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Cell cycle regulation and *in vitro* hybrid arrest analysis of the major human uracil-DNA glycosylase

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ABSTRACT

Uracil-DNA glycosylase (UDG) is the first enzyme in the excision repair pathway for removal of uracil in DNA. *In vitro* transcription/translation of a cloned human cDNA encoding UDG resulted in easily measurable UDG activity. The apparent size of the primary translation product was 34 kD. Two lines of evidence indicated that this cDNA encodes the major nuclear UDG. First, *in vitro* translation of human fibroblast mRNA isolated from S-phase cells resulted in measurable UDG activity and this UDG translation was specifically inhibited 90% by an anti-sense UDG mRNA transcript. Secondly, cell cycle analysis revealed an 8–12 fold increase in transcript level late in the G_1-phase preceding a 2–3 fold increase in total UDG activity in the S-phase. UDG degradation was found to be very slow ($T_{1/2} \approx 30h$), therefore, the rate of UDG synthesis could be derived from the rate of UDG accumulation, and was found to correlate temporarily and quantitatively with the transcript level. Inhibitor studies showed that RNA and protein synthesis was required for induction of UDG. However, specific inhibition of DNA replication with aphidicolin indicated that entrance of fibroblasts into the S-phase was not required for UDG accumulation.

INTRODUCTION

Uracil in DNA may be found either after incorporation of dUTP instead of dTTP (1–3) or as a result of deamination of cytosine (4, 5). The latter process can give rise to transition mutations (6). Uracil-DNA glycosylase initiates the base excision repair pathway for removal of uracil, and was first detected in *Escherichia coli* (7–9). An analogous enzyme activity has been found in a variety of prokaryotic and eukaryotic systems including mammalian cells (3, 10–13). All human tissues examined express uracil-DNA glycosylase activity, but at varying degrees (14–15). The most comprehensive studies on uracil-DNA glycosylase in subcellular organelles indicate that a minor mitochondrial form is different from a major nuclear form (16–17), although the biochemical properties of the two forms were rather similar in HeLa cells (18). The amount of the major nuclear form increases several-fold during the S-phase (19–20) in contrast to the mitochondrial form (21). Furthermore, a specific association with replicating SV40 chromatin suggested that the nuclear activity might occur as part of a replication complex (22).

Recently, uracil-DNA glycosylase from human placenta was purified and partially sequenced (23), the corresponding cDNA (UNG15 and UNG40) was cloned (24), and the gene assigned to chromosome 12 (25). A strong sequence similarity was observed to UDG from *E. coli* (26), *Streptococcus pneumoniae* (27), animal viruses (28–31) and yeast (32), ranging from 41 to 56% identical residues. Unexpectedly, the human protein showed highest similarity to the *E. coli* protein, and this similarity was 73% when conservative amino acid substitutions were included. Furthermore, only one insertion in the human sequence was required to perfectly align the two sequences. The two mature proteins were also almost equal in size, and the hydropathy profiles were very similar, suggesting that the two proteins have similar structures. Conserved sequences were to a large extent confined to 4 hydrophobic boxes, and these were conserved in all the species mentioned.

Using antibodies apparently specific for uracil-DNA glycosylase, Vollberg *et al.* (33) and Muller and Caradonna (34) isolated cDNA clones that might encode uracil-DNA glycosylase. These clones are different from UNG15/40 and also different from each other. In the present work, we show that anti-sense mRNA transcribed *in vitro* from the SP6 promoter of pUNG40 hybrid arrests *in vitro* translation of uracil-DNA glycosylase from mRNA isolated from human fibroblasts by approximately 90%. Furthermore, transcript levels of this mRNA during the cell cycle were those expected for a nuclear UDG. These results strongly indicate that the cDNA we have isolated, encodes the major form of UDG.

MATERIALS AND METHODS

Cell strains and culture

The diploid human skin fibroblast strains used were ST 1 (from a normal 16-year-old male) and SKB 1 (from a normal 6-month-old female), kindly provided by Tove Myhre at The National Hospital, Oslo. Fibroblasts used were from passages number

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7–15. Cells were cultured in Dulbecco's modified minimum essential medium supplemented with 10% FCS, 0.1 mg/ml gentamicin, 0.3 mg/ml glutamine and 2μg/ml fungizone in a water-saturated atmosphere of 5% CO_2 in air at 37°C. Cells, just prior to confluence, were serially subcultured by the 1:3 split ratio regimen. All cell culture media components were from GIBCO. Gen-Probe mycoplasma detection system (Gen-Probe, CA) was used to test for mycoplasma contamination. Both strains were negative.

