Inhibition of Laminin-5 Production in Breast Epithelial Cells by Overexpression of p300

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The transcriptional coactivator p300 is essential for normal embryonic development and cellular differentiation. We have been studying the role of p300 in the transcription of a variety of genes, and we became interested in the role of this coactivator in the transcription of genes important in breast epithelial cell biology. From MCF-10A cells (spontaneously immortalized, non-transformed human breast epithelial cells), we developed cell lines that stably overexpress p300. These p300-overexpressing cells displayed reduced adhesion to culture dishes and were found to secrete an extracellular matrix deficient in laminin-5. Laminin-5 is the major extracellular matrix component produced by breast epithelium. Immunofluorescence studies, as well as experiments using normal matrix, confirmed that the decreased adhesion of p300-overexpressing cells is due to laminin-5-deficient extracellular matrix and not due to loss of laminin-5 receptors. Northern blots revealed markedly decreased levels of expression of two of the genes (designated LAMA3 and LAMC2) encoding the α3 and γ2 chains of the laminin-5 heterotrimer in the cells that overexpress p300, whereas LAMB3 mRNA, encoding the third or β3 chain of laminin-5, was not markedly reduced. Transient transfection experiments with a vector containing a murine LAMA3 promoter demonstrate that overexpressing p300 down-regulates the LAMA3 promoter. In summary, overexpression of p300 leads to down-regulation of laminin-5 production in breast epithelial cells, resulting in decreased adhesion.

A phosphoprotein originally discovered by virtue of its binding to the adenovirus E1A transforming protein, p300 has subsequently been shown to function as a transcriptional coactivator for a very large number of transcription factors, bridging them with the basal transcription complex (1). p300 has enzymatic activity as a histone acetyltransferase that links chromatin remodeling with activation of transcription (2). p300 can mediate cross-talk among separate signaling pathways (1, 3), has important roles in several fundamental cellular processes, including differentiation (4), is essential for normal embryonic and fetal development (5), and may function as a tumor suppressor (6). The p300 protein has several distinct domains that interact specifically with diverse proteins. p300 shares many functions with a highly homologous protein, cAMP-response element-binding protein-binding protein (CBP). Whereas p300 and CBP seem to behave interchangeably for many functions, especially in vitro, there are also some specific functions for which one of the proteins cannot replace the other (7). Recently, experiments utilizing targeted gene disruption (knockout) technology have confirmed that p300 function is essential for normal embryonic cellular proliferation, morphogenesis, and development, with double knockouts resulting in 100% embryonic lethality (5). Even the haploinsufficiency of p300, as generated in the heterozygotes, resulted in severe developmental abnormalities and frequent embryonic lethality. Likewise, haploinsufficiency of CBP gives rise to the severe developmental abnormalities characteristic of the Rubinstein-Taybi syndrome, including mental retardation, craniofacial abnormalities, skeletal abnormalities, and increased cancer incidence (8). Thus, normal levels of p300 cannot replace CBP during embryonic development, and normal levels of CBP cannot replace p300 during embryonic development: both proteins are required.

In this paper, we describe the generation of stable cell lines from MCF10A cells that overexpress full-length p300 and the effects of this overexpression on production of laminin-5. Laminin-5 is the major extracellular matrix protein produced by MCF10A cells and is also the major protein in extracellular matrix of breast epithelium in vivo. The laminin-5 protein is a heterotrimeric glycoprotein consisting of the α3, β3, and γ2 chains with each chain the product of a separate gene, designated LAMA3, LAMB3, and LAMC2, respectively (9). Not much is known about the transcriptional regulation of these genes to date. The murine promoter for the LAMA3 gene has been cloned (10), and its regulation by transforming growth factor β in keratinocytes was found to involve three AP-1 sites (11). We show that p300 overexpression decreases the mRNA of both the LAMA3 and LAMC2 genes and can down-regulate the murine LAMA3 promoter in transient transfection assays. These results suggest that p300 may play an important role in the transcriptional regulation of the LAMA3 gene.

EXPERIMENTAL PROCEDURES

Cell Culture and Matrix Preparations—The MCF10A (12) normal breast epithelial cell line was obtained from the American Tissue Culture Collection (Manassas, VA) and was maintained in a 1:1 mix of Dulbecco’s modified Eagle’s medium and Ham’s F-12 medium (Life Technologies, Inc.) supplemented with 5% equine serum (Life Technologies, Inc.), 0.01 mg/ml insulin (Sigma), 20 ng/ml epidermal growth factor ( Collaborative Biomedical Products, Bedford, MA) 100 ng/ml factor.

