evidence of either feature, and this argues against the possibility that either an ion or dust arc was present.

32. Dust and small fragments are subject to gravity, radiation pressure, and outgassing. The gravitational force $F_G = GM_\odot[4\pi a^3\rho/3]r_h^{-2}$ is counterbalanced by radiation pressure $F_R = L_\odot Q_{pr} a^2/[4r_h^2 c]$ and a less defined outgassing force $F_J = mv_g Q_{H_2O}$, where $L_\odot$ and $M_\odot$ are the solar mass and luminosity, a is the radius of the particle, r_h is the heliocentric distance, Q_{pr} is the radiation interaction efficiency, Q_{H_2O} is the water production rate, m is the mass per outgassed molecule, c is the speed of light, and v_g is the vectored average velocity of outgassed volatiles (0 m/s for spherical symmetry). The motion of particles larger than several millimeters is dominated by gravity, and they tend to stay co-orbital with the nucleus until they get close to the Sun, where Q_{H_2O} increases to the point that outgassing becomes a significant force.
33. Successive runs of the DSMC model indicated that the contrast of these interaction regions is a maximum for a sun-comet-Earth (SCE) angle near 90°, and that visibility drops rapidly away from this geometry. The arc seen on 26 March would have been undetectable for viewing geometries of 45° > SCE > 135°.
34. As discussed (*26*), the dimensions of the contact discontinuity between the two flows is dependent on the local collisional mean free path, which increases as r^2 away from the nucleus. As a result, volatile sources close to the nucleus will generate interaction regions with more compact dimensions than more distant sources.
35. An arc associated with the secondary condensation at 3500 km would have a width at its apex of more than 3000 km, with its surface brightness reduced by a factor of 12 relative to what it would have been at the location of the bright arc. Assuming that the brightness of the visible dust condensation accurately mirrors gas production (15%), then this source would produce an arc with 1% of the surface brightness of the brighter source. This would not have been detectable in our images.
36. We acknowledge the generous contribution of time and effort by the staff at the WIYN observatory and, in particular, D. Sawyer for supporting such a large unexpected observing program. We also thank J. Percival for work in modifying the WIYN control system to permit tracking of a moving source, F. Roesler for donation of an OH filter, D. J. Lien for many useful conversations on the topic of halo debris, and J. Wisniewski for assisting in the reduction of the images. Supported by the WIYN consortium universities and NOAO, by NASA grants NAGW-3319, W18963, and NAG5-647 to the University of Wisconsin, and by NASA grant NASW-1907 to the University of Michigan.

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Failure of Parturition in Mice Lacking the Prostaglandin F Receptor

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Mice lacking the gene encoding the receptor for prostaglandin $F_{2\alpha}$ (FP) developed normally but were unable to deliver normal fetuses at term. Although these FP-deficient mice showed no abnormality in the estrous cycle, ovulation, fertilization, or implantation, they did not respond to exogenous oxytocin because of the lack of induction of oxytocin receptor (a proposed triggering event in parturition), and they did not show the normal decline of serum progesterone concentrations that precedes parturition. Ovariectomy at day 19 of pregnancy restored induction of the oxytocin receptor and permitted successful delivery in the FP-deficient mice. These results indicate that parturition is initiated when prostaglandin $F_{2\alpha}$ interacts with FP in ovarian luteal cells of the pregnant mice to induce luteolysis.

Prostaglandins (PGs) mediate various physiological processes such as fever generation and inflammation (*1*). Aspirin and related drugs act through inhibition of PG biosynthesis. The prostaglandin $PGF_{2\alpha}$ is implicated in reproductive functions such as ovulation, luteolysis, and parturition. Actions of $PGF_{2\alpha}$ are mediated by the PGF receptor (FP), which is a heterotrimeric guanosine triphosphate–binding protein (G protein)–coupled rhodopsin-type receptor specific to this PG (*2*). To examine the physiological function of $PGF_{2\alpha}$, we disrupted the gene encoding FP in mice by homologous recombination.

