Supplemental Materials

Molecular Biology of the Cell

Dilsaver et al.
Emerin induces nuclear breakage in *Xenopus* extract and early embryos

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**SUPPLEMENTAL FIGURE LEGENDS**

**Figure S1: Nuclear breakage control experiments.** (A) In the purified emerin lane, 2.5 µl of 500 nM recombinant emerin were loaded. For the embryo extract lanes, extracts were prepared from post-MBT embryos as previously described (Edens and Levy, 2014) and 10 µl of extract were loaded in each lane. The western blot was probed with an anti-emerin antibody. For (B) – (E) experiments were performed as in Figure 1 using *X. laevis* egg extract with the following modifications: (B) The recombinant emerin protein was heated at 100°C or 60°C for 5 minutes and cooled prior to being added to the extract. Representative images from one experiment of two are shown. (C) The emerin concentration was varied from 0.6 – 5 nM. Representative images from one experiment of two are shown. (D) The incubation period after emerin addition was varied from 15-90 minutes. Representative images from one experiment of two are shown. (E) After incubation with emerin, reactions were supplemented with 1 µM recombinant GFP-cGAS. Representative images from one experiment of two are shown.

**Figure S2: Emerin-treated nuclei stained for nucleoporins and Man1 or encapsulated in droplets.** Experiments were performed as in Figure 1 using *X. laevis* egg extract with the following modifications: (A) After incubating with emerin or dialysis buffer, nuclei were fixed, spun onto coverslips, and stained with mAb414 that recognizes FG-repeat containing nucleoporins and Hoechst. Representative images are shown. In some instances, very faint mAb414 staining was visible on DNA released from ruptured nuclei, however the intensity was orders of magnitude weaker than
mAb414 staining on intact nuclei and could represent non-specific binding. Intact emerin-treated nuclei exhibited continuous mAb414 staining around the rim similar to controls. (B) Prior to emerin addition, extract was supplemented with mAb414 conjugated to Alexa Fluor 594 (10 µg/ml) and incubated for ten minutes. After incubating with emerin or dialysis buffer, nuclei were fixed in a buffer containing Hoechst (Chen and Levy, 2018), applied to a slide, and overlaid with a coverslip. Representative images are shown. Staining for mAb414 on DNA released from ruptured nuclei was never observed with these preparations. It is worth noting that the background mAb414 staining was higher in emerin-treated samples, likely because ruptured nuclei released nucleoporins into the cytoplasm. This was not observed for spindowns in (A) because released nucleoporins would not have been spun down onto the coverslips. Intact emerin-treated nuclei exhibited continuous mAb414 staining around the rim similar to controls. (C) After incubating with emerin or dialysis buffer, nuclei were fixed, spun onto coverslips, and stained with an α-Man1 antibody and Hoechst. Representative images are shown. In some instances, very faint Man1 staining was visible on DNA released from ruptured nuclei, however the intensity was orders of magnitude weaker than Man1 staining on intact nuclei and could represent non-specific binding. It is worth noting that in these egg extract nuclei, Man1 predominantly localizes to the nucleoplasm, consistent with the idea that egg extract and early embryonic nuclei have a different structure and composition that is potentially simplified relative to later in development. (D) Nuclei were assembled in egg extract and labeled as shown in Figure 1. Emerin was added at 5 nM and extract droplets in oil were generated using microfluidic devices as previously described (Hazel et al., 2013). The top droplet shows an example of a nucleus that apparently ruptured and repaired, as evidenced by both cytoplasmic DNA and intranuclear GFP-NLS signal. The other droplets show examples of completely disrupted nuclei with stringy and punctate DNA.

Figure S3: Emerin still ruptures nuclei with blocked NPCs, does not grossly disrupt cytoplasmic membranes, requires cytoplasmic extract to induce nuclear breakage, and associates with demembranated sperm. (A) Experiments were performed as in Figure 1 using X. laevis egg extract except that after nuclear assembly,
WGA was added at 0.2 mg/ml followed by a 5-minute incubation to occlude nuclear pore complexes. Emerin addition and imaging were then performed as in Figure 1. Representative images from one experiment of two are shown. (B) *X. laevis* egg extract was driven into interphase and incubated with 1 µM Dil for 60 minutes at room temperature. 5 nM emerin or an equivalent volume of dialysis buffer was added and incubation was continued for an additional 30 minutes. Extract was applied to a slide and overlaid with a coverslip. Dil-stained membranes were then visualized (labeled “30 minutes”). Slides were incubated an additional 60 minutes and then re-imaged (labeled “90 minutes”). Representative images are shown. (C) A 25 µl reaction of nuclei assembled in *X. laevis* egg extract was diluted with 1 ml buffer (ELB = 250 mM sucrose, 50 mM potassium chloride, 2.5 mM magnesium chloride, 10mM HEPES, pH 7.8). After centrifuging at 1600g for 3 minutes at room temperature, the supernatant was discarded and pelleted nuclei were resuspended in 25 µl ELB or 25 µl interphasic egg extract (Edens and Levy, 2014). Emerin was added to each reaction at 5 nM. Reactions were incubated at room temperature for 30 minutes prior to imaging as shown in Fig. 1A. Representative images are shown. (D) Demembranated *Xenopus* sperm (100,000 sperm/µl) was diluted 1:1 in interphasic egg extract and supplemented with 0.4 µM SNAP-emerin conjugated to Janelia Fluor 549 and 10 µg/ml Hoechst. Imaging was performed after a ten-minute incubation. Representative images are shown.

