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Biochemistry

A human cellular sequence implicated in *trk* oncogene activation is DNA damage inducible

(Xeroderma pigmentosum/UV irradiation/ribosomal protein L7a/SOS response)

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ABSTRACT **Xeroderma pigmentosum cells, which are deficient in the repair of UV light-induced DNA damage, have been used to clone DNA-damage-inducible transcripts in human cells. The cDNA clone designated pC-5 hybridizes on RNA gel blots to a 1-kilobase transcript, which is moderately abundant in nontreated cells and whose synthesis is enhanced in human cells following UV irradiation or treatment with several other DNA-damaging agents. UV-enhanced transcription of C-5 RNA is transient and occurs at lower fluences and to a greater extent in DNA-repair-deficient than in DNA-repair-proficient cells. Southern blot analysis indicates that the C-5 gene belongs to a multigene family. A cDNA clone containing the complete coding sequence of C-5 was isolated. Sequence analysis revealed that it is homologous to a human cellular sequence encoding the amino-terminal activating sequence of the *trk-2*^{h} chimeric oncogene [Kozma, S. C., Redmond, S. M. S., Xiao-Chang, F., Saurer, S. M., Groner, B. & Hynes, N. E. (1988) *EMBO J.* 7, 147–154]. The presence of DNA-damage-responsive sequences at the 5′ end of a chimeric oncogene could result in enhanced expression of the oncogene in response to carcinogens.**

Evidence has accumulated for inducible responses to DNA damage in mammalian cells (for review, see refs. 1–3). However, the spectrum of induced functions and the mechanism of their induction appears to be quite different from the well-known SOS response by which bacterial cells respond to DNA-damaging agents (4, 5). In mammalian cells, DNA damage induces reactivation of mammalian viruses (6) as well as enhanced expression of proteins, such as plasminogen activator (7), DNA ligase (8), collagenase (9), metallothionein (10, 11), class I major histocompatibility complex antigen (12), and specific DNA-binding proteins (13). There is also evidence for enhanced expression of oncogenes (14–16) and cell type specific genes (17). In addition, UV light induces secretion of extracellular factors, such as EPIF (18) and UVIS (19), which elicit in nontreated cells some of the above-mentioned DNA-damage-inducible (DDI) functions, including a mutagenic response. Recently, two classes of DDI transcripts have been cloned from Chinese hamster cells (20). Induction of class I transcripts is agent specific, whereas class II transcripts are induced by a variety of DNA-damaging agents and stress conditions.

In human cells, DNA lesions are repaired efficiently by cellular mechanisms (for review, see ref. 21), and it has therefore been difficult to detect responses induced specifically as a consequence of lesions in DNA. We have used xeroderma pigmentosum (XP) cells, which are deficient in repair of UV-light-induced DNA damage (22), to identify, by cDNA cloning, genes that are induced in human cells by UV irradiation. The cDNA clone described in this paper* is of special interest because sequence analysis revealed that it is homologous to a human cellular sequence that directly participates in *trk* oncogene activation (23). The significance of these results for the carcinogenic process is discussed.

MATERIALS AND METHODS

Cell Cultures. The human cell strains used are listed in Table 1. The cells were grown in Eagle's minimal medium containing 2× concentrations of essential and nonessential amino acids and vitamins and supplemented with 10% fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). In general, cells were seeded at 6×10^5 cells per 10-cm plate and used when they reached subconfluency. For irradiation, cells were washed twice with phosphate-buffered saline (PBS) and irradiated in a thin layer of PBS with a Hanovia low-pressure mercury lamp (maximum emission at 254 nm) at a fluence rate of 0.15 $J \cdot m^{-2} \cdot sec^{-1}$ or a Mineralight lamp (model R-51; maximum emission at 254 nm; Ultraviolet Products, San Gabriel, CA) at 1.5 $J \cdot m^{-2} \cdot sec^{-1}$ at room temperature.

Materials. Oligo(dT)-cellulose was from Collaborative Research, and random primer pd(N)6 was from Pharmacia. Avian myeloblastosis virus reverse transcriptase was from Life Sciences (Saint Petersburg, FL), *Escherichia coli* DNA polymerase I and Klenow fragment as well as restriction endonucleases were from Boehringer Mannheim or New England Biolabs, Sequenase version 2.0 was from United States Biochemical, and radiochemicals were from Amersham. Dimethyl sulfate was purchased from Aldrich; cycloheximide, actinomycin D, and 4-nitroquinoline oxide (4NQO) were from Sigma.

