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COMMENTARY

RNA thermosensors: how might animals exploit their regulatory potential?

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ABSTRACT

The secondary and tertiary orders of RNA structure are crucial for a suite of RNA-related functions, including regulation of translation, gene expression and RNA turnover. The temperature sensitivity of RNA secondary and tertiary structures is exploited by bacteria to fabricate RNA thermosensing systems that allow a rapid adaptive response to temperature change. RNA thermometers (RNATs) present in non-coding regions of certain mRNAs of pathogenic bacteria enable rapid upregulation of translation of virulence proteins when the temperature of the bacterium rises after entering a mammalian host. Rapid upregulation of translation of bacterial heat-shock proteins likewise is governed in part by RNATs. Turnover of mRNA may be regulated by temperature-sensitive RNA structures. Whereas the roles of temperature-sensitive RNA structures similar to RNATs in Eukarya and Archaea are largely unknown, there would appear to be a potential for all taxa to adaptively regulate their thermal physiology through exploitation of RNA-based thermosensory responses akin to those of bacteria. In animals, these responses might include regulation of translation of stress-induced proteins, alternative splicing of messenger RNA precursors, differential expression of allelic proteins, modulation of activities of small non-coding RNAs, regulation of mRNA turnover and control of RNA editing. New methods for predicting, detecting and experimentally modifying RNA secondary structure offer promising windows into these fascinating aspects of RNA biochemistry. Elucidating whether animals too have exploited the types of RNA thermosensing tools that are used so effectively by bacteria seems likely to provide exciting new insights into the mechanisms of evolutionary adaptation and acclimatization to temperature.

KEY WORDS: RNA editing, RNA secondary structure, RNA thermometers, Temperature, Thermosensors

Introduction

The aim of this Commentary is to help acquaint readers with a topic that might be largely unfamiliar – the vast potential of temperature-dependent changes in RNA secondary and tertiary structures (see Glossary) to adaptively modulate the responses of cells to changes in temperature. Because almost all work on the thermosensory roles of RNA has been performed with bacteria, animal physiologists may be unfamiliar with the potentials of these mechanisms for fostering evolutionary adaptation and acclimatization to temperature. To illustrate this potential, I begin with a brief review of some of the key findings made with bacteria. I then discuss the potentials of RNA thermosensing for facilitating temperature-adaptive responses in animals. I hope that this Commentary inspires exciting new questions that can be tested in the near future as new ways of studying RNA secondary and tertiary structure are developed (for a review of methods, see Ignatova and Narberhaus, 2017).

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The 'bad' and 'good' sides of macromolecular sensitivity to temperature

To set the stage for an analysis of thermosensing by RNAs, I begin with a short overview of the important 'balancing act' that exists between macromolecular stability and flexibility, a relationship often termed 'marginal stability' (see Glossary) (Somero et al., 2017). The functional properties of macromolecules depend on the maintenance of a particular three-dimensional conformation established by the secondary and tertiary structures of the macromolecules (RNA: Vandivier et al., 2014; proteins: Somero et al., 2017; Dong et al., 2018). However, these higher-order structures must also be flexible enough to change conformation during function. The higher-order structures of proteins and RNA are readily perturbed by changes in temperature, threatening the 'balancing act' so critical for function. Thus, changes in temperature often are regarded as having negative influences on macromolecular stability. However, there is also a 'good' side to this thermal perturbation: the alteration in conformation of the macromolecule that is caused by a change in temperature can function as a thermosensing mechanism and lead to downstream changes that are adaptive to the cell. Indeed, our primary focus here is on the beneficial effects that follow from the high sensitivities of RNA secondary and tertiary structure to changes in temperature. These changes in structure serve as core elements of a variety of sensory and regulatory systems that govern the biochemical composition and function of the cell – at least in the domain Bacteria. Whether these RNA-based sensory and regulatory mechanisms might be employed by Archaea and Eukarya remains largely unknown. However, the simplicity, sensitivity, rapidity and reversibility of function of RNA-based thermosensor mechanisms cannot help but generate the expectation that these mechanisms will be found to play important roles in all three domains of life.

