Regulation of transcripts encoding ADAMTS-1 (a disintegrin and metalloproteinase with thrombospondin-like motifs-1) and progesterone receptor by human chorionic gonadotropin in equine preovulatory follicles

D Boerboom^{1,2}, D L Russell², J S Richards² and J Sirois¹

¹Centre de Recherche en Reproduction Animale, Faculté de Médecine Vétérinaire, Université de Montréal, CP 5000, Saint-Hyacinthe, Québec, Canada J2S 7C6

²Department of Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030, USA

(Requests for offprints should be addressed to J Sirois; Email: siroisje@medvet.umontreal.ca)

Abstract

One member of a new family of metalloproteinases, a disintegrin and metalloproteinase with thrombospondin-like motifs-1 (ADAMTS-1), has been found to be expressed and hormonally induced in granulosa cells of ovulating rodent follicles. Furthermore, the targeted disruption of the ADAMTS-1 gene resulted in ovarian defects associated with severely impaired fertility. While these data demonstrate the importance of ADAMTS-1 in rodent ovarian physiology, the potential role of ADAMTS-1 in the ovulatory process of monoovulatory species remains unknown. The objectives of this study were to clone the equine ADAMTS-1 primary transcript and to study its regulation during human chorionic gonadotropin (hCG)-induced ovulation. A 3573 bp follicular cDNA library clone was isolated and found to encode a nearly complete, highly conserved ADAMTS-1 homologue. Real-time RT-PCR analysis detected this transcript in diverse tissues, including previously unreported sites of ADAMTS-1 expression such as the male reproductive tract, the follicular theca interna and the mature corpus luteum. The tissue distribution of the progesterone receptor (PR), a known regulator of ADAMTS-1 expression in rodent preovulatory follicles, was found to overlap that of ADAMTS-1 in some tissues. A study of the regulation of follicular ADAMTS-1 and PR mRNAs during the hCG-induced ovulatory process revealed distinct patterns of regulation in granulosa cells and in theca interna. In granulosa cells, ADAMTS-1 mRNA was found to be induced at 12 h post-hCG ($P<0.05$), followed by a return to basal levels by 30 h and a re-increase at 33–39 h ($P<0.05$). A concomitant increase in PR mRNA ($P<0.05$) was observed at 12 h post-hCG. In theca interna, abundant ADAMTS-1 mRNA was detected at all timepoints, and levels increased transiently at 33 h post-hCG ($P<0.05$), whereas no significant change was observed in PR mRNA. Together, these data demonstrate for the first time the hormonally regulated ovarian expression of ADAMTS-1 in a monoovulatory species, and identify a novel biphasic regulation of ADAMTS-1 in granulosa cells and a regulated expression in theca interna that were not previously observed in rodents.

Journal of Molecular Endocrinology (2003) **31**, 473–485

Introduction

The expulsion of the oocyte at the moment of ovulation is dependent upon the localized breakdown of the ovarian follicle. For this to occur, it is thought that a proteolytic degradation mechanism activated by the luteinizing hormone (LH) surge acts to breach the connective tissues of the follicle

wall (Tsafriri & Reich 1999). This theory has led researchers to propose numerous different enzymes as potential direct mediators of proteolysis or components of cascades leading to their activation (Espey & Lipner 1994, Richards *et al.* 1998, Tsafriri & Reich 1999).

Among the first of these were the tissue-type and urokinase-type plasminogen activators (tPA and

uPA), which are induced by LH and could serve to activate collagenase (Richards *et al.* 1998, Tsafiriri & Reich 1999). However, the generation of tPA/uPA double knockout mice whose ovulation rate is only slightly reduced (Leonardsson *et al.* 1995) suggests either that these may not be essential for ovulation, or that other functionally redundant mechanisms can compensate for their loss. Attention has also been focused on the matrix metalloproteinase (MMP) family, many of which are expressed in the ovary (Curry & Osteen 2001). Gene expression and zymographic analyses of proteolytic activity during the periovulatory period have implicated interstitial collagenase (MMP-1, MMP-13 in rats), the gelatinases (MMP-2 and -9) and possibly the stromelysin-like MMP-19 in the breakdown of the follicle wall (Richards *et al.* 1998, Hagglund *et al.* 1999, Tsafiriri & Reich 1999, Robker *et al.* 2000b, Curry & Osteen 2001, Curry *et al.* 2001). In addition, MMP-14, which activates and is coordinately regulated with MMP-2 in the ovary, may also play a role (Sato *et al.* 1994, Liu *et al.* 1998, Hagglund *et al.* 1999). The significance of these data has, however, been blurred by additional findings, such as the expression and activity of many of these enzymes at other stages of follicular and luteal development (Robker *et al.* 2000b, Curry & Osteen 2001), and that MMP-2 and -9 null mice are fertile (Itoh *et al.* 1997, Vu *et al.* 1998, Richards *et al.* 2002). Furthermore, several tissue inhibitors of metalloproteinases (TIMPs) are expressed at various stages of the ovarian cycle, and TIMP-1 expression is induced by LH coordinately with several MMPs (Hagglund *et al.* 1999, Tsafiriri & Reich 1999, Curry & Osteen 2001, Simpson *et al.* 2001). The exact role (if any) of each of these genes in ovulation therefore remains largely speculative. A more recently identified proteolytic enzyme expressed in the ovarian follicle is a disintegrin and metalloproteinase with thrombospondin-like motifs-1 (ADAMTS-1) (Robker *et al.* 2000b). Originally described as being selectively expressed in a murine colon adenocarcinoma cell line and up-regulated during the inflammatory response (Kuno *et al.* 1997), ADAMTS-1 was subsequently shown to associate with the extracellular matrix (ECM) (Kuno & Matsushima 1998) and to possess angio-inhibitory activity (Vazquez *et al.* 1999). Insight into its physiological functions was gained by its targeted disruption, which resulted in multisystemic abnormalities including uterine and

ovarian defects associated with severely impaired fertility (Shindo *et al.* 2000).

A potential role for ADAMTS-1 in ovarian physiology was also revealed by other groups seeking to identify LH-induced genes and progesterone receptor (PR) transcriptional targets (Espey *et al.* 2000, Robker *et al.* 2000a,b). Progesterone has long been known to be a key paracrine mediator of ovulation in rodents, as its synthesis increases dramatically during the ovulatory process, along with ovarian PR expression (Richards 1994). Furthermore, pharmacological inhibition of its synthesis and action blocks ovulation (Tsafiriri & Reich 1999) and, most importantly, PR null mice show an anovulatory phenotype (Lydon *et al.* 1995). While many mechanisms could account for the importance of progesterone in ovulation, a current hypothesis maintains that the preovulatory increase in progesterone could trigger the proteolytic degradation of the follicle wall (Tsafiriri & Reich 1999). Consistent with this, differential display RT-PCR identified ADAMTS-1 as a gene that is transiently induced in the granulosa cell layer of ovarian follicles following the LH surge, and at a time also subsequent to PR induction (Espey *et al.* 2000, Richards *et al.* 2002). It was further demonstrated that ADAMTS-1 induction is sensitive to the progesterone synthesis inhibitor epostane (Espey *et al.* 2000) and is markedly impaired in PR null mice (Robker *et al.* 2000a,b), thereby situating this event downstream of the induction of progesterone synthesis and PR expression. While it can be inferred from these studies that ADAMTS-1 is a likely key player in the ovulatory process, its exact role in the follicle remains unknown (Richards *et al.* 2002). Furthermore, all studies of ovarian ADAMTS-1 expression reported thus far have been limited to rodents, rendering unknown the importance of ADAMTS-1 in the ovarian physiology of large monoovulatory species. In view of this, the general objective of this study was to determine if ADAMTS-1 is expressed and hormonally regulated in equine preovulatory follicles, thereby providing an important monoovulatory model system for the future study of ovarian ADAMTS-1 function. The specific objectives were to clone and characterize the equine ADAMTS-1 primary transcript, and to study its regulation as well as that of PR in preovulatory follicles during human chorionic gonadotropin (hCG)-induced ovulation.