Cell synchronization

Confluent cultures were maintained by changing the medium every 3 days. After 6–8 days, completely confluent cultures were trypsinated and plated in fresh medium at a density of 10^6 cells/90 mm dish to release the density inhibition. The degree of synchronization was measured by 1h pulse labelling at different time points after density release with 2 μCi/ml [^{3}H]dThd. Acid-insoluble precipitates kept at at 0°C for 2h in 5% trichloroacetic acid were collected on Whatman GF/C filters and radioactivity measured by liquid scintillation spectrophotometry. DNA-synthesis was inhibited by the addition of aphidicolin (2 μg/ml) to the medium 9h after density release. Transcription was inhibited by addition of actinomycin D (5 μg/ml) either at 8- or 17h after density release. Protein synthesis was inhibited by the addition of cycloheximide (100 μg/ml) either immediately after release or just prior to the S-phase.

Uracil-DNA glycosylase assay

Uracil-DNA glycosylase activity was assayed using [^{3}H]dUMP-containing DNA (spec. act. 0.5 mCi/μmole) as described (35). For detection of UDG-activity after *in vitro* translation, [^{3}H]dUMP-containing DNA of high specific activity was prepared by amplifying double stranded DNA spanning a 560 bp region of the H-ras gene (1656–2216) (36) by the polymerase chain reaction (PCR) with [^{3}H]dUTP (13.4 Ci/mmole) substituted for dTTP. In this case, 10^5 cpm per assay was used instead of 2×10^4 cpm in the standard assay, and the incubation time was increased from 10 min to 60 min. Cell free extracts for determination of enzyme activity were prepared by centrifugation of cell sonicates at 14 000×g for 10 min in a Heraeus Biofuge 28RS refrigerated microcentrifuge. The radioactive material released after incubation of translated proteins with PRC-generated [^{3}H]dUMP-containing DNA was identified by thin layer chromatography.

RNA isolation and Northern blot analyses

At defined times after release, synchronized cells were harvested, and total RNA was extracted with phenol in the presence of SDS at pH 5.0 and 55°C. After ethanol precipitation, the samples were analysed by Northern blotting of denaturing formaldehyde agarose gels onto Nytran nylon membranes (Schleicher & Schüell) as described by Fourney *et al.* (37). The membranes were hybridized to [^{32}P]dCTP-labelled UNG15 (24). Control hybridizations were performed with a 548-base cDNA fragment from human glyceraldehyde-3-phosphate dehydrogenase (G3PDH). pHuGAP was from American Type Culture Collection. Filters were exposed to Kodak AR film in the presence of Quanta III intensifying screens at −70°C. Poly(A)$^+$-mRNA was either prepared directly from cell supernatants or from total cellular RNA with Dynabeads Oligo $(dT)_{25}$ (Dynal, Norway).

In vitro transcription

The orientation of the cDNA clones pUNG15 and pUNG40 encoding uracil-DNA glycosylase is such that sense mRNA can be made by *in vitro* transcription by T7 RNA polymerase from the T7 promotor in the parental pGEM7Zf(+) vector (Promega), whereas transcription from the SP6 promotor using SP6 RNA polymerase gives anti-sense mRNA (24). pUNG15 was opened with *Sma* I, and transcribed with T7 RNA polymerase to yield a 2077 bases long sense RNA-fragment containing both the leader and the trailer region including a poly (A)-tail of 17 residues. In separate experiments a G(5')ppp(5')G cap was added to the transcripts to study the effect on translational activity. An anti-sense fragment was made from the cDNA clone UNG40 (24) containing the human uracil-DNA glycosylase cDNA sequence without the poly (A)-tail and with a shorter leader. The plasmid was opened with *Xba* I and transcribed with SP6 RNA polymerase to yield a 1815-base fragment. To demonstrate specificity of the anti-sense UDG-transcript pUNG40 in hybrid arrest, three control transcripts were synthesized and used in parallel experiments. The transcripts were synthesized from commercial vectors from Promega, and denoted pGEM3Zf(+)/T7 (1818b) and pGEM2/SP6 (1641 b) (transcript lengths are given in brackets).