Construction of a cDNA Library. Total cellular RNA was isolated from human cells by using the guanidinium/cesium chloride method, and poly(A)$^+$ RNA was purified by chromatography through an oligo(dT)-cellulose column (26). Poly(A)$^+$ RNA isolated from XP2096-SV9 cells 5 hr after UV irradiation was used for generating a cDNA library in pBR322 as described by Gubler and Hoffman (27). In brief, the method involves oligo(dT)-primed first-strand synthesis and RNase H/DNA polymerase I-mediated second-strand synthesis. After second-strand synthesis, the cDNA was tailed with dGTP and annealed to *Eco*RV-cut cytidine-tailed pBR322, thus creating a new *Bam*HI site. After annealing, the constructs were transformed into competent *E. coli* MM294 as described by Hanahan (28). Ampicillin-resistant colonies were grown on L-broth agar plates containing ampicillin (50 μg/ml) and transferred to nitrocellulose. Colony hybridization was performed on duplicate membranes with radioactive cDNA prepared from either poly(A)$^+$ RNA of

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Abbreviations: XP, xeroderma pigmentosum; DDI, DNA damage inducible; SV40, simian virus 40.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M36072).

Table 1. Cell lines studied

Phenotype	Cell line
Xeroderma pigmentosum	
Group A	XP12BE (GM4429)
Group A	XP12RO*
Group C	XP2096 (SV-9)†
Group D	GM434/MH3‡
Group D	GM8207
Normal	
Foreskin	AG1518
Skin fibroblasts	GM0637
Embryonic kidney cells	HEK§

Lines with identification numbers GM or AG were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ). All cell lines except AG1518 were transformed with simian virus 40 (SV40).
*Obtained from J. Cleaver (University of California, San Francisco).
†Immortalized with origin-defective SV40 (24); obtained from D. Canaani.
‡Immortalized by R. Moses at Baylor College; obtained from D. Canaani.
§Immortalized with the pW2 fragment of SV40 DNA (25); obtained from L. Chang.

nonirradiated cells or poly(A)$^+$ RNA isolated 5 hr after irradiation.

Probes were labeled with the Klenow fragment of DNA polymerase I by using random oligonucleotides as primers (29) and [^{32}P]dATP (specific activity of 3000 Ci/mmol; 1 Ci = 37 GBq) as the radioactive label. In general, specific activities of 10^9 cpm/μg of DNA were obtained, and hybridization mixtures contained 10^7 cpm/ml.

Northern and Southern Analysis. Five micrograms of poly(A)$^+$ RNA prepared as described above or 25 μg of total RNA, prepared as described by Birnboim (30), was applied to each lane and electrophoresed in 1.2% agarose containing 2.2 M formaldehyde. Transfer was to nitrocellulose or Hybond-C extra (Amersham), and prehybridization and hybridization with radioactive probe were carried out at 42°C for 12 and 48 hr, respectively, in a mixture containing 50% formamide, 5× Denhardt's solution (1× Denhardt's = 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin), 5× SSPE (1× SSPE = 0.18 M NaCl/10 mM phosphate, pH 7.4/1 mM EDTA), 0.1% SDS, denatured salmon sperm DNA (100 μg/ml), and poly(A) (1 μg/ml). After hybridization, the filters were washed as follows: 10 min at room temperature in 2× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7) plus 0.1% SDS, 1 hr at 60°C in 1× SSC plus 0.1% SDS, and 1 hr at 60°C in 0.2× SSC plus 0.1% SDS; then the filters were dried and placed on film (Fuji x-ray film) with an intensifying screen. Hybridization was quantified by densitometry of the autoradiographs. Blots were also analyzed for β-actin mRNA, which is not inducible by UV (20), to verify that equal amounts of RNA were electrophoresed. The sizes of mRNAs were estimated from mobilities of mammalian and *E. coli* ribosomal RNA. For Southern blot analysis, high molecular weight DNA was isolated from the cells, digested overnight with restriction enzymes, and electrophoresed on 0.7% agarose gels.

Sequence Analysis. The cDNA insert of clone pcD(C-5) was subcloned into the pTZ18U vector and sequenced by the dideoxy chain-termination method (31) using synthetic primers and Sequenase version 2.0. The National Biomedical Research Foundation and EMBL/GenBank DNA and protein data bases and the MICROGENIC program were used to search for sequence homologies. PC-GENE (Genofit, Geneva) was used for protein analysis.

