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Involvement of 26S Proteasome in Oocyte Maturation of Goldfish *Carassius auratus*

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ABSTRACT—Diisopropyl fluorophosphate (DFP), a serine protease inhibitor, inhibited the activity of 26S proteasome in goldfish oocytes and arrested the oocytes at two distinct stages of maturation. One was prior to the migration of germinal vesicle (GV) toward the animal pole, and the other was between the attachment of GV to the oocyte plasma membrane and GV breakdown (GVBD). The first DFP-sensitive period corresponded to the period during which the activity of proteasomes increased, but maturation-promoting factor (MPF) was still inactive. During the second DFP-sensitive period, MPF was abruptly activated, although the proteasome activity detectable in the oocyte cytosol extracted by ultracentrifugation reached the lowest level during this period. These results suggest the requirement of 26S proteasome for at least two stages of oocyte maturation, the early stage before GV migration and the later stage, including MPF activation immediately before GVBD.

INTRODUCTION

Oocyte maturation is triggered by maturation-inducing hormone (MIH), which acts on the oocyte surface and induces the activation of maturation-promoting factor (MPF) in the oocyte cytoplasm (Nagahama *et al.*, 1995). Although MPF-induced oocyte maturation is insensitive, MIH-induced oocyte maturation is prevented by various protease inhibitors. Protease inhibitors originated in microorganisms such as leupeptin, antipain and chymostatin, block 1-methyladenine-induced oocyte maturation in starfish (Kishimoto *et al.*, 1982). The same series of inhibitors and a synthetic chymotrypsin inhibitor (N-tosyl-L-phenylalanine chloromethyl ketone (TPCK) also block progesterone-induced oocyte maturation in the frog (Guerrier *et al.*, 1977; Ishikawa *et al.*, 1989). These results suggest that protease activity is involved in the early step of oocyte maturation from MIH reception on the oocyte surface to MPF activation in the oocyte cytoplasm.

A non-lysosomal multicatalytic protease named proteasome has been identified in various eukaryotes. Proteasomes possess three distinct proteolytic activities (chymotrypsin-like, trypsin-like and peptidylglutamyl peptidase activities), and are sensitive to serine and thiol protease inhibitors (Hough *et al.*, 1987; Orlowsky, 1990). In frog oocytes, both the proteasome activity and oocyte maturation are inhibited by serine protease inhibitors such as TPCK and diisopropyl fluorophosphate (DFP) (Azuma *et al.*, 1991; Takahashi *et al.*,1994), suggesting that proteasomes are probably responsible for inducing oocyte maturation. The involvement of proteasomes in oocyte maturation has also been suggested by the inhibition of starfish oocyte maturation with trypsin inhibitors (Takagi Sawada *et al.*, 1989, 1992).

We purified and characterized 20S and 26S proteasomes from goldfish oocyte cytosol (Tokumoto *et al.*, 1995a, b). During the course of that study, we showed that goldfish oocyte cytosol mainly contains 26S proteasome. In the present study, we examined the inhibitory effects of DFP, a proteasome inhibitor, on oocyte maturation in goldfish. We found that DFP arrests oocytes at the stage before the migration of germinal vesicle (GV), when the activity of the 26S proteasome (active form) increases abruptly after MIH exposure. These findings suggest the commitment of 26S proteasome to the early events of goldfish oocyte maturation. We also demonstrated the inhibition of oocyte maturation by DFP applied just before germinal vesicle breakdown (GVBD), and discuss the

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involvement of 26S proteasome in the later events of oocyte maturation through the activation of MPF.

MATERIALS AND METHODS

Oocyte preparation and in vitro *culture*

Goldfish were purchased commercially and raised at 15°C. Ovaries were isolated from sacrificed females and placed in fresh goldfish Ringer's solution (125 mM NaCl, 2.4 mM KCl, 0.28 mM $MgSO_4$, 0.89 mM $MgCl_2$, 2.4 mM $CaCl_2$, 2 mM HEPES, 5.6 mM glucose, 100 IU/ml penicillin, 0.2 mg/ml streptomycin, pH 7.5) and washed three times with the same solution. The maturation of full-grown immature oocytes was induced in vitro by incubating ovarian fragments (each containing 20-40 oocytes) in goldfish Ringer's solution containing 1 μg/ml of 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DP), an MIH of goldfish (Nagahama *et al.*, 1995) at room temperature with gentle agitation. To assess maturation processes, GV was morphologically examined under a binocular microscope after fixing the oocytes in clearing solution (Lessman and Kavumpurath, 1984). Nuclear state (%) at each time points was determined in twenty oocytes.

