Activation of Protein Kinase C in Human Uterine Smooth Muscle Induces connexin-43 Gene Transcription through an AP-1 Site in the Promoter Sequence*

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Myometrial connexin-43 gap junctions are scarce throughout gestation but appear in large numbers at term to facilitate contractions during labor. The mechanisms that regulate this process are incompletely characterized. This report investigates the effects of protein kinase C activation on the regulation of connexin-43 gene transcription in human uterine smooth muscle cells. In primary myometrial cells treated with phorbol ester, transient increases in c-Fos and c-Jun protein levels were observed at 2–4 h, followed by significant increases in connexin-43 protein levels at 6–8 h. Nuclear run-on transcription analysis showed an increase in connexin-43 transcription 3 h after phorbol ester treatment. AP-1 sites were identified in the sequence of the 5′-flanking promoter region of the human connexin-43 gene at 44 and 1000 base pairs upstream of transcription start. Transcription from a reporter plasmid containing the proximal human connexin-43 promoter was increased in transfected primary cultures treated with phorbol ester. Mutation of the proximal AP-1 site in the promoter abolished the phorbol ester-dependent transactivation. This work provides evidence that transcription of the human connexin-43 gene is induced through protein kinase C activation in uterine smooth muscle cells, and that the induction involves up-regulation and activation of c-Jun and c-Fos.

Connexins compose a family of transmembrane proteins, which form hexameric channel-structures in cell membranes. When hemi-channels of adjacent cells attach, an intercellular channel is created, which allows for the passive diffusion of ionic mediators and secondary messengers of less than 1,500 daltons (Da) and establishes direct communication between connected cells via such small molecules. A specialized region of the membrane containing connexin channels is called a gap junction (1). Gap junctions allow for coordinate function of the cells in a tissue.

Several mammalian connexins cDNAs have been sequenced (2–4), and the proteins, named according to their respective molecular weights, appear to be tissue-specific. The primary connexin in cardiac muscle, osteoblasts, granulosa cells of the ovary, and vascular and uterine smooth muscle is connexin-43 (Cx43) (43,000 Da) (3, 5–7). Additionally, during mouse fetal development, temporal and spatial patterns of connexin-43 gene (cx43) expression occur (8). There is little information concerning the mechanisms that control connexin-43 gene expression or that govern tissue-specific differences in gene expression.

In adult tissues such as the heart muscle and bone, cx43 expression is relatively stable. In myometrium, Cx43 gap junctions are not evident except just before parturition when connexin-43 (cx43) mRNA levels increase dramatically (9). Cx43 gap junctions subsequently become abundant in myometrium (10–12) and are believed to play an essential role in successful deliveries by synchronizing contractions during labor through the passage of molecules such as [Ca2+] (13–15).

The mechanisms that regulate the sparsity of myometrial gap junctions during pregnancy and their abundance during parturition are unknown. Prior to labor, myometrial cx43 expression is relatively stable. In myometrium, cx43 gap junctions are not evident except just before parturition when connexin-43 (cx43) mRNA levels increase dramatically (9). Cx43 gap junctions subsequently become abundant in myometrium (10–12) and are believed to play an essential role in successful deliveries by synchronizing contractions during labor through the passage of molecules such as [Ca2+] (13–15).

The mechanisms that regulate the sparsity of myometrial gap junctions during pregnancy and their abundance during parturition are unknown. Prior to labor, myometrial cx43 expression is regulated in part by the steroid hormones, estrogen, and progesterone (10, 13, 16). Progesterone suppresses cx43 gene transcription and inhibits the trafficking of Cx43 protein through the Golgi apparatus (12, 16). Estrogen can induce premature cx43 expression and gap junction formation in the myometrium of pregnant animals (14, 17). However, cx43 expression only remains high in estrogen-treated myometrial cells if internal uterine pressure is maintained after delivery (10). These data indicate that estrogen acts with a factor(s) produced through uterine distension to influence myometrial cx43 gene expression. It is plausible that such a factor or factors are bio-reactive moieties derived from phospholipid breakdown upon extension of the smooth muscle membrane.

