Induction of the Mouse Ribonucleotide Reductase R1 and R2 Genes in Response to DNA Damage by UV Light*

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Ribonucleotide reductase is responsible for the production of deoxyribonucleotides required for DNA synthesis and consists of two nonidentical subunits, proteins R1 and R2. Here we show that the R1 promoter can be induced up to 3-fold, and the R2 promoter is induced up to 10-fold by UV light in a dose-dependent manner. This was demonstrated using serum-starved, synchronized G0/G1 mouse fibroblast 3T3 cells stably transformed with different R1 and R2 promoter-luciferase reporter gene constructs. R2 promoter activation requires a minimal promoter, containing a TTTAAA element plus the transcription start, and either three upstream DNA-protein binding regions or one proximal, NF-Y binding region. This is different from proliferation-specific activation of the R2 promoter. Using Northern blotting we show a preferential accumulation of the minor, 1.6-kilobase R2 transcript in irradiated cells, whereas the levels of the major 2.1-kilobase transcript are unchanged. No R2 promoter activation was observed after treatment of mouse cells with agents reported to induce the ribonucleotide reductase genes in Saccharomyces cerevisiae such as hydroxyurea or methylimethane sulfonate. This indicates that activation of ribonucleotide reductase gene expression is specific for nucleotide excision repair in mammalian cells and not part of a general response to DNA damage.

Ribonucleotide reductase is a key enzyme in DNA synthesis because it catalyzes the direct reduction of ribonucleotides to the corresponding deoxyribonucleotides (1–3). The enzyme consists of two nonidentical subunits, proteins R1 and R2. Each subunit is inactive by itself, but together they form an active enzyme complex. Mammalian ribonucleotide reductase has been purified to homogeneity, and full-length cDNA clones encoding mouse R1 and R2 proteins have been isolated (4, 5). There are two R2 transcripts, a major one of 2.1 kb1 and a minor one of 1.6 kb, which differ only in the utilization of polyadenylation signals (5). Genomic clones covering the mouse R1 and R2 genes have also been characterized (6, 7).

There is a close correlation between mammalian ribonucleotide reductase activity and the rate of DNA synthesis with no detectable activity in G0/G1 cells and maximal activity in S-phase cells (1, 2). Holoenzyme activity is regulated during the cell cycle by S-phase-specific de novo synthesis and subsequent breakdown of the R2 subunit that is limiting for enzyme activity (8, 9). The R1 protein levels in proliferating cells are in excess and constant throughout the cell cycle. Expression of both the R1 and R2 mRNAs is S-phase-specific with no detectable transcripts in G0/G1 cells (10). The R2 gene expression is regulated by an early, proliferation-specific induction of the promoter in combination with a G1-phase-specific transcriptional block (11).

There are four DNA-protein interaction regions in the mouse R2 promoter.2 The three upstream elements, β, γ, and δ (from nt −672 to nt −527), are required for proliferation-specific induction of the R2 promoter. The region most proximal to the R2 transcription start, α (nt −93 to −56), contains a CCAAT-motif binding the NF-Y transcription factor that is required for continuous activity of the R2 promoter through the S-phase (12). An R2 promoter lacking the upstream regions is not serum-inducible but retains basal levels of transcription provided the proximal NF-Y binding element is present (12).

Exposure of mammalian cells to 254 nm UV light leads to the introduction of a number of photoproducts in their DNA. These are removed by a nucleotide excision repair repair mechanism involving excision of the damaged nucleotides together with a number of nucleotides adjacent to the damage (13). The resulting gaps are then filled in by a DNA polymerase and sealed by a ligase. It is reasonable to assume that ribonucleotide reductase may be involved in the repair, at least in nonproliferating cells, since deoxyribonucleotides are present in very low levels in cells outside S-phase (1, 2).

A number of human excision repair genes have been cloned (13), and an in vitro system capable of complete repair synthesis using purified protein components was recently described (14). However, most of these genes seem not to be significantly induced in response to DNA damage. In contrast, other mammalian genes encoding transcription factors, growth factors, and growth factor receptors are activated at the transcriptional level by UV irradiation (13). In some cases, the primary inducing signal is generated outside the nucleus possibly involving free oxygen radicals and membrane-associated tyrosine kinases, whereas other data suggest a direct role of DNA damage (13).