Preparation of oocyte cytosol

Oocyte cytosol was extracted as follows. Thirty oocytes manually isolated from ovarian fragments were washed in goldfish Ringer's solution. Excess medium was removed, then 150 μl of fresh Ringer's solution was added. The oocytes were crushed by ultracentrifugation (150,000 × g, 30 min, 4°C) and clear supernatant was collected.

Assay of proteasome activity

The activity of the 26S proteasome in oocyte cytosol was measured using the fluorogenic substrate, Suc-LLVY-MCA (Peptide Institute) in the absent SDS, as described previously (Tokumoto and Ishikawa, 1993; Tokumoto *et al.*, 1993).

Purification of 26S proteasome from goldfish oocyte cytosol

The 26S proteasome was purified from goldfish oocyte cytosol as described previously (Tokumoto *et al.*, 1995b).

RESULTS AND DISCUSSION

Changes in GV morphology and proteasome activity during goldfish oocyte maturation

During normal maturation, GV breaks down beneath the animal pole of the oocyte after migrating from the center to the animal pole. Changes in GV during oocyte maturation are classified as follows: GV located at the center of the oocyte (GVI), GV migrating to the animal pole (GVM), GV attached to the oocyte plasma membrane (GVA), and GVBD (Tokumoto *et al.*, 1993).

The activity of the 26S proteasome in the cytosol extracted from goldfish oocytes remarkably changed during oocyte maturation. The proteasome activity increased transiently within 1 hr of exposure to 17α,20β-DP, then gradually decreased to the lowest level at 5 hr thereafter. The oocytes started GV migration after the transient increase in proteasome activity, and completed GVBD, when the proteasome activity reached the lowest level. The 26S proteasome activity elevated again at 8 hr after completion of GVBD (Fig. 1).

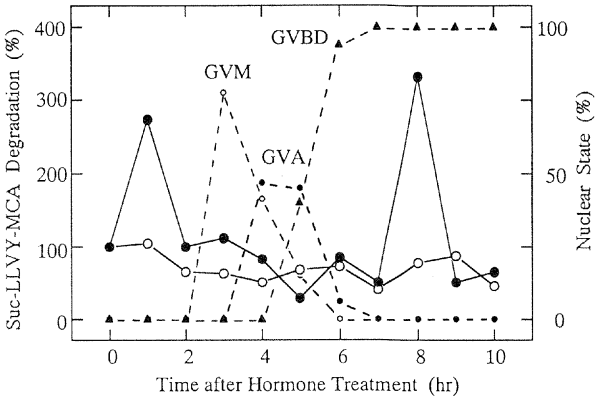

Fig. 1. Changes in the state of the germinal vesicle and the activity of the 26S proteasome during goldfish oocyte maturation. The 26S proteasome activity in the oocyte cytosol from oocytes exposed to 17α,20β-DP (●) or ethanol (vehicle of 17α,20β-DP) (○), was determined as described in Materials and Methods. The 26S proteasome activity is indicated in arbitrary units with values (%) for activity in the immature oocyte cytosol set at 100%. The status of germinal vesicle during the 17α,20β-DP-induced oocyte maturation was also examined (---). GVM, migrating germinal vesicle; GVA, attached germinal vesicle; GVBD, germinal vesicle breakdown.