A targeting vector was constructed in which the coding region of the second exon of the FP gene could be replaced with the β-galactosidase and neomycin resistance genes (Fig. 1A) (*3*). Heterozygous mice (−/+), when crossed, yielded homozygotes (−/−) (Fig. 1B) with a frequency of 25.6% (n = 262), indicating that mice lacking FP develop normally. X-Gal staining of β-galactosidase in homozygous mice showed that the targeted allele was expressed in the corpora lutea in ovaries (Fig. 1C) (*4*), as is FP (*2*). Disruption of the FP gene was verified in the ovary by both Northern (RNA) blot analysis and radioligand binding assay (*5*). FP mRNA expression was reduced in heterozygous mice and absent in homozygous mice (Fig. 1D). Specific $[^3H]PGF_{2\alpha}$ binding in a crude membrane preparation of the ovary (mean ± SEM, n = 8) was 121.3 ± 4.8 fmol per milligram of protein, 38.7 ± 1.6 fmol/mg, and below the limit for detection for wild-type, heterozygous, and homozygous mice, respectively. Homozygous FP mice also lacked the normal ileac contractile response to $PGF_{2\alpha}$ (Fig. 1E).

Homozygous mutant mice were healthy. No gross abnormalities were found in mutant animals in their general behavior, general appearance, or tissue histology, or from biochemical and hematological examination. Homozygous males were normal in their reproductive ability. Although $PGF_{2\alpha}$ is critical to luteal regression in ovine and sow (*6*) and FP is expressed in the corpora lutea of mice during the normal estrous cycle (*2*), no change was found in the estrous cycle in homozygous or heterozygous FP females compared with wild-type mice (Table 1). The homozygous females were also able to become pregnant. The number of corpora lutea and implants in the uterus

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Table 1. Estrous cycle, ovulation, and fertilization in wild-type and mutant mice. Female mice of either genotype were mated with wild-type males and examined at day 19 of pregnancy. Data are mean ± SEM.

Variable	Genotype		
	+/+	+/−	−/−
Estrous cycle, days	5.1 ± 0.35 (n = 19)	5.4 ± 0.37 (n = 14)	5.0 ± 0.32 (n = 17)
Number of corpora lutea	9.0 ± 0.62 (n = 6)	9.2 ± 0.72 (n = 5)	9.4 ± 0.45 (n = 7)
Number of implants	7.9 ± 0.96 (n = 6)	8.4 ± 1.10 (n = 5)	8.8 ± 0.51 (n = 7)

of these pregnant mice (Table 1) were normal, indicating that ovulation, fertilization, and implantation occur normally in FP-deficient mice. Although studies of aspirin-like drugs and cyclooxygenase-2–deficient mice suggest that ovulation requires PGs (*7*), our results suggest that, if a PG is involved in ovulation, it must be one other than $PGF_{2\alpha}$.

Through pregnancy, heterozygous mice like the wild type increased in body weight to day 20 or 21, when they delivered pups and lost weight (Fig. 2A) (*8*). FP-deficient homozygous mice also increased in body weight through pregnancy; however, their body weight increased until day 22 or 23 without parturition and then decreased slowly for about 1 week. No parturition was found in homozygous mice regardless of the paternal genotypes (Fig. 2B). Inspection of their uteri revealed that prolonged pregnancy caused degenerative changes in the placenta and that the fetuses died in utero and were reabsorbed. Fetuses could be rescued from homozygous mice by cesarean operation before or at the expected term (Fig. 2C) and appeared to develop normally. No abnormalities were observed in the weight or histology of placentas at this stage in FP-deficient mice. The homozygous females could become pregnant again several weeks after the expected term, but again failed to induce labor. Thus, induction of labor is lacking in the homozygous female mice.

