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Mitogen-Activated Protein Kinase Is Involved in the Progesterone-Mediated Induction of Baboon Glycodelin

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In the human and non-human primate the major secretory product of the uterine glandular epithelial cells is glycodelin. The expression of glycodelin is associated with elevated progesterone levels as its production peaks during the late luteal phase of the menstrual cycle and in early pregnancy. Consistent with our previous studies, we found that the majority of the progestin responsiveness of the baboon glycodelin promoter was retained in the –20/+48 region, a region devoid of progestin- and Sp1-response elements. Using serial 5' and 3' deletions of 10 basepairs of the promoter within the pGL3Basic vector, we identified the 5' and 3' limits required for progestin responsiveness as –22 and +18, respectively. When the same regions were cloned into the pGL3Promoter vector, a construct that contains the heterologous SV40 promoter, progestin did not enhance expression. Mutation of the DNA binding domain of the progesterone receptor, which disrupts its ability to activate the progesterone response element, does not obliterate its ability to induce expression via the baboon glycodelin promoter. Inhibitors of protein tyrosine kinases, genistein and AG18, blocked the progestin-mediated induction as did an inhibitor of MEK, PD98059, but not an inhibitor of p38 MAP kinase, SB202190. These findings imply that glycodelin induction in response to progestins involves a nongenomic mechanism through the ERK1/2 branch of the MAP kinase pathway. The ultimate target may be a factor involved in the initiation of glycodelin gene transcription.

Key Words: Baboon; gene regulation; glycodelin; uterus.

Introduction

During the late luteal phase of the menstrual cycle and in early pregnancy, glycodelin is the major secretory product of the glandular epithelial cells of the human uterus *(1)*. A similar pattern of production occurs in the baboon, a nonhuman primate *(2)*. Gene expression profiling of the human endometrium has shown that glycodelin is also the most highly expressed gene at this time *(3–5)*. Glycodelin has been proposed to function as an immunosuppressive agent during early pregnancy *(6)* or as an inhibitor of sperm binding to the zona pellucida *(7)*. In endometriosis, a condition associated with progesterone resistance, the expression of glycodelin is dramatically reduced, and it has been proposed that this contributes to the impaired implantation and low fertility rates frequently seen in patients with this disease *(8)*. A similar impairment in glycodelin production is seen in the baboon in which endometriotic lesions have been induced experimentally *(9)*.

Taylor et al. *(10)* showed that progestins are able to induce glycodelin expression in isolated human epithelial cells. Although computer analysis of the human glycodelin gene identified a potential progesterone response element *(11)*, functional analysis of both the human *(12)* and baboon *(13)* glycodelin promoters have demonstrated that it does not play a role in the progestin-mediated response. Gao et al. *(12)* identified several Sp1 sites in the human glycodelin gene as being key to the progestin-mediated induction. However, in the baboon promoter, these sites are not involved in the progestin-mediated induction of the glycodelin gene *(13)*. In this study, we have identified the minimal baboon promoter required for the progestin-mediated induction of the glycodelin gene. Furthermore, we show the mechanism by which the progesterone receptor acts on the baboon glycodelin gene is nongenomic and involves the MAPK pathway.

Results

The baboon glycodelin promoter region previously shown *(13)* to be sufficient for the progesterone-mediated induction (–68/+48) was analyzed for potential transcription factor binding sites (Fig. 1). Seven potential transcription factor binding sites were identified using TFSEARCH (http://www.cbrc.jp/research/db/TFSEARCH.html): A GC box (–59/–46), three ADR1 (alcohol dehydrogenase gene regulator 1) sites (–32/–37, –29/–34, and –15/–20), a Lyf-1 (lymphoid transcription factor-1) site (–12/–20), a NF-E2

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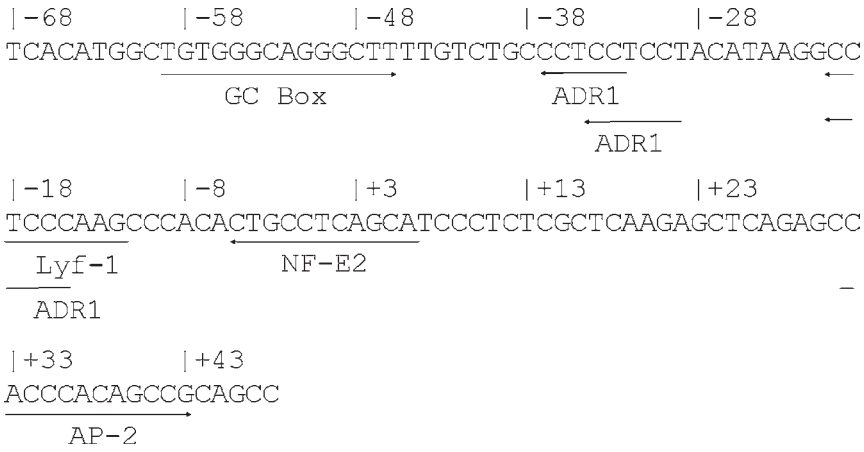

Fig. 1. Nucleotide sequence of baboon glycodelin promoter and potential enhancer elements. ADR1, alcohol dehydrogenase gene regulator 1; AP-2, activator protein 2; Lyf-1, lymphoid transcription factor-1; NF-E2, nuclear factor erythoid 2.

