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Some Aspects Affecting the Molecular Mechanisms of Eukaryotic Adaptation under Global Warming

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Abstract: Global warming is an irreversible process resulting in the deterioration of living conditions for various organisms, including the most important agricultural species. So-called ς^{32} factor of *Escherichia coli* is embedded into the RNA-thermosensor in the λ cIII gene and plays an important role in the regulation of bacterial response to hightened temperatures. Expression of heat/cold-shock genes and some virulence genes in response to temperature changes is coordinated by the genome. There are some known RNA-thermosensors with different structures that provide a functional control of the diversity of cellular processes. The most common RNA-thermosensor is the ROSE-element suppressing expression of heat-shock genes. A common feature is functionally important and it is elimination that makes the RNA-thermosensor insensitive to high temperatures. In this paper we describe molecular sequences (RNA-thermosensor) whose chemical compounds influence on the homeostatic temperature regulation, namely, on the corresponding enzymes. Though the data on RNA-thermosensors we obtained for microorganisms it is maybe possible in the long run to change the animal genome at the molecular level by the insertion of these sequences or cultivation of symbiotic microorganisms, which may be used for production of biologically active compounds. In addition, such insertions would probably be able to reduce the negative effect of high environmental temperatures on living organisms.

Key words: Global warming, RNA-thermosensors, DNA, ribonucleic acid (RNA), enzymes.

Planetary changes associated with global warming adversely affect the ontogenesis of eukaryotes, including the main agricultural species of flora and fauna. The heat shock proteins DnaK, DnaJ, GrpE and HflB are coordinated by the genome in response to changes in temperature. The most common ribonucleic acid (RNA) thermometer is the ROSE element, which suppresses the expression of heat shock genes. Heat shock proteins are molecular chaperones or proteases that can fold, translocate or degrade proteins (denaturation of proteins at elevated temperature). This work shows the molecular level sequences (RNA thermometers) and temperature regimes affecting the processes of molecular mechanisms inside the cell. Although the results on RNA thermometers were obtained on microorganisms, in our opinion there is a prospect at the molecular level to change the genome of higher plants and animals. Namely, the insertion of these sequences and the cultivation of symbiotic microorganisms can be used in biotechnology for obtaining biologically active substances, at elevated ambient temperatures, thus reducing the negative impact of high temperatures on living organisms.

In previous studies [1, 2], the main factors are shown: RNA thermometers, ROSE elements, and other genome sequences that are able to influence the

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resistance of organisms at elevated temperatures. Table 1 shows the temperature factors affecting some of the molecular mechanisms that occur in the cell.

Previously, material [10, 13, 14] was presented showing molecular biology methods that significantly accelerate the selection process for the creation of plant varieties, animal breeds, and strains of microorganisms, which are now based on classical methods of hybridization, introduction, and acclimatization.

Often the regulation of genes controlling the environment is carried out at the level of transcription, through the action of regulatory proteins. The expression of ς^{32} is regulated at the level of transcription,

Table 1 Temperature factors impacting some molecular mechanisms realized within a cell.

