Effects of UV, 4-NQO and TPA on gene expression in cultured human epidermal keratinocytes

Tonja Kartasova, Ben J.C.Cornelissen¹, Peter Belt and Pieter van de Putte

Laboratories of Molecular Genetics and ¹Biochemistry, State University of Leiden, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands

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ABSTRACT

An approach to study effects of UV light on gene expression in human epidermal keratinocytes, a cDNA library was constructed from poly(A)RNA isolated after UV irradiation from cultured keratinocytes. The cDNA library was differentially screened with labelled cDNA probes synthesized on poly(A)RNA isolated from UV irradiated or nonirradiated keratinocytes. Forty clones were selected and subjected to further analysis, 31 of them are described in this report. Whereas total mRNA synthesis is reduced after UV irradiation or treatment with 4-NQO Northern blot analysis revealed that there is an at least relative increase in the level of mRNAs corresponding to the majority of the isolated cDNA clones. Among these 15 were identified as corresponding to mRNAs for 50K and 56K keratins and for 50K- and 46K-related keratin. In addition, clones were found corresponding to the proteinase inhibitor cystatin A and to the glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Treatment of keratinocytes with the tumor promoter TPA had no effect on the mRNA level for most of the clones except those corresponding to keratins. Our results indicate that in keratinocytes UV irradiation leads to a relative increase in the level of some mRNAs.

INTRODUCTION

Irradiation with ultraviolet (UV) light has numerous effects on living cells. In bacteria a set of reactions known as SOS response is triggered by UV light (1). The induction of this process has been studied intensively in E.coli where the expression of a large number of enzymes involved in different steps of DNA replication, recombination, repair and cell division is under the control of the lexA-recA regulatory mechanism (2). In mammalian cells no direct evidence has been found for the existence of an SOS-like response (3). However, irradiation of cells prior to infection enhances both the survival and mutagenesis of UV irradiated virus (4). This enhancement resembles a similar phenomenon in E.coli where it has been shown to be part of the SOS response (1). Therefore, it is feasible that also in mammalian cells UV light induces new enzymatic activities which facilitate survival of irradiated virus and which are involved in processes of DNA replication and repair, and of cell division. This prompted us to initiate a study on the detection of genes activated in human cells by UV irradiation.

Most studies on the response of human cells to UV irradiation have been carried out with dermal fibroblasts. In situ the effects of UV
Irradiation on dermal fibroblasts is limited because of the high UV absorbance by the epidermis, the outermost cell layer of the skin, consisting predominantly of keratinocytes. Three other minor cell types of the epidermis are melanocytes, Langerhans cells and Merkel cells. Keratinocytes are functionally specialized to protect the organism against damaging effects of different external agents, including UV light. Therefore, they represent an attractive system to study the influence of UV irradiation on gene expression.

A cDNA library was prepared using poly(A)RNA isolated from cultured epidermal keratinocytes after UV irradiation. The method originally used by Scott et al. (5) for the isolation of mouse genes activated in SV 40-transformed cells was chosen to screen this library for cDNA clones corresponding to those mRNAs whose concentration increases in the cytoplasm after UV exposure. Forty clones were selected for further analysis, 31 of them are described here.

MATERIALS AND METHODS

Enzymes and chemicals

Terminal deoxynucleotidyl transferase, *E. coli* DNA ligase and ribonuclease H were from PL Biochemicals. *E. coli* DNA polymerase I, T4 DNA ligase and RNase inhibitor RNasin were from Promega Biotec. AMV reverse transcriptase was kindly provided by Dr. J.W. Beard (St. Petersburg) and was used in the initial stage of this work; later AMV reverse transcriptase was purchased from Promega Biotec. Other chemicals: SI nuclease (Sigma), 3H-labelled polyrU and 32P-labelled dCTP (Amersham), poly(U) Sepharose (Pharmacia), m-aminobenzyloxymethyl-cellulose (Miles laboratories). All cell culture media were from Gibco. Cholera toxin was from Schwarz/Mann, epidermal growth factor from Collaborative Research. TPA (12-tetradecanoylphorbol-13-acetate) was from Sigma and 4-NQO (4-nitroquinoline-1-oxide) from ICN K & K laboratories, Inc., Plainview, N.Y. USA.

