Identification of the ATP + Mg-dependent and polycation-stimulated protein phosphatases in the germinal vesicle of the *Xenopus* oocyte

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Two protein phosphatase activities were characterized in the germinal vesicle of *Xenopus laevis* oocytes after manual dissection of the nucleus. One enzyme can be classified as an active form of the ATP + Mg-dependent (AMD) phosphatase, the other as a polycation-stimulated (PCS) phosphatase. The activity of the PCS phosphatase is localized exclusively in the soluble compartment of the nucleus (nucleoplasm). The catalytic subunit of the AMD phosphatase activity is associated either with the nuclear particulate fraction or with an inhibitory subunit in the nucleoplasm.

INTRODUCTION

Fully grown *Xenopus laevis* oocytes are physiologically arrested in the prophase of the first meiotic division. Oocytes treated with progesterone progress through meiotic maturation and arrest at metaphase II. Post-translational modifications, rather than transcriptional regulatory devices, are presumed to play a major role in the transition through this cell cycle. Evidence supports the hypothesis that the prophase arrest of the oocyte is maintained by inhibitory phosphoproteins, the phosphorylation state of which is controlled by the cyclic AMP-dependent protein kinase and the ATP + Mg-dependent (AMD) phosphatase, also termed phosphatase 1 ([1–3] see ref. [4] for nomenclature). Many studies also imply that meiotic as well as mitotic cell division is controlled by a factor or factors, known as maturation promoting factor(s) (MPF), and that MPF activity and protein phosphorylation are closely related. The appearance of MPF activity and a major burst in total protein phosphorylation during progesterone-induced oocyte maturation are approximately coincident [5]; highly purified preparations of MPF contain protein kinase activity [6], and transition between inactive pre-MPF and active MPF could be due to the inactivation of a phosphatase [7].

An increase in protein phosphorylation can be triggered by several mechanisms: changes in substrate concentrations, increased protein kinase activity, increases in both phosphatase and kinase activities that would stimulate phosphate turnover, or decreased phosphatase activity. Many distinct activities of endogenous oocyte kinases are enhanced at the time of the appearance of MPF [8], among them the S6 kinase II activity [9] and a Ca^{2+} - and cyclic nucleotide-independent

histone kinase activity [10]. Different protein phosphatase activities were previously identified in *Xenopus laevis* oocytes, and although further work is necessary to fully characterize these phosphatases, the oocyte seems to possess a phosphatase equipment similar to most mammalian tissues examined [11,12]. The AMD phosphatase is present in different forms. It can be isolated from the cytosol as an inactive form that can be activated by protein kinase F_A , also present in the oocyte [11]. The activity of the AMD phosphatase in the cytoplasmic particulate fraction is largely suppressed. This activity can be stimulated by the addition of the deinhibitor protein (DI) plus *p*-nitrophenyl phosphate (*p*NPP), or can become expressed by partial trypsinolysis (S. Staquet & J. Goris, unpublished work). In addition to a Ca^{2+} /calmodulin-dependent phosphatase, an active form of the AMD phosphatase seems to function as S_6 phosphatase in oocytes and might be involved in the increase in S_6 phosphorylation observed after mitogenic stimulation [12]. The polycation-stimulated (PCS) phosphatases, also termed phosphatases 2A (see ref. [4]) are also present in the cytosol of *Xenopus laevis* oocytes, and the PCS_L phosphatase has recently been purified to homogeneity [13]. As in skeletal muscle, the Ser/Thr-specificity of the PCS_L phosphatase can be changed into a Tyr-specific phosphatase [13,14]. In addition, other Tyr-phosphatases ([14], P. Hendrix, unpublished work) and a Ca^{2+} /calmodulin-dependent phosphatase [11,12] were identified. However the regulation of the protein phosphatases in *Xenopus in vivo* is largely unknown.

The rate of phosphate turnover in proteins is increased in the M-phase [15], suggesting that both protein kinase and protein phosphatase activities are increased after breakdown of the nuclear envelope. Because of the simultaneous release of the contents of the germinal

Abbreviations used: MPF, maturation promoting factor; AMD phosphatase, ATP + Mg-dependent phosphatase; PCS phosphatase, polycation-stimulated phosphatase; DI, deinhibitor protein; *p*NPP, *p*-nitrophenyl phosphate.

