *Yersinia pestis* virulence gene activator LcrF was found to be much higher at 37 °C than at 26 °C, although transcription was insensitive to temperature (Hoe *et al.*, 1992; Hoe & Goguen, 1993). Temperature-dependent LcrF production was also observed in *E. coli* when transcription was driven from a T7 promoter, suggesting a post-transcriptional expression signal in the *lcrF* message itself (Hoe & Goguen, 1993). Although degradation of *lcrF* mRNA might play a role, differential translation of the message was suggested to be the main reason for the increase of the activator protein at high temperatures. A secondary structure in the *lcrF* mRNA was predicted to sequester the translation initiation region. Thermal destabilization of the stem loop allowing translation initiation was calculated to occur in the physiologically relevant temperature range between 33 and 37 °C (Hoe & Goguen, 1993).

A similar mechanism was shown to control virulence gene expression in the food-borne pathogen *Listeria monocytogenes*. The virulence gene activator PrfA is not present at low temperatures, although the *prfA* transcript is being made (Renzoni *et al.*, 1997). An extended stem-loop structure was predicted in the leader sequence of about 120 nucleotides (Fig. 6). Chemical probing experiments and the introduction of a series of mismatches, compensatory and stabilizing mutations suggested strongly that this structure is responsible for thermoregulation of *prfA* expression (Johansson *et al.*, 2002). *Listeria* mutants engineered to produce PrfA at

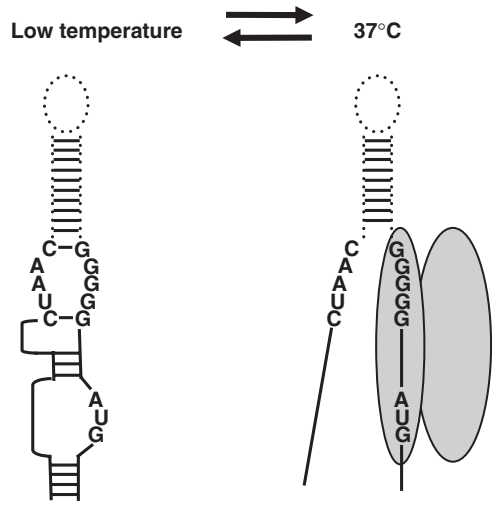

Fig. 6. Schematic drawing of the prfA thermometer from *Listeria monocytogenes*. The dotted loop indicates that the top of the extensive hairpin structure is not drawn to scale.

30 °C owing to destabilized leader sequences were able to invade cultured kidney cells. Translational control of the *prfA* gene was retained when it was transferred to *E. coli*, although *L. monocytogenes* (Gram-positive) and *E. coli* (Gram-negative) are only distantly related bacteria using quite different gene expression mechanisms. This observation provides strong evidence that no *Listeria*-specific factor is involved in translational control and that the mRNA alone functions as a thermometer.

In contrast to the heat-shock sensors discussed above, the *prfA* structure is not branched and consists of a single stem loop. Base-pairing with the SD sequence is weak and the AUG codon is not masked (Fig. 6). It is thus conceivable that the virulence thermometer is more sensitive than the others and functions at slightly lower temperatures. However, a detailed comparative expression analysis would be required to address this question. Unlike ROSE-containing transcripts, the *prfA* mRNA does not seem to be processed at low temperatures. Northern blot analysis revealed equivalent amounts of the monocistronic transcript at 20 and 37 °C (Johansson *et al.*, 2002).

