p38 MAP kinase negatively regulates cyclin D1 expression in airway smooth muscle cells

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Page, Kristen, Jing Li, and Marc B. Hershenson. p38 MAP kinase negatively regulates cyclin D1 expression in airway smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 280: L955–L964, 2001.—We have demonstrated that platelet-derived growth factor (PDGF) stimulates p38 mitogen-activated protein (MAP) kinase activation in bovine tracheal myocytes, suggesting that p38 is involved in growth regulation. We therefore examined whether p38 regulates expression of cyclin D1, a G1 cyclin required for cell cycle traversal. The chemical p38 inhibitors SB-202190 and SB-203580 each increased basal and PDGF-induced cyclin D1 promoter activity and protein abundance. Overexpression of a dominant negative allele of MAP kinase kinase-3 (MKK3), an upstream activator of p38α, had similar effects. Conversely, active MKK3 and MKK6, both of which increase p38α activity, each decreased transcription from the cyclin D1 promoter. Together, these data demonstrate that p38 negatively regulates cyclin D1 expression. We tested whether p38 regulates cyclin D1 expression via inhibition of extracellular signal-regulated kinase (ERK) activation. Chemical inhibitors of p38 induced modest ERK phosphorylation and activation. However, dominant negative MKK3 was insufficient to activate ERK, and active MKK3 and MKK6 did not attenuate platelet-derived growth factor-mediated ERK activation. These data are consistent with the notion that p38α negatively regulates cyclin D1 expression via an ERK-independent pathway.

There is ample evidence supporting the notion that excess airway smooth muscle mass contributes to the pathogenesis of airflow obstruction in asthma (6, 10–12, 19, 22, 49, 52, 55). Accordingly, we have examined the signaling pathways responsible for cell cycle traversal in primary bovine tracheal myocytes. Inhibition of mitogen-activated protein (MAP) kinase/extracellular signal-regulated kinase (ERK) kinase (MEK) reduced platelet-derived growth factor (PDGF)-induced DNA synthesis in a concentration-dependent manner, suggesting that catalytic activation of MEK-1 and ERK is required for DNA synthesis in these cells (28). In addition, we found that activation of MEK-1 is required and sufficient to activate transcription from the promoter of cyclin D1 (47), a critical regulator of G1 progression in these cells (66).

Growth factor treatment of airway smooth muscle cells also induces activation of the two stress-activated MAP kinases, p38 and c-Jun amino-terminal kinase (JNK) (41, 45, 50), consistent with the notion that these intermediates, like ERKs, play a role in the growth regulation. The precise role of p38 and JNK in airway smooth muscle cell cycle traversal has not been established, however.

The p38 MAP kinase family consists of four isoforms. p38α was originally identified in lipopolysaccharide-stimulated mouse macrophages and was found to have substantial homology to the yeast high-osmolarity glycerol kinase (9, 18, 36, 38). Since this original identification, three additional isoforms, β, γ, and δ, have been cloned (23, 24, 53, 60). p38α, -β, and -δ are somewhat ubiquitously expressed, whereas p38-γ is primarily restricted to skeletal muscle (60). p38α and -β are inhibitable by pyridinylimidazole compounds such as SB-202190 and SB-203580, whereas p38γ and -δ are not (14, 24, 31). Three MAP kinase kinases (MKKs) for p38 have been identified. MKK6 strongly activates all p38 isoforms, and MKK3 preferentially activates p38α and -δ (14, 21, 23, 37, 46, 57). MKK4 appears to phosphorylate and activate both JNK1 and p38α (9, 36). Few studies in any cell system have addressed the potential role of p38 in the regulation of cell growth. Overexpression of p38 inhibits serum-induced NIH/3T3 cell cycle progression (39). Mutations of the MKK4 gene have been found in human pancreatic, lung, breast, testicular, and colorectal cancer cell lines (54, 56). Expression of an active MEK kinase (MEKK)-3 inhibited cell cycle progression and reversed Ras-induced transformation in NIH/3T3 cells (13). This effect was blocked by coexpression of an inactive MKK6, suggesting that cell cycle arrest was mediated by p38.

The mechanism by which signaling through the p38 pathway may negatively regulate growth has not been well studied. p38 attenuates transcription from the cyclin D1 promoter in CCL-39 hamster lung fibroblasts (33) but is necessary for optimal induction of cyclin D1 promoter activity in the human breast cancer MCF-7 cell line (34).