RESULTS

Cloning of DDI Transcripts. A cDNA library was constructed using UV irradiation as the inducer of DDI transcripts. For this purpose a simian virus 40 (SV40)-transformed XP group C cell line (XP2096-SV9) was exposed to a fluence of UV light (1.5 J/m^2) that reduces survival to 5%, and poly(A)$^+$ RNA was isolated from cells collected 5 hr after irradiation. The cDNA library, consisting of 10^5 independent clones, was used for differential screening. Of 4500 clones screened, 3 gave a stronger hybridization signal when probed with cDNA prepared from UV-irradiated than from control cells. The clone, designated pC-5, containing the largest insert [750 base pairs (bp)] and giving the strongest signal, was chosen for further characterization.

Northern Blot Analysis. We examined the size and the abundance of the transcripts homologous to pC-5 (C-5 transcript) by Northern hybridization using the *Bam*HI 500-bp fragment of pC-5 as probe. The probe hybridized with a single RNA species of ≈1 kilobase (kb) in all the cell cultures tested (Fig. 1).

The time course for induction of the 1-kb C-5 transcript in XP group A, the most repair-deficient cell line employed, is shown in Fig. 2. At a fluence that reduced survival to 5%, induction was rapid, reaching maximal levels 4 hr after irradiation and gradually declining thereafter to basal levels at about 10–12 hr. The same time course was observed when cells of a repair-proficient line were irradiated with a dose reducing survival to 15%.

We have previously shown that DNA repair deficiency predisposes human cells to produce enhanced levels of plasminogen activator in response to UV-light damage; furthermore, the level of enzyme induced in such cells was correlated with the extent of repair deficiency (7, 32). The repair-deficient and repair-proficient cell strains described in Table 1 were employed to test whether this correlation also holds for UV induction of the C-5 transcript. The dose dependence for induction of this message in several DNA-repair-proficient and DNA-repair-deficient cell lines is shown in Figs. 1 and 3. In two different XP group A cell lines (XP12RO and XP12BE), the C-5 transcript was elevated 7- to 10-fold, respectively, after a UV dose of 3 J/m^2 (Fig. 3*A*).

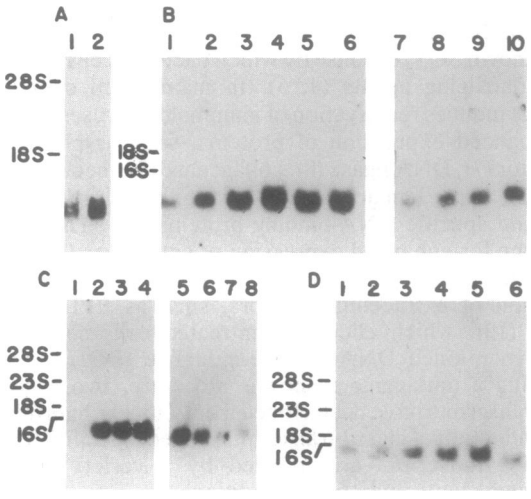

FIG. 1. UV induction of the C-5 transcript in DNA-repair-deficient and repair-proficient cell lines. Cells were irradiated with the fluence indicated, and RNA was isolated 4 hr after irradiation. (*A*) Northern blot hybridization of poly(A)$^+$ RNA from XP group C (XP2096 SV-9) cells that were unirradiated (lane 1) or irradiated (1.5 J/m^2) (lane 2). (*B*) Dose–response of XP group C cells exposed to fluences of 0, 0.75, 1.5, 3, 4.5, and 6 J/m^2 (lanes 1–6) and GM0637 cells exposed to 0, 3, 6, and 9 J/m^2 (lanes 7–10). (*C*) XP group A cells (XP12RO) exposed to 0, 1.5, 3, and 4.5 J/m^2 (lanes 1–4) and XP group D cells (GM8207) exposed to 0, 0.75, 1.5, and 2.5 J/m^2 (lanes 5–8). (*D*) Nontransformed foreskin cells (AG1518) exposed to 0, 3, 6, 9, 12, and 15 J/m^2 (lanes 1–6). In *B–D* equal amounts of total RNA were analyzed.

