Ion Compartmentalization in Frog Oocytes as Demonstrated by X-Ray Microanalysis

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Abstract. The distributions of K, Na, Mg and Ca within frog ovarian and oviduccal oocytes were studied by electron probe wavelength dispersive X-ray microanalysis. An important heterogeneity could be found both in nuclear and jelly coated oocytes. The highest K, Mg and, to a lesser extent, Na concentrations were found in the pigmented area of the peripheral cytoplasm. There is a certain correlation between the distribution of K and Mg. The concentration of K (but not of Na) in the nucleus was higher than that in the non-pigmented cytoplasm. The distribution of Ca was rather uniform. The high amounts of K, Na and S determined in the oocyte jelly coat seem to have become accumulated by ion-exchange mechanism. Oocyte pigment granules are believed to be the site of ion compartmentalization and to play a role in regulation of intracellular ionic composition.

Key words: K, Na, Mg, Ca distribution — Oocytes — X-ray microanalysis

Introduction

Kinetic analysis and ion-selective microelectrode measurements (Dick and McLaughlin 1969; Century and Horowitz 1974; Palmer et al. 1978) have suggested that in amphibian oocytes intracellular Na^+ and K^+ are heterogeneously distributed. A nonuniform Ca^{2+} and Mg^{2+} distribution was also observed in frog oocytes (Morril et al. 1971; Morril et al. 1980). This heterogeneity is likely to reflect subcellular compartmentalization of ions, i.e. an asymmetrical distribution of ions between the cytoplasm and the subcellular organelles. The identity of intracellular organelles responsible for ion compartmentalization within oocytes still remains obscure.

In this study the distribution of K, Na, Mg and Ca within frog oocytes in different stages of maturation was investigated by electron probe X-ray microanalysis. Special attention was paid to the question whether pigment granules may selectively accumulate some ions. Earlier, high concentrations of K, Na and Ca were found by X-ray microanalysis in melanin granules of the frog retina pigment

epithelium (Burovina et al. 1972a) as well as in melanophores of the frog skin (Govardovskii et al. 1976). High levels of these elements were also demonstrated in the pigmented zones of photoreceptors and pigmented cells of the cricket ommatidium (Gribakin et al. 1976; Burovina et al. 1978).

Materials and Methods

Experiments were carried out on pregnant females of *Rana temporaria* during February and May. The animals were killed and the ovaries were removed and immersed in Ringer's solution. For X-ray microanalysis small ovarian segments or individual eggs from the oviducts were mounted on small copper supporters and rapidly frozen in propane cooled with liquid nitrogen. The sectioning was performed with a freezing microtome at −25 °C. Ten μm thick slices were dried in vacuum at −25 °C for 3—4 h. Dry slices were mounted on silicon substrate plates and coated with carbon. During this procedure, the frozen oocytes or the dried slices had no contact with water, and all the tissue components, including the soluble ones, have thus been preserved (Burovina et al. 1972b). The elements may become redistributed due to tissue damage during freezing and drying. It was shown that such micro-damage does not exceed 0.2—1.0 μm (Burovina et al. 1978), i.e. much less than the oocyte areas under study.

For electron microscope studies, oocytes were fixed in 2.5 % glutaraldehyde in 0.1 mol/l cacodylate buffer (pH 7.2—7.4) for 2 h at 4 °C, dehydrated in ethanol and acetone and embedded in Epon-812. Ultrastructural studies were performed using an electron microscope JEM-100B.

X-ray microanalysis was performed with a scanning electron microscope-microanalyzer JSM-U3 (JEOL, Japan). K_α radiations of K, Na, Ca, Mg and some other elements were detected with wavelength dispersive spectrometers with RAP and PET crystals. The analysis was performed with an accelerating voltage of 25 kV and sample currents of 10—20 nA. Quantitative data were obtained by point analysis with an electron probe diameter of 1 μm, or by scanning in a 10 × 10 μm square raster. Crystals of NaCl, $KAlSi_3O_8$, $3[Ca_3(PO_4)_2].2\,H_2O$ and pure Mg were used as standards. Chemical concentrations were calculated using the computer program BICEP (Biological Intensity Correction for Electron Probe, Warner and Coleman 1973), with modified parameters. The accuracy of BICEP under our condition was verified by an analysis of freeze-dried albumin solution specimens with a given concentration of the element under study. Good results were obtained with bulk specimens while in thin slices the calculated concentrations were much higher than the actual ones. We experimentally measured and computed by BICEP the ratios of thin to bulk specimen X-ray counts (I_h/I_{bulk}) for slices of different thickness. The computed correction coefficients for thickness $P(h) = I_h/I_{bulk}$ were found to be significantly higher than those measured experimentally. To get the coincidence of experimental with computed data, parameters of the range equation given by Colby (1968) were used in our calculations by BICEP, while Warner and Coleman (1973) used parameters given by Andersen (1965). More details on the quantitative analysis were reported elsewhere (Burovina and Pivovarova 1978).

