Site-specific fluorescence dynamics in an 'RNA thermometer' reveals the mechanism of temperature-sensitive translation

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Although the information content of RNA primarily resides in its sequence, the structure and dynamics of RNA are emerging as regulators of its biological expression. These regulatory regions modulate the level of translation by a variety of modes such as binding to metabolites, temperature-sensitive alterations in local structure and dynamics etc. The ROSE (**R**epression **O**f heat **S**hock gene **E**xpression) element of mRNA present in the 5'-UTR of small heat-shock genes in many Gram-negative bacteria is known to function as a 'RNA thermometer' by controlling protein translation in a temperature range of 30 - 42° C where the translation is blocked till 30°C and allowed at 42°C and beyond, perhaps due to an unfolding transition of the ROSE hair-pin motif.

In this work, we have used site-specific fluorescence labeling and picosecond time-domain fluorescence spectroscopy to unravel the mechanism of temperature-sensitive translation. The 'ROSE RNA' was site-specially labeled with 2-aminopurine (2-AP), a sensitive fluorescent analog of adenine. Observables such as fluorescence lifetime, fluorescence anisotropy decay kinetics and dynamic fluorescence quenching revealed properties such as the level of base stacking, rotational motion of the bases, segmental dynamics of the backbone and the level of exposure of base to solvent. As expected of an RNA with a strong hairpin structure, all read-outs of 2-AP residue that were studied showed remarkable position dependence/sensitivity in the RNA sequence at 25°C. The striking result was the persistence of the same position-dependence of the parameters even at 45°C albeit at a measurably reduced levels. However the same position-dependence was nearly "wiped" out in the presence of urea at denaturing concentrations where all intra-molecular interactions in RNA are undone. These observations have prompted us to revise the existing model of ROSE RNA action: we now suggest that unlike proposed earlier, the thermometer action of ROSE emanates not from its unfolding structural transition between 25 and 45°C, but rather from its propensity to enhance structural dynamics without "melting" the structure. We believe that it is this dynamics that leads to full melting only up on the addition of a denaturant (urea in our experimental regime). We hypothesize that either the enhanced dynamics of the structure it self or its full melting due to an extrinsic factor (perhaps a protein interaction) might be the basis of its thermometer action.