I find this highly unlikely. Biological compensation mechanisms (*35*, *36*) are likely to limit the maximal effect of cocaine on neural systems, including the value representation. This can be modeled in a number of ways, one of which is to include a global effectiveness-of-dopamine factor, which multiplies all $R(s)$ and $D(s)$ terms. If this factor decreased with each drug receipt, the values of all states would remain finite. Simulations based on an effectiveness-of-dopamine factor that decreases exponentially with each drug receipt (factor = $0.99^n$, where $n$ is the number of drug receipts) showed similar properties to those reported here, but the values of all states remained finite.

Another important issue in reinforcement learning is what happens when the reward or drug is removed. In normal TDRL, the value of states leading to reward decay back to zero when that reward is not delivered (*6*). This follows from the existence of a strongly negative δ signal in the absence of expected reward. Although firing of dopamine neurons is inhibited in the absence of expected reward (*16*), the inhibition is dramatically less than the corresponding excitation (*7*). In general, the simple decay of value seen in TDRL (*6*, *39*) does not model extinction very well, particularly in terms of reinstantiation after extinction (*40*). Modeling extinction (even for natural rewards) is likely to require additional components not included in current TDRL models, such as state-space expansion.

A theory of addiction that is compatible with a large literature of extant data and that makes explicitly testable predictions has been deduced from two simple hypotheses: (i) dopamine serves as a reward-error learning signal to produce temporal-difference learning in the normal brain and (ii) cocaine produces a phasic increase in dopamine directly (i.e., neuropharmacologically). A computational model was derived by adding a noncompensable δ signal to a TDRL model. The theory makes predictions about human behavior (developing inelasticity), animal behavior (resistance to blocking), and neurophysiology (dual dopamine signals in experienced users). Addiction is likely to be a complex process arising from transitions between learning algorithms (*3*, *20*, *22*). Bringing addiction theory into a computational realm will allow us to make these theories explicit and to directly explore these complex transitions.

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41. This work was supported by an Alfred P. Sloan Fellowship. I thank M. Thomas, E. Larson, M. Carroll, D. Hatsukami, J. Jackson, A. Johnson, N. Schmitzer-Torbert, and Z. Kurth-Nelson for comments on the manuscript and helpful discussions.


**Supporting Online Material**

www.sciencemag.org/cgi/content/full/306/5703/1944/DC1
Materials and Methods
Figs. S1 to S7


6 July 2004; accepted 19 October 2004
10.1126/science.1102384


# The $G_s$-Linked Receptor GPR3 Maintains Meiotic Arrest in Mammalian Oocytes


**Lisa M. Mehlmann,[1]* Yoshinaga Saeki,[2]† Shigeru Tanaka,[2]† Thomas J. Brennan,[3]‡ Alexei V. Evsikov,[4] Frank L. Pendola,[4] Barbara B. Knowles,[4] John J. Eppig,[4] Laurinda A. Jaffe[1]***



**Mammalian oocytes are held in prophase arrest by an unknown signal from the surrounding somatic cells. Here we show that the orphan $G_s$-linked receptor GPR3, which is localized in the oocyte, maintains this arrest. Oocytes from *Gpr3* knockout mice resume meiosis within antral follicles, independently of an increase in luteinizing hormone, and this phenotype can be reversed by injection of *Gpr3* RNA into the oocytes. Thus, the GPR3 receptor is a link in communication between the somatic cells and oocyte of the ovarian follicle and is crucial for the regulation of meiosis.**


Meiosis, which reduces the oocyte's chromosome number in preparation for fertilization, begins long before fertilization occurs. In most species, including mammals, DNA replication, entry into meiosis, and chromosomal recombination occur early in oogenesis, but then at late prophase, meiosis arrests. Much later, shortly before ovulation, meiosis resumes: the nuclear envelope breaks down, the chromosomes condense, and a metaphase spindle is formed. In vertebrates, this occurs in response to luteinizing hormone (LH) from the pituitary, which acts on the somatic (granulosa) cells that surround the oocyte in the ovarian follicle (*1*, *2*).