**Figure S4: GFP-emerin localization in *X. laevis* embryos.** One-cell embryos were microinjected with 1500 pg GFP-emerin mRNA and 500 pg H2B-RFP mRNA and allowed to develop. Nuclei in microinjected embryos were visualized with H2B-RFP at the indicated stages. Representative images are shown. In stage 8 and 10 embryos, GFP-emerin puncta are apparent and some DNA masses are elongated and stringy, likely reflecting nuclear breakage. Some cells also show signs of lysis. In stage 12 embryos, GFP-emerin is still punctate or diffusely cytoplasmic in some cells, as in the top set of images. However in some cells, GFP-emerin is localized to the nuclear envelope, as well as to the cytoplasm and cell cortex, as in the bottom set of images.
VIDEO LEGENDS

Video 1: Emerin-induced breakage of *X. laevis* egg extract nuclei upon mixing. Nuclei assembled in egg extract and labeled with 10 µg/ml Hoechst were applied to a coverslip and imaged on an inverted confocal microscope. Emerin was added at a concentration of 5 nM. Initially nuclei remained intact as shown at the beginning of the video. Imaging was initiated and then the extract droplet was mixed using a pipet tip, corresponding to the middle frames in the video when the Hoechst-stained DNA goes out of focus. After mixing emerin into the reaction, stringy and punctate DNA is apparent, similar to what is shown in Figure 1 for emerin-treated nuclei sandwiched between a glass slide and coverslip. The length of the movie is 30 seconds.

Video 2: Emerin-induced breakage of *X. laevis* egg extract nuclei under flow in a microfluidic device. Nuclei assembled in egg extract and labeled with Sytox green are flowing from the left. Emerin at a concentration of 400 nM is flowing from the top. XB buffer is flowing from the bottom. Note that ruptured nuclei clog the device at the junction, leading to backflow of extract into the other channels. This explains why nuclei are periodically seen to flow from the top and bottom channels. Imaging was performed on an inverted fluorescence microscope, and the length of the movie is 40 seconds.

Video 3: Embryos microinjected with emerin mRNA cease development. One-cell embryos were microinjected with 1500 pg emerin mRNA (2 embryos on the right) or an equivalent volume of water as a control (1 embryo on the left) and allowed to develop. Bright-field imaging was performed at room temperature starting around stage 8. Images were acquired every 90 seconds for a total of ~19 hours.
REFERENCES
Chen, P., and Levy, D.L. (2018). Nucleus Assembly and Import in Xenopus laevis Egg Extract. Cold Spring Harb Protoc. doi:10.1101/pdb.prot097196
Edens, L.J., and Levy, D.L. (2014). cPKC regulates interphase nuclear size during Xenopus development. J Cell Biol 206, 473-483.
Hazel, J., Krutkramelis, K., Mooney, P., Tomschik, M., Gerow, K., Oakey, J., and Gatlin, J.C. (2013). Changes in Cytoplasmic Volume Are Sufficient to Drive Spindle Scaling. Science (New York, NY) 342, 853-856.
Figure S1

A) α-emerin western blot with emerin standards.

B) Emerin heated to 100°C and 60°C with Hoechst staining.

C) Emerin concentrations: 0.6 nM, 1.3 nM, 2.5 nM, and 5 nM with Hoechst staining.

D) Egg extract nuclei incubated with buffer or emerin for different lengths of time:
- Buffer: 15 min, 30 min, 45 min, 60 min, 75 min, 90 min
- Emerin: 15 min, 30 min, 45 min, 60 min, 75 min, 90 min

E) Emerin-treated egg extract nuclei with Hoechst, GFP-cGAS, and Merge staining.

Scale bars: 200 μm (B), 20 μm (C), 50 μm (D), 100 μm (E).
