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Progesterone-induced Maturation and Down Regulation of Membrane Bound Na^+, K^+-ATPase

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Abstract

Progesterone induces maturation by releasing oocyte from G2 of MI cell cycle arrest. This process is rate limiting as it produces fertilizable eggs. Therefore, it draws a lot of attention. Na^+, K^+-ATPase is a membrane bound enzyme molecule that has known to have various functions. One of these functions is to act as receptor for Progesterone. In oocyte maturation to egg entirely depends on progesterone, a hormone that is known to reduce risk of cancer. Using EM Histo-cytochemical novel technique, we have shown that membrane bound Na^+, K^+-ATPase are gradually down-regulated following Progesterone-induced maturation. By Germinal Vesicle Break down, Na^+, K^+-ATPase is completely down-regulated from oolemma and only present in the narrow region of Germinal Vesicle Break Down. This is an important phenonmena as this down-regulation coincides with cell entering M-phase. Here, I also briefly introduce you to a technique that only localizes phosphate cleaving membrane bound Na^+, K^+-ATPase.

Keywords: Na^+; K^+-ATPase; Down regulation; Progesterone

Introduction

Progesterone induces fully grown Stage VI oocytes to mature that leads to Nuclear Envelope Break Down and the appearance of white spot in the animal hemisphere [1-3]. Na^+, K^+-ATPase is a P-type ATPase that undergoes E1-E2 transition to translocate $3Na^+$ outside cell against electrochemical gradient. In all living animal cells it regulates cell pH. It is also involved in firing electrical current in neuronal cells. It has specialized functions in muscles and cardiac cells. The molecular structure and genetic expression of this protein has been extensively studied [4,5]. It is a multigene protein. It is heterodimer of a catalytic α-subunit of approximately 100-110 kD peptide and a β-sununit of 40 kD. Four isoforms of α-subunit have been reported [4,6,7]. Three isoforms of β-subunit have also been identified [6]. The third subunit of Na^+, K^+-ATPase is γ-subunit of 7-10 kD [8,9]. Experimental evidences suggest that the Na^+, K^+-ATPase activity is modulated by protein kinase A and protein kinase C by phosphorylation [10,11].

Na^+, K^+-ATPase is a key instigator of cell polarity [12]. We have shown that Na^+, K^+-ATPase activity is asymmetrically localized over the animal hemisphere of fully differentiated Stage VI Xenopus oocytes [12]. The Vegetal pole that holds the vegetal cortices comprising VegT, TGF-β and Cyclin-B lacks Na^+, K^+-ATPase activity completely. These Phosphate-cleaving membrane bound Na^+, K^+-ATPase sets up an internal gradient of Na^+ that is critical in establishing polarity [13]. One important aspect of Progesterone-induced down-regulation of membrane bound Na^+, K^+-ATPase from animal hemisphere is down-regulation fattens the internal Na+ gradient. Therefore, it releases vegetal cortices from its position and moves upward following Progesterone-induced maturation [14] due to complete down-regulation of Na^+, K^+-ATPase from the animal hemisphere [12]. This down-regulation causes complete flattening of internal Na^+ that is set due to asymmetric localization of Na^+, K^+-ATPase activity. This asymmetry sets up three internal Na^+ gradients [12]. The vegetal hemisphere that lacks Na^+, K^+-ATPase activity has a sink for Na^+. The animal hemisphere that has Na^+, K^+-ATPase activity has medium level of Na^+. While the equatorial region has negligible to low Na^+. We believe, the acidic proteins such as Tgf-β [15], Cyclin-B [16,17] and VegT [14] are localized in the vegetal cortices while the basic proteins such as AP-2 [18], Sox2 and Sox3 [19] are localized in the animal hemisphere and the neutral proteins such as Brachy [20] is localized in the equatorial region. Exceptions are there. As we know myoD is a basic protein and it is localized in the equatorial region possibly due to its binding partner. My estimation is Na^+ is the key instigator of cell polarity. Therefore, this asymmetry is critical [12].

Following Progesterone-induced maturation, Na^+, K^+-ATPase is localized in the animal pole region. It appears that the Dorsal-ventral axis is already set up in the matured eggs with presence of ATPase activity towards the dorsal side (Figure 1) [12]. Na^+, K^+-ATPase is key receptor molecule [1,2]. We believe, apart from Progesterone and Estradiole it also possibly act as receptor for sperm and we expect an up-regulation of ATPase activity at the sperm entry point. We think Na^+, K^+-ATPase may be the key receptor for hormones [1,2] and sperm. We are making greater stride to resolve the issue. As we know Na^+ is the binding factor between sperm and egg therefore such asymmetric localization is critical. Surely this will be a turning point. Another important point about the down-regulation of Na^+, K^+-ATPase following Progesterone-induced maturation is arrest of cell cycle at M-phase. Does that means Na^+, K^+-ATPase activity may have a role in metastasis? It would be interesting to observe Na^+, K^+-ATPase activity in uterine and cervical cancer by EM Histo-Cytochemical method, the technique that localizes phosphate cleaving Na^+, K^+-ATPase activity at the resolution of electron microscope. It may resolve what effect progesterone and estrogen have on uterine and cervical cancer (we are open to collaboration).

