Epithelial injury induces Egr-1 and Fos expression by a pathway involving protein kinase C and ERK

Dieckgraefe, Brian K., and Danielle M. Weems. Epithelial injury induces Egr-1 and Fos expression by a pathway involving protein kinase C and ERK. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G322–G330, 1999.—The signaling pathways activated in response to gastrointestinal injury remain poorly understood. Previous work has implicated the extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase as a mediator of wound-signal transduction and a possible regulator of epithelial restitution. Monolayer injury resulted in rapid activation of p42 and p44 ERK. Injury-induced ERK activation was blocked by protein kinase C inhibition or by disruption of the cell cytoskeleton. Significant increases in Fos and early growth response (Egr)-1 mRNA levels were stimulated by injury, peaking by 20 min. ERK activation and the induction of Egr-1 mRNA were inhibited in a dose-dependent fashion with PD-98059. Fos mRNA expression was partially blocked by PD-98059. Western blot analysis demonstrated strong expression and nuclear localization of Fos and Egr after wounding. Electrophoretic mobility shift assays demonstrated that nuclear extracts contained a protein that specifically bound double-stranded oligonucleotides containing the Egr consensus binding element. Gel supershift assays demonstrated that the protein-DNA complexes were recognized by anti-Egr antibody. Inhibition of injury-induced ERK activation by PD-98059 or direct interference with Egr by expression of a dominant negative mutant led to significantly reduced in vitro monolayer restitution; extracellular signal-regulated kinase; mitogen-activated protein kinase; early growth response-1; injury.

Sheets of epithelial cells line the alimentary tract and serve as a barrier to potentially toxic luminal contents. Maintenance of a continuous epithelial barrier in the gastrointestinal tract requires both dynamic and precise control of cell proliferation, differentiation, migration, and senescence. Continuous challenges to this barrier occur as a consequence of physiological (e.g., digestion) and disease-related (toxic, infectious, inflammatory, and mechanical) insults. Deep or extensive tissue injury requires cellular proliferation for replacement of lost tissue mass. In contrast, superficial injury is repaired rapidly over minutes to hours by a process known as restitution. Within minutes of epithelial injury, cells adjacent to a wound flatten, lose polarity, extend lamellipodia, and rapidly migrate over the denuded basal lamina to reestablish epithelial continuity (5, 28, 29, 34). The stimulatory role of growth factors in the regulation of these events, both by directly mediating cell proliferation and by the enhancement of cell migration characteristic of restitution, has been well established (7, 12–14). However, our understanding of epithelial wound-signal transduction pathways, with potential roles as efferent and afferent mediators of the response to gastrointestinal injury, remains poorly defined.

Epithelial injury is likely to initiate a complex series of changes in gene expression that mediate the downstream events required for tissue repair, including cell proliferation, migration, and remodeling of the extracellular matrix. Immediate early genes serve as mediators of membrane proximal second-messenger signaling cascades. Because many immediate-early genes encode transcription factor components, they are uniquely positioned to transactivate multiple selected secondary targets. Early growth response (Egr)-1 and Fos are important mediators of a broad range of biological responses. Egr-1 is a zinc finger nuclear phosphoprotein that binds with high affinity to the DNA sequence GCG(G/T)GGGCG. Exogenously expressed Egr-1 can transactivate promoters containing this element (19). The Fos transcriptional control region contains numerous cis regulatory elements responsive to a diverse group of extracellular stimuli (21). One example, the serum response element (SRE), is a target of activated extracellular signal-regulated kinase (ERK). ERK-mediated phosphorylation of the activation domain of the ternary complex factor protein Elk-1 results in increased DNA binding, formation of the ternary complex, and associated transcriptional activation of Fos and other SRE-dependent genes (42).

Although Egr-1 appears to be involved in a variety of cellular processes, one fundamental role for Egr-1 may be that of a general activator of injury-induced gene expression (22, 23). The response of vascular endothelium to injury has been characterized (23) and is strikingly similar to our reported results. Denuding injury to the aortic endothelium led to rapid increases in Egr-1 expression, specifically at the wound edge. Further support for this hypothesis is that many genes with potential relevance for tissue repair contain nucleotide recognition elements for Egr-1, including transforming growth factor-β1 (10, 25), tissue factor (9, 30, 31), urokinase-type plasminogen activator (41), intercellular adhesion molecule-1 (32), insulin-like growth factor II (15), and platelet-derived growth factor A and B (23, 24, 39).

