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A matter of timing and precision

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A recent paper in *Molecular Systems Biology* by de Lachapelle and Bergmann (2010) has renewed the claim—first made by Bergmann *et al* (2007)—that the Bcd morphogen gradient in the early *Drosophila* embryo is exerting its regulatory influence before reaching steady state. There are several significant problems with the data and reasoning presented to support this claim, which stands in stark contrast to previously published quantitative evidence.

The central problem affecting the assertions of de Lachapelle and Bergmann (2010) is that the data claimed to support them were obtained at the wrong developmental stage. Moreover, the reasoning used to interpret these data does not take into account new results obtained after the original study by Bergmann *et al* (2007) was published.

With respect to the data, the authors measure the variance of gap and pair-rule domain positions in 'cellularizing embryos displaying a distinct seven-stripe Eve pattern' (Bergmann *et al*, 2007), which covers a period of approximately 30 min during the late blastoderm stage, right before the onset of gastrulation (see Figure 1 in the study by Surkova *et al*, 2008). The expression patterns at this stage are not suitable to support the authors' claims for the following reasons:

Pertaining to Bcd itself, there is strong quantitative evidence that the nuclear concentration of Bcd (which exerts its effect as a transcription factor) not only remains stable during the late blastoderm stage, but actually starts to disappear shortly before gastrulation (Gregor *et al*, 2007; Surkova *et al*, 2008). This indicates that the Bcd gradient is not pre- but post-steady state at the time target domain boundaries were measured.

Regarding Bcd targets, there is a serious discrepancy between the developmental stage, at which measurements were taken, and the stage for which Bcd gradient formation is modeled. The Bcd gradient forms and positions its target domain boundaries during the cleavage and early blastoderm stages, more than an hour before domain precision was measured (Gregor *et al*, 2007; Jaeger *et al*, 2007). During this hour, gap and pair-rule gene products accumulate, and replace maternal gradients (such as Bcd) as the predominant regulators of domain boundary positions (Jaeger *et al*, 2004; Jaeger *et al*, 2007). Interactions among these downstream factors significantly increase precision of the system, which becomes progressively de-correlated from the spatial distribution of positional errors in the Bcd gradient (Holloway *et al*, 2006; Jaeger *et al*, 2007; Surkova *et al*, 2008; Manu *et al*, 2009). These studies demonstrate that measurements of expression domains at the late blastoderm do not measure direct response to Bcd, but rather the state of the entire—highly connected and complex—segmentation gene network.

The authors dismiss this problem by stating that variability at later stages merely reflects earlier variability in the Bcd gradient. As evidence to support this claim, they show that the amplitude and spatial distribution of positional errors in the measured target domains are similar to the amplitude and spatial distribution of positional errors in the Bcd gradient, as well as those predicted by their model, which does not take target interactions into account.

In the face of direct genetic evidence for cross-regulation among Bcd targets (Jaeger *et al*, 2004; Manu *et al*, 2009 and references therein), a counterargument based merely on similar levels of variance is not convincing. Beyond that, there exists reason to believe that the positional errors measured by de Lachapelle and Bergmann (2010) are themselves artifactual.

As acknowledged by the authors in their Figure S4, posterior gap domains and Eve stripes shift significantly to the anterior during the 30 min, over which measurements were taken, whereas no such shifts are observed in the central region of the embryo (Jaeger *et al*, 2004; Surkova *et al*, 2008). This renders the measured increase in variability toward the posterior insignificant. Moreover, analyses of more precisely staged embryos suggest that positional error in both anterior and posterior target domains is much lower than that shown here, and de-correlated from errors in the Bcd gradient at the late blastoderm stage (Holloway *et al*, 2006; Surkova *et al*, 2008). This discrepancy with previously published data—together with the artifactual nature of the increase in error toward the posterior—casts serious doubt on the claim that positional error in target domains mirrors the distribution of positional error in the Bcd gradient. This equivalence, however, is the keystone of the authors' argument.

Finally, it should be noted that pre-steady-state decoding of the Bcd gradient has been reported as implausible in a recent theoretical study by Saunders and Howard (2009), which analyzed pre-steady-state gradients of transcription factor morphogens, exhibiting time and spatial scales on the order of magnitude of those observed for Bcd. Such gradients show high levels of internal fluctuations, and are very sensitive to variability in the time window during which gradient concentration is measured. This is inconsistent with the observed precision and the rapid timing of Bcd gradient interpretation in the blastoderm.

Pre-steady-state decoding of morphogen gradients was an intriguing idea when it was first proposed by Bergmann *et al*, 2007, and some evidence exists that it may indeed occur in certain gradient-based patterning processes, such as the dorso-ventral system in the *Drosophila* embryo (Kanodia *et al*, 2009).

However, we currently lack any convincing data supporting pre-steady-state decoding of Bcd, while much existing quantitative evidence argues against it.

It has become clear that the main challenge for understanding the control of positional precision in the *Drosophila* blastoderm is to disentangle the many distinct and dynamic regulatory contributions to robustness. This challenge is best tackled at the early blastoderm stage, when regulation is simple, as cross-regulation among target genes has not yet become important (Jaeger *et al*, 2007). Unfortunately, a large majority of papers in the literature—including de Lachapelle and Bergmann (2010)—focus on later stages, at which cross-regulatory interactions between target genes must be considered, as they are an essential part of the system. Combining simplistic models of early stages with quantitative measurements at later ones is unlikely to contribute to the resolution of the issues at hand.

Conflict of interest

The author declares that he has no conflict of interest.

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