



**Oocyte-Specific Deletion of Pten Causes Premature Activation of the Primordial Follicle Pool**

Pradeep Reddy, *et al.*  
*Science* **319**, 611 (2008);  
DOI: 10.1126/science.1152257

***The following resources related to this article are available online at [www.sciencemag.org](http://www.sciencemag.org) (this information is current as of March 3, 2008):***

**Updated information and services**, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/cgi/content/full/319/5863/611>

**Supporting Online Material** can be found at:

<http://www.sciencemag.org/cgi/content/full/319/5863/611/DC1>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/cgi/content/full/319/5863/611#related-content>

This article **cites 10 articles**, 4 of which can be accessed for free:

<http://www.sciencemag.org/cgi/content/full/319/5863/611#otherarticles>

This article appears in the following **subject collections**:

Development

<http://www.sciencemag.org/cgi/collection/development>

Information about obtaining **reprints** of this article or about obtaining **permission to reproduce this article** in whole or in part can be found at:

<http://www.sciencemag.org/about/permissions.dtl>

# Oocyte-Specific Deletion of *Pten* Causes Premature Activation of the Primordial Follicle Pool

Pradeep Reddy,<sup>1</sup> Lian Liu,<sup>1,2\*</sup> Deepak Adhikari,<sup>1\*</sup> Krishna Jagarlamudi,<sup>1\*</sup> Singareddy Rajareddy,<sup>1\*</sup> Yan Shen,<sup>1</sup> Chun Du,<sup>1</sup> Wenli Tang,<sup>1</sup> Tuula Hämäläinen,<sup>3</sup> Stanford L. Peng,<sup>4</sup> Zi-jian Lan,<sup>5</sup> Austin J. Cooney,<sup>6</sup> Ilpo Huhtaniemi,<sup>3,7</sup> Kui Liu<sup>1†</sup>

In the mammalian ovary, progressive activation of primordial follicles from the dormant pool serves as the source of fertilizable ova. Menopause, or the end of female reproductive life, occurs when the primordial follicle pool is exhausted. However, the molecular mechanisms underlying follicle activation are poorly understood. We provide genetic evidence that in mice lacking PTEN (phosphatase and tensin homolog deleted on chromosome 10) in oocytes, a major negative regulator of phosphatidylinositol 3-kinase (PI3K), the entire primordial follicle pool becomes activated. Subsequently, all primordial follicles become depleted in early adulthood, causing premature ovarian failure (POF). Our results show that the mammalian oocyte serves as the headquarters of programming of follicle activation and that the oocyte PTEN-PI3K pathway governs follicle activation through control of initiation of oocyte growth.

Ovarian follicles are the basic units of a mammalian ovary, and each follicle contains an oocyte that is surrounded by somatic (granulosa) cells (1). Whereas the majority of follicles remain as dormant primordial follicles containing immature oocytes surrounded by a few flattened somatic cells for use in later reproductive life, a limited number of primordial follicles are recruited from the resting follicle reservoir into the growing follicle pool. The activation of primordial follicles is a progressive and highly regulated process. It ceases and female reproductive life ends when the follicle pool is exhausted at menopause (1, 2). The duration of fertility of a female is determined by the initial size of her primordial follicle pool and by the rate of its activation and depletion (3).

A landmark of follicular activation is the initiation of rapid oocyte growth within primordial follicles (4, 5). Activation is irreversible, such that activated follicles that are not selected for further development will undergo atresia. Although the initial follicular activation is believed to be independent of gonadotropin action, the molecular mechanisms are poorly defined (1, 2). Our recent studies (5, 6) suggest that the phosphatidylinositol 3-kinase (PI3K) pathway of oocytes may play a role in regulating the activation

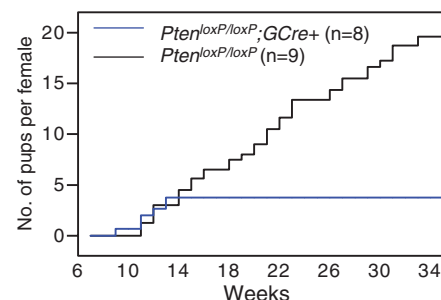
of primordial follicles; however, further functional evidence is required to support this notion.