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1 The abbreviations used are: CBP, cAMP-response element-binding protein-binding protein; AP, activator protein; CMV, cytomegalovirus; HA, hemagglutinin; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline.
cholera toxin (Sigma) and 500 mg/ml hydrocortisone (Collaborative Biomedical Products). Stably transfected clones were also supplemented with 50 μg/ml hygromycin (Sigma). 804G rat bladder carcinoma cells were maintained as described previously (15).

Matrix preparations from MCF10A and MOP1 cells were prepared as described previously (15). Matrix still attached to plastic surfaces was either collected in sample buffer and run on an SDS-PAGE gel for Western blotting or labeled with 0.5 mg/ml bismuthmethylacryloic acid 3-sulf-N-hydroxysuccinimide ester (Sigma) in PBS and then collected in sample buffer. Matrix preparations were resolved by 7.5% SDS-PAGE and blotted to nitrocellulose. Blots were incubated with streptavidin-horseradish peroxidase (Amersham Pharmacia Biotech) and detected with the ECL reagent (Amersham Pharmacia Biotech). To coat surfaces with matrix for immunofluorescence and adhesion studies, MCF10A cells or 804G cells were grown to confluence, treated with 20 mM ammonium hydroxide to destroy the cells but preserve the matrix on the desired surface, and washed well to remove cell debris (15). New test cells for experiments were then plated on this matrix-coated surface.

Plasmids and Transfection—For stable transfections, MCF10A cells were transfected either with a helium-pulse Accell (gene gun) device (Agracetum, Inc., Middleton, WI) or with Superfect (Qiagen, Inc., Valencia, CA) with the pTKHygro vector containing a hygromycin resistance gene under a thymidine kinase promoter and a vector designated pmet300 (4). The p300 for p300 was obtained from the p300 construct (p300CHA vector) and inserted into the M-H cells. M-H cells that express p300 in low enough amounts that the cells contain the p300 Overexpression Decreases Laminin-5

react with CBP, incubated with a secondary goat-anti-mouse antibody conjugated to horseradish peroxidase, and detected by ECL (Amersham Pharmacia Biotech).

Western analysis of laminin-5 subunits was performed on cell lysates and matrix preparations in SDS-urea gel. Antibody 10B5 to laminin-5 α3 chain (18), mouse monoclonal antibody 3D-5 to laminin-5 β2, and the commercially available mouse antibody to kalinin B1 (laminin-5 β3 subunit, Clone 17 (Transduction Laboratories, Lexington, KY) were used. The anti-vinulin antibody was the mouse monoclonal VIN-11–5 from Sigma. Goat anti-mouse horseradish peroxidase (Bio-Rad) was used as a secondary antibody. The primary antibody to actin (Amersham Pharmacia Biotech) was detected with a secondary goat-anti-mouse IgM (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD).

Northern Blotting—The probe for β3 laminin-5 chain, clone 5-5C, and the EP-1 plasmid containing the laminin-5 α3 chain probe were generous gifts of Drs. Robert E. Burgeson and Maureen C. Ryan. The LAMC2 probe was amplified by reverse transcription-polymerase chain reaction with MCF10A RNA using polymerase chain reaction primers specific for the LAMC2 gene (5'-AGATGTTGATGCTGGAAGCCGATG-3' and 5'-AGGTTCAATTGTTCAATGTA-3'). These primers create a fragment that runs from base pair 756 to base pair 1211 of the LAMC2 sequence obtained from GenBank™. RNA was harvested from cells using the RNEasy Kit (Qiagen, Inc.). Samples of 30 μg of total RNA were electrophoresed in denaturing 1% agarose/formaldehyde gels and transferred to a Hybond-N+ membrane (Amersham Pharmacia Biotech) in 20× SSC. Blots were fixed and prehybridized at 65 °C for 1 h in QuickHyb buffer (Stratagene, La Jolla, CA). 32P-Labeled probes were prepared by the random primer Redivue method (Amersham Pharmacia Biotech), and 10×106 cpm/ml of the LAMC2 probe was hybridized. This probe was hybridized for 1 h in QuickHyb buffer at 65 °C. The membrane was then washed twice in 2× SSC/0.1% SDS at room temperature for 5 min and once in 0.1× SSC/0.1% SDS for 30 min at 60 °C. This same blot was stripped and reprobed with the LAM3 and LAMC2 probes. Blots were visualized by autoradiography.