Translational hybrid arrest

In vitro translation experiments were performed in rabbit reticulocyte- and wheat germ lysates from Promega. Optimal translation of uracil-DNA glycosylase was obtained after addition of 0.4 mM spermidine, 50 mM potassium acetate, 5 mM methionine and 10 ng/μl mRNA. mRNA was isolated from synchronized normal human fibroblasts just prior to the S-phase. In the hybrid arrest experiments, 33 ng/μl each of cellular mRNA and mRNA transcripts were incubated in 10 mM tris-HCl (pH 7.5), 0.15 M KCl, 2 U/μl RNasin (Promega), 1 mM dithiothreitol in a total volume of 3 μl for 5 min at 67°C and immidiately cooled on ice. 1.5 μl was used in each translation experiment in a total of 10 μl, and the potassium acetate concentration was reduced to 27 mM to compensate for the potassium ions from the hybrid arrest mixture. Translation was performed at 30°C for 1h, immediately cooled on ice, and 5μl aliquots were subjected to

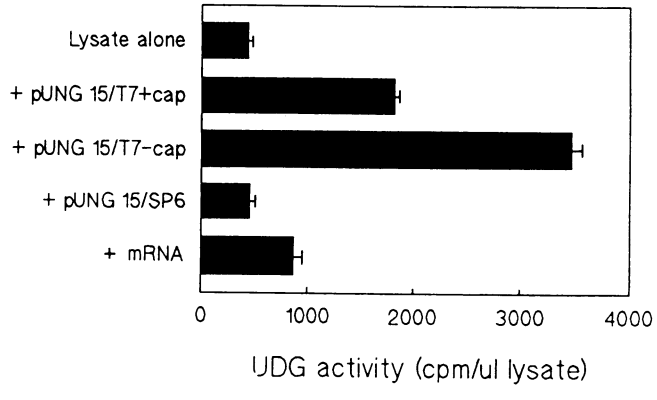

Fig. 1. Translation of active uracil-DNA glycosylase in rabbit reticulocyte lysates. Cellular mRNA or RNA transcripts were added to lysates to a final concentration of 10 ng/μl. 5 μl of the translation mixtures were used in uracil-DNA glycosylase assays as described. In the lysate controls, only mRNA dilution buffer (100 U/ml RNasin, 2 mM DTT) was added. The experiment was performed three times, each in duplicate.

uracil-DNA glycosylase assays as described. In separate tubes, 1μCi/μl [^{35}S]methionine (1200 Ci/mmole) was added prior to translation, and the labeled amino acid incorporation was determined according to the manufacturers protocol. Gel analysis of translation products was performed in discontinous polyacrylamide gels (5−15% separating gel) in the presence of 0.1% SDS. After fixation, gels were immersed in Amplify (Amersham) for 30 min, dried and exposed to Hyperfilm β-max (Amersham) for 4−5 days.

Cell cycle regulation

The rate of biosynthesis of active UDG was mathematically derived from the curve of best fit for the accumulated UDG activity using the kaleidagraph (Abelbeck software) data program. To compensate for the slow rate of UDG degradation as measured in the cycloheximide experiments, calculated amounts of degraded activity was added to the observed activity at each time point in uninhibited controls. The increase in cell number at the end of the experimental period was also corrected for to avoid false impression of UDG degradation.

RESULTS

In vitro translation of uracil-DNA glycosylase

T7 RNA polymerase transcripts from plasmid pUNG15 carrying cDNA encoding UDG were translated to enzymatically active uracil-DNA glycosylase *in vitro* in rabbit reticulocyte lysates. In contrast, anti-sense SP6 transcripts were not translated to active uracil-DNA glycosylase, as expected (Figure 1). Under the present experimental conditions, capping of the mRNA transcripts using the cap analog G(5′)ppp(5′)G reduced the amount of active uracil-DNA glycosylase translated by approximately 45% as compared to uncapped transcripts. The radioactivity released in the uracil-DNA glycosylase assay was identified as uracil by thin layer chromatography (35) proving that UNG15 encodes an active uracil-DNA glycosylase (data not shown). The NH_2-terminal end of uracil-DNA glycosylase identified in uracil-DNA glycosylase prepared from human placenta was Ala-Arg-Asn and the protein had an apparent molecular mass of 29 kD as determined by SDS-polyacrylamide electrophoresis (23). Ala-Arg-Asn was found in the amino acid sequence predicted from UNG15 at a position 78 amino acids downstream of the NH_2-terminal. The amino acid sequence indicated that the correct mass for this active form of the enzyme was 25.8 kD rather than 29 kD. A similar anomalous migration behaviour for another small positively charged DNA repair enzyme after SDS-PAGE was recently described (38). The estimated mass of the entire protein was 33.8 kD (24). When 5 ng/μl pUNG15/T7 transcript was translated in the presence of [^{35}S]methionine, two new bands were visible at 34 kD and 31 kD after SDS PAGE and fluorography. When the concentration of the T7 transcript was increased to 15 ng/μl, the intensity of the 34 kD band decreased, while the 31 kD band increased and a weak 28 kD band appeared (Figure 2).

