

NOTE TO USERS

This reproduction is the best copy available.

UMI[®]



uOttawa

L'Université canadienne
Canada's university

FACULTÉ DES ÉTUDES SUPÉRIEURES
ET POSTDOCTORALES



FACULTY OF GRADUATE AND
POSTDOCTORAL STUDIES

Ling Wang

AUTEUR DE LA THÈSE / AUTHOR OF THESIS

M.Sc. (Biochemistry)

GRADE / DEGREE

Department of Biochemistry, Microbiology and Immunology

FACULTÉ, ÉCOLE, DÉPARTEMENT / FACULTY, SCHOOL, DEPARTMENT

Mouse Oocyte Maturation: How Similar is it to Frog Oocyte Maturation?

TITRE DE LA THÈSE / TITLE OF THESIS

J. Lui

DIRECTEUR (DIRECTRICE) DE LA THÈSE / THESIS SUPERVISOR

CO-DIRECTEUR (CO-DIRECTRICE) DE LA THÈSE / THESIS CO-SUPERVISOR

EXAMINATEURS (EXAMINATRICES) DE LA THÈSE / THESIS EXAMINERS

I. Skerjane

K. Turksen

Gary W. Slater

LE DOYEN DE LA FACULTÉ DES ÉTUDES SUPÉRIEURES ET POSTDOCTORALES /
DEAN OF THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

**Mouse Oocyte Maturation:
How Similar is it to Frog Oocyte Maturation?**

by

Ling Wang

A thesis submitted to
the Faculty of Graduate and Postdoctoral Studies
in partial fulfillment of
the requirements for the degree of
Master of Science in Biochemistry

Department of Biochemistry, Microbiology and Immunology

University of Ottawa

Ottawa, Canada

January 2005

© Ling Wang, Ottawa, Canada, 2005



Library and
Archives Canada

Bibliothèque et
Archives Canada

Published Heritage
Branch

Direction du
Patrimoine de l'édition

395 Wellington Street
Ottawa ON K1A 0N4
Canada

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file *Votre référence*
ISBN: 0-494-11449-5
Our file *Notre référence*
ISBN: 0-494-11449-5

NOTICE:

The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protègent cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.


Canada

Abstract

In vertebrates, an immature oocyte is physiologically arrested at prophase of meiosis I and is enclosed in a follicle envelope. In response to the pre-ovulatory gonadotropin surge, the oocyte resumes meiosis and “matures” to the metaphase of meiosis II. Metaphase II-arrested oocytes, called eggs, are then released from the follicle envelope in ovulation. Oocyte maturation has been best studied in the amphibian species *Xenopus laevis*. However, it has long been argued that the hormonal regulation of oocyte maturation in mammals is significantly different from that in amphibian species. Most notably is that denuded mammalian oocytes undergo “spontaneous” maturation in vitro whereas denuded amphibian oocytes remain indefinitely in prophase until treated with progesterone or androgens. Furthermore, progesterone, while considered as the physiological inducer of oocyte maturation in frog oocytes, has no maturation inducing activities in follicle-enclosed mammalian oocytes.

In this study, I have attempted to address the similarities/differences between amphibian and mouse oocytes by focusing on two aspects of mouse oocyte maturation. In the first project, I investigated the ability of several antagonists of serotonin receptors to initiate follicle-enclosed mouse oocytes to undergo maturation. I demonstrated that ritanserin, but not any others, was capable of inducing oocyte maturation in the intact mouse follicles. Significantly, ritanserin is also capable of inducing frog oocyte maturation, as demonstrated by others in our lab. These results therefore suggested that a similar cAMP-elevating G protein coupled receptor, the target of ritanserin, is responsible for maintaining prophase arrest in both frog and mouse oocytes. In the second project, I

have investigated the ability brefeldin A (BFA), a specific inhibitor of a small G protein ARF1, to initiate mouse oocyte maturation, as it has been suggested that BFA is capable of inducing frog oocyte maturation. I demonstrated that BFA indeed was as potent as human chorionic gonadotropin (HCG) to initiate follicle-enclosed oocytes to undergo germinal vesicle breakdown. However, BFA-treated oocytes failed to complete maturation and, instead, were arrested at metaphase I with apparently normal bipolar spindles. We further demonstrated a dominant negative mutant of ARF1 (ARF1-T31N-HA) similarly arrested the maturing oocytes at metaphase I.

These studies helped reinforce the idea that oocyte maturation is fundamentally the same in mammals as it is in amphibians. The experimentally observed differences may not be very significant biologically. This concept will be discussed in conjunction with recently published literature.

Acknowledgements

I express great thanks to my supervisor Dr. X. Johné Liu for his guidance and patience throughout my M.Sc. project. The time in his laboratory gave me the opportunity to explore the reasons why I enjoy science, and opened my mind to new avenues of research. More importantly, I learned to think critically and scientifically. Also, the guidance that I received from my advisory committee (Dr. J Baltz and Dr. B Vanderhyden) was much appreciated.

I would also like to thank everybody in our lab, including Dr. Chunqi Ma, Dr. Ronald Booth, Dr. Yinglun Sheng, Jing Wang, X. Shawn Liu, Veronique Montplaisir and Yanwei Xi for their help and numerous discussions that aided in the completion of this thesis.

My thanks go out to all the staff and scientists of the LOEB Institute for their friendship and guidance. I would especially like to thank Dr. Andrew Ridsdale for his assistance of using confocal microscope.

Table of Contents

Abstract	ii
Acknowledgements	iv
Table of Contents	v
List of Abbreviation	vii
List of Figures	viii
1. Introduction	1
1.1 Development of the mammalian oocyte	1
1.2 The first meiotic arrest (prophase arrest)	7
1.2.1 Hypoxanthine	10
1.2.2 cAMP	10
1.2.3 G Protein-Coupled Receptor (GPCR)/G protein	12
1.2.4 The possible role of a serotonergic receptor system in the oocyte	13
1.3 Gonadotropin induced oocyte maturation	15
1.3.1 Steroids and LH-induced oocyte maturation	16
1.3.2 Reduction of cAMP and LH-induced oocyte maturation	18
1.4 Meiosis I (MI) to meiosis II (MII) transition	19
1.4.1 Cyclin B.....	20
1.4.2 APC/C (anaphase promoting complex/cyclosome)	20
1.4.3 Cytoskeletons (microtubule and actin)	22
2. Material and Methods	23
2.1 Chemicals and antibodies	23
2.2 Solutions	24
2.2.1 Oocyte handling and culture media	24
2.2.2 Solutions for fixation and immuno-staining	25
2.3 Mice and oocytes/follicles manipulations	25
2.3.1 Animals	26
2.3.2 Oocyte isolation and culture	26
2.3.3 Follicle isolation and culture.....	26
2.3.4 Examination of oocytes	27
2.4 Microinjection	28
2.5 Western blotting	29
2.6 In vitro translation	31
2.7 Immunofluorescence	31
2.8 Imaging	32
2.8.1 Confocal imaging	32
2.8.2 Brightfield and fluorescent imaging	33
2.9 Statistics	33
3. Results	34
3.1 A 5-HTR antagonist induces oocyte maturation in the mouse: evidence that a GPCR is responsible for maintaining prophase arrest	34
3.1.1 Ritanserin induced follicle-enclosed oocyte maturation	34

3.1.2 Exogenous serotonin did not alter oocyte maturation	39
3.1.3 Synthesis of serotonin is not required for prophase arrest	39
3.1.4 Attempts to identify ritanserin-antagonized receptor	46
3.2 BFA induces resumption of meiosis, but arrests mouse oocytes at metaphase I ...	51
3.2.1 Brefeldin A induces follicle-enclosed oocyte maturation	52
3.2.2 BFA arrests denuded oocytes at metaphase I	57
3.2.3 Inhibition of ARF1 arrests oocytes at metaphase I	57
3.2.4 Further characterization of BFA effects in mouse oocytes	62
4. Discussion	70
4.1 Ritanserin induces oocyte maturation in both frogs and mice: a common mechanism for maintenance of prophase arrest in vertebrate oocytes.....	71
4.2 The effect of BFA on oocyte maturation: a possible role for Golgi dynamics in regulation of oocyte maturation	73
5. References	76
Curriculum Vitae	89

List of Abbreviations

M	molar
mM	millimolar
μM	micromolar
nM	nanomolar
IU	international units
5-HT	5-hydroxytryptamine, serotonin
5-HTR	serotonin receptor
AC	adenylyl cyclase
ADP	adenosine diphosphate
APC/C	anaphase promoting complex/ <i>cyclosome</i>
ARF	ADP-ribosylation factor
BFA	brefeldin A
cAMP	cyclic adenosine-3',5'-monophosphate
CPA	p-chloro-phenylalanine
CSF	cytostatic factor
dBcAMP	dibutyryl cyclic adenosine-3',5'-monophosphate
FSH	follicle stimulating hormone
GFP	green fluorescence protein
GPCR	G-protein coupled receptor
GRK	G-protein coupled receptor kinase
GV	germinal vesicle
GVBD	germinal vesicle break down
HCG	human chorionic gonadotropin
IBMX	3-isobutyl-1-methylxanthine
LH	luteinizing hormone
LDM	lanosterol 14 α -demethylase
MAPK	mitogen activated protein kinase
MAS	meiosis activating sterols
MEM	minimal essential medium
MES	mesullergine
MET	methiothepine
MI	meiosis I
MII	meiosis II
MPF	m-phase (maturation) promoting factor
OMI	oocyte meiotic inhibitor
PB	polar body
PDE	phosphodiesterase
PKA	protein kinase A
RIT	ritanserine
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis

List of Figures

<i>Figure 1.</i> Development of the mammalian oocyte	3
<i>Figure 2.</i> Development of follicles in the mouse	6
<i>Figure 3.</i> Time course of oocyte maturation in the mouse	9
<i>Figure 4.</i> Light images of the typical oocyte	36
<i>Figure 5.</i> Ritanserin induced follicle-enclosed oocyte maturation	38
<i>Figure 6.</i> Concentration-dependent responses of ritanserin to induce follicle-enclosed oocyte maturation	41
<i>Figure 7.</i> Exogenous 5-HT did not inhibit HCG-induced GVBD in intact follicles	43
<i>Figure 8.</i> Exogenous 5-HT did not block spontaneous GVBD	45
<i>Figure 9.</i> CPA did not induce follicle-enclosed oocyte maturation	48
<i>Figure 10.</i> Microinjection of mouse oocytes within preantral follicles	50
<i>Figure 11.</i> BFA induced follicle-enclosed oocyte maturation	54
<i>Figure 12.</i> Confocal image of HCG and BFA-treated follicle-enclosed oocytes shown in Fig. 11	56
<i>Figure 13.</i> BFA arrested denuded oocytes at metaphase I	59
<i>Figure 14.</i> ARF1 protein expression	61
<i>Figure 15.</i> ARF1 dominant negative mutant injection arrested denuded oocytes at metaphase I	64
<i>Figure 16.</i> MAPK activity in BFA-treated oocytes	66
<i>Figure 17.</i> BFA did not induce GVBD in the presence of dBcAMP	69

1. INTRODUCTION

1.1 Development of the mammalian oocyte

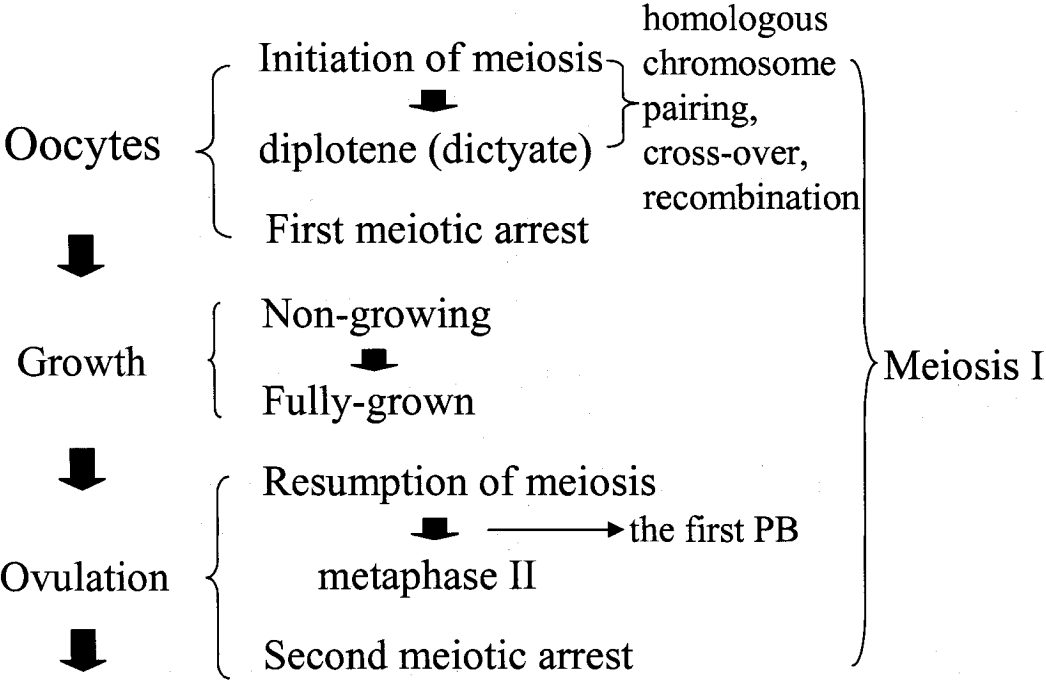
In vertebrates, a long-held view in reproductive biology is that females are born with a finite, non-renewing pool of germ cells (Borum, 1961; McLaren, 1984). As a result, oogenesis (the process of forming female gametes) was thought to begin early in fetal development and end in the sexually mature adult (Baker, 1982). According to the theory, in early stages of embryonic development, primordial germ cells differentiate into oogonia, then oogonia undergo multiple rounds of mitotic division. Following the last mitotic division, oogonia enter the interphase when DNA replication takes place in preparation for meiosis. This synthetic activity symbolizes transformation of oogonia to oocytes. After initiation of meiosis, oocytes progress through several stages (during which homologous chromosomes complete pairing, cross-over and recombination) before arresting at the diplotene (dictyate) stage. Oocytes remain in this first meiotic arrest until being stimulated to resume meiosis at the time of ovulation (Fig. 1) (Wassarman, 1988). However, this theory has been challenged by Johnson et al (2004), who provide evidence that proliferative germ cells exist in postnatal mouse ovary, thus suggesting that oogenesis could also occur in mammalian adults. The relative contribution of these postnatally generated germ cells to mammalian reproduction remains unknown.

Fig 1.
Development of the mammalian oocyte. See text for details. Modified from Wassarman,
1988.

