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Transcriptional Control of K5, K6, K14, and K17 Keratin Genes by AP-1 and NF-κB Family Members

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The expression of keratins K5 and K14 is restricted to the basal layers of the healthy epidermis, whereas the expression of K6 and K17 is induced in response to proliferative and inflammatory signals, respectively. The control of keratin expression occurs primarily at the transcriptional level. We studied the effects of transcription factors of the AP-1 and NF-κB families on the expression of those four keratin genes. We chose AP-1 and NF-κB proteins because they are activated by many extracellular signals, including those in hyperproliferative and inflammatory processes. DNA constructs expressing the transcription factors were, in various combinations, cotransfected with constructs containing keratin gene promoters and the CAT reporter gene into HeLa cells or keratinocytes. We found that the K5 and K14 promoters, which are coexpressed in vivo, are regulated in parallel by the cotransfected genes. Both were activated by the c-Fos and c-Jun components of AP-1, but not by Fral. On the other hand, the NF-κB proteins, especially p65, suppressed these two promoters. The K17 promoter was specifically activated by c-Jun, whereas the other transcription factors tested had no significant effect. In contrast, the K6 promoter was very strongly activated by all AP-1 proteins, especially by the c-Fos + c-Jun and Fral + c-Jun combinations. It was also strongly activated by the p65 NF-κB protein. AP-1 and NF-κB acted synergistically in activating the K6 promoter, although the AP-1 and the NF-κB responsive sites could be separated physically. These results suggest that the interplay of AP-1 and NF-κB proteins regulates epidermal gene expression and that the activation of these transcription factors by extracellular signaling molecules brings about the differential expression of keratin genes in epidermal differentiation, cutaneous diseases, and wound healing.

Keratins Transcriptional control AP-1 NF-κB

THE epidermis is composed of 10–20 layers of keratinocytes. In the normal epidermis, basal keratinocytes proliferate whereas the suprabasal ones cease to divide and terminally differentiate (6). This normal process of epidermal differentiation is altered in hyperproliferative conditions of the skin such as wound healing, psoriasis, and squamous cell carcinoma, when keratinocytes become activated (14). Activated keratinocytes are hyperproliferative, migratory, and respond to and produce proinflammatory cytokines and growth factors, including IL-1, TNF-α, EGF, and TGF-α (14).

Among the markers of keratinocyte differentiation is the expression of the different keratins, the intermediate filament proteins of the cytoskeleton, which contribute to the strength and structure of epithelial cells (26). There are more than 30

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identified keratin proteins, each encoded by its own gene. In the healthy epidermis, basal keratinocytes express K5 and K14, whereas suprabasal, differentiating keratinocytes express K1 and K10. However, in suprabasal keratinocytes of the hyperproliferative epidermis, the expression of K1 and K10 is suppressed and replaced by that of K6 and K16. Thus, the expression of K6 and K16 marks keratinocyte activation (11,26). In certain inflammatory processes, especially those characterized by the presence of Th-1 type lymphocytes, the K17 keratin is also expressed (13).

Regulation of keratin expression occurs primarily at the transcriptional level (23). Signals from the extracellular environment initiate enzymatic cascades, which lead to the activation of transcription factors. Activated transcription factors then regulate gene expression by diverse mechanisms that include binding to specific DNA sequences and interaction with other transcription factors or nuclear receptors. They can also induce the expression of additional regulatory factors as well as differentiation or hyperproliferation specific proteins.

Perhaps the most widely studied regulated transcription factors are those belonging to the AP-1 and NF-κB families (30). AP-1 is a nuclear transcription complex composed of dimers encoded by the *fos* and *jun* families of proto-oncogenes (1). Whereas Fos proteins only heterodimerize with members of the *jun* family, Jun proteins may hetero- or homodimerize with both Fos and other Jun proteins. AP-1 activity is induced by growth factors such as serum, EGF, and TGF-α, cytokines such as IL-1 and TNF-α, as well as tumor promoters such as TPA and UV light (1,12).

In the epidermis, AP-1 regulates cell growth, differentiation, and transformation (2,5,24,25). However, the expression of individual AP-1 proteins in epidermal layers is a controversial issue that awaits resolution. Certain authors find c-Fos in lower layers of the epidermis (2,8,31) whereas others do not find any c-Fos (24), which agrees with the lack of epidermal phenotype in *fos* knockout mice (25). The differing results could be explained by varied different epitopes of the antibodies used, or functional redundancy of Fos family members. Be that as it may, it is clear that the AP-1 proteins in keratinocytes can regulate positively the expression of differentiation markers (15,16,22) and may convey calcium- and PKC-dependent signals (24,31).

The NF-κB family includes the proteins p65, p50, and c/Rel, which both homo- and heterodimerize among themselves (20). Activation of these proteins is not dependent upon new protein synthesis; rather, they are stored in the cytoplasm bound to inhibitory proteins, such as IκB. Inflammatory processes that induce IκB phosphorylation and degradation result in the release, nuclear translocation, and activation of the NF-κB complex. Signaling by EGF, TNF-α, and IL-1 activates NF-κB (4). NF-κB proteins interact with AP-1 and other transcription factors, such as NF-IL6 (18,28).