There are few direct indications that mammalian ribonucleotide reductase is DNA damage-inducible. It has been shown that the DNA-damaging, alkylating agent chlorambucil induced expression of ribonucleotide reductase in mouse cells, but the drug also directly inhibited the activity of the enzyme (15). In contrast, there are a number of indirect indications that ribonucleotide reductase may be important for excision repair.

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1The abbreviations used are: kb, kilobase(s); PBS, phosphate-buffered saline; DMEM, Dulbecco’s modified Eagle’s medium; nt, nucleotide.

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These data are obtained from studies using UV irradiation in combination with the specific protein R2 inhibitor hydroxyurea and addition of deoxyribonucleosides to the medium of irradiated tissue culture cells (16–18).

Much more is known about the inducibility and possible role of ribonucleotide reductase in DNA repair in Saccharomyces cerevisiae. The RNR1 and RNR3 (large subunits) as well as the RNR2 (small subunit) genes in yeast are induced to different levels by DNA damaging agents such as UV light, 4-nitroquinoline-1-oxide, methyl methanesulfonate, and also by hydroxyurea (19). A DNA damage-responsive element in the yeast RNR2 promoter was identified together with four interacting protein factors likely to mediate the DNA damage response.

In the present study we demonstrate that the promoters of the mouse R1 and R2 genes are UV-inducible. In contrast, the alkylating agent methyl methanesulfonate has no effect on the R2 promoter activity indicating that the induction only occurs in response to DNA damage causing nucleotide excision repair. The UV induction observed in resting cells utilizes a different pathway from the proliferation-induced activation of the R2 promoter. We also demonstrate a preferential accumulation of the smaller 1.6-kb R2 transcript in logarithmically growing R2-overproducing mouse TA 3 cells after UV irradiation.

EXPERIMENTAL PROCEDURES

Cell Culture, Synchronization, Transfection, and Selection of Stable Transformants—Balb/3T3 cells (ATCC CCL 163) and hydroxyurea-resistant, R2-overproducing mouse TA 3 cells were grown as monolayer cultures in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated horse serum as described earlier (5). For serum synchronization, cells were plated at a density of 2 × 10^6 cells per 14-cm dish. After 24 h, cells were washed with phosphate-buffered saline (PBS); 25 ml of medium containing 0.6% fetal calf serum were added; and the cells were incubated for another 48 h. In experiments with serum-released cells, DMEM containing 20% horse serum was added to PBS-washed cells after 48 h of serum starvation.

Transfection of cells was made by electroporation as described (11), and stable transformants were selected in the presence of Geneticin base (Sigma). Resistant clones were expanded, tested for luciferase activity or for the presence of the luciferase gene fused to R2 promoter sequences by polymerase chain reaction, and frozen in liquid nitrogen as described (11, 12). Usually, two to three independent clones were tested for each particular construct.

Plasmid Constructs—The plasmids p19lucR2, p19lucR2Ex2-ATG (nt +1479 to +17 of the R2 promoter) and p19lucR2 0.5 (nt +470 to +17 of the R2 promoter) were described earlier (11).

The plasmid p19lucR2 0.11 was constructed by cleaving the p19lucR2 1.5 with BstDI that has one site in the R2 promoter exactly at the 5' end of the α region (nt −89) and two sites in the p19luc vector, and SacI that has one site in the polynkinter between the R2 promoter and the luciferase cDNA. The small BstDI-SacI fragment representing the region of the R2 promoter including the α region, the TTTAAA box, and the transcription start, and a small piece from the p19luc polylinker was isolated and ligated to p19luc cleaved with HincII and SacI (20). All constructs contain the R2 transcription start but not the ATG representing the translation start of the R2 gene.

The plasmid p19lucR2 (1.0–α) was made as described (12) and had 33 nucleotides (nt −80 to −47) deleted from the DNA-protein binding α region. This deletion abolished the specific binding of the NF-Y transcription factor to this region (12).

The plasmid p19lucR2 TTTAAA contains only the TTTAAA box and the transcription start, and a small piece from the R2 promoter (nt +47 to +17) ligated to the luciferase reporter gene (12).

The plasmid p19lucR2Ex2-ATG is described elsewhere.2 This plasmid contains the full-length R2 promoter, the first exon with the original ATG start codon mutated to ATC and the first intron ligated to the luciferase reporter gene. The plasmid therefore harbors the transcriptional block region located to the first intron of the R2 gene (11). All R2 plasmid constructs are summarized in Fig. 1.