Effect of DFP on proteasome activity

The 26S proteasome activity increased transiently after exposure to 17α,20β-DP (Fig. 1). To examine whether this increase is required for inducing oocyte maturation, we tested the effect of the proteasome inhibitor, DFP, on oocyte maturation. Although we showed that DFP inhibits proteasome activity in frog oocytes (Takahashi *et al.*, 1994), the effects of DFP on proteasomes in goldfish oocytes remained unknown. We first examined this point. When goldfish oocytes were externally exposed to DFP, the proteasome activity in the cytosol extracted from the treated oocytes decreased in accordance with the dose (Fig. 2A). In the cytosol fraction from non-DFP treated oocytes incubated with various concentrations of DFP, the proteasome activity was inhibited with similar dose-dependence (Fig. 2B). Furthermore, the 26S proteasome purified from goldfish ovary (Tokumoto *et al.*, 1995b) was inhibited by similar concentrations of DFP to those that inhibited the 26S proteasome activity in the oocytes and in the cytosol (Fig. 2C). The concentration required for 50% inhibition in each experiment ranged between 1 to 2 mM. These results confirmed that DFP permeates the oocyte cytoplasm, where it inhibits 26S proteasome activity.

Effects of DFP on oocyte maturation

Oocytes were incubated with various concentrations of DFP for 1 hr, then with 17α,20β-DP in the absence of DFP. Oocyte maturation was dose-dependently inhibited, being arrested at either GVI or GVA, but never at GVM (Fig. 3). To determine when oocytes are sensitive to DFP, 1 mM DFP was pulse-applied every 1 hr during the incubation, and the state of GV was examined 8 hr after exposure. The inhibitory effect of DFP was detected at two periods, before GVM (1-3

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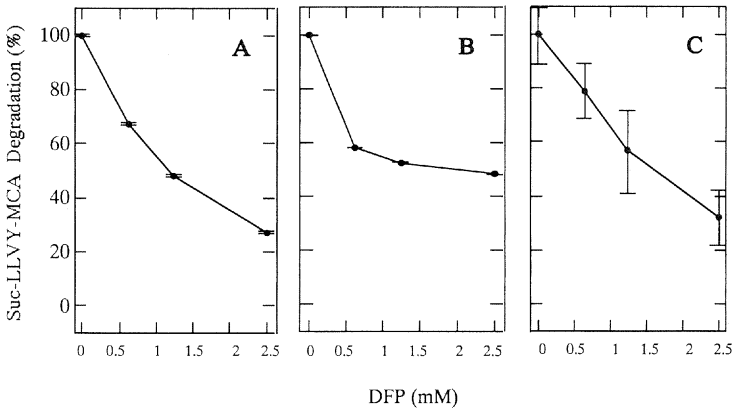

Fig. 2. Effects of DFP on Suc-LLVY-MCA hydrolyzing activity. (**A**) Oocytes were treated with various concentrations of DFP for 1 hr. After 3 washes in Ringer's solution, 30 oocytes were extracted and Suc-LLVY-MCA hydrolyzing activity was determined. (**B**) Fresh oocyte extract was incubated with various concentrations of DFP for 10 min. Suc-LLVY-MCA hydrolyzing activity was then determined. (**C**) Purified 26S proteasome fraction (100 μg/ml) was treated with various concentrations of DFP for 10 min, then Suc-LLVY-MCA hydrolyzing activity was determined.

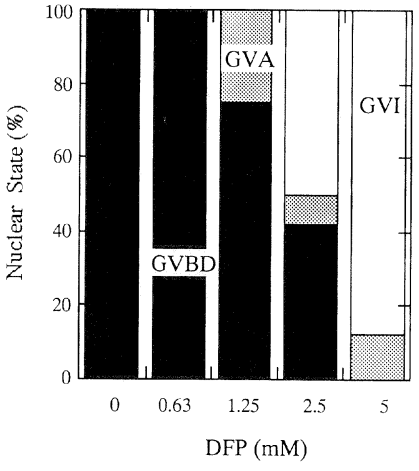

Fig. 3. Effects of DFP on 17α,20β-DP-induced oocyte maturation. Twenty oocytes were incubated in 3 ml Ringer containing various concentrations of DFP for 1 hr, then maturation was induced by 17α,20β-DP. After an 8 hr incubation, the status of germinal vesicle was examined. GVI, intact germinal vesicle; GVA, attached germinal vesicle; GVBD, germinal vesicle breakdown.

hr after the hormone treatment) and between GVM and GVA (4-5 hr after the hormone treatment) (Fig. 4). The first DFP-sensitive period corresponded to the period when the 26S proteasome activity increased after hormone exposure, whereas it was low during the second DFP-sensitive period (Fig. 1).

