Int. J. Dev. Biol. 40: 97-101 (1996)

Sea urchin embryology in the sixties

GERHARD CZIHAK*

Institute of Genetics and Developmental Biology, University of Salzburg, Austria

The development of sea urchins and other echinodermata is among the most fascinating ones: the differences in the morphology of developmental stages and the adult are greater than in any other developmental system. A radial symmetric embryo develops into a bilateral planctonic form (Fig. 1) which becomes transformed into a pentameric adult of the benthos after evagination of an imaginal disc and contraction of the remaining tissues (Czihak, 1960a, Fig. 1).

Careful examinations of the normal development in manifold variations (natural experiments) make plausible that a basically radial organization of the embryo is temporarily moulded into a bilateral one for the time of planctonic life only (Czihak, 1960b, 1962a,b).

The regulatory capacity of the sea urchin embryo is remarkable. Different parts of the embryo can be removed or added; the intrinsic plan of anlagen in most cases replace the missing parts or incorporate the added ones (Hörstadius, 1975).

The animal-vegetal axis is preformed during oogenesis; the mature egg has an animal plasmamembrane and animal cortex of denser cytoplasm as well as the vegetal counterparts. At fertilization not only processes of the cell cycle (DNA-replication, chromosome condensation etc.) are released; a cortical clock is triggered changing the most vegetal cortex 3 h p.f. (post fertilization). The microtubules of one aster in the third mitotic figure become more or less disintegrated which leads to an asymmetric cleavage and formation of micromeres, which later induce the archenteron (Hörstadius, 1975; Ransick and Davidson, 1993) and eventually form the mesenchyme (Davidson, 1993). Surgical removal of the vegetal cytoplasm and cortex abolishes the action of the cortical clock (Czihak, 1973).

The first cleavage plane determines the oral-aboral axis (Cameron *et al.*, 1989). Using a cytochemical reaction a gradient of cytochrome oxidase activity was demonstrated in the animal half, the maximum of activity being in the future oral field (Fig. 2). Under experimental conditions the cytochrome oxidase gradient can be suppressed and the resulting uniform distribution of the activity of this enzyme in the mitochondria of the animal half (Fig. 3) was found to be correlated with the development of a radial symmetric larva (Czihak, 1963). Such larvae have a pentameric organization (in many cases five regularly distributed spicules) and one coelomic sac at the tip of the archenteron (Fig. 4).

Early cell type specific activity of other enzymes was demonstrated cytochemically in blastula (Fig. 5) and gastrula stages (Fig. 6) most likely reflecting local gene activation (at that time the first such report in the sea urchin embryo, Czihak, 1962 a). Gene activities are now studied in several laboratories.

Development of organanlagen for the adult commences with the formation of two coelomic sacs which have no function in the pluteus since their variations and even absence, which were found in some rare cases (Figs. 10c, 11), have no influence on viability and growth of the larval stage (Czihak, 1965).

The coelomic sacs detach from the tip of the archenteron, one disk-like sac in radial larvae (Fig. 4) or two sacs of unequal size in the normal bilateral gastrula (Fig. 7). In most cases the left one is the bigger one, the ratio in cell numbers within the two coelomic sacs being close to 3:2 (Czihak, 1962a) suggesting that a pentameric anlage was divided into two parts under the influence of the oral field gradient (Czihak, 1961).

During migration of the coelomic sacs along the oesophagus towards the stomach the somatocoels separate from the remaining coelomic cells and spread on the surface of the now spherical stomach (Fig. 8). When one of the somatocoels was destroyed by local UV-irradiation (Czihak, 1965) the remaining one continued spreading to the side of the missing somatocoel which indicates that migration stops in the median only when a

---

*Address for reprints: Department of Genetics and Developmental Biology, University of Salzburg, Hellbrunner Str. 34, A-5020 Salzburg, Austria. FAX: 43-662804-5795 or 5010.

