Temperature-controlled Structural Alterations of an RNA Thermometer*

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Thermoresponsive structures in the 5′-untranslated region of mRNA are known to control translation of heat shock and virulence genes. Expression of many rhizobial heat shock genes is regulated by a conserved sequence element called ROSE for repression of heat shock gene expression. This cis-acting, untranslated mRNA is thought to prevent ribosome access to low temperature through an extended secondary structure, which partially melts when the temperature rises. We show here by a series of in vivo and in vitro approaches that ROSE is a sensitive thermometer responding in the physiologically relevant temperature range between 30 and 40 °C. Point mutations predicted to disrupt base pairing enhanced expression at 30 °C. Compensatory mutations restored repression, emphasizing the importance of secondary structures in the sensory RNA. Only moderate inducibility of a 5′-truncated ROSE variant suggests that interactions between individual stem loops coordinate temperature sensing. In the presence of a complementary oligonucleotide, the functionally important stem loop of ROSE was rendered susceptible to RNase H treatment at heat shock temperatures. Since major structural rearrangements were not observed during UV and CD spectroscopy, subtle structural changes involving the Shine-Dalgarno sequence are proposed to mediate translational control. Temperature perception by the sensory RNA is an ordered process that most likely occurs without the aid of accessory factors.

RNA can act as a sensory molecule to determine and respond to changes in the cellular environment (1–5). Temperature is one of the important parameters under constant vigilance in unicellular organisms. A rapid increase in it triggers the induction of heat shock proteins in all species investigated so far (6). Mammalian pathogens turn on virulence genes only at the body temperature of the warm-blooded hosts (7). Among the diverse regulatory systems, RNA thermometers have evolved to sense and transduce ambient temperature signals to the translation machinery (2, 5, 8). They operate posttranscriptionally by the formation of secondary structures that mask the ribosome binding site at low temperatures. Elevated temperatures disrupt base pairing and thereby facilitate ribosome entry and translation initiation.

In Escherichia coli, the cellular level of the heat shock transcription factor RpoH (σ32) is controlled at different stages of transcription, translation, protein activity, and stability (8). Computer predictions, mutational analyses, structure probing techniques, and toeprinting assays established that translation efficiency of the rpoH gene that codes for σ32 is modulated by two cis-acting regions within the 5′-coding sequence of its mRNA, which form an extended secondary structure occluding the ribosome binding site, in a temperature-dependent manner (8–10). A close inspection of rpoH sequences from other γ-proteobacteria strongly suggests that translational control of rpoH is conserved among these bacteria (11). Access of ribosomes to the liberated Shine-Dalgarno (SD) sequence at temperatures above 35 °C also permits translation of the virulence gene activators prfA in Listeria monocytogenes and lerF in Yersinia pestis (12, 13).

A 159-bp-long 5′-untranslated region (UTR)1 in the cspA mRNA, encoding for the major cold shock protein CspA in E. coli, works by a reverse mechanism by existing in different secondary structures at 37 and 15 °C (14). Here, in contrast to the earlier cases, the secondary structure at low temperature is thought to permit ribosome loading and translation. Another classical example in this category is λ ciI mRNA, which exists in dynamic equilibrium between two alternate conformations (15). At 37 °C, lysogeny is stimulated by production of ciI protein from the free translation initiation region of the mRNA. However, raising the temperature to 45 °C shifts the equilibrium toward the other structure shielding the ribosome binding site which impedes translation and thereby favors the lytic pathway. In a more complex mode of control, temperature-induced mRNA-mediated activation of translation occurs by pairing of trans-acting elements like the small regulatory DsrA RNA to the leader of the mRNA of the general stress sigma factor RpoS in E. coli (16).