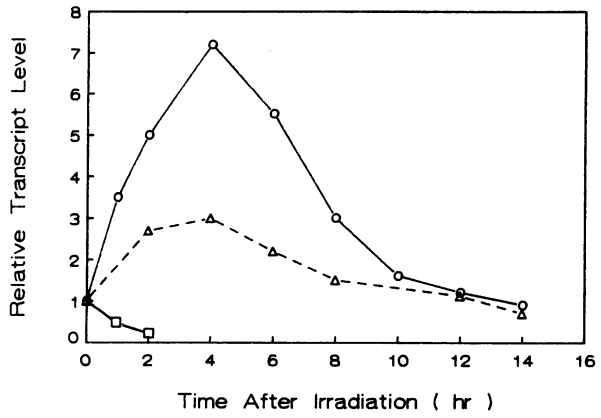

FIG. 2. Kinetics of induction of the C-5 transcript by UV irradiation. Cultures of XP12RO (○) and GM0637 (△) cells were exposed to fluences of 1.5 and 6 J/m^2, respectively, and RNA was analyzed on Northern blots after various times of postirradiation incubation. C-5 transcript levels in UV-irradiated cells were calculated relative to the abundance of the C-5 transcript in nonirradiated cells. Also shown is C-5 mRNA in cells treated with actinomycin D (5 μg/ml), added immediately after irradiation and present throughout postirradiation incubation (□).

Induction of this transcript by UV light was also enhanced in a repair-proficient SV40-transformed human fibroblast cell line as well as in primary cultures of foreskin (Fig. 3*B*). The extent of induction was, however, significantly lower in repair-proficient than in repair-deficient cells and induction occurred at much higher fluences.

Of all the cell cultures tested, XP group D cells (GM8207 and GM434/MH3) were exceptional in that substantial basal levels of the C-5 transcript were present in nonirradiated cultures. C-5 transcript levels decreased in XP group D cells rather than increasing after irradiation (Fig. 1*C*, lanes 5–8). It is noteworthy that significant and somewhat variable basal levels of the C-5 transcript were consistently observed in all nonirradiated cell cultures; they never equaled, though, those in XP group D cell lines. The factors affecting this variability are not yet known.

Effect of Various Agents on Induction of C-5 Transcripts. The effect of inhibitors of protein and RNA synthesis on C-5 mRNA induction was examined (Table 2). Cycloheximide at concentrations that inhibited protein synthesis to 5% of

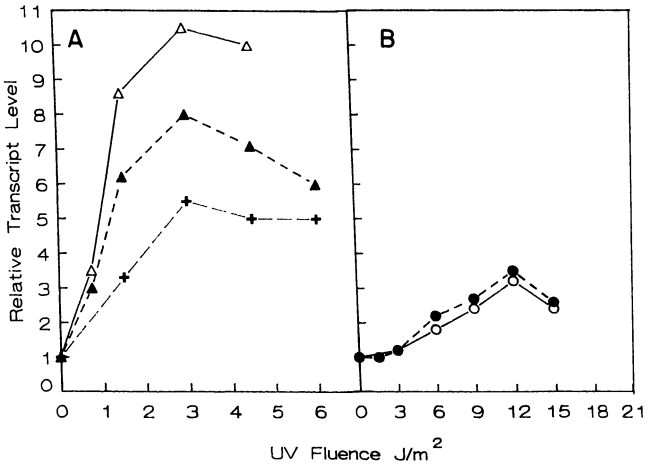

FIG. 3. Dose–response for UV induction of the C-5 transcript. Cells were irradiated as indicated, and total RNA was extracted 4 hr after irradiation and analyzed on Northern blots. (*A*) △, XP12RO cells; ▲, XP12BE cells; +, XP2096 cells. (*B*) ○, AG1518 cells; ●, GM0637 cells. Transcript levels were determined as in Fig. 2. All the cell lines except AG1518 were transformed with SV40.