Promoters of the keratin genes contain binding sites for the AP-1 and NF-κB transcription complexes (5,7,19,21). AP-1 and NF-κB are induced by many agents that regulate keratin genes and control epidermal processes that alter keratin expression. Therefore, using cotransfection experiments, we have examined the transcriptional control of keratin genes by AP-1 and NF-κB proteins. We focused our attention on four keratin genes, K5 and K14, because they are expressed in the basal layer of the healthy epidermis, and K6 and K17 because they are expressed in hyperproliferative and inflammatory conditions. We found characteristic and different patterns of keratin gene regulation by AP-1 and NF-κB proteins, patterns that explain, in part, the differential expression of keratin genes in basal and activated keratinocytes.

MATERIALS AND METHODS

DNA Constructs

The constructs containing keratin gene promoters have been described previously (11). The lengths of the keratin DNA are shown in Fig. 1. Deletions of the K5 promoter were prepared by Ohtsuki (21). Deletions of the K6 promoter were prepared by M. Komine (submitted), using PCR and a common proximal oligonucleotide K6R in conjunction with distal primers (Table 1). The proximal primer contains a *Sal*I, whereas the distal primers contain a *Bam*HI restriction site, both with three additional nucleotides to facilitate the

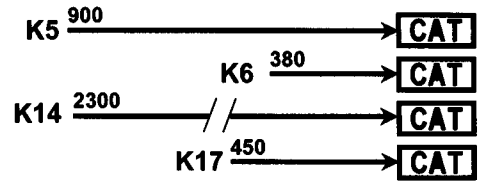


FIG. 1. Constructs containing keratin gene promoters. The arrows represent the promoters, drawn roughly to scale, with their lengths in base pairs shown above the arrows. Each promoter is linked to the CAT reporter gene (boxes).

TABLE 1
SEQUENCE OF THE NF-κB AND THE AP-1 CONSENSUS OLIGONUCLEOTIDES

K6R	TTTGTCGACCATGGTTCCAGAGATGAGAG
D268	TTTGGATCCAACTTCATGAATT
D193	TTTGGATCCACTAAAGGAAGCGAAA
D180	TTTGGATCCAAAAATGCAATCTCGG
D172	TTTGGATCCAATCTCGGTATTTCAT
D139	TTTGGATCCAGGTGTGAATCTCAC
D111	TTTGGATCCAGCCCTTCCCAAC
NF-κB	AGTTGAGGGGACTTTCCCAGGC
AP-1	CTAGTGATGAGTCAGCCGGATC

digestion. The constructs expressing AP-1 proteins, c-Fos, c-Jun, and Fra1, were a gift from E. Ziff, those expressing NF-κB proteins, p65, p50, and c/Rel from A. Beg (3). DNA encoding these transcription factors was linked to a RSV promoter. *E. coli*-containing plasmids were grown to stationary phase in LB and the plasmids were purified using maxi-preps (Promega).

Cells and Transfection

HeLa cells were grown in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% calf serum and were incubated at 37°C in a 5% CO_2 atmosphere. Subconfluent cultures were maintained in 100-mm dishes by periodically splitting cells with a solution of 0.25% trypsin in Hank's Balanced Salt Solution (JRH Biosciences). They were cotransfected with pK5-CAT, pK6-CAT, pK14-CAT, pK17-CAT, and pRSVZ-β-GAL, as well as with constructs expressing c-Fos, c-Jun, Fra1, p65, p50, and c/Rel, using the transfection protocol described previously (9,29). Briefly, the cell cultures were split into 2.5-ml wells and grown to 15–30% confluency 16 h later. DNA constructs were diluted in 100 μl of water. First 25 μl of 2 M $CaCl_2$, then 125 μl BES-buffer, pH 6.95 (50 mM *N*-bis 92-hydroxyethyl-2-aminoethanesulfonic acid, 250 mM NaCl, 1.5 mM Na_2HPO_4), was added drop by drop. The transfection mixture was then incubated at room temperature for 10–15 min before being added to the cells. Final concentrations of keratin promoter DNAs were 0.8 μg/ml for pK5-CAT and pK14-CAT, 1.0 μg/ml for pK6-CAT, and 1.5 μg/ml for pK17-CAT as well as for the K5 and K6 deletions. The constructs expressing the transcription factors were always added in a 1:3 ratio relative to the keratin promoter constructs. In addition, 0.3 μg/ml of pRSVZ was added into each transfection. For each well 250 μl of DNA solution was added to 2.25 ml of the growth medium. Cells were incubated with the transfection solution for 12 h, when fresh medium was added. Forty-eight hours following transfection, the cells were washed with phosphate-buffered saline and harvested by scraping.