Diacylglycerol is an early product of inositol phospholipid breakdown in the cell membrane. Diacylglycerol is the major activator of protein kinase C (PKC) (18). The phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) also activates PKC. The PKC signal transduction cascade causes activation of cell-specific transcription factors, which in turn bind cognate sequence elements in gene promoters and modulate gene expression. Several cis-acting elements in the DNA have been identified, which regulate gene expression after activation of

* This work was supported in part by National Institutes of Health (NIH) Grant HD 30482 (to J. A.), a grant from the March of Dimes Birth Defects Foundation (to R. E. G.), and NIH Grant HL02391 (to G. I. F.). (Received for publication, June 3, 1996, and in revised form, July 9, 1996)
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PKC (for review see Ref. 19). The sequence of the 5′-flanking promoter region of the human cx43 gene revealed two types of cis-acting elements associated with PKC activation, AP-1 and AP-2 sites.

The AP-1 site is the cognate binding sequence for the Jun and Fos families of transcription factors (20, 21). Jun and Fos, products of “immediate-early” genes, induce the expression of the “late” genes which contain AP-1 sites. C-Jun and c-Fos are the members of the families which are primarily expressed in uterine smooth muscle cells (22–24). In general, activation of PKC results in increased expression of jun and fos genes and AP-1-mediated transcription is stimulated.

In this report, the role of c-Jun and c-Fos in cx43 gene regulation in uterine smooth muscle was investigated using primary cultures of human uterine smooth muscle cells composed of nonpregnancy myometrial cells, as well as uterine leiomyoma cells, myometrial-like benign tumors which express many estrogen-regulated genes expressed in pregnancy myometrium (see Ref. 25 for discussion). Upon culturing, nonpregnancy myometrial cells, as well as uterine leiomyoma cells, were used to detect Cx43 protein was purchased from Zymed Laboratories Inc. (San Francisco, CA). The mouse monoclonal antibody used to detect desmin intermediate filament protein (55,000 Da) was used to detect desmin in the cultured myometrial and leiomyoma cells according to the procedure of Chomczynski and Sacchi (34). The transcription start was determined by the primary extension method as described (33) using 20 µg of total RNA of each type. The final sequencing gel was dried on Whatman paper and subjected to autoradiography.

**Mutation of AP-1 Site—** Mutation of an AP-1 site to 5′-TCGGCCG-3′ has been shown by others to silence the function of the site (35). The proximal AP-1 in the cx43 promoter was mutated using the Kunkel method (36), and the mutagenic oligonucleotide 5′-TCCGAGTTCGC-GTGGTGGC-3′ (changed bases are underlined). A 420-bp HindIII DNA fragment of the proximal cx43 promoter was subcloned into M13mp18 vector using standard protocols (33). The protocols described by Ausubel et al. (37) were used to create the mutant AP-1 site. The HindIII fragment containing the mutant AP-1 site was subcloned into the chloramphenicol acetyltransferase (CAT) vector, pCXV1-CAT. The final mutant clone was confirmed by DNA sequencing.

**Transient Expression Assays—** Reporter plasmids containing portions of the 5′-proximal cx43 promoter cloned upstream of the bacterial CAT gene (pBL-CAT) were used in transient expression assays (see Table I). A cryptic AP-1 site has been reported in the backbone of such vectors (38). This site was deleted by digesting pBL-CAT, with EcoO109 and NdeI, filling-in the digested ends with Klenow enzyme, and religating (33).

The primary cultures were transfected with 40 µg of reporter plasmid/10-cm plate using the calcium phosphate precipitation method as described previously (27). Expression vectors for c-jun or c-fos were co-transfected with the CAT reporter plasmids at 0.5 µg/10-cm plate. Transfected cells were treated with either 100 ng/ml TPA or reagent vehicle for 48 h before obtaining cell lysates. Lysates were normalized for protein concentration and analyzed for CAT activity as described previously (27).

**Statistical Analysis—** Statistical analysis of the results was made with GraphPad’s InStat computer software for small sample sets using a two-tailed Student’s t test, or with GraphPad’s Prism software using two way ANOVA analyses.

**RESULTS**

**AP-1 Sites Are Present Upstream of the cx43 Transcription Start—** The human cx43 gene transcription start has been determined for cardiac muscle (32). To determine the human cx43 transcription start in myometrial and leiomyoma primary cells, primer extension assays were performed using total RNA purified from myometrial and leiomyoma cells. The RNAs had

| Table I Plasmids used in the analysis |
|--------------------------------------|
| **Plasmid** | **Gene** | **Species** | **Ref.** |
| pRGAPDH-13 | gpd | Rat | 30 |
| pCMV-fos | c-fos | Rat | 31 |
| pHSV-c-jun | c-jun | Human | 31 |
| pGF-10 | cx43 | Human | 4 |
| p2400-LUX | cx43 | Human | 32 |
| pCVX1-CAT | cx43 | Human | This study |
| pV1-AP1 caps CATV | cx43 | Human | This study |
| pCXV1-CAT | cx43 | Human | This study |

<sup>a</sup> Glyceraldehyde-3-phosphate dehydrogenase.

