Progesterone receptor-dependent regulation of genes in the oviducts of female mice

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Akison LK, Boden MJ, Kennaway DJ, Russell DL, Robker RL. Progesterone receptor-dependent regulation of genes in the oviducts of female mice. *Physiol Genomics* 46: 583–592, 2014. First published June 10, 2014; doi:10.1152/physiolgenomics.00044.2014.—Oviducts play a critical role in gamete and embryo transport, as well as supporting early embryo development. Progesterone receptor (PGR) is a transcription factor highly expressed in oviductal cells, while its activating ligand, progesterone, surges to peak levels as ovulation approaches. Progesterone is known to regulate oviduct cilia beating and muscular contractions in vitro, but how PGR may mediate this in vivo is poorly understood. We used PGR null mice to identify genes potentially regulated by PGR in the oviducts during the periovulatory period. Histologically, oviducts from PGR null mice showed no gross structural or morphological defects compared with normal littersates. However, microarray analysis of oviducts at 8 h posthuman chorionic gonadotropin revealed >1,000 PGR-dependent genes. Using reverse-transcription polymerase chain reaction (RT-PCR) we selected 10 genes for validation based on their potential roles in oocyte/embryo transport and support. Eight genes were confirmed to be downregulated (Adamts1, Igf8, Edn3, Prlr, Pgfr, Des, Myocd, and Actg2) and one upregulated (Agtr2) in PGR null oviducts. Expression of these genes was also assessed in oviducts of naturally cycling mice during ovulation and day 1 and day 4 of pregnancy. Adamts1, Igf8, Edn3, Prlr, and Pgfr were significantly upregulated in oviducts at ovulation/mating. However, most genes showed basal levels of expression at other times. The exceptions were Prlr and Pgfr, which showed pulsatile increases on day 1 and/or day 4 of pregnancy. This is the first, comprehensive study to elucidate putative PGR-regulated genes in the oviduct and reveals key downstream targets potentially mediating oocyte and embryo transport.

ovulation; PRKO; PRlacZ; embryo transport; fallopian tube; P4

THE OVIDUCTS ARE ACTIVE AND highly specialized organs that are critical in bidirectional transport of gametes, as well as nourishment and precisely timed movement of the developing embryo to the uterus. They are the first point of contact for the newly ovulated cumulus oocyte complex (COC), with ciliated cells assisting in the pick-up and transport of the expanded COC to the site of fertilization (55). Secretory cells contribute a multitude of products to oviductal fluid, which facilitates transport, fertilization, and early embryo development. Contractions by smooth muscle cells in the oviductal wall also regulate transport of the embryo through the creation of peristaltic motion (11, 22). These processes are primarily regulated by ovarian steroid hormones, and thus the oviducts and ovaries are inextricably linked, both structurally and functionally. Progesterone (P4) production by the ovary increases dramatically in the hours leading up to ovulation in rodents, surpassing the levels of estradiol (54). In the oviducts, P4 has been shown to impact on oviduct ciliary, muscular, and nerve function and control the volume and composition of oviductal fluid, at least ex vivo (see Refs. 1, 22 for reviews).

Progesterone receptor (PGR) is a nuclear transcription factor involved in gene regulation, and Pgr mRNA and protein are highly expressed in the oviduct (23, 56). Constitutive expression of Pgr mRNA in the oviduct across the estrous cycle has been suggested by a lacZ reporter study in mice (23) and has also been reported for PGR protein in human fallopian tubes (2). However, other studies in the mouse (51, 56) and human (41) found variable expression of Pgr mRNA and/or protein regulated by hCG or stages of the cycle.

Two early studies demonstrated that PGR directly affects oviductal function in vivo (17, 58). In one study, rats were treated continuously with the PGR antagonist RU486 via osmotic pumps from day 1 of pregnancy, and oviducts and uteri were flushed at various times during early pregnancy. In the rats treated with RU486, embryos arrived prematurely in the uterus, ~11 h earlier than in control rats (17). In a later study, mice were injected subcutaneously with antagonists RU486, ZK98734, or vehicle daily for the first 3 days of pregnancy, and the uteri flushed on the afternoon of day 3. The PGR antagonist groups had large numbers of embryos in the uterus compared with very few in the vehicle-treated mice (58). This suggests that the nuclear PGR is involved in oviductal transport; however, the mechanism for this was not determined.

To date, the only putative PGR targets identified in the oviduct are prostaglandin receptors (specifically Ptger1, Ptger2, and Ptgfr) (59) and cytokines (specifically Tnfα and Il8) (27), which were identified as dysregulated in human oviduct in response to mifepristone (RU486) treatment. Thus, unlike the mammary gland, ovary, and uterus, very little is known about PGR-regulated genes in the oviduct. In addition, no studies have examined gene expression in the oviduct during the periovulatory period, a time when the oviduct is preparing to receive the newly ovulated COC.

