Histone deacetylase inhibition and progesterone act synergistically to stimulate baboon glycodeilin gene expression

Randal C Jaffe¹, Susan D Ferguson-Gottschall¹, Weihua Gao², Craig Beam² and Asgerally T Fazleabas³

Departments of ¹Physiology and Biophysics, ²Epidemiology and Biostatistics and ³Obstetrics and Gynecology, University of Illinois at Chicago, 835 South Wolcott, Chicago, Illinois 60612, USA

(Requests for offprints should be addressed to R C Jaffe; Email: rcjaffe@uic.edu)

Abstract

During the late luteal phase of the menstrual cycle and early pregnancy, the major secretory product of the uterine glandular epithelial cells in humans and non-human primates is glycodeilin. Previous studies using Ishikawa cells, a human endometrial cell line, have shown that a chimeric plasmid containing the baboon glycodeilin promoter responds to progestins but the response is modest compared with the induction of glycodeilin seen *in vivo* and in gene array analysis. A recent report indicating that the histone deacetylase inhibitor trichostatin A (TSA) promoted glycodeilin expression prompted us to examine its mechanism of action. In Ishikawa cells transfected with the baboon glycodeilin promoter, TSA and the synthetic progestin medroxyprogesterone acetate both stimulated expression of the reporter and the combined treatment produced a synergistic effect. The effect of TSA and progestin was absent when the same promoter constructs were transfected into COS-1 cells, a kidney cell line, and a TSA effect but no progestin effect was observed in T47D cells, a mammary cell line. Through deletion analysis, the TSA action was localized to the –67/–52 region of the baboon glycodeilin promoter, a region which contains the proximal Sp1 site. Deletions of this same region had no effect on progestin responsiveness. Our findings indicate that at least two regions of the glycodeilin promoter are important for the normal induction of glycodeilin expression. Non-target cells may lack factors which act on the response elements resulting in the restriction of expression to the appropriate target tissue.

Journal of Molecular Endocrinology (2007) **38**, 401–407

Introduction

Glycodeilin, a 28 kDa glycoprotein, is the most abundant secretory product of the human uterine glandular epithelial cells during the late luteal phase of the menstrual cycle and early pregnancy (Bell *et al.* 1985). In a non-human primate model, the baboon, glycodeilin synthesis begins in the mid-luteal phase of the menstrual cycle and increases dramatically during early pregnancy (Hausermann *et al.* 1998). Gene expression profiling has shown that glycodeilin is the most abundant transcript in the human endometrium during these phases (Kao *et al.* 2002, Borthwick *et al.* 2003, Riesewijk *et al.* 2003). In several conditions where the reproductive success rate is low, such as endometriosis, the expression of glycodeilin is drastically curtailed (Kao *et al.* 2003). Proposed functions for glycodeilin in reproduction include inhibition of sperm binding to the zona pellucida (Oehninger *et al.* 1995) and suppression of the immune response during early pregnancy (Bolton *et al.* 1987).

Progestins have been shown to induce glycodeilin expression in isolated human uterine epithelial cells (Taylor *et al.* 1998). Although computer analysis of the

human glycodeilin promoter identified a potential progesterone response element (Vaisse *et al.* 1990), functional studies have found that it is not involved in the progestin induction (Gao *et al.* 2001, Jaffe *et al.* 2003). In the human glycodeilin promoter, Gao *et al.* (2001) showed that the progestin-mediated induction is dependent on several Sp1 sites. In the structurally similar baboon glycodeilin promoter, we found that the Sp1 sites were not necessary for the progestin-mediated induction (Jaffe *et al.* 2003, 2006). The progestin-mediated induction was, however, blunted by inhibition of the extracellular signal regulated kinase (ERK)1/2 branch of the mitogen activated protein kinase (MAPK) pathway (Jaffe *et al.* 2006). In Ishikawa cells, the production of glycodeilin has also been shown to be induced by histone deacetylase (HDAC) inhibitors (Uchida *et al.* 2005).

