Cell Cycle Contraction Waves in *Xenopus* are Suppressed by Injecting Calcium Buffers

Andrew L. Miller (Marine Biological Laboratory), Richard A. Fluck and Lionel F. Jaffe

During the period of rapid metachronous cleavages that follow fertilization, the cortex of *Xenopus* eggs contracts before each cell division (1). These periodic events were termed "surface contraction waves." The wave that precedes the first cell division is most easily studied and has been described in detail. It is propagated in a circular manner from the animal pole, through the equator, and on to the vegetal pole. The surface contraction wave induces a change in the height of the egg. When the wave moves from the animal pole to the equator the egg rounds up, its maximum height occurring as the wave crosses the equator. In normally cleaving eggs, the maximum height of the egg in the rounding phase also corresponds to the appearance of the furrow at the animal pole. Eggs treated with antimitotic drugs (colchicine and vinblastine), although prevented from cleaving, undergo a series of periodic surface contraction waves timed closely to the cleavage cycle in untreated eggs. In addition, this cycle of contractions does not require the sperm nucleus, the egg nucleus, or the sperm centriole. Hara *et al.* proposed that a cytoplasmic biological clock present in vertebrate eggs operates independently of the nucleus and may be involved in regulating the cell cycle (1).

Our idea was to investigate the involvement of free cytosolic calcium elevation in the initiation and propagation of these surface contraction waves. To do this, we selected a relatively weak calcium buffer with a dissociation constant closely matched to localized calcium elevations already measured in large vertebrate eggs during early development and cytokinesis (2, 3). The buffer chosen was 5,5′-dibromo-BAPTA (K_D = 1.5 μM). Our experience in the use of such weak "shuttle" buffers led us to select a final buffer concentration of 2 mM for 5,5′-dibromo-BAPTA for these preliminary experiments. This assumes that 70% of the egg volume is inaccessible yolk, and that 70% of the remaining cytosol is water (4). As a control, we injected 5-mononitro-BAPTA (K_D = 40 μM) again to a final concentration of $\approx$2 mM. Facilitative diffusion theory predicts that for such a weak buffer, a final cytosolic concentration of 2 mM would be insufficient to suppress a local rise in free calcium in the 5 μM range (5). Sufficient calcium was added to the buffer injectates to set them at the resting pCa of the *Xenopus* cytosol. This was assumed to be 0.4 μM (6), which resulted in injectates having a 5:1 and a 100:1 ratio of buffer to calcium for 5,5′-dibromo-BAPTA and 5-mononitro-BAPTA, respectively.

Unfertilized *Xenopus* eggs were obtained by standard procedures and prick-activated. Fertilization membranes were then removed mechanically and the eggs mounted on agar platforms in optically clear cuvettes. These, in turn, were mounted in a modified low-power microscopy set-up that allowed simultaneous time-lapse video microscopy both from above the egg and from the side. Buffers were injected with a high pressure injection system (Medical Systems Corp. PLI-100) that accurately delivered precise, repeatable volumes. Eggs were allowed to relax and contract at least once before buffers were injected. We attempted to inject the buffers at the state of maximum contraction.

Figure 1A shows contractions that are similar, both in their periodicity and in their amplitude ($\approx$36 min and $\approx$18% of the maximum egg height, respectively), to those described by Hara *et al.* (1). In the 5-mononitro-BAPTA injection experiment illustrated in Figure 1B, the egg contracts to an average of 18.2% of its maximum height in the first two contractions before the buffer was injected. The mean figure for the eight contractions following the injection is 17.5%, indicating a minimal effect. When 5,5′-dibromo-BAPTA is injected at about the maximum state of contraction (Fig. 1C and D, respectively), it reduces the amplitude of the subsequent contractions by about two-fold, compared to that before the buffer was injected (*i.e.*, from 13.8% to a mean of 4.9% and from 15% to a mean of 9.2%, for Fig. 1C and D, respectively).

Although the degree of contraction was dramatically affected by the injection of 5,5′-dibromo-BAPTA, the periodicity of the cycle of contraction and relaxation clearly was not. The mean time between contraction peaks for Figure 1A, B, C and D are 36.0, 33.2, 33.0, and 34.5 min, respectively.

These preliminary buffer-injection experiments indicate that localized cytosolic calcium elevations do play a role in the propagation or modulation of surface contraction waves in parthenogenetically activated *Xenopus* eggs. The lack of any effect of the injected buffers on the periodicity of the contraction waves will require further investigation if we are to ascertain whether calcium plays a role in a basic cell cycle timing mechanism that resides in the cytoplasm, or more specifically, in the cortex of the egg.

