Sharp borders from fuzzy gradients

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Critical boundaries in the early *Drosophila* embryo are set by morphogenetic gradients. A new quantitative study shows that the placement of one such boundary is more accurate than the gradient thought to set it. Genetic analysis of the accuracy of the process implicates a gene not previously thought to be involved.

The regulation of embryo development was the central problem of the old Entwicklungsmechanik, or developmental mechanics. The most spectacular manifestations of regulation drove their discoverer, Driesch, to abandon experimentation for philosophy after concluding that chemical or physical explanation of the phenomenon was impossible. Modern molecular tools have enabled investigators to re-examine regulation at a quantitative level [1,2]. A provocative and incisive example of this 'new wave' of quantitative studies is the recent publication of a precise study of noise and robustness in the determination of body segments in the early embryo of the fruit fly, *Drosophila melanogaster* [3]. Houchmandzadeh *et al.* find phenomena that are almost as puzzling as those discovered by Driesch, but where Driesch encountered an experimental blank wall, these modern investigators use genetics to point the way to crucial new experiments.

Drosophila segmentation is arguably the best characterized morphogenetic system in molecular terms. At the time of segment determination, the embryo is a hollow shell of nuclei, as yet not separated by membranes. The genes required for segment determination are well characterized [4], and those zygotic genes that act early are all transcription factors. The simple geometry permits high quality quantitative imaging, and the absence of cell membranes allows transcription factors to interact directly, without the mediation of cell–cell signaling. In their study, Houchmandzadeh, Leibler and Wieschaus considered one of the earliest events in the segmentation cascade, in which a gradient of maternal Bicoid (Bcd) transcription factor triggers the expression of the zygotic *hunchback* (*hb*) gene in areas of the embryo containing a Bicoid concentration of higher than a critical threshold (Fig. 1). By characterizing the noise and robustness of this process, they shed some very modern light on very old embryological questions and demonstrate that the segmentation morphogenetic field has not run out of interesting genetic surprises for investigators.

At least three classic embryological questions have concrete molecular realizations in early *Drosophila* segmentation. First is the question of precision in specifying positional information by morphogenetic gradients. Second is the question of size regulation, or scaling. Size regulation occurs when embryos form pattern scaled by their size, regardless of how large or small they are. Third is the question of temperature compensation. Embryos of many organisms develop reliably at a range of temperatures, yet physicochemical processes, such as diffusion and reaction, are quite sensitive to temperature. In addition, the genetic analysis performed by Houchmandzadeh *et al.* has some truly unexpected features that call into question the validity of the current molecular paradigm where segmentation is regarded purely as a cascade of transcription factors [5,6].

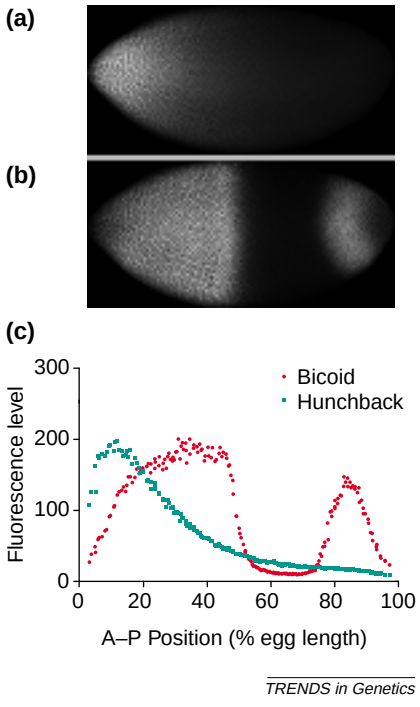

Fig. 1. Bicoid (a) and Hunchback (b) protein expression patterns in a fluorescently immunostained whole mount embryo. (c) Quantitative graph of Bicoid and Hunchback levels from the embryo above. Each symbol shows the expression level in the central 6% of dorsal (top)–ventral (bottom) values plotted against % egg length from the anterior pole. This is embryo hx21 from the FlyEx database (http://flyex.ams.sunysb.edu/FlyEx and http://urchin.spbcas.ru/FlyEx).

