Supporting Information for

A Precise Bicoid Gradient is Nonessential During Cycles 11-13 for Precise Patterning in the *Drosophila* Blastoderm

Elena M. Lucchetta, Meghan E. Vincent and Rustem F. Ismagilov*

Department of Chemistry and Institute for Biophysical Dynamics,

The University of Chicago, Chicago, Illinois 60637, USA

Supporting Figures

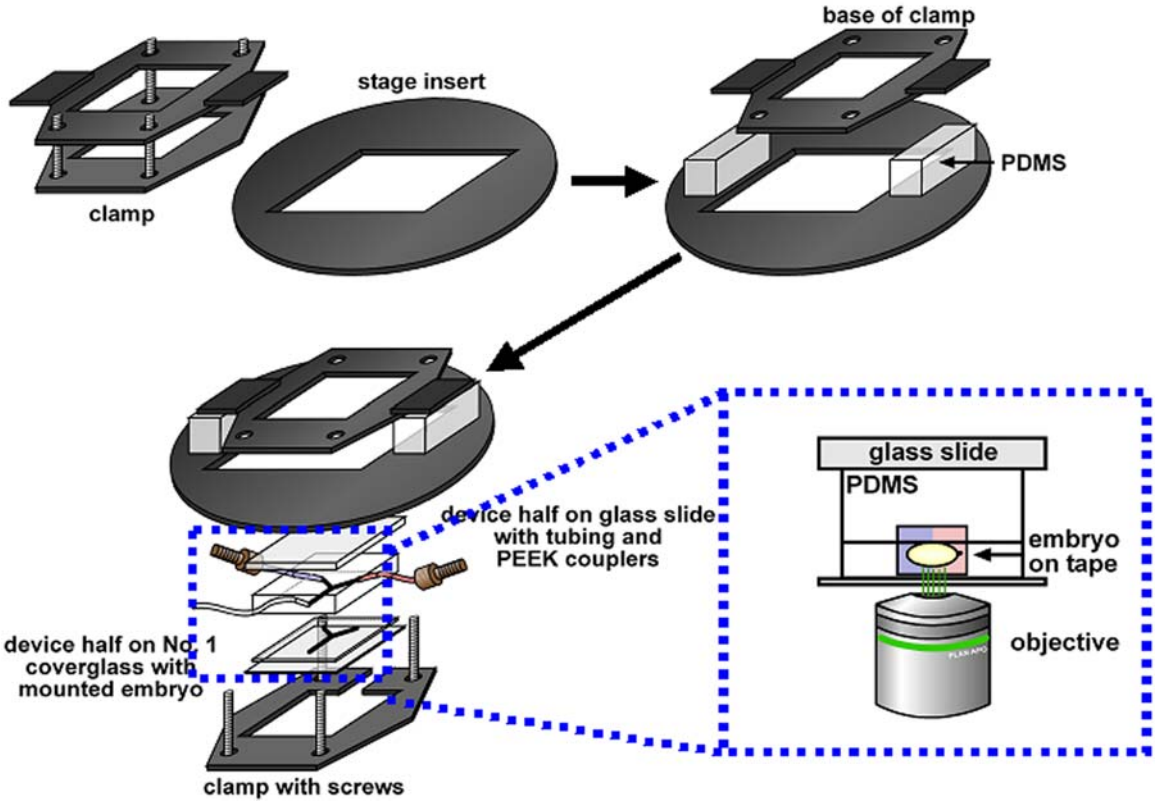


Figure S1. Schematic of microfluidic device coupled to confocal microscopy. The microfluidic device is clamped to a plate, which inserts into the motorized stage of the microscope, minimizing movement of the device relative to the microscope. A thin (~500 μm) device is fabricated to accommodate a higher numerical aperature objective (x20, 0.7 N.A.).

