C. R. Biologies xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Comptes Rendus Biologies

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Review

Xenopus laevis oocyte as a model for the study of the cytoskeleton

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ARTICLE INFO

Article history:
Received 7 March 2018
Accepted after revision 6 April 2018
Available online xxx

Keywords:
Oocyte
Xenopus
Mitochondrial cloud
Polarization
Spectrin
Cytokeratin
p4.1R

ABSTRACT

At the beginning of diplotene, the oocyte of *Xenopus laevis* is a cell of about 10–20 microns destined to increase 10,000-fold its size when the oocyte becomes filled with yolk platelets and has accumulated a great number of pigment granules in a half of its periphery. Its internal architecture is gradually accomplished during growth because of several factors, especially because of cytoskeletal changes. In the fully-grown oocyte, the cytoskeleton appears to sustain the eccentrically located germinal vesicle through arms radiating from the cortex to the germinal vesicle, a unique organization not to be found in other Amphibians. In this report, we summarized and analysed steps of cytoskeletal proteins and related mRNAs organization and function throughout diplotene stage, highlighting our studies in this animal model. The cytoskeletal proteins appear to exploit their activity with respect to ribosomal 60S subunit maturation and during translation. Most importantly, the polarity of the oocyte is achieved through a sophisticated and highly organized localization of mRNAs and cytoskeletal proteins in one side of the cell. This asymmetry will start the construction of the oocyte polarity that is instrumental for determining the characteristic of this cell, which will become an embryo. Moreover, in the same time membrane composition, conditioned by the underlying cytoskeletal organization, will acquire the prerequisites for sperm binding and fusion.

1. Introduction

Xenopus oocyte was first demonstrated to have the capacity to translate exogenous mRNAs, then it was shown that this cell is particularly suitable for other studies such as electrophysiology, Ca2+ signalling, the developmental and cellular mechanisms of RNA localization, axis specification and establishment of the cytoskeleton changes during oogenesis. The latter aspect constitutes the topic of this review. Indeed, the stage-dependent cytoskeletal organization of the *Xenopus* oocyte is unique with respect to the oocyte of other Amphibian genders (*Discoglossus*, *Bufo*, *Rana* etc.); this peculiarity constitutes a rewarding model for the study of the cytoskeleton in relation to functional stages of oogenesis. In the *Xenopus* oocyte, the cytoskeleton distribution is dynamically regulated during oogenesis. During diplotene, oocytes undergo important functional and molecular changes related to oogenesis itself or to the development of the prospective embryo. Indeed, the occurrence of spatial distribution of organelles and molecules, which is of fundamental importance for the organization of the oocyte, strictly depends upon the cytoskeleton and viscoelastic conditions of the cytoplasm. An actin-based cytoskeleton is present in the cortex starting from early stage 1 [1–3], when the oocyte does not display clear signs of polarization in the cytoplasm (Fig. 1a, b). At the end of oogenesis, stages 4–6, a complex and polarized cytoskeleton network is present in the

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https://doi.org/10.1016/j.crvi.2018.04.001
1631-0691/