Precision in reading a morphogenetic gradient

Houchmandzadeh *et al.*'s central result concerns the interpretation of the *bicoid* gradient. Wolpert [7] proposed in 1969 that cells in an embryonic field determine their position and hence their future fate by reading a 'morphogenetic gradient' of some instructive substance. In 1988, Driever and Nusslein-Volhard [8] exhibited the first example of such a gradient by showing that downstream gene expression was perturbed as predicted by Wolpert's model when the Bicoid protein gradient was rescaled by increasing the dose of the *bcd* gene. Nevertheless, the *even-skipped* stripes used as a fate marker did not move as far as a simple gradient model predicted, a result confirmed for *Kruppel* in a numerical model [9] and experimentally for *hb* by Houchmandzadeh *et al.*

Houchmandzadeh *et al.* break fundamentally new ground by considering the natural variability of this process and analyzing its genetic basis. They compared the variability of the position x_{Hb}, where Hb expression is 50% maximum, with that of the point x_{Bcd}, where Bcd is 23% of maximum (the approximate Bcd threshold concentration for Hb activation), and found that although x_{Bcd} varied by 30% egg length (E.L.), x_{Hb} varied by only 4% E.L.

This sharp decrease in variability is surprising. The generally accepted idea is that the Hb domain boundary is positioned by binding of Bcd to the Hb promoter. Even an infinitely precise reading of the Bcd concentration would only recapitulate the variability of x_{Bcd}, and would most likely amplify it because of the effects of molecular noise. In chemically well-characterized prokaryotic systems, these noise effects are

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substantial [10]. Although transcriptional mechanisms in *Drosophila* are still too poorly understood to allow precise calculation of noise effects, reasonably generic arguments appear to show that gradient reading processes amplify inherent concentration fluctuations [11,12]. By contrast, an experimental study indicated that positional noise decreases with time in the blastoderm [13].

Size invariance

The problem of size invariance, or scaling, is perhaps the best known and has attracted investigators' attention since Driesch demonstrated that half a sea urchin blastula can develop into a whole organism. Insect embryos exhibit regulative properties when their size is perturbed by tying a loop of thread around the embryo [14], but the molecular interpretation of these experiments is unclear. With molecular tools, natural variation or specific mutations can be used to obtain differently sized embryos, and the regulative properties of such embryos can be compared.

Houchmandzadeh *et al*. observed embryos that varied in size from 430 to 500 μm in length. This means that the low variance of x_{Hb}, expressed in %E.L., implies size invariance. When expressed in microns, x_{Hb} shows a high correlation with egg length, whereas x_{Bcd} does not. This implies that control of x_{Hb} is not local in the sense that the distance to the poles of the egg is factored into the placement of the Hb border. The Bcd gradient is exponential, and exponentials have a characteristic length scale (e.g. the distance over which concentration halves). Remarkably, Hb appears somehow to have converted its border placement from the scaling of the Bcd gradient to the scaling of the entire embryo.

Temperature compensation

Both diffusion and protein–DNA binding affinities vary with temperature, and so Houchmandzadeh *et al*. explored the results of varying temperature. Here they found that although the Bcd gradient showed marked dependence on temperature, the position of x_{Hb} was temperature compensated.

Genetic analysis

No story in *Drosophila* is complete without characterization of its genetics, which in this case yielded the most surprising results. Given that precise placement of x_{Hb} is first seen in cleavage cycle 13 [3], when zygotic genes are becoming active, it was logical to investigate other zygotic gap genes, such as *Kruppel* and *giant*, that are known to regulate *hb*. Although mutations in some of these genes shifted the position of x_{Hb}, none altered its variance. An *hb*-null mutant that nevertheless makes *hb* RNA and protein showed wild-type variance, eliminating *hb* autoregulation as a possible source of accuracy. Using special *Drosophila* constructs missing large pieces of chromosomes, the authors assayed 80% of the zygotic genome. None of these large deficiencies affected the variance of x_{Hb}. It is therefore unlikely that the accuracy of *hb* expression is under zygotic genetic control.

That of course leaves open the possibility of maternal control. *hb* itself has a dual maternal/zygotic role. Maternally deposited *hb* RNA that is initially distributed throughout the embryo is translationally regulated by a gradient of Nanos (Nos) protein, which is highest at the posterior pole. This regulation generates a gradient of maternal Hb protein in the presumptive abdomen of the embryo. Thus, *nos* and hb^{mat} are natural candidates for control of *hb* accuracy, particularly because hb^{mat} has been implicated in the non-Wolpertian behavior of shifted fate markers when the Bcd gradient is rescaled [9]. However, neither *nos*, hb^{mat} or five other maternal genes affected the variance of x_{Hb}.