Analysis of mice in which both isoforms of PGR are ablated affords the opportunity to identify gene targets specifically regulated by genomic PGR. Multiple reproductive defects have been reported for PGR null female mice, including uterine implantation failure, impaired mammary gland morphogenesis/differentiation, lack of sexual receptiveness, and anovulation (33), but this is the first examination of morphological and potential functional consequences of PGR ablation in the oviducts.

In this study, we examined whether Pgr mRNA expression is hormonally regulated in the mouse oviduct, for instance by
the luteinizing hormone (LH) surge that precedes ovulation or during preimplantation development. We next sought to identify putative PGR-regulated genes in the oviduct by microarray analysis and validated by RT-PCR the expression of a suite of genes with predicted roles in oviductal transport and embryo support. These genes were further characterized in oviducts from naturally cycling, mated wild-type (C57Bl/6J) mice over a detailed time course covering day 1 and day 4 of pregnancy. We show that genes important for regulating smooth muscle contraction, cilia beating, and cell adhesion were dysregulated in PR null mice, indicating a potentially important role for PGR in regulating oviductal function.

MATERIALS AND METHODS

Animals and tissue collection. All animal experiments were conducted in accordance with the National Health and Medical Research Council Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th edition, 2004) and were approved by the University of Adelaide Animal Ethics Committee (approval number M-2011-034). All mice were maintained at Laboratory Animal Services (University of Adelaide, SA, Australia) on a 12 h light-dark cycle, with room temperature between 18 and 24°C and humidity between 40 and 70%. Mice were housed in SealSafe Plus individually ventilated cages with EasyFlow air handling units (Tecniplast) and maintained under specific pathogen-free conditions. All cages contained corn cob bedding and Enrich-n' Nest for environmental enrichment and breeder cages also contained Crink-l’Nest nesting material (Tecniplast). Rodent chow (Meat-fre Rat and Mouse Diet, Specialty Feeds) and water were provided ad libitum.

Female PRlacz knock-in mice were generously provided by Assoc. Prof. John Lydon (Baylor College of Medicine, Houston, TX) and experimental mice generated via an in-house breeding colony. The lacZ insertion results in disruption of transcription of both isoforms of PGR (23), and therefore mice homozygous for the lacZ insertion are a phenocopy of the knockout strain (PRKO) described by Lydon et al. (33) and are hereafter designated as PRKO. Mice heterozygous for the lacZ insertion (PR/+ or PR+/−) exhibit normal fertility (23). At weaning, offspring were genotyped by a two-way PCR analysis of tail DNA using primers specific for the Pgr gene and the selectable neomycin gene: Pgr sense 5’ TAG ACA GTG TCT TAG ACT CTT GTG TG 3’; Pgr antisense 5’ GAT GGG CAC ATG GAT GAA ATC 3’; Neo 5’ CITT CCC CCA CGG GTA CCT TAC GTG GC 3’. The Neo primer was used with a primer from the Pgr gene to amplify a 110 bp product from the mutant allele. A 590 bp WT product was created using the two Pgr primers.

Oviducts were collected at carefully timed stages of the periovulatory period by using a standard hormonally controlled ovulation model; PRKO and PR+/− mice were injected via the intraperitoneal (ip) route at 21–23 days old with 5 IU of equine chorionic gonadotropin (eCG, National Hormone and Peptide Program) to stimulate follicle growth followed 44–47 h later by injection of 5 IU ip of human chorionic gonadotropin (hCG; Merck, Sharp & Dohme), which triggers ovulation at 11 h post-hCG in heterozygotes. Whole oviducts were collected from untreated mice and at eCG + 0 h, 4 h, 8 h, or 10 h post-hCG and snap-frozen in liquid nitrogen for RT-PCR analysis of gene expression (n = 5 samples per genotype per time point, total of 25 mice per genotype). A further 15 mice for each genotype were used for collection of oviducts at eCG + 8 h for microarray analysis. Oviducts from both genotypes were also collected at eCG + 8 h, 10 h, 12 h, or 14 h post-hCG for histology.

For assessments of gene expression changes over a time course in naturally cycling, mated mice, male and female C57Bl/6J mice at 8 wk of age were obtained from the Animal Resources Centre (Murdoch, WA, Australia) and housed at Laboratory Animal Services (University of Adelaide, Adelaide, SA, Australia) under the same conditions as described above. This strain was chosen as it most closely resembles the background strain for the PGR transgenic mice (129S7 × C57Bl/6J). Estrus cycle stage was assessed in females by vaginal smear. When in proestrus, as indicated by cytology containing primarily nucleated epithelial cells with some early cornified epithelial cells (8), females were housed 2:1 with a C57Bl/6J male and assessed for evidence of mating by the presence of a vaginal plug. Once mating was confirmed, females were housed singly.