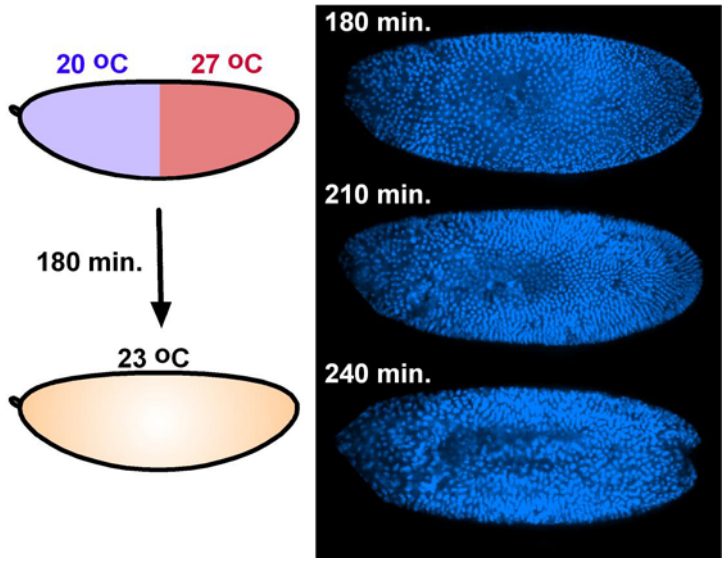


Figure S2. Embryos imaged from cycles 11-13 in a temperature step with anterior at 20 ^{o}C and posterior at 27 ^{o}C gastrulate and recover from the temperature step. Embryo shown is a later stage of that presented in Figure 3.

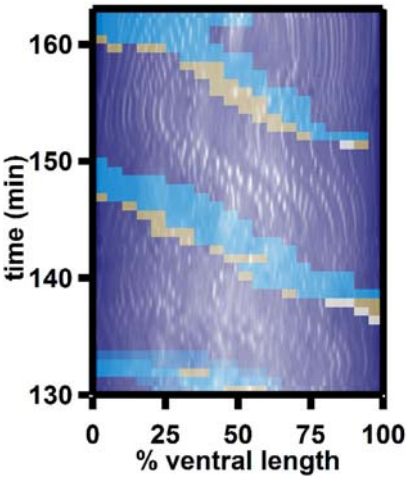


Figure S3. Correspondence of nuclear division cycles with the movement of nuclei in the embryo presented in Figure 2D,E, and F.

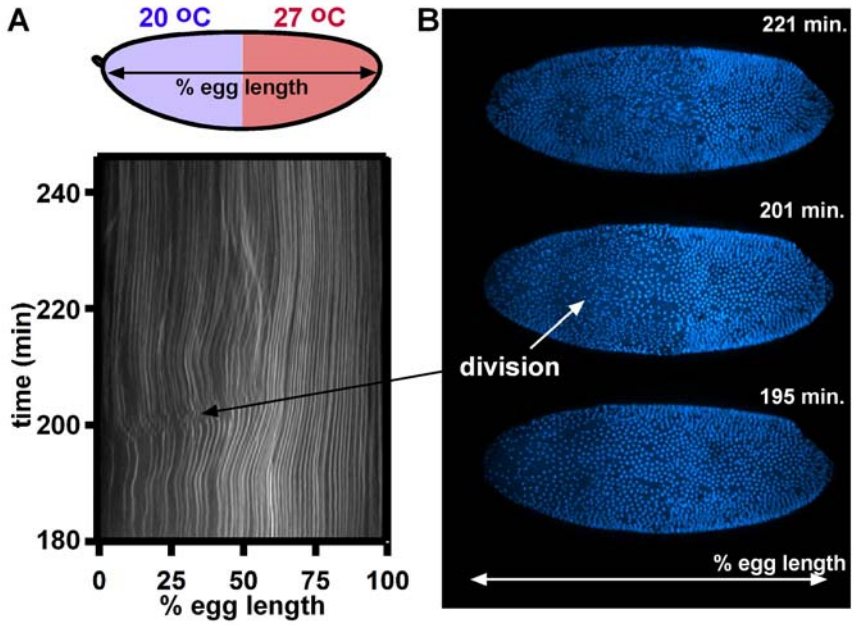


Figure S4. An embryo in a temperature step corrected for nuclear density by dividing only in the anterior half of the embryo. A) Space-time plot showing nuclear position over time. Nuclei in the anterior half of the embryo divided at ~200 minutes. B) Corresponding images from the time series.