Bacterial 'RNA thermometers' – basic roles and mechanisms

The importance of RNA thermosensing is well illustrated by bacterial RNA thermometers (RNATs) (see Glossary). Thermosensing by RNATs involves a temperature-induced change in RNA secondary structure (see Glossary) in defined ('thermometer') regions of an mRNA molecule that triggers rapid alteration in the capacity of the mRNA to undergo translation (Serganov and Patel, 2007; Kortmann and Narberhaus, 2012; Krajewski and Narberhaus, 2014; Mortimer et al., 2014). Responses to both heat- and cold-stress exploit RNATs, allowing effective translation of proteins needed at either high or low temperature. Pivotal to the RNAT response is exposure of the Shine–Dalgarno

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Glossary

Epitranscriptomic modification
Chemical modifications of transcribed RNAs, such as methylation, pseudouridylation and RNA editing, that can alter the translational ability, stability and protein coding of mRNAs.

Marginal stability
The condition of macromolecular structural stability that allows the conformation of the protein or nucleic acid to have the right geometry for recognizing and binding the ligand(s) with which it must interact and yet be flexible enough to allow the changes in conformation required for completion of function to occur. For RNA secondary and tertiary structure, temperature-sensing elements (thermosensor regions) must have the right stability to allow temperature-driven changes in shape to take place when (and only when) a signalling function is required, for example when increases or decreases in temperature occur that must be met by altered gene expression or translational activity.

Riboswitches
RNA sensors that bind specific molecules and undergo concomitant changes in conformation and functional state. Riboswitches occur in bacteria and eukaryotes and can be modulated by temperature through effects on RNA conformation.

RNA editing
The conversion of cytosine (C) to uridine (U) by cytidine deaminases, or adenosine (A) to inosine (I) by the enzyme ADAR. Editing may lead to non-synonymous codons in an mRNA, i.e. to changes in protein sequence. RNA editing is highly sensitive to temperature, and can lead to the production of temperature-specific protein sequences.

RNA secondary structure
Non-covalent interactions among proximal nucleobases within a single RNA molecule that lead to structures such as hairpin loops that may be important in RNA thermosensing activities. Secondary structures involve both canonical Watson–Crick base pairings and non-canonical base pairings, which typically have lower thermal stabilities than canonical types of base pairings. Melting temperatures of thermosensing RNA regions can be modified by minor changes in base composition.

RNA sensors
Regions of mRNAs that can modulate gene expression in response to changes in physical and chemical factors. RNA is able to bind inorganic ions (e.g. K^+ and Mg^{2+}) and small organic molecules such as amino acids, with concomitant changes in secondary or tertiary structure. Thus RNA sensors would appear to play a role in osmotic regulation as well as in responses to change in temperature. RNA sensors include riboswitches and RNA thermometers.

RNA tertiary structure
Non-covalent interactions between different elements of secondary structure within a single RNA molecule. Tertiary structure plays many roles in governing RNA function.

RNA thermometers (RNATs)
Portions of RNA molecules, notably mRNAs, whose temperature-sensitive melting or change in conformation leads to downstream changes in processes such as translation. RNATs are characterized by rapid rates of response to change in temperature and a high temperature sensitivity, such that changes in temperature of ∼1°C can lead to rapid activation or inhibition of the RNAT-mediated process. RNATs are important in both heat- and cold-induced changes in translation rate.

ROSE element
An RNA thermometer (repression of heat shock gene expression), that controls expression of small heat-shock proteins (HSPs) in bacteria.

Shine–Dalgarno (S–D) sequences
Six-base-long sequences (AGGAGG) within a bacterial mRNA that govern binding of the mRNA to the small (30S) ribosomal subunit. S–D sequences are commonly located eight bases upstream of the AUG start codon. Exposure of the S–D sequence is necessary for translation of the mRNA to be initiated, and the S–D exposure process is key to function of RNA thermometers.

Start codon
The base triplet (AUG) where translation commences.

Pseudouridylation
Conversion of uridine to its isomer pseudouridine, which leads to more stable base pairing with adenosine relative to U–A pairing.

(S–D) sequence (see Glossary) – the six-nucleobase (AGGAGG) region of a bacterial mRNA that governs binding of the mRNA to the small (30S) ribosomal subunit (Fig. 1). The structural changes that lead to exposure of the S–D sequence allow binding of the mRNA to the 30S subunit and rapid initiation of translation of the encoded protein. Moreover, initial binding of the mRNA to the ribosome can trigger further unwinding of the RNAT that enhances mRNA–ribosomal interactions (Meyer et al., 2017). The conformational changes in RNATs can involve melting of relatively small regions of the mRNA, for example hairpin structures, in response to elevated temperature (the 'zipper' mechanism; Fig. 1) or a shift between alternative conformations of the mRNA that involve larger regions of the molecule (the 'switch' mechanism) (Kortmann and Narberhaus, 2012). The latter mechanism is important in RNATs that activate translation upon a decrease in cell temperature, as occurs for cold-shock proteins of bacteria. RNATs, whether they involve zipper or switch mechanisms, are remarkably sensitive temperature detectors; they can sense changes in temperature of the order of 1°C and turn translation on or off, accordingly. The speed of response of RNATs is rapid, supporting the notion that RNATs enable 'translation on demand' when temperature changes (Kortmann et al., 2011).

