PEA3 Is Up-regulated in Response to Wnt1 and Activates the Expression of Cyclooxygenase-2*

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The inducible prostaglandin synthase cyclooxygenase-2 (COX-2) is aberrantly expressed in intestinal tumors resulting from APC mutation, and is also transcriptionally up-regulated in mouse mammary epithelial cells in response to Wnt1 expression. β-Catenin stabilization is a consequence of both APC mutation and Wnt signaling. We have previously observed coordinate regulation of the matrilysin promoter by β-catenin and Ets family transcription factors of the PEA3 subfamily. Here we show that while β-catenin only weakly activates the COX-2 promoter, PEA3 family transcription factors are potent activators of COX-2 transcription. Consistent with this, PEA3 is up-regulated in Wnt1-expressing mouse mammary epithelial cells, and PEA3 factors are highly expressed in tumors from Wnt1 transgenic mice, in which COX-2 is also up-regulated. Promoter mapping experiments suggest that the NF-IL6 site in the COX-2 promoter is important for mediating PEA3 responsiveness. The NF-IL6 site is also important for COX-2 transcription in some colorectal cancer lines (Shao, J., Sheng, H., Inoue, H., Morrow, J. D., and DuBois, R. N. (2000) J. Biol. Chem. 275, 33951–33956), and PEA3 factors are highly expressed in colorectal cancer cell lines. Therefore, we speculate that PEA3 factors may contribute to the up-regulation of COX-2 expression resulting from both APC mutation and Wnt1 expression.

Wnt1 is a mammary oncogene that encodes a secreted signaling factor. Targeted expression of Wnt1 in murine mammary glands results in epithelial hyperplasia with subsequent carcinoma formation (1). Wnt1 signaling leads to stabilization of a cytosolic pool of β-catenin, and consequently transcriptional activation by β-catenin/TCF complexes (2, 3). Additionally, β-catenin may induce TCF-independent transcription (4–6). Inappropriate activation of the Wnt signaling pathway has been detected in numerous tumors, arising as a consequence of Wnt gene misexpression, APC mutation, or mutation of other components of the pathway such as axin and β-catenin itself (7). Multiple transcriptional targets of Wnt signaling have now been identified, some of which are likely to contribute to tumorigenesis. Of these, several have been demonstrated to be directly activated by β-catenin, including cyclin D1, c-myc, matrilysin, and peroxisome proliferator activated receptor δ (8–12). In addition, we have shown transcriptional up-regulation of COX-2 in Wnt1-expressing mouse mammary epithelial cells (13), but did not determine whether this was due to direct regulation of the COX-2 promoter by β-catenin.

COX-2, the inducible isoform of prostaglandin synthase, is aberrantly expressed in human colorectal cancers, and also in tumors from mouse colorectal cancer models carrying germline Apc mutations (14–18). Additionally, COX-2 overexpression has now been detected in multiple human cancers including those of the skin, head and neck, lung, breast, and stomach (19–24). Strikingly, COX-2 overexpression in murine mammary gland is sufficient to induce tumorigenesis (25). Thus, considerable interest is focused on COX-2 as a potential therapeutic target for the prevention or treatment of cancer. Both genetic ablation and pharmacological inhibition of COX-2 have resulted in reduced tumorigenesis in several animal cancer models (26–30), and selective COX-2 inhibitors have also proved effective in reducing the number of colorectal polyps in familial adenomatous polyposis patients (31). Several mechanisms have been proposed to account for the role of COX-2 in tumorigenesis. COX-2 overexpression in epithelial cells is associated with enhanced invasiveness and suppression of apoptosis (25, 32, 33). Prostaglandin overproduction is likely to have pleiotropic consequences including stimulation of proliferation and local immunosuppressive effects that could facilitate tumorigenesis. Recent data also demonstrate a role for COX-2 in angiogenesis (34–39).

