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Receptor-mediated Transport of Vitellogenin during Oogenesis of a Polychaete, *Pseudopotamilla occelata*

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Receptor-mediated endocytosis has been suggested for a stage-specific transport mechanism of vitellogenin into the oocytes of a sabellid polychaete, *Pseudopotamilla occelata.* Membrane proteins of oocytes of three size classes, including small (30-70 μm in diameter), intermediate (70-140 μm in diameter) and large (180-200 μm in diameter), showed a stage-specific variation. Coelomic fluid proteins (CP), assumed to be vitellogenin, consists of several proteins, which showed quite a different pattern from that of yolk proteins. Incorporation of ^{125}I-CP into the oocytes of the intermediate size class almost linearly increases with time, showing a contrast to the pattern of the large size class, in which the incorporation is low and approaches a plateau, suggesting the vitellogenin transports by a regulated process only in the intermediate size class. Vitellogenin receptor proteins were identified to be 60 kDa and 68 kDa only in the intermediate size class by a ligand blotting test.

Vitellogenin, a yolk precursor protein, is taken up into oocytes by receptor-mediated endocytosis in chicken and coho salmon (Stifani et al., 1988; 1990), tilapia (Chan et al., 1991), rainbow trout (LeMenn and Nunez-Rodriguez, 1991; Tyler and Lancaster, 1993), perch (Tao et al., 1996), locust (Roehrkasten and Ferenz, 1986; Roehrkasten et al., 1989), and *Xenopus* (Opresko and Wiley, 1987). In the polychaete worm, transport of vitellogenin into oocytes shows a saturation kinetics (Fischer et al., 1991) and the receptor protein for vitellogenin was visualized by ligand blotting studies, indicating that the transport is performed by a regulated process (Hafer et al., 1992).

In sabellid polychaetes, coelomic fluid proteins, which are assumed to be vitellogenins, are taken up stage-specifically into the oocytes of the intermediate size class of 70-140 μm in diameter (Jang 1988; Lee and Kim 1993). The membrane proteins of oocytes, some of which are assumed to be receptor proteins, are mainly glycoproteins, which turn over very rapidly, indicating that receptor proteins of oocytes change during oogenesis, so that oocytes may carry receptor proteins specific to the stages of the intermediate size class (Lee, 1988).

In this paper we demonstrated that the transport of vitellogenin is mediated by receptor proteins, which are present specifically at the membrane of oocytes of the intermediate size class in a polychaete, *Pseudopotamilla occelata.*

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Materials and Methods

Collection and size separation of oocytes

Pseudopotamilla occelata Moore, a sabellid polychaete, which is abundant in the east coast of Korea, was used for the studies. Oocytes were directly collected from the coelomic sacs by dissecting the female worms along the body length at the lateral side with a sharp razor blade. The oocytes were then separated into three size classes by straining them through Nitex mesh of various pore sizes. The small size class ranges from 30 μm through 70 μm, the intermediate size class from 70 μm through 140 μm, and the large size class from 140 μm through 200 μm in diameter. The details of separation of oocytes into size classes are described by Nam et al. (1996).

Extraction and electrophresis of membrane proteins

Membrane proteins were extracted directly from the membranes isolated from the oocytes of each size class. Isolation of membranes, extraction of membrane proteins and gel elctrophoresis are described elsewhere (Lee, 1988).

Iodination of coelomic fluid and yolk proteins

Coelomic fluid proteins were extracted from concentrated coelomic fluid collected when the female worms were dissected. Yolk proteins were extracted directly from yolk granules of the large size class. Extraction and iodination of coelomic fluid and yolk proteins were carried out as described by Lee and Kim (1993).

