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Differential Regulation of Histone H1 and Ribosomal S6 Kinases during Sea Star Oocyte Maturation†

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Received May 8, 1987; Revised Manuscript Received July 23, 1987

ABSTRACT: In the preceding paper [Pelech, S. L., Meijer, L., & Krebs, E. G. (1987) *Biochemistry* (preceding paper in this issue)], at least three activated kinases were detected in soluble extracts from sea star oocytes induced to undergo maturation by 1-methyladenine (1-MeAde). Coincident with nuclear envelope breakdown (20 min after exposure to 1-MeAde), there was a rapid activation of a histone H1 kinase that eluted from DEAE-Sephacel with a conductivity of ~6 mmho. By contrast, 60-min treatment of the oocytes with 1-MeAde was required for maximal activation of two kinases, each of which phosphorylated a synthetic peptide, Arg-Arg-Leu-Ser-Ser-Leu-Arg-Ala (RRLSSLRA), patterned after a phosphorylation site sequence from ribosomal protein S6. These RRLSSLRA kinases were released from DEAE-Sephacel with elution conductivities of ~6 and ~10.5 mmho. The 1-MeAde dose–response curves for maturation induction and activation of the histone H1 and RRLSSLRA kinases were superimposable. Both oocyte maturation and the activation of the kinases required the presence of 1-MeAde during the hormone-dependent period. When 1-MeAde was removed after this period, full histone H1 kinase activation still occurred and maturation was induced. Forskolin pretreatment of the oocytes, by elevating the basal cAMP level more than 35-fold, doubled the hormone-dependent period and similarly delayed the onset of histone H1 kinase activation by 1-MeAde. However, postmaturation activation of the RRLSSLRA kinases was completely blocked by forskolin. The phorbol ester tumor promoter 12-*O*-tetradecanoylphorbol 13-acetate (TPA) inhibited both 1-MeAde-induced oocyte maturation and activation of the histone H1 kinase at equivalent phorbol ester concentrations, but only when introduced prior to the completion of the hormone-dependent period. On the other hand, TPA elicited modest activations of these kinases in the absence of 1-MeAde.

A fundamental problem in developmental biology is the delineation of the chain of intracellular molecular events that are responsible for oocyte maturation, i.e., the resumption of meiotic divisions. In most animals, oocytes are arrested in the first prophase stage of meiosis and feature a prominent nucleus or germinal vesicle. The rupture of the nuclear envelope or germinal vesicle breakdown (GVBD)[1] serves as a useful marker for oocyte maturation in response to 1-methyladenine

† S.L.P. is the recipient of a Medical Research Council of Canada 1967 Centennial Fellowship. This work was supported in part by a CNRS/NSF exchange program.
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[1] Abbreviations: cAMP, adenosine 3′,5′-monophosphate; GVBD, germinal vesicle breakdown; I_{50}, dose producing a 50% inhibition; 1-MeAde, 1-methyladenine; MPF, maturation-promoting factor; PB1, emission of the first polar body; RRLSSLRA, Arg-Arg-Leu-Ser-Ser-Leu-Arg-Ala; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; Mops, 4-morpholinepropanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid; ATPγS, adenosine 5′-*O*-(3-thiotriphosphate).

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(1-MeAde) in sea stars and progesterone in amphibians [reviewed in Masui and Clarke (1979) and Meijer and Guerrier (1984)]. Prior to this event, a number of biochemical changes have been temporally linked with the binding of these maturation-inducing hormones to the oocyte plasma membrane [reviewed in Meijer and Guerrier (1984) and Maller (1985)].

One phenomenon, the appearance of a proteinaceous factor, termed "maturation-promoting factor" (MPF), in the cytoplasm of maturing oocytes has attracted considerable attention (Meijer & Guerrier, 1984; Maller, 1985). Microinjection of impure preparations of MPF into immature oocytes triggers GVBD and completion of maturation in the absence of maturation-inducing hormones. MPF appears to be highly conserved across several phylla, since numerous transfer experiments have been successfully performed between yeast, mollusk, echinoderm, amphibian, and mammalian cells. Due to its lability during purification, the enzymatic or modulatory activity of MPF is not known. The production of active MPF may be an intermediate step in a universal regulatory cascade that culminates in nuclear envelope breakdown and the G2/M transition in mitosis of somatic cells as well as meiosis of oocytes.

The detection of MPF in maturing oocytes has been intimately correlated with the burst of total oocyte protein phosphorylation that precedes GVBD (Guerrier et al., 1977; Maller et al., 1977; Doree et al., 1981, 1983; Gerhart et al., 1984). The activation of a cascade system involving protein phosphorylation provides an attractive conceptual model for how the action of maturation-inducing hormones may be transduced and amplified. Partially purified MPF exhibits protein kinase activity, is autocatalytic, and is stabilized by ATP, ATPγS, and phosphatase inhibitors (Drury, 1978; Wu & Gerhart, 1980; Kanatani & Nagahama, 1980; Hermann et al., 1983). Regardless of whether or not MPF is a cause or consequence of the phosphorylation burst, enhanced protein phosphorylation appears mandatory for oocyte maturation (Doree et al., 1983; Hermann et al., 1984; LeGoascogne et al., 1984; Pondaven & Meijer, 1986; Meijer et al., 1986).