Modulation of COX-2 protein levels can be achieved via multiple mechanisms, including transcriptional activation, mRNA stabilization, and altered COX-2 protein stability. We have previously demonstrated transcriptional up-regulation of COX-2 in response to Wnt1 expression in mouse mammary epithelial cells (13). The goal of the current study was to elucidate this process.

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‡‡ The abbreviations used are: TCF, T cell factor; C/EBP, CCAAT/enhancer-binding protein; COX, cyclooxygenase; CRE, cyclic AMP response element; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LIP, liver-enriched inhibitory protein; MG, mammary glands; NF-IL6, nuclear factor interleukin-6; NF-κB, nuclear factor-κB; MOPS, 4-morpholinosopanesulfonic acid.
citate the mechanism(s) underlying Wnt1-mediated induction of COX-2. Given that COX-2 is also up-regulated in intestinal tumors resulting from APC mutation, we initially hypothesized that β-catenin might regulate the COX-2 promoter. Here we examine the effect of both β-catenin and Ets family transcription factors of the PEA3 subfamily on COX-2 promoter activity.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Generation, characterization, and culture of the control and Wnt1-expressing mouse mammary epithelial cell lines have been previously described (13). C57/MV7 and RAC/MV7 are control populations infected with MV7 retrovirus, while C57/Wnt-1 and RAC/Wnt-1 are Wnt1-expressing populations, and RAC/Wnt-1 #9 is a clonal subline selected for high level Wnt1 expression. Identical culture conditions were used for the parental cell line C57MG. 293 human embryonic kidney cells were grown in Dulbecco's modified Eagle's medium (4.5 g/liter D-glucose) containing 10% fetal bovine serum (Life Technologies, Inc.), 100 units/ml penicillin, and 100 µg/ml streptomycin.

**Cell Transfection and Luciferase Assays**—293 cells were transfected using LipofectAMINE (Life Technologies), according to the manufacturer's instructions. Briefly, cells were seeded in 24-well plates at 1 × 10⁵ cells/well. 20 h after cell seeding, 6 wells were transfected for 4 h using a transfection mixture consisting of 1.2 ml of serum-free Dulbecco's modified Eagle's medium, 3.6 µl of LipofectAMINE, and 2.2 µg of total plasmid DNA (including 0.2 µg of pRL-TK). Where necessary “empty” vectors were included to maintain constant amounts of DNA. Lysates were prepared 48 h after transfection, and Firefly and Renilla luciferase activities were measured using a Dual Luciferase Reagent kit (Promega) and a Monolight 2010 luminometer (Analytical Luminescence Laboratory). Firefly activity was normalized to the Renilla activity, and results were expressed as percentage of control activity. To transfect 293 cells for protein analysis or RNA preparation, cells were plated at 5 × 10⁵ cells per 10-cm plate, and transfections scaled-up proportionally to surface area. Similar conditions were used to transfect C57MG cells, except that cells were plated at 2.5 × 10⁶ cells/well, and 5.4 µl of LipofectAMINE was used for transfection.

**Mammary Tissues and Tumors**—A breeding colony of Wnt1 transgenic mice (1) was maintained by crossing Wnt1 transgenic B6SJL males (obtained from the Jackson Laboratory) with strain-matched females. Mice were genotyped by polymerase chain reaction analysis of tail-tip DNA. The primers used were: 5'-CCGAAACAGCAAGCTGCTCACAACG-3' and 5'-ACTCCACAGGGTGAGTGCTGTC-3'. These primers amplify a 425-base pair fragment present in the transgene, but not in the endogenous Wnt1 gene. Wnt1 transgenic animals were sacrificed when tumors were 1 cm in diameter, and wild type littermates were simultaneously sacrificed. Tumors and mammary glands were snap-frozen in liquid nitrogen and stored at −80 °C prior to use.