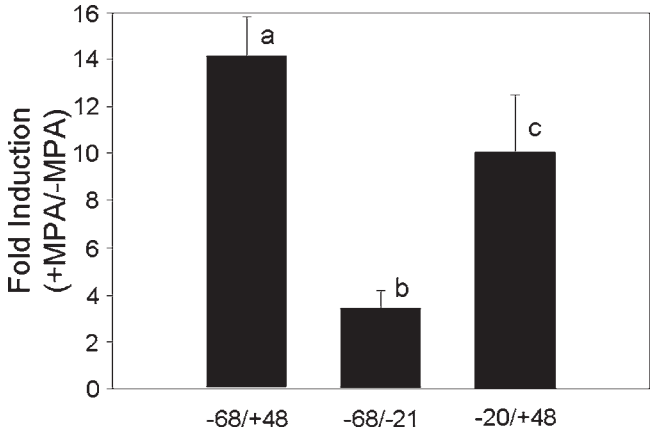

Fig. 2. MPA responsiveness of subfragments of the –68/+48 region of the baboon glycodelin promoter in pGL3-Basic. The –68/+48, –68/+21, or –20/+48 containing pGL3-Basic was co-transfected with the PRB expression plasmid and pCMV Sport-βgalactosidase into Ishikawa cells. The cells were treated with either 1 μmol/L medroxyprogesterone acetate (MPA) or the ethanol vehicle for 24 h. The fold increase was calculated by dividing the luciferase/β-galactosidase in the MPA-treated wells by the luciferase/β-galactosidase in the untreated wells and is expressed as the mean ± SD of three different experiments. The significance of the differences ($p < 0.05$) between the groups was determined by ANOVA and the Student–Newman–Keuls test. Bars with differing letters were significantly different from each other.

(nuclear factor erythroid 2) site (+6/–5), and an AP2 (activator protein 2) site (+35/+43).

In order to more precisely define the region necessary for the progesterone-mediated response, and thereby the potential transcription factors involved, we began by dividing the glycodelin promoter at a unique StuI site (–23/–18). Each of the parts (–68/–21 and –20/+48) were ligated into pGL3-Basic and analyzed for their ability to promote luciferase reporter production in a progestin-dependent fashion. As can be seen in Fig. 2, the –20/+48 region retained a much greater responsiveness to the synthetic progestin medroxyprogesterone acetate (MPA) than the –68/–21 region. Within the –20/+48 region, there are five computer-identified response elements—Lyf-1, NF-E2, AP-2, and ADR1 (Fig. 1). None of these elements have previously been identified as being involved in progesterone-mediated regulation of gene expression.

To precisely define the minimal region of the glycodelin promoter that promulgates the progesterone response, progressive 10 bp deletions from the 5' (Fig. 3) and 3' (Fig. 4) ends of the –68/+48 region in pGL3-Basic were made. As shown in Fig. 3, the –12/+48 region in pGL3-Basic of the glycodelin promoter is significantly ($p < 0.05$) weaker in promoting reporter production in response to MPA than the –22/+48 region in pGL3-Basic. Although the –12/+48 region in pGL3-Basic was significantly better at responding to MPA than the pGL3-Basic reporter plasmid without any part of the glycodelin promoter, it was not significantly better than any of the other constructs containing progressively less of the glycodelin promoter. Serial 10 bp deletions from the 5' end beyond –12 in pGL3-Basic were no better at promoting reporter expression in response to MPA than the empty pGL3-Basic reporter vector.

The MPA responsiveness of the reporter constructs containing the –68/+48, –68/+28, and –68/+18 regions of the glycodelin promoter in pGL3-Basic were not significantly different from each other (Fig. 4). A further removal of 10 bp leaving the region –68/+8 in pGL3-Basic resulted in a construct that was significantly weaker in responding to MPA than the full length (–68/+48) construct in pGL3-Basic but was not significantly different than the reporter plasmid containing the –68/+28 and –68/+18 regions of the baboon glycodelin promoter in pGL3-Basic. All the constructs containing even greater deletions at the 3' end of the glycodelin promoter (–68/–33, –68/–23, –68/–13, or –68/–3) in pGL3-Basic exhibited a progesterone responsiveness that was significantly less than that obtained with reporter constructs containing the –68/+18, –68/+28, and –68/+48 region of the glycodelin promoter in pGL3-Basic but they were not significantly different from each other.

