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Summary of Research Proposal/Résumé de la proposition de recherche

Regulation of CRP-S promoters in *Haemophilus influenzae* and *Escherichia coli*

Models of CRP action are foundational to understanding how proteins interact with DNA and how transcription factors recruit RNA polymerase, so it is surprising that a major mode of CRP action has been overlooked. We have recently identified the components of the *Haemophilus influenzae* CRP-dependent competence regulon (25 genes in 13 transcription units) and partially characterized its regulation by the competence-specific protein Sxy. Controlling the regulon is an entirely novel mode of regulation by CRP, in which Sxy enables CRP to activate transcription at a new class of CRP sites called CRP-S sites. This led us to discover similar but previously unrecognized CRP- and Sxy-dependent regulons of *Escherichia coli* and *Vibrio cholerae*. Because CRP-S promotors lack potential Sxy-binding sites and Sxy lacks features of known DNA-binding proteins, **we hypothesize that Sxy acts by modifying interactions between DNA, CRP and RNA polymerase**. The mechanism of this unprecedented regulation is one focus of this proposal.

The second focus is on transcriptional and especially post-transcriptional control of *sxy* expression, the limiting step in activation of the *H. influenzae* CRP-S regulon. Transcription of *sxy* is strongly induced by active CRP, but levels of Sxy protein are limited by one or more other regulatory factors that act through the secondary structure of *sxy* mRNA. The 5' end of *sxy* mRNA folds into a stem and complex loop; mutations that change the stability of the stem affect *sxy* expression by changing the translatability of the mRNA. Because transfer of cells to a medium lacking nucleotides enhances translatability, **we hypothesize that changes in nucleotide pools alter the rate of polymerase progression. This in turn determines whether base pairing blocks access of the ribosome to the *sxy* start codon and/or Shine-Dalgarno site, thus regulating translation by the rate of transcription**.

The CRP-S regulon appears to integrate two molecular signals: (1) CRP is the central regulator of carbon-and energy metabolism, activating transcription in response to depletion of preferred PTS sugars, and (2) Sxy is implicated in signaling the state of cellular nucleotide pools. The CRP-S regulon contains genes for both DNA uptake and DNA metabolism, and its dual regulation may optimize the cells' response to nucleotide depletion in times of energy shortage.

Our experiments in *H. influenzae* and *E. coli* will answer the following specific questions:

I. How is *sxy* regulated in *H. influenzae*?

I-A: Does the kinetics of transcription regulate *sxy* expression?
I-B: Which base interactions are functionally important for *sxy* expression?
I-C: How do new *sxy* mutations affect competence?

II. How is *sxy* regulated in *E. coli*?

II-A: What induces *sxy* transcription?
II-B: Where does *sxy* transcription initiate?
II-C: Can *sxy* mutations increase sxy expression?
II-D: Is *sxy* translation regulated?

III. How does Sxy activate transcription in *H. influenzae* and *E. coli*?

III-A: What are Sxy's effects on transcription?
III-B: Do *E. coli* and *H. influenzae* Sxy proteins reciprocally complement?
III-C: Do CRP and Sxy physically contact each other in vivo?
III-D: Does Sxy replace CRP-RNAP interactions?
III-E: Can CRP or RNAP mutations bypass the need for Sxy?