Results

Ion distribution was studied in frog oocytes taken in two different stages of maturation. The February oocytes, 1300—1600 μm in diameter, were obtained from frog ovaries. In this stage, the nucleus of the oocytes has an oval shape and is situated at the animal pole of the cell. The peripheral cytoplasm of the animal pole

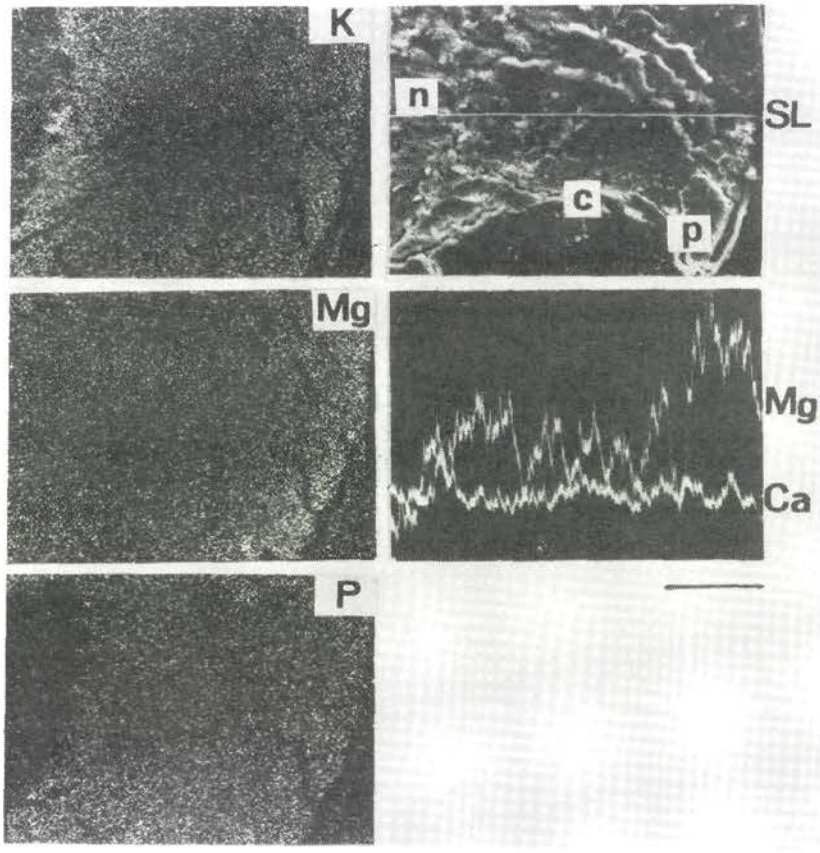

Fig. 1. Typical distributions of K, Mg, P and Ca in ovarian oocyte. Right: secondary electron image (SEI) of the oocyte analyzed area (n — nucleus, c — cytoplasm, p — pigmented layer) and Mg and Ca X-ray profiles obtained during the probe movement along the scan line SL. Left: K, Mg and P X-ray images of the same oocyte area. Calibration: 100 μm.

is heavily pigmented. The highest density of pigment granules is seen near the cell boundary and around the nucleus.

Typical pictures of K, Na, Mg, S and P distributions in this stage oocyte are shown in Fig. 1. A high level of K was found in the nucleus. In the pigmented region of the peripheral cytoplasm, high levels of K and Mg were found. There was no correlation between the distributions of K and P. The concentration of P in the nucleoplasm was found to be lower than that in the cytoplasm. The distribution of Ca (not shown) was uniform with the operating conditions used in this study.

The cytoplasmic concentration of K varied widely (Table 1). The range values

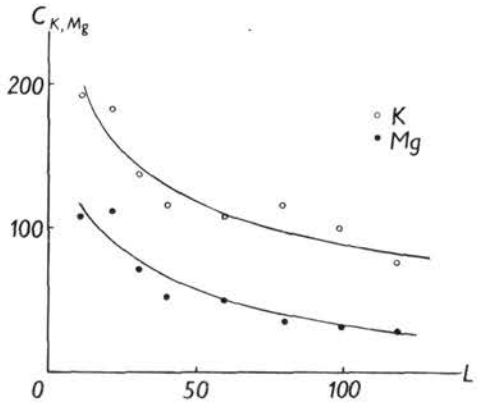

Fig. 2. Distribution of K and Mg in the pigmented area of the ovarian oocyte. Abscissa — the distance from the cell surface (μm); ordinate — concentrations of K (○) and Mg (●) (mmol/kg wet weight).

