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On the role of AP2 in epithelial-specific gene expression

Thierry Magnaldo, Ruben G. Vidal, Mamitaro Ohtsuki, Irwin M. Freedberg, and Miroslav Blumenberg

The Ronald O. Perelman Department of Dermatology and Department of Biochemistry, New York University Medical Center, New York, New York

Transcription factor AP2 plays an important role in transcription of keratin genes, and it has been suggested that AP2 confers epithelial specificity. Promoters of keratin genes contain AP2 sites, usually within tight clusters of binding sites for other nuclear transcription factors. The role of AP2 was examined by in vitro gel shift analysis, AP2 binding site mutagenesis, and stable and transient transfection experiments. Nonepithelial cells, such as GM10 fibroblasts and melanocytes, neither express keratin nor become phenotypically epithelial when transfected with an AP2-expressing vector. However, in 3T3 and HeLa cells, co-transfection of an AP2-expressing vector increases the level of transcription from keratin gene promoters. This increase requires an intact AP2 binding site. Thus, the role of AP2 in keratin gene expression is quantitative rather than qualitative. AP2 interacts with other transcription factors and may convey extracellular regulatory signals to the transcription complex in the promoters of keratin genes.

The activator protein AP2 was among the first transcription factors to be discovered (Mitchell et al., 1987). This 52 kDa protein can bind both promoter elements and enhancers; moreover, it is one of the transcription factors common to the SV40 promoter and enhancer and the human metallothionein IIa promoter (Lee et al., 1987). AP2, like most transcription factors, is built from domains that perform individual tasks: transactivation depends on the proline-rich amino-terminal domain (Williams et al., 1988), while the DNA-binding and the overlapping dimerization domains are in the carboxy-terminal region (Williams and Tjian, 1991a,b). AP2 binds a G+C-rich DNA sequence motif, the AP2 site, that contains, at a minimum, the sequence GCCNNNNGGC, although considerable deviation from this motif is allowed (Imagawa et al., 1987). Such consensus sequences have been found in the regulatory elements of many genes, although protein binding to these sequences has been demonstrated in only a few (Royer et al., 1991; Seto et al., 1990; Oka et al., 1991; Dominguez et al., 1991; Ekström et al., 1993). It must be noted that the mere presence of an AP2 consensus binding motif does not necessarily indicate a role for the AP2 transcription factor in regulation of expression (Lafyatis et al., 1991).

AP2 can interact synergistically with other transcription factors (Hyman et al., 1989), but more commonly AP2 acts antagonistically to transcription factors such as NF_KB, AP3, and NF-1 by competing for the DNA and causing mutual interference (Israël et al., 1989; Mercurio and Karin, 1989; Courtois et al., 1990). The

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Correspondence: Miroslav Blumenberg, The Ronald O. Perelman Department of Dermatology, New York University Medical Center, 550 First Avenue, New York, NY 10016 Tel (212) 263-5924 Fax (212) 263-8752

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transcriptional activity of the AP2 protein is regulated by cAMP and protein kinase C pathways (Imagawa et al., 1987), whereas the level of expression of AP2 is regulated by retinoic acid as well (Lüscher et al., 1989).

AP2 consensus binding motifs have been recognized in many keratin gene promoters (Ohtsuki et al., 1992, 1993; Leask et al., 1991) and other epidermis-specific genes (Tamai et al., 1993; Blumenberg, 1993). The AP2 binding sites in keratin gene promoters are commonly found in clusters that contain binding sites for additional transcription factors (Ohtuski et al., 1993; Magnaldo et al., 1993); therefore AP2 may be one of the components that define epidermal specificity of keratin gene expression (Leask et al., 1991).

However, as described above, AP2 protein is not exclusively epithelial (Lüscher et al., 1987; Buettner et al., 1993), and its expression is higher in cells derived from the neural crest (Mitchell et al., 1991). Furthermore, AP2 is not consistently a transcriptional activator; in some cases it suppresses expression of genes that contain AP2 sites. Rather than determining epithelial specificity, AP2 may modulate the level of expression of epithelial genes.

To examine the role of AP2 in keratin gene expression, we have (1) confirmed that the bona fide, in vitro-translated AP2 protein binds the AP2 sites in keratin gene promoters, (2) created stable transfectants of fibroblasts that express AP2 to examine their phenotype, (3) co-transfected a vector expressing AP2 with reporter constructs containing keratin gene promoters into several cell types, and (4) determined the levels of endogenous AP2 protein in various cell types. The results indicate that the levels of AP2 correlate well with the levels of expression of keratin genes, and that the role of AP2 in expression of keratin genes is quantitative and not qualitative.