Throughout much of mammalian oogenesis, prophase arrest is maintained by inherent factors in the oocyte and correlates with low levels of activity by cell cycle regulatory proteins, including cyclin B and CDK1 (*1*). However, once the oocyte reaches its full size and an antral space begins to form between the granulosa cells, prophase arrest in the oocyte becomes dependent on unidentified signals from the granulosa cells. Oocytes that are removed from antral follicles resume meiosis spontaneously (*3*, *4*).

The maintenance of prophase arrest in oocytes within antral follicles requires the activity of signaling molecules within the

oocyte. In particular, the heterotrimeric G protein $G_s$ is required; injection of oocytes with an inhibitory $G_s$ antibody, or a dominant-negative form of $G_s$, causes meiosis to resume within the follicle (*5*, *6*). $G_s$ stimulates adenylyl cyclase in the oocyte to keep cyclic adenosine monophosphate (cAMP) elevated (*7*), and this results, through activation of protein kinase A and subsequent incompletely understood steps, in the inhibition of the cyclin B–CDK1 complex that drives the prophase-to-metaphase transition (*8*–*11*). Because $G_s$ by itself has no detectable constitutive activity (*12*), it is likely that a receptor in the oocyte membrane is required to keep $G_s$ active.

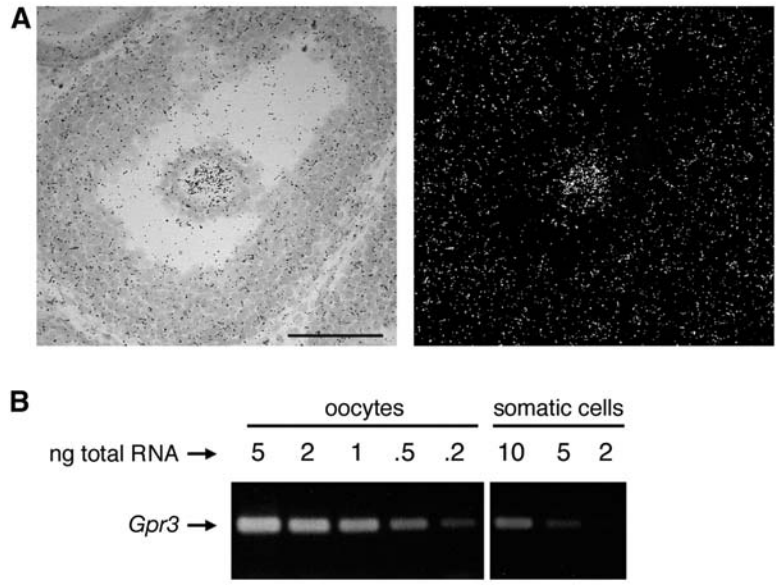


**Fig. 1.** *Gpr3* RNA is localized in the oocyte versus the somatic cells of antral follicles. (**A**) In situ hybridization of an ovarian section from a 22-day-old eCG-primed mouse, using a $^{33}$P-labeled *Gpr3* antisense riboprobe. Left: A transmitted light image of a follicle. Right: A reflected light image of the silver grains in the overlying autoradiographic emulsion. Scale bar, 100 μm. Eight of eight follicles showed a similar distribution. (**B**) RT-PCR comparing the relative expression of *Gpr3* RNA in oocytes and somatic cells dissected from antral follicles. RT-PCR for *Gpr3* was performed with cDNA derived from the indicated amounts of total RNA from each tissue. Normalized to total RNA, oocytes contained 14 ± 7 times as much *Gpr3* RNA as somatic cells (mean ± SD for six RT-PCR experiments with cDNA from two separately collected sets of oocytes and somatic cells) (*13*).