0214-6282/96/$03.00

Printed in Spain

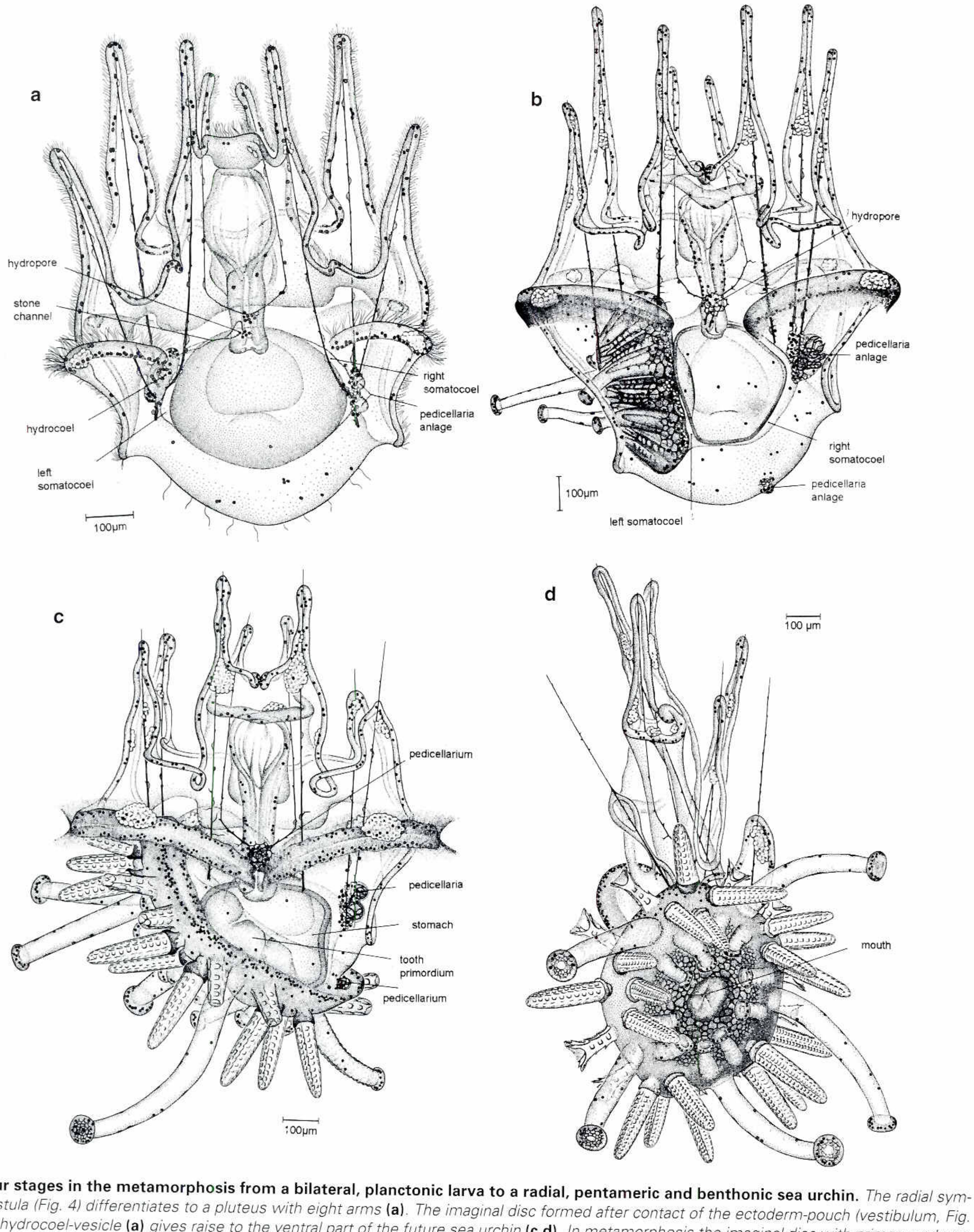

Fig. 1. Four stages in the metamorphosis from a bilateral, planctonic larva to a radial, pentameric and benthonic sea urchin. *The radial symmetric blastula (Fig. 4) differentiates to a pluteus with eight arms* **(a)**. *The imaginal disc formed after contact of the ectoderm-pouch (vestibulum, Fig. 8) and the hydrocoel-vesicle* **(a)** *gives raise to the ventral part of the future sea urchin* **(c,d)**. *In metamorphosis the imaginal disc with primary and secondary ambulacral feet and rows of spines unfold* **(c)** *and the ectodermal parts of the pluteus with anlagen of pedicellaria and underlying mesodermal cells shrink* **(c,d)** *to form the dorsal half of the young sea urchin (cf. Fig. 10).*