Cell culture

A primary culture of human epidermal keratinocytes was established essentially as described by Rheinwald and Green (6) with some modifications according to Ponec et al. (7). Cells were grown two or four passages, harvested after trypsinization by centrifugation at 900 rpm for 10 min. in a MSE 2 centrifuge and used immediately for RNA isolation.

Treatment of cells with UV, TPA and 4-NQO

For irradiation short wave (254nm) UV light was used at a dose rate of
1 J/m²/sec. Dose rates were determined with a UVX-radiometer (Ultraviolet Products, Inc., San Gabriel, California). Before irradiation, cells were washed twice with a pre-warmed phosphate-buffered saline (PBS) solution, then the PBS solution was removed and cells were irradiated with doses indicated in the text. TPA treatment was for 12 hours at a concentration of 20 ng/ml. 4-NQO was used at a concentration of 0.5 mM. Incubation of cells with 4-NQO was for 4 hours (5-10% survival), cells were washed and fresh medium was added. RNA was isolated 12 hours later.

**RNA isolation**

All operations concerning RNA isolation were carried out at 0-4°C. Total cytoplasmic RNA was isolated from 10-20 X 10⁶ cells as follows. After trypsinization cells were collected by centrifugation. The cell pellet was resuspended in 10 volumes of a buffer containing 10 mM Hepes (pH 7.5) and 1 mM EDTA. Cell membrane was disrupted in a Dounce homogenizer. The cytoplasmic fluid was clarified from cell debris, nuclei and mitochondria by centrifugation at 12,000 rpm for 20 min at 4°C in a Sorvall SS-34 rotor. The supernatant was extracted twice with an equal volume of water-saturated phenol in the presence of 0.5 % (w/v) SDS and 100 mM Tris pH 9.0, and twice with an equal volume of chloroform. RNA was precipitated by the addition of sodium acetate (pH 4.8) to 0.3 M and 2.5 volume of ethanol. The precipitate was stored at -20°C until required. RNA was collected by centrifugation at 10,000 rpm for 10 min. at 4°C in a Sorvall HB4 rotor, dried briefly and resuspended in a buffer containing 25 % formamide, 0.7 M NaCl, 10 mM Hepes (pH 7.4), 10 mM EDTA and 0.1 % SDS and applied three times to a poly(U)-Sepharose column. The column was extensively washed with the same buffer containing 50 % formamide and 0.5 M NaCl. The poly(A)RNA was eluted in 95 % formamide, 10 mM Hepes (pH 7.4), 5 mM EDTA and 0.1 % SDS. The RNA was ethanol-precipitated after addition of 0.1 volumes of 3 M sodium acetate, washed and dissolved in sterile water.

**Construction of pECVl vector**

Vector pECV1 is a derivative of pSV2-glo (8). In the SV40 part of the plasmid the sequence between the EcoRI and BamHI sites was deleted to remove an irrelevant part of the viral sequence (fig.1). In the pBR322 part the PstI-PvuII fragment was substituted by the PstI-AvaI fragment of pBR329. As a result the PvuII site was lost. In the BgIII site a KpnI linker was inserted. A HindIII-KpnI fragment containing a rabbit beta globin cDNA was substituted by the rabbit beta globin second intron derived from plasmid pBR327/RchR-
beta-IIIC4 (generously provided by Peter Diercks, University of Zurich). The KpnI site was used further for preparation of vector-primer and linker DNAs (9). The sequence between the EcoRI site in the globin intron and the KpnI site is as follows: GAATTCGTTACC.

The cDNA libraries

Double-stranded cDNA synthesized on poly(A)RNA isolated from keratinocytes 12 hours after UV irradiation with 50 J/m² or from nontreated cells was cloned in the pECV1 vector (fig.1) according to the procedure described by Okayama and Berg (9). pECV-1 cut with KpnI and tailed in a terminal transferase reaction with T's was used as primer for cDNA synthesis. The PstI-KpnI fragment containing rabbit beta globin intron and tailed at the KpnI site with C's was used as linker. For transformation E.coli MH 1 (10) was used. Transformants were grown overnight in the presence of ampicillin. The cells were pelleted and resuspended in 0.7% peptone and 30% glycerol and stored at -80°C until used. Two cDNA libraries were constructed: one of 250,000 clones, corresponding to poly(A)RNA from nontreated cells and another of 180,000 clones corresponding to poly(A)RNA from UV irradiated cells.