Materials and methods

Materials

Sigma Fast DAB tablets were purchased from Sigma Chemical Co. (St Louis, MO, USA); hCG was purchased from The Buttler Co. (Columbus, OH, USA); [α - 32 P]deoxycytidine 5'-triphosphate (dCTP) was obtained from Perkin-Elmer Life Sciences (Woodbridge, Canada); QuikHyb hybridization solution and the ExAssist/SOLR system were purchased from Stratagene Cloning Systems (La Jolla, CA, USA); Prime-a-Gene labeling system was purchased from Promega Corp. (Madison, WI, USA); synthetic oligonucleotides and TRIzol total RNA isolation reagent were purchased from Invitrogen Life Technologies (Burlington, Canada); dye-labeled hybridization probes were obtained from Synthesgen, LLC (Houston, TX, USA) and TIB Mol-Biol (Adelphia, NJ, USA); Vectastain Elite ABC Kit was obtained from Vector Laboratories, Inc. (Burlingame, CA, USA); Antigen Retrieval Citra Plus Solution was purchased from BioGenex Laboratories, Inc. (San Ramon, CA, USA); LightCycler RNA Amplification Kit for Hybridization Probes was obtained from Roche Diagnostics (Laval, Canada); QuantiTect Probe RT-PCR Kit was purchased from Qiagen Inc. (Mississauga, Canada).

Isolation of equine tissues and RNA extraction

Equine preovulatory follicles and corpora lutea were isolated from Standardbred and Thoroughbred mares at specific stages of the estrous cycle as previously described (Boerboom & Sirois 1998). Briefly, mares were teased daily with a pony stallion for detection of estrus, and ovarian follicular development was monitored by transrectal real-time ultrasonography. The ovulatory process was induced by administration of hCG (2500 IU, i.v.) once the dominant follicle had reached a diameter of 35 mm. Ovariectomy was performed via colpotomy on the standing animal under neuroleptanalgesia as described (Boerboom & Sirois 1998) at 0, 12, 24, 30, 33, 36 and 39 h post-hCG ($n=4$ or 5 follicles/timepoint). In this model, the normal expected time of ovulation in mares is 39–42 h after hCG treatment (Boerboom & Sirois 2001). Corpora lutea were isolated on day 8 of the estrous cycle ($n=3$; day 0=day of ovulation). Each follicular sample detailed in the text therefore

represents the single preovulatory follicle from a given estrous cycle of a single mare. All animal procedures were approved by the institutional animal care and use committee (Comité de Déontologie Animale de l'Université de Montréal) and were conducted in accordance with the guidelines described in *The UFAW Handbook on the Care and Management of Laboratory Animals* as well as FRAME's guidelines on papers involving the use of laboratory animals, as promulgated by the Society for Endocrinology.

The preovulatory follicles from recovered ovaries were dissected into preparations of follicle wall (theca interna with attached granulosa cells) or further dissected into separate isolates of granulosa cells and theca interna as described (Boerboom & Sirois 2001). In some instances, follicle wall, granulosa cell and theca interna samples were derived from the same follicle. Male gonadal tissues were obtained from the Centre Hospitalier Universitaire Vétérinaire (Université de Montréal) following routine castration procedures. Uterine tissues were endometrial biopsy samples removed from pregnant or nonpregnant mares at precisely cycle day 15, and were a gift from Dr Denis Vaillancourt (Université de Montréal, Canada). All other tissues were obtained at a local slaughterhouse. Total RNA was isolated from tissues using TRIzol reagent (Life Technologies) according to manufacturer's instructions, using a Kinematica PT 1200C Polytron Homogenizer (Fisher Scientific, Montréal, Canada) and quantified by spectrophotometry according to standard protocols (Sambrook *et al.* 1989).

Cloning and characterization of the equine ADAMTS-1 cDNA and protein

The equine ADAMTS-1 cDNA was isolated from a previously described expression library (Boerboom & Sirois 1998) made from RNA extracted from an equine preovulatory follicle isolated during estrus, 36 h after hCG treatment. Screening of approximately 150 000 phage plaques was performed as directed in the ZAP-cDNA Synthesis Kit instruction manual (Stratagene), using a 1.5 kb fragment of murine ADAMTS-1 genomic DNA (which includes a part of the 3' coding region (D L Russell & J S Richards, unpublished observation)). The probe was labeled with [α - 32 P]dCTP using the Prime-a-Gene labeling

system (Promega) to a final specific activity greater than 1×10^8 c.p.m./ μ g DNA, and hybridization was performed at 55 °C with QuikHyb hybridization solution (Stratagene). Of approximately 100 positive clones obtained, ten were selected for plaque purification through secondary and tertiary screening. The Ex-Assist/SOLR system (Stratagene) was then used to perform *in vivo* excision to obtain the cloned cDNAs inserted within the pBluescript plasmid. Sequencing was performed by the Service de Séquençage de l'Université Laval (Québec, Canada) using vector-based T7 and T3 oligonucleotide primers and ADAMTS-1 sequence-specific primers (Invitrogen).

Nucleotide analyses were performed using MacDNASIS software version 2.0 (Hitachi, Hialeah, FL, USA), and comparisons with GenBank databases were performed online by standard nucleotide–nucleotide BLAST analyses (Altschul *et al.* 1997) at the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov). Protein sequence comparisons were made online by standard protein–protein BLAST analyses (Altschul *et al.* 1997) at the same website; homologous proteins were aligned using the CLUSTALW v1.7 software (Thompson *et al.* 1994) online at the Protein Information Resource of the National Biomedical Research Foundation (pir.georgetown.edu/pirwww) (Wu *et al.* 2002). Functional domains within the ADAMTS-1 protein were identified by online BLAST analyses (Altschul *et al.* 1997) against the ProDom 2001.3 Protein Domain Database at the ProDom project website (<http://prodes.toulouse.inra.fr/prodom/doc/prodom.html>).