Cells were disrupted in an extraction buffer (250 mM sucrose, 10 mM Tris 7.8) with four freeze–thaw cycles and the transfection efficiency was measured by β-galactosidase assays (11). Briefly, 10 μl of each cell extract was incubated in microtiter wells with 160 μg *O*-nitrophenyl-β-D-galactosidase (Sigma) dissolved in 190 μl buffer (66 mM Na_2HPO_4, 33 mM NaH_2PO_4, 40 mM mercaptoethanol, 2 mM $MgSO_4$, and 0.1 mM $MnCl_2$). The reaction mixture was incubated at room temperature until an obvious yellow color developed. To stop the reaction 100 μl of 1 M Na_2CO_3 was added to each well. OD was measured at 420 nm. CAT protein concentration was measured using an ELISA kit, as described by the manufacturer (Boehringer-Mannheim).

Normal human foreskin epidermal keratinocytes, a generous gift from M. Simon, were grown in a defined keratinocyte growth medium (Gibco), in 60-mm plates. This medium was supplemented with cholera toxin, thyroid hormone, insulin, EGF, and bovine pituitary extract. After two passages, at 80% confluency, the cells were switched to basal medium. The basal medium lacks the supplements. This medium was removed after 2 h and a solution containing plasmid DNA with 1% Polybrene was added to the cells; 8 μg of pK5-CAT and pK14-CAT, 10 μg of pK6-CAT, 15 μg of pK17-CAT as well as of the K5 and K6 deletions were added to each plate. The transcription factor-expressing constructs were added in a 1:3 ratio relative to the keratin promoter constructs and 3 μg of p-RSVZ was added to each plate. The total volume of the DNA solution added per plate was 1 ml. Six hours later, this solution was aspirated off and the cells were shocked with 28% DMSO in DMEM for 3 min. The cells were washed three times with PBS and incubated with fresh basal medium for 24 h. Harvesting of keratinocytes and assays for β-galactosidase and CAT were done as described above.

All transfections were performed two to four times, each time in duplicate plates, and the average CAT values were normalized by calculating the ratio of CAT concentration to β-galactosidase activity.

Electrophoretic Mobility Shift Assays

The cell extracts were prepared from keratinocytes treated for indicated times with EGF or IL-1,

as described previously (17,19). The extracts were incubated in binding buffer containing 100 mM Tris, pH 7.8, 0.5 M NaCl, 5 mM DDT, 25 mM $MgCl_2$, 5% glycerol, and 0.5 mM EDTA with or without unlabeled competitor for 15 min on ice in the presence of 1.8 μg/ml of poly(dI-dC). The probes, end-labeled with [γ-^{32}P]ATP using T_4 polynucleotide kinase (80,000 cpm), were added and the binding solutions incubated for 30 min on ice. The sequence of the NF-κB and the AP-1 consensus oligonucleotides are shown in Table 1. The protein–DNA complexes were separated from the free probe by electrophoresis on 5% polyacrylamide gel (29:1 acrylamide:bisacrylamide) in 1 × TBE buffer. Dried gels were autoradiographed for 12–40 h at −70°C. The identity and specificity of the bands has been confirmed using nonspecific probes (Sp-1 consensus oligonucleotide and irrelevant segments from keratin promoters) and in supershift assays using antibodies (Santa Cruz).

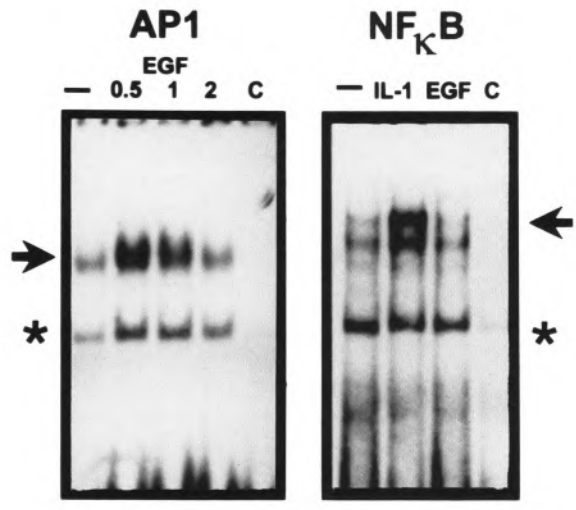


FIG. 2. AP-1 and NF-κB activities are induced by EGF and IL-1, respectively. Electrophoretic mobility shift assays using AP-1 (left) and NF-κB (right) sequences as DNA probes. Keratinocyte extracts were prepared from untreated cultures (−), or cultures treated with EGF for 0.5, 1, and 2 h (left panel) or with IL-1 or EGF for 1 h (right panel). Competitor DNA was added to show specificity (c). Arrows point to specific bands, those affected by corresponding antibodies (not shown). The asterisks mark nonspecific bands, present in whole-cell but not nuclear extracts.

