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Evaluating the *Drosophila* Bicoid morphogen gradient system through dissecting the noise in transcriptional bursts

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ABSTRACT

Motivation: We describe a statistical model to dissect the noise in transcriptional bursts in a developmental system.

Results: 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. 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.

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1 INTRODUCTION

Transcription is an inherently noisy molecular process that takes place as burst-like events (Boettiger and Levine, 2009; Eldar and Elowitz, 2010; Friedman *et al*., 2006; Ma, 2011; Raj *et al*., 2006; To and Maheshri, 2010). How the incoherent transcriptional bursts lead to coherent and precise gene expression patterns during development is a fundamental question and a subject of intense investigations (Martinez Arias and Hayward, 2006). It has been suggested that averaging over time and/or space allows a biological system to gain coherence and improve expression boundary precision (Erdmann *et al*., 2009; Gregor *et al*., 2007; He *et al*., 2010a; Okabe-Oho *et al*., 2009; Tostevin *et al*., 2007). To fully understand how precise gene expression patterns are achieved in response to morphogen inputs, it is necessary to have methods that can evaluate the experimentally detected transcriptional bursts before such averaging takes place. 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*., 2011a, b; 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 w^{1118} 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, 2010a) 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

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transcribing in a snapshot (see main text for details). The distributions of experimentally detected intron dot numbers suggest that the two copies of *hb* or *otd* 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 'intron 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 numbers of active copies in this cell are $\rho=2p$ and $\eta^2=(1-p)/2p$.

$$p(k)=\binom{2}{k}p^k(1-p)^{2-k}, \text{ for } k\in\{0,\ 1,\ 2\} \tag{1}$$

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).

$$\rho\equiv 2\langle p\rangle=\frac{2}{N}\sum_{1}^{i=N}p_i \tag{2}$$

$$\begin{aligned}\eta^2_{\text{tot}}&\equiv\frac{1}{\rho^2 N}\sum_{1}^{i=N}\sum_{0}^{k=2}\binom{2}{k}p_i^k(1-p_i)^{2-k}(k-2p_i)^2 \\ &=\frac{4}{\rho^2 N}\sum_{1}^{i=N}(p_i-\langle p\rangle)^2+\frac{2}{\rho^2 N}\sum_{1}^{i=N}p_i(1-p_i) \\ &=\frac{\left\langle p^2\right\rangle-\langle p\rangle^2}{\langle p\rangle^2}+\frac{\langle p\rangle-\left\langle p^2\right\rangle}{2\langle p\rangle^2}\equiv\eta^2_{\text{ext}}+\eta^2_{\text{int}}\end{aligned} \tag{3}$$

In Equation (3), the total noise of the stated system, η_{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, η_{ext}. The other contribution, which we define as internal noise, η_{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 $\langle p^2\rangle$ can be calculated as:

$$\left\langle p^2\right\rangle=\rho^2(1+\eta^2_{\text{tot}})-\rho. \tag{4}$$

By experimentally measuring the mean number of active copies (ρ) and its total noise (η_{tot}), we can obtain both η_{ext} and η_{int} as:

$$\eta^2_{\text{ext}}=-\frac{2}{\rho}+1+2\eta^2_{\text{tot}} \tag{5}$$

$$\eta^2_{\text{int}}=\frac{2}{\rho}-1-\eta^2_{\text{tot}}. \tag{6}$$

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 $\langle p^2\rangle$ cannot be measured and, consequently, η_{ext} and η_{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; Hilfinger and Paulsson, 2011; Raser and O'Shea, 2004; Raser and O'Shea, 2005; Swain *et al*., 2002; Thattai and van Oudenaarden, 2001), but it has several important features. In particular, the noise defined 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):

$$p(B)=\frac{p_{\max}}{1+(B/K)^{-h}}, \tag{7}$$

where $p_{\max}$, 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 (7) makes it possible, similar to propagation of errors (Gregor *et al*., 2007), to directly convert fluctuations in activator concentration, $\eta_B=\delta B/B$, to the variance of the burst probability of its target gene:

$$\delta_p=\delta B\left|\frac{d_p}{dB}\right|\approx\eta_B h\langle p\rangle(1-\frac{\langle p\rangle}{p_{\max}}). \tag{8}$$