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vesicle into the cytoplasm, it was of interest to investigate the protein phosphatases in the nucleus. We presently describe the intranuclear localizations of the AMD and PCS phosphatases.

MATERIALS AND METHODS

Phosphorylase [16], the active catalytic subunit of the AMD protein phosphatase [17], inhibitor-1 [18] and DI [17] were purified according to published methods. ^{32}P -labelled phosphorylase and the phospho form of inhibitor-1 were prepared as described [19]. Collagenase type I, dispase II and DNAase I (grade II) were obtained from Boehringer; phenyl-Superose and Superose-12 columns were from Pharmacia. *Xenopus laevis* adult females (SEREA-CNRS, Montpellier, France) were bred and maintained under laboratory conditions. Other materials were obtained from sources described in [20].

Ovaries removed from anaesthetized animals were transferred to medium A (88 mM-NaCl, 0.33 mM- CaNO_3 , 1 mM-KCl, 0.41 mM- CaCl_2 , 0.82 mM- MgSO_4 , 10 mM-Hepes, pH 7.4) and digested with dispase and collagenase as described [13]. Defolliculated full-grown stage VI [21] oocytes were carefully selected and enucleated manually, using a procedure adapted from [22]. After incubation for 20 min in medium A diluted 1:10 and containing an additional 10 mM- MgCl_2 , a small incision was made obliquely at the animal pole and the translucent germinal vesicle emerged from the slit. A standard preparation contained 200 nuclei.

The nuclei were transferred to 1 ml of medium R (1 mM-EDTA, 0.5 mM-dithiothreitol, 1 mM-phenylmethanesulphonyl fluoride, 1 mM-benzamidine, 250 mM-sucrose, 50 mM-Tris/HCl, pH 7.4) at 0–4 °C, and centrifuged for 1 min at 1000 g, in order to pellet the nuclei without rupturing. After removal of the supernatant, the nuclei were resuspended and rinsed first in 0.5 ml and then three more times in 0.25 ml of medium R. They were broken by hypotonic shock at 4 °C in 0.2 ml of medium H1 (50 mM-NaCl, 1 mM-EDTA, 1 mM-EGTA, 0.5 mM-dithiothreitol, 1 mM-phenylmethanesulphonyl fluoride, 1 mM-benzamidine, 0.01 mM-leupeptin, 50 mM-Tris/HCl, pH 7.4) and centrifuged (Beckman Airfuge, 207 kPa, 130 000 g, 10 min). The pellet was resuspended in 0.1 ml of medium H1 at 4 °C and rinsed four times in these conditions. The last pellet was resuspended at 4 °C in 0.1 ml of medium H1 containing 0.5 % Triton X-100, centrifuged at 207 kPa, 130 000 g, for 10 min, and the resulting pellet was resuspended in 0.1 ml of medium H1 containing 1 % Triton X-100. After centrifugation (207 kPa, 130 000 g, 10 min), the pellet was rinsed in 0.2 ml of medium H1, resuspended in 0.1 ml of medium H1 containing 0.6 M-NaCl and centrifuged at 207 kPa, 130 000 g for 10 min. All the supernatants and pellets resulting from this procedure were assayed for the presence of protein phosphatases.

To separate the protein phosphatases in the soluble nuclear fraction (i.e. the two pooled supernatants after hypotonic disruption and wash of the first pellet) this fraction was made 0.8 M- $(\text{NH}_4)_2\text{SO}_4$, applied on a f.p.l.c. phenyl-Superose column equilibrated with 0.8 M- $(\text{NH}_4)_2\text{SO}_4$ in medium H2 (1 mM-EDTA, 1 mM-EGTA, 0.5 mM-dithiothreitol, 1 mM-phenylmethanesulphonyl fluoride, 1 mM-benzamidine, 0.01 mM-leupeptin, 50 mM-Tris/HCl, pH 7.4), and eluted with a linear descending 0.8–0 M- $(\text{NH}_4)_2\text{SO}_4$ gradient in medium H2. After dialysis

against medium H2, the fractions obtained were concentrated against 20 % (w/v) poly(ethylene glycol) in medium H2 and assayed.