RESULTS

AP-1 and NF-κB Activities Can Be Induced in Keratinocytes

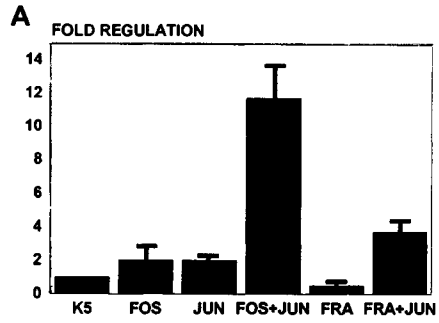
To determine whether AP-1 and NF-κB activities respond to extracellular stimuli in epidermal keratinocytes, we treated these cells in culture with EGF or IL-1, prepared protein extracts and compared them with the extracts of untreated cells as controls in electrophoretic mobility shift assays. Using the AP-1 consensus sequence as a probe, we found that EGF addition induces a strong increase in AP-1 binding activity within the first hour (Fig. 2). Later, this activity wanes. Using the NF-κB probe, we found that the addition of IL-1, but not of EGF, increases the binding activity (Fig. 2). In both cases the binding is specific, because it can be eliminated using the same, unlabeled probe and supershifted using corresponding antibodies (L. R. and W. Long, data not shown). The faster migrating band (Fig. 2, asterisk) is an artifact of whole-cell extract preparation. It is not found in nuclear extracts.

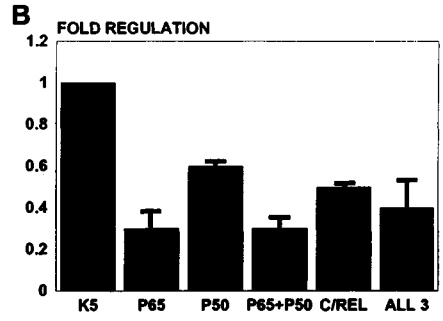
Transcriptional Regulation of the K5 Keratin Gene

To elucidate the transcriptional regulation of K5 keratin gene expression, constructs expressing the AP-1 proteins c-Fos, c-Jun, and Fra1 as well as the NF-κB proteins p65, p50, and c/Rel were cotransfected with the K5-CAT construct (Fig. 3). Although cotransfection of c-Fos and c-Jun individually led to only a twofold increase of the K5 promoter activity, simultaneous cotransfection of c-Fos and c-Jun produced a strikingly synergistic response of approximately 12-fold. Fra1, another member of the Fos family, suppressed K5 activity twofold. However, simultaneous cotransfection of Fra1 and c-Jun did not produce a response significantly different from that of c-Jun alone.

In contrast, the NF-κB proteins had a weakly suppressive effect on the K5 promoter (Fig. 3B). p65 individually and in combination with p50 or with both p50 and c/Rel suppressed K5 activity threefold. Overexpression of either p50 or c/Rel individually downregulated the K5 promoter twofold. Furthermore, when p65 and p50 were cotransfected with c-Fos and c-Jun, NF-κB suppressed the inductive activity of the c-Fos and c-Jun AP-1 dimer and reduced K5 activity to baseline levels (Fig. 3C).

The full-length K5-CAT construct contains 900 bp of the K5 promoter. To determine the DNA regions responsive to AP-1 and NF-κB, we transfected two K5 promoter deletions, K5-D560 and K5-D300, containing 560 and 300 bp of the K5 promoter, respectively, with c-Fos, c-Jun, p65, and p50. Whereas both the full-length and the K5-D560 construct were responsive to both AP-1 and





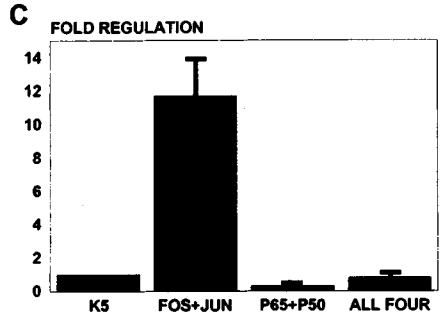
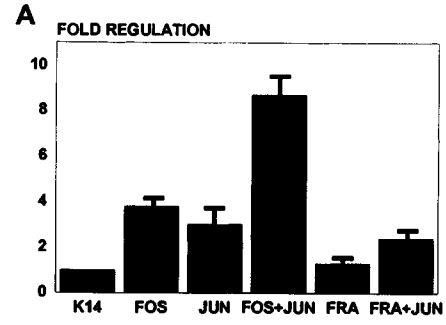


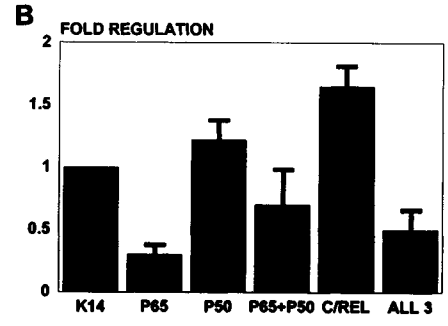
FIG. 3. Regulation of the K5 keratin gene promoter. Constructs containing the K5 promoter were cotransfected with constructs expressing (A) AP-1 proteins, (B) NF-κB proteins, and (C) both, into HeLa cells. The level of expression of the K5 promoter-CAT construct was designated as 1. Each transfection was performed two to four times, always with duplicate plates.