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Recent evidence suggests that p38 exerts its effects, in part, via the negative regulation of ERK. The triggering of stress-activated kinases, concomitant with the inhibition of the ERK pathway, has been observed in a number of cell systems undergoing apoptosis (4, 65). In rat-1 fibroblasts, stimulation of α1A-adrenoceptors by phenylephrine stimulated p38 activation while inhibiting ERK phosphorylation (3). Treatment with the chemical p38 inhibitors SB-202190 or SB-203580 has been demonstrated to increase ERK activation in mouse keratinocytes (48), HepG2 cells (51), and, in a preliminary report, canine tracheal myocytes (25). These data suggest that p38 may negatively regulate the ERK pathway, although the signaling intermediates linking p38 and ERK have not been established.

We tested the hypothesis that in primary bovine tracheal myocytes, p38 negatively regulates transcription from the cyclin D1 promoter. Our results suggest that p38 negatively regulates cyclin D1 expression in an ERK-independent manner.

METHODS

Materials. Anti-human α-smooth muscle actin, peroxidase-linked goat anti-rabbit IgG, protein A Sepharose beads, and myelin basic protein were purchased from Sigma (St. Louis, MO). SB-203580 and SB-202190 were from Calbiochem (La Jolla, CA). PDGF was obtained from Upstate Biotechnology (Lake Placid, NY). PD-98059 and recombinant activating substrate (43).

Western analysis of whole cell extracts. Extracts (10 µg) were resolved on 10% SDS-polyacrylamide gels and transferred to nitrocellulose by semidry transfer (Hoefer, San Francisco, CA). After incubation with the appropriate polyclonal antibody, the blots were incubated in 10% FBS-DMEM overnight. After being rinsed, the blots were incubated with a liposome solution consisting of serum- and antibiotic-free medium, plasmid DNA (total of 1.8 µg/plate), and LipofectAMINE (12 µl/plate). Cells were transiently cotransfected with plasmids encoding the human cyclin D1 promoter subcloned into a luciferase reporter and either the relevant expression vector or control vector (42). After 5 h, the liposome solution was replaced with 10% FBS-DMEM. The next day, the cells were serum starved in DMEM. After 24 h of serum starvation, selected cultures were treated with chemical p38 inhibitors and/or PDGF (30 ng/ml). Finally, 16 h after PDGF treatment, cells were harvested for analysis of luciferase activity with lysis buffer provided in the Promega luciferase assay system. Luciferase activity was measured at room temperature with a luminometer (Turner Designs, Sunnyvale, CA). Luciferase content was assessed by measuring the light emitted during the initial 30 s of the reaction, and the values are expressed in arbitrary light units. The background activity from cell extracts was typically >0.02 U compared with signals on the order of 102 to 103 U. Cyclin D1 promoter transcriptional activation was normalized for transfection efficiency by cotransfecting cells with a cDNA encoding β-galactosidase (30 ng/plate). β-Galactosidase activity was assessed by colorimetric assay with o-nitrophenyl-β-D-galactoside as a substrate (43).

We have found that cotransfection with viral promoter-driven expression vectors tends to suppress cyclin D1 promoter activity, perhaps due to a limitation in the transcription factors available for overall gene expression. This effect seems to be pronounced in primary cells. Concentration-response curves were therefore generated for each expression vector to determine optimal concentration. In all cases, concentrations of 30–50 ng expression vector/plate were used.

Preparation of cell extracts for immunoblotting. Cells were cultured in six-well plates and serum starved for 48 h before treatment. Selected cultures were treated with 3 µM SB-202190 or SB-203580 1 h before growth factor treatment or with 30 ng/ml of PDGF (for 10 min or 16 h for ERK or cyclin D1 immunoblots, respectively). Cells were washed in PBS (150 mM NaCl and 0.1 M phosphate, pH 7.5) and extracted in a lysis buffer containing 50 mM Tris, pH 7.5, 40 mM β-glycerophosphate, 100 mM NaCl, 2 mM EDTA, 50 mM NaF, 200 µM Na3VO4, 200 µM phenylmethylsulfonyl fluoride, and 1% Triton X-100. Insoluble materials were removed by centrifugation (13,000 rpm for 10 min at 4°C), and the supernatant was transferred to fresh microcentrifuge tubes.

