Biosynthesis of plasma membrane proteins from embryo cells of sea urchins at early embryogenesis

V.V. Sova and Yu.P. Chuguev

Kamchatka Branch, Institute of Marine Biology, Far East Research Center of the USSR Academy of Sciences, 683000 Petropavlovsk-Kamchatsky, USSR

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Studies on the biosynthesis of membrane proteins from embryo cells of *Strongylocentrotus intermedius* and *Scaphechinus mirabilis* sea urchins have revealed specificity in the synthesis of plasma membrane proteins at different stages of early embryogenesis (zygote, middle blastula, gastrula).

Sea urchin *Embryogenesis* *Plasma membrane* *Membrane protein* *Protein biosynthesis*

1. INTRODUCTION

It is known that in the process of early embryogenesis of sea urchins the rate of protein synthesis changes at each stage [1]. Moreover, at different stages of embryogenesis protein biosynthesis undergoes not only quantitative but also qualitative changes [2]. It was shown by electron microscopy studies that an increase of the cell surface just after fertilization occurs due to intensive growth of microvilli [3]. The growth of microvilli stops at the 8-blastomere stage. At the middle blastula stage the surface of the embryo cells must increase 10-fold [4]. Thus, it is probable that protein biosynthesis of plasma membrane cells from sea urchin embryo should have changed essentially in the process of evolution.

This paper is devoted to studies on biosynthesis of plasma membrane proteins from embryo cells of sea urchins to reveal the assumed differences.

2. MATERIALS AND METHODS

2.1. Reagents

We used standard electrophoresis sets (Reanal, Hungary), 2-mercaptoethanol (Serva) and ^{14}C-labelled amino acid hydrolyzate (Amersham).

2.2. Sea urchins and gametes

S.intermedius and *Sc.mirabilis* sea urchins were collected at the period of active spawning at Posiet Bay, Sea of Japan. Preparation of gametes, fertilization of egg cells and growth of embryos were performed as in [5]. Stages of evolution were determined using tables of normal evolution [6].

2.3. Incorporation of isotopic label

Seawater was passed through membrane filters (Synpore, Czechoslovakia) of 0.45 μm pore size. The antibiotics, penicillin and streptomycin (100 units/ml each) were added to the suspension of growing embryos. Thirty min before termination of incubation of embryos of the studied stage the ^{14}C-labelled amino acid hydrolyzate (final concentration 0.33 μCi/ml) was added to the suspension. After incubation the embryos were concentrated and washed with cold amino acid hydrolyzate.

2.4. Isolation of the plasma membrane fraction

This was performed as in [2].

2.5. Electrophoresis of plasma membrane proteins

Electrophoretic separation of proteins in PAGE (10–18%) was carried out according to [7].

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2.6. Autofluorography

The gels were treated as in [8], dried and exposed on X-ray Kodak XRP-6 film.

3. RESULTS

3.1. Isolation and characteristics of the plasma membrane fraction

Plasma membrane preparations were analyzed by electron microscopy (fig.1) and by determining the enzymic activity of cytochrome *c* oxidase. As seen from fig.1, the preparations are not contaminated by other organelles.

3.2. Characteristics of the protein moiety of plasma membranes

The data of electrophoretic separation of plasma membrane proteins from embryo cells of *S.intermedius* sea urchins are shown in fig.2. During development from the egg cell stage to the gastrula stage no changes occur in the polypeptide set of plasma membranes. But at the middle blastula stage and closer to the gastrula stage the amount of polypeptides with M_r 10 000–20 000 increases. The set of plasma membrane polypeptides from sea urchin embryo cells is represented by a wide range of M_r (10 000–200 000 and above).

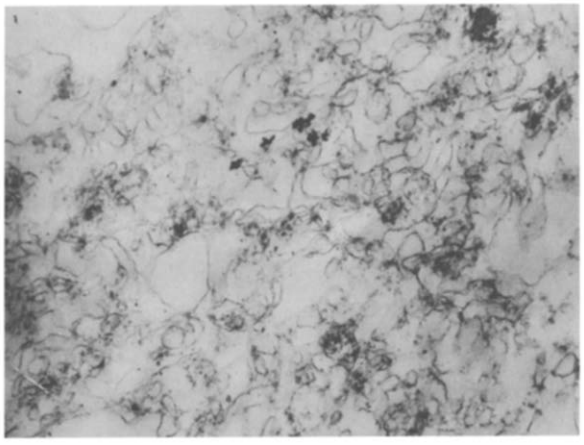

Fig.1. Electron micrograph of ultrathin section of a plasma membrane preparation of cells from *S.intermedius* at the middle blastula stage: 1% solution of osmic acid was used for fixation. Magnification ×20 000; JEM 100C electron microscope.