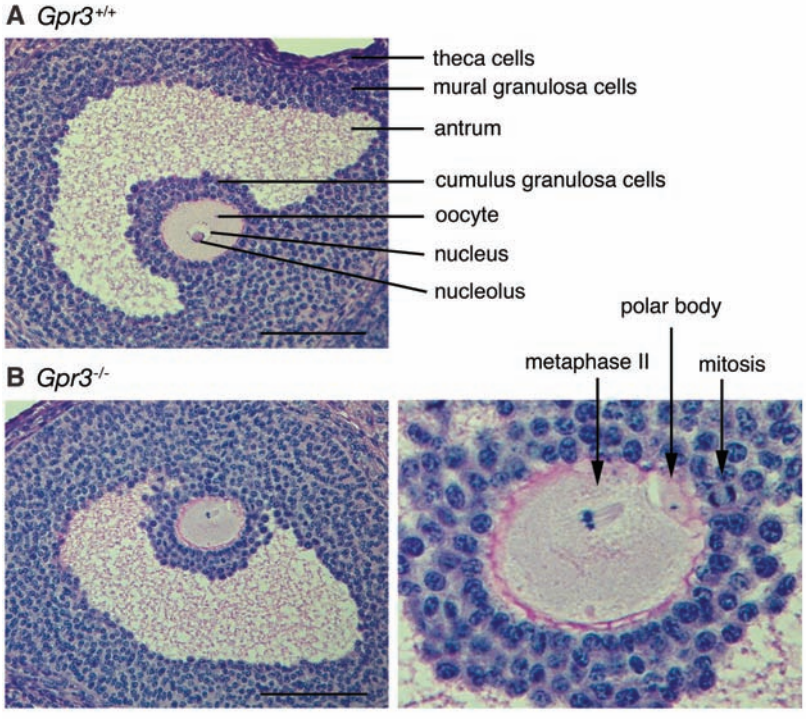


**Fig. 2.** Spontaneous resumption of meiosis in an oocyte within a $Gpr3^{-/-}$ antral follicle (adult mouse). (**A**) An antral follicle from a control $Gpr3^{+/+}$ ovary. The oocyte is arrested in prophase. (**B**) An antral follicle from a $Gpr3^{-/-}$ ovary. The oocyte has metaphase II chromosomes and a polar body. The right panel is an enlarged view of the left. Mitosis is ongoing in the somatic cells. Scale bars, 100 μm.

To identify such a receptor, we searched an expressed sequence tag (EST) database derived from a cDNA library of fully grown prophase-arrested mouse oocytes (*13*), looking for proteins predicted to be G protein–coupled receptors (GPCRs) because of their 7-transmembrane structure. Of the ~1000 mammalian genes that encode 7-transmembrane proteins, 15 were found in the oocyte EST database (table S1). One of these proteins, the orphan receptor GPR3, was of particular interest because it elevates cAMP when expressed in a variety of cultured cells (*14*–*16*), implying that GPR3 activates $G_s$.

*Gpr3* RNA is found predominantly in the ovary, testis, and brain; it has not been found elsewhere, except at very low levels in kidney and lung (*14*, *17*–*19*). In situ hybridization of mouse ovarian sections showed that *Gpr3* RNA is localized in the oocyte (Fig. 1A), and reverse transcription polymerase chain reaction (RT-PCR) measurements indicated that *Gpr3* RNA expression is ~14 times higher in the oocyte than in the surrounding somatic cells (Fig. 1B).

To investigate whether the GPR3 receptor is required to maintain meiotic prophase arrest in mouse oocytes, we examined histological sections of ovaries from *Gpr3* knockout mice (*13*). The $Gpr3^{-/-}$ animals were indistinguishable from $Gpr3^{+/+}$ animals in external morphology, growth, and activity. Their ovaries were of normal size and had a distribution of preantral, early antral, and antral follicles similar to that seen in $Gpr3^{+/+}$ mice (fig. S1). The appearance and organization of the somatic cells in the $Gpr3^{-/-}$ follicles was similar to that in the $Gpr3^{+/+}$ follicles (Figs. 2, A and B; 3, A and B; and fig. S1).

However, although all of the oocytes within antral follicles of $Gpr3^{+/+}$ ovaries were in prophase, with an intact nuclear envelope and nucleolus (Fig. 2A; Fig. 3, A and D; and fig. S2), most of the oocytes within antral follicles

[1]Department of Cell Biology, University of Connecticut Health Center (UCHC), Farmington, CT 06032, USA. [2]Massachusetts General Hospital, Charleston, MA 02129, USA. [3]Deltagen Inc., San Carlos, CA 94070, USA. [4]The Jackson Laboratory, Bar Harbor, ME 04609, USA.