Oviducts were collected at 20:00 on the day of proestrus and at 4 h intervals for the next 28 h (8 time points, n = 6 samples per time point, total of 48 mice). Ovulated COCs were removed by gently tearing the oviduct wall. Oviducts were also collected from mated mice at 4 h intervals from 20:00 on day 4 of pregnancy (6 time points, n = 6 samples per time point, total of 36 mice). The uterus and oviduct were flushed to assess embryonic development and confirm mating. Oviducts were only collected from mice with appropriately staged embryos and were immediately snap-frozen in liquid nitrogen and stored at −80°C.

Histology. All oviducts were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin (H&E). Oviducts from 4 animals per genotype per time point were examined (8–10 sections per animal). Images of sections were captured at ×40 magnification using the NanoZoomer HT Digital Pathology System (Hamamatsu Photonics), and one representative image from each genotype at 10 h and 14 h post-hCG was compiled for comparison of gross histology. All sections were also examined at ×60 under an Olympus BX51 microscope, and one representative image from each genotype at 10 h and 14 h post-hCG was captured with a SPOT RT-SE digital camera (Scitech) to show greater detail of the cells in the luminal epithelium. Where possible, images were taken from the proximal oviduct (infundibulum and ampulla), which has a highly folded mucosal layer and is adjacent to the ovary, rather than from the distal section (isthmus), which has a much narrower lumen and less plicate mucosal layer.

RNA extraction. Oviducts were homogenized in 1 ml Trizol (Sigma-Aldrich) using a Preccelys 24 tissue homogenizer (Sapphire Bioscience). Total RNA was extracted by a modified Trizol protocol, which included an overnight precipitation step at −20°C and the addition of 1 μl Ambion GlycoBlue (15 mg/ml, Applied Biosystems) hybridization precipitation to assist with visual detection of the RNA pellet. The final pellet was dissolved in 25 μl of ultrapure water. To eliminate potential contamination by genomic DNA, all samples were DNase-treated using Ambion DNA-free (Applied Biosystems) according to the manufacturer’s instructions. RNA concentration and purity was assessed using a Nanodrop ND-1000 Spectrophotometer (Biolab).

Microarray sample preparation, array hybridization, scanning, and analysis. Samples were made by pooling equal quantities of RNA from the oviducts of three animals collected at eCG + 8 h post-hCG (n = 5 samples per genotype, total of 15 mice per genotype). RNA integrity was assessed with an Agilent Bioanalyzer (Agilent Technologies) and all samples were within 8.1–9.6. Microarray analysis was conducted on 100 ng total RNA using Affymetrix GeneChip Mouse Gene 1.0 ST Arrays (Affymetrix). These arrays contain 28,853 genes with ~27 probes distributed evenly across each transcript, allowing unbiased analysis of expression. Gene-level analysis of multiple probes on different exons is summarized into an expression value representing all transcripts from the same gene. Sample preparation, array hybridization, and scanning were performed by the Adelaide Microarray Centre (Adelaide, SA, Australia) using Affymetrix GeneChip Kits (Affymetrix) according to the manufacturer’s instructions. Microarray data were analyzed using Partek Genomics Suite software (Partek). Background correction was performed by robust multichip averaging followed by probe affinity adjustment for the variable G-C content of probes. Quantile normalization was performed to remove systematic variations resulting from array preparation or sample hybridization conditions. A mean probe-set summary...
was obtained by averaging the intensity values for the multiple probes representing an individual gene. Full datasets have been deposited in the National Center for Biotechnology Information’s Gene Expression Omnibus (GEO) (15) and are accessible via GEO Series accession number GSE51499.

**Real-time RT-PCR.** For the PRKO and PR+/− oviduct samples, 1.2 μg of RNA was reverse-transcribed in a 60 μl reaction using random hexamers (250 ng/μl, Roche), dNTPs (10 mM, GIBCO Invitrogen Australia) and Superscript III reverse transcriptase (GIBCO Invitrogen Australia) for first-strand cDNA synthesis, according to the manufacturer’s instructions. We confirmed the absence of contaminating genomic DNA by running a control sample lacking Superscript III reverse transcriptase enzyme. For the C57Bl/6J time course oviduct samples, 1 μg of RNA was reverse-transcribed in a 40 μl reaction using random hexamers (100 ng/μg, Geneworks) and dNTPs and Superscript III as above.

