The nuclear concentration of kin17, a mouse protein that binds to curved DNA, increases during cell proliferation and after UV irradiation

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UV-irradiation induces, in mammalian cells, the expression of a set of genes known as the ‘UV-response’, which may be reminiscent of the bacterial response, called SOS system. The multifunctional protein RecA controls the expression of the SOS genes. We report the expression profile of a mouse gene conserved among mammals, called Kin17, that codes a DNA-binding protein of undetermined biochemical activity and which shares epitopes with the bacterial RecA protein. We demonstrate that the level of Kin17 RNA was 5-fold higher in mid-S phase of serum-stimulated BALB/c 3T3 fibroblasts than in quiescent cells. Cells in S-phase displayed a high level of kin17 protein with a marked nuclear localisation. The maximal level of Kin17 RNA was observed 18 h after serum stimulation, indicating that Kin17 gene is a new member of the late growth-related genes. The accumulation of kin17 protein during cell proliferation follows the increase in Kin17 RNA and correlates with DNA synthesis, which suggests a possible role of kin17 protein in a transaction related to DNA-replication. In quiescent fibroblasts, a 3-fold increase in Kin17 RNA was seen 13 h after UV irradiation. In parallel, kin17 protein accumulated in the nucleus, which suggests that it might be required after the stress produced by UV irradiation.

Introduction

Exposure of mammalian cells to UV radiation leads to the formation of DNA-lesions and to structural changes that perturb DNA metabolism. The constitutive expression of genes involved in the nucleotide excision repair system (NER*) allows the efficient removal of the great majority of the UV-induced lesions (1). In addition to the NER system, mammalian cells react to UV-irradiation by inducing the ‘UV-response’, which involves the activation of multiple and complex signal transduction pathways. The UV-activation of nuclear transcription factors such as AP-1 and p53 leads to the induction of several genes involved in a general host protective function (2). Several of these genes are rapidly induced, such as the immediate early genes c-jun, c-fos or c-myc. Other UV-responsive genes are induced over a longer time-course, e.g. collagenase, plasminogen activator (3). Some of the UV-activated pathways are also activated by growth factor/receptor kinase interactions at the cell membrane (4,5). This constitutes one of the primary signals that stimulates the UV-responsive genes. Nevertheless, the presence of DNA damage induces a nuclear signal that is also required for the stimulation of the ‘UV-response’ (6).

The mammalian ‘UV-response’ might be reminiscent of the bacterial SOS system. In bacteria, the SOS genes are induced after activation of the multifunctional RecA protein, which acts like a co-protease in the cleavage of LexA protein and leads to the induction of the SOS genes (7,8). Recently, several yeast proteins related to Escherichia coli RecA protein have been identified and shown to participate in a complex that is responsible for genetic recombination and recombinational DNA repair (9). In mammals, Rad51 protein is considered to be a functional analogue of RecA protein. The Rad51 protein has functional domains, homologous to the RecA protein, that allow the promotion of the DNA strand exchange reaction (10,11). Despite the identification of an increasing number of proteins that are involved in DNA transactions, such as replication, recombination and repair, the molecular basis of such processes remains to be elucidated in mammalian cells.

We have previously described a mouse protein of 45 kDa, called kin17, which displays common epitopes with RecA protein (12). The exact biochemical activity of kin17 protein remains undetermined, but the preferential binding to curved DNA of a bacterial promoter in vitro and in vivo has been clearly demonstrated, which suggests a role in gene expression regulation (13). Kin17 and RecA proteins have different primary structures and kin17 protein lacks the canonical strand exchange domain that is characteristic of RecA protein (11). However, kin17 and RecA proteins display a 47% homology over a 40-residue stretch in the RecA C-terminal region, which is involved in the regulation of DNA binding and in the SOS response in E.coli (14).

We have recently isolated and characterized the mouse Kin17 gene located on band B of chromosome 2. The Kin17 gene is conserved among mammals and the 1.7 kb Kin17 mRNA is ubiquitously expressed at a low level in adult mouse tissues. In contrast, several mouse tumorigenic cells display increased amounts of Kin17 RNA as compared with non-tumorigenic cells (15). Since tumorigenic cells display a perturbed growth control and often fail to inhibit growth at high cell density, we assumed a correlation between Kin17 gene expression and cellular growth state. To test this hypothesis, we have examined the effects of serum-depletion, cell contact and serum-stimulation on cellular expression of the Kin17 gene. We used non-tumorigenic BALB/c 3T3 fibroblasts in cell proliferation studies because they are very sensitive to serum