Materials and methods

DNA constructs and their purification

Plasmids containing keratin gene promoters used in this study (Fig. 1) have been described before (Jiang et al., 1993). Specific mutation of the AP2 site in the K5 keratin gene promoter was constructed using a two-round PCR procedure (Bernerd et al., 1993). The PCR was performed using T. aquaticus DNA polymerase under conditions suggested by the manufacturer (Perkin Elmer Cetus). Amplified DNA was digested with the appropriate restriction enzymes and ligated into similarly digested and gel-purified pGCAT vector. The sequences of all the fragments inserted into the pGCAT vector were confirmed by restriction analysis or sequenced using the dideoxy plasmid sequencing method. The effects of the mutation, both in gel retardation and in transfection assays, have been described (Ohtsuki et al., 1993).

DNA probes and competitors for gel retardation assays

Double-stranded synthetic DNA was obtained by slow annealing of complementary oligonucleotides after 5 minutes of boiling in 10 mM Tris-HCl (pH 7.0), 1 mM EDTA, 150 mM NaCl. The oligonucleotides used as radioactive probes were designed to contain recessed 3′ ends. They are listed in Table 1. Both synthetic and PCR-engineered DNA fragments were end-labeled in the presence of 40 μCi [α^{32}P]dCTP (Klenow Fill-in Kit, Stratagene). Radiolabeled probes were purified by gel filtration using Sephadex G50 columns (Excellulose GF5, Pierce). At times, end-labeled DNA fragments were isolated from polyacrylamide gels to remove contaminating radioactive material.

Nuclear extracts

Nuclear extracts from HeLa cells and human epidermal keratinocytes (HEK) were prepared essentially according to the method of Dignam et al. (1983). However, for HEK the following modification was used: pelleted cells (800×g,

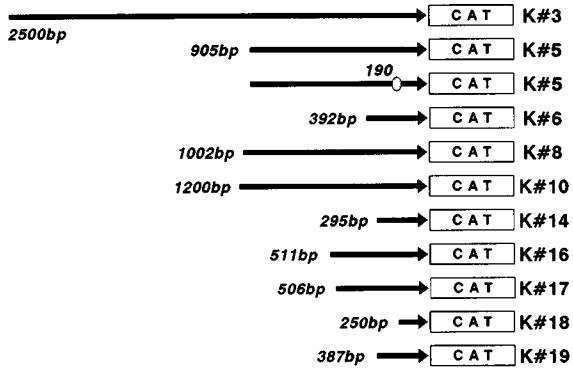

Figure 1. Structures of the DNA constructs containing keratin gene promoters. The numbers indicate the lengths, in base pairs, from the translational start site.

Table 1. Synthetic oligonucleotides used in gel retardation assays.

Description	Sequence $\left(\begin{smallmatrix}5'\to3'\\3'\to5'\end{smallmatrix}\right)$
K5 AP2	GGGACAGCTGCCCCCCAGGCATGGATC TGTCGACGGGGGGTCCGTACCTAGGGG
K5 Sp1	GGGGATCGCATGCCCAGCCCACTGATC CTAGCGTACGGGTCGGGTGACTAGGGG
K5 SITE A	GGGCCACTTAATCATTCACAGCTCGAC GGTGAATTAGTAAGTGTCGAGCTGGGG
K5 AP2 MUT	GGGCTGCCCTTCATGCATGCCCAGCCCACTTAATCATTCACAGCT GACGGGAAGTACGTACGGGTCGGGTGAATTAGTAAGTGTCGAGGG
K16 AP2-LIKE	GGGAGCTCCTTCCCCAGCTGCTATAAAGGTC TCGAGGAAGGGGTCGACGATATTTCCAGGGG
Mtlla AP2	GGGAACTGACCGCCCGCGGCCCGTGTGC TTGACTGGCGGGCGCCGGGCACACGGGG

5 minutes, 4°C) were resuspended in 2 volumes of cell-homogenization buffer (1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, 10 mM Hepes, pH 7.9) and broken by 20 to 30 strokes with a B pestle in a glass Dounce homogenizer. The yield of intact nuclei and the efficiency of cell breakage were monitored under a microscope. The nuclei were pelleted by a 20 minute, 25,000×g centrifugation at 4°C in a SW41 rotor and resuspended in 3 ml of nuclei homogenization buffer (420 mM NaCl, 225 mM EDTA, 1.5 mM $MgCl_2$, 25% glycerol, 20 mM HEPES, 0.5 mM PMSF, 0.5 mM DTT, 1 mM benzamidine, 0.5 µg/ml leupeptin, 1 µg/ml pepstatine A). Nuclei were broken with an additional 15 to 20 strokes in a homogenizer, as described above. The preparation was gently stirred at 4°C for 30 minutes. Cell debris was removed by a 20 minute, 25,000×g centrifugation at 4°C in a SW41 rotor. Clear supernatant was dialyzed for 5 hours at 4°C against 1 liter of storage buffer (200 mM EDTA, 100 mM KCl, 20% glycerol, 0.5 mM DTT, 20 mM HEPES, and the same concentrations of protease inhibitors as above). In vitro synthesized AP2 protein was a gift from T. Williams (Yale).