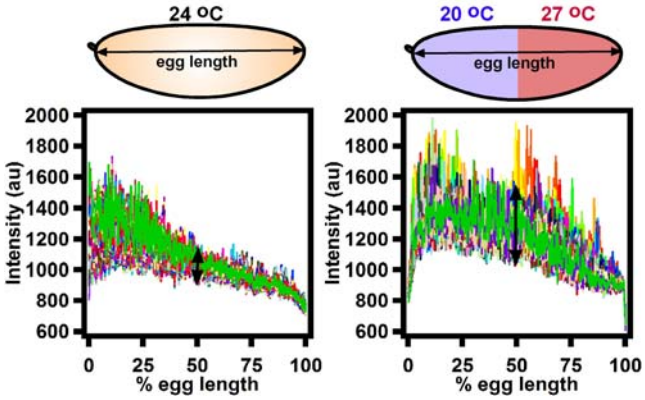


Figure S5. Projection of Bcd readout at 50% egg length over cycles 11-13. The embryo in a temperature step reads out drastically different concentrations of Bcd in comparison to the embryo developed at uniform temperature. Surprisingly, patterning is normal in both embryos.

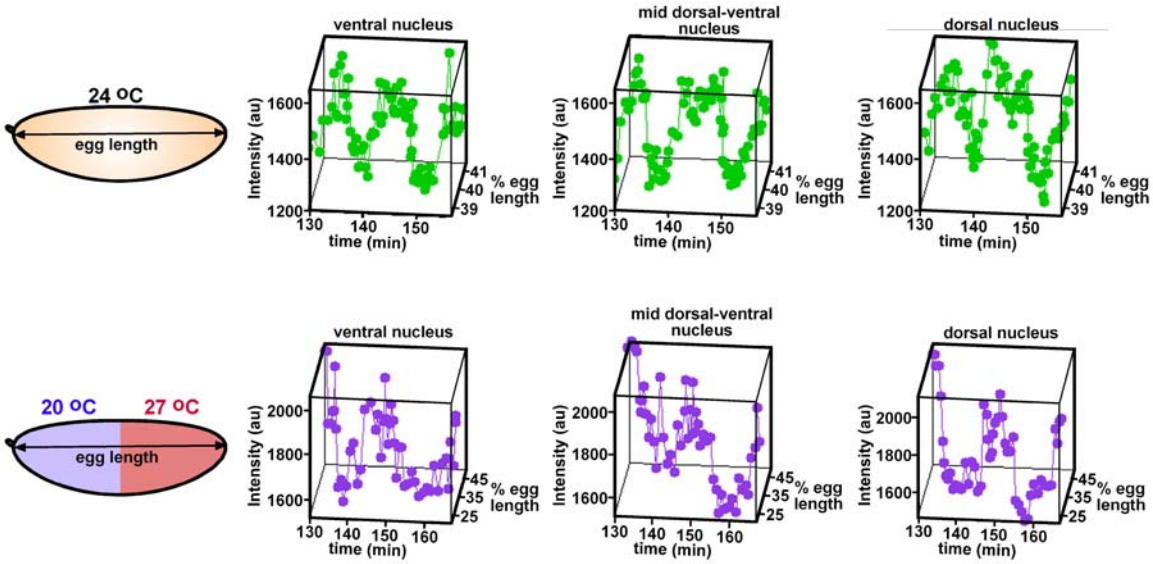


Figure S6. Bcd intensity within a given nucleus at 40% egg length remains the same in both embryos developing at uniform temperature and in a temperature step, despite drastic difference in nuclear motion. Nuclear motion of three nuclei in the embryo developed at uniform temperature was on the order of 2% egg length, while nuclear motion of three nuclei in the embryo developed in a temperature step was on the order of 20% egg length.