Immobilization of cDNA library on cellulose

Four milligrammes of plasmid DNA from the cDNA library made on poly(A)RNA isolated from nontreated keratinocytes were treated according to Scott et al. (5) and coupled to 70 mg (dry weight) of 4-aminoxyethyl-cellulose according to the procedures of Noyes and Stark (11). About 26% of the DNA was bound to cellulose.

Synthesis of 32P-labelled probe and control cDNAs

The cDNA used as a plus-probe in differential screening of the cDNA library was synthesized on poly(A)RNA isolated from epidermal keratinocytes 12 hours after UV irradiation with 50 J/m². cDNA synthesis was carried out in a buffer containing 100 mM Tris (pH 7.4), 10 mM MgCl₂, 50 mM KCl, 5mM DTT, 100 μg/ml oligo dT, 1.5 units/μl RNasin, 50 μg/ml poly(A)RNA, 500 μmolar dATP, dGTP and dTTP, 50 μmolar dCTP, 5μCi/ml alpha-32P-labelled dCTP (spec.act. 3000 Ci/mmol) and 1.25 U/μl AMV reverse transcriptase. Incubation was at 42°C for 2 hours. 32P-labelled cDNA was freed from its template RNA by incubation in 0.2 N NaOH at 65°C for 15 min., neutralized with 0.1 volumes of 3 M sodium acetate (pH 4.8) and passed over a 1 ml Sephadex G50 fine column in sterile water. The DNA in the excluded volume was ethanol-precipitated after addition of 0.1 volume of 3 M sodium acetate (pH 4.8). Poly(A)RNA isolated from nontreated cells was used as a template for the synthesis of the 32P-labelled minus-probe.
Hybridization to cDNA-cellulose

The hybridization to cDNA cellulose was done as described by Scott et al. (5). The hybridization mixture contained about 260 μg/ml of cDNA inserts bound to cellulose. The 32P-labelled cDNA probes were used at a concentration of 4 μg/ml. The hybridization was repeated four times (for details see Scott et al). Finally, nonhybridized cDNA probes were collected by ethanol precipitation.

Solution hybridization

The hybridizations in solution were performed in a buffer containing 10 mM Na PIPES (pH 6.5), 1 mM EDTA, 0.2 % SDS, 2XSSC, 83 μg/ml poly(A)RNA and about 10^6 cpm/ml 32P-labelled cDNA. The hybridization mixture was covered with parafin oil in order to prevent evaporation, boiled for 3 min. in a water bath, chilled on ice. Hybridizations were carried out at 65°C for 10 hours, corresponding to a R₀ₜ value of ten calculated assuming that an R₀ₜ corresponds to hybridization for 1 hour at a concentration of poly(A)RNA of 83 μg/ml. Reaction was stopped by addition of cold hybridization buffer without poly(A)RNA.

Nuclease S1 reaction

S1 reaction was performed in S1 assay buffer, containing 30 mM sodium acetate (pH 4.5), 250 mM NaCl, 4.5 mM ZnSO₄, 10 μg/ml denatured, fragmented salmon sperm DNA. S1 nuclease activity, sufficient to produce a limit digest of single-stranded DNA, was determined in advance. Incubation was at 20°C for 30 min. Reaction was stopped by addition of EDTA to 10 mM and ethanol precipitation.

Differential screening

Colony hybridizations were done according to Hanahan and Meselson (12). Four replica filters, with about 3000 colonies were differentially screened in the following way: filters number 1 and 3 were hybridized to the 32P-labelled plus-probe and filter number 2 and 4 were hybridized to the 32P-labelled minus-probe.

Northern blot analysis

The amount of poly(A)RNA for each RNA preparation was quantitated by hybridization to 3H-labelled polyrU (13). Equal amounts of poly(A)RNA, 0.1 μg per slot, were treated with glyoxal, electrophoresed in 1.5 % agarose gels, blotted onto Gene Screen filters (NEN) and hybridized to radiolabelled cDNA probes (14) by the procedures described by Sarachu et al. (15).
cDNA sequencing

The cDNA inserts were subcloned in M13 vectors and sequenced by the dideoxy chain terminating method of Sanger et al. (16).