NF-κB, construct K5-D300 was regulated only by AP-1 activity, demonstrating the loss of NF-κB regulation (data not shown). Therefore, the region of K5 responsive to NF-κB is located between 560 and 300 bp upstream of the start codon, whereas the region responsive to AP-1 is located within the proximal 300 bp.

Regulation of the K14 Keratin Gene

We examined the effects of the same transcription factors upon the regulation of K14, which is usually coexpressed with K5. The AP-1 proteins c-Fos and c-Jun induced the K14 promoter four- and threefold, respectively (Fig. 4A). As with K5, simultaneous overexpression of c-Fos and c-Jun produced a synergistic, ninefold upregulation. The K14 promoter activity did not respond to Fra1 and the K14 response to the combination of Fra1





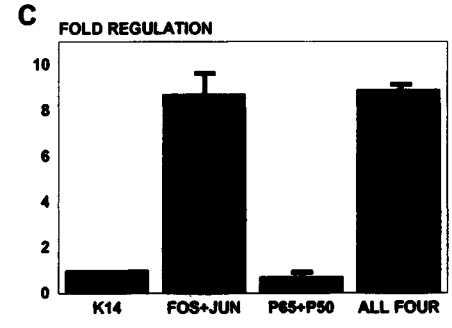


FIG. 4. Regulation of the K14 keratin gene promoter. K14 promoter-CAT construct was coexpressed as in Fig. 1 with (A) AP-1 proteins, (B) NF-κB proteins, and (C) both.

and c-Jun was similar to the response to c-Jun alone.

Although the K14 promoter was not regulated by the NF-κB proteins p50 and c/Rel, the p65 protein suppressed K14 promoter activity threefold. Overexpression of p65 with p50 or with p50 and c/Rel suppressed the K14 promoter approximately twofold (Fig. 4B). However, when c-Fos, c-Jun, p65, and p50 were cotransfected simultaneously, the effects of the AP-1 proteins were dominant (Fig. 4C). The responses of the K5 and K14 keratin genes to AP-1 and NF-κB proteins were strikingly similar, reflecting the coexpression of these two keratins in vivo. The only significant difference, the response to all four cotransfected transcription factors, although reproducible, may depend on the relative amounts of the overexpressed proteins.

Both K5 and K14 responded similarly to both AP-1 and NF-κB in keratinocytes as well (Fig. 5). Both promoters were induced by AP-1 proteins and suppressed by NF-κB proteins. However, in keratinocytes, the responses to these transcription factors were attenuated. This is perhaps due to incomplete activation of these proteins by the signal transduction pathways. In HeLa cells, which are transformed, the signal transduction pathways are more active, perhaps sufficiently to activate fully the transfected transcription factors.

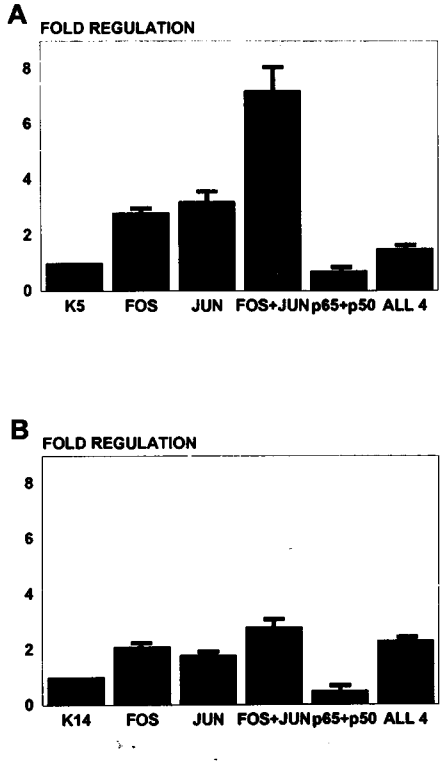


FIG. 5. The responses of K5 and K14 are attenuated in keratinocytes. Constructs containing (A) K5 and (B) K14 promoters were cotransfected into keratinocytes with the AP-1 and NF-κB constructs. Compared to the results in Figs. 3 and 4, the regulation in keratinocytes is similar, but weaker.

Regulation of the K17 Keratin Gene

We examined the effects of the same transcription factors upon the regulation of the K17 keratin, which is usually expressed in inflammatory processes. Our previous studies identified the STAT-1 binding site that confers interferon-γ responsiveness to the K17 gene (10). The AP-1 protein c-Jun induced the K17 promoter threefold, whereas c-Fos and Fra1 were without effect (Fig. 6A). Simultaneous overexpression of c-Fos or Fra1 with c-Jun did not affect the response to c-Jun. Apparently, K17 promoter activity can be increased by c-Jun, but does not respond to c-Fos or to Fra1.

Interestingly, the K17 promoter was not regulated by the NF-κB proteins at all (Fig. 6B). When c-Fos, c-Jun, p65, and p50 were cotransfected simultaneously, the effects of the AP-1 proteins were dominant (Fig. 6C). Note that the results in Fig. 6 were obtained with keratinocytes.