*Abbreviations: DMSO, dimethyl sulfoxide; BrdU, bromodeoxyuridine; DAB, 3,3′-diaminobenzidine tetrahydrochloride; NER, nucleotide excision repair system; MEM, minimum Eagle’s medium; FCS, fetal calf serum; UDS, unscheduled DNA synthesis; PKC, protein kinase C; TPA, 12-O-tetradecanoylphorbol 13-acetate; UV, UV irradiation at 254 nm; MOPS, 3-(N-morpholino) propane sulfonic acid; FITC, fluorescein isothiocyanate.
concentration and culture density. Furthermore, BALB/c 3T3 fibroblasts can be easily arrested for long periods in the G0 state and then stimulated to proliferate (16). We considered whether UV-irradiation is able to modify the expression of the Kin17 gene by analogy with the UV-inducible SOS system in bacteria.

We now report the detection of Kin17 RNA and of kin17 protein in mouse in cultured cells and show that the expression profile of this gene correlates with cell proliferation of cultured fibroblasts. The Kin17 gene seems to be part of the group of late growth-related genes. We report that after UV-irradiation of non-replicating BALB/c 3T3 fibroblasts, the Kin17 RNA and protein levels are enhanced during the late stages of DNA repair synthesis, which indicates that the Kin17 gene may be involved in a cellular response to the stress produced by UV irradiation.

**Materials and methods**

**Cells and culture conditions**

BALB/c 3T3 fibroblasts were routinely cultured in minimum Eagle’s medium (MEM) supplemented with 10% fetal calf serum (FCS) and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin). Cultures were maintained at 37°C in a humidified atmosphere that contained 5% CO2. During asynchronous growth, confluent arrested cells were seeded at 5 × 10^5 cells per 100 mm dish in MEM, 10% FCS. Cells were made quiescent by 72 h incubation in MEM that contained 0.25% FCS, and were thereafter re-stimulated to proliferate by increasing the FCS concentration to 10%. RNA samples were isolated at given intervals as described below.

**TPA treatment**

A 100 µg/ml stock solution of 12-O-tetradecanoylphorbol 13-acetate (TPA) from Sigma was prepared in dimethyl sulphoxide (DMSO) and aliquots were kept at –80°C. Stationary cell cultures were incubated for 72 h in MEM containing 0.25% FCS, treated with TPA at a final concentration of 10 ng/ml and incubated for 0.5, 1, 2, 6, 8, 10 and 24 h. Control cells were incubated with 0.1% DMSO. Thereafter, total RNA samples were collected as described below.

**UV-irradiation**

UV-irradiation at 254 nm was performed with a germicidal lamp at a fluence rate of 0.2 J/m² per s. Dosimetry was performed with the UV-radiometer CX-254 (Vilber Lourmat, Marne la Vallée, France). Prior to irradiation, confluent cells were incubated for 36 h in MEM, 0.25% FCS. The medium was removed and re-applied after irradiation. Control cells treated in the same way were not irradiated. The cells were harvested at the indicated time-points.

**Unscheduled DNA synthesis (UDS)**

Fibroblasts plated in complete medium (MEM, 10% FCS) in 35 mm dishes with a coverslip were incubated to confluence. Cells were further incubated for 36 h in MEM, 0.25% FCS and UV-irradiated at 15 J/m² without medium. Cells incubated with the same medium were labelled 1, 6, 11 and 19 h after UV-irradiation with 10 µCi/ml of [3H]-thymidine (83.2 Ci/mmol, Dupont-NEN) for 4 h and further incubated with cold thymidine for 1 h. Slides were finally processed for conventional UDS determination (17). Mock-treated cells were processed in the same way excepting UV-irradiation. The percentage of S-phase was evaluated by scoring at least 700 cells/culture. For the UDS, silver grains overlying 35 nuclei were counted for each sample.

**In vivo DNA synthesis**

Cells were grown to 30% confluence in MEM, 10% FCS. After 72 h incubation in MEM, 0.25% FCS, cells were re-stimulated to proliferate with MEM, 10% FCS. At the indicated times, cells that were pulse-labelled for 1 h with 10 Ci [3H]-thymidine (52.0 Ci/mmol, Amersham) were harvested and lysed with 1 M NaCl, 0.1 M EDTA, 0.5% SDS. The suspension obtained was finally deposited onto GF/C paper (Whatman, UK). After precipitation with ice-cold 5% trichloroacetic acid, the amount of [3H]-labelled DNA was determined using a liquid scintillation counter. DNA synthesis was expressed as the percent of [3H]-labelled acid-precipitable material present in stimulated cells compared with unstimulated cells. Each point represents the mean of two measurements.