One maternal gene did affect the accuracy, however. In two of three mutant alleles of the *staufen* (*stau*) gene that were tested, the variability of Hb was as large as that of Bcd, even accounting for Bcd gradient changes in *stau* mutants. *stau* had not been previously implicated in interactions with *hb*. It is a maternal protein that has been implicated in the transport of *bcd* and other maternal RNAs [15,16]. It is of interest that Stau is found chiefly at the two poles of the embryo, but in low quantities throughout. Moreover, Stau has both microtubule- and RNA-binding activities, which are presumably the means by which Stau acts to localize *bcd* message.

This surprising result gives a tantalizing indication that zygotic expression patterns could form in part by active transport as well as diffusion. The mechanism of such transport, if it occurs, remains to be elucidated, as does the nature of its coupling to the overall geometry of the embryo. Whatever the mechanism, our picture of how the segment determination field regulates now has an important new feature.

More generally, Houchmandzadeh *et al*.'s result could shed light on an area of longstanding tension between reaction-diffusion theoreticians, on the one hand, and experimentalists and detailed modelers of experimental data, on the other. Observations of the embryo lead experimentalists to consider the pattern that arises from the regulatory combinatorics of many different proteins [5,6] and some theoreticians model these experiments [9,17,18], but neither group can easily explain regulation coupled to embryo size and geometry. Classical reaction-diffusion models naturally show size regulation [19,20] and error-reduction [12], but the detailed diffusion-coupled mechanisms invoked have been criticized as unbiological because of the modeling assumptions they use. Perhaps the study of 'reaction-active-transport' equations will resolve this tension.

In any case, experimentalists and theoreticians have their work cut out for them, and we can confidently predict that any philosophical conclusions from this work will come in conjunction with further scientific investigations, and not instead of them.

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Connecting the DOTs: covalent histone modifications and the formation of silent chromatin

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Histone methylation has emerged as a significant regulator of chromatin structure and function. Two different classes of histone methyltransferase (HMT) have been described, which target either lysine or arginine residues in the histone N-terminal tails. A flurry of recent papers now describe a third class of HMT that affects chromatin silencing indirectly, not by methylation of histone tails, but instead by targeting a conserved lysine residue in the core domain of the nucleosome.

The past decade has witnessed the demise of two widely held assumptions regarding the regulation of eukaryotic gene expression. One is the idea that histone components of chromatin are metabolically inert, serving only to package DNA into condensed, higher-order structures. The other is that transcriptional control is strictly promoter-dependent, involving the interplay between specific DNA sequences and their cognate transcription factors. An integral role for histones in gene regulation was proven when several cofactors that control gene expression turned out to be proteins that alter chromatin structure – either ATP-dependent remodeling complexes or enzymes that catalyze covalent histone modifications [1,2]. Although promoter-specific control mechanisms are the hallmark of gene regulation, the identification of chromosome-position-dependent, promoter-independent gene repression – a phenomenon known as silencing – defined a mode of gene regulation that appears to mark silent chromatin domains for epigenetic inheritance [3].

Covalent histone modifications

The nucleosome is composed of DNA and two subunits each of the histone proteins H2A, H2B, H3 and H4 [4]. The histones interact to form a core domain around which the DNA is wrapped [5], with N- and C-terminal tail domains that can be modified in several ways to affect chromatin structure and function [6]. Histone tail acetylation led to the first direct link between histone structure and gene regulation: the *GCN5* and *RPD3* genes of *Saccharomyces cerevisiae* were initially identified as a coactivator and corepressor of gene expression, respectively, and were later found to encode a histone acetyltransferase and a histone deacetylase [7]. Thus, histone acetylation and deacetylation appeared to be a toggle between activation and repression.

This seemingly straightforward connection between histone tail modification and regulation of gene expression soon took on another level of complexity. Phosphorylation of histone H3 Ser10 was associated with gene activation, presumably by promoting a more open chromatin structure, yet the same modification was also involved in chromosome condensation, a condition associated with repression [8]. It appeared that histone tail modifications do not function alone, but instead act in combination to specify different outcomes. Indeed, H3 Ser10 phosphorylation stimulates acetylation of H3 Lys14, leading to gene activation, whereas deacetylation of Lys14 precedes methylation of H3 Lys9, leading to repression [9,10]. These observations are the basis for the 'histone code' hypothesis, which proposes that different patterns of histone tail modifications lead to either the association or the dissociation of distinct effector proteins that promote or restrict the formation of open chromatin [6,11] (Fig. 1).

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