Studies on the physiological function of spermine in the process of progesterone induced toad oocyte maturation

LI Runsheng and Jiake TSO
Shanghai Institute of Cell Biology, Academia Sinica

ABSTRACT

Spermidine or spermine but not putrescine inhibited progesterone induced *Bufo bufo gargarizans* oocyte maturation. The ID_{50} for spermine inhibition via intra - oocyte microinjection on maturation induced by progesterone was 6. 8 mM(100 nl). Spermine could inhibit MPF induced toad oocyte maturation with a much higher ID_{50}.

A 55 kD protein was dephosphorylated during the process of progesterone induced oocyte maturation. Spermine selectively promoted the level of phosphorylation ,of this protein in both progesterone- stimulated and hormone- untreated oocytes. The extent of its dephosphorylation was fairly correlated with the percentage of GVBD in the hormone stimulated oocytes.

The level of endogenous spermine was reduced by 28% between the period of 0. 40 $GVBD_{50}$ and 0. 60 $GVBD_{50}$, at which 55 kD protein was dephosphorylated.

Spermine inhibited progesterone- stimulated protein synthesis in almost the same dose dependent manner as its inhibitory effect on the hormone- induced maturation. The endogenous spermine regulated 55 kD protein dephosphorylation which may trigger the increase of protein synthesis and in turn promote the activation of MPF. It is possible that 55 kD protein may be one of the components of messenger ribonucleoprotein (mRNP) particles.

Key words: *spermine , toad oocyte, progesterone induced maturation..*

INTRODUCTION

Polyamines (putrescine, spermidine and spermine)are ubiquitous in biological systems. Cell growth, both *in vivo* and *in vitro*, is tightly coupled with the enhancement of ODC (ornithine decarboxylase) activity and the polyamines level[1,2].

Fully grown amphibian oocytes were arrested physiologically at the G_2/M border of

the first meiotic division. Upon exposure to progesterone, oocytes resumed meiotic cycle. The. most prominent event occurred in the process was the appearance of maturation promoting factor (MPF) activity, a nonspecific meiotic or mitotic inducer, which in turn directly triggered the entry of interphasic cell into mitotic phase without any further requirement of protein synthesis [3].

Protein synthesis was enhanced during the progesterone induced amphibian oocyte maturation. The synthesis of cyclins and pp[39mos] was sufficient to activate oocyte MPF [4]. Ribosomal S6 protein phosphorylation has been proposed to regulate the increased protein synthesis [5]. On the other hand, it is notable that the augmentation of protein synthesis resulted from special unmasking of mRNA stored in the oocyte cytoplasm [6]. The process of mRNA unmasking and its regulation during the oocyte maturation remained unclear.

During the process of progesterone induced amphibian oocyte maturation, the enhancement of ODC activity and the change in polyamine levels took place. No information has been available as regarding to the physiological roles of polyamine in the course of oocyte maturation [7,8].

This paper was oriented to probe into the effect of polyamine on oocyte maturation and its mechanism. The results revealed that spermine was able to inhibit protesterone induced toad oocyte maturation, probably resulting from spermine enhanced 55 kD protein phosphorylation in toad oocyte. Certainly, it is worthy to prove whether the 55 kD protein was one of the components of messenger ribonucleoprotein particles (mRNP) related to the unmasking process of mRNA.

MATERIALS AND MATHODS

Materials

Bufo bufo gargarizans were obtained from Jiangsu Province in December, and kept in cold room (4℃) to main tain the full - grown oocytes sensitive to the stimulation of progesterone. Some female toads were reared in an environment of higher temperature (28 - 30℃) all over the year in order to make oocytes inert to progesterone treatment. This means that oocytes from the "high temperature reared toads" will not undergo GVBD after hormone stimulation.

Progesterone, putrescine, spermidine and spermine were obtained from Sigma. γ-^{32}P -ATP and γ-^{32}P -GTP(10 Ci/mmol), ^{35}S - methionine (523 mCi/mmol) were purchased from Amersham.

Oocyte preparation

Full -grown oocytes (about 1.8 mm in diameter) were selected from toad's ovary fragments. Ovarian membrane and follicular membrane around the oocytes were divested manually with a pair of microforcps under a stereoscope. The nude oocytes were incubated in Ringer's solution at 18℃ for the experiments.

Criteria of oocyte maturation

Oocyte maturation was judged by the disappearance of germinal vesicle after 8 - 12 h of progesterone treatment or microinjection of 100 nl cytoplasm containinig MPF. The breakdown of the germinal vesicle was ascertained by the dissection of oocytes after 5 min of fixation in 10% trichloroacetic acid. All the experiments were performed with oocytes that have a potency to undergo germinal vesicle breakdown (GVBD) with a frequency of 100%. The time when 50% of oocyte population underwent GVBD was set as 1.0 on the relative time scale, i.e. 1.0 GVBD$_{50}$, so that different schedules can be compared.

Microinjection assay

Approximately 50−100 nl of sample or cytoplasm were microinjected into oocyte at the animal hemispere near the equatorial region. For microinjection, all the chemical reagents used were dissolved in 5 mM Tris−HCl buffer (pH 7. 4). 0. 1−0.2 μCi of r −^{32}p − ATP was used for each microinjection in the analysis of protein phosphorylation.

^{32}P− labeled protein determination

Oocytes were homogenized in cold homogenization buffer (50 mM Tris − HCl, pH 7.4, 200 mM sucrose, 10 mM β−glycerophosphate, 1 mM MgCl₂, 1 m M PMSF), then centrifuged at 2000×g for 20 min at 4℃. Aliquots (15−40μ) of the 2000×g supernatant were deposited on filler paper discs (Whatman No. 3 MM) and precipitated on the discs by 2−18 h of 10% trichloroacetic acid treatment. For qualitative analysis of ^{32}P−labeling, the 2000×g super−natant samples were diluted with an equal volume of SDS sample buffer and boiled at 100℃ for 3 min, then submitted to 5−13% or 7.5 %−15% SDS− polyacrylamide gel electrophoresis and autoradiography.