In sea star oocytes, phosphorylation of numerous proteins can be stimulated within 5 min of 1-MeAde addition and complete GVBD induced in under 20 min (Guerrier et al., 1977). With the possible exception of ribosomal protein S6 on the basis of size, none of these phosphorylated substrates have been identified. Guerrier et al. (1977) originally detected a 20–50% enhancement of endogenous protein kinase activity in 1-MeAde-treated *Marthasterias glacialis* oocytes. Using mixed histones as substrates, Picard et al. (1985)found a 3.5-fold increase in protein kinase activity during maturation. This activity later declined at the time of emission of polar bodies 1 and 2, as did the in vivo [^{32}P]phosphate labeling of proteins and MPF activity (Doree et al., 1983). The decrease in protein kinase activity was attributed to proteases, and its reappearance required protein synthesis (Picard et al., 1985). Sano (1985) detected a 7-fold increase in the activity of a calcium- and cAMP-independent protein kinase (or kinases) during maturation of *Asterina pectinifera* oocytes, and a strong specificity of the kinase for histone H1 was inferred.

In the preceding paper (Pelech et al., 1987), we described three major maturation-activated kinases that were selectively stimulated in soluble extracts from maturing sea star oocytes. One of these kinases preferentially phosphorylated histone H1, while the other two were shown to phosphorylate ribosomal protein S6 and a synthetic peptide, Arg-Arg-Leu-Ser-Ser-Leu-Arg-Ala (RRLSSLRA), patterned after a phosphorylation site in S6 (Gabrielli et al., 1984). In the present study, we have explored the relationship between these kinases and the maturation process to a greater depth. Our findings correlated the marked stimulation of the histone H1 kinase with the commitment of oocytes to GVBD. Full activation of this kinase was evident prior to GVBD. By contrast, optimum stimulation of the RRLSSLRA kinase activities occurred post-GVBD and did not correlate with oocyte maturation.

Experimental Procedures

Materials and Oocyte Processing. 8-Hydroxyeicosatetraenoic acid was a generous gift of Dr. A. Brash (Vanderbilt University, Nashville). 1-MeAde, dithiothreitol, arachidonic acid, forskolin, and TPA were obtained from Sigma. *Evasterias troschelii* were collected in the Seattle area, *Pisaster ochraceus* was from Friday Harbor, WA, and *Leptasterias hexactis* were collected from the Puget Sound area, WA.

Sea stars were kept either in closed-circuit aquaria or in running sea water at the Seattle Public Aquarium. The gonads were dissected out of the animals, gently torn open in ice-cold calcium-free artificial sea water, and filtered through cheesecloth. Oocytes were then washed 3 or 4 times in calcium-free artificial sea water to remove the 1-MeAde-producing follicle cells and were resuspended, as a 10% (v/v) suspension, in natural sea water until use. Oocyte maturation was recorded as the percentage of oocytes exhibiting germinal vesicle breakdown (GVBD) after 30–60 min (*E. troschelii*) or 60 min (*P. ochraceus*). Control (unstimulated) oocytes underwent a maximal spontaneous maturation rate of approximately 5–10%. The hormone-dependent period was determined by dilution of an oocyte suspension aliquot, at various times after 1-MeAde stimulation, to an inactive concentration of 1-MeAde (100 μL of a 10% oocyte suspension in 8 mL of natural sea water). Unless stated otherwise, all experiments were performed at 15 °C and with *E. troschelii* oocytes.

Preparation of Homogenates. To prepare homogenates, 1-mL aliquots of the 10% oocyte suspension were rapidly centrifuged (5 s at full speed in an Eppendorf microfuge). The sea water was removed by aspiration, and 300 μL of buffer A (60 mM β-glycerol phosphate, 30 mM *p*-nitrophenyl phosphate, 25 mM Mops, pH 7.2, 15 mM EGTA, 15 mM $MgCl_2$, 1 mM dithiothreitol, and 0.1 mM sodium vanadate) was added to the oocyte pellet. After rapid mixing, two variations of the following procedure were adopted. In the first case, the oocytes were resuspended in buffer A and immediately frozen at −70 °C. Prior to measurement of kinase activities, the samples were thawed and centrifuged for 15–20 min at 150000*g* at 5 °C in a Beckman airfuge to obtain cytosol. In the second variation, the oocytes were immediately ruptured in buffer A with a glass Dounce homogenizer and centrifuged for 15–20 min at 150000*g* at 5 °C in a Beckman airfuge, and the clear supernatant was stored at −70 °C until use in the kinases assays. The former approach was convenient for initial processing of a large number of samples within a short time period, but the latter approach yielded higher levels of kinase activation. The histone H1 and RRLSSLRA kinase assays were performed in the presence of a synthetic peptide inhibitor of cAMP-dependent protein kinase (Scott et al., 1986) as described in the preceding paper (Pelech et al., 1987).

