Chapter 25
Primary Thermosensory Events in Cells

Ilya Digel

Abstract Temperature sensing is essential for the survival of living organisms. Since thermal gradients are almost everywhere, thermoreception could represent one of the oldest sensory transduction processes that evolved in organisms. There are many examples of temperature changes affecting the physiology of living cells. Almost all classes of biological macromolecules in a cell (nucleic acids, lipids, proteins) can serve as a target of the temperature-related stimuli. This review is devoted to some common features of different classes of temperature-sensing molecules as well as molecular and biological processes involved in thermosensation. Biochemical, structural and thermodynamic approaches are discussed in order to overview the existing knowledge on molecular mechanisms of thermosensation.

Abbreviations

CSP	cold shock proteins
HSP	heat shock proteins
PIP2	phosphatidylinositol bisphosphate
TRP channels	*transient receptor potential* channels
TRPA	"ankyrin" subfamily
TRPM	"melastatin" subfamily
TRPV	"vanilloid" subfamily

Temperature changes are among the main stresses experienced by organisms from bacteria to plants and animals and therefore temperature is one of the environmental cues under constant vigilance in living cells. Several problems arise from exposing a cell to a sudden change in temperature [1]: firstly, membrane fluidity changes, that affects many membrane-associated vital functions; secondly, nucleic acid topology will be affected causing shifts in processes such as transcription and translation.

I. Digel (✉)
Laboratory of Cellular Biophysics, Aachen University of Applied Sciences, Juelich, Germany
e-mail: digel@fh-aachen.de

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Finally, the protein function is directly affected both from structural and catalytic points of view.

Hence, living cells need "devices" for sensing environmental temperature changes in order to adapt their biochemical processes accordingly. A successful adaptive thermotropic response cannot be performed only by corresponding changes in the rate and equilibrium of enzymatic reactions. Such a mechanism of adaptive reaction is too unspecific and uncontrollable. To cope with temperature variation, living organisms need sensing temperature alterations and translating this sensory event into a pragmatic gene response.

While such regulatory cascades may ultimately be complicated, they contain primary sensor machinery at the top of the cascade. The functional core of such machinery is usually that of a temperature-induced conformational or physico-chemical change in the central constituents of the cell. Hence, a specific sensory transduction mechanism is needed, including, as a key element, *a molecular sensor*, transforming certain physical parameter (temperature) into a biologically significant signal (change in membrane permeability, specific inhibition/stimulation of gene expression, etc.). In a sense, a living organism can use structural alterations in its biomolecules as the primary thermometers or thermostats. Thus, sensory transduction is a complex biological process aimed at integrating and decoding physical and chemical stimuli performed by primary sensory molecular devices. Furthermore, sensory perception of potentially harmful stimuli can function as a warning mechanism to avert potential tissue/organ damage.

Among temperature-controlled processes in living organisms, most well-known are the expression of heat-shock and cold-shock genes [2]. Relocation of a culture of *Escherichia coli* adapted to an optimal growth to a sudden temperature increase, or decrease, by some 10–15 °C results in adaptive shock responses. Such responses involve a remodeling of bacterial gene expression, aimed at adjusting bacterial cell physiology to the new environmental demands [3, 4]. The response of prokaryotic and eukaryotic systems to heat-shock stress has been thoroughly investigated in a large number of organisms and model cell systems. Notably, all organisms from prokaryotes to higher eukaryotes respond to cold and heat shock in a similar manner. The general response of cells to temperature stress (cold or heat) is the rapid over-expression of small groups of proteins, the so-called CSPs (cold-shock proteins) or HSPs (heat shock proteins), respectively, but the initial launching mechanism is different in both cases.

In bacteria, the heat response generally invokes some 20 heat-shock proteins, mostly chaperones, whose functions are primarily to help dealing with, and alleviate, the cellular stress imposed by heat [5]. Many of these proteins participate in reconstituting and stabilizing protein structures and in removing misfolded ones. The expression of this special chaperone system, which includes the proteins DnaK, DnaJ and GrpE, is activated by the appearence of misfolded, temperature-denatured proteins. Thus, one could implicate the binding of partially unfolded proteins by chaperones as the thermosensoric event regulating expression of heat-shock proteins, where the primary sensory element is constituted by some easily

denaturing proteins. This, in turn, demonstrates that even bacteria can practically utilize destructive changes in protein conformation as a means for temperature sensing.

