XENOPUS PUMILIO IS A SEQUENCE-SPECIFIC CYCLIN B1 MRNA-BINDING PROTEIN AND INTERACTS WITH CPEB

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Translational activation of cyclin B1 mRNA that preexists as a masked form in immature oocytes plays a key role in initiating or promoting oocyte maturation in many vertebrates. *Xenopus* oocytes contain a homolog of Pumilio which binds specifically to cyclin B1 mRNA, raising the possibility that this protein is involved in translational control of cyclin B1 mRNA during oocyte maturation. We identified the Pumilio-binding site in *Xenopus* cyclin B1 mRNA as the sequence UGUA (nucleotides 1335–1338), which is close to but not overlap with the cytoplasmic polyadenylation element (CPE). By injection of various cis-elements into oocytes, we also showed that the sequence responsible for masking cyclin B1 mRNA is located at the CPE (nucleotides 1353–1360) near the Pumilio-binding site. Protein pull-down and co-immunoprecipitation assays revealed the association of endogenous Pumilio with CPE-binding protein (CPEB), a key regulator of translational repression and activation of mRNAs stored in oocytes. These results demonstrate for the first time in any species that Pumilio is a sequence-specific RNA-binding protein and associates with CPEB, which might give a new insight into the mechanism of translational regulation of cyclin B1 mRNA during oocyte maturation.

OOGENESIS AND VITELLOGENIN OF THE ASTEROID.

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Annual changes in the morphology of gonads and proteins in the coelomic fluid were investigated in *Asterina pectinifera* and some other species. The ovaries and testes were observed by hematoxylin-staining and enzyme-antibody staining with anti-Aa150, a polyclonal antibody raised against vitellogen of *Asterias amurensis*. Immuno-staining of the gonads of both sexes indicated that vitellogen was accumulated not only in the ovary but also in testes. Immuno-reaction was observed in the basement membrane of testes throughout the year. Although some spermatocytes showed immuno-reaction, none of immuno-reaction was detected in the mature spermatozoa, which completed metamorphosis. In the female gonad, interestingly, the most intense staining with the antibody was observed in the immature oocytes of which were a half of the mature egg. The intensity of staining decreased as the full-grown oocytes. These results suggest that accumulation of vitellogen proceeds prior to that of lipids during oogenesis. Analysis of the protein composition in the coelomic fluid of female and male indicated that temporal increase of vitellogenin did not occur.

NORMAL EXPRESSION OF SEVERAL GROWTH FACTORS RELATED TO SURVIVAL OF PRIMORDIAL GERM CELLS IN SOMATIC CELLS FROM _ter_ MUTANT MICE

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The *ter* (teratoma, Chr.18) gene causes deficiency in the primordial germ cells (PGCs) in *ter/ter* mice from 8.0 days *post coitum* (dpc) to adulthood. The PGCs survived in vitro in the conditioned media (CM) prepared from +/+ somatic cells of the fetal gonads, but most of them did not in the CM from *ter/ter* somatic cells. The BrdU incorporation into the PGCs cultured in +/+ or *ter/ter* CM indicated that the *ter* gene does not affect S phase of the cell cycle of PGCs. The *ter/ter* PGCs were found to survive and proliferate by interaction with +/+ gonadal somatic cells in vitro as well as +/+ PGCs. Thus, it was suggested that +/+ gonadal somatic cells might produce a novel growth factor (designated as TERF), that might be encoded by the normal gene at the *ter* locus, to support survival of the PGCs. It was also suggested that *ter/ter* ones might produce a default factor to induce apoptotic death in PGCs.

To examine the function of the *ter* gene on several growth factors related to survival of the PGCs, in this study the gonads from the *ter* congenic strain were stained with each antibody against stem cell factor (SCF), leukemia inhibitory factor (LIF) and basic fibroblast growth factor (bFGF). There were no genotype differences in each expression of SCF, LIF and bFGF in 12.5 dpc gonads in vitro. Strong expressions of SCF and bFGF and weak expressions of LIF in Sertoli cells and the peritubular cells of the matured *ter/ter* testes in paraffin sections were similar to those of +/+ testes. Thus, it was suggested that the *ter* gene might act by a novel mechanism different from those of SCF, bFGF and LIF in the mouse gonads.