Our knowledge on mRNA-based translational control of heat shock genes derives mainly from two systems, the E. coli rpoH gene and the rhizobial ROSE (repression of heat shock gene expression) element. The expression of multiple small heat shock proteins in Bradyrhizobium japonicum and other rhizobia is controlled by ROSE (17). To date, 15 ROSE elements have been described (18). All these 70–120-nucleotide-long regulatory elements are located in the 5′-UTR of the heat shock operon in rhizobial species. According to our computer predictions, they form extended secondary structures with a highly conserved hairpin covering the SD sequence and AUG start codon. Base pairing in this region is imperfect due to the presence of internal loops and a highly conserved bulged residue. Nucleotide exchanges predicted to disrupt base pairing increased basal expression of the ROSE-lacZ fusions at 30 °C (19). On the
**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Growth Conditions—Escherichia coli** strains were grown in Luria-Bertani medium at 30 or 37 °C. *B. japonicum* 110bcpc4 was propagated aerobically at 30 °C in peptone-salts-yeast extract (PSY) medium (20) supplemented with 0.1% (w/v) arabinose. If appropriate, antibiotics were added in the following concentrations: spectinomycin, 100 μg/ml; chloramphenicol, 30 μg/ml (for counterselection against *E. coli* donor strains); tetracycline, 10 μg/ml for *E. coli* strains and 50 μg/ml for *B. japonicum* strains.

**Plasmid and Strain Constructions—**Standard protocols were used for recombinant DNA technology (21). All plasmids were constructed in *E. coli* DH5α. Point mutations in ROSE, and the Δ1–67 deletion to obtain Mini-ROSE constructs (ROSE, constructs consisting of stem loop IV only) were introduced by mutagenic oligonucleotides using the QuickChange mutagenesis kit (Stratagene) with the pUC18-derived plasmid pRJ5064 as a template. It carries a 718-bp PstI fragment containing ROSE1 flanked by a 264-bp upstream sequence and 352 bp downstream of the gene in the vector pSUP482 (22), which had been selected in *B. japonicum*.

Mutated inserts were isolated after restriction with PstI or PstI and SmaI (depending on the exact history of the plasmid) and transferred upstream of the *spc* gene in *B. japonicum* rpoN (23). The Δ1–67 deletion to obtain Mini-ROSE constructs (ROSE, constructs consisting of stem loop IV only) was introduced by mutagenic oligonucleotides using the QuickChange mutagenesis kit (Stratagene) with the pUC18-derived plasmid pRJ5064 as a template. It carries a 718-bp PstI fragment containing ROSE1, flanked by a 264-bp upstream sequence and 352 bp of the 5’-end of *hsa*A (17). For ROSE variants with two nucleotide exchanges, appropriate plasmids with single point mutations were selected for a second round of mutagenesis. The sequences of mutated ROSE elements were confirmed by automated sequencing.

**In Vitro Transcription—**PCRs containing the T7 promoter followed by the wild-type or mutated ROSE sequence were generated from corresponding plasmids. RNA was transcribed from the PCR templates with T7 RNA polymerase in a reaction mixture containing transcription buffer: 40 mM Tris-HCl (pH 7.9 at 25 °C), 6 mM MgCl2, 10 mM dithiothreitol, 10 mM NaN3, and 2 mM spermidine; 4 mM ribonucleoside triphosphates and RNase inhibitor. The reaction was incubated for 3 h at 37 °C, and RNA was precipitated with salt and ethanol.

**In vitro experiments were routinely performed with RNA redissolved in cacodylate buffer containing 50 mM sodium cacodylate, 50 mM KCl, and 10 mM MgCl2. Some CD experiments were carried out in the same buffer containing 1 mM MgCl2 or in water.**

**Spectroscopic Studies—**UV spectroscopy was carried out with a Hewlett Packard 8452A diode array spectrophotometer containing a Peltier device. Temperature was raised at 1 °C/min. The cuvette was incubated for 5 min at the temperature at which the A260 was measured to attain the specified temperature. A wavelength scan from 220 to 320 nm was recorded for each temperature.