Table 2. Effect of different treatments on the abundance of the C-5 transcript

Cell line*	Agents†	Treatment, min	Postincubation, hr	Relative C-5 RNA‡
HEK	CH (10 μg/ml)	240	0	0.9
	UV (15 J/m^2)	—	4	3.1
	CH and UV§	240	—	3.6
XP2096	4NQO (0.1 μM)	60	5	2.5
		60	20	1.2
HEK	4NQO (1 μM)	60	5	1.4
	4NQO (10 μM)	60	5	2.8
		60	20	1.3
XP2096	DMS (100 mM)	30	0	2.0
		30	2	2.5
		30	4	2.4
	DMS (200 mM)	30	0	4.2
		30	2	5.7
		30	4	6.0
XP2096	HS (44°C)	60	0	2.5
		60	1¶	2.6

*Transformed with SV40.
†Agents used: CH, cycloheximide; 4NQO, 4-nitroquinoline oxide; DMS, dimethyl sulfate; HS, heat shock.
‡Abundance of C-5 transcript in treated compared to nontreated cultures. Equal amounts of total RNA (25 μg) from chemically treated cultures or poly(A)$^+$ RNA (5 μg) of heat-treated cultures were subjected to Northern analysis, and the abundance of transcripts was determined by scanning autoradiographs.
§HEK cells were irradiated (15 J/m^2) and incubated for 4 hr in the presence of cycloheximide (10 μg/ml) prior to isolation of RNA.
¶After incubation for 60 min at 44°C, cells were incubated at 37°C for 1 hr.

normal, as judged by [^{35}S]methionine incorporation in nontreated cells, enhanced rather than reduced C-5 transcript levels after UV irradiation. On the other hand, UV induction of C-5 mRNA was blocked by actinomycin D (5 μg/ml) (Fig. 2). Experiments with actinomycin D also demonstrated that UV-induced and basal C-5 mRNA exhibited similar stability (half-life of ≈50 min). This suggests that the increase in C-5 RNA following irradiation was due to enhanced transcription.

The carcinogen/mutagen 4-nitroquinoline oxide produces bulky adducts in DNA, which, like pyrimidine dimers, are not repaired in XP cells (33). As shown in Table 2, exposure to 4-nitroquinoline oxide resulted in a 2.5-fold transient increase of C-5 RNA in XP group C cells; a 100-fold higher concentration of the carcinogen was required to get a similar response in repair-proficient cells. Dimethyl sulfate, an alkylating agent, affects XP and normal cells to a similar extent. Induction of C-5 mRNA was observed immediately after exposure to dimethyl sulfate, and it increased up to 2 hr after removal of the alkylating agent.

Several DNA-damage-responsive genes that are also induced by heat shock have been detected in mammalian cells (20). Our preliminary experiments have shown that elevated temperatures also enhance C-5 transcript levels in human cell lines, with maximum induction occurring after incubation for 60 min at 44°C. Under these conditions the C-5 transcript was about 2.5-fold more abundant than at 37°C, with no further increase being observed during recovery from heat shock (shown for XP group C cells in Table 2).

Southern Blot Analysis. Genomic DNA of UV irradiated and nonirradiated HEK cells was digested with restriction enzymes that do not cut within the cDNA insert of the pcD(C-5) clone described below. The results presented in Fig. 4 demonstrate that the C-5 probe hybridizes with numerous bands of human DNA. Similar results were obtained with genomic DNA of XP group C cells and hybridization to

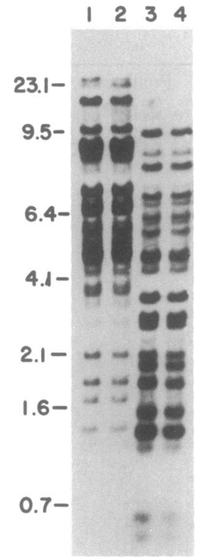

FIG. 4. Southern blot analysis of human genomic DNA. DNA was isolated from nonirradiated HEK cells (lanes 1 and 3) or from HEK cells 5 hr after irradiation (22.5 J/m^2) (lanes 2 and 4). DNA was cleaved with *Eco*RI (lanes 1 and 2) or *Msp* I (lanes 3 and 4) and probed with the 500-bp fragment of pC-5.

C-5 fragments of smaller size (data not shown). These results suggest that C-5 might belong to a multigene family.

