Comment on "Precise domain specification in the developing *Drosophila* embryo"

Christof M. Aegerter[1] and Tinri Aegerter-Wilmsen[2]
[1]*Fachbereich Physik, University of Konstanz, Universitätstrasse 10, 78457 Konstanz, Germany*
[2]*Institute for Molecular Systems Biology, ETH Zürich, Wolfgang Pauli-Strasse 16, 8093 Zürich, Switzerland*
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In a recent paper, Houchmandzadeh *et al.* [Phys. Rev. E **72**, 061920 (2005)] introduce a correlated bigradient model in order to explain the robust scaling of the boundary of hunchback (hb) expression in the early *Drosophila* embryo. In particular, they stress that recent experiments by Lucchetta *et al.* [Nature (London) **434**, 1134 (2005)], where embryos whose anterior and posterior halves develop at different temperatures still show excellent precision in the hb boundary, are in good agreement with such a model. We would like to show here that this conclusion is unwarranted. This is because the experiments of Lucchetta *et al.* were done at different temperatures from those studied in the model. Since in other temperature combinations the model does not produce precise boundaries and there are no systematic trends in these deviations, a comparison to the experiment is not possible. Furthermore, we would like to point out that any correlated bigradient model should also take into account the fluctuations of the bicoid profile within an embryo. When forming correlated bigradients of experimental profiles from an online library, we observe that these intraembryo variationss destroy robustness of the hb boundary even in the wild-type situation.

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I. BASICS OF THE BIGRADIENT MODEL

In the version of the bigradient model proposed by Houchmandzadeh *et al.* [1], the boundary of hunchback (hb) expression is given by the point where the concentrations of bicoid (bcd) and a hypothetical posterior repressing protein are equal [2]. One can then analytically calculate the properties of the hb boundary supposing an exponential gradient for the two proteins [3], which is reasonable as this is the stationary solution of diffusion including breakdown. For instance one finds that the hb boundary scales with embryo length (EL) [3], as is found experimentally [4]. With these analytically known properties it is then possible to study the influence of fluctuations in either the decay lengths or the amplitudes of the protein concentrations on the precision of the hb boundary. When studying uncorrelated fluctuations in the best possible case (i.e., only fluctuations of one of the two proteins), we have found that the precision of the hb boundary is increased with respect to bcd [3], but is still far beyond that experimentally observed [4]. Similar results have been found for a slightly different version of a bigradient model recently [5]. The key point of the model now proposed by Houchmandzadeh *et al.* is that when the fluctuations in both proteins are correlated, the system will naturally find the center of the embryo, such that robust precision is achieved. In the absence of the identification of the posterior protein (pp), it is difficult to obtain an experimental measure for the degree of correlation of the two proteins. There are, however, two ways to achieve this discussed in the literature.

Lucchetta *et al.* [6] have recently measured the development of the hb boundary when the anterior and posterior halves of the embryo are set to develop at different temperatures (20 and 27 °C) using a microfluidics device. They find that such an unnatural development does not alter the scaling and precision of the hb boundary (i.e., a hb boundary at 0.49 EL with a precision to a standard deviation of 0.01 EL). Intuitively, one would argue that the different temperatures of the two halves lead to a different posterior gradient and hence uncorrelated changes, which would lead to reduced precision and robustness. Lucchetta *et al.* thus also argue against the validity of a bigradient model in the formation of the hb boundary [6]. Houchmandzadeh *et al.* [1], show, however, that for a combination of temperatures of 18 and 25 °C a combined change in the amplitude and the decay length can lead to a compensation of the temperature changes. We will demonstrate below that for many other temperature combinations the model does not show such a compensation without any systematic trends. Hence, the temperature compensation mechanism cannot be considered robust. This means that due to the different temperatures used by Lucchetta *et al.* [6] and Houchmandzadeh *et al.* [1], the experiments cannot be used to test the correlated bigradient model directly.

Due to the fact that in a correlated model the position of the hb boundary is mainly determined by the anterior properties of bcd and the posterior properties of pp, another test is possible. When the two proteins are perfectly correlated, as proposed by Houchmandzadeh *et al.*, fluctuations of, e.g., the decay length *within* the embryo will lead to different relevant decay lengths of pp and bcd [3]. Thus one can use the full experimentally observed bcd profile as a measure of the relevant parts of bcd and pp and obtain an estimate of the precision of a correlated bigradient model. Due to the large extent of the intraembryo variations, shown below, the best possible estimate of the hb boundary precision is much worse (by a factor of 4) than the experimental one even in the correlated case [3].