PI3Ks are lipid kinases that phosphorylate the 3'-OH group on the inositol ring of inositol phospholipids. PTEN (phosphatase and tensin homolog deleted on chromosome 10), a lipid phosphatase, reverses this process and thus functions as a major negative regulator of PI3K action (7). The PI3K pathway is a fundamental signaling pathway for the regulation of cell proliferation, survival, migration, and metabolism (7). To study the functional roles of the oocyte PI3K pathway in mammalian follicular activation, we deleted the *Pten* gene from mouse oocytes by crossing *Pten*<sup>loxP/loxP</sup> mice (8) with transgenic mice expressing *growth differentiation factor 9* (*Gdf-9*) promoter-mediated Cre recombinase (referred to as *GCre* mice); this recombinase is active specifically in oocytes (9) (fig. S1). We found that during a testing period from 6 to 34 weeks of age, the *Pten*<sup>loxP/loxP</sup>; *GCre*<sup>+</sup> females produced a maximum of one normal-sized litter but became infertile in early adulthood (i.e., after 12 to 13 weeks of age) (Fig. 1).

To study how the loss of *Pten* from oocytes impedes mouse fertility, we compared the first wave of postnatal follicular development in *Pten*<sup>loxP/loxP</sup>; *GCre*<sup>+</sup> and *Pten*<sup>loxP/loxP</sup> mice. We found no apparent morphological difference in postnatal day 5 (PD5) ovaries of *Pten*<sup>loxP/loxP</sup>; *GCre*<sup>+</sup> and *Pten*<sup>loxP/loxP</sup> mice. The ovaries of both genotypes had mostly primordial follicles containing small oocytes surrounded by flattened pregranulosa cells (Fig. 2, A to C, arrows) and some activated follicles containing enlarged oocytes (Fig. 2, A to C, arrowheads), with comparable numbers (fig. S3A). At PD8, however, the *Pten*<sup>loxP/loxP</sup>; *GCre*<sup>+</sup> ovaries appeared larger (fig. S2, A and B) with more activated follicles (fig. S3B), including transient follicles containing enlarged oocytes surrounded by flattened pregranulosa cells (fig. S2C, yellow arrows), primary (type 3b) follicles with enlarged oocytes sur-

rounded by one layer of cuboidal granulosa cells, and some secondary (type 4) follicles with two layers of granulosa cells (fig. S2, B and C, black arrows), whereas the *Pten*<sup>loxP/loxP</sup> ovaries had mostly primordial (fig. S2A, red arrows) and type 3b follicles (fig. S2A, arrowheads). Accordingly, the percentage of primordial follicles in *Pten*<sup>loxP/loxP</sup>; *GCre*<sup>+</sup> ovaries at PD8 (49.6%) was significantly lower than that in *Pten*<sup>loxP/loxP</sup> ovaries (83.6%) (fig. S3B). By PD23 and 35, the *Pten*<sup>loxP/loxP</sup>; *GCre*<sup>+</sup> ovaries (Fig. 2E and fig. S2E) remained larger than the *Pten*<sup>loxP/loxP</sup> ovaries (Fig. 2D and fig. S2D) and contained significantly more activated follicles (Fig. 2F, fig. S2F, and fig. S3, C and D). By PD23, virtually no primordial follicles could be identified in mutant ovaries (figs. S2E and S3C), whereas 69.2% of the follicles in control ovaries were still at the primordial stage (fig. S2D, inset, and fig. S3C). Therefore, the entire pool of primordial follicles had been activated in *Pten*<sup>loxP/loxP</sup>; *GCre*<sup>+</sup> ovaries. This also resulted in reduced follicle death and clearance before and around the time of sexual maturity in the mutant mice (10) (fig. S3F). The growth dynamics of the activated transient follicles seemed different: Some follicles appeared to remain at the transient stage (Fig. 2F and fig. S2F, yellow arrows; fig. S3, C to E), whereas others appeared to develop further (fig. S3, C to E).