The plasmid p19lucR1 1.0 contains a fragment from the mouse R1 promoter starting at nt −812 and ending at nt +242 relative to the major transcription start (21). All plasmid constructs were verified by double-stranded DNA sequencing.

Oligonucleotides—All oligonucleotides were synthesized on an Applied Biosystems 392 RNA/DNA synthesizer and purified by chromatography on Sephadex G-25 columns.

UV Induction, Methyl Methanesulfonate, and Hydroxyurea Treatment—Before irradiation, cells were washed with phosphate-buffered saline (PBS) prewarmed to 37 °C. Then the saline was removed, and the cells were irradiated at a distance of 25 cm using a Universal UV lamp (CAMAG, Typ TL-900/U) preferentially emitting 254 nm UV light (0.8 J/m2 from this distance). The UV dose was monitored using a radiometer UVX-25 (Ultra-Violet Products, Inc., USA). Immediately after irradiation, the same conditioned prewarmed medium was added back to avoid serum stimulation, and the cells were incubated at 37 °C for the indicated time before harvesting. In the experiment where UV irradiation was combined with release of the cells into S-phase, the G0/G1-synchronized cells were washed with PBS after 48 h of starvation in DMEM containing 0.6% fetal calf serum and irradiated, and prewarmed DMEM containing 20% horse serum was added.

In the case of methyl methanesulfonate treatment, the cells were washed as described above and then incubated for 30 min at 37 °C in the presence of prewarmed PBS containing the drug at 2.36–118 μM concentrations. The drug was removed, and the cells were washed twice with prewarmed PBS before the same conditioned prewarmed medium was added. The cells were then incubated for different periods at 37 °C.

In experiments with hydroxyurea, the drug was added directly to the medium at a final concentration of 0.5 to 2 mM and was present in the medium during the course of the experiment. The cells were harvested every 30 min during a 5-h period.

Flow Cytometry Analyses—Aliquots of 0.2–1.0 × 10^6 cells were removed for fixation in a phosphate-buffered 4% formaldehyde solution and subsequent flow cytometry analyses of DNA content (8).

Luciferase Assay—Harvesting of cells and preparation of cell extracts for luciferase assays were performed as described (11). Luciferase activity was measured as the number of light units emitted during a 10-s period per μg of total protein, determined by the Bradford protein assay (22).

DNA Single Strand Break Measurements—Cells were grown in a medium containing [3H]thymidine to label the DNA. After serum starvation and treatment with UV light, DNA single strand breaks were measured by the method developed by Erxen and Ahnstro¨m (23). This involves alkali treatment, neutralization, and sonication and then single- and double-stranded DNA are separated by hydroxylapatite chromatography at 60 °C. Radioactivity in the eluted fractions were measured in a Beckman LS 6000 SE counter. Before use, the elution profile of the hydroxylapatite column was tested using a linear gradient of potassium phosphate buffer, pH 6.8.

RNA Extraction and Northern Blot Analyses—Total cellular RNA was extracted from cells by the guanidinium thiocyanate method (24). The RNA was denatured with glyoxal, electrophoresed on a 1% agarose gel, blotted to a nitrocellulose filter, and hybridized to a full-length R2 cDNA probe as described (3, 25).

Colony Forming Ability Studies—Cells were plated on 8.5-cm dishes.

UV Induction of Mouse Ribonucleotide Reductase Genes

FIG. 1. The R2 promoter-luciferase reporter gene constructs used in the transfection experiments. The upper panel represents the promoter region of the mouse R2 gene with DNA-protein interacting regions indicated by black boxes. The numbering is relative to the start of transcription that is +1 (7). The restriction endonucleases used to make the different constructs are indicated.

![Diagram](https://example.com/diagram.png)
at a density of $10^5$-10⁶ cells per dish in 10 ml of DMEM plus 10% horse serum. After 10 h, the cells were irradiated as described above and incubated for 7 days. Colonies were counted and compared with nonirradiated control cultures.