Binding test and autoradiography

Membrane proteins, which were extracted and electrophoresed without addition of β-mercaptoethanol and dithiothreitol or without heat treatment, were transferred to nitrocellulose membrane from the gel and blocked with 5% non-fat milk at 4℃ for 24 h. Nitrocellulose membrane containing membrane proteins was incubated with ^{125}I-labeled coelomic fluid proteins in a buffer (25 mM Tris-HCl, pH 8.0, 50 mM NaCl, 2 mM $CaCl_2$) for 2 h at room temperature. At the end of incubation, free ^{125}I or non-specifically bound ^{125}I were washed out of the membrane three times with washing buffer (10 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.1% Tween 20). The nitrocellulose membrane was autoradiographed with Kodak X-ray film at -70℃

Results

Characterization of membrane proteins of oocytes

Membrane proteins varied stage-specifically during oogenesis; in the oocytes of intermediate size class, 21 different proteins of 31 kDa, 33 kDa, 35 kDa, 39 kDa, 41 kDa, 45 kDa, 47 kDa, 52 kDa, 54 kDa, 66 kDa, 73 kDa, 82 kDa, 88 kDa, 90 kDa, 97 kDa, 106 kDa, 116 kDa, 138 kDa, 160 kDa, 175 kDa and 190 kDa were detected, whereas only 8 proteins of 45 kDa, 55 kDa, 62 kDa, 63 kDa, 65 kDa, 66 kDa, 90 kDa and 190 kDa and 14 proteins of 31 kDa, 35 kDa, 45 kDa, 52 kDa, 60 kDa, 66 kDa, 69 kDa, 76 kDa, 90 kDa, 97 kDa, 110 kDa, 116 kDa, 160 kDa and 190 kDa were detected in the small and large size classes, respectively (Fig. 1). Some of these proteins were identical throughout the stages, but 11 proteins of 33 kDa, 39 kDA, 41 kDa, 47 kDa, 54 kDa, 73 kDa, 82 kDa, 88 kDa, 106 kDa, 138 kDa and 175 kDa were specific to the intemediate size class, whereas a protein of 55 kDa and 3 proteins of 69 kDa, 76 kDa and 110 kDa were specific to the small and the large size classes, respectively. Membrane proteins varied greatly in kind and in quantity during oogenesis, strongly suggesting that the oocyte membranes are functionally differentiated throughout oogenesis.

Characterization of coelomic fluid and yolk proteins

Coelomic fluid proteins (CP) and yolk proteins (YP) were analyzed and compared to each other. Eight proteins of 55 kDa, 61 kDa, 73 kDa, 89 kDa, 100 kDa, 115 kDa, 140 kDa and 170 kDa were identified from coelomic fluid (Fig. 2A). These proteins are, however, quite different from the yolk proteins, which were identified to be 34 kDa, 37 kDa, 39 kDa, 44 kDa, 59 kDa, 65 kDa, 66 kDa, 76 kDa, 104 kDa, 108 kDa and 200 kDa, although the proteins used for the analysis were only the portion of the yolk proteins which were dissolved in bicarbonate buffer and most of yolk proteins were not dissolved in the buffer probably because of the low solubility of the large molecular size. The difference between CP and YP was also confirmed by autoradiograms of ^{125}I-CP and ^{125}I-YP (Fig. 2B). The results strongly suggest that CP may be processed and positioned into yolk granules in the forms of proteins different from the original CP after transport into oocytes.

Stage-specific transport of coelomic fluid proteins

Transport of ^{125}I-CP into the oocytes of the intermediate and the large size classes were quantitatively assayed with various time intervals. For this, 600 oocytes of relatively homogeneous size were incubated with

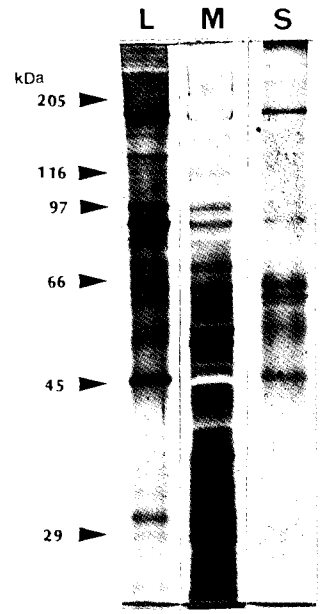

Fig. 1. Membrane proteins of oocytes of large (L), intermediate (M) and small size classes (S) analyzed by polyacrylamide gel electrophoresis. Proteins were stained with silver nitrate.