Gel retardation assays

Five µg of nuclear extract protein were incubated with approximately 5×10^4 cpm of end-labeled, double-stranded DNA fragment in the presence of 2 µg of poly(dI-dC)·poly(dI-dC) (Pharmacia) in a final volume of 25 µl. The binding reactions contained 20 mM Tris-HCl (pH 8), 100 mM NaCl, 1 mM DTT, 5 mM $MgCl_2$, 10% glycerol, 2% polyvinyl alcohol, and 0.1 mM EDTA. Incubations were carried out at room temperature for 30 minutes. For competition, the mixture of extracted protein and a 100-fold molar excess of unlabeled competitor DNA was preincubated at room temperature for 15 minutes before probe was added. Samples were subjected to electrophoretic separation at room temperature on a 6% nondenaturing polyacrylamide gel (29:1 acrylamide:bis-acrylamide), at 125 V for 1.5 to 2 hours. Gels were exposed to X-ray film at room temperature or −70°C for 6–16 hours. Densitometry of autoradiograms was performed with Image 1 AT (Universal Imaging Co.).

Cell transfection and enzyme assays

3T3 and HeLa cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% calf serum. All cells were grown at 37°C in a 5% CO_2 atmosphere in medium containing penicillin and streptomycin. The transfection procedure of Jiang et al. (1991) was slightly modified. One day before transfection, cells were plated onto 100 mm dishes. Four hours before transfection, the medium was changed. For each transfection, a mixture of 5 to 10 µg of one of the plasmids with CAT inserts, 10 µg of AP2 expression vector, 2 µg of pRSVZ, and 3 µg of carrier (25 µg total DNA) was prepared. pRSVZ contains the *lac Z* gene downstream from the Rous sarcoma virus regulatory region and serves as an internal control for efficiency of transfection. The cells were harvested after 48 hours and disrupted by sonication.

HEK from foreskin were purchased from Clonetics (San Diego). They were passaged twice by 1:5 dilution at seeding densities of 2,500–5,000 cells/cm^2. Cells were grown in keratinocyte-SFM medium (GIBCO). Subconfluent cultures were transfected at 80% confluency. Cells were washed with PBS on plates, harvested 48 hours after transfection by scraping into 15 ml of PBS, collected by centrifugation, and washed once more in PBS. Each pellet was resuspended in 150 µl of 0.25 M Tris buffer, pH 7.8. Cells were disrupted by 3 to 5 cycles of freezing and thawing in liquid nitrogen and 37°C water bath, respectively. Cell debris was removed by centrifugation, and 50 µl of the supernatant was used in the β-galactosidase assay. The remainder of the supernatant was heated at 65°C for 10 min-

utes, clarified by centrifugation, and stored at −20°C until 30–60 µl were used in the CAT assay as described.

Murine melanocyte line melan-a (Bennett et al., 1987) was a gift from Dr. S. Orlow (New York University Medical Center). It was grown in MEM pH 6.9 with mM glutamine, 1.1 mg/L sodium pyruvate, 200 nM tetradecanoyl phorbol acetate (TPA), nonessential amino acids and 10% fetal calf serum, 0.1 mM mercaptoethanol, penicillin, and streptomycin. Melanocytes were transfected using $Ca_3(PO_4)_2$ co-precipitation method (Jiang et al., 1991).

Protein purification and Western blotting

Cell pellets were homogenized in buffer containing 25 mM Tris-HCl, pH 7.6, 1 mM EGTA, 1 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride, 50 µg/ml antipain, 2 µg/ml aprotinin, and 1 µg/ml pepstatin A. The homogenate was centrifuged at 10,000× g for 20 minutes, and the insoluble protein pellets were again homogenized and centrifuged as described above. The keratin proteins were obtained by extracting the insoluble pellets with 25 mM Tris-HCl and 9 M urea. Protein concentrations were determined using a Bio-Rad assay. Gel electrophoresis and immunoblot analysis with the basic keratin specific antibody AE3 were performed as described (Cooper and Sun, 1986).

Results

AP2 protein binds to the sites in keratin gene promoters

DNA sequences of the promoters of keratin genes contain sites that resemble the AP2 consensus motif (Fig. 2). However, AP2 can bind to sites that significantly deviate from the consensus; at the same time, not all AP2 site-like sequences function as binding sites. Furthermore, transcription factors other than AP2 can bind DNA at AP2 sites; therefore the mere presence of the sites does not prove functional binding of the protein.

Nuclear proteins from both HeLa and HEK bind the AP2 site of K16 promoter. The amount of binding protein is significantly higher in HEK (see below). A mutant sequence that does not bind AP2 was tested (Ohtsuki et al., 1993), and it was found that this sequence was not bound by nuclear proteins from HeLa or HEK cells. The AP2 site from the K16 keratin gene promoter was efficiently bound by the native nuclear proteins from both cell types (Fig. 3A).