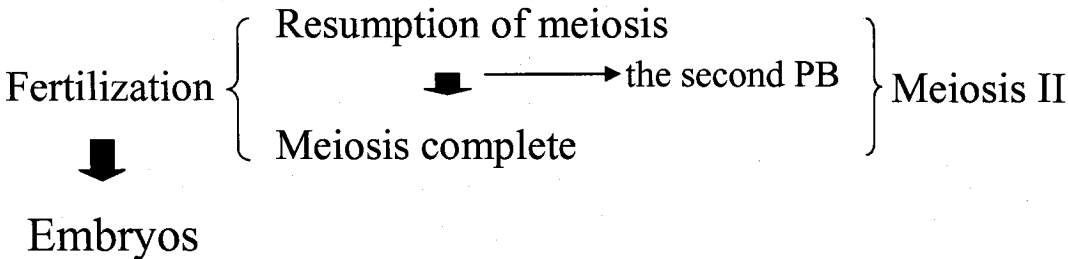
Primordial germ cells

↓
Oogonia

↓ Multiple mitotic divisions, DNA replication

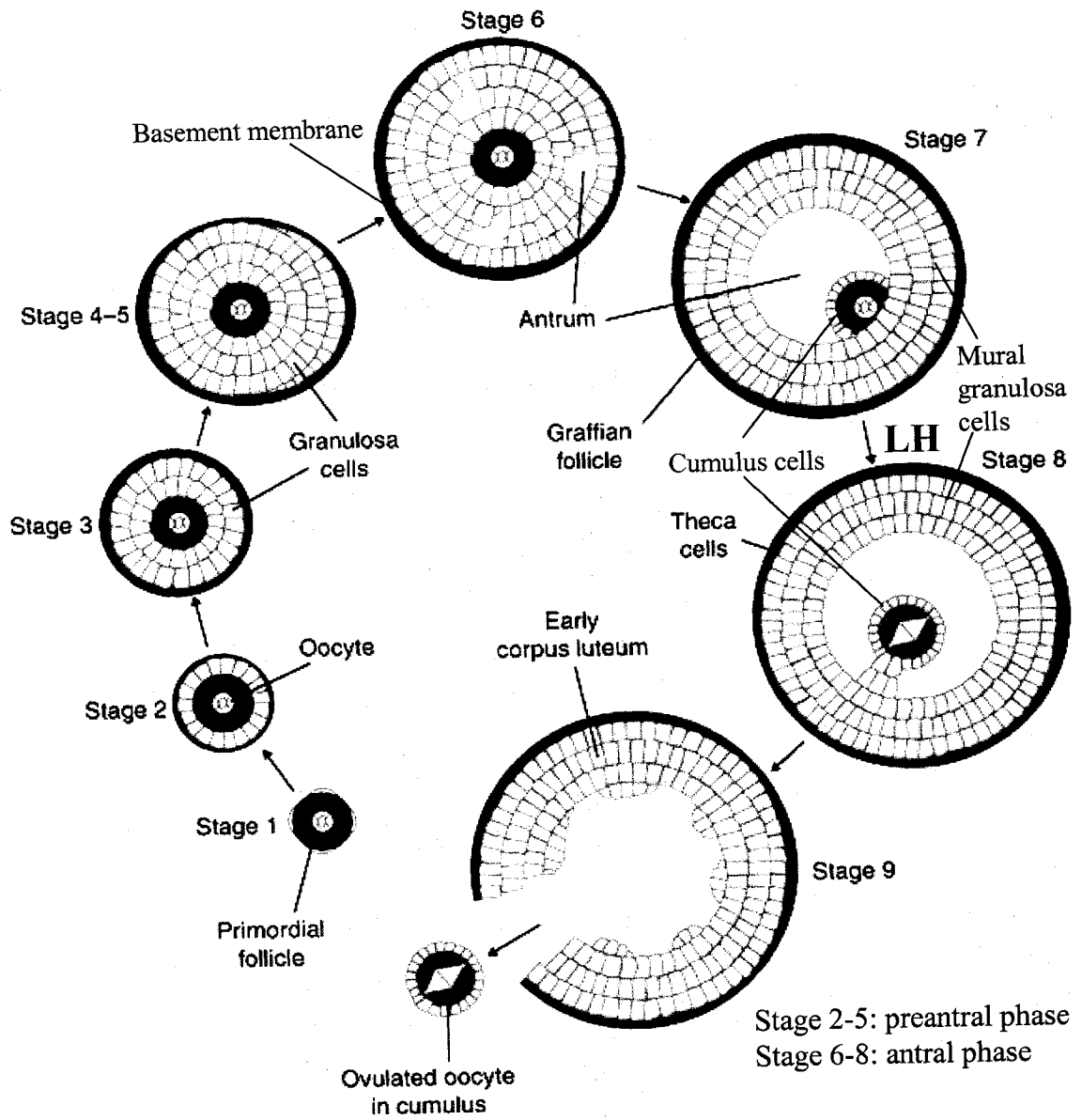


Unfertilized eggs



In the mouse, shortly after birth, the oocyte grows from a diameter of about $12\mu\text{m}$ to a final diameter of about $80\mu\text{m}$, the completion of oocyte growth takes 2 to 3 weeks (Wassarman, 1988). Each oocyte is surrounded by somatic cells that grow concomitantly with the oocyte, from a single layer of flattened cells to three layers of cuboidal granulosa cells by the time the oocyte has completed its growth (Blandau, 1970). This oocyte-containing structure in the ovary is termed the follicle. At the same time, the cells surrounding the follicle differentiate into theca cells. Theca cells are separated from granulosa cells by a basement membrane. At stage 6 of follicle development (2000 to 3000 granulosa cells), some small spaces between granulosa cell layers develop and are called antra (Fig. 2). Fluid filtered from the blood supply starts to accumulate within these antra and eventually form a large antral cavity. The occurrence of the antra is a landmark to classify follicles into preantral and antral follicles (before and after antra formation respectively). The oocyte completes growth before formation of the antra. Consequently, the majority of follicle growth occurs after the oocyte stops growing. From stage 6, granulosa cells rapidly proliferate and differentiate into two populations, the mural granulosa cells, which are adjacent to the basement membrane, and the cumulus cells, which surround the oocyte (Murray et al, 2000). During the late preantral to early antral period (stage 5 to 6 of follicle development), the oocyte starts to synthesize molecules necessary for the resumption of meiosis and has become competent to resume meiosis when removed from the follicle. However, in vivo, the oocyte continually arrests in

Fig 2.
Development of follicles in the mouse. See text for details. Modified from Murray et al,
2000.



dictyate of the first meiotic prophase (GV (germinal vesicle) stage) before sexual maturation (Grondahl et al, 2000).

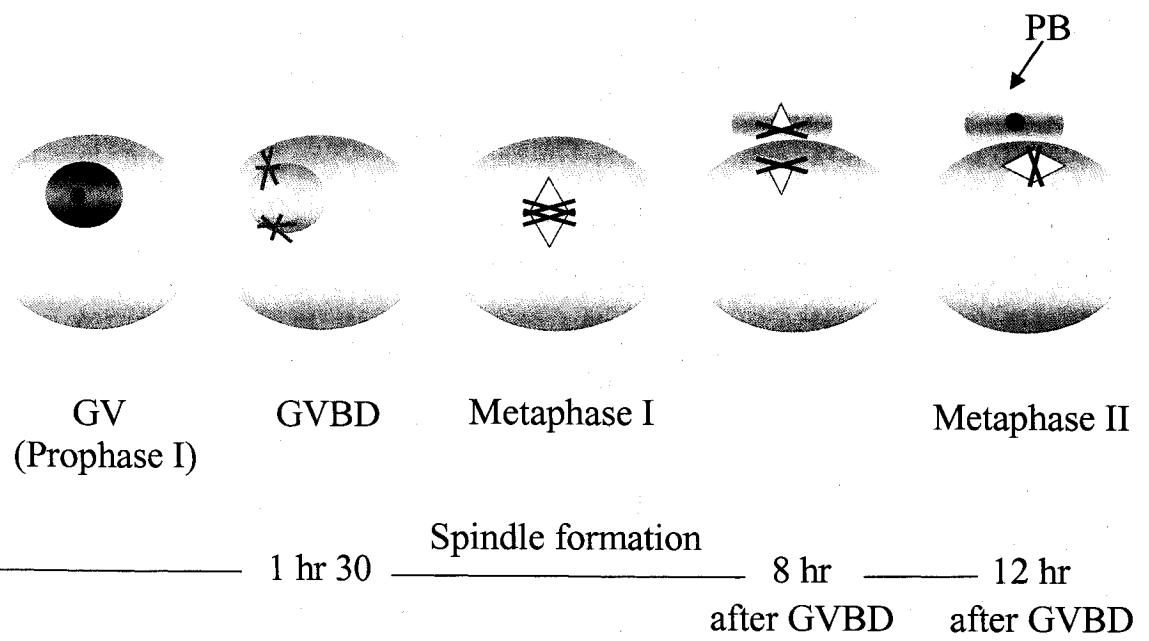
In sexually mature females, a periodic surge of pituitary gonadotropin triggers re-initiation of meiosis. This resumption of meiosis is characterized by chromosome condensation, dissolution of the nuclear membrane or germinal vesicle breakdown (GVBD), metaphase I spindle formation, chromosome segregation and extrusion of a polar body. The mature eggs become arrested at metaphase II (second meiotic arrest) until penetration by a spermatozoa. The progression from the dictyate stage to metaphase II of meiosis is called oocyte maturation (Fig.3) (Tsafiriri, 1978). A cyostatic factor (CSF) (Masui et al, 1971), of which Mos kinase is an essential component (Sagata et al, 1989), is responsible for maintaining the metaphase II arrest. Only a metaphase II-arrested egg can develop into an embryo when fertilized by spermatozoa.

1.2 The first meiotic arrest (prophase arrest)

The finding of Pincus and Enzmann (1935) that oocytes liberated from follicles (so-called denuded oocytes) undergo spontaneous maturation in rabbit has been confirmed in many mammalian species (Edwards, 1965; Schuetz, 1974). These evidence suggest that the follicular environment prevents oocyte maturation until ovulation. Furthermore, it was reported that follicular fluid alone inhibited resumption of meiosis of denuded mouse oocytes (Tsafiriri and Dekel, 1994), which led to the hypothesis that mural granulosa cells

Fig 3.

Time course of oocyte maturation in the mouse. See text for details. Modified from Terret et al, 2003.



produce an oocyte meiotic inhibitor (OMI) (Conti et al, 2002). Many potential candidates have been put forward, I will only discuss a few here.

1.2.1 Hypoxanthine

One earlier candidate for OMI is hypoxanthine, an intermediate of purine nucleotide biosynthesis. Hypoxanthine is widely used to inhibit hydrolysis of cyclic adenosine monophosphate (cAMP). Several studies found that follicular fluid from mouse and pig follicles contained hypoxanthine at 1-2 mM, a concentration sufficient to prevent GVBD in denuded mouse oocytes (Downs et al, 1985b). However, the physiological significance of hypoxanthine is questionable. First, hypoxanthine accumulates in several body fluid as a consequence of anoxia (Gardiner et al, 1989). So it is possible that high levels of hypoxanthine measured in follicular fluid are generated, at least in part, by postmortem hypoxia because the long distance from the vasculature located in theca layers outside the basal lamina may cause hypoxic in the inner region of the follicle (Eppig, 1993). Second, HCG-induced oocyte maturation in vivo was not accompanied by a decrease in the concentration of hypoxanthine in the follicular fluid (Eppig et al, 1985; Lavy et al, 1990).

1.2.2 CAMP

On the other hand, studies in species as diverse as starfish, *Xenopus*, and mammals have reached the identical conclusion that high levels of the second messenger cAMP in

the oocyte play a critical role in maintaining the first meiotic arrest (Ferrell, 1999; Taieb et al, 1997). First, membrane permeable analogs of cAMP prevented denuded oocytes from undergoing spontaneous GVBD (Cho et al, 1974; Wassarman et al, 1976a). Second, a decline of cAMP concentration was detected in oocytes immediately preceding GVBD both in vivo and in vitro (Schultz et al, 1983; Racowsky, 1984). Third, forskolin, a direct stimulator of adenylate cyclase (AC), prolonged GV stage in denuded oocytes (Ekholm et al, 1984; Bornslaeger et al, 1985; Racowsky, 1985). Fourth, cAMP phosphodiesterase (PDE) inhibitors, such as 3-isobutyl-1-methylxanthine (IBMX), prevented spontaneous GVBD (Schultz et al, 1983; Conti et al, 1998). Finally, microinjection of mouse oocytes with the catalytic subunit of cAMP-dependent protein kinase (protein kinase A (PKA)) inhibited spontaneous GVBD (Bornslaeger et al, 1986).

It is clear that cAMP in the oocyte plays a dominant role in maintaining prophase arrest. However, the source of accumulated cAMP in the oocyte is still not clear. It has been proposed that elevated levels of cAMP in the oocyte are provided by cumulus cells through gap junctions. In this hypothesis, a decrease of cAMP levels in the oocyte may be caused by closure of the gap junctions between cumulus cells and the oocyte during ovulation or experimental isolation of the oocyte from the follicle. Although gap junctions play an important role in oocyte-cumulus cell communication, as demonstrated by studying connexin 37-knockout mouse model (Simon et al, 1997), however, oocyte development in these mice arrests before meiotic competence is achieved. So this model does not provide

information on the role of gap junctions at the time of meiotic resumption (Carabatsos et al, 2000). Alternatively, the oocyte produces sufficient cAMP to maintain prophase arrest, perhaps in response to external factors (Eppig, 1993). This hypothesis is supported by several recent studies. It was observed that inhibitors of PDE3 (the major PDE form in rat and mouse oocytes (Shitsukawa et al, 2001)) substantially elevated cAMP levels in denuded oocytes and inhibited spontaneous maturation (Richard et al, 2001; Tsafiriri et al, 1996). Similarly, denuded oocytes from PDE3A knock-out mice contained high levels of cAMP and failed to undergo spontaneous maturation (Masciarelli et al, 2004). Furthermore, adenylyl cyclase 3 (AC3) was reported to be highly expressed in rat and mouse oocytes. Oocytes from the mice deficient in AC3 failed to arrest at prophase I in vivo, indicating that AC3 contributed to the control of cAMP levels in the oocyte and the maintenance of the prophase arrest (Horner et al, 2003). Consistent with these studies was the observation that microinjection of antibodies against Gs α into mouse oocytes caused meiotic resumption in intact follicles (Mehlmann et al, 2002).

1.2.3 G Protein-Coupled Receptor (GPCR)/G protein

The function of G protein in oocyte maturation was suggested by the evidence that microinjection of mouse oocytes with a nonhydrolyzable GTP analog, GTP γ S, suppressed GVBD (Downs et al, 1992). In addition, cholera toxin, which constitutively activates adenylate cyclase (Cassel et al, 1978; Moss et al, 1979) by catalyzing the adenosine

diphosphate (ADP)-ribosylation of the α subunit of stimulatory G proteins (Gs), augmented the meiosis-arresting action of cAMP-elevating agents in mouse oocytes (Downs et al, 1992; 1985a; Vivarelli et al, 1983). All of these early evidence suggest G protein (especially Gs protein)-cAMP pathway is involved in the first meiotic arrest.

Mehlmann et al (2002) showed that prophase arrest can be released in the mouse by microinjecting the oocyte within the antral follicle with antibodies against $Gs\alpha$, which indicated that follicle cells might keep the cell cycle arrested by activating oocyte-resident Gs. Interestingly, in frog oocytes, the same $Gs\alpha$ antibodies were also able to cause GVBD (Gallo et al, 1995), indicating that Gs activity may be a conserved mechanism for maintaining prophase arrest in vertebrate oocytes. On the other hand, in frog oocytes, inhibitors of G protein $\beta\gamma$ subunit ($G\beta\gamma$) lowered cAMP levels and caused spontaneous oocyte maturation. Overexpression of $G\beta\gamma$ increased cAMP levels and inhibited progesterone-induced oocyte maturation (Sheng et al, 2001). Furthermore, overexpression of a G protein coupled receptor kinase (GRK3) or β -arrestin-1 caused hormone-independent oocyte maturation in the frog (Wang and Liu, 2003). GRK3 and β -arrestin were thought to target an endogenous GPCR and cause its desensitization and inhibition. All these studies suggest that an activated GPCR circuit in both frog and mouse oocytes is responsible for maintaining high levels of cAMP and prophase arrest. However, the GPCR responsible for maintaining prophase arrest has not been identified yet.

1.2.4 The possible role of a serotonergic receptor system in the oocyte

The involvement of neurotransmitters in the control of reproduction in vertebrates, through the hypothalamo-hypophysial system, is well known. However, some evidence has emerged during the last decade suggesting that biogenic amines, particularly serotonin (5-hydroxytryptamine, 5-HT), also influences reproductive tissues directly, either by promoting the ability of follicle cells to secrete maturation-inducing substance in response to gonadotropins, or by affecting the oocyte maturation in response to the maturation-inducing substance (Cerda et al, 1998; Sirotkin and Schaeffer, 1997; Stricker and Smythe, 2001).

In the mammalian ovary, the presence of serotonin and its receptors have been well documented. High levels of serotonin are present in bovine corpora lutea (Battista et al, 1987), rat reproductive tract (Amenta et al, 1992), and human ovarian follicular fluid (Bodis et al, 1993). Serotonin is produced, accumulated, and transported to target tissues by blood leukocytes (Rudnick and Humphreys, 1992) or nerve terminals (Lawrence and Burden, 1976). Serotonin also can be locally produced by mast cells within the rat ovary, oviduct and uterus, but not by ovarian follicular cells (Amenta et al, 1992). It was found that serotonin stimulated progesterone and estradiol secretion by culturing rat and hamster preovulatory follicles (Tanaka et al, 1993; Terranova et al, 1990), porcine and human granulosa cells (Schaeffer and Sirotkin, 1995; Bodis et al, 1992).

Buznikov et al (1993) described that two antagonists (made in house) of serotonin

receptors (5-HTR) were able to induce frog oocyte maturation, suggesting that 5-HTRs might be responsible for maintaining prophase arrest in frog oocytes. However, isotype specificities of these antagonists were not reported. Furthermore, these reagents are no longer available. Pharmacological analyses and molecular cloning have revealed at least fourteen 5-HTR subtypes in diverse tissues (Hoyer et al, 1994). All 5-HTRs except 5-HT3R belong to the family of seven transmembrane domain receptors that are coupled to different intracellular effectors via G-proteins (i.e. GPCR). Among these subtypes, 5-HT4R, 5-HT6R and 5-HT7R are linked to a Gs-protein-mediated stimulation of adenylate cyclase (Graveleau et al, 2000). It was reported that human granulosa cells expressed a 5-HT7R involved in the regulation of steroidogenesis via adenylate cyclase activation. Moreover, pre-treating human granulosa cells with 5-HT desensitized LH receptor (see later), suggesting 5-HT7R down-regulated the LH receptor-coupled cAMP signaling system, maybe by hetero-dimerization (Graveleau et al, 2000). Whether 5-HT7R is also expressed in the oocyte or whether 5-HT7R plays a role in oocyte maturation is not known.

1.3 Gonadotropin induced oocyte maturation

Pituitary-derived gonadotropins are the physiological hormones for the induction of oocyte maturation. Pincus and Enzmann (1935) first reported that the resumption of oocyte meiosis in rabbits followed copulation (which stimulates gonadotropin release) or

administration of “pituitary hormones”. Similarly, resumption of oocyte meiosis was also found following injection experiments carried out in many other species including mouse (Edwards and Gates, 1959), rat (Vermeiden and Zeilmaker, 1974), hamster (Iwamatsu and yanagimachi, 1975) and pig (Hunter and Ploge, 1966). Furthermore, administration of pentobarbitone sodium (which blocks gonadotropin surge) (Freeman et al, 1970; Ayalon et al, 1972) or hypophysectomy (Vermeiden and Zeilmaker, 1974) of rats prevented not only ovulation but also oocyte maturation.