In this manuscript, we report that in Ishikawa cells an HDAC inhibitor and progestin act synergistically on the baboon glycodeilin promoter. We find that the most proximal Sp1 element is sufficient and required for the trichostatin A (TSA) effect. In COS-1 cells, a kidney cell line from African green monkeys, both the synthetic progestin medroxyprogesterone acetate (MPA) and

TSA are ineffective while in T47D cells, a human mammary tumor cell line containing the progesterone receptor, TSA was effective but MPA was not in inducing reporter gene expression and there was no synergism between TSA and MPA. Thus, maximal expression of the glycodeclin gene relies on factors involved in the non-genomic pathway of progestin stimulation and inhibition of HDAC either of which may be restricted to target tissues providing a mechanism for the target tissue specific expression of glycodeclin in high amounts.

Materials and methods

Materials

pGL3Basic, β -galactosidase enzyme assay system, and luciferase assay system were purchased from Promega. pCMVSPORT- β -galactosidase, Dulbecco's modified Eagle medium (DMEM), phenol red-free DMEM, fetal bovine serum (FBS), and other tissue culture supplies were obtained from Invitrogen. TSA and bovine insulin were obtained from Sigma-Aldrich. The human progesterone receptor B (PRB) expression plasmid and progesterone response element (PRE)-luciferase plasmids were a gift from Dr Ming-Jer Tsai (Baylor College of Medicine, Houston, TX, USA).

Plasmid constructions

The generation of the 5' deletions was carried out by introducing NheI restriction sites into the $-68/+48$ region (the transcriptional start site numbered +1) of the baboon glycodeclin promoter within the pGL3Basic reporter plasmid as described previously (Jaffe *et al.* 2006) using the procedure of Ho *et al.* (1989). Each of the constructs was confirmed by sequencing. The 5' deletions were created by digesting with NheI and MluI and then the Klenow fragment of DNA polymerase I was used to fill in the overhangs. The plasmids were purified by agarose gel electrophoresis and ligated. All plasmids were sequenced to verify that the promoter had the expected deletions by the DNA Sequencing Facility of the UIC Research Resources Center.

Cell culture, transfection, and luciferase assay

Ishikawa and COS-1 cells were cultured in DMEM supplemented with 10% FBS, 100 IU/ml penicillin, and 100 μ g/ml streptomycin in a 37°C incubator with a humidified atmosphere and 5% CO₂. T47D cells were cultured in RPMI 1640 media containing 10% FBS, 0.2 U/ml bovine insulin, 100 IU/ml penicillin, and 100 μ g/ml streptomycin. On the day before the transfections, the cells were plated at a concentration of 1×10^5 cells/cm² in 12-well plates in phenol red-free DMEM

supplemented with 2% charcoal-stripped FBS (Ishikawa and COS-1 cells) or 5% charcoal-stripped FBS plus 0.2 U/ml bovine insulin (T47D cells), 100 U/ml penicillin, and 100 μ g/ml streptomycin. The cells were transfected in triplicate with plasmids using the calcium phosphate precipitation method (Sambrook *et al.* 1989) as we have described previously (Jaffe *et al.* 2003, 2006) with each well receiving 0.25 μ g pCMVSPORT- β -galactosidase, 1.25 μ g luciferase reporter plasmid, and 1.25 μ g PRB expression plasmid. We have previously shown that under our culture conditions our line of Ishikawa cells do not exhibit functional progesterone receptor activity (Jaffe *et al.* 2003, 2006). After a 4-h incubation with the plasmid, the cells were washed, glycerol shocked, and placed in the treatment media which included either 1 μ M MPA or an equal volume of the vehicle, ethanol, and either TSA at the indicated concentration or its vehicle, DMSO, for 24 h. The cell lysates were prepared in reporter lysis buffer and the luciferase activity was measured with the luciferase assay system and the β -galactosidase with the β -galactosidase enzyme assay system.

Statistical analysis

Data are expressed as the mean $\pm$ s.d. of three independent experiments. To stabilize the variance, log-transformed data were analyzed. To compare different conditions, ANOVA as well as Tukey's multiple comparison test were used. Differences were considered statistically significant at $P < 0.05$. Analyses were performed using statistical analysis system (SAS), mainly PROC ANOVA (version 9.1, SAS Institute, Cary, NC, USA).