Results

Activation of Histone H1 Kinase and RRLSSLRA Kinases during Oocyte Maturation. The preceding paper (Pelech et al., 1987) demonstrated that the activities of at least three

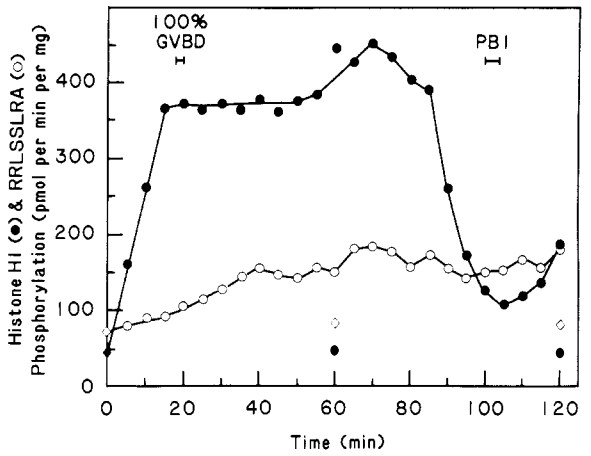

FIGURE 1: Time course of activation of histone H1 and RRLSSLRA kinases during oocyte maturation. Oocytes were treated (○, ●) or not treated (◇, ◆) with 1 μM 1-MeAde for up to 2 h prior to harvesting. Histone H1 (1 mg/mL) (●, ◆) or RRLSSLRA (0.25 mM) (○, ◇) phosphorylating activities were determined in soluble extracts prepared from aliquots of oocytes removed at various times. Values are the mean of duplicate determinations, and similar results were obtained in three independent experiments. One hundred percent of the treated oocytes underwent GVBD at 18–20 min after 1-MeAde addition, and the first cell division (PB1) occurred after 100–105 min as indicated.

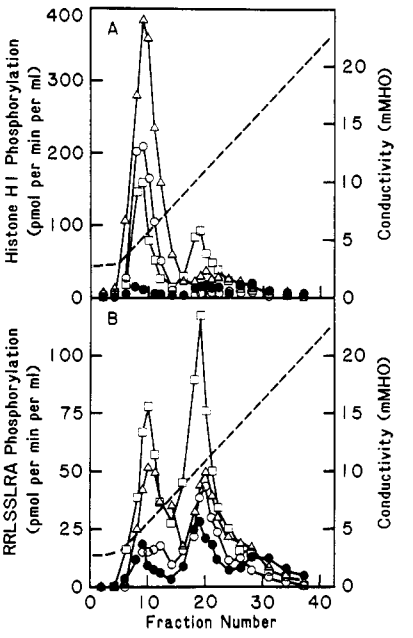

FIGURE 2: DEAE-Sephacel chromatography of histone H1 and RRLSSLRA phosphorylating activities in sea star oocyte cytosol. Cytosolic protein (2.5 mg) in buffer B (30 mM β-glycerolphosphate, 20 mM Mops, pH 7.2, 2 mM EGTA, 2 mM EDTA, 1 mM dithiothreitol, and 0.5 mM vanadate) from sea star oocytes treated with 2 μM 1-MeAde for 0 (●), 15 (○), 45 (△), and 90 (□) min was loaded onto a 2.5-mL DEAE-Sephacel column (Sigma) equilibrated in buffer B. The column was washed with 5 mL of buffer B and developed with a 40-mL linear gradient of 0–450 mM NaCl in buffer B with a flow rate of 0.4 mL/min. The collected fractions (~1.0 mL) were assayed with 1 mg of histone H1/mL (panel A) and 0.25 mM RRLSSLRA (panel B) for kinase activity with 50 μM [γ-^{32}P]ATP. No histone H1 or RRLSSLRA phosphorylating activity was detected in the wash-through fractions. The conductivity gradient (---) is shown. One hundred percent GVBD occurred 25–30 min after the addition of 1-MeAde to this population of oocytes.

protein kinases were elevated in soluble extracts of maturing sea star oocytes as compared to prophase-arrested oocytes. These maturation-activated kinases represented the major phosphorylating activities toward histone H1 and the synthetic S6 peptide RRLSSLRA in unfractionated cell extracts when precautions were adopted to inhibit cAMP-dependent protein kinase and protein kinase C. The following experiments were designed to further investigate the relationship between these kinases and the oocyte maturation process more precisely.