Protein synthesis determination

160 oocytes were incubated in 2 ml of Ringer's solution containing approximately 30 μ Ci/ml of ^{35} S−labeled me−thionine for 10 h. After extensive washing with incubation medium, groups of 40 oocytes were injected individually with 100 nl of the indicated concentration (5, 10 or 20 mM) of spermine. 2 h later,each group was subdivided into 2 portions (20 oocytes each), and submitted to the following treatment : incubated in Ringer's solution as control ; incubated in Ringer's solution containing 1 μM progesterone. The acid−insoluble radioactivity from 2000 ×g supernatant was measured for the determination of protein synthesis as described by Stith et al. [9].

Quantitative determination of the contents of polyamine by HPLC

A group of 10 oocytes were homogenized in 100 μl of 0. 5N HClO₄. The supernatant was obtained after centrifu−gation and was directly used for quantitative determination of the oocyte polyamine according to the method proposed by Seiler and Knodgen [10].

Determination of protein concentration

Protein concentration was determined by the method described by Bradford [11] with bovine serum albumin serv−ing as standard.

Determination of the levels of oocyte cAMP

Using the method proposed by Thibier et al. [12].

RESULTS

I. Effects of spermine on the process of progesterone induced oocyte maturation

Previous experiments concerning physiological functions of polyamines were all per−formed under the condition of endogenous depletion of polyamines [13−15]. We were, however,interested in the effects of exogenous spermine on oocyte maturation without any perturbation of endogenous spermine. As shown in Fig. 1, spermine inhibited pro−gesterone−induced oocyte maturation when 100 nl of the polyamine was injected into oocytes 2 h prior to progesterone stimulation. The effective dose of spermine to inhibit 50% of progesterone induced oocyte maturation (ID₅₀) was 6.8 mM (100 nl). The in−hibitory effect of spermine on maturation was also observed when the injected spermine was dissolved in either water (pH 7.4) or Ringer's solution, but with a slightly higher ID₅₀ when dissolved in Tris buffer. In all circumstances the oocytes that were unable to undergo GVBD seemed to be still healthy after 20 h of hormone stimulation, as judged by the presence of normal germinal vesicle under stereoscopic analysis.

Effect of spermine on oocyte maturation

Spermidine has also been found to be capable of repressing progesterone induced oocyte maturation. Its ID_{50} was about 20 mM (Fig 1). However, putrescine could not inhibit the process of maturation induced by progesterone ,even when a higher dose was used.

To further explore the inhibition of oocyte maturation by spermine, we made an attempt to characterize the action of spermine in detail on the process of maturation. From
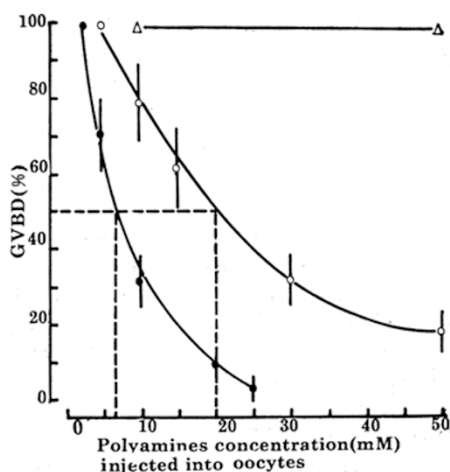

Fig 1. Effect of polyamines on the progesterone−induced oocyte maturation

Groups of 15−20 oocytes were injected with 100 nl of various concentrations of either putrescine (△), spermine (●) or spermidine (○) 2 h prior to the stimulation with 1 μM progesterone. GVBD was scored 11−12 h after the addition of hormone. Each point represented the mean of results of 5 groups with its standard deviation.
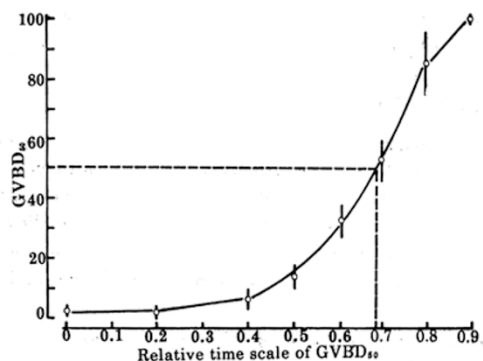
Fig 2. Inhibitory effect of spermine in the process of progesterone induced oocyte maturation. Oocytes (15 − 20 per group) were injected with 100 nl of 25 mM spermine at the indicated relative time of $GVBD_{50}$ after progesterone treatment. GVBD was scored 12 after exposure to 1 μM progesterone. Vertical bars indicated the mean ± S. E. M. of 5 in dependent observations.

the results shown in Fig 2, it was obvious that the later the time of spermine treatment was applied in the course of maturation, the higher the frequency of GVBD was obtained. The time at which 50% oocytes became resistant to spermine inhibition, designated as 50% spermine−sensitive transition (SST_{50}), on average, occurred at 0.68 $GVBD_{50}$.

Progesterone induced maturation was also inhibited when oocytes were cultured in Ringer's solution containing 10 mM spermine (pH 7.4). The efficiency of maturation inhibition caused by spermine was observed to vary among oocytes derived from individual animals, possibly indicating the difference in the capacity of oocytes to uptake exogenous spermine.

