SI Appendix

Compartmental model of the syncytial blastoderm: The early embryo is modeled as a periodic array of compartments (Fig. 3C). Each compartment consists of a cytoplasmic region and a single nucleus (Fig. 3Aii). FRAP experiments have shown that transport within a compartment occurs at a much faster time scale than transport between adjacent compartments (1). We therefore assume that the cytoplasmic and nuclear regions are mixed uniformly; i.e. the concentration of cytoplasmic species is uniform within the cytoplasmic region of a compartment and the concentration of nuclear Dl is uniform within a nucleus.

The cortical region of the syncytium is approximated as the space between two ellipsoids. The nuclei are assumed to be spherical and are contained within uniformly distributed cuboidal compartments in the cortex. The total volume of the cortical region is determined by experimental measurements of the embryo at cycle 14 (2). Since it has been determined that there are about 6000 nuclei at the last nuclear cycle, the volume of a single compartment at cycle 14 ($V^{14}_{compart}$) is calculated by dividing the total volume of the cortical region into 6000 equivalent compartments. We assume that the total volume of all the compartments remains constant from one cycle to another. Accordingly, doubling of nuclei leads to halving of individual compartmental volume. Hence, the volume of a compartment during cycle 'i' is given by:

$$V^{i}_{compart} = 2^{14-i} V^{14}_{compart} \qquad i \in [10,14]$$

From one cycle to the next, the compartments are modeled to divide in a way such that, the width (w) of a compartment remains the same while the length (l) and the breadth (b) decrease by a factor of $\sqrt{2}$ to account for the halving of compartmental volume (Fig. 3C). Accordingly, the surface area at the boundary shared by adjacent compartments reduces by a factor of $\sqrt{2}$. Note that the volume of a single compartment remains constant within a nuclear cycle. However, the volumes of the cytoplasmic and nuclear regions within an individual compartment changes continuously during interphase. Specifically, nuclear volume increases and consequently the volume of the cytoplasmic region decreases. We approximate a time-dependent function for nuclear radius from the measurements by Gregor et. al (2). The volume of the cytoplasmic region within a compartment is the difference between the total volume of a single compartment ($V_{compart}$) and the volume of the nucleus.

$$V(t)_{nucleus} = \frac{4}{3}\pi r(t)^3_{nucleus}, \qquad V(t)_{cytoplasm} = V(t)_{compart} - V(t)_{nucleus}$$

The model considers a one-dimensional (1D) axis along the DV cross-section of the embryo (Fig. 3Ai). Owing to bilateral symmetry, simulating one half of the embryo with no flux boundary conditions is sufficient. According to the model for compartmental division, as the number of nuclei doubles in the 3D monolayer of the syncytium, the number of nuclei along the 1D axis of the model increases by $\sqrt{2}$. Thus, considering the DV cross-section close to the middle of the embryo along the AP axis, 13, 18, 26, 37 and 51 nuclei were considered at cycle 10, 11, 12, 13 and 14 respectively.

Model equations and parameters: Fig. 3B illustrates the proposed model for reaction kinetics and transport processes. Transport from one compartment to another is proportional to the shared surface area between adjacent compartments. Nuclear import and export are modeled as surface reactions on the surface of the nucleus. Production and degradation of Cactus as well as association and dissociation of the Dl-Cact complex is restricted to the cytoplasmic region of the compartment. Dissociation of the Dl-Cact complex depends on the gradient of Toll activation. Since this gradient is unknown, the dissociation reaction rate gradient is parameterized using a family of curves with three parameters: R, S and ξ. For each cycle, the equations are solved numerically for a time interval based on the detailed measurements by Foe and Alberts (3, 4).