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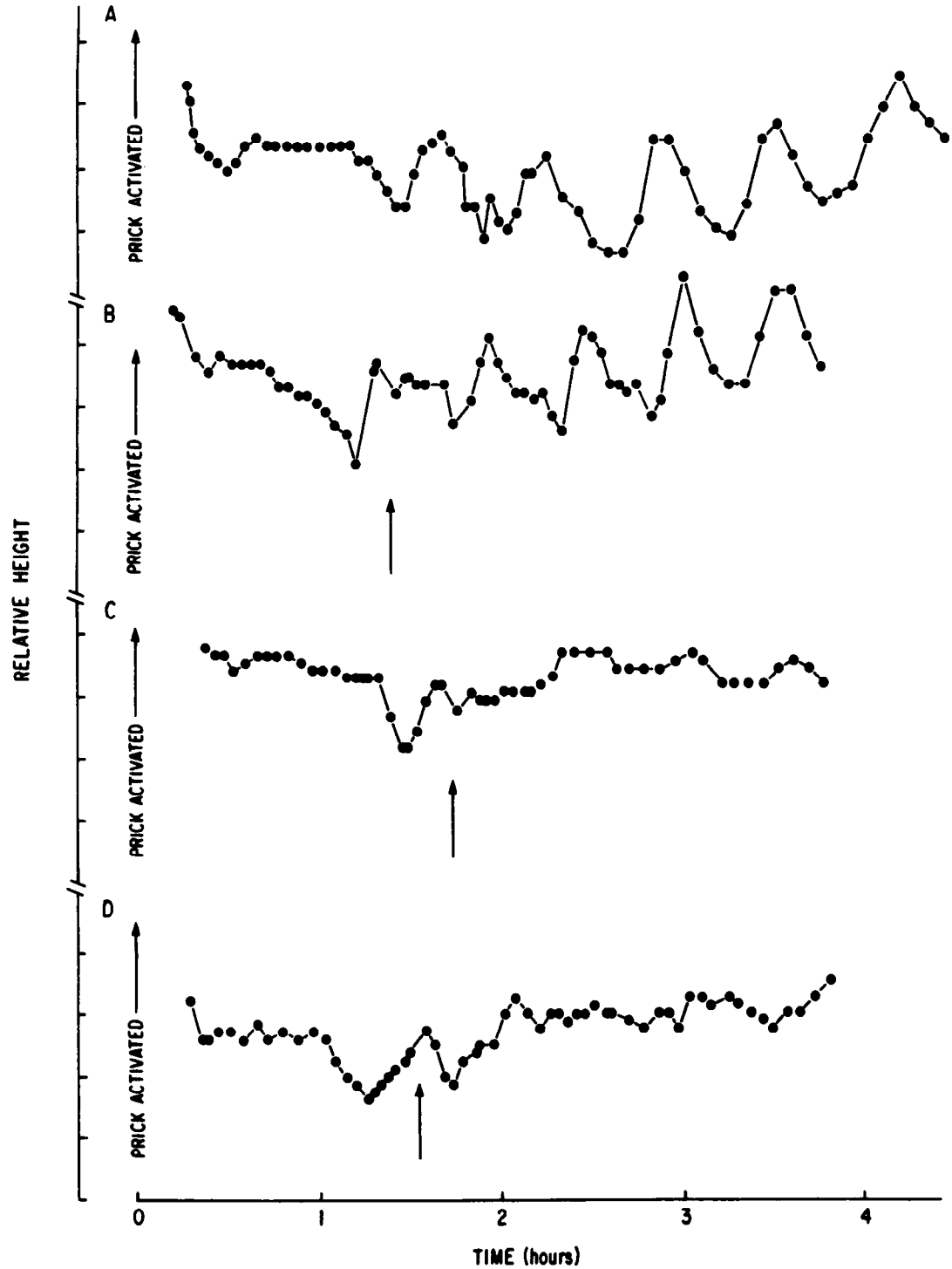

Figure 1. *Periodic changes in the height of parthogenetically activated* Xenopus *eggs. Arrows mark the time of buffer injection. Eggs were allowed to proceed through one relaxation and contraction cycle before injection. (A) Uninjected control; (B) egg injected with 5-mononitro-BAPTA to a final cytosolic concentration of 2 m*M*; (C, D) Eggs injected with 5,5'-dibromo-BAPTA to a final cytosolic concentration of 2 m*M.