Transcription is an inherently noisy molecular process that takes place as burst-like events (Boettiger and Levine, 2009; Eldar and Maheshri, 2010). How the incoherent transcriptional bursts lead to cell-to-cell differences including fluctuations in activator concentration. We use our developed methods to analyze the Drosophila Bicoid (Bcd) morphogen gradient system. For its target gene hunchback (hb), the noise properties can be recapitulated by a simplified gene regulatory model in which Bcd acts as the only input, suggesting that the external noise in hb transcription is primarily derived from fluctuations in the Bcd activator input. However, such a simplified gene regulatory model is insufficient to predict the noise properties of another Bcd target gene, orthodenticle (otd), suggesting that otd transcription is sensitive to additional external fluctuations beyond those in Bcd. Our results show that analysis of the relationship between input and output noise can reveal important insights into how a morphogen gradient system works. Our study also advances the knowledge about transcription at a fundamental level. We assume that, at any given moment of time, each copy of a native gene inside a cell can exist in either a bursting (active) or non-bursting (inactive) state. The experimentally measured total noise in the transcriptional states of a gene in a population of cells can be mathematically dissected into two contributing components: internal and external. While internal noise quantifies the stochastic nature of transcriptional bursts, external noise is caused by cell-to-cell differences including fluctuations in activator concentration. Here we develop a statistical model for such a purpose, where we mathematically dissect the experimentally measured total noise into the internal and external components.

We use our model to analyze our experimental data extracted from early Drosophila embryos (He et al., 2011), where both the nuclear concentrations of the activator Bicoid (Bcd) and the transcriptional states of its target genes hunchback (hb) and orthodenticle (otd) are simultaneously measured. We show that the dissected external noise in hb transcriptional bursts can be implicitly derived from the measured Bcd input noise. In contrast, additional input noise is required to account for both the measured total noise and the dissected external noise in otd transcription. These results suggest that, although Bcd acts as a direct and sustained input for both otd and hb transcription (He et al., 2011; Liu et al., 2011), these two target genes are distinct from each other. Our findings are consistent with recent studies (Kim et al., 2011b; Lohr et al., 2009; Ochoa-Espinosa et al., 2009), suggesting that, in addition to Bcd, otd transcription also responds to other input(s) and, thus, is sensitive to additional external fluctuations. Our study provides an example illustrating the use of dissecting experimentally measured transcriptional noise in understanding the mechanistic operations of a native morphogen gradient system.

# 2 METHODS

To obtain our published dataset (He et al., 2011), we combined fluorescence in situ hybridization with immunostaining on 1–4 h z11B embryos. Here, we used flattened embryos to maximize the number of nuclei per embryo and we took ~6 Confocal z-section images in 0.5 μm intervals to capture all the intron dots. Our imaging setting was maintained the same for different embryos on different slides with all images captured in a single imaging cycle. Thus the datasets under our experimental conditions have the same molecule number-to-intensity rescaling factors, which make it possible to combine the data extracted from different embryos and group nuclei according to their A–P positions. In our detection for intron dots, the definition of thresholds is according to He et al. (2011) and the threshold setting was optimized to detect a stable pattern of the intron dot expression. A comparison between machine-recognized intron dots and human-recognized dots indicates an uncertainty of 8% in our analysis. See He et al. (2011) for further technical details and intron dot data quality, and He et al. (2008, 2011a) for Bcd data quality under our experimental conditions. In our current analysis, we use each detected intron dot to denote a gene copy that is actively
transcribing in a snapshot (see main text for details). The distributions of experimentally detected intron dot numbers suggest that the two copies of hh or od in a nucleus are independent (not shown).