IEC-6 cells, derived from fetal rat duodenal crypt cells, have been one of the best-studied in vitro models for epithelial restitution (7). The use of cultured IEC-6 cells for our studies allows investigation of isolated epithelial wound-signal transduction pathways in the absence of cell-to-cell communication with other cellu-
lar elements found in the more complex normal mucosa. We have previously shown that ERK and p38 mitogen-activated protein (MAP) kinase pathways undergo rapid activation in this injury model. The involvement of MAP kinase cascades in the transduction of stress or injury-induced signals is a response that appears to have been largely conserved throughout evolution. Activation of MAP kinase cascades has been described in a variety of settings, ranging from intestinal epithelial injury (11), tissue hypoxia (33), neural damage (1), mechanical injury to plants (38), and to varied environmental stresses in yeast (20). Our present studies demonstrate that rapid ERK activation by injury involves protein kinase C (PKC) and leads to the rapid expression of Fos and Egr-1 mRNA. This expression immediately precedes the nuclear localization of Fos and Egr-1 proteins and cis element DNA binding activity. The importance of the ERK-Egr-1 pathway in regulating injury-induced cell migration is demonstrated by two inhibitory approaches. Directly blocking ERK activation or interference with Egr-1 function both resulted in diminished cell migration after injury.

MATERIALS AND METHODS

Materials and reagents. [γ-32P]ATP and [α-32P]dCTP were obtained from Amersham Life Sciences (Arlington Heights, IL). cDNA clone for mouse Egr-1 was obtained from the American Type Culture Collection (ATCC) DNA probe repository. A plasmid containing rat c-Fos was a gift of Dr. Jeffery Milbrandt. cDNA fragments were gel purified and labeled by random priming (Prime-a-Gene; Promega, Madison, WI). Polyclonal antibodies to Egr-1, Fos, and ERK2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Phosphospecific anti-ERK antibodies were kindly provided by New England Biolabs (Beverly, MA). The MAP kinase kinase (MEK) I and -2 inhibitor, PD-98059, was provided by Parke-Davis (Ann Arbor, MI), and the phosphor inhibitor, SB-203580, was provided by SmithKline Beecham (King of Prussia, PA). Double-stranded oligonucleotides containing consensus element binding sequences for activator protein (AP)-1, Egr, or sequences containing mutations in AP-binding sequences were labeled with [γ-32P]ATP, using T4 polynucleotide kinase. Binding reaction mixtures, consisting of 0.5 ng of the labeled oligonucleotide, 1 μg of poly(dI-dC) (Pharmacia Biotech), and 10 μg of nuclear extract proteins, were incubated at room temperature for 20 min in 10 mM Tris, pH 7.9, 5% glycerol, 33 mM KCl, 50 mM NaCl, 1 mM dithiothreitol, and 1 mM EDTA. The protein-DNA complexes were resolved by 4% polyacrylamide gel electrophoresis under low ionic conditions (7 mM Tris, pH 7.9, 3 mM sodium acetate, 1 mM EDTA). After electrophoresis, gels were dried and subjected to autoradiography. Gel supershift assays were performed as described above with the inclusion of 2 μg of affinity-purified antibody. Reaction mixtures were incubated for 45 min at room temperature for Egr element-containing oligonucleotides. Reactions containing control antibodies were treated identically.

Stable transfection of cells with a dominant interfering Egr-1 mutant. The plasmid, pBX-EGRΔNH2-neo, was a gift of Dr. John Monroe. Complete details of the plasmid design and dominant interfering function have been published previously (6). IEC-6 cells were split, grown to 70% confluence in T75 flasks, and transfected with 5 μg of pBX-EGRΔNH2, using a Dospel liposomal transfection reagent (Boehringer Mannheim) in a 6:1 (μl Dospel/μg DNA) ratio. After 4 days of growth in nonselective media, media was switched to complete media containing 400 μg/ml G418 sulfate (GIBCO BRL). After 10 days of antibiotic selection, donal colonies were isolated and subcloned. Cultures were permanently maintained under 400 μg/ml G418 selection.