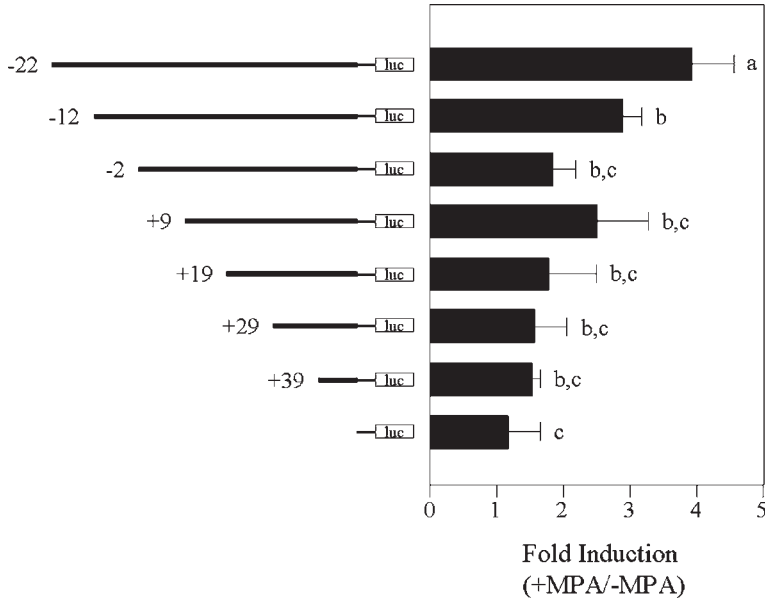

Fig. 3. Effect of progressive 5' deletions of the baboon glycodelin gene in the pGL3-Basic vector on MPA responsiveness in Ishikawa cells. pGL3-Basic plasmids containing progressive 5' deletions of the baboon glycodelin promoter–luciferase plasmid were cotransfected with the PRB expression plasmid and pCMV Sport-βgalactosidase into Ishikawa cells. The cells were treated either with 1 μmol/L MPA or ethanol vehicle for 24 h. The luciferase/β-galactosidase from the MPA treated group was divided by the luciferase/β-galactosidase for the ethanol vehicle group. Each bar represents the mean ± the SD of three different experiments. Significant differences ($p < 0.05$) were determined by ANOVA and Student–Newman–Keuls test. Bars with differing letters denote significant differences.

