AN ULTRASTRUCTURAL STUDY OF THE EFFECTS OF WHEAT GERM AGGLUTININ (WGA) ON CELL CORTEX ORGANIZATION DURING THE FIRST CLEAVAGE OF XENOPUS LAEVIS EGGS

II. CORТICAL WOUND HEALING

M. GEUSKENS AND R. TENCER
Laboratoire de Cytologie et d'Embryologie moléculaires,
Université libre de Bruxelles, Belgium

SUMMARY
Uncleaved fertilized eggs of Xenopus laevis treated with wheat germ agglutinin (WGA) have been pricked at the animal pole both inside and outside the regressed furrow region. The wounded cortex of both regions has been studied with the electron microscope and compared with the same region of wounded, untreated eggs. In all 3 cases, filaments are organized in an annular zone in the damaged cortex. When the surface is pricked outside the regressed furrow of WGA-treated embryos, bundles of microfilaments radiate from the ring and extend in deep folds which form a 'star' around the wound at the surface of the embryo. However, when the surface is pricked in the new membrane of the regressed furrow, filaments are intermingled with internalized portions of the plasma membrane. It is suggested that, when the surface is pricked outside the furrow region, more filaments are mobilized to counteract the tangential retraction of the membrane which has acquired more rigidity after WGA binding.

INTRODUCTION
The observation that wheat germ and soybean agglutinins block cleavage of Xenopus eggs, possibly by disorganizing the microfilament system involved in surface constriction (Tencer, 1978a, b), has led us to examine with the electron microscope the effects of lectin treatment on cortical wound healing in uncleaved fertilized eggs. Indeed, a contractile system situated beneath the surface of the embryo and around the wound is apparently involved in this process (Gingell, 1970; Luckenbill, 1971; Bluemink, 1972). Previous results have shown that wound healing does occur in WGA-treated eggs, but that the closure of the hole is incomplete (Tencer, 1978b). Moreover, the wound rapidly becomes surrounded by deep radial folds which form a 'star' and probably result from tension in the egg surface.

In the present paper, we have studied the organization of the microfilaments under the wounded surface in order to obtain more information on the possible interference of lectins with the contractile system.

Correspondence to: Dr M. Geuskens, Laboratoire de Cytologie et d'Embryologie moléculaires, Université libre de Bruxelles, 67 rue des Chevaux, 1640-Rhode-St-Genèse, Belgium.
MATERIALS AND METHODS

Collection of the eggs and artificial fertilization were carried out as described by Tencer (1978a, b). The pricking experiments were performed in full-strength Niu and Twitty solution (Niu & Twitty, 1953) after removal of the fertilization membrane with watchmaker’s forceps and transfer of the fertilized eggs onto 1% agar in small Petri dishes. Cortical wounds were made with sharpened tungsten needles in the animal region of control eggs during first furrow formation, approximately 1.5 h after fertilization. Treatment with WGA (50 and 100 μg/ml) of eggs lacking fertilization membranes was begun 1 h after fertilization and continued for 30 min to 1 h. The wound was made in the animal pole after transfer of the eggs to Niu & Twitty solution without lectin. Wounds were also made in the regressed furrow region. Treatment with WGA (50 μg/ml) was then begun approximately 1-5 h after fertilization at the stripe stage, and pricking in the regressed groove was made 20-30 min later.

Approximately 2 min after insertion of the needle into the cortex, embryos were fixed in 2.5% glutaraldehyde in 0.1 M Sorensen phosphate buffer pH 7.4. In some experiments, fixation took place 10 min to 1 h after wounding. After overnight fixation at room temperature, the wound region was dissected out under a binocular microscope and the cortical fragments fixed in 1% OsO4 in the same buffer for 2 h at room temperature. After dehydration in ethanol, embryo fragments were embedded in Epon. Sections were ‘stained’ with uranyl acetate and lead citrate and the grids observed in an AEI EM6B electron microscope.

RESULTS

Wounded control embryos

Our general ultrastructural observations concerning the presence of surface folds around a cytoplasmic exudate and the aggregation of pigment granules in the wound region are in agreement with those of Luckenbill (1971) and Bluemink (1972). In frontal (horizontal), slightly oblique, sections of the wound region, part of a dense ring of material is clearly visible in the cortex between the exovate and the highly pigmented cytoplasm (Fig. 1). At a higher magnification, oriented microfilaments are observed in this annular region (Fig. 2), while a few groups of microfilaments radiate from the ring. Surface folds have an enlarged extremity filled with β-glycogen particles and a narrow lower part where microfilaments are visible (Figs. 3, 4).

WGA-treated embryos

Wound made outside the furrow region. The surface of the animal pole around the wound is densely folded (Fig. 6), these folds being more convoluted and in closer contact with each other than when a wound has been made in the cortex of a control egg. Moreover, these folds are covered with a coat (Fig. 6) and occasionally, their membrane appears to be interrupted. Microfilaments are less frequently observed inside them than when a wound has been made in the cortex of control eggs. Micro-

Fig. 1. Pricked animal cortex of a control embryo. An annular zone of dense material is visible around the exovate. × 15000.