Real-time RT-PCR analysis

Quantification of ADAMTS-1, PR and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts was performed by real-time RT-PCR analysis using the LightCycler system (Roche) and either the LightCycler RNA Amplification Kit for Hybridization Probes (Roche) or the QuantiTect Probe RT-PCR Kit (Qiagen), following the manufacturer's instructions. RT and PCR reactions were conducted sequentially in a single capillary reaction tube using 100 ng of each RNA sample and the equine gene-specific oligonucleotide pairs 5'-TCGTG TAACA TCGAG GACTG TCC-3' and 5'-AGATC CATTT CCTCC

GCAAA TACC-3' for ADAMTS-1, 5'-ACTTC TTAAT ACAAT TCCTT TGGAA G-3' and 5'-GAAC AGAAA CAACT CCTTT TGGCC-3' for PR (GenBank accession number AF007798) and 5'-ATCAC CATCT TCCAG GAGCG AGA-3' and 5'-GTCTT CTGGG TGGCA GTGAT GG-3' for GAPDH (GenBank AF157626), resulting in the generation of 348, 126 and 341 bp products respectively. Cycling conditions using the kit supplied by Roche were one cycle of 55 °C for 20 min (RT step), one cycle of 95 °C for 30 s (denaturation step), 45 cycles of 95 °C for 5 s, 59 °C (ADAMTS-1) or 55 °C (GAPDH) for 15 s and 72 °C for 20 s (PCR step), and one cycle of 40 °C for 30 s (cooling step). Cycling conditions using the Qiagen kit were one cycle of 55 °C for 20 min, one cycle of 95 °C for 15 min, 35 (PR) or 45 (ADAMTS-1 and GAPDH) cycles of 95 °C for 5 s, 59 °C for 30 s and 72 °C for 30 s, and one cycle of 40 °C for 30 s. The accumulation of PCR products was quantified at every cycle by use of the dye-labeled, gene-specific oligonucleotide hybridization probes 5'-AGGGC AGTGT GTAAA AGCTG GCTG (fluorescein)-3' and 5'-(LC red 640) GACCG CATCA TAGAC TCCAA AAAGA AG (phosphate)-3' for ADAMTS-1, 5'-CAGTT TGAAG AGATG AG ATC AAAC ACA(fluorescein)-3' and 5'-(LC red 640) TAGAG AGCTC ATCAA GGCGA TTGGT (phosphate)-3' for PR and 5'-CCAAG GTCAT CCATG ACCAC TTTG(fluorescein)-3' and 5'-(LC red 640) CATCG TGGAG GGACT CATGA CCAC (phosphate)-3' for GAPDH. Binding of the probes to their complementary sequences on the PCR products permits fluorescence resonance energy transfer between donor (fluorescein) and acceptor (LC red 640) dye molecules. Fluorescence vs cycle number data curves were compared with standard curves generated by the PCR amplification of plasmid DNA samples of known concentration, thereby permitting the calculation of an absolute number of copies of target RNA molecules in each sample.

Statistical analysis

All mRNA quantification data were normalized by dividing ADAMTS-1 or PR transcript copy numbers by their corresponding GAPDH copy numbers, which were quantified in separate

real-time RT-PCR reactions. In the case of follicular tissues, for which multiple samples were analyzed for each timepoint, results are expressed as means $\pm$ S.E.M. ($n=3-5$ follicles (i.e. mares)/timepoint). When Levene's test demonstrated homogeneity of variance, one-way ANOVA was used to test the effect of time (after hCG) on ADAMTS-1:GAPDH or PR:GAPDH transcript level ratios. When ANOVAs indicated significant differences ($P<0.05$), Dunnett's test was used for multiple comparisons with the control (0 h post-hCG). If Levene's test showed heterogeneity of variance, data were transformed to logarithms before ANOVA analysis. In cases when logarithmic transformation did not result in homogeneity of variance, the nonparametric Wilcoxon test was used for multiple comparisons with the control (0 h post-hCG). By this method, the nonparametric test was applied to all data sets except those presented in Fig. 4A and the PR data in Fig. 5B. Statistical analyses were performed using JMP software (SAS Institute, Inc., Cary, NC, USA).

Results

Cloning and characterization of the equine ADAMTS-1 transcript and protein

To clone the equine ADAMTS-1 primary transcript, a fragment of the murine ADAMTS-1 gene was used to probe a previously described equine follicular expression library (Boerboom & Sirois 1998). This resulted in the isolation of ten cDNA clones encoding the equine ADAMTS-1 homologue, the longest of which (clone A5) was sequenced extensively (Fig. 1, GenBank accession number AF541975). The 3573 bp cDNA included 1292 bp of 3'-untranslated region, preceded by an open reading frame of 2281 bp that was found to be incomplete at its 5' end, when aligned with its human homologue (Fig. 1A). Attempts to isolate the 5' end of the equine ADAMTS-1 transcript using a variety of library cloning, 5'-RACE and RT-PCR techniques proved to be unfruitful. This was attributed to the $>80\%$ G-C content of the first 100 bp of the equine cDNA clone, a structural feature that would easily block efficient reverse transcription. By homology with the known murine ADAMTS-1 gene structure (GenBank accession number AB001735), clone A5 apparently lacks most of the sequences derived from exon I.

When translated, cDNA clone A5 was found to encode a nearly complete ADAMTS-1 protein which, by comparison with its species homologues, lacks only the propeptide and signal sequence regions (Fig. 2). The region encompassing the key structural features (catalytic domain, disintegrin-like domain, spacer region and thrombospondin type I domains) was shown to be highly conserved, demonstrating 95, 92 and 92% identity to the human, mouse and rat ADAMTS-1 proteins respectively. Sites of proteolytic processing of the ADAMTS-1 protein by furin and MMPs previously identified in other species (Kuno *et al.* 1999, Rodriguez-Manzanique *et al.* 2000) were found to be conserved in the equine homologue. In addition, three of the four putative N-linked glycosylation sites identified in the murine homologue (Kuno *et al.* 1997) were also found to be present in equine ADAMTS-1.

Tissue distribution of ADAMTS-1 and PR transcripts

To study the distribution of ADAMTS-1 transcripts in equine tissues, steady-state mRNA levels were quantified by real-time RT-PCR. ADAMTS-1 transcripts were detected in a wide variety of non-ovarian tissues, several of which had not been previously identified as sites of ADAMTS-1 expression in any species, including lymph nodes, epididymis and testis (Fig. 3). As PR had previously been identified as an upstream regulator of ovarian ADAMTS-1 expression (Espey *et al.* 2000, Robker *et al.* 2000a,b), analyses of its transcript levels were performed on the same tissues. While the distribution of PR was found to overlap that of ADAMTS-1 in some tissues, PR but not ADAMTS-1 was detected in the glandular stomach. Conversely, in the liver and skin, ADAMTS-1 mRNA was detected in absence of PR. Furthermore, it should be noted that while PR and ADAMTS-1 mRNA levels were comparable in uterine and ovarian tissues (Figs 3 and 4), PR transcripts were generally two orders of magnitude less abundant than ADAMTS-1 in all other tissues, as indicated in the labels of the axis in Fig. 3.