During the periodic preovulatory gonadotropin surge, both LH (luteinizing hormone) and FSH (follicle stimulating hormone) are secreted. In the mouse, either injection of LH into the mouse or incubation of intact graafian follicles (preovulatory antral follicle) with LH, but not FSH, induces oocyte maturation (Neal and Baker, 1975). Furthermore, administration to rats of antiserum to the β chain of LH prevented oocyte maturation and ovulation (Tsafiriri et al, 1976). Hence, it has been concluded that LH is the physiological trigger of oocyte maturation and ovulation (Tsafiriri and Dekel, 1994).

However, the action of LH on the oocyte is indirect since oocytes lack LH receptors (Dekel et al, 1988; Byskov et al, 1997). LH receptors are expressed only in theca cells and mural granulosa cells (Zhang et al, 2001; Eppig et al, 1998). Exactly what mediates LH action to induce oocyte maturation is not known.

1.3.1 Steroids and LH-induced oocyte maturation

The role of steroids in mediating LH-induced meiosis resumption in amphibians has been well documented (Maller, 1985). In mammals, the preovulatory LH induces a prompt rise in follicular steroidogenesis (Channing and Tsafirri, 1977). However, the physiological role of steroids in mammalian oocyte maturation remains doubtful. First, addition of steroids to the culture medium did not induce GVBD in follicle-enclosed mouse oocytes (Tsafirri et al, 1972). Furthermore, inhibitors of steroidogenesis such as cyanoketone, aminoglutethimide or ketoconazole did not affect LH-induced oocyte maturation despite preventing LH-induced rise in steroids accumulation (Lieberman et al, 1976; Tsafirri et al, 1998).

In contrast, a family of C29 sterols was found to induce denuded mouse oocytes to resume meiosis in the presence of hypoxanthine (Byskov et al, 1995). These sterols termed MAS or meiosis activating sterols, which were extracted from human preovulatory follicular fluid, have attracted significant attention in the last decade. Since the original report on MAS induction of meiosis in isolated mouse oocytes (Byskov et al, 1995), conflicting results have been reported by several other laboratories. First, MAS-induced oocyte maturation was remarkably delayed as compared with spontaneous (Downs et al, 2001) or LH-induced (Cao et al, 2004) maturation. Second, MAS levels appeared to peak post-meiotically and GVBD was triggered when MAS level was <20% of its maximum (Baltsen, 2001). Third, inhibition of MAS synthesis did not prevent GVBD within the

follicle (Tsafiriri et al, 1998; Xie et al, 2004). Fourth, LH/HCG did not increase ovarian lanosterol 14 α -demethylase (LDM, the key enzyme during synthesis of MAS) expression at a time relevant for the resumption of meiosis (Vaknin et al, 2001). Finally, immunocytochemical study confirmed that LDM localized in the oocyte (Vaknin et al, 2001), which was not compatible with its role as a mediator of LH-induced oocyte maturation. Thus, it remains doubtful that MAS accumulation is a natural part of the signaling pathway that LH employs to induce oocyte maturation (Tsafiriri et al, 2002).

1.3.2 Reduction of cAMP and LH-induced oocyte maturation

Despite the uncertainty of the physiological mediator of LH action, cAMP is known to play a pivotal role in LH-induced oocyte maturation. LH receptor is a Gs-coupled receptor (GPCR) and therefore LH induces a large increase in cAMP levels in granulosa cells (Hsueh et al, 1984). On the other hand, cAMP levels in the oocyte decrease in response to LH actions (Schultz et al, 1983). It is thought that LH dismantles the gap junctions between granulosa cells and the oocyte, so that cAMP can no longer be transferred to the oocyte. Therefore, the increase in cAMP in granulosa cells is translated into the decrease in cAMP in the oocyte (Conti et al, 2002). This notion is supported by the finding that specific inhibitors of PDE3 (expressed in the oocyte, but not follicle cells) block LH-induced oocyte maturation, whereas inhibition of PDE4 (expressed in granulosa cells, but not the oocyte) produces meiotic resumption and ovulation (Tsafiriri et al, 1996). However, other

studies indicated that the loss of communication via gap junctions lag behind resumption of meiosis by several hours when maturation was induced by LH/HCG in vivo or in intact follicles in vitro (Moor et al, 1980; Eppig, 1982). Alternatively, the action of LH may inhibit the ability of the oocyte to produce cAMP. For example, the action of LH could reverse the action of the GPCR (in the oocyte) mentioned above (Mehlmann et al, 2002; Sheng et al, 2001; Wang and Liu, 2003).

1.4 Meiosis I (MI) to Meiosis II (MII) transition

Oocyte maturation is also an interesting and unique cell cycle problem. During oocyte maturation, two successive cell divisions occur without an intermediate S phase, so that haploid gametes are produced (Brunet et al, 1999). In meiosis I, homologous chromosomes (two sister chromatids from maternal source and two chromatids from paternal source) are segregated while the cohesion between sister chromatids is maintained. In contrast, the second meiotic division is effectively a mitotic division as two sister chromatids joined at the centromeres segregate.

Early studies found that applying protein synthesis inhibitors to fully grown oocytes blocked MI to MII transition (Wassarman et al, 1976a; Clarke and Masui, 1983), indicating that certain newly synthesized protein(s) controlled this transition. Furthermore, Clarke(1998) found that when protein synthesis was inhibited in prophase-arrested oocytes or oocytes that just completed GVBD, maturing oocytes arrested at metaphase I. In

contrast, if protein synthesis was inhibited at metaphase I, oocytes completed their first meiotic division and entered interphase (formation of a nucleus with decondensed chromosomes). These studies suggested that multiple protein synthesis events were required to complete oocyte maturation.

1.4.1 Cyclin B

It is generally accepted that cyclin B is a key protein involved in the control of this transition. Cyclin B and p34^{cdc2} protein kinase (Cdk1) are components of maturation-promoting factor (MPF) (Lohka et al, 1988). MPF was first described as a cytoplasmic factor that induced complete maturation when injected into immature frog oocytes (Masui and Markert, 1971). In mouse oocytes, MPF activation precedes GVBD (Choi et al, 1991), reaching a plateau at metaphase I (Verlhac et al, 1994). MPF drops sharply when the homologous chromosomes separate and the first polar body is emitted (Hashimoto and Kishimoto, 1986). After that, MPF increases again and peaks in metaphase II. Ubiquitination-mediated degradation of cyclin B is responsible for this fall in MPF activity (Hampl and Eppig, 1995; Murray, 1995). In support of the role of cyclin B degradation, cyclin B1 mRNA injection arrested mouse oocytes at metaphase I (Ledan et al, 2001).

1.4.2 APC/C (anaphase promoting complex/cyclosome)

A multi-protein complex, APC/C is thought to be a key regulator of protein

ubiquitination and degradation during the cell cycle. The activated APC/C ubiquitinates key regulators of mitosis and targets them for degradation via the 26S proteasome. At the metaphase-to-anaphase transition, the activated APC/C ubiquitinates cyclin B and securin, a protein that sequesters a protease named separase and keeps it inactive (Peters, 2002; Nasmyth, 2002). Once securin is degraded, separase is liberated and becomes active to cleave Scc1, a subunit of the cohesion complex holding sister chromatids together (Peters, 2002). Then the sister chromatids separate to the opposite poles. In somatic cells, the metaphase-to-anaphase transition takes place only when all sister chromatids have been properly attached to the bipolar spindle and are aligned at the metaphase plate. When this is not the case, the “spindle checkpoint” will be activated which causes cell cycle arrest at the metaphase-to-anaphase transition with high levels of cyclin B, high mitotic kinase activity, and condensed chromosomes (Gorbsky, 2001; Musacchio and Hardwick, 2002). The checkpoint protein Mad2 has been shown to directly interact with and inhibit APC/C (Wassmann and Benezra, 2001). Whether such a checkpoint exists during the first meiotic division in mammalian oocytes when homologous chromosomes are segregated remains controversial. Recently, two papers (Wassmann et al, 2003; Terret et al, 2003) showed that overexpression of Mad2 or application of 26S proteasome inhibitor caused maturing mouse oocytes to arrest at metaphase I. Securin mRNA injection also arrested denuded mouse oocytes at metaphase I. In contrast, in frog oocytes, inhibition of APC/C or ubiquitination-mediated protein degradation did not prevent metaphase I-to-anaphase

transition. These treatments however inhibited second anaphase initiation upon egg activations (Peter et al, 2001; Taieb et al, 2001). Therefore, it is concluded that APC/C is not required for the first meiotic anaphase in frog oocytes.

1.4.3 Cytoskeletons (microtubule and actin)

In addition to the changes in MPF activity, meiosis I to meiosis II transition also depends on the appropriate assembly of the first meiotic spindle in relation to the oocyte cortex. An early study showed that cytochalasin B, an inhibitor of actin polymerization, arrested denuded mouse oocytes at metaphase I with a bipolar spindle in the center of the oocyte (Wassarman et al, 1976b), suggesting disruption of actin system in the oocyte blocked spindle migration to the cortex and subsequent cell cycle progression. Consistent with this idea are two recent studies that demonstrated the requirement of components of the actin cytoskeleton in regulating spindle movement, cortical attachment and meiosis I to meiosis II transition (Leader et al, 2002; Weber et al, 2004).

2. MATERIALS AND METHODS

2.1 Chemicals and Antibodies

Leibovitz's L-15 medium, all components of KSOM and KFHM media (see solution), fetal bovine serum (FBS), ascorbic acid, selenium, serotonin (5-HT), human chorionic gonadotropin (HCG), ritanserin, methiothepine, mesullergine, brefeldin A (BFA), p-chlorophenylalanine (CPA), dibutyryl cyclic AMP (dBcAMP), Tris-HCl, formaldehyde, Triton X-100, taxol, aprotinin, glycine, beta-mercaptoethanol (β -Me), leupeptin, pepstatin, phenylmethylsulfonyl fluoride (PMSF), glycerophosphate, sodium orthovanadate, transferring, insulin, Tween 20 and mineral oil were purchased from Sigma (St. Louis). Minimal essential medium (MEM) alpha medium, Penicillin/Streptomycin, were purchased from Invitrogen (Burlington, ON). In vitro translation kit was obtained from Amersham Biosciences (Baie d'Urfe, QC). Follicle stimulating hormone (FSH) was purchased from Pituitary hormone & antisera center, NIDDK (Torrance, CA)

Rat anti-HA antibody was purchased from Roche Diagnostics (Laval, QC). Anti-mouse beta-tubulin DM1B was obtained from Developmental Studies Hybridoma Bank at University of Iowa (Iowa city, IA). Anti-sheep phospho-MAPK was purchased from Upstate Biotechnology (Lake Placid, NY). Rat anti-alpha tubulin monoclonal antibody was obtained from Chemicon International (Temecula, CA). Peroxidase-conjugated donkey anti-sheep antibody was from Jackson Immunoresearch

Laboratories (West Grove, PA). Peroxidase-linked sheep anti-mouse and anti-rat antibodies were obtained from Amersham Biosciences (Baie d'Urfe, QC). Alexa-594 goat anti-rat antibody, Sytox green were obtained from Molecular Probes (Eugene, OR).

ARF1-T31N-HA construct was kindly provided by Dr. Donaldson JG (Cell Biology and Metabolism Branch, National Institutes of Health, Bethesda, MA).

2.2 Solutions

2.2.1 Oocyte handling and culture media

KSOM medium (Lawitts and Biggers, 1993) – to culture denuded oocytes in an incubator containing 5% CO₂:

104mM NaCl, 2.5mM KCl, 0.35mM KH₂PO₄, 0.2mM MgSO₄, 1mM Na lactate, 0.2mM glucose, 0.2mM Na pyruvate, 25mM NaHCO₃, 1.7mM CaCl₂, 1mM glutamine, 0.01mM tetrasodium EDTA, 0.03mM streptomycin SO₄, 0.16mM K penicillin G. Medium was supplemented with 1mg/ml bovine serum albumin (BSA) and equilibrated with 5% CO₂ by placing in the incubator before use.

KFHM medium (Lawitts and Biggers, 1993) – to handle denuded oocytes outside of the incubator (5% CO₂):

Same as KSOM, except 21mM Hepes replaces 21mM NaHCO₃ in KSOM. Medium was

supplemented with 1mg/ml BSA, adjusted pH value to 7.4 before use.

Follicle-handling medium:

Leibovitz's L-15 medium supplemented with 2mM glutamine, 50 μ g/ml ascorbic acid, 2ng/ml selenium and 0.3% BSA. Medium was adjusted pH value to 7.2 before use.

Follicle-culture medium:

MEM α medium (containing L-glutamine) supplemented with 5% FBS, 1% penicillin/streptomycin, 50 μ g/ml ascorbic acid, 2ng/ml selenium and 0.01mM EDTA.

2.2.2 Solutions for fixation and immuno-staining

These solutions were according to Carabatsos et al (2000).

Fixation solution:

2% formaldehyde, 0.5% Triton X-100, 1 μ M taxol, 10 μ g/ml aprotinin and Tris Buffered Saline (TBS: 10mM Tris, 150mM NaCl).

Blocking solution:

2% BSA, 2% FBS, 0.1M glycine, 0.01% Triton X-100 and TBS buffer.

2.3 Mice and oocytes/follicles manipulations

2.3.1 Animals

All mice were from Charles River, Canada. Immature CF1 female mice, 21-27d old, were used for all experiments. Mice were provided with water and mouse chow ad libitum and housed in air-conditioned rooms illuminated for 14h/day.

2.3.2 Oocyte isolation and culture

Mice were killed by cervical dislocation. Ovaries were immediately removed and transferred to 2-3ml KFHM medium in a 35mm tissue culture dish (Fisher, Pittsburgh, PA). Antral follicles were pierced with a 25-gauge sterile needle under a dissecting microscope. Deduded oocytes were obtained by repeated pipetting with a Pasteur pipette to mechanically remove cumulus cells. Only oocytes showing clear nuclear membrane (GV oocytes) were collected. Oocytes were cultured using microdrop culture (Lawitts and Biggers, 1993). Briefly, 3-7 droplets of KSOM medium (approximately 50 μ l/drop) were placed on 35mm tissue culture dish and overlaid with 2-3ml of KSOM-washed mineral oil. The medium was pre-equilibrated in an incubator with 5% CO₂, 5% O₂, 90% N₂/air at 37°C and 100% humidity before use. Oocytes were washed through 2-3 drops of KSOM using a mouth-operated pipette, then cultured in the last drop of KSOM, and the dish was returned to the incubator. Culture time is specified in figure legends.

2.3.3 Follicle isolation and culture

Antral follicles (Downs, 2000): Ovaries from immature CF1 mice were placed in a Petri dish containing 2-3ml follicle-handling medium. Individual antral follicles (usually 30-40/mouse) were dissected from the ovary using a forcep and a sterile 30-gauge needle attached to a syringe. Care was taken to remove as much interfollicular tissue as possible. Only follicles exhibiting a clear, transparent antrum were selected for further experiments. Follicles were washed and transferred by Pasteur pipette to 0.5ml follicle-culture medium in center-well organ culture dish at 37°C with 5% CO₂.

Preantral follicles (O'Brien et al, 2003): The procedure of dissecting preantral follicles is the same as antral follicles. Follicles selected for culture had no antrum, but with at least two layers of granulosa cells, appeared healthy, pale in colour and translucent, and contained a spherical oocyte locating in the center of the follicle. A theca layer was retained around the follicle. Individual follicles were washed and then cultured in 100µl follicle-culture medium (supplemented with 100mIU/ml FSH, 5µg/ml transferrin and 5µg/ml bovine insulin) in V-shaped wells of a 96-well tissue culture plate at 37°C with 5% CO₂. Follicle diameter was measured daily using a calibrated eyepiece micrometer. The survival rate of follicles was checked by evaluation of degeneration or atresia (blackening of the follicle) and bursting (loss of oocytes). Follicles were moved to fresh medium daily and were cultured for up to 6 days.

2.3.4 Examination of oocytes

At the end of culture, denuded oocytes were collected under a dissecting microscope for examination. Follicle-enclosed oocytes were released from the follicle by a small incision of follicle wall and collected after removing cumulus cells. Oocytes were examined for their meiotic stage by Hoffman modulation contrast microscope (Nikon). Oocytes were classified as GV (presence of an intact germinal vesicle), GVBD (no nuclear structure, no PB) and metaphase II (presence of a PB). Data from the whole mouse were excluded if dead oocytes accounted for more than 40% in any treatment group. Since we did not observe any significant difference in death rate among all groups, dead oocytes were excluded from the percentage calculations. In case of follicle culture, data were also excluded if spontaneous matured oocytes account for over 50% in the control group.

2.4 Microinjection

Denuded oocytes were collected as described above. Then oocytes were transferred to a drop of KFHM supplemented with 100 μ M dBcAMP (used to maintain meiotic arrest) covered with paraffin oil to prevent evaporation. The oocyte was immobilized by a holding pipette. Microinjection was carried out using micropipettes (about 1 μ m diameter opening, pulled from Borosilicate glass by Flaming/brown micropipette puller (Sutter instrument, Novato, CA)) mounted on Pico-injector (PL1-100, Harvard apparatus, Holliston, MA) adapted to an Axiovert S100 inverted microscope (ZEISS, Thornwood, NY). A pressure

pulse of 5-7 psi of 50-60 ms duration was delivered by using a picopump to inject RNA into the oocyte. About 10pl of the RNA solution was injected per oocyte. After microinjection, oocytes were washed and cultured in KSOM with 100 μ M dBcAMP for 6h. Following 6h culturing, oocytes were washed 3 times and transferred to KSOM for overnight culture. Meiotic status of oocytes were checked at next morning.