Cold-shock gene expression

Like heat, cold is a physical parameter that severely affects multiple processes in a living cell. A temperature drop influences membrane fluidity as well as the conformation and functionality of nucleic acids and proteins. As a protective measure, most bacteria are able to mount a cold-shock response inducing so-called cold-shock proteins (CSPs), which have diverse and partly unknown functions (Graumann & Marahiel, 1996; Phadtare *et al.*, 2000). Regulation of CSP genes occurs primarily at the post-transcriptional level. Low stability of CSP mRNAs at normal temperatures plays a major role, but various layers of translational control are also involved (Gualerzi *et al.*, 2003). Certain proteins, including initiation factors and CSPs, some of which act as RNA chaperones, might contribute to the preferential translation of cold-shock genes at low temperatures (Phadtare *et al.*, 2000; Gualerzi *et al.*, 2003). In the context of this review, it is interesting that a conspicuously long 5′-UTR consisting of 159 nucleotides in the *E. coli cspA* mRNA has been postulated to act as an RNA thermometer (Yamanaka *et al.*, 1999). A deletion analysis in the 5′-UTR revealed that *cspA* expression did not correlate with the amount of transcript, suggesting that the leader sequence controls translation efficiency. Alternative RNA structures at high and low temperatures are thought to modulate the accessibility of the ribosome-binding site (Yamanaka *et al.*, 1999). Stability control of the *cspA* mRNA might also be because of alternative RNA structures. Rapid degradation of the transcript by RNase E occurs at high temperatures when the recognition site is predicted to be single-stranded and accessible (Fang *et al.*, 1997).

Thermoregulation by the small noncoding RNA DsrA

So far, we have discussed inbuilt *cis*-acting thermosensors that control mRNA translation upon thermal signals. A segment of RNA complementary to the ribosome-binding site is located in more or less close proximity, allowing intramolecular base pairing. A different regulatory mode is carried out by *trans*-encoded antisense RNAs. Here, a small noncoding RNA is expressed as an independent transcription unit from its target. Often these antisense RNAs have multiple targets, and some targets are regulated by multiple noncoding RNAs, thereby generating a network of global regulation (Wassarman *et al.*, 1999; Gottesman, 2002; Storz, 2002).

The only documented example of temperature-regulated translation by a *trans*-encoded antisense RNA is found in the regulation of RpoS. To combat environmental challenges, *E. coli* triggers expression of many genes involved, both in temporary emergency responses and in long-term adaptation processes. The transcription of many of these genes is dependent on the stationary phase or starvation σ factor, RpoS (σ^{38}). RpoS is more abundant at 25 °C than at higher temperatures, and RpoS-dependent promoters are expressed efficiently at low temperatures (Sledjeski *et al.*, 1996). The abundance of RpoS at low temperatures results from thermal regulation of its translation by an 85-nucleotide small noncoding RNA, the DsrA RNA. Temperature is one of the environmental factors that determine the amount of DsrA present in the cell at two stages: transcription initiation and stability of the antisense RNA. As a consequence, the amounts of DsrA are higher at lower temperatures (Sledjeski *et al.*, 1996; Repoila & Gottesman, 2001).

The full-length 85-nucleotide long DsrA primary transcript (F form) is processed to a 60- or 61-nucleotide form (T form) lacking the 5′-region which interacts with the *rpoS* mRNA. The F form is more stable at 25 °C than at 37 or 42 °C, thus outscoring the T form at low temperatures (Repoila & Gottesman, 2001; Repoila *et al.*, 2003). Interaction of the F form with the leader sequence of the *rpoS* transcript relieves translational repression by disrupting complementary base pairing of the region flanking the SD sequence (Fig. 1B). Removal of the 5′-end of the F form might occur at an RNase E cleavage site between two stem loops in the DsrA RNA (Moll *et al.*, 2003). Exactly how temperature influences processing of the small RNA is unclear. However, because temperature reportedly controls the abundance and stability of the active conformation of DsrA it has been considered an RNA thermometer of the RpoS regulon (Repoila *et al.*, 2003).

Conclusions and perspectives

The chemical nature of RNA provides a vast repertoire of options for intra- and intermolecular interactions which can be used for regulatory purposes. RNA can utilize reversible temperature-dependent conformational alterations to tune gene expression. Also, the inherent short half-life of mRNA makes it an attractive regulatory candidate for transient temperature changes.

In terms of a rapid response, translational control is one step ahead of transcriptional control as it acts on already-existing RNA. Economically, it may not be the cheapest solution to produce a transcript that is not translated (or even degraded). However, the option to induce stress responses or virulence genes at appropriate temperatures very rapidly might be worth the expense. To avoid further biosynthetic costs caused by undesired cotranscriptional translation, most thermometers are placed in the 5′-UTR rather than in the coding sequence.

