Correlating molecular structure and cellular function using microarrays

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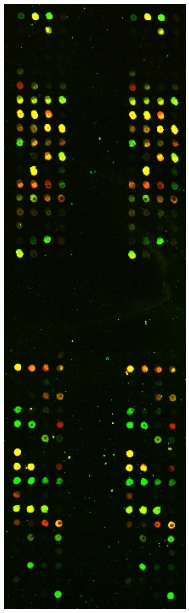
Introduction & objectives

The control of RNA levels is a key process in cellular growth and development and is mediated at the levels of transcriptional initiation and mRNA decay. We are in the process of examining the fine details of aspects of both in *E. coli*, using the latest gene array technology and the insights available to us from extensive *in vitro* structural and functional studies. Of particular interest is the major transcriptional regulators of the methionine, arginine and tryptophan pathways, namely MetJ, ArgR (AhrC in *B. subtilis*) and TrpR, respectively. These proteins have been examined in molecular detail by crystallography and represent distinct classes of regulatory protein; a ribbon-helix-helix DNA binding motif, activated by a long range electrostatic co-repressor effect, for MetJ, and an unusual hexameric organisation of helix-turn-helix motifs for ArgR/AhrC. They have also been studied extensively *in vitro* to determine their operator binding properties, and, at least partially, *in vivo* using site-directed mutants of both protein and operator components. TrpR is a "classic" member of the HTH family of dimeric transcription factors with many similarities to MetJ in its operator binding, although its mechanism of co-repressor action is distinct. These three repressors control major biosynthetic pathways in *E. coli* and offer an unprecedented opportunity to correlate *in vitro* structure and function studies with *in vivo* physiology. They still constitute the major examples of main stream regulatory proteins in the PDB and are ideal systems for such studies given the wealth of *in vitro* data available. We are also in the process of examining the global impact of enzymes involved in controlling the stability of RNA in *E. coli*. Mutations that affect the activity of RNase E (see other report) and other players in RNA turnover have been isolated. In conjunction with microarray technology, these offer an exciting opportunity to dissect the nature and regulation of mRNA decay on a genome-wide scale and the possibility that it is integrated with the control of transcriptional initiation.

Progress

We have established robust methodology and background data sets for gene knock-outs of transcription and decay factors. Of particular value has been the construction of mini-arrays containing multiple samples of limited number of gene probes. For each experiment, we obtain ratios of gene expression that are statistically significant. We predict that even small changes (<1.5 or >0.7) will detected. Consistent with our recent biochemical analysis of RNaseG, transcriptome analyses suggest that this enzyme does have a role in the processing of 9S RNA and initiating the decay of *ompA* mRNA.



Array results

Collaborators

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Reference

Tock, M.R., Walsh, A.P., Carroll, G.& McDowall, K.J. (2000) The CafA protein required for the 5'-maturation of 16S rRNA is a 5'-end-dependent ribonuclease that has context-dependent broad sequence specificity. *J. Biol. Chem*. **275,** 8726-32.

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