Microinjection of oocytes within preantral follicles is technically similar to but harder than that of denuded oocytes. The micropipette has to penetrate the follicle wall in order to inject RNA into the oocyte. DBcAMP was not necessary in the injection or culture medium since oocytes are not competent to undergo maturation. After injection, preantral follicles were kept culturing for up to 6 days until they reached preovulatory stage. Then follicles were dissected to check meiotic status of enclosed oocytes.

2.5 Western blotting

Proteins from 40-50 denuded mouse oocytes were extracted using 10 μ l 2 \times SDS sample buffer containing 10% β -Me. The lysates were immediately heated to 100 $^{\circ}$ C for 4 min, then cooled on ice for 4 min and centrifuged at 13,000 rpm for 5 min (Su et al, 2002). For frog oocytes, oocytes were lysed by pipetting up and down in ice-cold PBS lysis buffer (10mM sodium phosphate pH 7.5, 150mM NaCl, 0.1% Triton X-100, 10 μ g/ml of leupeptin, 1mM phenylmethylsulfonate, 10 μ l lysis buffer per oocyte). The lysates were centrifuged immediately at 13000 rpm for 5 min, the clarified extract was removed and mixed with

2×SDS sample buffer.

Proteins were separated by SDS-PAGE in 15% polyacrylamide gels. The fractionated proteins were transferred onto Protran pure nitrocellulose membrane (Scheicher & Schuel, Keene, NH) (voltage 9V for 1h). Nonspecific binding was minimized by blocking the membrane in Blotto (5% skim milk in TBST (TBS supplemented with 0.02% Tween-20)) for 30 min before incubating with primary antibody. The membrane was incubated overnight with sheep polyclonal anti-phospho-MAPK (1:300) or rat anti-HA (1:1000) depending on the experiment. Then the membrane was washed in TBST at least 3 times (10min each) to remove nonspecific antibody binding. The blot was then incubated for 1h with peroxidase-conjugated donkey anti-sheep antibody (1:5000) or sheep anti-rat antibody (1:3000) depending on the experiment. The blot was washed again at least 5 times (5min each) in TBST. Immunoreactive proteins were detected by ECL western blotting detection reagents (Amersham Pharmacia Biotech, Buckinghamshire, England) followed by exposure to film.

For re-probing, the blot was washed twice in TBST and stripped in 10ml stripping buffer (1M Tris-HCl 625 μ l, pH 6.8, 10% SDS 2ml, β -Me 79.72 μ l) at 65°C for 30 min to remove primary and secondary antibodies. The blot was then washed twice in TBST (10min each) and re-blocked with 5% milk in TBST for 30-60min. The blot was re-probed by mouse anti- β -tubulin DM1B (1:1000) and peroxidase-linked sheep anti-mouse antibody (1:3000).

2.6 In vitro translation

1 μ l ARF1-T31N-HA mRNA was incubated with 35 μ l rabbit reticulocyte lysate, 5 μ l [³⁵S] methionine and 1 μ l amino acid (no Met) at 30°C for 1h. Then 2 μ l sample was added into 20 μ l 2xSDS sample buffer and boiled for at least 3 min. The mixture was loaded onto a 15% SDS-polyacrylamide running gel overlaid with a 5% SDS- polyacrylamide stacking gel. The mixture was separated by electrophoresis until the dye-front just ran out of the gel (200V for 45-60 min). The stacking gel was removed and the running gel was stained and fixed in 45% methanol, 10% acetic acid, 2.5mg/ml coomassie brilliant blue and water for 15-20 min. Excess stain was removed by washing in a series of solutions containing 45% methanol, 10% acetic acid and water until the protein bands were seen. The gel then was dried on filter paper under vacuum (70°C, 3h). Densitometric analysis was performed utilizing Typhoon 8600 variable mode laser imager (Molecular Dynamics, Sunnyvale, CA) supported by Typhoon scanner control software (Amersham Biosciences, Baie d'Urfe, QC).

2.7 Immunofluorescence

All procedures of fixation and immuno-staining were done in 37°C-heat box (unless otherwise specified) in 100 μ l solution in 96-well culture plate. Oocytes were fixed by pre-warmed (37°C) fixing solution for 20 min, then rinsed 3 times in blocking solution (10min each). Oocytes were then incubated overnight with rat anti- α -tubulin (1:50 in

blocking solution) at 4°C. Oocytes were rinsed at least 5 times in blocking solution (10 min each) to minimize nonspecific binding followed by incubation with Alexa-594 goat anti-rat antibody (1:200 in blocking solution) for 3h in the dark. The remaining procedure was carried out such that oocytes were exposed to as little light as possible. Unbound secondary antibodies were removed by 6 times rinses in blocking solution (10 min each). Then oocytes were incubated with Sytox green (1:1000 in 1:1 Triton-TBS:water) for at least 3h. Oocytes were rinsed once with 0.5% Triton X-100 in TBS and once with water. Then oocytes were stuck to size-0 coverslips and mounted onto glass slides with 75mg/ml n-propyl gallate in 1:1 glycerol:water. To prevent distortion of their shape by the coverslip, tape with a big hole in the middle formed a well on the slide into which oocytes could be mounted. The sides of the coverslip were sealed with nail polish and stored in the dark until microscopic analysis.

2.8 Imaging

2.8.1 Confocal imaging

Confocal images were collected using an Olympus IX70 inverted microscope equipped with a BioRad MRC-1024 confocal laser-scanning unit. For the Alexa 594 conjugates, a helium-neon laser produced excitation at 594nm with emission at 600nm. For Sytox green staining, the fluorescent excitation was produced by a 488nm Argon-ion laser and collected through a band pass filter centered at 522nm. Images were collected using an

Olympus UApo 40x water immersion lens and Laser sharp MRC-1024 confocal system
BioRad v 3.2 software (Bio-Rad Laboratories, Mississauga, ON).

2.8.2 Brightfield and fluorescent imaging

Images were obtained by using a Leica MZ FLIII microscope (Leica Microsystem Inc. Wetzler GmbH, Germany) equipped with Qimaging Retiga 1300 digital camera (W. Nuhsbaum Inc, McHenry, IL) and Openlab 3.0.4 software (Improvision Inc, Lexington, MA). Fluorescence was from Ebq-100 light source with GFP (FITC) filter.

2.9 Statistics

Every experiment involving follicle or oocyte culturing was performed at least 5 times. The number of mice for each experiment is indicated in the figure legend. Data are presented as the mean \pm the standard error of the mean (SEM). In all cases, $P < 0.05$ was considered significant. All SEM calculations were performed by Microsoft Excel 2002. Statistical comparisons between several groups were performed by ANOVA followed by Tukey-Kramer's Multiple Comparisons Test (by SPSS 12.0 for Windows, SPSS Inc, Chicago, IL). Student t-tests were used when there were only two groups of data.

3. RESULTS

3.1 A serotonin receptor antagonist induces oocyte maturation in the mouse: Evidence that a G protein-coupled receptor is responsible for maintaining prophase arrest

3.1.1 Ritanserin induced follicle-enclosed oocyte maturation

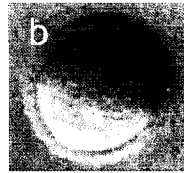
To test whether a serotonergic system is involved in the maintenance of the first meiotic arrest, we incubated isolated antral follicles with several antagonists (methiothepine, mesullergine, ritanserin) of serotonin receptors. Antral follicles were chosen because mouse oocytes require intact follicles to maintain prophase arrest. Following overnight incubation, follicles were dissected to determine the cell cycle status of enclosed oocytes. As shown in Fig. 4, oocytes showing a clear nuclear membrane were classified as GV. Oocytes that did not show any nuclear structures were classified as GVBD. Oocytes showing a polar body (PB) were considered as fully mature (arrested at metaphase II, see later). Dead oocytes appeared flat, as compared to the spherical shape of live oocytes. We found that ritanserin, but not the other serotonin antagonists, significantly induced GVBD and PB formation within the follicle ($P < 0.005$) (Fig. 5), albeit with reduced efficiency compared to HCG (Human chorionic gonadotropin). HCG activates the LH receptor and was therefore used as a positive control. Most ritanserin-treated oocytes,

Fig 4.

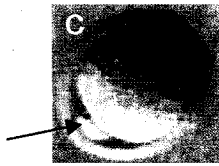
Light images of the typical oocyte. a) GV oocyte. b) Oocyte that has undergone GVBD. c) mature oocyte (arrow indicates a polar body). d) Dead oocyte.



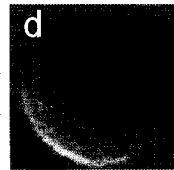
GV



GVBD



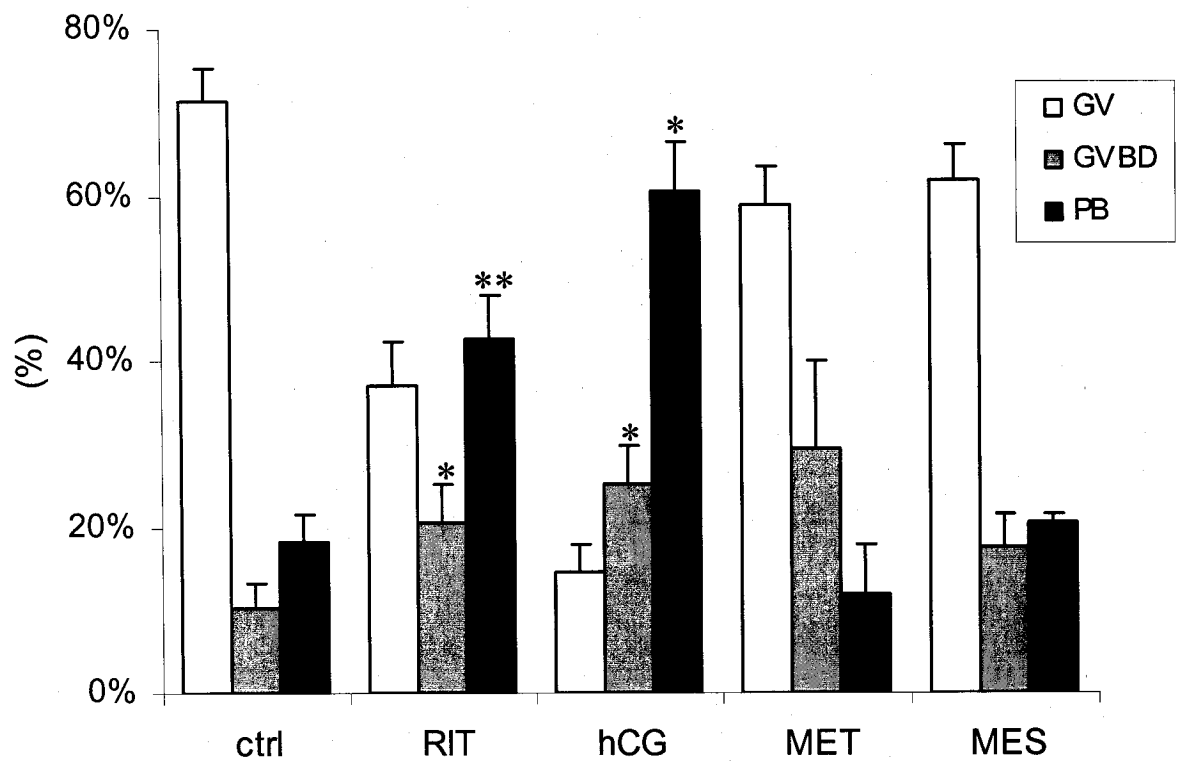
mature oocyte
(PB)



Dead

Fig 5.

Ritanserlin induced follicle-enclosed oocyte maturation. Antral follicles were incubated with MEM (ctrl), 50IU/ml HCG, 10 μ M ritanserlin (RIT), mesullergine (MES) or methiothepine (MET) for 16-20h. Follicles were dissected and enclosed oocytes were assessed for cell cycle status. Shown are averages of GV, GVBD, PB ratio with SEM in each group. Oocytes for each mouse were divided into 5 groups. In total 9 mice were used. *, P<0.005. **, P<0.05.



like HCG-treated oocytes, proceeded to metaphase II, as indicated by the presence of a polar body ($P < 0.05$). Ritanserin-induced oocyte maturation was concentration-dependent (Fig. 6), with optimal concentrations of about $10\mu\text{M}$.

3.1.2 Exogenous serotonin did not alter oocyte maturation

The ability of an antagonist of serotonin receptor (ritanserin) to induce oocyte maturation suggested that a serotonin receptor might be the meiosis inhibitory GPCR. To determine if exogenous serotonin might influence oocyte maturation, we took two approaches. In the first approach, we treated antral follicles with serotonin for an hour before the addition of HCG, then follicles were further cultured for 6h. As shown in Fig. 7, HCG induced a significant percentage of oocyte maturation ($P < 0.001$, compared to control group). The presence of exogenous serotonin did not affect HCG-induced oocyte maturation. In the second approach, we applied serotonin to denuded oocytes. We found that none of the concentrations of serotonin inhibited spontaneous oocyte maturation (Fig. 8). Whereas as a control, dBcAMP significantly blocked spontaneous oocyte maturation ($P < 0.001$).

3.1.3 Synthesis of serotonin is not required for prophase arrest

These results indicated that exogenously added serotonin did not influence oocyte maturation. To eliminate the possibility that the exogenous serotonin did not reach the

Fig 6.

Concentration-dependent responses of ritanserin to induce follicle-enclosed oocyte maturation. Antral follicles were incubated with MEM, 50IU/ml HCG or the indicated concentrations of ritanserin for 16-20h, then follicles were dissected, enclosed oocytes were assessed for cell cycle status. Black bar represents death rate. Maturation rate includes GVBD and PB rate. *, $P < 0.005$; **, $P < 0.001$.

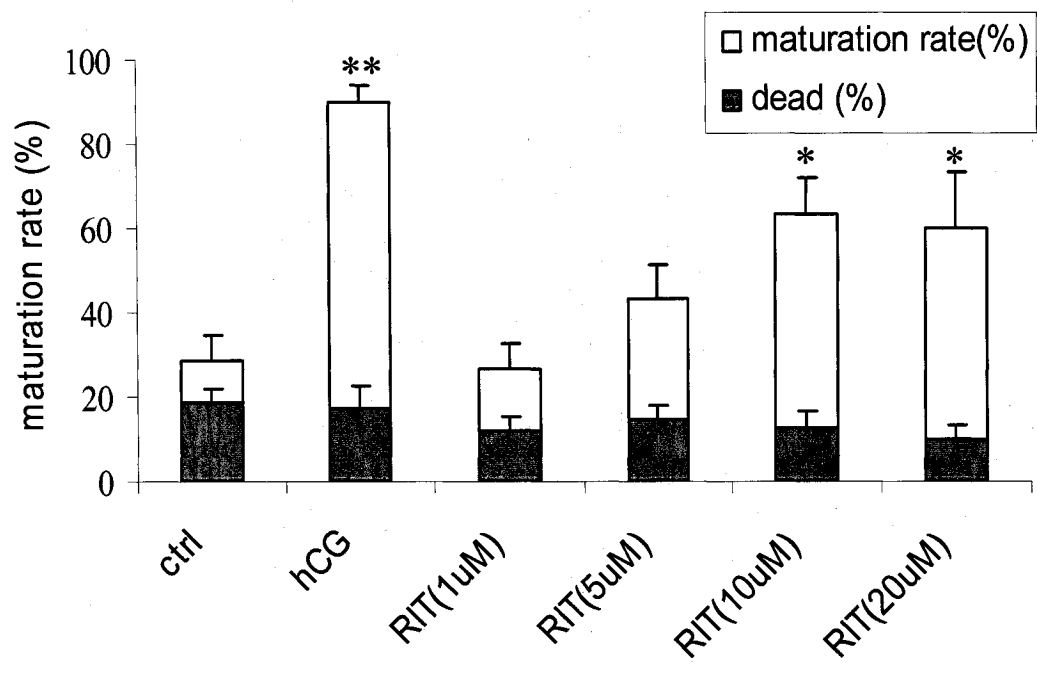


Fig 7.

Exogenous 5-HT did not inhibit HCG-induced GVBD in intact follicles. Antral follicles were incubated with $10 \mu\text{M}$ 5-HT 1h before the addition of HCG (50IU/ml), then follicles were cultured for a further 6h. Follicles were dissected and enclosed oocytes were assessed for cell cycle status. Oocytes from each mouse were divided into 3 groups. 12 mice were used totally. *, $P < 0.001$.

