Noise in Gene Expression Reveals Cellular Pathways

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Short Abstract — **A population of isogenic cells exhibits substantial variation in gene expression, often described as noise. Part of this noise is due to variation in signaling pathways in individual cells across a population. This form of noise correlates the expression of genes which share regulatory motifs. Such noise-induced correlations can be quantitatively measured using fluorescent reporters, and their information content exploited to extract regulatory relationships. Here, we focus on the Msn2/4 general stress pathway in budding yeast and measure the correlation in individual cells between reporters of different branches of this pathway and a large fraction of the genome. By combining these quantitative correlation measurements with targeted gene deletions, we dissect how different aspects of the pathway are regulated in complex and non-redundant ways by several systems including PKA, Tor, and PKC.**

Keywords **— noise in gene expression, stress response, gene regulation**

An isogenic population of cells exhibits wide variation in size, cell cycle state, and protein copy number. This variation is inevitable, and is the compounded effect of many sources such as stochastic variation in transcription, growth rate, and stress state of individual cells. At the level of any individual gene, fluctuations arise due to "global" variation in transcriptional/translational capacity, stochastic variation in transcriptional activity at a given promoter, and "pathway" noise propagating from upstream transcription factors and signaling [1,2,3]. Importantly, this pathway noise is shared among downstream targets of a signaling pathway, causing the expression of genes which share regulatory elements to be correlated within a given cell [4].

In this work, we exploit these noise-induced correlations as a probe for pathway membership and organization. We use synthetic and natural networks in *S. cerevisiae* to demonstrate that correlation in gene expression can expose co-regulation and analytically show that, given a faithful reporter of a pathway of interest, the degree of sensitivity of a gene to that pathway can be quantified. Such sensitivities can be globally measured by construction of dual-fluorophore libraries where RFP reporters of a pathway of interest can be crossed to subsets of the available yeast GFP library in which open reading frames are fluorescently tagged at the endogenous locus.

Acknowledgements: This work was supported by NSERC (J.SO), the Howard Hughes Medical Institute (J.S.W), and the Packard Foundation (H.ES)

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Using this sensitivity metric, we probe the yeast *S. cerevisiae* general stress pathway, which is defined by those genes responsive to the homologous transcription factors Msn2 and Msn4 [5]. We demonstrate that noise in gene expression and sensitivity of each gene to this general stress pathway can be quantitatively measured, and that these sensitivities are predictive of the response of any gene to heat stress. Our investigations found that the information content of such single cell data-derived metric of sensitivity changes across conditions, making it possible to observe how relationships between genes change as a function of the environment and stimuli—in contrast to population based correlation measurements which might obscure these relationships. Taking advantage of this feature, we examined how the sensitivity to stress pathways changes in low compared to high stress conditions, and also how growth promoting pathways reorganize in response to different carbon sources.

Using the same approach in combination with gene deletions, we were able to dissect the strong and non-redundant influence of several pathways—including PKA, Tor, PP2 and PKC—on Msn2/4, positioning these transcription factors as integrators of stress and growth signals. Subsequent experiments validated these results and describe the complex regulation of Msn2 localization and, more surprisingly, abundance. These results point to the potential general use of noise and fluctuations in cellular pathways as quantitative discovery and analysis tools.

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