**For CD measurements, RNA was quantified by UV absorbance at 260 nm and used at a concentration of 0.7 μg/ml. A wavelength scan from 200 to 320 nm was done in a Bio-Kine 32 scanning spectrophotometer MS450 with an acquisition of 10 s. The desired temperature was maintained with the cuvette chamber being connected to a water bath provided within the instrumental setup. The CD spectra at different temperatures were found to be reproducible for several experiments.**

**RNAseH Treatment—**A 32P-labeled transcript of ROSE was incubated with the oligonucleotides A (5'-GGCCGGGCGCTTGGCCGGCCGGCCGGG-3'), B (5'-CTTGGGCGCGCGCTTCGCTGCCC-3'), C (5'-CTCCTGATAGCCAAAATCTCCTCC-3'), or D (5'-GAATGGTAGTGGCGACCAAGGGGGCTGATG-3') at the specified temperatures for 10 min in cacodylate buffer. 0.5 units of RNAseH were added to the reaction mixture followed by a further incubation of 5 min. The products were then loaded on 8% denaturing PAGE containing 8 M urea. Transcripts of known size were run as size standards. The experiment with the mutants of ROSE was slightly modified by annealing the oligonucleotide at the specified temperature. All reactions were then incubated with RNAseH at 20 °C to maintain uniformity of the enzymatic action.

**RESULTS**

**Temperature-responsiveness of ROSE-type Thermosensors in Vivo—**To assess temperature control of ROSE-regulated genes, we chose two translational *lacZ* fusions, namely *B. japonicum* ROSE,-*hsa*P-*lacZ* and *Bradyrhizobium* sp. (Parasponia) ROSE1,-*hsa*D,-*lacZ*. Both ROSE sequences have been shown to be functionally interchangeable repressor elements in rhizobia (18). In the present study, expression of the plasmid-borne fusions was measured in *E. coli*. Thermally controlled expression cannot be monitored under steady-state conditions in the homologous backgrounds because most rhizobia are unable to grow at temperatures higher than 34 °C. However, they survive a prolonged heat shock even at 42 °C (24). As one protective measure, ROSE-controlled heat shock proteins are strongly induced at non-permissive growth temperatures ranging from 37 to 42 °C in *B. japonicum* (25). From previous studies, we know that thermostability by ROSE can be simulated in *E. coli* (19) (see also Figs. 3 and 4).

**As in rhizobial backgrounds (18), both *lacZ* fusions showed different basal expression levels in *E. coli*. The ROSE1 fusion produced a very low β-galactosidase activity of ~0.2 Miller units at 25 °C, whereas the ROSE1 fusion generated 24.4 megunits. Despite this initial difference, both fusions exhibited a very similar temperature response (Fig. 1). Induction started around 33 °C and led to ~10- and 35-fold elevated expression levels at 35 and 37 °C, respectively. As a control, we used a *B. japonicum* *rpoN*-*lacZ* fusion (26), which was expected not to be thermally controlled. Starting from 59 megunits at 25 °C, β-galactosidase activity indeed increased only slightly with increasing temperatures.**

**Depression by Point Mutations in ROSE—**Previous mutational studies revealed a number of point mutations in ROSE1 that altered its regulatory properties (19). Two additional single exchange variants, U70A and A78U, were constructed and assayed in comparison with the existing U98A and A106U.
mutations (Fig. 2). All four mutations are predicted to reduce the free energy of the ultimate hairpin of ROSE1 without directly interfering with base pairing of the SD sequence and translational start codon. Expression of translational lacZ fusions on pSUP482-derived plasmids in E. coli or of chromosomally integrated fusions in B. japonicum was determined. The point mutations partially relieved ROSE-mediated repression at 30 °C in both backgrounds, reinforcing the idea that correct stem loop formation is critical for regulation (Fig. 3, A and B). Although significantly derepressed at 30 °C, expression of point-mutated ROSE variants was further enhanced at 37 °C. The introduction of two simultaneous mismatches in ROSE (U70A,U98A and A78U,A106U) resulted in massive expression at 30 °C (Fig. 3, A and B). Some additional stimulation was observed when the E. coli cultures were grown at 37 °C (Fig. 3 A).