Western analysis of whole cell extracts. Extracts (10 µg) were resolved on a 10% SDS-polyacrylamide gel and transferred to nitrocellulose by semidry transfer (Hoefer, San Francisco, CA). After incubation with the appropriate polyclonal antibody, signals were amplified and visualized with anti-rabbit IgG and enhanced chemiluminescence.

Measurement of ERK and p38 activation. Cells were transiently cotransfected with cDNAs encoding HA-tagged ERK2 or p38α and the expression vector of interest. Forty-eight hours after transfection, the cells were serum starved in DMEM. The next day, selected cultures were treated with PDGF (30 ng/ml for 10 min) or anisomycin (50 µg/ml for 30 min). Activation of ERK2 or p38α was then assessed by immunoprecipitation of the epitope tag followed by an in vitro phosphorylation assay with major basic protein (MBP) and ATF-2 as substrates, respectively, as described (28, 41).
Treated cells were washed twice with PBS and incubated in a lysis buffer consisting of 50 mM Tris·HCl, pH 7.5, 1% Triton X-100, 40 mM β-glycerophosphate, 100 mM NaCl, 50 mM NaF, 2 mM EDTA, 200 μM Na2VO4, and 0.2 mM phenylmethylsulfonyl fluoride (30 min at 4°C). Insoluble materials were removed by centrifugation (13,000 rpm for 10 min at 4°C). Cell lysates were then incubated for 3 h with 30 μl of protein A Sepharose beads precoupled with the 12CA5 anti-HA antibody. Immunoprecipitates were washed three times with lysis buffer and twice with kinase buffer containing 20 mM HEPES (pH 7.4), 10 mM MgCl2, 1 mM dithiothreitol, 200 μM Na3VO4, and 10 mM p-nitrophenyl phosphate. Immune complexes were resuspended in a final volume of 30 μl of kinase buffer and incubated (20 min at 30°C) with 5 μCi of [α-32P]ATP and either 0.25 mg/ml of MBP or 0.2 mg/ml of ATF-2. Reactions were terminated by adding Laemmli buffer and boiling. Samples were resolved on a 10% SDS gel, and the proteins were transferred to a nitrocellulose membrane by semidy transfer. After Ponceau staining, the membrane was exposed to film and substrate phosphorylation assessed by optical scanning (Jandel Scientific, San Rafael, CA).

To confirm that apparent differences in ERK or p38 activity were not related to alterations in expression of the epitope-tagged ERK or p38, nitrocellulose membranes were probed with the anti-HA antibody 12CA5. Signals were amplified and visualized with peroxidase-linked rat anti-mouse x light-chain IgG and enhanced chemiluminescence.

Statistical analysis. When applicable, significance was assessed by one-way analysis of variance (ANOVA). Differences identified by ANOVA were pinpointed by the Student-Newman-Keuls multiple range test.

RESULTS

Chemical inhibition of p38 negatively regulates cyclin D1 transcription and protein abundance. Inhibition of p38 has been demonstrated to enhance cyclin D1 promoter activity and protein abundance in Chinese hamster lung fibroblasts (33). To determine the effect of p38 inhibition on cyclin D1 expression in bovine tracheal myocytes, cells were transiently transfected with a cDNA encoding the full-length cyclin D1 promoter subcloned into a luciferase reporter gene (2). SB-202190 or SB-203580 (3 μM each) each increased basal and PDGF-induced transcription from the cyclin D1 promoter (Fig. 1A). Furthermore, SB-203580 increased basal and PDGF-induced cyclin D1 protein abundance (Fig. 1B).

MKK3 and MKK6 regulate transcription from the cyclin D1 promoter and cyclin D1 protein abundance. MKK3 and MKK6 phosphorylate and activate p38 (14, 21, 23, 37, 46, 57). To determine whether MKK3 regulates cyclin D1 expression, cells were transiently transfected with cDNAs encoding mutant alleles of MKK3 and the luciferase-tagged cyclin D1 promoter. Similar to the chemical p38 inhibitors, overexpression of a dominant negative form of MKK3 (MKK3-AL) increased transcription from the cyclin D1 promoter (Fig. 2A). Overexpression of a constitutively active MKK3 (MKK3(glu)) reduced basal and PDGF-induced cyclin D1 promoter activity, and overexpression of active MKK6 (MKK6(glu)) had similar effects (Fig. 2B).