Although most RNA thermometers function by a conceptually simple melting mechanism, the underlying molecular details are far from being understood. Some of the open questions that remain to be addressed in the future are as follows:

(1) *Structure of known RNA thermometers*. Computer-aided secondary structure predictions have been extremely helpful in establishing the RNA thermometer concept. However, it appears from preliminary NMR studies that the actual ROSE structure around the SD sequence deviates, at least in part, from the computer-calculated secondary structure. Hence, a detailed analysis of a number of structurally and functionally different RNA thermometers by structure probing techniques, NMR and/or X-ray crystallography is required in order to comprehend fully their mode of action.
(2) *The melting process*. Matters are complicated by the fact that most RNA thermometers exhibit a complex architecture consisting of several hairpins whose molecular interplay is not yet understood. As described in previous sections, mutational studies have implied that the modular design of the *rpoH* and ROSE thermometers is functionally important. The calculated melting temperature of entire thermometer sequences is usually far above the relevant physiological temperature. It is possible that translation initiation requires only local destabilization around the ribosome-binding site and/or depends on the coordinated action of individual stem loops.

Another complication is that RNA thermometers probably do not have two distinct alternative conformations and therefore, in contrast to riboswitches, they are unlikely to act as an on/off switch. Instead, RNA folding–unfolding dynamics seems to play an important role in this case. RNA thermometers presumably use a dimmer-type mechanism progressing through multiple subtly different structures along a temperature gradient. Resolving the exact secondary and tertiary interactions and their dynamic response to temperature will be a major challenge for future studies.

Another important task will be to provide direct experimental proof for the presumed translational control

mechanism. Except for the *rpoH* mRNA (Morita *et al.*, 1999b), it remains to be demonstrated that binding of RNA thermometers to the ribosome correlates directly with temperature. To establish whether accessory factors other than the transcript and the 30S particle are required for thermosensing, toe-printing studies with purified components are needed.

(3) *Stability of thermometer containing transcripts*. Some RNA thermometers are postulated to decay when they are not translated (Nocker *et al.*, 2001a); why the cell produces a transcript simply to degrade it is unknown. Interestingly, disposal of unused RNA sensors seems to be a common principle, as several riboswitch-containing transcripts also are intrinsically unstable (Hoe & Goguen, 1993; Nou & Kadner, 1998; Winkler *et al.*, 2004). Neither the identity of the RNAses in action nor the decay mechanism is known.

(4) *Novel RNA thermometers*. The most speculative aspect is whether there are new RNA thermometers to be discovered. A particularly interesting question relates to the existence of RNA thermometers in psychrophilic or thermophilic microorganisms. Entirely new structures are to be expected. Also, we still do not know whether regulation of genes not coding for heat-shock, cold-shock or virulence proteins can be mediated by similar RNA elements.

The low sequence and structure conservation among the known RNA thermometers poses a principal challenge, as it provides little prospect for homology-based searches. To this end, the ongoing development of an advanced bioinformatic analysis tool using improved structure modeling algorithms is promising (Mathews *et al.*, 1999; Zuker *et al.*, 1999; Hofacker, 2003; Gardner & Giegerich, 2004; Voss *et al.*, 2004).

(5) *Novel mechanisms*. It will also be interesting to discover whether the regulatory capacity of RNA thermometers extends beyond translational control. All known thermosensors act by controlling access to the ribosome-binding site. So far, examples for control of transcription termination or ribozyme activity as is used in some riboswitches (see Fig. 1a) have not been found.

The concept of gene regulation by RNA sensors has emerged only very recently. It is surprising that 2% of all Bacillus genes have been predicted to be regulated by riboswitches, most of them controlling fundamental metabolic processes (Mandal *et al.*, 2003). New RNA sensors are being discovered in rapid succession, demonstrating that our knowledge on the regulatory potential of RNA is just beginning to unfold. Evidently, there is a message in many untranslated regions. We just need to read it.

Acknowledgements

Previous support by the ETH Zürich (TH 0-20037-02) and present funding by the German Research Foundation (DFG NA240/4-1) is gratefully acknowledged.

References

Ahmed R & Duncan RF (2004) Translational regulation of Hsp90 mRNA. AUG-proximal 5′-untranslated region elements essential for preferential heat shock translation. *J Biol Chem* **279**: 49919–49930.