To determine if ADAMTS-1 transcripts are present in mature ovarian follicles and whether their levels are regulated by the LH surge, preovulatory follicles were isolated before and after the administration of an ovulatory dose of hCG,

A

B

```

1  GGATGACGAG  ACCCAGCCCT  CGAGGGGCGC  GGAGCCGGAG  GGCCAGGACG
51  CCGGGACGCA  GTGGGCGCCG  CGGGACAGGG  CCGCGCAGCG  CGCCGGACGG
101  CCAACAGGAA  CTGGAAGCAT  AAGAAAGAAG  CGATTGTGTG  TCAGCCCGCG
151  ATATGTGGAA  ACCATGCTTG  TGGCTGACCA  GTCTATGGCA  GAGTTCACAG
201  GCAGTGGTTT  AAAGCACTAC  CTTCTCACCT  TGTCTCTCGT  GCGGCGCCGG
251  TTATACAAAC  ACCCCAGCAT  TCGGAATFCA  GTTAGCTCGG  TGGTGGTGAA
301  GATCCTATGC  ATCTATGAGG  AACAGAAGGG  GCCCGAAGTG  ACTTCCAATG
351  CTGCTCTCAC  TCTGCGGAAT  TTCTGCAACT  GGCAAAAGCA  GCACAACCCG
401  CCCAGTGACC  GGGATGCGGA  GCACTATGAC  ACAGCGATTG  TTTTCACCCG
451  ACAGGATTGG  TGTGGGGCCC  AGACATGTGA  TACTCTTGGG  ATGGCTGATG
501  TTGGAACAT  ATGTGATCCC  AGCAGAAGCT  GCTCGGTCAT  AGAAGATGAT
551  GGTTTACAAG  CTGCCTTCAC  CACAGCCCAT  GAATTAGGCC  ACGTGTTTAA
601  CATGCCACAT  GATGATGCAA  AGCAATGTGC  CAGCATTAAT  GGTGTCAACC
651  GGGATTCCCA  CATGATGGCG  TCAATGCTTT  CCAATTGGGA  CCGCAGCCAG
701  CCTTGGTCTC  CTGCACGGC  CTACATGATT  ACATCATTTT  TGGATAATGG
751  TCACGGTGAA  TGTTTGATGG  ACAAGCCCCA  GAGCCCCATA  CAGCTCCCCT
801  CTGATCTCCC  CGGGACCTTG  TATGATGCCA  ACCGGCAATG  CCAGTTCACG
851  TTTGGGGAGG  AGTCCAACCA  CTGCCCGGAT  GCAGCCAGCA  CGTGACGACG
901  CCTCTGGTGC  ACTGGCACTT  CTGGCGGGTT  GCTGGTGTGC  CAACACCAAC
951  ACTTCCCTTG  GGCAGATGGC  ACCAGCTGTG  GAGAGGGGAG  ATGGTGTGTG
1001  AATGGCAAGT  GTGTGAACAA  GACCGACAGG  AAGCATTTTG  ATACTCCTGT
1051  TCATGGAAGC  TGGGGGCCGT  GGGGCCCTGT  GGGAGACTGT  TCGAAGACAT
1101  GTGGTGGAG  AGTTCAAGT  ACAATGAGGG  AATGTGACAA  CCCAGTGCCA
1151  AAGAATGGAG  GGAAGTACTG  TGAAGGCAAG  CGCGTGCGCT  ACAGGTCTGT
1201  TAACATCGAG  GACTGTCCGG  ATAATAATGG  GAAAACCTTT  AGAGAGGAAC
1251  AATGTGAGGC  TCACAATGAA  TTTTCGAAAG  CTTCTTTGG  GAGTGGCCCT
1301  GCGGTGGAGT  GGACACCCAA  GTATGCTGGA  GTCTCACCAG  AGGACAGGTG
1351  CAAGCTCATC  TGTCAAGCCA  AAGGCATTGG  CTACTTCTTC  GTTTTGCAGC
1401  CCAAGTGGT  AGATGGTACT  CCATGTAGCC  CCGATTCCAC  CTCTGTCTGC
1451  GTGCAAGGGC  AGTGTGTAAA  AGCTGGCTGT  GACCGCATCA  TAGACTCCAA
1501  AAAGAAGTTT  GATAAATGCG  GTATTTCGGG  AGGAAATGGA  TCTACATGCA
1551  AGAAAATATC  CGGATCAGTT  ACTAGTGCAA  AACCTGGATA  TCATGATATC
1601  GTCACAATTC  CAACTGGAGC  CACAAACATT  GAAGTGAAAC  AACGGAATCA
1651  GAGGGGATCC  AGAAATAATG  GAAGCTTTCT  TGCCATCAAA  GCTGCTGATG
1701  GCACATACAT  CCTGAATGGT  GACTTCACTT  TGTCCACTTT  AGAACCAAGC
1751  ATTACGTACA  AAGGTAGTGT  CTTGAGGTAC  AGTGGCTCTT  CTGACGCTGT
1801  GGAAGAAGTT  CGCAGCTTTA  GTCCTCTCAA  AGAGCCCTTA  ACCATCCAGG
1851  TCCTTACAGT  GGGCAATGCC  CTTGACCGCA  AAATTAATA  CACATCTTTT
1901  GTGAAGAAGA  AGAAGGAATC  TTCAATGCC  ATCCCTACTT  TCTCAGAATG
1951  GGTCAATGAA  GAGTGGGGCG  AATGTTCCAA  GTCATGTGGA  CAGGGTTGGC
2001  AGAGAAGACT  GGTAGAGTGC  CGAGACATCA  ATGGCGAGCC  AGCTTCCGAG
2051  TGTGCGAAGG  AAGTGAAGCC  AGCCAGCACC  AGACCTTTGT  CAGACCTGCC
2101  TTGTCCCGCG  TGGCAGCTGG  GGGATTGGTC  GCCATGTTCC  AAGACTTTGT
2151  GGAAGGGTTA  CAAAAGAGA  ACCTTGCACT  GTCTGTCCCA  CGATGGGGGG
2201  GTGTTGTCTC  AAGAGAGCTG  CGATCTTTTA  AAGAAACCCA  AACATTACAT
2251  AGACTTTTGC  ACCATGGCAG  AATGCAGTTA  Agcaaatgtca  gtgttgaggg
2301  aaaagtaaa  tgtggaagg  ctgatgcact  gaaatcaaga  aggtgtgagg
2351  aatccagtg  acctgccc  tgcatacaaa  ggtgtgtcta  tgagttagga
2401  gactataggt  aggtagaaaa  gaagttagat  cgtcagaata  tctgtccagt
2451  tacaaaactg  atagggtagt  taatgaggt  tattaatctc  tgagaactga
2501  tatagcatga  taaggccca  gggcattatt  atcatccctt  ttgttacatc
2551  tatgacaagt  taacaaaaaa  atagaaacaa  ttgtcaaaat  aatttttaag
2601  aatattacaa  tccctgtttc  ctggtactga  ttaataactt  tgatcatagg
2651  ggattaggaa  atgaaaagca  ggaagaaagt  gagaatttta  gtttaagatg
2701  tggcttattt  cacctcgcta  acaatagagg  gagaaggagg  tatgtatagg
2751  gtctttggcc  agcactgcca  tggctgtct  ggtttcagag  aatgctatct
2801  tttctaccca  gcagtaagaa  ttcaacattg  accagcatga  gagaatgtg
2851  taacaacgtc  caatgactcc  gctgtacttt  tctgtgtgca  ttctgtgtgc
2901  tttatttgtc  aattcathtt  gacaaggaaa  caattccgtg  tatttgttaag
2951  aatgcattga  gtctacaaga  gggaaaaaaa  gcagcaatgt  atcagatgct
3001  gggaaaaata  actagaggag  gcaggatgag  gtcagtaacc  cctaccattc
3051  gttcctcctt  atgtaagcct  gctttaggaa  tgtggatgtg  gaaaaacagt
3101  gtgtgtctca  aggaagtcag  taacaccaca  caaaaaggat  gtaatgccag
3151  aataagtatg  ggggtcatag  aatgggggtc  ccagtttttg  gggacactgc
3201  agtcaactgt  ctccagtg  ggaggctact  gaggggtagc  ggggtccatc
3251  ccagcagctg  gtccaacagt  catatcctgg  tgaatgtctg  ttcagctctt
3301  caactatgaa  agagaatatg  actttttcca  tgtgtatata  gtaaaaatata
3351  ttactataaa  ttctatgtac  ttataagata  ttggtttgtg  tgttctctct
3401  aagaaggact  atagtttgtg  ataaatgcc  ataataacat  atttattttt
3451  atacatttct  ttctaagtat  aaatctttta  agttatatcg  cttttgtaaa
3501  agaacatata  aaaaatagag  tatttatata  atatatgtta  ctagaataaa
3551  taaaaagaca  ctttttgaaa  aaa