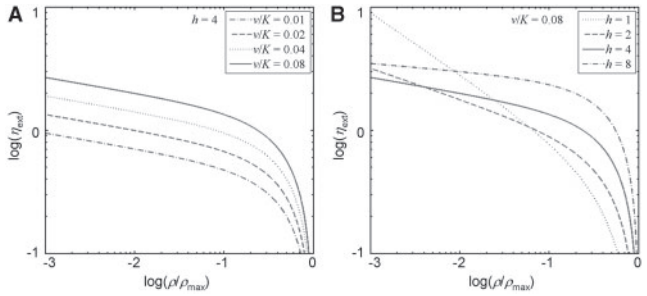

Fig. 1. Parameters affecting noise propagation. Activator input noise is converted to external noise (η_{ext}) in target gene transcription based on Equation (10) in a simplified gene regulatory model. Here, η_{ext} is shown as a function of ρ_i, the mean number of active copies. The effects of parameters that affect noise propagation are also shown. Such parameters include the activator noise strength (v), the activator threshold concentration (K) and the Hill coefficient (h). Figure 1A shows that η_{ext} increases as the normalized noise strength (v/K) increases. Figure 1B shows that, at a given v/K, the effect of h on η_{ext} is sensitive to ρ.

As defined in Equation (3), the external noise (η_{ext}) in the transcriptional bursts of the target gene is the noise in p:

$$\eta_{ext} \equiv \frac{\delta_p}{\langle p \rangle} \approx \eta_B h (1 - \frac{\rho}{\rho_{max}}), \tag{9}$$

where $\rho_{max} = 2\, p_{max}$.

To facilitate calculations in a theoretical analysis, we assume (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 η_B to Equation (9) leads to the expression of η_{ext} as a function of ρ:

$$\eta_{ext}^2 = \frac{v h^2}{K} (\frac{\rho_{max}}{\rho} - 1)^{1/h} (1 - \frac{\rho}{\rho_{max}})^2. \tag{10}$$

Figure 1 shows η_{ext}, converted from activator input noise according to Equation (10), as a function of ρ/ρ_{max}. Figure 1A and B show the effects of v/K and h, respectively, on η_{ext}. Thus, η_{ext} 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 η_{ext} using the input–output relationship in Equation (10). These two calculations should arrive at the same η_{ext} 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 η_{ext} 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 *hb* transcription data suggests a dominant role of Bcd input

We now evaluate experimental data extracted from early *Drosophila* embryos (He *et al*., 2011). Bcd is a transcriptional activator that forms an anterior-to-posterior (A–P) gradient (Driever and Nusslein-Volhard, 1988; Houchmandzadeh *et al*., 2002). It 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, nuclear 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 *hb* 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 (ρ) and noise (η_{tot}) of active copies for nuclei within the bins. Using Equations (5) and (6), we dissect η_{tot} into η_{ext} and η_{int} for each bin. Figure 2A shows a scatter plot (on log–log scale) of the measured η_{tot} (blue circles) and the dissected η_{ext} (green diamonds) and η_{int} (red squares) as a function of ρ. Figure 2A also shows our theoretical predictions (solid lines) calculated from Equation (10) 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 Fig. 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 η_{ext} using Equation (9) without such an assumption. Figure 2B shows a scatter plot of η_{ext} 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

