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The role of Bicoid cooperative binding in the patterning of sharp borders in *Drosophila melanogaster*

Francisco J.P. Lopes[a,b,*], Alexander V. Spirov[c,d], Paulo M. Bisch[a]

[a] Laboratório de Física-Biológica, Instituto de Biofúsica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
[b] Polo de Xerém, Universidade Federal do Rio de Janeiro, Duque de Caxias, Brazil
[c] Laboratory of Evolutionary Modelling, the Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, Saint-Petersburg, Russia
[d] Computer Science Department and Center of Excellence in Wireless & Information Technology, State University of New York at Stony Brook, 500 Stony Brook Road, Stony Brook, NY, USA

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ABSTRACT

In *Drosophila* embryonic development, the Bicoid (Bcd) protein establishes positional information of downstream developmental genes like *hunchback* (*hb*), which has a strong anterior expression and a sharp on-off boundary in the mid-embryo. The role of Bcd cooperative binding in the positioning of the Hb pattern has been previously demonstrated. However, there are discrepancies in the reported results about the role of this mechanism in the sharp Hb border. Here, we determined the Hill coefficient (n_H) required for Bcd to generate the sharp border of Hb in wild-type (WT) embryos. We found that an n_H of approximately 6.3 (*s.d.* 1.4) and 10.8 (*s.d.* 4.0) is required to account for Hb sharpness at early and late cycle 14A, respectively. Additional mechanisms are possibly required because the high n_H is likely unachievable for Bcd binding to the *hb* promoter. To test this idea, we determined the n_H required to pattern the Hb profile of 15 embryos expressing an hb^{14F} allele that is defective in self-activation and found n_H to be 3.0 (*s.d.* 1.0). This result indicates that in WT embryos, the *hb* self-activation is important for Hb sharpness. Corroborating our results, we also found a progressive increase in the required value of n_H spanning from 4.0 to 9.2 by determining this coefficient from averaged profiles of eight temporal classes at cycle 14A (T1 to T8). Our results indicate that there is a transition in the mechanisms responsible for the sharp Hb border during cycle 14A: in early stages of this cycle, Bcd cooperative binding is primarily responsible for Hb sharpness; in late cycle 14A, *hb* self-activation becomes the dominant mechanism.

Contents

* Corresponding author. Fax: +55 21 2280 8193.
E-mail addresses: fjplopes@gmail.com, flopes@ufrj.br (F.J.P. Lopes).

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Introduction

It has been shown that cooperative binding plays a central role in pattern formation and in the interpretation of morphogenetic positional information during embryonic development. One classical example is the Bicoid (Bcd) protein that establishes the anterior–posterior (AP) developmental axis during embryonic development in *Drosophila melanogaster*. This morphogenetic protein determines positional information for downstream developmental genes such as *hunchback* (*hb*), *Krüppel* (*Kr*) and *knirps* (*kni*), which also show cross-regulation (Berleth et al., 1988; Crauk and Dostatni, 2005; de Lachapelle and Bergmann, 2010; Driever and Nusslein-Volhard, 1988; He et al., 2010a; Jäckle et al., 1986; Jaeger et al., 2004; Manu et al., 2009; Nusslein-Volhard et al., 1987; Papatsenko and Levine, 2011). It has been shown that cooperative binding is critical for Bcd transcriptional activity (Burz and Hanes, 2001; Burz et al., 1998; Driever et al., 1989; Lebrecht et al., 2005; Lopes et al., 2005, 2008; Ma et al., 1996) and plays a central role in reducing transcriptional noise during *Drosophila* development (Holloway et al., 2011).

The *hb* gene encodes a morphogenetic protein (Lehmann and Nussleinvolhard, 1987; Papatsenko and Levine, 2008; Tautz et al., 1987) and exhibits anterior and posterior expression patterns (Fig. 1). The *hb* regulatory region has two distinct promoters, P1 (distal) and P2 (proximal); each controls the expression of specific transcripts that encode the same protein (Margolis et al., 1995; Spirov et al., 2002; Spirov et al., 2000; Tautz et al., 1987)[1]. In the anterior region of the embryo, the regulation of *hb* by Bcd and Hb proteins (Driever and Nusslein-Volhard, 1989; Lukowitz et al., 1994; Margolis et al., 1995; Simpson-Brose et al., 1994; Struhl et al., 1989; Treisman and Desplan, 1989) produces a uniform expression pattern with a sharp on–off boundary at mid-embryo (Fig. 1). It was shown that an ~300 bp region upstream of the *hb* coding region, the core part of the proximal promoter, that contains 6 main Bcd sites is sufficient to confer full regulation of *hb* by Bcd (Driever et al., 1989; Schroder et al., 1988; Struhl et al., 1989). Cooperative binding of Bcd to these six sites has also been previously demonstrated (Burz et al., 1998; Lopes et al., 2005; Ma et al., 1996). Moreover, to show that Bcd cooperative binding could determine the positioning of the *hb* pattern, Driever et al. (1989) used a series of lacZ artificial constructs that were driven by fragments of the native *hb* promoter and contained different numbers of Bcd binding sites. It was then suggested that this mechanism could account for the sharpness of the *hb* pattern because the sharpness of the lacZ patterns increased with the number and strength of Bcd sites. However, none of the lacZ constructs analyzed achieved wild-type (WT) Hb sharpness. This result was found even when an artificial construct was driven by six strong and six weak Bcd sites, which showed the strongest level of expression. Since these experiments, many efforts have been dedicated to characterizing the role of Bcd cooperative binding in *hb* pattern formation (Burz et al., 1998; Crauk and Dostatni, 2005; Gregor et al., 2007a; Lebrecht et al., 2005; Lopes et al., 2005, 2008; Ma et al., 1996).