```

Figure 1 Molecular cloning and characterization of the nucleotide sequence of the equine ADAMTS-1 primary transcript. (A) Schematic representation of the isolated cDNA clone, aligned with its human homologue (GenBank accession number NM_006988). ORF=open reading frame, UTR=untranslated region. (B) Complete nucleotide sequence of the equine ADAMTS-1 cDNA clone. The ORF is indicated by uppercase letters, the translation termination codon (TAA) is highlighted in bold, the 3'-UTR is indicated by lowercase letters, putative polyadenylation signals are underlined, and numbers on the left refer to the first nucleotide on that line. The nucleotide sequence has been assigned the GenBank accession number AF541975.

Figure 2 Domain structure of the equine ADAMTS-1 protein and comparison with known homologues. The deduced amino acid sequence of the equine ADAMTS-1 protein is aligned with the human (GenBank accession number AF207664), mouse (NM_024400) and rat (NM_009621) homologues. Identical residues are indicated by a printed period. Specific regions and domains are bordered by vertical lines with arrows and identified overhead in boldface type. Putative furin (RKRR) and MMP (AKPG) proteolytic processing sites are double-overlined, and the specific point of cleavage is indicated with an inverted arrow. The neutral zinc metalloprotease zinc-binding region contained within the metalloprotease domain is indicated with a dashed double overline. Putative N-linked glycosylation sites are indicated with asterisks. Numbers on the right refer to the last amino acid residue on that line.

and steady-state ADAMTS-1 mRNA levels were quantified (Fig. 4A). In contrast to previous reports in rodents (Espey *et al.* 2000, Robker *et al.* 2000a),

expression of ADAMTS-1 mRNA was detected in equine follicles before the LH surge (i.e. 0 h post-hCG). Although ADAMTS-1 transcript levels

Figure 3 ADAMTS-1 and PR transcripts in various tissues. ADAMTS-1, PR and GAPDH mRNA levels were quantified by real-time RT-PCR using the LightCycler system, and data were normalized by calculating ADAMTS-1:GAPDH or PR:GAPDH copy number ratios. Three RNA samples were used for all tissues except for lung and thymus, for which $n=2$. Data are expressed as means (bars) $\pm$ S.E.M. (error bars), except where $n=2$ (expressed as means only).

appeared to increase approximately 2-fold at 12 h post-hCG, no statistically significant differences could be detected when compared with 0 h ($P>0.10$). Also unexpectedly, the highest amounts of ADAMTS-1 mRNA of all tissues analyzed were detected in the mature (day 8) corpus luteum, a tissue that had not been previously reported to express ADAMTS-1 in any species. Unlike ADAMTS-1, a significant increase in PR transcript levels was noted at 12 h post-hCG ($P<0.05$), after which they rapidly decreased, and reached their

Figure 4 Regulation of ADAMTS-1 and PR mRNAs in equine preovulatory follicles during hCG-induced ovulation. ADAMTS-1, PR and GAPDH mRNA levels were quantified by real-time RT-PCR using the LightCycler system, and data were normalized by calculating ADAMTS-1:GAPDH or PR:GAPDH copy number ratios. Five RNA samples were used for all timepoints issues except for $t=0$ h ($n=4$) and corpora lutea (CL) ($n=3$). Data are expressed as means (columns) $\pm$ S.E.M. (error bars). Follicle wall mRNA preparations were obtained from preovulatory follicles at the indicated times after the administration of an ovulatory dose of hCG, CL were isolated on day 8 of the estrous cycle. Statistically significant difference ($P<0.05$) from the untreated preovulatory follicle (i.e. 0 h post-hCG) is indicated with an asterisk (*).

A. Granulosa Cells

B. Theca Interna

Figure 5 Regulation of ADAMTS-1 and PR mRNAs in isolated granulosa cells and theca interna during hCG-induced ovulation. Isolated granulosa cell (A) and theca interna (B) mRNA preparations were obtained from preovulatory follicles at the indicated times after the administration of an ovulatory dose of hCG. ADAMTS-1, PR and GAPDH mRNA levels were quantified using the LightCycler system, and the data were normalized by calculating ADAMTS-1:GAPDH or PR:GAPDH copy number ratios. Data are represented as the means of these ratios (columns) $\pm$ S.E.M. (error bars). Four samples were analyzed per timepoint, except (A) t=33 h (ADAMTS-1 and PR), t=36 h (PR only) and t=39 h (PR only), and (B) t=0 h and t=33 h (ADAMTS-1 and PR), where $n=3$. Statistically significant difference from 0 h post-hCG ($P < 0.05$) is indicated with an asterisk (*).

lowest point at 36 h (Fig. 4B). PR mRNA was also readily detectable in the corpus luteum, although at much lower abundance than what was observed in 12 h post-hCG follicle wall.

Regulation of ADAMTS-1 and PR mRNAs in granulosa cells and theca interna

To investigate the unexpected absence of ADAMTS-1 mRNA regulation in the follicle wall during the ovulatory process, real-time RT-PCR analyses were performed on separate isolates of granulosa cells and theca interna. In granulosa cells, ADAMTS-1 mRNA was found to be markedly induced at 12 h post-hCG ($P < 0.05$), followed by a return to basal levels by 30 h (Fig. 5A). Interestingly, mRNA levels then exhibited a secondary increase that reached a plateau at 36 h ($P < 0.05$). Consistent with its established role as a regulator of ADAMTS-1 expression in rodent granulosa cells, PR mRNA levels were also found to increase significantly at 12 h ($P < 0.05$), and to subsequently decrease to their lowest point at 30 h. However, no significant secondary re-increase in PR mRNA levels ($P > 0.10$) was observed thereafter, even though levels appear to rise after 30 h

post-hCG (Fig. 5A). Unlike ADAMTS-1, PR transcripts were detected at t=0 h.

