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Association between $p34^{cdc2}$ levels and meiotic arrest in pig oocytes during early growth

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Summary

The molecules involved in determining meiotic competence were determined in porcine oocytes isolated from preantral and antral follicles of different sizes. Oocytes isolated from preantral follicles had a mean diameter of 78 μm, contained diffuse filamentous chromatin in the germinal vesicle and were incapable of progressing from the G_2 to the M phase of the cycle even after 72 h in culture. Oocytes from early antral follicles had a mean diameter of 105 μm, showed a filamentous chromatin configuration and about half resumed meiosis but arrested at metaphase I (MI) when cultured. Oocytes from mid-antral (3–4 mm) and large antral follicles (5–6 mm) had mean oocyte diameters of 115 and 119 μm respectively, contained condensed chromatin around the nucleolus and progressed to metaphase II (MII) in 48% and 93% of instances respectively. Analysis of $p34^{cdc2}$, the catalytic subunit of maturation promoting factor (MPF), by immunoblotting indicates that the inability of small (78 μm) oocytes to resume meiosis is due, at least in part, to inadequate levels of the catalytic subunit of MPF. On the other hand, the inability of intermediate-sized (105 μm) oocytes from antral follicles to complete the first meiotic division by progressing beyond MI appears not to be limited by levels of $p34^{cdc2}$, which are maximal by this stage. We postulate that an inadequacy of molecules other than $p34^{cdc2}$ limits progression of MI to MII; the acquisition of these molecules during the final stages of growth may be correlated with the formation of the perinucleolar chromatin rim in the germinal vesicle.

Key words: Catalytic subunit, Growth, Meiosis, $p34^{cdc2}$, Pig oocyte

Introduction

Whilst pig oocytes increase in diameter from approximately 30 to 120 μm during the growth phase, nuclear function in these cells is blocked at the diplotene stage of the first meiotic prophase throughout this period. After reaching full size, oocytes either resume meiosis in response to gonadotrophic stimuli or else undergo degeneration during the process of follicular atresia. Meiotic resumption in oocytes destined for ovulation is manifested by the disassembly of the nuclear membrane (germinal vesicle breakdown: GVBD), condensation of chromatin, assembly of the metaphase I (MI) spindle, first meiotic division and progression to metaphase II (MII) where a second period of cell cycle arrest is imposed (Wassarman, 1988). These sequential *in vivo* processes can also be induced *in vitro* by culturing fully grown follicular oocytes under appropriate conditions (Edwards, 1965). In an extension of this work, *in vitro* culture systems have been used to determine the meiotic potential of growing oocytes in a variety of species including the mouse (Sorensen & Wassarman, 1976), rabbit (Jelinkova *et al.*, 1994), pig (Motlik *et al.*, 1984) and cow (Sato *et al.*, 1990). These studies revealed that the capacity to complete the G_2-to M-phase transition of meiosis is first acquired in

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oocytes at an intermediate stage of growth; intermediate-sized oocytes do not progress beyond MI. The ability to progress to MII is only acquired in oocytes approaching full size. Hence, it is thought that acquisition of meiotic competence of oocytes consists of two steps: (1) the G_2- to M-phase transition and (2) progression from MI to MII. Acquisition of meiotic competence during the oocyte growth phase has been correlated either with changes in nuclear morphology (Crozet *et al.*, 1981; Motlik *et al.*, 1984; Wickramasinghe *et al.*, 1991) or with inadequacies in the biochemical characteristics of growing oocytes (Schultz *et al.*, 1979; Bornslaeger *et al.*, 1988).