To prepare the cellular fractions, oocytes were rinsed three times with medium R, homogenized in 3 vol. of the same buffer, and 180 μl per tube were centrifuged for 30 s at minimal speed in the Airfuge (about 1000 g). This procedure pelleted the nuclei and the bulk of the vitellus. The layer between the floating lipids and the pellet (about 130 μl , crude extract) was recentrifuged for 10 min at 207 kPa, 130 000 g. The clear supernatant (about 100 μl , cytosol) was carefully removed, and the pellet containing glycogen, ribosomes and microsomes was resuspended in 130 μl of medium R.

Phosphorylase phosphatase was assayed by measuring the release of ^{32}P phosphate from labelled phosphorylase *a* as described in [13]. PCS phosphatase activity [19] was detected by including 35 μg of protamine hydrochloride/ml in the assay mixture. It was observed that the polycation stimulation in the crude cellular fractions could be obtained only with protamine concentrations five times higher (35 $\mu\text{g}/\text{ml}$) than the optimum concentrations for the purified enzymes. In order to avoid the protamine inhibition observed at these same high concentrations [19], 16 mM- $(\text{NH}_4)_2\text{SO}_4$ was added in the assay to completely block the inhibition of the PCS phosphatases by polycations. Under these conditions, the active forms of the AMD phosphatase are inhibited more than 95 % (J. Goris, unpublished work). The specific assay of active forms of the AMD phosphatase was based on the characteristic stimulation of the enzyme by the DI and *p*NPP [20,23].

RESULTS

Protein phosphatase activity in the nuclear fractions

The amount of protein phosphatase activity was determined in the nuclear fractions prepared as described in the Materials and methods section, using ^{32}P -labelled phosphorylase *a* as the substrate. Spontaneously active phosphorylase phosphatase activity (assayed without added effectors) was recovered in different fractions (Fig. 1). In the supernatants of the sucrose-containing rinse medium R, this activity probably originated from both contaminating cytoplasm (see below), and material which had escaped through nuclear pores despite the high osmotic strength. After five washes of the nuclei, only a slight activity was detected in the last supernatant (Fig. 1), suggesting that the activity did not originate from the outer surface of the nuclear membrane. To reinforce this view, the outer nuclear membrane was removed by washing resuspended nuclei in medium R containing 0.5 % Triton X-100, without causing nuclear lysis [24]. This additional step in the procedure did not extract phosphatase activity (results not shown), implying that no phosphatase activity was associated with the outer nuclear membrane. When nuclei disrupted by osmotic shock in the hypotonic medium H1 were centrifuged, a strong activity (Fig. 1) was detected in the supernatant, representing the soluble nuclear proteins. After four washes of the insoluble pellet with medium H1, no more activity was released (Fig. 1). When the final pellet was treated with up to 1 % Triton X-100 in medium H1, no protein phosphatase activity was released, suggesting that the nuclear protein phosphatases were not

Fig. 1. Subnuclear distribution of protein phosphatase activity in *Xenopus laevis* oocytes

Germinal vesicles (200) were prepared and fractionated as described in the Materials and methods section. The phosphorylase phosphatase activity was measured without effectors (▨), or after preincubation with DI/*p*NPP (□) or in the presence of protamine (■).

membrane-bound (Fig. 1). Protein phosphatase activity could be further extracted from the insoluble pellet with 0.6 M-NaCl (Fig. 1), a treatment which solubilizes most of the chromatin proteins [24]. Therefore the intranuclear protein phosphatases appear to be localized in the nucleoplasm and associated with the chromatin or other insoluble material.

