Detection of an Intrinsic Transcriptional Frequency Across the Human Genome

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***Short Abstract* —We present a spectral 'noise mapping' approach to measure both dynamics and magnitude of fluctuations in gene expression at thousands of distinct human genomic loci. The results demonstrate that virtually all loci exhibit transcriptional bursting with a frequency clustered around a period of one hour. The one-hour burst period is exhibited by both strong and weak reporter promoters. Cell-signaling molecules (e.g. TNFα) modulate and 'whiten' the transcriptional-frequency spectrum, providing genome-wide evidence of competition for shared transcriptional resources within single cells. Overall, the results suggest that cellular states may be associated with distinct transcriptional frequencies, allowing characterization of unique activity signatures.**

I. Purpose

Gene expression is an episodic process characterized by bursts (1, 2) in transcription and translation. Evidence for transcriptional bursting has been found in yeast (3, 4), fly (5), and for specific human promoters (6, 7) and recent models suggest that bursts arise due to stochastic 'waiting times' inherent in the formation of active transcriptional complexes (8). The physiological importance of transcriptional bursting lies in its ability to generate beneficial noise for stress responses (9) and fate determination (10), and provide a mechanism for regulatory control over gene expression via modulation of burst frequency (11). Recent reports have shown that cells that tune the frequency of transcriptional bursts to match environmental fluctuation frequencies exhibit enhanced fitness (12), suggesting that favored or intrinsic burst frequencies may exist. However, to date, transcriptional bursting has not been demonstrated to be a predominant mode of gene expression and genome-wide surveys of transcriptional burst frequency have not been performed. Here, we present a high-throughput 'noise-mapping' approach to globally survey real-time single-cell expression kinetics and test (i) whether transcriptional bursting is widespread throughout the human genome and (ii) whether intrinsic, or favored, burst frequencies exist.

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II. Methods & Results

To screen for transcriptional bursting across the human genome, we capitalize on the semi-random pattern of integration exhibited by the HIV-1 lentivirus where the vast majority of integrations (~70%) occur within transcriptionally-active regions. T cells and B cells were infected with HIV-based lentiviral vectors encoding a short-lived fluorescent protein (i.e. the two-hour half-life version of GFP, d_2GFP) to generate a polyclonal library where each individual cell contains the vector integrated at a distinct genomic position. Cells were then fluorescently imaged for 12-hours and the resulting fluorescence intensity trajectories analyzed by 'noise mapping'. Overall, we analyzed over 7,000 individual cells with three different promoters integrated throughout the genome. Surprisingly, virtually all examined cells exhibit significant bursting kinetics at virtually all genomic loci. Furthermore, all cells (i.e. integration sites) analyzed exhibit a common mean frequency of gene-expression clustered around a rapid characteristic period of 1 hour.

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