Supplemental Data for Gregor et al 2007,

"Stability and nuclear dynamics of the Bicoid morphogen gradient"

Information for Figure 2

Here we provide further details concerning the experimental methods and implications of the data in Figure 2E-H:

E GFP-antibody intensities vs. Bcd-antibody intensities for 15 doubly stained *bcd* null-mutant embryos expressing Bcd-GFP. Observed linear relationship argues that both the GFP and Bcd regions of the fusion protein are detectable at the same time after translation (both antibodies are attached to different epitopes of the exact same protein) and that the GFP region remains associated with the Bcd region of the fusion protein. GFP does not persist after the Bcd epitope is degraded and miscellaneously cleaved Bcd or GFP protein are present in the egg, suggesting that Bcd and GFP are degraded simultaneously. **F** GFP-antibody intensities vs. eGFP-autofluorescence intensities for 17 GFP-stained *bcd* null-mutant embryos expressing Bcd-GFP. The linear relationship is a strong indication that the fluorescence intensities obtained in antibody stainings for GFP linearly reflect the actual concentration of eGFP present at a given location in the embryo, and hence via E of Bcd protein. The linearity also suggests that an eGFP maturation delay does not influence Bcd-GFP expression in embryos fixed at this stage of development. The larger scatter around the diagonal compared to E presumably stems from the fact that under these conditions natural eGFP fluorescence is very weak. **G** Bcd-antibody intensities for 20 randomly selected pairs of embryos from a Bcd-stained batch of 21 wild-type embryos. Linearity implies that this method of comparing profiles is also viable for comparing profiles of embryos of different genotypes with each other. **H** Bcd-antibody intensities of 14 pairs of embryos randomly chosen from a batch of 15 Bcd-stained embryos expressing Bcd-GFP vs. embryos randomly chosen from a batch of 18 Bcd-stained *bcd* null-mutant embryos expressing Bcd-GFP. Clustering around the diagonal indicates that the spatial profiles of Bcd-GFP have diffusion and degradation behavior similar to those of the endogenous protein.

Slowing of diffusion by fixed binding sites

Let's express the total Bcd concentration in the cytoplasm as

$$C_{tot} = C_{\text{free}} + \rho f, \tag{S1}$$

where $C_{\text{free}}(\vec{r}, t)$ is the unbound freely diffusing Bcd concentration and ρf is the bound Bcd concentration with ρ being the concentration of binding sites for Bcd in the cytoplasm and $f(\vec{r}, t)$ the fractional binding site occupancy whose dynamics is given by

$$\frac{\partial f}{\partial t} = k_{\text{on}}(1 - f)C_{\text{free}} - k_{\text{off}} f, \tag{S2}$$

with dissociation constant $K_D = k_{\text{off}}/k_{\text{on}}$. When the binding/unbinding of Bcd to cytoplasmic constituents is locally equilibrated ($\partial f/\partial t = 0$) we can express the steady state fractional occupancy as

$$f(t) = \frac{C_{\text{free}}}{C_{\text{free}} + K_D}. \tag{S3}$$

Hence for weak binding ($C_{\text{free}} \ll K_D$) Eq. (S1) becomes

$$C_{tot} = C_{\text{free}} \left(1 + \frac{\rho}{K_D}\right), \tag{S4}$$

which leads to a ratio of bound to unbound Bcd molecules.

The cytoplasmic binding/unbinding dynamics of Bcd introduces a simple modification to the time evolution of the free Bcd concentration and Eq. (5) in the main text becomes

$$\frac{\partial C_{\text{free}}}{\partial t} = D\nabla^2 C_{\text{free}} - \rho \frac{\partial f}{\partial t} - \frac{1}{\tau} C_{\text{free}}, \tag{S5}$$

which introduces a coupling between Eq. (S2) and Eq. (S5). Let's find the steady state solution of this system within the approximation of negligible concentration variation perpendicular to the antero-posterior axis. For low fractional occupancy ($f \ll 1$), the Fourier transform of Eq. (S2) is given by

$$-i\omega \tilde{f} = k_{\text{on}} \tilde{C}_{\text{free}} - k_{\text{off}} \tilde{f} \tag{S6}$$

where $\tilde{f}(q, \omega)$ and $\tilde{C}(q, \omega)$ are the Fourier transforms of f and C, respectively, with conjugate variables q and ω. For times larger than the correlation time for binding/unbinding $\tau_c = 1/k_{\text{off}} + k_{\text{on}}\tilde{C}_{\text{free}}$ we have $\omega \ll k_{\text{off}}$. This approximation is justified by the much larger time scales of the dynamics of the Bcd gradient. Thus Eq. (S6) becomes

$$\tilde{f} = \frac{\tilde{C}_{\text{free}}}{K_D} \left(1 + \frac{i\omega}{k_{\text{off}}}\right), \tag{S7}$$

where we retained the first order term of the Taylor expansion in ω on the right hand side. Inserting Eq. (S7) in the Fourier transform of Eq. (S5) results in

