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

Lisa K. Akison, Michael J. Boden, David J. Kennaway, Darryl L. Russell, and Rebecca L. Robker

The Robinson Research Institute, School of Paediatrics & Reproductive Health, University of Adelaide, Adelaide, South Australia, Australia

Submitted 2 April 2014; accepted in final form 8 June 2014

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 littersmates. 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 (Adams1, Iga8, Edn3, Prlr, Ptgfr, Dsc, 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. Adams1, Iga8, Edn3, Prlr, and Ptgfr were significantly upregulated in oviducts at ovulation/mating. However, most genes showed basal levels of expression at other times. The exceptions were Prlr and Ptgfr, 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 cells assisting in the pick-up and transport of the expanded newly ovulated cumulus oocyte complex (COC), with ciliated embryo to the uterus. They are the first point of contact for the critical in bidirectional transport of gametes, as well as nourishment and pregnancy, and the uteri 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 preovulatory 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-free 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+/-) 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 CGT TGT TG 3’; Pgr antisense 5’ GAT GGG CAT ATC GAT GAA ATC 3’; Neo 5’ CTT CAC CCA CGA GTA CCT TAC GCT TC 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 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 prepared 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/μl, Geneworks) and dNTPs and Superscript III as above.

cDNA templates were analyzed by semi-quantitative 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 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 threshold of 1.5 and considering only relationships that have been experimentally demonstrated. A functional analysis was conducted to identify all cellular functions significantly associated with the gene set. This included a downstream effects analysis, which identified processes predicted to increase or decrease, given the expression changes in the experimental dataset. Finally, we compiled lists of genes associated with specific keywords from the function annotations to identify candidate genes associated with specific processes important for embryo and oocyte transport in the oviduct.

All gene expression results are presented as means ± SE (n = 5 or 6 per time point). Before analysis, data sets were tested for a normal distribution with the Kolmogorov-Smirnov test and equality of variances between groups. Time points and/or genotypes were compared 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 SigmaPlot 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

---

**Fig. 1.** Pgr mRNA expression in mouse oviducts. A: oviductal tissues were collected over a periovulatory time course from PR+/− mice injected with equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG); n = 5 animals for each time point. B: gene expression in oviducts collected from naturally cycling and mated C57Bl/6J mice at 4 h intervals during day 1 and day 4 of pregnancy; n = 4–6 animals from each time point genotype. Hatched bars at the top indicate the dark cycle and white bars the light cycle. *When ovulation/mating should occur.
revealed that the global gene expression patterns in PRKO and PR+/-/H11002 correlated from PRKO vs. PR+/-/H11001 changes were identified by microarray of whole oviducts isolated from PRKO at eCG and distribution at all time points. Again, representative images of the periovulatory time points (eCG + 8 h, 10 h and 12 h post-hCG) or postovulation (eCG + 14 h post-hCG). Representative images at eCG + 10 h and 14 h post-hCG are shown (Fig. 2A). Ovulated COCs were observed in the oviduct sections at eCG + 10 h and 14 h post-hCG (Fig. 2A). At higher magnification, the luminal epithelial cells of the PRKO oviducts showed normal appearance, with ciliated cells and secretory “peg” cells visible in seemingly equivalent numbers and distribution at all time points. Again, representative images at eCG + 10 h and 14 h post-hCG are shown (Fig. 2B). All sections imaged were from the proximal oviduct (infundibulum and ampulla) as this is the site of initial oocyte pickup and transport and has the greatest surface area of luminal epithelium to compare between genotypes. Therefore, no obvious developmental or structural abnormalities were detected in this region of the PRKO oviducts and further detailed morphological assessments were not performed.

Microarray analysis of PRKO oviducts. Transcriptional changes were identified by microarray of whole oviducts isolated from PRKO vs. PR+/-/ mice at 8 h post-hCG. PCA plot revealed that the global gene expression patterns in PRKO and 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 Supplemental Dataset 1) and 265 genes at the step-up P < 0.01 level of significance. The vast majority of these genes were downregulated 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.
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; prostanoid 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/6j 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 Agr2 was induced by hCG or the endogenous LH surge; however, Actg2 was downregulated and Agr2 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 gene expression in the mouse used X-gal staining of tissues from a PRlacZ reporter mouse as an indirect measure of gene expression changes in PRKO relative to PR+/− oviducts. Negative fold change indicates downregulation and positive fold change indicates upregulation. P value is the adjusted, step-up P value following false discovery rate correction. PGR, progesterone receptor; ND, no data.