**Protein Analysis**—Transfected 293 cells were lysed, and lysates analyzed for expression of Myc-epitope-tagged proteins and ERK2, using 9E10 monoclonal and 122 polyclonal antibodies, respectively, as previously described (40). Cox-2 protein in mammary glands and tumors was assayed using a coupled immunoprecipitation/immunoblotting assay. 10 mg of mammary gland or tumor tissue was sonicated in 1 ml of RIPA buffer prior to resuspension in Laemmli sample buffer. Cox-2 protein was detected by Western blotting after running the immunoprecipitates on SDS-polyacrylamide gels as described previously (13).

**Northern Blot Analysis**—RNA was prepared from confluent cells and from mammary glands and tumors using RNezol B (Tel-Test, Inc.) according to the manufacturer's instructions. Total RNA from tissue was isolated briefly prior to homogenization in RNezol B. Northern blot analysis was performed as described previously using MOPS/formaldehyde gels (13).

**Plasmids**—Northern probes used were as follows: human COX-2 (S. M. Prescott, University of Utah, Salt Lake City, UT), murine PEA3 (41), murine ERK (42), murine ERβ (42), and GAPDH (13). The COX-2 promoter reporter construct COX-2-LUC contained nucleotides −1432/−59 of the human COX-2 promoter linked to luciferase (43). In addition, the following truncated COX-2 promoter constructs were used: −327/−59, −220/−59, −124/−59, and −52/−59 (43). Constructs were also utilized in which mutations had been introduced into the −327/−59 backbone. KMB, ILM, and CRM have mutagenized NF-κB (−233/−214), NF-IL6 (−132/−124), and CRE (−59/−53) sites, respectively (43). The stromelysin-1 promoter construct p754TR-Luc (42) was used to compare activation by Ets factors with that of the COX-2 promoter. p754TR-Luc was co-transfected with expression vectors encoding Ets factors plus c-Jun, since Ets factors alone were insufficient to induce stromelysin-1 promoter activity. The TOPFLASH vector, an artificial β-catenin/TCF-responsive promoter reporter (44), was used to confirm that overexpressed β-catenin could drive transcription. The following
Transfected with: PEA3 β-catenin

Volume of lysate: 4μl 2μl 1μl 8μl 4μl 2μl

β-catenin

PEA3

ERK2

Fig. 2. Comparison of β-catenin and PEA3 expression. 293 cells were transfected with expression vectors encoding β-catenin or PEA3, and lysates prepared as described under “Experimental Procedures.” Equivalent amounts of each expression vector were used as in the experiments shown in Figs. 1A and 4. To compare the relative expression levels of β-catenin and PEA3, varied amounts of each lysate were electrophoresed on the same polyacrylamide gel, and analyzed by Western blotting sequentially with the anti-Myc epitope antibody 9E10 (upper panel) and the anti-ERK2 antibody 122 (lower panel). 9E10 detected both PEA3 (lanes 1–3) and β-catenin (β-cat; lanes 4–6), since both are Myc-epitope-tagged. Anti-ERK2 antibody was used as a loading control. Comparison of lane 5 with lanes 2 and 3 suggests that β-catenin is present at 2–4-fold lower levels than PEA3.

Fig. 3. The COX-2 promoter is selectively activated by PEA3 factors. 293 cells were transfected with expression vectors encoding PEA3, ER81, ERM, ETS-1, or ETS-2, together with COX-2-LUC and pRL-TK. In parallel, cells were transfected with the Ets expression vectors, pRL-TK, a stromelysin promoter luciferase reporter construct (p754TR-Luc), and a c-Jun expression vector. Luciferase assays were performed as described above. Results shown are the mean ± S.D. of six replicates.

expression vectors were used: pMT2β-catenin (myc-tagged; 45), pCANmycPEA3 (42), pDNA-ER81 (42), pCANmycERM (42), Ets-1 (S. Hiebert, Vanderbilt University, Nashville, TN), pSG5-Ets-2 (D. Watson, Medical University of South Carolina, Charleston, SC), pCMV-c-jun (R. Wisdom, Vanderbilt University), and pCMV-LIP, encoding dominant negative C/EBPβ (46, 47). In addition, expression vectors encoding C/EBPα, C/EBPβ, and C/EBPδ were obtained from S. McKnight (University of Texas, Southwestern, TX).