Immunofluorescence—M-H, MOP1, and MOP2 cells were grown on glass coverslips and fixed with 3.7% formaldehyde for 5 min, washed with PBS, permeabilized with 0.5% Triton X-100 at 4 °C for 7 min, and washed again in PBS. The coverslips were incubated with primary antibody GoH3 (Immunotech, Inc., Westbrook, ME) for α2 integrin staining, and antibody P1B5 (Life Technologies, Inc.) for α3 staining, diluted in PBS at 4 °C in a humid chamber overnight, washed three times in PBS, and incubated with an appropriate fluorochrome-conjugated secondary antibody for 1 h at 37 °C.

RESULTS

Characterization of MCF10A Clones Overexpressing p300—MCF10A cells were stably transfected with the pmet300 plasmid expressing full-length p300 cDNA from the metallothionein promoter (17). The p300 in this construct was tagged with a helix-loop-helix/leucine zipper domain (18). This construct was transfected into MCF10A cells, and two clones, MCF10A-overexpressing p300 1 and 2 (MOP1 and MOP2), were isolated that express p300 in low enough amounts that the cells contain...
continue growing in long term culture. Fig. 1A shows an immunoprecipitation of [35S]methionine-labeled cell lysates with an anti-HA antibody that was analyzed by SDS-PAGE. A band migrating at 300 kDa was seen only in the MOP1 cells, confirming expression of p300 from the pmet300 vector in MOP1 cells. B, whole cell lysates were collected from M-H and MOP2 cells, immunoprecipitated with HA tag antibody as in A, resolved by SDS-PAGE, transferred to nitrocellulose, and blotted with p300CT power clone antibody (Upstate Biotechnology Inc.). This method confirms the identity of a band at 300 kDa, as p300 was expressed only in MOP2 cells and not in control M-H cells. C, total p300 expression is greater in MOP1 (lane 2) and MOP2 (lane 3) cells than in M-H (lane 1) cells. Whole cell extracts were analyzed by 7.5% SDS-PAGE, transferred to nitrocellulose membrane, and blotted with p300N15 antibody (Santa Cruz Biotechnology).

FIG. 1. Expression of p300 in M-H control and MOP cells. A, M-H and MOP1 cells were labeled with [35S]methionine. Radiolabeled whole cell lysates were immunoprecipitated with 12CA5 antibody to the HA tag and analyzed by SDS-PAGE. A band migrating at 300 kDa was seen only in the MOP1 cells, confirming expression of p300 from the pmet300 vector in MOP1 cells. B, whole cell lysates were collected from M-H and MOP2 cells, immunoprecipitated with HA tag antibody as in A, resolved by SDS-PAGE, transferred to nitrocellulose, and blotted with p300CT power clone antibody (Upstate Biotechnology Inc.). This method confirms the identity of a band at 300 kDa, as p300 was expressed only in MOP2 cells and not in control M-H cells. C, total p300 expression is greater in MOP1 (lane 2) and MOP2 (lane 3) cells than in M-H (lane 1) cells. Whole cell extracts were analyzed by 7.5% SDS-PAGE, transferred to nitrocellulose membrane, and blotted with p300N15 antibody (Santa Cruz Biotechnology).

FIG. 2. Cellular morphology. Photos of cells growing in tissue culture at a magnification of × 200 taken with an Olympus model SC35 camera. A, typical epithelial cobblestone morphology of M-H control cells. B and C, MOP1 (B) and MOP2 (C) cells exhibit an altered, more stellate morphology.

FIG. 3. Growth of clones overexpressing p300 is slower than control cells. 2.5×10⁴ cells were seeded per well in 6 well plates. Cells were trypsinized, resuspended and counted with a hemacytometer on days indicated. Error bars represent standard deviation of 4 determinations.