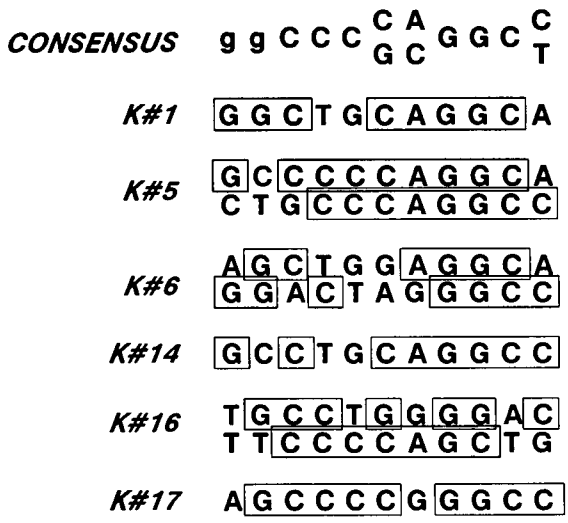

Figure 2. Sequences of the AP2 sites in keratin gene promoters. The top line shows the consensus AP2 binding site compiled from published sequences. Below are the sequences of the putative and demonstrated AP2 sites in the keratin gene promoters, with the nucleotides matching the consensus sequence in boxes. Note that the promoters of K5, K6, and K16 keratin genes have two AP2 sites.

To determine whether AP2 protein can bind the AP2 site in the promoter of the K16 keratin gene, AP2 protein was produced in vitro using reticulocyte lysates with AP2 mRNA template (provided by Dr. T. Williams). The in vitro-produced AP2 bound the AP2 sites, as shown in gel shift experiments (Fig. 3B). The AP2 sites from the human metallothionein IIa gene and from the K16 promoter both bound the in vitro-produced protein, the latter with lower efficiency, and they competed with each other. Nuclear protein bound to the same two DNA fragments showing identical migration patterns (data not shown). These results demonstrate that the bona fide AP2 protein binds the bona fide AP2 site in the K16 keratin gene promoter.

Stable transfectants

If expression of AP2 were sufficient to confer epithelial phenotype and induce keratin synthesis, then nonepithelial cells stably transfected with a vector expressing the AP2 gene might be expected to contain keratin protein and perhaps appear more epithelial than fibroblastic. Similar transfections with *MyoD* gene impart myoblastic phenotype, but in perusing the lit-

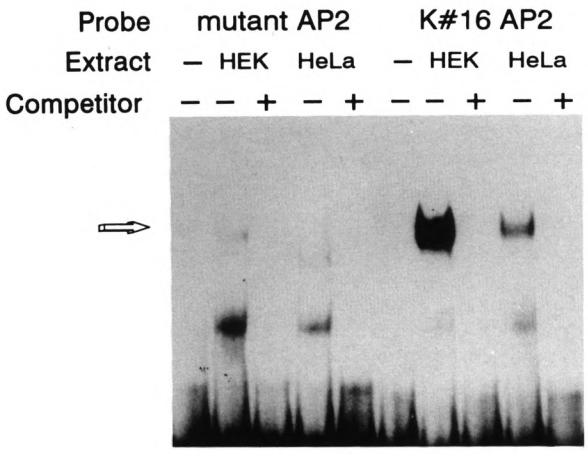

Figure 3A. Both HeLa and keratinocyte extracts contain AP2 activity. Nuclear extracts from both cell types were prepared, and equal amounts of total protein were used in gel shift assays. The AP2-specific band is marked with an arrow. The mutant AP2 site probe contains a sequence reported specifically not to bind the AP2 protein, whereas the K16 AP2 probe contains the oligonucleotide described in Table 1. Nonlabeled DNA fragments were used as competitors.

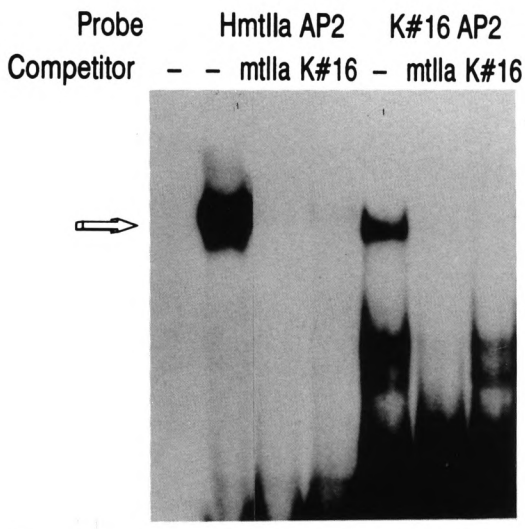

Figure 3B. Bona fide AP2 protein binds to the AP2 site. AP2 produced in vitro from primed reticulocyte lysate was used as a source of protein in gel shift assays. The AP2-specific band is marked with an arrow. Two DNA probes were used—one derived from the human metallothioneine gene (HMtIIa), the other from the K16 promoter. The same DNA fragments were used as nonlabeled competitors.

erature, we have not observed any indication that AP2 imparts an epithelial character. Nevertheless, to test this hypothesis, a vector plasmid that expresses AP2 protein was co-transfected with a pSVneo plasmid into 3T3 fibroblasts, and stable transformants were selected. As a control, the parent vector without the AP2 gene was used. Twenty-four clones of transformants were chosen for further analysis. None of them—and none of the other colonies—exhibited epithelial character. Their phenotype and growth characteristics were indistinguishable from those of the parental control, 3T3 cells, and from the six control clones.