RESULTS

Isolation of cDNA clones

A primary culture of epidermal keratinocytes derived from human foreskin was established essentially as described by Rheinwald and Green (6). A cDNA library of 180,000 clones was prepared on poly(A)RNA isolated from keratinocytes 12 hours after UV irradiation with 50 J/m². Keratinocytes when grown in vitro under standard conditions undergo differentiation and form a multilayer of cells. Thus, during irradiation only the uppermost cell layer will receive the calculated UV dose. The dose of 50 J/m² was chosen as the approximate equivalent of the UV dose (17,18) shown to be optimal for UV induction of proteins in normal human fibroblasts, growing in monolayers (19). A second cDNA library was constructed from poly(A)RNA isolated from nonirradiated cells. This library was used for the depletion of the cDNA probes from abundant sequences (see below). Double-stranded cDNA was synthesized according to Okayama and Berg (9) and cloned in the mammalian expression vector pECV1 (fig.1), a derivative of pSV2-glo (8). The pECV1

![Figure 1. Construction of the vector pECV1. For details, see materials and methods.](image)
vector lacks the pBR322 "poison" sequences (20) and has the rabbit beta-globin second intron (21) situated just downstream from the SV 40 early promoter. The cDNA was cloned in such a way that it could be expressed directly from the early SV 40 promoter upon transfection to mammalian cells (fig.1).

For the isolation of clones corresponding to mRNAs which are relatively abundant after UV irradiation as compared to mRNAs from nonirradiated cells the differential screening procedure described by Scott et al. (5) was used with some modifications. Two $^{32}$P-labelled cDNA probes were prepared; the one synthesized on poly(A)RNA isolated from keratinocytes 12 hours after UV irradiation with 50 J/m$^2$ was designated "plus-probe" and the other synthesized on poly(A)RNA isolated from nontreated keratinocytes "minus-probe". Both probes were treated further to remove abundant sequences. DNA from the cDNA library synthesized on poly(A)RNA from nonirradiated cells was coupled to m-aminobenzyloxymethyl cellulose and hybridized to the plus- or minus-probes. The sequences which were left after four rounds of hybridization

<table>
<thead>
<tr>
<th>clone number</th>
<th>related clones</th>
<th>identification</th>
<th>size of cDNA insert (nucleotides)</th>
<th>length of mRNA (nucleotides)</th>
<th>increase or decrease of mRNA level</th>
</tr>
</thead>
<tbody>
<tr>
<td>426</td>
<td>5</td>
<td>56K keratin</td>
<td>543$^a$</td>
<td>2000-2200$^a$</td>
<td>++$^b$</td>
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<tr>
<td>8a</td>
<td>5</td>
<td>50K keratin</td>
<td>552</td>
<td>1550-1650</td>
<td>++$^b$</td>
</tr>
<tr>
<td>266</td>
<td>5</td>
<td>50K- &amp; 46K-</td>
<td>936</td>
<td>1550-1650</td>
<td>+++$^d$</td>
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<td>2</td>
<td>GAPDH</td>
<td>725</td>
<td>1100-1300</td>
<td>+++$^c$</td>
</tr>
<tr>
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<td>1</td>
<td>-</td>
<td>480$^e$</td>
<td>1000-1200</td>
<td>++$^f$</td>
</tr>
<tr>
<td>216</td>
<td>2</td>
<td>-</td>
<td>560</td>
<td>600-800</td>
<td>+++$^f$</td>
</tr>
<tr>
<td>264</td>
<td>1</td>
<td>-</td>
<td>480$^e$</td>
<td>600-800</td>
<td>+++$^f$</td>
</tr>
<tr>
<td>345</td>
<td>1</td>
<td>-</td>
<td>458</td>
<td>500-700</td>
<td>++$^f$</td>
</tr>
<tr>
<td>408</td>
<td>1</td>
<td>-</td>
<td>490$^e$</td>
<td>500-700</td>
<td>++$^f$</td>
</tr>
<tr>
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<td>1</td>
<td>-</td>
<td>167</td>
<td>500-700</td>
<td>++$^f$</td>
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<tr>
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<td>1</td>
<td>-</td>
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<td>500-700</td>
<td>++$^f$</td>
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<tr>
<td>148</td>
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<td>-</td>
<td>286</td>
<td>500-700</td>
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<tr>
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<td>400-600</td>
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</tr>
<tr>
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<td>1</td>
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<td>n.d.</td>
<td>600-800</td>
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</tr>
<tr>
<td>313</td>
<td>1</td>
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<td>n.d.</td>
<td>500-700</td>
<td>++$^f$</td>
</tr>
<tr>
<td>77</td>
<td>1</td>
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<td>600$^e$</td>
<td>1500-1700</td>
<td>0$^e$</td>
</tr>
<tr>
<td>23</td>
<td>1</td>
<td>-</td>
<td>766</td>
<td>1000-1200</td>
<td>0$^e$</td>
</tr>
</tbody>
</table>