<sup>b</sup> Connexin-43.
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Previous studies have shown that activation of PKC in human primary myometrial cells results in increased Cx43 protein levels. To test if the TPA-induced increases in myometrial Cx43 protein levels were the result of increased gene transcription, nuclear run-on transcription analyses were performed. Nascent radiolabeled nuclear transcripts were made from fresh nuclei isolated from primary human myometrial cells either treated with TPA or reagent vehicle, and were hybridized to specific cDNAs immobilized on nitrocellulose membrane. Relative transcription from the glyceraldehyde-3-phosphate dehydrogenase (gapdh) gene, a dramatic increase in de novo transcription from the cx43 gene and the c-fos genes was evident in myometrial cells after 3 h of TPA treatment (Fig. 2, lane marked TPA). These data indicate that activation of PKC induces increased cx43 gene transcription in human primary myometrial cells.

Transcription of the cx43 Gene Is Induced through the AP-1 Site following Activation of PKC—The role of PKC in the regulation of myometrial cx43 gene expression has been previously studied in myometrial cells treated with the phorbol ester TPA, an activator of protein kinase C. The relative levels of Cx43, desmin, c-Fos and c-Jun proteins were then determined by immunoblot analysis (Fig. 3). The levels of Cx43, c-Jun, and c-Fos, normalized to the intermediate filament protein desmin, gradually increase with time in the vehicle-treated cultures after the change to serum-free medium. The increase in Cx43 protein with time in serum-free medium is consistent with previous observations. The reasons for these increases are presently unknown. When compared to the time control samples, the amount of Cx43 protein increased significantly in cultures treated for 6–8 h with TPA (n = 3, p < 0.0001) (Fig. 1B). The increase in Cx43 protein levels followed transient increases at 2–4 h in the levels of c-Fos and c-Jun proteins, the transcription factors that bind AP-1 sites (Fig. 3C). In contrast, the cell-specific, 49.5-kDa transcription factor AP-2, which can also be induced through TPA treatment, was not detected (data not shown). Two forms of c-Jun were observed in the myometrial cells after TPA treatment: a 39-kDa form, which is a dormant form, and a slower mobility 46-kDa form, which presumably is an active form due to phosphorylation of serines 63 and 73 (39). Both forms increased with time after TPA treatment, indicating both an increase in c-Jun gene expression and activation of Jun protein.

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Activation of PKC Results in Increased cx43 Expression in Myometrial Cells—Transcription regulatory through AP-1 and AP-2 sites are induced through the PKC cascade. To investigate the role of PKC in the regulation of myometrial cx43 gene expression, primary human uterine smooth muscle cells were treated with the phorbol ester TPA, an activator of protein kinase C. The relative levels of Cx43, desmin, c-Fos and c-Jun proteins were then determined by immunoblot analysis (Fig. 3). The levels of Cx43, c-Jun, and c-Fos, normalized to the intermediate filament protein desmin, gradually increase with time in the vehicle-treated cultures after the change to serum-free medium. The increase in Cx43 protein with time in serum-free medium is consistent with previous observations (16). The reasons for these increases are presently unknown. When compared to the time control samples, the amount of Cx43 protein increased significantly in cultures treated for 6–8 h with TPA (n = 3, p < 0.0001) (Fig. 1B). The increase in Cx43 protein levels followed transient increases at 2–4 h in the levels of c-Fos and c-Jun proteins, the transcription factors that bind AP-1 sites (Fig. 3C). In contrast, the cell-specific, 49.5-kDa transcription factor AP-2, which can also be induced through TPA treatment, was not detected (data not shown). Two forms of c-Jun were observed in the myometrial cells after TPA treatment: a 39-kDa form, which is a dormant form, and a slower mobility 46-kDa form, which presumably is an active form due to phosphorylation of serines 63 and 73 (39). Both forms increased with time after TPA treatment, indicating both an increase in c-Jun gene expression and activation of Jun protein.