cDNA templates were analyzed by semiquantitative real-time PCR in triplicate using the Rotor-Gene 6000 Real-time Rotary Analyzer (Qiagen). Each reaction included 10 ng of cDNA in a 20 μl reaction with Power SYBR Green PCR Master Mix (Applied Biosystems) and Quantitect Primer Assays (Qiagen). Ribosomal protein L19 (Rpl19) was used as an internal control for every sample and this Quantitect assay was confirmed to have comparable amplification efficiency with Quantitect assays for each gene of interest and was stably expressed across all samples (data not shown). Real-time RT-PCR data were analyzed using the ΔΔCt method (30) for quantitation relative to a calibrator or reference sample. The calibrator was a pooled sample of 6 C57Bl/6J oviducts collected at eCG + 10 h post-hCG that was run on every plate. For comparison of PR+/− and PRKO gene expression, fold-change data were normalized to the untreated (PGR) or 0 h post-hCG (all other genes) PR+/− samples, such that the mean fold-change for this group was 1.0. For the C57Bl6/J time course, fold change data were normalized to the first time point (day 1, 20:00), such that the mean fold-change for this group was 1.0.

**Statistical analyses.** Microarray data were analyzed by a one-factor analysis of variance (ANOVA) with post hoc false discovery rate (FDR) corrections made for multiple testing to reduce the chance of type I errors (i.e., false positives) by the Benjamini-Hochberg step-up procedure (3). Therefore, gene expression was considered significantly different between genotypes at step-up P < 0.05 and is presented as fold change of PRKO samples relative to PR+/− samples. Negative fold change indicates downregulation; positive fold change indicates upregulation. Principal component analysis (PCA) was used to summarize the gene expression data for each sample array and to examine the variability among arrays within genotype. ANOVA, FDR, and PCA analyses were all performed with the Partek Genomics Suite v6.5 software package (Partek). A volcano plot was used to highlight genes of interest with large magnitude fold changes (i.e., high biological significance) and/or high statistical significance (12). Data sets containing genes that were differentially expressed between the two genotypes were imported into the Ingenuity Pathway Analysis software (IPA, Ingenuity Systems) to identify significant gene associations with biological functions. In brief, a core analysis of the PGR regulated gene set was conducted in IPA with fold-change gene associations with biological functions. In brief, a core analysis of the PGR regulated gene set was conducted in IPA with fold-change for each gene by a one- or two-way ANOVA, followed by a pairwise multiple comparison procedure (Holm-Sidak method) to determine significant differences between groups. Data were log or square-root transformed where required to normalize the distribution. When these transformations were unsuccessful in normalizing the data, a nonparametric one-way ANOVA on ranks (Kruskal-Wallis test) was performed with the Student Newman Keuls method (for equal sample sizes) or Dunn’s multiple comparison procedure (for unequal sample sizes) to determine significant differences between groups. For the C57Bl/6J time course, days 1 and 4 were analyzed separately. For all analyses, statistical significance was set at P < 0.05. All analyses were performed with the Sigmamaplot 11.0 graphing and statistical package (Systat Software).

**RESULTS**

**Pgr mRNA expression in the oviduct.** Across the periovulatory period Pgr expression was generally high in the oviduct, but there was a nonsignificant trend for a decrease in Pgr mRNA at 10 h post-hCG (P = 0.057, Fig. 1A). A lack of Pgr mRNA expression in the PRKO oviducts was also confirmed (data not shown). The trend for a slight decrease in Pgr expression at the time of ovulation was also seen in the C57Bl/6J naturally cycling time course, although there were no statistically significant changes in gene expression across day 1 or day 4 of pregnancy (P = 0.147, Fig. 1B).

**Histology of PRKO oviducts.** Histological sections revealed grossly normal morphology in the PRKO compared with PR+/− oviducts in terms of the degree of epithelial layer folding or thickness of the underlying muscle wall layers at any...
revealed that the global gene expression patterns in PRKO and
\[\text{H11001}/\text{H11002}\] isolated from PRKO vs. PR
changes were identified by microarray of whole oviducts iso-
eCT\text{H1105}\text{H11021}\text{H11005}
ated at eCG and distribution at all time points. Again, representative images
secretory “peg” cells visible in seemingly equivalent numbers
oviducts showed normal appearance, with ciliated cells and
higher magnification, the luminal epithelial cells of the PRKO
developmental or structural abnormalities were detected in this
region of the PRKO oviducts and further detailed morpholog-
ical assessments were not performed.

Microarray analysis of PRKO oviducts. Transcriptional
changes were identified by microarray of whole oviducts iso-
lated from PRKO vs. PR+/- mice at 8 h post-hCG. PCA plot
revealed that the global gene expression patterns in PRKO and
\[\text{PRKO}/\text{H11001}/\text{H11002}\]
PR+/- biological replicates clustered separately (Fig. 3). There was a profound effect of the PRKO genotype in the
oviduct, with 1,003 genes differentially expressed in PRKO
compared with PR+/- (step-up \[P < 0.05\]; Fig. 3 and Supple-
mental Dataset 1) and 265 genes at the step-up \[P < 0.01\] level
of significance. The vast majority of these genes were down-
regulated in PRKO mice (83% at step-up \[P < 0.05\] and 93% at
step-up \[P < 0.01\]).

