Use Of Single-Molecule FRET And Fluorescence Correlation Spectroscopy To Probe RNA Folding And Transcriptional Initiation

P. Stockley, C. Gell, T. Sabir, R. Leach, C. Adams (University of Leeds), M. Buck (Imperial College), and A. Smith (University of Leeds)

A major hurdle to a better understanding of the molecular mechanisms underlying complex biological processes, such as RNA folding and transcriptional initiation, is the lack of sensitivity of traditional biochemical techniques that consequently require relatively concentrated samples for study. Measurements made on ensembles of molecules in concentrated solutions result in an averaging of multiple conformational equilibria that may exist and this critical molecular detail is lost.

Single-molecule studies offer a route around these problems because they can in principle identify the discrete conformational equilibria and correlate the behaviour of individual molecules over time, thus clearly defining molecular mechanisms. Indeed, the single-molecule technique may be the only approach that satisfactorily probes the behaviour of RNA molecules under conditions that approach those found *in vivo*, i.e. where most biomolecules would function independently and at very low concentrations. Recently, with the advent of suitable light sources and detectors, it has become feasible to study nucleic acids by well established fluorescence labelling strategies with single molecule sensitivity.

Our philosophy in applying single molecule techniques to the study of RNA structure/folding is straightforward. We have begun to work with a simple model system, namely the (TR) RNA that initiates assembly of coat protein (CP) from the RNA bacteriophage MS2, a stem-loop of just 19 nts in length, in order to develop and validate our technology. Both single molecule FRET and FCS have been used to extract information about the rates of folding and unfolding, and the use of sequence varaints has begun to reveal the key barriers to these processes. The approaches that have succeeded with the TR RNA can now be applied to ever more complex RNA targets. These include the self-assembly packaging (prohead) RNA (pRNA) from the bacteriophage ϕ29. The latest data from these two model systems will be described. In addition, we are studying the process of transcriptional initiation in bacteria using the same technology but with fluorophores on both the DNA template and the RNA polymerase molecule or its activators. These data are being correlated with structural stduies of the isolated molecules area their complexes.