To determine whether the synthesis of keratin proteins occurred in the absence of alterations in cell phenotype, cultures of transfected cells were harvested, and their cytoskeletal components isolated and analyzed by Western blot. Figure 4 shows results of 7 of the 24 analyzed clones: none contained detectable amounts of keratin protein. Thus, the expression of AP2 transcription factor in 3T3 fibroblasts is not sufficient to induce an epithelial phenotype or synthesis of keratin protein.

Effects of co-transfected AP2 gene on keratin gene promoters

The inability of the AP2 protein in stable transfectants to induce expression of keratin genes could be due to the lack of an essential coregulator in 3T3 cells or to the silencing—e.g., by methylation—of the entire keratin gene loci. The second possibility is less likely, in view of the low but detectable level of expression of

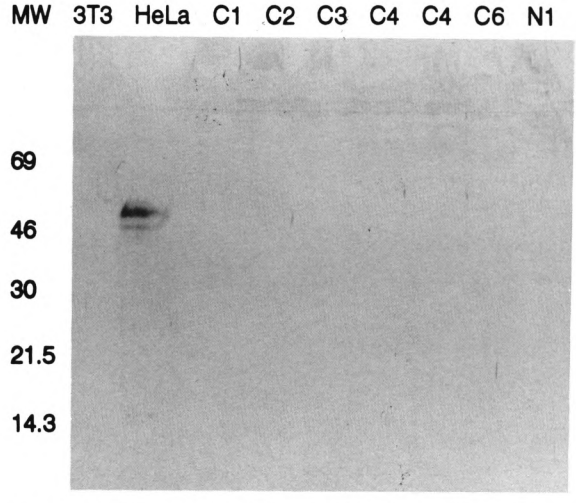

Figure 4. Western blot showing the lack of keratin protein in cells stably transformed with the AP2 expression vector. The parental 3T3 cells and HeLa cells served as controls. The molecular weights of proteins are indicated.

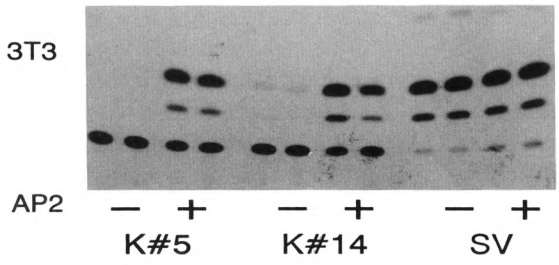

Figure 5. Co-transfection into 3T3 fibroblasts. Constructs containing K5, K14, or SV40 virus promoters were co-transfected into 3T3 cells with the AP2 expressing vector or the parent vector as a control. Note that the K5 and K14 gene promoters show increased levels of CAT expression due to the AP2 protein.

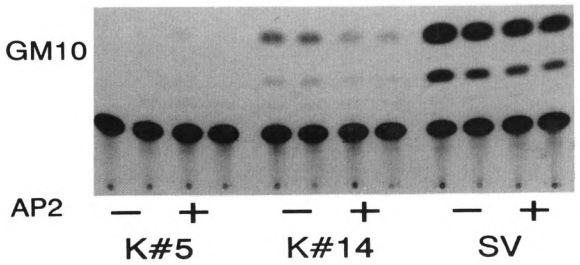

Figure 6. Co-transfection into GM10 fibroblasts. Co-transfection with AP2 expressing vector has no effect on the expression of K5 or K14 promoters in GM10 cells.

endogenous keratin in some strains of 3T3 cells (Guidice and Fuchs, 1987). However, the first possibility is also disproved by the co-transfection experiments shown in Figure 5. When constructs containing K5 or K14 keratin gene promoter were co-transfected with the AP2 expression vector into 3T3 cells, we observed a strong increase in the promoter activities. The effect was specific for these two promoters; SV40 promoter was slightly but consistently suppressed (Fig. 5), whereas promoters of K3, K6, K10, K16, K17, and K19 genes were not affected at all (not shown). Although these promoters contain AP2 sites, raising the levels of AP2 does not influence their transcription, either because the low endogenous levels are sufficient to saturate the promoters, or because the promoters do not depend on AP2 for initiation of transcription in the cell lines tested.