II. TEMPERATURE SECTIONS

One of the main points of Houchmandzadeh *et al.* [1] is that their model can naturally explain the recent data of Luc-

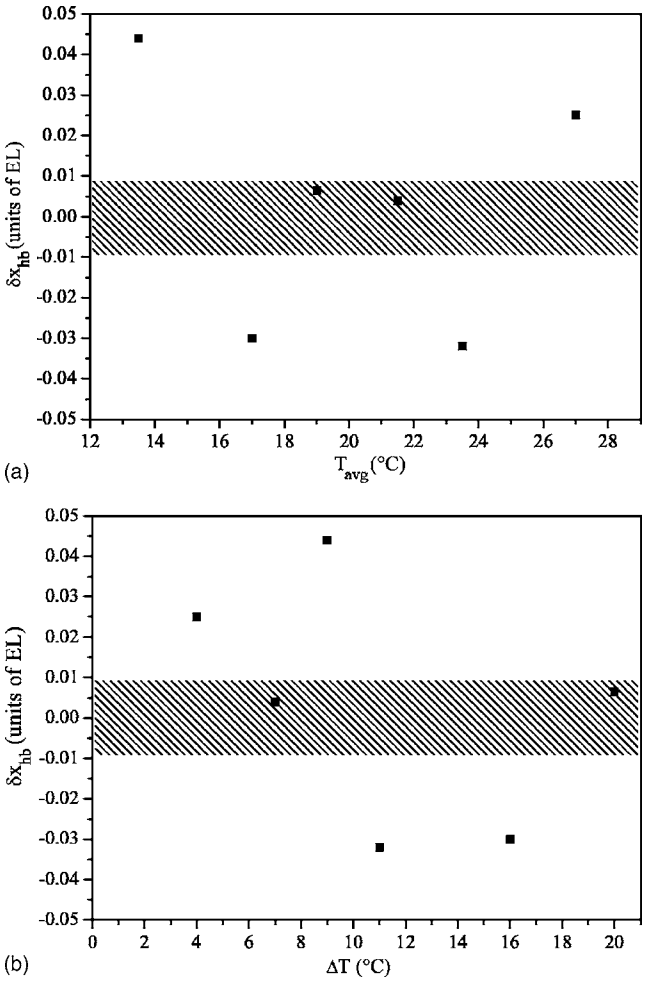

FIG. 1. Shifts of the hb boundary when different temperatures are applied to the two halves of the embryo. Taking the temperatures at which Houchmandzadeh *et al.* have determined the amplitude and decay length of bcd, one can obtain the hb boundary for six different T combinations. In (a), the shift is given as a function of the average temperature, whereas in (b) it is given as a function of the temperature difference. As can be seen, there are large fluctuations in the shift without any systematic dependence. The data of Lucchetta *et al.* correspond to points at $\Delta T=7$ °C and $T_{av}=23.5$ °C and fall into the wild-type variation of 0.01 EL indicated by the shaded area. This does not correspond to a temperature combination in the model of Houchmandzadeh *et al.*, so that the data can not be compared to the model.

chetta *et al.* [6] discussed above. These experiments were done at a temperature combination of 20 and 27 °C, where a precision of the hb boundary as in the wild type (0.01 EL) was observed. Houchmandzadeh *et al.* [1], on the other hand, calculate the properties of their model for a temperature combination of 18 and 25 °C. While these temperatures are close to the experimental ones, one may wonder whether this discrepancy in temperatures is relevant or not. In addition to the temperatures where they explicitly calculate the properties of the model, Houchmandzadeh *et al.* also give the relevant information (i.e., amplitudes and decay lengths of the bcd profiles) at two more temperatures which were characterized in [4]. Thus six possible temperature combinations can be calculated. In order to test whether the temperature compensation mechanism proposed by Houchmandzadeh *et al.* is robust and whether a difference in the temperatures used by Lucchetta *et al.* and Houchmandzadeh *et al.* is important, we have calculated the properties of the correlated bigradient model at all these temperature combinations. While some of these temperature combinations are not biologically relevant, we are trying to test properties of the model itself, such as a robust temperature compensation and whether there are systematic deviations, which allow a comparison of experiment and theory at different temperatures. The biological relevance of each single case is therefore not important.