Oocytes from the sea star *E. troschelii* typically undergo GVBD approximately 20 min after initial exposure to 1-MeAde; the first polar body is extruded at 100 min and the second one 60 min later. When cytosols were prepared from populations of oocytes treated with 1-MeAde for 0–2 h and assayed for phosphorylating activity with histone H1 and RRLSSLRA, very different activation time courses were recorded (Figure 1). The histone H1 phosphorylating activity was clearly stimulated within 3–5 min after 1-MeAde addition. It reached a maximum at GVBD and transiently declined just prior to the emission of the first polar body. Depending on the method of extract preparation, up to a 30-fold stimulation was detectable at GVBD (H1 phosphorylating activity in extracts of 1-MeAde-treated oocytes versus unstimulated oocytes). By contrast, there was a lag in the activation of RRLSSLRA phosphorylation; maximal activity was reached after GVBD (60–80 min after 1-MeAde addition). The RRLSSLRA phosphorylating activity remained elevated during first cell division in six separate experiments, despite a decline in the histone H1 phosphorylating activity in each instance. Stimulation of the RRLSSLRA phosphorylating activity was not as dramatic as the enhanced histone H1 phosphorylating activity; a 2–3-fold stimulation was generally noted (Figure 1).

DEAE-Sephacel chromatography of oocyte cytosol facilitates the separation of the major stimulated histone H1 kinase from the major activated RRLSSLRA kinase (Pelech et al., 1987). To specifically examine the time course of activation of these kinases, cytosols from oocytes exposed to 1-MeAde for 0, 15, 45, and 90 min were fractionated on DEAE-Sephacel and the column fractions were assayed for histone H1 and RRLSSLRA phosphorylating activity (Figure 2). In this experiment, 100% GVBD occurred 25–30 min after addition of 1-MeAde to the oocytes. Full activation of the histone H1 kinase (elution conductivity ≃ 6 mmho) was achieved within 45 min of 1-MeAde treatment and subsequently declined by 90 min (Figure 2A). However, during the 45–90-min period of hormonal treatment, the activities of two RRLSSLRA kinases (with elution conductivities of ~6 and ~10.5 mmho) continued to increase (Figure 2B). Since the histone H1 phosphorylating and RRLSSLRA phosphorylating activities in the region of the ~10.5-mmho peak followed a similar time course of stimulation, it is possible that both substrates were phosphorylated by the same kinase. The early rise in the RRLSSLRA phosphorylating activity in the region of the ~6-mmho peak may have been due, in part, to phosphorylation of the synthetic peptide by the histone H1 kinase. Nevertheless, these findings verified that the increased phosphorylation of histone H1 by cytosol from maturing oocytes largely reflected the ~6-mmho histone H1 kinase activity and the increased phosphorylation of RRLSSLRA arose from both RRLSSLRA kinases.

Stimulated cytosolic histone H1 and RRLSSLRA phosphorylating activities were observed in 1-MeAde-treated oocytes from all three sea star species examined, and in *E. troschelii* with all mimetics of 1-MeAde tested (data not shown); these included dithiothreitol (Kishimoto & Kanatani, 1973), arachidonic acid (Meijer et al., 1984), and 8-hydroxyeicosatetraenoic acid (Meijer et al., 1986a).

The 1-MeAde concentration dependence of the histone H1 and RRLSSLRA kinase activations and their correlation with

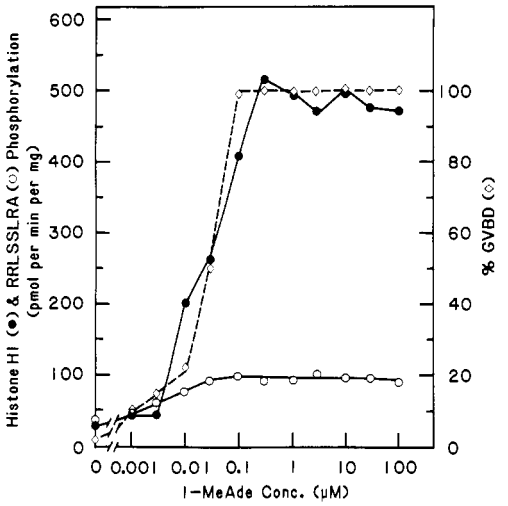

FIGURE 3: 1-MeAde dose–response curves for GVBD and activation of histone H1 and RRLSSLRA kinases. Oocytes were treated for 30 min with 0–100 μM 1-MeAde, and the histone H1 (●) and RRLSSLRA (○) kinase activities were subsequently determined in soluble extracts from these cells. Oocytes from the same batch, treated similarly with various 1-MeAde concentrations, were scored for percent GVBD after 60 min (◇). Similar results were obtained in two independent experiments.