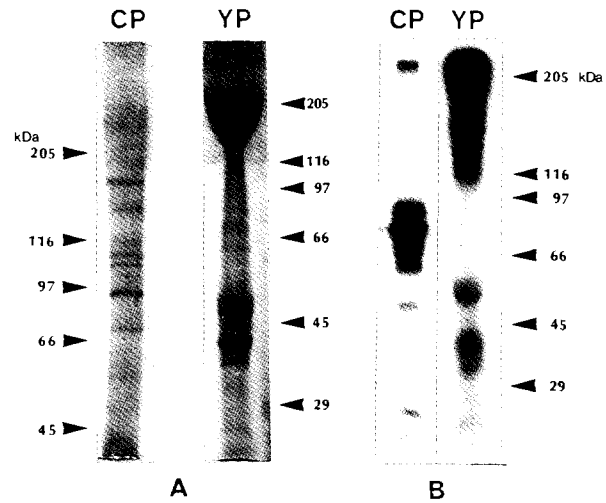

Fig. 2. Coelomic fluid proteins (CP) and yolk proteins (YP) analyzed by polyacrylamide gel electrophoresis (A) and autoradiography (B). For autoradiography, ^{125}I-CP was used.

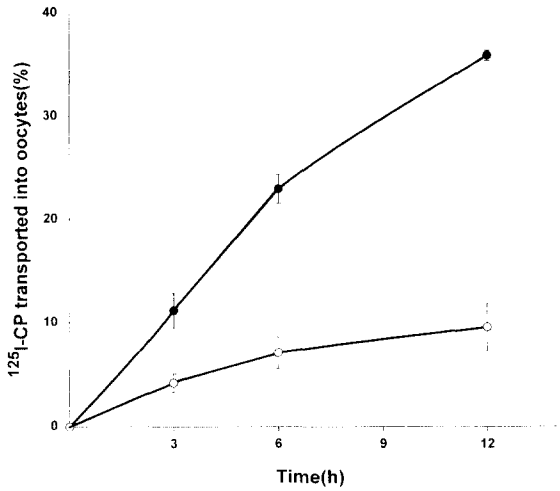

Fig. 3. Transport of ^{125}I-CP into the oocytes of intermediate (●) and large size classes (○). Six hundred oocytes of relatively homogeneous size were incubated with ^{125}I-CP of 1.2×10^5 CPM in a total volume of 600 μl and 100 μl of the suspension was taken at each time and radioassayed. The amount of ^{125}I-CP transported was plotted as percent of the total input.

^{125}I-CP of 12×10^5 CPM in a total volume of 600 μl and 100 μl of the suspension containing about 100 oocytes was taken at each time and radioassayed (Fig. 3). According to the results, the transport of ^{125}I-CP into the intermediate size class increased rapidly and almost linearly up to 12 h of incubation, whereas the transport into the large size class increased very slowly, approaching a plateau at 12 h. The intermediate size class took up more than 35% of the total input of ^{125}I-CP by 12 h, whereas the large size class took up only 7.3% by the same incubation period.

A competition test in which the transport of ^{125}I-CP was competed with unlabeled CP showed that the amount of ^{125}I-CP transported into the oocytes decreased with increasing amounts of unlabeled CP (Fig. 4).

A binding test, in which membrane proteins of the oocytes of three size classes were manipulated to bind to ^{125}I-CP, showed that this binding was achieved specifically with the membrane proteins of the oocytes of the intermediate size class (Fig. 5). The molecular weights of the proteins were 60 kDa and 68 kDa.

