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CONTACT PLATE OF *AURELIA AURITA* IS A FUNCTIONAL ANALOGUE OF MAMMALIAN ZONA PELLUCIDA

L. ADONIN* & O. PODGORNAYA

Institute of Cytology RAS, Saint-Petersburg, Russia

All cnidarians are thought to possess two tissue layers: endoderm and ectoderm, which are separated by mesoglea in medusa. The determination of the composition of the *Aurelia aurita* jellyfish mesoglea was performed. A new protein »mesoglein« was determined as one of the main components of mesoglea. Deduced amino acid sequence of mesoglein contains the Zona Pellucida (ZP) domain and DSL domain. Antibodies against mesoglein stain the plate in the place of contact of germinal epithelium and oocyte. The structure found was named the »contact plate«. The contact plate could be the precursor and functional analogue of the ZP.

Key words: *Aurelia aurita*, Zona Pellucida, extracellular matrix

Cnidaria are lower multicellular animals with a body consisting of two epithelial layers. An extracellular substance – mesoglea – is situated between the epidermal and gastrodermal layers of these animals. The object of the present work is *Aurelia aurita*. *A. aurita*'s habitat is very wide: from the northern latitudes (White Sea and Barents Sea) to the south-east coast of Australia and New Zealand, *A. aurita* inhabits a vast spectrum of possible ecosystems, including anchialine lagoons or ponds (TOMASCIK *et al.*, 1997; BAKRAN-PETRICIOLI *et al.*, 1998).

A. aurita medusa is the sexual adult stage in a complex animal life cycle. The medusa possesses a huge extracellular matrix (ECM) – mesoglea, in between two cell layers. Two types of fibers, which are embedded in a jelly-like ground substance, have been described at the morphological level in cnidarian mesoglea: collagen-like and the so-called »elastic« or vertical fibers (CHAPMAN, 1959; ELDER, 1973; WEBER & SCHMID, 1985). The main part of *A. aurita* mesoglea is ECM but it also contains a number of mesogleal cells (Mc). The population of Mc inside mesoglea was observed in other species of Scyphozoa and Anthozoa (CHAPMAN, 1966). This feature is not unique but rather rare. Little was known about their functions. We determined the polypeptide composition of Mc, and mesoglea raised antibodies (AB) against one of the major mesogleal protein and checked the antibodies' specificity. Using light and electron microscopy immunostaining we showed that Mc are involved in the formation of mesogleal fibres (SHAPOSHNIKOVA *et al.*, 2005).

The apparent molecular mass ($M_{r)}$ of the polypeptide against which the AB was raised is 45/47 kDa (p47). A protein with a similar M_r was observed in the *Hydra* ECM, but it did not react with any antibodies (AB) against known vertebrate ECM proteins, and no suggestion was made about its nature (SARRAS & MADDEN, 1991). We supposed that p47 of *A.aurita* mesoglea could be an unknown protein and we

* leo.adonin@gmail.com

made an attempt to clone its gene (MATVEEV *et al.*, 2007). The merged sequences acquired by 3' and 5' RACE produced mRNA sequence 1421 bp long. NCBI BLAST (ALTSCHUL *et al.*, 1997) search for homologous nucleotide and protein sequences revealed that the mRNA sequence was novel. It was submitted to GenBank (Accession No. DQ467654) and named mesoglein (MATVEEV *et al.*, 2007).

The search for known domains and motifs in the deduced protein sequence reveals similarity of amino acid positions 43-84 aa to Delta/Serrate/Lag-2 (DSL) domain and similarity of positions 93-337 aa to Zona pellucida (ZP) domain. Mesoglein happens to belong to the ZP-domain protein family and therefore we paid attention to *A. aurita* oogenesis.

The *A. aurita* medusa, known worldwide as the moon jellyfish, reproduces sexually during summer. Gametes are released from the gonads into the gastric pouches. Ova, produced fast by oogenesis, remain in the female body but spermatozoa exit via the mouth into the sea (WONG & WESSEL, 2006). They enter the mouth of a female and make their way to the gonad where they fertilize the eggs. Embryos are released from the mouth and brooded on the oral arms.

AB RA47 against mesoglein in fact stain the plate in the place where germinal epithelium contact oocyte on the paraffin sections. According to its position, we named the structure found the »contact plate«. The description of the *A. aurita* contact plate morphological formation is the subject of the current work

Mesoglein belongs to the family of extracellular matrix proteins that contain a zona pellucida (ZP) domain and it is an early metazoan member of the ZP-domain--containing protein family.

It is known that such ZP-domain proteins as ZP1, ZP2 and ZP3 in mouse ZP play an important role in fertilization (JOVINE *et al.*, 2005). However, nothing is known about oogenesis-related ZP-domain proteins in the lower multicellular animals. Mesoglein happens to belong to the ZP domain protein family and therefore we paid attention to *A. aurita* oogenesis.

Antibodies RA47 do stain the plate in the contact place of germinal epithelium and oocyte on the paraffin sections. According to its position, we named the structure found the »contact plate«. The description of *A. aurita* oogenesis is far from being complete. It is known that oocytes arise from the germinal epithelium – the derivate of the bottom wall of the gastric pocket. Some adjacent cells probably take part in the nutrition of the growing oocyte (ECKELBARGER & LARSON, 1988). At late stages, these cells build up funnelform cavities in the germinal epithelium just under mature oocytes. These cavities serve as cages for spermatozoids (IVANOVA-KAZAS, 1975). But no specific structures have been described in the region of contact between oocyte and adjacent epithelium. We split oogenesis into seven stages for convenience of description. The main feature of the stage is the oocyte size – from 10 mm (stage I) up to 150–170 mm at the end of maturation (stage VII).