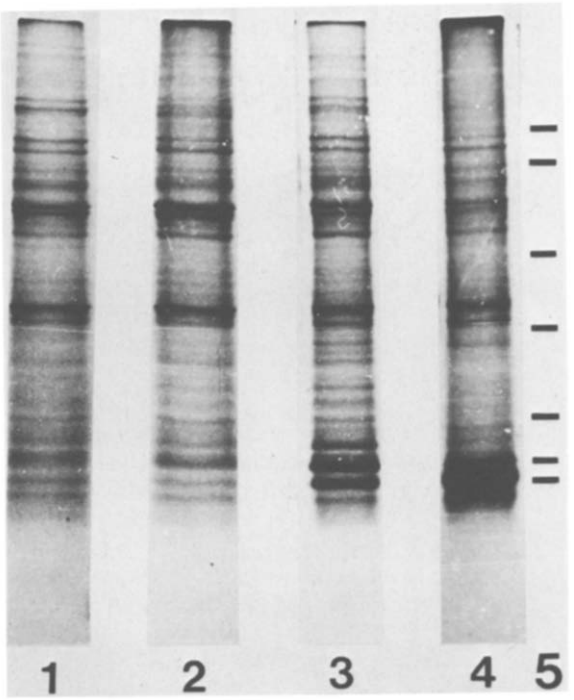

Fig.2. Data of electrophoretic separation of plasma membrane polypeptides from embryo cells of *S.intermedius* sea urchins at different stages of evolution in a PAGE gradient (10–18%) in the presence of SDS. Lanes: (1) mature egg cell; (2) zygote; (3) middle blastula; (4) gastrula; (5) marker proteins [from top to bottom] cytochrome *c*, 12 400 Da; pancreatic ribonuclease, 13 363 Da; soybean trypsin inhibitor, 20 000 Da; carbonic anhydrase, 29 000 Da; ovalbumin, 43 000 Da; bovine serum albumin, 67 000 Da; phosphorylase *b*, 94 000 Da.

3.3. Biosynthesis of plasma membrane proteins from sea urchin embryo cells

Fig.3 presents autofluorography data from electropherograms of plasma membrane proteins from sea urchin embryo cells at stages from zygote to gastrula. Intensive synthesis of polypeptides with M_r 30 000 and above proceeds at the zygote stage (40 min period of growth).

The middle blastula stage is characterized by a primary synthesis of polypeptides with M_r 10 000–13 000. Three polypeptides can be distinguished in this group of which polypeptide 'b' is synthesized most intensively. At the gastrula stage the synthesis of the polypeptides noticeably slows down which is clearly seen in the low-molecular-mass region (fig.3, lane 3).

Fig.3. Autofluorography data from electropherograms of membrane polypeptides from embryo cells of *S.intermedius* sea urchins at different stages of development in a PAGE gradient (10–18%) in the presence of SDS. Lanes: (1) zygote; (2) middle blastula; (3) gastrula, (a, b, c) membrane polypeptides of 10–13 kDa.

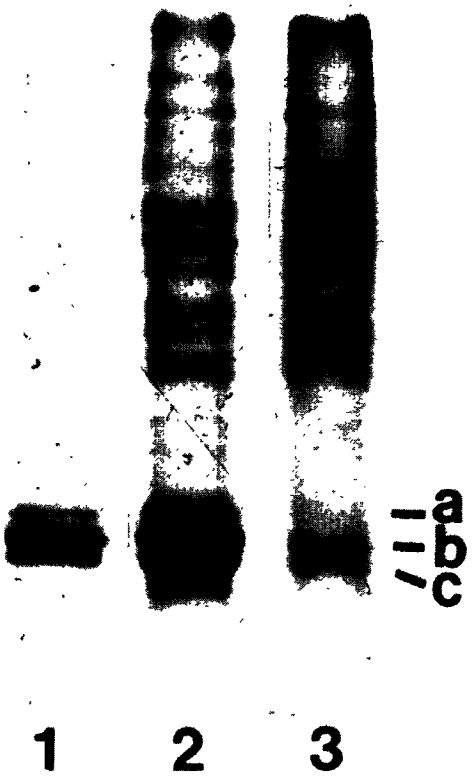

Fig.4. Autofluorography data from electropherograms of membrane polypeptides from embryo cells of *S.intermedius* sea urchins at different stages of development in a PAGE gradient (10–18%) in the presence of SDS after different periods of exposure on X-ray film. Lanes (1) middle blastula, 7 days; (2) middle blastula, 15 days; (3) gastrula, 15 days.

