The Temporal and Spatial Relationships between Cortical Contraction, Sperm Trail Formation, and Pronuclear Migration in Fertilized *Xenopus* Eggs

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**Summary.** The cortical contraction begins 4 min after insemination and one minute after prick activation. During the next 4 min, the pigment margin moves 15 degrees toward the animal pole. The cortex then relaxes to the prefertilization level over the next 10 min. Contrary to earlier estimations, the cortical contraction occurs during the same time span as the wave of cortical granule exocytosis. We suggest that the two events may result from a common stimulus. The sperm trail (ST) forms during the relaxation of the cortex. The ST first appears as a conically-shaped trail of pigment in the cytoplasm; it then elongates into a funnel-shaped trail as the male pronucleus migrates into the egg. The base of the cytoplasmic ST can be seen on the surface of the egg as a circular condensation of pigment. The male and female pronuclei migrate at a constant rate of 12 μm per minute. The male pronucleus migrates by the enlargement of its aster, whereas, it appears that the female pronucleus is dependent on the male aster for its motion.

**Key words:** Cortical contraction – Pronuclear migration – Sperm trail – *Xenopus* – Amphibians

**Introduction**

Shortly after fertilization a single, distinct condensation of pigment appears near the surface of the animal hemisphere in a monospermic *Xenopus* egg. The site of pigment condensation has been called the "sperm entry point" by Paleček et al. (1978); we shall use the designation "sperm trail" (ST), for reasons that will be presented subsequently. In recent studies the ST has served as a marker for determining the future dorsoventral axis of the embryo (Kirschner et al. 1980; Gerhart et al. 1981), the future plane of the first cleavage (Gerhart 1980), and the degree of polyspermy (Grey et al. 1982).

Several events of major significance occur immediately before and after the appearance of the ST in *Xenopus* (see Gerhart 1980, for a complete review). Events that precede ST formation include: cortical granule breakdown and formation of the fertilization envelope (Grey et al. 1974; Wolf et al. 1976), the activation wave (Hara and Tydeman 1979), the cortical contraction (Gurdon 1960; Hara and Tydeman 1979; Ortolani and Vanderhaeghe 1965; Paleček et al. 1978; Rzehak 1972), and the rotation of the egg within the perivitelline space (Hara and Tydeman 1979; Paleček et al. 1978; Rzehak 1972). After the ST has formed, the pronuclei migrate and meet within the cytoplasm (Graham 1966; Paleček et al. 1978), the dorsoventral axis is established (Kirschner et al. 1980; Gerhart et al. 1981), and the plane of the first cleavage is determined (Gerhart 1980).

Although the relationship between the ST and the cortical contraction has been described in detail for *Rana pipiens* (Elinson 1975), this relationship, as well as the relationship between ST formation and pronuclear migration, have been characterized less thoroughly in *Xenopus*. In this paper, we describe the temporal and spatial relationship between the cortical contraction, ST formation, and pronuclear migration.

**Materials and Methods**

**Gametes and Fertilization**

Gametes were obtained according to the methods described previously (Grey et al. 1982). In order to obtain synchronized fertilization, high concentrations of sperm were prepared as follows. One testis was macerated in 1.0 ml of cold 3X F1 saline solution (made according to Hollinger and Corton 1980); the suspension was handcentrifuged to remove testicular debris. The sperm suspension was warmed to room temperature and mixed with the eggs in four times the volume of F1 to initiate motility. The percentage of fertilization was scored at first cleavage.

**Light Microscopy and Data Analysis**

Samples were collected by two methods. (1) In order to study migration of pronuclei, we dejellied eggs in 2% cysteine in F1 (pH 7.8) 10 min after sperm addition. Eggs were fixed in Smith's fixative (Humason 1972) at five-minute intervals starting with the appearance of the ST and ending when the ST was no longer visible. Some eggs were allowed to develop to ascertain the time of first cleavage. Time intervals were then normalized, according to the method of Gerhart et al. (1981), from insemination (0.0) to first cleav-
The cortical contraction is the first observed change in the pigment pattern that occurs in the fertilized egg. The salient feature of this event is the movement of pigment, localized in the cortex, toward the animal pole (Fig. 1B, C). At the peak of the contraction the margin of the pigmented cortex is shifted 15 degrees. As in Rana pipiens (Elgin 1975), we assume the contraction involves the entire cortex and not just the pigment granules; the magnitude of the contraction, however, is less in Xenopus than in R. pipiens.

The cortical contraction begins about 4 min after insemination (0.05) (Fig. 1A). Maximum contraction occurs 4 min later (0.10), at which time the cortical granule reaction is completed as evidenced by the elevation of the fertilization envelope and rotation of the egg within the perivitelline space. The cortex then slowly relaxes to the pre-fertilization level over the next 10 min (0.20). In prick-activated eggs, the contraction is initiated 1 min after pricking; the durations of the contraction and relaxation phases are the same as in fertilized eggs. Since our measurements were on the whole egg, the delay between the stimulus and the observed contraction could be a result of the time necessary for this response to become sufficient to measure; a longer delay has been seen in the larger egg of Rana pipiens (Elgin 1975).

In summarizing data from several observers, Gerhart (1980, Table II) put the relative time of the exocytotic wave at 0.08-0.12 and the cortical ("activation") contraction at 0.12-0.20. We found, however, that the cortical contraction began considerably earlier (ca. 0.05) and reached its maximum by 0.10, i.e., it is concomitant with the exocytotic event (Grey et al. 1974, 1982). We propose that at a given location on the egg the wave of cortical granule breakdown is followed immediately by a wave of cortical contraction, the latter event culminating in the shift of pigment toward the animal pole. We are presently investigating this possible relationship.

The mechanism of the cortical contraction is unknown. But the cortex of an oocyte contains actin (Franke et al. 1976), and a distinct layer of microfilaments forms beneath the plasma membrane in the wake of the advancing front of cortical granule exocytosis (see Fig. 12, in Grey et al. 1974). It seems probable that these microfilaments are responsible for the contraction of the cortex toward the animal pole. This relationship between the exocytotic event and the cortical contraction predicts that one should observe an asymmetry to the cortical contraction in those cases in which the sperm enter near the equator. We have observed that a transitory horseshoe-shaped concentration of pigment usually appears, opposite the side of sperm entry, around the margin of the maturation spot at the peak of the cortical contraction. This pattern is consistent with the notion that the cortical contraction first begins at the point of sperm entry and spreads in the wake of the exocytotic wave.

In view of their temporal relationship, it seems probable that the cortical exocytosis and the cortical contraction are responses to a common stimulus. Both a cortical contraction and cortical granule exocytosis occur when calcium is injected into the cortex (Gingell 1970; Hollinger et al. 1979) or when the egg is exposed to the calcium ionophore A23187 (Schroeder and Strickland 1974; Steinhardt et al. 1974). If there is a rise in free calcium at fertilization in