**RESULTS**

**DNA Single Strand Breaks**—It has been reported that murine cells such as Balb/3T3 fibroblasts have very low but detectable levels of excision repair (26). Fig. 2 shows the amount of single-stranded DNA in G₀/G₁-synchronized Balb/3T3 cells after exposure to two different doses of 254 nm UV light. The amount of single-stranded DNA is directly proportional to the amount of single strand breaks in a DNA molecule after UV exposure (23). However, we had difficulties detecting significant levels of breaks because only about 7% (that is very close to the lower level of detection by this method) and about 13% of single-stranded DNA was detected using 3 and 10 J/m², respectively. The finding that the amount of single-stranded DNA increased with increasing UV dose indicates that the cells do not perform excision repair but at a very low level.

Hydroxyurea treatment causes single-strand break accumulation in human cells after UV irradiation (17). When 2 mM hydroxyurea was added to the medium 3 h prior to UV irradiation, the percent of single-stranded DNA increased also in our murine cells and reached 21 and 28% for 3 and 10 J/m², respectively (Fig. 2). Hydroxyurea treatment without irradiation did not affect the amount of single-stranded DNA (data not shown). This indicates that in the presence of hydroxyurea there is an accumulation of excised gaps in DNA that cannot be filled in with newly synthesized DNA most probably due to lack of deoxyribonucleotides.

**R2 Promoter Activity in Resting Balb/3T3 Cells after UV Irradiation**—Fig. 3A shows luciferase activity after UV light irradiation of G₀/G₁-synchronized mouse fibroblast cells stably transformed with the p19ucR2 1.5 plasmid (Fig. 1). The R2 promoter-controlled luciferase activity increased immediately to peak at 5 and 8 h after irradiation with a dose of 3 and 10 J/m², respectively. The 3 J/m² dose resulted in about 90% cell survival, and the 10 J/m² dose resulted in about 12% survival when measured after 7 days. The luciferase values represented a 2- and 5–6-fold increase, respectively, compared with the nonirradiated control cells. A dose response could be observed where increasing dose resulted in increasing promoter activity (Fig. 3A) and the higher dose of UV irradiation delayed the maximal response compared with the lower dose. The increase in promoter activity was transient, and the luciferase activity returned back to control levels after 9 and 15–25 h after UV irradiation, using 3 and 10 J/m², respectively.

The UV-induced activation of the R2 promoter was not caused by stimulation of the resting G₀/G₁ synchronized cells to proliferate since flow cytometry analyses of the cells after UV irradiation showed no changes in cell cycle distribution. About 85% G₀/G₁- and 10% S-phase cells were detected within 15 h after exposure to 10 J/m² (Fig. 3B), and the same result was obtained for cells irradiated at 3 J/m² (data not shown).

**R2 Promoter Activity in Logarithmically Growing Balb/3T3 Cells after UV Irradiation**—Logarithmically growing Balb/3T3 cells stably transformed with the p19ucR2 1.5 plasmid (Fig. 1) were irradiated with a dose of 10 J/m². Luciferase activity was measured at different time points during a 12-h period after irradiation and compared with the activity in nonirradiated control cells (Fig. 4). A clear increase in activity was observed throughout the experiment. However, the increase was less pronounced than the one observed in nonproliferating cells with the same construct, and no clear peak was evident (cf. Fig. 3A). In the logarithmically growing cells, about 40% of the cells were in G₀-phase and 50% in S-phase as shown by flow cytometry (data not shown).

**R2 Promoter Activity in UV-irradiated G₀/G₁ Cells Stimulated to Proliferate**—To further analyze the mechanism of R2 promoter activation by UV irradiation, we measured luciferase activity in UV-irradiated, serum-synchronized G₀/G₁ cells stably transformed by the p19ucR2 1.5 construct after the readmission of serum to start proliferation. Nonirradiated, synchronized cells were used as a control. As shown earlier, the R2 promoter was activated very early when cells pass from quiescence to proliferation (Fig. 5). However, UV irradiation in—
Figure 4. R2 promoter-controlled luciferase activity in logarithmically growing Balb/3T3 cells stably transformed with the p19LucR2 1.5 plasmid after UV irradiation, 10 J/m². Circles, UV-irradiated cells; squares, control, nonirradiated cells.

Figure 5. R2 promoter-controlled luciferase activity in G₀/G₁-synchronized Balb/3T3 cells stably transformed with the p19LucR2 1.5 plasmid after serum release and UV irradiation with a dose 10 J/m². Closed circles, serum-released cells; open circles, UV-irradiated and serum-released cells.