Alternatively, cells that were grown to confluence were incubated for 72 h in MEM, 0.25% FCS and thereafter re-stimulated to proliferate with MEM, 10% FCS. At the indicated times, cells pulse-labelled with 10 µM bromodeoxyuridine (BrdU) for 1 h were fixed with methanol/aceticone (1:3) for 10 min at –20°C and rehydrated in PBS for 15 min at room temperature. DNA synthesis was detected using the FITC-conjugated anti-BrdU monoclonal antibody according to the manufacturer’s instructions (Boehringer Mannheim). Specimens were examined under a Zeiss Axioshot2 microscope. The percent age of BrdU-labelled nuclei was evaluated by scoring at least 200 cells/culture.

**Probes**

Radiolabelled DNA fragments were:

1. A 996-bp fragment of mouse Kin17 cDNA obtained by PCR using oligonucleotides 5’ GAGCCCCAAGCCATCGCAAT 3’ and 5’ TTCTC-GCCGTCCTAATCCCTCA 3’.
2. A 2.0-kb BamHI/EcoRI fragment of human β-actin cDNA (Clontech).
3. A 2.1-kb BamHI-BamHI fragment of pRSV-c-jun carrying murine c-jun cDNA, kindly provided by Drs M.Yaniv and F.Mehta.
5. A 1.7-kb Sacl–SacII fragment of the exon 2 carried by pKH4-c-myc genomic DNA, kindly provided by Dr Goubin.
6. The oligonucleotide 5’ AAGATCACAGTAGTTGATTTCC 3’ that corresponded to human 28S rRNA (4011–4036) (19).

DNA probes were radiolabelled using the Random Priming DNA Labelling Kit (Boehringer) and oligonucleotides were labelled using T4 kinase. The specific activities of the probes ranged from 3 to 8 × 10⁶ c.p.m./µg of DNA.

**RNA isolation and analysis**

After cell lysis, the RNAs isolated by the acid guanidinium thioconate–phenol–chloroform extraction method were separated in a 1.2% agarose gel that contained 6% formaldehyde and 1× MOPS buffer and then transferred onto nitrocellulose membranes (NY 13. Schleicher & Schuell) by capillary transfer (20). Membranes UV-irradiated at 1200 J/m² in a cross-linker (Spectronlink, Spectronics Corporation, NY, USA) were hybridized with a DNA probe radiolabelled as indicated above. The final concentrations of the hybridization media were 50% formamide, 0.5 M sodium phosphate pH 7.4, 5× Denhardt’s solution, 0.5% SDS. After 16 h of hybridization at 42°C, the membranes were washed twice for 20 min in 2× SSC, 0.1% SDS at room temperature followed by 15 min in 0.5× SSC, 0.1% SDS at 42°C and then 15 min in 0.1× SSC, 0.1% SDS at 60°C, and were exposed for different times to autoradiographic film. To rehybridize the filters, probes were stripped off by incubation in 5 mM Tris–HCl pH 8.0, 0.2 mM EDTA, 0.1× Denhardt’s solution for 2 h at 65°C. The hybridization signals, quantified with a Molecular Imager (GS-250, Bio-Rad), were normalized with the signals corresponding to GAPDH or 28S rRNA.

**Protein preparation and Western blot analysis**

Cells scraped from culture dishes were washed with cold PBS and centrifuged. All subsequent steps were performed essentially as described (21).

**Immunocytochemistry**

Fibroblasts washed twice in PBS were immediately fixed in methanol-acetone (1/3) at –20°C for 10 min. The pAb2064 serum directed against kin17 protein (or the respective pre-immune serum) was diluted 1/100 in PBS. The biotinylated goat anti-rabbit IgG secondary antibody was diluted 1/200 in 3.3% dianinosobenzilidene tetrachlorohydrochloride (DAB) as described by the supplier (Vectastain ABC Elite Kit, Vector Laboratories, Burnimgame, USA). All subsequent steps were performed as already described (21).

**Results**

**Variation in Kin17 RNA levels during cellular proliferation**

To test a possible correlation between Kin17 gene expression and cell proliferation, we performed a set of experiments using BALB/c 3T3 cells in order to determine the effect produced by the stimulation or arrest of cell division or by cell contact inhibition on Kin17 RNA levels by Northern blots.

