The surface contraction waves of *Xenopus* eggs reflect the metachronous cell-cycle state of the cytoplasm

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Activated *Xenopus laevis* eggs undergo a series of surface contractions in response to cell-cycle progression but fail to cleave unless the sperm centrosome is present. These surface contraction waves (SCWs) begin at the animal pole and progress around the egg, occur every cell cycle and precede cleavage [1–3]. The SCWs are biphasic, comprising a relaxation phase (SCWa) and a contraction phase (SCWb). To investigate how these events are linked to the underlying cell cycle, we studied the temporal and spatial relationship between the SCWs and previously characterized biochemical markers of cell-cycle progression. We found that the relaxation phase was a response to activated maturation-promoting factor (MPF). In contrast, the contraction phase required inactivation of MPF and was blocked when MPF activity was maintained at elevated levels. We also found that a wave of MPF activity traveled within the cell from the animal to the vegetal hemisphere. Taken together, these experiments suggest that the SCWs are a local response to a wave of MPF activation and inactivation. The egg cytoplasm, therefore, is metachronous in terms of cell-cycle progression; multiple cell-cycle states are present and spatially distinct within the egg at the same time.

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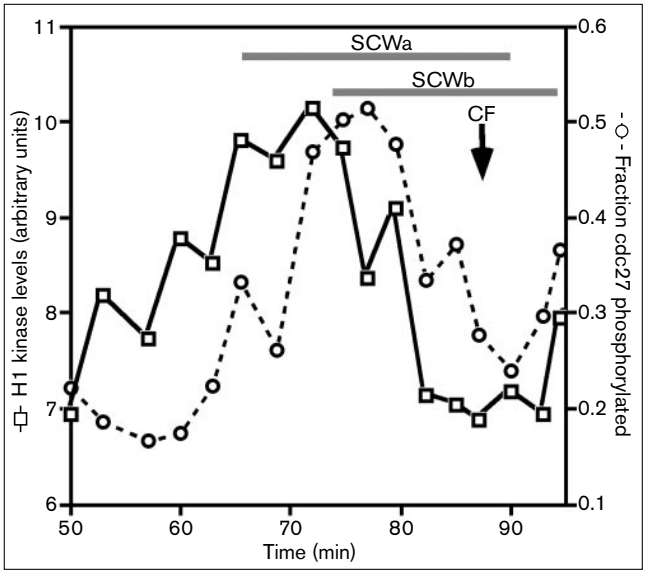
Figure 1



Temporal relationship between the phases of the cell-division cycle and the phases of the SCWs following activation of eggs with ionophore. Histone H1 kinase levels and the fraction of cdc27p phosphorylated are depicted on the graph. Also shown are the approximate times of SCWa and SCWb, as determined by analysis of four eggs from the same batch that were filmed over the time period indicated and in which the SCWs were synchronous to within 2 min. The relative time of cleavage-furrow initiation (determined by reviewing films of fertilized eggs in separate experiments) is indicated by CF.

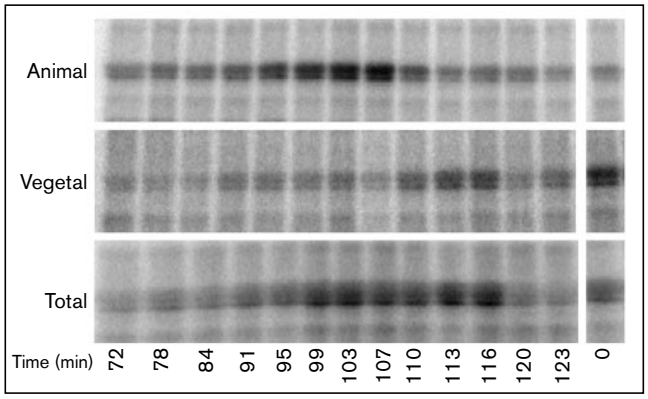
Results and discussion

SCWs and cell-cycle progression

Surface contraction waves (SCWs) occur in artificially activated eggs with the same periodicity as cleavage in fertilized eggs from the same clutch [1]. The exact temporal relationship between the onset of the SCWs and entry into mitosis was determined by synchronously activating eggs, harvesting samples at various times after activation to measure histone H1 kinase activity (as an indicator of maturation-promoting factor (MPF) levels [4]) and comparing these data with filmed SCWs in eggs from the same clutch. As shown in Figure 1, the onset of SCWa occurs when the H1 kinase activity rises. The contraction phase, SCWb, begins before the kinase levels drop, and continues until well after the kinase activity falls to baseline. As has been shown previously, the relaxation and contraction phases overlap temporally [2,5].