II. *Effects of spermine on MPF activation and MPF action*

MPF activity in maturing oocytes, as determined by the ooplasm transfer method, appeared shortly before 0.50 $GVBD_{50}$, and reached its maximal level at 0. 85 $GVBD_{50}$. The SST_{50} occurred at 0.68 $GVBD_{50}$(Fig 2), just in the period when MPF activity devel-

oped rapidly. Such an apparent relation forced us to probe into the effect of spermine on MPF activation.

The toad oocyte maturation was blocked by 96% when the oocytes were microinjected with 100 nl of 25 mM spermine 2 h before progesterone stimulation (Tab 1). Then one would ask : Is MPF activation repressed, or its action to elicit GVBD is inhibited by spermine ? To elucidate these questions, oocytes treated with spermine and progesterone were microinjected with cytoplasm containing MPF. The recipient oocytes underwent GVBD with the same frequency as that of the donor oocytes(Tab 2). The results were in favor of our suggestion that progesterone induced MPF activation other than the action of MPF is inhibited by spermine treatment.

It has been found, however, that spermine could repress MPF−induced oocyte maturation, but the ID$_{50}$ is approximately 25 mM. much higher than that of spermine required for inhibiting progesterone induced maturation (unpublished data). a fact which suggested that different mechanisms may be involved in these two kinds of spermine inhibition.

Tab 1. Effect of spermine on the appearance of MPF activity

Series	Incidence of GVBD[1]		
	Donor oocytes[2]	Recipient oocytes[3]	Control oocytes[4]
Female 1	1/20	0/20	11/11
Female 2	1/14	1/14	10/10
Female 3	1/16	1/15	10/10
Female 4	0/14	0/14	9/10
Average of GVBD%	4.5%	3.4%	97.5%

(1) Number of oocytes undergoing GVBD/Total number of tested oocytes
(2) Donor oocytes were pretreated with spermine (by microinjecting 100 nl of 25mM spermine 2 h prior to hormone stimulation) and then treated with 1 μM progesterone for 14 h.
(3) Recipient oocytes were oocytes recieved 100 nl of cytoplasm from the donor oocytes by microtransfer method
(4) Oocytes treated simply with 1 μM progesterone for 14 h were referred to control oocytes

Tab 2. Release of spermine inhibitory effect by MPF on progesterone−induced oocyte maturation

Series	Incidence of GVBD*		
	Progesterone treated oocytes[1]	Spermine plus progesterone[2]	MPF transferred[3]
Female 1	14/14	0/20	18/18
Female 2	15/15	1/12	12/12
Female 3	14/14	1/15	15/15
Female 4	17/17	1/15	14/14

* Number of oocytes undergoing GVBD/Total number of tested oocytes
(1) Ooeytes treated with l μM progesterone for 14 h
(2) Oocytes were microinjected with 100 μl 25 mM spermine 2 h prior to the 14 h treatment of 1 μM progesterone
(3) Ooeytes of the category (2) indicated above were microinjected with 100 nl cytoplasm containing MPF (i. e. cyto−plasm from matured oocytes)

Effect of spermine on oocyte maturation

III. The level of cAMP in spermine treated oocytes

The level of cAMP has been shown to fall in the early stage of progesterone induced amphibian oocyte maturation [16]. Some agents that elevate the cAMP level of oocytes, for example, cholera toxin, could inhibit the hormone induced maturation and activation. We have investigated the effcet of spermine on cAMP of the toad oocytes. It has been found that spermine significantly decreased the level of cAMP in intact oocytes (Fig 3). The results strengthened the assumption that the inhibitory effect of spermine on oocyte maturation did not result from the change of cAMP content.
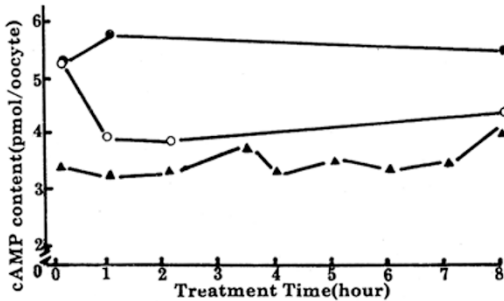

Fig 3. Effect of spermine on endogenous level of oocyte cAMP

●---●   oocytes without any treatment

○---○   oocytes stimulated by $1 \mu M$ progesterone

▲---▲   oocytes microinjected with 100 nl of 25 mM spermine

Each point represented the average value of 2 independent determinations.

IV. Analysis of protein synthesis in spermine treated oocytes

Spermine has been shown to repress protein synthesis in mammalian cells [17]. It has also been shown that progesterone enhanced protein synthesis was coupled with MPF activation in the course of oocyte maturation. We have analyzed the changes of protein synthesis in spermine−treated oocytes in order to further clarify the mechanism of its inhibition.

The amount of ^{35}S−labeled methionine incorporated into proteins was 21,000 cpm per $100 \mu g$ of $2,000 \times g$ supernatant from unstimulated toad oocytes. The magnitude of incorporation fell considerrably in spermine−treated oocytes, and a decrease of 49% as compared to the control was obtained in oocytes injected with 20 mM spermine (Fig 4) which seemed almost a saturated dose. Protein synthesis was elevated by more than 9 0% in progesterone−stimulated oocytes than that of untreated oocytes . Spermine
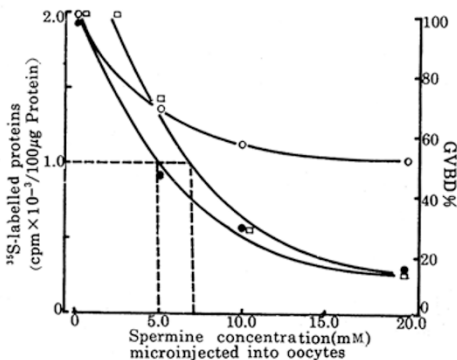

Fig 4. The inhibitory effect of spermine on oocyte protein synthesis

(○---○)   the amount of ^{35}S−methionine incorporated into proteins in the control oocytes

(●---●)   the net augmentation of ^{35}S−methionine incorporated into oocytes after progesterone treatment as compared with the control

(□---□)   GVBD%, scored 10 h after hormone treatment.