Regulation of the K6 Keratin Gene Promoter

Each AP-1 protein, including Fra1, individually induced K6 activity in HeLa cells. Both c-Fos and c-Jun, when overexpressed individually, activated the K6 promoter twofold. K6 activity increased fivefold when cotransfected with Fra1. Simultaneous overexpression of c-Jun with either c-Fos or Fra1 showed unexpectedly strong synergism and induced the K6 promoter approximately 40-fold (Fig. 7A).

Importantly, the overexpression of the p65 NF-κB protein also resulted in a strong activation of the K6 promoter (Fig. 7B). Cotransfection of p65 alone increased K6 activity 45-fold, and cotransfection with p50 and c/Rel increased it even more. Neither p50 nor c/Rel affected K6 activity when overexpressed individually. Furthermore, AP-1 and NF-κB proteins interacted synergistically, resulting in close to a 10-fold increase in promoter activity compared to the effects of either the AP-1 or the NF-κB transcription complex alone (Fig. 7C).

Using deletion analysis of the K6 promoter, we identified the regions responsive to AP-1 and NF-κB (Fig. 8). Although the K6 promoter contains a consensus NF-κB site and two consensus

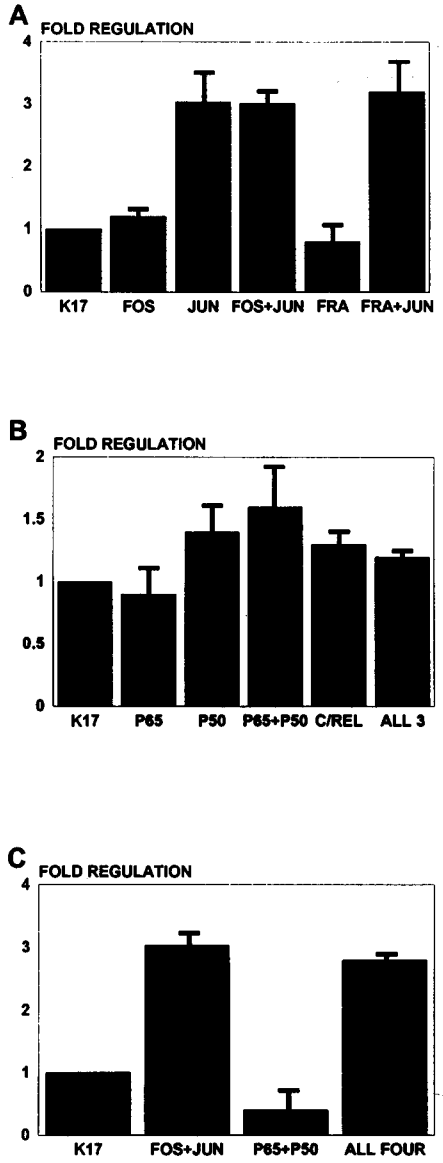


FIG. 6. Regulation of the K17 keratin gene promoter. K17 promoter–CAT construct was coexpressed as in Fig. 1 with (A) AP-1 proteins, (B) NF-κB proteins, and (C) both. Note that the induction by c-Jun is insensitive to either c-Fos or Fra1. Also note that the NF-κB proteins do not have a significant effect on the K17 promoter.

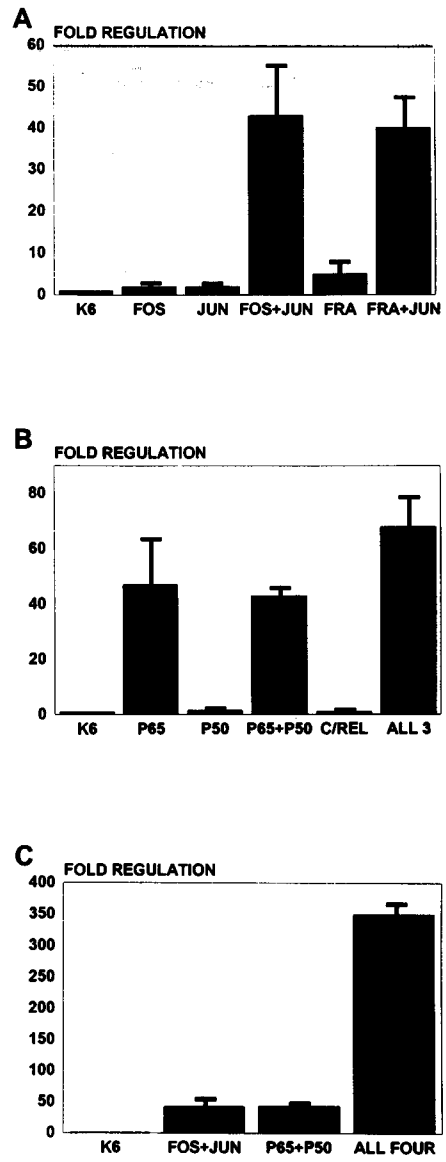


FIG. 7. Regulation of the K6 keratin gene promoter. K6 promoter–CAT construct was coexpressed as in Fig. 1 with (A) AP-1 proteins, (B) NF-κB proteins, and (C) both. Note that the scales on the ordinate differ from previous figures, especially in (C), showing stronger induction and synergistic effect.