Evidence from a variety of studies shows that the meiotic cycle is driven by a protein kinase, generically known as maturation promoting factor (MPF), which consists of a 34 kDa catalytic subunit ($p34^{cdc2}$) and a cyclin B regulatory subunit (see Murray & Hunt, 1993). That inadequacies in these cell cycle molecules limit meiotic competence in growing oocytes has been postulated by Motlik & Kubelka (1990) and demonstrated by Christmann and colleagues (1994). Using growing pig oocytes, these latter workers showed that oocytes of 90 μm diameter were unable to induce MPF activation despite containing the two subunits of $p34^{cdc2}$ kinase in amounts comparable to those found in fully grown oocytes. The purpose of the present study was to extend this work to earlier phases of oocyte growth. We report on the nuclear morphology, meiotic competence and catalytic subunit levels in small oocytes (78 μm) from preantral follicles and from different larger-sized oocytes from antral follicles. Our results show that levels of $p34^{cdc2}$ are limiting in small oocytes from preantral follicles; in growing oocytes from antral follicles we confirm that inadequacies in MPF kinase activation rather than in the absolute levels of $p34^{cdc2}$ limit meiotic progression.

Materials and methods

Collection of oocytes

Pig ovaries were obtained from prepubertal gilts slaughtered at a local abattoir. Following three washes in Dulbecco's phosphate-buffered saline containing 0.1% polyvinylalcohol (PBS-PVA), preantral and antral follicles were dissected in PBS-PVA from the ovaries using the techniques described by Moor & Trounson (1977). The follicles were classified into four categories according to diameter: preantral (0.2–0.4 mm, class A), small antral (0.5–1.5 mm, class B), mid-antral (3.0–4.0 mm, class C), and large antral (5.0–6.0 mm, class D). Follicles in each class were opened in HEPES-buffered medium 199 (Nissui Pharmaceutical, Tokyo, Japan) and oocyte cumulus complexes (OCC) were isolated from the follicles. After three washes in medium 199, the diameters of oocytes (excluding zona pellucida) were measured with an ocular micrometer attached to an inverted microscope. Some oocytes were denuded of cumulus cells immediately by pipetting, mounted on slides, fixed in acetic alcohol, stained with 1% orcein and examined for chromatin and nucleolar structure using differential interference optics.

Culture of oocytes

Maturational competence was assessed by culturing growing oocytes (OCC) in microdrops (20 μl) of the culture medium covered with paraffin oil for up to 72 h at 38.5°C in 5% CO_2 and 95% air. Bicarbonate-buffered TCM 199 supplemented with 10% fetal calf serum (FCS; Bio Cell, CA), 100 μg/ml sodium pyruvate, 0.1 μg/ml kanamycin (Sigma, St Louis, MO) and 0.1 IU/ml hMG (Pergonal, Teikoku Zoki, Tokyo, Japan) was used for culture. After 24, 48 or 72 h of culture, OCCs were collected and treated with 0.05% hyaluronidase (Sigma) to remove expanded cumulus cells. The oocytes were then denuded completely by pipetting, mounted on slides, fixed and stained as described above, and examined under the differential interference microscope. Oocytes which, after fixation, showed evidence of nuclear or other forms of degeneration were excluded from further analysis.

Statistical differences were analysed by chi-squared test. A p value < 0.05 was considered statistically significant.

Electrophoresis and Western blotting

Oocytes were isolated from preantral, small, mid- and large antral follicles in the same manner as described above. They were denuded mechanically by pipetting in PBS-PVA, transferred into acid Tyrode's solution (pH 2.5) for 2–3 min and then gently pipetted using a small-bore pipette to remove the last remnants of the zona pellucida. Resulting zona-free oocytes were washed three times in PBS-PVA and dissolved in SDS-PAGE sample buffer (Laemmli, 1970). The total mass of oocytes for each experimental group was adjusted to be approximately equal to each other by decreasing the number of oocytes as oocyte size increased. Parietal granulosa cells from large antral follicles (class D) were also collected and dissolved in sample buffer as a control.