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W & Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**: 3389–3402.

Altuvia S & Oppenheim AB (1986) Translational regulatory signals within the coding region of the bacteriophage λ cIII gene. *J Bacteriol* **167**: 415–419.

Altuvia S, Locker-Giladi H, Koby S, Ben-Nun O & Oppenheim AB (1987) RNase III stimulates the translation of the cIII gene of bacteriophage lambda. *Proc Natl Acad Sci USA* **84**: 6511–6515.

Altuvia S, Kornitzer D, Teff D & Oppenheim AB (1989) Alternative mRNA structures of the cIII gene of bacteriophage λ determine the rate of its translation initiation. *J Mol Biol* **210**: 265–280.

Altuvia S, Kornitzer D, Kobi S & Oppenheim AB (1991) Functional and structural elements of the mRNA of the cIII gene of bacteriophage lambda. *J Mol Biol* **218**: 723–733.

Avedissian M, Lessing D, Gober JW, Shapiro L & Gomes SL (1995) Regulation of the *Caulobacter crescentus dnaKJ* operon. *J Bacteriol* **177**: 3479–3484.

Balsiger S, Ragaz C, Baron C & Narberhaus F (2004) Replicon-specific regulation of small heat shock genes in *Agrobacterium tumefaciens*. *J Bacteriol* **186**: 6824–6829.

Chowdhury S, Ragaz C, Kreuger E & Narberhaus F (2003) Temperature-controlled structural alterations of an RNA thermometer. *J Biol Chem* **278**: 47915–47921.

Cuesta R, Laroia G & Schneider RJ (2000) Chaperone hsp27 inhibits translation during heat shock by binding eIF4G and facilitating dissociation of cap-initiation complexes. *Genes Dev* **14**: 1460–1470.

Epshtein V, Mironov AS & Nudler E (2003) The riboswitch-mediated control of sulfur metabolism in bacteria. *Proc Natl Acad Sci USA* **100**: 5052–5056.

Fang L, Jiang W, Bae W & Inouye M (1997) Promoter-independent cold-shock induction of *cspA* and its derepression at 37 °C by mRNA stabilization. *Mol Microbiol* **23**: 355–364.

Gardner PP & Giegerich R (2004) A comprehensive comparison of comparative RNA structure prediction approaches. *BMC Bioinformat* **5**: 140.

Gottesman S (2002) Stealth regulation: biological circuits with small RNA switches. *Genes Dev* **16**: 2829–2842.

Graumann P & Marahiel MA (1996) Some like it cold: response of microorganisms to cold shock. *Arch Microbiol* **166**: 293–300.

Gross CA (1996) Function and regulation of the heat shock proteins. *Escherichia coli and Salmonella: Cellular and Molecular Biology* (Neidhardt FC, *et al.*, eds), pp. 1382–1399. American Society for Microbiology, Washington, DC.

Grundy FJ & Henkin TM (2004) Regulation of gene expression by effectors that bind to RNA. *Curr Opin Microbiol* **7**: 126–131.

Grundy FJ, Lehman SC & Henkin TM (2003) The L box regulon: lysine sensing by leader RNAs of bacterial lysine biosynthesis genes. *Proc Natl Acad Sci USA* **100**: 12057–12062.

Gualerzi CO, Giuliodori AM & Pon CL (2003) Transcriptional and post-transcriptional control of cold-shock genes. *J Mol Biol* **331**: 527–539.

Henkin TM & Yanofsky C (2002) Regulation by transcription attenuation in bacteria: how RNA provides instructions for transcription termination/antitermination decisions. *Bioessays* **24**: 700–707.

Herman C, Thévenet D, D'Ari R & Bouloc P (1995) Degradation of σ^{32}, the heat shock regulator in *Escherichia coli*, is governed by HflB. *Proc Natl Acad Sci USA* **92**: 3516–3520.

Herman C, Thévenet D, D'Ari R & Bouloc P (1997) The HflB protease of *Escherichia coli* degrades its inhibitor λcIII. *J Bacteriol* **179**: 358–363.