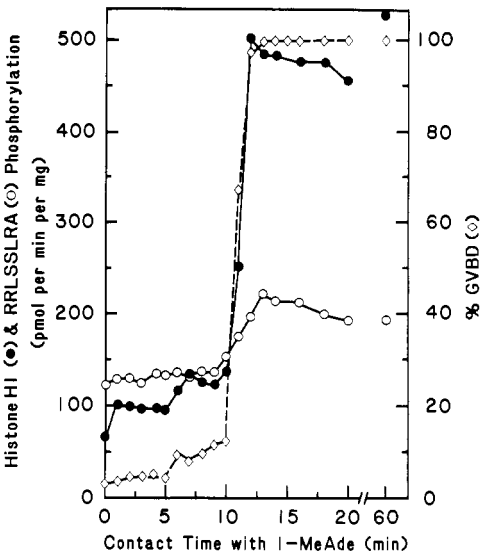

FIGURE 4: Effects of contact time with 1-MeAde on GVBD and activation of histone H1 and RRLSSLRA kinases. Oocyte suspensions were treated with 0.5 μM 1-MeAde, and at various times, aliquots of 500 μL were removed and injected into 15 mL of natural sea water. After 65 min, the oocytes were harvested for determination of the histone H1 (●) and RRLSSLRA (○) kinase activities. Aliquots were also removed in parallel for hormone-dependent period determination.

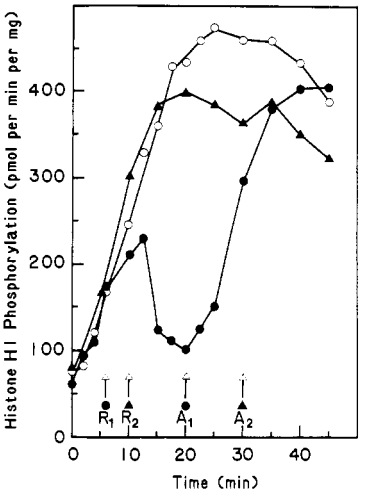

FIGURE 5: Effects of removal of 1-MeAde during or after the hormone-dependent period. Three batches of oocytes were treated with 1 μM 1-MeAde added at time 0. In the first batch (○), 1-MeAde was present throughout the experiment and 100% GVBD was reached within 20 min. In the second batch (●), 1-MeAde was removed by washing the oocytes at time 6–10 min (R_1). These oocytes underwent only 3% GVBD, showing that 1-MeAde had been effectively removed prior to completion of the hormone-dependent period. 1-MeAde was then added back at 20 min (A_1). In the third batch (▲), 1-MeAde was removed between 10 and 15 min (R_2). These oocytes underwent 100% GVBD, showing that 1-MeAde had been removed after the hormone-dependent period. 1-MeAde was added back at 30 min (A_2). At various times, aliquots of the three oocyte suspensions were removed and processed for measurement of the histone H1 kinase activity.

the triggering of GVBD is illustrated in Figure 3. The 1-MeAde dose–response curves for maturation induction and for activation of the histone H1 kinase were very similar; at subthreshold[2] concentrations of 1-MeAde, the degree of histone H1 kinase stimulation was proportional to the 1-MeAde concentration. It became maximal and independent of the 1-MeAde concentration once the threshold 1-MeAde concentration for complete GVBD had been reached. Even a further 1000-fold increase in the 1-MeAde concentration did not affect the kinase activity. The RRLSSLRA kinases also attained maximal activation at the threshold concentration of 1-MeAde (Figure 3).

Activation of Histone H1 and RRLSSLRA Kinases Depends on the Hormone-Dependent Period. To become irreversibly committed to maturation, oocytes must be in continuous contact with 1-MeAde for a discrete length of time known as the hormone-dependent period. When 1-MeAde is removed during the this period, maturation is not induced. However, if it is removed after the hormone-dependent period, maturation may still proceed (Guerrier & Doree, 1975; Nemoto, 1982). Due to the importance of this time interval, the relationship between it and the stimulation of the maturation-activated kinases was investigated. The kinase activities were measured after the addition of 1-MeAde for various periods of times, and almost no stimulation of the kinase activities occurred when 1-MeAde was removed during the hormone-dependent period (Figure 4). On the contrary, full activations were achieved despite the removal of 1-MeAde after this period (Figure 4). The hormone-dependent periods for GVBD and stimulation of the histone H1 and RRLSSLRA phosphorylating activities were superimposable. Time course experiments (Figure 5) revealed that when 1-MeAde was removed during the hormone-dependent period (Figure 5, R_1), the histone H1 kinase activity declined to its basal level. A second addition of 1-MeAde (Figure 5, A_1) triggered a reactivation of the enzyme. When 1-MeAde was removed after the hormone-dependent period (Figure 5, R_2), the activated state was maintained. Further addition of 1-MeAde (Figure 5, A_2) did not induce a greater stimulation of the kinase.

Another approach to investigate the correlation between the hormone-dependent period and the activation of the histone H1 and RRLSSLRA kinases was the use of forskolin. Treatment of sea star oocytes with forskolin has been shown to elevate the level of cAMP 35-fold, double the duration of the hormone-dependent period, and delay the onset of GVBD without inhibiting this event (Meijer & Zarutskie, 1987) (Figure 6A). The effect of forskolin on the time course of

[2] The threshold concentration of 1-MeAde was the concentration that triggered 100% germinal vesicle breakdown.

