Oogenesis in neotenic cave salamander: Ultrastructure of previtellogene oocytes in *Proteus anguinus* (Amphibia, Urodela, Proteidae)

Lilijana Bizjak Mali and Boris Bulog

Department of Biology, Biotechnical Faculty, University of Ljubljana, Vecna pot 111, 1000 Ljubljana, Slovenia

lila.bizjak@bf.uni-lj.si
Keywords: *Proteus anguinus*, oogenesis, ultrastructure

Background: *Proteus anguinus* is a cave-dwelling amphibian salamander, inhabits only underground waters in Dinaric karst regions. It is obligate neotene, with very long life span, possibly more then 70 years and late sexual maturity. The sexual mature appears in 11 years old males and 15 years old females at 11-12ºC (Juberthie et al., 1996). The studies of reproductions of *Proteus* lasting many years in the Cave-laboratory of Moulis in France (Juberthie et al., 1996), revealed that female laying eggs at 6 years interval. This indicates on extremely slower oogenesis of *Proteus* in comparison with the other amphibians and pointed our study to the oocytes development in this species.

The classification of developing oocytes of amphibians has been carried out by many researchers (Dumont, 1972; Sretarugsa et al., 2001). Developing oocytes in amphibians have been divided into six stages based on their external appearance, histology, color and size. In addition, oocytes can be classified by the uptake of vitellogenin or yolk protein into three phases: previtellogenesis, vitellogenesis and postvitellogenesis or mature oocytes. The same criteria we have used in our preliminary study to classify developing oocytes of *Proteus anguinus*. In all examined ovaries of sexual mature specimens of *Proteus* oogonium and previtellogene phases (stage I and II) of oocytes were present and in some specimens larger vitellogenic (stage III and IV) oocytes too, but rarely (Tabaler, 2008). No mature oocytes (postvitellogenic) or ovum were found. Most ovaries sampled showed atretic oocytes. In present paper the ultrastructure of the *Proteus* previtellogenic stage I oocytes is represent.

Results: The stage I oocytes of *Proteus anguinus* are previtellogenic with diameter ranging from about 100 to 300 μm and can be identified by their transparent cytoplasm. They are present in all ovaries analyzed. The nucleus is large, with numerous nucleoli. The most predominant ultrastructural characteristics of previtellogene oocytes are extensive quantities of smooth membrane system, numerous mitochondria and lipid droplets distributed throughout the cytoplasm, as well as abundance of free ribosomes (Fig. 1). The abundance of free ribosomes in the cytoplasm is a basic characteristic of anuran oocytes, they are the site where various proteins are synthesized for usage within the cell (Sretarugsa et al., 2001). A multiple Golgi complexes of flattened tubules and small vesicles with spare luminal content are present. Very frequent are myeline-like structures made of numerous densely packed membranous layers (Fig. 2). For myeline-like structure has been supposed that may play the role of membrane stocks to be used eventually for the formation of nascent endoplasmic membranes in the oocytes (Semakova and Kiseleva, 2003). The ooplasm of *Proteus* previtellogene oocytes contains remarkable annulate lamellae of closely packed membrane stacks (Fig. 3). Each lamella consists of two parallel membranes separated by a cisternal space (Fig. 4). Annulate lamellae are commonly found in the cytoplasm of both invertebrate and vertebrate germ cells, embryonic cells and some tumor cells (cit. in Kessel, 1965; Ghadially, 1997). For amphibian oocytes they have been characteristic prior to vitellogenesis

DOI: 10.3217/978-3-85125-062-6-327,

(Ganion, 1991). Although annulate lamellae are widespread occurrence, the functional significance of this organelle is still unknown. They possess certain structural characteristics of both nuclear envelope and granular endoplasmic reticulum (Kessel, 1965). Some researchers speculate that they have some role in cell growth and differentiation, other suggest that may be involved in the transfer of RNA from the nucleolus to the cytoplasm (Ghadially, 1997).

Oogenesis in *Proteus anguinus* has not been studied yet and our preliminary study provides the first histological and ultrastructural detail describing growth of the oocytes, and leads to the acquisition of basic knowledge of their reproductive biology.