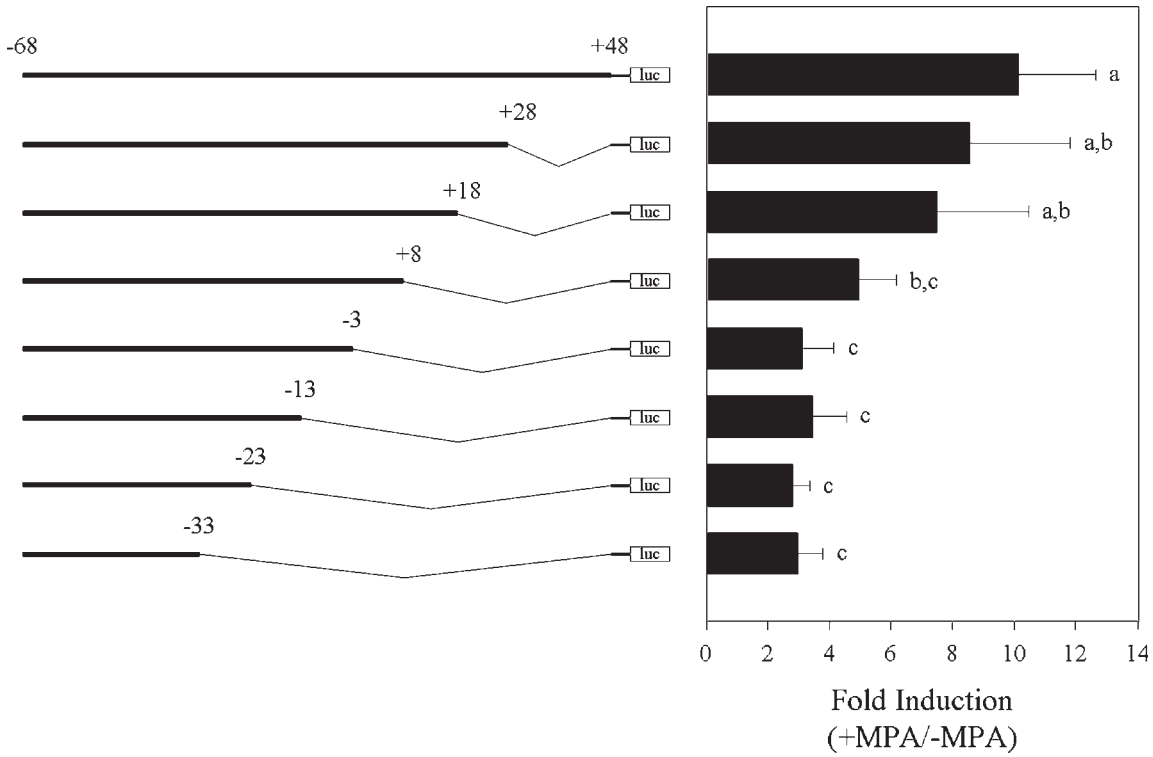

Fig. 4. Effect of progressive 3' deletions of the baboon glycodelin gene in the pGL3-Basic vector on MPA responsiveness in Ishikawa cells. pGL3-Basic plasmids containing progressive 3' deletions of the baboon glycodelin promoter–luciferase plasmid were cotransfected with the PRB expression plasmid and pCMV Sport-βgalactosidase into Ishikawa cells. The cells were treated either with 1 μmol/L MPA or ethanol vehicle for 24 h. The luciferase/β-galactosidase from the MPA-treated group was divided by the luciferase/β-galactosidase for the ethanol vehicle group. Each bar represents the mean ± the SD of four different experiments. Significant differences ($p < 0.05$) were determined by ANOVA and Student–Newman–Keuls test on log-transformed values to normalize the variance. Bars with differing letters denote significant differences.

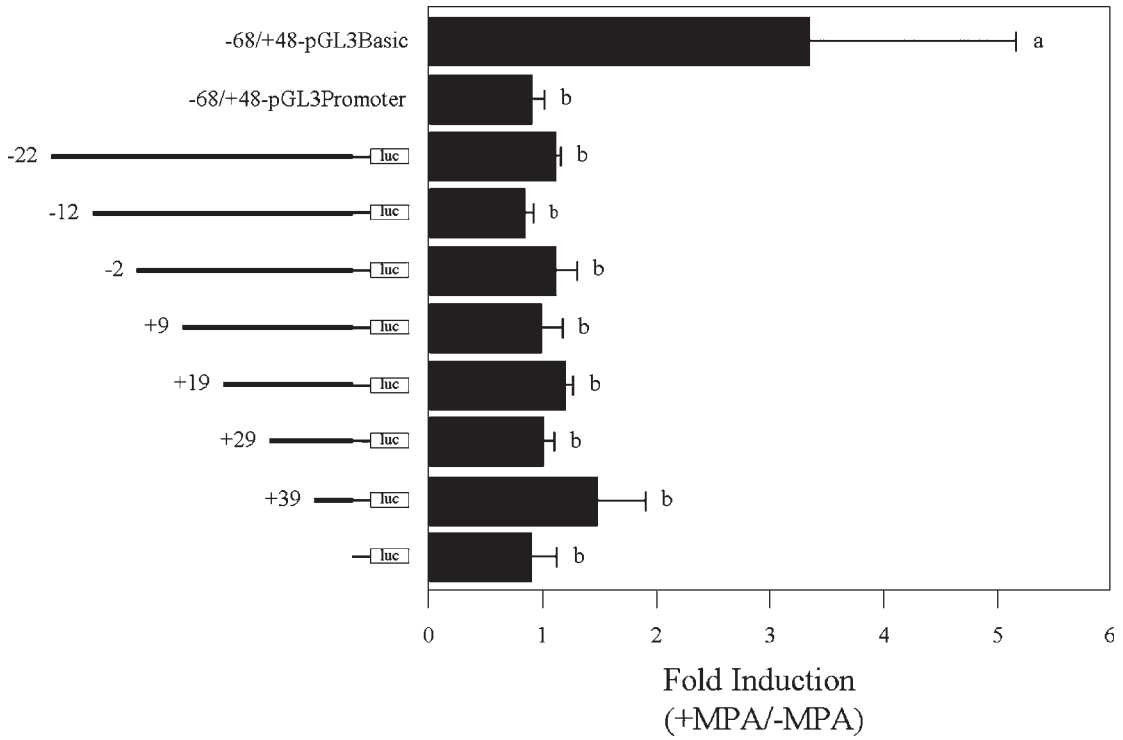

Fig. 5. Effect of progressive 5' deletions of the baboon glycodelin gene in the pGL3-Promoter vector on MPA responsiveness in Ishikawa cells. pGL3-Promoter plasmids containing progressive 5' deletions of the baboon glycodelin promoter–luciferase plasmid or the –68/+48 glycodelin promoter in pGL3-Basic were co-transfected with the PRB expression plasmid and pCMV Sport-βgalactosidase into Ishikawa cells. The cells were treated either with 1 μmol/L MPA or ethanol vehicle for 24 h. The luciferase/β-galactosidase from the MPA treated group was divided by the luciferase/β-galactosidase for the ethanol vehicle group. Each bar represents the mean ± the SD of three different experiments. Significant differences ($p < 0.05$) were determined by ANOVA and Student–Newman–Keuls test. Bars with differing letters denote significant differences.