Results

In Ishikawa cells, a human endometrial cell line, co-transfected with the $-67/+48$ Glycodeclin-pGL3Basic plasmid and PRB expression plasmid, the addition of the HDAC inhibitor TSA to the media for 24 h produced a concentration-dependent increase in luciferase expression (Fig. 1a). When compared with vehicle-treated cells (0 nM TSA), the addition of 250 or 500 nM TSA to the media produced a significant increase in luciferase expression. At the highest concentration of TSA utilized, 500 nM, there was an approximately 13-fold induction in luciferase levels. As depicted in the right half of Fig. 1a, the same concentration of TSA had no effect on cells transfected with the pGL3Basic plasmid and only a slight effect on cells transfected with the PRE plasmid (Fig. 1a, right panel). When Ishikawa cells were co-transfected with the $-67/+48$ Glycodeclin-pGL3Basic and PRB expression plasmids and treated with MPA, there was a greater than sixfold increase in luciferase expression (0 nM TSA lane Fig. 1c). Addition of both MPA and

Figure 1 Effect of TSA concentration on the responsiveness of the -67/+48Glycodeclin-pGL3Basic, pGL3Basic, and PRE reporter plasmids in the presence and the absence of MPA in Ishikawa cells. Ishikawa cells were co-transfected with the PRB expression plasmid, pCMVSPORT- β -galactosidase, and either the -67/+48Glycodeclin-pGL3Basic luciferase reporter plasmid, the pGL3Basic luciferase reporter plasmid, or the PRE luciferase reporter plasmid. The cells were treated with either (A) the indicated concentration of TSA or (B) and the indicated concentration of TSA and 1 μ M MPA for 24 h. The luciferase/ β -galactosidase was divided by the luciferase/ β -galactosidase for the cells treated alone for each of the reporter plasmids. (C) The ratio of the luciferase expression in the presence of MPA to the expression in the absence of MPA at each concentration of TSA is presented. Each bar represents the mean $\pm$ s.d. of the results from three independent experiments. Significant differences ($P < 0.05$) were determined by Tukey's multiple comparison test on log-transformed values. Bars with differing letters were significantly different from each other.

various concentrations of TSA to the media produced a synergistic induction of luciferase expression (Fig. 1b) with a 136-fold induction at the highest level of TSA tested. The response was dependent on the glycodeclin promoter as in Ishikawa cells transfected with the pGL3Basic vector, there was only a minor effect of TSA and MPA together (Fig. 1b). The response of the Ishikawa cells co-transfected with the -67/+48Glycodeclin-pGL3Basic and PRB expression plasmids to the synthetic progestin MPA was significant at all levels of TSA tested and the magnitude of the response was only slightly increased, from 6- to 11-fold, as the concentrations of TSA increased (Fig. 1c). This difference was only significant for the cells treated with 250 nM TSA as compared with the cells treated with vehicle alone. A reporter plasmid containing a PRE when co-transfected into the Ishikawa cells with the PRB expression

plasmid was highly responsive to progesterone (Fig. 1c, right-hand panel) but displayed no synergism between TSA and MPA (Fig. 1b, right-hand panel).

Computer analysis of the human (Vaisse *et al.* 1990) and baboon (Jaffe *et al.* 2003) glycodeclin promoter identified several potential response elements upstream of -67. We have previously shown (Jaffe *et al.* 2003) that this region is not required for the progestin response. To examine if this region plays a role in the responsiveness of the glycodeclin promoter to the HDAC inhibitor TSA, we examined the effects of MPA and TSA on larger constructs of the glycodeclin promoter (Fig. 2). The effect of TSA alone on the -2007/+48 and -368/+48Glycodeclin-pGL3Basic constructs of the baboon glycodeclin promoter was similar to the effect of TSA on the response of the -67/+48Glycodeclin-pGL3Basic construct. These