He et al. (2010b) contributed to the above discussion with immunofluorescence to determine Bcd and Hb protein profiles from 28 WT embryos at early cycle 14A and found that a Hill coefficient (n_H) of approximately 5.1 ± 2.7 in the dorsal and 4.9 ± 2.7 in the ventral side was sufficient to account for Hb sharpness. Using the same technique with 9 early embryos at cycle 14A and visual inspection, Gregor et al., 2007a found an n_H of approximately 5.0. This result indicates that Bcd cooperative binding is sufficient to account for *hb* regulation. However, the coefficients required are higher than those that are observed experimentally. Using DNAse footprint assays *in vitro*, an estimated n_H of 3.6 was found for Bcd binding to a 250 bp fragment of the native *hb* promoter containing the six Bcd strong sites (Ma et al., 1996). Using gel-shift assays, the n_H was estimated as 3.0 (*standard deviation*, *s.d.* 0.031) for the binding of a homeodomain-containing fragment of Bcd (called Bcd_{89-154}) to a 230 bp *hb* element also containing the six Bcd strong sites (Burz et al., 1998). Using immunofluorescence and fluorescent *in situ* hybridization (FISH), early embryos at cycle 14A (He et al., 2011) were used to show that the n_H required for Bcd to pattern the *hb* transcriptional profile (6.1 ± 2.6, calculated from 14 embryos) was higher than the coefficient required to pattern the Hb protein profile (5.2 ± 0.4, calculated from 5 embryos). In addition, Houchmandzadeh et al. (2002) have suggested that Bcd alone cannot account for Hb sharpness because an n_H of more than 10 is required based on their estimations. Finally, the idea that Hb sharpness is caused by *hb* self-activation through bistable kinetics was proposed based on work using a systems biology approach that combined immunofluorescence and a reaction-network model (Lopes et al., 2008).

During cycle 14A, the *hb* expression pattern exhibits a significant variation of approximately 30% (Lopes et al., 2008; Surkova et al., 2008) and reaches a maximum level of expression around mid-cycle. Bcd protein concentration, in turn, decreases continuously from its maximum level at the beginning of the cycle (Gregor et al., 2007b; Surkova et al., 2008). These patterns of temporal variation indicate that the contribution of both Bcd and Hb proteins must be temporally modulated and that a precise characterization of Bcd cooperative binding and *hb* self-activation in Hb sharpness must account for the variations in different stages of cycle 14A.

Here, we analyzed Bcd cooperativity levels using 30 WT Bcd and Hb profiles. We found that an n_H of 6.3 (*s.d.* 1.4) is required to account for Hb sharpness at early cycle 14A. However, at late stages of this cycle, an n_H of approximately 10.8 (*s.d.* 4.0) is required. Bcd binding is not likely to reach this level of cooperativity. Thus, we investigated additional regulation that could be taking place by determining the n_H required to pattern the Hb profile of 15 embryos expressing an hb^{14F} allele that is defective in self-regulation. We found an n_H of 3.0 (*s.d.* 1.0), which is in agreement with previous *in vitro* results (Burz et al., 1998; Ma et al., 1996). This result indicates that *hb* self-activation contributes to Hb sharpness in WT embryos, which was suggested in earlier studies (Lopes et al., 2008; Simpson-Brose et al., 1994). We verified our results using an independent set of data (Poustelnikova et al., 2004; Surkova et al., 2008) to follow the progressive increase in the n_H required to account for Hb sharpness from early to late cycle 14. Taken together, our results indicate that there is a transition in the mechanisms responsible for *hb* sharpness during cycle 14A: in early stages of this cycle, Bcd cooperative binding is mainly responsible for *hb* sharpness; in late cycle 14A, *hb* self-activation becomes the dominant mechanism.

[1] For details http://www.evol.nw.ru/spirov/hox_pro/hunchback.html.