

Dispatches

Gene Regulation: Boundaries within Limits

Quantitative measurements of the Hunchback transcription factor in *Drosophila* embryos show that its target genes can respond with a high degree of precision to the exact level of the protein, simulating a continuous, analog readout, while other target genes show a combinatorial effect, resembling a Boolean logic element.

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A central problem in developmental biology has been the action of morphogens, which are diffusible substances that set up a spatial concentration gradient and contribute to fate determination of developing tissues in a concentration-dependent manner [1]. At a molecular level, different morphogen concentrations appear to determine different developmental fates at least in part through selective regulation of different target genes. *Drosophila* has proved to be a fruitful system for investigation of molecular mechanisms of morphogens, and in particular the blastoderm embryo, in which nuclei share a common cytoplasm, has provided several examples of diffusing transcription factors that exhibit morphogen-like properties: The Bicoid protein, which forms an anterior-to-posterior concentration gradient and is required for the specification of the head and thorax region of the fly embryo, was the first transcription factor to be characterized as a morphogen. Bicoid directs anterior development by activating the zygotic transcription of *hunchback*, as well as other segmentation genes encoding transcription factors such as Krüppel, Giant, and Knirps. Initial studies [2] had considered a portion of the *hunchback* transcriptional regulatory region to explain the differential interpretation of the Bicoid protein gradient: The number and quality of Bicoid binding sites could be changed to accordingly alter the responsiveness of a reporter gene to the gradient, suggesting a simple differential readout mechanism [2]. Later work, however, indicated that additional factors were important for dictating response of a gene to the Bicoid gradient, including functional synergy with the Hunchback

protein and cooperative binding [3,4]. Overall, how the Bicoid morphogen gradient is interpreted remains a conundrum, as examination of a number of Bicoid-responsive cis-regulatory elements indicated that differential thresholds for this transcription factor are dictated by features not readily apparent using simple bioinformatic analysis [5].

The *bicoid* gene is an evolutionary innovation, present only in *Drosophila* and higher flies, and seems to be associated with the evolution of the unique syncytial blastoderm embryo. *hunchback*, on the other hand, is an ancient, conserved transcriptional regulator found in a wide diversity of insect orders, including those that feature a short germband mechanism of development, in which segmental gene expression is played out largely in a cellularized environment [6]. Hunchback plays a key regulatory role in early *Drosophila* development and, like Bicoid, is expressed in an anterior-to-posterior gradient that is a product of both translational and transcriptional regulation. Hunchback is required for the correct expression of the gap genes *Krüppel* (*Kr*), *giant* (*gt*) and *knirps* (*kni*), whose expression defines broad, non-repetitive domains of the embryo at the top of the segmentation hierarchy, as well as of pair-rule genes (*even-skipped* (*eve*), *hairy*) which are expressed in transverse stripes that define the segmental subdivision of the fly embryo [7–12].

In some settings, Hunchback protein is found to act as a transcriptional repressor, and in others, as an activator, but in both cases Hunchback is thought to act directly through specific cis-regulatory elements. Previous studies tested the response of endogenous *gt*, *kni*, and *Kr* genes to systematic manipulation of Hunchback concentrations, demonstrating that

Hunchback acts as a classical morphogen controlling expression of different target genes in a concentration-dependent manner [7,8,11]. These experiments were carried out using mutant backgrounds that eliminated key patterning regulators such as *bicoid*, with severe consequences for later patterning events [7]. Nonetheless, the picture that emerged was that in *Drosophila* gradients of Hunchback provide critical patterning information, exerting a morphogen-like effect on downstream gap genes. These earlier studies, however, did not analyze the cis-regulatory elements that were presumably targeted by Hunchback, but a later study from Steve Small's laboratory [12] did exactly that, focusing on two enhancers from the *eve* locus that are repressed at different levels of Hunchback. The *eve* stripe 4/6 enhancer is strongly repressed by low levels of Hunchback, while the *eve* stripe 3/7 enhancer is repressed only at higher concentrations of the protein. Bioinformatic analysis indicated that the more sensitive enhancer was predicted to possess more Hunchback binding sites of higher affinity, suggesting that the simple model originally proposed for Bicoid gradient interpretation might also apply to this protein. In the recent paper by Yu and Small published in *Current Biology* [13], this system is revisited, with three further refinements. First, the relative levels of Hunchback protein are determined using confocal laser-scanning microscopy; second, a greater palette of target genes is considered (six genes repressed by Hunchback, including two enhancers of *eve*); and third, more subtle perturbation analysis is employed, involving measurements of target genes in response to naturally-occurring shifts in the Hunchback gradient during blastoderm development, and targeted misexpression of the protein in ventral regions. The authors raise two important questions: do different target genes consistently show regulation at the same relative levels