$^a$ length in nucleotides; $^b$ the symbols +, ++, +++ indicate the degree of increase of the mRNA level; $^c$ the symbols -, --, --- indicate the degree of decrease of the mRNA level; $^d$ the symbol 0 indicates no effect; $^e$ these clones were only partially sequenced; n.d. = not determined.
were then hybridized at $R_0t=10$ to poly(A)RNA isolated from keratinocytes 12 hours after UV irradiation with 50 J/m$^2$. Nonhybridized cDNA sequences were removed by nuclease S1 treatment and the RNA in the DNA:RNA hybrids was degraded by alkali. At the end one-two percent of the probes was left. When at this stage of purification the probes were used for differential screening of replica filters, the hybridization with minus probe was still too high. Therefore an additional step was introduced in the purification procedure: after S1 treatment and alkaline hydrolysis of the RNA, the probes were hybridized at $R_0t=10$ to poly(A)RNA from nontreated cells and then used directly in differential screening. Now only nonhybridized cDNA molecules participate in the colony hybridizations. Almost no hybridization of the minus-probe to the replica filter could be detected, whereas the plus-probe gave clear hybridization signals. Following this strategy 40 clones which reacted positively after hybridization with the plus-probe but not with the minus-probe were selected for further analyses and 31 of them are described below (table 1). They constitute 0.2-0.8 % of the screened cDNA population. cDNA clones were characterized by Northern blot analysis, cross-hybridizations and sequencing.

**Northern blot analysis**

After UV irradiation total synthesis of mRNA is reduced. Therefore, when equal amount (0.1 µg) of poly(A)RNA from keratinocytes before and after irradiation were analysed on Northern blots, no discrimination could be made between absolute or relative changes in mRNA concentration after UV irradiation. For most of the selected clones a relative increase in the level of mRNAs was detected in the cytoplasm of keratinocytes 12 hours after UV irradiation with 50 J/m$^2$ or 6 hours after UV irradiation with 35 J/m$^2$ (fig.2, compare lane b with lanes a and d). The induction ranges between two and five times. It is also apparent from figure 2 that the initial concentration of the mRNAs varies considerably. There is no relative increase in the cytoplasmic mRNA level after UV irradiation for clones 23 and 77. The latter was identified later as corresponding to the alpha subunit of the human elongation factor 1 (EF-1-alpha) (22). Apparently, these two clones which correspond to abundant mRNAs slipped through the selection procedure. Hybridization with EF-1-alpha was used in Northern blots as an internal standard for mRNA concentration. Northern blot hybridizations of the cDNA clones with poly(A)RNA isolated from keratinocytes derived from a different skin donor gave for UV the same result as in figure 2 (data not shown). In these hybridizations a mouse gamma-actin cDNA was used as an internal standard.
Figure 2. Northern blot analysis of poly(A)RNA isolated from keratinocytes 12 hours after UV irradiation with 50 J/m² (a), from nonirradiated cells (b), from keratinocytes 12 hours after a 4 hour treatment with 0.5 mM 4-NQO (c), from keratinocytes 6 hours after UV irradiation with 35 J/m² (d). Only the part of the gel containing the bands of RNA recognized by each particular probe is shown. In case of clones 266 and 8A subclones which do not hybridize to each other were used for hybridizations. The concentration of poly(A)RNA was determined by hybridization to ³²H-labelled polyrU (2).