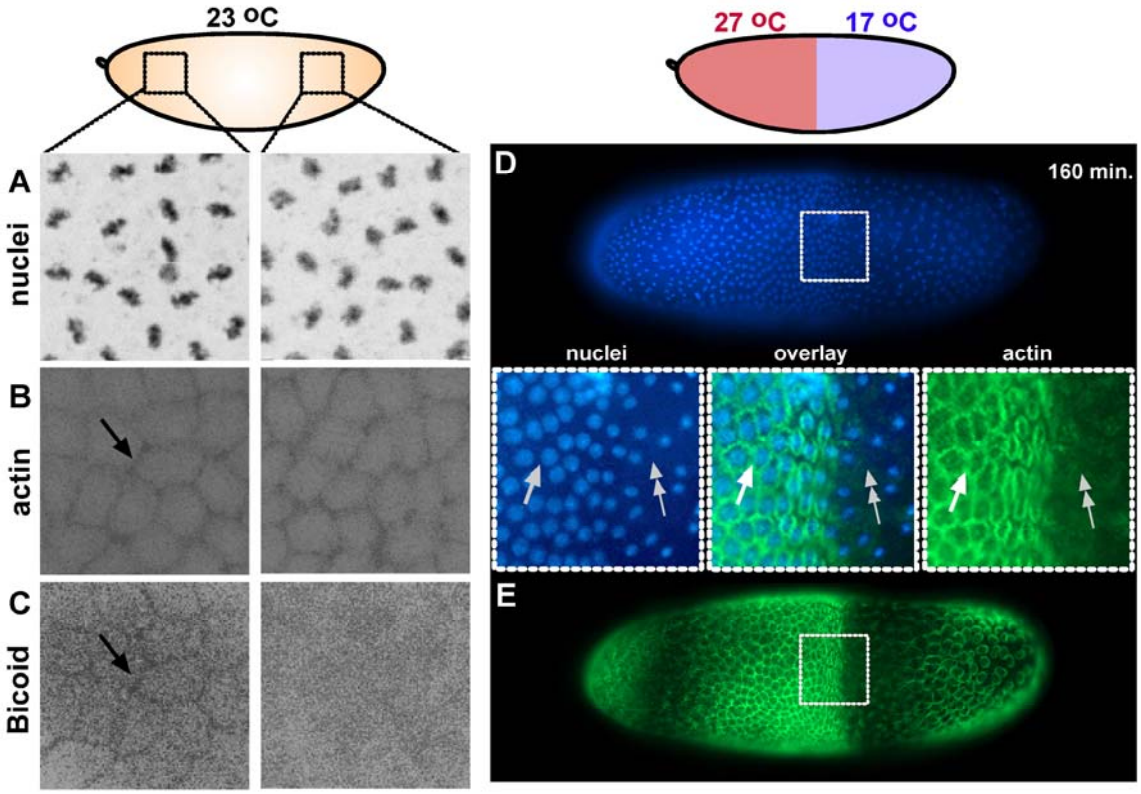


Figure S6. Images from the anterior and posterior halves of an embryo developed at uniform 23 ^{o}C and stained for nuclei, actin, and Bicoid. (A) Regions with nuclei in metaphase/anaphase.

(B) Actin in these regions forms hexagonal rings around individual nuclei. (C) As the nuclei divide the Bicoid protein, localized in the head, appears diffuse and partially overlapping actin. (D-E) The actin network is disrupted at the boundary between high and low density nuclei in embryos exposed to a temperature step. (D) Nuclei detected by DAPI staining. A boundary is observed between high and low densities of nuclei. (E) Actin detected by phalloidin. The actin network is disrupted at the boundary between high and low density nuclei and appears to be highly compressed in the region of high density of nuclei.