A brief description of two types of bacterial RNATs illustrates the important functions of these thermosensing mechanisms. One is the FourU thermosensor of the pathogen *Salmonella typhimurium* (Rinnenthal et al., 2011). This RNAT is termed a FourU thermometer because four consecutive uridines form base pairs with part of the S–D sequence. The FourU RNAT is utilized to enable the bacterium to sense when it has entered a mammalian host – that is, to sense that its cell temperature has risen from a lower ambient temperature to one near 37°C, and that conditions are now appropriate for rapidly activating translation of pathogenic proteins. A second type of RNAT functions in the regulation of bacterial heat-shock responses. The most common type of RNAT serving this function is termed a ROSE element (for 'repression of heat-shock gene expression') (see Glossary), which governs expression of small heat-shock proteins (HSPs) (Kortmann and Narberhaus, 2012). The hairpin structure of the ROSE element melts above a certain temperature, which, as in the case of the FourU RNAT, exposes the S–D sequence and allows a rapid upregulation of synthesis of HSPs from pre-existing mRNA, a response that can be vital for cell survival. This rapid production of HSPs using pre-existing mRNA can provide initial protection of the proteome and allow the bacterium time to initiate the slower process of upregulating transcription of heat-shock genes. Furthermore, the rapid loss of the translational competence of HSP mRNA that occurs when cell temperature decreases to non-stressful levels allows the production of HSPs to be curtailed at the cessation of heat stress. Continued production of large amounts of HSPs during recovery periods can be detrimental to the cell (Kortmann et al., 2011). Thus, both the 'on' and 'off' aspects of RNAT thermoswitching are advantageous.

The discussion above has focused on mRNAs that encode one specific type of protein. RNATs also can influence the relative levels of expression of different proteins that are synthesized at different temperatures through the control of differential expression of multiple genes present in a single mRNA. In bacteria, proteins that have linked functions are commonly encoded within a single operon, within which occur individual S–D sequences for each encoded protein. Similar levels of expression are commonly found for all of the proteins encoded by an operon, but in some cases there are large differences among proteins in expression levels (Krajewski and Narberhaus, 2014). These different expression levels might