Involvement of 26S proteasome in the early phase of oocyte maturation

As found in the oocytes exposed to DFP (Fig. 3), those incubated with 1 mM DFP during the first DFP-sensitive period were arrested at either GVI or GVA (Fig. 4), indicating that the first protease-dependent critical point exists between GVI and GVM and that if oocytes pass this point, they can reach GVA without interruption. Between GVI and GVM, oocytes have relatively high 26S proteasome activity and are sensitive to DFP. Thus, 26S proteasome activity should be necessary for oocytes to pass this early step of maturation, including the GV migration.

Possible involvement of 26S proteasome in the later phase of oocyte maturation

When oocytes were treated with 1 mM DFP at the second DFP-sensitive period, GV continued to migrate toward the animal pole and attached to the oocyte plasma membrane, but did not break down (Fig. 4). This result indicates that the period between GVA and GVBD is also protease-dependent. MPF is abruptly activated during this period (Hirai *et al.*, 1992; Yamashita *et al.*, 1992, 1995; Katsu *et al.*, 1993). Moreover, an injection of active MPF into immature goldfish oocytes induced GVBD without the preceding migration of GV towards the animal pole, resulting in GVBD in the center of the oocytes (Yamashita *et al.*, unpublished observation). This finding indicated that when MPF is precociously activated, GV will be broken down irrespective of its position within the oocyte. Therefore, it is most likely that the point between GVA and GVBD is mainly controlled by MPF.

DFP-induced arrest of oocyte maturation should be caused by the failure to activate MPF. We examined whether or not the 26S proteasome is involved in MPF activation. Since proteasome activity in the extracted cytoplasm is low during this period (Fig. 1), the 26S proteasome may not be positively involved in MPF activation. DFP inhibits various serine proteases, leaving the possibility that proteases other than proteasomes play a role in inducing oocyte maturation during this period via the activation of MPF. However, it should be

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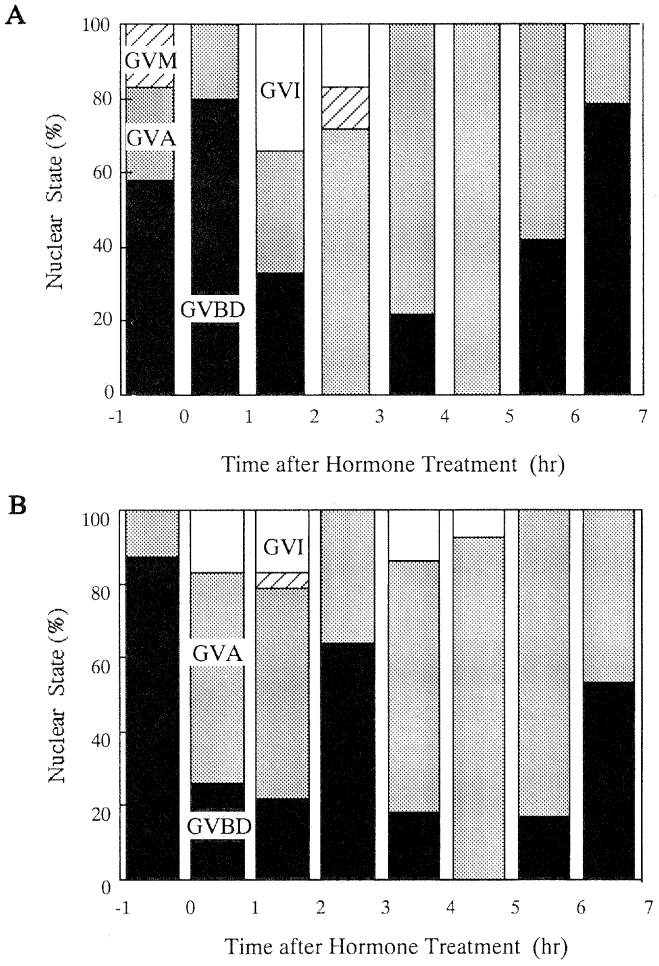

