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A three-state balancing act

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Abstract

How do pathogens survive temperature variations? At a molecular level, one bacterial species seems to regulate gene expression in response to temperature through structural equilibria in corresponding RNA sequences.

In bacteria, many messenger RNA molecules carry a regulatory segment called a riboswitch. Specific binding of small ligand molecules, such as adenine, to this segment determine whether the riboswitch mRNA will be translated into a protein^{1–4}. On page 355 of this issue, Reining *et al.*⁵ show that regulation of gene expression through such ‘riboswitching’ is coupled to temperature sensing. The authors investigate the adenine-sensitive riboswitch from the pathogenic bacterium *Vibrio vulnificus*⁶, and demonstrate that efficient RNA regulation at different temperatures — those of the bacterium’s marine habitat and its human host — requires a change in the riboswitch’s structural behaviour from a two-state pattern to a three-state one.*

A typical riboswitch consists of two domains at the 5’ end of the mRNA: a ligand-binding aptamer and an adjoining expression platform, which can have one of two mutually exclusive structures depending on whether the aptamer is in the ligand-bound or ligand-unbound state. The structural change in the expression platform signals that gene expression should be turned on or off.

During gene transcription, a polymerase enzyme synthesizes first the aptamer and then the expression platform⁷. The sequential release of riboswitch domains from the polymerase is especially meaningful for transcription-controlling riboswitches, which act under kinetic control. To direct the folding of the expression platform, the aptamer domain of the growing RNA chain must bind rapidly to its ligand, which requires high ligand concentrations⁸; otherwise, the resulting full-length mRNA becomes trapped in a default fold that cannot respond to the ligand⁹. In transcription-controlling riboswitches, the two mutually exclusive structures are generally referred to as terminator and antiterminator folds, causing cessation of polymerase activity and continuation of mRNA synthesis, respectively.

Bacterial transcription is tightly coupled to translation. In translation-controlling riboswitches, the molecular mechanism relies on either sequestration or liberation of the

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Shine–Dalgarno sequence — the mRNA site that binds to cellular organelles known as ribosomes to initiate translation. In contrast to transcription-controlling riboswitches, most translation-controlling riboswitches act under thermodynamic control⁹. Consequently, ligand-dependent control of translation is maintained even for a full-length mRNA. For these riboswitches, therefore, two states (ligand-bound and -unbound) seem sufficient to turn translation on and off.

Reining and colleagues have found that, for robust functioning, their translation-controlling adenine-sensing riboswitch must occur in three structural states. The authors investigated the full-length (more than 100-nucleotide) riboswitch domain at single-nucleotide resolution. They also determined a complete set of thermodynamic and kinetic parameters for folding and ligand-binding of this RNA, under conditions that involved varying the concentrations of RNA, magnesium ions (a factor mediating structure formation) and adenine, as well as, importantly, temperature.

The researchers find that a ligand-free (apo) form of the riboswitch exists in a pre-equilibrium of two structurally distinct aptamer folds (Fig. 1). One of the structures (apoA) can bind to the ligand, exhibiting a structure that resembles a third structural state — the adenine-bound holo form. The other structure (apoB) adopts a different fold and cannot interact with the ligand. But why does this bistable sequence element exist?

Reining *et al.* provide a clear answer: this pre-equilibrium is markedly temperature dependent and counterbalances the temperature-dependent changes in ligand affinity. At low temperatures, when adenine has a high affinity for the RNA, only a small population of the adenine-binding apoA form is available, but it is sufficient to achieve high riboswitching efficiency (Fig. 1). At higher temperatures, this population is significantly increased and compensates for the lower RNA affinity of the ligand. Thus, whereas in a pure two-state model switching efficiency would vary drastically with environmental changes, in this three-state model the switching efficiency remains robust.

The authors further perform a coupled transcription–translation assay to underline the significance of their biophysical findings. They show that a mutant riboswitch that cannot adopt the apoB structure and exists only in the binding-competent apoA conformation lacks sensitivity to changes in adenine concentration and so cannot control gene expression in response to varying ligand doses. This mutant is also insensitive to changes in temperature and is always in an ‘on’ state.

Nature probably contains further examples of three-state structural behaviour for RNA that balances RNA-regulated gene expression. Bistable elements are widespread in RNA sequences and have been thoroughly characterized in at least one other riboswitch system — a preQ₁ class I riboswitch from the human pathogen *Fusobacterium nucleatum*¹⁰. Moreover, temperature-sensitive RNAs that control gene expression, ‘RNA thermometers’¹¹, are prevalent. Coupling ligand and temperature sensitivities seems logical, although the three-state mechanism discovered by Reining *et al.* compensates for temperature fluctuations rather than using this parameter to directly trigger a gene response.

As the present paper shows, mechanistic insights into riboswitch function can provide a deeper understanding of the potential adaptive strategies used by bacteria in different environments. Such insights may also be of fundamental interest for exploring the possibility of targeting riboswitch RNAs with antibacterial drugs — for example, by interfering with the delicate balance of the RNA-structure equilibria. Furthermore, these findings will influence the design of RNA biosensors, which represent emerging tools for live-cell imaging¹². They will also affect the use of riboswitches in synthetic-biology applications for reprogramming cells to autonomously perform complex tasks, such as ‘seek-and-destroy’ herbicides¹³.

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Figure 1. Three states of a riboswitch.

Riboswitches consist of an aptamer and an expression platform. Reining *et al.*⁵ find that, in contrast to the accepted two-state model, a three-state model exists for the structural behaviour of an adenine-sensing bacterial riboswitch. They show that the ligand-free apo form of this riboswitch can exist in two pre-equilibrium states: an apoA state that can bind a ligand (adenine), and an apoB state that cannot. On binding adenine, the apoA structure adopts a third (holo) state. The structural pre-equilibrium between apoA and apoB states counterbalances the temperature-dependent equilibrium between the apoA and holo states, guaranteeing effective regulation, over a broad temperature range, of the gene corresponding to the RNA sequence that carries the riboswitch.