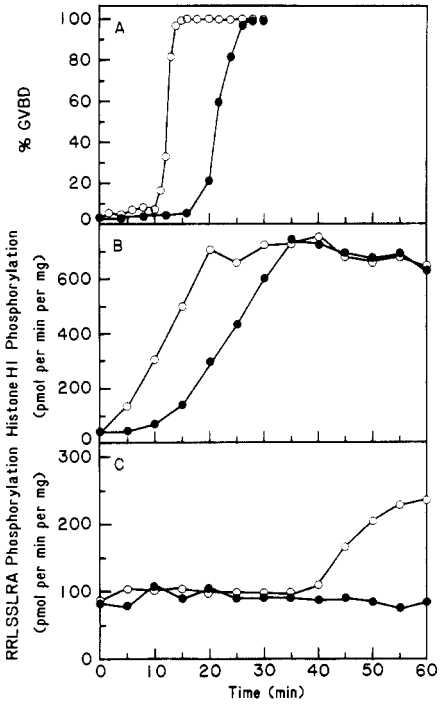

FIGURE 6: Effect of forskolin on the hormone-dependent period and histone H1 and RRLSSLRA kinase activities. Oocytes were pretreated (●) or not (○) with 100 μM forskolin for 60 min prior to addition of 1 μM 1-MeAde for 0–60 min. Panel A: The hormone-dependent period was assessed as described under Experimental Procedures. Histone H1 (panel B) and RRLSSLRA (panel C) kinase activities were assayed in soluble extracts prepared at various times from oocytes incubated with 1-MeAde. Identical results were obtained in two independent studies.

histone H1 kinase activation was striking; a delay occurred that corresponded to the increase in the hormone-dependent period length (Figure 6B). The effect of forskolin on the RRLSSLRA kinases was even more severe; 1-MeAde-induced phosphorylation of RRLSSLRA was totally abolished (Figure 6C). Despite this, GVBD and emission of the polar bodies were still induced (data not shown).

To further study the relationship between the hormone-dependent period and the activation of the histone H1 and RRLSSLRA kinases, an inhibitor of oocyte maturation that is effective only prior to completion of the hormone-dependent period, i.e., TPA, was tested (Kishimoto et al., 1985). The dose-dependent inhibitor effects of this phorbol ester on both 1-MeAde-induced maturation and the histone H1 kinase activation are shown in Figure 7A. The I_{50} for both events was approximately 0.4 μM. The effect of TPA on oocyte maturation was strictly limited to the hormone-dependent period; addition of TPA after this period failed to inhibit the maturation process (Figure 7B). In a similar manner, the effect of TPA on the histone H1 kinase activity was also specifically restricted to the hormone-dependent period. TPA added after the hormone-dependent period had no effect on the activation of the histone H1 kinase (Figure 7B).

Although TPA treatment inhibited 1-MeAde-induced GVBD, the stimulation of the RRLSSLRA kinase activities was apparently unaffected (Figure 7). This may have been because these enzymes are activated independently by TPA via protein kinase C, which may compensate for loss of the 1-MeAde-induced stimulation. TPA treatment of mammalian and avian cells has previously been shown to activate S6 kinases via a protein kinase C dependent mechanism (Blenis & Erikson, 1986; Pelech et al., 1986; Pelech & Krebs, 1987). To test for TPA-induced activation of the two RRLSSLRA kinases, cytosols from sea star oocytes exposed to 1 μM TPA for 30 min and untreated oocytes were chromatographed on DEAE-Sephacel and the column fractions were assayed for histone H1 and RRLSSLRA phosphorylating activity (Figure 8). TPA treatment of the oocytes resulted in moderate stimulation of the RRLSSLRA kinases, with elution conductivities of ~6 and ~10.5 mmho, and possibly the major histone H1 kinase (~6 mmho). The activation of the ~10.5-mmho RRLSSLRA kinase following phorbol ester treatment of the oocytes lends additional support for the possibility that this kinase may be related to the mitogen-activated S6 kinase in Swiss mouse 3T3 cells (Pelech & Krebs, 1987).