Isolation and Sequence of Full-Length Human C-5 cDNA. Initial screening of the pC-5 clone for sequence similarity did not reveal any significant homology to any known DNA sequences or proteins. In view of our results that C-5 mRNA is moderately abundant in all the human cell lines tested, we attempted to isolate a full-length C-5 cDNA clone from an Okayama–Berg SV40-transformed human fibroblast pcD cDNA library that had been enriched for cDNA of 1–1.5 kb in size (34). Of 10^5 colonies screened with the labeled 500-bp *Bam*HI fragment of the pC-5 insert, 16 positive clones were identified and 4 were characterized. All of these clones hybridized with the C-5 probe and contained inserts with a size of about 1 kb. The cDNA of one of these clones, designated pcD(C-5), was subcloned in pTZ18U and sequenced. Its nucleotide and derived amino acid sequence are shown in Fig. 5. The C-5 cDNA sequence contains 891 bp and has an open reading frame extending 798 bases from the first ATG initiation codon. The deduced amino acid sequence of C-5 cDNA is unusual because of its high proportion of basic amino acids (14.6% lysine and 8.6% arginine). A search of the EMBL/GenBank data base (Release 20, August 1989) revealed almost complete homology of the nucleotide sequence of C-5 cDNA to a human cellular sequence that contributed its 5′ end to generate a *trk* chimeric oncogene (23). It is noteworthy that the pcD(C-5) clone contains the entire C-5 cDNA including the sequences at the 5′ end, which were absent in the clone originally isolated by Kozma *et al.* (23).

DISCUSSION

This paper describes the identification of a DDI gene (designated C-5), which may represent an additional type of response to DNA damage. As observed for some other DDI genes, induction of C-5 mRNA by UV irradiation occurs at lower fluences and to a greater extent in repair-deficient XP cells than in DNA-repair-proficient human cells. These results suggest that persistent DNA lesions are necessary intermediates in C-5 induction. This conclusion is also supported by experiments with other DNA-damaging agents (Table 2). UV-enhanced transcription is transient, with the largest increase in C-5 RNA being observed 4 hr after irradiation. This time course indicates C-5 induction to be a relatively early response to UV-light damage.

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                                             1
-31 TTTTTTTTTCTCTCTCCTCCCGCCGCCCAAGATGCCGAAAGGAAAGAAGGCCAAGGGAAAGAAGGTGGCTCCGGCCCCAG
                                    M  P  K  G  K  K  A  K  G  K  K  V  A  P  A  P    16

 50 CTGTCGTGAAGAAGCAGGAGGCTAAGAAAGTGGTGAATCCCCTGTTTGAGAAAAGGCCTAAGAATTTTGGCATTGGACAG
    A  V  V  K  K  Q  E  A  K  K  V  V  N  P  L  F  E  K  R  P  K  N  F  G  I  G  Q  43

130 GACATCCAGCCCAAAAGAGACCTCACCCGCTTTGTGAAATGGCCCCGCTATATCAGGTTGCAGCGGCAGAGAGCCATCCT
     D  I  Q  P  K  R  D  L  T  R  F  V  K  W  P  R  Y  I  R  L  Q  R  Q  R  A  I  L 70

210 CTATAAGCGGCTGAAAGTGCCTCCTGCGATTAACCAGTTCACCCAGGCCCTGGACCGCCAAACAGCTACTCAGCTGCTTA
      Y  K  R  L  K  V  P  P  A  I  N  Q  F  T  Q  A  L  D  R  Q  T  A  T  Q  L  L   96

290 AGCTGGCCCACAAGTACAGACCAGAGACAAAGCAAGAGAAGAAGCAGAGACTGTTGGCCCGGGCCGAGAAGAAGGCTGCT
    K  L  A  H  K  Y  R  P  E  T  K  Q  E  K  K  Q  R  L  L  A  R  A  E  K  K  A  A  123

370 GGCAAAGGGGACGTCCCAACGAAGAGACCACCTGTCCTTCGAGCAGGAGTTAACACCGTCACCACCTTGGTGGAGAACAA
     G  K  G  D  V  P  T  K  R  P  P  V  L  R  A  G  V  N  T  V  T  T  L  V  E  N  K 150

450 GAAAGCTCAGCTGGTGGTGATTGCACACGACGTGGATCCCATCGAGCTGGTTGTCTTCTTGCCTGCCCTGTGTCGTAAAA
      K  A  Q  L  V  V  I  A  H  D  V  D  P  I  E  L  V  V  F  L  P  A  L  C  R  K   176

530 TGGGGGTCCCTTACTGCATTATCAAGGGAAAGGCAAGACTGGGACGTCTAGTCCACAGGAAGACCTGCACCACTGTCGCC
    M  G  V  P  Y  C  I  I  K  G  K  A  R  L  G  R  L  V  H  R  K  T  C  T  T  V  A  203

610 TTCACACAGGTGAACTCGGAAGACAAAGGCGCTTTGGCTAAGCTGGTGGAAGCTATCAGGACCAATTACAATGACAGATA
     F  T  Q  V  N  S  E  D  K  G  A  L  A  K  L  V  E  A  I  R  T  N  Y  N  D  R  Y 230