RESULTS

Transient transfections were performed to determine whether the COX-2 promoter was susceptible to regulation by β-catenin. The 293 human embryonic kidney cell line was selected for these experiments based on ease of transfection (4). Transfected 293 cells were cotransfected with a β-catenin expression vector and with COX-2-LUC, a luciferase reporter construct containing 1.4 kilobase pairs of the human COX-2 promoter (43). In addition, we tested the effect of the Ets family transcription factor PEA3 since we had previously observed synergistic activation of another target gene, matrilysin, by PEA3 and β-catenin (42). Overexpression of β-catenin stimulated the activity of TOPFLASH, an artificial β-catenin/TCF-responsive promoter reporter (44), by 5–10-fold (data not shown). However, we observed only very weak activation of the COX-2 promoter construct COX-2-LUC by β-catenin (Fig. 1A). The mean increase observed in six experiments in response to β-catenin was 28% (p < 0.03). In contrast, PEA3 activated COX-2-LUC up to 20-fold (Fig. 1, A and B). Together PEA3 and β-catenin elicited a greater than additive response in some experiments (Fig. 1A), although the observed trend was not statistically significant.

One potential explanation for the contrasting potencies of PEA3 and β-catenin in activating the COX-2 promoter could be different expression levels of the two proteins. To directly compare expression levels, lysates were prepared from 293 cells transfected with expression vectors for PEA3 or β-catenin (both of which were Myc epitope-tagged) and analyzed by Western blotting with anti-Myc epitope antibody 9E10. On this basis, PEA3 was expressed at 2–4-fold higher levels than β-catenin (Fig. 2). However, transfection of 50-fold less PEA3 than used in this experiment was sufficient to potently activate the COX-2 promoter (Fig. 1B), and it therefore seems unlikely that this small difference in expression levels is sufficient to explain the differential responsiveness of the COX-2 promoter to β-catenin and PEA3.

We also examined the response of the COX-2 promoter to the Ets factors Ets-1 and Ets-2, in connection with the PEA3 subfamily members PEA3, ER81, and ERM. Of these, PEA3 was the most potent activator of the COX-2 promoter (Fig. 3). ER81 and ERM were also capable of inducing significant activation, in contrast with the weaker responses elicited by Ets-1 and Ets-2. It was not possible to compare expression levels of the various factors directly, since the cDNAs were not uniformly epitope-tagged. However, since Ets-2 caused much stronger activation of the stromelysin-1 promoter than did
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PEA3 in the same experiment, we conclude that the COX-2 promoter is preferentially responsive to PEA3 family members (Fig. 3). Strikingly, PEA3 alone was also sufficient to activate transcription of the endogenous COX-2 gene. Transient transfection of PEA3 into 293 cells caused accumulation of COX-2 (Fig. 3). Strikingly, PEA3 alone was also sufficient to activate promoter regulation in these cells. To test this, the effects of PEA3 and β-catenin on COX-2 promoter activity in C57MG cells were compared. PEA3 overexpression increased COX-2 promoter activity to 205% (p < 0.03, Fig. 6). A smaller degree of activation was observed in response to β-catenin in several experiments, but was not statistically significant. These experiments were limited by the poor transfection efficiency associated with C57MG cells (4). Nevertheless, our data suggest that, both in 293 and C57MG cells (Figs. 1 and 6), PEA3 is a more potent activator of the COX-2 promoter than β-catenin.

In addition to being up-regulated in Wnt1-expressing C57MG cells, PEA3 was highly expressed in all tumors examined from Wnt1 transgenic mice (Figs. 5, B and C), contrasting with the very low expression level in normal virgin mammary gland (41) (Fig. 5B). ERM was also highly expressed in all Wnt1 tumors tested, while no expression was detected in wild type mammary gland (Fig. 5E, data not shown). Comparison of all three factors revealed that, in contrast to PEA3 and ERM, ER81 was expressed at a relatively low level in the tumors (Fig. 5E, data not shown), albeit at higher levels than in virgin mammary gland (Fig. 5F).