Compensatory Mutations Restore Repression—If base pairing rather than the exact nucleotide sequence was important for regulation, double mutations introducing a complementary nucleotide opposite to an unpaired point mutation should restore wild-type properties. This prediction was tested by construction of the A78U,U98A and A106U,U70A mutants (Fig. 2). In fact, E. coli or B. japonicum strains carrying these complementary mutations fully regained repression at 30 °C (Fig. 3, A and B), pointing out that base pairing is the major determinant for ROSE-mediated repression.

Importance of Stem Loops I–III for Thermosensing—A length of around 100 nucleotides and the complex structure of all known ROSE elements raise the question of whether the predicted stem loop structures at the 5’/H11032-end are functionally important. A Mini-ROSE construct was compared with the full-length version. Expression of corresponding hspA-lacZ fusions in E. coli or B. japonicum showed that the Mini-ROSE variant was capable of repression at 30 °C (Fig. 4, A and B). Interestingly, heat induction in E. coli was less pronounced than in the full-length context (Fig. 4 A). Introduction of nucleotide exchanges equivalent to U98A or A106U into Mini-ROSE led to enhanced expression at 30 °C in E. coli and B. japonicum (Fig. 4, A and B). The overall expression levels in E. coli at 30 and 37 °C were ~10 times lower than in the full-length mutants (compare with Fig. 3A). These results clearly show that Mini-ROSE consisting of stem loop region IV is a functional repressor element. However, temperature responsiveness appears less efficient in the absence of region I–III.

Subtle Temperature-mediated Alterations in the RNA Thermometer—To further investigate the thermal response of ROSE, we performed several in vitro experiments. ROSE-containing transcripts were synthesized in vitro from corresponding PCR-generated templates carrying the T7 RNA polymerase promoter sequence. Thermally induced structural changes in the wild-type ROSE RNA were recorded by UV spectroscopy. Only a moderate, gradual increase in A260 values was observed with the increase in temperature from 20 to 60 °C (Fig. 5).
Melting of an RNA Thermometer

Hyperchromicity was reversible with decreasing temperature. The mild transition between 35 and 40 °C might be indicative of local melting in a subregion of ROSE.

Melting studies were also carried out by CD spectroscopy. Fig. 6A shows the profile of a wavelength scan for wild-type ROSE from 200 to 320 nm with increasing temperature from 25 to 80 °C. The decrease in peak intensities around 270 nm and a peak shift toward 275 nm with rising temperatures indicates loss of base stacking interactions in the RNA molecule. Again, drastic melting of the structure was not observed in the physiologically relevant temperature range. When the maxima around 270 nm were plotted against temperature, it became evident that subtle structural changes progressed gradually up to a temperature of 60 °C with a very mild transition around 35–40 °C (Fig. 6, B and D). Complete melting resulting in the loss of base stacking (between 270 and 290 nm) and large conformational changes (between 240 and 260 nm) was evident at 80 °C (Fig. 6A, dark red line). The study was further extended with the G83Δ and U98A mutants of ROSE and with the wild-type Mini-ROSE fragment. From the wavelength profiles, the highest peak intensity obtained at a particular temperature around 270 nm was plotted against that temperature in Fig. 6B. It appears that the G83Δ RNA is more resistant to a temperature increase up to about 50 °C than the wild-type species, agreeing well with its predicted higher conformational stability. On the contrary, the height of the peaks from RNA of the derepressed U98A mutant dropped more steadily with increasing temperature, suggesting a looser structure. Only minor changes in peak intensities with increasing temperature were registered for the Mini-ROSE RNA lacking stem loops I, II, and III. Consistent with the in vivo data, this reflects that it is not as effective a thermosensor as the full-length structure.