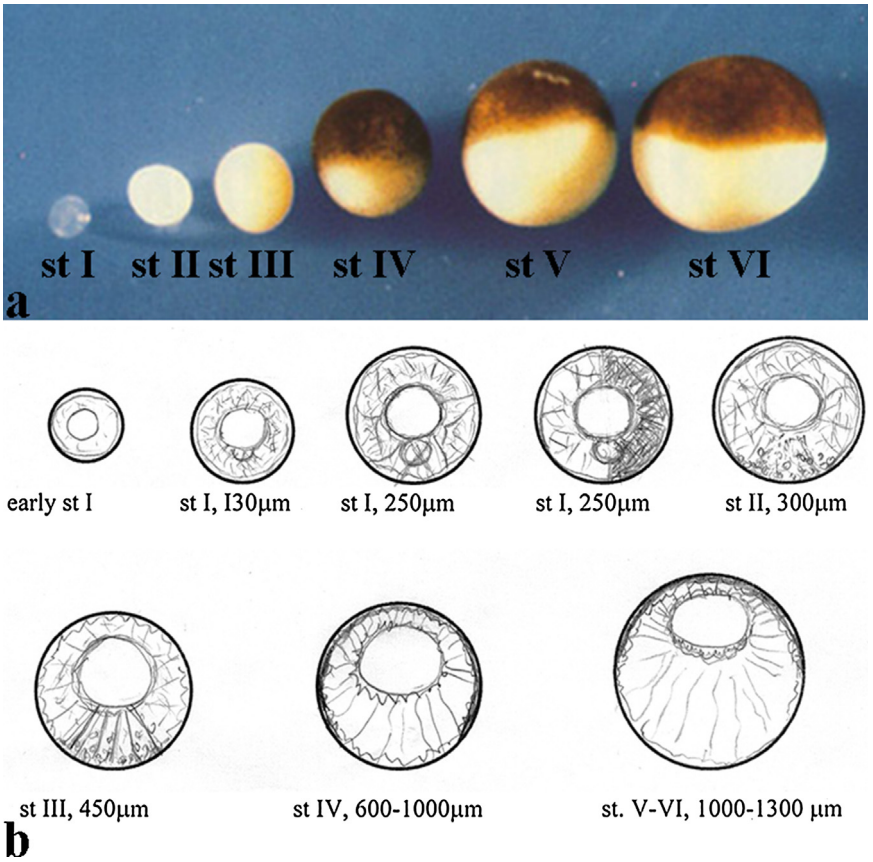

Fig. 1. *Xenopus laevis* oogenesis; a: photograph of oocytes stages according to [35]; b: schematic drawing of steps of cytoskeleton organization during *Xenopus laevis* oogenesis. In early stage I, the CSK proteins, in particular actin and spectrin, are sparsely present in the cortex. When the oocyte reaches about 130 μm in diameter, the CSK is organized also around the GV. At 250 μm, the CSK is widely present in the cortex and in the cytoplasm, where in particular tubulin, spectrin, and cytokeratin are found. In the cortex, p4.1R is also present. The CSK filaments permeate the MC. In this stage of growth, a transient asymmetrical localization of α-spectrin (RNA and protein) bisects the oocyte cytoplasm in two halves. At stage II, when the CSK is abundantly present in all oocyte districts, the MC disaggregates, and its content, including CSK components, is transported to the fronting cortex, the vegetal pole. At stages II–III, yolk deposition occurs at the oocyte periphery. The microtubule array sustaining the "late pathway", radiates from the nuclear envelope to the site where the MC has disaggregated. At stage IV, the crown of microtubule, cytokeratin, actin, spectrin has extended in all the cytoplasm, from the cortex to the nuclear envelope. The pigment granules are in almost all the peripheral cytoplasm, whereas later, at stages V–VI, they are found only in the animal half. The stage VI fully grown oocyte displays a polarized organization of all its constituents, including the cortex and around the GV.

cytoplasm. As a result, a radial distribution of filaments extends from the cortex to the Germinal Vesicle (GV) periphery (Fig. 1a, b). In addition to actin, several cytoskeletal proteins have been detected in the growing oocyte, including tubulin [4–12], vinculin [13], vimentin [14–20], talin [1], cytocheratin [21–26], spectrin [26–29] and myosin [2,30–32]. When the oocyte reaches final stages of growth (stages 4–6)[1], it acquires a defined polarization with respect to its virtual animal/vegetal axis (A/V axis). The eccentrically located germinal vesicle marks the animal side, and several cytoplasmic constituents, such as yolk platelets and ribosomes, distribute along the A/V axis (Fig. 1a, b). Immunocytochemistry coupled with disruptive drugs showed that actin, intermediate filaments, and microtubules have a fundamental role in the establishment and maintenance of this animal/vegetal polarity. In particular, in fully grown oocytes, cortical and perinuclear actin is linked to microtubules through their minus end, displaying the mechanical and functional scaffolding of the oocyte [33,34].

2. The early oocyte

2.1. Cytoskeleton organization

In stage-I oocytes, in which the yolk has not yet been deposited, the CSK forms a supple network that gradually spreads throughout the cell and is primarily composed of actin microfilaments, microtubules, vimentin, cytokeratin and spectrin [3,21,34,36]. Actin-binding spectrins of 239 and 100 kDa associate with the cell membrane, the nuclear envelope and the growing mitochondrial cloud (MC) [26,29], similarly to the organization achieved by the intermediate filaments (Fig. 1 and Fig. 2)

[1] Staging of the *Xenopus* oocytes. The growth stages of the *X. laevis* oocyte are: stage I (50–300 mm), stage II (300–450 mm), stage III (450–600 mm), stage IV (600–1000 mm), stage V (1000–1200 mm), and stage VI (1200–1300 mm) according to [35].

Please cite this article in press as: R. Carotenuto, M. Tussellino, *Xenopus laevis* oocyte as a model for the study of the cytoskeleton, C. R. Biologies (2018), https://doi.org/10.1016/j.crvi.2018.04.001