Through its interaction with p53, p300 has been suggested to play a role in p53-regulated cell growth (21). In muscle cells, p300 is required for Myo-D-dependent cell cycle arrest (22). Based on these findings, we hypothesized that overexpression of p300 in MOP cells may have altered the growth rates of these cells. Fig. 3 shows that M-H cells maintained a faster growth rate than MOP1 and MOP2 cells. Growth of M-H cells is similar to that of parental MCF10A cells (data not shown). Saturation densities at day 8 were 31.2, 9.1, and 5.4×10⁴ cells/cm² for M-H, MOP1, and MOP2 cells, respectively.

Overexpressing p300 Alters Cellular Adhesion—We observed that the MOP1 and MOP2 cells required less time for trypsinization when passaging the cells. This observation led us to test the adhesion of cells overexpressing p300 on tissue culture and three extracellular matrix substrates. Fig. 4 shows the results of 3-h adhesion assays. MOP1 and MOP2 cell adhesion was 75 and 41% of the M-H control cell adhesion, respectively. The adhesion of cells on fibronectin improved by 14,
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37, and 230% for M-H, MOP1, and MOP2 cells, respectively. The increase in adhesion of M-H, MOP1, and MOP2 cells to laminin-5 was 56, 25, and 154%, respectively. When plated on laminin-1, the adhesion of all three cell lines decreased by approximately 40%.

Adhesion experiments were performed in which cells were treated with 20 μg/ml cycloheximide at the time of plating for the adhesion assay to inhibit protein synthesis in the cells. Cells were incubated for 3 h and stained as above. Adhesion of control cells was decreased by 72%, and MOP1 and MOP2 adhesion was reduced 100%, to the cell-free baseline. This shows that protein synthesis is necessary for maximal adhesion in all of the cells tested.

Overexpressing p300 Results in a Decrease of Laminin-5 Extracellular Matrix—MCF10A cells secrete abundant extracellular matrix, of which laminin-5, a heterotrimeric protein with a molecular mass of greater than 400 kDa, makes up more than 80%. Laminin-5 consists of three subunits, α3, β3, and γ2, which in their processed forms are 160, 140, and 105 kDa, respectively. We analyzed more closely the laminin-5 production by M-H, MOP1, and MOP2 cells in the 190-kDa unprocessed and the 160-kDa processed forms (Fig. 6A). The α3 subunit was present in cell lysates and matrix from MCF10A and M-H cells in the 190-kDa unprocessed form and the 160-kDa processed form (Fig. 6B). The α3 subunit was not detectable by Western blot in the MOP1 and MOP2 cell lysates or matrix (Fig. 6B). The β3 subunit was present in the lysates of all cell types but was not seen in the MOP1 and MOP2 cell matrices (Fig. 6C). We simultaneously blotted the β3 blot with an antibody to actin to control for protein loading. Densitometric analysis of the β3 blot in Fig. 6B revealed that the amount of β3 protein present in the cell lysates from MCF10A and M-H cells contained 3.5-fold the amount present in MOP1 and MOP2 cells. This is different from the levels of RNA, which do not change significantly, but the difference is probably due to decreased β3 protein stability in MOP cells due to the lack of the other laminin-5 subunits. The γ2 subunit has a 155-kDa unprocessed and 105-kDa processed form. The Western analysis for the γ2 subunit showed mainly the unprocessed form in cell lysates of MCF10A and M-H cells but no γ2 in MOP1 or MOP2 cell lysates (Fig. 6D). The processed form was more evident in the matrix of MCF10A and M-H cells. The unprocessed form was still present because matrix samples were collected from subconfluent cells. Fig. 6E shows Western analysis of the blot shown in Fig. 6D, with an antibody to vinculin as a loading control for the cell lysates.

p300 Down-regulates the LAMA3A Promoter—Because the laminin-5 α3 subunit was dramatically reduced, we considered it possible that this gene is down-regulated at the transcriptional level. We performed experiments using a clone, designated pGalA, containing the promoter of the murine LAMA3A gene linked to the β-galactosidase reporter (8, 36). The laminin α3 chain in mice has two isoforms, designated 3A and 3B, each using its own promoter. This clone contains the promoter of the 3A isoform. Transient transfection experiments placing pGalA into MCF10A cells revealed that the mouse LAMA3A promoter drove transcription of beta galactosidase in these human cells (Fig. 7A). Cotransfection of MCF10A cells with pGalA and a plasmid expressing p300 driven by a CMV promoter resulted in inhibition of pGalA-driven transcription by about 50% (Fig. 7A). Cells were cotransfected with a CMVLuc construct, and results are shown normalized to luciferase activity. There was no evidence of a general repression of transcription in these cells, as the control CMV-luciferase plasmid expression was unchanged from controls that were not transfected with CMVp300 (data not shown).

