PROTEIN KINASE ACTIVITIES DURING MATURATION IN XENOPUS LAEVIS OOCYTES.

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SUMMARY.

Protein kinase activities have been compared in ovarian oocytes and in ovulated eggs of Xenopus laevis.

In ovaries and ovarian oocytes, we have detected, in addition to an already known (1) cyclic AMP stimulated phosphoprotein kinase, a second very active phosphoprotein kinase which is cAMP-independent.

Besides these two activities, a third protein kinase activity becomes detectable after maturation and ovulation : it is a cAMP and cGMP-dependent histone kinase.

INTRODUCTION.

At the end of oogenesis, amphibian oocytes are blocked in the diplotene stage of meiotic prophase. During maturation, they proceed until metaphase of the second meiotic division; they remain arrested at that stage until fertilization. Pituitary hormones and progesterone can induce the processus of maturation and ovulation, both in vivo and in vitro (2).

In Rana pipiens, Morrill and Murphy (3) have reported that maturation is characterized by an intense phosphorylation of proteins. They suggested that the hormone-induced release from the prophase block might result from either the induction of a new protein kinase, or the activation of a preexistant enzyme (possibly via cAMP).

Tenner and Wallace (1) have identified a cAMP-dependent phosphoprotein kinase activity in the ovary of Xenopus laevis.

The present results show the existence, in ovarian oocytes, of an additional phosphoprotein kinase which is, on the contrary, cAMP independent. Besides these two activities, a different cAMP-stimulated kinase activity, with a high specificity for histone, appears during maturation.

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MATERIALS AND METHODS.

Large (stage 6) ovarian oocytes were isolated out of ovaries which had been treated during 15 h. at 20°C with a 0.1 % collagenase (Worthington Biochemical Corporation) in a modified (4) Barth's medium. Oocytes which had undergone maturation and ovulation by in vitro treatment of ovaries with progesterone and pituitary extract, and unfertilized eggs were obtained according to Hanocq et al (5).

Calf thymus histone (type II A), egg vitellin phosvitin and cyclic nucleotides were purchased from Sigma Corp. γ-[^{32}P]ATP was obtained from New England Nuclear.

Protein kinase activity was tested in an incubation volume of 0.35 ml, containing : 17.5 μmoles sodium glycerophosphate buffer (pH 6), 0.7 μmole theophyllin, 2.5 μmoles NaF, 0.1 μmole EDTA, protein substrate (variable), enzyme (variable), 3.5 μmoles Mg acetate, 2nmoles γ-[^{32}P]ATP 10^6-2.10^6cpm/nmole), with or without 1.75 nmole cAMP. Incubations were carried out for 30 min. at 30°C. Aliquots of the reaction mixtures (100 μl) were delivered on filter paper discs (Whatman No. 3 MM, 2.3 cm $\emptyset$). The discs were transferred into cold 10% trichloroacetic acid (TCA) - 0.1 M KH_2PO_4, washed three times with the same medium, then three times with 5% TCA - 0.05 M KH_2PO_4. The discs were rinsed in 90% ethanol, dried and counted in 5 ml of toluene-Omnifluor scintillation liquid with a Packard Tri-Carb 3380.

RESULTS.

The isolation and fractionation of protein kinases from the ovarian oocytes or the hormone-treated oocytes, was performed according to the procedure developed by Tenner and Wallace for amphibian ovaries (1).
After elution from the DEAE-cellulose column, each fraction was tested for protein kinase activity towards both histone (part a of all figures) and phosvitin (part b of all figures), as substrates, in the presence and in the absence of cAMP.

Protein kinase activities in ovaries and ovarian oocytes.

Fig. 1 shows the elution profile obtained when a homogenate from total

ovaries, precipitated with $(NH_4)_2SO_4$ between 0.20 and 0.35 g/ml, was submitted to chromatography on DEAE-cellulose. Three peaks of protein kinase activities can be detected. The peaks A_1 and A_2 probably correspond to the single peak of kinase activity described by Tenner and Wallace [1], which cata-