$$-i\omega \left(1 + \frac{\rho}{K_D}\right) \tilde{C}_{\text{free}} = (-q^2 D - 1/\tau)\tilde{C}_{\text{free}}, \tag{S8}$$

which is the Fourier transform of the linearized Eq. (5) in the main text with an additional factor of $(1-\rho/K_D)$ on the left hand side. Hence, the steady state solution is again given by Eq. (5) with a renormalized diffusion constant

$$D \longrightarrow D' = \frac{D}{\left(1+\frac{\rho}{K_D}\right)}, \tag{S9}$$

and a renormalized protein lifetime

$$\tau \longrightarrow \tau' = \tau\left(1+\frac{\rho}{K_D}\right). \tag{S10}$$

The smaller diffusion constant results from the binding/unbinding dynamics and can be interpreted as an effective diffusion constant of Bcd molecules in a "buffer" of cytoplasmic binding sites. The longer lifetime could be due to molecules only being degraded if they are not bound. Hence the length constant is not affected by the binding/unbinding dynamics of Bcd to cytoplamic constituents, i.e. $\lambda = \lambda' = \sqrt{D'\tau'}$, and in principle, an adiabatic transition from the fast diffusing regime to a slow diffusing regime in the cortical cytoplasmic region of the egg is possible without length constant changes.

Could diffusion be faster than we observe?

The simplest resolution of our paradox would be if diffusion is faster in the bulk of the embryonic cytoplasm than it is in the cortex, or the immediate neighborhood of the nuclei, where we make our measurements. For technical reasons, we were unable to measure D for Bcd in the bulk cytoplasm of fertilized eggs. Our cut sections demonstrated that Bcd is found in this space, and the radial variations in Bcd concentrations are consistent with the possibility that diffusion is an order of magnitude faster in the core because there are an order of magnitude fewer binding sites (see Methods). On the other hand, our measurements in the cytoplasm of unfertilized eggs–as an approximation to the bulk cytoplasm in fertilized eggs–did not show a larger diffusion constant. Thus, the evidence for spatial variations in diffusion constant remains equivocal.

An alternative is that diffusion constants are different on different time scales. Our direct measurements of Bicoid diffusion using photobleaching are limited to the $\sim 1\,\text{min}$ time scale (Figure 5), and this also is the relevant time scale for exchange between nuclei and the surrounding cytoplasm (Figure 4B). But the steady state is established on the time scale $\sim 90\,\text{min}$, and more generally the Bicoid profile is "used" by the system on the time scale of hours. When we injected inert molecules of molecular weight comparable to Bcd into the embryo, and monitored their spatiotemporal dynamics on the scale of hours, we found that the results were consistent with the diffusion equation and with $D \sim 10-15\,\mu\text{m}^2/\text{s}$ (Gregor et al 2005). Further, there was a component of this diffusion that was not consistent with the Stokes-Einstein relation, but instead had the form expected if the cytoplasm were being randomly stirred by some active process. If this process occurs on time scales longer than one minute but shorter than one hour, it could account for the discrepancy between the measured diffusion constants of Bcd and the diffusion constants needed to explain the rapid approach to a steady state gradient.

More quantitatively, if the cytoplasm moves with a typical velocity v and tends to keep moving in the same direction for a time $\sim \tau_c$ before becoming randomized, then if we look at long times we will see a contribution to the effective diffusion constant $D_{\text{eff}} = v^2\tau_c$. Our previous results (Gregor et al 2005) suggest that this term is $6.2 \pm 1.0\,\mu\text{m}^2/\text{s}$. If, for example, $\tau_c \sim 10\,\text{min}$, then the typical velocities would be very small, $v \sim 0.1\,\mu\text{m/s}$. Such small velocities would not significantly perturb the recovery from photobleaching in the volumes that we studied, nor would they significantly change the flux into the nucleus (Berg & Purcell 1977). Thus, motions on this scale would not contribute to our diffusion measurements or to the analysis of nuclear-cytoplasmic exchange, but would be sufficient to explain how the Bcd profile reaches steady state on the observed time scale. It is even possible that the relevant motions, rather than being continuous, are episodic, perhaps associated with the mitotic cycles themselves.

A potential limitation of our experiments is that we are unable to measure Bcd during the first hour after fertilization. It therefore remains a possibility that the Bcd diffusion coefficient is significantly greater during this period than it is during cycles 9–12, facilitating anterior to posterior transport and resolving the apparent inconsistency between the slow diffusion coefficient measured later and the rapidity of gradient stabilization. A decline in synthesis rate could also enhance the efficacy of early fast transport; however, existing evidence (Salles et al 1994) suggests that the Bcd translation rate actually increases during the first two hours after egg fertilization, rendering this contribution unlikely. In addition to these experimental limitations, our estimates for equilibration times using the SDD model have assumed that synthesis, diffusion and degradation are not under the control of Bcd activity; the advantages of such feedback control in the generation of robust gradients in other morphogen gradient systems has been examined (Eldar et al 2003).

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