3 RESULTS

3.1 A statistical model for evaluating transcriptional noise

Let us consider a standard diploid cell that has two identical copies of a gene. At a given moment of time, i.e. in a snapshot of the cell, each copy can exist in either an actively-transcribing (bursting) or non-transcribing (non-bursting) state. Experimentally, actively transcribing gene copies can be detected as ‘introns dots’ (see Section 2 and further on for experimental details). We define gene copies that are actively transcribing as active copies and those that are not as inactive copies. We assume that both copies of the gene in this cell are independent of each other. They have an identical probability $p$ of existing as active copies. Thus, the probability of this cell to have $k$ active copies of the gene in a snapshot can be expressed as in Equation (1). The mean and noise (i.e. fractional variance) of the number of active copies in this cell are $p = 2p$ and $\eta^2 = (1-p)/2p$.

We then consider a population of $N$ cells. We assume that all copies of the gene in this population are independent. For the $i$-th cell in this population, we denote the probability of its gene copies existing as active copies by $p_i$. We now evaluate all copies of the gene in all the cells in this population. The mean and noise of number of active copies per cell can be expressed, respectively, as in Equations (2) and (3).

$$p = 2p = \sum_{i=1}^{N} p_i$$

\[\eta_{\text{tot}}^2 = \frac{1}{p^2N} \sum_{i=1}^{N} \sum_{k=0}^{2} \binom{2}{k} p_i^k (1-p_i)^{2-k} (k-2p_i)^2 \]

In Equation (4), the total noise of the stated system, $\eta_{\text{tot}}$, is a simple sum of two components. One component is from the noise in $p$, which is induced by cell-to-cell differences that we define as external noise, $\eta_{\text{ext}}$. The other contribution, which we define as internal noise, $\eta_{\text{int}}$, is proportional to the variance of the intrinsic binomial distribution of the binary states, which is calculated for each cell and then averaged over the entire population of cells. The quantity $(p^2)$ can be calculated as:

\[\langle p^2 \rangle = \rho^2 (1 + \eta_{\text{tot}}^2) - \rho.\]

By experimentally measuring the mean number of active copies ($\rho$) and its total noise ($\eta_{\text{tot}}$), we can obtain both $\eta_{\text{ext}}$ and $\eta_{\text{int}}$ as:

$$\eta_{\text{ext}}^2 = \frac{2}{\rho} + 1 + 2\frac{\delta_{\text{tot}}}{\rho}$$

$$\eta_{\text{int}}^2 = \frac{2}{\rho} - 1 - \eta_{\text{tot}}^2.\]

In Supplementary Material, we present a general case of noise dissection in cells with $c$ identical copies of a gene. As discussed there, for genes with a single copy per cell ($c = 1$), such as $X$-linked genes in Drosophila males, the quantity $p^2$ cannot be measured and, consequently, $\eta_{\text{ext}}$ and $\eta_{\text{int}}$ cannot be dissected in our model.

The approach described above for noise dissection is broadly analogous to the previous concepts developed for single-cell systems (Elowitz et al., 2002; Hiltlinger and Paulsson, 2011; Raser and O’Shea, 2005; Swain et al., 2002; Thattai and van Oudenaarden, 2001), but it has several important features. In particular, the noise described here is based on the active and inactive copies of a gene, data that can be extracted from snapshots without the need to acquire live-imaging data. In addition, it is based on the endogenous copies of the gene (see Section 2 and further on for experimental details) and does not require engineered reporters such as dual reporters. The dual-reporter analysis requires an assumption that the two reporters and their products are identical because differences between the two reporters and products can falsely contribute to the internal noise component. The use of the endogenous gene copies as in our study does not require this assumption because both copies and their products are identical.

Furthermore, as discussed below, our experimental system is an entire embryo, where technically cells have not yet been formed at the time of analysis. Thus, the external source of fluctuations estimated in our study includes both embryo-to-embryo and nucleus-to-nucleus variances. For this reason, we use the terms ‘internal’ and ‘external’ as opposed to ‘intrinsic’ and ‘extrinsic’.