Hernandez G, Vazquez-Pianzola P, Sierra JM & Rivera-Pomar R (2004) Internal ribosome entry site drives cap-independent translation of *reaper* and *heat shock protein 70* mRNAs in *Drosophila* embryos. *RNA* **10**: 1783–1797.

Hess MA & Duncan RF (1994) RNA/protein interactions in the 5′-untranslated leader of HSP70 mRNA in *Drosophila* lysates. Lack of evidence for specific protein binding. *J Biol Chem* **269**: 10913–10922.

Hess MA & Duncan RF (1996) Sequence and structure determinants of I Hsp70 mRNA translation: 5′-UTR secondary structure specifically inhibits heat shock protein mRNA translation. *Nucleic Acids Res* **24**: 2441–2449.

Hoe NP & Goguen JD (1993) Temperature sensing in *Yersinia pestis*: translation of the LcrF activator protein is thermally regulated. *J Bacteriol* **175**: 7901–7909.

Hoe NP, Minion FC & Goguen JD (1992) Temperature sensing in *Yersinia pestis*: regulation of *yopE* transcription by *lcrF*. *J Bacteriol* **174**: 4275–4286.

Hofacker IL (2003) Vienna RNA secondary structure server. *Nucleic Acids Res* **31**: 3429–3431.

Holcik M, Sonenberg N & Korneluk RG (2000) Internal ribosome initiation of translation and the control of cell death. *Trends Genet* **16**: 469–473.

Hurme R & Rhen M (1998) Temperature sensing in bacterial gene regulation – what it all boils down to. *Mol Microbiol* **30**: 1–6.

Jarzembowski JA, Rajagopalan LE, Shin HC & Malter JS (1999) The 5′-untranslated region of GM-CSF mRNA suppresses translational repression mediated by the 3′ adenosine-uridine-rich element and the poly(A) tail. *Nucleic Acids Res* **27**: 3660–3666.

Johansson J, Mandin P, Renzoni A, Chiaruttini C, Springer M & Cossart P (2002) An RNA thermosensor controls expression of virulence genes in *Listeria monocytogenes*. *Cell* **110**: 551–561.

Kaempfer R (2003) RNA sensors: novel regulators of gene expression. *EMBO Rep* **4**: 1043–1047.

Kim YK & Jang SK (2002) Continuous heat shock enhances translational initiation directed by internal ribosomal entry site. *Biochem Biophys Res Commun* **297**: 224–231.

Klemenz R, Hultmark D & Gehring WJ (1985) Selective translation of heat shock mRNA in *Drosophila melanogaster* depends on sequence information in the leader. *EMBO J* **4**: 2053–2060.

Konkel ME & Tilly K (2000) Temperature-regulated expression of bacterial virulence genes. *Microbes Infect* **2**: 157–166.

Kornitzer D, Teff D, Altuvia S & Oppenheim AB (1989) Genetic analysis of bacteriophage λ cIII gene: mRNA structural requirements for translation initiation. *J Bacteriol* **171**: 2563–2572.

Kubodera T, Watanabe M, Yoshiuchi K, Yamashita N, Nishimura A, Nakai S, Gomi K & Hanamoto H (2003) Thiamine-regulated gene expression of *Aspergillus oryzae thiA* requires splicing of the intron containing a riboswitch-like domain in the 5′-UTR. *FEBS Lett* **555**: 516–520.

Lai EC (2003) RNA sensors and riboswitches: self-regulating messages. *Curr Biol* **13**: 285–291.

Lamphear BJ & Panniers R (1991) Heat shock impairs the interaction of cap-binding protein complex with 5′ mRNA cap. *J Biol Chem* **266**: 2789–2794.

Landick R, Turnbough CL & Yanofsky C (1996) Transcription attenuation. *Escherichia coli and Salmonella: Cellular and Molecular Biology* (Neidhardt FC, *et al.*, eds), pp. 1263–1286. ASM Press, Washington, DC.

Lee MG (1998) The 3′-untranslated region of the *hsp70* genes maintains the level of steady state mRNA in *Trypanosoma brucei* upon heat shock. *Nucleic Acids Res* **26**: 4025–4033.