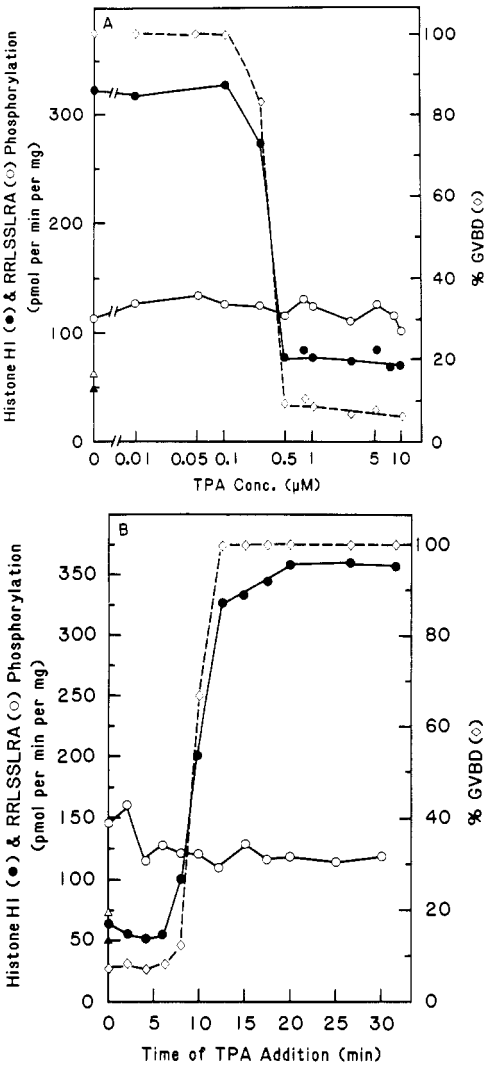

FIGURE 7: Effect of TPA on 1-MeAde-induced GVBD and histone H1 and RRLSSLRA kinase activities. Panel A: Oocytes were treated for 40 min simultaneously with 1 μM 1-MeAde and 0–10 μM TPA and subsequently processed for histone H1 (●) and RRLSSLRA (○) kinase activity determinations or scored for GVBD (◇). The histone H1 (▲) and RRLSSLRA (△) kinase activities of untreated control oocytes are shown on the y axis. Similar results were obtained in three independent experiments. Panel B: Oocytes were exposed to 1 μM 1-MeAde at time 0, and 5 μM TPA was introduced at various times. After 40 min, oocytes were scored for GVBD (◇) and processed for histone H1 (●) and RRLSSLRA (○) kinase activity determinations. The histone H1 (▲) and RRLSSLRA (△) kinase activities of untreated control oocytes are shown on the y axis.

DISCUSSION

Activation of Distinct Protein Kinases during Oocyte Maturation. In the preceding paper (Pelech et al., 1987), at least

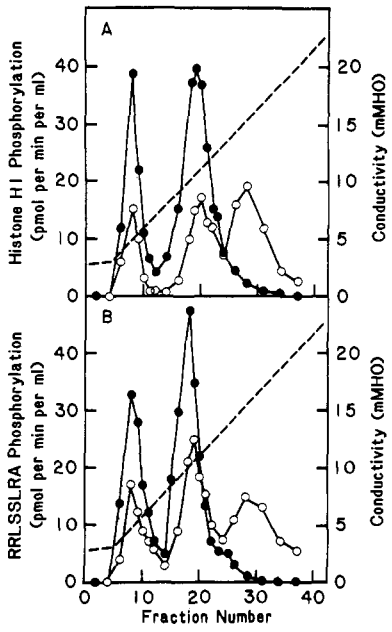

FIGURE 8: DEAE-Sephacel chromatography of histone H1 and RRLSSLRA phosphorylating activities in cytosol from TPA-treated sea star oocytes. Cytosol (7.5 mL of 2.5 mg of protein in buffer B) from untreated (○) and 1 μM TPA treated for 30 min (●) oocytes was subjected to DEAE-Sephacel chromatography, and the column fractions were assayed for histone H1 (panel A) and RRLSSLRA (panel B) activity under the conditions described in the legend to Figure 2.

three kinases, which are activated during 1-MeAde-induced sea star oocyte maturation, could be distinguished on the basis of behavior on various columns, substrate preference, and inhibition by various agents. In this study, the differential activation of these enzymes during the maturation process provided additional biological arguments for their distinction.

The major stimulated histone H1 kinase in maturing sea star oocytes elutes from DEAE-Sephacel as an ~6-mmho peak and exhibits an apparent M_r of ~90 000 on Sephacryl S-300 (Pelech et al., 1987). Presumably, this is the same histone kinase that was described by Sano (1985) in maturing sea star oocytes and by Picard et al. (1987) in early sea star embryos. In addition, this kinase resembles the "growth-associated histone kinase" (Langan, 1978), a mitosis stage specific histone H1 kinase, active during late G_2, which has been described in many types of dividing somatic cells (Lake, 1973; Gurley et al., 1974; Langan, 1978; Chambers et al., 1983; Quirin-Stricker, 1984; Woodford & Pardee, 1986).

In most cases, but with a few exceptions, histone H1 phosphorylation, thought to be catalyzed by the growth-associated histone kinase, closely correlates with chromosome condensation during late G_2 and metaphase and with the initiation of mitotic cell division [for review see Wu et al. (1986)]. Chromosome condensation is a major event during sea star oocyte maturation and occurs near the time of GVBD. It is feasible that this event is mediated by the histone H1 kinase, which is maximally activated at this time. It is possible that the histone H1 kinase also plays a fundamental role in cell division initiation by phosphorylating non-histone proteins (Adlaka et al., 1985).

Whatever the role, several lines of evidence imply that activation of this histone H1 kinase is undissociable from sea star oocyte maturation and may be critical for its induction. (i) The 1-MeAde dose–response curves for the stimulation of the histone H1 kinase and GVBD were identical. (ii) Like GVBD, activation of the histone H1 kinase required the presence of 1-MeAde for a defined length of time, the hormone-dependent period. (iii) Maximal histone H1 kinase activation slightly preceded 100% GVBD. (iv) Forskolin delayed histone H1 kinase activation and GVBD in parallel. (v) The TPA dose–inhibition curves for 1-MeAde-induced histone H1 kinase activity and GVBD were identical, as was the TPA-sensitive periods for both events. (vi) Like GVBD, histone H1 kinase activation was independent of macromolecular synthesis (Pelech et al., 1987).