3.2 Propagation of activator input noise to transcriptional noise

We now consider the external noise in an ideal, simplified gene regulatory system where an activator is the only input for its target gene’s transcriptional output. As before (Gregor et al., 2007; He et al., 2008, 2011), we use a Hill function to describe the relationship between the expected probability of a gene copy to exist as an active copy ($p$) and the activator concentration inside the nucleus of a cell ($B$):

\[p(B) = \frac{p_{\text{max}}}{1 + (B/K)^n}\]

where $p_{\text{max}}$, $K$ and $h$ are, respectively, the maximal burst probability, the activator concentration at half maximal burst probability and the Hill coefficient. These values can be adjusted in theoretical studies for evaluating the behavior of different target genes or different transcription system. They can also be calculated directly from experimental data (see below). Equation (4) makes it possible, similar to propagation of errors (Gregor et al., 2007), to directly convert fluctuations in activator concentration, $\eta_B = dB/B$, to the variance of the burst probability of its target gene:

\[\delta_p = dB \frac{dp}{dB} \approx \eta_B h \rho (1 - \rho) / p_{\text{max}}.\]

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We now evaluate experimental data extracted from early Drosophila embryos (He et al., 2010a; Tkacik et al., 2008; Tostevin et al., 2007) that the noise in the nuclear concentration of this activator, B, is Poissonian, $\eta_B^2 = v/B$, where $v$ is referred to as the noise strength. Applying this distribution of $\eta_B$ to Equation (5) leads to the expression of $\eta_{tot}$ as a function of $\rho$:

$$\eta_{tot}^2 = \frac{v}{K} (\frac{\rho_{max}}{\rho} - 1) [1 - (1 - \frac{\rho}{\rho_{max}})^2].$$

(9)

Figure 1 shows $\eta_{tot}$, converted from activator input noise according to Equation (10), as a function of $\rho/\rho_{max}$. Figure 1A and B show the effects of $v/K$ and $h$, respectively, on $\eta_{tot}$. Thus, $\eta_{tot}$ can be calculated by two distinct methods: one that is based on the active and inactive states of the gene copies with the noise in transcription dissected according to Equation (5), and another that is based on the activator input noise that is converted to $\eta_{tot}$ using the input–output relationship in Equation (10). These two calculations should arrive at the same $\eta_{tot}$ value in the ideal, simplified gene regulatory system. We will use this property of an ideal, simplified system to evaluate those of the real biological systems. In particular, if the $\eta_{tot}$ values calculated from these two methods are similar, it would suggest that the actual biological system can be approximated by the simplified gene regulatory system, but a significant deviation between these two values would be indicative of oversimplification of the real biological system.

3.3 Evaluation of hh transcription data suggests a dominant role of Bcd input

We now evaluate experimental data extracted from early Drosophila embryos (He et al., 2010a; Tkacik et al., 2008; Tostevin et al., 2007). Bcd is a transcriptional activator that forms an anterior-to-posterior (A–P) gradient (Driever et al., 1991), instructs embryonic patterning by activating its target genes in a concentration-dependent manner (Ephrussi and St Johnston, 2004; Grimm et al., 2010; Liu et al., 2011; Porcher and Dostatni, 2010). During the period of Bcd action (i.e. at the syncytial stage of embryonic development), all nuclei undergo synchronous mitotic divisions and, thus, Bcd concentration represents a primary source of nucleus-to-nucleus differences relevant to Bcd target gene transcription. In our simplified gene regulatory model, cell-to-cell differences arise solely from activator concentration differences. In addition, our previous analyses of hh expression profiles in embryos with perturbed Bcd gradient properties suggest that Bcd acts as a dominant input at early nuclear cycle 14, a time for our current analysis (Cheung et al., 2011; He et al., 2008, 2010a, 2010b, 2011; Liu and Ma, 2011; Liu et al., 2011). At earlier cycles, maternal HB acts as an additional input for zygotic $hb$ transcription (Porcher et al., 2010). In our simplified gene regulatory model, the activator is the only input for the active copies of its target gene. These considerations suggest that $hb$ represents an excellent candidate of a native Bcd target gene suitable for evaluation against the simplified gene regulatory model.