Within the sequence identified above as the progestin-responsive region using progressive deletions from the 5' and 3' ends of the glycodelin promoter in pGL3-Basic of the glycodelin promoter is the transcription start site. To isolate the effect of the deletions on the initiation of transcription from an enhancer activity, we analyzed the consequence of progressive deletions within the context of the pGL3-Promoter vector. As can be seen in Fig. 5, none of the glycodelin promoter constructs in the pGL3-Promoter vector exhibited a progestin-enhanced expression. In the same experiments, the –68/+48 glycodelin promoter in pGL3-Basic enhanced the luciferase expression in a progestin-dependent manner.

The absence of a progesterone response element (PRE) in the region of the glycodelin promoter sufficient to impart a progesterone-mediated response could mean that the progesterone receptor acts on another gene whose product induces glycodelin expression. We examined this possibility by determining whether the ability of the progesterone receptor to mediate the progesterone response was compromised if the DNA binding region was mutated. As expected, the mutated progesterone receptor was drastically weaker than the wild-type progesterone receptor at inducing reporter gene expression driven by a PRE in a progesterone-dependent manner (Fig. 6). The mutant progesterone receptor,

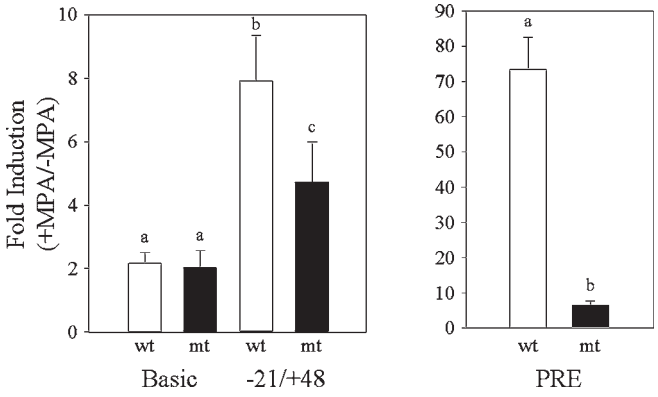

Fig. 6. Effect of mutating the DNA binding domain of the progesterone receptor on the MPA induction of reporter gene expression. Ishikawa cells were cotransfected with the –21/+48 glycodelin promoter–pGL3 Basic luciferase chimeric plasmid (left panel) or PRE luciferase chimeric plasmid (right panel), pCMV Sport-βgalactosidase, and either the wild-type (wt) PRB or mutant (mut) PRB expression plasmid and treated with either 1 μ*M* MPA or the ethanol vehicle for 20 h. The luciferase/β-galactosidase from the MPA-treated group was divided by the luciferase/β-galactosidase for the ethanol vehicle group. Each bar represents the mean ± the SD of three different experiments. Significant differences ($p < 0.05$) were determined by ANOVA and Student–Newman–Keuls test. Bars with differing letters denote significant differences.

