

## Are Yolk Phosvitins Carriers for Specific Cations? Comparative Microanalysis in Vertebrate Yolk Platelets

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Qualitative X-ray microanalysis of frozen-dried cyclostome, teleost and amphibian yolk platelets has demonstrated the general and undoubtable presence of P, S, Cl (!) and K, the probable presence of Na and the irregular presence of Ca and Mg. Iron occurred only in trace amounts if at all.

Details of the process of yolk utilization by the embryo are still enigmatic. Indirect evidence obtained from particular features of the glycolipophosphoprotein molecules building up the yolk platelets as main constituents has, therefore, become a matter of recent interest. Thus, the highly phosphorylated phosvitins ("cation exchange-resin") may play a role as carrier of cations essential for embryonic growth (Taborsky [1]); studies on cation-binding as well as on the entailing conformational changes of the phosvitin molecule are numerous (Taborsky [1, 2]). Recent observations on yolk platelets in this laboratory, however, do not fit into the frame of a very specific role of yolk-phosphoproteins in the transport of selected cations. Although the investigation has been performed only on the usual qualitative basis, the broad collection of material and the regularity of findings render the observations significant.

Cryosections of whole eggs (*Lampetra planeri* [Bloch], Cyclostomata; *Pelvicachromis pulcher*, Cichlidae, Teleostei; *Triturus* sp., Salamandridae, Amphibia) or isolated yolk platelets (*Myxine glutinosa* L., Cyclostomata) were dried at subzero temperatures onto pure carbon specimen supports for scanning electron microscopy. Lamprey and teleost material fixed in Na-phosphate buffered glutaraldehyde was used for comparison. Elemental analysis was carried out at 25 kV in a Philips PSEM 500 scanning elec-

tron microscope equipped with energy-dispersive Edax system (3–4 spectra – 100 000 to 400 000 total counts each – per species, point probe diameter 1  $\mu\text{m}$ ).

Ooplasmic inclusions corresponding both in size and frequency to yolk platelets of the various species were identified in the scanning mode and a point probe was then positioned on their interior. Elements markedly and undoubtedly present in all unfixed samples and absent in the carbon support between single platelets were: P, S, Cl, and K. Ca was also present in *Lampetra*, *Pelvicachromis* and *Triturus* (as judged from its clear K  $\beta$  peak at 4.01 keV, while Ca-K  $\alpha$  at 3.69 keV fuses with K-K  $\beta$  at 3.59 keV). Due to the peculiar background spectrum (Lange and Blödorn [3]) we shall not enter into detailed discussion of the probable presence of some Na and Mg. Fe was never unequivocally demonstrated, but traces might have occurred in the cyclostomes.

Whereas P and S probably represent covalently bound protein constituents, the presence of chloride (only irregularly present following fixation, absolutely absent from neutral underground) in this highly anionic protein ambience is remarkable. Results with respect to iron were disappointing, especially considering recent biochemical work (Taborsky [2]).

It is concluded that K<sup>+</sup> is regularly and undoubtedly present in considerable amounts in yolk platelets; this holds true for Ca<sup>2+</sup>, but to a lesser degree (not found in *Myxine*). The occurrence of Na<sup>+</sup> (and in *Lampetra* some Mg<sup>2+</sup>) is probable but evidence is at present unsatisfactory for technical reasons. The presence of traces of Fe cannot be excluded. Due to the presence of chloride, the above cations can only in part be bound by the phosphate groups of the highly anionic yolk-platelet proteins.

By combining the recent results in yolk-platelet crystal research (Lange [4]; Ohlendorf *et al.* [5]) with morphometry of the ooplasm and attempts at microanalytic quantitation, important quantitative data on both a relative and absolute scale will eventually become available.

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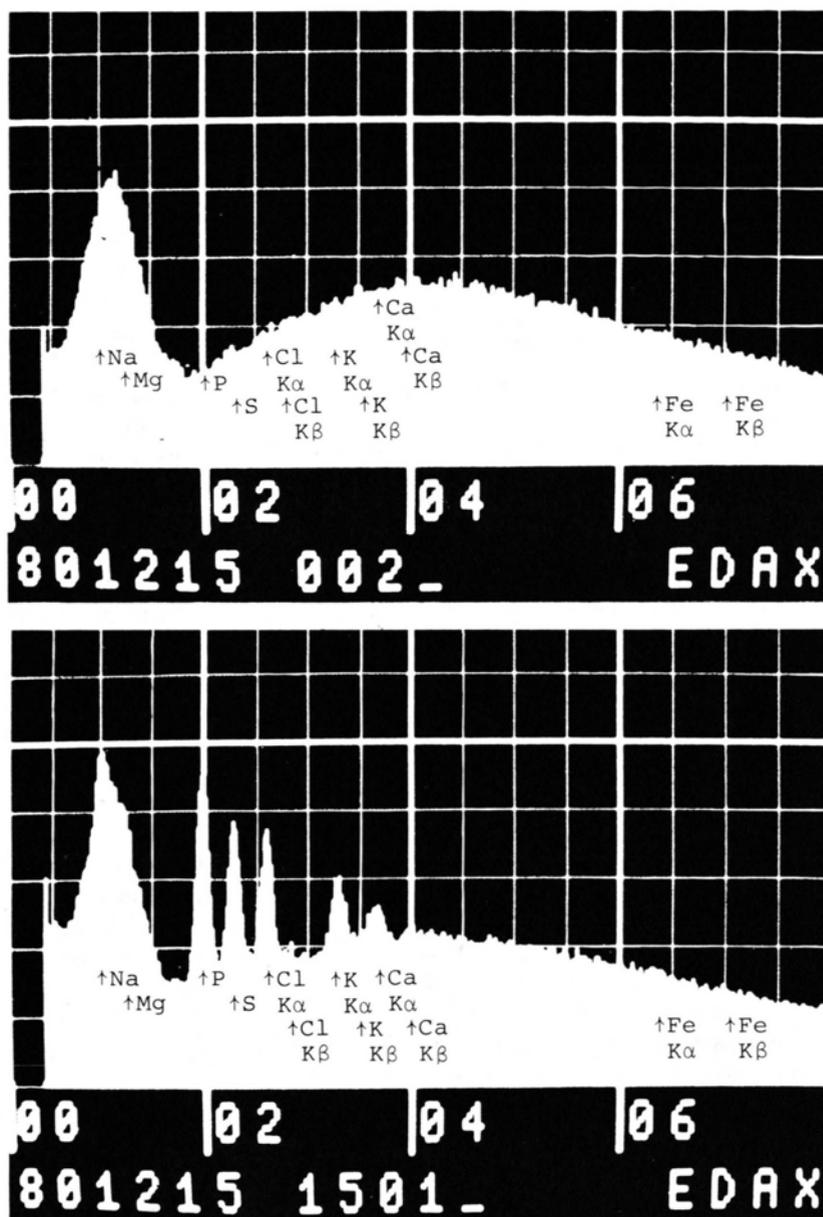
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Fig. 1. Microanalysis of a lamprey yolk platelet. Upper half: Carbon background between yolk platelets (point probe). Lower half: Representative yolk-platelet spectrum (point probe).  $K\alpha$  and  $K\beta$  lines are indicated separately only if their energies differ by more than 150 eV. Both spectra have a basis of approximately 400000 counts and are displayed on the same scale (vertical grid spacing 5000 counts, horizontal grid spacing 0.5 keV).



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