As detailed previously (He et al., 2011), our experimental dataset consists of the Bcd concentration (in arbitrary units) and the $hb$ transcriptional states of individual nuclei of fixed wild-type (wt) embryos. Currently this is the only high-quality combined dataset that contains both the Bcd input data and the $hb$ transcriptional states in embryos. In our experiments, we used an intronic probe to detect the nascent $hb$ transcripts, with a simultaneous detection of Bcd through immunostaining. Images of these embryos thus capture, as in snapshots, distinct fluorescent dots, referred to as the $hb$ intron dots (He et al., 2011). The reliability of both Bcd input data (He et al., 2008, 2010a) and the intron dot data (He et al., 2011) under our experimental conditions has been described previously. For our current analysis, we count the number of the $hb$ intron dots in individual nuclei from the relevant parts of individual embryos. Here, we use a detected $hb$ intron dot to denote an active copy of the $hb$ gene. We sort our data by binning them according to the A–P position, and calculate the mean ($\rho$) and noise ($\eta_{tot}$) of active copies for nuclei within the bins. Using Equations (4) and (6), we dissect $\eta_{tot}$ into $\eta_{tot}$ and $\eta_{ext}$ for each bin. Figure 2A shows a scatter plot (on log-log scale) of the measured $\eta_{tot}$ (blue circles) and the dissected $\eta_{ext}$ (green diamonds) and $\eta_{int}$ (red squares) as a function of $\rho$. Figure 2A also shows our theoretical predictions (solid lines) calculated from Equation (12) using the following values that are derived from fitting the experimental data (see legends): $h=6$, $v=0.5$ and $K=6$. Our results show a resemblance between the measured/dissected noise profiles and those calculated theoretically. They suggest that the experimentally measured (and dissected) noise properties for $hb$ transcription are broadly similar to those predicted theoretically in a simplified gene regulatory model (see Figure 2 legend for adjusted $R^2$-values and below for an experimental system with contracting behaviors).

In our theoretical predictions shown as solid lines in Figure 2A, a Poissonian distribution of Bcd concentration fluctuations is assumed. To evaluate whether our conclusions are dependent on this assumption, we directly converted experimentally measured Bcd input noise to $\eta_{tot}$ using Equation (5) without such an assumption. Figure 2B shows a scatter plot of $\eta_{tot}$ values calculated by two distinct methods (see above): one from the measured noise in $hb$ intron dot number through Equation (5) (shown as the dissected
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Precise target gene expression boundaries. Our results show that the hh boundary position (marked by an arrowhead in Fig. 2A) coincides with a convergence of noise profiles that results in a more balanced contribution of $\eta_{\text{ext}}$ and $\eta_{\text{int}}$. These results suggest that, based on Saunders and Howard (2009), the Bcd gradient system in wt embryos may be optimal for the hh boundary precision.

3.4 Dissection of otd transcriptional noise suggests a role of additional input(s)

In addition to hh, Bcd also activates the expression of another target gene otd in early Drosophila embryos (Gao and Finkelstein, 1998). Unlike hh, the Bcd-activated otd expression boundary position is much closer to the anterior of the embryo. Although Bcd also acts as a direct and sustained input for otd transcription (He et al., 2011), recent studies suggest that otd expression is subject to regulation by both the Bcd gradient and the terminal system inputs mediated by a mitogen-activated protein kinase (MAPK) pathway (Kim et al., 2011a, b; Lobe et al., 2009; Ochon-Espinosa et al., 2009; Porcher and Dostatni, 2010). Thus, the otd transcriptional system may significantly deviate from our simplified gene regulatory model, where an activator is assumed to be the only input for its target gene transcription. To test this idea, we analyze our otd data in a manner identical to our hh analysis described above. As discussed in Supplementary Material and above, male embryos have a single copy of otd (i.e. $c = 1$) and, thus, such data are not suitable for the noise dissection. Here, we present our analysis of the otd intron dot data from female embryos.