Samples were run in 13% SDS-polyacrylamide gels, and proteins were transferred to nitrocellulose membranes (Hybond-C super, Amersham Life Science, UK) in a Trans-Blot SD semi-dry transfer cell (Bio-Rad Laboratories, Hurcules, CA) for 1 h at 2 mA/cm^2 in transfer buffer (Bjerrum & Schafer-Neilsen, 1986). The membranes were blocked with 10% FCS in PBS containing 0.1% Tween 20 (PBS-Tween) for 3 h, then

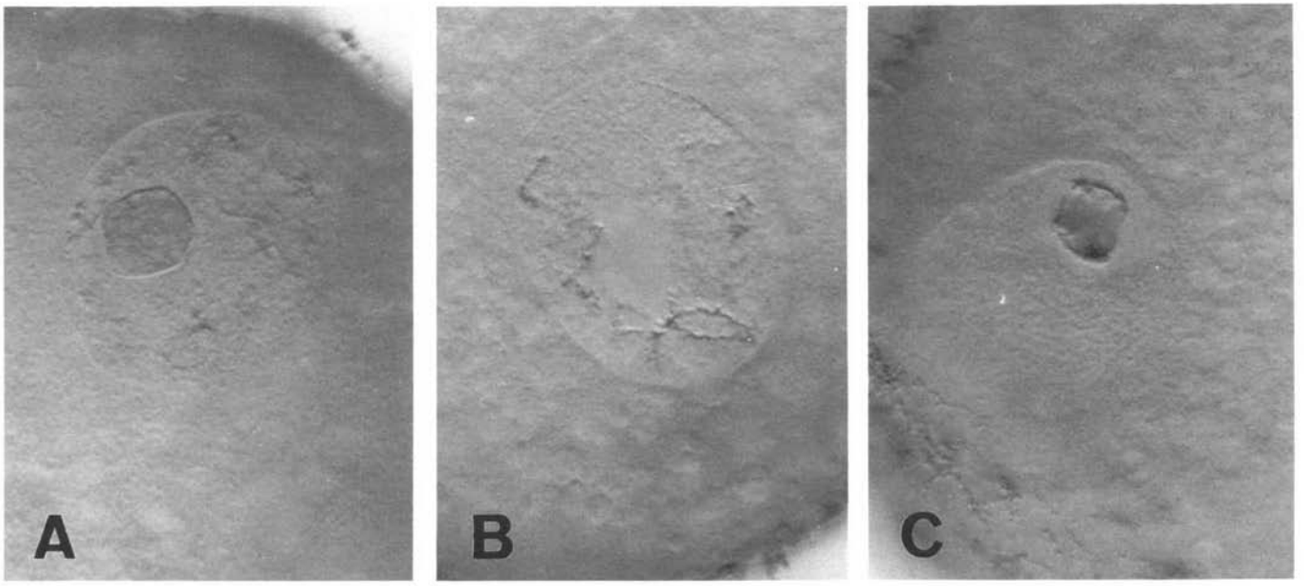

Figure 1 Nuclear morphology of pig oocytes at various stages of growth. The oocytes were fixed in acetic alcohol, stained with 1% aceto-orcein and prepared as whole mounts. (*A*) Growing oocyte 105 μm in diameter isolated from an early antral follicle. Decondensed filamentous chromatin is distributed throughout the germinal vesicle. The nucleolus is stained by orcein and has many vacuoles. (*B*) Growing oocyte 115 μm in diameter isolated from an intermediate-sized antral follicle. Chromatin has begun to condense forming thick chromatin strings. (*C*) Fully grown oocyte 120 μm in diameter from a large antral follicle. A well-condensed chromatin ring is localised around a translucent nucleolus.

Table 1 Chromatin configuration in pig oocytes isolated from follicles of various sizes

Follicular diameter (mm)	No. of oocytes examined	No. (%) of oocytes at stage of:				No. (%) of oocytes degenerating	No. (%) of oocytes with orcein-positive nucleolus
		FC	SC	GV I	GV II		
A: 0.2–0.4	36	32 (89)^{a}	3 (8)^{b,c}	0 (0)	0 (0)	1 (3)	35 (97)^{a}
B: 0.5–1.5	89	81 (92)^{a}	6 (6)^{c}	0 (0)	0 (0)	2 (2)	81 (92)^{a}
C: 3.0–4.0	87	6 (7)^{b}	68 (78)^{a}	13 (15)^{b}	0 (0)	0 (0)	5 (6)^{b}
D: 5.0–6.0	89	0 (0)	23 (26)^{b}	62 (70)^{a}	4 (4)	0 (0)	0 (0)