Increased the luciferase activity about 24-fold compared with the 9–10-fold increase in the nonirradiated cells.

Measurements of R2 mRNA Levels in Logarithmically Growing Mouse TA 3 Cells after UV Irradiation—Our data so far demonstrate UV-induced activation of the R2 promoter. To study if this activation also results in increased R2 mRNA levels, logarithmically growing, hydroxyurea-resistant, R2-overproducing mouse TA 3 cells were irradiated with a dose of 10 J/m². Total RNA was extracted at 0, 4, and 7 h after irradiation and analyzed by Northern blotting using an R2 cDNA probe (Fig. 6). Interestingly, in these cells a preferential accumulation of the minor 1.6-kb R2 transcript was seen 4 h after irradiation, whereas the levels of the major 2.1-kb transcript were relatively constant in repeated experiments.

Methyl Methanesulfonate and Hydroxyurea Have No Effect on the R2 Promoter Activity in Balb/3T3 Cells—In the yeast Saccharomyces cerevisiae the expression of the R2 gene is induced by UV light, hydroxyurea, and by the alkylating agent methyl methanesulfonate (19). We tested the effects of methyl methanesulfonate on the R2 promoter activity in G₀/G₁-synchronized p19LucR2 1.5-transformed mouse cells but could not detect any significant changes in luciferase activity within 5 h of treatment. The same concentrations of drug as those reported by Elledge and Davis (27) and those reported in the literature to cause a considerable amount of alkylated bases in mammalian DNA were used (28) (Table I). Only a very slight increase in luciferase activity was detected 3 and 4 h after treatment of G₀/G₁-synchronized cells with three different doses of drug (2.36, 11.8, and 118 µM). Furthermore, no significant increase was observed when logarchimally growing Balb/3T3 cells were exposed to different doses of drug (80–1700 µM, data not shown).

Hydroxyurea is a specific inhibitor of ribonucleotide reductase acting by reducing and labilizing the iron center of mammalian R2 and also by a direct 1-electron reduction of the tyrosyl free radical (29, 30). In our stably transformed cell lines, hydroxyurea alone did not affect the R2 promoter activity in any significant way. Addition of hydroxyurea to a G₀/G₁-synchronized culture of p19LucR2 1.5-transformed mouse fibroblasts did not increase the luciferase expression within 5 h of treatment. Moreover, addition of hydroxyurea (0.5–2 mM) to logarithmically growing cultures of the same cells did not result in any significant increase in luciferase activity up to 3 h after the addition of the drug (data not shown).

Finally, addition of 2 mM hydroxyurea to the medium 3 h prior to UV irradiation of serum-synchronized cells stably transformed with the p19LucR2 1.5 construct did not result in any increased luciferase activity compared with cells irradiated in the absence of hydroxyurea (data not shown).

R2 Promoter Elements Important for UV Induction—To localize regions in the R2 promoter responsible for the UV-induced activation, the experiments with resting cells were repeated with cells stably transformed with the p19LucR2 0.5 plasmid (Fig. 7). This plasmid contains the first 470 base pairs upstream from the transcription start of the R2 gene including the proximal DNA-protein binding α region but not the β, γ, and δ regions (Fig. 1). This construct has been shown to main-
tained basal levels of transcription but is not serum-inducible. The same qualitative pattern of luciferase activity was observed with this reporter construct as with the p19lucR2 1.5. However, the induction was more pronounced with a 3- and 9–10-fold increase after 3 and 10 J/m², respectively (Fig. 7).

To further localize the region of the R2 promoter important for UV activation, another luciferase reporter gene construct, p19ucR2 0.11 (Fig. 1), containing only the first 93 base pairs upstream from the transcription start of the R2 gene was used in similar experiments as above. The p19ucR2 0.11 construct, like the p19ucR2 0.5, was shown to maintain basal levels of transcription, could not respond to serum stimulation, but showed UV induction similar to p19ucR2 0.5 (data not shown).

A reporter gene construct, p19ucR2 1.0–α, where the α region is deleted but the upstream β, γ, and δ elements are still present (Fig. 1) was earlier shown to respond to serum stimulation, but the response was transient and started to decay before cells entered the S-phase (12). Surprisingly, this construct showed a similar UV induction of the R2 promoter activity in G0/G1-synchronized cells as the induction obtained with the full-length promoter (data not shown).