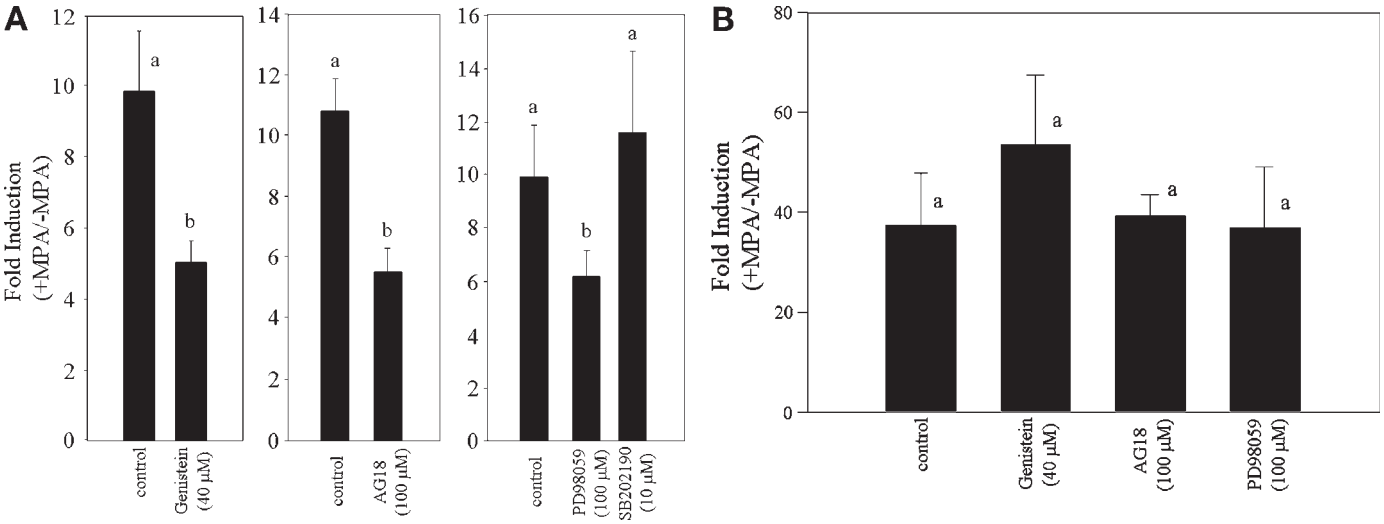

Fig. 7. Effect of protein kinase inhibitors on the MPA induction of baboon glycodelin promoter–driven reporter gene expression. Ishikawa cells were co-transfected with the **(A)** –21/+48 baboon glycodelin promoter–pGL3-Basic luciferase reporter plasmid or **(B)** the PRE luciferase reporter plasmid, the PRB expression plasmid, and pCMV Sport-βgalactosidase. The cells were treated with either 1 μ*M* MPA or the ethanol vehicle and the indicated inhibitor for 20 h. The luciferase/β-galactosidase from the MPA-treated group was divided by the luciferase/β-galactosidase for the ethanol vehicle group. Each bar represents the mean ± the SD of three different experiments. Significant differences ($p < 0.05$) were determined by ANOVA and Student–Newman–Keuls test. Bars with differing letters denote significant differences.

however, was still able to induce reporter gene expression in a progestin-dependent manner when the pGL3-Basic reporter plasmid contained –21/+48 of the glycodelin promoter. The progesterone-dependent induction obtained with the mutant progesterone receptor from the –21/+48 region of the glycodelin promoter in pGL3-Basic was significantly lower, however, than that obtained with the wild-type progesterone receptor.

Finally, we examined if a nongenomic mechanism could explain the progesterone receptor–mediated glycodelin induction. Two broad spectrum protein tyrosine kinase inhibitors, genistein and AG18, when added to the media of Ishikawa cells transfected with PRB and –21/+48–pGL3-Basic, at least partially blocked the MPA-dependent induction of the reporter luciferase (Fig. 7A). The inhibition could be duplicated by a specific inhibitor of MAP kinase kinase (MEK), PD98059, but not by a specific inhibitor of p38 MAP kinase, SB202190. The protein kinase inhibitors genistein and AG18 and the MEK inhibitor PD98059 had no effect on the MPA induction of the PRE-containing reporter plasmid (Fig. 7B).

Discussion

In our previous study *(13)*, we found that the –68/+48 region of the baboon glycodelin promoter was sufficient for the progesterone response. Within this region lies a GC box to which the nuclear transcription factor Sp1 can bind. This site was shown *(12)* to be important for the progesterone regulation of the human glycodelin gene. However, our results *(13)* using site-directed mutagenesis indicated that this potential Sp1 binding site is not involved in progestin-mediated induction of the baboon glycodelin promoter. Our finding in this article that the region from –68/–21 retains little of the promoter activity is consistent with our previous conclusion that the GC box in the baboon glycodelin promoter is not involved in the progesterone-mediated induction. Within the region (–20/+48) that retains the progesterone responsiveness, computer analysis identified several potential response element motifs but none of these motifs have previously been associated with progesterone-mediated gene expression.

Serial deletions from the 5' and 3' ends of the –68/+48 fragment in pGL3-Basic were used to pinpoint the region of the baboon glycodelin promoter responsible for the progesterone responsiveness. The minimal element identified, –22/+18, is rather broad which may indicate that more than one transcription factor is involved in the induction of the baboon glycodelin gene. Computer analysis of this region revealed that it contains potential binding sites for Lyf-1, ADR1, and NF-E2. We could find no evidence in the literature that any of these transcription factors are involved in the mechanism of action of progesterone. Because this region encompasses the transcription start site, the deletions may have reflected the impairment of transcription initiation. To analyze this, we cloned the –68/+48 region of the glycodelin promoter and various 5' deletions within the context of the pGL3-Promoter vector where the glycodelin promoter is upstream of the SV40 promoter. Within this vector, transcription of the luciferase gene would be initiated from the SV40 promoter rather than the glycodelin transcription initiation site. All the constructs of the glycodelin

promoter within the pGL3-Promoter vector were unresponsive to progestin. We interpret this to mean that the factor involved in the progestin induction plays a role in the initiation of transcription from the glycodelin promoter.

In the absence of an apparent progesterone response element or a response element for transcription factors with which the progesterone receptor is known to interact a potential mechanism to account for the progesterone responsiveness of the baboon glycodelin gene is that the progesterone receptor induces a transcription factor which in turn induces expression of the glycodelin gene. This was explored by studying how mutation of the DNA binding domain of the progesterone receptor affected hormone-mediated induction of our reporter–pGL3-Basic constructs. The mutation that we introduced within the DNA binding domain of the progesterone receptor by site-directed mutagenesis dramatically reduced the ability of the synthetic progestin MPA to induce reporter gene expression driven by the progesterone responsive element. This was expected because the amino acids that were altered are important for direct genomic induction. However, this same mutated progesterone receptor was still able to drive reporter expression in a hormone-dependent manner when the –21/+48 region of the baboon glycodelin promoter was present in the pGL3-Basic vector. This would seem to rule out the possibility that the absence of an effect on the PRE was due to the mutated form of the PRB misfolding. The slight but significant reduction in effectiveness of the mutated progesterone receptor on the glycodelin promoter as compared to the effectiveness of the wild-type receptor could indicate that a portion of the induction is dependent on the binding of the progesterone receptor to its response element. However, we cannot completely rule out a technical explanation such as a lower level of expression of the mutated progesterone receptor or its presence as a less stable form.