Fig. 4. Effects of a pulse-application of DFP on oocyte maturation. In the continuous presence of 17α,20β-DP, twenty oocytes were treated with 1 mM DFP for 1 hr during the indicated periods, and the status of germinal vesicle was examined 8 hr later. GVI, intact germinal vesicle; GVM, migrating germinal vesicle; GVA, attached germinal vesicle; GVBD, germinal vesicle breakdown. Panels **A** and **B** indicate results from different females.

noted that the proteasome activity referred to in this study was that found in oocyte cytosol extracted by ultracentrifugation. It is uncertain whether or not the level of 26S proteasome activity in intact oocytes is really low during the second DFP-sensitive period. Proteasomes are colocalized in nucleus and cytoplasm (Tanaka *et al.*, 1992) and accumulate into GV before GVBD in amphibian oocytes (Arrige *et al.*, 1988; Gautier *et al.*, 1988). Cell cycle-dependent accumulation of proteasome into the nucleus is also detected in ascidian embryos (Kawahara and Yokosawa, 1992). Therefore, it is likely that the decrease in the 26S proteasome activity in the extracted cytosol before GVBD is due to transportation of the 26S proteasome to GV, which will be easily precipitated by centrifugation. Thus its contents, including proteasomes were not sufficiently extracted. In other words, substantial 26S proteasome activity may be present in GV before GVBD.

In mammalian cultured cells (Pines and Hunter, 1991) and starfish oocytes (Ookata *et al.*, 1992), MPF accumulates into the nucleus just before its breakdown. Proteasomes also accumulate in the nucleus prior to nuclear envelope breakdown in liver cells (Tanaka *et al.*, 1992) and in amphibian oocytes (Gautier *et al.*, 1988). Although not confirmed in the same organism, the simultaneous accumulation of proteasomes and MPF to GV before GVBD implies their interaction within it, which may be prerequisite for subsequent GVBD. Detailed examinations on the localization and the activity of proteasome during oocyte maturation are required to clarify the involvement of proteasomes in MPF activation.

The level of 26S proteasome activity increases again after GVBD. The timing of the second peak apparently corresponds to that of the first polar body extrusion, between the first and second meiotic metaphase. We reported that in goldfish oocytes, H1 histone kinase (MPF) activity transiently decreased when the first polar body was eliminated (Yamashita *et al.*, 1992). Thus, it is possible that activation of the 26S proteasome activity in this period is responsible for the decrease in MPF activity, probably by inducing the degradation of the regulatory subunit of MPF, cyclin B.

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REFERENCES

Arrige AP, Tanaka K, Goldberg AL, Welch WJ (1988) Identity of the 19S 'Prosome' particle with the large multifunctional protease complex of mammalian cells (the proteasome). Nature 331: 192–194

Azuma Y, Tokumoto T, Ishikawa K (1991) High molecular weight-multicatalytic proteinases in premature and mature oocytes of *Rana pipiens*. Mol Cell Biochem 100: 171–181

Gautier J, Pal JK, Grossi de SA, Beetshen JC, Scherrer K (1988) Differential cytolocalization of prosomes in axolotol during oogenesis and meiotic maturation. J Cell Sci 90: 543–553

Guerrier P, Moreau M, Doree M (1977) Inhibition de la reinitiation de la meiose des ovocytes de *Xenopus laevis* partrois antiproteases naturelleas, l'antipain, la chymostatine et la leupeptine. C R Acad Sci Paris 284: 317–319

Hirai T, Yamashita M, Yoshikuni M, Lou YH, Nagahama Y (1992) Cyclin B in fish oocytes: Its cDNA and amino acid sequences, appearance during maturation, and induction of $p34^{cdc2}$ activation. Mol Reprod Dev 33: 131–140

Hough R, Pratt G, Rechsteiner M (1987) Purification of two high molecular weight proteases from rabbit reticulocyte lysate. J Biol Chem 262: 8303–8313

Ishikawa K, Schuetz AW, San Fransisco SK (1989) Induction and inhibition of amphibian (*Rana pipiens*) oocyte maturation by protease inhibitor (TPCK). Gamete Res 22: 339–354