4-NQO introduces lesions in DNA which in E.coli can be removed by the same excision mechanism as UV lesions (23). We have analysed whether 4-NQO has an effect similar to UV irradiation on the levels of mRNA corresponding to our clones. Keratinocytes were treated with 0.5 mM 4-NQO during 4 hours. Twelve hours after the treatment poly(A)RNA was isolated and used for Northern blotting. As shown in figure 2 lane c, an at least relative increase in mRNA levels after 4-NQO treatment is detectable on Northern blots after hybridization with most of the ³²P-labelled cDNA clones.

In another set of experiments TPA was used as an external agent. This tumor promoting agent has been shown to stimulate terminal differentiation in the majority of normal cultured human and mouse keratinocytes (24,25). Keratinocytes were treated with 20 ng/ml TPA during 12 hours and used directly for poly(A)RNA isolation. For most of the clones no difference could be observed in poly(A)RNA concentrations in TPA treated and nontreated cells (data not shown). However, at least a relative increase in mRNA level after TPA treatment was found for clone 216 and a decrease in mRNA level after TPA treatment was demonstrated for clones 8A, 426 and 266 (fig.3). Hybridization to the gamma-actin mRNA served as an internal standard.
Identification of keratin cDNA

The cDNA clones were further characterized by cross-hybridization and sequencing. By these analyses fifteen related clones were found which could be divided into three groups of five clones each. After comparison with DNA sequences of GenBank (Los Alamos, USA) by computer analysis using UWCGG programmes, the sequences of these 15 clones were identified as sequences of keratin genes. One group of five cDNA clones was derived from a mRNA encoding 56K keratin (26), five other clones were derived from mRNA encoding 50K keratin (27). The third group of five clones represent a keratin whose sequence is homologous to 50K and 46K keratins (27,28). The sequence of the longest cDNA clone from the third group, clone 266, is shown in figure 4. This clone has an insert of 956 nucleotides which represents about 60% of the mRNA. The first 687 nucleotides of clone 266 show homology with the DNA sequences of 50K and 46K keratins of 92% and 84.3%, respectively. This leads to a homology on the protein level of 90.8% between clone 266 and 50K keratin and of 88.2% between clone 266 and 46K keratin (fig.5). Beyond nucleotide 687 a homology of 41.7% is found between the deduced protein sequence from clone 266 and 50K keratin, but only when a deletion of 9 amino acids in the C-terminus of the protein sequence of clone 266 is postulated (fig.5). There is almost no (6.8%) homology on the protein level between clone 266 and 46K keratin in this region. This indicates that the C-termini of these three keratins are heterogenous beyond the alpha-helical domain predicted from the amino acid sequence of 46K and 50K keratins (28,27) (fig.5). In the 3'-
Figure 4. Nucleotide sequence of the insert of clone 266. The amino acid sequence corresponding to the open reading frame is indicated (the standard one-letter code is used). A possible polyadenylation signal occurring in the 3'-noncoding region is underlined.

noncoding region clone 266 has 40% homology with 50K and 46K keratin mRNAs.

Other identified sequences

The protein sequence deduced from the DNA sequence of clone 200 appeared to be highly homologous to the protein sequence of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) from human muscles (29).

Clone 283 contains an insert of 438 nucleotides the sequence of which is shown in figure 6. The first ATG at nucleotides 46-48 is the start of an open reading frame which terminates with a TAG at positions 340-342. The sequence AAAATGA around the ATG-codon is in a good agreement with the consensus sequence PuNNATGPu of Kozak (30). The reading frame encodes a protein of 98 amino acids. A possible polyadenylation site (31) is located 15 nucleotides upstream from the polyA tail. However, the sequence CAYTG which is found adjacent to the polyadenylation site in many eukaryotic mRNAs (32) is not found in the 3'part of the sequence of clone 283. On a Northern blot, clone
Figure 5. Comparison of the amino acid sequence predicted from DNA sequence of clone 266 with amino acid sequences of 50K and 46K keratins (13,31). Arrow indicates the end of the alpha-helical domain.