We first analysed asynchronously growing cells. Fibroblasts were plated at 6.7 × 10^5 cells/cm² in MEM + 10% FCS and incubated for various times up to 110 h. RNAs were then extracted and analysed by Northern blot. The signal produced by the 28S rRNA confirmed that equal amounts of total RNAs were transferred onto the membrane. Kin17 RNA was quantified and normalized. In parallel, we determined the number of cells. We observed a 3-fold relative increase in
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**Fig. 1.** *Kin17* RNA levels increase during asynchronous growth of mouse fibroblasts. Fibroblasts BALB/c 3T3 plated at $6.7 \times 10^3$ cells/cm$^2$ were grown in complete medium (MEM + 10% FCS) and harvested at the indicated times. (A) Total RNAs were extracted and each sample (10 µg) was analysed by Northern blot hybridization using a radiolabeled *Kin17* cDNA probe (upper panel) or a synthetic oligonucleotide complementary to 28 S rRNA (middle panel). The autoradiographs are shown. The signal of *Kin17* quantified with a Molecular Imager (Bio-Rad GS-250) was normalized using the 28 S rRNA signal as described in Materials and methods. The levels of *Kin17* RNA were expressed in arbitrary units and plotted (lower panel). (B) The number of cells per plate was monitored throughout the experiment and plotted. (C) Alternatively, exponentially growing cells (lane E) were starved with MEM + 0.25% FCS and harvested at 24, 48 and 72 h and compared with cells grown in MEM + 10% FCS and harvested at confluence (lane C). *Kin17* and 28 S RNA levels were determined by Northern blot (upper panel) and quantified as described in (A) and shown in the lower panel. (D) The number of cells per plate during the experiment is shown. The histograms are representative of three independent experiments.

*Kin17* RNA 24 h after plating. The level remained unchanged up to 72 h and had decreased to the basal level 88–110 h after plating (Figure 1A). The cell number increased during the proliferating period (24–72 h) and then reached a plateau at 80 to 120 h (Figure 1B). *Kin17* RNA levels increased with the growth rate of cells between 24 and 72 h. They then dropped when cells stopped proliferating because of the high cell density (Figure 1).

We sought to determine whether down-regulation of the *Kin17* gene occurred during the arrest of cell division or was due to an inhibitory effect produced by cell contact.

**Serum-starvation of exponentially growing cells leads to a decrease in *Kin17* RNA levels**

Exponentially growing BALB/c 3T3 fibroblasts at nearly 30% confluence were serum-deprived in MEM, 0.25% FCS for 72 h. We detected the highest levels of *Kin17* RNA before serum-starvation. The levels decreased progressively over 72 h after serum-starvation (Figure 1C), whereas cell density remained at ~60%, which indicates that *Kin17* RNA levels were correlated with a decrease in cell division rate rather than with cell density (Figure 1D).

**Stimulation of cell proliferation leads to the accumulation of *Kin17* RNA**

Exponentially growing fibroblasts were synchronized by starvation in 0.25% FCS for 72 h as described above. These starved cells were further stimulated to proliferate by adding fresh medium supplemented with 10% FCS and were incubated for 2 to 32 h after stimulation. Measurement of [Me-3 H]thymidine incorporation, performed in parallel cultures, showed that cells initiated DNA synthesis 12 h after serum addition, and reached a maximum rate 20 h later (Figure 2A). These kinetics of DNA synthesis are predictive for cell-cycle progression of BALB/c 3T3, i.e. G1-phase within 6–10 h, S-phase 10–22 h later, followed by G2-M. We observed a clear 3-fold increase in *Kin17* RNA 6 h after stimulation, when most of the cells were in G1 phase. We detected a 5-fold maximal increase in mid-S phase cells 18 h after stimulation. Afterwards, the *Kin17* RNA level slowly decreased during the end of the S and the
Fig. 2. *Kin17* RNA levels correlate with DNA synthesis after serum-stimulation of synchronised fibroblasts. (A) Exponentially growing BALB/c 3T3 fibroblasts serum-starved for 3 days as in Figure 1C, were subsequently stimulated with fresh medium containing 10% FCS, labelled at the indicated times with [3 H]thymidine for 1 h and processed for determination of *in vivo* levels of DNA synthesis. (B) In parallel, the levels of *Kin17*, *c-myc* and 28 S RNA were determined by Northern blot as described in Figure 1. The autoradiographs are shown. The signals corresponding to *Kin17* RNA were quantified as in Figure 1, expressed in arbitrary defined units and represented as a histogram (lower panel). (C) Confluent cells, serum-deprived for 3 days with MEM, 0.25% FCS, were serum-stimulated and RNA was isolated at the indicated times and analysed for *Kin17*, *c-jun* and 28S RNA by Northern blot as described in Figure 1. The quantification and normalization of the *Kin17* RNA signal are expressed in arbitrary units. In parallel, cells grown under the same conditions were pulsed for 1 h with BrdU and processed for immunofluorescence detection as described in Materials and methods. The histograms are representative of three independent experiments.