In case of cold shock, the primary sensing event is more obscure. Various reports have now shown that when in vitro cultivation temperature is lowered, the increase of the cell membrane rigidity results in compromised membrane-associated cellular functions. Furthermore, cold stress dramatically hinders membrane-bound enzymes, slows down diffusion rates and induces cluster formation of integral membranous proteins [6]. In mammalian cells, the five known mechanisms by which cold-shock-induced changes affect gene expression are: (i) a general reduction in transcription and translation, (ii) inhibition of RNA degradation, (iii) increased transcription of specific target genes via elements in the promoter region of such genes, (iv) alternative pre-mRNA splicing, and (v) via the presence of cold-shock specific internal ribosome entry segments in mRNAs that result in the preferential and enhanced translation of such mRNAs upon cold shock [7].

It has been pointed out that cold stress exposes cells not to one but to two major stresses: those related to changes in temperature and those related to changes in dissolved oxygen concentration at decreased temperature, and it is therefore necessary to study responses to each, either independently or as part of a coordinated response. Separating the relative effects of temperature and oxygen as a result of decreased temperature is difficult and has not been extensively addressed to date. Both changes in dissolved oxygen and temperature reduction result in similar changes in cultured mammalian cells [7].

The shock response systems briefly mentioned above belong to ultimate mechanisms aimed to survival under extreme temperature conditions. However, the ability to express certain factors can be affected by reasonably small temperature changes. Less drastic changes in temperature may not induce shock responses, but can be sufficient to modulate the expression of bacterial virulence genes, for example in *Shigellae* [8] and *Yersiniae* [9]. While one might be surprised that organisms built on such minimalist approaches as bacteria respond to temperature changes, the consequence of these observations is that even bacteria actually sense temperature shifts in order to control gene expression accordingly. Investigators have now been studying the moderate temperature sensation in a variety of organisms for at least several decades or more. Recently, a number of reports have shown that exposing bacteria, yeasts or mammalian cells to sub-physiological temperatures invokes a coordinated cellular response manifesting itself as alterations in transcription, translation, metabolism, the cell cycle and the cell cytoskeleton [7, 10–13]. Nevertheless, very little is known so far about the molecular mechanisms that govern initial response on small thermal stimuli, particularly the primary sensory transduction mechanisms.

Below, we have tried to uncover some aspects of the biophysical basis of temperature sensing by biological molecular thermometers, summarizing some most general ideas concerning the primary components of temperature signal transduction.

25.1 Temperature Sensing Biomolecules

In addition to specificity and sensitivity, successful thermoresponse should be one that is reversible and controlled. Thus, complexity of thermosensing and thermoregulation on the organism level may reflect the demands to handle and fine-tune responses to an important environmental factor in a dynamic fashion. However, ultimately, it seems that basic and rather simple (bio) chemical processes are serve as primary sensory events and, for that purpose thermotropic changes in physico-chemical state of biological molecules appear highly suitable. While the information available is somewhat scant, the picture emerging shows that cells can use signals generated through changes in nucleic acid or protein conformation, or changes in membrane lipid behavior, as sensory devices. Bellow we make a short overview of temperature-sensing properties of most important groups of biological macro-molecules.

It is worthy to note that probably even water alone could serve as a primitive temperature sensor. In the middle of the twentieth century Oppenheimer and Drost-Hansen [14] reported that a number of more or less abrupt changes in the properties of water and aqueous solutions occur when the temperature is increased from 0 to 60° C. These changes or "kinks" occured within a rather narrow temperature range (±2° C) near 15, 30, 45, and 60° C, respectively and most probably caused by changes in the hydrogen bond network of the water. The authors argued that the temperature-induced structural changes in water and aqueous solutions exert a direct influence on biological phenomena. In a later work W. Drost-Hansen [15] suggested some mechanisms how these structural changes happening with *vicinal* (adjacent to surfaces) water can affect the behavior or activity of biological systems. It was argued that optimal conditions for a complex physiological activity (such as, for instance, growth) will occur somewhere near the middle of the interval between two consecutive kinks. This issue has been discussed in literature very controversially and has not received wide recognition.