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>Reference Sequence</th>
<th>Fold Change</th>
<th>P Value</th>
<th>Studies in the Oviduct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Itga8</td>
<td>integrin alpha 8</td>
<td>NM_001001309</td>
<td>−9.87</td>
<td>&lt;0.001</td>
<td>ND</td>
</tr>
<tr>
<td>Des</td>
<td>desmin</td>
<td>NM_010043</td>
<td>−3.55</td>
<td>&lt;0.001</td>
<td>42</td>
</tr>
<tr>
<td>Edn1</td>
<td>endothelin 3</td>
<td>NM_007903</td>
<td>−3.43</td>
<td>0.001</td>
<td>24</td>
</tr>
<tr>
<td>Prlr</td>
<td>prolatin receptor</td>
<td>BC096586</td>
<td>−3.39</td>
<td>0.002</td>
<td>50</td>
</tr>
<tr>
<td>Adamtsl</td>
<td>ADAM metallopestidase with thrombospondin type 1 motif, 1</td>
<td>NM_009621</td>
<td>−3.18</td>
<td>0.001</td>
<td>ND</td>
</tr>
<tr>
<td>Actg2</td>
<td>actin, gamma 2, smooth muscle, enteric</td>
<td>NM_009610</td>
<td>−2.52</td>
<td>&lt;0.001</td>
<td>ND</td>
</tr>
<tr>
<td>Ptgr</td>
<td>prostaglandin F receptor</td>
<td>NM_008966</td>
<td>−2.28</td>
<td>0.013</td>
<td>59</td>
</tr>
<tr>
<td>Myocd</td>
<td>myocardin</td>
<td>NM_145136</td>
<td>−0.29</td>
<td>0.003</td>
<td>ND</td>
</tr>
<tr>
<td>Agr2</td>
<td>angiotensin II receptor, type 2</td>
<td>NM_007429</td>
<td>2.18</td>
<td>0.003</td>
<td>46, 62</td>
</tr>
<tr>
<td>Ptg2</td>
<td>prostaglandin-endoperoxide synthase 2</td>
<td>NM_011198</td>
<td>−1.83</td>
<td>0.014</td>
<td>57, 60</td>
</tr>
</tbody>
</table>

Fold change is from microarray assessment of gene expression changes in PRKO relative to PR+/− oviducts. 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 progestrone 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, Ovgp1 (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 oviducal 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 Ptgfr, 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, Iga8 and Adamt8, were downregulated in PRKO oviducts. Adamt8 is a PGR-regulated gene in

---

**Fig. 5.** RT-PCR validation of putative PGR-regulated genes in the oviduct. **Left:** validation of gene expression in PR+/− and PRKO oviducts collected over a periovulatory time course from mice injected with eCG and hCG; n = 5 animals from each time point/genotype. Different letters denote significant differences from 2-way ANOVAs and post hoc Holm-Sidak multiple comparison procedures (P < 0.05). Data were log-transformed where required to normalize the distribution. **Right:** gene expression in oviducts collected from naturally cycling and mated C57Bl/6j mice at 4 h intervals during day 1 and day 4 of pregnancy; n = 6 animals from each time point/genotype. Different letters denote significant differences from 1-way ANOVAs and post hoc Holm-Sidak multiple comparison procedures (P < 0.05) conducted separately on day 1 and day 4 data. Data were log- or sqrt-transformed where required to normalize the distribution. Where transformation did not normalize the distribution, a nonparametric Kruskal-Wallis test was performed (Edn3 day 1; Prlr day 4; Ptgfr day 1 and 4). Hashed bars at the tops of each panel indicate the dark cycle and white bars the light cycle. *When ovulation/mating should occur. A: PGR-regulated genes induced by luteinizing hormone (LH), upregulated in naturally cycling/mated mice around the time of ovulation but with constant levels of expression on day 4 of pregnancy. B: PGR-regulated genes induced by LH and with some upregulated expression on both days 1 and 4 of pregnancy. C: PGR-regulated genes induced by LH but with constant levels of expression in unstimulated mice. D: PGR-regulated genes with constitutive expression across both time courses.
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 *Agtr2* 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 been shown to regulate bovine oviductal contractions in vitro (62), and its main receptor, *Agtr2*, 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 *Agtr2* 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 oviductal transport of early embryos into the uterus. *Prlr* is known to be important for preimplantation embryo development, with embryo-transfer experiments in *Prlr−/−* and wild-type mice confirming that the oviductal environment is deficient in *Prlr−/−* mice during embryo development (39). In rodents, cervical stimulation at mating induces semicircadian surges 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 P4 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.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Laura Watson, Kate Frewin, Emily Alvino, Kylie Dunning, Kara Cashman, Athena Voultsios, and Tamara Varcoe (Discipline of Obstetrics & Gynaecology, University of Adelaide) for assistance with collection of oviducts. Mark van der Hoek and Rosalie Kenyon (Discipline of Anatomy and Pathology, University of Adelaide) provided technical assistance with sectioning and staining oviducts for histology.

GRANTS

This work was supported by research grants from the National Health and Medical Research Council (NHMRC) of Australia (to D. L. Russell and R. L.
DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES

42. Popescu LM, Ciontea SM, Cretoiu D, Hinescu ME, Radu E, Ionescu Peluso JJ.
45. Popescu LM, Ciontea SM, Cretoiu D, Hinescu ME, Radu E, Ionescu Peluso JJ.