Measurement of wound-induced cell migration. Wounds were created in confluent monolayers of IEC-6 cells using the razor blade injury model established by others (7). After injury, IEC-6 monolayers were pretreated for 2 h in plain DMEM with varying concentrations of PD-98059 or the dimethyl sulfoxide (DMSO) vehicle. Immediately after injury, the old media and detached cells were aspirated and replaced with fresh DMEM containing inhibitor. After an overnight incubation, injured monolayers were fixed in 10% buffered Formalin, and phase-contrast images were taken and digitized for analysis. The area of the wound, newly covered by migrating cells, was analyzed using the area measurement function of National Institutes of Health Image 1.61/ppc. The relative wound-induced cell migration of IEC mock trans-
fected or IEC cells transfected with pBX-EGRΔNH2 was determined in the same manner as outlined above. Confluent monolayers were preincubated for 12 h in plain DMEM. Immediately after injury, the DMEM was aspirated and replaced with fresh DMEM or DMEM "preconditioned" by an 8-h incubation with an uninjured confluent IEC-6 cell monolayer. After overnight incubation, wounded monolayers were fixed and analyzed as described above.

RESULTS

Epithelial injury results in MEK-mediated activation of ERK in IEC-6 cells. Previous studies have shown that mechanical injury to confluent monolayers resulted in the rapid activation of ERK1/2 and p38 MAP kinase pathways (11). The present experiments were carried out to better define events that precede and follow ERK activation. ERK activation was measured using a phosphospecific anti-ERK antibody. This reagent specifically recognizes ERK after catalytic activation by phosphorylation (Tyr204). This method yielded comparable degrees of ERK activation in our wounding model compared with mobility shift and immune complex kinase assays (11). As shown in Fig. 1, top, monolayer injury resulted in detectable increases in phospho-ERK by 2 min, peaking at 5 min and returning nearly to baseline by 15 min. Pretreatment with PD-98059 (2'-amino-3'-methoxyflavone), a potent specific cell-permeable inhibitor of MEK1 and -2 (MAP kinase kinase), blocked, in a dose-dependent manner, wound-induced ERK activation. By densitometry scanning, the PD-98059 IC50 is ~20–30 µM, in close agreement with the known IC50 of 10 and 50 µM for MEK1 and MEK2, respectively. Wound-induced ERK activation was inhibited >96% by 60 µM PD-98059. This inhibitor concentration was chosen for subsequent experiments.

Neither wounding nor treatment with PD-98059 resulted in any change in the level of total ERK (Fig. 1, bottom), suggesting that ERK activity in our model was regulated by catalytic phosphorylation by MEK, rather than by changes in total ERK protein expression. DMSO, the vehicle for PD-98059, did not alter ERK activation at the concentrations used in these studies (data not shown).

ERK activation by wounding is sensitive to inhibitors of PKC and microfilament structure. After the identification that injury leads to rapid activation of ERK MAP kinase, we undertook experiments to examine the effect of inhibitors of PKC, contractile actin microfilaments, prostaglandin endoperoxide synthase, and lipoxygenase pathways. As shown in Fig. 2, in the absence of inhibitors, monolayer injury resulted in rapid transient activation of ERK, peaking at 5 min. By 15 min, levels of activated ERK had returned nearly to baseline. Calphostin C, a highly selective inhibitor of PKC, was utilized to examine the potential role of PKC in wound-induced ERK activation. Calphostin C-mediated inhibition of PKC is light dependent (4). Treatment with 100 nM calphostin C in association with illumination resulted in a >80% reduction in ERK activation 5 min after wounding as determined by densitometry scanning. Conversely, treatment of IEC-6 monolayers with phorbol myristate acetate resulted in rapid ERK activation (data not shown). Cytochalasin D inhibits actin filament structure without the effect on glucose transport associated with other cytochalasins. Treatment of monolayers with 5 µM cytochalasin D also strongly inhibited the injury-induced increase in activated ERK at 5 min. In contrast, even high concentrations of indomethacin to block cyclooxygenase or NDGA to...
block lipoxygenase failed to significantly reduce induction of activated ERK by monolayer injury.