25.2 Membrane Lipids Fluidity

The physical state of phospholipid membranes does change in response to temperature shifts in phase-transition manner [16], but the temperature-induced changes in real biological membranes are not sharp because many kinds of fatty acids and cholesterol-like molecules present, having different characteristic temperature points of phase transition. Thus, it would not be surprising if cells (even those of bacteria) could utilize the changes in membrane fluidity as a thermometer device, assisted by protein helpers, playing a role of switchers, "sharpening" the temperature response. Microorganisms counteract the membrane propensity to rigidify at lower temperature and are able to maintain a more-or-less constant degree of membrane fluidity (*homeoviscous adaptation*). The cyanobacterium *Synecocystis* responds to decreased temperature by increasing the *cis*-unsaturation

of membrane-lipid fatty acids through expressing acyl-lipid desaturases [17–19]. Lipid unsaturation would then restore membrane fluidity at the lower temperature. In *B. subtilis*, this lipid modification is initiated through the activity of a so-called "two-component regulatory system" consisting of the DesK and DesR proteins [17]. Prokaryotic two-component regulatory systems usually consist of protein pairs: a sensor kinase and a regulatory protein [20].

It appears that it is a combination of membrane physical state and protein conformation that is able to sense temperature and even to translate this sensing event into proper gene expression. However, sensing of temperature through direct alteration in nucleic acid conformation might be more efficient temperature-mediated mechanism of gene expression.

25.3 RNA and DNA Thermotropic Reactions

Theoretically, RNA molecules have a strong potential as temperature sensors, in that they can form pronounced secondary and tertiary structures [21], and through their ability to form intermolecular RNA: RNA hybrids [22]. Both of these processes greatly depend on the formation of complementary base pairing, and consequently one would anticipate these to be dependent on environmental temperature. Indeed, messenger RNAs, apart from carrying their coding information for protein generation are also rapidly emerging as regulators of expression of the encoded message. With unique chemical and structural properties, sensory RNAs perform vital regulatory roles in gene expression by detecting changes in the cellular environment either alone or through interactions with small ligands [23, 24] and proteins [25, 26].

Regulatory RNA elements, "riboswitches", have been reported recently, responding to intracellular signals by conformational changes. Riboswitches are conceptually divided into two parts: an *aptamer* and an expression platform. The aptamer directly binds the small molecule, and the expression platform undergoes structural changes in response to the changes in the aptamer. The expression platform is what regulates gene expression. Riboswitches demonstrate that naturally occurring RNA can specifically response on versatile physical and chemical stimuli, a capability that many previously believed was the domain of proteins or artificially constructed RNAs [27].

RNA thermometers operate at the post-transcriptional level to sense selectively the temperature and transduce a signal to the translation machinery via a conformational change. They have usually a highly structured 5′-end that shields the ribosome binding site at physiological temperatures [28–31]. Changes in temperature are manifested by the liberation of the Shine–Dalgarno (SD) sequence, thereby facilitating ribosome binding and translation initiation.

It is known that both in prokaryotic and eukaryotic cells the geometry and tension of DNA is highly dynamic and corresponds to its functional activity. In the bacterial cell, chromosome and plasmid DNAs are contained in a "twisted" superhelical conformation [32, 33], where the degree of supercoiling varies in response to

changes in the ambient temperature. The expression of many genes is dependent on DNA conformation, and temperature-dependent gene regulation is mastered through changes in DNA supercoiling [3, 34, 35].

Examples of pure DNA-related temperature sensitivity are rare if ever reported. In most cases, genomic thermo-sensitivity appears to be a result of certain interplay among DNA, RNA and proteins. Some bacteria carry a DNA-plasmid which shows a controlled constant plasmid copy number at one temperature and a much higher or totally uncontrolled copy number at a different temperature The high copy number phenotype of pLO88 plasmid maintained in *Escherichia coli* (HB101) is observed only at elevated temperatures, (above 37° C), and is due to the precise position of a Tn5 insertion in DNA, but the exact mechanism remains obscure [36].

Recent experiments show [37] that artificial thermoresponsive devices may be constructed based on the temperature-dependence of the relative populations of left- and right-handed nucleic acid helical conformations. The authors reported that "upon an increase in temperature, particular sequences of DNA oligonucleotide duplexes in high salt conditions switch from a left-handed (Z) form to a right-handed (B) one, while RNA responds inversely by switching from a right- (A) to a left-handed (Z) form . . . Calculations revealed a complex interplay between configurational, water, and ionic entropies, which, combined with the sequence-dependence, rationalize the experimentally observed transitions from A- to Z-RNA and Z- to B-DNA in high salt concentrations and provide insight that may aid future developments of the use of nucleic acids oligomers for thermal sensing at the nanoscale in physiological conditions." [37]

The role of DNA-binding proteins has been established for plant thermosensitivity too. Kumar and Wigge [38] have revealed that eviction of the histone H2A.Z from nucleosomes performs a central role in plant thermosensory perception. Using purified nucleosomes, they showed that H2A.Z displays distinct responses to temperature in vivo, independently of transcription events.