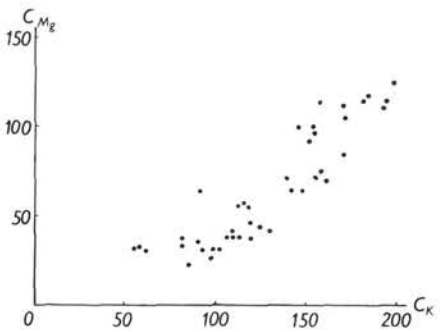

Fig. 3. Concentrations of K and Mg (mmol/kg wet weight) in various parts of the ovarian oocyte pigmented cytoplasm (around the nucleus and near the cell surface).

given in the Table illustrate the great nonuniformity, even inside certain parts of the cell. The highest K concentration was found in the pigmented area of the cytoplasm (100—190 mmol/kg w. w.), with the lowest one (50—120 mmol/kg w. w.) being observed in the light part of the cytoplasm. The pigmented area of the peripheral cytoplasm also contained the highest concentrations of both Mg and Na (Table 1). The concentration of K in the nucleus (100—160 mmol/kg w. w.) was higher than that in the non-pigmented cytoplasm, however no Na gradient could be revealed on the nuclear membrane (35 and 30 mmol/kg w.w. in the nucleus and the non-pigmented cytoplasm, respectively). We failed to measure the concentration of Ca because the intensity of the Ca K_α-line was almost identical with that of the background. The distribution of K and Mg in the pigmented area of the

Table 1. Concentrations of K, Na, Mg and Ca in oocytes and skin melanophores of the frog *R. temporaria*.

Stage	Cell compartment	Concentrations (mmol/kg wet weight)			
		K	Na	Mg	Ca
Ovarian oocytes (February)	Nucleus	100—160	35	30—35	*)
	Cytoplasm (light part)	50—120	30	10—20	*)
	Pigmented area of cytoplasm	100—190	50	80—100	*)
Oviduccal oocytes (April)	Cytoplasm (light part)	30—90	20	10—20	*)
	Pigmented area of cytoplasm	100—200	30	70—120	*)
	Jelly coat	130—145	45	20—40	*)
Skin melanophores		140—250	170—340	60—240	60—200

*) — concentration of Ca is less than 5 mmol/kg wet weight, the sensitivity limit of the method. Range values of concentrations in 10 oocyte sections from 3 animals are given for each developmental stage. Five skin melanophore cells in 2 sections from one animal have been measured and range values of concentration are given.

cytoplasm was studied by step point analysis. An electron probe of 2 μm in diameter was placed at certain distances from the cell boundary and the X-ray line intensities of K and Mg were simultaneously recorded by two spectrometers (Fig. 2). The concentrations of both elements increased towards the layer with the highest density of pigment granules. A correlation between K and Mg distributions could be found. The same correlation was observed in measuring the concentrations of these two elements at differents points of the pigmented cytoplasm (Fig. 3).

The April oocytes taken from the frog oviducts are covered by a jelly coat. The oocyte diameter is approximately 2000 μm, the nucleus is disintegrated, the animal pole is intensively pigmented. Spherical pigment granules occur predominantly in the layer below the cell surface. The granules are surrounded by a membrane and consist of an electron-dense unhomogeneous matrix (Fig. 4).

The typical distributions of K, Mg, Ca, P and S along the chosen scan line in the animal hemisphere of the egg are presented in Fig. 5. Also, ion distribution in oocytes in this stage of maturation was very unhomogeneous. The highest levels of K and Mg were observed in the layer showing the highest density of pigment granules. The concentration of K in the pigmented region of the cytoplasm varied from 100 to 200 mmol/kg w.w. and the concentration of Mg from 70 to 120 mmol/kg w.w. (Table 1). The lowest concentration of K, Mg and Na were

