Calcium Waves Accompany Contraction Waves in the *Oryzias latipes* (Medaka) Blastoderm

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Soon after the onset of epiboly in the medaka embryo, the blastoderm begins to contract rhythmically (1). Contraction waves, which propagate in a sub-epithelial (stellate) layer (2), arise in a pacemaker region and propagate across the blastoderm at about 15 μ/s at 20°C (1). The contractions continue even after the blastoderm is separated from the yolk, and the rhythmic contraction of such detached blastoderms is optimal in a balanced saline solution (BSS) containing ca. 1 mM Ca^{2+} and is inhibited in BSS containing higher (7.2 mM) or lower (0.18 mM) concentrations of Ca^{2+} (3).

To determine whether these contraction waves are accompanied by calcium waves, we microinjected recombinant aequorin into medaka eggs, fertilized them, and monitored their luminescence after about 24 h, by which time the embryos had begun to contract rhythmically. Photon emission was monitored with an ultrasensitive imaging photon detector, and the contraction waves were recorded by time-lapse video microscopy (4).

We examined 15 embryos and saw regular, periodic pulses of light in 9 of them. These pulses arose in the pacemaker region, spread across the blastoderm, and ended on the opposite side of the blastoderm, near the embryonic axis. We saw no pulses in two eggs that had not quite developed to the stage at which they begin to contract rhythmically. The interval between calcium pulses at the pacemaker (155 ± 11 s, $\bar{x}$ ± SEM, n = 9 embryos) was estimated by counting photons for 9–32 min in a 15-pixel-square box drawn over this region (Fig. 1); the interval between mechanical pulses at the pacemaker (146 ± 7 s, $\bar{x}$ ± SEM, n = 6 embryos) was estimated from an analysis of time-lapse video tape recordings. The velocity of the calcium wave (11.9 ± 1.1 μ/s, $\bar{x}$ ± SEM, n = 7 embryos) was estimated by comparing the timing of the pulses at the pacemaker with those near the embryonic axis. This velocity is in the range of the fast calcium waves that are propagated by calcium-induced calcium release or by some related reaction-diffusion process (5).

The calcium pulses and waves described herein are similar to the contraction waves in the following ways: 1) they both originate in the pacemaker region of the blastoderm, propagate across the blastodisc, and extinguish near the embryonic axis; 2) they have similar periods; and 3) they propagate at similar velocities.

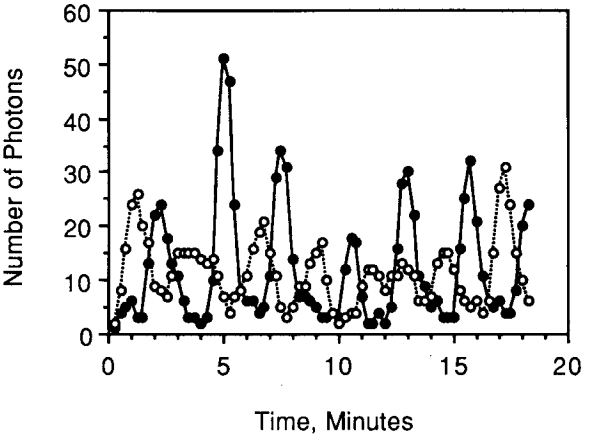

Figure 1. *Calcium pulses during rhythmic contractions of the medaka blastoderm. Photons were counted in 15-square-pixel boxes drawn over the pacemaker region (● —— ●) and near the embryonic axis (O · · · O), using a 45-s window and advancing it at 15-s steps for 18.25 min. These two regions represent the beginning and end, respectively, of the calcium (and contraction) wave; thus, for example, the calcium pulse in the pacemaker region at 2.25 min gave rise to a wave that reached the embryonic axis at about 3.75 min.*

Thus, we conclude that a wave of elevated Ca^{2+} accompanies the contraction wave.

We thank Dr. Osamu Shimomura for his generous gift of recombinant aequorin, Richard Sanger for engineering assistance, Eric Karplus for data analysis software, and Jane McLaughlin for technical assistance. Supported by NSF grant DCB-9017210 to RAF and by NSF DCB-8811198 (Amendment 01) and NIH RRO1395 to LFJ.

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