Each point represented the average value of two independent measurements.

blocked the augmentation of protein synthesis promoted by progesterone in a dose dependent manner, and its ID_{50}(5.0 mM) was close to the ID_{50}(6.8 mM) of spermine inhibition on GVBD. The experimental results suggested that the inhibitory effect of spermine on protein synthesis may result in the failure of GVBD.

V. *Protein phosphorylation in spermine treated oocytes*

It is necessary to study the inhibitory mechanism of spermine on oocyte protein synthesis from the point of view of protein phosphorylation, since it has been shown that some phosphorylated proteins could regulate mRNA translation in the process of maturation. In order to characterize ^{32}P−labeled proteins ,the 2,000 ×g supernatant fractions were submitted to SDS polyacrylamide gel electrophoresis. Several proteins were readily phosphorylated in oocytes treated with spermine (Fig 5, lanes A−D). Also,a series of proteins were phosphorylated in the course of oocyte maturation (Fig 5, lanes A and E), but no significant change was observed about the pattern of phosphorylation as compared with those of spermine treated oocyte, suggesting that their mode of phosphorylation was independent of oocyte maturation. It seems that the effect of spermine on these proteins is not a prerequisite for the inhibition of oocyte maturation.
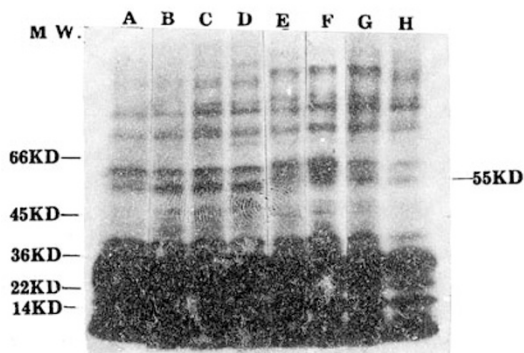

Fig 5. One -dimentional gel analysis of the oocyte protein phosphorylation changes after the microinjection of 100 nl different concentration of spermine

Lane A: 0 m*M* spermine

Lane B: 5 m*M* spermine

Lane C: 10 m*M* spermine

Lane D: 20 m*M* spermine

Lane E: 1 *μM* progesterone stimulation

Lane F: 5 m*M* spermine plus 1 *μM* progesterone stimulation

Lane G: 10 m*M* spermine plus 1 *μM* progesterone stimulation

Lane H: 20 m*M* spermine plus 1 *μM* progesterone stimulation

On the other hand, a 55 kD protein vas dephosphorylated in mature oocytes induced by progesterone (Fig 5, lane E). Moreover, the extent of phosphorylation of this protein was correlated with the dose of spermine injected (Fig 5, lanes A to D), and the inhibition of the percentage of GVBD in spermine−treated oocytes was correlated with its injected concentration (Fig 1). It is interesting to note that the protein underwent dephosphorylation during the period of 0.40−0.70 $GVBD_{50}$(Fig 6), which just coincided with the moment that maturing oocytes were going to become insensitive to spermine inhibition (Fig 2). Such results gave strong support to the view that the inhibition by

spermine on progesterone induced maturation was probably due to the spermine depen-
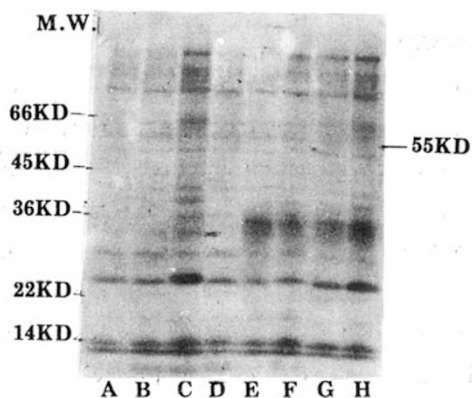dent phosphorylation of 55 kD protein.

Fig 6. The change of protein phosphorylation and dephos-
phorylation in oocytes during the course of proges-
terone induced maturation

Lane A: injected with Tris HCl buffer

Lane B: injected with 100 nl of 20 mM spermine

Lane C: treated with 1 μM progesterone until 1, 2 $GVBD_{50}$

Lane D: treated with 1 μM progesterone 2 h after the injec-
tion of spermine until 1.2 $GVBD_{50}$

Lane E: treated with the hormone until 0.2 $GVBD_{50}$

Lane F: treated with the hormone until 0.4 $GVBD_{50}$

Lane G: treated with the hormone until 0. 7 $GVBD_{50}$

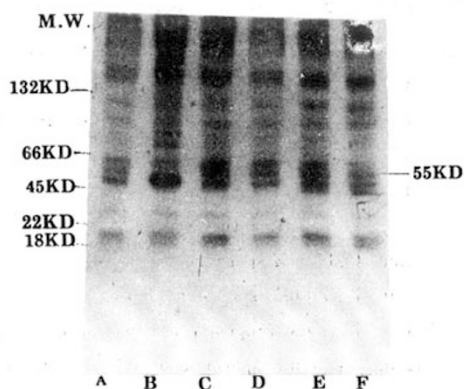Lane H: treated with the hormone until 0.9 $GVBD_{50}$

Fig 7. 55 kD protein dephosphorylation in progesterone
stimulated oocytes derived from"hibernant toad"