Because of the relatively low transfection efficiency of primary bovine tracheal myocytes, we could not determine whether inhibition of p38 by dominant negative MKK3 alters cyclin D1 protein abundance in whole cell lysates. We therefore examined this question in the A7r5 rat aortic smooth muscle cell line and in COS cells. Overexpression of MKK3-AL increased cyclin D1 protein abundance (Fig. 3).

Chemical p38 inhibitors inhibit p38 and activate ERK in primary bovine tracheal myocytes. In certain cell systems, chemical inhibition of p38 (with SB-202190 or SB-203580) increases ERK activity (3, 25, 51). As in other cell types (2, 33, 62), it has been demonstrated in bovine tracheal myocytes that ERK activation is an upstream activator of transcription from the cyclin D1 promoter (47). Thus inhibition of p38 could increase cyclin D1 accumulation via the activation of ERK. We therefore investigated the potential interactions between the p38 and ERK signaling pathways in bovine tracheal smooth muscle cells.

To test the effects of chemical inhibitors on p38 activity, cells were transiently transfected with a HA-tagged p38α (64), and p38α activity was assessed by immunoprecipitation of the epitope tag followed by in vitro phosphorylation with ATF-2 as a substrate. As
expected, pretreatment with either SB-202190 or SB-203580 (each 3 µM) attenuated anisomycin-induced p38α activation (Fig. 4). To test whether p38 inhibitors increase endogenous ERK activity, we first assayed for ERK phosphorylation with an antibody against dually phosphorylated ERK. Both SB-202190 and SB-203580 modestly increased ERK phosphorylation (Fig. 5A). Next, we transiently transfected cells with a HA-tagged ERK2 (20) and assessed ERK activity by immunoprecipitation of the epitope tag followed by in vitro phosphorylation assay with MBP as a substrate. Both SB-202190 and SB-203580 were sufficient to induce modest activation of ERK2 (Fig. 5, B and C). ERK2 activation by SB-202190 was reduced by pretreatment with PD-98059, a specific inhibitor of MEK (Fig. 5D). Taken together, these data suggest that chemical p38 inhibitors activate ERK in bovine tracheal smooth muscle cells.

Fig. 2. Effect of mutant alleles of mitogen-activated protein kinase/extracellular signal-regulated kinase kinase (MKK)-3 and MKK6 on cyclin D1 transcription. A: cells were transiently cotransfected with cDNAs encoding the human full-length cyclin D1 promoter subcloned into a luciferase reporter gene (~1745CD1LUC) and the dominant negative allele of MKK3 (MKK3-AL). Selected cultures were treated with PDGF (30 ng/ml for 16 h). Data are means ± SE for 4 experiments. *Significantly greater than control, P < 0.05 by ANOVA. **Significantly greater than PDGF, P < 0.05 by ANOVA. B: cells were transiently cotransfected with cDNAs encoding the cyclin D1 luciferase reporter gene and constitutively active forms of MKK3 [MKK3(glu)] or MKK6 [MKK6(glu)]. Data are means ± SE for 4 experiments. *Significantly less than PDGF, P < 0.05 by ANOVA.

Fig. 3. Effect of a dominant negative allele of MKK3 on cyclin D1 protein abundance. A7r5 vascular smooth muscle cells and COS cells were transfected with MKK3-AL. Selected cultures were treated with PDGF (30 ng/ml for 16 h) or epidermal growth factor (EGF; 30 ng/ml for 16 h). Whole cell lysates were resolved by SDS-PAGE, transferred to nitrocellulose, and probed with an anti-cyclin D1 antibody (1:1,000 dilution). This experiment was repeated twice with similar results.

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Fig. 4. Effects of SB-202190 and SB-203580 on anisomycin-induced p38α phosphorylation and activity. A: cells were transiently transfected with a cDNA encoding a hemagglutinin (HA)-tagged p38α. After serum starvation, cells were pretreated with 3 µM SB-202190 or SB-203580 for 1 h before activation with anisomycin (50 µg/ml for 30 min). Cell lysates were immunoprecipitated with an antibody against HA. Top: p38α activity was assessed by in vitro phosphorylation assay with activating transcription factor (ATF)-2 as a substrate, Bottom: level of expression of epitope-tagged p38α as determined by immunoblotting. B: in vitro phosphorylation assays. Data are means ± SE; n = 4 experiments. *Significantly less than anisomycin, P < 0.05 by ANOVA.
muscle cells and that they do so by stimulating an upstream activator of MEK.