283 hybridizes to a messenger of about 500 nucleotides in length, indicating that the insert might represent a full length or nearly full length copy of the mRNA. Screening of the library with the 5'-part of the insert of clone 283 (nucleotides 1 to 306) resulted in several additional clones. Restriction analysis revealed that most of them were of a length similar to clone 283. The sequence of two cDNA clones, clones 242 and 301, was determined and appeared to be identical to the sequence of clone 283, except for some minor variations which are indicated in figure 6A. The difference in the predicted amino acid sequence between clone 283 (242) and clone 301 is shown in figure 6B. The variation in the primary structure between the related cDNA clones may reflect expression of different alleles or related genes and requires further investigation. The predicted protein sequence was compared with the amino acid sequences of the NBRF data bank by computer analysis using UWGCG programmes. It appeared that the amino acid sequence deduced from the DNA sequence of clone 283 was completely identical to the amino acid sequence of stefin. This inhibitor of cysteine proteinases has been isolated from human polymorphonuclear granulocytes and its primary structure has been determined by amino acid sequence analysis (33). Immunologically, stefin has been found to be identical to cystatin A, a cysteine proteinase inhibitor isolated from human skin (34,35).

Non identified sequences

The sequences of clones 23, 92, 216, 264, 345, 408, 418, D21 and 148 have no homology to sequences from GenBank (Los Alamos, USA) or to amino acid
sequences from the NBRF data bank. The sequence of clone 64 and 313 has not been determined due to the long C-tail at the 5'-termini of these clones and a lack of suitable internal restriction enzyme sites.

**DISCUSSION**

The method originally developed by Scott et al. (5) for the isolation of mouse genes activated in SV 40-transformed cells was slightly modified and used in this study to isolate cDNA clones corresponding to mRNAs with elevated levels after UV irradiation as compared to the nonirradiated situation. Most of the clones isolated from our cDNA library corresponded to mRNAs that are already abundant in nontreated cells. Possibly, after UV irradiation the increased level of a large set of abundant mRNAs interferes with the detection of clones corresponding to the UV inducible mRNAs of lower abundance. It has been shown recently for some genes such as the oncogenes c-abl and c-mos and the dihydrofolate reductase gene that actively transcribed
genes are repaired faster than genes which are transcribed with a lower efficiency (36,37). Our finding could be the result of such a differential repair if it holds for the majority of the actively transcribed genes and also explain why most of the clones are derived from mRNAs which are already abundant in untreated cells as they are presumably more actively transcribed. However, we can not exclude the possibility that some of the isolated cDNA clones correspond to mRNAs which are preferentially transcribed in the basal layer of proliferating cells. RNA synthesis in these cells is less affected by UV irradiation than in cells of the upper layers. This may lead to a relatively increased level of proliferation specific mRNAs in the poly(A)RNA pool after irradiation.

The potent carcinogen 4-NQO mimics UV effects on living cells. The same uvrABC excision complex which repairs UV induced lesions in the chromosome of E.coli restores DNA after the damage produced by 4-NQO (23). It is likely that an in this respect similarly operating excision repair system exists in men, since DNA repair deficient cells of xeroderma pigmentosum patients are abnormally sensitive to both UV exposure and treatment with 4-NQO (38). For most cDNA clones a relative increased level of corresponding mRNAs as a response to 4-NQO treatment was detected on Northern blots, indicating that the DNA damage might be responsible for this reaction.

Keratin proteins are very abundant intermediate filaments of epithelial cells. More than 17 different keratins have been identified, but usually only a subset of 2-10 keratins is expressed in any given epithelium. Based on immunological cross-reactivity and isoelectric point the keratin family has been subdivided into type I or acidic keratins and type II or basic keratins. During hyperproliferation, all stratified squamous epithelial cells express a common set of five keratins: 46K, 48K and 50K keratins of type I and 56K and 58K keratins of type II (39). Complete nucleotide sequences are known for 46K, 50K and 56K keratins (28,27,26). Cultured epidermal cells express all five keratins, though the production of 48K keratin is reduced (40). Clone 266 with a sequence highly homologous to 50K and 46K keratins might correspond to the mRNA encoding 48K keratin since this keratin has been shown to be related to 50K and 46K keratins by peptide mapping (41). Additional support for this assumption comes from the observation that when a subclone containing a part of the 3'-noncoding region of clone 266 with no homology to 50K and 46K keratins was used in Northern blot hybridization, a mRNA of 1500-1700 nucleotides in length was detected in the human cell line A431 (data not shown), which expresses the 48K keratin (42). The at least relative increase
in the level of mRNAs encoding 50K and 56K keratins and 50K- and 46K-related keratin may be the result of either an increase in the keratin mRNA synthesis and processing or a higher stability of these mRNAs. None of the clones found corresponds to 46K and 58K keratins, although these keratins are two abundant keratins in cultured keratinocytes. This might suggest that the reaction to UV irradiation is not uniform for all keratin genes.