Lindquist S & Craig EA (1988) The heat-shock proteins. *Annu Rev Genet* **22**: 631–677.

Mandal M & Breaker RR (2004a) Adenine riboswitches and gene activation by disruption of a transcription terminator. *Nat Struct Mol Biol* **11**: 29–35.

Mandal M & Breaker RR (2004b) Gene regulation by riboswitches. *Nat Rev Mol Cell Biol* **5**: 451–463.

Mandal M, Boese B, Barrick JE, Winkler WC & Breaker RR (2003) Riboswitches control fundamental biochemical pathways in *Bacillus subtilis* and other bacteria. *Cell* **113**: 577–586.

Mandal M, Lee M, Barrick JE, Weinberg Z, Emilsson GM, Ruzzo WL & Breaker RR (2004) A glycine-dependent riboswitch that uses cooperative binding to control gene expression. *Science* **306**: 275–279.

Mathews DH, Sabina J, Zuker M & Turner DH (1999) Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure. *J Mol Biol* **288**: 911–940.

McDaniel BA, Grundy FJ, Artsimovitch I & Henkin TM (2003) Transcription termination control of the S box system: direct measurement of S-adenosylmethionine by the leader RNA. *Proc Natl Acad Sci USA* **100**: 3083–3088.

McGarry TJ & Lindquist S (1985) The preferential translation of *Drosophila hsp70* mRNA requires sequences in the untranslated leader. *Cell* **42**: 903–911.

Mironov AS, Gusarov I, Rafikov R, Lopez LE, Shatalin K, Kreneva RA, Perumov DA & Nudler E (2002) Sensing small molecules by nascent RNA: a mechanism to control transcription in bacteria. *Cell* **111**: 747–756.

Moll I, Afonyushkin T, Vytvytska O, Kaberdin VR & Bläsi U (2003) Coincident Hfq binding and RNase E cleavage sites on mRNA and small regulatory RNAs. *RNA* **9**: 1308–1314.

Morimoto RI (1998) Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. *Genes Dev* **12**: 3788–3796.

Morita M, Kanemori M, Yanagi H & Yura T (1999a) Heat-induced synthesis of σ^{32} in *Escherichia coli*: structural and functional dissection of *rpoH* mRNA secondary structure. *J Bacteriol* **181**: 401–410.

Morita MT, Tanaka Y, Kodama TS, Kyogoku Y, Yanagi H & Yura T (1999b) Translational induction of heat shock transcription factor σ^{32}: evidence for a built-in RNA thermosensor. *Genes Dev* **13**: 655–665.

Münchbach M, Nocker A & Narberhaus F (1999) Multiple small heat shock proteins in rhizobia. *J Bacteriol* **181**: 83–90.

Nagai H, Yuzawa H & Yura T (1991) Interplay of two *cis*-acting messenger RNA regions in translational control of σ^{32} synthesis during the heat shock response of *Escherichia coli*. *Proc Natl Acad Sci USA* **88**: 10515–10519.

Nahvi A, Sudarsan N, Ebert MS, Zou X, Brown KL & Breaker RR (2002) Genetic control by a metabolite binding mRNA. *Chem Biol* **9**: 1043–1049.

Nakahigashi K, Yanagi H & Yura T (1995) Isolation and sequence analysis of *rpoH* genes encoding σ^{32} homologs from Gram negative bacteria: conserved mRNA and protein segments for heat shock regulation. *Nucleic Acids Res* **23**: 4383–4390.

Narberhaus F (1999) Negative regulation of bacterial heat shock genes. *Mol Microbiol* **31**: 1–8.

Narberhaus F (2002a) mRNA-mediated detection of environmental conditions. *Arch Microbiol* **178**: 404–410.

Narberhaus F (2002b) α-Crystallin-type heat shock proteins: socializing minichaperones in the context of a multichaperone network. *Microbiol Mol Biol Rev* **66**: 64–93.

Narberhaus F, Weiglhofer W, Fischer HM & Hennecke H (1996) The *Bradyrhizobium japonicum rpoH*$_1$ gene encoding a σ^{32}-like protein is part of a unique heat shock gene cluster together with *groESL*$_1$ and three small heat shock genes. *J Bacteriol* **178**: 5337–5346.