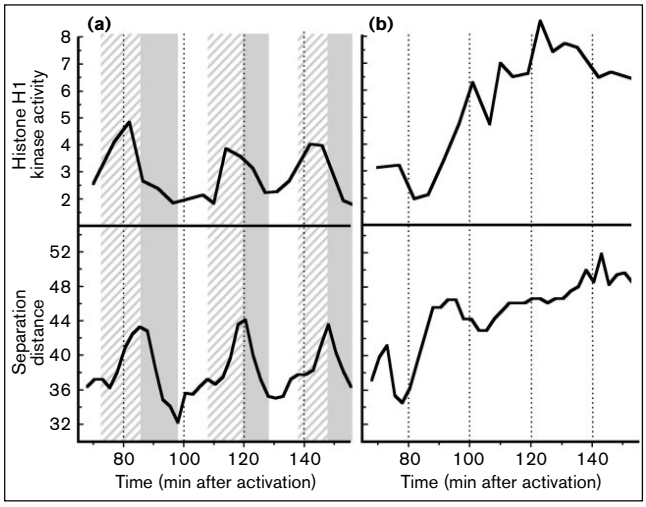
We also determined the relationship between the SCWs and biochemical markers that indicate exit from mitosis. The cdc27 protein (cdc27p) is one of several subunits of the multisubunit anaphase-promoting complex that are phosphorylated as the complex is activated at the metaphase–anaphase transition [6]. The anaphase-promoting complex mediates the ubiquitination and subsequent degradation of substrate proteins required to complete mitosis [6–10]. The reduction in electrophoretic mobility of cdc27p by phosphorylation is at present the best biochemical marker for entry into anaphase [6]. In Figure 1, the phosphorylation state of cdc27p is plotted in relation to both histone H1 kinase levels and the two phases of the SCW. As expected, the peak in cdc27p phosphorylation occurs after the peak in histone H1 kinase levels, and is maintained for several minutes after H1 kinase levels have dropped. The temporal correlation

Figure 2



Time course of histone H1 kinase activity in animal and vegetal hemispheres. At timed intervals after activation of eggs with ionophore, two egg halves (one egg equivalent per assay) were collected, frozen, and subsequently assayed for histone H1 kinase. The figure shows the labeled histone, with the time of sample collection indicated under each lane. Samples were generated by severing eggs with glass rods 45–60 min after activation as described in the text. Similar results were obtained with eggs severed with forceps immediately before freezing (see Figure S1 in Supplementary material).

Figure 3



Comparison of apparent cortical movements with MPF levels. Eggs were activated with ionophore and injected with either **(a)** buffer or **(b)** *cyc*Δ90. The upper graph in each panel shows the histone H1 kinase activity of individual eggs harvested at the indicated times. The lower graphs show the cortical movements of eggs maintained at the same temperature filmed over the indicated period. The cortical movements are recorded as the separation distance between two radially placed particles on the egg surface. The phases of the SCW in (a) are indicated by shading in each panel: SCWa, hatched shading; SCWb, solid shading.

between phosphorylation of cdc27p and the onset of SCWb suggests that SCWb may be either a response to cdc2 inactivation, or perhaps a result of the cell-cycle-dependent proteolysis of a substrate other than cyclin. The approximate time of cleavage furrow formation (CF) is also shown in Figure 1; the cleavage furrow forms after the contractile phase (SCWb) has progressed about halfway around the egg.