Mass balance equations:

$$\frac{d(V_n C^h_{Dl,n})}{dt} = A_n k_i C^h_{Dl,c} - A_n k_e C^h_{Dl,n}$$

$$\frac{d(V_C C^h_{Dl,c})}{dt} = \Gamma A_m (C^{h+1}_{Dl,c} - 2C^h_{Dl,c} + C^{h-1}_{Dl,c}) + k_D C^h_{Dl-cact,c} V_C - k_b C^h_{Dl,c} C^h_{cact,c} V_C - A_n k_i C^h_{Dl,c} + A_n k_e C^h_{Dl,n}$$

$$\frac{d(V_C C^h_{Dl-cact,c})}{dt} = \Gamma A_m (C^{h+1}_{Dl-cact,c} - 2C^h_{Dl-cact,c} + C^{h-1}_{Dl-cact,c}) - k_D C^h_{Dl-cact,c} V_C + k_b C^h_{Dl,c} C^h_{cact,c} V_C$$

$$\frac{d(V_C C^h_{cact,c})}{dt} = \Gamma A_m (C^{h+1}_{cact,c} - 2C^h_{cact,c} + C^{h-1}_{cact,c}) + k_D C^h_{Dl-cact,c} V_C - k_b C^h_{Dl,c} C^h_{cact,c} V_C + P_{cact} V_C - k_{Deg} C^h_{cact,c} V_C$$

$$where, k_D = \frac{R}{S + x^{\xi}}$$

Boundary conditions $\forall t \in (0, T_{\text{int}})$

$$C^0_{sp,c} = C^2_{sp,c} \qquad @\, x = 0, \quad C^{n+1}_{sp,c} = C^{n-1}_{sp,c} \qquad @\, x = L, \quad sp \in [Dl, Cact, Dl - Cact]$$

Initial conditions $\forall x \in (0, L)$

Cycle 10 $C^h_{cact,c} = C^o_{cact} \equiv {P_{cact}}/{k_{Deg}}$, $C^h_{Dl-cact,c} = C^o_{Dl-Cact}$, $C^h_{Dl,c} = C^h_{Dl,n} = 0$

Cycle 11-14 -- Based on the concentration profiles established at the end of previous cycle.

When the syncytium undergoes a transition from interphase to mitosis, nuclei dissolve and thus, the system is governed by the dynamics of cytoplasmic species alone with no nuclei and no nuclear Dorsal. The transition is modeled as follows: (i) Breaking of the nuclear envelope takes place instantaneously at the transition between interphase and mitosis, (ii) nuclear Dl is distributed instantaneously and uniformly in its corresponding cytoplasmic region, (iii) the cytoplasmic region occupies the entire compartment with constant volume. We also assume the transition from the mitosis of one cycle to the interphase of the next cycle to be instantaneous. The gradients established at the end of mitosis provide the initial conditions for interphase of the next cycle. This transition is explained in Figure S1 A. For instance, consider the transition from mitosis to interphase for Dl-Cact complex. Blue circles represent the concentration of Dl-Cact at the end of cycle 11 mitosis. Then, a gradient (red curve) connecting these points is constructed and

the concentration in each compartment at the beginning of cycle 12 interphase is derived from the gradient (red curve) and is represented by green triangles.

During interphase, the dynamics of four species in N compartments is governed by a system of 4N coupled, nonlinear and non-autonomous differential equations. During mitosis, when the nuclei dissolve, the system is governed by 3N equations and there is no nuclear Dorsal, nor nucleocytoplasmic shuttling. Interphase of the next cycle is governed by 4N' equations where N' is the increased number of compartments as described above.

Nondimensionalization: The system of equations is rendered dimensionless using the following set of scaling factors. Nuclear, cytoplasmic and bound Dl is scaled using the concentration of bound Dl at the beginning of cycle 10. Similarly, free cactus is scaled using its steady state concentration. Time is scaled using the total time from cycle 10 to cycle 14 and space is scaled using the estimate for the half-circumference of the DV cross-section of the embryo.

$$\tau = t/T, \quad z = x/L, \quad \overline{C}^h_{Dl,n} = C^h_{Dl,n}/C^o_{Dl}, \quad \overline{C}^h_{Dl,c} = C^h_{Dl,c}/C^o_{Dl}, \overline{C}^h_{Dl-cact,c} = C^h_{Dl-cact,c}/C^o_{Dl}, \quad \overline{C}^h_{cact,c} = C^h_{cact,c}/C^o_{cact}$$