Identification of two types of nuclear protein phosphatases

In order to determine the types of the nuclear protein phosphatases, the assays were performed in the presence of their effectors (Fig. 1). The AMD phosphatase activity is inhibited by protamine and enhanced by DI and *p*NPP, while the PCS phosphatases are stimulated by protamine and inhibited by *p*NPP. Checking the effect of protamine and DI/*p*NPP on the phosphorylase phosphatase activity in the first sucrose/50 mM-NaCl washes and in the 0.6 M-NaCl wash, substantial differences can be observed. (1) Whereas in the first sucrose wash, protamine stimulates the phosphatase activity about 3-fold, there is only a slight stimulation in the first 50 mM-NaCl wash (1.3-fold), and an inhibition in the 0.6 M-NaCl wash. (2) With DI/*p*NPP, there is only a slight stimulation in the first sucrose wash (1.35-fold), while they stimulate the phosphatase activity by about 3-fold in the first 50 mM-NaCl wash and only by 1.8-fold in the 0.6 M-NaCl wash. Based on these observations and the known effects on purified phosphatases, one can assume that the ratio of PCS/AMD phosphatases in the first sucrose wash (cytoplasm) and the first 50 mM-NaCl (nucleoplasm) is different. In the cytoplasm (Table 1) as in the first sucrose wash (Fig. 1), the basal activity can largely be ascribed to PCS phosphatases(s). Since there is

still a stimulation by DI/*p*NPP, the AMD phosphatase is certainly present. This could be further substantiated, since after a high-speed centrifugation the stimulation by DI/*p*NPP is recovered specifically in the pellet, resulting in an increased protamine-stimulation of the phosphatase activity in the supernatant (Table 1). In the nucleoplasm, the ratio of PCS/AMD phosphatases is in favour of the AMD phosphatases, which is revealed by a pronounced stimulation by DI/*p*NPP and a weak stimulation by protamine, inhibiting the AMD phosphatase. These assumptions are proven to be correct since both phosphatases could be separated by phenyl-Superose chromatography and partially by gel-filtration (see

Table 1. Identification of the protein phosphatases present in the cytoplasm of *Xenopus laevis* oocytes

Cellular fractions were prepared and assayed for phosphorylase phosphatase activity as described in the Materials and methods section. Note: the stimulation of the phosphorylase phosphatase activity in the cellular extract by protamine in the standard assay conditions [35 µg of protamine per ml/16 mM-(NH₄)₂SO₄] is very low and variable, probably through interaction of protamine with other cellular components.

Fraction	Activity (munits/oocyte)		
	Basal	+ DI/ <i>p</i> NPP	+ Protamine
Crude extract	25.4	91.5	—
High-speed supernatant	11.8	15	459
High-speed pellet	5.4	40.9	3

Table 2. Identification of protein phosphatase activity in the nuclear particulate fraction of *Xenopus laevis* oocytes after treatment with high salt concentration and DNAase

The nuclear particulate fraction of 333 nuclei was prepared without the Triton washes (see the Materials and methods section) and resuspended in 0.1 ml of medium H1. After addition of 10 μ l of 3.6 M-NaCl or 10 μ l of 10 mg of DNAase/ml to 50 μ l of the resuspended pellet, the incubation was carried out for 20 min at 30 °C, followed by a centrifugation for 10 min at 207 kPa in the Airfuge. The pellet obtained after the DNAase treatment was re-extracted with 60 μ l of 0.6 M-NaCl in medium H1 under the same conditions. Phosphorylase phosphatase was measured in the supernatants after a preincubation in the presence or absence of DI/pNPP. Results are expressed in munits of phosphatase activity per oocyte.

Treatment	Activity (munits/oocyte)	
	Basal	+ DI/pNPP
0.6 M-NaCl supernatant	0.68	1.60
DNAase supernatant	0.22	0.44
0.6 M-NaCl supernatant of DNAase pellet	0.32	0.52

below). Furthermore, the sensitivity to inhibitor-1 and trypsin treatment (see below) are in agreement with the assumption that two types of phosphatases are present in the nucleoplasm.

The protein phosphatase released from the nuclear particulate fraction by high salt concentration (Table 2) appears to be exclusively the AMD phosphatase, since the enzyme activity, which is completely inhibited by protamine, is stimulated by DI/pNPP. In the residual pellet, no phosphatase activity could be detected. The AMD phosphatase could also be partially released from the insoluble pellet by treatment with DNAase 1 (Table 2), indicating that this enzyme is associated with chromatin. After DNAase extraction, the remaining pellet still contained AMD phosphatase activity, which could then be released by 0.6 M-NaCl (Table 2). This suggests that this enzyme could be associated with non-chromatin material such as the lamin network.