Inhibition of ERK activation resulted in a partial inhibition of Fos and total inhibition of Egr-1 mRNA transcript appearance after wounding. Activated ERK is known to phosphorylate the ternary complex factor Elk-1, whose phosphorylation coincides with transcriptional activation of immediate-early genes through activation of the SRE. Figure 3 examines the induction and putative role of the MEK/ERK pathway in wound induction of Fos and Egr-1. Fos and Egr-1 mRNA levels peak at 20 min after injury. Pretreatment with 60 µM PD-98059 resulted in only a slight (~30% by densitometry scanning) decrease in Fos 20 min after wounding. Equal RNA loading and membrane transfer were confirmed (within 7%) by quantification of ethidium bromide-stained 18S and 28S rRNA band intensity. This approach was felt to be the most accurate method because of the potential unknown effects of metabolic inhibitors on commonly used housekeeping genes. Egr-1 mRNA induction by injury was partially inhibited by 20 µM and completely blocked by 60 µM PD-98059. These results identify differences in the regulation of Fos and Egr-1 and suggest the contribution of additional pathways to Fos expression. Pretreatment with SB-203580, a specific inhibitor of p38 MAP kinase, also did not significantly reduce Fos mRNA expression 20 min after injury (data not shown).

Monolayer injury resulted in nuclear accumulation of Fos and Egr-1 protein. To examine the functional consequence of the rapid, but transient, ERK activation and increased Egr-1 and Fos mRNA levels, studies were carried out to examine Egr-1 and Fos protein expression and cellular location. Western blotting was used to examine nuclear and cytoplasmic extracts isolated from IEC-6 monolayers at various times after injury. Figure 4, top, shows the expression of Fos. The induction of p62 Fos protein was first evident in the nuclei 15 min after injury. Progressive accumulation of Fos was detected 45 and 90 min after injury. The majority of the Fos protein was directed to the nuclei, although some was detectable in 60- and 120-min cytoplasmic extracts. In the experiment shown, no Fos was detectable immediately after injury (time 0). However, in some duplicate experiments, a small amount of Fos was present at baseline, perhaps reflecting subtle differences in the culture conditions or rapid cellular synthesis (even at 4°C) after scraping the cell monolayer to isolate the nuclear extracts. Figure 4, bottom, looks at the expression of the p88 Egr-1 protein after monolayer injury. Egr-1 protein also was induced in nuclear extracts, peaking by 45 min. Continued Egr-1 expression was evident at least through 90 min. In contrast to Fos, little cytoplasmic accumulation was detected at any of the times examined.

Nuclear expression of Fos was partially, and Egr-1 protein expression completely, inhibited by pretreatment with PD-98059, but not SB-203580. The availability of potent and specific inhibitors for both ERK and p38 MAP kinase pathways allowed us to conduct studies to look for additional effects of the p38 pathway on the regulation of Fos and Egr-1 expression. Nuclear extracts were isolated from uninjured IEC-6 cells (0), or monolayers wounded 90 min earlier (90), or 90 min in the presence of 60 µM PD-98059 (90/PD-98059), or 25 µM SB-203580 (90/SB-203580). Nuclear Fos expression was stimulated 90 min after injury (Fig. 5, top). Mone-
layers wounded in the presence of PD-98059, but not SB-203580, had a significant reduction in Fos expression. Figure 5, bottom, is a Western blot performed using antibody directed against Egr-1. Egr-1 protein expression was induced by injury at 90 min, an effect that was nearly eliminated by treatment with PD-98059, but not SB-203580. Collectively, these results suggest that activation of the MEK/ERK pathway accounts for the wound-induced regulation of Egr-1 expression and contributes, at least in part, to nuclear Fos expression. These studies fail to identify a clear role for the p38 pathway in the expression of these genes.

Monolayer injury leads to expression of nuclear proteins that specifically bound double-stranded oligonucleotides containing the Egr consensus element. By supershift analysis, this protein-DNA complex was found to contain Egr-1 or an immunologically similar protein. After demonstration of nuclear Egr-1 protein expression after injury, studies were carried out to determine if this protein was capable of binding to the Egr consensus element. As shown in Fig. 6, nuclear extracts taken from injured IEC-6 cells bound to oligonucleotides containing the consensus Egr binding site or a similar oligonucleotide containing a mutation that is known not to support Egr-1 binding, resulting in two nonspecific (with respect to Egr element binding) protein-DNA complexes. A third complex, specific for the Egr consensus oligonucleotide, appeared in lanes containing nuclear extracts isolated from IEC-6 cells 45 and 90 min after monolayer injury. Supershift assays were performed to determine if Egr-1 was contained within the protein-DNA complex. Addition of antibody directed against Egr-1, but not a matched control antibody directed against α1-antitrypsin, resulted in a further reduction in the electrophoretic mobility of the Egr consensus element-specific protein-DNA complex in 45- and 90-min nuclear extracts. These results suggest that Egr-1 or an immunologically similar protein is contained in this DNA binding complex.