G2-M phases but remained three times the basal level at up to 30 h after stimulation (Figure 2B). In the same experiment, we detected the expression of *c-myc*, a well-known serum-inducible gene. We observed a marked increase in *c-myc* RNA 2 h after serum stimulation and a sudden decrease, which is in agreement with previous results (16) (Figure 2B). We concluded that *Kin17* RNA levels increase progressively during cell-cycle progression, particularly in the transition G1/S and during the first half of the S phase, and that they correlate with the rate of DNA synthesis in cultured cells.

**Serum-stimulation of confluent cells increases *Kin17* RNA level**

We addressed the question of whether confluent cells, which displayed the lowest levels of *Kin17* RNA, were sensitive to serum stimulation. We cultivated BALB/c 3T3 fibroblasts in MEM + 10% FCS until confluence, and then lowered the serum concentration to 0.25%. After 3 days, the medium was supplemented again with 10% FCS. Cells were harvested 1, 2, 6, 12, 24 and 32 h later and *Kin17* RNA was detected. The levels detected in confluent serum-starved cells remained low until 2 h after serum-stimulation. A significant increase was clearly detected at 6 h after stimulation. *Kin17* RNA progressively increased further and remained three times the level detected in quiescent unstimulated cells for up to 32 h (Figure 2C). In the same experiment, we detected *c-jun* RNA, a well-characterized serum-inducible gene. As expected, *c-jun* RNA transiently increased 1 h after stimulation, which is in agreement with previous results (22) (Figure 2C). Under our experimental conditions, 42% of confluent BALB/c 3T3 fibroblasts underwent replicative DNA synthesis after serum-stimulation as determined by BrdU incorporation (Figure 2C). This rate is in agreement with the 44% observed in other laboratories (16). These observations suggest that the increase in *Kin17* RNA is independent of cell density and is correlated with the mitogenic activity of the serum.

Therefore, the mitogenic effect of serum on cells at low or high density, increases *Kin17* RNA levels with similar kinetics. In all the experiments, the increase in *Kin17* RNA started 6 h after stimulation and peaked at ~18 h, and was followed by a progressive decrease, although levels remained high for at least 12 more hours.
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**Fig. 3.** Immunocytochemical detection of kin17 protein in proliferating BALB/c 3T3 fibroblasts. We observed the cellular distribution of kin17 protein in quiescent cells and during serum-induced proliferation by using anti-kin17 antibody. Fibroblasts plated at $6.7 \times 10^3$ cells/cm$^2$ were grown in MEM, 10% FCS. After 48 h of incubation, exponentially growing cells were incubated in MEM, 0.25% FCS, for 3 days. At this point, some coverslips were processed for immunocytochemical detection with the pAb2064 antibody diluted 1/100 as described in Materials and methods. Quiescent cells were washed, fixed and the anti-kin17 antibodies revealed with DAB. A representative image at $\times 300$ magnification is presented in panel (A). Other coverslips were further incubated in MEM, 10% FCS for 20 h. Serum-stimulated cells were washed, fixed and processed as above. Panel (B) shows a $\times 300$ magnification of the observed staining.

**Variation in kin17 protein levels during cellular proliferation**

We investigated whether the amount of kin17 protein reflected the changes observed in *Kin17* RNA levels under the conditions described above. We detected kin17 protein by immunocytochemical experiments using rabbit anti-kin17 antibodies, as previously described (21). Quiescent cells incubated with pAb2064 antibody displayed weak staining (Figure 3A). At 20 h after serum-stimulation, when the cells were essentially in the S phase, we observed intense staining in the nuclei of $>60\%$ of the cells (Figure 3B). The detected pattern that corresponded to kin17 protein localization was in complete agreement with the localization of kin17 protein in mouse rat and human cells indicated by other immunofluorescence approaches (21,23,24). The immunocytochemical observations indicated that in proliferating cultured mouse cells, kin17 protein has a preferential nuclear localization, and revealed that the amount of kin17 protein follows the pattern of *Kin17* RNA synthesis.