The failure to demonstrate a direct genomic effect of the progesterone receptor on the baboon glycodelin promoter led us to entertain the possibility that a nongenomic mechanism is involved. To address this we performed inhibitor studies and found two different broad spectrum inhibitors of protein tyrosine kinases, genistein and AG18, significantly reduced the ability of progesterone to induce reporter gene expression. A comparable level of inhibition was seen in cells incubated with the MEK inhibitor PD98059 but not the p38 MAPK inhibitor SB202190.

Much of the focus on the mechanism of action of members of the steroid receptor superfamily, which includes the progesterone receptor, has been the receptor–response element interaction. However, there is increasing evidence in the literature that steroids may also act via a nongenomic mechanism. Using a knock-in model *(14)* in which the DNA binding domain of the estrogen receptor α was mutated, certain actions associated with estrogen acting through the estrogen receptor α were not altered, implying that the estrogen receptor may have nongenomic actions in addition to its genomic actions. Part of the nongenomic actions of the estrogen receptor may be due to effects on the mitogen-activated protein (MAP) kinase pathway *(15,16)*. The progesterone receptor has also been reported to affect certain processes through the MAP kinase pathway via an interaction with the estrogen receptor *(17)*. In addition, it was recently reported that the progesterone receptor may affect the MAP kinase pathway via interaction with c-Src *(18)*. Our data lead us to hypothesize that the progesterone receptor controls the expression of at least one progestin responsive gene, glycodelin, via the MAP kinase pathway.

As computer and functional analysis of the promoter region of progesterone responsive genes is carried out, more cases may be found where the responsive gene either does not contain a progesterone response element or the elimination of the putative progesterone responsive element does not impact the progesterone responsiveness of the gene. Our results point to the ERK1/2 branch of the MAPK family pathway as another potential mechanism. Our focus is now directed toward identifying the member of the MAPK pathway with which the progesterone receptor interacts and the target of ERK1/2 that directs the induction of the baboon glycodelin promoter.

Materials and Methods

Materials

pGL3-Basic, pGL3-Promoter, β-Galactosidase Enzyme Assay System, and Luciferase Assay System were purchased from Promega (Madison, WI). pCMV Sport-βgalactosidase, DMEM, phenol-red-free DMEM, fetal bovine serum, and other tissue culture supplies were obtained from Invitrogen (Carlsbad, CA). The QuikChange Site-Directed Mutagenesis Kit was purchased from Stratagene (La Jolla, CA). Genistein, AG18, PD98059, and SB202190 were purchased from Calbiochem (La Jolla, CA). The human PRB expression plasmid and PRE-luciferase plasmids were a gift from Dr. Ming-Jer Tsai (Baylor College of Medicine, Houston, TX).

Plasmid Constructions

*Nhe*I restriction sites were introduced into the –68/+48 region (the transcriptional start site numbered +1) of the glycodelin promoter within the pGL3-Basic reporter plasmid in 10 bp steps using the procedure of Ho et al. *(19)*. Each of the constructs was confirmed by sequencing. Deletions from the 5' end were generated by digestion with *Nhe*I and *Mlu*I and deletions from the 3' end were generated by digestion with *Nhe*I and *Bgl*II. After filling in the overhangs with the Klenow fragment of DNA polymerase I, the plasmids were purified by agarose gel electrophoresis and ligated. All plasmids were sequenced by the DNA Sequencing Facility of the Research Resources Center of UIC to verify that the promoter had the expected deletions. The unmodified –68/

+48 glycodelin promoter and promoters modified by the insertion of a *Nhe*I site generated above were excised from the pGL3-Basic plasmid by digestion with *Bgl*II and *Mlu*I. After gel purification, the modified and unmodified glycodelin promoters were ligated into pGL3-Promoter digested with *Bgl*II and *Mlu*I to generate compatible ends. Deletions from the 5' end were generated as above by digestion with *Nhe*I and *Bgl*II, filling in the overhangs with the Klenow fragment of DNA polymerase I, purification by agarose gel electrophoresis and ligation. The plasmids were sequenced to verify the deletions.

The mutant PRB was generated from the PRB expression plasmid using the QuikChange II Site-Directed Mutagenesis Kit. The sense primer began with nucleotide 1913 of GenBank accession number NM_000926 and was GGTG TCCTTACCTGTGCGGCCTGTAAGGTCTTCTTTAAG AGGGC and the antisense primer began with nucleotide 1956 and was GCCCTCTTAAAGAAGACCTTACAGGCCGCA CAGGTAAGGACACC (the changed bases are underlined). This converts the GSCKV of the DNA binding domain to AACKV. The plasmid was sequenced to verify the change.