Several hormones are known to be involved in parturition. Oxytocin (OT) has been proposed as a key regulator of parturition (*9*). Uterine sensitivity to OT increases markedly at term, although OT concentrations, both in maternal plasma and in intrauterine tissues, do not change significantly before or during parturition. The increase in OT sensitivity is associated with increased uterine expression of OT receptors (*10*), which has led to a proposal that

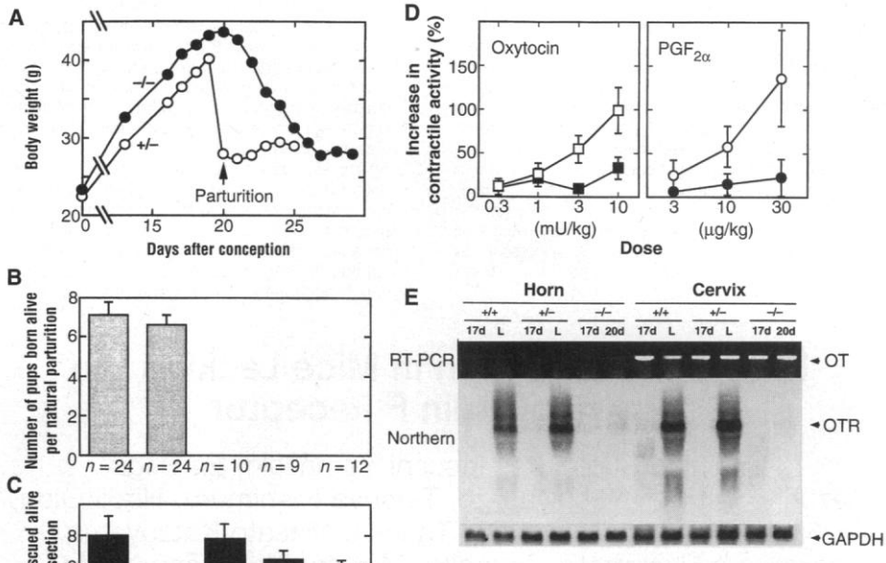

Fig. 2. Failure of parturition in mice lacking FP. (**A**) Change of maternal body weight during pregnancy in mutant mice. Typical patterns of body weight change in heterozygous (open symbols) and homozygous (closed symbols) mice are shown. Parturition observed in a heterozygote (usually on day 20) is indicated by an arrow. (**B**) Average number of pups born alive at days 20 and 21 per natural parturition and (**C**) rescued alive at day 19 or 20 by cesarean section. Number of crosses (*n*) and parental genotypes are indicated. Data are shown as mean ± SEM; n.d., not done. (**D**) Contractile responses of the pregnant uterus to $PGF_{2\alpha}$ or oxytocin (OT) in wild-type and FP-deficient mice. Various doses (per kilogram of body weight) of $PGF_{2\alpha}$ or OT were administered intravenously to day 20 pregnant wild-type (open symbols) and FP-deficient mice (closed symbols). Values show percentage increase of contraction induced by each drug compared with the pre-administration level and represent the mean ± SEM (n = 5). (**E**) Expression of OT and OT receptor (OTR) mRNA. Total RNA was isolated from uterine horn and cervix on day 17 (17d) or 20 (20d) of pregnancy or during labor (L) and subjected to RT-PCR and Northern analyses for OT and OTR mRNA, respectively.