Inhibition of the ERK pathway by PD-98059 reduced injury-induced IEC-6 monolayer restitution. A similar defect in postinjury cell migration is present in IEC-6 cells transfected with a dominant interfering mutant of Egr-1. Wounds were created in confluent IEC-6 cell monolayers as described in MATERIALS AND METHODS. Before injury, monolayers were pretreated for 2 h in DMEM with varying concentrations of PD-98059 or the DMSO vehicle. After overnight incubation to allow cell migration, injured monolayers were fixed, and phase contrast images were digitized for analysis. The relative area of the wound, freshly covered by migrating cells, is shown in Fig. 7A. PD-98059 resulted in a dose-dependent inhibition of IEC-6 monolayer migration. Inhibitor concentrations resulting in a 50% reduction in injury-induced cell migration are in very close agreement with inhibition of injury-induced ERK activation. PD-98059 concentrations >100 µM could not be tested due to compound insolubility in cell culture media. To examine the hypothesis that ERK-induced expression of Egr-1 regulates postinjury cell migration, IEC-6 cells were mock transfected or transfected with a dominant-interfering mutant of Egr-1, pBX-EGRΔNH2. The relative area of the wound, freshly covered by migrating IEC-6 cells, either mock transfected or transfected with the dominant-interfering mutant, is shown in Fig. 7B. Transfection with pBX-EGRΔNH2 reduced postinjury cell migration by ~50%. Addition of serum-free conditioned media from uninjured (shown in Fig.
or injured (data not shown) IEC-6 monolayers restored cell migration nearly to baseline. Addition of conditioned media to injured mock-transfected monolayers did not significantly further increase cell migration (data not shown). These results suggest that Egr-1 may mediate the expression of a factor or factors necessary for the augmented cell migration after injury and that this effect, at least in part, is mediated by a factor released in the culture media.

**DISCUSSION**

Epithelial integrity is subjected to continuous challenges, ranging from superficial injury associated with...
the process of normal digestion and absorption to pathological injury from infectious, toxic, mechanical, or inflammatory causes. The initial step in triggering an appropriate response must be activation of key wound-signal transduction pathways, leading to changes in gene expression, proliferation, differentiation, and cell migration. Epithelial wound-signal transduction is mediated, in part, by activation of the p42 and p44 ERK MAP kinase signaling cascade. This and other members of the MAP kinase superfamily of signal transduction pathways are attractive candidates for directing gene transcription in the context of injury. These pathways transduce a diverse array of extracellular events or signals in a response by acting on key cellular enzymes and protein kinases, cytoskeletal proteins, and transcription factors (8). IEC-6 monolayer injury resulted in very rapid MEK-dependent activation of the ERK pathway. Despite the transient nature of ERK activation, downstream effects, such as the induction of transcription factors, were more prolonged. This narrow window for the detection of ERK activation suggests that ERK induction may occur in response to a wider range of stimuli than is currently appreciated. The use of specific pathway inhibitors, such as PD-98059, provides one mechanism to implicate these pathways in the control of rapid or complex responses.

Inhibition of PKC by the highly specific inhibitor calphostin C prevented ERK pathway activation. We have also found that treatment with phorbol 12-myristate 13-acetate rapidly activated ERK and augmented cell migration in our injury model (data not shown). PKC inhibitors also reduced injury-induced migration in a conditionally immortalized murine small intestinal cell line (MSIE) cell injury model (35). PKC-δ activation of ERK via a Raf-dependent pathway has recently been described (40) and is distinct from Ras-dependent ERK activation by members of the epidermal growth factor (EGF) family. PKC activation was required for ERK activation and immediate-early gene expression in a bile acid injury model using hepatic stellate cells (3). Interestingly, inhibitors of PKC activation did not block wound-induced activation of p38 MAP kinase in our injury model, suggesting that p38 MAP kinase activation occurred by a different mechanism (data not shown). A similar distinction between the role of PKC in ERK and stress-activated protein kinase activation in stretched cardiac myocytes has been reported (26). Disruption of the actin microfilament cytoskeleton by cytochalasin D strongly inhibited ERK activation. Mechanical stress-induced activation of ERK has also been described in other systems (26, 27, 43, 44). Because intact epithelial membranes are maintained under continuous tension, linking ERK activation to alterations in changes in cytoskeletal stress would provide a sensitive mechanism to activate epithelial wound-induced gene expression.