*To whom correspondence should be addressed. E-mail: lmehlmann@neuron.uchc.edu (L.M.M.); ljaffe@neuron.uchc.edu (L.A.J.)

†Present address: Ohio State University, Columbus, OH 43210, USA.

‡Present address: Toccata Therapeutics, Menlo Park, CA, 94025, USA.

of *Gpr3*$^{-/-}$ ovaries contained metaphase chromosomes, indicating that meiosis had resumed (Fig. 2B; Fig. 3, B and D; and fig. S2). For these studies, we primarily used ovaries from prepubertal (22- to 24-day-old) mice to avoid the complexity of the cycling adult ovary (*13*). In the prepubertal ovaries, 82% of the antral follicles contained oocytes that had resumed meiosis, compared with 0% in *Gpr3*$^{+/+}$ controls (Fig. 3D). The one adult *Gpr3*$^{-/-}$ ovary that we analyzed also showed that the majority of oocytes in antral follicles had resumed meiosis (Fig. 2B and fig. S2).

Among the *Gpr3*$^{-/-}$ antral follicles containing oocytes that had resumed meiosis, 42% were clearly at metaphase II, as indicated by the presence of a polar body (Figs. 2B and 3B). Oocytes that contained a metaphase spindle but lacked a visible polar body could have been at metaphase I or at metaphase II with a degenerated polar body. All oocytes within preantral follicles of *Gpr3*$^{+/+}$ and *Gpr3*$^{-/-}$ mice were arrested in prophase (Fig. 3D and fig. S2).

During the transition from preantral to antral, follicles go through an "early antral" stage, in which pockets of fluid appear between the granulosa cells; these pockets eventually coalesce to form the antrum. Near the early antral stage, oocytes become dependent on the somatic cells (*4*) and on cAMP (*7*, *20*), for the maintenance of prophase arrest. In the early antral follicles of prepubertal *Gpr3*$^{-/-}$ mice, 37% of the oocytes had resumed meiosis, as indicated by the presence of condensed chromosomes (Fig. 3, C and D); within the early antral category, oocytes in larger follicles showed a larger fraction of meiotic resumption (table S3). Most of these oocytes showed a metaphase spindle, although a few appeared to be at prometaphase; polar bodies were not observed (fig. S2 gives results from early antral follicles in adult mice).

To determine whether the oocytes in *Gpr3*$^{-/-}$ early antral and antral follicles that did not resume meiosis were competent to do so if removed from the follicle, we dissected follicles of comparable size ranges from *Gpr3*$^{-/-}$ ovaries (*13*). Thirty-six percent of the oocytes from *Gpr3*$^{-/-}$ early antral follicles had resumed meiosis at the time of isolation, and only a few additional oocytes had resumed meiosis by 5 hours after isolation (Fig. 3E). Thus, during follicle development, the maintenance of meiotic arrest becomes dependent on the GPR3 receptor at close to the same time that arrest becomes dependent on the somatic cells. Of the oocytes from *Gpr3*$^{-/-}$ antral follicles, 89% had resumed meiosis at the time of isolation, and this percentage increased to 99% by 5 hours after isolation (Fig. 3E). Possibly another $G_s$-linked receptor functions to maintain arrest in the small subgroup of *Gpr3*$^{-/-}$ oocytes that do not resume meiosis in their antral follicles.

The resumption of meiosis in *Gpr3*$^{-/-}$ follicles did not appear to result from an increase in LH, as could occur in a cycling adult mouse or potentially even in a prepubertal mouse if pituitary function was affected by the absence of GPR3. LH action on the mural granulosa cells causes extracellular matrix material to be deposited around the cumulus granulosa cells, resulting in an expansion of the cumulus mass in preparation for ovulation; cumulus expansion is evident by the time the oocyte reaches metaphase I (*21*, *22*) (Fig. 4A). However, no spontaneous cumulus expansion was seen in the *Gpr3*$^{-/-}$ follicles (Figs. 2B and 3B). Likewise, there was no spontaneous expansion of the follicle diameter (table S4), as is seen in response to LH (*23*). Thus, the presence of chromosomes aligned on metaphase I or II spindles in an oocyte within an unexpanded follicle with an unexpanded cumulus mass is not consistent with a response of the follicle to LH. Expression of an LH-dependent gene that encodes one of the proteins produced during cumulus expansion, chondroitin sulfate proteoglycan