All of these results indicate the presence of two types of protein phosphatase activities in the nucleus: most of the PCS phosphatase activity is localized in the soluble nucleoplasm, while the AMD phosphatase activity is distributed between soluble and particulate material.

Separation of the two types of soluble nuclear protein phosphatases

After five washes in medium R containing 250 mM-sucrose, nuclei were homogenized in medium H1 and centrifuged. The pellet was resuspended in the same medium, centrifuged, and the two supernatants were combined. This soluble fraction was chromatographed to separate the two protein phosphatase activities previously detected. When the extract was applied to a phenyl-Superose column the AMD phosphatase activity, inhibited by protamine and stimulated by DI/pNPP, eluted in the breakthrough, while the PCS phosphatase activity, stimulated by protamine and inhibited by

Table 3. Separation of two types of protein phosphatases present in the nucleoplasm of *Xenopus laevis* oocytes by hydrophobic column chromatography

The nucleoplasm of 500 germinal vesicles (combined first two 50 mM-NaCl washes) was prepared, applied and eluted from a phenyl-Superose column (5 cm $\times$ 0.5 cm) as described in the Materials and methods section and in the text. The phosphorylase phosphatase activity detected in the breakthrough fraction [0.8 M-(NH₄)₂SO₄] and at the end of the descending salt gradient (15 ml, flow rate 0.5 ml/min) at about 50 mM-(NH₄)₂SO₄ was assayed as described in the legend to Fig. 1.

Fraction	Activity (munits/ml)		
	Basal	+ DI-pNPP	+ Protamine
0.8 M-break-through	380	625	117
50 mM eluate	93	14	272

DI/pNPP, was recovered as a single peak at the end of the linear descending gradient (Table 3).

Characterization of the different protein phosphatases

Following analysis of the soluble nuclear fraction by Superose-12 column chromatography (Fig. 2a), and further characterization of the phosphatases based on the effects of protamine, DI and pNPP on their activities, a single 185 kDa PCS phosphatase and two AMD phosphatases, a minor 185 kDa and a major 90 kDa enzyme, could be identified. This result suggests that PCS phosphatase in the nucleoplasm is an oligomeric form of the enzyme. The nature of the nucleoplasmic AMD phosphatase was further investigated since its molecular mass and the effects of the DI in the absence of pNPP are indicative of an active catalytic subunit suppressed by an inhibitor subunit. Limited trypsinization (Table 4) resulted in an increased enzyme activity, a well known characteristic of the active forms of the AMD phosphatase [25], since the active catalytic subunit is extremely resistant to trypsinization in contrast

Table 4. Effect of trypsin treatment on phosphatases present in the nucleoplasm and nuclear particulate fraction of *Xenopus laevis* oocytes

The nucleoplasm (N) and 0.6 M nuclear particulate extract (PE) were or were not treated with 25 μ g of trypsin/ml for 3 min at 30 °C. Soybean trypsin inhibitor (166 μ g/ml) was added to stop the reaction and phosphorylase phosphatase was assayed after preincubation with the indicated effectors as described in the Materials and methods section.

Frac-tion	Phosphatase activity (munits/oocyte)							
	No trypsin				+ Trypsin			
	Basal	pNPP	DI	DI/pNPP	Basal	pNPP	DI	DI/pNPP
N	1.08	1.59	2.20	4.36	2.60	4.79	3.40	6.47
PE	0.49	1.09	0.74	1.69	0.71	1.34	0.91	1.65

Fig. 2. Gel filtration of the protein phosphatases present in (a) the nucleoplasm and (b) the nuclear particulate fraction from *Xenopus laevis* oocytes