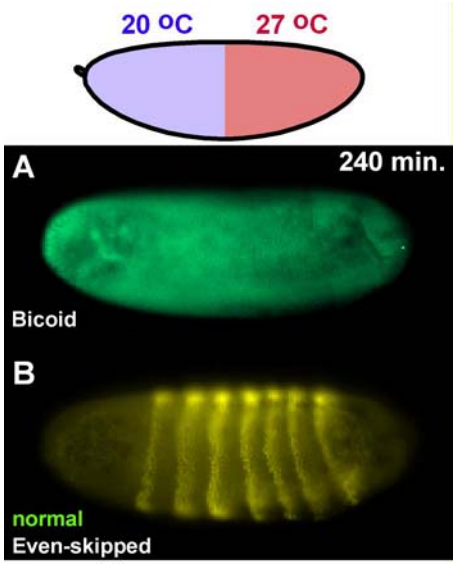


Figure S8. An embryo exposed to a temperature step and imaged in real-time displays normal Even-skipped patterning during cycle 14, as detected by removing the embryo from the microfluidic device at cycle 14 and immunostaining.

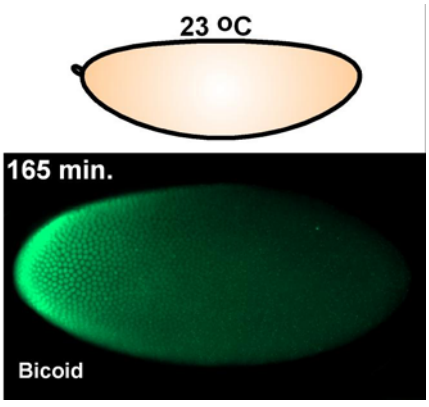


Figure S9. Control embryos developed at 23 ^{o}C and fluorescently immunostained for Bcd displayed a normal Bcd profile.