**MKK3 and MKK6 regulate p38 but not ERK in primary bovine tracheal myocytes.** To examine the effects of mutant forms of MKK3 and MKK6 on p38 activity, bovine tracheal myocytes were transiently cotransfected with cDNAs encoding a HA-tagged ERK2 (top). After serum starvation, selected cultures were treated for 1 h with 3 μM SB-202190 or SB-203580. Selected cultures were treated with PDGF (30 ng/ml for 10 min). Cell lysates were immunoprecipitated with an antibody against HA. ERK activity was assessed by in vitro phosphorylation with major basic protein (MBP) as a substrate. The level of HA-ERK2 expression was determined by immunoblotting (bottom). C: in vitro phosphorylation assays. Data are means ± SE; n = 4 experiments. *Significantly greater than control, P < 0.05 by ANOVA. D: after transfection (as described above), selected cultures were pretreated with PD-98059 (30 μM for 1 h) and stimulated with SB-202190 (3 μM for 1 h). A positive control is also shown (PDGF, 30 ng/ml for 10 min). ERK activity was assayed by in vitro phosphorylation with MBP as a substrate (top). The level of expression of epitope-tagged ERK2 was determined by immunoblotting (bottom). This experiment was repeated twice, with similar results.

**DISCUSSION**

ERK activation has been shown to be required for DNA synthesis in a wide variety of cell systems including airway smooth muscle (28, 35, 40, 59, 63). On the other hand, activation of p38 MAP kinase has been associated with growth inhibition (33) and apoptosis (4,
We have demonstrated that PDGF induces activation of both ERK and p38 in bovine tracheal myocytes (41) and that ERK activation is required for PDGF-induced cyclin D1 expression and DNA synthesis in these cells (28, 41, 47). If p38 negatively regulated cyclin D1 expression, then PDGF-induced activation of p38 might limit ERK-mediated cell growth. Because excess airway smooth muscle proliferation has been noted in patients with fatal asthma, the concurrent regulation of growth-stimulatory and growth-inhibitory pathways by growth factors might constitute an important physiological mechanism limiting airflow obstruction in these patients.

In this report, we show that inhibition of p38, either by chemical inhibitors or by overexpression of a dominant negative allele of MKK3, increases cyclin D1 promoter transcription and protein abundance in cultured primary airway smooth muscle cells. These data imply a basal level of p38 activation in serum-starved cells (Figs. 4 and 6). Furthermore, selective activation of p38 by overexpression of either MKK3 or MKK6 attenuated both basal and PDGF-induced transcription from the cyclin D1 promoter. These data suggest that p38 negatively regulates cyclin D1 expression in airway smooth muscle cells. Our data confirm previous experiments in CCL-39 fibroblasts in which overexpression of MKK3 decreased basal and serum-induced cyclin D1 expression, whereas treatment with a chemical p38 inhibitor or a dominant negative form of MKK3 increased transcription from the cyclin D1 promoter (33). Furthermore, our data are consistent with recent studies suggesting that p38 negatively regulates cyclin G1 expression. In PC12 cells, hypoxia increased p38 activation while decreasing cyclin D1 levels (8).
bryonic kidney cells, chemical inhibition of p38 allowed Rac1 to induce anchorage-dependent cyclin A transcription (44).

In PC12 (65) and HeLa cells (4), p38 activation occurred concomitantly with inhibition of the ERK pathway, suggesting that p38 negatively regulates ERK. Also, treatment with the pyridinyl imidazole compounds SB-203580 and SB-202190 has been noted to increase ERK activation in mouse keratinocytes (48), canine tracheal myocytes (25), and HepG2 cells (51), suggesting that inhibition of p38 activates the ERK pathway (51). We therefore examined the regulation of ERK activity by p38 in bovine tracheal myocytes. We found that treatment of bovine myocytes with the chemical p38 inhibitors SB-202190 and SB-203580 increased ERK activation. However, overexpression of a dominant negative form of MKK3, which inhibited anisomycin-induced p38α activation, did not increase basal or PDGF-stimulated ERK activity. Furthermore, when we overexpressed active forms of MKK3 and MKK6, both of which increase p38α activation, there was no reduction of either basal or PDGF-induced activation of ERK. Taken together, these data suggest that the selective inhibition or activation of p38α by upstream activators does not regulate ERK activation. Furthermore, they suggest that the pyridinyl imidazole compounds SB-202190 and SB-203580 may activate ERK via a MEK-dependent, p38α-independent pathway.