We failed to observe any staining when cells were incubated only with the secondary anti-rabbit antibody or with pre-immune serum [(21) and data not shown].

**Kin17 RNA remains constant after phorbol ester treatment of fibroblasts**

Several serum-inducible genes are transcriptionally controlled by the pathway governed by the protein kinase C (PKC). The phorbol esters such as 12-O-tetradecanoylphorbol 13-acetate (TPA) are able to activate the PKC directly in the absence of serum (25). We investigated whether the *Kin17* gene is part of the pathway controlled by PKC, by testing the effect of TPA treatment on the levels of *Kin17* and c-*jun* RNAs in serum-deprived confluent fibroblasts. Cells treated with 100 ng/ml TPA were harvested 0.5, 1, 2, 6, 8, 10 and 24 h later and the amounts of *Kin17* and c-*jun* RNA were determined by Northern blot as described above. The induction of the c-*jun* gene by TPA was easily detected under our experimental conditions, and confirms the previously described transcriptional control of c-*jun* by PKC (26). Under our experimental conditions, c-*jun* RNA increased 30 min after TPA-treatment and remained at a relatively high level for at least 10 h as compared with the level detected in mock-treated cells (Figure 4, middle panel). In the same experiment, we did not detect any variation in *Kin17* RNA levels in TPA- or mock-treated cells (Figure 4, upper panel). We observed a similar effect of TPA in cells, which were arrested by serum starvation during exponential growth (data not shown). Therefore, the TPA-activation of the PKC alone was unable to induce the accumulation of *Kin17* RNA.

**UV-irradiation enhances Kin17 RNA and kin17 protein levels**

We investigated whether the stress produced by UV irradiation at 254 nm (UVC) affects the expression level of the *Kin17* gene in confluent BALB/c 3T3 fibroblasts.

**Detection of Kin17 RNA**

We irradiated quiescent fibroblasts to avoid possible interference with the increase in *Kin17* RNA inherent to cell proliferation. Fibroblasts serum-deprived for 36 h were UV-irradiated at a dose of 15 J/m$^2$ and further incubated with the same medium. Cells harvested at different times were analysed by Northern blot as described above. Mock-irradiated cells were manipulated in the same way but were not irradiated. The relative concentration of *Kin17* RNA remained constant for 6 h after irradiation in three independent experiments, thus
Fig. 4. Stable levels of Kin17 RNA after TPA-treatment of fibroblasts. Confluent BALB/c 3T3 fibroblasts serum-starved for 72 h (lane C) were treated with TPA (100 ng/ml) or DMSO (0.1% w/v). Cells incubated further with the same medium (MEM + 0.25% FCS) were harvested at the indicated times and RNAs were analysed by Northern blot (10 µg of each sample). The membranes, which were sequentially hybridized with Kin17, c-jun and GAPDH radiolabelled probes, were exposed for autoradiography for 60 h, 12 h and 4 h, respectively. The autoradiographs are shown.

Fig. 5. UVC-irradiation increases Kin17 gene expression in BALB/c 3T3 fibroblasts. Confluent fibroblasts that were serum-starved for 36 h were irradiated at 15 J/m² with a UVC lamp at 254 nm or were mock-irradiated. Cells further incubated using the same medium were harvested at the indicated times and processed for Northern blot analysis using Kin17, c-jun and GAPDH radiolabelled probes. The membranes were exposed for 60, 12 and 4 h, respectively, and the corresponding autoradiographs are shown in the upper, middle and lower panels. The Kin17 signals were quantified, normalized, expressed in arbitrary units and plotted. The histogram is representative of three independent experiments.

UVC radiation affects replication and induces unscheduled DNA synthesis (UDS) (1). Under particular conditions, it can also induce a mitogenic effect (28). Therefore we measured UDS levels at different post-irradiation times to monitor the repair synthesis. To test the possibility that the UV-induction of Kin17 expression is correlated with the UV-induction of replicative DNA synthesis in quiescent cells, we scored the fraction of S-phase cells revealed autoradiographically after [3H]thymidine incorporation. Confluent fibroblasts were serum-deprived for 36 h, and then irradiated and processed at different post-irradiation times as described in Materials and methods. The results are summarized in Table I. We detected the maximal repair synthesis rate within the first pulse period after UV-irradiation. The UDS was stimulated >10-fold, which is typical for repair-proficient fibroblasts. Subsequently, the UDS rate decreased in parallel with the disappearance of UV-photoproteins in genomic DNA. The percent of S-phase cells in the same samples ranged between 1.1 to 1.4% in unirradiated fibroblasts and between 0.28 to 0.85% in irradiated ones. Therefore, the accumulation of Kin17 RNA and protein after UVC irradiation could not be caused by proliferative stimuli. In fact, although the fraction of S-phase cells progressively increased after irradiation, it was <1% and therefore inconsis-
Effect of UV light on \textit{Kin17} gene expression in BALB/c cells