If co-transfection of the AP2 expression vector can induce transcription from the keratin gene promoters in 3T3 cells, then other fibroblast cell types may respond similarly. However, when the DNA was co-transfected into the human fibroblast cell line GM10, the effect of AP2 could not be demonstrated (Fig. 6). The slight suppression of K14 and SV promoters was not consistently observed. It was seen only with some preparations of the AP2 plasmid DNA that suppress the pRSVZ to the same extent. Thus, the finding of response to co-transfected AP2 expression vector in 3T3 cells cannot be generalized to all fibroblasts.

AP2 protein is expressed at high levels in cells of neural crest origin (Mitchell et al., 1991), which may contain AP2-associated co-regulators or modification systems that are absent from fibroblasts. However, we did not observe any effect of AP2 co-transfection in melanocytes, nonepithelial cells that originate in the neural crest (Fig. 7).

In HeLa cells, which express AP2 (see Fig. 3), co-transfection of AP2-expressing vector increased significantly the level of expression of the K5 keratin gene (Fig. 8). The K14CAT construct, which is expressed in HeLa cells at a very high level, could not be further induced by co-transfection (Fig. 8). The endogenous amounts of AP2 in HeLa cells are apparently sufficient for maximal transcription of the K14 keratin gene promoter.

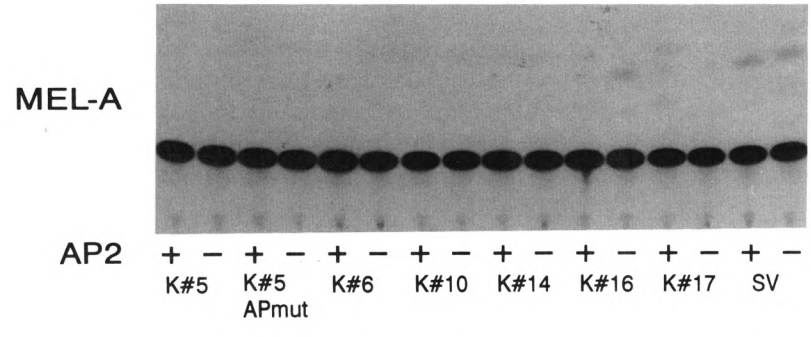

Figure 7. Co-transfection into melanocytes. Co-transfection with AP2 expressing vector has no effect on the expression of any keratin gene promoter tested in the MEL-A melanocyte line.

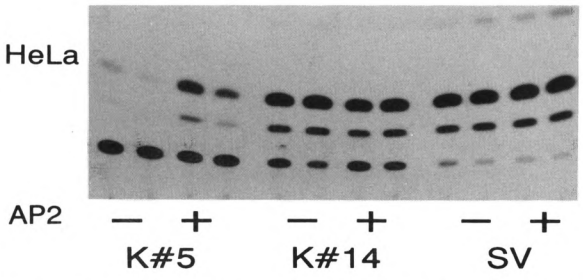

Figure 8. Co-transfection into HeLa cells. Co-transfection with AP2 expressing vector has no effect on the expression of K14 keratin gene promoter, but it does increase the level of transcription of K5 promoter.

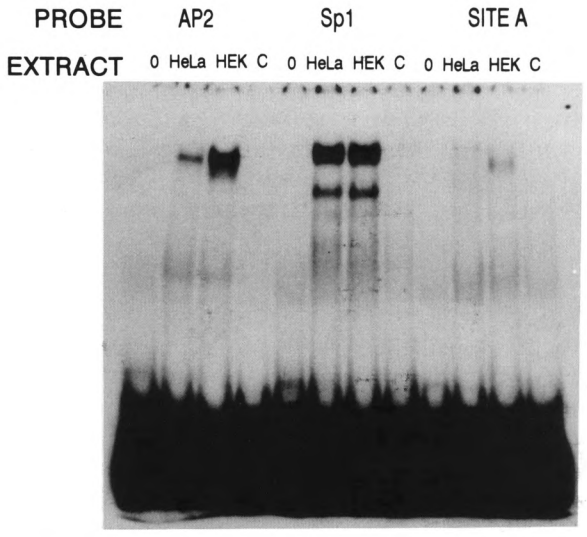

Figure 9. Relative levels of three transcription factors in HeLa cells and in human epidermal keratinocytes. Note that the AP2 site containing probe derives its sequence from the K5 keratin gene promoter, different from the K16 gene sequence in Figure 3. In preliminary experiments, the relative levels of Sp1 transcription factor were determined in the two cell types. Then, the amounts of protein that result in same levels of Sp1 site binding were used in gel retardation assays with three different probes derived from the K5 promoter: AP2, Sp1, and "site A" (Ohtsuki et al., 1993). The levels of Sp1 and "site A" binding proteins are identical in the two cell types, whereas HEK contain a much higher level of AP2 protein.