Journal of Experimental Biology (2018) 221, jeb162842. doi:10.1242/jeb.162842

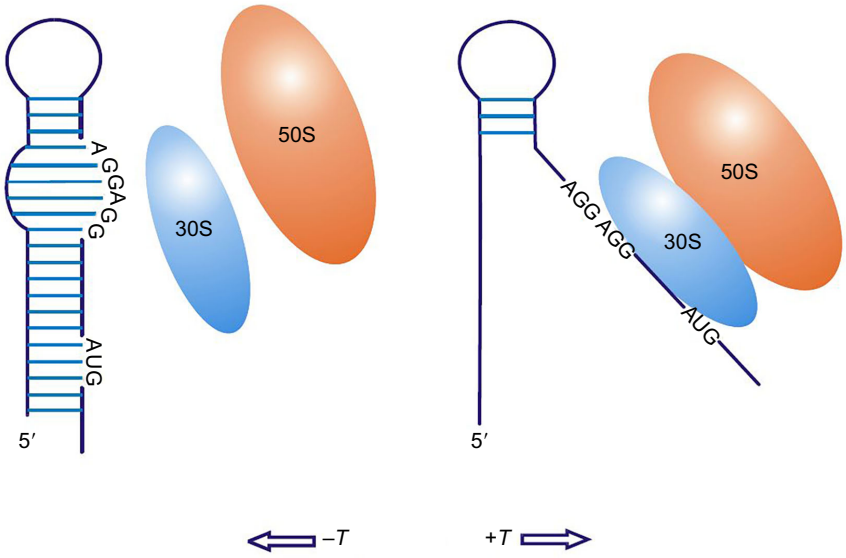

Fig. 1. Basic characteristics of a zipper-type bacterial RNA thermometer. The 5′ end of the mRNA contains a thermally labile sequence region that 'melts' if cell temperature rises above a certain value (rise in temperature: $+\Delta T$; fall in temperature: $-\Delta T$). Light blue lines between strands of the mRNA depict hydrogen bonds between bases. The RNA thermometer (RNAT) contains the Shine–Dalgarno (S–D) sequence (AGGAGG) that, when fully exposed, can bind to the small (30S) ribosomal subunit and allow translation to commence. The start codon (AUG) is often located eight nucleotides downstream from the S–D sequence. Thus melting of the 'thermometer' allows the S–D sequence and start codon to interact with the 30S subunit, promoting translation of the mRNA. For structures of other RNATs, e.g. switch-type RNATs and temperature-regulated riboswitches (see Glossary), see illustrations given in Serganov and Patel (2007), Kortmann and Narberhaus (2012) and Reining et al. (2013).

reflect variation in the accessibility of the S–D sequences of different protein-coding regions to the 30S ribosomal binding site. Changes in temperature can alter the relative expression levels of proteins encoded by a single operon, and these effects are consistent with the occurrence of RNATs with different thermal responses (Krajewski and Narberhaus, 2014).

The complex influences of temperature on translation that are mediated through RNAT effects provide a further ground for caution in assuming a tight linkage between the transcriptome and the proteome. Relationships between changes in the amount of a particular mRNA and its corresponding protein are complex and variable among proteins and across time (Buckley et al., 2006; Schwanhäusser et al., 2011). Temperature effects on the translational competence of mRNAs merit integration into analyses that attempt to correlate mRNA levels with protein abundance.

Studies of genetically engineered bacterial RNATs have provided insights into the evolution of these switches, notably the amount of change in base sequence needed to modify the on–off temperature. For example, a single base substitution is able to modify the on–off temperature of a FourU RNAT (Rinnenthal et al., 2011). These laboratory manipulations of thermometer melting temperature suggest that evolutionary adaptation of the on–off temperatures of RNA thermosensors, to enable them to function in the particular range of temperatures at which temperature-sensitive gene expression is required, is likely to be rapid. Laboratory manipulation of RNATs has also shown that it is possible to modify the sharpness of the RNAT's response to temperature, which can range from a gradually responding rheostat to a sharp on–off response to a slight change in temperature (Neupert et al., 2008). Most natural RNATs appear to have a rheostat mechanism, but engineered RNATs with sharper on–off responses might prove useful in biotechnological contexts.

Do RNATs help to regulate heat-shock responses in animals?

The important roles played by RNATs in regulating the heat-shock response and other aspects of the proteome's composition in bacteria raise the question as to whether this type of thermosensing mechanism is employed in eukaryotes as well. There is no definitive answer to this question at the present time, but there is some evidence that at least two types of RNAT are employed by animals to regulate their heat-shock responses. One of these conjectured RNATs appears to function in a manner similar to the RNAT used by bacteria to control translation of HSP mRNAs. In *Drosophila*, there is evidence that translation of the mRNA encoding heat-shock protein 90 (HSP90) is partially regulated by an RNAT-like mechanism (Ahmed and Duncan, 2004). HSP90 differs from most molecular chaperones by playing multiple roles in the cell. These include, in addition to chaperoning of proteins, regulation of steroid hormone receptors, protein turnover in the proteasome and intracellular trafficking. Because of the need for HSP90 under normal (non-stressful) thermal conditions, its message is transcribed constitutively, but translation can be somewhat muted. However, when *Drosophila* are heat stressed, the protein chaperoning function of HSP90 assumes greater importance and a rapid upregulation of HSP90 synthesis occurs. There is evidence that melting of portions of the 5′ mRNA sequence that lie close to the AUG start codon (see Glossary) enhances the rate of translation of the existing message, leading to a rapid heat-induced increase in HSP90 levels. It is noteworthy that the mRNAs for other heat-shock proteins of *Drosophila* have 5′ regions with minimal secondary structure, whereas the 5′ region of the message for HSP90 has a complex secondary structure, one that seems suited for supporting an RNAT function. The genetic tractability of *Drosophila* might permit alteration of the HSP90 mRNA sequence to test whether an RNAT-type function is indeed present. In trypanosomes, there is also some evidence that temperature-mediated changes in secondary structure of an mRNA encoding a heat-shock protein (HSP83) might function as an RNAT (Kramer, 2012). However, as in the case of *Drosophila*, definitive proof of this putative RNAT is absent.

