Cell-cycle dominates noisy gene expression

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***Short Abstract* — The large variability in mRNA and protein levels found from single cell measurements has been largely attributed to random periods of transcription, often occurring in bursts. We use real-time fluorescent protein measurements and static single-cell mRNA measurements to show that variable transcription from the synthetic tetO promoter in *S. cerevisiae* is dominated by its dependence on the cell cycle, with random pulses of expression restricted to the S/G2/M phases at low expression levels. Our results suggest highly variable mRNA distributions in yeast may be largely driven by differences in transcriptional activity between G1 and S/G2/M.**

***Keywords* — stochastic gene expression, mRNA FISH, tetO promoter**

I. Background

At the single-cell level, variable and long-tailed mRNA and protein distributions from regulable genes are well-described by stochastic models of transcriptional bursting [1-4]. Real-time observations of transcription in multiple organisms appear consistent with this behavior [5]. Thus the observed mRNA variability is attributed to stochasticity oin reactions intrinsic to transcription and standard stochastic models are widely used to infer steady-state dynamics [6].

However, earlier studies found protein variability depends also on extrinsic sources [7]. Global extrinsic factors have been suggested to largely impact translation [1], but their influence on transcription and bursting is unclear. In particular, the cell cycle has global effects on total protein and RNA synthesis that should play a role in transcription.

II. Results

We developed a method to infer the instantaneous transcription rate of a gene averaged over a ~20 minute time window from fluorescent time series of single yeast cells [8-9]. This enables us to monitor how transcription changes as cells progress from G1 (unbudded) to S/G2/M (budded) phases. For a several constitutive, housekeeping genes we find a ~2-fold increase in transcription rate between G1 and S/G2 consistent with the increased gene dosage. The same is true for the regulable synthetic tetO promoter when expressed at high levels. But at lower expression levels where previous mRNA FISH measurements suggested a burst frequency of < 1 per cell cycle [2], transcription is restricted to S/G2, does not occur every cell cycle, and is partially correlated between identical promoters when using a dual reporter assay.

In previous work, the measured mRNA distributions of tetO promoters and other noisy genes in yeast fit a negative binomial distribution as predicted by the standard model [2,6]. We used bud size to analyze these distributions by cell-cycle progression. The standard bursting model cannot qualitatively describe these disaggregated distributions, whereas assuming Poisson expression occurs but there are large differences in the G1 and S/G2/M transcription rate can describe the variability. These differences for tetO promoters are not a feature of low expression levels. The constitutive *DOA1* gene has similar mean expression but ~2-fold increase in transcription rate through the cell cycle.

Finally, using a novel method to measure the kinetics of activation [9], we found that the delay time for both tetO promoters and the native *PHO5* promoter to activate depends strongly on their cell-cycle phase, with a permissive period that appears in early S/G2.

III. Conclusion

This work suggests differences in transcriptional activity between G1 and S/G2/M appear to drive much of the mRNA variability for at least a few noisy promoters in yeast, rather than transcriptional bursting. This alters the interpretation of studies where static mRNA/protein distributions are fit to stochastic models of gene expression to infer steady-state dynamics. The prevalence, origins and consequences of this finding is currently being pursued.

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