In contrast with *in situ* hybridization data reported in rodents (Espey *et al.* 2000, Robker *et al.* 2000a), theca interna was found to be an important site of equine ovarian ADAMTS-1 expression (Fig. 5B). Appreciable amounts of ADAMTS-1 mRNA were detected at 0 h, accounting for the unexpected levels detected in follicle wall at the same timepoint (Fig. 4A). The pattern of ADAMTS-1 mRNA regulation by hCG in theca interna was found to be distinct from what was observed in granulosa cells, with a single, sharp increase in mRNA levels occurring at 33 h ($P < 0.05$), followed by a return to basal levels by 36 h. An apparent 4-fold increase in PR transcript abundance in theca interna was also found to occur late in the ovulatory process (30–33 h post-hCG), but mRNA levels were not found to be significantly different from 0 h post-hCG ($P > 0.10$).

Discussion

Many of the important advances in our understanding of molecular ovarian physiology have

been gained through the use of well-established rodent models (Richards 1994). This is clearly due to several technical and practical advantages of working with rodents, not the least of which is the possibility of genetic manipulations (Nishimori & Matzuk 1996). Despite these advantages, however, the information derived from rodents does not always serve as an accurate predictor of what occurs in large monoovulatory species. This has been demonstrated notably by a recent series of studies in equine preovulatory follicles, which showed that several genes involved in steroidogenesis and ovulation exhibit spatial and temporal expression patterns within the follicle that are different from what has been reported in rodents (Boerboom & Sirois 1998, 2001, Boerboom *et al.* 1999, 2000, Kerban *et al.* 1999a). The present study further emphasizes these differences by demonstrating distinct patterns of ovarian ADAMTS-1 and PR expression and regulation. Notably, the prolonged, biphasic regulation of ADAMTS-1 transcripts by hCG in equine granulosa cells contrasts with its rapid, monophasic induction observed in rodents (Pinter *et al.* 1996, Espey *et al.* 2000, Robker *et al.* 2000a,b). Also, while the theca interna is clearly an important site of ADAMTS-1 and PR expression in equine preovulatory follicles, *in situ* hybridization studies suggest their expression in the rodent ovary is limited to granulosa cells (Pinter *et al.* 1996, Espey *et al.* 2000, Robker *et al.* 2000a,b). These data may reflect distinct roles for these genes in follicular development and luteinization in each species, and further highlight the importance of studying both mono- and polyovulatory animal models of ovarian physiology.

The analyses of the tissue distribution of ADAMTS-1 mRNA reported herein feature several unexpected findings. Most notably, this is the first report of the detection of ADAMTS-1 mRNA in lymph nodes, the male reproductive tract, the mature corpus luteum and the theca interna cell layer of the ovarian follicle (Vazquez *et al.* 1999, Miles *et al.* 2000). The presence of high levels of ADAMTS-1 mRNA were particularly unexpected in the two latter tissues, considering that the corpus luteum undergoes remodeling that necessitates extensive neovascularization, and ADAMTS-1 mRNA levels increase in the theca interna at a time associated with the presence of increased numbers of thecal blood vessels (Kerban *et al.* 1999b). These data are thus in apparent

contradiction with the proposed anti-angiogenic activity of ADAMTS-1 (Vazquez *et al.* 1999). Ovarian ADAMTS-1 would somehow need to exert its biological function without inhibiting angiogenesis, perhaps by post-translational modification. Alternatively, its anti-angiogenic properties may simply be overwhelmed at certain times during the ovulation/luteinization process by a preponderance of pro-angiogenic activities present in the follicle wall and corpus luteum. It should be noted, however, that other biological evidence downplaying the anti-angiogenic properties of ADAMTS-1 has been reported. For instance, ADAMTS-1 expression has no apparent impact on vascular density in certain tumors (Masui *et al.* 2001), and the ADAMTS-1^{-/-} mouse features a paradoxical deficiency in adrenal medulla capillary formation (Shindo *et al.* 2000, Tang 2001). The role of ADAMTS-1 in angiogenesis thus may be complex and tissue-specific.

The present study is the first to report the primary structure of the ADAMTS-1 transcript and protein in a monoovulatory nonprimate species. Among the most highly evolutionarily conserved elements identified in the equine ADAMTS-1 protein is the region encompassing the thrombospondin type I domains and the spacer region. This region is found in ADAMTS proteins and the ECM-associated glycoproteins punctin (ADAMTSL-1), lacunin and papilin, and has recently been termed the 'papilin cassette' (Nardi *et al.* 1999, Kramerova *et al.* 2000, Hirohata *et al.* 2002). As the murine ADAMTS-1 papilin cassette has been demonstrated to function as an ECM-binding domain (Kuno & Matsushima 1998, Kuno *et al.* 1999), this cassette may permit the ADAMTS, ADAMTSL, lacunin and papilin protein families to associate with the ECM. In the case of ADAMTS-1, binding to the ECM presumably serves to target its proteolytic activity to where its substrates are located. As ADAMTS-1 has been shown to degrade the large aggregating proteoglycans aggrecan, brevican and versican (Sandy *et al.* 2001, Richards *et al.* 2002), it has been proposed that ADAMTS-1 could act upon follicular proteoglycans which inhibit certain growth factors and gonadotropins, and could thereby increase their bioactivity (Richards *et al.* 2002). Alternatively, proteoglycan cleavage could also impact upon cell migration by modulating cell-cell and cell-ECM adhesion, a function that

the ADAMTS-1 disintegrin domain may also perform by disrupting the binding of cellular integrins to ECM proteins (Salustri *et al.* 1999, Evans 2001). Consistent with this, equine granulosa cells become noticeably detached from each other and from their basement membrane within 12 h post-hCG, in a manner concurrent with ADAMTS-1 induction (Kerban *et al.* 1999b). This could serve to disperse the luteinizing granulosa cells within the mucoid web that accumulates in the antrum during the ovulatory process (Kerban *et al.* 1999b), thereby permitting proper formation of the corpus luteum. Perhaps ADAMTS-1 expression then persists in the corpus luteum so as to permit its formation, remodeling and/or regression. Validation of these hypotheses will obviously require the identification of the biologically relevant ovarian substrates of ADAMTS-1.