1 The online version of this article contains supplemental material.

Fig. 2. Oviductal histology in PR+/- and PRKO mice. Photomicrographs of
oviduct sections stained with hematoxylin and eosin, collected from PR+/-
and PRKO mice at eCG + 10 h and 14 h post-hCG (i.e., 2 h pre-
and 2 h postovulation). One representative section is shown at each time point for each
genotype from 32–40 sections examined across 4 animals per genotype. A:
sections shown at \(\times 20\) magnification. Arrow indicates cumulus cells from
an oculated cumulus oocyte complexes (COCs). B: higher power (\(\times 60\))
photomicrographs. Arrows indicate eosinophilic ciliated cells, and arrowheads indicate large nucleated secretory peg cells.

Fig. 3. Microarray analysis of oviducts from PRKO and PR+/- mice. Oviducts were collected from PR+/- or PRKO mice at 8 h post-hCG and
mRNA isolated and used for analysis of global gene expression differences.
A: principal components analysis (PCA) plot of oviduct microarray samples.
Red spheres are PR+/- samples \((n = 5)\) and blue spheres are PRKO samples
\((n = 5)\). B: volcano plot of oviduct microarray data. All 28,853 genes from the
Affymetrix GeneChip Mouse Gene 1.0 ST Array are plotted. The red dashed
line represents step-up \[P = 0.05\] with points above the line having step-up
\(P < 0.05\) (i.e., significantly dysregulated in PRKO relative to PR+/- samples;
\(n = 1,003\)) and points below the line with step-up \(P > 0.05\) (i.e., not
significant). The green dashed line represents step-up \(P = 0.01\). The plot is
centered around genes with a fold change of 1 (log2 1 = 0), with genes to the
right of the vertical black line upregulated in PRKO and genes to the left of the
vertical black line downregulated in PRKO compared with PR+/-.

Genes with a fold change <2 are shown in gray. Some of the genes mentioned in the
text are shown in green.
Integrin alpha 8 (Itga8) was the most downregulated gene in the PRKO oviducts (almost 10-fold difference to PR+/−). The zinc finger protein, Zbtb16, was also highly downregulated in PRKO oviducts (>4-fold difference), as were genes previously identified to be regulated by PGR in the ovary (Adams1, Hif3a). Three genes of particular interest to oviductal transport, the contractile proteins actin, gamma 2, smooth muscle, enteric (Actg2) and desmin (Des) and the vasoactive peptide endothelin 3 (Edn3), were all highly downregulated in PRKO oviducts (2.5-, 3.5-, and 3.4-fold, respectively). Also, genes involved in prostaglandin synthesis and activity (prostaglandin synthase 1, Ptgs1; prostaglandin synthase 2, Ptgs2; prostaglandin E receptor 1, Ptger1; and prostaglandin F receptor, Ptgfr) were dysregulated in the PRKO oviducts. This confirms previous reports of PGR-regulation of prostaglandin receptors in the ovary (59).

Of the 7% of genes that were highly upregulated (i.e., step-up \( P < 0.01 \)) in the PRKO oviducts, an ion transporter from the solute carrier family, SLC26A4, also known as pendrin, showed the highest upregulation (2.7-fold) and angiotensin II receptor, type 2 (Agtr2) showed an upregulation of 2.2-fold.

IPA identified biological functions significantly enriched among the dysregulated genes in the PRKO oviducts. Downstream functional effects analysis repeatedly predicted significant downregulation of cell migration and contractility and upregulation of cell morphology among the top 10 processes altered in PRKO oviducts (Fig. 4). We then identified suites of genes that we predicted could be important in oviductal transport of oocytes and embryos. These were genes with functional annotations of “cell movement,” “cell spreading,” “migration,” “pathfinding,” or “invasion,” with 93 dysregulated genes represented by these keywords, including Itga8, Edn3, prolactin receptor (Prlr), Adams1, Agtr2, and Ptgs2. Genes with functional annotations of “vasoconstriction/vasodilation” or “muscle contraction/contractility” (33 genes) included Des, Edn3, Actg2, myocardin (Myocd), Agtr2, and Ptgs2. Itga8 was also the top gene on the list with functional annotations of “adhesion,” “attachment of cells,” or “binding of cells” (41 genes).