The result of this is given in Fig. 1, where we show the shift in the hb boundary as a function of both temperature difference and mean temperature. Similarly, the shifts in the hb boundary do not show a systematic dependence on the average of the discrepancies in ΔT and T_{av} between model and experiment. As can be seen, only two of these six possible temperature combinations are consistent with the observed wild-type precision of 0.01 EL and there is no systematic dependence on either temperature difference or mean temperature. Thus an extrapolation to correct the small temperature error is not possible. Furthermore, Fig. 1 shows that small changes of the order of the mismatch in temperatures used by Lucchetta *et al.* [6] and Houchmandzadeh *et al.* [1] can lead to a significant change in the hb boundary of several times the wild-type standard deviation. Consider, for instance, the point at $\Delta T=4$ °C and $T_{av}=27$ °C. It has been shown in [4] that in the absence of a temperature gradient (i.e., $\Delta T=0$) the position of the hb boundary does not depend on T_{av}. It would therefore be reasonable that in the case of $\Delta T=4$ °C the precision should be at least as good as in the case of $\Delta T=7$ °C in spite of the difference in T_{av}. We therefore have to conclude that the temperature compensation at the combination of 18 and 25 °C studied by Houchmandzadeh *et al.* is more likely accidental, such that the temperatures would have to match exactly in order to be compared to the experiments of Lucchetta *et al.* [6]. Unfortunately, however, the two temperature sets do not match up, so that a comparison of theory and experiments seems unwarranted. Hence the observation of precision in the model at temperatures of 18 and 25 °C cannot be used as explanation of the experiments of Lucchetta *et al.* at 20 and 27 °C.

III. INTRAEMBRYO FLUCTUATIONS

Moreover, as explained above, any model proposing a correlated bigradient model in order to produce a precise and scaled hb boundary should take account of intraembryo fluctuations. As has been noted before [3,5] and by Houchmandzadeh *et al.* [1], a robust finding of the middle in a bigradient model only works as long as the gradients are perfectly correlated. Thus the properties of the pp gradient, such as the decay length and the initial concentration, have to match those of bcd exactly. Houchmandzadeh *et al.* have used this property to study the temperature compensation mechanism discussed above [1]. This, however, also implies that if there are variations in, say, the decay length of bcd

within the embryo, they will appear at corresponding positions for pp. This effectively destroys the correlation as the variations do not appear at the symmetric positions but at the same embryo position. Therefore the precision is greatly reduced as well [3]. As an extreme example, one can consider a situation where variation is such that the anterior and posterior halves have different decay lengths. This would correspond to the situation discussed by Houchmandzadeh *et al.* [1], where the initial concentrations of bcd and pp are the same. Since the anterior half of the bcd gradient and the posterior half of the pp gradient mainly determine the hb boundary, this will lead to a shift in the hb boundary. In order to investigate the importance of such intraembryo fluctuations, we have studied the bcd profiles in the relevant cycle of development available at the online library flyex [7] (114 embryos). By performing a nonlinear Levenberg-Marquardt fit to these profiles for $I(x)=A\exp(-x/\lambda)+B$, we obtain an average decay length of bcd of $\lambda=0.21$ EL with a variation of 0.046 EL. Here, the error arising from the fit is 0.004 EL, such that the variation does not stem from the experimental noise in the staining data. This is consistent with the results of Houchmandzadeh *et al.* [4] for their data. Similarly to Houchmandzadeh *et al.*, we have restricted the fit to the interval starting at $x=0.15$ as systematic deviations associated with the position of the bcd source within the embryo and a corresponding peak in the bcd concentration lead to a misrepresentation at smaller x. Fixing the background level thus determined, we can now also compare the decay lengths in different parts of the embryo using the same fitting procedure. When determining the decay lengths separately in the intervals of [0.25,0.5] and [0.5,0.75], we find that they vary greatly with a standard deviation of 20% over all embryos. Note that these variations again do not appear from noise in the data and a corresponding uncertainty in the fit as this error is at the 2% level. Similarly, the parameters are well determined also in the interval [0.5, 0.75], as the signal-to-noise ratio of the data (with the background subtracted) always exceeds 3 and is 11 on average. In addition, the size of the variation of the anterior and posterior decay lengths does not depend on the size and position of the interval. We find a variation of 18% and 22% for interval lengths of 0.3 and 0.2, respectively, which is within the uncertainty of the fit. Thus, using this estimation for the strength of the intraembryo fluctuations between the anterior and posterior halves, one can then obtain a determination of the corresponding variation of the hb boundary in a correlated bigradient model. From this we obtain an uncertainty of $\delta x_{hb}=0.045$ when the gradients are perfectly correlated and when there are variations only in the decay length and thus this constitutes a lower limit. This is more than a factor of 4 above the observed wild-type variation and in good accord with the result of [3], where we performed a similar analysis.