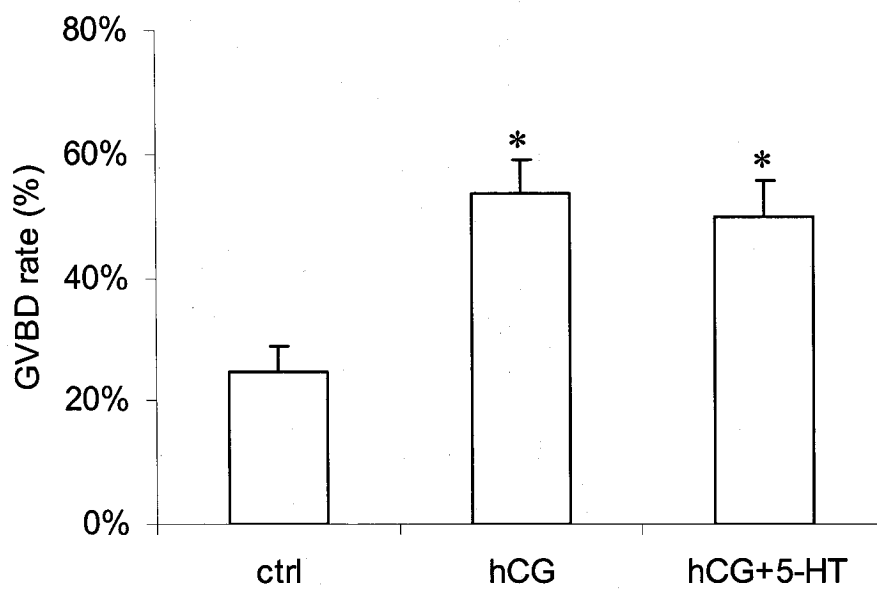
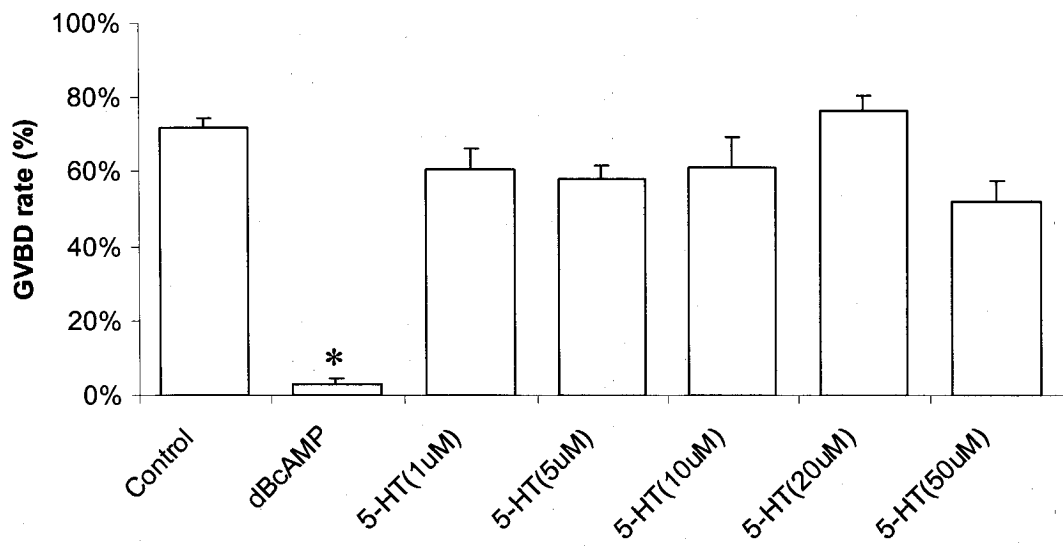


Fig 8.

Exogenous 5-HT did not block spontaneous GVBD. Denuded oocytes were cultured with different concentrations of 5-HT for 6h. GVBD was scored at the end of 6h. *, $P < 0.001$.



proper site or was too quickly metabolized, we attempted to inhibit endogenous serotonin biosynthesis. We incubated antral follicles with a high concentration of p-chloro-phenylalanine (CPA), an inhibitor of tryptophan hydroxylase (rate-limiting enzyme in serotonin biosynthesis from tryptophan) overnight. As shown in Fig. 9, CPA did not induce oocyte maturation within the follicle ($P > 0.05$, compared to control group). Taken together, these data strongly suggest that an ovary-based serotonergic system is unlikely the major factor in oocyte meiotic arrest.

3.1.4 Attempts to identify ritanserin-antagonized receptor

In our attempt to identify the target of ritanserin in mouse oocytes, we sought to develop a model of microinjecting gene-specific morpholino antisense oligos into the oocyte within preantral follicles, followed by culturing these follicles until they reach preovulatory stage (Fig. 10A). Preantral follicles contain only a few layers of follicle cells and hence the enclosed oocytes are more accessible for microinjection. In addition, the week-long culture time (O'Brien et al, 2003) (from preantral to antral follicle) provides a physiological opportunity for protein synthesis/turnover, which is critical when antisense oligos are involved. Specifically, we were interested in the possibility that injection of antisense oligos against candidate GPCR might result in the removal of the meiosis inhibitory GPCR and hence result in spontaneous oocyte maturation within the follicle once the oocyte becomes competent.

Fig 9.

CPA did not induce follicle-enclosed oocyte maturation. Antral follicles were incubated with 50 μ M CPA or CPA+5-HT for overnight. Follicles were dissected and enclosed oocytes were assessed for cell cycle status. Oocytes from each mouse were divided into 4 groups. 5 mice were used totally. *, $P < 0.001$.

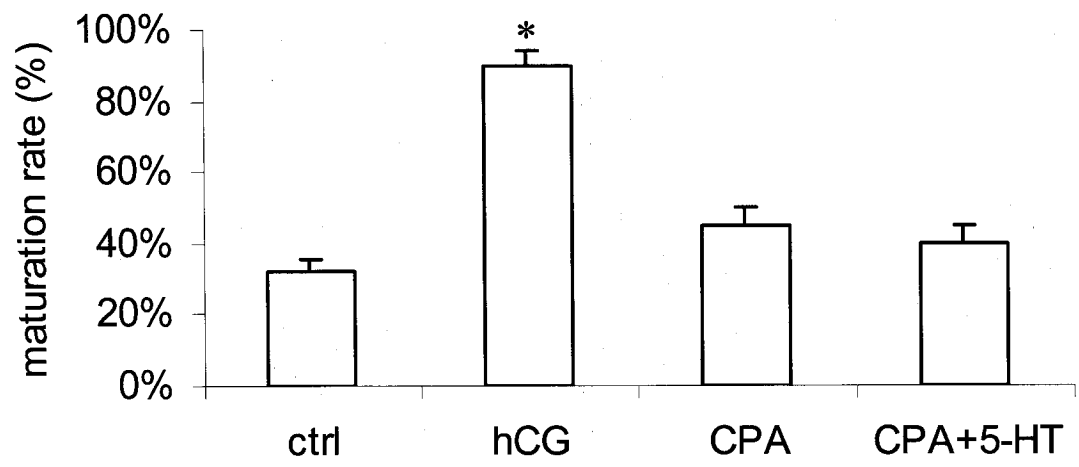
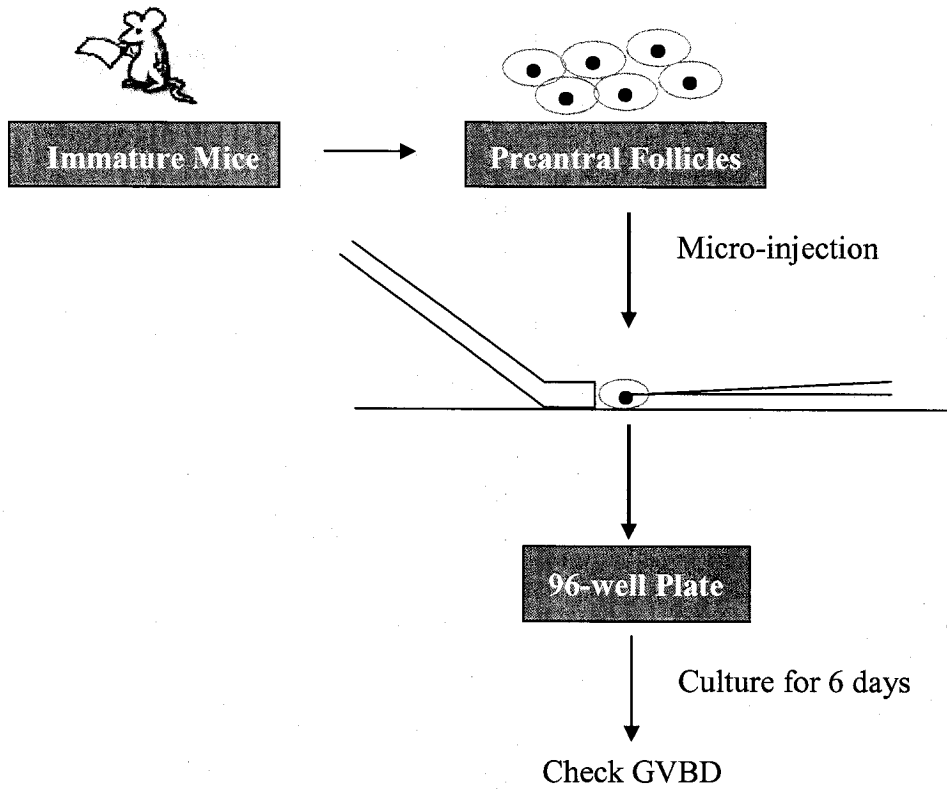


Fig 10.

Microinjection of mouse oocytes within preantral follicles. A. Microinjection scheme. B. GFP expressed in oocytes within preantral follicles. 2h after GFP mRNA injection, pictures were taken under fluorescence microscopy. a) control preantral follicles injected with H₂O. b) preantral follicles injected with GFP (oocyte is glowing in the follicle).

A



B



Although several groups have cultured preantral mouse follicles to a stage of meiotic competence (Hu et al, 2002; O'Brien et al, 2003), none of them has reported injection of oocytes within follicles prior to the culture. We successfully expressed GFP in mouse oocytes within preantral follicles (Fig. 10B). Unfortunately, we were unable to culture preantral follicles injected with GFP mRNA to antral follicles. All follicles underwent atresia before the full 6-day protocol.

3.2 Brefeldin A induces resumption of meiosis, but arrests mouse oocytes at metaphase I.

In our search for a unified “maturation inducer” of vertebrate oocytes, we were intrigued by an early report that brefeldin A (BFA) was capable of inducing *Xenopus* oocyte maturation (Mulner-Lorillon et al, 1995). BFA, a specific and potent inhibitor of the small G protein ARF1, is widely used to inhibit membrane trafficking (Lippincott-Schwartz et al, 1989). Specifically, BFA blocks anterograde (ER-Golgi (Lee et al, 2004)) membrane fusion without inhibiting retrograde (Golgi-ER (Lee et al, 2004)) membrane fusion, therefore cells treated with BFA eventually lose any visible Golgi apparatus (Lippincott-Schwartz et al, 1990; Sciaky et al, 1997). Interestingly, an emerging theory has suggested Golgi breakdown may play an active role in initiation of mitosis (Sutterlin et al, 2002). We therefore wished to test whether Golgi disruption induced by BFA could initiate meiosis in mouse oocytes.

3.2.1 Brefeldin A induces follicle-enclosed oocyte maturation

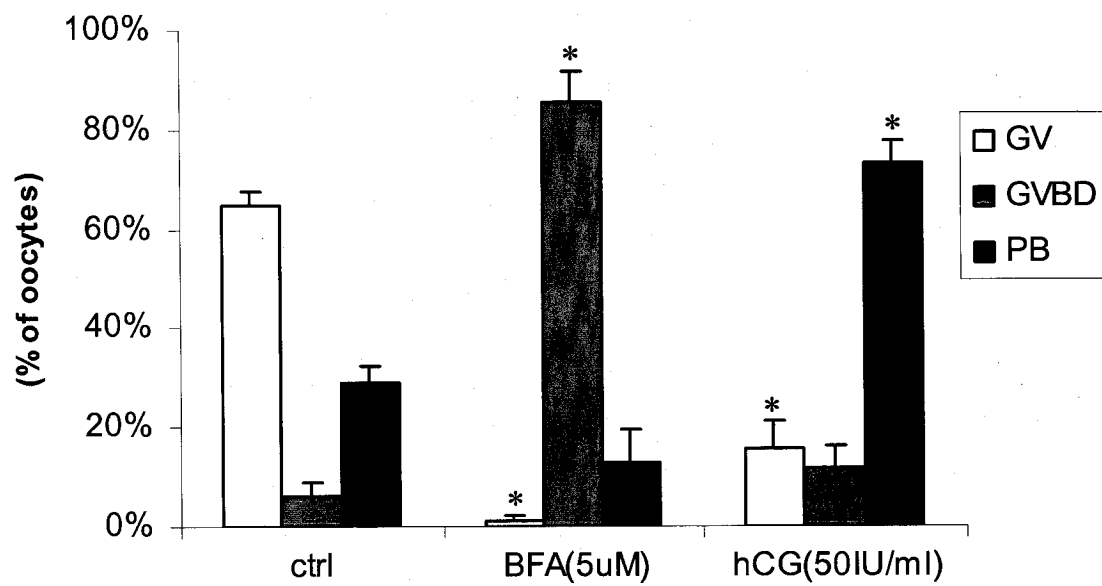
To explore if BFA can induce mouse oocyte maturation within the follicle, we incubated antral follicles with 5 μ M BFA for overnight. Upon dissection of the follicles, we found that almost 100% BFA-treated follicles contained a GVBD positive oocyte ($P < 0.001$), whereas only 30% follicles containing such oocytes in the control group (Fig. 11A). However, unlike HCG-treated oocytes which were fully mature (with a polar body), most BFA-treated oocytes had no polar bodies (Fig. 11B).

After dissection of follicles, we collected and fixed oocytes, followed by staining them with Alexa 594-anti-tubulin and Sytox green (chromosome dye). Analysis of microtubule and chromosome configurations by confocal microscopy (Fig. 12) showed that the HCG-treated oocyte had a polar body and a barrel-shaped metaphase II spindle. The spindle was bipolar and peripherally located near the cortex with its long axis parallel to the cortex. Condensed chromosomes were aligned along the equator of the spindle (metaphase plate). In contrast, the BFA-treated oocyte had no polar body, but had a somewhat “blunt-ended” bipolar spindle. The spindle was located in the center of the oocyte with condensed chromosomes aligned at the metaphase plate. These analyses indicated that most BFA-treated oocytes were arrested at metaphase I.

Fig 11.

BFA induced follicle-enclosed oocyte maturation. Antral follicles were incubated with MEM (ctrl), 5uM BFA or 50IU/ml HCG for 16-20h. Follicles were dissected and enclosed oocytes were assessed for cell cycle status. A) Shown are averages of GV, GVBD, PB rate with SEM in each treatment. Oocytes from each mouse were divided into 3 groups. 8 mice were used totally. *, $P < 0.001$. B) Light image of HCG and BFA-treated oocytes. HCG-treated oocytes (a) progressed to metaphase II with normal-appearing first polar body (as indicated by an arrow). BFA-treated oocytes (b) had no polar bodies.

A



B

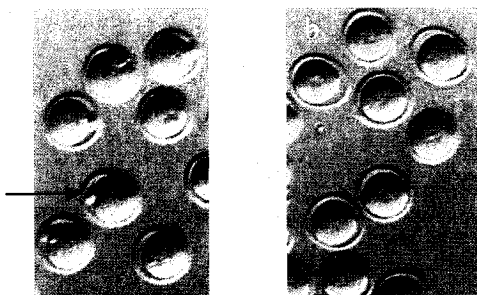
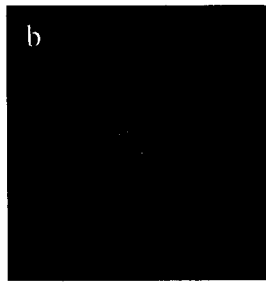
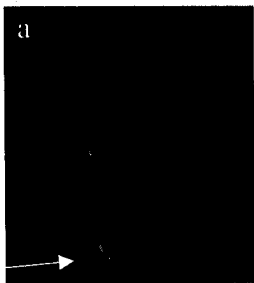


Fig 12.

Confocal image of HCG and BFA-treated follicle-enclosed oocytes shown in Fig. 11. After isolation from follicles, oocytes were fixed and stained with Alexa 594-anti-tubulin (red) and Sytox green (green). Oocytes were observed by confocal microscopy. a) HCG-treated oocytes (metaphase II stage, arrow indicates a PB) b) BFA-treated oocytes (metaphase I stage).



3.2.2 BFA arrests denuded oocytes at metaphase I

These results suggested that BFA had two distinct effects – to initiate oocyte maturation and to prevent anaphase initiation. To test whether BFA had a similar effect on denuded oocytes undergoing spontaneous maturation, we incubated denuded oocytes with 5 μ M BFA for overnight. We found that almost none of BFA-treated oocytes had PB, compared to more than 90% in the control group ($P < 0.001$) (Fig. 13). Spindle morphology in BFA-treated denuded oocytes was similar to that in BFA-treated follicle-enclosed oocytes, which confirmed that BFA-treated denuded oocytes were also arrested at metaphase I (data not shown).

3.2.3 Inhibition of ARF1 arrests oocytes at metaphase I

To further establish the specificity of BFA effect in oocytes, we tested whether a dominant-negative mutant of ARF1 (ARF1-T31N-HA) would have a similar effect on oocyte maturation. We employed denuded oocytes for this purpose as it is technically difficult to inject oocytes within antral follicles. We first confirmed ARF1-T31N-HA expression by in vitro translation and by immunoblotting following mRNA injection into frog oocytes. A constitutively activated mutant (ARF1-Q71L-HA) was also analyzed (Fig.14). After microinjection, we incubated denuded mouse oocytes in the presence of dBcAMP (used for maintaining prophase arrest to allow expression of the protein from the injected mRNA) for 6h. Oocytes were then transferred to fresh medium without dBcAMP

Fig 13.