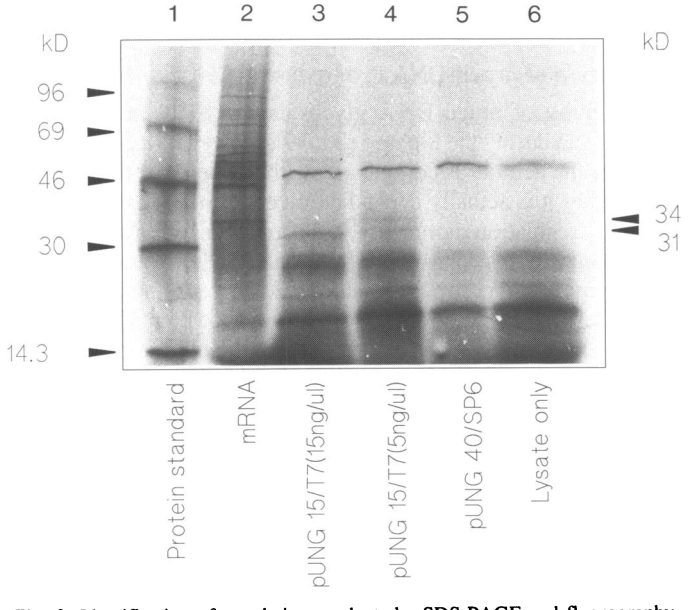

Fig. 2. Identification of translation products by SDS-PAGE and fluorography. 10 ng/μl fibroblast mRNA (lane 2), 15 ng/μl pUNG15/T7 transcript (lane 3), 5 ng/μl pUNG15 T7-transcript (lane 4), 10 ng/μl pUNG15/SP6 transcript (anti-sense) (lane 5), lysate control (lane 6), [^{14}C]methylated protein standard (lane 1). Bands appearing after translation of pUNG15/T7 transcripts are indicated by arrows.

Hybrid arrest of *in vitro* translation of human mRNA by anti-sense mRNA from pUNG-40

To be able to detect translation of mRNA for UDG from S-phase fibroblasts, we optimized translation conditions and used high specific activity of [^{3}H]uracil-containing DNA. Rabbit reticulocyte lysates were best suited for such experiments, as they

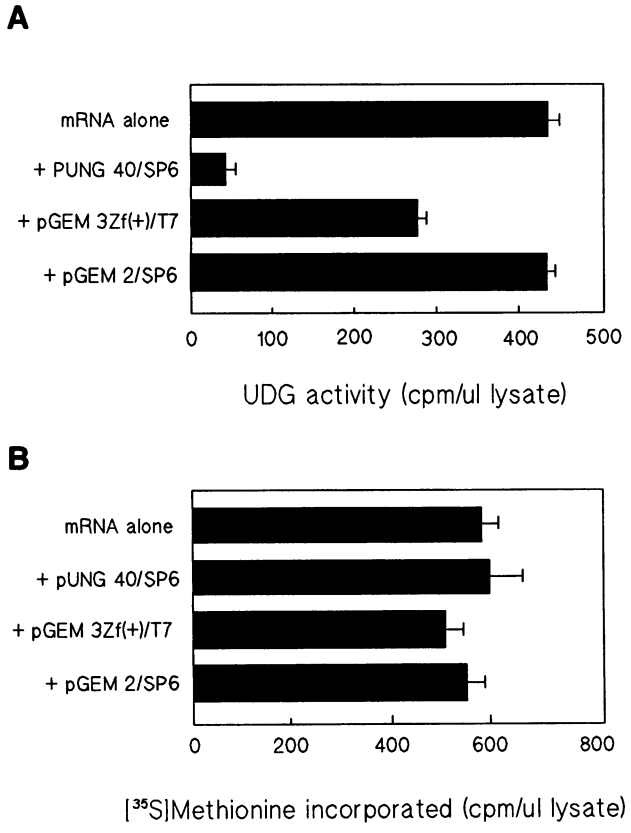

Fig. 3. Translational hybrid arrest of UDG activity. Cellular mRNA was hybridized with the anti-sense uracil-DNA glycosylase RNA transcript pUNG40 SP6 and two control transcripts pGEM3Zf(+)/T7 and pGEM2/SP6 (see materials and methods). (A): After translation, reactions were subjected to uracil-DNA glycosylase assays as described. (B): As (A), but [^{35}S]methionine was included in the translation mixtures and total translational efficiency was measured as described. The histograms represent the results normalized for the endogenous lysate UDG activity (average of 5 independent experiments).