Relative levels of AP2 protein in epithelial cell types

If AP2 is the sole determinant of keratin gene expression, then those cells that express K5, such as human epidermal keratinocytes, should contain AP2, whereas cells that do not express K5, such as HeLa cells, should not. That this is not the case is supported by the results presented in Figure 3. Alternatively, if AP2 plays a quantitative role in regulation of keratin expression, then its levels may be different in different epithelial cell types. To compare the levels of AP2 protein, extracts from nuclei of HeLa cells and HEK were prepared. In preliminary gel shift experiments, it appeared that the level of AP2 binding activity is much higher in HEK (Fig. 3), which is consistent with the fact that HEK express K16, but HeLa cells do not. However, experiments presented in Figure 3 are subject to artifacts of protein purification procedures, e.g., variations in yields and in proteolysis. Therefore, the binding to the AP2 site in the promoter of the K5 gene was compared with binding to the associated Sp1 and "A" sites that serve as internal controls (Ohtsuki et al., 1993). When the amount of nuclear extract was adjusted for the equivalent levels of Sp1 and Site A binding proteins, we again found several-fold higher levels of AP2 in HEK than in HeLa nuclear extracts (Fig. 9). These results demonstrate major quantitative difference in the levels of AP2 in the two epithelial cell types, which may determine the level of keratin gene expression.

Discussion

The results presented here indicate that the transcription factor AP2 plays a significant role in the expression of keratin genes K5 and K14: AP2 functions directly, i.e., by binding to the AP2 sites in the promoters of keratin genes. The role is a quantitative one, i.e., AP2 determines the level of transcription but is not a sole determinant of the epithelial specificity of keratin gene expression.

Forced expression of AP2 in stable transformants does not by itself confer epithelial phenotype. Thus, AP2 is not the epithelial equivalent of MyoD, a transcription factor sufficient for cell-type specificity. Clearly, however, AP2 plays a quantitative role in keratin gene expression: mutations in the AP2 sites of the K5 and K16 keratin gene promoters abolish transcription (Ohtsuki et al., 1993; Magnaldo et al., 1993), and in certain cell types co-transfection with AP2-expressing vector increases transcription from keratin gene promoters.

The cell lines used, epithelial and nonepithelial, were selected very carefully and deliberately to represent a wide variety of phenotypes.

For example, 3T3 are pre-transformed cells shown to change their phenotype easily in response to MyoD and could be expected to behave similarly in response to a hypothetical "EpiD." These cells already express low level of keratin mRNA (Guidice and Fuchs, 1987). On the other hand, GM10 (which are immortal) are purely fibroblastic—clearly a nonepithelial control. Melanocytes were chosen because they are not epithelial but are of neuroepithelial origin and produce endogenous AP2. Finally, we decided to use the two most extreme examples of human epithelial cells, HeLa and HEK. HeLa was chosen because it is a highly transformed immortal cell line that does not express endogenous K5 keratin. HEK, on the other hand, are primary cells that retain their in vivo characteristics, including expression of K5, when cultured. We expect that additional epithelial cell lines would fall between these two extremes.

One of the roles of AP2 may be to mediate signaling by the extracellular milieu. Two signal transduction pathways, cAMP and protein kinase C, can be mediated through AP2 (Imagawa et al., 1987; Hyman et al., 1989). These two pathways are essential for keratinocyte differentiation, and we note that keratinocytes, which strongly respond to both pathways, express copious amounts of AP2, whereas HeLa cells, which are fully transformed, do not. The role of AP2 in signal transduction will be examined in future experiments.

Retinoic acid is known to induce AP2 expression (Luscher et al., 1989), which may indirectly alleviate the suppression of K5 and K14 keratin gene expression by retinoic acid and its receptor (Tomic et al., 1990 and 1992). In this respect, dimerization of AP2 (Williams and Tjian, 1991b) and its interaction with other transcription factors gain interest (Mitchell et al., 1987; Courtois et al., 1990; Mercurio and Karin, 1989), because AP2 is involved in mediating extracellular signals—e.g., by tumor necrosis factor (Israël et al., 1989)—that profoundly affect keratinocyte physiology (Kupper, 1990; Nickoloff et al., 1990). Significantly, AP2 binding sites are found within clusters of other transcription factor binding sites in keratin gene promoters (Ohtsuki et al., 1993). The role of AP2 in regulating keratin expression, once fully understood, will prove to be far more complicated and more interesting than initially thought.

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Thierry Magnaldo is currently in the Department of Biology, Laboratory of Epithelial Cell Differentiation, École Normale Supérieure, 46 rue d' Ulm, 75230 Paris Cedex 05, France.

Mamitaro Ohtsuki is currently in the Department of Dermatology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan.

References

D. C. Bennett, P. J. Cooper, and I. R. Hart (1987), Int J Cancer 39, 414–418.

F. Bernerd, T. Magnaldo, I. M. Freedberg, and M. Blumenberg (1993), Gene Expr 3, 187–199.

M. Blumenberg (1993), in Molecular Biology of the Skin: The Keratinocyte (M. Blumenberg and M. Darmon, eds.), Academic Press, Inc., San Diego, pp. 1–32.