At 7 weeks, *Pten*<sup>loxP/loxP</sup>; *GCre*<sup>+</sup> ovaries still appeared larger than *Pten*<sup>loxP/loxP</sup> ovaries (fig. S2, G and H), with significantly higher numbers of transient and preantral type 5 follicles (with three or more layers of granulosa cells) accumulated (fig. S3E). Corpora lutea (CL), which are temporary endocrine structures developed from the remnants of ovulated follicles, were present (fig. S2H, arrows), indicating that ovulation had taken place. Furthermore, in ovaries of 12-week-old *Pten*<sup>loxP/loxP</sup>; *GCre*<sup>+</sup> mice, follicular structure was largely deformed (fig. S2K) and CL were undergoing degeneration (luteolysis) (fig. S2K, arrows). In ovaries of both 7- and 12-week-old *Pten*<sup>loxP/loxP</sup>; *GCre*<sup>+</sup> mice, many transient follicles containing degraded oocytes were observed (fig. S2, I and L, arrows), which suggests that some of the prematurely activated follicles undergo atre-



**Fig. 1.** Comparison of the cumulative number of pups per *Pten*<sup>loxP/loxP</sup>; *GCre*<sup>+</sup> female (blue line) and per *Pten*<sup>loxP/loxP</sup> female (black line);  $n = 9$  for *Pten*<sup>loxP/loxP</sup> mice,  $n = 8$  for *Pten*<sup>loxP/loxP</sup>; *GCre*<sup>+</sup> mice. All *Pten*<sup>loxP/loxP</sup>; *GCre*<sup>+</sup> females became infertile in young adulthood (after week 12 to 13).

<sup>1</sup>Department of Medical Biochemistry and Biophysics, Umeå University, SE-901 87 Umeå, Sweden. <sup>2</sup>Department of Chemotherapy, Cancer Center, Qilu Hospital, Shandong University, Jinan 250012, China. <sup>3</sup>Department of Physiology, Institute of Biomedicine, University of Turku, 20520 Turku, Finland. <sup>4</sup>Clinical Research and Exploratory Development, Roche Palo Alto, Palo Alto, CA 94304, USA. <sup>5</sup>Birth Defects Center, Department of Molecular, Cellular and Craniofacial Biology, University of Louisville Health Sciences Center, Louisville, KY 40202, USA. <sup>6</sup>Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030, USA. <sup>7</sup>Department of Reproductive Biology, Imperial College London, Hammersmith Campus, London W12 0NN, UK.

\*These authors contributed equally to this work.

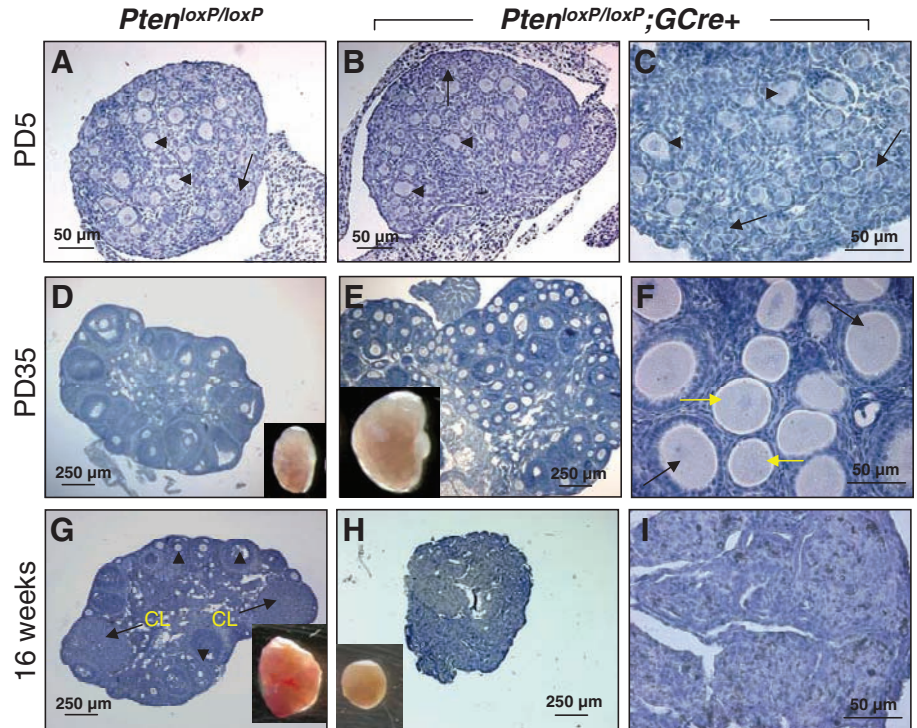
†To whom correspondence should be addressed. E-mail: kui.liu@medchem.umu.se