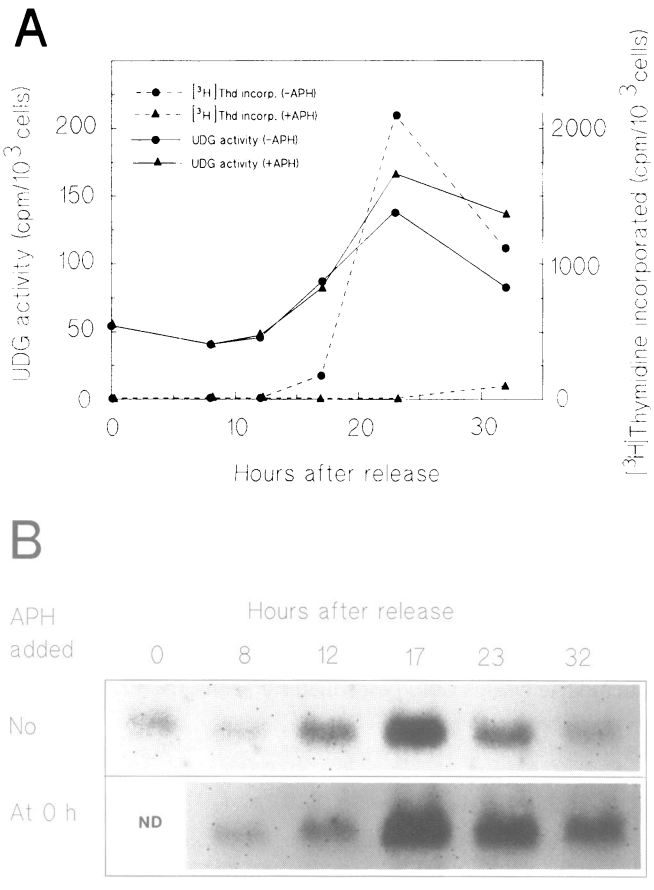

Fig. 4. Induction of uracil-DNA glycosylase activity, DNA synthesis and mRNA levels in the cell cycle. Normal human skin fibroblasts were synchronized by density inhibition release as described. At defined times after release, cells were collected for uracil-DNA glycosylase assays, measurement of [^{3}H]thymidine incorporation and total RNA isolation. (A): Uracil-DNA glycosylase activity and [^{3}H]thymidine incorporation. When aphidicolin was added, this was done immediately after release. (B) Northern analysis of UDG mRNA levels at different times after release.

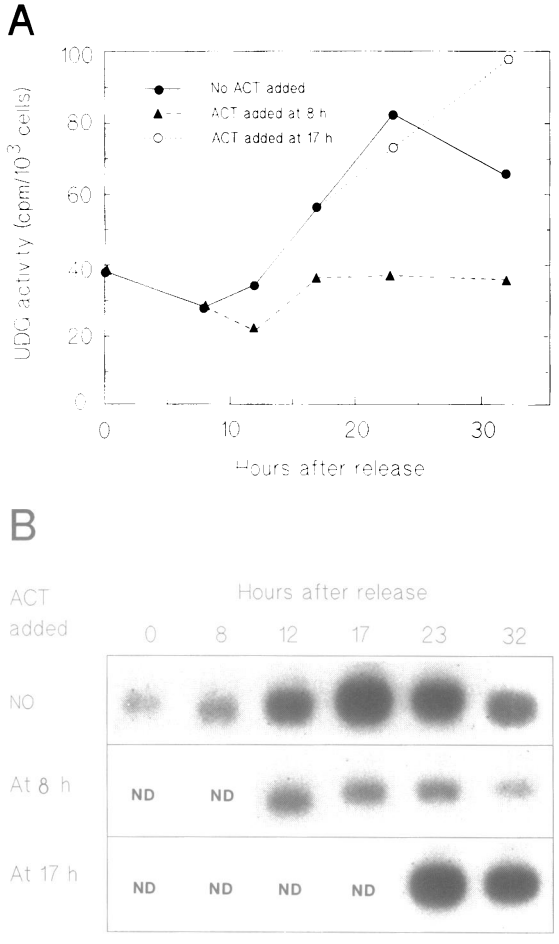

Fig. 5. Effect of actinomycin D on induction of uracil-DNA glycosylase activity and mRNA level after density inhibition release. (A): Uracil-DNA glycosylase activity. When actinomycin D was added, this was done at 8h or 17h, respectively. (B): Northern analysis of UDG mRNA levels at different times after release.