Narberhaus F, Käser R, Nocker A & Hennecke H (1998) A novel DNA element that controls bacterial heat shock gene expression. *Mol Microbiol* **28**: 315–323.

Nocker A, Hausherr T, Balsiger S, Krstulovic NP, Hennecke H & Narberhaus F (2001a) mRNA-based thermosensor controls expression of rhizobial heat shock genes. *Nucleic Acids Res* **29**: 4800–4807.

Nocker A, Krstulovic NP, Perret X & Narberhaus F (2001b) ROSE elements occur in disparate rhizobia and are functionally interchangeable between species. *Arch Microbiol* **176**: 44–51.

Nou X & Kadner RJ (1998) Coupled changes in translation and transcription during cobalamin-dependent regulation of *btuB* expression in *Escherichia coli*. *J Bacteriol* **180**: 6719–6728.

Nou X & Kadner RJ (2000) Adenosylcobalamin inhibits ribosome binding to *btuB* RNA. *Proc Natl Acad Sci USA* **97**: 7190–7195.

Nudler E & Mironov AS (2004) The riboswitch control of bacterial metabolism. *Trends Biochem Sci* **29**: 11–17.

Parsons LM, Waring AL, Limberger RJ & Shayegani M (1999) The *dnaK/dnaJ* operon of *Haemophilus ducreyi* contains a unique combination of regulatory elements. *Gene* **233**: 109–119.

Phadtare S, Yamanaka K & Inouye M (2000) The cold shock reponse. *Bacterial Stress Responses* (Storz G & Hengge-Aronis R, eds), pp. 33–45. ASM Press, Washington, DC.

Quijada L, Soto M, Alonso C & Requena JM (2000) Identification of a putative regulatory element in the 3′-untranslated region that controls expression of HSP70 in *Leishmania infantum*. *Mol Biochem Parasitol* **110**: 79–91.

Renzoni A, Klarsfeld A, Dramsi S & Cossart P (1997) Evidence that PrfA, the pleiotropic activator of virulence genes in *Listeria monocytogenes*, can be present but inactive. *Infect Immun* **65**: 1515–1518.

Repoila F & Gottesman S (2001) Signal transduction cascade for regulation of RpoS: temperature regulation of DsrA. *J Bacteriol* **183**: 4012–4023.

Repoila F, Majdalani N & Gottesman S (2003) Small non-coding RNAs, co-ordinators of adaptation processes in *Escherichia coli*: the RpoS paradigm. *Mol Microbiol* **48**: 855–861.

Schumann W (2003) The *Bacillus subtilis* heat shock stimulon. *Cell Stress Chaperones* **8**: 207–217.

Servant P & Mazodier P (2001) Negative regulation of the heat shock response in *Streptomyces*. *Arch Microbiol* **176**: 237–242.

Shapira M, McEwen JG & Jaffe CL (1988) Temperature effects on molecular processes which lead to stage differentiation in *Leishmania*. *EMBO J* **7**: 2895–2901.

Shotland Y, Koby S, Teff D, *et al.* (1997) Proteolysis of the phage λ CII regulatory protein by FtsH (HflB) of *Escherichia coli*. *Mol Microbiol* **24**: 1303–1310.

Sledjeski DD, Gupta A & Gottesman S (1996) The small RNA, DsrA, is essential for the low temperature expression of RpoS during exponential growth in *Escherichia coli*. *EMBO J* **15**: 3993–4000.

Soukup JK & Soukup GA (2004) Riboswitches exert genetic control through metabolite-induced conformational change. *Curr Opin Struct Biol* **14**: 344–349.

Storz G (2002) An expanding universe of noncoding RNAs. *Science* **296**: 1260–1263.

Straus DB, Walter WA & Gross CA (1987) The heat shock response of *E. coli* is regulated by changes in the concentration of σ^{32}. *Nature* **329**: 348–351.

Straus D, Walter W & Gross CA (1990) DnaK, DnaJ, and GrpE heat shock proteins negatively regulate heat shock gene expression by controlling the synthesis and stability of σ^{32}. *Genes Dev* **4**: 2202–2209.