sia directly from the transient stages. Mice at this or older ages showed completely irregular estrous cycles. By the age of 16 weeks, *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* ovaries no longer displayed normal ovarian morphology (Fig. 2, H and I). The ovaries were smaller, rounded, and yellow (Fig. 2H, inset) in comparison to the pink-colored *Pten<sup>loxP/loxP</sup>* ovaries, which contained CL (Fig. 2G, arrows) and follicles (Fig. 2G, arrowheads).

Moreover, in sera of 12- to 20-week-old *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* mice, elevated levels of follicle-stimulating hormone (FSH) (Fig. 3A) and luteinizing hormone (LH) (Fig. 3B) were observed relative to *Pten<sup>loxP/loxP</sup>* control mice. Thus, activation of the pool of primordial follicles leads to follicle depletion; this causes premature ovarian failure (POF) in *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* mice. The phenotype observed in these mice resembles that of human POF (11).

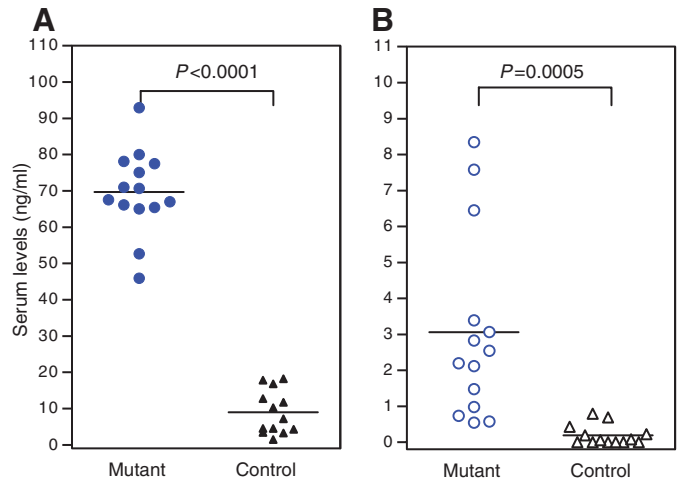
To elucidate the molecular mechanisms underlying the accelerated oocyte enlargement in *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* ovaries, we studied Akt signaling in oocytes isolated from ovaries of PD12 to 14 *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* and *Pten<sup>loxP/loxP</sup>* mice. We found that the level of phospho-Akt (p-Akt, Ser<sup>473</sup>) was elevated in *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* oocytes that were cultured in vitro and starved of serum (Fig. 4A). In addition, Kit ligand (KL), which can activate the PI3K pathway in growing oocytes through its oocyte surface receptor Kit (6), activated Akt to a greater extent in *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* oocytes than in *Pten<sup>loxP/loxP</sup>* oocytes (Fig. 4B). Thus, the loss of *Pten* in oocytes leads to enhanced oocyte PI3K-Akt signaling.