Figure 2 Effect of TSA and MPA on the responsiveness of the $-2007/+48$, $-368/+48$ and $-67/+48$ Glycodeclin-pGL3Basic reporter plasmids in Ishikawa cells. Ishikawa cells were co-transfected with the PRB expression plasmid, pCMVSPORT- β -galactosidase, and either the $-2007/+48$, $-368/+48$ or $-67/+48$ Glycodeclin-pGL3Basic luciferase reporter plasmid. The cells were treated with either 500 nM TSA or the DMSO vehicle and either 1 μ M MPA or the ethanol vehicle for 24 h. The luciferase/ β -galactosidase was divided by the luciferase/ β -galactosidase for the cells treated with the vehicles alone for each of the reporter plasmids in each of three separate experiments. Each bar represents the mean $\pm$ s.d. Significant differences ($P < 0.05$) were determined by Tukey's multiple comparison test on log-transformed values. For each of the constructs, bars with differing letters were significantly different from each other.

constructs were also responsive to MPA as we have previously reported (Jaffe *et al.* 2003). A synergistic response was observed when TSA and MPA were added to the media of Ishikawa cells co-transfected with the construct and the PRB. However, the $-2007/+48$ and the $-368/+48$ constructs exhibited no greater response than the $-67/+48$ region of the glycodeclin promoter.

COS-1 cells, a kidney cell line derived from African green monkeys, co-transfected with various constructs of the glycodeclin promoter ($-2007/+48$, $-368/+48$, and $-67/+48$) in pGL3Basic and the PRB expression vector do not exhibit a response to MPA (Fig. 3) as we have previously reported (Jaffe *et al.* 2003). TSA treatment of the COS-1 cells co-transfected with any of the glycodeclin promoter constructs and the PRB expression vector did not produce any response greater than controls. A synergistic response to TSA and MPA was obtained with the $-2007/+48$ and the $-67/+48$ Glycodeclin-pGL3 constructs but it was minor compared with that seen with the same constructs in Ishikawa cells. Similarly, MPA had no effect on the same glycodeclin promoter constructs in T47D cells, a human mammary ductal cell line (Fig. 4, left panel). This cell

Figure 3 Effect of TSA and MPA on the responsiveness of the $-2007/+48$, $-368/+48$ and $-67/+48$ Glycodeclin-pGL3Basic reporter plasmids in COS-1 cells. COS-1 cells were co-transfected with the PRB expression plasmid, pCMVSPORT- β -galactosidase, and either the $-2007/+48$, $-368/+48$ or $-67/+48$ Glycodeclin-pGL3Basic luciferase reporter plasmid. The cells were treated with either 500 nM TSA or the DMSO vehicle and either 1 μ M MPA or the ethanol vehicle for 24 h. The luciferase/ β -galactosidase was divided by the luciferase/ β -galactosidase for the cells treated with the vehicles alone for each of the reporter plasmids in each of three separate experiments. Each bar represents the mean $\pm$ s.d. Significant differences ($P < 0.05$) were determined by Tukey's multiple comparison test on log-transformed values. For each of the constructs, bars with differing letters were significantly different from each other.

line contains an endogenous PR and MPA greatly increases luciferase levels in T47D cells transfected with the PRE promoter (Fig. 4, right panel). Unlike in the COS-1 cells, the various baboon glycodeclin reporter constructs exhibited an increase in luciferase expression in response to TSA, but there was no synergism between TSA and MPA when the media contained both factors.

To determine the region of the glycodeclin promoter responsible for the TSA responsiveness in the Ishikawa cells, we made serial deletions from the 5' end of the $-67/+48$ Glycodeclin promoter. Deletion of 15 bp from the 5' end producing the $-52/+48$ glycodeclin promoter completely abolished the TSA responsiveness (Fig. 5). All further reductions had a response to TSA identical to that of the pGL3Basic vector alone. Removal of the region between -67 and -52 of the glycodeclin promoter had no effect on the responsiveness to MPA (Fig. 6). Further 10 bp deletions of this region of the baboon glycodeclin promoter had little effect on the responsiveness to MPA with only the deletion to -22 having a significantly lower response to the synthetic progestin than the $-52/+48$ Glycodeclin construct.