Sudarsan N, Barrick JE & Breaker RR (2003) Metabolite-binding RNA domains are present in the genes of eukaryotes. *RNA* **9**: 644–647.

Sudarsan N, Wickiser JK, Nakamura S, Ebert MS & Breaker RR (2003) An mRNA structure in bacteria that controls gene expression by binding lysine. *Genes Dev* **17**: 2688–2697.

Tilly K, Erickson J, Sharma S, Georgopoulos C (1986) Heat shock regulatory gene *rpoH* mRNA level increases after heat shock in *Escherichia coli*. *J Bacteriol* **168**: 1155–1158.

Tomoyasu T, Gamer J, Bukau B, *et al*. (1995) *Escherichia coli* FtsH is a membrane bound, ATP-dependent protease which degrades the heat-shock transcription factor σ^{32}. *EMBO J* **14**: 2551–2560.

Vanhamme L & Pays E (1995) Control of gene expression in trypanosomes. *Microbiol Rev* **59**: 223–240.

Vitreschak AG, Rodionov DA, Mironov AA & Gelfand MS (2004) Riboswitches: the oldest mechanism for the regulation of gene expression? *Trends Genet* **20**: 44–50.

von der Haar T, Gross JD, Wagner G & McCarthy JE (2004) The mRNA cap-binding protein eIF4E in post-transcriptional gene expression. *Nat Struct Mol Biol* **11**: 503–511.

Voss B, Meyer C & Giegerich R (2004) Evaluating the predictability of conformational switching in RNA. *Bioinformatics* **20**: 1573–1582.

Wassarman KM, Zhang A & Storz G (1999) Small RNAs in *Escherichia coli*. *Trends Microbiol* **7**: 37–45.

Winkler WC & Breaker RR (2003) Genetic control by metabolite-binding riboswitches. *Chembiochem* **4**: 1024–1032.

Winkler WC, Cohen-Chalamish S & Breaker RR (2002a) An mRNA structure that controls gene expression by binding FMN. *Proc Natl Acad Sci USA* **99**: 15908–15913.

Winkler W, Nahvi A & Breaker RR (2002b) Thiamine derivatives bind messenger RNAs directly to regulate bacterial gene expression. *Nature* **419**: 952–956.

Winkler WC, Nahvi A, Sudarsan N, Barrick JE & Breaker R.R. (2003) An mRNA structure that controls gene expression by binding S-adenosylmethionine. *Nat Struct Biol* **10**: 701–707.

Winkler WC, Nahvi A, Roth A, Collins JA & Breaker RR (2004) Control of gene expression by a natural metabolite-responsive ribozyme. *Nature* **428**: 281–286.

Yamanaka K, Mitta M & Inouye M (1999) Mutation analysis of the 5′ untranslated region of the cold shock *cspA* mRNA of *Escherichia coli*. *J Bacteriol* **181**: 6284–6291.

Yost HJ & Lindquist S (1991) Heat shock proteins affect RNA processing during the heat shock response of *Saccharomyces cerevisiae*. *Mol Cell Biol* **11**: 1062–1068.

Yueh A & Schneider RJ (2000) Translation by ribosome shunting on adenovirus and hsp70 mRNAs facilitated by complementarity to 18S rRNA. *Genes Dev* **14**: 414–421.

Yura T, Kanemori M & Morita MT (2000) The heat shock response: regulation and function. *Bacterial Stress Responses* (Storz G & Hengge-Aronis R, eds), pp. 3–18. ASM Press, Washington, DC.

Zilka A, Garlapati S, Dahan E, Yaolsky V & Shapira M (2001) Developmental regulation of heat shock protein 83 in *Leishmania*. 3′ processing and mRNA stability control transcript abundance, and translation is directed by a determinant in the 3′-untranslated region. *J Biol Chem* **276**: 47922–47929.

Zuker M (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res* **31**: 3406–3415.

Zuker M, Mathews DH & Turner DH (1999) Algorithms and thermodynamics for RNA secondary structure prediction: a practical guide. *RNA Biochemistry and Biotechnology* (Barciszewski J & Clark BFC, eds.) Kluwer Academic Publishers, NATO ASI Series.