To investigate the cause of accelerated oocyte growth in *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* ovaries, we studied whether the enhanced PI3K-Akt signaling led to increased activation of ribosomal protein S6 (rpS6). At PD5—a developmental stage with no apparent morphological differences between *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* and *Pten<sup>loxP/loxP</sup>* ovaries (Fig. 2, A to C)—the activation of Akt had already been elevated in *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* oocytes (Fig. 4C, PD5, p-Akt). This result correlated with enhanced expression (Fig. 4C, PD5, rpS6) and phosphorylation (indicating activation) of rpS6 (Fig. 4C, PD5, p-rpS6, Ser<sup>235/6</sup>). Such a result suggests that enhanced protein translation had already started when the *Gdf-9*-Cre-mediated *Pten* deletion in oocytes had just taken place (9). Similarly, in oocytes isolated from *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* ovaries at PD12 to 14, enhanced PI3K-Akt signaling led to elevation of both expression and phosphorylation of rpS6 (Fig. 4C, PD12 to 14). However, activation of the mammalian target of rapamycin (mTOR)—p70 S6 kinase (S6K) cascade was not increased by loss of *Pten*, as the levels of phospho-mTOR (p-mTOR, Ser<sup>2448</sup>), phospho-tuberin/TSC2 (p-TSC2, Thr<sup>1462</sup>), and phospho-S6K (p-S6K, Thr<sup>389</sup>) at PD5 and PD12 to 14 remained similar in *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* and *Pten<sup>loxP/loxP</sup>* oocytes (Fig. 4C). Thus, the enhanced activation of rpS6 was caused by elevated rpS6 expression per se (Fig. 4C). However, the phosphorylation of rpS6



**Fig. 2.** Activation of the primordial follicle pool followed by premature ovarian failure (POF) in *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* mice. (A to F) Overactivation of primordial follicles in *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* mice. Ovaries from PD5 and PD35 *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* mice and their *Pten<sup>loxP/loxP</sup>* littermates were embedded in paraffin, and sections of 8- $\mu$ m thickness were prepared and stained with hematoxylin. (G to I) POF caused by depletion of all follicles in *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* mice. Ovaries from 16-week-old *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* mice and *Pten<sup>loxP/loxP</sup>* littermates were used. The experiments were repeated more than three times, and for each time and each age, ovaries from one mouse of each genotype were used.

**Fig. 3.** Elevated levels of FSH (A) and LH (B) in adult *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* mice (mutant) and *Pten<sup>loxP/loxP</sup>* mice (control);  $n = 14$  for *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* mice,  $n = 13$  for *Pten<sup>loxP/loxP</sup>* mice. Female mice of both genotypes (12 to 20 weeks old) were killed and sera were collected for measurement of FSH and LH levels (10).  $P$  values are shown in each panel.

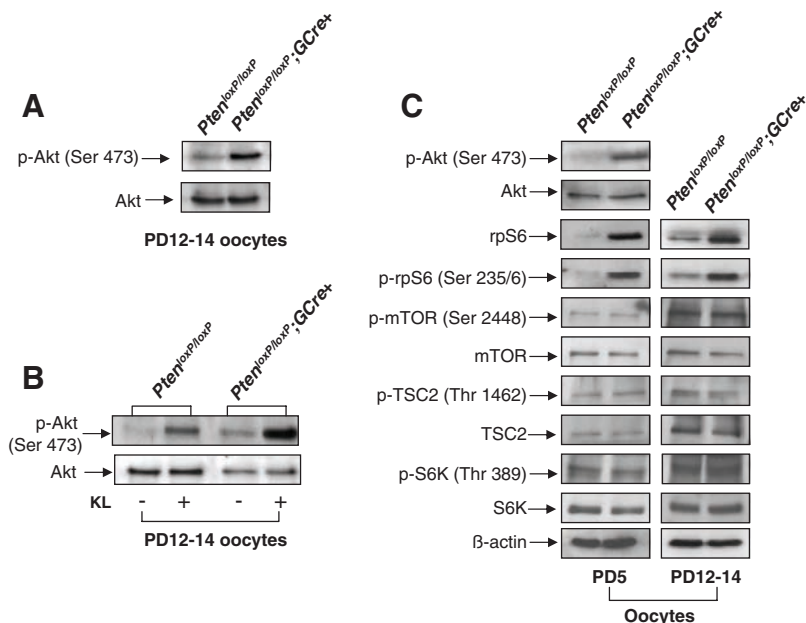


and S6K in mutant oocytes was sensitive to the PI3K-specific inhibitor LY294002 and the mTOR-specific inhibitor rapamycin (fig. S4A), indicating that activation of rpS6 in *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* oocytes is dependent on the activities of PI3K and mTOR.