Fig. 2. Same embryo as in Fig. 1. At higher magnification, a bundle of oriented microfilaments is observed in the dense ring. × 60000.

Fig. 3. Filaments are visible in the surface folds formed along the wound made in the animal cortex of a control embryo. × 50000.
Wound healing in lectin-treated eggs
filaments form a ring beneath the folds (Fig. 5) and their parallel orientation into bundles around the wound is visible in frontal sections (Fig. 6). In more deeply cut sections, the ring seems to be smaller and the arrayed organization of filamentous material is less obvious. Bundles of microfilaments forming a ring are also observed when the eggs are fixed 1 h after pricking. Radial bundles of microfilaments, originating from different places in the ring, bifurcate in the direction of the deep folds which form a 'star' around the wound. These bundles extend into deep folds which are themselves fringed by smaller folds covered with a patchily distributed coat (Fig. 7). Some microtubules are observed in the radial deep folds, but they are not parallely oriented.

Wound made in the regressed furrow region. Under the binocular microscope, a wound made in the regressed furrow region has the same general appearance as one
M. Geuskens and R. Tencer

Fig. 7. Frontal section of one of the deep folds which radiate from the prick-hole at the surface of a WGA-treated embryo wounded outside the furrow region. A microfilament bundle is observed inside the deep fold which is fringed by patchily covered smaller folds. ×33 000.

made in the animal cortex of control embryos. At the ultrastructural level, frontal sections made in the cortex of the pricked region show that filamentous material has assembled around the cytoplasmic exudate (Fig. 8). This material is intermingled with membranes and large vesicles (Fig. 9). Frequent contacts between double membranes and vesicles are observed (Fig. 9). Microfilaments attached to the convoluted intracortical membranes are not oriented in a parallel direction (Fig. 9). When serial sections of the ring are made, such an orientation of microfilaments in bundles is more obvious at other levels. As is the case in wounds made in control eggs, some small bifurcating bundles are observed. Double trilaminar membranes and vesicles associated with filamentous material are observed in the furrow region outside the annular zone bordering the wound. Indentations of the plasma membrane into the

Fig. 8. Frontal section of pricked cortex in the regressed furrow region of a WGA-treated embryo. Membranes and vesicles are intermingled with filaments in an annular zone around the wound. ×8 400.

Fig. 9. Enlargement of part of Fig. 8. Double trilaminar membranes and vesicles intermingled with filaments are observed in the annular zone which surrounds the wound. ×44 000.
Wound healing in lectin-treated eggs

---

Image 1: 

Image 2: 
cortex, where they form a network, and areas of glycogen particles and chains partially surrounded by small vesicles containing flocculent material have been already described in the companion paper (Geuskens & Tencer, 1979).

DISCUSSION

The way in which a wound made in the animal cortex of uncleaved fertilized *Xenopus* eggs heals has been extensively studied and discussed by Bluemink (1972). We offer here additional evidence in support of the idea that microfilaments organize in a ring-shaped area which probably contracts in the same manner as a purse string in order to reduce the size of the wound. The binding of WGA to its receptors on the membrane of the egg does not impede the organization of microfilaments in an annular zone surrounding the exovate, but it apparently confers more rigidity to the cell surface. The radial distribution of the microfilaments beneath the surface and around the ring, corresponds to the large folds of tension which form externally around the wound. These radiating bundles of filaments could impede normal healing by counter-balancing the action of the ring. Their presence apparently results from an increased reaction of the cortex to the pricking in WGA-treated eggs. Indeed, small groups of radiating filaments exist when wounds are made in control eggs and the annular wall of microfilaments itself is apparently wider in the WGA-treated eggs; however, this is difficult to ascertain because of differences in section angle and depth with regard to the filament ring in treated and control egg sections. All the filaments situated in the pricked region of the cortex are apparently mobilized for counteracting the tangential retraction of the membrane which has bound WGA molecules and consequently is more rigid. The more densely folded surface could also result from increased rigidity of the membrane due to the cross-linking action of the WGA molecules.

When a wound is made in the region of the regressed furrow after WGA treatment, microfilaments intermingled with membranes and vesicles are observed around the exovate. This apparently results from stronger binding of the filaments to the internalized portions of the membrane rather than to passive dragging of these structures during the reorganization of the microfilaments into a ring. Indeed, isolated double membranes associated with microfilaments are observed in the vicinity of the wound and are not directly connected with the annular zone. Numerous internalized membranes and vesicles are present in the blocked or regressed furrow region (Geuskens & Tencer, 1979). These structures are characteristic of this region and exist independently of the pricking, as a consequence of the WGA treatment. Their presence within the filament ring confirms that the reaction of the fertilized egg to the wound is a local phenomenon and apparently concerns only the filaments situated in the pricked region. Despite the presence of these membranes and vesicles, the filaments can coordinate their action sufficiently to thwart spreading of the wound and to reduce the size of the hole.

Thanks are due to Dr J. Osborn for his help with the English version. M. Geuskens is ‘Maitre de recherches’ of the Belgian National Fund for Scientific Research.
REFERENCES


(Received 6 November 1978)