Characterization of putative PGR-regulated genes in the oviduct. Based on their involvement in the significantly affected functions suggestive of a potential role in oocyte/embryo transport, as well as high fold-change regulation by PGR indicated by microarray, a suite of 10 genes was selected for further analysis (Table 1). These genes were examined across the periovulatory time course to determine if expression levels were regulated in normal oviducts and to validate a temporal expression pattern consistent with regulation by P4/PGR. These genes were also assessed in oviducts from wild type, naturally cycling, and mated C57Bl/6d mice to examine expression during a natural ovulatory cycle and preimplantation embryo development (Fig. 5).

Only one gene, Ptgs2, was not confirmed to be significantly dependent on Pgr expression in the oviduct by RT-PCR (data not shown). The remaining nine genes were validated to be PGR dependent, with most showing fold-change differences between genotypes in excess of differences seen by microarray and exhibiting interesting temporal patterns of expression, particularly during ovulation. Three genes were specifically induced in response to the LH surge and regulated by PGR (Fig. 5A). In PR+/− oviducts, Adams1 upregulated within 4 h after hCG treatment and, consistent with this, was also induced after the endogenous LH surge in cycling mice. In PRKO oviducts, Adams1 was decreased at each periovulatory time point, and no induction after hCG was evident. Itga8 was significantly induced within 4 h after LH/hCG, peaking ~3.5-fold 8 h and 10 h post-hCG compared with oviducts from eCG-treated mice. In cycling mice, Itga8 increased sevenfold in oviducts after the endogenous LH surge (Fig. 5A). Itga8 was reduced in PRKO oviducts at each time point examined, with again no evidence for the periovulatory induction (Fig. 5A). Edn3 was induced by both hCG treatment and the endogenous LH surge, peaking fourfold higher at 8–10 h after hCG. Edn3 mRNA was also dramatically reduced before hCG treatment and failed to be induced in the periovulatory period in oviducts of PRKO mice (Fig. 5A).

Two genes were induced by LH and also exhibited some regulated expression during preimplantation development (Fig. 5B). Prlr and Ptgfr were increased by both hCG and the endogenous LH surge, and both genes were significantly reduced in PRKO oviducts. Interestingly, both genes also appeared to be regulated in the oviduct in the early stages of pregnancy. There was some evidence for an increase in Prlr and Ptgfr expression at 04:00 and 12:00 on day 4 of pregnancy, although these increases were not statistically significant (Fig. 5B).

Myocd and Des have distinct roles in muscular contraction and were also hormonally regulated in the oviduct (Fig. 5C).
Both Des and Myocd were significantly induced by hCG in the oviduct although this periovulatory induction was modest (2- to 2.5-fold, respectively) and the induction was not statistically significant in the naturally cycling mice. The induction of both genes was also PGR dependent (Fig. 5C).

Other genes were constitutively expressed in wild-type oviducts. Neither Actg2 or Agrp2 was induced by hCG or the endogenous LH surge; however, Actg2 was downregulated and Agrp2 was upregulated in PRKO oviducts relative to PR+/− (Fig. 5D) consistent with the microarray results.

**DISCUSSION**

This study shows that the nuclear transcription factor PGR is not required for oviductal development but is required for a host of genes to be expressed during ovulation and preimplantation development.

Previous studies localizing PGR in the oviduct detected maximum levels of PGR protein at 48 h post-eCG and a decrease in both isoforms of PGR after hCG injection in the mouse (51). This contrasts with another study in mouse showing increased PGR protein in response to hCG, particularly in ciliated cells (56). The only previous study of Pgr mRNA expression in the mouse used X-gal staining of tissues from a PRlacZ reporter mouse as an indirect measure of Pgr expression, and although nonquantitative, indicated constitutively high expression of Pgr in the oviduct from 48 h post-eCG until 24 h post-hCG (23). Our results report the first direct quantification of Pgr mRNA in mouse oviducts during the periovulatory period and indicate a relatively constant level of expression with some suggestion of a moderate decrease just prior to ovulation. However, the constitutive expression of Pgr from ovulation and during day 4 of pregnancy indicates that it has important functions at all stages of preimplantation development. In human fallopian tubes, Pgr mRNA has been reported to show similar expression dynamics across the ovulatory cycle to that seen by us in the mouse, with relatively stable but high expression during the late prophase and a decrease during the luteal phase (21, 49). This suggests that the mouse is an applicable model for investigating PGR-regulated genes that may also be important for oviductal function in the human.