Temperature	Molecular mechanisms
20-40 °C	Trimerization and DNA binding of purified HSF can be directly induced by heat shock temperatures *in vitro* so that a higher percentage of trimers is observed at 40 °C compared to 20 °C [3].
22-43 °C	Gel mobility shift assays showed that the DNA-binding activity of purified TlpA is sensitive to temperature with less binding observed at 43 °C than at 22 °C [4].
25 °C	*RpoS* 038 is more abundant at 25 °C than at higher temperatures; *RpoS*-dependent promoters are efficiently expressed at low temperatures [5].
25 °C	F form (a full-size 85-nucleotide primary DsrA transcript) is transformed into 60- or 61-nucleotide form (T form) via the loss of its 5'-region; interaction of the T form with mRNA is more stable at +25 °C than at 37 or 42 °C, which results in the displacement of this form at low temperatures [6].
26-37 °C	LcrF encodes a transcription factor responsible for inducing the expression of plasmid-encoded virus genes in response to temperature. A comparison of the amount of LcrF protein produced per unit of message at 26 °C compared to 37 °C indicated that the efficiency of LcrF mRNA translation increased with temperature [7].
30 °C	*Listeria* mutants constructed to obtain PrfA because of destabilization of leader sequences [6].
30 °C	Full-size reverse transcripts initiated from the primer complimentary to the encoding *rpoH* region are obtained [7].
30-40 °C	Optimum pairing with the Shine-Dalgarno (SD) sequence in this mutant prevents unfolding of the folded structure within the temperature range of 30-40 °C [9].
30-42 °C	Accumulation and direction of RNA polymerase to the promoters of the heat shock genes [10] (Missiakas).
30-42 °C	*RpoH* transcription is controlled from four different promoters [11].
30-42 °C	*RpoH* mRNA translation, ς^{32}stability, and ς^{32} activity are all induced by temperature shifts from 30 °C to 42 °C [11].
30-42 °C	Gained insight into the translational regulation of *rpoH* by constructing an *rpoH*-lacZ translational fusion that carried ~650 bp of promoter sequence and most of the *rpoH* coding sequence. This fusion was strongly induced within 2 min after a shift from 30 °C to 42 °C [9, 11].
30-42 °C	For the wild-type *rpoH* RNA, no toeprint was observed at 30 °C, indicating that formation of the ternary complex is prevented at this temperature. In contrast, a toeprint appeared within 5 min of incubation at 42 °C, demonstrating that 30S binding does occur at the higher temperature [11].
33-37 °C	Thermal destabilization of the rod loop providing translation initiation to occur within the physiological range of temperatures [12].
37 °C	A shift of the balance in relation to the structure involved into the protein binding and cIII protein production occurs [3, 9].
37 °C	Cell content of the promoter of the *Yersinia pestis* LcrF gene (virus gene) is significantly higher at 37 °C than at 26 °C [3, 7, 12].
37- 45 °C	*In vitro* experiments showed that the cIII mRNA can exist in two conformations. High temperatures (45 °C) and mutations that increase cIII expression promoted the formation of one structure in which ribosome binding is efficient. By contrast, low temperatures (37 °C) and mutations that reduce cIII expression promoted the formation of a second structure in which the translation region is occluded and ribosome binding is reduced [1] (Altuvia).
37- 45 °C	TlpA, which is encoded on a virulence plasmid, is a transcriptional repressor of its own synthesis. Expression of a tlpA-lacZ transcriptional fusion is elevated 13.2-fold between 37 °C and 45 °C, and this regulation is dependent on TlpA [13] (Hurme).
42 °C	Primer prolongation was blocked by the assembling of a triple complex including the *rpoH* transcript, 30S ribosome, and tRNAfMet [14].
45 °C	Influences on the balance between the cIII and ell structures [8, 9].