BFA arrested denuded oocytes at metaphase I. Oocytes were isolated from ovary with 100 μ M dBcAMP in isolation medium. Meiotic progression was assessed after 16h-culturing with or without BFA. Oocytes from each mouse were divided into 2 groups. 6 mice were used in total. *, $P < 0.001$.

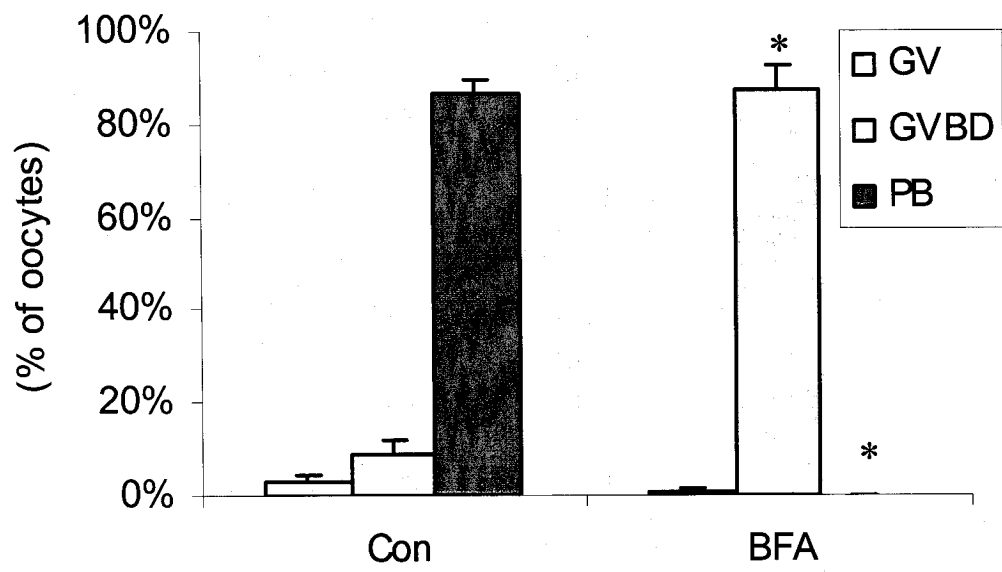
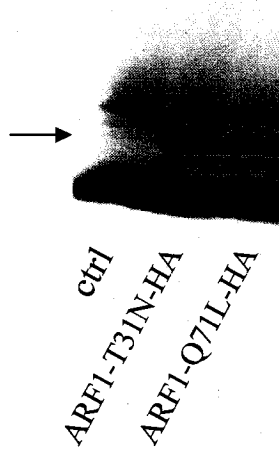


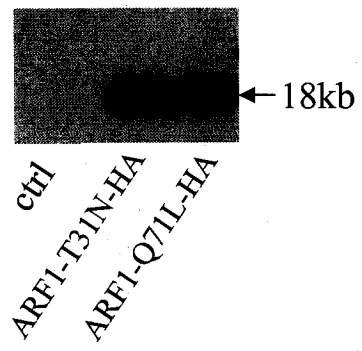
Fig 14.

ARF1 protein expression. A) *in vitro* translation (SDS-PAGE separation and autoradiography) of two ARF1 RNAs. ARF1-T31N-HA: dominant negative construct. ARF1-Q71L-HA: constitutively activated construct. B) Western analysis of expression of two ARF1 proteins in *Xenopus* oocytes. Both of them were identified as 18kDa bands by reaction with the anti-rat HA antibody.

A



B



for overnight. The oocytes were then examined for their cell cycle status. We found that about 50% of denuded oocytes injected with ARF1-T31N-HA were arrested at metaphase I (as confirmed by spindle morphology), compared to about 20% in oocytes injected with GFP or ARF1-Q71L-HA ($P < 0.005$) (Fig. 15). A bipolar spindle peripherally located near the cortex was observed by confocal microscopy in ARF1-T31N-HA injected oocytes. These results suggested that endogenous ARF1 was the target for BFA.

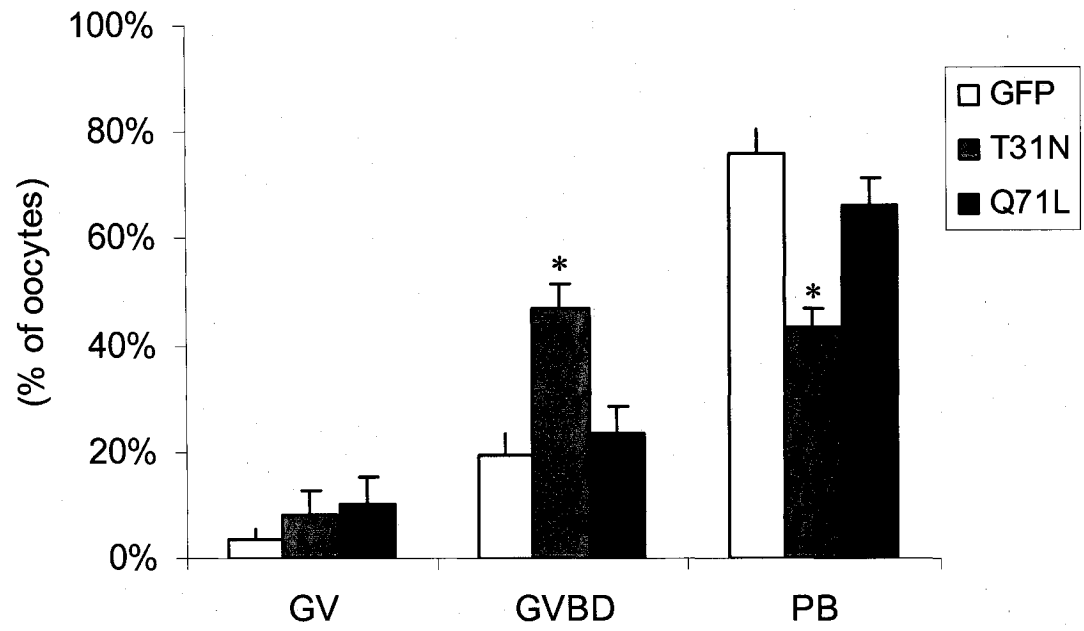
3.2.4 Further characterization of BFA effects in mouse oocytes

One of the most important protein kinase cascades involved in oocyte maturation is the Mos/MAP Kinase pathway (Nebreda and Ferby, 2000). MAPK is activated prior to GVBD in *Xenopus* oocytes. However, MAPK is activated about 2-3 hours after GVBD in mouse oocytes (Verlhac et al, 1993). This delayed activation of MAPK in mammalian oocytes suggests that MAPK does not regulate early meiotic events such as GVBD. In both species, MAPK activity remains high between meiosis I and meiosis II. This high activity is thought to be responsible for suppressing DNA replication between the two M-phases (Josefsberg et al, 2003). We were curious whether BFA-treated oocytes contained activated MAPK, as they were arrested at metaphase I. Following overnight incubation of denuded oocytes in the presence or absence of BFA, we extracted both groups of oocytes for immunoblotting using antibodies against phosphorylated (activated) MAPK (Widmann et al, 1999). As shown in Fig.16, MAPK is phosphorylated in both control (metaphase II

Fig 15.

ARF1 dominant negative mutant injection arrested denuded oocytes at metaphase I. A) oocytes arrested at metaphase I after ARF1 dominant negative RNA injection. Denuded oocytes were injected with ARF1-T31N-HA, ARF1-Q71L-HA or GFP in the presence of dBcAMP, then were cultured in medium containing dBcAMP for 6h. At the end of culture, oocytes were washed for 3 times and then were transferred to maturation medium for overnight culture. Meiotic progression was scored at the next morning. Oocytes from each mouse were injected with one RNA. 5 mice were used for each RNA injection. *, $P < 0.005$. B) confocal image of ARF1-T31N-HA injected oocytes. After overnight culturing, oocytes were fixed, stained with Alexa 594-anti-tubulin (red) and Sytox green (green). a) GFP-injected oocytes arrested at metaphase II (arrow indicates a PB). b) ARF1-T31N-HA injected oocytes arrested at metaphase I.

A



B

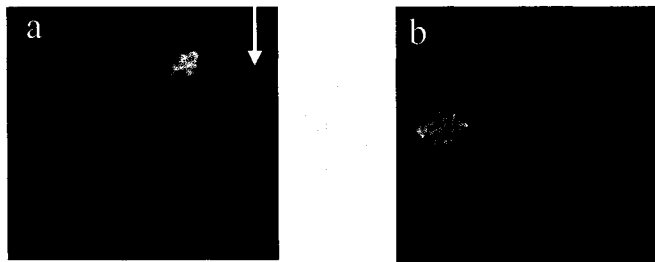


Fig 16.

MAPK activity in BFA-treated oocytes. Oocytes were collected after overnight culture with or without BFA, then 40 oocytes from GV, control and BFA groups were lysed to run gel, blotted by anti-phosphor-MAPK or anti-tubulin antibody respectively.

P-MAPK

beta-tubulin

GV
Con (metaphase II)
BFA (metaphase I)

— — —

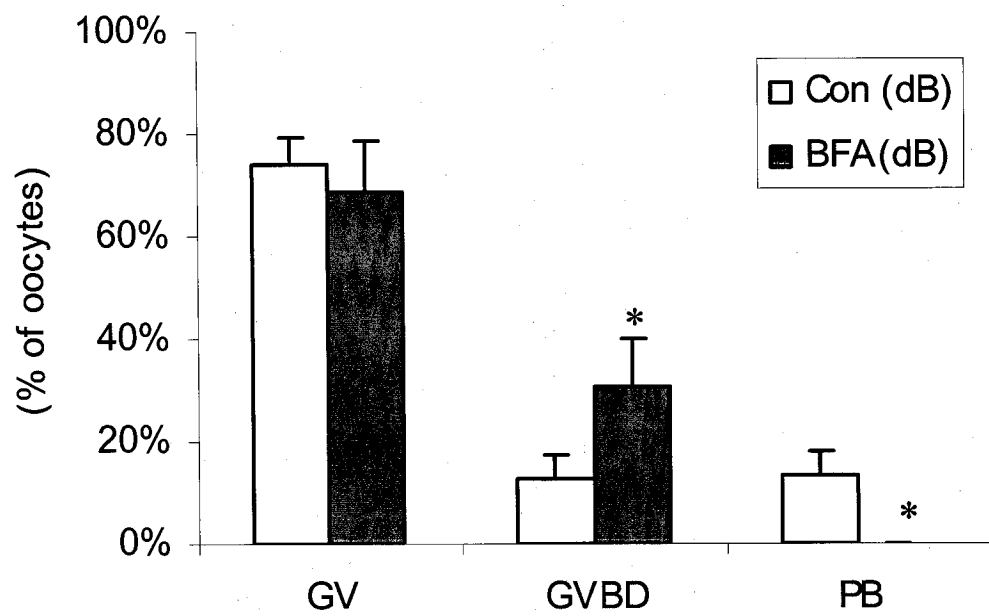
— — —

arrested oocytes) and BFA (metaphase I arrested oocytes) group. Immunoblotting by anti-tubulin confirmed that similar amounts of protein were loaded. These results indicated that MAPK was activated in BFA-induced oocyte maturation.

Finally, we wished to determine whether BFA could overcome prophase arrest induced by exogenous cAMP. We treated denuded oocytes with BFA in the presence of or absence of dBcAMP. We found that most BFA-treated oocytes arrested at GV stage, similar to control oocytes which were only incubated with dBcAMP ($P > 0.05$) (Fig. 17). The fact that BFA did not overcome the inhibitory effect of cAMP in oocyte maturation suggests that factors involved in BFA-induced oocyte maturation are upstream of cAMP reduction. Interestingly, among the ~30% oocytes in each group that “escaped” from cAMP inhibition, half of “escaped” oocytes in control group were arrested at metaphase II. However, all “escaped” oocytes in BFA group were arrested at metaphase I ($P < 0.05$, compared to control group). This further confirms the effect of BFA to block metaphase I to anaphase transition.

Fig 17.

BFA did not induce GVBD in the presence of dBcAMP. Oocytes were cultured with 5 μ M BFA and 100 μ M dBcAMP for overnight. Meiotic progression was scored at the next morning. 5 mice were used in total. Oocytes from each mouse were divided into 2 groups. *, P<0.05.



4. DISCUSSION

Our lab has a longstanding interest in studying mechanisms of vertebrate oocyte maturation using the amphibian model (*Xenopus laevis*) (Bayaa et al, 2000; Sheng et al, 2001; Ma et al, 2003; Wang and Liu, 2003; Wang and Liu, 2004). Relative to mouse oocyte maturation, the *Xenopus* model is much better developed (Schmitt and Nebreda, 2002; Karaiskou et al, 2001; Maller, 2003). However, it has long been debated how much, mechanistically, oocyte maturation in amphibians resembles that in mammals, particularly in the mouse (the best studied mammalian species). The uncertainty was largely based on two experimental observations: firstly isolated frog oocytes maintain prophase arrest until treated with maturation-inducing hormones but isolated mouse oocytes undergo spontaneous oocyte maturation; secondly steroids (particularly progesterone and androgens) are potent maturation-inducing hormones for amphibian oocytes but they have no apparent stimulatory effect on mouse oocytes, whether tested in antral follicles or in denuded oocytes. Despite these apparent differences, oocyte maturation in both species (*Xenopus laevis* and the mouse) is fundamentally the same. Firstly, immature oocytes in both species physiologically arrest at prophase I in the ovary before sexual maturation. Secondly, under the influence of gonadotropins (particularly LH), oocytes of both species resume meiosis and progress to metaphase II when they arrest again until fertilization. Thirdly, high levels of cAMP in the oocytes of both species are thought to be essential for maintaining prophase arrest and a drop in oocyte cAMP is thought to be essential for

oocyte maturation. Fourthly, MPF activation is critically important to initiate the first physical sign of oocyte maturation, GVBD, in both species and MPF undergoes a transient inactivation during meiosis I to meiosis II transition. My research project therefore was designed to find a common mechanism to explain the apparent difference in hormonal regulation of oocyte maturation in the two species.

4.1 Ritanserin induces oocyte maturation in both frogs and mice: a common mechanism for maintenance of prophase arrest in vertebrate oocytes

We discovered that a well characterized serotonin receptor antagonist, ritanserin, was capable of inducing mouse oocyte maturation within intact preovulatory (antral) follicles. Like the physiological inducer of oocyte maturation, LH (or its mimic HCG), ritanserin stimulated the complete maturation such that the treated follicles contained mature eggs arrested at metaphase II, as indicated by the presence of a polar body (the first polar body) and a bipolar metaphase II spindle. Interestingly, similar concentrations of ritanserin were also capable of inducing isolated frog oocytes to undergo oocyte maturation, as demonstrated by others in our lab (Sheng et al, 2005). Noticeably, ritanserin was less efficient than HCG in the mouse. We suggest that ritanserin acted directly on the oocyte to trigger oocyte maturation, as its action on frog oocytes was restricted to the oocytes (Sheng et al, 2005). However, the somatic follicle cells in the mouse follicles may somehow

“blunt” the oocyte response in intact follicles. Consistent with this explanation, Gravelleau et al (2000) showed that human granulosa cells expressed 5-HT₇R which is a G_s-coupled receptor. Antagonizing this 5-HT₇R in the follicle cells would be expected to reduce cAMP levels in the follicle cells and therefore negatively regulate oocyte maturation since the increase of cAMP levels in follicle cells is essential for LH-induced oocyte maturation (Conti et al, 2002). Indeed, in the frog, ritanserin induced almost 100% GVBD in follicle cell-free oocytes. However, the effect of ritanserin in oocyte-follicle cell complexes were much more variable (Sheng et al, 2005).

The identity of the ritanserin target, a putative G_s-coupled GPCR, remains unknown. All 5-HT₁Rs except 5-HT₃ belong to the family of seven transmembrane receptors that are coupled to different intracellular effectors via G proteins (i.e. GPCR). 5-HT₄R, 5-HT₆R and 5-HT₇R are coupled to G_s protein and therefore may be considered as candidate GPCR in the oocytes. Indeed, ritanserin is a potent antagonist of 5-HT₇R. However, several pieces of evidence make it unlikely that the oocyte target of ritanserin is any of typical 5-HT receptors. First, among the several serotonin receptor antagonists tested, ritanserin is the only one that was capable of inducing oocyte maturation. No known 5-HT receptors are uniquely sensitive to ritanserin. Indeed, in experiments performed by others in the lab, ritanserin, mesullergine and methiothepine were all capable of inhibiting *Xenopus* 5-HT₇R-mediated cAMP elevation in transfected COS-7 cells (Sheng et al, 2005). Second, the concentrations (~5 μM) required to trigger oocyte maturation were much

higher than would be expected if a typical 5-HT receptor were involved (by at least 100 fold). Third, exogenous 5-HT did not inhibit HCG-induced oocyte maturation within intact follicles or spontaneous maturation in denuded mouse oocytes, nor did it inhibit progesterone-induced frog oocyte maturation (Sheng et al, 2005). Finally, high concentrations of tryptophan hydroxylase inhibitor (CPA), which were expected to inhibit serotonin synthesis in the follicles, did not induce oocyte maturation. Taken together, these results strongly argued against a prominent role for an endogenous serotonergic system in maintaining prophase arrest in the mouse or in the frog.