It has been hypothesized that unknown intra-ovarian factors stimulate some primordial follicles to initiate growth while the rest of the follicles remain quiescent. On the other hand, it has also been suggested that follicular activation is triggered by the release of inhibitory mechanisms that maintain the primordial follicles in their

resting state (1). Our data show that the oocyte governs follicular activation and that oocyte PTEN functions as a suppressor of this process. The intra-oocyte PTEN-PI3K signaling cascade appears to play a role in the initiation of oocyte growth. We propose that activation of the PI3K pathway in each individual oocyte may be essential in determining the fate of the primordial follicle—whether it remains dormant, whether it becomes activated at a certain time, or whether it undergoes atresia directly from the primordial stage.

Furthermore, we report the distinctive ovarian phenotype of POF in mice with oocyte-specific



**Fig. 4.** Enhanced Akt signaling in *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* oocytes leads to elevated expression and activation of rpS6. Oocytes were isolated from ovaries of *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* and *Pten<sup>loxP/loxP</sup>* mice at PD5 and PD12 to 14, and Western blots were performed (10). (A) Levels of p-Akt (Ser<sup>473</sup>) and total Akt in PD12 to 14 *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* and *Pten<sup>loxP/loxP</sup>* oocytes. (B) Activation of Akt (p-Akt, Ser<sup>473</sup>) by KL treatment (100 ng/ml, 2 min) in PD12 to 14 *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* and *Pten<sup>loxP/loxP</sup>* oocytes. Levels of KL were used as internal controls. (C) Signaling studies in *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* and *Pten<sup>loxP/loxP</sup>* oocytes at PD5 and PD12 to 14, showing levels of p-Akt (Ser<sup>473</sup>), rpS6, p-rpS6 (Ser<sup>235/6</sup>), p-mTOR (Ser<sup>2448</sup>), p-TSC2 (Thr<sup>1462</sup>), and p-S6K (Thr<sup>389</sup>). Levels of total Akt, mTOR, TSC2, S6K, and β-actin were used as internal controls. All experiments were repeated at least three times. For isolation of PD5 oocytes for Western blot, 10 to 15 *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* or *Pten<sup>loxP/loxP</sup>* mice were used for each lane. For isolation of PD12 to 14 oocytes, 3 to 5 *Pten<sup>loxP/loxP</sup>;GCre<sup>+</sup>* mice or 6 to 10 *Pten<sup>loxP/loxP</sup>* mice were used per lane. In each lane, 30 to 40 μg of protein sample was loaded.

ablation of *Pten*, which is caused by excessive activation and depletion of primordial follicles. Thus, our findings may have broad physiological and clinical implications, contributing to in-depth understanding of both normal ovarian physiolo-

gy and the development of ovarian diseases. In humans, POF is defined as a primary ovarian defect characterized by absent menarche (primary amenorrhea) or by premature depletion of ovarian follicles or arrested folliculogenesis be-

fore the age of 40 years (secondary amenorrhea), with an estimated incidence of 1% (11). We hypothesize that genetic variations leading to over-activation and depletion of follicles may be among the possible causes of POF in humans. On the other hand, the retardation of follicle activation and/or excessive primordial follicle atresia, both of which may be caused by underactivation of the PI3K pathway in oocytes, can also lead to POF, albeit from opposite directions. Recognition of the importance of the PTEN-PI3K signaling network in oocytes opens up new prospects for our understanding of the physiological and pathological processes of the mammalian ovary.