MPF activation and SCWs in egg fragments

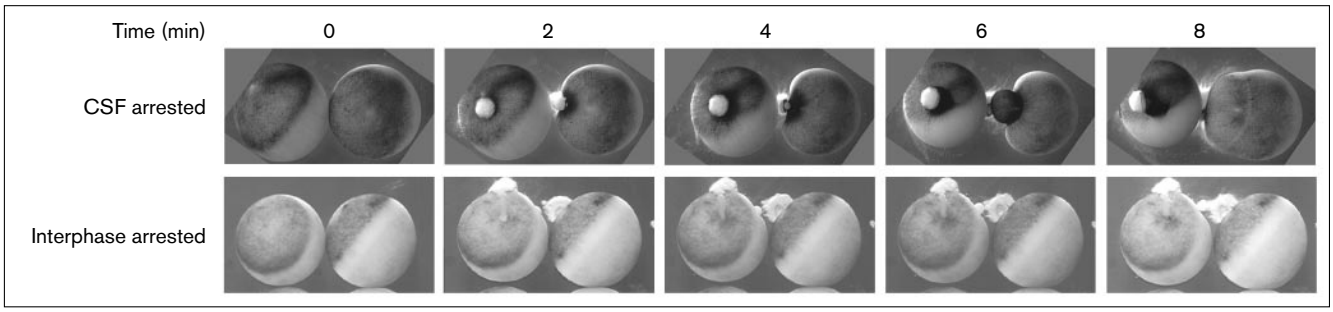
Although the timing of the SCW is related to the underlying cell cycle, the wave of structural response might be explained by either of two general models. In the first, a self-propagating wave is initiated in the animal pole when the activity of MPF reaches a threshold level. The periodic contraction is then a prolonged and asymmetric response to a uniform signal. In the second model, the response of the egg to the signal is more or less uniform, but the signal (for example, MPF activity) is propagated through the egg from the animal to the vegetal pole. To test these models, eggs were activated with ionophore, and separate and intact animal and vegetal egg fragments were generated by laying a glass rod across the egg at the equator (see [11]); the fragments were filmed as they progressed through the cell cycle. Both animal and vegetal fragments underwent several serial SCWs after separation (data not shown) indicating that there is no threshold level of activity triggering a self-propagating wave to originate solely in the animal hemisphere, and is therefore not consistent with the first model. Although the vegetal fragments did not always round up as obviously as the animal fragments [12] (perhaps due to the large and abundant yolk platelets in the vegetal hemisphere), we could easily discern a wave of cortical movement. Indeed, the vegetal fragment can undergo serial waves even after it is completely separated from the animal fragment [12].

To test the second model, activated eggs were cut in half, either with glass rods as described above, or with forceps, and the halves were assayed for histone H1 kinase levels (Fig. 2 and Supplementary material). The levels of histone H1 kinase activity peaked first in the animal halves, then in the vegetal halves, regardless of the method of separating the hemispheres, indicating that there is a traveling wave of kinase activity that originates in the animal half of the egg, and then proceeds to the vegetal half. This wave of kinase activity correlates with the appearance of the SCW suggesting that the observed waves are indeed a local response to the activation of MPF, consistent with the second model described above.

Phases of the SCW and cell-cycle state

The temporal correlation between the onset of SCWa and the activation of MPF (Fig. 1) suggests that SCWa may depend, either directly or indirectly, upon substrate phosphorylation by MPF. To test this proposal, eggs were injected with a cyclin mutant, *cyc*Δ90, which can activate the cdc2 kinase like wild-type cyclin, but which is not

Figure 4



Contraction of the egg is dependent upon the cell-cycle state. Eggs were fertilized and induced to enter CSF-induced arrest by cytoplasmic transfer (upper panels). Arrested eggs were then injected with $CaCl_2$ to trigger resumption of the cell cycle, and filmed during this transition. Within several minutes of calcium injection, the eggs underwent a robust contraction (the number of min elapsed following injection is indicated above the figure). Eggs that had been arrested in interphase by activation in the presence of protein synthesis inhibitors (lower panels) did not contract in response to calcium, although the cortical wound healed effectively. See Supplementary material for a movie showing the responses of the eggs to calcium injection.

degraded in a cell-cycle-dependent manner [13,14]. As shown in Figure 3, histone H1 kinase levels oscillated for several cycles in the control eggs but rose steadily without oscillation in the *cyc*Δ90-injected eggs, eventually reaching levels higher than those seen in cycling eggs.

The SCWs are represented graphically in the lower panels of Figure 3 as the separation distance between two radially placed carbon particles on the surface of the animal hemisphere of the egg. In the mock-injected egg, the two phases of the SCW were seen as an increase in the distance between the particles (SCWa) and as a decrease in the distance between the particles (SCWb). A comparison of histone H1 kinase activity levels to the cortical movement plot in these control eggs (Fig. 3a) reveals that SCWa correlates with the increase in histone H1 kinase levels and that SCWb occurs after the histone H1 kinase levels have dropped. The *cyc*Δ90-injected eggs, however, relax and do not subsequently contract (Fig. 3b), suggesting that the egg has undergone the first phase of the wave, relaxation, but not the subsequent contraction.