Since RNA conformation may be influenced by divalent cations and overall ionic strength, we carried out similar CD experiments with wild-type ROSE RNA dissolved in water or in cacodylate buffer containing high or low magnesium concentrations. The traces recorded under all three conditions were superimposable (Fig. 6C). In accordance with the measurements at high magnesium concentrations, a comparative analysis in water showed that the G83Δ RNA was more stable up to 45 °C and that the U98 RNA was more labile at 30 °C than the wild-type RNA (Fig. 6D). As in the UV experiment, structural changes in the RNA were reversible between 25 and 50 °C (Fig. 6E). When shifted beyond 70 °C, the ROSE RNA was unable to restore its conformation upon cooling (data not shown).

RNaseH Protection Experiments Reveal Temperature-controlled Base Pairing—In yet another approach, the accessibility of the individual stem loops to complementary DNA oligonucleotide was assessed at different temperatures. Oligonucleotides A, B, and C were targeted against stem loops II, III, and IV, respectively (Fig. 2). Oligonucleotide D is complementary to the coding sequence of the hspA gene 11 nucleotides downstream of the translation start codon. Hydrogen bonding between the synthetic RNA and the complementary oligonucleotides is assumed to be possible only in unpaired RNA stretches. As a consequence, the resulting DNA-RNA hybrids will become a substrate for RNaseH, which will cleave the transcript into smaller products.

Fig. 7A shows full-length ROSE RNA in lane 1. Cleavage by RNaseH in the presence of oligonucleotide A was obtained at 30 and 42 °C. With oligonucleotide B, RNaseH cleavage started even as low as 0 °C followed by enhanced cleavage at higher temperatures. Interestingly, prominent protection of region IV against RNaseH attack was observed with oligonucleotide C at 20 and 30 °C (Fig. 7B), whereas strong cleavage was obtained at 42 °C. As one might expect, the region downstream of ROSE was accessible to oligonucleotide D and hence processed by RNaseH already at 0 and 30 °C.

To gain further detailed insight into the structural features of ROSE, a comparative RNaseH analysis was performed with the wild-type transcript and the G83Δ and U98A variants. With oligonucleotides A, B, and D, the mutated RNAs behaved similarly to the wild-type transcript, indicating that mutations in stem loop IV do not affect the conformation of other stem loops (data not shown). The results obtained with oligonucleotide C complementary to stem loop IV are presented in Fig. 8. The wild-type ROSE and G83Δ transcripts were protected from

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**Fig. 4. Expression of Mini-ROSE<sub>hspA-lacZ</sub> fusions.** Mini-ROSE fusions lack nucleotides 1–67 of ROSE, (Fig. 2). Expression was measured as indicated in the legend for Fig. 3. WT, wild type. MU, Miller units.

**Fig. 5. Structural changes of ROSE RNA shown by UV spectroscopy.** A<sub>260</sub> values of wild-type ROSE were plotted against temperature. The RNA preparation was first treated up to 60 °C and then cooled down again.

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[Image and text related to the figure are included here.]
degradation by RNaseH at 20 and 30 °C. In accordance with our previous observation, the wild-type RNA was cleaved by RNaseH at 42 °C. However, even at this temperature, the G83/H9004 RNA did not base-pair with oligonucleotide C and re-

FIG. 6. Thermal response of ROSE RNAs demonstrated by CD spectroscopy. In A, wild-type ROSE RNA in cacodylate buffer containing 10 mM MgCl₂ was subjected to a wavelength scan from 200 to 320 nm. Measurements were taken at 25, 30, 35, 40, 45, 50, 60, 70, and 80 °C. The traces recorded with increasing temperatures are color-coded from dark blue (25 °C) over yellow (45 °C) to dark red (80 °C). The decreasing peak intensity around 275 nm with increasing temperature is indicated by a downward arrow. The shift of the profile in the opposite direction in the range of 240–260 nm is indicated by the upward arrow. In B, peak maxima (in millidegrees, derived from A) of ROSE and its variants around 275 nm were plotted against temperature. C, comparison of structural changes in ROSE wild-type RNA under different buffer conditions. The maxima around 275 nm were plotted against temperature as in B. In D, temperature profiles of ROSE variants were recorded in water and plotted as above. E, reversible structural changes of wild-type ROSE RNA (in cacodylate buffer with 10 mM MgCl₂) at temperatures between 25 and 50 °C. The temperature was first increased up to 50 °C before the RNA sample was cooled down again.