Fig.4 presents autofluorography data for electropherograms of analogous stages of development but with a longer exposure on X-ray film. At the middle blastula stage we can clearly observe biosynthesis of the whole spectrum of plasma membrane polypeptides, but the portion of newly synthesized polypeptides with M_r 10 000–13 000 is at least 90%. Practically all the polypeptides are also synthesized at the gastrula stage, however, in the range 10–13 kDa the synthesis is comparable with that of other polypeptides, while the synthesis of the low-molecular-mass polypeptides has ceased completely (fig.4, lane 3a).

Electrophoretic data on plasma membrane proteins fom different species of *S.intermedius* and *Sc.mirabilis* sea urchins are given in fig.5. The protein composition of plasma membranes varies in different species of sea urchins. Nevertheless, a the middle blastula stage polypeptides of M_r 10 000–13 000 are similar in their electrophoretic mobility and compose an essential portion of the spectrum of membrane proteins in the two species of sea urchins. Their biosynthesis proceeds analogously. The synthesis of polypeptide 'a' is more intensive in *Sc.mirabilis* than in *S.intermedius*.

4. DISCUSSION

Sea urchin embryos are unique objects for studying plasma membrane biogenesis due to their availability in preparative amounts, simultaneous development at early stages of embryogenesis and ability to incorporate isotope labels. At early stages of embryogenesis blastomere cells are large enough for obtaining a high degree of purification of the plasma membrane fraction by differential centrifugation. The use of a hypotonic buffer at

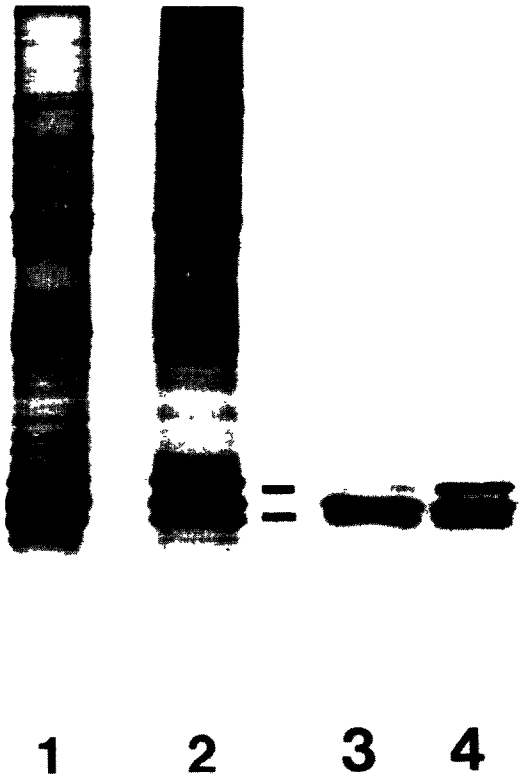

Fig.5. Data of electrophoretic separation and autofluorography of plasma membrane polypeptides from embryo cells of different species of sea urchins at the middle blastula stage in a PAGE gradient (10–18%) in the presence of SDS. Lanes (1) electropherogram of membrane polypeptides from *S.intermedius*; (2) electropherogram of membrane polypeptides from *Sc.mirabilis*; (3) autofluorography of membrane polypeptides from *S.intermedius*; (4) autofluorography of membrane polypeptides from *Sc.mirabilis*.

one of the purification stages completely eliminated mitochondrial contamination. Centrifugation in a 3% sucrose gradient density concentration range yielded highly purified preparations of plasma membranes.

Up to now the molecular bases of the processes occurring during fertilization of the egg cell and early embryogenesis have been little studied. At early embryogenesis of sea urchins an increase of the plasma membrane surface occurs not only as a result of cell division but also due to the formation of a significant amount of finger-like microvilli on the fertilized egg cell surface [3,10]. It has been assumed that microvilli consist mainly of a high-M_r actin-like protein [10]; however, our data show that a whole set of polypeptides with $M_r > 30\,000$ is synthesized at the zygote stage. The present results and data on the biosynthesis of membrane polypeptides from hybrid embryos [11,12] allow us to conclude that mRNAs for these polypeptides were accumulated during oogenesis of sea urchins.

The anomalous synthesis of the polypeptide a, b, c group at the middle blastula stage is of particular interest. The synthesis of these polypeptides essentially decreases at the gastrula stage. The function of the studied proteins has not yet been established, but their clearly pronounced synthesis specificity at this stage of embryo development allows one to suggest a role in the formation of the blastomere cell surface, which is probably connected with the regulation of early embryogenesis at the level of intercellular interactions.

Further studies of these proteins and the mechanism of their synthesis can shed light on the role of the surface blastomere cells in the mechanism of differentiation.

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