Our observation of high level expression of PEA3 factors in tumors from Wnt1 transgenic mice, coupled with the demonstration that PEA3 activates the COX-2 promoter, prompted us to test whether COX-2 was also up-regulated in Wnt1-expressing mammary tumors. Levels of COX-2 protein were compared in normal mammary glands from wild type mice and in tumors from Wnt1 transgenic mice. We observed a significant increase in COX-2 protein in the three tumors tested compared with virtually undetectable basal levels in wild type mammary gland (Fig. 7). These data suggest that increased expression of PEA3 factors may contribute to COX-2 up-regulation in these tumors.

In order to map the PEA3-responsive elements in the COX-2 promoter, a range of promoter reporter constructs were used in...
which deletions or site-specific mutations had been introduced (43). Progressive truncation of the promoter sequentially deletes binding sites for NF-κB, NF-IL6, and finally the CRE (Fig. 8A). No significant diminution in PEA3 responsiveness was observed until residues −220 to −125 were deleted (Fig. 8B). The −124/+59 construct was virtually unresponsive to PEA3, suggesting that the PEA3 response element(s) lie between −220 and −125. Since an NF-IL6-binding site is present in this region of the promoter, we next tested the responsiveness to PEA3 of various site-specific promoter mutants, including the ILM construct in which the NF-IL6 site is mutated. This experiment was primarily intended to rule out involvement of this site in the PEA3 response. However, to our surprise, we found that mutation of the NF-IL6 site completely and specifically abolished both basal and PEA3-stimulated COX-2 promoter activity (Fig. 9). In contrast, mutation of the NF-κB site had no effect on PEA3 responsiveness. Mutation of the CRE site reduced both basal and PEA3-stimulated activity, such that the index of stimulation exhibited by the CRM construct was not reduced relative to the wild type construct. Collectively, these data strongly implicate the NF-IL6 site in mediating PEA3 responsiveness. Since we have previously found mutation of the NF-IL6 site to have little effect on COX-2 promoter responsiveness to the lipid ceramide (49), we believe these data to reflect a specific involvement of the NF-IL6 site in mediating PEA3 responsiveness.

The NF-IL6 site is a consensus binding site for transcription factors of the C/EBP family. Several C/EBP factors have been identified (50). C/EBPα is generally associated with differentiation, while C/EBPβ and C/EBPδ are primarily implicated in mediating gene activation during inflammation and cell proliferation. Our data demonstrating the importance of the NF-IL6 site for PEA3 responsiveness suggested that C/EBP factors might be involved in PEA3-mediated activation of the COX-2 promoter. To test this hypothesis we used several C/EBP expression constructs, including a dominant negative variant, LIP (liver-enriched inhibitory protein). Consistent with previous observations in other cell types (43, 51), both C/EBPα and C/EBPδ stimulated COX-2 promoter activity, while C/EBPβ had little effect on basal promoter activity (Fig. 10A). None of the C/EBP isoforms tested enhanced the response to PEA3 (data not shown). However, LIP, which functions as a dominant negative C/EBP due to the absence of a transactivation domain (47), caused dose-dependent inhibition of the PEA3 response (Fig. 10B). This inhibitory effect of LIP suggests that C/EBP factors may be involved in mediating COX-2 promoter responsiveness to PEA3 (see “Discussion”).

Transcriptional up-regulation of COX-2 has previously been observed under conditions of nuclear β-catenin accumulation, such as in intestinal tumors and Wnt1-expressing cell lines. The ability of β-catenin to activate transcription in complex with other transcription factors has led to speculation that β-catenin might directly regulate the COX-2 promoter. As an initial test of this hypothesis, we examined the regulation of COX-2 promoter activity by overexpressed β-catenin. We also tested the potential role of PEA3 factors, since we had previously observed coordinate regulation by β-catenin and PEA3 of the matrilysin promoter (42). We observed that β-catenin caused only very weak activation of a COX-2 promoter reporter construct (Figs. 1A and 6). Although it remains possible that

![Image](https://example.com/image1)

**FIG. 6.** PEA3 stimulates COX-2 promoter activity in C57MG cells. C57MG cells were transfected with expression vectors encoding β-catenin or PEA3, together with a COX-2 promoter luciferase reporter construct (COX-2-LUC), and with pRL-TK as an internal control. Luciferase activities were performed as described above. Results shown are the mean ± S.D. of five replicates.

