

Research Statement, Robert Osuna

Our current work involves the regulation of DksA in *E. coli*. Bacteria rely on numerous global gene regulators to rapidly control the activity of many of its genes in their attempt to protect themselves or benefit from a sudden change in their immediate environment. DksA, a fairly recently discovered bacterial gene regulator, plays an essential role in the regulation of the transcription of ribosomal RNA (necessary for the synthesis of proteins in the cell) and of numerous other genes involved in a variety of important cellular processes. DksA differs from most bacterial gene regulators in that it functions by binding directly to the RNA polymerase enzyme (the enzyme responsible for carrying out transcription) rather than to DNA. Little is known about how DksA itself is regulated. Therefore, this project focuses on attaining a detailed understanding of how *Escherichia coli* cells control the production of DksA in response to different growth conditions. Preliminary work indicated that the *dksA* gene is regulated at the level of transcription (i.e. RNA synthesis). The regulation involves two transcription initiation sites (i.e. promoters), and several other transcription factors (including DksA itself). The *dksA* gene expression was also found to be controlled at the level of translation (i.e. protein synthesis), and this control required a specific portion of the *dksA* mRNA referred to as the 5'-untranslated region (5'-UTR). One aim of this project is to investigate how *dksA* is transcriptionally controlled under various different growth conditions. The *dksA* promoters, including a newly discovered promoter, which is most active under conditions of starvation, will be more precisely defined. The *dksA* regulatory effects by several suspected transcription factors will be investigated in detail. The relative transcription activity from all *dksA* promoters will be simultaneously measured while in their native chromosomal contexts during different growth conditions and in different bacterial strains lacking any of suspected transcriptional regulators. Another aim is to investigate how *dksA* gene expression is controlled at a post-transcriptional step (i.e. after the *dksA* mRNA has been made). The project will examine the hypothesis that changes in the rate of breakdown of *dksA* mRNA during different phases of cell growth contribute to the regulation of *dksA* expression. The relative DksA protein levels in various *E. coli* strains and several other bacterial species will be measured during different growth conditions. The effects of suspected *dksA* transcription regulators on the cellular levels of DksA protein will also be examined. The PI will probe the RNA structure in the 5'-UTR of the *dksA* mRNA and carefully examine its role in translation control. The project will also aim to detect, capture, and identify potential cellular factors that may associate with the 5'-UTR to affect translation control of DksA. Together, these experiments will provide a broad and extensive understanding of the cellular processes used to regulate this important global gene regulator in response to different bacterial growth conditions.

Our lab has had a longstanding interest in studying some of the functions of the DNA binding and bending protein Fis in *E. coli*, as well as understanding the mechanisms involved in its growth phase-specific regulatory pattern. Fis (Factor for Inversion Stimulation) is involved in several different biological processes such as stimulation of DNA inversion reactions mediated by the Hin, Gin, and Cin family of recombinases, stimulation of lambda phage DNA integration and excision from the bacterial chromosome, stimulation of transcription of ribosomal and tRNA operons and other genes, autoregulation and repression of several other genes, and modulation of DNA topology.



Fis is subject to a complex set of transcriptional control mechanisms. Together, they allow adequate

Fis expression levels in response to sudden changes in the nutritional environment. Upon a nutritional upshift, Fis protein and mRNA levels rapidly increase, reaching a peak within the time that is required for the cells to shift to a faster growth rate, and then decreases to very low or undetectable levels during late logarithmic growth and early stationary phases. Understanding the molecular mechanism(s) responsible for this peculiar growth phase-dependent regulation pattern is a subject of investigation in our lab. Fis is also subject to negative transcriptional control in response to conditions of starvation, otherwise known as stringent control. We have shown that stringent control and growth phase-dependent regulation require different and separable molecular mechanisms. Between the two, levels of Fis mRNA become tightly coupled to the nutritional environment. Two additional mechanisms of transcriptional control include stimulation by the integration host factor (IHF) and transcriptional repression by Fis. The IHF protein binds to a site centered at about 116 bp upstream of the *fis* promoter transcriptional start site to stimulate transcription three to four-fold. The transcription stimulation occurs in a helical phasing-dependent manner, suggesting that its action may require an interaction with the promoter-bound RNA polymerase. Negative regulation by Fis requires at least two Fis binding sites that flank the *fis* promoter region. Because neither the stringent control, IHF stimulation, or the Fis autoregulation are required for the growth-phase-dependent regulation observed for Fis, and because the *fis* promoter region is sufficient to generate its growth phase regulation pattern, we have focused on the *fis* promoter region to more closely investigate the molecular details surrounding this process. Thus far, we have been able to link the growth phase dependent control with the use of a poor match to the -35 and -10 sigma 70 promoter consensus sequences, combined with the use of CTP as the primary transcription initiation nucleotide.

To learn more about the role of Fis in *E. coli*, we became interested in identifying new genes that are subject to Fis regulation. We have performed 2-dimensional gel electrophoresis and observed that there are a number of proteins (>25) that are more highly expressed in the presence of Fis and another set of proteins that are repressed in the presence of Fis. So far we have been able to identify 9 of them by partial microsequencing. We have also performed DNA microarray analysis and have uncovered many more genes that appear to be subject to Fis regulation, directly or indirectly. Current research efforts are focusing on Fis as a global gene regulator.

We are also interested in understanding how a Fis dimer specifically interacts with DNA and other proteins to carry out its functions. Genetic and Biochemical studies have shown that Fis contains at least two functional regions. A carboxy-terminal region (amino acids 74-94) contains a helix-turn-helix DNA binding motif and is required for proper DNA binding and bending. Another region closer to the amino terminus (amino acids 17 to 44) is required for the ability of Fis to stimulate Hin-mediated DNA inversion, but is not required for other functions such as DNA binding, DNA bending, stimulation of lambda DNA excision, and autoregulation.