**References and Notes**

1. E. A. McGee, A. J. Hsueh, *Endocr. Rev.* **21**, 200 (2000).
2. A. N. Hirshfield, *Int. Rev. Cytol.* **124**, 43 (1991).
3. G. F. Erickson, *J. Soc. Gynecol. Investig.* **8**, S13 (2001).
4. P. M. Wassarman, D. F. Albertini, in *The Physiology of Reproduction*, E. Knobil, J. D. Neill, Eds. (Raven, New York, 1994), vol. 1, pp. 79–122.
5. K. Liu *et al.*, *Dev. Biol.* **299**, 1 (2006).
6. P. Reddy *et al.*, *Dev. Biol.* **281**, 160 (2005).
7. L. C. Cantley, *Science* **296**, 1655 (2002).
8. M. Groszer *et al.*, *Science* **294**, 2186 (2001); published online 1 November 2001 (10.1126/science.1065518).
9. Z. J. Lan, X. Xu, A. J. Cooney, *Biol. Reprod.* **71**, 1469 (2004).
10. See supporting material on Science Online.
11. P. Beck-Peccoz, L. Persani, *Orphanet. J. Rare. Dis.* **1**, 9 (2006).
12. Supported by the J. C. Kempe and Seth M. Kempe Memorial Foundation, the Swedish Research Council, the Swedish Cancer Foundation, Lion's Cancer Research Foundation at Umeå University, and the Novo Nordisk Foundation (K.L.).

**Supporting Online Material**

www.sciencemag.org/cgi/content/full/319/5863/611/DC1

Materials and Methods

SOM Text

Figs. S1 to S6

References

29 October 2007; accepted 17 December 2007

10.1126/science.1152257

# The Maternal Nucleolus Is Essential for Early Embryonic Development in Mammals

Sugako Ogushi,<sup>1,2,3\*</sup> Chiara Palmieri,<sup>5</sup> Helena Fulka,<sup>3,4</sup> Mitinori Saitou,<sup>2</sup> Takashi Miyano,<sup>1</sup> Josef Fulka Jr.<sup>3</sup>

With fertilization, the paternal and maternal contributions to the zygote are not equal. The oocyte and spermatozoon are equipped with complementary arsenals of cellular structures and molecules necessary for the creation of a developmentally competent embryo. We show that the nucleolus is exclusively of maternal origin. The maternal nucleolus is not necessary for oocyte maturation; however, it is necessary for the formation of pronuclear nucleoli after fertilization or parthenogenetic activation and is essential for further embryonic development. In addition, the nucleolus in the embryo produced by somatic cell nuclear transfer originates from the oocyte, demonstrating that the maternal nucleolus supports successful embryonic development.

To create a totipotent zygote, the oocyte and spermatozoon combine not only their nuclear DNA but also certain RNAs, proteins, and organelles. Some zygotic material is

strictly of maternal or paternal origin. For example, mitochondria originate exclusively from the oocyte (1), whereas the centriole in most mammals (excluding rodents) comes from the sper-

matozoon (2, 3). Another zygotic organelle that seems to be supplied by the oocyte is the nucleolus. The nucleoli in fully grown oocytes are compact and transcriptionally inactive (4–7). It is not known how or to what extent this nucleolar material contributes to the construction of zygotes and early embryos. Our results demonstrate that the nucleolus in the zygote and early embryo is exclusively maternally inherited and originates from the material that is present in the oocyte germinal vesicle. Moreover, using nuclear transfer experiments, we demonstrate that nucleoli originating from a somatic cell or even from an embryonic stem (ES) cell cannot substitute for the original oocyte nucleolar material.

The scheme for our analysis is shown in fig. S1. Nucleoli were microsurgically removed from fully grown oocytes before gonadotropic stimulations in pigs and mice (Fig. 1, A and B, fig. S2, and movies S1 and S2) (8). The oocytes from which we aspirated a small amount of nucleoplasm served as controls [fig. S1, sham-operated (Sham)]. The success of enucleolation was checked immunocytochemically (Fig. 1C and fig.