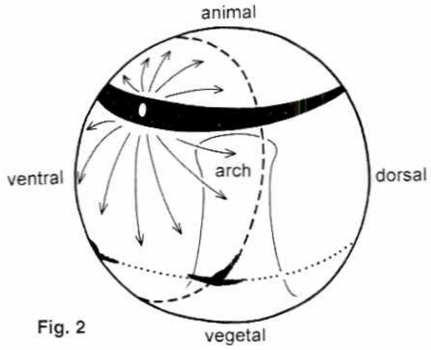

Fig. 2

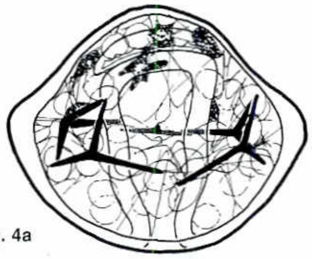

Fig. 4a

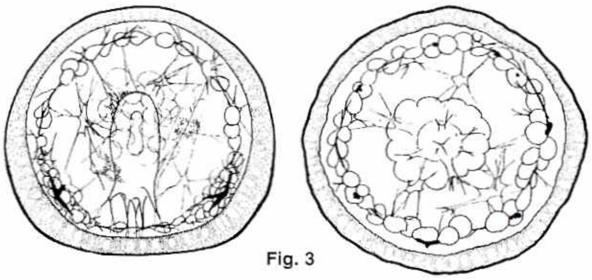

Fig. 3

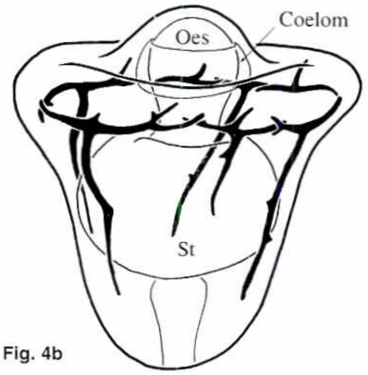

Fig. 4b

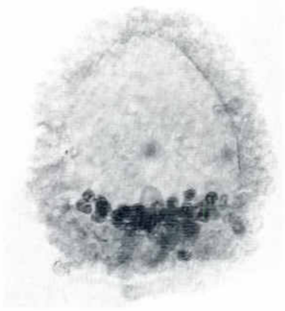

Fig. 5a

Fig. 5b

Fig. 6a

Fig. 6b

Fig. 2. Schematic representation of the determination and development of the oral field. *Cytochrome-oxidase activity was found to be highest in the part of the animal half where the oral field develops under the influence of a hypothetical gradient and the mouth is formed in contact with the archenteron (arch). At the border of the oral field (dashed line) the ciliary band develops. At the intersection of this border and the ring of primary mesenchyme cells (stippled line) the triradiate spicules are formed.*

Fig. 3. Vital staining of mitochondria in a normal (a) and a gastrula of Ps(ammechinus) mil(iaris) radialized by treatment with 8-chloroxanthin (b). *Density of cytochrome-oxidase active mitochondria is higher in the future oral field and evenly distributed in a radial larva.*

Fig. 4. Radialized more or less pentameric larvae (a) *of Ps. mil. after the animal half of the blastula was destroyed by UV-irradiation; there is one terminal coelomic sac; rather five spicules are formed than two as in bilateral gastrulae;* **(b)** *of a LiCl-treated 3.5 day-old larva of Paracentrotus lividus in which a ring-like coelomic sac was formed resembling the hydrocoeanlage in early development of holothurians (Oes = Oesophagus, St = stomach).*

Fig. 5. Cytochemical demonstration of the activity of cytochrome-c-reductase almost exclusively in primary mesenchyme cells in a blastula (a) and a gastrula (b) of Ps. mil.