translation, protein stability, and activity of ς^{32}. Although *rpoH* transcription is not induced by temperature shifts from 30 °C to 42 °C, *rpoH* transcription is controlled from four different promoters. One of these promoters is recognized by ς^{E}—RNA polymerase, which also acts as an inducer of *rpoH* expression at extreme temperatures of > 50 °C. However, several posttranscriptional mechanisms based on an RNA molecule have recently been discovered. According to date in Ref. [11], the secondary structure of *rpoH* mRNA itself is a thermosensor. At low temperatures, the *rpoH* mRNA is folded into a secondary structure that closes the ribosome binding site and the initiation codon. During heat shock, this structure unfolds, allowing ribosome binding and increased synthesis of ς^{32}. It became clear that certain tRNA molecules are not only a substat for ribosomes, but also contain control elements that modulate their own expression, depending on environmental conditions. Structural changes in such sensory RNAs are caused by specific changes in the environment of existence. There are two fundamentally different classes: cis-acting RNA elements, the regulatory potential of which is located inside the mRNA sequence and trans-acting, small, non-coding RNA molecules, which function by pairing nucleotides with complementary mRNA sequences located in other loci of the genome. In contrast to classical attenuators that regulate the structure of the leader sequence of RNA according to the position of the transmitting ribosome, the cis-acting RNA changes its conformation in response to physical or chemical signals. The so-called riboswitches monitor the metabolic state of the cell through binding with high specificity and affinity with the metabolites. They are located in the 5'-UTR region of the genes coding for the biosynthesis, absorption, or degradation of small metabolites and provide feedback control for these metabolic pathways. Their structure consists of the receptor region (8-membered oligonucleotide), which is characterized by a consensus sequence specific to the substrate molecule, and the parent region (basis for expression). Binding of a small molecule "pushes" a conformational switch; it alters the gene expression by one of three possible mechanisms—premature transcription termination; translation initiation; or mRNA processing. Most riboswitches exclude expression in a bound state. However, a small number of switches, including gene expression, were found. In contrast to specific riboswitches that bind to metabolites, a closely related type of sensory mRNA, RNA thermometers act in response to a common physical signal, namely, intracellular temperature, which is an important parameter affecting, in particular, the expression of genes encoding heat and cold shock proteins, the expression of virus genes, is under the constant control of duplexes. A well-known characteristic of nucleic acids is that they melt with increasing temperature. Therefore, the temperature shift is able to modulate the conformation of the regulatory molecules of RNA. That is, the transition of the molecule from the configuration of the intramolecular pin to the single-stranded state. Interest in the possibility of formation of pin structures appeared due to the fact that they regulate the stability of mRNA of microorganisms.

Previous studies show that increasing synthesis and stability lead to increased levels of ς^{32}. Activity of ς^{32} and its association with RNA polymerase are also modulated by heat shock. An increased resistance of ς^{32} at high temperature was detected. In the normal growth process, the half-life of ς^{32} is 1 min; as the temperature rises, the half-life increases to ~5 min. Interestingly, the heat shock proteins DnaK, DnaJ, GrpE and HflB, whose expression are regulated by the function of ς^{32}, destabilize ς^{32}. These proteins interact with ς^{32}, isolate it from RNA polymerase, and cause it to degrade. Disordered proteins that accumulate after heat shock appear to titrate the DnaK, DnaJ, and GrpE chaperones and the HflB protease far from ς^{32}. Thus, a pool of incorrectly folded proteins are considered one

of the indicators of increased temperature in the cell. Increased synthesis of ς^{32} often occurs at the translation level. However, although the *E. coli* heat shock response has been studied for many years, a thermometer that signals an increase in translation has not been known.

ς32 factor is regulated at the level of transcription, translation, protein stability, and activity of ς^{32}. In Ref. [11], the secondary structure of *rpoH* mRNA itself is a thermosensor. At low temperatures, *rpoH* mRNA folds into a secondary structure that closes the ribosome binding site and the initiation codon. During heat shock, this structure unfolds, allowing ribosome binding and increased synthesis of ς^{32}.

One of the possible mechanisms of mRNA stabilization is associated with the formation of a noncanonical (hairpin) structure at the 3’ or 5’ ends of the mRNA [15].

A number of RNA thermometers are known that differ structurally and functionally and control the diversity of cellular processes. All open molecular thermometers, which may be cis- or trans-acting, control translation through the isolation of the ribosome-binding site, and most of them are located in the 5'-UTR of bacterial heat shock genes or virus genes. At low temperature, the SD sequence (5'-AAGGAG-3'; 5'-rraggak-3' is the consensus sequence for prokaryotes; 5'-uygcu-3' for gram-negative bacteria) is masked (located inside the pin structure). An increase in temperature destabilizes the pin structure so that the ribosome binding site (SD sequence) becomes accessible, which allows translation initiation (AUG is the translation initiation start codon).