Apparently, the temperature-induced conformational changes in DNA are mainly controlled through the presence of "nucleotid-associated" proteins, of which H–NS is the best characterized [32, 39]. In *E. coli*, creating and maintaining conformational structures in the DNA molecule are mainly regulated through the balance of two opposing topoisomerase activities, mainly those of topoisomerases II and I [40, 41]. The abovementioned examples of membrane- and nucleic acid-based temperature sensitivity imply that these systems often include proteins as a key regulatory component. Therefore, from the point of view of molecular temperature sensation, protein-based molecular "thermometers" represent an extremely interesting group.

25.4 Protein Thermometers

Many sensory pathways in living organisms use structural changes in proteins as a primary perceptive event, activating further signaling cascades. *E. coli* being exposed to an oxidative agent such as hydrogen peroxide, responds by the activation

of a transcriptional regulator protein OxyR [42]. Activation of OxyR is achieved through the formation of a disulphide bound within the protein, upon which OxyR induces the expression of a set of genes adapting the bacterial cell to oxidative stress. This illustrates how it is possible both to "sense" and respond to an abrupt change in a specific environmental factor in a simple, yet elegant mode.

One would expect the organisms and cells to be similarly elegant when sensing temperature shifts. Indeed, a striking example is the temperature-controlled switching of the flagellar rotary motor of *E. coli* between the two rotational states, clockwise (CW) and counterclockwise (CCW) [43]. The molecular mechanism for switching remains unknown, but seems to be connected to the response regulator protein CheY-P. Two models of CheY-P action proposed so far explain shifting the difference in free energy between CW and CCW states in terms of (1) conformation-related differential binding [44, 45] and (2) thermodynamic changes in dissociation constants [46].

Further studies on the thermosensory transducing system in *E. coli* revealed that two major chemoreceptors, *Tar* and *Tsr*, which detect aspartate and serine, respectively, also function as thermoreceptors, together with *Trg* and *Tap* receptors [47]. Interestingly, in spite of different specificity and sensitivity, amino acid sequences of all these four chemoreceptors have a significant homology. They are transmembrane proteins having two functional domains acting as chemoreceptors: one is a ligand-binding domain located in the periplasm and the other is a signaling domain located in the cytoplasm. Thus, it is suggested that a temperature change induces a conformational change in these two receptors and that this conformational change triggers the signaling for thermoresponse. In the simplest model of thermoreception by these receptors, two conformational states of these receptors are assumed: a low-temperature state and a high-temperature state [48]. The swimming pattern of the *Trg*- and *Tap*-containing cells is determined simply by the temperature of the medium, indicating that these cells under nonadaptive conditions sense the absolute temperature as the thermal stimulus, and not the relative change in temperature.

The understanding of protein thermotropic sensory transductions in terms of their underlying molecular mechanism is fast-advancing thanks to the discovery and functional characterization of the *transient receptor potential* (TRP) channels. This protein family, first identified in *Drosophila*, is at the forefront of the sensory stem, responding to both physical and chemical stimuli and, thus having diverse functions [49, 50].

The family of TRP channels currently comprises around 30 members grouped into seven related subfamilies: TRPC, TRPV, TRPA, TRPP, TRPM, TRPN and TRPML. In higher organisms, TRPV channels are important polymodal integrators of noxious stimuli, mediating among all, thermosensation and nociception (pain sensation) [51].

To characterize thermal sensitivity of cells, molecules and processes, the $\boldsymbol{Q_{10}}$ **(temperature coefficient)** is used. Q_{10} reflects the rate of change of a biological or chemical system as a consequence of increasing the temperature by 10°C. This coefficient is used, for example, for the characterization of the nerve conduction velocity.

The Q_{10} is calculated as:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{10/(T_2-T_1)}$$

where R is the rate of change and T is the temperature.

For biological systems, the Q_{10} value is generally between 1 and 3 but a subset of TRP channels, the thermo-TRPs, characterized by their unusually high temperature sensitivity (Q_{10} >10): TRPV1–TRPV4 are heat activated [52–54], whereas TRPM8 [54, 55] and TRPA1 [56] are activated by cold. With a Q_{10} of about 26 for TRPV1 [57] and about 24 for TRPM8 [58, 59], they far surpass the temperature dependence of the gating processes characterized by other ion channels ($Q_{10} \approx 3$) [57]. In spite of the great advances made in last years the molecular basis for regulation by temperature remains mostly obscure because of the lack of *native* structural information. Nevertheless, deeper understanding of dynamics and thermodynamics of these proteins will bring us closer to revelation of universal principles of thermal sensation.