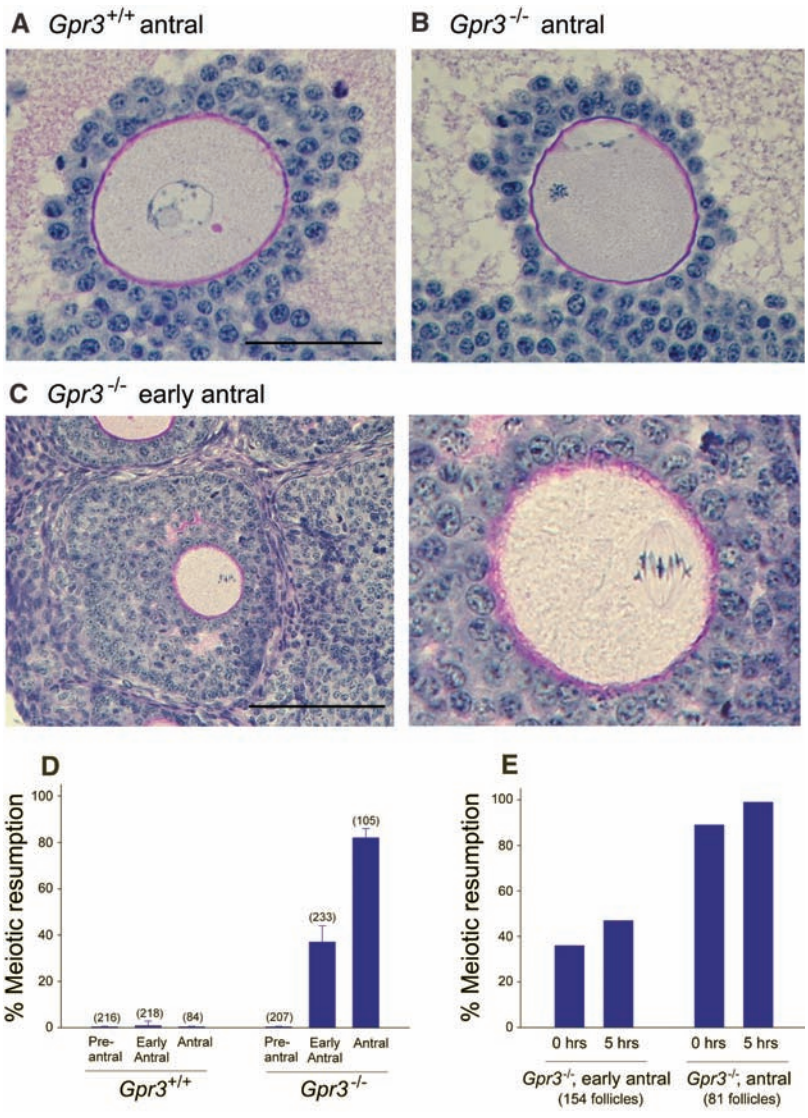


**Fig. 3.** Spontaneous resumption of meiosis in oocytes within *Gpr3*$^{-/-}$ antral and early antral follicles (of 22- to 24-day-old prepubertal mice). (**A**) A prophase-arrested oocyte within an antral follicle of a control *Gpr3*$^{+/+}$ ovary. Scale bar, 50 μm. (**B**) An oocyte with metaphase II chromosomes and a polar body, within an antral follicle of a *Gpr3*$^{-/-}$ ovary. Scale bar, 50 μm. (**C**) An early antral follicle from a *Gpr3*$^{-/-}$ ovary, showing metaphase I chromosomes. An enlarged view is on the right. Scale bar, 100 μm. (**D**) Percentages of oocytes that had resumed meiosis, counted in sections of ovaries from 22- to 24-day-old *Gpr3*$^{+/+}$ and *Gpr3*$^{-/-}$ mice. The graph shows the mean ± SD from analysis of ovaries from four *Gpr3*$^{+/+}$ and four *Gpr3*$^{-/-}$ mice; numbers above the bars indicate how many follicles were examined (*13*) (tables S3 to S5). (**E**) Percentages of *Gpr3*$^{-/-}$ oocytes that had resumed meiosis, at the time of removal from their follicles or 5 hours later (data obtained from five 22- to 24-day-old mice). For (E), "antral" includes follicles >250 μm in diameter; "early antral" includes follicles 140 to 250 μm in diameter.