Lanes A−D: Supernatants of oocytes derived from hibernat-
ed toad

Lane A: from hormone untreated oocytes

Lane B: treated with 1 μM progesterone

Lane C: microinjected with 50 ng Trichosanthin

Lane D: microinjected with the Trichosanthin,then submit-
ted to the treatment of progesterone

Lanes E−F: Supernatants of oocytes derived from toad
reared at high temperature

Lane E: form hormone untreated oocytes

Lane F: treated with 1 μM progesterone

We have further investigated the possible relationship between the maintenan of
phosphorylation of 55 kD protein and the inhibition of spermine on progesterone in-
duced MPF activity. Trichosanthin, an inhibitor of protein synthesis at translation lev-
el, has been found to inhibit the appearance of MPF activity in the toad oocytes stimu-
lated by progesterone (unpublished data). Trichosanthin was shown to inhibit proges-
terone induced 55 kD protein phosphorylation (Fig 7, lanes C and D).

Previous experiment results from our laboratory revealed that progesterone never
induded MPF activity in oocytes derived from "high temperature reared toads", although
it stimulated the appearance of an active substance which was able to promote the acti-
vation of MPF in the hormone untreated oocytes obtained from "hibernated females"
[18]. As we have prejudged, the 55 kD protein was prominently phosphorylated in the
former kind of oocytes, and was not dephosphorylated when they were submitted to the

hormone treatment (Fig 7, lanes E and F), which was in good agreement with the absence of active MPF in the oocytes. A conclusion may thus derived that dephosphorylation of 55 kD protein coupled tightly with the appearance of MPF activity.

VI. Intracellular levels of polyamines in the process of progesterone induced oocyte maturation .

In each hormone untreated toad oocyte, 1.08 nmol of $HClO_4$ soluble putrescine, 0.42 nmol of $HClO_4$ soluble spermidine and 0. 28 nmol of $HClO_4$ soluble spermine were estimated using HPLC method [10] (Tab 3). During the course of progesterone induced oocyte maturation, a consecutive increase of intracellular putrescine level was observed as illustrated in Fig 8a. The largest amount found in mature oocytes,was higher than that of hormone untreated oocyces. The level of spermidine did not change in the process of maturation (Fig 8 b) indicating that the metabolism in oocytes of this
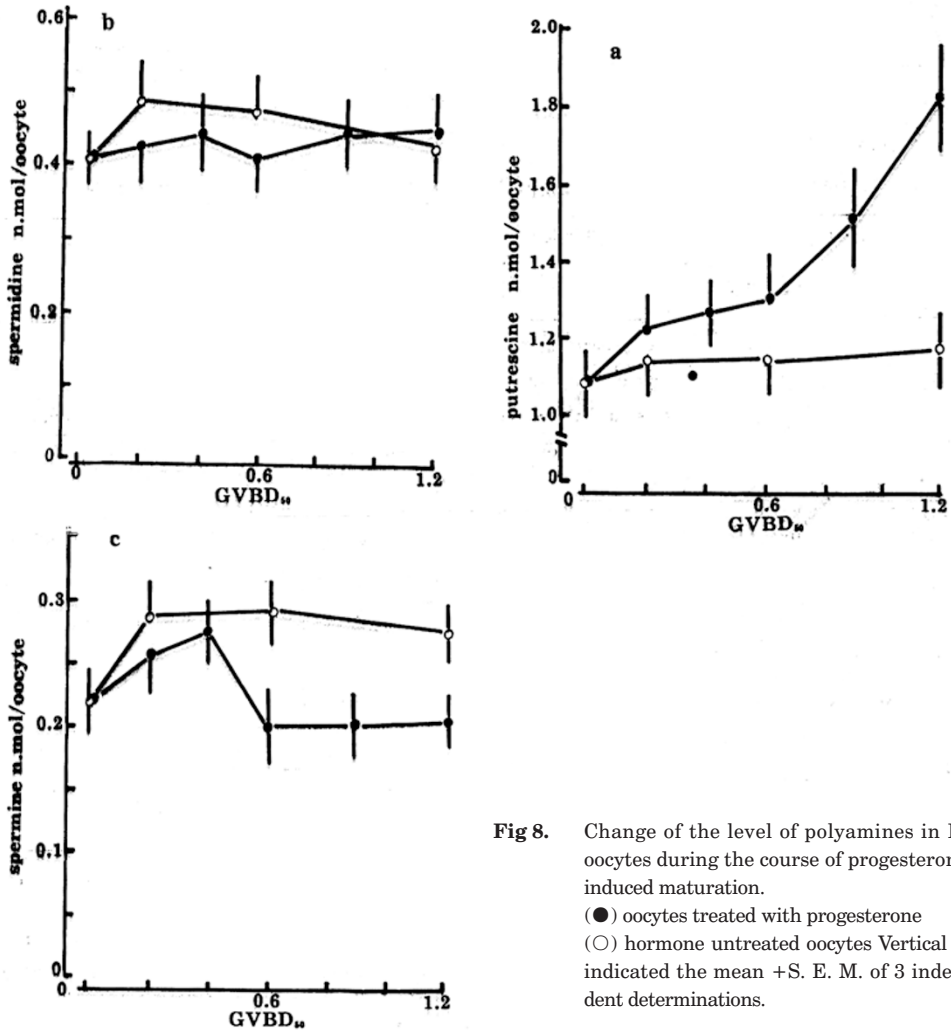

Fig 8.   Change of the level of polyamines in Bufo oocytes during the course of progesterone. induced maturation.
(●) oocytes treated with progesterone
(○) hormone untreated oocytes Vertical bars indicated the mean +S. E. M. of 3 independent determinations.

polyamine was insusceptible to the hormone stimulation. However,the intracellular level of spermine dropped by 28% during the period of $0.40-0.60$ $GVBD_{50}$(Fig 8c).