25.5 Biophysical Aspects of Protein Thermosensitivity

It appears from the above mentioned examples of protein participation in temperature sensing events that sudden conformational changes, "structural transitions" play essential role on the primary conversion of physical stimulus into biologically relevant signal.

Phase transitions and other "critical" phenomena continue to be the subject of intensive experimental and theoretical investigation. In this context, systems consisting primarily of well characterized proteins and water can serve as particularly valuable objects of study. The importance of studies of specific phase transitions in protein/water solutions derives also from their physiological relevance to the supramolecular organization of normal tissues and to certain pathological states. For example, such phase transitions play the main role in the deformation of the erythrocyte in sickle-cell disease [25, 60] and in the cryoprecipitation of immunoglobulins in cryoglobulinemia and rheumatoid arthritis [61].

Discussions about protein stability and temperature-induced structural transitions are usually limited to the stability of the native state against denaturation. Yet the native state may include different functionally relevant conformations characterized by different Gibbs energies and therefore different stabilities (e.g. the R and T states of hemoglobin). At biological temperatures, proteins alternate between well-defined, distinct conformations. In order to those conformational states to be distinct, there must be a free-energy barrier separating them (Fig. 25.1). Adaptive alterations of protein conformation in response to signaling events might reflect corresponding changes in free-energy profile. From this point of view, temperature as a stimulus does not differ physically from, for example, a ligand-binding event. The experimental observation of distinct conformational populations by IR-spectroscopy is possible but usually requires the existence of at least two spectrally different but overlapping components of the amide I band [62].

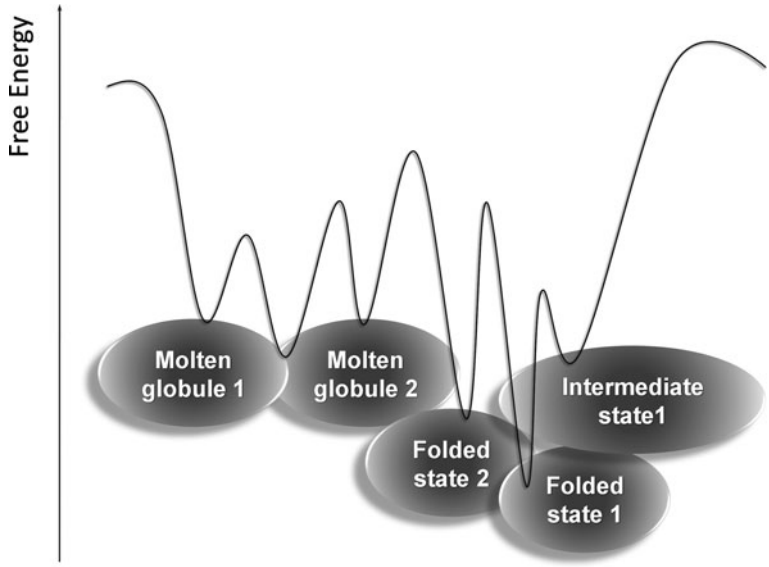

Fig. 25.1 A hypothetical model of a free energy profile of a globular protein. The threshold character of the protein reaction means that the resting states and the active states are different thermodynamic states of the system, separated by an energy barrier. Often, several protein states are thermodynamically stable and prevail in appropriate conditions. Transitions between different protein states take place in the cell in response to external stimuli

The motions involved to get from one state to another are usually much more complex than the oscillation of atoms and groups about their average positions. In proteins, because most of the forces that stabilize the native state are non-covalent, there is enough thermal energy at physiological temperature for weak interactions to break and reform frequently. Thus a protein molecule is more flexible than a molecule in which only covalent forces dictate the structure.

Recently, it became clear that natively unfolded proteins also play an important role in the cell. Dunker et al. [63] proposed to widen the notion of functional protein types in the cell: to the "classical" proteins with well defined tertiary structure, they added molten globules and proteins with unfolded conformations. Uversky [64] has suggested to supplement this list with a fourth, relatively stable protein conformation – the premolten globule, which might be called the boiling globule, as in the coordinates of the unfolding reaction it follows the globule and molten globule and precedes the completely unfolded conformation. Apparently, all these protein states are thermodynamically stable, although to different degrees.

Even when the native protein does not undergo a conformational change, it is still characterized by the occurrence of a large number of local unfolding events that give rise to many sub-states. Thus, the native state itself needs to be considered as a statistical ensemble of conformations rather than unique entity. These distinctions are very important from the functional point of view since different conformations are usually characterized by different functional properties.