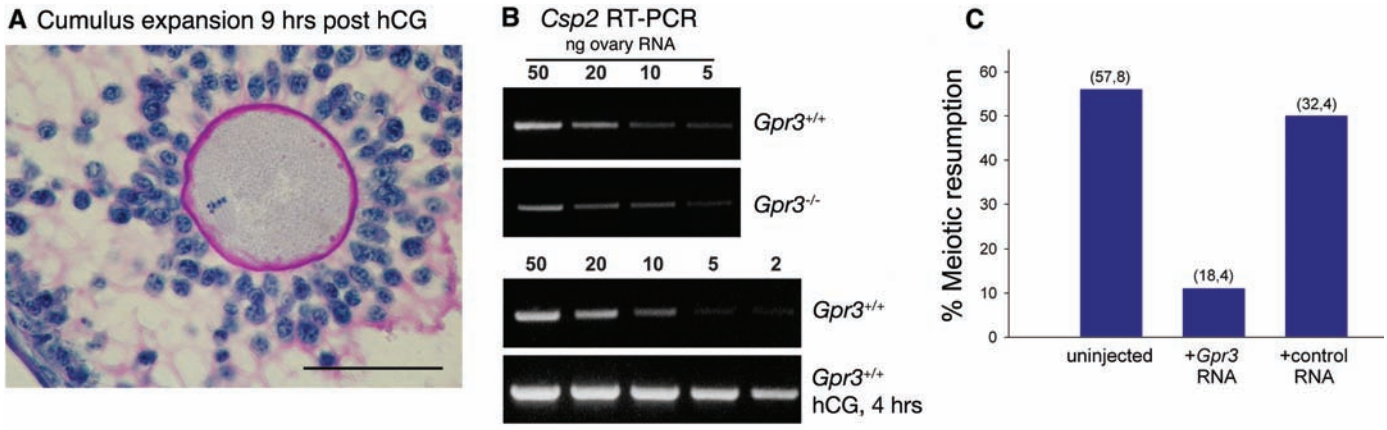


**Fig. 4.** Resumption of meiosis in *Gpr3*$^{-/-}$ ovaries is not due to elevation of LH and is prevented by injection of *Gpr3* RNA into the oocyte. (**A**) For comparison with the *Gpr3*$^{-/-}$ follicle shown in Fig. 3B, cumulus expansion in an antral follicle from a 22-day-old wild-type mouse injected 9 hours previously with the LH receptor agonist, hCG. Metaphase I chromosomes are present. Scale bar, 50 μm. In the *Gpr3*$^{-/-}$ follicles, oocytes reached metaphase II without cumulus expansion. (**B**) There was no increase in expression of an LH-dependent gene, *Csp2*, when *Gpr3*$^{-/-}$ and *Gpr3*$^{+/+}$ ovaries were compared. RT-PCR for *Csp2* was performed using cDNA derived from the indicated amounts of ovary RNA. Similar results were obtained in two experiments. For comparison, the lower panels show the increased expression of *Csp2* RNA in a *Gpr3*$^{+/+}$ ovary collected 4 hours after injection of the mouse with hCG, versus an unstimulated *Gpr3*$^{+/+}$ ovary; LH receptor stimulation increased expression of *Csp2* RNA by a factor of ~25. Previous work has shown that *Csp2* RNA increases by 2 hours after LH receptor stimulation and remains elevated for 16 hours (*22*). (**C**) Rescue of the ability to maintain meiotic arrest by injection of *Gpr3* RNA into prophase-arrested *Gpr3*$^{-/-}$ oocytes within preantral or very early antral follicles. After 4 days in culture, when antrum formation was occurring in most follicles, oocytes were isolated to determine if meiosis had resumed. Numbers above the bars indicate how many follicles and mice were analyzed. The percent meiotic resumption in the *Gpr3* RNA-injected oocytes was significantly different from that in uninjected oocytes and in control oocytes injected with RNA encoding red fluorescent protein ($P$ = 0.0009 and 0.007, respectively, Fisher's exact test).