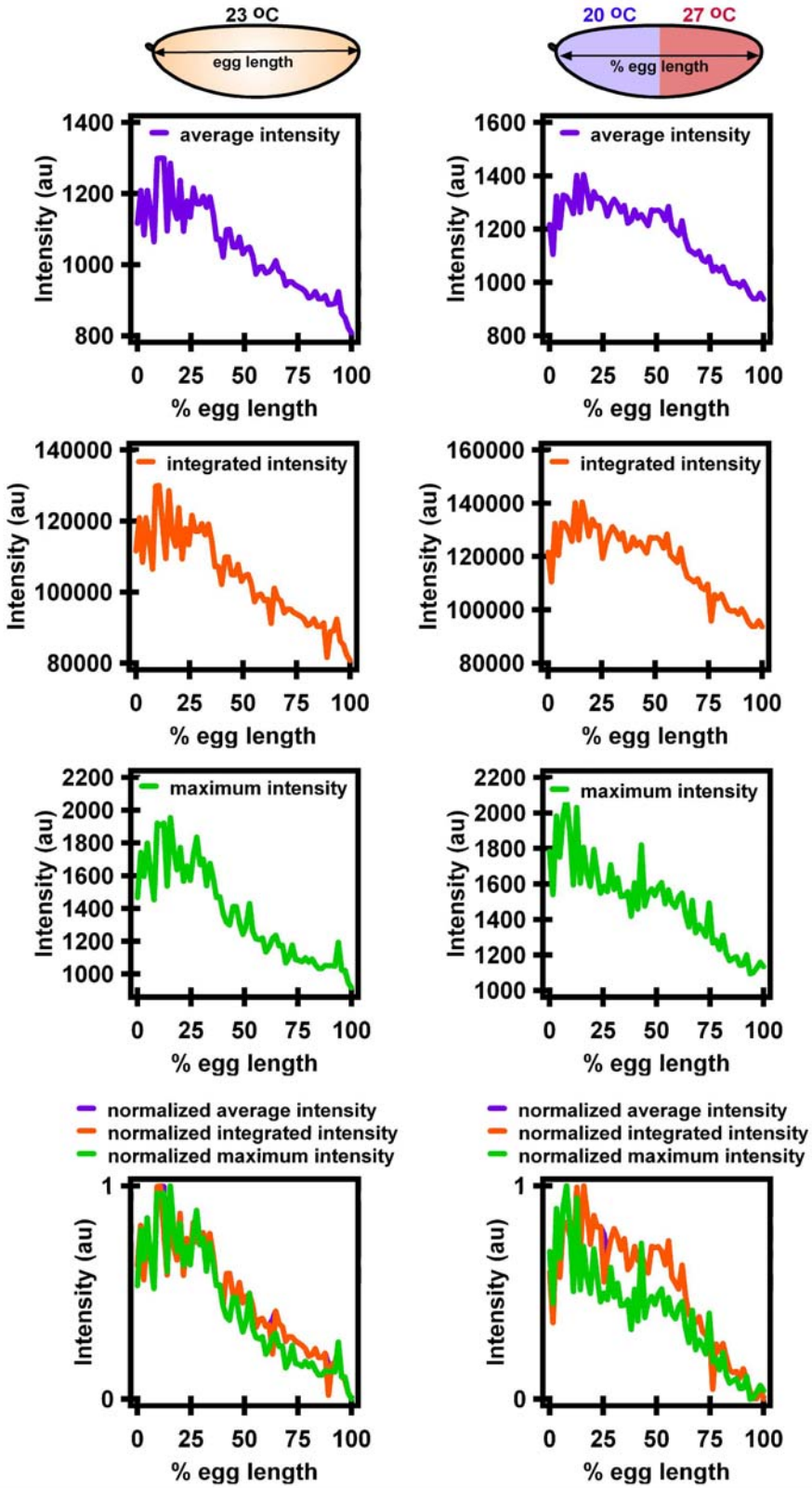