had a 20-fold lower endogenous activity of uracil-DNA glycosylase than wheat germ extracts. Under optimized reaction conditions, translation of mRNA to active uracil-DNA glycosylase was reproducibly detected. (Figure 3 A). mRNA isolated from other phases of the cell cycle yielded less, or no detectable increase in uracil-DNA glycosylase activity (data not shown). Translation of mRNA from HeLa S3 cells also resulted in measurable uracil-DNA glycosylase activity (data not shown). When cellular mRNA was hybridized to anti-sense UNG40/SP6 transcripts prior to translation, measurable uracil-DNA glycosylase decreased approximately 90% (Figure 3 A). No detectable decrease in total [^{35}S]methionine incorporation was observed in parallel incubations, thus excluding a general inhibition of translation by the anti-sense transcript (Figure 3 B). Control experiments with other transcripts demonstrated that the inhibitory effect was specific for the uracil-DNA glycosylase anti-sense transcript. These results clearly indicate that mRNA corresponding to cDNA clones UNG15 and UNG40 is encoded by a major gene for biosynthesis of uracil-DNA glycosylase. Alternatively, other potential uracil-DNA glycosylase genes must be closely related to the cDNA clones we have isolated.

Regulation of uracil-DNA glycosylase activity in the cell cycle

The activity of uracil-DNA glycosylase activity increases just prior to and during the S-phase (20, 39). However, it is not known whether the activity is coregulated with the onset of the S-phase, or whether it is actually dependent on cells entering the S-phase. To study regulation more closely, normal human fibroblasts were synchronized by density inhibition release (Figure 4 A). [^{3}H]thymidine incorporation started to increase approximately 12 h after release, and reached a maximum at 23 h after release. Uracil-DNA glycosylase activity was induced just prior to the S-phase and reached a maximal 2–3 fold increase at the peak of the S-phase. However, when aphidicolin was added at 9h after release to specifically block DNA synthesis by inhibiting the replicative polymerase (40), uracil-DNA glycosylase was induced irrespective of a total block of DNA synthesis. In fact, we reproducibly found a slight increase in uracil-DNA glycosylase activity after blocking DNA synthesis by aphidicolin. This indicates that induction of uracil-DNA glycosylase is normally coregulated with the S-phase, but is independent of ongoing DNA replication. To examine whether uracil-DNA glycosylase is under transcriptional control, total RNA was isolated at a number of points during the cell cycle and subjected to Northern analysis using randomly primed cDNA inserts from pUNG40 as probe

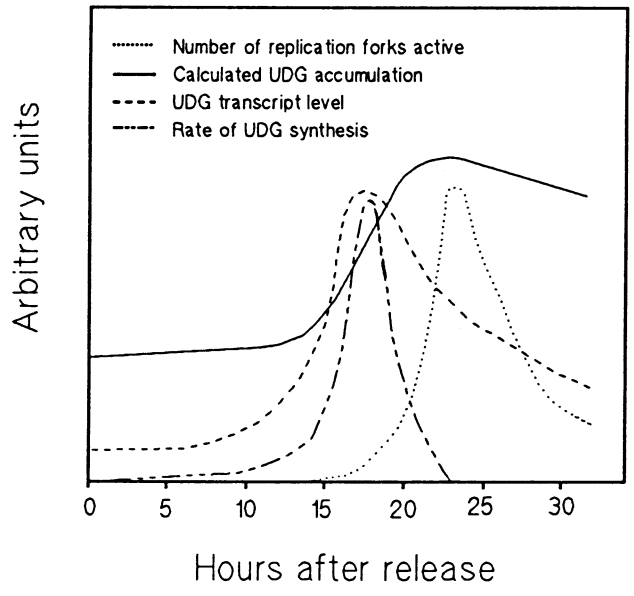

Fig. 6. Rate of synthesis of uracil-DNA glycosylase in the cell cycle. The curve for UDG activity was a result of 6 cell cycle experiments covering 11 different time points (a total of 70 measurements) and corrected for a slow rate of degradation as described in materials and methods. A curve for transcript levels was obtained by densitometric scanning in 5 different experiments, and the best fit curve established. [^{3}H]thymidine incorporation in three separate experiments, each in duplicate, was used to obtain the relative number of replication forks active.

