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Precise Developmental Gene Expression Arises from Globally Stochastic Transcriptional Activity

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SUMMARY

Early embryonic patterning events are strikingly precise, a fact that appears incompatible with the stochastic gene expression observed across phyla. Using single-molecule mRNA quantification in *Drosophila* embryos, we determine the magnitude of fluctuations in the expression of four critical patterning genes. The accumulation of mRNAs is identical across genes and fluctuates by only ~8% between neighboring nuclei, generating precise protein distributions. In contrast, transcribing loci exhibit an intrinsic noise of ~45% independent of specific promoter-enhancer architecture or fluctuating inputs. Precise transcript distribution in the syncytium is recovered via straightforward spatiotemporal averaging, i.e., accumulation and diffusion of transcripts during nuclear cycles, without regulatory feedback. Common expression characteristics shared between genes suggest that fluctuations in mRNA production are context independent and are a fundamental property of transcription. The findings shed light on how the apparent paradox between stochastic transcription and developmental precision is resolved.

INTRODUCTION

A fundamental question in biology concerns the degree of precision that cellular systems exhibit in their responses to a given set of environmental conditions, extracellular signals, or other input stimuli (Lagha et al., 2012; Lander 2013, Little and Wieschaus, 2011). Production of and interactions between molecules are intrinsically stochastic, limiting the ability of cells to control gene expression and biochemical activities (Raser and O'Shea, 2005), but the propensity of cellular systems to achieve appropriate phenotypic behavior constrains the tolerable magnitude of molecular fluctuations (Rao et al., 2002). In most contexts, it is unknown how closely cellular activity and phenotypic behavior rely on precise control of gene expression.

Many features of *Drosophila* embryogenesis suggest that strict control of gene expression determines reproducible and precise cell fate establishment. In *Drosophila* embryos, patterned gene expression in the early syncytium of ~6,000 nuclei is triggered by asymmetrically distributed, maternally supplied cues (Sauer et al., 1996). Among these is the transcription factor Bicoid (Bcd), the anterior-posterior (AP) concentration gradient of which shows remarkably reproducible distributions between embryos (Gregor et al., 2007). Moreover, within an embryo, the nuclei at similar AP coordinates differ in Bcd concentration by less than 10% (SD over mean), a degree of precision sufficiently high for each row of cells along the AP axis to discern its position from its immediate neighbors (Gregor et al., 2007). Bcd precision correlates with highly precise protein distribution of zygotically expressed target genes (Dubuis et al., 2013; Gregor et al., 2007) that confer cells with distinct gene expression programs within under 3 hr following fertilization (Gergen et al., 1986; Kornberg and Tabata, 1993).

These observations suggest a model in which tightly regulated transcriptional inputs give rise to rapidly established, highly precise outputs. However, the degree of precision in developmental transcription is largely unexplored. In all contexts assayed from prokaryotes to mammalian cells, absolute levels of a given transcript differ by at least ~50% between genotypically identical cells, and for a majority of genes, this variability is even higher (Cohen et al., 2009; Gandhi et al., 2011; Golding et al., 2005; Paré et al., 2009; Taniguchi et al., 2010; Raj et al., 2006, 2010; Reiter et al., 2011; Sigal et al., 2006; Zenklusen et al., 2008). Quantitative observations support the idea that the process of transcription is intrinsically stochastic (Kaern et al., 2005; Li and Xie, 2011). In developmental contexts, it is unknown whether relatively small input transcription factor fluctuations impact the transcriptional output and whether embryogenesis requires the activity of specialized filtering and/or feedback mechanisms to ensure fidelity in the rapid establishment of gene expression programs.