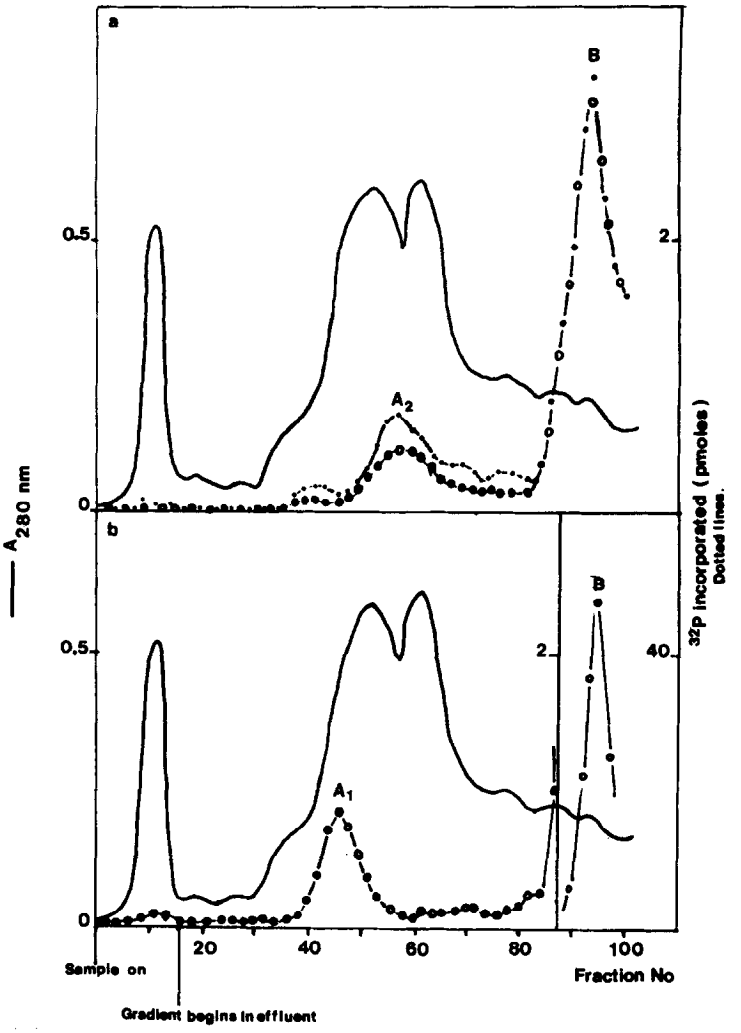

Fig. 1 - DEAE-cellulose chromatography profile of kinases from 3 ovaries. The DEAE-cellulose column (14 x 3.5 cm) was equilibrated with 5 mM potassium phosphate - 2 mM EDTA and eluted with 150 ml of the same buffer, followed by a continuous concave gradient ranging from 5 mM potassium phosphate - 2 mM EDTA (pH 7.0)(2 x 335 ml in each mixing chambers) to 500 mM potassium phosphate - 2 mM EDTA (pH 7.0)(330 ml in the reservoir) (6). Flow rate of the column was 80 ml/h; 10 ml fractions were collected, 50 µl of which were assayed for protein kinase activities as indicated in Methods. a. Histone kinase activity (0.25 mg/ml histone). b. Phosvitin kinase activity (3mg/ml phosvitin). o—o Without cyclic AMP. •—• With cyclic AMP (5 µM).

lyzes the phosphorylation of both histone and phosvitin. On the contrary, peak B, which elutes at a higher ionic strength (potassium phosphate 0.4 M), is a cAMP independent phosphoprotein kinase. It has a much higher activity than peaks A_1-A_2 and is more stable upon dialysis, concentration by ultrafiltration and storage at -20°C. Peak B is still present when the extract is made from isolated large ovarian oocytes instead of whole ovaries (fig. 2).