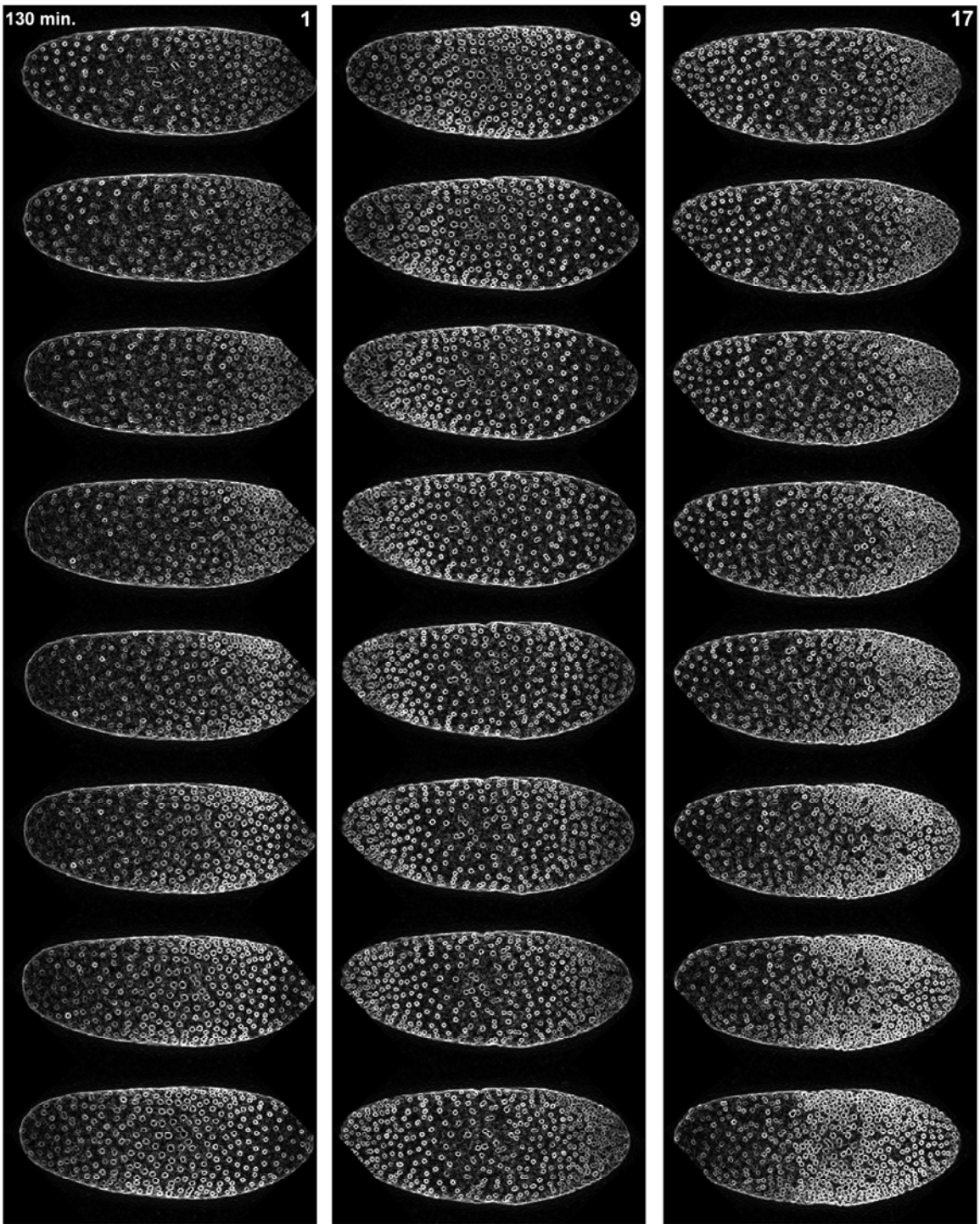