Activation of the RRLSSLRA kinases (elution conductivities of ~6 and ~10.5 mmho from DEAE-Sephacel) continued after completion of GVBD in the sea star oocytes and was maximal much later on during the maturation process. This is in contrast to the situation with *Xenopus laevis* ooctyes induced to mature with progesterone (Cicirelli et al., 1987). In the frog oocytes, peak activation of the amphibian equivalents of the major stimulated histone H1 and RRLSSLRA kinases from sea star oocytes occurred near the time of GVBD. Forskolin pretreatment completely abolished 1-MeAde-induced RRLSSLRA phosphorylating activity in sea star oocytes without preventing GVBD and emission of the polar bodies. Therefore, these RRLSSLRA kinases do not appear to be critical in the induction of oocyte maturation, although they may be crucial for later stages in embryonic development.

It is likely that the pathways leading to 1-MeAde-induced stimulation of the histone H1 and RRLSSLRA kinases are complex, since it takes some time to reach full activation (20 min for the histone H1 kinase, 60 min for the RRLSSLRA kinases). The experiments in which 1-MeAde was removed from the oocytes during or after the hormone-dependent period indicated that the histone H1 kinase (and presumably the other maturation-activated kinases) activation pathway involves at least two steps: a reversible step (during the hormone-dependent period) and a committed step (after the hormone-dependent period). It is tempting to speculate that the first, reversible step is controlled by the production of a labile, as yet unknown second messenger and that the second, committed step arises from the activation by this second messenger of a kinase, possibly MPF, that phosphorylates and activates the histone H1 kinase (Pelech et al., 1987). Future studies should clarify the relationship between the histone H1 kinase and MPF.

ACKNOWLEDGMENTS

We thank Professor B. Shapiro for many discussions and continuous support during a sabbatical year spent in Seattle (by L.M.). We are grateful to Roland Anderson of the Seattle Public Aquarium, who provided most of the sea stars used in this study.

Registry No. 1-MeAde, 5142-22-3; TPA, 16561-29-8; ribosomal protein S6 kinase, 90698-26-3; histone H1 kinase, 70431-11-7; forskolin, 66575-29-9.

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Two-Dimensional Crystals of Enzyme–Effector Complexes: Ribonucleotide Reductase at 18-Å Resolution†

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Received March 11, 1987; Revised Manuscript Received June 18, 1987

ABSTRACT: The B1 subunit of ribonucleotide reductase formed two-dimensional crystals when bound to an effector nucleotide linked to lipids in planar layers at the air/water interface. The effector lipid consisted of dATP coupled through the γ-phosphoryl group and an ε-aminocaproyl linker to phosphatidylethanolamine. Two-dimensional crystals of B1 reductase, like those of antibodies and cholera toxin obtained previously, formed under physiologic conditions of pH and ionic strength, with no precipitant added to the solution. There was, however, a requirement for dTTP in the solution, presumably to ensure binding of the dATP–lipid at only one of two effector sites on the enzyme. Diffraction from the crystals extended to 18-Å resolution in negative stain, with unit cell parameters a = 110 Å, b = 277 Å, and γ = 90°. Image analysis revealed the B1 dimer as a pair of roughly cylindrical objects, each 105–109 Å in length and 31–34 Å in diameter.

Ribonucleoside-diphosphate reductase is an enzyme of broad regulatory significance, present in both prokaryotes and eukaryotes (Thelander & Reichard, 1979; Martin & Gelfand, 1981; Follman, 1982; Reichard & Ehrenberg, 1983). It catalyzes and controls the reduction of ribonucleoside diphosphates to deoxyribonucleotides and thus partitions nucleotides between RNA and DNA synthesis. The catalytic and regulatory properties of the enzyme derive from the interaction of two types of subunit in a double dimer and from a remarkable set of allosteric effects. In *Escherichia coli*, the dimers are known as B1 (177 000 daltons) and B2 (87 000 daltons) and contain binding sites for effectors (nucleoside triphosphates) and a source of reducing power (dithiols and a stable free radical), respectively. Mechanistic studies have been limited by a lack of structural information. The dimers separate during purification, and single crystals of each dimer have been obtained, but X-ray diffraction analyses have not been completed (Joelson et al., 1984). We report here on imaging the B1 dimer by formation of two-dimensional crystals

† This research was supported by NIH Grants AI21144 and GM30387 to R.D.K. and by a grant from the Swedish Medical Research Council to P.R. H.O.R. received support from Cellular and Molecular Biology Training Grant GM07276-12 from the National Institutes of Health.

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0006-2960/87/0426-7974$01.50/0