Tumor promoters such as TPA have many direct effects on gene expression, membrane composition and cytoskeleton organization in mammalian cells. In cell cultures this agent has been shown to affect expression of cellular microfilaments. TPA treatment of in vitro cultured chicken fibroblasts leads to reduction of beta actin synthesis (43), whereas in chicken chondrocytes it stimulates beta actin, fibronectin and type I collagen production (44). In the majority of normal cultured human and mouse keratinocytes TPA induces terminal differentiation (24,25). The at least relative decrease in the level of mRNAs encoding 50K and 56K keratins and 50K- and 46K-related keratin in the cytoplasm of TPA-treated keratinocytes might be the result of this switch from proliferation to differentiation. Protein analysis of different sections of epidermis has demonstrated the disappearance of these keratins from terminally differentiated keratinocytes (40).

Clone 200 shows homology to glyceraldehyde 3-phosphate dehydrogenase from human muscles (29). This glycolytic enzyme has been found to be inducible in cultured rat fibroblasts by epidermal growth factor (EGF) and by serum stimulation (45). In this study we found a relative increase in the level of the GAPDH mRNA after UV irradiation. Recently, a similar observation has been reported for the metallothionein mRNA whose induction has been shown in primary human fibroblasts after treatment with UV light (46) or by serum growth factors (47).

Among the isolated clones a cDNA clone was identified as corresponding to the cysteine proteinase inhibitor cystatin A or stefin (33). Cystatin A was originally purified from human epidermis (34). It was localized in the cytoplasm of upper cell layers of keratinized cells in the epidermis by an immunohistochemical method (48). In the present study a mixed population of dividing and differentiating cells has been used and as consequence no discrimination in the cystatin A mRNA expression has been made between these two cell fractions. Therefore, the possibility exists that the change in the level of the cystatin A mRNA after UV irradiation or treatment with 4-NQO is only occurring in a subset of the cells used for mRNA isolation. The role of cystatin A in the development of the cellular response to UV induced skin
damage is still to be established. Lysosomal rupture which occurs in epidermal cells after exposure to UV light (49) can lead to release into the cytoplasm of lysosomal proteinases such as cathepsins B, H and L (50). The leakage of proteinases from lysosomes into the cytoplasm or through the cellular membrane has been thought to be one of the causes of inflammation of the skin induced by UV irradiation (51). The presence of inhibitors of these proteinases such as cystatin A and the relative increase in its mRNA level after UV irradiation suggest that this protein may play a role in reduction of tissue damage after irradiation. Cysteine proteinases belong to a group of endopeptidases which may contribute to tumor cell invasion (for review, see ref.52). Their inhibitors such as cystatin A may play an important role in regulation of proteinase activity both in normal and pathological situations. Certain proteinase inhibitors have been shown to block SOS response in bacterial cells (53). In mammalian cells proteinase inhibitors prevent malignant transformation in vitro (54) and carcinogenesis in vivo (55). One of the possible mechanisms of their action as suggested by Kennedy and Little (54) may be an inhibition of error-prone DNA repair which in bacterial cells is induced after proteolytic cleavage of SOS repressors (1). Recently, purified cystatin A has been shown to inhibit cell proliferation when added to epidermal cell cultures (56). This finding suggests a possible role of the cystatin A induction by UV light in the protective reaction.

The level of the mRNA corresponding to clone 216 changes in a similar way after UV irradiation and after treatment with either 4NQ0 or TPA. It is interesting to find out whether heat shock or any other "stress inducer" stimulates the expression of clone 216 corresponding gene as well.

The results of the experiments described here do not allow to discriminate between absolute or relative increase (or decrease) in mRNA concentration after different treatments. Therefore, it remains to be established on what level the expression of genes whose cDNAs were isolated by differential screening is affected by UV light or by treatment with other DNA damaging or stress inducing agents. It might be the result of differential expression on the level of transcription, but as discussed above, the preferential repair of some UV-damaged genes can not be excluded. The mRNA processing and stability can also play a role in the changes of cytoplasmic concentrations of certain mRNAs.

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