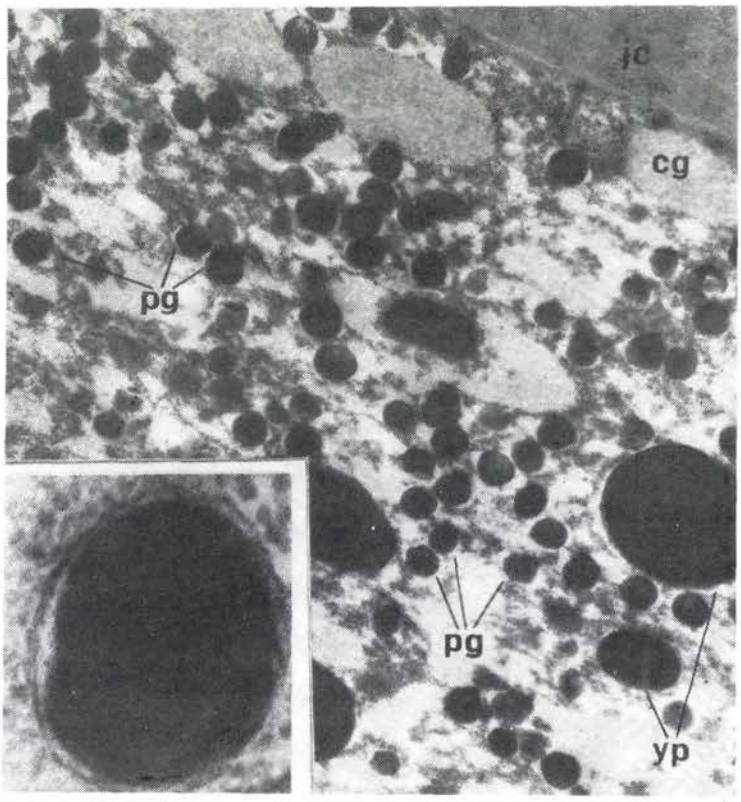

Fig. 4. Transmission electron micrograph of the peripheral cytoplasm of the oviduccal egg. Small round highly electron opaque pigment granules (pg), cortical granules (cg), yolk platelets (yp), jelly coat (jc). x 8000. Inset: higher magnification of a single pigment granule coated by a membrane. x 77,000.

found in the light part of the cytoplasm, although essential variation in the concentration of K could be observed (from 30 to 90 mmol/kg w.w.). Surprisingly high concentrations of K, Na and S were found in the oocyte jelly envelope (Fig. 5, Table 1). The measured distribution of Ca was rather uniform, and Ca concentration in oocytes was too low to be measured quantitatively by X-ray microanalysis. As distinguished from the oocyte pigment granules, the skin melanophores contained high concentrations of all elements under study, including Ca (Table 1).

Discussion

The highest concentrations of K, Mg and, to a lesser extent, Na were found in the pigmented area of the peripheral cytoplasm of both ovarian and oviduccal eggs.

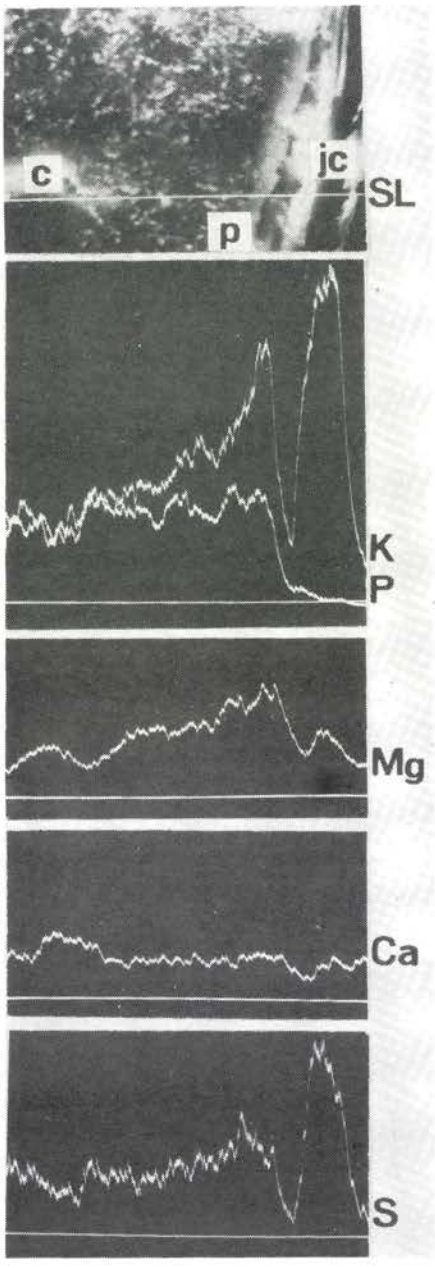

Fig. 5. Typical distributions of K, P, Mg, Ca, S in the oviduccal egg with a jelly coat. Secondary electron image of the egg analyzed area (c- cytoplasm, p- pigmented layer, jc- jelly coat) and X-ray profiles of K, P, Mg, Ca, S obtained during the probe movement along the scan line SL. Calibration: 25 μm.

