# Robust extrinsic noise in the *SSA1* promoter

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*Short Abstract* — **Quantitative models suggest that the stochasticity in gene expression informs on molecular mechanism, for example, by distinguishing between fast or slow promoter dynamics. To validate these models, we measured the fundamental rate constants for the heat shock promoter *SSA1* in *S. cerevisiae*. We show that even accounting for cell volume, protein distributions measured are inconsistent with the generally accepted random telegraph model of gene expression, likely due to 70% of the noise, or more, deriving from extrinsic sources. Finally, we show that destabilizing our reporter gene fails to increase intrinsic noise as predicted.**

GENE expression is known to be a stochastic process, first revealed by the wide variation of protein levels observed among genetically identical *E. coli* [1]. Subsequent measurements of single cell eukaryotes and mammalian cells confirm the universality of stochastic gene expression in living organisms [2-4].

Quantitative biologists have proposed a variety of models explaining the origin of observed gene expression noise. The prevailing "random telegraph" model imagines stochastic switching of DNA promoters between an active and inactive state [2-4]. Transcription occurs from the active state, RNA is further translated into protein, and both RNA and protein degrade with first-order rates. Single-molecule RNA-FISH experiments support the random telegraph model for RNA production [2-4]. At the protein level, we [5] and others [6,7] have proposed computational methods for inferring the fundamental rate constants of the random telegraph model from steady-state or perturbed protein distributions. We sought to use these state-of-the-art methods to understand the regulatory mechanisms underlying basal and induced expression in the heat shock promoter *SSA1* in *S. cerevisiae*.

We cloned the *SSA1* promoter in front of an eGFP reporter and integrated our gene chromosomally at the ΔHIS3 locus in the yeast laboratory strain BY4741. Using flow cytometry, we measured population variability in protein expression in high-throughput. Since significant additional information can be obtained from distributions of absolute numbers of proteins per cell, we coupled flow cytometry with single molecule counting by fluorescence correlation spectroscopy (FCS).

A significant body of work postulates relationships between the random telegraph rate constants and population stochasticity [5-7]. However, few of these methods have been verified experimentally. Using a combination of heat-shock and cyclohexamide block experiments, we measured the bulk rate constants for *SSA1* under inducing (37°C) and non-inducing (22°C) conditions.

With estimates for the fundamental rate constants in hand, we were surprised to find our model, and others, failed to predict the actual rate constants; equivalently, Gillespie-simulation of the random telegraph model with measured rate constants failed to reproduce measured protein distributions. Given the ubiquity of extrinsic variance [3,6,9], we asked whether our predictions could be improved by measuring and accounting for extrinsic noise. Remarkably, at least 70% of the noise arose from extrinsic sources. This noise was robust to normalization by forward and side scatter, in contrast to the findings in a previous study [8].

To reduce the impact of extrinsic noise, we destabilized our GFP reporters by various magnitudes using the N-end pathway. Despite predictions to the contrary [6,9], we found that reporter destabilization marginally impacts the balance of intrinsic and extrinsic noise for *SSA1*.

The random telegraph model of gene expression is inconsistent with *SSA1* expression stochasticity at the protein level. This observation is robust to the common methods for reducing extrinsic noise.

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