FC, filamentous chromatin stage; SC, stringy chromatin stage; GV I and GV II, germinal vesicle I and II stage as defined by Motlik & Fulka (1976).
$^{a-c}$ Values with different superscripts in the same column differ significantly ($p < 0.05$).

incubated at a dilution of 1:200 for 2 h at room temperature with one of the three anti-$p34^{cdc2}$ antibodies described below. After three washes in PBS-Tween, the membranes were incubated for 1 h at room temperature in blocking buffer containing horseradish-peroxidase-labelled anti-rabbit (1:1000, Amersham Life Science, UK) or anti-mouse immunoglobulins (1:1000, Dako Japan, Tokyo, Japan) depending on the origin of the first anti-$p34^{cdc2}$ antibody. After three washes of 10 min each with PBS-Tween, peroxidase activity was visualised using the ECL Western blotting detection system (Amersham Life Science, UK).

Antibodies against $p34^{cdc2}$

Three different anti-$p34^{cdc2}$ antibodies were used in this study. Polyclonal antibodies were prepared in rabbits either against the PSTAIRE motif (peptide sequence EEGV**PSTAIR**EISLLKELRH), or against the site of ATP binding on $p34^{cdc2}$ (peptide sequence EKIGEG**TYG**VVYKGRGKTT) (Moor *et al.*, 1992). Monoclonal antibody, raised in mice against the PSTAIRE sequence (EGV**PSTAIR**EISLLKE), was a generous gift from Dr M. Yamashita (Yamashita *et al.*, 1991).

Results

Morphology of growing pig oocytes

The mean diameters of oocytes isolated from preantral follicles (class A) and three sizes of antral follicles (classes B, C and D) were 78.1 ± 5.5 ($n = 62$), 105.5 ± 7.0 ($n = 89$), 115.5 ± 5.1 ($n = 87$) and 119.3 ± 5.2 μm ($n = 89$), respectively. The differences among the four classes were statistically significant ($p < 0.05$). After orcein staining, several clearly different patterns of chromatin configuration were observed (Table 1). The typical pattern found in the small oocytes (classes A and B) is shown in Fig. 1*A*. The decondensed filamentous chromatin was distributed throughout the germinal vesicle and has been designated as the 'filamentous chromatin (FC)' stage. In class C oocytes, the chromatin was clearly thicker than in oocytes in classes A and B; the term 'stringy chromatin (SC)' has been used to describe this stage (Fig. 1*B*). As the oocytes approached full size a condensing chromatin ring formed around the nucleolus (Fig. 1C), showing the morphology of 'GV I' staged oocytes as characterised by Motlik & Fulka (1976). A few oocytes had a chromatin configuration which characterised the initial stages of GVBD; these were classified into three categories – GV II, GV III and GV IV – again based on the criteria described in detail by Motlik & Fulka (1976). Nucleolar morphology also altered as oocytes approached full size; the nucleoli in small oocytes from class A and B follicles were both intensely stained by orcein and contained many small vacuoles (Fig. 1*A*). As oocyte diameter increased, the vacuoles became larger in size and fewer in number. The nucleoli in oocytes of classes C and D were not stained by orcein (Fig. 1*B*, *C*).

Meiotic competence

Meiotic competence was evaluated in the oocytes after 24, 48 and 72 h in culture. After 24 h, expansion of cumulus cells was observed around oocytes in classes B, C and D: in class A, no cumulus expansion was observed and, instead, the cells surrounding each oocyte became attached to the surface of the dish and spread thereafter to form a monolayer. Table 2 shows the maturational stages of the cultured oocytes isolated from each follicular class. No significant meiotic resumption was observed in oocytes from preantral follicles (class A). Most of the oocytes from preantral follicles (class A) that had not degenerated after 24, 48 or 72 h culture contained filamentous chromatin and vacuolated nucleoli. In all other classes, meiotic resumption was observed as early as 24 h of culture. In follicles from small, mid- and large antral follicles (classes B, C and D) 22%, 14% and 29% of oocytes, respectively, underwent GVBD and reached