Cell Culture, Transfection, and Luciferase Assay

Ishikawa cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 μg/mL streptomycin in a 37°C incubator with a humidified atmosphere and 5% CO_2. On the day before the transfections, the cells were plated at a concentration of 1.1×10^5 cells/cm^2 in 12-well plates in phenol-red-free DMEM supplemented with 2% charcoal stripped FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were transfected in triplicate with plasmids using the calcium phosphate precipitation method *(20)*. Each well received 0.25 μg pCMV Sport-βgalactosidase, 1.25 μg of the luciferase reporter plasmid, and 1.25 μg of the PRB expression plasmid. In our line of Ishikawa cells, MPA will not produce an induction of luciferase when the cells are transfected with a glycodelin promoter containing reporter plasmid unless the progesterone receptor is introduced by cotransfection, indicating our line lacks an endogenous progesterone receptor. After a 4 h incubation with the plasmid, the cells were washed, glycerol shocked, and placed in media containing either 1 μ*M* medroxyprogesterone acetate (MPA) or an equal volume of the vehicle, ethanol, for 24 h. In the experiments using kinase inhibitors, the compounds at the indicated concentration were added when the hormone treatment was initiated. The inhibitor concentrations utilized are approximately 10 times their IC_{50} values according to their manufacturer (Calbiochem). The cell lysates were harvested by scraping in reporter lysis buffer and the luciferase activity 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 ± SD of three or four separate experiments as indicated. When significant differences, $p < 0.05$, between the groups were present as determined by ANOVA, post-hoc analysis was performed with the Student–Newman–Keuls test. In cases where the variance failed a normalcy test, the data were subjected to log transformation before analysis.

Acknowledgments

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References

1. Bell, S. C., Hales, M. W., Patel, S., Kirwan, P. H., and Drife, J. O. (1985). *Br. J. Obstet. Gynaecol.* **92,** 793–803.
2. Hausermann, H. M., Donnelly, K. M., Bell, S. C., Verhage, H. G., and Fazleabas, A. T. (1998). *J. Clin. Endocrinol. Metab.* **83,** 1226–1233.
3. Kao, L. C., Tulac, S., Lobo, S., et al. (2002). *Endocrinology* **143,** 2119–2138.
4. Riesewijk, A., Martin, J., van Os, R., et al. (2003). *Mol. Hum. Reprod.* **9,** 253–264.
5. Borthwick, J. M., Charnock-Jones, D. S., Tom, B. D., et al. (2003). *Mol. Hum. Reprod.* **9,** 19–33.
6. Bolton, A. E., Pockley, A. G., Clough, K. J., et al. (1987). *Lancet* **1,** 593–595.
7. Oehninger, S., Coddington, C. C., Hodgen, G. D., and Seppala, M. (1995). *Fertil. Steril.* **63,** 377–383.
8. Kao, L. C., Germeyer, A., Tulac, S., et al. (2003). *Endocrinology* **144,** 2870–2881.
9. Fazleabas, A. T., Brudney, A., Chai, D., Langoi, D., and Bulun, S. E. (2003). *Fertil. Steril.* **80(Suppl. 2),** 820–827.
10. Taylor, R. N., Savouret, J. F., Vaisse, C., et al. (1998). *J. Clin. Endocrinol. Metab.* **83,** 4006–4012.
11. Vaisse, C., Atger, M., Potier, B., and Milgrom, E. (1990). *DNA Cell Biol.* **9,** 401–413.
12. Gao, J., Mazella, J., Seppala, M., and Tseng, L. (2001). *Mol. Cell Endocrinol.* **176,** 97–102.
13. Jaffe, R. C., Donnelly, K. M., and Fazleabas, A. T. (2003). *Mol. Hum. Reprod.* **9,** 35–40.
14. Jakacka, M., Ito, M., Martinson, F., Ishikawa, T., Lee, E. J., and Jameson, J. L. (2002). *Mol. Endocrinol.* **16,** 2188–2201.
15. Migliaccio, A., Di Domenico, M., Castoria, G., et al. (1996). *EMBO J.* **15,** 1292–1300.
16. Improta-Brears, T., Whorton, A. R., Codazzi, F., York, J. D., Meyer, T., and McDonnell, D. P. (1999). *Proc. Natl. Acad. Sci. USA* **96,** 4686–4691.
17. Migliaccio, A., Piccolo, D., Castoria, G., et al. (1998). *EMBO J.* **17,** 2008–2018.
18. Boonyaratanakornkit, V., Scott, M. P., Ribon, V., et al. (2001). *Mol. Cell.* **8,** 269–280.
19. Ho, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K., and Pease, L. R. (1989). *Gene* **77,** 51–59.
20. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). *Molecular cloning: a laboratory manual*. Cold Spring Harbor Press: Cold Spring Harbor, NY.