I N T R O D U C T I O N

One of the most striking aspects of biology is in the diversity of cellular life. Although much of this variability has been attributed to genetic and environmental factors, recent studies have shown4 that genetically identical organisms in the same environment exhibit heterogeneity in gene expression. This phenomenon, gene expression noise, has been observed and measured across species as divergent as prokaryotes and mammalian cells.[1-4]

We have been keenly interested in understanding more about the origin of this heterogeneity as well as wondering what potential functional consequences it may have. We have begun addressing these questions with a suite of experiments described in the following chapters.

In Chapter 1, we examine the effects selection may have on gene expression noise *in silico*. We find that under fairly general conditions, directional selection for the value of a quantitative phenotypic trait can yield an increase in the noise in that trait.

In Chapter 2, we begin to look experimentally at the effects *cis*-regulatory mutations have on gene expression noise. Through the creation of promoter mutant libraries, we look for mutations that change noise; furthermore, by also looking at how these same mutations affect mean levels of gene expression noise, we test the independence of these two parameters. By measuring mean and noise levels of gene expression in three mutant yeast promoter libraries, we see that the two parameters are independent in some cases. More importantly, we see a wide variety of mean and noise values, suggesting that *cis*-regulatory mutations can control gene expression noise independently of mean.

Upon finding out that specific promoter mutations can impact gene expression noise, we were interested in finding out if the binding and unbinding of transcription factors to promoter regions had a similar impact. In Chapter 3, we observe the localization dynamics of a calcium-responsive yeast transcription factor, Crz1. We find that it localizes to the nucleus in short coherent bursts in response to calcium. The frequency, but not duration, of Crz1 localization bursts increases with extracellular calcium. This frequency-modulated nature of the bursts enables proportional control of target genes. Interestingly, these localization bursts also lead to transcriptional bursts of target genes, leading to noisy gene expression. This is especially intriguing because it suggests a trade-off of sorts. The

bursts lead to noisy gene expression but they also lead to proportional regulation. Hence the localization dynamics of a yeast transcription factor provide a mechanism to increase gene expression noise and provide proportional control of a regulon of target genes.

Since the strategy of looking at protein localization dynamics had yielded such interesting results, we decided to take a more comprehensive look at protein dynamics in yeast. In Chapter 4, we describe a screen in which we take movies of all available tagged proteins in yeast. We find that there are a few other fast bursting proteins, like Crz1. However, several other types of localization behavior exist, including slow bursts, amplitude modulation, and static heterogeneity. Amongst the other fast bursting proteins, we have found two transcription factors, Msn2 and Mig1, which burst in response to glucose deprivation. When observing both proteins in the same cell, it is clear that their bursts are not completely independent, as seen in the cross-correlation function. We hope to use chemical perturbations to track down this lack of independence and measure expression levels of combinatorial target genes, hoping it will help uncover mechanistic insights into the observed fast bursts and their downstream consequences.

To summarize, we have looked at different potential mechanisms for generating gene expression noise, namely selection, *cis*-regulatory mutations, and the localization of *trans*-acting factors. All appear as if they could contribute to gene expression noise; most notably, the bursting of transcription factors in and out of the nucleus, in addition to leading to increased amounts of gene expression noise, also yields proportional control of a suite of genes. These results also provide a mechanism for proportional control, a phenomenon which may be important in several contexts and may even provide a rationale for some of the gene expression noise that we observe. We anticipate that further investigation of the functional consequences of noise will continue to provide insights into cellular behavior.