Table 2 Maturational stage of pig oocytes isolated from follicles of various sizes and cultured thereafter *in vitro* for 24, 48 or 72 h

Follicular diameter (mm)	Time of culture (h)	No. of oocytes examined	No. (%) of oocytes at stage of:							No. (%) of oocytes degenerating
			FC	SC	GV I	GV II–IV	D	MI	MII	
A: 0.2–0.4	0	27	26 (96)^{a}	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4)^{b,c}
	24	38	33 (87)^{a,b}	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (13)^{b,c}
	48	47	33 (71)^{b,c}	2 (4)^{e}	0 (0)	0 (0)	2 (4)^{b}	0 (0)	0 (0)	10 (21)^{a,b}
	72	43	22 (51)^{c}	3 (7)^{d,e}	0 (0)	0 (0)	1 (2)^{b}	0 (0)	0 (0)	17 (40)^{a}
B: 0.5–1.5	0	56	40 (72)^{b}	11 (20)^{c,d}	2 (4)^{c}	1 (2)^{c}	0 (0)	0 (0)	0 (0)	2 (2)^{c}
	24	46	14 (30)^{d}	17 (37)^{b,c}	0 (0)	3 (7)^{b,c}	4 (9)^{a,b}	6 (13)^{c}	0 (0)	2 (4)^{c}
	48	46	2 (4)^{e}	7 (15)^{d,e}	2 (4)^{b,c}	7 (15)^{a,b}	0 (0)	23 (51)^{a}	2 (4)^{c}	3 (7)^{b,c}
	72	48	0 (0)	7 (15)^{d,e}	4 (8)^{b,c}	10 (21)^{a,b}	1 (2)^{b}	18 (38)^{a,b}	4 (8)^{c}	4 (8)^{b,c}
C: 3.0–4.0	0	48	4 (8)^{e}	40 (84)^{a}	3 (6)^{b,c}	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)^{c}
	24	37	0 (0)	0 (0)	22 (59)^{a}	7 (19)^{a,b}	4 (11)^{a,b}	0 (0)	1 (3)^{c}	3 (8)^{b,c}
	48	37	0 (0)	0 (0)	7 (19)^{b}	3 (8)^{b,c}	2 (5)^{a,b}	5 (14)^{c}	17 (46)^{b}	3 (8)^{b,c}
	72	39	0 (0)	0 (0)	6 (15)^{b,c}	5 (13)^{b,c}	1 (3)^{b}	7 (18)^{b,c}	19 (48)^{b}	1 (3)^{c}
D: 5.0–6.0	0	45	0 (0)	9 (20)^{c,d}	25 (55)^{a}	11 (25)^{a}	0 (0)	0 (0)	0 (0)	0 (0)
	24	41	0 (0)	0 (0)	19 (46)^{a}	8 (20)^{a,b}	9 (22)^{a}	3 (7)^{c}	0 (0)	2 (5)^{b,c}
	48	42	0 (0)	0 (0)	3 (7)^{b,c}	0 (0)	1 (2)^{b}	7 (17)^{c}	31 (74)^{a}	0 (0)
	72	44	0 (0)	0 (0)	2 (5)^{b,c}	0 (0)	1 (2)^{b}	0 (0)	41 (93)^{a}	0 (0)

FC, filamentous chromatin stage; SC, stringy chromatin stage; GV I–GV IV, germinal vesicle I–IV stage as defined by Motlik & Fulka (1976); D, diakinesis stage; MI, metaphase I stage; MII, metaphase II stage.

$^{a-e}$ Values with different superscripts in the same column differ significantly ($p < 0.05$).

diakinesis or MI within the first 24 h. Most of the other oocytes from small antral follicles (class B) retained a stringy chromatin configuration, while those from larger follicles (classes C and D) that had not undergone GVBD after 24 h were at GV I. After 48 h of culture, the percentages of oocytes that had undergone GVBD increased to 54%, 65% and 93% for the oocytes from follicle classes B, C and D, respectively. Progression to MII was observed in only 4% of class B oocytes, whilst those in classes C and D progressed to MII in 46% and 74% of cases respectively. After 72 h in culture virtually no further progression to MII was observed in oocytes from small and mid-antral follicles (classes B and C). However, in class D the percentage of MII oocytes increased from 74% to 93% when the culture interval was increased from 48 to 72 h.