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The human 5'-proximal \textit{cx43} promoter. \textbf{A}, sequence of the human 5'-proximal \textit{cx43} promoter region with several cis-acting elements marked as described in the text. The analyzed proximal AP-1 site is boxed, and the position of the transcription start site are indicated by an arrow and +1. \textbf{B}, a restriction map of about 3000 bp of \textit{cx43} promoter region and exon I. \textit{P} is Pst\textit{I}, \textit{H} is Hind\textit{III}, \textit{B} is Bgl\textit{II}, \textit{E} is Eco\textit{RV}, and \textit{A} is Acc\textit{I}. The position of the AP-1 sites and transcription start are indicated in the figure. Appropriately positioned under the restriction map are plasmids that were used in the transient expression assays (see text). \textit{X} denotes mutation of the proximal AP-1 site in the promoter.
from the EcoRV site 700 bp upstream of transcription start to a created BglII (32) site 170 bp downstream of the transcription start was primarily used in this study (see pCXV1-CAT3 in Fig. 2B). This portion of the promoter contains the natural transcription start, two of the half-palindromic EREs, two of the AP-2 sites, the proximal AP-1 site, and most of exon I. Transient expression assays using reporter construct pCXHB-CAT3 (see Fig. 2B), which contains 580 bp of the cx43 5′-flanking promoter region and lacks the two half-palindromic EREs produced similar results (data not shown) to those using pCXV1-CAT3.

Primary myometrial and leiomyoma cells were co-transfected with the CAT reporter construct pCXV1-CAT3 and/or expression vectors for c-Jun and c-Fos. Subsequently the cultures were either treated with TPA or reagent vehicle for 2 days, the amount of time needed for effective transient transfection of the primary cultures. CAT activity in each cell lysate was determined and interpreted as reflecting the transcriptional activity from the reporter plasmids. We did not see a difference between the responses of myometrial and leiomyoma cells (Fig. 5). The CAT activity in lysates from transfected primary cells that were not treated with TPA was the same whether or not they were co-transfected with c-Jun and c-Fos expression vectors. Increases in CAT activity were observed in the lysates of TPA-treated cells that were not co-transfected with expression vectors for c-Jun or c-Fos; however, the increases were not statistically significant (p = 0.125; n = 5). Significant increases in CAT activity were observed in the lysates of TPA-treated cells that were not co-transfected with expression vectors for c-jun and c-fos expression vectors. Increases in CAT activity were observed in the lysates of TPA-treated cells that were not co-transfected with expression vectors for c-jun or c-fos; however, the increases were not statistically significant (p = 0.125; n = 5). Significant increases in CAT activity were observed in the lysates of TPA-treated cells that were not co-transfected with expression vectors for c-Jun and c-Fos.

**Fig. 3. Analysis of cx43, c-Fos, and c-Jun protein levels in myometrial primary culture after TPA treatment.** A, autoradiograph of a representative immunoblot showing the relative levels of Cx43, desmin, c-Fos, and c-Jun in the cell lysates of myometrial primary culture cells after TPA treatment. Lanes marked with (+) contain lysates from cells treated with 100 ng/ml TPA for indicated times in hours. Lanes marked with (-) contain lysates from the controls (no TPA), which were treated with reagent vehicle, 0.05% Me2SO. Lane P shows levels of Cx43, desmin, c-Fos, and c-Jun in pregnancy myometrial tissue from a different gel. B, summary of three separate analyses that compare the levels of Cx43 protein levels in TPA treated and control myometrial cells for 2, 4, 6, 8, and 18 h. Controls and treated cells were harvested at the same time points. Cx43 protein levels were normalized to the levels of desmin, a muscle-specific intermediate filament. C, summary of Western blot analysis shown in panel A comparing the levels of C-Fos and two forms of c-Jun (46 and 39 kDa) proteins in TPA-treated and control myometrial cells for 2, 4, 6, and 18 h. Controls and treated cells were harvested at the same time point. C-Fos and c-Jun protein levels were normalized to the levels of desmin.

