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

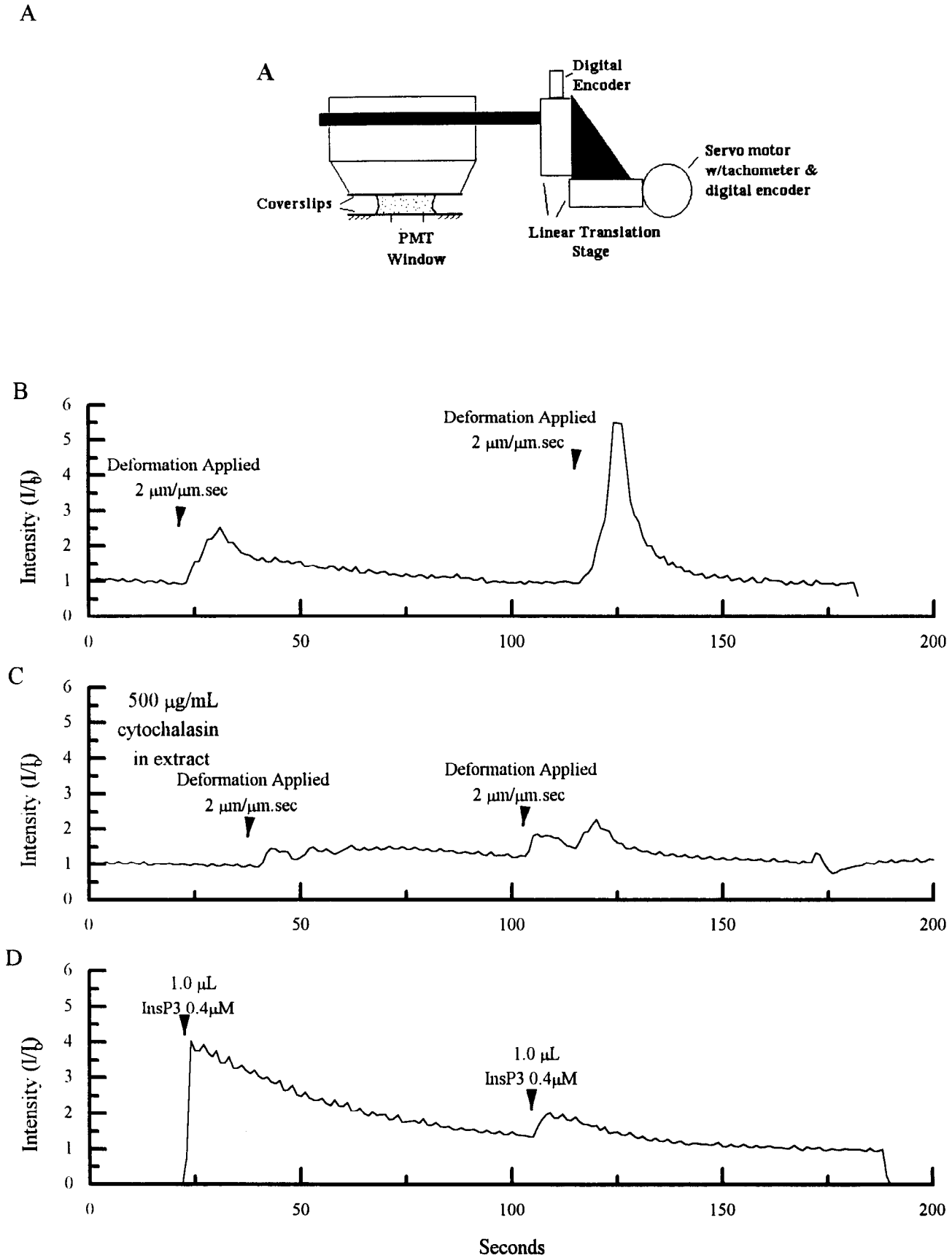

Figure 1. *Figure 1A is a schematic diagram of the device used to apply mechanical shear to extracts. Figure 1B shows increases in luminescence resulting from the application of mechanical shear to an aequorin-loaded extract. Figure 1C illustrates how this mechanically induced calcium release can be dramatically reduced in the presence of cytochalasin (500 μg/ml). Figure 1D is an example of an equivalent magnitude of calcium release induced by addition of 1 μl of IP_3 (0.4 μM).*

can be dramatically reduced in the presence of cytochalasin (500 μg/ml). Figure 1D illustrates the magnitude of release induced by the addition of 1 μl of IP_3 (0.4 μ*M*).