(Figure 4B). Filters were also probed with a randomly primed cDNA fragment from human glyceraldehyde-3-phosphate dehydrogenase (G3PDH), and the G3PDH mRNA level was found to be nearly constant during the cell cycle as determined from densitometric scanning (data not shown). This probe also served as a control to ensure correct quantification of cellular RNA during denaturing gel electrophoresis.

A marked increase in UDG transcript levels was observed approximately 12h after release of the cells, reached a maximum at 17h (which is prior to the peak of the S-phase) and then gradually decreased during the S-phase. Similar kinetics were observed in aphidicolin-treated cultures, but the maximal UDG transcript level was higher, and the decrease in transcript level during the S-phase was slower than in the controls (Figure 4B).

When RNA transcription was blocked by actinomycin D at 8h after release, UDG activity remained at G_1-level during the next 24h (Figure 5A). During the same period, UDG mRNA levels gradually decreased through the cell cycle with an apparent $T_{1/2}$ of approximately 15h (Figure 5B). Arrest of transcription at 17h after release did not significantly affect UDG transcript levels as compared to the controls, but UDG enzyme activity continued to rise even after the S-phase. This increase was essentially explained by the influence of actinomycin D on cell division. At 32h after release, approximately 30% of the control cells had divided, giving a significant drop in the *per cell* UDG level. In the inhibited cultures, no cell divisions were observed during the same period.

Inhibition of protein synthesis by addition of cycloheximide immediately after release completely arrested induction of UDG (data not shown). The enzymatic activity slowly decreased during the cell cycle with an apparent $T_{1/2}$ of at least 30h. A gradual decrease of UDG mRNA was also observed, but a significant enzyme activity still remained in the cells even when UDG message had decreased to the limit of detection. Arrest of protein synthesis at 17h after release also arrested UDG induction, although in this case no effect was observed at the mRNA level.

The rate of UDG synthesis in relation to UDG mRNA levels and accumulated UDG activity in the cell cycle is given in Figure 6. The results show that the rate of UDG synthesis peaks at approximately 30−60 min after the maximal transcript level, and support the results from the inhibitor experiments in that UDG induction is under transcriptional control. However, since the rate of UDG synthesis decreases faster than the UDG mRNA level, UDG expression may also be controlled at the level of translation during the S-phase. Alternatively, lack of post-translational modification may result in a less active form of the enzyme during this period. In summary, the cell cycle kinetics support that UNG15/40 encode a major nuclear form of UDG in human fibroblasts.

DISCUSSION

The high degree of conservation of genes for uracil-DNA glycosylase from bacterial, viral, lower eukaryotes and mammals makes this gene family an interesting subject for evolutionary as well as biochemical studies. In general, DNA repair genes frequently appear to be conserved, although generally less than in the case of uracil-DNA glycosylase. Thus, in some regions, human O^6-methylguanine-DNA methyltransferase also shows a high sequence similarity with the *E.coli ada* and *ogt* genes encoding the same enzymatic activity (38, 41). A significant similarity was also found for rat 3-methyladenine-DNA glycosylase and the *E.coli* Tag II protein in some regions (42). The conservation of various genes for DNA repair enzymes from phylogenetically distant species indicate that each of them have evolved from a common ancestor. However, there are also examples of DNA repair enzymes from the same species catalyzing the same reaction, that bear little resemblance. Thus the amino acid sequence of *E.coli* Tag I and Tag II proteins are not related (43).

Although no sequence data are available, the cDNA clone for uracil-DNA glycosylase isolated by Vollberg *et al.*, (33), is clearly different from UNG15/40. First, it recognizes a transcript of 1.6 kb, which is different from the 2.2−2.4 kb transcript hybridizing to UNG15/40. Secondly, the Southern blot patterns are clearly different. The interpretation of this work relies heavily on the specificity of the monoclonal antibodies used for characterization of the gene product and isolation of the cDNA clone. As far as we can understand, it has not been excluded that the monoclonal antibodies may recognize a protein associated with uracil-DNA glycosylase, rather than the glycosylase itself. The cDNA isolated by Muller and Caradonna (34) is also clearly different from UNG15/40 as judged from nucleotide sequence, predicted amino acid sequence, Southern blot patterns and chromosomal assignment. Furthermore, the predicted amino acid sequence from the cDNA isolated by Muller and Caradonna reveals a striking similarity to conserved regions of Drosophila cyclin A and human cyclin B, the significance of which is presently not known.