Figure S10. Comparison of Bcd intensity measured as an average intensity, integrated intensity or maximum intensity for one time point.


130 min.
1
9
17

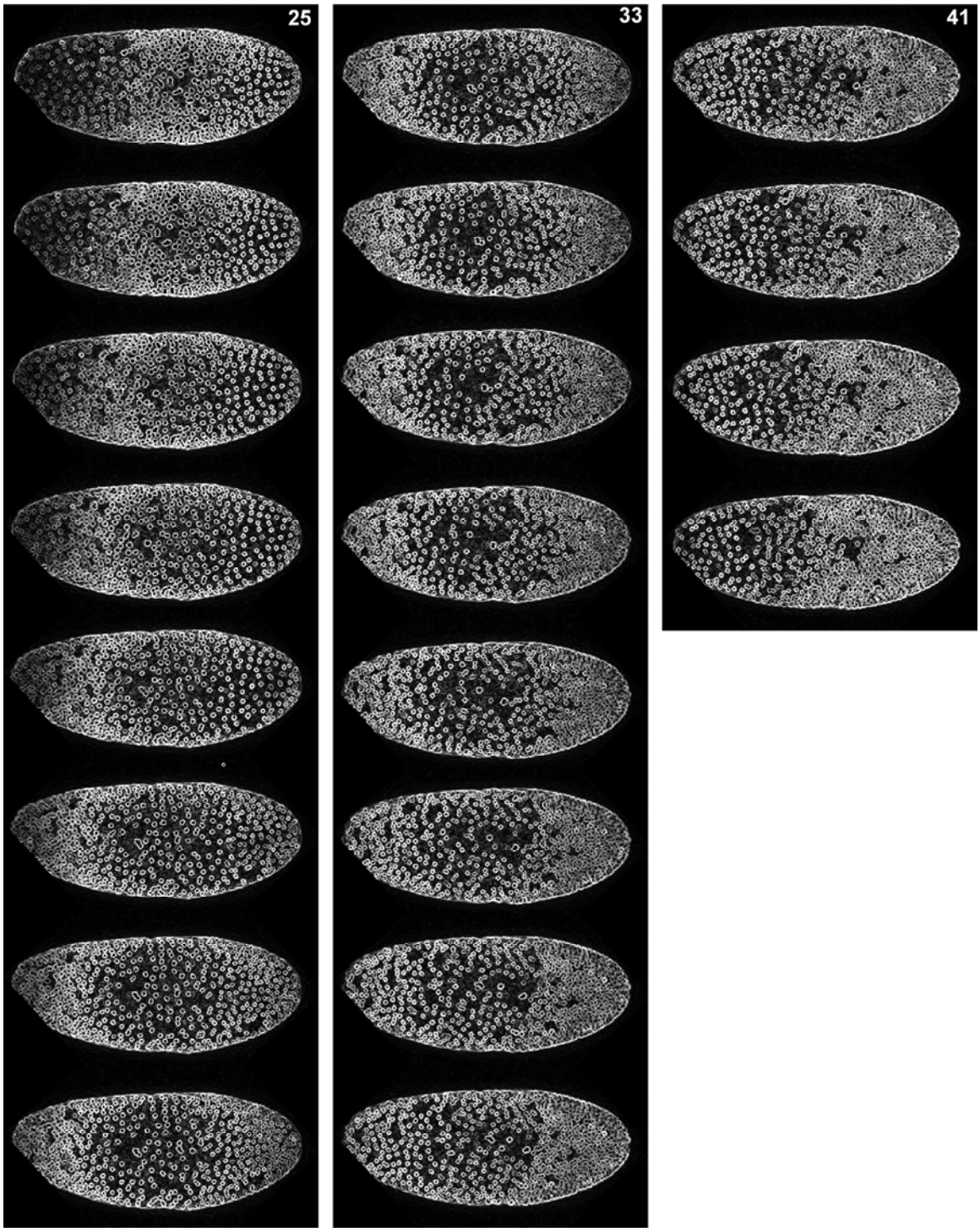


Figure S11. Sobel filter used to track nuclei over time in embryos exposed to a temperature step.

Supporting Movies

Movie S1. Movies show the dynamics of cytoplasmic movement in developing embryos. To facilitate visualization of the movement at the surface of the embryo, black particle tracers were added to the images by using the image analysis software. (Top) In embryos developed in a microfluidic device at uniform 23 ^{o}C, minimal cytoplasmic movement is observed. (Bottom) In embryos developed in a microfluidic device exposed to a temperature step with anterior (left) at 20 ^{o}C and posterior (right) at 27 ^{o}C, dramatic cytoplasmic movement is observed, with net movement from the warm to the cool half of the embryo.

Movie S2. A movie shows the dynamics of nuclear movement in a developing embryo in a microfluidic device at uniform temperature of 23 ^{o}C. The nuclei were visualized by using the expression of histone-eGFP (blue).

Movie S3. A movie shows the dynamics of nuclear movement in a developing embryo in a microfluidic device exposed to a temperature step with anterior (left) at 20 ^{o}C and posterior (right) at 27 ^{o}C. The nuclei were visualized by the expression of histone-eGFP (blue).

Movie S4. A movie shows a partial nuclear division cycle only in the anterior (left, cool) half of an embryo developing in a microfluidic device exposed to a temperature step with the anterior (left) at 20 ^{o}C and posterior (right) at 27 ^{o}C. The nuclei were visualized by using the expression of histone-eGFP (blue).

Movie S5. A movie shows the dynamics of the Bcd gradient in a developing embryo in a microfluidic device at uniform temperature of 23 ^{o}C. Embryos were expressing Bcd-eGFP (green).

Movie S6. A movie shows the dynamics of the Bcd gradient in a developing embryo in a microfluidic device exposed to a temperature step with anterior (left) at 20 ^{o}C and posterior (right) at 27 ^{o}C. Embryos were expressing Bcd-eGFP (green).