Maximal levels of K and Mg seemed to correspond to the maximal density of pigment granules, i.e. high amounts of these elements are accumulated in melanin pigment granules. As mentioned above, melanin granules of the frog retina pigment epithelium (Burovina et al. 1972a) as well as melanophores of the frog skin (Govardovskii et al. 1976) were found to contain high concentrations of K, Na and Ca. Also, the frog skin melanophores have high concentrations of Mg (Table 1). High concentrations of Ca were reported for melanin-containing tissues of frog, cat and rabbit (Panessa et al. 1977), whereas the melanin granules of the frog eggs contain much lower Ca concentrations and their Na concentration does not differ significantly from that of the cytoplasm (Table 1, Fig. 5).

Pigment granules of amphibian oocytes are known to differ from those of the retina and skin by both their ultrastructure (Noda et al. 1977) and enzyme activity (Harsa-King 1980). The peculiarity of the oocyte pigment granules in respect to ion accumulation may be due to their structural and biochemical specifity, though the nature of ion deposition still remains obscure. Melanin granules have the capacity to act as a cation-exchanger material (White 1958; Bruenger et al. 1967; Sarna et al. 1976), but the in vitro melanin-affinity of the alkali and alkaline earth metals appears to be rather low (Potts and Au 1976; Larrson and Tjalve 1978). The accumulation coefficients estimated from Table 1 and Fig. 5 seem to be much higher.

Selective accumulation of ions by pigment granules is probably due to both the cation-exchange activity of melanin and properties of the membrane of pigment granules. Different accumulation of Ca and Na by melanin granules contained in different tissues of the same animal species allows us to suggest the participation of pigment granules in the regulation of intracellular ionic composition. High levels of K, Na, Ca and Mg as mentioned above were found in the pigments of visual and pigment cells of the cricket eye (Gribakin et al. 1976; Burovina et al. 1978). Ca is supposed to be a structural unit of the pigment granules in the insect visual cell ommochromes because the levels of bound Ca in these structures are different for insects of different species (White and Michaud 1980). In this case, the replacement of Ca by another element, e.g. Mg, can change both the structure of the pigment and its capacity to accumulate or release ions at some functional states.

The high amount of K in egg jelly coats, which is correlated with a high level of S (Fig. 5), may be accounted for by the accumulation of K by the ion-exchange mechanism with the participation of sulphur hydroxyl groups (rather than carboxyl ones as is the case of melanin granules). Being a barrier between the developing cell and external media, the egg jelly coat seems to play an important role in the regulation of intracellular ionic composition. Selective accumulation of ions by the egg jelly coat supports the idea on the participation of mucopolysaccharide layers in the regulation of intracellular ionic composition (Bennett 1963) and provides a perfect model to study the mechanism of such a regulation.

The considerable heterogeneity of K and Na distributions in frog eggs does not allow us to compare the concentrations of these elements in certain cell parts with mean intracellular concentrations reported in literature. The intracellular concentration of Na was reported to increase from 20 to 120 mmol/l and that of K to decrease from 120 to 15 mmol/l during the growth of the toad *Bufo bufo* oocytes, while in *Rana temporaria* oocytes, these concentrations vary from 9 to 190 mmol/l for Na and from 126 to 28 mmol/l for K (Cannon et al. 1974). Lower levels of intracellular Na (69 and 74 mmol/l in frog ovary oocytes and ovulated eggs, respectively) were reported by Morrill, while in the same stages of maturation the concentrations of K were found to be 70 and 108 mmol/l, respectively (Morrill 1965). The reason for variations of Na and K during the egg growth requires further investigation, taking into account ion compartmentalization. It is remarkable that Na and K activity coefficients in toad immature oocytes are 0.36 and 0.73, respectively (Dick and McLaughlin 1969), while in mature frog oocytes, these coefficients are 0.08 for Na and 1.15—1.29 for K (Palmer et al. 1978). This great difference in the activity coefficients may be accounted for by both species differences and the different extent of ion compartmentalization at a certain developmental stage. We could not find any significant Na or K gradient on the oocyte nuclear membrane, although the concentration of K in the nucleus is a little higher than that in the light part of the cytoplasm (Table 1). The nucleus is unlikely to play a dominating role in ion sequestration because of its small volume as compared to that of cytoplasm. Earlier data of high Na concentration in the nucleus of frog oocytes (Naora et al. 1962) could not be confirmed, only a high concentration of K was found (Dick 1978a, b; Dick and Ibrahim 1979). According to our data, pigment granules are believed to be more likely the site of ion compartmentalization.

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