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RECEPTORS MEDIATED ENDOCYTOSIS OF VTG IN FISH FOLLICLE.

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Introduction

Oocyte vitellogenesis is one of the best examples of cell specialization for specific endocytosis of proteins. Indeed, vitellogenin, a hepatically synthesized lipophosphoglycoprotein which constitutes the main plasma yolk precursor reaches ovaries by the blood stream to be selectively taken up by the oocytes.

Vitellogenin enters the oocyte follicle by capillary vessels located in the theca, the outer layer surrounding the oocyte. It reaches the germinal cell passing through meso-epithelial cells of the theca and basement lamina, through intercellular spaces of granulosa cells, then into the extra-cellular matrix located between granulosa and the oocyte plasmic membrane, and finally along the oocyte microvillosities in the channels of the zona radiata until oolemma (Abraham et al.,1984 ; Selman & Wallace, 1989). Internalization occurs on specialized areas of the oolemma leading to formation of coated pits pinching off the oolemma and entering the peripheral ooplasm. They give rise to coated vesicles fusing into irregular shaped yolk granules also called multivesicular bodies (Busson-Mabillot, 1984) where proteolytic cleavage of vitellogenin into yolk protein subunits, phosvitin and lipovitellin, occurs. The granules then fuse into yolk globules intermingled with lipid globules to form the yolk. The specificity of vitellogenin internalization has been investigated in Gobius niger vitellogenic follicles, by ultra-structural autoradiography using ^{3}H-vitellogenin showing specific accumulation of vitellogenin in newly formed yolk globules and by photonic immunocytochemistry.

Vitellogenin internalization in amphibian (Opresko & Wiley, 1987), bird (Stifani et al., 1990) and insect (Roehrkasten et al., 1989) ovary has been shown to be a receptor-mediated mechanism, probably acting at the oolemma level just prior endocytosis.

The goal of the present study is to characterize the vitellogenin receptor system in fish.

Material and methods

Fish used were carp Cyprinus carpio, coho salmon Oncorhynchus kisutch, goldfish Carassius auratus, rainbow trout Oncorhynchus mykiss, sole Solea vulgaris and Siberian sturgeon Acipenser baeri.

Our experiments have been performed with homogenized follicles devoid of yolk, frozen in liquid nitrogen and stored for up to several months at - 20°C.

Purification of vitellogenin from plasma of estradiol pre-treated fish was obtained with one-step DEAE cellulose chromatography using Tris buffer at pH 7.8 in presence of calcium chloride and PMSF. Elution was performed with a linear gradient of sodium chloride (0-150 mM). Fractions of the main peak were identified as vitellogenin on polyacrylamide electrophoresis, pooled and concentrated on Amicon cell until the desired protein concentration.

Iodination of vitellogenin with ^{125}I was performed by the iodogen method, with specific activities not exceeding 100,000 cpm/pM.

Fish membrane receptors were solubilized with octyl-b-glucoside (Stifani et al., 1990) and the octyl-glucoside extracts subjected to one-dimentional electrophoresis on SDS polyacrylamide gels followed by transfer to nitrocellulose.

Identification of the VTG receptor was performed by the ligand blotting technique. Western blots were carried out by incubation in Tris buffer containing 5% of non-fat dry milk as blocking agent of aspecific sites.

Characterization of binding was performed using filter assay (Stifani et al., 1990).

Results

a - Visualization of vitellogenin receptor.

Autoradiograms of ligand blotting obtained with ^{125}I-goldfish VTG and carp oocyte membrane extracts, under non-reducing conditions, identified as a protein with an apparent molecular weight of 90 kDa as VTG receptor. The binding is completely abolished in presence of a fifty-fold excess of cold goldfish vitellogenin. Ligand blotting of the vitellogenin receptor from coho salmon and from chicken as comparative control in presence of ^{125}I-trout VTG gave for salmon VTG receptor an

apparent molecular weight of 100 kDa. Fifty fold excess of cold trout VTG completely extinguished the signal, demonstrating the specificity of the binding.

b - Characterization of vitellogenin receptor.

Characterization of the VTG receptor was performed by binding of ^{125}I-trout vitellogenin to crude salmon oocyte membranes using a solid-phase filtration assay.
Adjunction of suramine which blocks receptors gives rise to a linear non specific binding. Specific binding is determined by substraction of non specific binding from total binding and is saturable (Fig.1a). Transformation of data to Scatchard plot indicates the existence of a single class of binding site for vitellogenin (Fig.1b).