**Fig. 4. Analysis of transcriptional response of cx43 and c-fos genes to TPA treatment.** Nuclear run-on transcription assays were performed with nuclei from primary myometrial cells treated for 3 h with reagent vehicle (0) or with 100 ng/ml TPA (TPA). Radiolabeled nascent transcripts purified from cells that underwent each treatment were hybridized to a molar excess of indicated cDNA probes immobilized onto nitrocellulose membrane strips: human cx43, human c-fos, and rat gapdh cDNAs.
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FIG. 5. Induction of CAT activity with TPA in primary culture cells. A, representative autoradiograph of a thin-layer chromatography plate on which monoacetylated ([14C]N-acetylthiocholine) products (MAP) have been separated from the unacetylated substrate (US). The CAT activities shown are from lysates of leiomyoma primary cultures transfected with pCXV1-CAT3 and/or expression vectors for c-fos and/or c-jun, and treated with 100 ng/ml TPA or with reagent vehicle as indicated at the bottom of the autoradiograph. B, bar graph summarizing the results for five transfections each of myometrial and leiomyoma cultures as shown in A. All transfections were with pCXV1-CAT3 and were treated with TPA, except that the one marked 0 did not receive TPA. J, F, and J/F indicate co-transfection with expression vectors for c-jun, c-fos, or both, respectively. Standard deviations are shown by the error bars. The results from co-transfection with pCXV1-CAT3 and expression vectors for c-fos and c-jun without TPA treatment were the same as for the transfection marked 0 and have not been included in the bar graph. C, representative autoradiograph as in panel A, except cells were transfected with pV1-AP1mut-CAT3, which contains the mutant AP-1 site. In the last lane on the right, the cells were co-transfected with pERE/TK/CAT and an expression vector for human ER (see Ref. 28) and were treated with 10 nm estrogen as a positive control.


duction of c-fos and/or c-jun expression with time after TPA treatment (39, 40). Decreases in c-Fos and c-Jun levels are observed in myometrial cells treated with TPA for 18 h and are similar to the levels in untreated cells (see Fig. 3). This suggests that a negative feedback mechanism exists, which diminishes the levels of the transcription factors despite continued TPA treatment.

Mutation of the proximal AP-1 site to 5'-TCGGCCG-3' results in loss of TPA-induced transcription (Fig. 5C). No increase in CAT activity was detected in the lysates from cultures treated with TPA if they were co-transfected with c-fos and/or c-jun expression vectors. These results clearly establish that the AP-1 site in the most proximal region of the human cx43 promoter has a role in inducing transcription upon activation of PKC in myometrial and leiomyoma primary cells.

Discussion

Cx43 gap junctions play a crucial role in the function of the myometrium during labor by propagating action potentials during muscle contractions. The steroid hormones regulate cx43 expression in myometrial tissue during pregnancy, but it is evident that other factors such as uterine distention are also involved in the regulation. Here we show that after treatment with TPA, primary uterine smooth muscle cells exhibit a dramatic increase in levels of c-Fos and c-Jun proteins, the factors that bind to AP-1 sites. The increases in c-Jun and c-Fos are followed by an increase in Cx43 protein levels. Expression of c-Jun and c-Fos is negligible in myometrial tissue from non-pregnant women (this study and Ref. 43) and is further suppressed in the myometrium of women in late pregnancy (43). We are able to detect c-Jun and c-Fos proteins in active-labor myometrial tissue, which also expressed high levels of Cx43 protein. We show too that TPA induces increased transcription from the cx43 gene. Results from transient expression assays clearly demonstrate that the proximal AP-1 site in the human cx43 promoter is functional in inducing transcription in primary cultures of uterine smooth muscle cells upon activation of PKC. This work then supports the concept that up-regulation of cx43 expression in human myometrium at parturition is in part induced by c-Jun and c-Fos binding to the AP-1 site after activation of PKC. Activation of PKC may occur through release of diacylglycerol during uterine distention.

The 5'-proximal promoter region of the cx43 gene also contains two AP-2 sites just upstream of transcription start. The cognate AP-2 transcription factor is expressed in a tissue-specific manner; is regulated by retinoic acid, PKC, and cAMP (19, 42); and is induced upon activation of PKC in cells that express this factor (44). Its expression is developmentally regulated during embryogenesis (45, 46). We found no evidence that AP-2 expression can be induced in myometrial or leiomyoma primary cells through TPA treatment. We could not detect this protein in pregnancy myometrium. Additionally, mutation of the AP-1 site abrogates TPA-induced transcription in the presence of intact AP-2 sites. The AP-2 site in the cx43 5'-proximal promoter does not appear to regulate cx43 expression in myometrial cells after activation of PKC.