Figure 2C shows the measured $\eta_{\text{tot}}$ (blue circles) and dissected $\eta_{\text{tot}}$ (red squares) and $\eta_{\text{ext}}$ (green diamonds) in otd intron dot number. It also shows the theoretically predicted noise profiles (solid lines) for otd transcription based on our simplified gene regulatory model using the following values derived from fitting the experimental data (see Fig. 2 legend): $h = 4$, $v = 0.5$ and $K = 15$. These results show that, in contrast to hh (Fig. 2A), the external noise in otd transcription dissected from experimental data (green diamonds) is significantly higher than what is theoretically predicted (green line). As discussed above, these results suggest that, in addition to the Bcd input noise, otd transcription is sensitive to other source(s) of input (external) noise missing in our simplified gene regulatory model. Figure 2C further shows that, unlike the hh transcriptional system, theoretically predicted $\eta_{\text{int}}$ and $\eta_{\text{tot}}$ also deviate from the measured/dissected values.

To further evaluate the otd transcriptional system, we plot in Figure 2D the dissected $\eta_{\text{tot}}$ against the converted $\eta_{\text{ext}}$ values calculated by the two different methods for different A–P positions (see above). While dissected $\eta_{\text{tot}}$ is much higher than converted $\eta_{\text{tot}}$ in absolute values, they are correlated with each other. These results suggest that the proposed additional input (external) noise for otd transcription is correlated with Bcd input noise. We currently do not know the exact source of this noise. It is interesting to note that recent studies have revealed a retroactive regulatory mechanism where Bcd, a MAPK substrate, can in turn affect MAPK activity and its availability to other substrates (Kim et al., 2011a, b). It remains to be investigated whether the retroactivity of Bcd on MAPK may represent a potential mechanism that can lead to an ‘amplification’ of the Bcd input noise in term of otd transcription.
4 CONCLUSIONS

Our analysis of the noise properties of transcriptional bursts in response to an activator input provides a useful framework for investigating how developmental decisions are made. An important feature of our statistical model is that it does not require knowledge about, or modeling of, the specific molecular steps leading to such stochastic bursts. The feasibility to capture the actively-transcribing copies of a variety of native genes in early Drosophila embryos (Boettiger and Levine, 2009; Pare et al., 2009; Wilkie et al., 1999) suggests that our model may be of general use in evaluating other transcriptional systems. As shown in our current study, dissection of noise in transcription, coupled with the simultaneous measurement of the activator input noise, can provide critical insights—at a systems level—into whether the activator in hand is a dominant input for a gene’s transcription in a native developmental system. Understanding the relationship between the morphogen input and target genes’ transcriptional output is crucial to advancing the morphogen concept, a cornerstone of developmental biology (Kerszberg and Wolpert, 2007; Lander, 2007; Wartlick et al., 2009). These results suggest that, consistent with our recent studies (Cheung et al., 2011; He et al., 2008, 2010a, 2010b; Liu et al., 2011), Bcd is a dominant input for hb expression at the time of our analysis. The observed properties for hb contrast with those of otd, suggesting the contribution of another input(s) for otd transcription. Together, our findings represent an important step toward enhancing our knowledge of morphogen action at a systems level.

Our study also advances our knowledge about transcription at a fundamental level. As discussed recently (Hilfinger and Paulsson, 2011), in dual-reporter studies performed in single cell systems, cell-to-cell differences other than activator concentration differences, e.g. cell cycle stage differences, are also included in the extrinsic component of transcriptional noise. The nuclei in the embryos that we analyzed are nearly synchronous with regard to cell cycle stage. This special property has contributed to our ability to document experimentally, for the first time to our knowledge, that the external noise of a gene’s transcription can be explained by the activator input noise.

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