However, our data do support the involvement of a Gs-coupled GPCR as the target of ritanserin. Perhaps the strongest evidence is that ritanserin causes rapid reduction of cAMP in frog oocytes and that elevation of cAMP blocked the action of ritanserin (Sheng et al, 2005). Therefore, our data suggested for the first time that a common GPCR (the oocyte target of ritanserin) is responsible for maintaining prophase arrest in both frog oocytes and in mouse oocytes. The identity of this GPCR and its regulation in prophase oocytes are currently under investigation by others in the lab. We would suggest that the action of LH in both species is to overcome the action of this GPCR and therefore to release prophase arrest.

4.2 The effect of brefeldin A (BFA) on oocyte maturation: a possible role for Golgi dynamics in regulation of oocyte maturation

The “Golgi poison” brefeldin A was of interest to us for two reasons. First, two separate studies on BFA reported its ability to induce GVBD in frog oocytes (Mulner-Lorillon et al, 1995) but arrest maturing mouse oocytes at metaphase I (Moreno et al, 2002). Neither study explained the mechanism of BFA action. In particular, it is not even clear whether the effect of BFA is mediated through its known target, the small G protein ARF-1. As my research interest was to seek common mechanisms of vertebrate oocyte maturation, BFA was a very appealing tool. However, the second and more important reason was the emerging new theory that Golgi fragmentation during mitosis may be a regulatory step that controls cell cycle progression. Although it is well known that the Golgi undergoes dramatic fragmentation during cell division, it has long been thought that Golgi fragmentation is the necessary consequence of cell cycle progression (the need to partition Golgi into daughter cells) (Shorter et al, 2002). Several recent studies appear to suggest that Golgi fragmentation may play an active role in controlling entry into mitosis (Sutterlin et al, 2002; Colanzi et al, 2003).

We demonstrated in this study that BFA had two distinct effects in mouse oocytes. The first was to overcome the physiological prophase arrest and the second was to arrest maturing mouse oocytes at metaphase I. We further demonstrated that BFA likely targeted endogenous ARF-1 as injection of dominant negative ARF-1 mutant in denuded oocytes caused similar metaphase I arrest. Unfortunately, we cannot confirm the ability of this mutant ARF-1 to initiate oocyte GVBD in intact follicles, as injecting mRNA into oocytes

enclosed in antral follicles was technically too difficult.

Although we have not studied Golgi dynamics during HCG-induced mouse oocyte maturation or after BFA treatment, others have reported ultra-structural analyses of Golgi dynamics during oocyte maturation. Specifically, Golgi apparatus appeared as many stacks in prophase-arrested oocytes and underwent fragmentation following GVBD (Mulner-Lorillon et al, 1995; Moreno et al, 2002; Assey et al, 1994). It is clearly an intriguing possibility that Golgi fragmentation and reassembly during oocyte maturation play active roles in initiation of GVBD and meiosis I to meiosis II transition, respectively. In other words, disruption of Golgi apparatus may be an integral step in the signaling cascade of LH-induced GVBD in the oocytes. On the other hand, reassembly (even partially) of Golgi apparatus at the end of metaphase I may provide another signal for anaphase initiation and the subsequent polar body formation.

How might Golgi apparatus be involved in controlling cell cycle progression? Golgi apparatus is a known host of numerous cytoplasmic proteins in a cell cycle-dependent manner. These include heterotrimeric G proteins, phosphatidylinositol 3-kinase, N-Ras, cyclin B2, cdc42 (Donaldson et al, 2000a; Donaldson and Jackson, 2000b), to name just a few. There are clearly many exciting possibilities to explain the role of Golgi dynamics in controlling oocyte maturation and some of these are under investigation by others in our lab.

5. REFERENCE

- Amenta F, Vega JA, Ricci A, Collier WL. 1992. Localization of 5-hydroxytryptamine-like immunoreactive cells and nerve fibers in the rat female reproductive system. *Anat Rec.* 233, 478-84.
- Assey R.J, Hyttel P, Greve T, Purwantara B. 1994. Oocyte morphology in dominant and subordinate follicles. *Mol. Reprod. Dev.* 37, 335-44.
- Ayalon D, Tsafiri A, Lindner HR, Cordova T, Harell A. 1972. Serum gonadotrophin levels in proestrous rats in relation to the resumption of meiosis by the oocytes. *J. Reprod. Fertil.* 31, 51-8,
- Baker T. 1982. Oogenesis and ovulation. In: *Reproduction in Mammals, Vol. 1*, edited by C. Austin and R. Short, pp. 17-45. Cambridge University Press, Cambridge.
- Baltsen M. 2001. Gonadotropin-induced accumulation of 4,4-dimethylsterols in mouse ovaries and its temporal relation to meiosis. *Biol Reprod.* 65, 1743-50.
- Battista PJ, Rexroad CE Jr, Condon WA. 1987. Mechanisms involved in the action of serotonin-induced stimulation of progesterone production by bovine luteal cells in vitro. *Mol Cell Endocrinol.* 51, 145-51.
- Bayaa, M., Booth, R.A., Sheng, Y., and Liu, X.J. 2000. The classical progesterone receptor mediates *Xenopus* oocyte maturation through a nongenomic mechanism. *Proc Natl Acad Sci USA.* 97, 12607-12.
- Blandau R. 1970. Growth of the ovarian follicle and ovulation. *Prog. Gynecol.* 5, 58-76.
- Bodis J, Torok A, Tinneberg HR, Hanf V, Hamori M, Cledon P. 1992. Influence of serotonin on progesterone and estradiol secretion of cultured human granulosa cells. *Fertil Steril.* 57, 1008-11.
- Bodis J, Hartmann G, Torok A, Bognar Z, Tinneberg HR, Cledon P, Hanf V. 1993. Relationship between the monoamine and gonadotropin content in follicular fluid of preovulatory graafian follicles after superovulation treatment. *Exp Clin Endocrinol.* 101, 178-82.

Bornslaeger EA, Schultz RM. 1985. Adenylate cyclase activity in zona-free mouse oocytes. *Exp Cell Res.* 156, 277-81.

Bornslaeger EA, Mattei PM, Schultz RM. 1986. Involvement of cAMP-dependent protein kinase and protein phosphorylation in regulation of mouse oocyte maturation. *Dev Biol.* 114, 453-62.

Borum K. 1961. Oogenesis in the mouse. A study of meiotic prophase. *Exp Cell Res.* 24, 495-507.

Brunet S, Maria AS, Guillaud P, Dujardin D, Kubiak JZ, Maro B. 1999. Kinetochore fibers are not involved in the formation of the first meiotic spindle in mouse oocytes, but control the exit from the first meiotic M phase. *J Cell Biol.* 146, 1-11.

Buznikov GA, Nikitina LA, Galanov AYu, Malchenko LA, Trubnikova OB. 1993. The control of oocyte maturation in the starfish and amphibians by serotonin and its antagonists. *Int J Dev Biol.* 37, 363-4.

Byskov AG, Andersen CY, Nordholm L, Thogersen H, Xia G, Wassmann O, Andersen JV, Guddal E, Roed T. 1995. Chemical structure of sterols that activate oocyte meiosis. *Nature.* 374, 559-62.

Byskov AG, Yding Andersen C, Hossaini A, Guoliang X. 1997. Cumulus cells of oocyte-cumulus complexes secrete a meiosis-activating substance when stimulated with FSH. *Mol Reprod Dev.* 46, 296-305.

Cao X, Pomerantz SH, Popliker M, Tsafiri A. 2004. Meiosis-activating sterol synthesis in rat preovulatory follicle: is it involved in resumption of meiosis? *Biol Reprod.* 71, 1807-12.

Carabatsos MJ, Sellitto C, Goodenough DA, Albertini DF. 2000. Oocyte-granulosa cell heterologous gap junctions are required for the coordination of nuclear and cytoplasmic meiotic competence. *Dev. Biol.* 226, 167-79.

Cassel D, Pfeuffer. 1978. Mechanism of cholera toxin action: covalent modification of the guanyl nucleotide-binding protein of the adenylate cyclase system. *Proc. Natl. Acad. Sci. USA.* 75, 2669-73.

Cerda J, Subhedar N, Reich G, Wallace RA, Selman K. 1998. Oocyte sensitivity to serotonergic regulation during the follicular cycle of the teleost fundulus heteroclitus. *Biol Reprod.* 59, 53-61.

Channing CP, Tsafiri A. 1977. Mechanism of action of luteinizing hormone and follicle stimulating hormone on the ovary in vitro. *Metabolism*. 26, 413-68.

Cho WK, Stern S, Biggers JD. 1974. Inhibitory effect of dibutyryl cAMP on mouse oocyte maturation in vitro. *J Exp Zool*. 187, 383-6.

Choi T, Aoki F, Mori M, Yamashita M, Nagahama Y, Kohmoto K. 1991. Activation of P34cdc2 protein kinase activity in meiotic and mitotic cell cycles in mouse oocytes and embryos. *Development*. 113, 789-95.

Clarke HJ, Masui Y. 1983. The induction of reversible and irreversible chromosome decondensation by protein synthesis inhibition during meiotic maturation of mouse oocytes. *Dev Biol*. 97, 291-301.

Clarke HJ. 1998. Cytoplasmic factors that control nuclear behavior in mammalian oocytes: A re-evaluation of studies performed as a student of Yoshio Masui. *Biol Cell*. 90, 549-55.

Colanzi A, Suetterlin C, Malhotra V. 2003. Cell-cycle-specific Golgi fragmentation: how and why? *Curr Opin Cell Biol*. 15, 462-7.

Conti M, Andersen CB, Richard FJ, Shitsukawa K, Tsafiri A. 1998. Role of cyclic nucleotide phosphodiesterases in resumption of meiosis. *Mol Cell. Endocrinol*. 145, 9-14.

Conti M, Andersen CB, Richard F, Mehats C, Chun SY, Horner K, Jin C, Tsafiri A. 2002. Role of cyclic nucleotide signaling in oocyte maturation. *Mol. Cell. Endocrinol*. 187, 153-9.

Dekel N, Galiani D, Beers WH. 1988. Induction of maturation in follicle-enclosed oocytes: the response to gonadotropins at different stages of follicular development. *Biol Reprod*. 38, 517-21.

Donaldson J.G, Lippincott-Schwartz J. 2000a. Sorting and signaling at the Golgi complex. *Cell*. 101, 693-6.

Donaldson J.G, Jackson C.L. 2000b. Regulators and effectors of the ARF GTPases. *Curr Opin Cell Biol*. 12, 475-82.

Downs SM, Eppig JJ. 1985a. A follicular fluid component prevents gonadotropin reversal of cyclic adenosine monophosphate-dependent meiotic arrest in murine oocytes. *Gamete Res*. 11, 83-97.

Downs SM, Coleman DL, Ward-Bailey PF, Eppig JJ. 1985b. Hypoxanthine is the principal inhibitor of murine oocyte maturation in a low molecular weight fraction of porcine follicular fluid. *Proc Nat Acad Sci USA*. 82, 454-8.

Downs SM, Buccione R, Eppig JJ. 1992. Modulation of meiotic arrest in mouse oocytes by guanyl nucleotides and modifiers of G-proteins. *J Exp Zool*. 262, 391-404.

Downs SM. 2000. Adenosine blocks hormone-induced meiotic maturation by suppressing purine de novo synthesis. *Mol Reprod Dev*. 56, 172-9.

Downs SM, Ruan B, Schroepfer GJ. 2001. Meiosis-activating sterol and the maturation of isolated mouse oocytes. *Biol Reprod*. 64, 80-9.

Edwards RG, Gates AH. 1959. Timing of the stages of the maturation, division, ovulation, fertilization and the first cleavage of eggs of adult mice treated with gonadotrophins. *J. Endocrinol*. 18, 292-304.

Edwards, R.G. 1965. Maturation in vitro of human ovarian oocytes. *Lancet*. 2, 926-9.

Ekholm C, Hillensjo T, Magnusson C, Rosberg S. 1984. Stimulation and inhibition of rat oocyte meiosis by forskolin. *Biol Reprod*. 30, 537-43.

Eppig JJ. 1982. The relationship between cumulus cell-oocyte coupling, oocyte meiotic maturation, and cumulus expansion. *Dev Biol*. 89, 268-72.

Eppig JJ, Ward-Bailey PF, Coleman DL. 1985. Hypoxanthine and adenosine in murine ovarian follicular fluid: concentrations and activity in maintaining oocyte meiotic arrest. *Biol Reprod*. 33, 1041-9.

Eppig.J.J. 1993. Regulation of mammalian oocyte maturation. In: Adashi, E.Y, Leung, P.C.K. *The ovary*. Raven Press, New York, pp.185-208.

Eppig JJ, Pendola FL, Wigglesworth K. 1998. Mouse oocytes suppress cAMP-induced expression of LH receptor mRNA by granulosa cells in vitro. *Mol Reprod Dev*. 49, 327-32.

Ferrell, J.E.Jr. 1999. *Xenopus* oocyte maturation: new lessons from a good egg. *Bioessays*. 21, 833-42.

Freeman ME, Butcher RL, Fugo NW. 1970. alteration of oocytes and follicles by delayed ovulation . *Biol. Reprod*. 2, 209-15.

Gallo CJ, Hand AR, Jones TL, Jaffe LA. 1995. Stimulation of *Xenopus* oocyte maturation by inhibition of the G-protein alpha S subunit, a component of the plasma membrane and yolk platelet membranes. *J Cell Biol.* 130(2):275-84.

Gardiner EE, Newberry RC, Keng JY. 1989. Postmortem time and storage temperature affect the concentrations of hypoxanthine, other purines, pyrimidines, and nucleosides in avian and porcine vitreous humor. *Pediatr Res.* 26, 639-42.

Gorbsky GJ. 2001. The mitotic spindle checkpoint. *Curr Biol.* 11, R1001-4.

Graveleau C, Paust HJ, Schmidt-Grimminger D, Mukhopadhyay AK. 2000. Presence of a 5-HT7 receptor positively coupled to adenylate cyclase activation in human granulosa-lutein cells. *J. Clin. Endocrinol. Metab.* 85, 1277-86.

Grondahl C, Lessl M, Faerge I, Hegele-Hartung C, Wassermann K, Ottesen JL. 2000. Meiosis activating sterol-mediated resumption of meiosis in mouse oocytes in vitro is influenced by protein synthesis inhibition and cholera toxin. *Biol Reprod.* 62, 775-80.

Hampl A, Eppig JJ. 1995. Analysis of the mechanisms of metaphase I arrest in maturing mouse oocytes. *Development.* 121, 925-33.

Hashimoto N, Kishimoto T. 1986. Cell cycle dynamics of maturation –promoting factor during mouse oocyte maturation. *Tokai J Exp Clin Med.* 11, 471-7.

Horner K, Livera G, Hinckley M, Trinh K, Storm D, Conti M. 2003. Rodent oocytes express an active adenylyl cyclase required for meiotic arrest. *Dev Biol.* 258, 385-96.

Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PP. 1994. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol Rev.* 46, 157-203.

Hsueh AJ, Adashi EY, Jones PB, Welsh TH Jr. 1984. Hormonal regulation of the differentiation of cultured ovarian granulosa cells. *Endocr Rev.* 5, 76-127.

Hu Y, Cortvrindt R, Smits J. 2002. Effects of aromatase inhibition on in vitro follicle and oocyte development analyzed by early preantral mouse follicle culture. *Mol Reprod Dev.* 61, 549-59.

Hunter RHF, Polge C. 1966. Maturation of follicular oocytes in the pig after injection of human chorionic gonadotrophin. *J. Reprod. Fertil.* 12, 525-31.

- Iwamatsu T, Yanagimachi R. 1975. Maturation in vitro of ovarian oocytes of prepubertal and adult hamsters. *J. reprod. Fertile.* 45, 83-90.
- Johnson J, Canning J, Kaneko T, Pru JK, Tilly JL. 2004. Germline stem cells and follicular renewal in the postnatal mammalian ovary. *Nature.* 428, 145-50.
- Josefsberg LB, Galiani D, Lazar S, Kaufman O, Seger R, Dekel N. 2003. Maturation-promoting factor governs mitogen-activated protein kinase activation and interphase suppression during meiosis of rat oocytes. *Biol Reprod.* 68, 1282-90.
- Karaiskou A, Dupre A, Haccard O, Jesus C. 2001. From progesterone to active Cdc2 in *Xenopus* oocytes: a puzzling signalling pathway. *Biol Cell.* 93, 35-46.
- Lavy G, Behrman HR, Polan ML. 1990. Purine levels and metabolism in human follicular fluid. *Hum Reprod.* 5, 529-32.
- Lawitts JA, Biggers JD. 1993. Culture of preimplantation embryos. *Methods Enzymol.* 225, 153-64.
- Lawrence IE Jr, Burden HW. 1976. The autonomic innervation of the interstitial gland of the rat ovary during pregnancy. *Am J Anat.* 147, 81-94.
- Leader B, Lim H, Carabatsos MJ, Harrington A, Ecsedy J, Pellman D, Maas R, Leder P. 2002. Formin-2, polyploidy, hypofertility and positioning of the meiotic spindle in mouse oocytes. *Nat Cell Biol.* 4, 921-8.
- Ledan E, Polanski Z, Terret M, Maro B. 2001. Meiotic maturation of the mouse oocyte requires an equilibrium between cyclin B synthesis and degradation. *Dev Biol.* 232, 400-13.
- Lee MCS, Miller EA, Goldberg J, Orci L, Schekman R. 2004. Bi-directional protein transport between the ER and Golgi. *Annu Rev Cell Dev Biol.* 20, 87-123.
- Lieberman ME, Tsafiriri A, Bauminger S, Collins WP, Ahren K, Lindner HR. 1976. Oocytic meiosis in cultured rat follicles during inhibition of steroidogenesis. *Acta Endocrinol.* 83, 151-7.
- Lippincott-Schwartz J, Yuan L.C, Bonifacino J.S, Klausner R.D. 1989. Rapid redistribution of Golgi proteins into the ER in cells treated with brefeldin A: evidence for membrane cycling from Golgi to ER. *Cell.* 56, 801-13.