Two unique regulatory elements, which bind protein factors found in the nuclear extract of several cell lines, have been identified in the 5'-proximal promoter of the mouse cx43 gene (47). Homologous sequences can be found in the human cx43 promoter in similar positions but were not analyzed in the present study. An activator element (5'-CTCTAGGCCCC) was located at −72 to −62 and appears to be needed for basal transcriptional activity. A repressive element (5'-CTCTCCCGGCC) was located from −102 to −92, which appears to inhibit transcription in an analysis of the most 5'-proximal region of the mouse cx43 promoter. It is perplexing, however, that the repressive element binds to nuclear proteins from active-labor rat pregnancy myometrium, which has high cx43 expression, and not to nuclear proteins from prelabor pregnancy myometrium, which has suppressed cx43 expression (see Ref. 47). Additionally, the element was not repressive when larger regions of the promoter were analyzed. In contrast, in the human cx43 promoter region, the 5'-proximal region of the promoter is the most active compared to larger pieces of the promoter in cardiac myocytes, which express cx43 (32). This
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part of the promoter is also the most active (data not shown) in the primary uterine smooth muscle cells, which also express cx43. It is logical that cx43 promoter activity is cell type-specific. Analyses of the cx43 promoter may differ depending on whether the cells or cell lines are actively expressing the cx43 gene.

Interestingly, the repressive element identified in the mouse cx43 promoter sequence resembles an SP-1 element, although binding of its cognate protein(s) was not significantly competed for with a consensus SP-1 binding site oligonucleotide (14). SP-1 sites can be bound by repressive members of the SP-1 family and have been shown to be necessary to attenuate transcription of the fos gene (15). A trimer of putative SP-1 sites appears 1388–1459 bp upstream of transcription start in the human cx43 promoter. It is possible that the SP-1 sites are involved in repressing cx43 promoter activity in the cardiac myocytes and in the uterine smooth muscle cells.

Animal models indicate that during pregnancy, myometrial gap junction expression is up-regulated by estrogen and down-regulated by progesterone (see Ref. 13 for review). Estrogen and progesterone activate nuclear receptors, which are transcription factors themselves. The activated receptors bind as dimers to enhancer hormone response elements (HREs). HREs can function in a number of positions relative to the gene they regulate, but usually appear in the 5′-flanking region of the promoter. Unexpectedly, the sequence of the 5′-flanking cx43 promoter (this study) did not contain full EREs or PREs. Several putative ERE and PRE half-palindromic sites were identified among several hundred base pairs upstream of the gene. An estrogen-responsive half-palindromic ERE (5′-TGGGTCA) found in the ovalbumin gene has been shown to be a target for TPA-induced c-Jun/c-Fos transactivation independent of the binding of its cognate protein(s) was not significantly competed with a consensus SP-1 binding site oligonucleotide (14). The function of the AP-1 activity (50–55). For example, expression of c-jun and c-fos is regulated by sex steroids in a tissue-specific manner (22–24). The functional AP-1 site in the human cx43 promoter opens the possibility that transcriptional regulation of the cx43 gene by the steroid hormones in female reproductive tissues may involve the Fos/Jun transcription complex. The role of potential interactions of c-Jun/c-Fos with the steroid hormone receptors in the regulation of cx43 expression needs to be explored.

Acknowledgments—We thank Drs. Paul Webb, Peter J. Kushner, and Rossalie M. Uht for the expression vectors for c-fos and cjun. We thank Dr. Xiaoying Gao for initiating the sequencing of the promoter. We are also greatly indebted to Dr. John C. Chumas for help with tissue acquisition.

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11. Yu et al. (29) reported that the 5′-flanking promoter of the rat cx43 gene contains estrogen-responsive elements. They observed striking induction of ER-mediated transcription in HeLa cells co-transfected with an expression vector for ER, however, paradoxically they did not observe the same induction in the ER-containing rat osteosarcoma cell line, R5 17c2,8. even when co-transfected with an ER expression vector. Perhaps the reason for the different results between the two cell lines lies in different endogenous HeLa and ROS transcription factors. Paradoxically, HeLa cells do not normally express cx43 and R5 cells do (7). Thus, the difference in results cannot be accounted for by the presence of factors which repress cx43 expression in the ROS cells. This suggests that ER needs to interact with certain positive factors to induce transcription of the cx43 gene, and these are present in HeLa cells but not R5 cells.

In steroid-responsive tissues, cross-talk has been shown to occur between steroid hormone regulation and the regulation of AP-1 activity (50–55). For example, expression of c-jun and c-fos is regulated by sex steroids in a tissue-specific manner (22–24). The functional AP-1 site in the human cx43 promoter opens the possibility that transcriptional regulation of the cx43 gene by the steroid hormones in female reproductive tissues may involve the Fos/Jun transcription complex. The role of
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Activation of Protein Kinase C in Human Uterine Smooth Muscle Induces connexin-43 Gene Transcription through an AP-1 Site in the Promoter Sequence
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*J. Biol. Chem.* 1996, 271:23667-23674.
doi: 10.1074/jbc.271.39.23667

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