As shown in Tab 4, 60% of $^{14}C-$labeled spermine was soluble in 0.5 N $HClO_4$ which was out of our expectation. The radioactivity which escaped from the oocytes and entered into the incubation medium represented less than 10% of the total. An estimated amount of 30% of the radioactive spermine injected into oocytes bound tightly in the pellets, obtained from oocyte homogenate after centrifugation, and composed mainly of yolk platelets. In the meantime, the binding amount of labeled spermine was found to be slightly enhanced in oocytes injected with 100 nl of 5 mM spermine. As from data shown in Tab 4, $HClO_4$ $-$soluble radioactivity of spermine stimulated oocyte and the hormone$-$untreated oocyte was comparable.

Tab 3. The polyamine contents in hormone - untreated and mature oocytes

Polyamines	The polyamine content (nmol/oocyte)	
	Hormone-untreated oocyte	Mature oocyte
Putrescine	1.08±0.09*	1.80±0.14
Spermidine	0.42±0.03	0.39±0.04
Spermine	0.28±0.03	0.20±0.03

∗ Represented the mean ± S. E.M. of 3 independent measurements.

Tab 4. The amount of spermine in oocytes and culture medium after injection*.

Oocyte	$^{14}C-$spermine mixed with 4 μl of solution indicated below for injection	$HClO_4-$soluble radioactivity (cpm×10^{-5})	Radioactivity in the medium (cpm×10^{-5})
Hormone$-$untreated	Tris buffer	1.83±0.48	0.21±0.06
	Tris buffer containing 5 mM spermine	1.47±0.44	0.19±0.06
Mature	Tris buffer	1.72±0.41	0.26±0.08
	Tris buffer containing 5 mM spermine	1.45±0.40	0.27±0.08

∗ 4 μl of the original solution of ^{14}C $-$labeled spermine ($3.0×10^5$cpm) mixed with equal volume of the' indicated solution was injected into 40 hormone -untreated oocytes. Having been incubated in Ringer's solution for 10 h, the oocytes were homogenated with 150 μl of 0. 5 N $HClO_4$ after extensive washing with incubation medium, and samples of 50 μl of the 2000×g supernatant together with 25 μl of 1.0 N NaOH were dropped on to filter paper (Whatman No. 3 MM. lcm²) for the determination of radioactivity (mean±S. E. M. of 3 independent measurements).

DISCUSSION

I. Spermine inhibits progesterone induced oocyte maturation

It is obvious from our data that spermine was the most potent polyamine capable of repressing the progesterone induced oocyte maturation (Fig 1). 0. 28 nmol of $HClO_4$ soluble spermine could be evaluated in each resting oocyte by HPlC method (Fig 8) which represents 60% of the total endogenous spermine (Tab 3). Since the amount of the total endogenous spermine per oocyte was 0.39 nmol and the ID_{50} of injected spermine inhibition was 0.34 nmol (Fig 1), it is difficult to imagine that the inhibition of exogenous spermine on oocyte maturation was an effect caused by non physiological dose. This viewpoint was strengthened by the fact that MPF could relieve the inhibitory effect of spermine on oocyte maturation (Tab 2).

II. Spermine blocks progesterone induced MPF activation and acts as an intracellular regulator to control oocyte maturation

The occurrence of SST_{50} at 0.68 $GVBD_{50}$ (Fig 2) indicated that the putative endogenous target for spermine inhibition might play a crucial role in the midst of the course of oocyte maturation. The decline of oocyte cAMP content, as one of the most important event observed in the early stage of maturation, was promoted rather than inhibited by spermine treatment (Fig 3). Accordingly, we paid much attention to the effect of spermine on MPF activity. The MPF activity was hardly detectable in oocytes whose GVBD was totally inhibited by spermine. In cytoplasm microtransfer experiments, the percentage of GVBD in spermine treated donor oocytes was almost the same as that in the recipient oocytes (Tab 1), showing that spermine inhibition of progesterone induced maturation was due to the lack of MPF activation instead of GVBD triggering action of MPF. In consistent with the conclusion, MPF was found to be able to release the inhibition of maturation by spermine (Tab 2). However, we have indeed found that spermine could inhibit MPF induced oocyte maturation, but the ID_{50}(25 mM) was much higher than that of progesterone induced oocyte maturation, indicating that different mechanisms might exist in these 2 different cases of inhibition.

Sunkara et al. [8] have detected a 5 fold reduction of spermine content in mature *Xenopus* oocytes induced by HCG. However, they did not find any change of spermine level in the process of progesterone induced maturation. Now we have disclosed the fact that endogenous level of spermine was reduced by 28% in the course of progesterone induced toad oocyte maturation (Fig 8). As putrescine inhibited spermine synthesis at its physiological concentration, any increased level of putrescine could lead to a drop of spermine level[19]. The same situation, however, did not occur in the course of normal oocyte maturation, since a significant increase of putrescine was observed at 0.90 $GVBD_{50}$, nor microinjection of 100 nl 20 mM putrescine changed the basal level of oocyte spermine significantly (unpublished data).

We have found that the level of spermine dropped in the process of progesterone induced toad oocyte maturation (Fig 8) and exogenous spermine has been shown to inhibit oocyte maturation (Fig 1). We have also found that the reduced level of endogenous spermine could lower casein kinase G activity which, in turn, resulted in decrease of its

substrate (the 55 kD protein) phosphorylation (unpublished data). Therefore, spermine may act as a negative intracellular regulator in modulating the toad oocyte maturation induced by progesterone. As evidence supporting this tentative hypothesis, Russell and Durie [20] suggested that the rate of cell growth parallels with the ratio of intracellular content of spermidine to that of spermine. Further investigation might ascertain whether or not an agent which could specifically lower endogenous level of spermine could accelerate progesterone induced toad oocyte maturation.