degradation by RNaseH at 20 and 30 °C. In accordance with our previous observation, the wild-type RNA was cleaved by RNaseH at 42 °C. However, even at this temperature, the G83Δ RNA did not base-pair with oligonucleotide C and re-

FIG. 7. Antisense-oligonucleotide (oligo) induced RNaseH cleavage. RNaseH-treated products were run on 8% denaturing PAGE. Lane 1 in A shows untreated full-length wild-type ROSE RNA as a control. Lanes 2–4 show RNaseH-treated ROSE RNA in the presence of oligonucleotide A at 0, 30, and 42 °C, respectively. The expected products are 188 and 17 nucleotides. The smaller product is not visible. Lanes 5–7 show RNaseH-digested products in the presence of oligonucleotide B. The products are 169 and 31 nucleotides. Again, the smaller fragment is not visible. In B, RNaseH products of ROSE RNA obtained at 0, 30, and 42 °C in presence of oligonucleotide C and oligonucleotide D are shown. The respective cleavage products are indicated.

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mained resistant to RNaseH. The U98A RNA, on the other hand, was already quite efficiently cleaved at 20 °C, providing further evidence that its translation initiation region is already accessible at low temperatures.

**DISCUSSION**

Sensing of environmental parameters by mRNA molecules is being recognized as a common, relatively simple regulatory strategy. The present study demonstrates that expression of ROSE-containing heat shock genes is tightly controlled by the ambient temperature. A series of *in vivo* and *in vitro* experiments shows that the ROSE RNA meets the requirement for a sensitive cellular thermometer. It registers even subtle changes in a temperature range between 30 and 40 °C and adjusts gene expression accordingly. Major structural changes in the ROSE RNA were not observed in this temperature range, suggesting that local changes are sufficient to trigger a strong temperature response. As a consequence, ribosomes are assumed to gain easier access to the liberated binding site.

Mismatch mutations in stem loop IV of the ROSE element still remained heat-inducible, as one might expect since only one of 14 base pairs is affected in single exchange mutants (Fig. 2). Introduction of double mutations that destroyed one additional base pair (U70A,U98A and A78U,A106U) drastically lowered the expression levels at 30 °C. Nevertheless, some inducibility under heat stress conditions was retained. It is easily conceivable that the regulatory hairpin in these mutated 5'-UTRs is only loosened up and not completely melted at low temperatures. This is supported by the CD spectrum of the synthetic U98A RNA. Restoration of repression by compensatory mutations (A78U,U98A and U70A,A106U) further strengthens the mechanistic importance of base pairing for temperature sensing. Optimized base pairing in the anti-SD sequence by eliminating the bulged G83 residue, on the other hand, has been shown to completely abolish thermally induced expression of a corresponding lacZ fusion (19). The present *in vitro* data demonstrate that the mutated RNA structure masking the SD sequence is too tight to melt at temperatures below 50 °C. Ribosome access to the SD sequence at relevant temperatures between 30 and 40 °C apparently requires a labile structure as provided by the bulged G83 residue and two immediately flanking loops (Fig. 2). Only local melting in this highly unstable region is proposed to be sufficient for the ribosomal RNA to gain access. Adding a complementary oligonucleotide in RNase H experiments probably mimics the effect of the 16 S rRNA, explaining why melting of the structure in the RNase H approach appears much more evident than the UV and CD studies would suggest.