**Fig. 4.** Cellular attachment assay. Cells resuspended in serum-free medium were seeded at a density of 2 × 10^4 cells/well in 96-well tissue culture plates or plates coated with fibronectin, laminin-5, or laminin-1. Cells were incubated for 3 h, washed with PBS, and stained with crystal violet. Normalized absorbance at 540 nm is shown for three experiments with six determinations each. (Note that results with laminin-5 are from a representative experiment with four determinations.) Error bars represent S.E. except for laminin-5 samples, which are S.D. *p = 0.042 by Student’s t test; **p = 0.0023 by Student’s t test.

**Fig. 5.** Northern analysis of the genes for the three laminin-5 chains. A, 30 μg of total RNA was electrophoresed on a formaldehyde/agarose gel, transferred to Hybond-N, and probed with a 32P-radiolabeled probe to the LAMA3 chain. B, Northern blot for the β3 chain C, Northern blot of the γ2 chain. A glyceraldehyde-3-phosphate dehydrogenase (GAPDH) internal control is included in each blot.
We next transiently transfected MCF10A, M-H, MOP1, and MOP2 cells with the pGalA vector and compared β-galactosidase activity between the different cell lines. We found that the MOP1 and MOP2 cells that overexpress p300 had decreased pGalA promoter activity compared with MCF10A and M-H controls (Fig. 7B).

**Organization of Laminin-5 Integrin Receptors**—The major receptors for laminin-5 on breast cells are the integrins α6β4 and α3β1. The α3β1 integrin localizes primarily in and near areas of cell-to-cell contact. M-H, MOP1, and MOP2 cells all displayed similar cell-to-cell contact accumulation of the α3 integrin (Fig. 8). The α6β4 integrin heterodimer has been localized primarily to the basal surface of the cell, where it forms part of a complex multiprotein adhesive structure called the hemidesmosome (5, 11). In vivo, hemidesmosomes are found attaching cells of epithelia to the basement membrane. Immunofluorescence studies of the α6 integrin revealed mottled patches with a “Swiss cheese” pattern on the basal surface of the cells, as seen in M-H cells on tissue culture (Fig. 9A). However, the p300-overexpressing cells displayed virtually no hemidesmosomal pattern staining of α6 in tissue culture (Fig. 9C). The α6 integrin staining in MOP cells is more diffuse, with accumulation of α6 staining seen in cell-cell contacts. The normal hemidesmosomal staining pattern was restored when the MOP2 cells were plated on a laminin-5-rich matrix (Fig. 9E). Staining for the β4 integrin co-localized with the α6 staining in all cells (data not shown).
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Laminins are a family of large heterotrimeric glycoproteins consisting of three related but different chains held together by coiled-coil interactions and disulfide bonds (23, 24). There are at least 11 laminin isoforms, resulting from a variety of combinations of α, β, and γ chains (25). To date, five α, three β, and two γ chains have been described. One of these isoforms, laminin-5, is an extracellular matrix protein that is important in the processes of epithelial cell adhesion and migration (26–28). In breast cells, laminin-5 has been shown to play a role in branching morphogenesis. Function blocking antibodies to laminin-5 inhibited branching morphogenesis of MCF10A cells on Matrigel (29). The role of laminin-5 in tumor progression appears to be tissue type-dependent, with laminin-5 highly expressed at the invasive edge in gliomas, colon carcinomas, gastric carcinomas, and squamous cell carcinomas (30–34), but it is reduced in basal cell, prostate, and breast carcinomas (35–39).

Laminin-5 is found primarily in the basement membranes of epithelial cells, which suggests that its expression is highly regulated and tissue-specific. The expression of p300 is ubiquitous and has been shown to activate transcription in a large variety of cell types. Accumulation of p300 has been shown in undifferentiated embryonal carcinoma cells and mediates the transcriptional repression of the SV40 enhancer in these cells (40). There is not much known about the functional activation of p300 itself. Differences in phosphorylation during the cell cycle suggest that phosphorylation of p300 may be important in its activity (41). It is not known at this time what temporal relationship may exist between laminin-5 production and p300 activity.