R. Buettner, P. Kannan, A. Imhof, R. Bauer, S. O. Yim, R. Glockshuber, M. W. Van Dyke, and M. A. Tainsky (1993), Mol Cell Biol 13, 4174–4185.

D. Cooper and T.T. Sun (1986), J Biol Chem 261, 4646–4654.

S. J. Courtois, D. A. Lafontaine, F. P. Lemaigre, S. M. Durviaux, and G. G. Rousseau (1990), Nucleic Acids Res 18, 57–64.

J. D. Dignam, P. L. Martin, B. S. Shastry, and R. G. G. Roeder (1983), Methods Enzymol 101, 582–598.

P. Dominguez, K. Ibaraki, P. G. Robey, T. E. Hefferan, J. D. Termine, and M. F. Young (1991), J Bone Miner Res 6, 1127–1136.

T. J. Ekström, W. M. Klump, D. Getman, M. Karin, and P. Taylor (1993), DNA Cell Biol 12, 63–72.

G. J. Guidice and E. Fuchs (1987), Cell 48, 453–463.

S. E. Hyman, M. Comb, J. Pearlberg, and H. M. Goodman (1989), Mol Cell Biol 9, 321–324.

M. Imagawa, R. Chiu, and M. Karin (1987), Cell 51, 251–260.

A. Israël, O. Le Bail, D. Hatat, J. Piette, M. Kieran, F. Logeat, D. Wallach, M. Fellous, and P. Kourilsky (1989), EMBO J 8, 3793–3800.

C.-K. Jiang, H. S. Epstein, M. Tomic, I. M. Freedberg, and M. Blumenberg (1991), J Invest Dermatol 96, 162–167.

C.-K. Jiang, T. Magnaldo, M. Ohtsuki, I. M. Freedberg, F. Bernerd, and M. Blumenberg (1993), Proc Natl Acad Sci USA 90, 6786–6790.

T. S. Kupper (1990), in Immunophysiology (J. J. Oppenheim and E. M. Shevach, eds.), Oxford University Press, New York, pp. 285–305.

R. Lafyatis, F. Denhez, T. Williams, M. Sporn, and A. Roberts (1991), Nucleic Acids Res 19, 6419–6425.

A. Leask, C. Byrne, and E. Fuchs (1991), Proc Natl Acad Sci USA 88, 7948–7952.

W. Lee, A. Haslinger, M. Karin, and R. Tjian (1987), Nature 325, 368–372.

B. Lüscher, P. J. Mitchell, T. Williams, and R. Tjian (1989), Genes Dev 3, 1507–1517.

T. Magnaldo, F. Bernerd, I. M. Freedberg, M. Ohtsuki, and M. Blumenberg (1993), DNA Cell Biol 12, 911–923.

F. Mercurio and M. Karin (1989), EMBO J 8, 1455–1460.

P. J. Mitchell, C. Wang, and R. Tjian (1987), Cell 50, 847–861.

P. J. Mitchell, P. M. Timmons, J. M. Hébert, P. W. J. Rigby, and R. Tjian (1991), Genes Dev 5, 105–119.

B. J. Nickoloff, C. E. M. Griffiths, and J. N. W. N. Barker (1990), J Invest Dermatol 94, 151S–157S.

M. Ohtsuki, S. Flanagan, I. M. Freedberg, and M. Blumenberg (1993), Gene Expr 3, 201–213.

M. Ohtsuki, M. Tomic-Canic, I. M. Freedberg, and M. Blumenberg (1992), J Invest Dermatol 99, 206–215.

T. Oka, A. Rairkar, and J. H. Chen (1991), Oncogene 6, 2077–2083.

H. D. Royer, M. P. Freyaldenhoven, I. Napierski, D. D. Spitkovsky, T. Bauknecht, and N. Dathan (1991), Nucleic Acids Res 19, 2363–2371.

E. Seto, P. J. Mitchell, and T. S. B. Yen (1990), Nature 344, 72–74.

K. Tamai, K. Li, and J. Uitto (1993), J Invest Dermatol (Abstr) 101, 498.

M. Tomic, C.-K. Jiang, H. S. Epstein, I. M. Freedberg, H. H. Samuels, and M. Blumenberg (1990), Cell Regul 1, 965–973.

M. Tomic-Canic, I. Sunjevaric, I. M. Freedberg, and M. Blumenberg (1992), J Invest Dermatol 99, 842–847.

T. Williams, A. Admon, B. Lüscher, and R. Tjian (1988), Genes Dev 2, 1557–1569.

T. Williams and R. Tjian (1991a), Genes Dev 5, 670–682.

T. Williams and R. Tjian (1991b), Science 251, 1067–1071.