Figure 4 Effect of TSA and MPA on the responsiveness of the -2007/+48, -368/+48 and -67/+48 Glycodeclin-pGL3Basic reporter plasmids in T47D cells. T47D cells were co-transfected with the PRB expression plasmid, pCMVSPORT- β -galactosidase, and either the -2007/+48, -368/+48 or -67/+48 Glycodeclin-pGL3Basic luciferase reporter plasmid. The cells were treated with either 500 nM TSA or the DMSO vehicle and either 1 μ M MPA or the ethanol vehicle for 24 h. The luciferase/ β -galactosidase was divided by the luciferase/ β -galactosidase for the cells treated with the vehicles alone for each of the reporter plasmids in each of three separate experiments. Each bar represents the mean $\pm$ s.d. Significant differences ($P < 0.05$) were determined by Tukey's multiple comparison test on log-transformed values. For each of the constructs, bars with differing letters were significantly different from each other.

Discussion

Glycodeclin is the major secretory product of the human (Bell *et al.* 1985) and non-human (Hausermann *et al.* 1998) primate uteri during the late luteal phase of the menstrual cycle and early pregnancy. Analysis of the expression profile using gene arrays (Kao *et al.* 2002, Borthwick *et al.* 2003, Riesewijk *et al.* 2003) shows that glycodeclin mRNA steady-state levels are also elevated. During the luteal phase of the menstrual cycle and in early pregnancy progesterone levels are elevated. Taylor *et al.* (1998) have shown in isolated human epithelial cells that progesterone is able to induce glycodeclin expression. In our previous studies (Jaffe *et al.* 2003, 2006) and those of Gao *et al.* (2001), it was shown that the progestin induction of the baboon and human glycodeclin promoter required the progesterone receptor and did not depend upon a canonical progesterone response element. The induction was retained when the DNA-binding domain of the progesterone receptor was mutated and did not depend on Sp1 response elements (Jaffe *et al.* 2006). Because the MAPK inhibitor PD98059 blunted the progestin responsiveness of the glycodeclin promoter, we proposed that the induction of the glycodeclin expression by the PR in a progestin-dependent fashion was non-genomic.

In our previous studies (Jaffe *et al.* 2003, 2006) and those of Gao *et al.* (2001), the glycodeclin promoter response to progestins was quite modest in comparison with the robust changes in expression that occur *in vivo* as seen on gene arrays (Kao *et al.* 2002, Borthwick *et al.*

Figure 5 Effect of progressive 5' deletions of the baboon glycodeclin promoter in pGL3Basic on the responsiveness to TSA in Ishikawa cells. pGL3Basic plasmids containing progressive 5' deletions of the baboon glycodeclin promoter were co-transfected into Ishikawa cells with pCMVSPORT- β -galactosidase. The cells were treated with either 500 nM TSA or the DMSO vehicle for 24 h. The luciferase/ β -galactosidase from the TSA-treated group was divided by the luciferase/ β -galactosidase from the DMSO vehicle-treated group. Each bar represents the mean $\pm$ s.d. from three different experiments. Significant differences ($P < 0.05$) were determined by Tukey's multiple comparison test on log-transformed values. Bars with differing letters were significantly different from each other.

Figure 6 Effect of progressive 5' deletions of the baboon glycodeilin promoter in pGL3Basic on the responsiveness to MPA in Ishikawa cells. pGL3Basic plasmids containing progressive 5' deletions of the glycodeilin promoter were co-transfected into Ishikawa cells with the PRB expression plasmid and pCMVSPORT- β -galactosidase. The cells were treated with either 1 μ M MPA or the ethanol vehicle for 24 h. The luciferase/ β -galactosidase from the MPA-treated group was divided by the luciferase/ β -galactosidase from the ethanol vehicle-treated group. Each bar represents the mean $\pm$ s.d. from three different experiments. Significant differences ($P < 0.05$) were determined by Tukey's multiple comparison test on log-transformed values. Bars with differing letters were significantly different from each other.

2003, Riesewijk *et al.* 2003). The recent report of Uchida *et al.* (2005) that the HDAC inhibitor TSA induced glycodeilin expression in Ishikawa cells prompted us to explore the interaction between these factors on the glycodeilin promoter. Our hypothesis was that the induction of glycodeilin expression seen *in vivo* was due to the multiple factors acting together.