- Lippincott-Schwartz J, Donaldson J.G, Schweizer A, Berger E.G, Hauri H.P, et al. 1990. Microtubule-dependent retrograde transport of proteins into the ER in the presence of brefeldin A suggests an ER recycling pathway. *Cell*. 60, 821-36.
- Lohka MJ, Hayes MK, Maller JL. 1988. Purification of maturation-promoting factor, an intracellular regulator of early mitotic events. *Proc Natl Acad Sci USA*. 85, 3009-13.
- Ma C, Cummings C, Liu XJ. 2003. Biphasic activation of Aurora-A kinase during the meiosis I- Meiosis II transition in *Xenopus* oocytes. *Mol Cell Biol*. 23, 1703-16.
- Maller JL. 1985. Oocyte maturation in amphibians. *Dev Biol*. 1, 289-311.
- Maller JL. 2003. Signal transduction. Fishing at the cell surface. *Science*. 300, 594-5.
- Masciarelli S, Horner K, Liu C, Park SH, Hinckley M, Hockman S, Nedachi T, Jin C, Conti M, Manganiello V. 2004. Cyclic nucleotide phosphodiesterase 3A-deficient mice as a model of female infertility. *J Clin Invest*. 114, 196-205.
- Masui, Y., Market C.L. 1971. Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes. *J. Exp. Zool*. 177, 129-45.
- McLaren A. 1984. Meiosis and differentiation of mouse germ cells. *Symp Soc Exp Biol*. 38, 7-23.
- Mehlmann, L.M., Jones, T.L.Z., Jaffe L.A. 2002. Meiotic arrest in the mouse follicle maintained by a Gs protein in the oocyte. *Science*. 297, 1343-5.
- Melo J, Toczyski D. 2002. A unified view of the DNA-damage checkpoint. *Curr Opin Cell Biol*. 14, 237-45.
- Moor RM, Smith MW, Dawson RM. 1980. Measurement of intercellular coupling between oocytes and cumulus cells using intracellular markers. *Exp Cell Res*. 126, 15-29.
- Moreno R.D, Schatten G, Ramalho-Santos J. 2002. Golgi Apparatus dynamics during mouse oocyte in vitro maturation: effect of the membrane trafficking inhibitor brefeldin A. *Biol Reprod*. 66, 1259-66.
- Moss J, Vaughan M. 1979. Activation of adenylate cyclase by cholera toxin. *Ann. Rev. Biochem*. 48, 581-600.

- Mulner-Lorillon O, Belle R, Cormier P, Drewing S, Minella O, Poulhe R, Schmalzing G. 1995. Brefeldin A provokes indirect activation of cdc2 kinase (MPF) in *Xenopus* oocytes, resulting in meiotic cell division. *Dev Biol.* 170, 223-9.
- Murray A. 1995. Cyclin ubiquitination: the destructive end of mitosis. *Cell.* 81, 149-52.
- Murray A, Biol M.I, Spears N, Phil D. 2000. Follicular development in vitro. *Seminars in Reprod Med.* 18, 109-22.
- Musacchio A, Hardwick KG. 2002. The spindle checkpoint: structural insights into dynamic signaling. *Nat Rev Mol Cell Biol.* 3, 731-41.
- Nasmyth K. 2002. Segregating sister genomes: the molecular biology of chromosome separation. *Science.* 297, 559-65.
- Neal P, Baker TG. 1975. Response of mouse Graafian follicles in organ culture to varying doses of follicle stimulating hormone and luteinizing hormone. *J. Endocrinol.* 65, 27-32.
- Nebreda, A.R., Ferby, I. 2000. Regulation of the meiotic cell cycle in oocytes. *Curr Opin Cell Biol.* 12, 666-675
- O'Brien MJ, Pendola JK, Eppig JJ. 2003. A revised protocol for in vitro development of mouse oocytes from primordial follicles dramatically improves their developmental competence. *Biol Reprod.* 68, 1682-6.
- Peter M, Castro A, Lorca T, Le Peuch C, Magnaghi-Jaulin L, Doree M, Labbe JC. 2001. The APC is dispensable for first meiotic anaphase in *Xenopus* oocytes. *Nat Cell Biol.* 3, 83-7.
- Peters JM. 2002. The anaphase-promoting complex: proteolysis in mitosis and beyond. *Mol Cell.* 9, 931-43.
- Pincus G, Enzmann E.V. 1935. The comparative behavior of mammalian eggs in vivo and in vitro. *J. Exp. Med.* 62, 665-75.
- Racowsky C. 1984. Effect of forskolin on the spontaneous maturation and cyclic AMP content of rat oocyte-cumulus complex. *J Reprod Fertil.* 72, 107-16.

Racowsky C. 1985. Effect of forskolin on maintenance of meiotic arrest and stimulation of cumulus expansion, progesterone and cyclic AMP production by pig oocyte-cumulus complexes. *J Reprod Fertil.* 74, 9-21.

Richard FJ, Tsafiriri A, Conti M. 2001. Role of phosphodiesterase type 3A in rat oocyte maturation. *Biol Reprod.* 65, 1444-51.

Rudnick G, Humphreys CJ. 1992. Platelet serotonin transporter. *Methods Enzymol.* 215, 213-24.

Sagata N, Watanabe N, Vande Woude GF, Ikawa Y. 1989. The c-mos proto-oncogene product is a cytostatic factor responsible for meiotic arrest in vertebrate eggs. *Nature.* 342, 512-8.

Schaeffer HJ, Sirotkin AV. 1995. Melatonin and serotonin directly regulate oxytocin, insulin-like growth factor-I and progesterone secretion by cultured human granulosa cells. *Adv Exp Med Biol.* 395, 547-8.

Schmitt A, Nebreda AR. 2002. Signalling pathways in oocyte meiotic maturation. *J Cell Sci.* 115, 2457-9.

Schuetz A.W. 1974. Role of hormones in oocyte maturation. *Biol. Reprod.* 10, 150-78.

Schultz RM, Montgomery R, Belanoff J. 1983. Regulation of mouse oocyte maturation: implication of a decrease in oocyte cAMP and protein dephosphorylation in commitment to resume meiosis. *Dev Biol.* 97, 264-73.

Sciaky N, Presley J, Smith C, Zaal K.J.M, Cole N, et al. 1997. Golgi tubule traffic and the effects of brefeldin A visualized in living cells. *J. Cell Biol.* 139, 1137-55.

Sheng YL, Tiberi M, Booth RA, Ma C, Liu XJ. 2001. Regulation of *Xenopus* oocyte meiosis arrest by G protein betagamma subunits. *Curr. Biol.* 11: 405-16.

Sheng YL, Wang L, Liu XS, Tiberi M, Baltz JM, Liu XJ. 2005. A serotonin receptor antagonist induces oocyte maturation in both frogs and mice: evidence that the same G protein-coupled receptor is responsible for maintaining meiosis arrest in both species. *J Cell Physiol.* 202, 777-86.

Shitsukawa K, Andersen CB, Richard FJ, Horner AK, Wiersma A, van Duin M, Conti M. 2001. Cloning and characterization of the cGMP-inhibited phosphodiesterase PDE3A expressed in mouse oocyte. *Biol. Reprod.* 65, 188-96.

Shorter J, Warren G. 2002. Golgi architecture and inheritance. *Annu. Rev. Cell Dev. Biol.* 18, 379-420.

Simon, A.M, et al. 1997. Female infertility in mice lacking connexin 37. *Nature.* 385, 525-9.

Sirotkin and Schaeffer. 1997. Direct regulation of mammalian reproductive organs by serotonin and melatonin. *J Endocrinol.* 154, 1-5.

Stricker SA, Smythe TL. 2001. 5-HT causes an increase in cAMP that stimulates, rather than inhibits, oocyte maturation in marine nemertean worms. *Development.* 128, 1415-27.

Su YQ, Wigglesworth K, Pendola FL, O'Brien MJ, Eppig JJ. 2002. Mitogen-activated protein kinase activity in cumulus cells is essential for gonadotropin-induced oocyte meiotic resumption and cumulus expansion in the mouse. *Endocrinol.* 143, 2221-32.

Sutterlin C, Hsu P, Mallabiabarrena A, malhotra V. 2002. Fragmentation and dispersal of the pericentriolar Golgi complex is required for entry into mitosis in mammalian cells. *Cell.* 109, 359-69.

Taieb FE, Gross SD, Lewellyn AL, Maller JL. 2001. Activation of the anaphase-promoting complex and degradation of cyclin B is not required for progression from meiosis I to II in *Xenopus* oocytes. *Curr Biol.* 11, 508-13.

Tanaka E, Baba N, Toshida K, Suzuki K. 1993. Serotonin stimulates steroidogenesis in rat preovulatory follicles: involvement of 5-HT₂ receptor. *Life Sci.* 53, 563-70.

Terranova PF, Uilenbroek JT, Saville L, Horst D, Nakamura Y. 1990. Serotonin enhances oestradiol production by hamster preovulatory follicles in vitro: effects of experimentally induced atresia. *J Endocrinol.* 125, 433-8.

Terret ME, Wassmann K, Waizenegger I, Maro B, Peters JM, Verlhac MH. 2003. The meiosis I-to meiosis II transition in mouse oocytes requires separase activity. *Curr Biol.* 13, 1797-1802.

Tsafri A, Lindner HR, Zor U, Lamprecht SA. 1972. In vitro induction of meiotic division in follicle-enclosed rat oocytes by LH, cyclic AMP and prostaglandin E₂. *J Reprod Fertil.* 31, 39-50.

Tsafri A, Lieberman ME, Koch Y, Bauminger S, Chobsieng P, Zor U, Lindner HR. 1976. Capacity of immunologically purified FSH to stimulate cyclic AMP accumulation and steroidogenesis in Graafian-follicles and to induce ovum maturation and ovulation in the rat. *Endocrinology.* 98, 655-61.

Tsafri A. 1978. Oocyte maturation in mammals. In: *The vertebrate ovary*, edited by R. Jones. Plenum Press, New York. pp. 409-42.

Tsafri A, Dekel N. 1994. Molecular mechanisms in ovulation. In: Findlay, J.K. (Ed.), *Molecular Biology Female Reproductive system*. Academic Press, San Diego, pp. 207-58.

Tsafri A, Chun SY, Zhang R, Hsueh AJ, Conti M. 1996. Oocyte maturation involves compartmentalization and opposing changes of cAMP levels in follicular somatic and germ cells: studies using selective phosphodiesterase inhibitors. *Dev Biol.* 178, 393-402.

Tsafri A, Popliker M, Nahum R, Beyth Y. 1998. Effects of ketoconazole on ovulatory changes in the rat: implications on the role of a meiosis-activating sterol. *Mol Hum Reprod.* 4, 483-9.

Tsafri A, Cao X, Vaknin KM, Popliker M. 2002. Is meiosis activating sterol (MAS) an obligatory mediator of meiotic resumption in mammals. *Mol Cell Endocrinol.* 187, 197-204.

Vaknin KM, Lazar S, Popliker M, Tsafri A. 2001. Role of meiosis-activating sterols in oocyte maturation: effects of specific inhibitors and changes in the expression of lanosterol 14 α -demethylase during the preovulatory period. *Biol Reprod.* 64, 299-309.

Verlhac MH, de Pennart H, Maro B, Cobb MH, Clarke HJ. 1993. MAP kinase becomes stably activated at metaphase and is associated with microtubule-organizing centers during meiotic maturation of mouse oocytes. *Dev Biol.* 158, 330-40.

Verlhac MH, Kubiak JZ, Clarke HJ, Maro B. 1994. Microtubule and chromatin behavior follow MAP kinase activity but not MPF activity during meiosis in mouse oocytes. *Development.* 120, 1017-25.

Vermeiden JPW, Zeilmaker GH. 1974. Relationship between maturation division, ovulation and luteinization in the female rat. *Endocrinology*. 95, 341-51.

Vivarelli E, Conti M, De Felici M, Siracusa G. 1983. Meiotic resumption and intracellular cAMP levels in mouse oocytes treated with compounds which act on cAMP metabolism. *Cell Diff*. 12, 271-6.

Wang, J., and Liu, X.J. 2003. A G protein-coupled receptor kinase induces *Xenopus* oocyte maturation *J. Biol. Chem.* 278:15809-14.

Wang, J. and X. J. Liu. 2004. Progesterone inhibits protein kinase A (PKA) in *Xenopus* oocytes: demonstration of endogenous PKA activities using an expressed substrate. *J Cell Sci*. 117, 5107-5116.

Wassarman PM, Josefowicz WJ, Letourneau GE. 1976a. Meiotic maturation of mouse oocytes in vitro: inhibition of maturation at specific stages of nuclear progression. *J Cell Sci*. 22, 531-45.

Wassarman PM, Albertini DF, Josefowicz WJ, Letourneau GE. 1976b. Cytochalasin B-induced pseudo-cleavage of mouse oocytes in vitro: asymmetric localization of mitochondria and microvilli associated with a stage-specific response. *J Cell Sci*. 21, 523-35.

Wassarman P.M. 1988. The Mammalian ovum. In: *The Physiology of Reproduction*, edited by E. Knobil and J. Neill et al. Raven Press, New York. pp. 69-102.

Wassmann K, Benezra R. 2001. Mitotic checkpoints: from yeast to cancer. *Curr Opin Genet Dev*. 11, 83-90.

Wassmann K, Niaux T, Maro B. 2003. Metaphase I arrest upon activation of the Mad2-dependent spindle checkpoint in mouse oocytes. *Curr Biol*. 13, 1596-1608.

Weber KL, Sokac AM, Berg JS, Cheney RE, Bement WM. 2004. A microtubule-binding myosin required for nuclear anchoring and spindle assembly. *Nature*. 431, 325-9.

Widmann C, Gibson S, Jarpe MB, Johnson GL. 1999. Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. *Physiol Rev*. 79, 143-80.

Xie H, Xia G, Byskov AG, Andersen CY, Bo S, Tao Y. 2004. Roles of gonadotropins and meiosis-activating sterols in meiotic resumption of cultured follicle-enclosed mouse oocytes. *Mol Cell Endocrinol.* 218, 155-63.

Zhang M, Shi H, Segaloff DL, Van Voorhis BJ. 2001. Expression and localization of luteinizing hormone receptor in the female mouse reproductive tract. *Biol Reprod.* 64, 179-87.

Curriculum Vitae

Education

- September 2002 to Present **University of Ottawa, Department of Biochemistry, Microbiology and Immunology**
Master of Science in Biochemistry
Explored two distinct signaling pathways regulating mouse oocyte maturation. 1) A serotonin receptor antagonist induces oocyte maturation in mouse: evidence that a G protein-coupled receptor is responsible for maintaining meiosis arrest. 2) Brefeldin A induces resumption of meiosis, but arrests oocytes at metaphase I.
- September 1999 to July 2001 **Shanghai Second Medical University, Department of Medicine**
Master of Medicine in Molecular Genetics
Studied the mitochondrial tRNA^{Leu(UUR)} gene 3243 A → G mutation in type 2 diabetes pedigrees.
- September 1994 to June 1999 **Shanghai Second Medical University, Department of Medicine**
Bachelor of Medicine

Scientific Publications

Sheng Y, Wang L, Liu XS, Montplaisir V, Tiberi M, Baltz JM, Liu XJ. 2005. A Serotonin Receptor Antagonist Induces Oocyte Maturation in Both Frogs and Mice: Evidence That the Same G Protein-Coupled Receptor Is Responsible for Maintaining Meiosis Arrest in Both Species. *Journal of Cellular Physiology*. 202, 777-86.

Scientific Presentations

Chunqi Ma, Ling Wang, Pei Pei Zheng, and X. Johné Liu. "Frog Oocyte Maturation: A Model for Asymmetric Cell Division." Abstract in 10th International Xenopus Meeting. September 2004, Woods Hole, USA.

Wang L, Liu XJ. “Brefeldin A Induces Resumption of Meiosis, but Arrests Oocytes at Metaphase I.” In Abstract of Cell Cycle Regulation of Meiosis – BSCB Autumn Meeting. September 2004, Newcastle, UK, p.22.

Wang L, Liu XJ. “The Role of Serotonin Receptor in Mouse Oocyte Maturation.” No. 13 in Abstract of Canadian Workshop on Human Reproduction & Reproductive Biology. May 2004, Ottawa, Canada.

Wang L, Wu SH. “Study of Mitochondrial tRNA^{Leu(UUR)} Gene 3243 A→G Mutation in Type 2 Diabetes Pedigrees.” Symposium of Sixth Chinese Congress of Endocrinology. December 2001, Hong Kong, China, p.135.