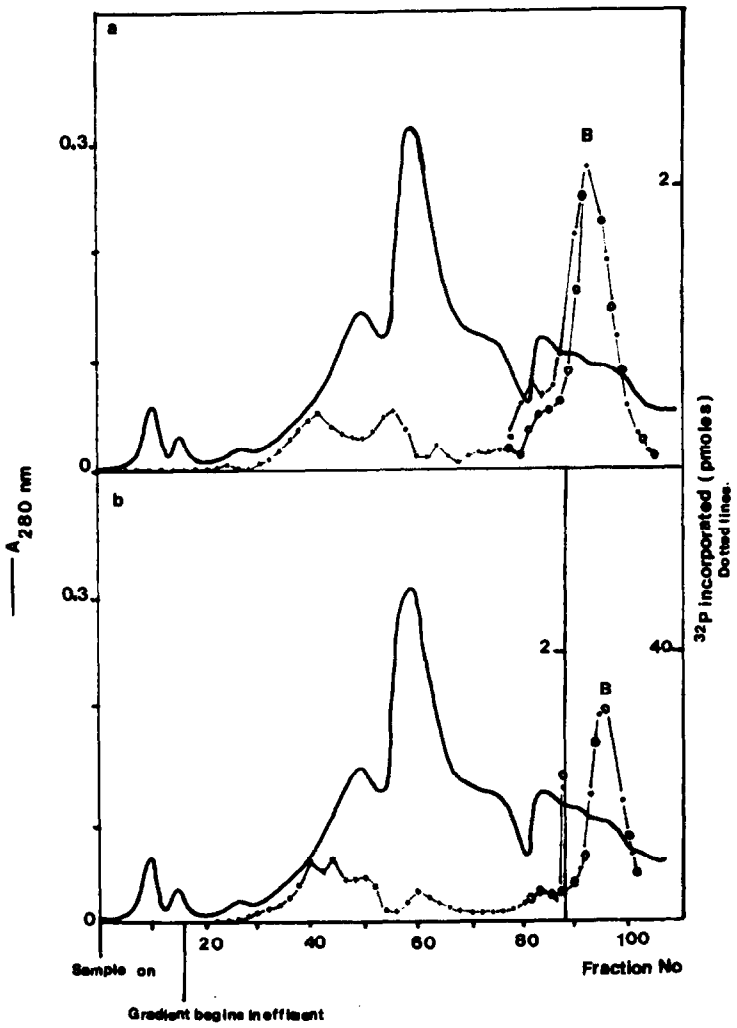

Fig. 2 - Chromatography profile of protein kinases from 30.000 large oocytes separated from the ovary by collagenase treatment. Same conditions as those described in fig.1.a. Histone kinase activity (0.25 mg/ml histone). b. Phosvitin kinase activity (3 mg/ml phosvitin). •——• Without cyclic AMP. •——• With cyclic AMP (5 M).

TABLE I. PROTEIN KINASE ACTIVITY IN $(NH_4)_2SO_4$ 0.20-0.35 g/ml FRACTIONS (pmoles $min.^{-1}mg^{-1}$).[x]

Origin of the preparation	Histone		Phosvitin	
	No cAMP	With cAMP	No cAMP	With cAMP
Ovarian large oocytes	5.7	7.1	2.6	2.6
Id. + progesterone	5.3	11.7	6.1	5.5
Id. + (progesterone (pituitary extract	4.8	12.4	4.6	4.6
In vitro ovulated oocytes	4.0	10.7	---	5.5

[x] All date have been corrected for protein kinase activity detected in the absence of substrate. The concentrations were 2 mg/ml of substrate, 25 μM [γ^{32}P]ATP (10^6 cpm/nmole) and 5 μM cAMP. The amount of preparation was 80 to 160 μg/100μl. The oocytes from three different animals were pooled in equal proportions. The same pool was used for each preparation.

Protein kinase activities in hormone-treated oocytes : appearance of a different cAMP dependent histone kinase, at maturation.

Table I shows that after hormone-induced maturation, the specific activity of protein kinase found in the $(NH_4)_2SO_4$ 0.20-0.35 g/ml fraction becomes more elevated when the substrate used is histone in the presence of cAMP.

The chromatogram obtained from in vitro ovulated oocytes (fig. 3) shows that a new kinase activity (peak M) elutes close to the B peak, at 0.25 M potassium phosphate. It differs clearly from the latter by its specificity for histone and its dependance towards cAMP. The two distinct peaks of activity (B and M), which elute at high ionic strength, were also detected in extracts from unfertilized eggs.

Table II shows the difference existing between the protein kinase activity of peaks B and M, both obtained from in vitro ovulated oocytes, with respect to substrate specificity and stimulation by c-nucleotides (cAMP and cGMP).

Although histone is a good substrate for protein kinase B, the enzyme

seems to phosphorylate phosvitin more efficiently; neither cAMP, nor cGMP stimulates these reactions. In constrast, protein kinase M is a specific histone kinase, the activity of which is markedly increased in the presence of 5 μM of either cAMP or cGMP. With phosvitin as substrate, the activity is much lower