690 CGATGAGATCCGCCGTCACTGGGGTGGCAATGTCCTGGGTCCTAAGTCTGTGGCTCGTATCGCCAAGCTCGAAAAGGCAA
      D  E  I  R  R  H  W  G  G  N  V  L  G  P  K  S  V  A  R  I  A  K  L  E  K  A   256

770 AGGCTAAAGAACTTGCCACTAAACTGGGTTAAATGTACACTGTTGAGTTTTCTGTACATAAAAATAATTGAAATAATACA
    K  A  K  E  L  A  T  K  L  G                                                      266

850 AATTTTCCTTC
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FIG. 5. Nucleotide sequence and deduced amino acid sequence of C-5 cDNA. The pcD(C-5) clone contains the entire C-5 cDNA sequence including the sequence at the 5′ end, which is bracketed (see text).

It is noteworthy that the C-5 message is moderately abundant in nontreated cells. Of all the cell lines tested, XP group D cells were exceptional in that they contained very high basal levels of the C-5 transcript. These results might indicate that XP group D cells are in a condition of constant stress, perhaps similar to that reported for cells of Bloom Syndrome patients, which express high levels of some DDI genes constitutively (35).

We have isolated a full-length C-5 cDNA clone and found that its sequence is homologous to a cDNA isolated by Kozma *et al.* (23) from a human placental cDNA library. Furthermore, these authors have shown that this sequence activates the *trk* oncogene by contributing the 5′ amino-terminal activating sequence to the receptor kinase domain of *trk*, thereby generating a chimeric oncogene (*trk-2^h*). Martin-Zanca *et al.* (36) first discovered that a *trk* oncogene detected in a human colon carcinoma originated from a somatic rearrangement of a receptor kinase sequence with the 5′ end of the tropomyosin gene. The *trk-2^h* oncogene was formed by recombination during the course of transfection of NIH 3T3 cells with DNA of a human breast carcinoma cell line.

The predicted amino acid sequence of the putative C-5 protein is unusual in its high proportion of basic amino acids, and it does not show sequence homology with other proteins of similar amino acid composition (e.g., histones and high mobility group proteins). Computer-assisted protein analysis also did not reveal nuclear targeting sequences or DNA-binding motifs. The 5′ sequence, comprising 41 amino acids, is highly charged and hydrophilic, and in this respect, it resembles other unrelated *trk* activating sequences (37, 38). There is now evidence that the C-5 cDNA sequence encodes the ribosomal large subunit protein L7a (39). The *Surf-3* gene, which encodes this protein, is a member of a multigene family containing many processed pseudogenes (40). This explains the results we obtained by Southern blot analysis.

The surfeit locus in mammals is unusual in that it contains a cluster of at least four unrelated genes (*Surf-1–Surf-4*) that alternate in the direction of transcription (41). It is therefore feasible that this organization of the surfeit genes facilitates the response of the *Surf-3* gene to DNA damage.

It has been assumed that DNA damage causes neoplastic transformation by means of induction of complex genetic changes involving mutations, gene amplification, recombination, and genomic rearrangement. Evidence for DNA-damage-induced nonhomologous recombination has been obtained from studies on the integration of plasmid DNA into mammalian chromosomes (42, 43). On the other hand, evidence for a role of DNA damage in activation of protooncogenes by gene rearrangement is limited. To the best of our knowledge, this is the first report identifying a cellular sequence involved in chimeric oncogene formation as being DDI. We are of course aware that the *trk-2^h* oncogene was formed during transfection; nevertheless, our results could be of significance to the understanding of carcinogenesis if an increase in transcription of a DDI gene correlates with an increase in nonhomologous recombination. A correlation between transcription and gene rearrangements has been observed for immunoglobulins (44), and it has also been reported that UV-enhanced transcription of the *Ty* element enhances transposition in yeast (45).

It is not known at present whether chimeric oncogenes of the *trk-2^h* type are formed at low rates during cellular growth, and it is not clear what the consequence of such an isolated event could be. However, one might expect that the presence of DNA-damage responsive sequences at the 5′ end of such an oncogene would result in its enhanced expression following carcinogen treatment. Elevated expression of oncogenes is one of the mechanisms believed to be involved in initiation of cellular transformation (2).

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