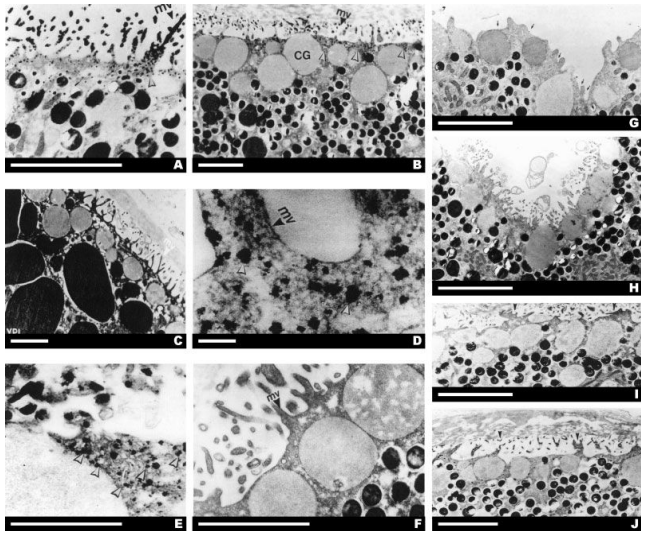

Figure 1: Localization of Na^+, K^+-ATPase activity following progesterone-induced maturation of stage-VI Xenopus oocytes. We see slight up-regulation (A) and then immediate complete down-regulation of Na^+, K^+-ATPase activity (B-H). A: An up-regulation of Na^+, K^+-ATPase activity within 1 hr after progesterone-induction. B: A substantial and near complete down-regulation of Na^+, K^+-ATPase activity following 3 hr of progesterone induction. C: Complete absence of Na^+, K^+-ATPase activity over the vegetal hemisphere. (D, E) Presence of vesicle associated reaction deposits in the sub cortical region of the oocyte following 3 hr of progesterone induction, suggesting endocytotic removal of the membrane Na^+, K^+-ATPase molecules from the plasma membrane. G: Presence of countable number of Na^+, K^+-ATPase molecules over the animal pole region of polar body extrusion following GVBD. Na^+, K^+-ATPase activity is completely down-regulated by GVBD, and vesicle-associated reaction deposits are absent from the cytoplasm of subcortical region. We believe the side that has maximum ATPase activity forms the dorsal side of the fertilized egg. This observation was only made possible by EM enzyme Histo-cytochemical technique: (I) Complete absence of reaction deposits, when K+ is completely removed. (F, J) Complete absence and near complete absence of Na^+, K^+-ATPase activity over two different regions over the animal hemisphere in the presence of 10 μM Ouabain. (H) The presence of countable number of Ca^{2+}, Mg^{2+}-ATPase activity at the region of polar body extrusion over animal pole region following GVBD. Magnifications/bar sizes: (A) 15,000/1 mm; (B) 6,000/1 mm; (C) 5,000/1 mm; (D) 5,000/100 nm; (E) 30,000/1 mm; (F) 15,000/1 mm; (G-I) 10,000/1 mm; (J) 8,000/1 mm. (Adopted from Mohanty and Gupta, 2012).

Progestrone is a steroid hormone involved in female menstrual cycle, pregnancy (supports gestation) and embryogenesis of human and other species [20-22]. In clinical term, reduced progesterone adversely affects endomatrium maturation and results in subfertility and pregnancy miscarriage [23,24]. On the contrary Estrogen exposure induces breast and endometrial cancer [25]. It also proliferated epithelial cells to develop into cancer [26]. Progesterone, on the otherhand, inhibits this estrogen-induced cell proliferation and stimulates epithelial differentiation [27]. Consequently, progesterone is used therapeutically to inhibit the proliferation of estrogen-dependent endometrial cancers [28-30].

It is also important to know why a down-regulation is necessary before fertilization. The reason could be very rewarding. I believe the vegetal cortices released from vegetal hemisphes eggs following Progesterone-induced maturation has a significant role. Cyclin B moves upward and enters into the nucleus to complete division. Similarly, down-regulation makes eggs quiescent and prepares eggs to hostile environment and any damage to DNA. In Xenopus, eggs are laid in wet-land that remains hostile. We were the first to show a detailed analysis of Na^+, K^+-ATPase activity in any vertebrate oocytes and in maturing eggs [12] and the experiment using EM Histo-cytochemical technique was repeated 7 times with constistant results. In the process we realized Na^+, K^+-ATPase activity can be different if localize by EM Histo-cytochemical technique and immune-cytochemical localization as later can localize epitope both from active and inactive Na^+, K^+-ATPase [31]. This protection allows organogenesis and patterning to proceed normally following fertilization to production of normal embryos.

EM Histo-cytochemical localization is the best technique to localize Na^+, K^+-ATPase activity. It gives most consistent results compare to immunocytochemical localization. The biochemical localization only shows presence of Na^+, K^+-ATPase activity but not their spatial distribution. For present study, we have used Mayahara et al. 1980 technique. It's a direct one-step method. In this technique we fixed the progesterone-induced and uninduced stage stage VI oocytes in 2% paraformaldehyde and 0.005% glutaraldehyde for 30 mins and then both group of oocytes were processed as method completely described in Mohanty and Gupta [12]. The technique is so versetile that one can count individual phosphate cleaving ATPase molecules. We estimated upto 20 times higher ATPase activity over the animal hemisphere than the vegetal hemisphere.

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