A p19ucR2 TTTAAA construct contains only the TTTAAA element and the transcription start, whereas all the DNA-protein binding sites (α, β, γ, and δ) are deleted (Fig. 1). No luciferase activity could be measured in extracts from serum-stimulated, -synchronized cells stably transformed with this construct or in logarithmically growing cells (12). Likewise, no UV induction was observed (data not shown). The presence of the R2 promoter fragment linked to an intact luciferase gene in the stably transformed cells was confirmed by polymerase chain reaction (12).

The G1-specific Transcriptional Block of the R2 Gene Does Not Interfere with the UV Induction—We were interested to study how the G1-specific transcriptional block earlier located to the first intron of the R2 gene would influence UV induction of the R2 expression in resting cells. Therefore, serum-starved cells stably transformed with the p19ucR2Ex2-ATG construct (Fig. 1) were UV-irradiated, and the luciferase expression was measured. The same pattern of UV-induced luciferase activity was seen as the one observed for the p19ucR2 1.5 (Fig. 3A and data not shown).

R1 Promoter Activity in Balb/3T3 Cells after UV Irradiation—The plasmid p19ucR1 1.0 contains the R1 promoter (nt –812 to +242) ligated to the luciferase reporter gene (21). G0/G1-synchronized cells stably transformed with p19ucR1 1.0 were irradiated with a dose of 10 J/m², and luciferase activity was measured during a 25-h period (Fig. 8). The luciferase activity started to increase 4 h after UV irradiation and peaked at 10 h showing a 2–3-fold stimulation of the R1 promoter activity. The activation was transient and the luciferase activity returned to control values after 15–20 h. Like the results with the R2 promoter-reporter gene constructs, addition of 2 mM hydroxyurea to logarithmically growing cultures of p19ucR1 1.0-transformed cells did not result in any significant increase in luciferase activity (data not shown).

**DISCUSSION**

Nonproliferating mammalian cells contain no detectable ribonucleotide reductase activity and have no detectable levels of R1 protein as shown using immunohistochemical techniques (31). Furthermore, after serum starvation, G0/G1 cells contain less than two molecules of R1 and R2 mRNAs per cell (10). Nucleotide excision repair is used by the cell to repair DNA damages caused by UV irradiation. This repair may occur outside of S-phase when the pools of deoxyribonucleoside triphosphates are very low (2). In this case the cell must have mechanisms by which it can supply the repair DNA synthesis with precursors. The only way to synthesize the precursors de novo is to induce ribonucleotide reductase.

In yeast cells ribonucleotide reductase expression is induced outside of S-phase in response to many different DNA-damaging agents (19). Using mouse R1 and R2 promoter-luciferase reporter gene constructs, we now demonstrate activation of the mouse R1 and R2 gene promoters by UV light. In serum-starved G0/G1 cells, the UV-stimulated luciferase expression increased up to 3-fold for the R1 promoter and up to 10-fold for the R2 promoter using 10 J/m². Maximal values were obtained 8–12 h postirradiation and then declined to values obtained in nonirradiated cells. This should be compared with up to a 10-fold increase in R2 promoter-controlled luciferase activity after serum release of G0/G1-synchronized cells (11). The increase occurred without any measurable perturbation in the distribution of cells in the different cell cycle phases as shown by flow cytometry. Therefore, our data show that also in mouse cells, ribonucleotide reductase can be induced outside S-phase by UV-induced DNA damage.

Only 93 base pairs of the proximal R2 promoter together with the transcriptional start were required to give a UV response. This includes the proximal DNA-protein binding α region (nt –93 to –56) that has been shown to bind the NF-Y transcription factor. Together with the upstream β, γ, and δ elements, the α region is absolutely required for S-phase-specific expression of the R2 gene (12). No UV induction was observed for a promoter-luciferase construct containing only the TTTAAA box and the transcriptional start, and this con-
struct did not support any basal levels of R2 transcription.