(a) Nuclei (500) were prepared and extensively washed as described in the Materials and methods section. Homogenization and high-speed centrifugation were carried out in 160 μ l of medium H1, and the supernatant was applied to a Superose-12 column (29 cm $\times$ 1 cm) equilibrated in 20 mM-Tris/HCl, pH 7.4/0.5 mM-dithiothreitol/0.1 M-NaCl/1% (v/v) isopropanol. The flow rate was 0.4 ml/min and fractions of 0.5 ml were collected. Phosphorylase phosphatase activity was measured as indicated in the Materials and methods section without effectors ($\circ$), in the presence of protamine ($\square$), or after preincubation with DI ($\blacktriangle$) or DI/pNPP ($\bullet$). The arrows show the elution position of the marker proteins in control runs: 1, aldolase (158 kDa); 2, bovine serum albumin (67 kDa); 3, the catalytic subunit of the AMD phosphatase (33 kDa); 4, chymotrypsinogen (25 kDa); 5, ribonuclease (13.7 kDa). The V_0 and V_i were 7.7 ml and 19.5 ml respectively. (b) The nuclear particulate pellet obtained as described above was extensively washed, treated with Triton X-100 and extracted with 0.6 M-NaCl (160 μ l) as described in the Materials and methods section. This extract was applied to the column, eluted and assayed as described above.

with other (inhibitory) subunits. The different PCS phosphatases are also destroyed by the same treatment [26]. Together with this activation by trypsin treatment, the effect of the DI was lost [the slight increase still observed after trypsin treatment (Table 4), is probably due to incomplete proteolysis]. Notwithstanding the presence of an inhibitory subunit, this enzyme form is less sensitive to the phospho-form of inhibitor-1 than the free catalytic subunit (Fig. 3).

When the phosphatase extracted from the nuclear particulate fraction was chromatographed on a Superose-12 column, a different pattern was obtained (Fig. 2b) and only the 35 kDa subunit, migrating at the same position as the catalytic subunit of the AMD phosphatase, could be detected. This 35 kDa subunit was only slightly stimulated by the DI (Fig. 2b, Table 4) or by trypsin treatment (Table 4), and was almost equally sensitive to phospho-inhibitor-1 as the purified catalytic subunit of the AMD phosphatase (Fig. 3). As previously shown for the active catalytic subunit of the AMD phosphatase [23], pNPP alone was still stimulatory (Table 4). No inactive form of AMD phosphatase that could be activated by protein kinase F_A , and neither the latter enzyme nor the modulator protein could be detected in any of the nuclear compartments.

Fig. 3. Sensitivity of the protein phosphatases present in the nucleoplasm (Δ) and nuclear particulate fraction ($\square$) from *Xenopus laevis* oocytes and the catalytic subunit of the AMD phosphatase ($\circ$) to inhibitor-1

Phosphorylase phosphatase assays were carried out as described in the Materials and methods section, in the presence of the indicated concentrations of the phospho-form of inhibitor-1. Data are expressed as percentage of control activity obtained in the absence of inhibitor-1.

DISCUSSION

In this study, we have investigated the different forms of protein phosphatases present in the germinal vesicle of the *Xenopus laevis* oocyte. One of these, localized in the nucleoplasm, is stimulated by DI and by pNPP, is activated by limited trypsinization and is inhibited by phospho-inhibitor-1, and can therefore be considered as an active form of the AMD protein phosphatase. The second enzyme, also nucleoplasmic, is stimulated by protamine and can therefore be identified as a PCS phosphatase. A third phosphatase could be extracted from the particulate fraction and is identified as the catalytic subunit of the AMD phosphatase. Previous studies have described phosphatases in the nuclei of somatic cells [24,27,30]. In all these cases, the enzymes were solubilized from crude chromatin using high NaCl concentrations, and no protein phosphatase activities were detected in the soluble compartment of the nuclei. However we now have detected high levels of soluble protein phosphatase activities in the nucleoplasm of the germinal vesicle. The method of preparation of somatic nuclei, based on the method of Blobel & Potter [28], possibly cannot prevent the escape of soluble material through nuclear pores. The homogenization technique clearly affects the distribution of phosphorylase phosphatase activities between pelleted nuclei and supernatant [29]. Such loss could explain why the previous works failed to recover phosphatase activities in the nucleoplasm. The manual enucleation procedure allows for a better control of the integrity of the nuclei. Another explanation could be that the presence of these activities in the nuclear soluble compartment could be typical for the oocyte, a germinal cell, in contrast with somatic cells (liver cell and HeLa cells) previously studied.