III. The failure of 55 kD protein dephosphorylation causes inhibition of progesterone induced maturation

55 kD protein was dephosphorylated in the process of progesterone induced toad oocyte maturation. Spermine selectively enhanced the basal level of phosphorylated 55 kD protein so that the hormone -stimulated dephosphorylation was incapable of reaching the same extent as observed in the control oocytes. The extent of dephosphorylation of 55 kD protein coupled positively with the percentage of GVBD in the polyamine treated toad oocytes (Fig 5 and Fig 1). We have shown that casein kinase G could inhibit progesterone induced oocyte maturation by phosphorylating its substrate 55 kD protein (unpublished data). It is worthy to notice that 55 kD protein dephosphorylation and the SST_{50} occurred at the same stage of maturation around 0. 7 $GVBD_{50}$(Fig 2 and Fig 6).

We have shown that spermine inhibited progesterone induced MPF activation. Then, what is the relation between 55 kD protein dephosphorylation and MPF activation? Neither 55 kD protein dephosphorylation nor MPF activity was detected in Trichosanthin treated oocyte (Fig 7) Progesterone was unable to induce these 2 events in toad oocytes derived from "high temperature reared females"(Fig 7), although it stimulated the production of an active substance which could induce MPF activity in oocytes obtained from"hibernated females"[18].

However, 55 kD protein has been sufficiently dephosphorylated early before MPF activity reached to its maximal level (Fig 6). It can be considered, therefore, that 55 kD protein dephosphorylation may be involved in MPF activation.

IV. A possible relationship between 55 kD protein dephosphorylation and the regulation of protein synthesis in the process of oocyte maturation

We are very interested in the mechanism with which 55 kD protein dephosphorylation resulted in MPF activation. It has recently been found that cyclin,which was synthesized during meiotic maturation and early embryonic cell cycle, activated MPF activity in cell free system [4]. Otherwise, c- mos oncoprotein was shown to be synthesized during progesterone induced oocyte maturation [21]. We do not know whether spermine inhibited cyclin and/or c−mos oncoprotein synthesis in toad oocytes, but we have indeed found that spermine interfered with protein synthesis both in progesterone stimulated and unstimulated toad oocytes (Fig 4). The effect of spermine on protein synthesis may cause failure of GVBD since progesterone induced increment of the amount of protein synthesis paralleled with the percentage of GVBD in spermine treated oocyte (Fig 4). Although the ID_{50} was higher for the spermine inhibition on GVBD than for the reduction of progesterone induced protein synthesis, the discrepancy may well be explained by

the synthesis of some other proteins, e.g. that of ODC [22] which was not involved in the inhibition of progesterone induced oocyte maturation by spermine.

Concerning the augmentation of protein synthesis resulted from specific unmasking of mRNP particles of ooplasm, there was evidence that the protein components of mRNP particles were oocyte specific and inhibited reversibly the translation of the mRNA [23]. The recruitment of mRNA might be predicted to require the removal of proteins from stored mRNA particles. The inhibitory proteins have been investigated from the angle of phosphorylation. Dearsly et al. [24] have found that a pp^{60} and pp^{56} of mRNP particles were heavily phosphorylated at developmental stages of *Xenopus* oocytes when mRNA translation was maximally repressed. Dephosphorylation of pp^{60} destabilized the mRNA/protein complex making mRNA accessible for translation[25]. In amphibian oocytes and eggs, both casein kinase G and its endogenous substrate pp^{54} are RNA binding proteins, and pp^{54} was phosphorylated in partially purified kinase preparation[26]. Casein kinase G may be ubiquitous in mRNP or informosomes[27]. We also demonstrated that 55 kD protein was dephosphorylated during progesterone induced toad oocyte maturation and it was an endogenous substrate for casein kinase G of toad oocytes (unpublished data). Moreover, microinjection of the kinase inhibited progesterone induced toad oocyte maturation and progesterone stimulated protein synthesis (unpublished data). It has recently been discovered by Kandrov et al. [28] that a considerable amount of microinjected casein kinase G was tightly associated with mRNP particles in frog oocytes. Altogether the data prompted us to speculate that 55 kD protein of toad oocytes may inhibit mRNA recruitment when they are phosphorylated, and may unmask mRNA for translation when dephosphorylated. In fact, the amount of a 56 kD masking protein of mRNP particles decreased during developmental stages of full grown *Xenopus* oocytes to 4 cell stage embryos[29]. The decrease in the magnitude of a protein in prosomes has been observed during axolotl meiotic maturation[29], which contained some components inhibiting protein synthesis in vitro[30].

The present study has, for the first time, demonstrated that spermine inhibited the process of progesterone induced amphibian oocyte maturation. In this process, the level of endogenous spermine was reduced, an event which may be considered as a negative intracellular control to trigger the recruitment of mRNA for protein synthesis via the way of 55 kD protein dephosphorylation. A significant fraction of endogenous spermine was most probably sequestered from the sites of protein synthetic machinery which, when occupied by a polyamine, permitted optimal protein synthesis[31].

REFERENCES

[1]   Pegg AE. Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. Cancer Res 1988; 48:759 74.

[2]   Nagaragam S, Ganem B, Pegg AE. Studies of nonmetabolizable polyamines that support growth of SV 3T3 cells depleted of natural polyamines by exposure to a alpha difluoromethylornithine. Biochem J 1988; 254: 373 78.

[3]   Lohka MJ. Mitotic control by metaphase promoting factor and cdc proteins. J Cell Science 1989;92:131 5.

[4]   Murray AW, Solomon MJ, Kirschner MW. The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. Nature (London) 1989; 280 6.