The smallest rate of movement of the upper coverslip that gave a substantial calcium release was 10 μm/s. When this is divided by the 50-μm gap between the two coverslips, it yields a deformation rate of 2 μm/μm · s. We estimated that the deformation rate during furrow progression in an intact frog egg would be only 0.001 μm/μm · s. Thus we are applying a much greater degree of deformation to an extract than would occur naturally during cytokinesis. This difference could be accounted for by the immense differences between the cytoskeletal architectures of an intact cell and a cycling extract. The degree of deformation we applied is, however, much smaller than that which occurs in routine handling of extracts during experimental procedures; *e.g.*, pipetting. One should be cautious, therefore, in interpreting results that could be unknowingly influenced by a mechanically induced release of calcium.

In summary, we have demonstrated that mechanical deformation of extracts induces a reproducible calcium release that is rapidly resequestered by a functional ER. In addition, the order of magnitude of this mechanical release is similar to that induced by IP_3. This suggests that the mechanical forces applied are not merely breaking up the ER and dumping vast amounts of calcium into the cytosol, but are opening stretch-activated ER channels. Our next step will be to image mechanically induced calcium release from intact eggs.

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Ca^{2+}-Induced Axosome Formation in Internally Dialyzed Giant Axons of *Loligo pealei*

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Axosomes are large vesicles (20–50 μm diameter) that form by fusion of induced, smaller vesicles within 30 min after transection of cephalopod giant axons in Ca^{2+}-containing artificial seawater (ASW) (1). Electron microscopy (Fig. 1a) shows hypertrophy of different types of membrane within cut axons and modifications of the cytoskeleton associated with these membranes. Vesiculation of glia (Schwann cells) and association of these vesicles with those of the axon are also observed. We used internal dialysis of excised giant axons of *Loligo pealei* to determine whether induced vesiculation is caused by direct structural damage (transection) or by elevated concentrations of Ca^{2+} and other external ions.

A method for dialyzing axoplasm within a giant axon (2) was modified. The bottom of the nerve chamber (trough) incorporated a glass slide so that axons could be observed during dialysis under phase-contrast light microscopy. A 4-cm length of isolated giant nerve fiber was cannulated by the insertion of separate, collinear glass capillaries (300 μm diameter) 3 mm into both transected ends. The ends were then tied against the outer surface of each capillary. A 160-μm diameter dialysis tube (cellulose acetate) with porous length of 1.5 cm was advanced through the proximal glass cannula until the porous length was midway into the axon and the tip protruded from the distal cannula (Fig. 1b). Flow through the dialysis tube was maintained at 0.5 ml/h with an infusion pump.

Control solution (lacking NaCl, $CaCl_2$, and $MgCl_2$) [(m*M*): 440 K glutamate, 2.5 EGTA-Tris, 5 TrisCl] was used as the external solution. The pH of the control and other solutions was adjusted to 7.4, and the osmolarity was kept constant at 974 mOs*M* by addition of glycine. Axoplasmic dialysis for 1 h with control solution produced no observable vesiculation. Addition of 3.5 m*M* $CaCl_2$ to the dialysate, which yielded a free [Ca^{2+}] of 1 m*M*, resulted in axosome production only in the dialyzed portion of the axon 30 min to 1 h after the change of dialysate (Fig. 1c). Replacement of glutamate with Cl^- in the control dialysate produced a small amount of vesiculation compared to the control. Dialysis with a divalent cation-free ASW (430 NaCl, 10 KCl, 2.5 EGTA-Tris, 5 TrisCl) produced a further increase in vesiculation over that produced by Cl^-, but axosomes formed only when Ca^{2+} was present in the dialysate at free concentrations $\geq$100 μ*M*. Mg^{2+} acted similarly to Ca^{2+} but at higher concentrations (>10 m*M*). The effectiveness of Ca^{2+} in inducing axosome formation was enhanced by the presence of Na^+, Cl^-, and Mg^{2+} in the dialysate; *i.e.*, dialysis with ASW produced more axosomes than did dialysis with the control solution at the same free [Ca^{2+}]. Thus, all ions in the extracellular fluid, except for