The expression of *cyc*Δ90 results in a metaphase–anaphase transition-like state, in which both MPF and the anaphase-promoting complex are fully activated [14]; for example, cdc27p is fully phosphorylated in the *cyc*Δ90 state. Processes specific to anaphase may be due to either the inactivation of MPF *per se*, or the cell-cycle-dependent proteolysis of a substrate other than cyclin [15]. In the *cyc*Δ90-injected eggs, the cortex relaxes, but never enters the contractile phase, consistent with the correlation between SCWa and elevated levels of MPF (Fig. 1). Although activation of MPF is normally transient, in the presence of *cyc*Δ90 it is sustained and the egg remains in a relaxed state. SCWa is thus apparently dependent upon the activation of MPF, whereas SCWb requires at least the inactivation of MPF, and may also depend upon the proteolysis of a substrate other than cyclin.

When eggs injected with *cyc*Δ90 were cut in half and the animal and vegetal halves were assayed for H1 kinase activity, the levels were seen to rise simultaneously in the two halves (see Figure S2 in Supplementary material), in contrast to the results seen with cycling eggs in Figure 2. Inhibition of cell-cycle-dependent proteolysis but not MPF activation [15,16] by the injection of excess peptide containing the cyclin amino-terminal destruction box also inhibited SCWb, but did not abrogate the wave pattern of SCWa (data not shown).

We have been able to induce a cortical contraction reminiscent of SCWb by inducing anaphase in eggs artificially arrested in metaphase [3]. In unfertilized eggs, MPF is fully active and the anaphase-promoting complex is fully phosphorylated. Ubiquitination by this complex is inhibited by cytostatic factor (CSF) and the egg is therefore maintained in metaphase arrest. Fertilized eggs can be induced to re-enter CSF-dependent metaphase arrest by injection with cytoplasm from an unactivated egg. When the embryo is subsequently injected with calcium, CSF is inactivated and cell-cycle progression and SCWs resume [3]. We used this system to test the model that SCWb correlates with entry into anaphase. Fertilized eggs were arrested in metaphase in the first cell cycle by cytoplasmic transfer; this arrest was confirmed by a failure of the eggs to cleave. When these eggs were injected with calcium to trigger anaphase, they immediately underwent a strong contraction (Fig. 4 and see Supplementary material for a movie of the contraction). To ascertain that the contraction was due to the cell-cycle state of the egg, and not simply a response to calcium, eggs were arrested in interphase by activation in the presence of cycloheximide (to inhibit

cyclin synthesis) and injected with calcium 60 minutes after activation: interphase eggs did not contract in response to calcium injection (Fig. 4 and see Supplementary material for movie). Although it is possible that artificial CSF-induced arrest does not mimic the relaxation events in response to normal MPF activation (SCWa), the results are consistent with the observation that SCWb correlates with entry into anaphase. It is also possible that the action of MPF might alter the responsiveness of the contractile machinery to calcium. In this case, entry into anaphase would be only indirectly related to the contractile event.

We do not yet know whether the SCWs are an active cortical event, or whether the apparent cortical waves are in fact the outward manifestation of changes occurring in the underlying cytoplasm. Elinson [17] has shown that the consistency of the egg cytoplasm changes dramatically in a cell-cycle-dependent manner, alternating between gelled and liquid states. The cortex may respond passively to this change in cytoplasmic consistency, resulting in an apparent wave of cortical activity. Conversely, the cortex of the egg, which is capable of regulated contractile activity, could be responding to cell-cycle progression by relaxation and contraction. The SCWs could even result from some combination of these two mechanisms, in which both cortical and cytoplasmic events contribute to what are observed as the SCWs. Similarly, the molecules that contribute to the cell-cycle-dependent cortical changes have not been identified. Although the interactions between actin and myosin have previously been shown to be susceptible to cell-cycle regulation, the insensitivity of the SCWs to cytochalasins ([18]; S.R. unpublished observation) suggests that actin filamentogenesis is not directly involved, but does not exclude a role for the crosslinking of pre-existing actin filaments in SCW activity.

Sawai [19] has shown that regions of the cortex with furrow-inducing activity may be transferred to a non-cleaving cell and induce furrow formation in the recipient. Interestingly, a furrow is only formed when the furrow-inducing activity is transferred to a cell that is in the 'rounded-up' state. Cleavage furrow and contractile ring formation occur after inactivation of MPF and activation of APC-dependent proteolysis. The SCWs, however, are responses to earlier events in the cell cycle, and may indeed be required to prepare the cell for cytokinesis. It is possible that the surface contraction waves in *Xenopus* eggs are equivalent to the rounding-up that cultured cells undergo prior to dividing. Cells may round up actively in response to the cdc2 kinase activation and inactivation, either through cortical contraction, or gelation of the cytoplasm, or both.

Supplementary material

A movie showing the contractions of the eggs in response to calcium injection, two supplementary figures and the Materials and methods section are published with this paper on the internet.

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