The coordinate regulation of PR and ADAMTS-1 mRNAs by hCG in equine granulosa cells during the initial, transient induction phase at 12 h post-hCG is in apparent agreement with the proposed function of PR as a regulator of ADAMTS-1 expression in rodents (Espey *et al.* 2000, Robker *et al.* 2000a,b). However, the absence of a significant secondary rise in PR in granulosa cells and of a significant increase of PR mRNA in theca interna preclude any definitive association between the two transcripts in the equine follicle. Additional studies involving larger numbers of follicles and/or investigating the regulation of ADAMTS-1 and PR proteins will help resolve this issue. Interestingly, a recent study in cattle, another monoovulatory species with a long ovulatory process (28–30 h post-gonadotropin-releasing hormone (GnRH)), demonstrated a biphasic pattern of PR mRNA regulation in cows, with primary and secondary increases observed at 6 and 24 h post-GnRH respectively (Jo *et al.* 2002). These data differ from what is reported herein in that the kinetics of PR mRNA regulation in equine granulosa cells were more drawn-out, likely reflecting the longer equine ovulatory process in mares (39–42 h post-hCG). Also, the elevated levels of PR expression observed before hCG treatment in mares (i.e. at 0 h) were not observed in cows. This may be caused by the unusually progressive preovulatory rise in circulating gonadotropin levels that occurs in horses (Daels & Hughes 1993), which could result in PR expression before the ovulatory process is induced. Why this does not in turn lead

to appreciable ADAMTS-1 expression in early preovulatory follicle granulosa cells is unknown, and may involve a transcriptional repression mechanism to prevent premature ADAMTS-1 expression.

In summary, this study describes for the first time the structure of the equine ADAMTS-1 transcript, as well as its distinct regulatory patterns in two separate follicular cell types during the hCG-induced ovulatory process. The biphasic pattern of ADAMTS-1 mRNA regulation in granulosa cells suggests the presence of both early and late hormonally induced gene regulatory events, at least in species with long ovulatory processes. In this regard, the exceptionally protracted equine ovulatory process permitted a high-resolution timecourse analysis of gene regulation. This study therefore highlights the value of the horse as a model system for the study of ovarian gene regulation, a model which will certainly serve to further elucidate the role of ADAMTS-1 in monoovulatory ovarian physiology.

Acknowledgements

The authors would like to thank Dr D Vaillancourt (Université de Montréal, Canada) for his generous gift of uterine biopsy samples, as well as Dr A K Goff (Université de Montréal, Canada) for his assistance with statistical analyses. This study was supported by Natural Sciences and Engineering Research Council of Canada grant OPG0171135, and National Institutes of Health grants NIH-HD-16229 and SCCPRR-HD-07495. D B is supported by a Fellowship from the Canadian Institutes of Health Research (CIHR), J S is supported by a CIHR Investigator Award.

References

- Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W & Lipman DJ 1997 Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25** 3389–3402.
- Boerboom D & Sirois J 1998 Molecular characterization of equine prostaglandin G/H synthase-2 and regulation of its messenger ribonucleic acid in preovulatory follicles. *Endocrinology* **139** 1662–1670.
- Boerboom D & Sirois J 2001 Equine P450 cholesterol side-chain cleavage and 3 beta-hydroxysteroid dehydrogenase/delta(5)-delta(4) isomerase: molecular cloning and regulation of their messenger ribonucleic acids in equine follicles during the ovulatory process. *Biology of Reproduction* **64** 206–215.