STUDIES ON THE CATALYTIC SITES OF THE PROTEASOME INVOLVED IN STARFISH OOCYTE MATURATION AND ON SIGNALOSOME-LIKE COMPLEX

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We have previously reported that the chymotrypsin-like activity of the proteasome plays a key role in starfish oocyte maturation. Here, we purified the 20S proteasome from oocytes of the starfish, *Asterina pectinifera*, and attempted to identify the catalytic subunits of the proteasome involved in the oocyte maturation. By comparing the effects of proteasome inhibitors (MG115, MG132, and PSI) as well as various leupeptin analogs on three catalytic sites of the proteasomes and on GVBD, we found that not only the chymotrypsin-like activity but also the trypsin-like activity of the proteasome plays a key role in oocyte maturation.

We previously reported that the 530-kDa proteasome-associable complex (RSC) may be a counterpart of the regulatory subunit complex contained in the 26S proteasome. However, from the viewpoint of subunit compositions (105-, 70-, 50-, 34-, 30-, and 23-kDa), this complex appears to be a novel proteasome-associable complex. Therefore, we designated this complex PC530. Starfish oocyte PC530 appears to be similar to mammalian signalosomes on the basis of subunit composition and molecular size.

EXPRESSION AND LOCALIZATION OF TESTICULAR IBA-1 ISOLATED BY DIFFERENTIAL DISPLAY.

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Mammalian spermatogenesis is an excellent model system to study regulation of gene expression during differentiation. Postnatal development of the rat seminiferous tubule can be subdivided into three parts: (1) a premeiotic phase characterized by mitotic division of diploid spermatogonia; (2) a meiotic phase, which leads to the formation of haploid round spermatids; and (3) a post-meiotic phase, which includes the remarkable morphological changes required for spermatozoa formation (spermiogenesis). To understand the mechanisms of testicular germ cell differentiation, it is necessary to isolate genes which are specifically expressed in a certain stage of testis development. Using differential display, we have analyzed the expression patterns of more than one hundred genes, and isolated several novel genes as well as known genes which are developmentally up-regulated in rat testis. One of those genes, Iba-1 (ionized calcium binding adaptor molecule-1) was found to become detectable at 4-week old testis and continue to increase thereafter. We will demonstrate the expression and localization of the Iba-1 protein in rat testis by immunohistochemistry with anti-Iba-1 specific antibody.

PHOSPHORYLATION OF GOLDFISH CYTOPLASMIC POLYADENYLATION ELEMENT-BINDING PROTEIN (CPEB) BY EG2 AND MPF.

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Cyclin B, the regulatory component of MPF (maturation-promoting factor) belongs to a family of protiens involved in the regulation of the cell cycle. In goldfish (*Carussius auratus*), 17α,20β-dihydroxy-4-pregnen-3-one, a natural maturation-inducing hormone in fish) induces oocyte maturation by stimulating the *de novo* synthesis of cyclin B. However, the exact mechanism of the initiation of cyclin B synthesis during oocyte maturation is uncertain. Previously, we found that cyclin B mRNA is polyadenylated during goldfish oocyte maturation, and this polyadenylation is regulated by CPEB (cytoplasmic polyadenylation element-binding protein), an RNA-binding protein that interacts with the U-rich cytoplasmic polyadenylation elements (CPEs) of maternal mRNAs. Furthermore, CPEB is phosphorylated during goldfish oocyte maturation, suggesting that this phosphorylation regulates the CPEB activity. So, we analyzed the protein kinase(s) that can phosphorylate CPEB. First, we checked wether MPF can phosphorylate CPEB in vitro. We found that MPF phosphorylates CPEB and induces its phosphoritic mobility shift. Second, we examined whether MAP kinase and Eg2 kinase that have the crucial role for oocyte maturation, can phosphorylates CPEB in vitro. Intriguingly, Eg2 but not MAP kinase can phosphorylate CPEB. These results suggest that MPF is responsible for CPEB phosphorylation during the later phase of oocyte maturation, while Eg2 is involved in early CPEB phosphorylation.