The stabilizing contributions that arise from the hydrophobic effect and hydrogen bonding are largely offset by the destabilizing configurational entropy. The hydrophobic effect is strongly temperature-dependent, and is considerably weaker and perhaps even destabilizing at low temperatures than at elevated temperatures. The contribution of various interactions for a "typical" protein is reported in many works [65–69]. Apparently, the transition from stabilizing to destabilizing conditions is achieved by relatively small changes in the environment. These can be changes in temperature, pH, addition of substrates or stabilizing co-solvents. While the exact contribution of different interactions to the stability of globular proteins remains a question, our understanding seems to be refined enough to allow for the reasonable prediction of the overall folding thermodynamics applying the second law of thermodynamics for free energy changes between folded and unfolded states [68, 69]. Important to mention that both the enthalpy end entropy changes are not constant but increasing functions of temperature, and that the Gibbs (free) energy stabilization of a protein can be written as:

$$\Delta \mathrm{G(T)} = \Delta \mathrm{H(T_R)} + \Delta C_P(T - T_R) - T\Delta S(T_R) + \Delta C_P \ln(T/T_R)$$

where T_R is a reference temperature. $\Delta \mathrm{C_p}$ is the heat capacity change, and $\Delta \mathrm{H(T_R)}$ and $\Delta \mathrm{S(T_R)}$ are the enthalpy and entropy values at that temperature, correspondingly. The temperature dependency of ΔH and ΔS is important because it transforms the Gibbs energy function from a linear into a parabolic function of temperature. This equation has only limited applicability since it does not consider the change of the solvent's entropy, which is, without doubt, an important contributor to the thermodynamics of protein behavior in solution.

For large values of $\Delta \mathrm{C_p}$, the Gibbs energy crosses zero point twice – one at high temperature (heat denaturation) and one at low temperature (cold denaturation). The native state is thermodynamically stable between those two temperatures and ΔG exhibits a maximum at the temperature at which $\Delta \mathrm{S} = 0$. The peculiar shape of the free energy function of a protein does not permit a unique definition of protein stability. For example, having a higher denaturation temperature does not necessarily imply that a protein will be more stable at room temperature.

Within the context of the structural parameterization of the energetics, the free energy of protein stabilization is approximated by the equation:

$$\Delta G = \Delta G_\mathrm{gen} + \Delta G_\mathrm{ion} + \Delta G_\mathrm{tr} + \Delta G_\mathrm{other}$$

where $\Delta \mathrm{G_{gen}}$ contains the contributions typically associated with the formation of secondary and tertiary structure (van der Waals interactions, hydrogen bonding, hydration, and conformational entropy), $\Delta \mathrm{G_{ion}}$ comprises the electrostatic and ionization effects, and $\Delta \mathrm{G_{tr}}$ reflects the contribution of the change in translational degrees of freedom existing in oligomeric proteins. The term $\Delta \mathrm{G_{other}}$ includes interactions unique to specific proteins that cannot be classified in a general way (e.g. prosthetic groups, metals, and ligands) and must be treated on a case-by-case basis.

B. Nilius and co-workers have recently applied this simple thermodynamic formalism to describe the shifts in voltage dependence of protein channels due to changes in temperature [70, 71], where the probability of the opening of a channel is defined as a function of temperature, Faraday's constant, the gating charge, and the free energy difference between open and closed states of the channel.

In order to understand the nature of dynamic transitions in proteins, it is also important to consider solvent effects. Solvent can affect protein dynamics by modifying the effective characteristics of the protein surface and/or by frictional damping. Changes in the structure and internal dynamics of proteins in dependency on solvent conditions at physiological temperatures have been found by using several experimental techniques [72–74]. It follows from the works of G. Büldt, G. Artmann, J. Zaccai, A. Stadler and others that solvent affects protein dynamics differently at different temperatures and salt concentrations [74–77] Therefore, a solvent dependence of the dynamic transition might be expected. Indeed, measurements on ligand binding to myoglobin indicated that dynamic behavior of the protein is correlated with a glass transition in the surrounding solvent [78]. Recent molecular dynamics analysis of hydrated myoglobin also indicates a major solvent role in protein dynamic transition behavior [79].