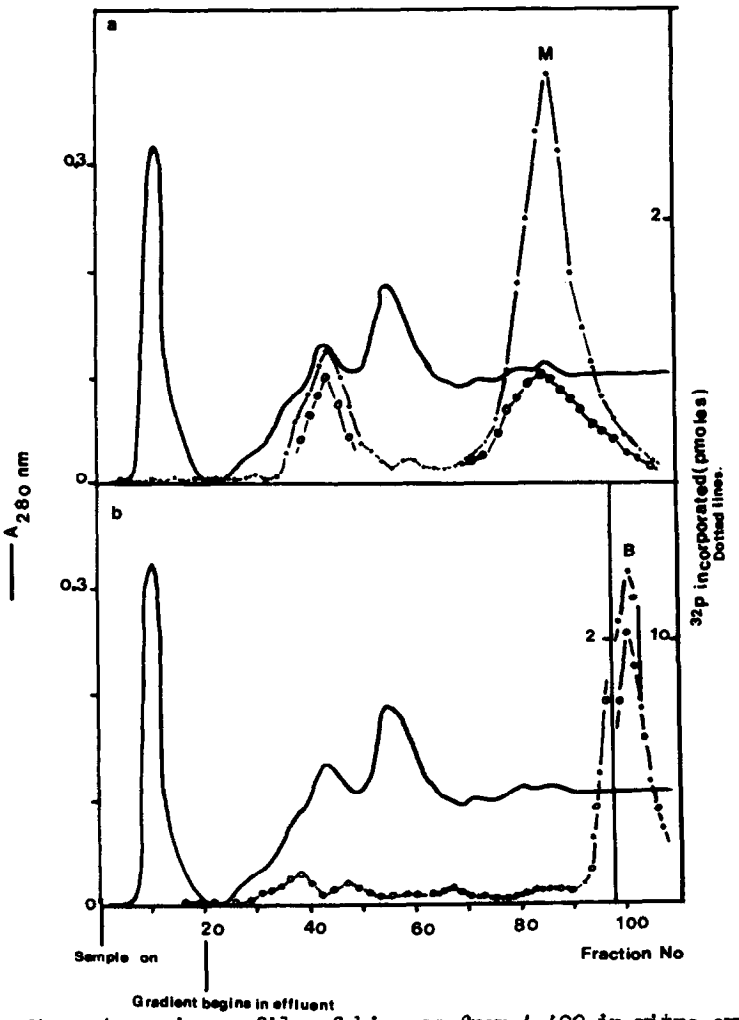

Fig. 3 - Chromatography profile of kinases from 4,400 in vitro ovulated oocytes. The DEAE-cellulose column (10 x 1,5 cm) was equilibrated with 5 mM potassium phosphate - 2 mM EDTA and eluted with 20 ml of the same buffer, followed by a continuous concave gradient ranging from 5 mM potassium phosphate - 2 mM EDTA (pH 7.0)(2 x 66 ml in each mixing chambers) to 500 mM potassium phosphate - 2 mM EDTA (pH 7.0)(66 ml in the reservoir). Flow rate was 20 ml/h; 2 ml fractions were collected and 50 μl were assayed for protein kinase activity with the standard mixture. a. Histone kinase activity (0,25 mg/ml histone). b. Phosvitin kinase activity (3 mg/ml phosvitin). o——o Without cyclic AMP. •——• With cyclic AMP (5 μM).

TABLE II. PROTEIN KINASE ACTIVITY OF PARTIALLY PURIFIED ENZYMES FROM IN VITRO OVULATED OOCYTES (pmoles $min.^{-1}mg.^{-1}$).[x]

Substrate	Conc. (mg/ml)	Peak M (fig. 3a).			Peak B (fig. 3b).		
		Without c-nucleotides	With cAMP	With cGMP	Without c-nucleotides	With cAMP	With cGMP
Histone	0.25	56.8	134.0	130.0	49.6	47.8	48.5
Phosvitin	0.25	8.5	4.7	4.8	200.6	198.3	211.5
Phosvitin	3.00	8.4	-	-	298.7	-	-

[x] All data have been corrected for protein kinase activity detected in the absence of substrate. The amount of enzyme preparation used in the assays was 6 μg/100 μl. The c-nucleotide concentration was 5 μM.

and is not enhanced by the cyclic nucleotides. Peak M has thus been characterized as a cAMP and a cGMP dependent histone kinase.

DISCUSSION.

The present data and those of Tenner and Wallace (1) indicate that X.laevis ovarian oocytes possess various protein kinases which differ in their dependance towards cAMP. The main contribution of our work was to demonstrate that another kinase activity appears after the hormone-induced maturation : the presence of this cAMP and cGMP stimulated histone kinase could be demonstrated in oocytes which had undergone in vitro maturation and ovulation, as well as in unfertilized eggs. Whether this new histone kinase of X.laevis ovulated oocytes represents a de novo synthesized enzyme remains to be investigated. It might be related to the protein kinases already present in ovarian oocytes, and differ from the latter only by a newly synthesized catalytic or regulatory subunit. Alternatively, it might correspond to a different macromolecular assembly of preexisting subunits, which would affect the activity of a preexisting enzyme.

It is possible that cAMP or cGMP-dependent protein kinases might play an

important role, if not in the induction of maturation itself, at least in cellular events associated with maturation or fertilization : for instance, it has been often suggested that histone phosphorylation might be responsible for the condensation of chromatin (7). It might be that the histone which becomes detectable during oocyte maturation plays a role in the extensive condensation undergone by the chromosomes during that period of development.

Further work on the properties of the protein kinases present in X.laevis eggs is needed before one can appreciate their exact role in oogenesis and early development.

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