Another temperature-sensing RNA, termed heat-shock RNA-1 (HSR1), has been described in mammalian cells (Shamovsky et al., 2006) and, in fact, appears to be widely distributed among eukaryotes and bacteria (Choi et al., 2015). This small RNA, which does not encode a protein, is synthesized constitutively and is a necessary component of the regulatory system that governs the trimerization and binding of heat-shock factor-1 (HSF-1) to the heat-shock element that controls transcription of heat-shock genes. HSR1 undergoes a temperature-dependent change in conformation, and this RNAT-like response might be instrumental in triggering the activation of HSF-1 and, then, transcription of heat-shock genes. HSR1 has an interesting evolutionary history – it probably originated in bacteria (Choi et al., 2015), and its broad occurrence

in animals and plants is consistent with an early horizontal transfer of the gene from bacterial to eukaryotic genomes.

RNA editing in animals: potentials for temperature-adaptive change in protein sequence

Modification of the chemical structure of RNAs is increasingly being shown to play important roles in controlling the transcriptome and proteome of the cell. These changes, termed epitranscriptomic modifications (see Glossary), include chemical modifications of RNA such as methylation and pseudouridylation (see Glossary), and RNA editing (see Glossary), the conversion of one type of nucleobase to another through the activities of specific enzymes (Licht and Jantsch, 2016). Pseudouridylation leads to base pairing that is more stable than canonical U–A pairing. Thus pseudouridylation during heat stress can lead to more heat-resistant RNA secondary and tertiary structures (Licht and Jantsch, 2016). Modification of RNA stability through pseudouridylation would seem to have the potential for conserving the optimal states of RNA stability in the face of changing body temperature. Epitranscriptomic modifications such as methylation can modify the proteome by affecting the rates of translation and RNA turnover. RNA editing, by contrast, has the potential to lead to changes in the sequences of proteins, resulting, in at least some cases, in proteins with improved functional capacity (Garrett and Rosenthal, 2012; Rosenthal, 2015). RNA editing involves two deamination reactions, conversion of cytidine (C) to uracil (U) and adenosine (A) to inosine (I), the latter being the more common form of editing. C-to-U editing involves cytidine deaminases. A-to-I editing involves activity of the enzyme ADAR ('adenosine deaminase acting on RNA'). A-to-I editing essentially converts A to guanosine (G) because the translational machinery reads I as G. These conversions can lead to non-synonymous codons – that is, to changes in protein primary structure. It has been estimated that A-to-I (=G) editing can reprogram approximately one-half of all codons (Rosenthal, 2015). Codons where ADAR editing generates non-synonymous codons are more heavily edited than sites where editing produces synonymous codons (Rosenthal, 2015).

The broad occurrence and diverse roles of RNA editing in animals are only now being revealed (Rosenthal, 2015; Buchumenski et al., 2017; Tan et al., 2017; Yablonovitch et al., 2017a,b). Recent studies of mammals have found that RNA editing occurs in all species studied and exhibits tissue- and species-specific patterning (Tan et al., 2017). Environmental changes can influence RNA editing in mammals. For example, both C-to-U and A-to-I editing appear important in mammalian cellular responses to hypoxia (Nevo-Caspi et al., 2011). Ectothermic species also exhibit RNA editing that has tissue- and developmental stage-specific patterning and is strongly affected by changes in body temperature (Rosenthal, 2015; Buchumenski et al., 2017; Yablonovitch et al., 2017a,b). Below, I focus on A-to-I editing in ectotherms because of its demonstrated temperature sensitivity and its potential roles in generating protein variants adapted to different temperatures.