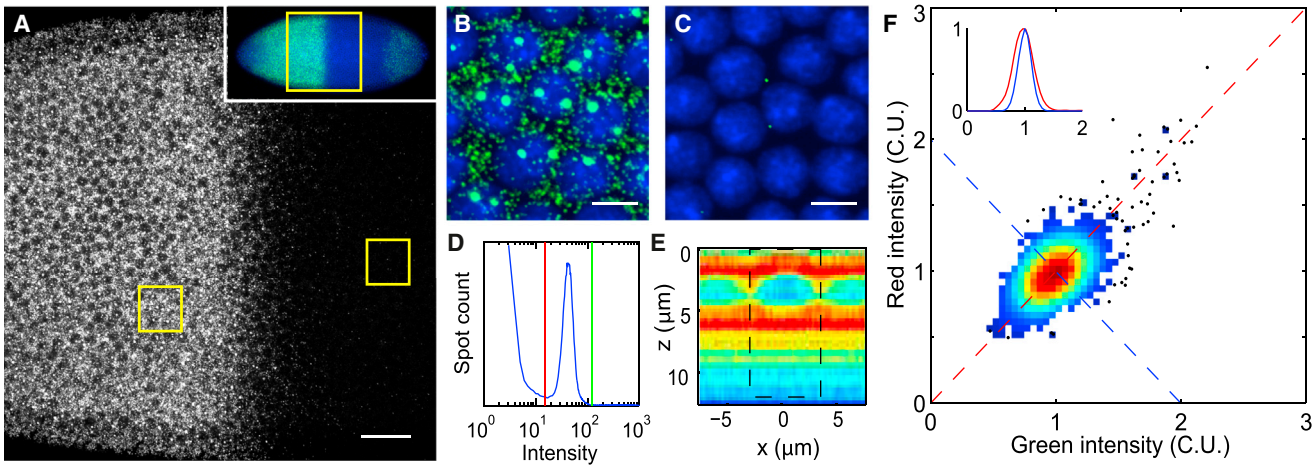

Figure 1. Counting of Absolute Transcript Number in *Drosophila* Embryos

(A) Confocal section through the nuclear layer of a WT embryo during interphase 13 labeled with 114 fluorescent oligonucleotide probes against *hb*, oriented anterior to the left, is shown. Scale bar, 25 μm. Inset is a low-magnification image identifying the region shown in (A).

(B and C) Magnified views of anterior (B) and posterior (C) boxed regions in (A) are presented. hb FISH probes (green) and DAPI staining of DNA (blue). Scale bars, 5 μm.

(D) Particle intensity histogram shows thresholds separating transcripts from noise (red line) and from the long tail of bright transcription sites (green line).

(E) *hb* transcript distribution in axial cross-section through a nucleus centered at x = 0 is shown. z = 0 represents apical surface. Color indicates mean particle density in relative units (red shows high; blue shows low). Dashed box indicates cylindrical summation volume.

(F) Intensity scatterplot in two channels using probes of alternating colors is illustrated. Data point density is given by color; black dots show single point outliers. Inset presents cross-sections of scatterplot in (F) along the correlated (red) and anticorrelated direction (blue) showing Gaussian distributions with σ = 20% (red) and σ = 12% (blue) after normalization to mean cytoplasmic particle intensity (1 "cyto unit" [C.U.]).

See also Figures S1 and S2.

Here, we address these questions with an enhanced method of fluorescence in situ hybridization (FISH) and accompanying image analysis (Little et al., 2011) to label and detect individual zygotically expressed mRNA molecules. We measure in absolute molecular counts the magnitude and fluctuations in the earliest gene expression events of the *Drosophila* embryo. To separate input fluctuations from variability intrinsic to transcription, we focus on those spatial domains in which gene expression is maximally unconstrained. Here, patterning inputs do not determine expression output levels, and thus, input fluctuations cannot impact output variability. These regions thereby reveal the greatest degree of precision achievable by the system. We show that in these regions, the earliest expressed genes share common expression characteristics: despite their expression in spatially distinct territories, their rates of production are identical, and all display intrinsically stochastic transcriptional activity. These similarities suggest that expression rate and variability result from fundamental, global features of transcriptional regulation that limit the attainable degree of precision. Nevertheless, the stochastic expression results in precise and nearly uniform transcript accumulation, achieved by straightforward spatiotemporal averaging.

RESULTS

Measuring Absolute Numbers of mRNA Transcripts in Early *Drosophila* Embryos

Previous work in *Drosophila* embryos has documented that nuclei at similar AP coordinates express nearly equivalent protein amounts of the gap gene Hunchback (Hb) with fluctuations of <10% (Gregor et al., 2007). The transcriptional activator of Hb, Bcd, displays variability on the same order as Hb (Gregor et al., 2007). A precise transcriptional response of the *hb* locus presents the most straightforward though as yet untested explanation of minimal Hb variation. To quantitatively evaluate transcription of *hb*, we adapted a FISH method developed previously (Little et al., 2011) to label *hb* mRNAs using multiple fluorescently labeled antisense DNA oligonucleotides (Figure 1A). By scanning confocal microscopy, we detect two broad classes of objects: sparse, bright spots representing sites of nascent transcription (e.g., Wilkie et al., 1999); and numerous diffraction limited spots, ~90% of which are located in the internuclear space that we refer to as cytoplasmic particles (Figures 1A–1C). These particles exhibit sufficiently high contrast to be readily distinguished from background imaging noise using automated image processing (Figure 1D). Each particle is found on at least three adjacent 250 nm confocal imaging sections with three-dimensional structure identical to the measured point spread function (PSF; Figures S1A–S1D available online). To test detection efficiency, we applied probes with alternating fluorophore colors. A minimum of 85% of cytoplasmic particles detected in one channel are found in the other, indicating that >94% of mRNAs are detected in at least one channel (Figures S1E–S1G).

Tight unimodal clustering around mean intensity suggests that the cytoplasmic particles are similar in mRNA content (Figure 1D). Deviation from mean intensity results from at least two phenomena: particles can be bound by different probe numbers, and multiple particles can overlap and be detected as single

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