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Cell-to-cell transcript variability—seeing signal in the noise

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SUMMARY

How stochastic is gene expression in mammalian cells? Not very, according to Battich et al. (2015), who report that single-cell variability in cytoplasmic mRNAs is remarkably predictable given measurements of a cell's phenotypic state and microenvironment. The noise from transcriptional bursts is buffered by a hallmark of eukaryotes—the nucleus.

Transcription of a gene is noisy business. Permissive chromatin states must combine with transcription factors, coactivators, and polymerase at a single locus to make RNA. When these conditions are right, transcription from the DNA template yields a stochastic burst of primary RNA copies with burst size and frequency depending on the genomic context (Dar et al., 2012). Transcriptional noise gives rise to cell-to-cell variability that is exploited by viruses, bacteria, and fungi (Raj and van Oudenaarden, 2008). By contrast, the intrinsic noise of transcription is ordinarily a problem for metazoans that must reproducibly coordinate cellular functions during tissue morphogenesis and homeostasis (Wang et al., 2012). When isogenic mammalian cells are cultured in vitro, there is a loss of coordination, and transcript abundances appear highly variable (Figure 1). Such cell-to-cell heterogeneity could reflect uncontrolled transcriptional bursting or more-predictable regulation from extrinsic factors that are specific to the context of each individual cell. By combining single-cell counting of cytoplasmic transcripts with various data-driven modeling approaches, Pelkmans and coworkers argue strongly for the latter (Battich et al., 2015). The noise from transcriptional bursts is dampened by retention of primary RNAs in the nucleus, allowing contextual inputs to act as the predominant source of transcript variability in the cytoplasm.

To quantify cytoplasmic transcripts, the authors use a variant of single-molecule FISH that uses branched DNA (bDNA) for signal amplification. The amplified signal and modularity of bDNA enable high-throughput one-color fluorescence detection of 932 transcripts at single-molecule resolution across thousands of cells per transcript. Other fluorescence channels are used to track the nucleus, mitochondria, and cell body, giving convenient indications of cellular regulatory states that may relate to transcript count. For example, overall nuclear fluorescence is proportional to DNA content and thus cell cycle status, whereas mitochondrial features conceivably give clues about cellular metabolism. Going beyond cell-autonomous indicators, Battich et al. (2015) also include cellular-state measures of nearby cell neighbors to compile a signature of 183 image-derived features per cell. These features are used to estimate the fraction of transcript variability that is attributable to a cell's

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extrinsic regulatory state and compare it to the competing hypothesis of stochastic transcriptional bursting.

For primary human keratinocytes and a karyotypically stable HeLa clone, the results are clear. With stochastic bursting, a classic three-state model of transcription does no better than random guessing at cytoplasmic mRNA abundances. By comparison, image surrogates of regulatory state predict upwards of 50–60% of the measurable variance, with most of the leftover variability attributable to Poissonian "counting noise" (e.g., counting 14 average transcripts per cell will only give exactly 14 transcripts less than 11% of the time; Figure 1). The mapping from cell phenotypic state to transcript copy number appears mechanistic and directional, because micropatterned substrates that homogenize cell size and nearest neighbors cause decreased cell-to-cell heterogeneity. Most provocatively, when the authors cluster their single-gene models based on the weights of the various image metrics, they uncover networks of transcripts with strong functional ties to the cellular features weighted most heavily. For instance, genes with strong mitochondrial-feature weightings are enriched in mitochondrially encoded transcripts, and those with strong nearest-neighbor weightings are enriched in autocrine-paracrine effectors. These findings suggest that the steady-state abundance of mature mRNAs is largely dictated by microenvironmental and morphometric inputs that vary from cell to cell in the population.

Predictability of transcripts in the cytoplasm is not incompatible with stochastic transcriptional bursting in the nucleus. If primary RNAs reside in the nucleus for a time before export, then this residence time can dampen the consequences of bursting. Indeed, both Battich et al. (2015) and a forthcoming paper from the Itzkovitz group (Bahar Halpern et al., 2015) show by different computational approaches that nuclear retention attenuates noisy cytoplasmic fluctuations, which would otherwise result from transcriptional bursting. Bahar Halpern et al. uncover multiple examples of transcripts that are strongly retained in the nucleus and suggest that certain 3′ UTR sequences may have evolved to promote buffering from noise. Battich et al. (2015) provide a direct test of the nuclear-retention hypothesis, showing by genetic perturbation that global delays in mRNA export reduce noise in cytoplasmic transcripts without affecting overall copy number. Thus, by splitting the Central Dogma into two subcellular compartments, eukaryotes can prevent transcriptional noise from impacting the coordinated cellular functions of translated proteins.

The work of Battich et al. (2015) is an exemplar of modern systems biology, wherein large-scale experiments enable computational models to uncover cell-biological principles that extend beyond the systems field (Janes and Lauffenburger, 2013). Prior work on RNA sequencing of single-cell isolates has demonstrated that cell-cycle status confers a dominant pattern of cell-to-cell variability (Buettner et al., 2015). Battich et al. (2015) go further by collecting transcriptomic profiles in situ and illustrating the strong influence that local context has on a cell's transcript abundance. The work thereby puts a disclaimer on single-cell transcriptomic methods that require the dissociation of adherent cells. Although adequate for lineage mapping, single-cell RNA sequencing of detached cells likely distorts transcript abundances that adapt to cellular context (Wang et al., 2014).