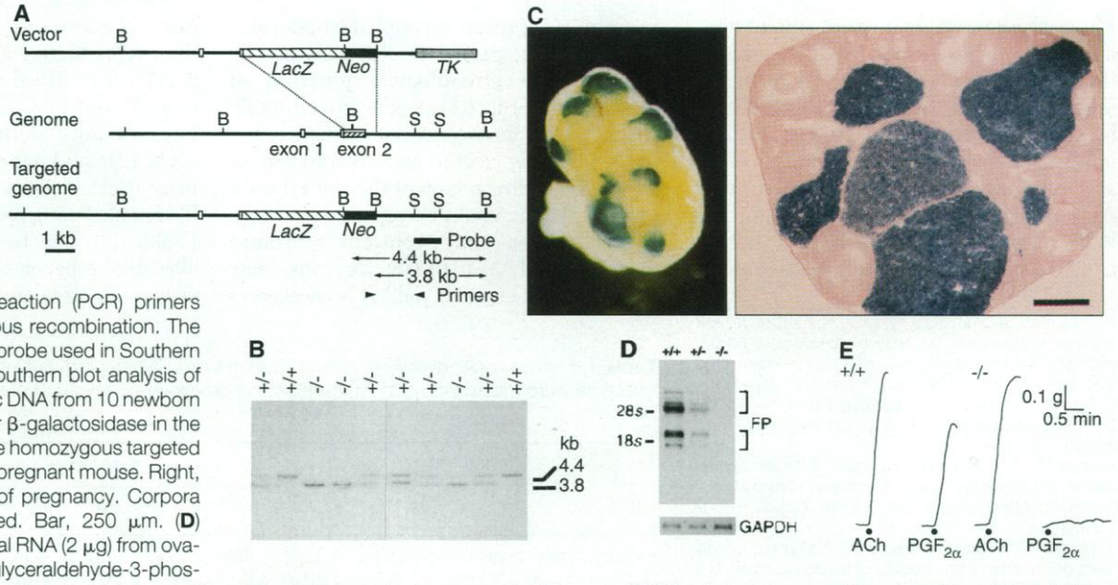

Fig. 1. Disruption of the gene encoding FP. (**A**) Strategy used for FP gene targeting (*3*). Construct of the targeting vector, organization of the FP gene, and structure of the targeted genome are shown. Restriction sites are indicated: B, Bam HI; and S, Spe I. Arrowheads indicate polymerase chain reaction (PCR) primers used to identify homologous recombination. The thick line indicates a DNA probe used in Southern (DNA) hybridization. (**B**) Southern blot analysis of Bam HI–digested genomic DNA from 10 newborn littermates. (**C**) Staining for β-galactosidase in the ovaries of mice carrying the homozygous targeted allele. Left, ovary from nonpregnant mouse. Right, ovary section on day 17 of pregnancy. Corpora lutea are positively stained. Bar, 250 μm. (**D**) Northern blot analysis. Total RNA (2 μg) from ovaries was used. GAPDH, glyceraldehyde-3-phosphate dehydrogenase mRNA; +/+, wild type; +/−, heterozygote; −/−, homozygote. (**E**) Loss of $PGF_{2\alpha}$-induced contraction of the ileum in FP-deficient mice. Acetylcholine (ACh), 10 μM, and $PGF_{2\alpha}$, 1 μM, were used to induce contraction.

the induction of OT receptors is a trigger for parturition (*11*). In FP-deficient mice, administration of OT at term did not produce uterine contraction as was seen in wild-type mice (Fig. 2D) (*12*). The expression of mRNA for OT and OT receptor was examined in the uterus at days 17 and 20 of pregnancy (Fig. 2E) (*13*). Consistent with previous reports (*14*), OT mRNA expression did not change during this period, and no significant difference was found among wild-type, heterozygous, and homozygous mice. On the other hand, expression of OT receptor mRNA increased at term in both wild-type and heterozygous mice. In contrast, no increase was observed in the homozygous mice. These results indicate that expression of the uterine OT receptor is not induced in the FP-deficient mice.

Parturition in mammals is preceded by a decline in progesterone concentrations in the maternal plasma (*15*), which leads to an increase in myometrial contractility (*16*). The plasma concentration of progesterone decreased progressively from day 19 to day 21 in wild-type mice (Fig. 3A) (*17*). In homozygous mutant mice, progesterone concentrations did not decline during this period. Plasma concentrations of estradiol were not significantly different between the two types of mice. These results suggest that failure of parturition in the FP-deficient mice may be due to the persistent production of progesterone. To induce a decline in progesterone concentration, we removed the ovaries from the mutant mice at day 19 of pregnancy because the corpora lutea in the ovary is the principal source of progesterone in mice. The ovariectomized mice delivered their pups alive after 24 hours. No delivery occurred in sham-operated animals (Fig. 3B). Uterine expression of OT receptor mRNA was also induced within 12 hours of ovariectomy; no OT receptor mRNA was detected in sham-operated mice (Fig. 3C). These results suggest that the failure of parturition in the FP-deficient mice is due to a failure of the corpora lutea to cease progesterone production.