It should be noted that a view in which thermally induced melting of hydrogen bonds masking the SD sequence is the key to temperature control might be too simplistic. First of all, the contribution of *trans*-acting factors can still not be excluded although all attempts to identify such factors have failed (9, 19, 27). Functioning of the rhizobial ROSE element in *E. coli* indicates that, if at all, a universal factor, e.g. ribosomal RNA or protein, may play a supportive role in the melting process. Secondly, the marked decrease in thermal induction of the Mini-ROSE constructs *in vitro* by about 10-fold as compared with full-length ROSE suggests that stem loops I–III play a regulatory role. CD data demonstrate that region IV alone does barely change its conformation at temperatures up to 45 °C. Stem loops I–III might serve two important consecutive functions: (i) they might promote correct folding of region IV while the RNA is being synthesized ("zipper" function). The requirement of base pairing in region III for repression by ROSE (19) together with the fact that spontaneous refolding of full-length ROSE RNA was not possible upon complete unfolding at 80 °C (CD data) supports this assumption; (ii) once the 5'-UTR is folded, local melting in the SD region is necessary for ribosome entry. Not yet defined interactions with stem loops I–III presumably facilitate the unfolding process, thereby increasing temperature sensitivity of ROSE ("wedge" function). Interestingly, the *rpoH* and *pfra* thermometers are also comprised of rather complex structures (9, 12). The inability of an *rpoH* derivative lacking an internal stem (stem III) to respond to heat shock was also interpreted as wedge function of the deleted structure (10). Tertiary interactions between stem loop regions of an mRNA that detects adenosyl-cobalamin have been reported previously (27). Apparently, fidelity of various sensory RNAs is achieved by a complex architecture.

It remains an open question whether RNA thermometers are able to shut off the heat shock as the temperature drops. Our CD and UV spectroscopic studies suggest that it might be possible. Base stacking interactions that were lost upon temperature increase were regained when the temperature was lowered. Complete and irreversible melting was obtained only at unphysiological temperatures above 70 °C. Reversion of the peak in CD spectra recorded between 25 and 50 °C was associated with a peak shift to higher wavelengths (from 278 to 282 nm, data not shown). The reason for this slight shift is unclear, but it might indicate a diversion from the original conformation. Nevertheless, regain of base stacking interactions at decreasing temperatures might well suffice to inhibit ribosome entry to the SD region, thus stalling translation and shutting off heat shock protein synthesis. Multiple rounds of the structural transition in an RNA thermometer are certainly not relevant in the cellular context due to generally short half-lives of mRNA in prokaryotes. There is in fact evidence that at least some sensory RNAs are degraded if they are not in a translation-competent conformation (19, 28).

Regulation of gene expression mediated by conformational changes in mRNA molecules is widely prevalent in biological systems. Apart from temperature, small molecules such as precursors of the vitamins cobalamin, thiamine, riboflavin can be detected by similar RNA-based sensors whose translation is blocked in the presence of these compounds (27, 29–31). Translational feedback regulation of the *Thermus thermophilus* ribosomal protein S15 occurs via a strikingly similar mechanism. Binding of S15 to its own mRNA induces a conformational change that masks the ribosome binding site (32). In an alternate mechanism, certain mRNA sensors trigger premature transcription termination by conformational changes upon binding of the vitamin precursors thiamin pyrophosphate and flavin mononucleotide or of S-adenosyl-methionine, thus re-
pressing transcription of biosynthetic genes in abundance of these molecules (29, 33–35).

All these mechanisms have in common that they seem to function without the aid of accessory factors, such as regulatory proteins or small antisense RNAs. The major difference lies in the fact that RNA thermometers do not act as a switch, like those sensors that bind other molecules which initiate a shift between two competing alternative conformations. In RNA thermosensors, distinct alternate conformations were not observed. Only subtle structural changes are probably sufficient to promote ribosome access, which is facilitated by imperfect base pairing around the SD In either case, RNA proved to be a simple, yet diverse, flexible and versatile molecule that offers sufficient chemical complexity to have broad functional capacity. Assigning a sensory function to mRNA itself in a coupled transcription-translational system as it occurs in the bacterial cytoplasm might have been a very successful early invention in evolution.

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