![Image](https://example.com/image2)

**FIG. 7.** Cox-2 protein is increased in Wnt1-expressing mammary tumors. Cox-2 protein was analyzed in lysates prepared from mammary tumors from three Wnt1 transgenic female mice (lanes 4–6) and from mammary glands isolated from three strain-matched wild type female mice (lanes 1–3). Lysates were prepared from 10 mg of each tissue sample. Cox-2 protein was immunoprecipitated, and immunoprecipitates were analyzed for Cox-2 by Western blotting as described under “Experimental Procedures.” The position of a Cox-2 standard is indicated by the arrow. Very little Cox-2 protein was detectable in the wild type mammary glands (lanes 1–3). In contrast, significant Cox-2 protein was observed in all three tumor samples (lanes 4–6).
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In this study, we found the NF-IL6 site to be essential for PEA3-mediated COX-2 promoter induction (Figs. 8 and 9). C/EBPα and C/EBPβ, but not C/EBPβ, caused modest stimulation of the COX-2 promoter (Fig. 10A), as has previously been reported for other cell types (43, 51), but overexpression of wild type C/EBPs did not enhance PEA3 responsiveness. In contrast, a dominant negative C/EBP diminished both basal and PEA3-stimulated promoter activity (Fig. 10B). Several models can be proposed to explain these data. PEA3 might bind directly to the NF-IL6 site, in which case the truncated dominant negative C/EBP protein could be antagonistic via competition with PEA3 for the NF-IL6 site. However, the NF-IL6 site does not resemble a consensus PEA3 site (5'-CCGGA(A/T)GC-3') (61). Alternatively, simultaneous binding of a C/EBP factor to the NF-IL6 site and of PEA3 to a cognate binding site might be required for activation. Several potential Ets-binding sites are present in the minimal PEA3-responsive fragment of the COX-2 promoter (Fig. 11), although none corresponds exactly to a consensus PEA3 site. Precedents for synergistic transcriptional activation by Ets factors and C/EBF factors have previously been described (62, 63). Thus it seems likely that binding of C/EBP and PEA3 to proximal sites may be required for induction of COX-2 transcription in our system. However, we have not excluded the possibility that PEA3 might bind to C/EBP proteins at the NF-IL6 site without itself binding to DNA.

As discussed above, NF-IL6 sites in the COX-2 promoter have previously been implicated in the response to multiple agents (43, 53–55). Interestingly, NF-IL6 sites are also implicated in COX-2 up-regulation in mouse skin tumors (51), and mutagenesis of the NF-IL6 site reduces COX-2 promoter activity in the human colon cancer cell lines HCA7 and LS-174 (64).

FIG. 8. Mapping the site of PEA3 responsiveness in the COX-2 promoter. A, human COX-2 promoter and deletion constructs. The transcription start site is indicated by an arrow, the TATA box at −31/−25 is shown as a white rectangle, and three well characterized transcription factor binding sites lying between −327 to +59 of the human COX-2 promoter are depicted as black ovals. The presence of these three sites in the truncated constructs used in the experiment shown in B are also shown. B, promoter truncation analysis. 293 cells were transfected with COX-2-LUC (−1432/+59) or with the truncated COX-2 promoter constructs shown in A, plus pRL-TK, and with or without PEA3 expression vector. Luciferase assays were performed as described above. Results shown are the mean ± S.D. of six replicates. Solid bars, −PEA3; hatched bars, +PEA3.