Fig. 6. Cytochemical demonstration of the increase of an esterase activity during the differentiation of the oesophagus in Ps. mil. *Note the separation of the coelomic sacs and the bigger one left to the oesophagus!*

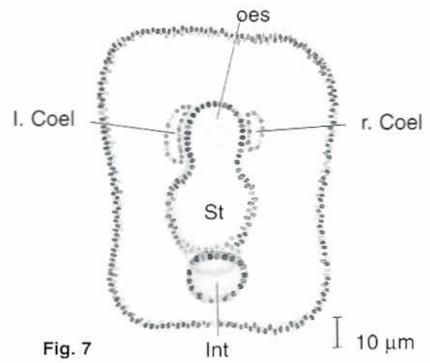

Fig. 7

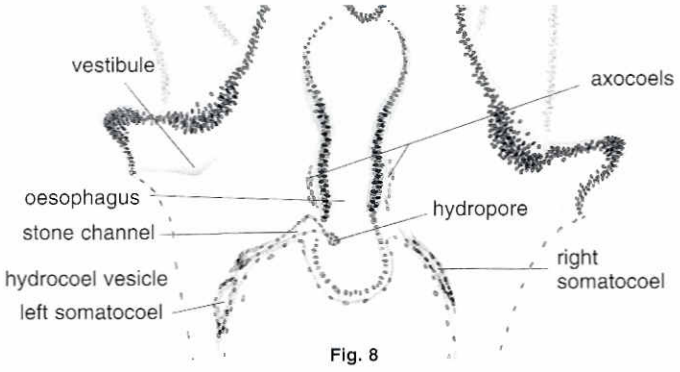

Fig. 8

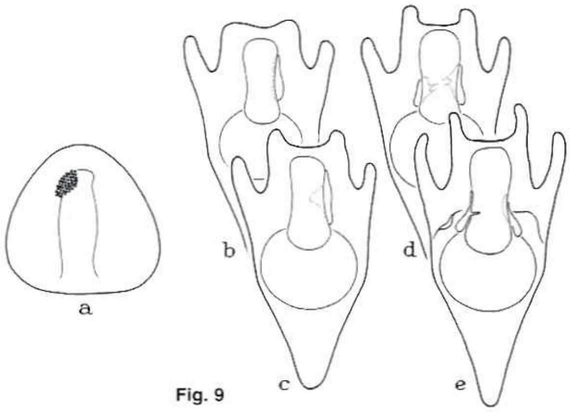

Fig. 9

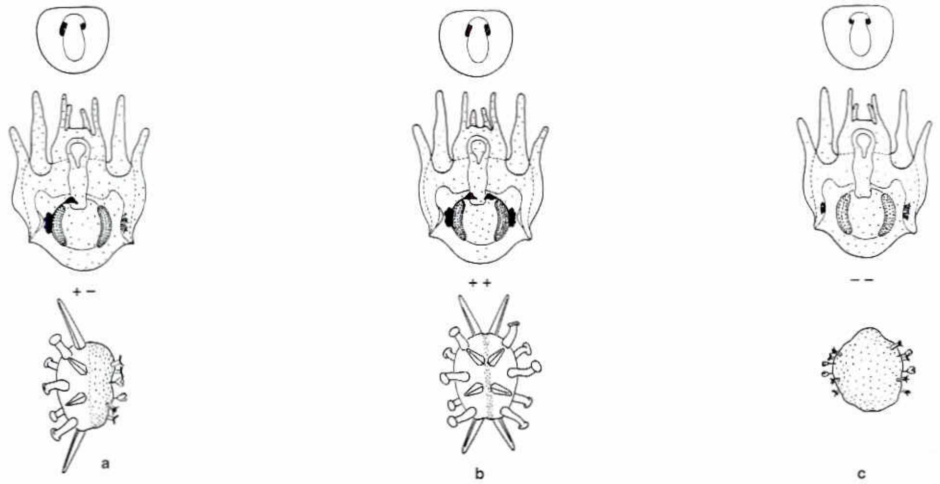

Fig. 10

Fig. 7. Optical section through a late gastrula (tetraeder) of Ps. mil. after Borax-carmin staining, showing the remarkable size difference of the coelomic sacs.