AP-1 sites, deletion of these sequences did not disrupt the induction by the corresponding transcription factors. For example, construct K6-D268, which lacks the NF-κB site, and K6-D193, as well as the shorter constructs K6-D172 and K6-D139, which lack both AP-1 sites, were still induced by the AP-1 proteins 30- to 45-fold. AP-1 regulation was completely lost in K6-D111 (Fig. 6B). There is a progressive decrease in NF-κB activity and shift from induction to suppression as the constructs become progressively shorter from K6-D193 to K6-D139 (Fig. 6C). Whereas NF-κB induced K6-D193 close to 60-fold, K6-D180 was induced less than 20-fold, K6-D172 CAT activity was not affected, and in K6-D139 it was even suppressed 10-fold by NF-κB. Thus, the AP-1 responsive and the

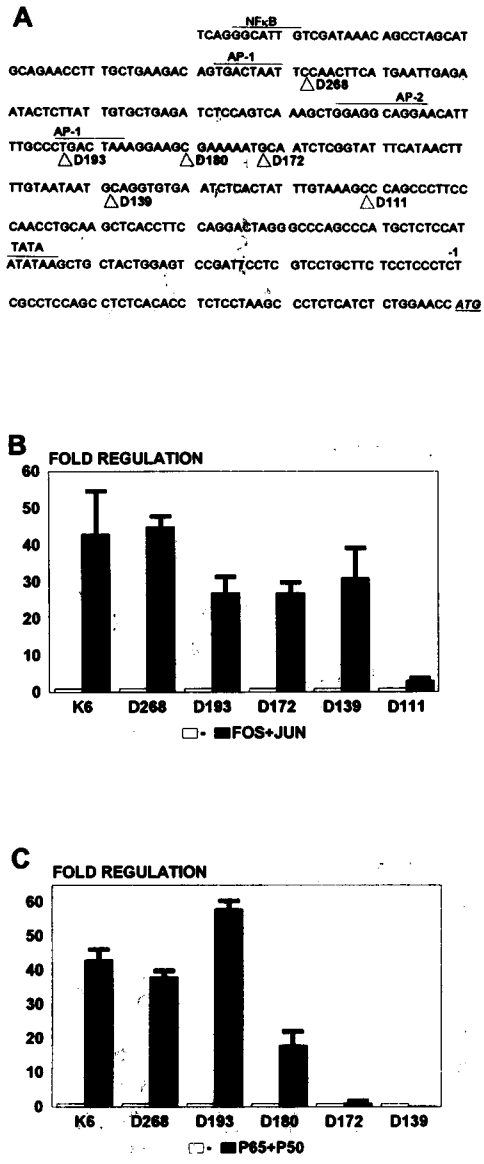


FIG. 8. Mapping the AP-1 and NF-κB responsive sites in the K6 promoter. (A) The sequence of the K6 promoter. Consensus binding sites for NF-κB, AP-1, AP-2, and TBP transcription factors are indicated. Triangles mark the sites of deletions constructed. (B) Cotransfection of c-Fos and c-Jun with the deletion constructs localizes the AP-1 responsive region between bp 111 and 139. (C) Cotransfection of p65 and p50 with the deletion constructs localizes the NF-κB responsive region between bp 139 and 193. Construct D172 is partially responsive to NF-κB. Note that construct D139, although fully responsive to AP-1, no longer responds to NF-κB.

NF-κB responsive sites can be separated from one another: the NF-κB responsive sites are between 139 and 193, whereas the AP-1 responsive sites are between 111 and 139. Interestingly, these segments do not contain obvious binding sequences for NF-κB and AP-1 proteins.

DISCUSSION

Transcription factors of the AP-1 and NF-κB families differentially regulate epidermal keratin genes. All four keratin genes—K5, K6, K14, and K17—are strongly induced by the AP-1 proteins, with subtle differences. The NF-κB proteins, on the other hand, strongly induce the K6 keratin gene, suppress K5 and K14, but leave K17 unaffected.

The regulation of the transfected promoters correlates well with the known features of regulation of endogenous keratin genes by the extracellular signals and the intracellular signaling pathways (Fig. 9). Wounding of the skin releases prestored IL-1, which triggers activation of the NF-κB proteins and consequently induction of K6 keratin expression. In contrast, the AP-1 proteins have been implicated in epidermal differentiation and consequently regulation of the K5 and K14 keratin genes. However, in hyperproliferative conditions, activation of the EGF receptor pathway triggers strong activation of the AP-1 proteins (11), which synergize with the NF-κB proteins in induction of K6 keratin expression. Independently of these signaling pathways, interferon-γ activates STAT-1 and consequently induces the expression of K17 (10,13). AP-1 and NF-κB do not affect this regulation.