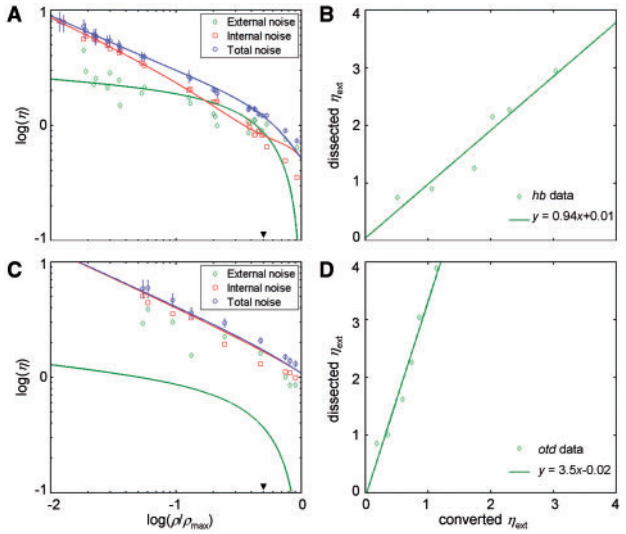

Fig. 2. *hb* and *otd* have distinct noise properties in relation to the Bcd input noise. The experimentally measured η_{tot} (blue circles) in *hb* (A) and *otd* (C) intron dot numbers is dissected into η_{ext} (green diamonds) and η_{int} (red squares) according to Equations (5) and (6), and the results are plotted against ρ. Solid lines are theoretical predictions based on Equation (10) in the simplified gene regulatory model. The adjusted R_2-values of fitting the experimental data with the model for η_{ext},η_{int} and η_{tot} of *hb* are, respectively: 0.85, 0.99 and 1.00; the adjusted R_2-values for η_{ext}, η_{int} and η_{tot} of *otd* are, respectively: −0.48, 0.97 and 0.98. The negative R_2 suggests that, constrained by the measured parameter values, the model cannot predict η_{ext} of otd. Arrowheads show the boundary positions of $\rho = \rho_{max}/2$. Here, K–values are measured as the mean Bcd concentrations at the marked boundary positions and *h*–values are extracted by fitting Equation (7) with the experimentally measured *B*–ρ profiles (He *et al.*, 2011). Since fluctuations in Bcd concentration are dominated by Poisson-like molecular noise (He *et al.,* 2010a), we perform a simple fitting of the experimentally measured Bcd noise to Poissonian distribution for extracting *v*-values. Figure 2B and D are scatter plots of dissected η_{ext} (same as in Figures 2A and C but showing data only from the activation boundary regions for better data spread) against converted η_{ext} (see text for details). Here, Figures 2B and C are for hb and *otd*, respectively. A linear regression line is also shown (with the equation in the inset box) for each panel.

η_{ext} in figure) and another converted from the experimentally measured Bcd noise through Equation (9) (shown as the converted η_{ext} in figure). The two η_{ext} values at individual A–P positions exhibit good agreement with each other, suggesting that, consistent with a previous study (He *et al*., 2010a), molecular (Poissonian) fluctuations in Bcd concentration are a dominant source of the measured Bcd noise in relevant parts of the embryos under our experimental conditions. Furthermore, as discussed above, the experimentally measured (and dissected) noise properties of the *hb* transcription system are well approximated by our simplified gene regulatory model. Together, they further support the suggestion that the nucleus-to-nucleus fluctuations in Bcd concentration are a dominant source of the external noise in *hb* transcriptional bursts (see also Holloway *et al*., 2011).

A recent theoretical study (Saunders and Howard, 2009) has suggested that an exponential morphogen gradient may be evolutionarily advantageous because of its ability to balance between the external and internal sources of noise for achieving precise target gene expression boundaries. Our results show that the *hb* boundary position (marked by an arrowhead in Fig. 2A) coincides with a convergence of noise profiles that results in a more balanced contribution of η_{ext} and η_{int}. These results suggest that, based on Saunders and Howard (2009), the Bcd gradient system in wt embryos may be optimal for the *hb* boundary precision.

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

In addition to *hb*, Bcd also activates the expression of another target gene *otd* in early *Drosophila* embryos (Gao and Finkelstein, 1998). Unlike *hb*, 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; Lohr *et al*., 2009; Ochoa-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 *hb* 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 η_{tot} (blue circles) and dissected η_{int} (red squares) and η_{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 *hb* (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 *hb* transcriptional system, theoretically predicted η_{tot} and η_{int} also deviate from the measured/dissected values.

To further evaluate the *otd* transcriptional system, we plot in Figure 2D the dissected η_{ext} against the converted η_{ext}, values calculated by the two different methods for different A–P positions (see above). While dissected η_{ext} is much higher than converted η_{ext} 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, 2011; 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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