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Calcium Pulses During the Activation of a Protostome Egg

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Transient increases in free cytoplasmic Ca^{2+} pass through the cytosol of a wide variety of fertilizing deuterostome eggs and do so from the sperm entry point to its antipode. These Ca^{2+} waves occur through the release of stored Ca^{2+}, and they provide most or all of the activating stimulus for these eggs. Based on several indirect lines of experimental evidence, it was proposed (1,2)

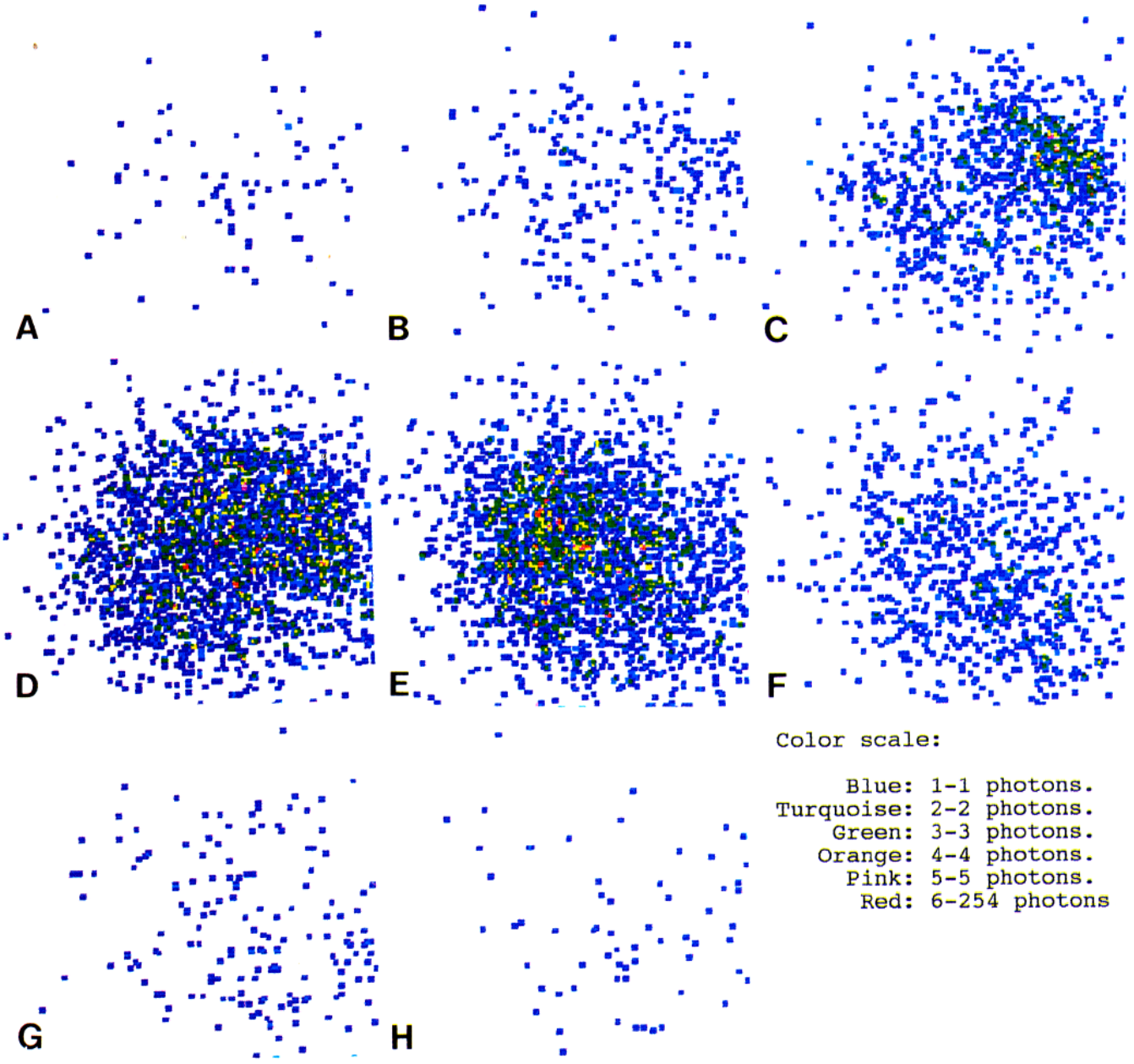

Figure 1. *An example of a wave of Ca^{2+} release in a fertilized* Chaetopterus *egg. Panels A through H show the photons accumulated during successive 3-s intervals. This wave moves from the upper right to the lower left. Panels A and H show the resting levels before and after the wave, which took approximately 6 s to traverse the 105 μm diameter egg, indicating a velocity of around 30 μm/s. The eggs was injected with f-recombinant aequorin prior to insemination.*