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More generally, there are many interesting questions raised by the authors' findings. For example, extensive protein bursting is observed when a reporter downstream of the HIV LTR is randomly inserted in a mammalian genome (Dar et al., 2012). Was the excess variability here caused by the lentiviral promoter or by the fact that the lymphocytic recipient cells lacked most of the contextual features used by Battich et al. (2015)? One also ponders the extensibility of the findings to 3D tissues, where cellular context is likely to be an even greater contributor to transcriptional regulatory heterogeneity. The bDNA FISH method of Battich et al. (2015) has not been adapted to tissue sections as achieved by Bahar Halpern et al. (2015) with standard oligonucleotide-based FISH. However, the 100-fold improved brightness of bDNA FISH opens up the possibility of confocal or two-photon microscopy of thick tissue slices in the future.

Stochastic bursts of activity arise outside of biology; namely, in telecommunication systems that transmit information as network packets. Communication channels stay within specified bandwidth limits by implementing a "leaky bucket" algorithm, which collects incoming packets and transmits them at a fixed rate regardless of when activity surges (Parekh and Gallager, 1993). RNAs do not leak through nuclear pore complexes, but active transport is slow enough to endow the nucleus with bucket-like characteristics. This noise-buffering analogy may even hold for the smaller nuclei of simpler eukaryotes, such as yeast, whose protein expression noise is mostly well behaved (Bar-Even et al., 2006). The results of Battich et al. (2015) and Bahar Halpern et al. (2015) should motivate further study into nuclear mRNA retention and the contextual drivers of cytoplasmic mRNA abundance.

REFERENCES

Bahar Halpern K, Caspi I, Lemze D, Levy M, Landen S, Elinav E, Ulitsky I, Itzkovitz S. Nuclear retention of mRNA in mammalian tissues. Cell Rep. 2015 in press.

Bar-Even A, Paulsson J, Maheshri N, Carmi M, O'Shea E, Pilpel Y, Barkai N. Noise in protein expression scales with natural protein abundance. Nat Genet. 2006; 38:636–643. [PubMed: 16715097]

Battich N, Stoeger T, Pelkmans L. Control of transcript variability in single mammalian cells. Cell. 2015 this issue.

Buettner F, Natarajan KN, Casale FP, Proserpio V, Scialdone A, Theis FJ, Teichmann SA, Marioni JC, Stegle O. Computational analysis of cell-to-cell heterogeneity in single-cell RNA-sequencing data reveals hidden subpopulations of cells. Nat Biotechnol. 2015; 33:155–160. [PubMed: 25599176]

Dar RD, Razooky BS, Singh A, Trimeloni TV, McCollum JM, Cox CD, Simpson ML, Weinberger LS. Transcriptional burst frequency and burst size are equally modulated across the human genome. Proc Natl Acad Sci U S A. 2012; 109:17454–17459. [PubMed: 23064634]

Janes KA, Lauffenburger DA. Models of signalling networks - what cell biologists can gain from them and give to them. J Cell Sci. 2013; 126:1913–1921. [PubMed: 23720376]

Parekh AK, Gallager RG. A Generalized Processor Sharing Approach to Flow Control in Integrated Services Networks: The Single-Node Case. IEEE ACM T Network. 1993; 1:344–357.

Raj A, van Oudenaarden A. Nature, nurture, or chance: stochastic gene expression and its consequences. Cell. 2008; 135:216–226. [PubMed: 18957198]

Wang CC, Bajikar SS, Jamal L, Atkins KA, Janes KA. A time- and matrix-dependent TGFBR3-JUND-KRT5 regulatory circuit in single breast epithelial cells and basal-like premalignancies. Nat Cell Biol. 2014; 16:345–356. [PubMed: 24658685]

Wang CC, Jamal L, Janes KA. Normal morphogenesis of epithelial tissues and progression of epithelial tumors. Wiley Interdiscip Rev Syst Biol Med. 2012; 4:51–78. [PubMed: 21898857]

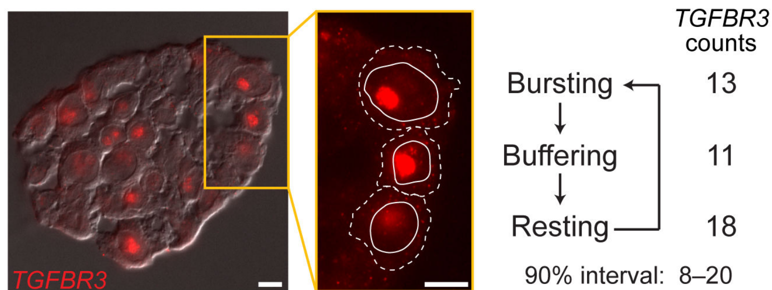

Figure 1. Nuclear buffering of a transcriptional regulatory heterogeneity

Basal-like breast epithelia were cultured as 3D spheroids, cryosectioned, and stained for *TGFBR3* by RNA FISH as in Wang et al. (2014). Nuclear (solid) and plasma membrane (dashed) borders were highlighted and cytoplasmic transcripts counted visually. The 90% confidence interval is shown for a Poisson random variable with mean of 14.

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