Change in $p34^{cdc2}$ during oocyte growth

The results presented in Fig. 2 show the relative levels of the 32 kDa and 34 kDa oocyte proteins recognised by the different anti-$p34^{cdc2}$ antibodies. Rabbit anti-$p34^{cdc2}$ antibodies reacted with both the 32 kDa and 34 kDa proteins (Fig. 2*B*, *C*), while the mouse antibody reacted at all stages with only the 34 kDa upper band proteins (Fig. 2*A*). All three antibodies reacted with 34 kDa protein in granulosa cells (lane 5). The immunoblots show that there is little 32 or 34 kDa protein in oocytes from class A preantral follicles (lane 1). The intracellular levels of immunoreactive protein increased with increasing oocyte size until the mid-antral follicle stage (class C; lane 3), when levels of both the 32 and 34 kDa proteins were comparable to those of fully grown oocytes from large antral follicles (class D; lane 4).

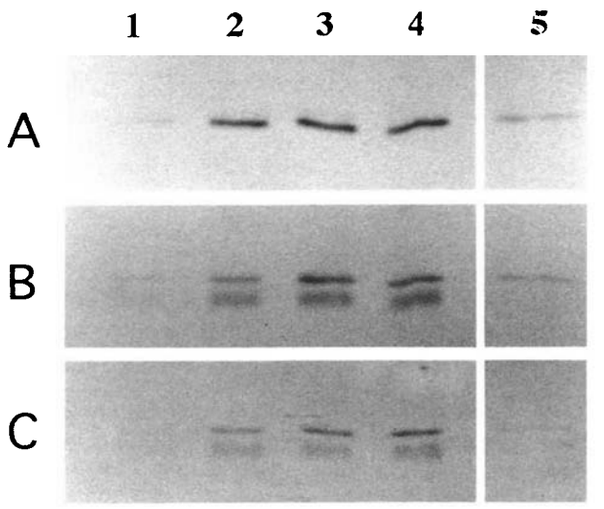

Figure 2 Immunoblotting analysis of the relative levels of $p34^{cdc2}$ in pig oocytes at various stages of growth. Lanes 1, 2, 3 and 4 contain a total of 182–219 oocytes from preantral (0.2–0.4 mm, class A), 108 from small antral (0.5–1.5 mm, class B), 84 from mid-antral (3.0–4.0 mm, class C) and 75 from large antral (5.0–6.0 mm, class D) follicles, respectively. These numbers were calculated to ensure that the total oocyte mass in each lane was similar to that in all other lanes. Lane 5 contains 5×10^4 cells granulosa cells. $p34^{cdc2}$ protein was detected by three independently raised antibodies – mouse PSTAIRE (*A*), rabbit PSTAIRE (*B*) and rabbit TYG (*C*) – as described in Materials and Methods.

Discussion

The growth of the pig oocyte and its associated follicle occur in a closely coordinated manner until the oocyte reaches its maximum diameter of approximately 120 μm; thereafter follicular growth progresses in the absence of further oocyte growth (Motlik *et al.*, 1984). The smallest oocytes (78 μm) examined in our experiments (class A: 0.2–0.4 mm follicle diameter) were already more than twice their non-growing diameter; these oocytes were nevertheless still totally incapable of reinitiating meiosis and progressing beyond the diplotene stage reached by them during fetal life. In contrast, during the next phase of growth (from 78 μm to 105 μm) more than half the oocytes become competent to undergo the G_2- to M-phase transition. It is noteworthy that the nuclear morphology of the meiotically incompetent class A oocytes and those in class B was both similar and characterised by the presence of diffuse chromatin and vacuolated nucleoli. Thus, it is clear that the competence to undergo the G_2- to M-phase transition in pig oocytes is acquired before the chromatin changes from a diffuse state to that of perinucleolar condensation. This is in marked contrast to the situation in the mouse, where the acquisition of meiotic competence during oocyte growth is closely correlated with a change in nuclear morphology from the diffuse to the perinucleolar condensed state (Wickramasinghe *et al.*, 1991).