A luciferase reporter gene construct containing only the upstream β, γ, and δ DNA-protein binding regions but lacking the proximal DNA-protein binding α region showed activation of the R2 promoter in G1/S- or G2/S-synchronized cells after UV irradiation. It has been shown that the R2 promoter lacking the CCAAT box-containing α region is serum-inducible in G1/S cells, but its activity starts to decay before cells enter the S-phase, and the transcriptional block is released (12). Taken together these data indicate that the UV inducibility of the R2 promoter requires only a basal level of transcription that can be maintained by either the three upstream β, γ, and δ regions or the single proximal DNA-protein binding α region of the R2 promoter in combination with the TTTAAA box and the transcriptional start. Therefore, the induction of the R2 promoter in resting cells after UV treatment is clearly different from the proliferation-specific response of the same promoter requiring all four DNA-protein binding regions. This result and the finding that the UV response is additive to the normal proliferation response suggest that the UV-induced activation of the R2 promoter uses different mechanisms than the proliferation-specific induction. Furthermore, the transcriptional block that regulates the S-phase-specific expression of the R2 gene seems not to be involved in regulating the UV-induced activation of R2 expression in nonproliferating cells.

UV induction of the R2 promoter was observed also in logarithmically growing cells. This may reflect activation of inactive R2 promoters in early G1 cells or further stimulation of already activated promoters in late G1/S-phase cells or a combination of both.

Gel shift experiments did not reveal any additional protein binding to the four regions of the R2 promoter after UV irradiation (data not shown). This is similar to the finding that no additional DNA-protein binding can be observed to the c-jun promoter after UV irradiation, although this promoter is induced about 100-fold in UV-irradiated cells (32). It has been suggested that the transcription factors that regulate UV response in mammalian cells undergo posttranscriptional modifications, such as phosphorylation, to be able to induce transcription after UV irradiation. Based on our data we favor the hypothesis that the R2 gene in mouse cells requires only a minimal promoter, supporting basal levels of transcription, for UV induction. However, this does not answer the question about the specificity of the UV response, and only a certain set of genes are induced after UV irradiation of mammalian cells (13).

It is difficult to measure the levels of R2 mRNA in normal mouse cells because of very low concentrations of the transcripts (10). Therefore, we used hydroxyurea-resistant, R2-overproducing mouse mammary tumor TA3 cells that have an amplified R2 gene and overexpress the R2 mRNA to about 40 times higher levels compared with native TA3 cells. However, the S-phase-specific expression of R2 mRNA is still retained (5, 8, 10). When the R2 mRNA levels vary during the normal cell cycle, the minor 1.6-kb transcript is always around 10% of the major 2.1-kb transcript (5). Exposure of logarithmically growing, hydroxyurea-resistant, TA3 cells to UV light unexpectedly led to a preferential transient accumulation of the minor 1.6-kb R2 transcript. A similar preferential accumulation of the smaller of two transcripts, differing in the utilization of polyadenylation signals, has been reported for the rat ornithine decarboxylase gene after induction by UV light (33). This may indicate similar mechanisms for the UV induction of these two genes.

The R2 promoter activation was not increased when the amount of single strand breaks was increased by combining UV irradiation with hydroxyurea. This indicates that the gaps themselves are not part of the cellular signaling pathway for R2 promoter induction. Also, the absence of any clear effect of hydroxyurea on the R1 or R2 promoter activity in logarithmically growing cells indicates that decreasing pools of deoxyribonucleotides are not the immediate signal to activate ribonucleotide reductase expression. This is quite different from the situation in yeast where hydroxyurea is a strong inducer of ribonucleotide reductase expression (19).

Methyl methanesulphonate unlike UV light does not cause bulky lesions in DNA but causes base damages by alkylation. These type of damages are thought to be repaired by short-patch repair mechanisms or dealkylation requiring very little if any deoxyribonucleotides (13). Methyl methanesulphonate did not activate the mouse R2 promoter indicating that in mammalian cells ribonucleotide reductase is specifically induced only by DNA damage requiring nucleotide excision repair. Again this differs from the situation in yeast where a number of different agents such as UV light, methyl methanesulphonate, and hydroxyurea all induce ribonucleotide reductase in a more general type of response (19). Our results are in agreement with previous reports on the lack of such a general response in mammalian cells (13).

Taken together our data show that both the R1 and R2 genes of mouse ribonucleotide reductase are UV-inducible by mechanisms different from the proliferation-specific promoter activation. Clearly, more studies are required to identify the signaling pathways in mammalian cells that can specifically induce genes such as ribonucleotide reductase in response to DNA damage by UV irradiation to allow repair of DNA even outside of S-phase.

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