Fig. 8. Part of a 9 day-old pluteus of Ps. mil. after separation of the coelomic sacs. *(cf. Fig. 6). The larger, left one had differentiated to axocoel, hydrocoel and somatocoel; the hydrocoel to hydroporus, stone channel and hydrocoel vesicle. The smaller, right coelomic sac formed axocoel and somatocoel only.*

Fig. 9. Development of the coelom when the left sac was completely destroyed by UV-irradiation during separation from the tip of the archenteron. *The missing left coelom* **(b)** *is regenerated by migration of cells from the right coelom* **(c,d)**. *Eventually all parts of the coelom differentiate and a vestibulum in contact with the hydrocoel forms an imaginal disc for the ventral half of the adult.*

Fig. 10. Correlation between the size of the coelomic sacs, differentiation of a hydrocoel and imaginal disc (black); somatocoels are stippled and the ectoderm of the pluteus developing to the dorsal half of the young sea urchin is marked by small circles. *The normal +- -pluteus has a hydrocoel on the left side; in the ++ -pluteus hydrocoels and imaginal discs develop on both sides and the -- -type has no hydrocoel on either side but two clusters of pedicellaria characteristic for the side on which no imaginal disc develops. In the normal pluteus (a) the imaginal disc unfolds and forms the ventral side of the young sea urchin; in the ++ -type (b) a siamese twin, namely two fused ventral sides form after metamorphosis, whereas a -- -pluteus metamorphoses into a reduction sphere consisting mainly of dorsal ectoderm with pedicellaria and lacking the ambulacral system and large spines.*

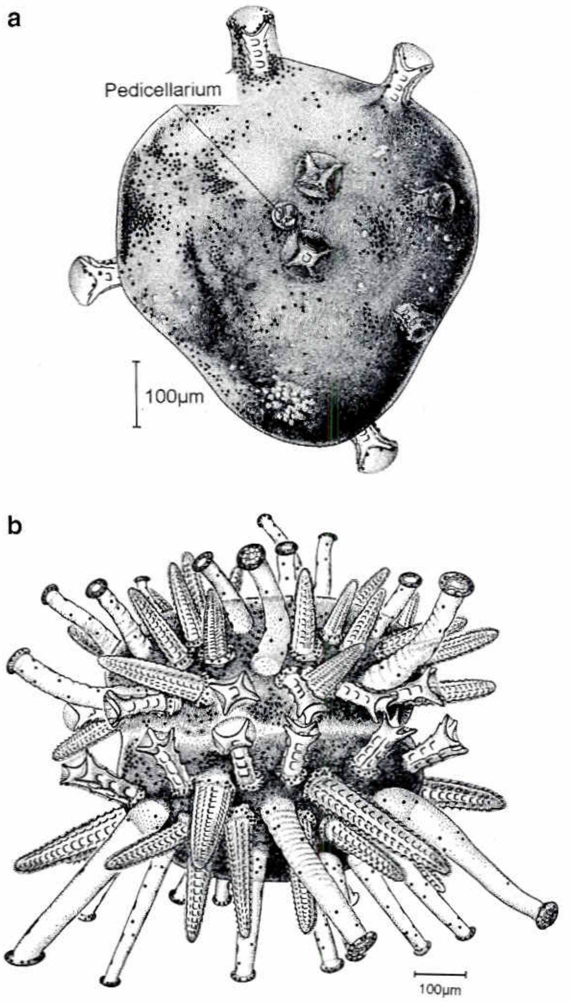

Fig. 11. Result of metamorphosis in plutei of Ps. mil. with double (a) or missing hydrocoel-anlagen (b) and imaginal discs. *Double imaginal discs (++ -type) develop into a siamese twin composed of two ventral sides, two mouths, two nervous systems but without pedicellaria. The -- -type* **(b)** *forms a reduction sphere with pedicellaria and some spines with four tips lacking a mouth, the nervous system, ambulacral feet and larger spines.*

somatocoel of the other side is encountered, the contact zone of the two somatocoels giving raise to the mesentery.