The effects of temperature on ADAR-mediated RNA editing arise from several sources. Expression of ADAR is temperature dependent; ADAR levels commonly decrease with rising exposure temperature (Buchumenski et al., 2017). ADAR is auto-editing, and the extent of editing rises with temperature (Rosenthal, 2015). Higher levels of auto-editing are associated with decreases in the enzymatic activity of ADAR. Because the editing activity of ADAR is directed by elements of RNA secondary and tertiary structure (Rieder et al., 2013, 2015; Buchumenski et al., 2017; Yablonovitch et al., 2017a,b) some of the temperature dependence of RNA editing could be due to thermally induced changes in RNA conformation. ADAR acts only on double-stranded RNA, so temperature-driven changes in RNA conformation at editing sites could be important in governing the extent to which a site undergoes editing. Using five species within the genomically well-characterized genus *Drosophila*, Rieder and colleagues (2015) provided evidence that thermal disruption of RNA secondary and tertiary structures did indeed lead to changes in access of ADAR to editing sites. In *Drosophila*, the temperature dependence of RNA editing driven by thermal effects on mRNA structures is both qualitative and quantitative (Rieder et al., 2015; Buchumenski et al., 2017). At low exposure temperatures (18 or 25°C), the extent of editing at specific editing sites was greater than at higher temperatures (29°C) (Buchumenski et al., 2017). However, at 29°C a much larger number of sites exhibited at least moderate levels of editing, and the editing process was characterized as being somewhat 'stochastic' (Buchumenski et al., 2017). In addition, low-temperature editing in *Drosophila* is most prevalent in non-coding (non-exonic) regions of mRNAs, whereas high-temperature editing involves more A-to-I changes in exonic regions. The higher level of stochasticity in RNA editing and the greater number of edited sites within exons at high temperature might have maladaptive consequences for the organism, e.g. by producing altered protein sequences that lead to impaired protein function. Some of the conclusions drawn from laboratory studies of *Drosophila* have been supported by field experiments. Recent studies of populations of *Drosophila* collected at warm south-facing and cool north-facing sites in Evolution Canyon, Israel, provide strong evidence for the importance of temperature-modulated RNA editing. In keeping with laboratory studies, the more warm-exposed populations exhibited lower amounts of RNA editing (Yablonovitch et al., 2017b). However, genetically based differences in propensities for RNA editing were also found between populations, such that south-facing populations had a reduced likelihood of RNA editing.

Although recent studies have greatly expanded our understanding of the scope and temperature sensitivity of ADAR-mediated RNA editing in ectotherms, the adaptive significance of temperature-dependent editing is not well understood. Changes in protein sequence caused by non-synonymous base changes could in principle lead to proteins with altered thermal characteristics that allow improved performance at a new body temperature. These effects could be important in the contexts of evolutionary adaptation and phenotypic acclimatization. There is, in fact, good evidence for this type of adaptive response in cephalopod molluscs adapted to widely different temperatures (polar, temperate and tropical) (Garrett and Rosenthal, 2012; Rosenthal, 2015). The edited protein studied is an ion channel protein (a delayed rectifier K^+ channel protein) that is important in neural transmission. As seen in other studies of ADAR-mediated RNA editing, the extent of editing at a single site varied with temperature. In an Antarctic octopus, editing at the codon for amino acid sequence position 321 was found in more than 90% of the transcripts analyzed, whereas in a tropical octopus this site exhibited only 30% editing. The changes in primary structure (a shift from a valine to an isoleucine) due to editing was conjectured to lead to alterations in protein flexibility in active site regions. These modifications in flexibility of the active site could facilitate optimal protein function at the temperatures the animal experiences. Thus, the rate of channel closing, a rate-governing step in ion transport, shows clear temperature compensation (Garrett and Rosenthal, 2012). The adjustment of protein flexibility through ADAR A-to-I editing seems likely in view of the fact that editing tends to lead to replacement of large

amino acid side-chains with smaller ones, notably glycyl residues, a sequence shift that favors more flexible protein conformations (Rosenthal, 2015). Thus, many of the amino acid substitutions introduced by high levels of editing at low temperatures would increase protein flexibility in a temperature-compensatory way (Dong et al., 2018). The discoveries made with octopus species adapted to widely different temperatures raise the question of whether during acclimatization to a new temperature the extent of editing at functionally important sites is modulated through temperature effects on ADAR activity. This type of phenotypic plasticity – producing different amounts of ADAR-edited protein variants in response to changes in body temperature – would seem to have enormous potential for acclimatization of the proteome. This 'rheostat'-like shift in the balance of different protein sequence variants might be of special importance to eurythermal ectotherms, whose proteins must function over wide ranges of temperature. How widely occurring this type of acclimatization response is remains an open question that warrants close examination.