In vitro translation of pUNG15 T7 transcripts strongly indicate that this cDNA encodes an uracil-DNA glycosylase. The 34 kD translation product is most probably the primary translation product, since there are no codons for Met in the cDNA that could explain the smaller products observed. It is presently not clear whether the smaller products arise from artificial

degradation *in vitro* or processing. Different rabbit reticulocyte lysates gave different amounts of primary translation product relative to the smaller forms, and in some experiments mainly the 34 kD form could be seen, indicating that this form is enzymatically active. The form of 29 kD, as measured by SDS-PAGE, is clearly an active form with a specific activity and turnover number essentially similar to uracil-DNA glycosylase from E. coli (8, 23).

When we purified human uracil-DNA glycosylase for amino acid sequencing, subcellular organelles were not fractionated, although the major enzyme activity was isolated (23). It was therefore not rigourously proven that the enzyme we subsequently cloned was the major nuclear form. Hybrid arrest of *in vitro* translation strongly indicates that UNG15 and the shorter cDNA UNG40 encodes the major nuclear form of human uracil-DNA glycosylase. An alternative and less likely explanation would be that the major mRNA has been lost or degraded during purification, or that it is not easily translated *in vitro*. Furthermore, the strong homology to the yeast nuclear uracil-DNA glycosylase gene (32) also indicates that UNG15/40 encode the human nuclear gene. Muller and Caradonna (34) argue that in the original purification of uracil-DNA glycosylase by Wittwer *et al.* (23), the mitochondrial enzyme may actually have been followed. In the original purification by Wittwer *et al.*, a 26.5 kD peptide copurified with the 29 kD UDG peptide through all purification steps. Amino acid sequencing revealed that the NH_2-terminals of these proteins were different. Interestingly, we have recently identified sequences identical to the NH_2-terminal of the 26.5 kD peptide in the internal regions of two other proteins. These are a 25 kD DNA-binding mouse nuclear protein (mmp25K) (44) and a 62 kD human polypyrimidine tract-binding protein (pPTB) (45). The molecular mass of pPTB is calculated from the cDNA sequence, and the mass of the *in vivo* form is still not known. Although a truncated form of pPTB may have copurified with uracil DNA-glycosylase purely because of the similar biochemical characteristics or an artificial association during purification, it is tempting to speculate that a part of pPTB may be functionally associated with the glycosylase in the nucleus. The function of the former might then be to bring the glycosylase in close physical association with U-rich regions of DNA.

Synchronization of cell cultures by density inhibition release gives information on the cellular processes taking place when cells enters active proliferation from quiescence. The pattern of UDG regulation may be different in freely cycling cells. To examine UDG regulation in freely cycling cells, would require a different method for obtaining cells from various stages of the cell cycle, for instance elutriation centrifugation or separation by flow cytofluorometry and cell sorting. However, in the *in vivo* situation, human fibroblasts are not normally freely cycling, with the possible exception of early fetal stages. Instead, resting fibroblasts are triggered to proliferate after some kind of injury or other stimuli, thus resembling the situation after release from contact inhibition.

Gupta and Sirover (21) found that only nuclear UDG was induced in WI-38 cells during proliferation, while the specific activity of mitochondrial UDG remained constant. These findings support that pUNG15 encodes a nuclear uracil-DNA glycosylase, as a 8–12 fold increase in specific message recognized by a UNG15 probe was observed prior to the onset of S-phase. We observed that uracil-DNA glycosylase transcripts preceded the peak of enzymatic activity by approximately 5h in the cell cycle. Interestingly, both transcript levels and enzyme activity increased after nearly complete block of the S-phase with aphidicolin. This may be explained by the cells staying for a prolonged time in late G1-phase, when UDG mRNA synthesis is high. Alternatively, inhibiting DNA synthesis may give rise to a higher free energy pool in the cells which may be utilized for RNA and protein synthesis. The results show that induction of uracil-DNA glycosylase late in the G_1-phase and maximal increase during the S-phase is not dependent on DNA synthesis. Probably many genes induced at the G_1/S-phase border are independent of DNA synthesis, thus proliferating cell nuclear antigen (PCNA) is fully induced after an aphidicolin block (46). In contrast, there is a strong interdependence between histone and DNA synthesis (47).

In conclusion, we have presented evidence indicating that the human cDNA clones for uracil-DNA glycosylase isolated previously (24) represent the gene encoding the major uracil-DNA glycosylase activity. We are now preparing antibodies against recombinant uracil-DNA glycosylase to study transport and subcellular localization of the enzyme. Furthermore, we are doing site-directed mutagenesis of the cDNA to map domains important for catalytic activity, DNA binding and protein stability. The high degree of conservation in some regions of the gene will probably be helpful for such mapping studies.

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