# mRNA diffusion as a mechanism of morphogenesis in Drosophila early development

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# mRNA diffusion as a mechanism of morphogenesis in *Drosophila* early development

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**In *Drosophila* early development, bicoid mRNA of maternal origin is deposited in one of the poles of the egg, determining the anterior tip of the embryo and the position of the head of larvae[1,2,3,4]. The deposition of mRNAs is done during oogenesis by the mother ovary cells and is transported into the oocyte along microtubules[5]. Initially, the oocyte has only one nucleus, but after fertilization and deposition of the egg, nuclear duplication by mitosis is initiated without the formation of cellular membranes[6]. During the first 14 nuclear divisions of the developing embryo, *bicoid* mRNA of maternal origin is translated into protein in the ribosomes and accumulates near the external nuclear walls of the recently formed nuclei. Here, we show that mRNA diffusion is the main morphogenesis mechanism explaining consistently the establishment of Bicoid protein gradients. Moreover, we show that if diffusion for both *bicoid* mRNA and Bicoid protein were assumed, a steady distribution of Bicoid protein would result, with a constant concentration along the embryo, contradicting observations.**

During the interphases following the 11th nuclear division up to the 14th, the concentration of Bicoid protein distributes non-uniformly along the antero-posterior axis of the syncytial blastoderm of the embryo of *Drosophila*. Bicoid protein has higher concentration near the anterior pole of the embryo and its local concentration decreases as the distance to the anterior pole increases. This is called the Bicoid protein gradient[2,4]. Recently, *bicoid* mRNA gradients along the antero-posterior axis of the embryo of Drosophila have been observed[7], clarifying our current views about Drosophila early development.

To infer about the mechanism of establishment of antero-posterior protein gradients in *Drosophila* early development, several models have been proposed. Some are based on the hypothesis of protein diffusion along the antero-posterior axis of the embryo[8,9], others are based on the diffusion of mRNA of maternal origin[10,11,12,13,14].

To clarify the role of diffusion in *Drosophila* early development, we extend a pattern formation model[11] to include diffusion for both *bicoid* mRNA and Bicoid protein. With this model, we evaluate the relative role of the diffusion coefficients of mRNA and protein.

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We denote by $R(x,t)$ and $B(x,t)$ the local concentrations of *bicoid* mRNA and of Bicoid protein, respectively, where $x$ denotes the longitudinal coordinate of the embryo ($x \in [0,L]$) and $t$ represents time. The typical embryo length is $L = 0.5$ mm. We consider that mRNA degrades with a fixed ratio $d$ and the ratio of production of protein is proportional to the local concentration of mRNA. Assuming a diffusion mechanism for both mRNA and protein, the model equations describing the synthesis of Bicoid protein from mRNA are,

$$\begin{aligned} \frac{\partial R}{\partial t} &= -dR + D_R \frac{\partial^2 R}{\partial x^2} \\ \frac{\partial B}{\partial t} &= aR + D_B \frac{\partial^2 B}{\partial x^2} \end{aligned} \qquad (1)$$

where $D_R$ and $D_B$ are the diffusion coefficients of both *bicoid* mRNA and Bicoid protein, respectively. The rate of production of protein is measured by the proportionality constant $a$. In model equations (1), both *bicoid* mRNA and Bicoid protein diffuse. We further assume that there are no fluxes of mRNA and protein from the interior to the exterior of the embryo (zero flux boundary conditions). At the beginning of Drosophila development, *bicoid* mRNA is deposited in some region of the antero-posterior axis of the embryo, and the initial concentration of Bicoid protein along the embryo is zero.

For a given initial distribution of *bicoid* mRNA and after some time, a steady nonzero concentration of the Bicoid protein is reached. At this equilibrium state, the time derivatives in the model equations (1) are zero. As the first equation in (1) is independent of protein $B$ and after reaching the steady nonzero concentration of Bicoid protein, it can be shown[11] that the *bicoid* mRNA concentration is zero, $R = 0$. At the steady state, the *bicoid* mRNA has been converted into protein and degraded. One of the features of model (1) is the formation of a transient mRNA gradient that decays to zero at the steady state[11].