1. Dumont J. N. J. Morph. **136** (1972) p153-180.
2. Juberthie C., Durand J., Dupuy M. Mémoires de Biospéologie, **XXIII** (1996) p53-56.
3. Kessel R.G. J. Cell. Biol. **24** (1965) p471-487.
4. Ganion L.R. Anat. Rec. **230**1(1991) p218-24.
5. Ghadially F.N. (1997). 4th Ed.,,Butterworth-Heinemann, Boston.
6. Semakova K. N., Kiseleva E. V. Tsitologiya. **45** (2003) p746-757.
7. Sretarugsa P., et al. ScienceAsia (2001) p1-14.
8. Tabaler I. (2008) Graduation thesis, Univ. in Ljubljana.

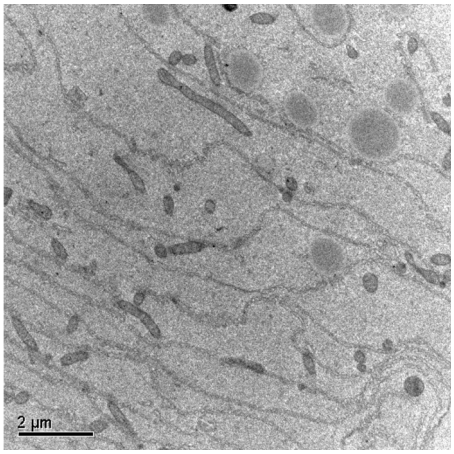

Figure 1. Oocyte cytoplasm of *Proteus anguinus* at low magnification with extensive smooth membrane system, mitochondria and lipids.

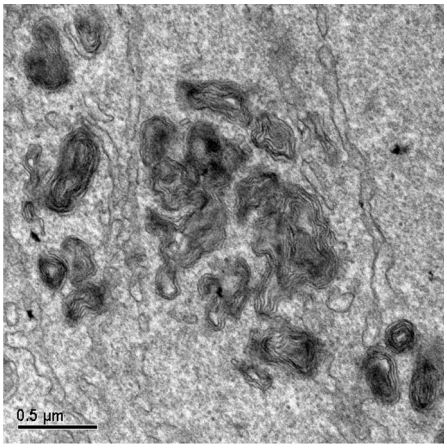

Figure 2. Myeline-like structures of numerous densely packed membranous layers in the ooplasm of *Proteus anguinus* oocyte.

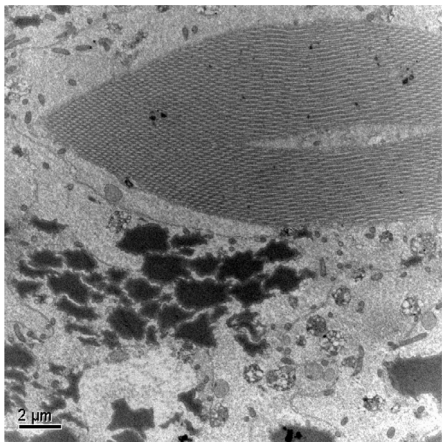

Figure 3. Annulate lamellae of *Proteus anguinus* oocytes.

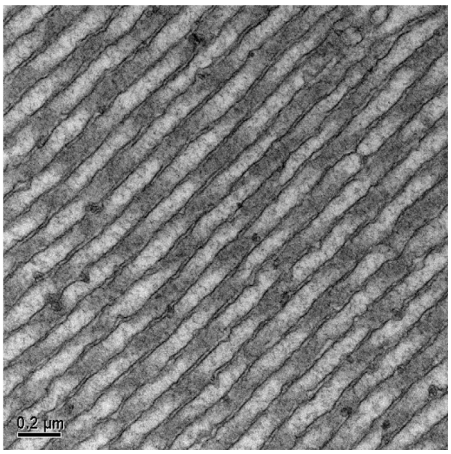

Figure 4. Annulate lamellae at higher magnification.

M.A. Pabst, G. Zellnig (Eds.): MC2009, Vol. 2: Life Sciences,
DOI: 10.3217/978-3-85125-062-6-327, © Verlag der TU Graz 2009