One interesting aspect of thermosensation is conversion of the code conformational changes into the code of cellular signaling. In our opinion, strong methodological basis for the understanding of these events was provided by studies by the scientific schools of Dmitrii Nasonov and Gilbert Ling that have gained new appreciation over the last 20–30 years owing to advances in protein physics, and thank to series of works by Vladimir Matveev [80, 81]. The latter has postulated that when an action (for instance, temperature change) on a cell or a cell structure exceeds the threshold, (i) formation of secondary structures begins in natively unfolded proteins (or unfolded regions of proteins), while (ii) secondary structures of molten globules start to become accessible for interaction with secondary structures of other proteins and with nucleic acids. Such secondary structures induced by the external action were called by the author *centers (sites) of native aggregation* (personal communication). Thus, the first event in the activated cell is the appearance of new secondary structures able to interact selectively with each other to form tertiary, quaternary, etc. structures. Proteins whose secondary structures appear under such circumstances lose their previous inertia and become reaction-capable.

This point of view on understanding the mechanisms of cellular reactions poses the question of native and denatured protein states in a new way. According to it, in the native state the key cell proteins are inert, non-reaction-capable; they do not interact with each other or with other biopolymers. Loss of the state of inertia is partial denaturation, where new secondary structures can appear, or can be modified, or can "float up" to the surface from the hydrophobic nucleus. In all cases the secondary structures are ready to interact. Numerous intermediate protein species, corresponding to different free energy minima provide basis for native aggregation.

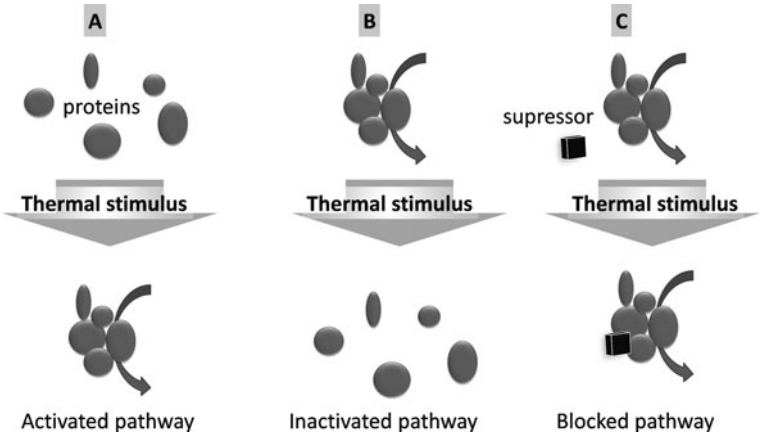

Fig. 25.2 Some possible strategies in converting protein structural information (shape, hydrophobicity, charge, domain organization) into cellular signaling events. Thermal stimulus provides energy for transferring protein molecule from the one energy state to the other, which results in changing protein surface. The induced appearance/disappearance of recognition sites on the protein surface leads to establishing inter- and intramolecular protein contacts. These native protein aggregation/disaggregation events can be interpreted as a key mechanism of signal propagation in the cell

Together with native aggregation, several other possibilities can be visualized to explain how thermo-induced conformational changes in proteins can be converted into signaling event (Fig. 25.2).

Kim et al. [82] and later Sourjik [83] studied the dynamics of the cytoplasmic domains of the *E. coli* chemotaxis receptor on interaction with repellent and attractant. It was concluded that an attractant decreases the number of secondary structures in the domain, which blocks signal transmission into the cytoplasm. A repellent produces the opposite effect: it increases the amount of secondary structures in the domain, which makes the signal function of the receptor possible. In terms of the hypothesis of native aggregation, repellent converts the domain into the excited state, enabling interactions necessary for signal transmission.

Since native aggregation results in the appearance of signaling and regulatory structures, it is obvious that as biological organization becomes more complicated during evolution, where novel mechanisms of regulation of the cell activity are needed.

25.6 Structural Features of Protein Thermometers

From the point of view of structural biophysics, thermosensation can be regarded a special case of mechanosensation and therefore many theoretical models and considerations developed for protein mechanosensors are also applicable for thermosensors. The difference between mechanosensitive channels and thermosensitive

molecules is only the size and the organization of the "exciting" agents – a lot of non-coordinated events (thermal stimuli) versus a net stretch (mechanical stimuli). Therefore, nor surprisingly, many members of thermosensing TRPV family are also known as osmo- and mechano-sensors. Because mechanical stimuli are omnipresent, mechanosensation could represent one of the oldest sensory transduction processes that have evolved in living organisms. Similarly to thermal sensors, what exactly makes these channels respondent to membrane tension is unclear. The answer will not be simple because both thermal- and mechano-sensors are very diverse [84, 85]. However, there are interesting parallels in structural composition of different classes of known temperature-sensory proteins, pending comprehension.