Our finding that TSA increased luciferase expression in Ishikawa cells transfected with the $-67/+48$ region of the baboon glycodeilin promoter in a dose-dependent manner up to concentrations of 500 nM are consistent with the report of Uchida *et al.* (2005) on the effect of various concentrations of TSA on glycodeilin protein and mRNA levels in Ishikawa cells. The effect of TSA was not a promiscuous one as it had little effect on pGL3Basic, the vector in which the glycodeilin promoter resided, no effect on the glycodeilin promoter in COS-1 cells or on a reporter containing a PRE. At the maximum levels of TSA tested, 500 nM, the effect of TSA alone on the $-67/+48$ Glycodeilin-pGL3Basic reporter plasmid in Ishikawa cells was slightly greater than the effect of the progestin alone. When both MPA and TSA were added to the media there was a significant synergistic effect. This effect was not due to a change in the effectiveness of MPA on the promoter. Within the $-67/+48$ region of the baboon glycodeilin promoter is a single Sp1 site at $-55/-50$. In various systems, HDACs have been shown to tether to the Sp1 transcription factor at the Sp1 response element (reviewed in Li *et al.* 2004). Our deletion analysis reveals that the region of the baboon glycodeilin

promoter containing the Sp1 element is critical for the TSA effect. In the larger fragments ($-2007/+48$ and $-368/+48$) of the baboon glycodeilin promoter tested there are two additional Sp1 elements. These elements do not seem to be important for the effect of TSA or for the synergism between MPA and TSA as neither of the larger fragments gave a better response than the $-67/+48$ fragment which contains a single Sp1 element.

Interestingly, in COS-1 cells, the same baboon glycodeilin promoter, previously shown in this cell line to be unresponsive to MPA, failed to show a response to TSA or a synergism between MPA and TSA. We previously showed that while the glycodeilin promoter will not respond to progestin in COS-1 cells, a PRE is still responsive to progestin (Jaffe *et al.* 2003). In T47D cells, a mammary ductal tumor cell line, which contains a PR there is also no response of the glycodeilin promoter to MPA. However, unlike the COS-1 cells, the T47D cells do exhibit a response to TSA but there is no synergism between MPA and TSA. We believe these findings imply that the cell specific expression of glycodeilin is due to specific factors in both the pathway by which progesterone is able to activate the glycodeilin gene, absent in both COS-1 and T47D cells, as well as the components responsible for increased glycodeilin expression in response to HDAC inhibition, absent in the COS-1 cells but not in the T47D cells. This may account for the previous observation that glycodeilin is expressed in the human mammary gland but it is not regulated by progesterone (Kamarainen *et al.* 1999).

It has been shown in a number of other genes that HDAC may interact with Sp1 which in turn is bound to the Sp1 element (reviewed in Li *et al.* 2004). Our results of the progressive deletion studies demonstrating that the deletion of the region between –67 and –52 of the glycodeilin promoter completely obliterates the response to TSA suggest this is the mechanism by which HDAC inhibition affects glycodeilin gene expression. That the TSA and progestin effects are independent is supported by the finding that deletions in this same region do not alter the responsiveness to progestin. Furthermore, since the level of expression of the reporter is elevated when the HDAC activity is inhibited but is not elevated when the site to which the HDAC is tethered is removed, we would hypothesize that the TSA induction of glycodeilin expression is not simply the removal of the HDAC but requires the subsequent recruitment of a histone acetyltransferase with subsequent acetylation of histones of the nucleosome.

Acknowledgements

We thank Dr Bruce Lessey for providing us with the Ishikawa cells. These studies were supported in part by NIH grant HD42280 to ATF. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References