Other known or potential effects of temperature on RNA editing merit attention as well. RNA editing can create or destroy splice sites, and if this type of editing is affected by temperature, an additional mechanism for generating temperature-dependent variation in the proteome exists (Rueter et al., 1999). There is, in fact, a close link between mRNA splicing and RNA editing processes (Rieder et al., 2015). Many of the sites where editing can occur are found near exon–intron boundaries, and some of the RNA regions that direct editing involve intronic *cis* elements. Thus ADAR editing must be performed before excision of the intronic elements needed for editing takes place. RNA editing appears to occur cotranscriptionally in most species (Rosenthal, 2015), which would help to ensure that intron-influenced editing could occur. As discussed below, temperature can change patterns of RNA splicing, which adds a further level of complexity to temperature–RNA–protein interactions. Lastly, it is worth noting that ADAR is also involved in editing small RNAs that are involved in RNA inhibition (RNAi) processes. Thus widespread effects of editing on gene expression are likely (Rosenthal, 2015). Further exploration of RNA editing is almost certain to provide important new insights into temperature effects on a wide range of processes.

Temperature-modulated RNA splicing: another mechanism for generating adaptive shifts in the proteome?

Another way in which temperature effects on RNA secondary structure can lead to changes in the proteome involves effects on splicing of pre-mRNAs (Meyer et al., 2011). The activity of the spliceosome complex that carries out the removal of introns and the joining of exons is affected by the secondary structure found at intron–exon boundaries (splice sites). In the budding yeast *Saccharomyces cerevisiae*, maturation of the pre-mRNA that encodes an amino peptidase enzyme exhibits alternative splicing patterns at different temperatures (Yassour et al., 2009). Genetic and computational studies point to a stem structure in an intron of the pre-mRNA that can serve as an RNAT and influence the choice of splice site when temperature changes (Meyer et al., 2011). How commonly temperature-driven choice of splice sites occurs is not known. Nor is it known whether the temperature-dependent splicing patterns generate protein variants with adaptive differences in thermal properties. Temperature-dependent splicing thus seems another exciting frontier for investigation.

Temperature-dependent splicing may also involve more complex regulatory steps than direct temperature-driven changes in RNA secondary structure. For example, in mice normal circadian changes in body temperature of only ∼1°C control alternative splicing of many exons, and these rhythms in splicing are regulated by the reversible, temperature-regulated phosphorylation of specific types of proteins involved in the splicing process (Preußner et al., 2017).

Differential translation of allelic mRNAs: another way to modulate the proteome?

The importance of allelic protein polymorphism in adaptation to temperature has been well established in a large number of ectothermic species (Watt and Dean, 2000). Allelic protein variants (allozymes) with different thermal optima can provide a eurythermal species with an opportunity to establish populations with adaptively different thermal optima in regions of its biogeographic range where temperatures differ. Thus a cold-optimized allozyme might be more common in populations living in colder regions of a species' range, whereas the warm-optimized allozyme would be dominant in warmer regions. Another scenario can be visualized as well – for a heterozygous individual whose genome encodes both alleles for the protein in question, temperature-modulated differential translation of allelic mRNAs could shift the balance of translation between the mRNAs encoding the two allozyme forms, allowing the organism to preferentially synthesize the allozyme better suited for the conditions at hand. Differential expression of alleles has been observed in a large number of systems (Pastinen, 2010), but its roles in temperature adaptation remain to be investigated.

Based on what is known about the roles of RNA secondary structure in influencing translation of mRNAs, it would seem feasible for slight changes in base composition to alter the thermally sensitive mRNA structures that govern translational ability in a way that ensures differential translation of the two allelic messages. Thus for the mRNA encoding the high-temperature-optimized allozyme, selection might favor more heat-resistant secondary structure in 'zipper' regions that must be melted to allow translation. This would lead to an absence or at least a lower rate of translation of the warm-optimized allozyme at temperatures below the melting temperature of the RNA thermometer. Conversely, the mRNA for the cold-optimized allozyme might have a switching mechanism akin to that found for cold-shock proteins in bacteria (Kortmann and Narberhaus, 2012), which would allow cold-induced enhancement of translational ability. Although studies of these types of mechanisms for allowing differential translation of allelic mRNAs remain to be performed, it might be possible in the near future to use algorithms that predict RNA secondary structure to screen sequences of allelic mRNAs for warm- and cold-adapted allozymes to learn whether there are differences in secondary structure in non-coding regions that might influence the temperature dependence of translation (Wan et al., 2012; Righetti and Narberhaus, 2014; Ignatova and Narberhaus, 2017; Qi and Frishman, 2017).

Temperature-modulated regulation by small non-coding RNAs – another mechanism for controlling gene expression and mRNA turnover?