The SRE is activated by the ERK pathway by phosphorylation of the ternary complex factor Elk-1 (42). In our model, both Fos and Egr-1 mRNA levels markedly increased after injury. Egr-1 induction was largely blocked by PD-98059 in response to injury, whereas only a partial reduction in Fos mRNA levels was demonstrated. Our data provided a direct link between injury-dependent ERK activation and Egr-1 induction and support the contribution of additional mechanisms for Fos transcriptional activation in the context of wounding. Differences between types of cells or injury may exist. Hypoxia-induced Fos induction in HeLa cells was blocked completely by PD-98059 (33). The presence of mRNA for Fos and Egr-1 coincided with subsequent expression and nuclear localization of these proteins. Although changes in nuclear expression and DNA binding activity provide suggestive data in support of a possible role in transcriptional regulation, DNA binding activity does not always reflect transcriptional activation. Future studies directed toward examination of the ability of Egr-1 to transactivate specific wound-responsive genes are necessary.

Rapid cellular migration is critical for repair in many injury models. Exactly what role the ERK pathway plays and which downstream targets are necessary for increased cell migration remain unclear. Our data demonstrated that inhibition of the ERK pathway by PD-98059 led to a dose-dependent reduction in IEC-6 migration. Similar results were recently reported in a model of vascular smooth muscle injury (18). ERK can act on multiple downstream targets. The availability of a dominant-negative Egr-1 protein made it possible to directly test the role for this specific transcription factor in injury-induced cell migration (6). Egr-1 is a nuclear phosphoprotein containing three zinc finger motifs that mediate specific binding to the Egr consensus element. Several studies have mapped distinct functional domains within this molecule (6, 16, 37). Phosphorylation of the activation domain between amino acid 174 and 270 provides a negative charge to a region responsible for interfacing with other transcriptional coactivators or members of the transcription complex (36). Mutants lacking this region still retain nuclear localization and DNA-binding domains but function in a dominant-negative fashion to block transcriptional activity of endogenous or exogenously expressed Egr-1 (6). The defect in postinjury migration of dominant-negative Egr-1 transfected cells was corrected by the addition of conditioned media. Together, these results suggest that Egr-1 was required for the expression and/or release of a soluble factor(s) necessary for cell migration after injury. Members of the EGF family are synthesized as large transmembrane precursors that potentially serve as a source for rapidly released soluble growth factors. Polk (35) reported that tyrosine phosphorylation of the EGF receptor rapidly followed MSIE monolayer injury. In fact, increases in EGF family members may be responsible for some degree of ERK activation that follows injury (17). These results suggest that our soluble factor may be a member(s) of the EGF family. It is interesting to speculate that the defect in our dominant negative clones may be the inability to release, by proteolytic cleavage, a soluble growth factor.
In summary, our data suggest that ERK activation may serve as a general mechanism to transcriptionally regulate pathophysiologically significant genes in wound repair. Because activation of the ERK MAP kinase pathway and indeed accumulation of Egr-1 have been shown to occur as a result of a large number of stimuli, additional factors must act to shape a response appropriate in the context of epithelial injury. Temporal and spatial differences in the activating stimuli, as well as concurrent activation of additional signaling pathways (e.g., p38), may be critical in determining the final transcriptional response. Ongoing efforts are focused on addressing several questions naturally arising from our results. First, what is the nature of the defect in Egr-1 dominant-negative cells, and what is the identity of the soluble factor in conditioned media that restores migration? Second, what is the mechanism for PKC activation in the context of injury, and which PKC isoform is responsible for ERK activation? Finally, what is the role of other signaling pathways [e.g., AP-1 (Fos) or p38 MAP kinase-induced transcription] in the cellular response to injury? Identification of genes temporally induced by specific repair programs may ultimately lead to novel targeted therapeutic agents for many diseases involving the epithelial barrier.

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