- Boerboom D, Kerban A & Sirois J 1999 Dual regulation of promoter II- and promoter I-derived cytochrome P450 aromatase transcripts in equine granulosa cells during human chorionic gonadotropin-induced ovulation: a novel model for the study of aromatase promoter switching. *Endocrinology* **140** 4133–4141.
- Boerboom D, Pilon N, Behdjani R, Silversides DW & Sirois J 2000 Expression and regulation of transcripts encoding two members of the NR5A nuclear receptor subfamily of orphan nuclear receptors, steroidogenic factor-1 and NR5A2, in equine ovarian cells during the ovulatory process. *Endocrinology* **141** 4647–4656.
- Curry TE Jr & Osteen KG 2001 Cyclic changes in the matrix metalloproteinase system in the ovary and uterus. *Biology of Reproduction* **64** 1285–1296.
- Curry TE Jr, Song L & Wheeler SE 2001 Cellular localization of gelatinases and tissue inhibitors of metalloproteinases during follicular growth, ovulation, and early luteal formation in the rat. *Biology of Reproduction* **65** 855–865.
- Daels PF & Hughes JP 1993 The normal estrous cycle. In *Equine Reproduction*, pp 121–132. Eds AO McKinnon & JL Voss. Philadelphia, PA: Lea & Febiger.
- Espey LL & Lipner H 1994 Ovulation. In *The Physiology of Reproduction*, pp 725–780. Eds E Knobil & JD Neill. New York: Raven Press.
- Espey LL, Yoshioka S, Russell DL, Robker RL, Fujii S & Richards JS 2000 Ovarian expression of a disintegrin and metalloproteinase with thrombospondin motifs during ovulation in the gonadotropin-primed immature rat. *Biology of Reproduction* **62** 1090–1095.
- Evans JP 2001 Fertilin beta and other ADAMs as integrin ligands: insights into cell adhesion and fertilization. *Bioessays* **23** 628–639.
- Hagglund AC, Ny A, Leonardsson G & Ny T 1999 Regulation and localization of matrix metalloproteinases and tissue inhibitors of metalloproteinases in the mouse ovary during gonadotropin-induced ovulation. *Endocrinology* **140** 4351–4358.
- Hirohata S, Wang LW, Miyagi M, Yan L, Seldin MF, Keene DR, Crabb JW & Apte SS 2002 Punctin, a novel ADAMTS-like molecule, ADAMTSL-1, in extracellular matrix. *Journal of Biological Chemistry* **277** 12182–12189.
- Itoh T, Ikeda T, Gomi H, Nakao S, Suzuki T & Itohara S 1997 Unaltered secretion of beta-amyloid precursor protein in gelatinase A (matrix metalloproteinase 2)-deficient mice. *Journal of Biological Chemistry* **272** 22389–22392.
- Jo M, Komar C & Fortune J 2002 Gonadotropin surge induces two separate increases in messenger RNA for progesterone receptor in bovine preovulatory follicles. *Biology of Reproduction* **67** 1981–1988.
- Kerban A, Boerboom D & Sirois J 1999a Human chorionic gonadotropin induces an inverse regulation of steroidogenic acute regulatory protein messenger ribonucleic acid in theca interna and granulosa cells of equine preovulatory follicles. *Endocrinology* **140** 667–674.
- Kerban A, Dore M & Sirois J 1999b Characterization of cellular and vascular changes in equine follicles during hCG-induced ovulation. *Journal of Reproduction and Fertility* **117** 115–123.
- Kramerova IA, Kawaguchi N, Fessler LI, Nelson RE, Chen Y, Kramerov AA, Kusche-Gullberg M, Kramer JM, Ackley BD, Sieron AL, Prockop DJ & Fessler JH 2000 Papilin in development; a pericellular protein with a homology to the ADAMTS metalloproteinases. *Development* **127** 5475–5485.
- Kuno K & Matsushima K 1998 ADAMTS-1 protein anchors at the extracellular matrix through the thrombospondin type I motifs and its spacing region. *Journal of Biological Chemistry* **273** 13912–13917.
- Kuno K, Kanada N, Nakashima E, Fujiki F, Ichimura F & Matsushima K 1997 Molecular cloning of a gene encoding a new type of metalloproteinase-disintegrin family protein with thrombospondin motifs as an inflammation associated gene. *Journal of Biological Chemistry* **272** 556–562.
- Kuno K, Terashima Y & Matsushima K 1999 ADAMTS-1 is an active metalloproteinase associated with the extracellular matrix. *Journal of Biological Chemistry* **274** 18821–18826.
- Leonardsson G, Peng XR, Liu K, Nordstrom L, Carmeliet P, Mulligan R, Collen D & Ny T 1995 Ovulation efficiency is reduced in mice that lack plasminogen activator gene function: functional redundancy among physiological plasminogen activators. *PNAS* **92** 12446–12450.
- Liu K, Wahlberg P & Ny T 1998 Coordinated and cell-specific regulation of membrane type matrix metalloproteinase 1 (MT1-MMP) and its substrate matrix metalloproteinase 2 (MMP-2) by physiological signals during follicular development and ovulation. *Endocrinology* **139** 4735–4738.
- Lydon JP, DeMayo FJ, Funk CR, Mani SK, Hughes AR, Montgomery CA Jr, Shyamala G, Conneely OM & O'Malley BW 1995 Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes and Development* **9** 2266–2278.
- Masui T, Hosotani R, Tsuji S, Miyamoto Y, Yasuda S, Ida J, Nakajima S, Kawaguchi M, Kobayashi H, Koizumi M, Toyoda E, Tulachan S, Arii S, Doi R & Imamura M 2001 Expression of METH-1 and METH-2 in pancreatic cancer. *Clinical Cancer Research* **7** 3437–3443.
- Miles RR, Sluka JP, Halladay DL, Santerre RF, Hale LV, Bloem L, Thirunavukkarasu K, Galvin RJ, Hock JM & Onyia JE 2000 ADAMTS-1: a cellular disintegrin and metalloprotease with thrombospondin motifs is a target for parathyroid hormone in bone. *Endocrinology* **141** 4533–4542.
- Nardi JB, Martos R, Walden KK, Lampe DJ & Robertson HM 1999 Expression of lacunin, a large multidomain extracellular matrix protein, accompanies morphogenesis of epithelial monolayers in *Manduca sexta*. *Insect Biochemistry and Molecular Biology* **29** 883–897.
- Nishimori K & Matzuk MM 1996 Transgenic mice in the analysis of reproductive development and function. *Reviews of Reproduction* **1** 203–212.
- Pinter JH, Deep C & Park-Sarge OK 1996 Progesterone receptors: expression and regulation in the mammalian ovary. *Clinical Obstetrics and Gynecology* **39** 424–435.
- Richards JS 1994 Hormonal control of gene expression in the ovary. *Endocrine Reviews* **15** 725–751.
- Richards JS, Russell DL, Robker RL, Dajee M & Alliston TN 1998 Molecular mechanisms of ovulation and luteinization. *Molecular and Cellular Endocrinology* **145** 47–54.
- Richards JS, Russell DL, Ochsner S & Espey LL 2002 Ovulation: new dimensions and new regulators of the inflammatory-like response. *Annual Review of Physiology* **64** 69–92.
- Robker RL, Russell DL, Espey LL, Lydon JP, O'Malley BW & Richards JS 2000a Progesterone-regulated genes in the ovulation process: ADAMTS-1 and cathepsin L proteases. *PNAS* **97** 4689–4694.
- Robker RL, Russell DL, Yoshioka S, Sharma SC, Lydon JP, O'Malley BW, Espey LL & Richards JS 2000b Ovulation: a multi-gene, multi-step process. *Steroids* **65** 559–570.
- Rodriguez-Manzanique JC, Milchanowski AB, Dufour EK, Leduc R & Iruela-Arispe ML 2000 Characterization of METH-1/ADAMTS1 processing reveals two distinct active forms. *Journal of Biological Chemistry* **275** 33471–33479.
- Salustri A, Camaioni A, Di Giacomo M, Fulop C & Hascall VC 1999 Hyaluronan and proteoglycans in ovarian follicles. *Human Reproduction Update* **5** 293–301.
- Sambrook J, Fritsch EF & Maniatis T 1989 *Molecular Cloning: a Laboratory Manual*, edn 2. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Sandy JD, Westling J, Kenagy RD, Iruela-Arispe ML, Verscharen C, Rodriguez-Mazaneque JC, Zimmermann DR, Lemire JM,

- Fischer JW, Wight TN & Clowes AW 2001 Versican V1 proteolysis in human aorta *in vivo* occurs at the Glu441-Ala442 bond, a site that is cleaved by recombinant ADAMTS-1 and ADAMTS-4. *Journal of Biological Chemistry* **276** 13372–13378.
- Sato H, Takino T, Okada Y, Cao J, Shinagawa A, Yamamoto E & Seiki M 1994 A matrix metalloproteinase expressed on the surface of invasive tumour cells. *Nature* **370** 61–65.
- Shindo T, Kurihara H, Kuno K, Yokoyama H, Wada T, Kurihara Y, Imai T, Wang Y, Ogata M, Nishimatsu H, Moriyama N, Oh-hashii Y, Morita H, Ishikawa T, Nagai R, Yazaki Y & Matsushima K 2000 ADAMTS-1: a metalloproteinase-disintegrin essential for normal growth, fertility, and organ morphology and function. *Journal of Clinical Investigation* **105** 1345–1352.
- Simpson KS, Byers MJ & Curry TE Jr 2001 Spatiotemporal messenger ribonucleic acid expression of ovarian tissue inhibitors of metalloproteinases throughout the rat estrous cycle. *Endocrinology* **142** 2058–2069.
- Tang BL 2001 ADAMTS: a novel family of extracellular matrix proteases. *International Journal of Biochemistry and Cell Biology* **33** 33–44.
- Thompson JD, Higgins DG & Gibson TJ 1994 CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22** 4673–4680.
- Tsafirri A & Reich R 1999 Molecular aspects of mammalian ovulation. *Experimental and Clinical Endocrinology and Diabetes* **107** 1–11.
- Vazquez F, Hastings G, Ortega MA, Lane TF, Oikemus S, Lombardo M & Iruela-Arispe ML 1999 METH-1, a human ortholog of ADAMTS-1, and METH-2 are members of a new family of proteins with angio-inhibitory activity. *Journal of Biological Chemistry* **274** 23349–23357.
- Vu TH, Shipley JM, Bergers G, Berger JE, Helms JA, Hanahan D, Shapiro SD, Senior RM & Werb Z 1998 MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. *Cell* **93** 411–422.
- Wu CH, Huang H, Arminski L, Castro-Alvares J, Chen Y, Hu ZZ, Ledley RS, Lewis KC, Mewes HW, Orcutt BC, Suzek BE, Tsugita A, Vinayaka CR, Yeh LS, Zhang J & Barker WC 2002 The Protein Information Resource: an integrated public resource of functional annotation of proteins. *Nucleic Acids Research* **30** 35–37.

Received 8 May 2003

Accepted 6 August 2003