FIG. 9. Mutation of the NF-IL6-binding site in the COX-2 promoter destroys PEA3 responsiveness. 293 cells were transfected with promoter constructs containing residues −327 to +59 of the human COX-2 promoter, plus pRL-TK, with and without PEA3 expression vector. WT is the wild type promoter sequence, KBM has the NF-κB site mutated, ILM has the NF-IL6 site mutated, and CRM has the CRE mutated. Luciferase assays were performed as described above. Results shown are the mean ± S.D. of six replicates. Solid bars, −PEA3; hatched bars, +PEA3.
This latter result is particularly intriguing since we have previously detected high level PEA3 expression in intestinal tumors and colorectal cancer-derived cell lines (42), and have also observed PEA3-induced COX-2 promoter activation in colorectal cancer cell lines.\(^2\) Thus PEA3-mediated regulation of the COX-2 promoter via the NF-IL6 element may explain the importance of this site in human colorectal cancer lines (64). The correlation between PEA3 expression and COX-2 expression both in intestinal tumors and in Wnt1-expressing cells and tumors (Figs. 5 and 7) suggests that PEA3 contributes to COX-2 up-regulation in response to both Wnt1 expression and APC mutation.

The coincidence of PEA3 expression in intestinal tumors resulting from APC mutation and in mammary tumors caused by Wnt1 expression suggests that PEA3 up-regulation is a common consequence of activation of the Wnt/\(\beta\)-catenin signaling pathway. This raises the intriguing possibility that PEA3 itself may be a target of \(\beta\)-catenin. To address this directly, the effect of \(\beta\)-catenin on a PEA3 promoter reporter construct has been assayed. Preliminary data indicate that \(\beta\)-catenin overexpression in Cos cells can stimulate PEA3 promoter activity up to 5-fold.\(^5\) If PEA3 transcription can be regulated by \(\beta\)-catenin, it is unclear why ectopic expression of \(\beta\)-catenin was insufficient to induce COX-2 transcription in 293 cells, when PEA3 was sufficient (Figs. 1 and 4). One potential explanation could be differing PEA3 expression levels achieved by transfection with \(\beta\)-catenin and PEA3. Since PEA3 is known to positively regulate its own transcription (65), ectopic PEA3 expression may lead to much greater levels of PEA3 expression than can be achieved in the same time frame by expression of \(\beta\)-catenin. Interestingly, the PEA3 promoters of human, mouse, and chicken all contain a TCF-binding site, conserved in both sequence and position relative to the transcriptional start site,\(^3\) suggesting that PEA3 is most likely a direct target of \(\beta\)-catenin.

PEA3 family members have recently been shown to regulate the promoter of another gene, matrilysin, in synergy with \(\beta\)-catenin (42). Aberrant matrilysin expression is detected in intestinal tumors, and matrilysin is also up-regulated in Wnt1-expressing mammary cell lines and tumors.\(^4\) Thus both COX-2 and matrilysin are responsive to Wnt signaling and are regulatable by PEA3 factors. In addition, we have recently observed synergistic activation by \(\beta\)-catenin and PEA3 of the murine Twist gene.\(^5\) Finally, we note that the promoters of several genes known to be \(\beta\)-catenin-responsive also contain consensus

\[^{2}\text{H. C. Crawford and L. R. Howe, unpublished data.}^\]
\[^{3}\text{C. Messier and J. A. Hassell, unpublished data.}^\]
\[^{4}\text{L. R. Howe, O. Watanabe, J. Leonard, and A. M. C. Brown, unpublished data.}^\]
\[^{5}\text{L. R. Howe and H. C. Crawford, manuscript in preparation.}^\]
Ets-binding sites. For example, the *Drosophila* gene *Even-skipped* is coordinately regulated via TCF and Ets-binding sites (66), and the *catenin* promoter can be activated cooperatively by PEA3, β-catenin, and c-Jun. Together these data lead us to speculate that PEA3 factors may contribute to regulation of several target genes of the Wnt/β-catenin pathway.

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