Since $R = 0$ at the steady state, by (1), the concentration and spatial distribution of Bicoid protein obeys to $D_B \frac{\partial^2 B}{\partial x^2} = 0$. From this equation and from the zero flux boundary conditions, it follows that the steady state distribution of the Bicoid protein is constant along the embryo. Thus, the assumption that both mRNA and protein diffuse implies that the concentration of Bicoid protein is constant along the embryo, contradicting the existence of a gradient of Bicoid protein.

With the choice $D_B = 0$, the *bicoid* mRNA diffusion model (1) explains the observed gradients of the protein Bicoid[11], fitting the experimental data with high accuracy, both in time and along the antero-posterior longitudinal axis of the embryo. The results of the mathematical model and the analysis just did lead to the conclusion that mRNA diffusion is the main mechanism responsible for the establishment of protein gradients.

Experimental observations of protein localization during cleavage stages 11-14, corroborate the model conclusions just described. The images in Figure 1 show

that Bicoid protein is always localized around the syncytial nuclei where ribosomes are positioned. This protein localization feature is seen in all the embryo data sets of the FlyEx database[15,16,17,18,19] (data sets ab18, ab17, ab16, ab12, ab14, ab9, ad13, ab8). The images show that Bicoid protein is not present in the inter-nuclear spaces in the cytoplasm, showing that diffusion of Bicoid protein cannot have a role in the establishment of the Bicoid gradient. Since diffusion is a dispersal effect homogenizing concentrations in a media, the mathematical model with the theoretical arguments presented here are consistent with the mechanistic interpretation of diffusion[20] and with the observed data of Figure 1.

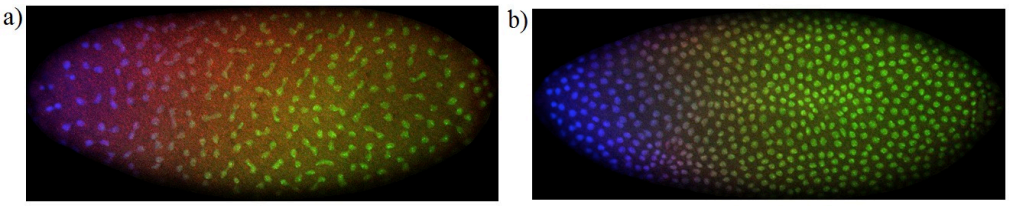


Figure 1: **Bicoid protein gradients:** Distribution of Bicoid (blue) and Caudal (green) proteins in the embryo of *Drosophila*, during interphase following the cleavage stages 11 (a) and 12 (b). The images were obtained from the FlyEx[15,16,17,18,19] database, and correspond to datasets ab18 (a) and ab17 (b). These proteins are translated from mRNA of maternal origin and accumulate around the syncytial nuclei. The absence of Bicoid and Caudal proteins in the inter-nuclear regions in the cytoplasm shows that Bicoid and Caudal proteins have a very low mobility, contradicting the hypothesis of diffusion for Bicoid and Caudal proteins.

Several authors have analysed experimentally the mRNA localization mechanisms in the embryo of *Drosophila*. Recently, the *bicoid* mRNA gradient has been observed experimentally[7]. The observation of rapid saltatory movements in injected *bicoid* mRNA into the embryo of *Drosophila*, followed by dispersion without localization has been reported[21]. Other diffusion effects observed on the mRNA motion have been reported for the *nanos* mRNA[22]. From a theoretical point of view, Saxton argued that the relative small size of mRNA suggests that random diffusion and specific anchoring to the cytoskeleton in a target area might suffice for localization in the syncytial blastoderm[5].

Further extension of the mRNA diffusion model has been applied to the description of morphogenetic gradients in other maternal and gap-gene families of mRNAs and proteins. The same modeling with mRNA diffusion has been applied to the description of gradient formation of Caudal[10,14], Tailless[12,13], Hunchback[12,13] and Knirps[12,13] proteins. Calibration of a mRNA diffusion model with experimental data enabled to predict the spatial position of the segment of the Huckebein protein[13] along the embryo of *Drosophila*. In all these cases, the

model predictions fit accurately the experimental data, both in time and along the antero-posterior longitudinal axis of the embryo, with relative errors below 5%.

We conclude that, in *Drosophila* early development, mRNA diffusion is the main morphogenesis mechanisms explaining consistently the establishment of Bicoid protein gradients.

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