We show that p300 overexpression leads to a decrease in the laminin-5 present in the extracellular matrix and that this is due to a decrease in the production of the α3 and γ2 subunits but not the β3 subunit. This decreased production is shown both at the mRNA and the protein level. The α3 laminin subunit is found in the laminin-5, laminin-6 (α3, β1, and γ1) and laminin-7 (α3, β2, and γ1) isoforms, whereas the γ2 laminin subunit is unique to the laminin-5 isoform. Laminin-5 and laminin-6 are both present in the basal lamina of stratified epithelia (42). Expression of the β2 subunit, a component of laminin-7, is believed to be restricted to the motor neuron synapse, blood vessels, and the kidney glomerulus (43–45). The regulation of the α3 and γ2 chains by p300 may also affect the production of these laminin isoforms.

MOP cells exhibit decreases in adhesion to tissue culture plastic compared with control cells. We show that plating MOP cells on a laminin-5 surface improves their adhesion but also improves the attachment of M-H control cells. In fact, the improvement of attachment of M-H cells to laminin-5 is greater than the change seen in MOP1 cells between tissue culture adhesion and laminin-5 adhesion. We believe that the decreased adhesion of MOP cells to tissue culture plastic may in part be due to their decreased laminin production. There may be additional factors that are contributing to this phenomenon.

There are two integrin receptors, αβ1 and αβ6, for which laminin-5 has been reported to be a ligand. We show that cells that produce less laminin maintain normal αβ1 integrin receptors. The αβ6 integrin has an altered distribution pattern in MOP cells but has normal distribution when in the presence of a laminin-5-rich matrix. The laminin-5 adhesion data do support the hypothesis that MOP cells express the integrin receptors necessary for attachment to laminin-5, which is confirmed by immunofluorescence.

We find that plating all cell types on fibronectin greatly increases their adhesion. Fibronectin is present in very small quantities in the extracellular matrix of MCF10A cells but is found in whole cell extracts of MCF10A, M-H, MOP1, and MOP2 cells (data not shown), and overexpressing p300 does not result in a reduction of fibronectin production. It is not surpris-
that plating on fibronectin increases the adhesion of these cells because breast epithelial cells make the α5β1 and the α6β1 integrin fibronectin receptors. Plating the cells on laminin-5 or fibronectin increases the adhesion of all of the cell types. This confirms that MOP cells are able to adhere to a laminin-5 or fibronectin substrate when it is present but are not able to produce laminin-5. All cell types will adhere strongly to fibronectin despite the low amounts of fibronectin found in their extracellular matrix. It is not clear why laminin-1 is inhibitory to adhesion in all of the cell types, but others have also reported that laminin-1 can be antiadhesive for epithelial cells (46).

Decreased levels of laminin-5 matrix in MOP cells may also be playing a role in the decreased growth rates of these cells. Cell signaling through laminin-5 can regulate proliferation of epithelial cells (19). It may also be possible for p300 to be affecting cell cycle through interactions with p53. We see no detectable laminin-5 in either MOP cell line, but the growth rate of these cell lines is different. These data suggest that although it may be possible that laminin-5 is involved in the decreased rate of cell growth, there are probably other factors that are modulating the growth rates of these cells.

We were very interested in the decrease in LAMA3 expression. We are not the first to report repression of a promoter element when p300 is in excess. Repression of the SV40 enhancer was seen with an accumulation of p300 in undifferentiated embryonal carcinoma cells (40). Results from transient transfection experiments (Fig. 7) suggest that overexpressing p300 has a down-regulating effect on the murine LAMA3A promoter activity. The murine LAMA3A promoter has three AP-1 sites that are important in its regulation (11). AP-1 has been shown to interact with p300 (47, 48) and may be important in mediating the down-regulation of the LAMA3A gene when p300 is overexpressed possibly by sequesterment of AP-1.

It has recently been discovered that CBP acts as a corepressor for TCF-1 in Drosophila (49). This discovery suggests that p300/CBP may not only act as a coactivator but also as a corepressor. Cloning and further analysis of the promoter will be necessary to determine the mechanism by which overexpression of p300 results in repression of the LAMA3A promoter. The decrease in laminin-5 production appears directly related to p300 through a transcriptional mechanism. In future studies, we hope to determine the specific mechanism(s) by which p300 decreases human LAMA3 transcription.

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