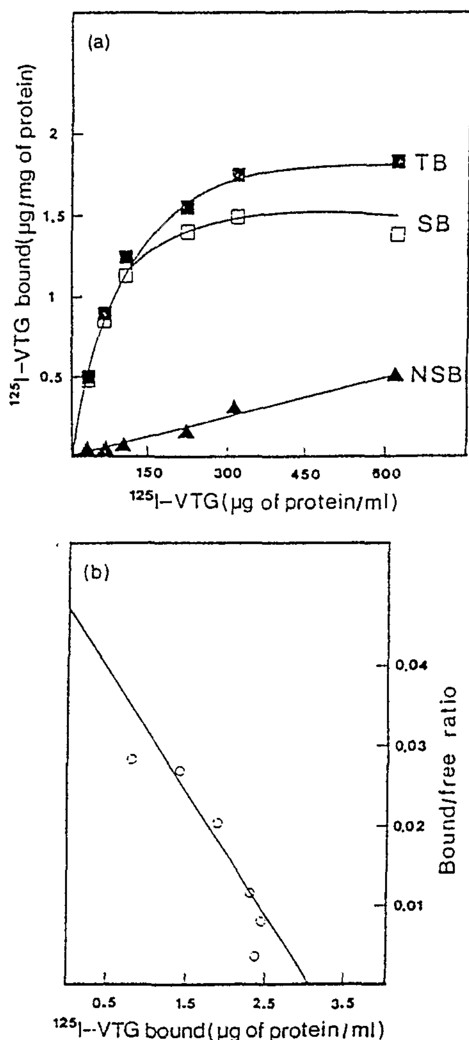

Fig. 1. a- Saturation curves. b - Scatchard transformation.

Competition binding studies were performed with increasing concentration of cold ligand. The binding is significantly reduced by adjunction of increasing amounts of unlabelled trout VTG or unlabelled chicken VTG demonstrating the specificity of the binding. In other hand competition with high concentration of HDL is ineffective (Fig.2).

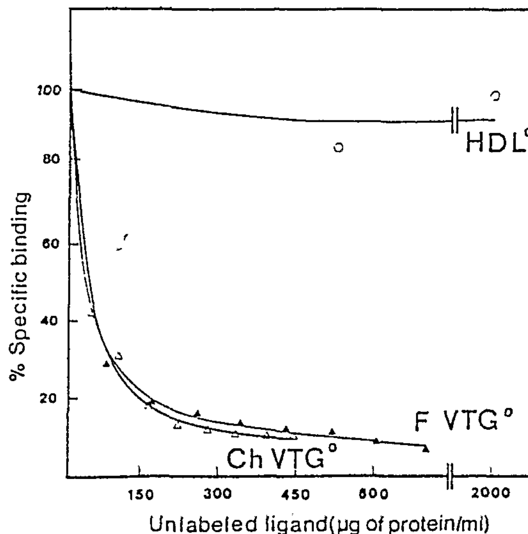

Fig. 2. Competition Binding.

c - Kinetics of vitellogenin receptor during vitellogenesis in trout.

In order to follow the kinetics of vitellogenin receptors in rainbow trout follicle we have tested different batches of oocyte membrane preparations during vitellogenesis of a winter strain. Seven samples were taken during type II vitellogenesis (exogenous vitellogenesis) from July to November just before spawning. Each time twenty five follicles were removed from the ovaries tested, carefully dissected out and measured. An average of oocyte diameters was taken to define the vitellogenesis stage : from 1.11 mm in July corresponding to gonadosomatic index of 0.57 to 3.97 mm just prior to ovulation corresponding to a gonadosomatic index of 9.84. The average ovule diameter is of 4.45 mm.

Binding of ^{125}I-trout vitellogenin to crude oocyte membrane preparations in presence (non specific binding) or in absence (total binding) of suramine (5 mM) allows the determination, using filter assay, of the affinity of the ligand for membrane preparations

If total binding is expressed as femtomoles of ligand per square millimeter surface unit versus

oocyte diameter, the number of receptors increases slightly during vitellogenesis but decreases dramatically at maturation. (Fig.3).