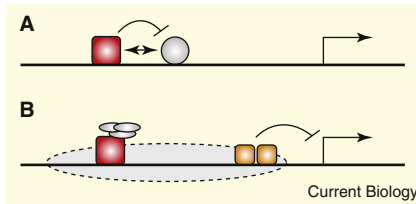


Figure 1. Models for ‘analog’ and ‘Boolean’ transcriptional switches controlled by the Hunchback morphogen.

(A) Promoters, such as *eve*, *pdm2*, and *kni*, that are programmed to respond to precise amounts of Hunchback (red square) may have activators (gray circle) positioned at specific distances from the repressors, providing a tunable spectrum of repression outputs for this distance-dependent short-range repressor, as has been shown previously for the Giant repressor [16]. (B) Promoters on which Hunchback acts as a permissive, but not sufficient, factor, such as those of *Kr* and *gt*, may involve Hunchback interaction with catalytic chromatin remodeling/modifying activities (gray ovals), setting the context for other repressors (orange squares) to act.

of Hunchback, and is Hunchback by itself sufficient to repress target genes? The results show that specific Hunchback concentrations — ranging from 4–40% of the maximum levels depending on the gene involved — set the positions of the target genes. Consistent with these results, the authors [13] also find that in a heterozygous *hunchback* mutant, which produces a shallower protein gradient, the spacing of *eve* stripes is altered, exactly as one would anticipate if these enhancers are hard-wired to respond to specific concentrations of the repressor. The use of an active range of 4–40% of maximal levels of Hunchback for setting boundaries explains why this gene is recessive, as the heterozygote would still supply ~50% of normal protein levels, more than sufficient for these thresholds. This apparent excess capacity in Hunchback may provide buffering against genetic or environmental variations, a feature that is presumably common to other gradient-forming, recessive patterning genes such as *knirps* and *giant*.

Interestingly, the boundary of *Kr* expression was not found to be positioned at a single concentration of Hunchback: In the early blastoderm embryo, the *Kr* anterior border formed at 48% of the maximum Hunchback concentration, and at a slightly later stage, when the expression pattern shifts anteriorly, the border formed at

a concentration threshold of more than 90%. Unlike with the other genes examined, misexpression of Hunchback in ventral regions was found to have little impact on *Kr* repression, while the combined misexpression of Giant and Hunchback potentially inhibited *Kr*. This result suggests a combinatorial mode of action on the *Kr* gene, whereby Hunchback sets up a permissive condition for Giant’s repressive action on the promoter. A similar situation is observed for Hunchback regulation of the posterior domain of *giant*: Here, very low levels of Hunchback are necessary, but not sufficient for, repression. The combinatorial action of Krüppel is required for efficient regulation of this boundary.

What further insights are gained from this analysis of Hunchback as a morphogen? By measuring precise levels of the protein gradient, this study [13] shows for the first time that two general types of transcriptional switches are triggered by the Hunchback morphogen: in one case, positional information is read out directly from the levels of the protein gradient, resembling an analog output. In the other, Hunchback serves to establish a permissive environment for Giant and Krüppel, but the specific level is less instructive, as with a digital either/or readout (Figure 1).

No specific features of the Hunchback binding sites on the relevant cis-regulatory regions seem to explain the analog-type direct readout; in fact, the identified differences in binding-site number and affinity for *eve* 3/7 and *eve* 4/6 enhancers in this study [13] seem less robust than suggested in the earlier study [12], possibly because of differences in the bioinformatic analyses employed. The simple model of overall binding-site affinity and number thus does not seem to provide much fine-scale information, despite the heavy reliance on these features for fractional occupancy models of enhancer activity [14]. One consideration is that Hunchback appears to function as a short-range repressor, and previous studies have indicated that small changes in spacing of binding sites for such proteins with respect to nearby activators can have dramatic effects on function, providing a sensitive tuning mechanism [15,16]. It is likely that in addition to the number and affinity of binding sites, repressor–activator spacing features

designed into enhancers contribute to the observed threshold responses.