To this point, we have focused on *cis*-acting RNA sensors (see Glossary) such as the 5′ untranslated region (UTR) RNATs of bacteria and, perhaps, of the Hsp90 mRNA in *Drosophila*. *Trans*-acting RNAs can also play a role in regulating genes in a temperature-dependent manner. Thus, in bacteria, a non-coding RNA (ncRNA) with 85 nucleotides (DsrA RNA) binds to the 5′ UTR region of certain mRNAs with RNATs and enhances exposure of the S–D sequence (de la Fuente et al., 2012). The RNAT is thus affected not only by temperature *per se*, but also by a *trans*-acting ncRNA that itself probably has a temperature-dependent interaction

with the 5′ UTR region. DsrA RNA not only activates translation, but also reduces the susceptibility of the mRNA to degradation by RNAses, allowing a given amount of message to be translated many times. RNA degradation by RNAses might be governed in part by exposure of degradation sites to RNAses, so stabilizing the mRNA structure may extend the half-life of the molecule.

Temperature-dependent ncRNA effects on cellular function have received little attention in animals. There is some evidence that certain ncRNAs in *Drosophila* and humans confer temperature sensitivity of protein synthesis through sequestering RNA processing factors (de la Fuente et al., 2012), but the scope of these effects is not known. In general, temperature effects on ncRNA functions would seem to have the potential to provide animals with another thermosensing mechanism for governing translation and mRNA turnover. One promising study area is the effect of temperature on interactions between mRNAs and the microRNAs that govern mRNA turnover. In organisms that shift gene expression in concert with short-term changes in temperature – for example, during diurnal thermal cycles – controlling mRNA degradation might be as important for regulating mRNA populations as modulating transcription of the mRNAs (Podrabsky and Somero, 2006).

RNA–temperature interactions: how might they influence Q_{10} values of physiological processes?

Temperature-induced changes in RNAT secondary structure would appear to have the potential to create a very high temperature dependence (Q_{10} value) for certain physiological processes. Thus, if a 1–2°C rise in temperature can essentially convert an mRNA from untranslatable to translatable, there would seem to be a potential for very large Q_{10} values in RNAT-regulated processes such as protein translation. Not only would a rise in temperature accelerate translation owing to normal Q_{10} effects, but the amounts of mRNA available for translation would rapidly rise as well.

Concluding thoughts: what to look for, and where and how to look?

Studies of biochemical adaptation to temperature by animals have focused largely on systems fabricated from proteins and lipids (Somero et al., 2017). In this Commentary, I have emphasized that another fertile field to investigate in animal thermal biology involves the effects of temperature on RNA structure and function. Based on what has been discovered in studies with different classes of bacterial RNAs, there appears to be a wide variety of temperature–RNA interactions that could play important roles in evolutionary adaptation and acclimatization to temperature. Investigating these relationships will involve a wide range of experimental approaches and animal study systems. Studies of RNA editing that involve a creative blend of genomic/bioinformatic analysis and protein structure–function studies provide guidance as to how some of this new research might evolve (Rosenthal, 2015). New bioinformatic tools for identifying regions of secondary structure in mRNAs and estimating their thermal stabilities are becoming available to comparative biologists (Wan et al., 2012). Through use of these tools, it may be interesting to determine whether the patterns of marginal stability noted for proteins have parallels in RNA structure, notably in regions having secondary structure that serve a thermosensing role. In this context, it seems noteworthy that, whereas the overall G+C content of DNA and most classes of RNAs does not increase with rising optimal temperatures for growth in bacteria and archaea, smaller RNAs that play functional roles governed by conformational changes do exhibit a positive correlation between adaptation temperature and G+C content (reviewed in Somero et al., 2017). This correlation suggests that regions of RNA molecules that possess secondary structures important for thermosensing functions could exhibit temperature-compensatory differences in stability that lead to conservation of the appropriate marginal stability of higher-order structure. These sites, which might include sequence regions important for RNA editing, regulation of translation and control of splicing would seem to be important foci for studying the evolution of RNA–temperature interactions. With the new tools for studying RNA structures that are becoming available, and with rapid growth in genome sequences and gene-editing technologies for non-model organisms, including terrestrial and aquatic ectotherms, we might soon discover whether animals and other eukaryotes are as inventive as bacteria in exploiting the potentials offered by temperature-sensitive RNA secondary and tertiary structures for governing adaptive responses to changes in temperature.

Acknowledgements

I thank Steve Hand and Donal Manahan for their helpful comments on the manuscript, and Freya Sommer for creating the figure.

Competing interests

The author declares no competing or financial interests.

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