Although the p34 band, recognised by all three antibodies in our immunoblot analysis, has been definitively identified as the catalytic subunit of MPF kinase, we are unable to state with certainty whether the 32 kDa lower band detected by the two polyclonal antibodies is a dephosphorylated form of $p34^{cdc2}$ or a related family member such as cdk2 (Meyerson *et al.*, 1992). Nevertheless both the upper $p34^{cdc2}$ band which is recognised by all three antibodies and the p32 lower band show a parallel incremental increase in intensity during oocyte growth. Irrespective of the nature of the lower band, the focus of the present study has been on changes in the catalytic subunit of MPF kinase during the ontogeny of the oocyte. We show that small oocytes (78 μm) from preantral follicles contain very low levels of $p34^{cdc2}$ and are

incapable of meiotic progression. By contrast all sizes of growing oocytes from antral follicles contained high levels of the catalytic subunit even though the capacity of the intermediate-sized oocytes (90–105 μm) to progress beyond the G_2 stage of the cycle is absent or limited (see Christmann *et al.*, 1994 and present data). Indeed, the comparison of our data with those of Christmann and colleagues (1994) who worked on oocytes (< 90 μm) from late preantral follicles is particularly illuminating. As pig oocytes increase in diameter from 70 to 90 μm their volume doubles and both the $p34^{cdc2}$ and B-type cyclins reach levels almost comparable to those found in fully grown oocytes. Despite the high levels of MPF kinase subunits, oocytes of 90 μm are incapable of undergoing the G_2- to M-phase transition under normal conditions *in vitro*. These oocytes do, however, undergo GVBD when treated with an inhibitor (okadaic acid) of protein phosphatase 1 and 2A which is known to bypass the phosphorylation cascade required for the activation of MPF (Christmann *et al.*, 1994). It is noteworthy that treatment by us of the smaller class of oocytes (78 μm) with okadaic acid was similarly incapable of inducing meiotic progression (data not shown). Thus, whilst small oocytes (78 μm) are incapable of undergoing GVBD because of inadequate levels of MPF kinase subunits, the next size category of oocytes (< 90 μm) is likewise inhibited from meiotic progression but by an entirely different set of mechanisms. The results of Christmann and colleagues (1994) indicate that this second arrest mechanism relates directly to the phosphatase kinase cascade that determines the phosphorylation status of tyrosine 15 of the $p34^{cdc2}$ molecule. That meiotic arrest in growing mouse oocytes similarly involves a sequence of different mechanisms is supported by the observation that the microinjection of okadaic acid induces GVBD in larger categories of growing mouse oocytes (55–60 μm) whilst smaller oocytes (50 μm) are entirely unresponsive (Gavin *et al.*, 1991). Moreover, it has been reported quite recently that growing mouse oocytes which are incompetent to resume meiosis (from 15-day-old mice; 51 μm) contain a much smaller amount of $p34^{cdc2}$ than meiotically competent oocytes (from 21-day-old mice; 59 μm) (Chesnel & Eppig, 1995). Taking these facts together, we postulate that inadequate levels of the subunits of MPF kinase block meiotic progression in early growing mammalian oocytes; during the intermediate stages of growth, oocytes contain an inactive pool of pre-MPF which cannot be mobilised because of lesions in the phosphorylation cascade required to activate the molecule. In the late growth phase oocytes have the capacity to progress from G_2 to M phase but are incapable of inducing chromosome segregation and the formation of a second metaphase plate.