P4 has been shown, predominantly in vitro, to have an impact on oviduct epithelial cell morphology and differentiation (9) and ciliary (34, 36, 37) and muscular (20, 52) function. In addition, the volume and composition of oviductal fluid are influenced by ovarian steroids across the cycle (22). Both genomic and nongenomic progesterone receptors are likely to be involved. PGRMC1 has been localized to the luminal epithelia and muscle cells of the bovine oviduct, where it remains at constant levels across the estrous cycle (31). Also, the newly described membrane progestin receptors β and γ (mPRβ and γ) have been found to be exclusively expressed in ciliated cells of the oviduct and to be hormonally regulated (38). Several studies have suggested that P4 may be acting in the oviduct via these nongenomic pathways (32, 40, 61). For example, Wessel et al. (61) showed that cilia beat frequency (CBF) was inhibited in bovine oviductal explants as soon as 15 min after treatment with P4, but that mifepristone pretreatment did not affect this result in the short term yet prevented the inhibitory influence of P4 after 24 h. However, a recent study confirmed that the classical progesterone receptor mediated the rapid reduction of CBF by P4 in oviductal explants treated with mifepristone as well as oviducts from PRKO mice in vitro (7). The coexpression of nuclear Pgr and membrane receptors in oviductal cells provides the possibility of a co-operative relationship in mediating cilia function.

Detailed histological examination of oviducts from PRKO mice at various time points leading up to ovulation, as well as immediately postovulation, revealed no obvious structural defects in whole oviducts or in the cells of the luminal epithelium. Ciliated cells and secretory cells were evident in H&E-stained sections, and their distribution and abundance did not suggest any marked differences between the genotypes. In support of this, two genes specific to secretory peg cells and not found in ciliated cells, Ovgl (6) and Pax8 (4), were not significantly different in PRKO compared with PR+/− oviducts by RT-PCR (data not shown). We focused our investigations for morphological anomalies on sections from the proximal oviduct as this is the site of initial oocyte pickup and transport and has the greatest surface area of luminal epithelium to compare between genotypes. However, due to the natural folding of the tissue, at least four or five sections per genotype per time point also contained regions from the distal oviduct, and examination of these regions did not reveal any obvious morphological abnormalities.

Although the morphology of oviducts from PRKO mice was grossly normal, there was a profound effect of PGR ablation on gene expression in the oviduct indicating disrupted functions of oviductal cells. As we did not test for direct regulation by PGR
on the promoter regions of these genes, our list undoubtedly includes secondarily affected and/or indirectly regulated genes, which are still, nonetheless, PGR dependent. Significant biological functions associated with these PGR-dependent genes in the oviduct were adhesion, attachment, and binding of cells, perhaps involved in the initial capture or transport of the newly ovulated COC; cell migration, movement, and invasion, which may play a role in transporting the oocyte/embryo along the oviductal lumen; and vasoconstriction/vasodilation and muscle contraction, which may also contribute to embryo transport as well as the movement of transudates from oviductal vessels into the luminal fluid. Importantly, further studies are required to examine the protein levels of each PGR-regulated gene as well as the functional consequences of these gene expression dynamics in the oviduct. However, given PGR is a transcription factor, investigation of the transcriptome is an appropriate starting point to ascertain direct effects of PGR activity. Validation and detailed analysis of a suite of genes identified from the microarray study by RT-PCR revealed that oviductal gene expression is clearly complex, with some genes responding to the LH surge independently of PGR, some regulated by PGR, and some regulated by both. It is also interesting that some genes were only regulated in the periovulatory window, while others, such as Prlr and Ptgr, were also dynamic at day 4 of pregnancy, suggesting regulation by circadian or other endocrine signals such as prolactin. To our knowledge, this study provides the most comprehensive assessment of PGR-dependent gene expression in the oviduct across the periovulatory and preimplantation periods. Our experimental design does not allow for conclusions to be made about gene expression within specific oviductal regions or cell types; however, it identifies a comprehensive list of genes required for such detailed future studies.

Two genes with well-known roles in cell adhesion and motility in other tissues, Itga8 and Adamts1, were downregulated in PRKO oviducts. Adamts1 is a PGR-regulated gene in
the ovary (44) and plays a well-established role in extracellular matrix remodeling in the ovary at ovulation (5), as well as in other tissues where it facilitates cell migration and invasion (43). Its specific role in the oviduct is unknown, although *Adams1* is known to cleave collagen type I (19), which is prevalent in the oviduct (26), and thus it may play a role in the dramatic morphological changes seen as the newly ovulated oocyte passes through the oviductal lumen, similar to its proposed role in softening the birth canal (45). *Iga8* is involved in the adhesion of cells to extracellular matrix, particularly tenascin, fibronectin, and vitronectin (48). Its expression was induced at the time of ovulation and was dramatically repressed in PRKO oviducts. *Iga8* has been identified in the human fallopian tube and found to be highest in the periovulatory phase (13). Although a role for *Iga8* in the oviduct has not yet been demonstrated, it may play a role in facilitating the initial pick-up of the COC after ovulation through interaction of the oviductal epithelium and COC, given that cumulus cells in the mature COC produce the integrin α8 ligands fibronectin and tenascin (16).