2 (CSP2 or versican V0) (*22*), was also not elevated in *Gpr3*$^{-/-}$ ovaries (Fig. 4B).

The resumption of meiosis in *Gpr3*$^{-/-}$ ovaries cannot be attributed to follicle atresia, because all of the *Gpr3*$^{-/-}$ follicles included in Fig. 3D appeared to be morphologically healthy, and mitosis was ongoing in the granulosa cells (Figs. 2B and 3B). A normal number of ovulated eggs was found in the oviducts of a *Gpr3*$^{-/-}$ mouse that had been injected 13 hours previously with the LH receptor agonist, human chorionic gonadotropin (hCG) (*24*), and sections of an ovary from an adult *Gpr3*$^{-/-}$ mouse showed corpora lutea (fig. S1). Thus, the pathways leading to ovulation and luteinization appear to be unimpaired in *Gpr3*$^{-/-}$ mice. The only detected defect in the *Gpr3*$^{-/-}$ ovary was the absence of the ability to maintain meiotic prophase arrest.

The predominant expression of *Gpr3* RNA in the oocyte versus the somatic cells of the follicle, and the dependence of meiotic arrest on oocyte $G_s$ (*5*, *6*), support the conclusion that it is the GPR3 receptor in the oocyte, rather than in the somatic cells, that is primarily required to maintain meiotic arrest. To examine whether the spontaneous resumption of meiosis could be prevented by introducing GPR3 protein into *Gpr3*$^{-/-}$ oocytes, we injected prophase-arrested *Gpr3*$^{-/-}$ oocytes within preantral or very early antral follicles with *Gpr3* RNA and then cultured them under conditions that allowed antral formation (*25*) (Fig. 4C and fig. S3). After the culture period, about half of control *Gpr3*$^{-/-}$ oocytes had resumed meiosis, but only 11% of the *Gpr3*$^{-/-}$ oocytes that had been injected with *Gpr3* RNA had resumed meiosis (Fig. 4C). The reversal of the knockout phenotype by *Gpr3* RNA injection into the oocyte further indicates that the GPR3 receptor that maintains meiotic arrest is located primarily in the oocyte itself.

Although GPR3 has some ligand-independent activity (*14*–*16*), additional activation of GPR3 in the oocyte by an agonist from or on the granulosa cells could explain why meiosis remains arrested only if the oocyte is surrounded by granulosa cells. A GPR3 ligand that increases $G_s$ activation remains to be discovered (table S1). Nevertheless, identification of GPR3 as a negative regulator of meiotic progression, through its activation of $G_s$ and thus adenylyl cyclase, provides a key for identifying how the follicle acts to maintain meiotic arrest. It also provides clues about how LH might relieve the arrest and reinitiate meiosis.

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26. We thank N. Ryan, S. J. Borch, L. Freudzon, L. Ross, and K. Kasai for technical assistance; A. F. Parlow and the National Hormone and Peptide Pituitary Program for providing equine chorionic gonadotropin (eCG) and follicle-stimulating hormone; R. Tsien for providing the mRFP1 red fluorescent protein cDNA and B. Kalinowski, G. Mandel, T. Jones, B. White, R. Berlin, and M. Terasaki for their continuing interest and advice. Supported by grants from the UCHC Center for Women's Health and the Patterson Trust (L.M.M.), from the Human Frontier Science Program (L.A.J.), and from NIH (L.M.M., Y.S., A.V.E., F.L.P., B.B.K., J.J.E., and L.A.J.).


**Supporting Online Material**

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12 August 2004; accepted 25 October 2004
10.1126/science.1103974