Virtually all the fully grown oocytes (41/42) that underwent GVBD progressed thereafter to MII. By contrast, 78% (18/23) of the oocytes (105 μm diameter) from early antral follicles remained blocked at MI even after 72 h in culture. As has been pointed out previously (Motlik *et al.*, 1984), as oocytes approach maximum diameter (120 μm) their capacity to complete meiosis increases in a parallel fashion; whilst only 22% of 105 μm diameter oocytes reached MII in culture, more than 70% of 115 μm diameter oocytes and over 90% of fully grown oocytes formed MII plates. It will be recalled that intracellular levels of active MPF kinase constitute the central machinery that drives the cell cycle from G_2 to M phase (Murray & Hunt, 1993). In pig oocytes, MPF kinase activity is low until just before nuclear membrane breakdown, when it rises sharply, remains high during MI, declines at anaphase and rises again before the formation of the second metaphase plate (Mattioli *et al.*, 1991; Christmann *et al.*, 1994). Fluctuations in the MPF kinase pattern in fully grown mouse oocytes during the MI to MII transition are broadly similar to those in pigs (Hashimoto & Kishimoto, 1988; Fulka *et al.*, 1992). However, Hampl & Eppig (1995) report that the MI block observed in mouse oocytes cultured during the late phase of growth is correlated with a sustained elevation of MPF kinase activity. In addition, these authors report that growing oocytes blocked in MI do not undergo the expected rate of cyclin B degradation that occurs in fully grown oocytes at the MI to AI transition (Murray, 1992). It is therefore tempting to conclude that the MI block observed in growing oocytes (105 μm diameter) from early antral follicles is a consequence of deficiencies in the ubiquitin-mediated proteolytic system involved in cyclin B degradation. That this may not be the primary cause of MI arrest is, however, suggested by the finding that separation of homologous chromosomes provides the trigger for cyclin degradation and the decline in MPF kinase levels in fully grown oocytes from a variety of species including the mouse, fruit fly and yeast (Fulka & Moor, 1993; Fulka *et al.*, 1994; Surana *et al.*, 1993; McKim *et al.*, 1993). Whilst in growing oocytes we are unable to identify with certainty the initial cause of MI arrest, we nevertheless favour the hypothesis that growing oocytes (> 105 μm diameter) are blocked in MI primarily because of their inability to initiate chromosome separation. We postulate further that this primary lesion leads in turn to failures in other components of the machinery involved in chromosome separation at anaphase. These secondary failures would include inadequate cyclin proteolysis and the consequent maintenance of elevated levels of active MPF kinase.

The capacity of growing oocytes (> 105 μm diameter) to maintain meiotic progression beyond MI

appears to be closely correlated also with chromatin changes during early culture. Chromatin configuration in the majority of growing oocytes (115 μm diameter) from mid-antral follicles shifts from a diffuse to a perinucleolar condensed pattern in the first 24 h in culture; interestingly, the proportion of oocytes that undergo this type of early chromatin condensation parallels that of oocytes in this class that reach MII. Almost all oocyte (105 μm) from smaller-sized antral follicles retain a diffuse pattern of chromatin during the first day in culture and all are blocked in MI. It is possible that perinucleolar chromatin reorganisation is indicative of early biochemical events which are, in turn, directly responsible for the MI to MII transition. However, it is equally possible that chromosome separation at anaphase is directly dependent on the structural organisation of chromatin during its early condensation around the nucleolus. It is notable that growing oocytes are never arrested at anaphase or telophase. This suggests that the full complement of molecules required for cytokinesis and for MII arrest is present in all oocytes that are capable of progressing from MI to AI.

Important questions can now be asked about the separate mechanisms that inhibit meiotic progression in growing oocytes of different sizes. It will be interesting to establish whether the earliest suppressor mechanism, namely inadequate synthesis of $p34^{cdc2}$ protein, is regulated at the transcriptional or translational level. The major question about the second suppressor mechanism concerns the precise point at which deficiencies in the $p34^{cdc2}$ phosphorylation cascade occur in intermediate-sized (90–100 μm) growing oocytes. Finally, identifying the primary causes of the MI block in the larger growing oocytes (> 105 μm) represents the central challenge to the understanding of the third type of growth-related meiotic suppressor.

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