Several genes involved in regulating smooth muscle function and contractility were also dysregulated in PRKO oviducts. In addition to its putative role in cell adhesion in the oviduct, *Iga8* may also be involved in regulating oviductal contractility as it has been localized to smooth muscle cells in other tissues (47) and maintains the contractile smooth muscle phenotype in vascular smooth muscle cells (63). *Myocd* is a transcriptional coactivator and master regulator of gene expression in vascular smooth muscle cells (28). This is the first report of *Myocd* expression in the oviduct to show moderate regulation by PGR, peaking in hCG-stimulated animals just prior to ovulation. *Des* is found in skeletal, cardiac, and smooth muscle cells (29), where it is essential for maintaining structural integrity during contractions. It has also been isolated to the interstitial Cajal-like (ICL) cells in the oviduct (42), which express PGR (10) and are thought to contribute to the contractility of the oviduct via their connections to smooth muscle cells (42). Damage to these ICL cells, for example during infection with *Chlamydia*, results in a loss of ICL cell activity and an increased risk of ectopic pregnancy in mice (14), emphasizing their importance in oviductal transport of the early embryo. Additionally, both *Actg2* and *Agrt2* have distinct roles in muscular contraction. *Actg2* has been shown to be expressed in rat smooth muscle cells, predominantly in the gastrointestinal and urogenital tracts (35), and is highly induced in the presence of *Myocd* in vitro (53). This is also the first report of *Actg2* expression in the oviduct, which was constitutively expressed over the periovulatory time course and downregulated in PRKO oviducts. Angiotensin II has previously been shown to regulate bovine oviductal contractions in vitro (62), and its main receptor, *Agrt2*, has previously been found in human fallopian tube (25). Together the reduced levels of these smooth muscle cell specification factors and functional regulators in PRKO oviducts indicate a role for periovulatory progesterone via PGR in regulating the active smooth muscle contractions that contribute to changing oviduct structure and the passage of ovulated COCs.

Ciliary activity is also recognized to be important for oviductal transport and has previously been shown, in vitro, to be regulated by steroid-induced local production of prostaglandins (57) and angiotensin II (46). The prostaglandin F receptor gene, *Ptgfr*, was the only prostaglandin pathway gene confirmed to be PGR dependent by RT-PCR. *Ptgfr* has previously been shown to be downregulated in human fallopian tubes after treatment with the PGR antagonist RU486 (59), consistent with our results. Prostaglandins likely play a dual role in oviductal transport, as they have also been shown to induce muscular contractions in human oviductal explants in vitro (60). Interestingly, *Ptgfr* was one of only two genes also induced in the oviducts from day 4 pregnant mice, suggesting ongoing regulation, perhaps by prolactin or circadian signals. Angiotensin II also potentially plays multiple roles in regulating oviductal contractility, as in addition to its proposed effects on oviductal contractility, it has been shown to regulate CBF in the oviduct in vitro (46). Therefore, both *Ptgfr* and *Agrt2* are likely to play a fundamental role in steroid-induced oviductal transport.

Finally, two genes previously proposed to support early embryo development, *Edn3* and *Prlr*, were both induced at the time of ovulation and were downregulated in PRKO oviducts. Although endothelins are primarily associated with vasconstriction in other tissues, an earlier study has suggested that *Edn3* plays a noncontractile role in the oviduct, regulating oviductal epithelial cell secretions (24). Mice treated with an endothelin receptor antagonist at eCG + 14 h hCG (i.e., after ovulation) had significantly fewer two-cell embryos flushed from the oviducts, and expression of the receptor subtypes A and B was specifically localized to the luminal epithelium, suggestive of a local role of *Edn3* on epithelial cell function (24). However, in addition to its proposed role in regulating oviductal secretions, it may also impact on ciliary activity in these cells, further affecting oviducal transport of early embryos into the uterus. *Prlr* is known to be important for preimplantation embryo development, with embryo-transfer experiments in *Prlr*−/− wild-type mice confirming that the oviductal environment is deficient in *Prlr*−/− mice during embryo development (39). In rodents, cervical stimulation at mating induces semicircular surge of prolactin secretion, which assist the maintenance of the corpus luteum and thus support pregnancy maintenance (18). The seemingly pulsatile expression and PGR dependence of *Prlr* in oviducts demonstrate that *Prl* plays a role in coordinating the response to prolactin in the oviduct.

This study identifies putative PGR-regulated genes in the oviduct during the periovulatory period, including many with potential roles in oocyte pick-up, fertilization, and early embryo transport. Thus, the results are a valuable resource for understanding the molecular mechanisms underlying P4 regulation of oviductal function and support the well-documented roles of P4 in eliciting functional changes such as ciliary beating and muscle contractility in the oviduct.

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AUTHOR CONTRIBUTIONS

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