**Fig. 6.** Nuclear kin17 protein increases after UVC-irradiation of BALB/c 3T3 fibroblasts. Confluent cells were irradiated as described in Figure 5 and harvested at the indicated times. Kin17 protein was determined (A) by Western blotting or (B) by immunocytochemistry. Total protein extracts prepared 1, 11 and 15 h after UVC-irradiation were separated by 11% SDS–PAGE, transferred to a membrane, and kin17 protein was detected with serum pAb2064 diluted 1/2000. The antibodies were revealed by chemiluminescence as described in Materials and methods (A). At 13 h after UVC-irradiation, cells were processed for immunocytochemistry, using the pAb2064 serum as described in Figure 3, and analysed at a magnification of ×1000 (B.2). Mock-irradiated cells were processed using the same conditions (B.1).

**Table I.** UDS levels and number of S-phase cells in UV-irradiated quiescent fibroblasts

<table>
<thead>
<tr>
<th>Time of labelling</th>
<th>UDS (grains/nucleus ± SEM)</th>
<th>% S-phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>-UV</td>
<td>+UV</td>
<td>-UV</td>
</tr>
<tr>
<td>1–5</td>
<td>4.6 ± 0.4</td>
<td>53.9 ± 6.1</td>
</tr>
<tr>
<td>6–10</td>
<td>3.8 ± 0.7</td>
<td>38.6 ± 2.6</td>
</tr>
<tr>
<td>11–15</td>
<td>3.9 ± 0.5</td>
<td>18.2 ± 5.3</td>
</tr>
<tr>
<td>19–23</td>
<td>4.6 ± 0.5</td>
<td>16.9 ± 3.5</td>
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aBALB/c 3T3 fibroblasts irradiated at 15 J/m$^2$ under the conditions described in Figure 6 were labelled for 4 h after irradiation at the indicated times and processed as described in Materials and methods.

bSEM, standard error of the mean.

Discussion

In bacteria, the genomic lesions produced by UV-irradiation activate RecA protein and lead to the induction of the SOS system (8). In mammalian cells, UV-irradiation induces a complex response that is initiated by membrane-associated proteins and leads to a transcriptional response via the activation of several nuclear transcription factors (29).

We describe here the expression profile of \textit{Kin17}, a mouse gene that is conserved among mammals and encodes a protein that is immunologically related to RecA. We provide the first evidence that the \textit{Kin17} gene is preferentially expressed in rapidly dividing BALB/c 3T3 fibroblasts and that the expression of this gene is enhanced after UV-irradiation. The molecular mechanism underlying this enhancement remains to be elucidated, but these data suggest that the \textit{Kin17} gene may be a new component of the late response to UV.

The idea that \textit{Kin17} expression is enhanced after stimulation of cell division is based on the fact that the levels of \textit{Kin17} RNA change under the following conditions: (i) the lowest levels are detected in quiescent cells, independently of whether cells came from confluent or subconfluent cultures; (ii) the increased expression during the exponential phase of asynchronous growing cultures; (iii) an increased expression in synchronized cells after stimulation of proliferation; (iv) the highest expression levels in the mid S-phase of synchronous cultures. Nevertheless, \textit{Kin17} RNA levels remain relatively high when cells are in G2 and mitosis, as compared with the level observed in quiescent cells. S-phase cells contain higher amounts of kin17 protein than quiescent cells and its localization is preferentially nuclear, in agreement with the previous identification of an active bipartite nuclear localization signal in kin17 protein (15). Several genes involved in DNA replication have expression patterns similar to that of \textit{Kin17} gene. Some of them display a sharp increase when cells are stimulated to proliferate, e.g. histones H2b and H3 (30). Others, such as...
PCNA, fluctuate 2-fold throughout the cell cycle (31). Curiously, the mouse Rad51 gene, a homologue of the E.coli recA gene, is expressed preferentially at the late G1/S boundary and might participate in DNA replication (32). Our results indicate maximal Kin17 RNA levels 18 h after serum stimulation, when the cells are mid-S phase, which suggests that Kin17 is a new member of the late growth-related genes family.