Using the initial fit results of the decay length, the initial concentration, and the background level, it is also possible to give a direct visualization of the importance of the intraembryo fluctuations. Due to the fact that bcd and pp are fully correlated, it is possible to calculate the profile of pp given that of bcd via $I_{pp}(x)=A^2\exp(-1/\lambda)/(I_{bcd}-B)$. When plotting both of these profiles, the position of the hb boundary can be read directly from the graph as the crossing point.

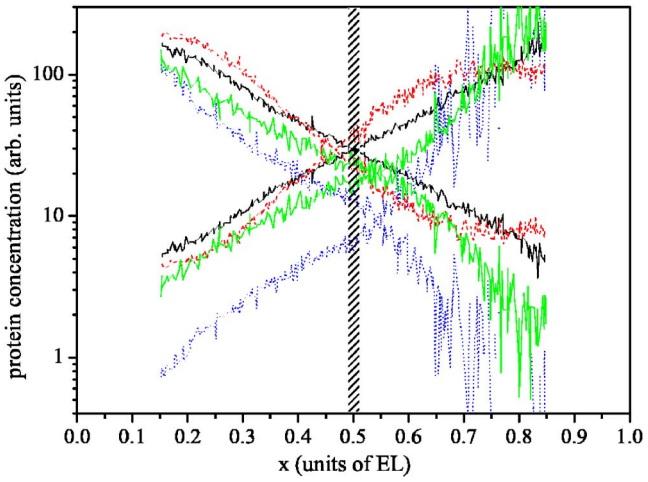

FIG. 2. (Color online) Profiles of the bcd concentrations during cycle 14 of the *Drosophila* development from the flyex database [7]. In order to study the effect of intraembryo variations on the position of the hb boundary in a bigradient model, we have calculated the shape of the gradient of the proposed posterior protein. This is given by the inverse of the bcd profile multiplied by the values of the bcd concentration at the posterior and anterior ends. As can be seen from the figure, intraembryo fluctuations can lead to a crossing point of bcd and the proposed posterior protein far away from the embryo center. Thus such a model is inconsistent with the experimentally found precise and robust scaling of the hb boundary (indicated by the shaded area) due to the intraembryo fluctuations in the bcd profiles.

This is shown in Fig. 2 for a few embryos, where it can be seen that due to intraembryo fluctuations the hb boundary can indeed shift by 0.05 EL, which is inconsistent with the wild-type data of Houchmandzadeh *et al.* [4]. In addition, the plot shows that the fluctuations are bigger than the noise level and also appear on long length scales so that it is reasonable that the quantitative analysis above did not find an influence of the noise on the result.

These shortcomings of a bigradient model have prompted us to develop a fundamentally different model [3], which does not propose the existence of any hypothetical protein. Furthermore, the model explicitly takes into account the role of the protein staufen in the process. Staufen was found to be the only protein influencing the hb boundary in an extensive genetic screen [4]. Due to the fact that this model relies on different mobilities of unbound hb RNA and a complex of hb RNA formed with staufen protein, it is highly robust against fluctuations in the bcd profile and hence also against intraembryo variations.

IV. CONCLUSION

To summarize, we have strong doubts about the applicability of a correlated bigradient model to the development of the hb boundary in the early *Drosophila* embryo. Not only can the temperatures used in the experiments of Lucchetta *et al.* [6] not be compared with those studied by Houchmandzadeh *et al.* [1,4], but there is a strong and seemingly random

variation of the supposed temperature compensation at different temperatures. Furthermore, Houchmandzadeh *et al.* [1] have completely neglected the presence of intraembyo variations, which are vital if one studies a correlated bigradient model [3]. Experimental bcd profiles show strong intraembryo variations, which lead to a reduction of precision of the hb boundary comparable to that which would be observed in the uncorrelated case [3].

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