Jakes *et al.* [30] have shown that rat liver nuclei contain two protein phosphatases identified as AMD and PCS phosphatases, whereas Kuret *et al.* [24] demonstrated in the same material the presence of the AMD phosphatase, without mentioning the presence of PCS phosphatases. Friedman [27] showed in HeLa cell nuclei the presence of a protein phosphatase activity partially inhibited by the modulator protein and stimulated by spermine. He concluded that the nuclei contained an AMD phosphatase activated by spermine. However these results are compatible with the presence of both AMD and PCS phosphatase activities. Our data show, at least in oocytes, that both of these activities are present in the germinal vesicle: a PCS protein phosphatase soluble in the nucleoplasm, and AMD-type phosphatases, localized both in the nucleoplasm and in the insoluble compartment. A further analysis of the phosphatases present in HeLa cell nuclei would probably have also revealed the presence of two types of phosphatase, thus avoiding the conclusion that the AMD phosphatase is activated by spermine. Since the AMD phosphatase activity extracted by 0.6 M-NaCl from the insoluble nuclear pellet can also be released by treatment with DNAase I, it is probable that this enzyme is associated with the chromatin.

The AMD phosphatase from the rat liver nuclei has been shown to exist as a monomeric catalytic subunit [24,30]. We showed here that two different forms of the AMD phosphatase are present in the germinal vesicle of *Xenopus laevis* oocytes: one nucleoplasmic form that exists as a complex between the catalytic subunit and an

inhibitory protein, and a particulate form which is solubilized by high salt concentrations. The nucleoplasmic form resembles very closely the recently-identified spontaneously-active DI-stimulated AMD phosphatase present in dog liver [20]. This enzyme exists as a two-subunit structure, consisting of a 35 kDa catalytic subunit and a 72 kDa inhibitory subunit. It is tempting to speculate that this 72 kDa subunit would represent a carrier protein essential for the transport of the catalytic subunit towards different cellular localizations. Indeed, in addition to the nuclear particulate forms of the enzyme, the same active catalytic subunit of the AMD phosphatase can be found associated with the glycogen particle [31–33], myosin [34] or microsomes [31,35], or in its inactive form in the cytosol [25]. The hypothesis can be formulated that this same catalytic subunit would shuttle between these different cellular localizations under different physiological conditions. The local concentration would be determined by the relative affinity of the phosphatase towards these different anchors. The finding that the AMD phosphatase is also in the nucleoplasm complexed with inhibitory components would only substantiate this hypothesis. Based on a nucleus/cytoplasm volume index of 12% in *Xenopus* oocytes [36], the concentration of the phosphatase stimulated by DI/pNPP is in the same range in the cytoplasm as in the nuclear compartment. Some nuclear proteins have been shown to require signal sequences for entry into the nucleus, but the nuclear targeting signals show a high degree of variability from protein to protein [37]. None of the known nuclear targeting signals were found in the sequences of the isoforms of the catalytic subunit of the AMD phosphatase from rabbit skeletal muscle [38], nor in the two isoforms of the catalytic subunit of the PCS phosphatases [39]. Nevertheless it is possible that the nuclear targeting signal is present in the regulatory subunits that are associated with the catalytic subunit.

The presence of nuclear protein phosphatases is important, since the cell nucleus is rich in phosphoproteins characterized by a rapid turnover of the phosphate groups [40]. It is probable that phosphorylation in the nucleus plays an important role in chromosome condensation, lamin polymerization, transcription via RNA polymerase II or via the nuclear phosphoprotein CREB, polyamine synthesis and other nuclear functions [41–43]. The presence of nuclear protein phosphatases is especially important in those cells which progress through mitotic cell division. The contents of the nucleus are released and dispersed in the cytoplasm at each G₂–M transition. Oocytes treated with progesterone progress through meiotic maturation, and extensive changes in protein phosphorylation occur at the exact time of germinal vesicle breakdown. It is interesting to speculate on how nuclear protein phosphatases are involved at the different steps of this meiotic cell division.

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