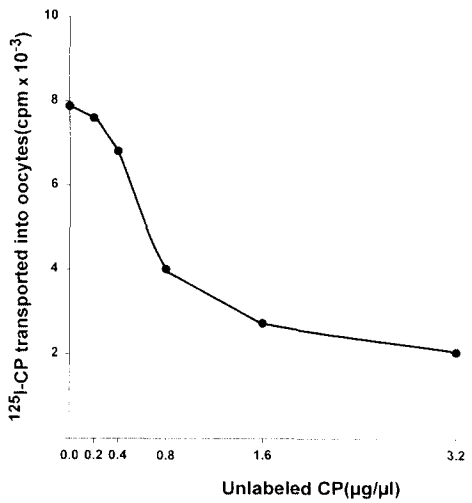

Fig. 4. Competitive transport of ^{125}I-CP into the oocytes of intermediate size class with unlabeled CP. ^{125}I-CP of 1.3×10^5 CPM was incubated with different amount of unlabeled CP for 12 h.

Discussion

Receptor-mediated endocytosis has been demonstrated to be a general mechanism of transporting vitellogenin into oocytes in various species, but the molecular sizes of the receptor proteins vary from 96 kDa in chicken (Stifani et al., 1988), 100 kDa in the coho salmon (Stifani et al., 1990), 150 kDa in the locust (Roehrkasten et al., 1989), and to 190-220 kDa in the polychaete (Hafer et al., 1992) and in the rainbow trout (Tyler and Lancaster, 1993; Tyler and Lubberink, 1996). Molecular characterization of the vitellogenin receptor genes of *Drosophila melanogaster* and mosquito revealed that the proteins encoded by the gene was predicted to be about 210 kDa (Schonbaum et al., 1995; Sappington et al., 1996).

Presence of receptor protein for vitellogenin in the oocytes of a sabellid worm, *Pseudopotamilla occelata*, was strongly suggested from the previous observations that vitellogenin labeled with fluorescein isothiocyanate was transported specifically into the oocytes of the intermediate size class ranging from 70 μm through 140 μm in diameter and furthermore, the transport was blocked by saturating the carbohydrate residues of the glycoproteins with specific lectin or by digesting the

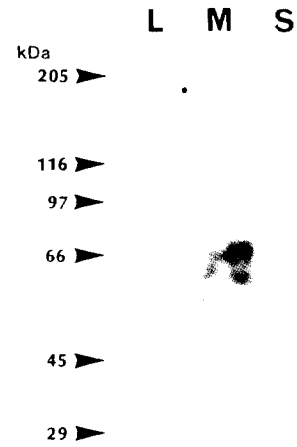

Fig. 5. Ligand blotting of oocyte membrane protein of various size classes. Total amount of 150 μg membrane proteins of large (L), intermediate (M) and small (S) size classes was subjected to electrotophoresis on 8% SDS polyacrylamide gel under non-reducing condition. The nitrocellulose membrane, to which membrane proteins were transferred from the gel, was incubated in 10 ml of buffer containing 50 μg of ^{125}I-CP (2.5×10^4 CPM) and autoradiographed for 18 h.

carbohydrate residues with endoglycosidase (Lee and Kim, 1993). Stage-specific CP transport was again confirmed by the assay of incorporation of ^{125}I-CP into the oocytes of the intermediate size class compared to that of the large size class. A linear increase in the transport of ^{125}I-CP into the intermediate size class is interpreted to be an indication that the transport is a regulated process, whereas a slow increase in the transport of ^{125}I-CP into the large size class approaching a plateau appears to be a consequence of non-specific binding.

The results of the ligand blotting studies indicate that the transport is mediated by receptor proteins, which are present only in the oocytes of the intermediate size class, a stage equivalent to the vitellogenesis, and are absent in the oocytes of the early previtellogenic and of the late postvitellogenic stages.

Receptor proteins in the oocytes of *Pseudopotamilla occelata* were identified to be 60 kDa and 68 kDa, which are rather small compared to those of other species and particularly to the molecular size of receptor protein of *Nereis virens* (Hafer et al., 1992). These receptor proteins were not detected in the electrophoretic pattern of the membrane proteins of the intermediate size class probably due to the low concentration of the proteins. The significance of size variation of receptor proteins among species has not been elucidated in spite of strong homology of receptor genes among different species (Schonbaum et al., 1995; Sappington et al., 1996).

Acknowledgements

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