These resulting dimensionless parameters correspond to different biophysical processes of the system, parameterization of Toll activation and stoichiometry: (i) σ – Nuclear Import, (ii) μ - Nuclear Export, (iii) λ – Transport across compartments, (iv) γ – Association of complex, (v) α – Degradation of Cactus, (vi) Ψ - Relative levels of Dl and Cact (vii-ix) β, φ, ξ – the parameters that characterize the spatial pattern for complex dissociation rate, which in turn reflect the spatial pattern of Toll activation (k_d in the mass balance equations). Thus, by specifying the values of these parameters, the model can be used to predict the spatiotemporal patterns of all the species considered in the system.

$$\sigma = \frac{A_n^{14} k_i}{V_n^{14}} T \quad \mu = \frac{A_n^{14} k_e}{V_n^{14}} T \quad \lambda = \frac{\Gamma A_m^{14}}{V_n^{14}} T \quad \gamma = k_b C^o_{cact} T \quad \alpha = k_{\deg} T \quad \psi = \frac{C^o_{Dl}}{C^o_{cact}} \quad \beta = \frac{RT}{L^\xi} \quad \varphi = \frac{S}{L^\xi} \quad \xi$$

Distribution of parameters consistent with the experimentally observed dynamics: An exhaustive sensitivity and robustness analysis of the set of nine-parameter vectors obtained is beyond the scope of this paper. We looked at the marginal distribution function of the individual parameters (Figure S1 B-D). Most notably, we found that the distribution function for dimensionless degradation of Cact is bounded from below, which means that once the degradation of Cact is fast enough, the precise rate of degradation does not matter (Figure S1 B). The distribution function for stoichiometry, the relative levels of Dl and Cact, is peaked close to one, which implies that the amounts of Dl and Cact in the system should be comparable (Figure S1 C). This in turn implies that this ratio must be tightly controlled in the wild type.

One of the most striking features observed is the peak of dimensionless transport coefficient close to one, which corresponds to a lateral displacement of about one compartment over the period of cycle 14 (Figure S1 D). This implies that the system operates close to the cell-autonomous regime. To further verify this hypothesis, we

considered the case with perfect compartmentalization and solved the differential equations at the ventral and the dorsal end for steady state analysis. Based on this analysis, the constraints applied on relative levels of Dl in the nucleus and the cytoplasm at the dorsal and ventral sides can be expressed as follows:

Constraint 1: At ventral end: Nuclear Dl > Cytoplasmic Dl: $1 > \frac{\mu}{\sigma}\left(1+\frac{\gamma\varphi}{\beta}\right)$

Constraint 2: At dorsal end: Nuclear Dl < Cytoplasmic Dl: $1 < \frac{\mu}{\sigma}\left(1+\frac{\gamma(\varphi+1)}{\beta}\right)$

We verified that even though the model does not impose perfect compartmentalization, >99% of the parameter vectors satisfy the steady state cell-autonomous form of the constraints described above. The result that the system operates close to the cell-autonomous regime is consistent with the photobleaching experiments with Dorsal-GFP by DeLotto et. al.(16).

REFERENCES

1. DeLotto R, DeLotto Y, Steward R, & Lippincott-Schwartz J (2007) *Nucleocytoplasmic shuttling mediates the dynamic maintenance of nuclear Dorsal levels during Drosophila embryogenesis Development* **134,** 4233-4241.
2. Gregor T, Wieschaus E, McGregor AP, Bialek W, & Tank DW (2007) *Stability and Nuclear Dynamics of the Bicoid Morphogen Gradient Cell* **130,** 141-153.
3. Foe VE (1989) *Mitotic domains reveal early commitment of cells in Drosophila embryos Development* **107,** 1-22.
4. Foe VE & Alberts BM (1983) *Studies of Nuclear and Cytoplasmic Behavior During the 5 Mitotic-Cycles That Precede Gastrulation in Drosophila Embryogenesis Journal of Cell Science* **61,** 31-70.

Figure Legends

Figure S1. (A) Illustration of the gradient mapping from cycle 11, with 19 compartments to cycle 12, with 26 compartments.
(B-D) Marginal Distribution functions for dimensionless rate of Cact degradation (B), stoichiometry (C), and dimensionless transport coefficient (D).