A structure similar to the contact plate was observed earlier (ECKELBARGER & LARSON, 1988), but the authors paid most attention to vitellogenesis. Their ultrastructural data suggest that different types or classes of yolk precursors enter the oocyte through the trophocytes and via the surrounding mesoglea. On semi-thin sections, the structure is clearly visible. Following the logic of the article, the authors claim that the material of the structure is the small granules of yolk precursors provided by trophocytes. Our histochemical data and immunostaining gives evidence

that contact plate composition differs from the surrounding yolk granules (ADONIN *et al.*, 2009). The question of whether the contact plate material is synthesized by the oocyte itself or comes from the trophocytes remains to be elucidated. The internal position of the granules at oogenesis early stages and the position of the plate itself suggest that trophocytes are not involved in the synthesis of their material.

We still do not know how many ZP proteins the *A.aurita* genome contains. The germinal epithelium subjected to immunoblot with RA47 against mesoglein revealed charged proteins with Mr even higher than that of Mesogleal cells (ADONIN *et al.*, 2009). Mesoglein acid-urea PAGE indicates that mesoglein is highly positively charged (MATVEEV *et al.*, 2007). The titration curve deduced from the mesoglein aa sequence shows pI 9.03 and charge 30 (calculated using an EMBOSS IEP tool) at pH 7.5 which is in agreement with experimental data. The high positive charge of mesoglein is unlike that of most other ZP domain-containing proteins. Only 12.5% of ZP domain proteins have predicted pI higher than pI 8. The mean predicted pI value of ZP-domain-containing proteins is pI 6.3, and the mean pI of ZP domains is pI 6.2. The proteins, recognized by RA47 in gonads, are also charged, which indicates their relation to mesoglein, though their charge is not as strong (ADONIN *et al.*, 2009). ZP-domain occupies about half of the whole aa sequence of the mesoglein. Antibodies RA47 could be directed against ZP-domain, at least against part of it. If so, ZP-domain-containing proteins are definitely members of the contact plate.

ZP-proteins of the contact plate could play a similar role in *A. aurita* fertilization, taking into consideration their position and fertilization features.

REFERENCES

ADONIN, L. S., PODGORNAYA, O. I., MATVEEV, I. V. & SHAPOSHNIKOVA, T. G., 2009: The plate in zone of oocyte and germinal epithelium contact in scyphomedusa *Aurelia aurita* binds antibodies to ZP-domain containing protein mesoglein. Cell Tissue Biol 3, 283–288.

ALTSCHUL, S. F., MADDEN, T. L., SCHAFFER, A. A., ZHANG, J., ZHANG, Z., MILLER, W. & LIPMAN, D. J., 1997: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

BAKRAN-PETRICIOLI, T., PETRICIOLI, D. & POZAR-DOMAC, A., 1998: Influence of isolation and peculiar ecological properties on biodiversity – An example of marine lake Zmajevo oko near Rogoznica (Adriatic Sea). Rapp. Comm. int. Mer Médit. 35, 518–519.

CHAPMAN, G., 1959: The mesoglea of *Pelagia noctiluca.* Quart J Micr Sci 100, 599–610.

ECKELBARGER, K. J. & LARSON, R. J., 1988: Ovarian morphology and oogenesis in *Aurelia aurita* (Scyphozoa: Semaeostomae): ultrastructural evidence of heterosynthetic yolk formation in a primitive metazoan. Mar Biol 100, 103–115.

ELDER, H. Y., 1973: Distribution and functions of elastic fibers in the invertebrates. Biol Bull 144, 43–63.

IVANOVA-KAZAS, O. M., 1975: Comparative embryology of invertebrate animals. Moscow: Nauka 15–120.

JOVINE, L., DARIA, C. C., LITSCHER, E. S. & WASSARMAN, P. M., 2005: Zona Pellucida domain proteins. Annu Rev Biochem 74, 83–114.

MATVEEV, I. V., SHAPOSHNIKOVA, T. G. & PODGORNAYA, O. I., 2007. A novel *Aurelia aurita* protein mesoglein contains DSL and ZP domains. Gene 399, 20–25.

MATVEEV, I. V., ADONIN, L. S., SHAPOSHNIKOVA, T. G. & PODGORNAYA, O. I., 2012: *Aurelia aurita* – cnidarian with a prominent medusiod stage. J. Exp. Zool. (Mol. Dev. Evol.) 318, 1–12.

SARRAS, M. P. JR, MADDEN, M. E., 1991: Extracellular matrix (mesoglea) of *Hydra vulgaris*. I. Isolation and characterization. Dev Biol 148, 481–494.

SHAPOSHNIKOVA, T., MATVEEV, I., NAPARA, T. & PODGORNAYA, O., 2005: Mesogleal cells of the jellyfish Aurelia aurita are involved in the formation of mesogleal fibres. Cell Biol Int 29, 952–958.

TOMASCIK, T., MAH, A. J., NONTJI, A. & MOOSA, M. K., 1997: The Ecology of the Indonesian Seas (Part II). Published by Periplus Editions (HK) Ltd.

WEBER, C. & SCHMID, V., 1985: The fibrous system in the extracellular matrix of hydromedusae. Tissue & Cell 17, 811–822.

WONG, J. L. & WESSEL, G. M., 2006: Defending the Zygote: search for the ancestral animal block to polyspermy. Curr. Top. Dev. Biol. 72, 1–115.