It has recently been demonstrated that SB-203580 inhibits Raf-1 in vitro (17). However, in vivo, inhibition of Raf-1 by SB-203580 is counterbalanced by a novel

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Fig. 7. Effect of mutant alleles of MKK3 and MKK6 on ERK activation. A: cells were transiently cotransfected with MKK3-AL and a HA-tagged ERK2. ERK2 was assessed by in vitro phosphorylation with MBP as a substrate (top). Selected cultures were treated with PDGF (30 ng/ml for 10 min) before harvest. Level of expression of epitope-tagged ERK2 was determined by immunoblotting (bottom). B: in vitro phosphorylation assays. Data are means ± SE for; n = 4 experiments. C: cells were transiently cotransfected with MKK3(glu) or MKK6(glu) and a HA-tagged ERK2. ERK2 activity was assessed by in vitro phosphorylation with MBP as a substrate (top). Selected cultures were treated with PDGF (30 ng/ml for 10 min) before harvest. Level of expression of epitope-tagged ERK2 was determined by immunoblotting (bottom). D: in vitro phosphorylation assays. Data are means ± SE for; n = 4 experiments.
feedback loop, leading to Raf-1 activation (16, 27). Our study, in which ERK activation was induced by both SB-202190 and SB-203580 but not a dominant negative inhibitor of MKK3, supports the notion that pyridinyl imidazole inhibitors may have p38-independent effects. Interestingly, neither chemical inhibitor increased ERK activation in COS cells (Page and Hershenson, unpublished results), indicating that the level of compensatory Raf-1 activation may vary with cell type. Our laboratory has previously shown (29) that in bovine tracheal myocytes, an alternative MEK activator besides Raf is responsible for MEK phosphorylation. Thus the finding that pyridinyl imidazole compounds activate ERK in our system suggests that these agents may inhibit other MAP kinase kinase kinases besides Raf.

Other possible explanations for the differential effects of chemical p38 inhibitors and the dominant negative MKK3 need to be considered. The pyridinyl imidazole compounds selectively inhibit p38α and -β, and therefore it is conceivable that the activation of ERK by these compounds relates to inhibition of p38β (31). Because p38β is activated by MKK6 but not by MKK3, experiments that employed a dominant negative mutant of MKK6 would address this possibility. However, the two dominant negative MKK6 mutants we tested, although they attenuated anisomycin-induced activation of p38, also significantly increased basal p38 activity. Nevertheless, our finding that a constitutively active MKK6 mutant that activates all isoforms of p38 (14, 23, 24, 60) had no effect on ERK activation suggests that p38β does not regulate ERK in our system.

It is important to note potential limitations of our approach, which employed the overexpression of constitutively active and dominant negative signaling intermediates. First, it is possible that overexpression of a constitutively active protein could induce a supra-physiological outcome. For example, it is conceivable that although selective activation of p38 by MKK3/6 inhibits cyclin D1 expression in our system, p38 activation alone may not be sufficient for this effect under normal conditions and may require the activation of other negative regulatory pathways. Second, dominant negative proteins may bind downstream signaling intermediates, thereby blocking the activity of other upstream activators that bind at the same site. However, due to the difficulty of selectively activating p38, we saw no alternative but to use these reagents. For example, chemical activators of p38 such as anisomycin, hyperosmolarity, and UV irradiation tend to cause multiple stress-related effects that would likely influence our results. Nevertheless, chemical inhibitors of p38 increased cyclin D1 promoter activation and protein abundance in our system, confirming the enhancing effects of the dominant negative MKK3 on transcription from the cyclin D1 promoter. Finally, we did not examine the effects of p38 on the transcription or protein abundance of other G1 cyclins, cyclin-dependent kinases, or cyclin-dependent kinase inhibitors. Thus while the negative regulation of cyclin D1 by p38 would tend to attenuate cell cycle traversal, the net effect of p38 activation on airway cell DNA synthesis and proliferation remains to be determined.

In conclusion, our data strongly suggest that in bovine tracheal myocytes, p38α negatively regulates transcription from the cyclin D1 promoter via an ERK-independent pathway. Furthermore, chemical inhibitors of p38 may activate ERK in a p38α-independent manner.

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REFERENCES


48. Saetta M, Di Stefano A, Rosina C, Thiene G, and Fabbri LM. Quantitative structural analysis of peripheral airways and


