

Research Interests

I am fascinated by a fundamental question in biology; How is regulation of gene expression as a response to external stimulus achieved? Expression of approximately seventy genes in *E. coli* are affected by increasing osmolality of growth medium. A current model of osmoregulation in *E. coli* suggests that the cell strives to maintain the pressure exerted by the cytoplasmic membrane on the cell wall (turgor) at some fixed set point. Upon osmotic upshock, potassium, glutamate and trehalose concentrations in the cytoplasm all increase dramatically and putrescine levels decrease. The changes in solute composition result from a complex network of gene expression which is carefully regulated. At least two signals appear to be important in initiating changes in gene expression which help maintain turgor; turgor pressure itself and cytoplasmic K^+ activity. These signals are sensed and relayed to regulator factors which influence the transcription and the translation of genes which are involved in synthesizing or transporting cytoplasmic solutes.

Both transcription and translation can be broken into initiation, elongation and termination phases, and within each phase are an indeterminate number of mechanistically important steps. A regulatory factor, can in principle affect any or all of these steps to ultimately reduce or increase expression of a gene product by as much as several orders of magnitude. Much of the research done on osmotic regulation has been aimed at identifying components important for regulation. The choreography of the interactions essential for regulation is not well understood. As a biological chemist, I find this an extremely challenging and interesting problem to investigate.

Small differences in the strength of an interaction between a regulatory effector and transcriptional or translational components may have huge effects on the levels of gene expression, or none at all. *In vitro*, most of the macromolecular interactions required for translation and transcription are extremely sensitive to salt concentration and ion species. Ultimately I am interested in trying to understand how changes in the cytoplasmic environment introduced by one regulatory response influence other regulatory responses.

Regulation of expression of RpoS, σ^S

The expression of many osmotically regulated genes is dependent upon the σ^S subunit of RNA polymerase. Upon osmotic upshock, expression of rpoS mRNA remains steady (Lange and Henge-Aronis, 1994, *Genes Dev.*, 8:1600-1612), however protein levels increase. This results from reduced turnover of σ^S protein and increased translation of the mRNA. I am particularly interested in understanding the translational regulation of this gene.

Translational initiation in prokaryotes is a complicated process. The mechanisms by which messenger RNA (mRNA) sequence and structure, and regulatory effectors influence translational initiation is just beginning to be understood. The expression of rpoS, which encodes an RNA polymerase subunit required for expression of genes during stationary phase and osmotic upshock (σ^S) is regulated during translation, apparently as a result of changes in its mRNA secondary structure (Muffler et al., 1996, *Genes Dev* 10:1143-51). The RNA binding protein, HF-1 has been found to be essential for translation of rpoS (Muffler et al., 1996, *Genes Dev* 10:1143-51), presumably because it

stabilizes a conformation of mRNA which is more readily translated. I am interested in understanding how intermolecular and intramolecular interactions compete kinetically to regulate translation. Intramolecular interactions of RNA can be extremely sensitive to salt concentration and ion type. It is likely that changes in the intracellular environment resulting from osmotic stress will alter the distribution of mRNA conformations, perturbing the regulation of this gene.

Translational initiation requires binding of the 30S subunit, containing the 16S ribosomal RNA (16S rRNA) and 21 ribosomal proteins, and initiating transfer RNA (tRNA f^{met}) to the messenger RNA (mRNA) to form a preinitiation complex. This complex rapidly equilibrates with the free components of the reaction. Formation of the preinitiation complex, and the binary 30S-mRNA complex, involves base pairing of a specific mRNA sequence (the Shine Dalgarno sequence) with its complement in the 16S rRNA in the 30S subunit. The preinitiation complex undergoes a slow, rate limiting isomerization to form an initiation competent complex which involves base pairing of the transfer RNA anticodon to the messenger RNA initiation codon. Formation of this stable initiation complex is essentially irreversible. The concentration of this complex is proportional to the rate of protein synthesis (Gualerzi and Pon, 1990, *Biochemistry* 28:742-747).

The kinetics of unfolding mRNA structure may influence the rate of formation of the stable initiation complex, and therefore the rate of protein synthesis. van Duin and coworkers have shown that the stability of mRNA secondary structures which trap the Shine Dalgarno sequence correlates strongly with the amount of translation of their products (de Smit and van Duin, 1994, *J. Mol. Biol.*, 235:173-184). The steps in translational initiation which are affected by secondary structure are unknown. It seems likely that structures which occlude the Shine Dalgarno sequence, either through base-pairing to other regions of the mRNA or by folding into conformations which block the sequence from interacting with the 16S rRNA in the 30S subunit, would affect formation of preinitiation complex. Alternatively, structures which allow the Shine-Dalgarno sequence to be exposed may not allow the isomerization from the preinitiation complex to the initiation complex.

I am interested in mapping the region of *rpoS* mRNA secondary structure which are important for translational regulation, and then in determining which steps in initiation are affected by the mRNA secondary (and tertiary) structure. This will involve making a series of mutants and compensatory mutants in sequences predicted to be involved in secondary structure formation, and characterizing the effects of the mutations on the kinetics of formation of preinitiation complexes and initiation complexes. Additionally, I am interested in determining whether the 30S ribosomal subunit assists melting these structures or whether it plays a passive role in the melting of secondary and tertiary structures. Examination of the rates of folding and unfolding mRNA structures will be necessary to understand whether the 30S subunit acts as an RNA helicase.

Regulatory factors which bind to mRNA may perform a variety of functions: they can directly block 30S binding, they may prevent tRNA anticodon:mRNA codon binding or they may stabilize RNA structures which bury or expose sequences necessary for translational initiation (Draper et al. RNA Structure and Function in press). HF-1 probably affects translational initiation by stabilizing a RNA conformation which is either more readily recognized by the 30S subunit or which can undergo the

isomerization to form an initiation complex more rapidly. I propose to examine the kinetics of formation of initiation complexes as functions of 30S and HF-1 concentrations to distinguish between these possible modes of translation regulation. I am also interested in developing model systems of mRNA regulation by using antisense oligonucleotides to positively and negatively affect formation of the preinitiation and initiation complexes. Oligonucleotides which bind to different regions of the mRNA would be expected to have very different mechanisms of regulation. Examination of the kinetics of initiation complex formation as functions of 30S and oligonucleotide concentrations would permit a mechanistic analysis of the effects of the oligonucleotides on translational initiation.

Cytoplasmic concentrations of intracellular cations (K^+ , Mg^{2+} and putrescine $^{2+}$) all change as a function of external osmolarity. These ions may have specific binding sites on the rpoS mRNA. Examining the effects of ion concentration and type on the mechanistic steps involved in the regulation of rpoS translation will be useful in attempting to understand the crossregulation of osmotically regulated genes.