How might Hunchback act as a permissive factor on the *Kr* and *gt* promoters, providing a less concentration-dependent, ‘Boolean’ response so that the enhancer senses the presence or absence of the factor to generate a permissive (1) or nonpermissive (0) readout? Hunchback has been found to interact with dMi-2, a component of the NURD chromatin remodeling and deacetylation complex [17]. This activity may be important for establishing a chromatin environment in which Krüppel and Giant are able to act. If such chromatin modifications act through positive feedback mechanisms, a catalytic amount of Hunchback would be sufficient to initiate the process, and specific thresholds would be less critical. A similar ‘facilitated activity’ model has been proposed for the long-range repressor Hairy [18]. Such mechanistic speculations remain to be tested, however. Functional characterization of Hunchback’s activity on diverse cis-regulatory elements promises greater rewards than just determining how a protein can be harnessed to produce distinct regulatory output. The control of gap genes is apparently an evolutionary innovation for Hunchback, correlating with the evolution of the syncytial *Drosophila* embryo, where transcription factor gradients play a unique role [6]. Quantitative understanding of the molecular mechanisms of this protein in the context of specific enhancers may provide a better understanding of morphogen activities and the evolution of novel regulatory networks [19].

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Evolutionary Biology: Parasite, Know Thyself

Studies of sex allocation provide some of the best evidence for Darwinian adaptation in nature. A new study of malaria parasites provides striking support for this cornerstone of evolutionary biology, with important implications for both evolutionary and medical biology.

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Sex allocation theory attempts to explain how natural selection shapes the way organisms divide resources between male and female offspring. What is often referred to as ‘fisherian’ sex allocation, after the explanation in R.A. Fisher’s key text [1], is the existence of an evolutionarily stable sex allocation where resources are equally divided into males and females. This applies only for the special case where there is random mating, and no inbreeding, as was first pointed out by W.D. Hamilton in 1967 [2]. Hamilton realised that, as mating became increasingly less random, natural selection would favour sex ratios skewed increasingly more strongly towards females, because this would reduce wastage of resources on males that compete with each other for the same fertilisations. Life cycles that favour this type of sex allocation behaviour are quite widespread, and while much of the work in this field has been on arthropods, it was pointed out some time ago by Read *et al.* [3] that the life cycle of malaria parasites selects

for the evolution of ‘hamiltonian’ sex ratio behaviour. A new study by Reece *et al.* [4], using elegant experimental and molecular techniques, has now provided the first conclusive evidence that several key assumptions and predictions of sex allocation theory are upheld in malaria parasites: these parasites appear to show considerable sophistication in their ability to adjust sex ratio in response to the prevailing conditions within their host. The findings also have some fascinating implications for our understanding of the fundamental biology and within-host behaviour of malaria parasites.

Hamilton’s sex ratio theory is often illustrated using the example of fig-pollinating and parasitoid wasps (Figure 1A), where newly emerged females are commonly confined to a patch in which the only potential mates are their brothers. The resulting ‘local mate competition’ leads to the optimal offspring sex ratio being very strongly female-biased: a mother’s fitness is maximized if she produces just enough sons to mate with all her daughters. At the other extreme, in a fully outbred (fisherian) population the optimal strategy is to produce equal

numbers of sons and daughters. Hamilton showed that, under local mate competition, the unbeatable sex allocation strategy (proportion of males produced, r^*) depends on the inbreeding rate according to the equation $r^* = (1-f)/2$, where f is Wright’s coefficient of inbreeding. An extension of this theory would predict that, if the level of inbreeding offspring experience is variable, natural selection favours a facultative sex allocation strategy, in which females adjust their offspring sex ratio to maximize the production of grandchildren; such facultative sex allocation has been observed in a wide range of taxa [5,6].

Just like fig-pollinating wasps, malaria parasites (Figure 1B) experience variable levels of inbreeding as a result of mating within a small ‘patch’ shortly after reaching sexual maturity. Sex in malaria (*Plasmodium*) and related Apicomplexan parasites (such as *Haemoproteus* and *Leucocytozoon*) occurs when sexual stages called gametocytes that circulate within the vertebrate bloodstream are taken up by a vector when it takes a blood meal. Mating occurs inside the vector gut, when sperm-like gametes are released from male gametocytes to seek out and fuse with the larger female gametes. Since most malaria infections are made up of only a few of the many genotypes present in the whole population, malaria parasites generally experience some degree of inbreeding. In accordance with local mate competition theory, their sex ratios are