[5]   Maller JL, Pike L J, Freidenberg GR, Codera R, Stith BJ, Olefsky JM, Krebs EG. Increased phosphorylation of ribosomal protein S6 following microinjection of insulin receptor kinase into *Xenopus* oocytes. Nature

(London) 1986;320:459- 61.

[6] Smith LD. Regulation of translation during amphibian oogenesis and oocyte maturation. In: Gall JG,ed. Ga metogenesis and early embryo. Alan R Liss Inc. 1986;131-50.

[7] Yanglai EU, Goudeau F, Mester J, Baulieu EE. Increased ornithine decarboxylase activity during progesterone induced meiotic maturation of *Xenopus* oocytes. Biochem Biophys Res Commun 1980; 96 : 1274 - 89.

[8] Sunkara PS, Wright DA. ,Nishioka K. An essential role for putrescine biosynthesis during meiotic maturation of amphobian oocytes. DevBiol 1981;87:351-5.

[9] Stith R, O'Conner C, Keen K, Smith LD. Kinetic analysis of amino acid pools and protein synthesis in amphibian oocytes. Dev Biol 1978;66:172-82.

[10] Seiler N, Knogen B. High pressure liquid chromatography procedure for the stimulation of the natural polyamines and their monoacetyl derivative. J Chromat 1980; 221: 227-35.

[11] Bradford M. A rapid and sensitive method for the qualititation for microgram of protein utilizing the principle of protein - dye binding. Anal Biochem 1976 ; 72 : 248 - 54.

[12] Thibier C, Mulner O, Ozon R. In vitro effects of progesterone and 17B - estradiol on choleragen activated *Xenopus* oocyte adenulate cyclase. J Ster Biochem 1982;17:191-96.

[13] Pegg AE, McCann PP. The metabolism and physiological functions of polyamines. Am J Physiol 1982;243; C212 -C21.

[14] Casero RA, Raymond JR, Bergerson J, Porter CW. Treatment with alpha -difluoromethylornithine plus a spermidine analog leads to spermine depletion and growth inhibition in cultured L1210 leukemia cell. J Cell Physiol 1984;121: 476 - 82.

[15] McGovern KA, Clark JL, Pegg AE. Effect of 1,3,6 - triaminohexane and 1,4,7 - triaminoheptane on growth and polyamine synthesis in SV- 3T3 cells treated with alpha -difluoromethylornithine. J Cell Physiol 1986; 127:311 -16.

[16] Maller JL. Interaction of steroids with cyclic nucleotide system of amphibian oocytes. Adv Cycl Nucleot Res 1983;15:295 - 336.

[17] Goldstein J. The effect of spermine on the accumulation of nuclei acid and protein in mammalian cells. Exp Cell Res 1965;37:494- 7.

[18] Tso J, Zhu G, Hsu KG, Wang YL, A hibernation factor -dependent maturation promoting substance appeared in progesterone treated toad oocytes. Cell Differ 1985 ; 16 (Suppl.) : 20S.

[19] Janne J, Poso H, Raina A. Polyamines in rapid growth and cancer. Biochem Biophys Acta 1978;473:241-78,

[20] Russell DH, Durie BGM. Polyamines and their accumulation in tumor cells. In: Russell DH, Durie BGM, eds. Polyamines as biochemical marker of normal and malignant grouwth. Raven Press, New York. 1978;15 -29.

[21] Sagata N, Daar I,Oskarsson M, Showalter SD, Van de Woude GF. The product of the mos proto -oncogene as a candidate "initiator" for oocyte maturation. Science 1989 ;245:643- 646.

[22] Li RS, Tso J. Increased ornithine decraboxylase activity is not essential for progesterone - induced Bufo oocyte maturation. Acta Biol Exper Sinica 1990; 23:405 -11.

[23] Richter JD, Smith LD. Reversible inhibition of translation by *Xenopus* oocyte -specific protein. Nature (London) 1984;309:378-380.

[24] Dearsly AL, Johnson RM, Barrett P, Sommervilli J. Identification of a 60kD phosphoprotein that binds stored messenger RNA of *Xenopus* oocytes. Eur J Biochem 1985 ;150 : 95 - 103.

[25] Kick D, Barrett P,Cummings A, sommervilli J. Phosphorylation of a 60kD polypeptide from *Xenopus* ooeytes blocks messenger RNA translation. Nucleic Acid Res 1987 ; 15 : 4099 - 4109.

[26] Stepanov AS, Kandrov KV, Elizarov SM. Protein kinase activity in RNA−binding protein of amphibian oocytes FEBS Lett 1982; 142:33 - 7.

[27] Cummings A, Sommervilli J. Protein kinase activity associated with stored messenger ribonucleoprotein particles of *Xenopus* oocytes. J Cell Biol 1988 ;107 : 45 - 56

[28] Kandrov KV, Benumov AO, Stepanov AS. Casein kinase II from *Rana temporaria* oocytes. Eur J Biochem 1989;180:441 - 8.

[29] Gautier J, Pal JK, Grossi de Sa MF, Beetschen JC, Scherrer K. Differential cytolocalization of prosomes in axolotl during oogenesis and meiosis maturation. J Cell Sci 1989;90:543- 53.

[30] Akhajat O, Infante D, Martins de Sa C, Grosal de Sa MF, Scherrer K. Isolation and characterisation of a particular prosome composed of RNA and multimers of a 21 kD protein that inhibits protein synthesis. Eur J Biochem 1987;170:23 - 33.

[31]   Rudkin BB, Mamont PS, Nikolaus P,Seiler B. Decreased protein synthetic activity is an early consequence of spermidine depletion in rat hepatoma tissue- cultured cells. Biochem J 1984; 217:731 -41.