- Bell SC, Hales MW, Patel S, Kirwan PH & Drife JO 1985 Protein synthesis and secretion by the human endometrium and decidua during early pregnancy. *British Journal of Obstetrics and Gynaecology* **92** 793–803.
- Bolton AE, Pockley AG, Clough KJ, Mowles EA, Stoker RJ, Westwood OM & Chapman MG 1987 Identification of placental protein 14 as an immunosuppressive factor in human reproduction. *Lancet* **1** 593–595.
- Borthwick JM, Charnock-Jones DS, Tom BD, Hull ML, Teirney R, Phillips SC & Smith SK 2003 Determination of the transcript profile of human endometrium. *Molecular Human Reproduction* **9** 19–33.
- Gao J, Mazella J, Seppala M & Tseng L 2001 Ligand activated hPR modulates the glycodeilin promoter activity through the Sp1 sites in human endometrial adenocarcinoma cells. *Molecular and Cellular Endocrinology* **176** 97–102.
- Hausermann HM, Donnelly KM, Bell SC, Verhage HG & Fazleabas AT 1998 Regulation of the glycosylated beta-lactoglobulin homolog, glycodeilin (placental protein 14:PP14) in the baboon (*Papio anubis*) uterus. *Journal of Clinical Endocrinology and Metabolism* **83** 1226–1233.
- Ho SN, Hunt HD, Horton RM, Pullen JK & Pease LR 1989 Site-directed mutagenesis by overlap extension using the polymerase chain reaction. *Gene* **77** 51–59.
- Jaffe RC, Donnelly KM & Fazleabas AT 2003 The induction of baboon glycodeilin expression by progesterone is not through Sp1. *Molecular Human Reproduction* **9** 35–40.
- Jaffe RC, Ferguson-Gottschall SD & Fazleabas AT 2006 Mitogen-activated protein kinase is involved in the progesterone-mediated induction of baboon glycodeilin. *Endocrine* **29** 121–127.
- Kamarainen M, Halttunen M, Koistinen R, von Boguslawsky K, von Smitten K, Andersson LC & Seppala M 1999 Expression of glycodeilin in human breast and breast cancer. *International Journal of Cancer* **83** 738–742.
- Kao LC, Tulac S, Lobo S, Imani B, Yang JP, Germeyer A, Osteen K, Taylor RN, Lessey BA & Giudice LC 2002 Global gene profiling in human endometrium during the window of implantation. *Endocrinology* **143** 2119–2138.
- Kao LC, Germeyer A, Tulac S, Lobo S, Yang JP, Taylor RN, Osteen K, Lessey BA & Giudice LC 2003 Expression profiling of endometrium from women with endometriosis reveals candidate genes for disease-based implantation failure and infertility. *Endocrinology* **144** 2870–2881.
- Li L, He S, Sun JM & Davie JR 2004 Gene regulation by Sp1 and Sp3. *Biochemistry and Cell Biology* **82** 460–471.
- Oehninger S, Coddington CC, Hodgen GD & Seppala M 1995 Factors affecting fertilization: endometrial placental protein 14 reduces the capacity of human spermatozoa to bind to the human zona pellucida. *Fertility and Sterility* **63** 377–383.
- Riesewijk A, Martin J, van Os R, Horcajadas JA, Polman J, Pellicer A, Mosselman S & Simon C 2003 Gene expression profiling of human endometrial receptivity on days LH + 2 versus LH + 7 by microarray technology. *Molecular Human Reproduction* **9** 253–264.
- Sambrook J, Fritsch EF & Maniatis T 1989. *Molecular Cloning: a Laboratory Manual*. 2, Cold Spring Harbor: Cold Spring Harbor Press.
- Taylor RN, Savouret JF, Vaisse C, Vigne JL, Ryan I, Hornung D, Seppala M & Milgrom E 1998 Promegestone (R5020) and mifepristone (RU486) both function as progestational agonists of human glycodeilin gene expression in isolated human epithelial cells. *Journal of Clinical Endocrinology and Metabolism* **83** 4006–4012.
- Uchida H, Maruyama T, Nagashima T, Asada H & Yoshimura Y 2005 Histone deacetylase inhibitors induce differentiation of human endometrial adenocarcinoma cells through up-regulation of glycodeilin. *Endocrinology* **146** 5365–5373.
- Vaisse C, Atger M, Potier B & Milgrom E 1990 Human placental protein 14 gene: sequence and characterization of a short duplication. *DNA and Cell Biology* **9** 401–413.

Received in final form 22 November 2006

Accepted 22 December 2006

Made available online as an Accepted Preprint 3 January 2007