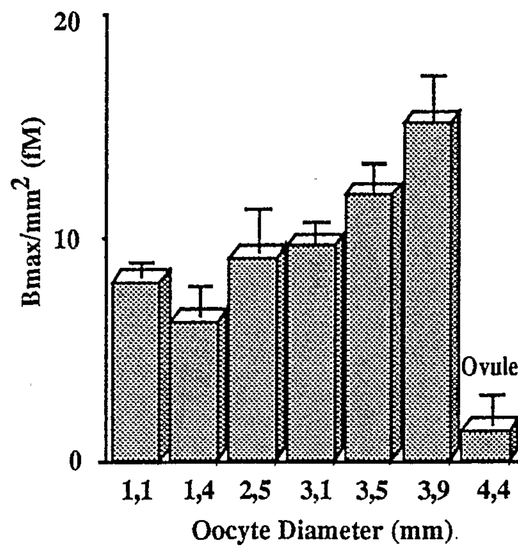

Fig.. 3. Maximum Binding per mm^2

The slope of linear regression of binding data per surface unit versus diameter indicates a two fold increase of the number of receptors between the beginning of vitellogenesis and the end, prior to maturation.

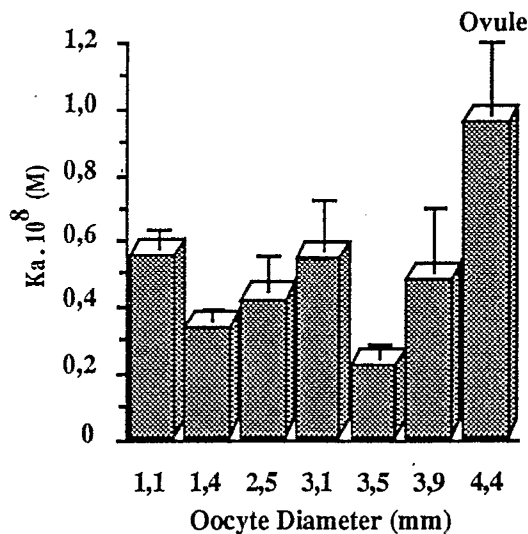

Fig. 4. Variations of Ka with oocyte diameter.

Ka expressed in fM of vitellogenin/mm^2 is almost constant (Fig.4).

Maximum binding expressed in fM of ligand per follicule is regularly increasing until maturation. Ovules exhibit a decrease of VTG binding (Fig.5).

Conclusion

Our experiments demonstrate the existence of vitellogenin receptors in fish oocytes characterized by low affinity and high capacity. These characteristics correspond to the definition of type II receptors.

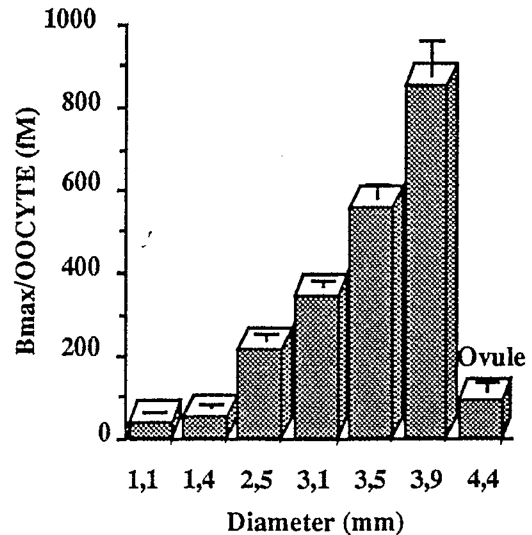

Fig. 5. Maximum Binding per follicle.

References

Abraham M., Hilge V., Lison S. & H. Tibika, 1984. The cellular envelope of oocytes in teleosts. Cell Tiss. Res. 235 : 403-410.

Busson-Mabillot S.,1984. Endosomes transfer yolk proteins to lysosomes in the vitellogenic oocyte of the trout. Biol. Cell, 51: 53-66.

Opresko L.K. & H.S. Wiley, 1987. Receptor-mediated endocytosis in *Xenopus* oocytes. J. Biol. Chem. 262 : 4109-4115.

Roehrkasten A., Ferenz H-J., Buschmann-Gebhardt B. & J. Hafer, 1989. Isolation of the vitellogenin-binding protein from locust ovaries. Arch. Ins. Biochem. Physiol. 10,: 141-149.

Selman K. & R.A. Wallace, 1989. Cellular aspects of oocyte growth in teleosts. Zool. Sc. 6 : 211-231.

Stifani S.,Le Menn F., Nuñez Rodriguez J. & W. Schneider, 1990. Regulation of oogenesis : the piscine receptor for vitellogenin. Biochim. Biophys. Acta 1045 : 271-279.

Tyler C.R., Sumpter J.P. & R.M. Handford, 1990. The dynamics of vitellogenin sequestration into vitellogenic ovarian follicles of the rainbow trout, *Salmo gairdneri*. Fish Physiol. Biochem. 8 : 211-219.