that protostome eggs are activated by a prolonged uptake of Ca^{2+} from the medium due to sperm-induced membrane depolarization, and that this uptake then starts an activation wave similar to those in deuterostomes, except that it moves inward from the whole surface rather than across the egg from pole to pole. To test these hypotheses, we microinjected *Chaetopterus pergamentaceus* oocytes with semisynthetic recombinant aequorins (3) and measured light emission in response to both fertilization and artificial activation by excess K^+.

Both fertilization and K^+-activation induced multiple, brief Ca^{2+} transients in the eggs (Fig. 1). Generally, the first transient was localized to one sector of the egg. This was followed by one or more global waves of Ca^{2+}. The waves passed through the egg at about 30 μm/s (speed calculated along the cell surface to allow direct comparison with wave speeds through other cells). After the global waves, more non-propagating pulses were observed; sequential waves and pulses originated at different points on the egg surface.

These new data are consistent with the hypothesis that the activation of protostome eggs is initiated by voltage-gated entry of Ca^{2+} through much or all of the egg surface. It is likewise consistent with the hypothesis that this entry then triggers a Ca^{2+} wave through the egg. But the notion that such waves are radial is clearly incorrect. All the waves that we have seen in *Chaetopterus* moved from pole to pole like those through deuterostome eggs.

Moreover, the speed of these waves—about 30 μm/s at room temperature—is the same as the speed exhibited by Ca^{2+} waves through 50 other, highly varied, active cells (2,4). Indeed, no clear exception to this conserved velocity is known for Ca^{2+} waves that penetrate deeply into normal cells. Since all these so-called "fast" waves are believed to be propagated by Ca^{2+}-induced Ca^{2+} release from the endoplasmic reticulum, we propose that the activation waves through *Chaetopterus* eggs are typical fast Ca^{2+} waves propagated in the same way. Moreover, the rapidity with which the pulses and waves were extinguished indicates that the eggs have powerful mechanisms with which to pump Ca^{2+} out of the cytosol.

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Mechanically Induced Calcium Release From *Xenopus* Cell Cycle Extracts

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We previously reported the discovery of slow (0.5 μm/s) calcium waves that accompany cytokinesis in both frog and fish eggs (1,2). The low velocity of these waves shows that they are unlikely to be propagated by calcium-induced calcium release (3). So we proposed that they are propagated mechanically and belong in a small but well-documented class of 0.3 to 1.0 μm/s waves that traverse a variety of cells during normal development (4,5). Such propagation does not require the influx of calcium through the plasma membrane from the extracellular medium (1). A likely candidate for an intracellular calcium repository is the cortical endoplasmic reticulum (ER), which is deformed during the organization and contraction of the contractile arc. One mechanism of release might be stretch-activated channels in the surface of the ER. In theory, the contracting actomyosin band and the cortical ER network might be connected by actin "cables" that would stretch the ER in front of the advancing furrow, releasing calcium and thus organizing and advancing the contractile band.

We sought, therefore, to induce the release of calcium mechanically from the ER in plasma-membrane-free cell cycling extracts from activated *Xenopus* eggs, and to prevent this release by adding an agent that disassembles actin microfilaments. We also sought to examine pharmacologically the nature of the channels involved in calcium release and to compare the magnitude of mechanically induced calcium release to that induced by inositol triphosphate (IP_3).

We used recombinant aequorin, a bioluminescent photoprotein that generates light when it binds with free calcium (6); the light was detected with a photomultiplier tube (PMT). Absolute resting levels of calcium in the extracts were determined with calcium-sensitive microelectrodes and were found to vary between 300 and 400 n*M*.

We designed and built a device that could apply reproducible deformations to a 30-μl droplet of extract compressed to a distance of 50 μm between two 22-mm-square, #1 thickness, horizontal glass coverslips (Fig. 1A). Shear forces were applied to the extract by moving the upper coverslip with a computer-controlled servomotor equipped with a tachometer-feedback speed-control system and a digital position encoder. This device allowed us to vary the direction, velocity, and duration of applied shear in a controlled and reproducible manner. The lower coverslip was fixed above the window of the PMT; thus the light emitted on application of a shear force could be monitored over time.

Figure 1B shows the effect of applying mechanical shear to the extract, and Figure 1C illustrates how the calcium release