An alternative sigma factor, *RpoS*, plays a central role in the regulation of the virulence-associated external surface proteins OspC and OspA in Lyme disease caused by the spirochete *Borrelia burgdorferi*. Temperature is one of the key environmental parameters controlled by *RpoS*, and the small non-coding RNA molecule, DsrABb, controls the increase in *RpoS* as a result of temperature changes. It was hypothesized that DsrABb is in a stable secondary structure at T = 23 °C, at which nucleotides do not pair with the *rpoS* transcript. After a temperature shift, the secondary structure of the small RNA molecule melts, which leads to the binding of the anti-SD region of *rpoS* mRNA. This can stimulate translation using the output of the SD sequence and the translation initiation point from the secondary structure to *rpoS* mRNA under the influence of a virus (37 °C).

Perhaps the most common bacterial RNA thermometer is the ROSE element, which suppresses the expression of heat shock genes. It was found in numerous A- and B-proteobacteria, including for *E. coli* and *Salmonella*. The ROSE element, having a length of 60 to 100 nucleotides (n.), is usually located in the 5^{f}-UTR of heat shock genes. Its rather complex structure includes 2-4 pin structures, one of which contains an SD sequence and, in some cases, also the AUG start codon. Another common RNA thermometer is the 4U element, which was first found in the small *agsA* gene, in the heat shock gene, *Salmonella*. The provided structure contains two hairpins, while four uridine residues form nucleotide pairs with an SD sequence. It was experimentally confirmed that it depends on the melting temperature of one of the hairpins, and ribosome binding to the SD sequence occurs only at heat shock temperatures.

The 4U element is often used to control heat shock genes and virulence of bacteria, since it can vaguely bind to a fragment of the 5'-AGGA-3' SD sequence. Thus, the hypothesis of control using an RNA thermometer for the IcrF (virF) *Yersinia* gene, which encodes a virulence revocation regulator, is fully confirmed. Gene translation does not occur at a temperature of 26 °C, but is induced at T = 37 °C. An analysis of the regulatory principles of known RNA thermometers showed that for their functioning it is necessary that only a few nucleotides form incomplete bonds with the nucleotides of the SD sequence or

flanking regions to prevent ribosome binding. This means that in nature there must be other, yet undiscovered types of RNA thermometers.

A minimal *rpoH-lac*Z construct was developed for further study of thermoregulation of *rpoH* translation. These workers show that mutations that were supposed to decrease stability cause an increase in expression, and mutations that were supposed to increase stability lead to a decrease in expression. Subsequently, they used circular dichroism (CD) to directly measure the melting temperature profiles of various RNAs. The measured thermal stability correlated with the expression levels observed on fused structures. For example, a mutant RNA carrying the substitution G→ A at position +15 (15A), which showed increased expression at 30 °C and, therefore, reduced thermal induction, was denatured at a lower temperature than the control RNA. Mutant RNA carrying 15A and a compensatory mutation at position +124 (15A-124U) show thermal regulation and thermal stability of an almost "wild" species [11].

Using bio-informatics analysis, confirmation of this hypothesis has been obtained [10]. The synthesis of effective artificial RNA thermometers has become another evidence of this assumption [11, 16]. But in many cases, the molecular details of the mechanism of eukaryotic sensitivity to temperature changes are not yet fully understood. Therefore, the possibilities of stabilizing the metabolism of living organisms using their own genetic resources (enzymes, RNA thermometers) and the possibility of their use in biotechnology, specifically in the preparation of biologically active substances that increase the resistance of organisms to stress temperatures, demonstrate the possibility of solving priority issues of the genotype response to global warming factors and solving the question of the existence of eukaryotes in abiotic environments.

Thus, it is obvious from the material presented that there are sequences that determine the body's resistance to high temperatures and play the role of protectors in their ontogenesis. This indicates that it is possible at the molecular level to alter the animal's genome, namely, by inserting these sequences, or by culturing symbiotic microorganisms that can be used in biotechnologies for the production of biologically active substances, which at elevated ambient temperatures reduce the negative effect of high temperature on living organisms.

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