Katsu Y, Yamashita M, Kajiura H, Nagahama Y (1993) Behavior of the components of maturation-promoting factor, cdc2 kinase and cyclin B, during oocyte maturation of goldfish. Dev Biol 160: 99–107

Kawahara H, Yokosawa H (1992) Cell cycle-dependent changes of

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proteasome distribution during embryonic development of the ascidian *Halocynthia roretzi*. Dev Biol 151: 27–33

Kishimoto T, Clark TG, Kondo HK, Shirai H, Kanatani H (1982) Inhibition of a starfish oocyte maturation by some inhibitors of proteolytic enzymes. Gamete Res 5: 11–18

Lessman CL, Kavumpurath S (1984) Cytological analysis of nuclear migration and dissolution during steroid-induced meiotic maturation in vitro of follicle-enclosed oocytes of the goldfish (*Carassius auratus*). Gamete Res 10: 21–29

Nagahama Y, Yoshikuni M, Yamashita M, Tokumoto T, Katsu Y (1995) Regulation of oocyte growth and maturation in fish. In "Current Topics in Developmental Biology Vol 30" Ed by RA Pederson, GP Schatten, Academic Press, California, pp 103–145

Ookata K, Hisanaga S, Okano T, Tachibana K, Kishimoto T (1992) Relocation and distinct subcellular localization of $p34^{cdc2}$-cyclin B complex at meiosis reinitiation in starfish oocytes. EMBO J 11: 1763–1772

Orlowsky M (1990) The multicatalytic proteinase complex, a major extralysosomal proteolytic system. Biochemistry 29: 10289–10297

Pines J, Hunter T (1991) Human cyclins A and B1 are differentially located in the cell and undergo cell cycle-dependent nuclear transport. J Cell Biol 115: 1–17

Takagi Sawada M, Someno T, Hoshi M, Sawada H (1989) Inhibition of starfish oocyte maturation by leupeptin analogs, potent trypsin inhibitors. Dev Biol 133: 609–612

Takagi Sawada M, Someno T, Hoshi M, Sawada H (1992) Participation of 650-kDa protease (20S proteasome) in starfish oocyte maturation. Dev Biol 150: 414–418

Takahashi M, Tokumoto T, Ishikawa K (1994) DFP-sensitive multicatalytic protease complexes (proteasomes) in the control of oocyte maturation in the toad, *Bufo japonicus*. Mol Reprod Dev 38: 310–317

Tanaka K, Tamura T, Yoshimura T, Ichihara A (1992) Direct evidence for nuclear and cytoplasmic colocalization of proteasomes (multiprotease complexes) in liver. J Cell Physiol 139: 34–41

Tokumoto T, Ishikawa K (1993) A novel "active" form of proteasomes from *Xenopus laevis* ovary cytosol. Biochem Biophys Res Commun 192: 1106–1114

Tokumoto T, Yamashita M, Yoshikuni M, Nagahama Y (1993) Changes in the activity and protein levels of proteasomes during oocyte maturation in goldfish (*Carassius auratus*). Biomed Res 14: 305–308

Tokumoto T, Yamashita M, Yoshikuni M, Kajiura H, Nagahama Y (1995a) Purification of latent proteasome (20S proteasome) and demonstration of active proteasome in goldfish (*Carassius auratus*) oocyte cytosol. Biomed Res 16: 173–186

Tokumoto T, Yoshikuni M, Yamashita M, Kajiura H, Nagahama Y (1995b) Purification and characterization of active proteasome (26S proteasome) from goldfish ovaries. Biomed Res 16: 207–218

Yamashita M, Fukada S, Yoshikuni M, Bulet P, Hirai T, Yamaguchi A, Lou YH, Zhao Z, Nagahama Y (1992) Purification and characterization of maturation-promoting factor in fish. Dev Biol 148: 8–15

Yamashita M, Kajiura H, Tanaka M, Onoe S, Nagahama Y (1995) Molecular mechanisms of the activation of maturation-promoting factor during goldfish oocyte maturation. Dev Biol 168: 62–75

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