Despite significant evolutionary distances and apparent differences of primary structure, all temperature-sensitive proteins known so far display some remarkable similarities in their tertiary/quaternary structure. The ability of a big protein TlpA responsible in *S. typhimurium* for temperature regulation of transcription undoubtedly resides in its peculiar structural design. Two-thirds of the C-terminal portion of TlpA is folded in an alpha-helical-coiled-coil structure that constitutes an oligomerization domain. The sensory capacity is consealed in this coiled-coil structure, which illustrates the means of sensing temperature through changes in protein conformation. As the temperature increases, the proportion of DNA-binding oligomers decreases, leading to a de-repression of the target gene. At moderate temperatures, the concentration of TlpA increases, shifting the balance to the formation of DNA-binding oligomers and, in part, restoring the repression potential of TlpA. Thus, TlpA undergoes a reversible conformational shift in response to temperature alteration, leading to an alteration in the oligomeric structure [48].

The coiled-coil protein structure is a very versatile and a flexible motif in mediating protein-protein interactions. In vertebrates, the thermosensitive elements of transcriptional mechanism typically contain such coiled-coil folding motifs, like those in leucine zipper family.

TRPV channel subunits have a common topology of six transmembrane segments (S1–S6) with a pore region between the fifth and sixth segment, and cytoplasmic N- and C-termini. In these thermo-TRP channels, it has been proposed that the structural rearrangement leads to a change in tension on the helical linker connecting the C-terminal domains with S6 segment. This tension on the linker provides the energy necessary to move the S6 inner helix to the open conformation [58, 59]. Indeed, partial deletions performed in the C-terminal domain of TRPV1 resulted in functional channels with attenuated heat sensitivity, whereas truncation of the whole TRPV1 C-terminal domain completely hindered channel expression [57]. Another possibility could be that temperature affects the interaction between a particular portion of the proximal C-terminal and some other region of the channel, probably an intracellular loop. Finally, it might be that independent arrangements induced by temperature on C-terminal domains directly promote gate opening [57].

Bernd Nilius' group in their study on the voltage dependence of TRP channel gating by temperature pointed out that the small gating charge of TRP channels compared to that of classical voltage-gated channels could lie at the basis of the large shifts of their voltage-dependent activation curves, and may be essential for their

gating versatility [70, 71]. Thus, small changes of the free energy of activation of these channels can result in large shifts of their voltage-dependent activation curves, and concomitant gating of these channels.

In membrane, TRP channels assemble into tetramers of identical subunits [86]. Recently obtained data indicate that the homo-oligomer, modular nature of the structures involved in activation processes allow different stimuli (voltage, temperature, and agonists) to promote thermo-TRP channel opening by different interrelated mechanisms, for example, in the form of allosteric interaction [58, 59, 87].

The very interesting aspect resides in the observation that some bacterial proteins like H–NS and StpA may form not only homo-oligomers but also hetero-oligomers exactly the same way as TRPV thermosensory channels of higher animals sometimes do [32, 58]. In this context, it is important to note that the temperature-sensitive H–NS function is also associated with oligomerization and that the H–NS oligomerization domain most evidently relies on the formation of coiled-coil oligomers [33, 75].

Together with polymerization, an interesting but still pending problem is modulation of thermotropic reactions by low-molecular weight compounds. A common feature shared by many TRPM8 channels is binding of phosphatidylinositol bisphosphate (PIP2), that leads to channel activation [88]. Binding and activation by capsaicin, ADP-ribose, menthol, eucalyptol etc. are classical examples of polymodality of temperature-sensitive proteins but the field still lacks systematical study. A pool of these and related questions will be generally addressed by a quickly developing discipline, *chemical genetics*, whose subject can be defined as "a selective interaction of a small molecule with a protein that may be regarded as functionally equivalent to mutation of the protein" [89].

The molecular dynamics and organization of the temperature-sensing proteins signaling complexes are still elusive, although fast-advancing progress in this arena is uncovering the molecular identity of these elements. A series of papers published by G. Artmann and coworkers, revealed intriguing temperature-related structural transitions phenomena in hemoglobins (Hb) and myoglobins of different species [65, 90, 91]. The reported non-linearity in hemoglobin temperature behavior is determined by physiological body temperature of the given species, is strongly influenced by many small molecules (ATP, PIP2 etc.) and therefore might surprisingly imply the role of Hb as a molecular thermometer [92].

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