The Kin17 gene is ubiquitously expressed at low levels in adult mouse tissues in which the great majority of the cells are in G0 or differentiated and arrested in GD (12). This observation agrees with the low expression of Kin17 gene in quiescent cultures in which 99% of cells are in G0. Our results suggest that Kin17 gene participates in a ubiquitous mechanism linked to cell proliferation.

Taking into account: (i) the increased expression of Kin17 gene during cell proliferation and in tumorigenic cell lines; (ii) the preferential binding of Kin17 protein to curved DNA in vitro and in vivo; (iii) the apparent role of curved DNA regions in replication (33), it is reasonable to postulate that Kin17 may participate in an essential DNA transaction.

The idea that the Kin17 gene is involved in DNA metabolism is further confirmed by the nuclear accumulation of Kin17 protein after UV-irradiation. UV-induced DNA lesions are eliminated via biphasic kinetics (34). In human cells, kinetic, 50–75% of the lesions are eliminated within 2 h by NER in the so-called 'early phase' (35). The remaining lesions are processed during a late phase by another mechanism (34,36). In rodents, the early phase of repair is slower than in human cells (37). Considering that Kin17 RNA levels: (i) begin to increase 7 h after irradiation and remain at high levels for 8 h in BALB/c 3T3 fibroblasts; and (ii) increase 3-fold 6 h after treatment of mouse lymphoma cells with 8-methoxy-psoralen plus UV A (Boesen and Simons, personal communication), we hypothesize that the enhancement of Kin17 RNA takes place in the late phase of repair, thereby raising the possibility of an involvement in the process of repair of the remaining DNA lesions not resolved by NER. Nevertheless the possibility that this increase reflects another cellular response cannot be discounted. Although the accumulation of Kin17 RNA is relatively weak, it is comparable with the 2- to 3-fold enhancement of the amount of DNA ligase I and PCNA RNAs after UV-irradiation (27,38).

The expression of Kin17 gene during cell proliferation and after UV-irradiation presents different kinetics, and suggests that gene expression is modulated via two different mechanisms. Indeed, in quiescent cultures with 99% of cells arrested, kin17 protein is virtually undetectable. Stimulation of cell division increases the number of cells in the S-phase to 70% and the amount of kin17 protein in the nucleus of proliferating cells. In contrast, after UV-irradiation of quiescent cells, the number of arrested cells remains constant, but kin17 protein levels increase as they do after stimulation of proliferation.

Low doses of UV radiation (from 3 to 6 J/m²) cause 6–18% of the quiescent cells to enter a state similar to the S-phase of the normal cell cycle, and induces replicative DNA synthesis. We exclude the possibility that the enhancement of Kin17 gene expression is because of such a mitogenic effect, because under our experimental conditions, the UV dose of 15 J/m² does not increase the number of dividing cells, which confirms previous results (28). Taken together, these observations indicate that the UV-induced increase in Kin17 RNA level is independent of proliferation.

UV-irradiation and serum stimulation do not cause the additive enhancement of Kin17 RNA, thus suggesting tight regulation of transcript levels (data not shown). In view of all the data presented here, we are currently investigating the possible involvement of kin17 protein in DNA-replication and in the cellular response to UV. The UV-responsive genes are induced by a variety of signal transduction pathways. The key event involves the activation of one of three transcription factors: AP-1, NFκB or p53 (29). Phorbol esters, such as TPA, induce most UV-responsive genes by activating the nuclear factors AP-1 and NFκB via the PKC pathway (2.3). In our hands, the expression of the Kin17 gene is not modified by TPA. We deduce that the Kin17 gene is not under the control of AP-1 or NFκB. Our results are in agreement with previous observations that Kin17 gene expression remains unchanged after TPA treatment of a mouse lymphoma cell line (Boesen and Simons, personal communication) (39). These observations are further supported by the lack of the TPA responsive element (TRE) in the promoter of Kin17 gene (12). In contrast, the promoter of the Kin17 gene has several p53 putative binding sites (Tissier and Bourdon, personal communication) (40). Alternatively, the accumulation of Kin17 RNA could be due to a post-transcriptional modification that leads to RNA stabilization. Further experiments should elucidate the mechanism of this accumulation.

In mammals, several conserved genes induced by the stimulation of cell division and by UV radiation have been identified. Only a limited number of them are involved in DNA-replication and have thus far been shown to be involved in DNA repair, e.g. DNA-ligase 1, polymerase δ and PCNA (27,38). Further investigation will be needed to confirm the possible participation of Kin17 gene in a DNA-repair process or in a more general protective function.

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