The induction of K5 and K14, the basal layers-specific keratins, by AP-1 proteins correlates well with the suggested role for AP-1 proteins in regulating gene expression in keratinocyte differentiation, in particular for the negative regulation of the AP-1 transcriptional activity in differentiating keratinocytes (24). The negative regulation may be due in part to induction of Fra1 in the suprabasal layers. K5 and K14 are induced by c-Fos + c-Jun, which predominates in the basal, but much less by Fra1 + c-Jun in the suprabasal cells (Figs. 3 and 4). In c-Fos knockout mice (25) there is no epidermal phenotype. The role of c-Fos in the basal lay-

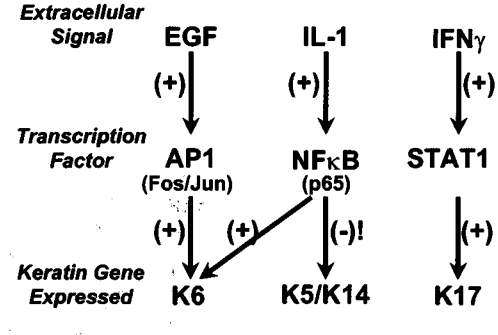


FIG. 9. Scheme of regulation of keratin genes by extracellular signals. Arrows with (+) signs indicate activation, whereas those with (−) indicate suppression. Note the cross-talk between EGF/AP-1 and IL-1/NF-κB in regulation of the K6 gene, and the independence of the K17 gene regulation.

ers may be accomplished by an understudy from the same family. Alternatively, the level of expression of K5 and K14 in the basal layer, even in the absence of c-Fos, may be sufficient to prevent major changes in phenotype.

In contrast, Fra1 does not affect the induction of K17 by Jun (Fig. 6). This correlates well with the exclusive suprabasal expression of K17 in inflammatory dermatoses. Therefore, in suprabasal keratinocytes, Fra1 may suppress the c-Fos + c-Jun-dependent expression of the basal keratins, K5 and K14, but permit the c-Jun-dependent expression of K17. Even more dramatic is the role of Fra1 in K6 keratin expression, where Fra1 by itself, and even more with c-Jun, is a strong inducer (Fig. 7). The role of AP-1 proteins in regulating keratin gene expression is quite complex: in the basal layer c-Fos and c-Jun induce the expression of K5 and K14; in the differentiating cells, as Fra1 replaces c-Fos, K5 and K14 are suppressed, K6 is induced, whereas K17, which responds to c-Jun alone, remains unchanged.

At present we cannot explain the dominant effect of NF-κB over c-Fos and c-Jun in regulating K5 and the exact reverse in regulating K14. Although these results are reproducible, they may depend very much on the relative concentrations of the cotransfected proteins, which we plan to examine in the future.

The AP-1 proteins are clearly just one of the components of keratin gene regulation, evidenced for example by the lack of expression of K17 and K6 in the basal layers. These two keratin genes are suppressed in the basal layer by hitherto unidentified mechanisms and, conversely, induced only when needed in suprabasal cells. Our previous work identified transcription factor STAT-1 as the primary inducer of K17 in inflammatory dermatoses associated with the Th-1 type lymphocytes and cytokines (10,13). Here we identify NF-κB proteins as strong inducers of K6 and show that they synergize with the AP-1 proteins.

NF-κB proteins are activated by a large number of stimuli, including viruses, bacterial products, mitogens, cytokines, UV and ionizing radiation, and chemical and oxidative stress (27). Many of these are associated with cutaneous induction of K6 keratin expression. Some of these also activate AP-1 proteins. We were particularly impressed by the synergistic effects of AP-1 and NF-κB, the effect that may cause very rapid and copious production of K6 keratin in activated keratinocytes.

Interestingly, the p65 protein component of NF-κB seems to be the predominant activator of K6, whereas p50 and c/Rel seem less active. This holds true for K5 and K14 as well, but importantly the effects of NF-κB on K5 and K14 are suppressive. Although the suppressive effects are not very strong (threefold), they may help to switch the keratin expression when keratinocytes become activated. The suppressive effect on K5 and K14 expression contrasts with the case of K17, which is completely unaffected by NF-κB proteins.

In summary, keratin gene expression is regulated by the intricate dance of the transcription factors activated in different cell types. Specifically, in the basal layer keratins K5 and K14 are expressed in part due to the members of the AP-1 family. As the cells differentiate, Fra1 + c-Jun replaces the c-Fos + c-Jun set and K5 and K14 are no longer expressed. If the keratinocytes are activated (e.g., as in psoriasis or wound healing), the p65 NF-κB protein is activated, which inhibits K5 and K14 expression, but greatly induces K6. In inflammation, IFN-γ activates STAT-1, which with c-Jun induces K17. Obviously, these are just the preliminary results; the entire process of keratin gene expression regulation is more complicated and probably many other transcriptional factors play important roles that have to be elucidated.

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