The larger cell mass on the left side splits again. Some cells remain on the oesophagus, forming the axocoel. This cluster has a counterpart on the right side. Most likely both contain primordial germ cells. The other part of coelomic cells on the left side, the hydrocoel-anlage continue to migrate onto the stomach (Fig. 8). In the subsequent differentiation the hydrocoel vesicle, gliding over the left somatocoel and a stone channel, opening with a hydroporus on the dorsal outer epithelium are formed (Czihak, 1960a).

The hydrocoel vesicle most likely induces the formation of an ectodermal pouch by invagination (Fig. 8). The ectodermal vestibulum together with the mesodermal hydrocoel form the imaginal disc for the ventral part of the future sea urchin starting with five primary tentacles.

Using UV-irradiation of different parts of the hydrocoel and of the ectodermal vestibulum the interrelationships of embryonic anlagen was analyzed (Czihak, 1965). As a rule missing parts were replaced by remaining cells. The most remarkable case was the replacement of a hydrocoel after UV-irradiation of the left coelomic sac during separation from the archenteron (Fig. 9). Cells of the cluster of coelomic material on the right side of the esophagus migrated to the left side where they differentiated to a normal hydrocoel participating in the formation of a imaginal disc in combination with ectodermal material of the vestibulum. This case of regulation is certainly unique even in embryonic development (Czihak, 1965).

Variations in the development of the coelomic sacs (Czihak, 1960b) include the situs inversus, a mirror image of the normal size difference, when the coelomic sac on the right side is the larger one and in consequence the imaginal disc develops on the right side, furthermore imaginal discs on both sides of the stomach leading to the formation of "siamese twins" (Fig. 11a) and third a lack of hydrocoels and in consequence of an imaginal disc on both sides resulting in a "reduction sphere" after metamorphosis consisting of two dorsal halves of a sea urchin (Fig. 11b).

Acknowledgments

The original work was supported by several grants of the DFG (Deutsche Forschungsgemeinschaft).

References

CAMERON, R. A., FRASER, S. E., BRITTEN, R. J., and DAVIDSON, E. H. (1989). The oral-aboral axis of a sea urchin embryo is specified by first cleavage. *Development 106:* 641-647.

CZIHAK, G. (1960a). Untersuchungen über die Coelomanlagen und die Metamorphose des Pluteus von *Psammechinus miliaris. Zool. Jb. Anat. 78:* 235-256.

CZIHAK, G. (1960b). Pseudoradiärsymmetrische Seeigelplutei. *Roux Arch. Entw.Mech. 152:* 593-601.

CZIHAK, G. (1961). Ein neuer Gradient in der Pluteusentwicklung. *Roux Arch. Entw.Mech. 153:* 353-356.

CZIHAK, G. (1962a). Entwicklungsphysiologische Untersuchungen an Echiniden (Topochemie der Blastula und Gastrula, Entwicklung der Bilateral- und Radiärsymmetrie und der Coelomdivertikel). *Roux Arch. Entw.Mech. 154:* 29-55.

CZIHAK, G. (1962b). Entwicklungsphysiologie der Echinodermen. *Fortschr. Zool. 14:* 238-267.

CZIHAK, G. (1963). Entwicklungsphysiologische Untersuchungen an Echiniden (Verteilung und Bedeutung der Cytochromoxydase). *Roux Arch. Entw.Mech. 154:* 272-292.

CZIHAK, G. (1965). Entwicklungsphysiologische Untersuchungen an Echiniden (Experimentelle Analyse der Coelomentwicklung). *Roux Arch. Entw.Mech. 155:* 709-729.

CZIHAK, G. (1973). The role of astral rays in early cleavage of sea urchin eggs. *Exp. Cell Res. 83:* 424-426.

DAVIDSON, E. H. (1993). Later embryogenesis: regulatory circuitry in morphogenetic fields. *Development 118:* 665-690.

HÖRSTADIUS, S. (1975). Isolation and transplantation experiments. In *The Sea Urchin Embryo, Biochemistry and Morphogenesis* (Ed. G. Czihak). Springer Heidelberg, pp. 364-423.

RANSICK, A. and DAVIDSON, E. H. (1993). A complete second gut induced by transplanted micromeres in the sea urchin embryo. *Science 259:* 1134-1138.