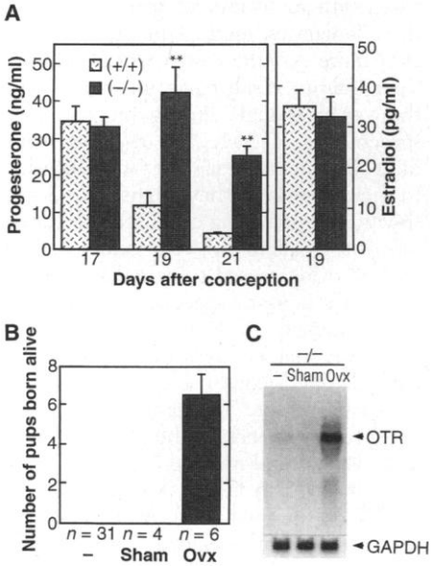

Fig. 3. Loss of luteolysis and restoration of parturition by ovariectomy in mice lacking FP. (**A**) Plasma concentrations of progesterone and estradiol in FP-deficient mice. Blood was collected from wild-type and mutant mice (n = 4 to 5) at the indicated days of gestation, and plasma concentrations of progesterone (left) and estradiol (right) were measured. Values are expressed as the mean ± SEM. The double asterisks indicate $P < 0.01$. (**B**) Restoration of parturition. FP-deficient mice at day 19 of pregnancy were sham-operated (sham) or ovariectomized (ovx) or left without treatment (−). The number of pups born alive at day 20 were counted. (**C**) Induction of OT receptor mRNA by ovariectomy in mutant mice. FP-deficient mice were treated as in (B). Uterine horn was obtained 12 hours after treatment and RNA was extracted.

Ever since the observation that aspirin-like drugs delay parturition (*18*), the role of PGs in this process has remained a mystery. A decrease in plasma progesterone concentrations occurs in many species before parturition begins. Although exogenous $PGF_{2\alpha}$ induces a decline in the progesterone concentration in some species and mimics the parturition process (*19*), it has long been suggested that the function of $PGF_{2\alpha}$ in parturition is downstream of OT (*11*). Indeed, OT added exogenously will induce PG production in uterine tissues both in vivo and in vitro (*11*). In this study, we have shown that $PGF_{2\alpha}$ works upstream of OT to induce luteolysis and that its uterotonic action in myometrium is not essential for parturition. In addition, OT receptor induction was well correlated with the appearance of parturition, although it is not clear that the former is essential in the latter process. Two previous reports demonstrated that parturition remains unaffected in the OT-deficient mice (*20*). $PGF_{2\alpha}$ may induce luteolysis through apoptosis of luteal cells, as suggested by the correlation of FP mRNA expression with luteal cell apoptosis in pseudopregnancy (*21*). Whether the mechanism identified in this study works in species other than the mouse remains to be tested.

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3. A 9.8-kb genomic fragment for FP was subcloned into pBluescript (Stratagene). A 1.3-kb fragment containing the region encoding the NH_2-terminus to Leu^{266} in the sixth transmembrane domain (*2*) was replaced by *LacZ* and the neomycin resistance gene (*Neo*) so that the FP-derived Met-Ser residues could be fused to the NH_2-terminus of β-galactosidase. The MC1-herpes simplex virus thymidine kinase (*HSV-TK*) gene was inserted into the Spe I site at the 3′ end of the homologous region. Two lines of resultant embryonic stem (E14-1) cells injected into C57BL/6 blastocysts gave rise to chimeric offspring, which in turn were mated with C57BL/6 females.
4. Ovaries or their sections (10 μm thick) were fixed with glutaraldehyde for 1 hour and stained with X-Gal (5-bromo-4-chloro-3-indoxyl-β-D-galactopyranoside) at 30°C. Sections were then counterstained with eosin.
5. Gonadotropin-synchronized ovary was subjected to RNA isolation for Northern blot analysis or crude membrane preparation for binding assay. A Pst I–Bgl II fragment [690 base pairs (bp)] of FP cDNA (*2*) was used as an exon 2–specific probe.
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13. Northern blot analysis for OT receptor mRNA was performed with a PCR-amplified 700-bp fragment of mouse cDNA corresponding to the coding region from the second to the sixth putative transmembrane regions as a probe. Uterine expression of OT was analyzed by reverse transcriptase PCR (RT-PCR) with primers specific for mouse OT. Three independent experiments were performed for each analysis.
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