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Regulation of the ribonucleotide reductase small subunit gene by DNA-damaging agents in *Dictyostelium discoideum*

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ABSTRACT

In *Escherichia coli*, yeast and mammalian cells, the genes encoding ribonucleotide reductase, an essential enzyme for *de novo* DNA synthesis, are up-regulated in response to DNA damaging agents. We have examined the response of the *rnrB* gene, encoding the small subunit of ribonucleotide reductase in *Dictyostelium discoideum*, to DNA damaging agents. We show here that the accumulation of *rnrB* transcript is increased in response to methyl methane sulfonate, 4-nitroquinoline-1-oxide and irradiation with UV-light, but not to the ribonucleotide reductase inhibitor hydroxyurea. This response is rapid, transient and independent of protein synthesis. Moreover, cells from different developmental stages are able to respond to the drug in a similar fashion, regardless of the basal level of expression of the *rnrB* gene. We have defined the *cis*-acting elements of the *rnrB* promoter required for the response to methyl methane sulfonate and 4-nitroquinoline-1-oxide by deletion analysis. Our results indicate that there is one element, named box C, that can confer response to both drugs. Two other boxes, box A and box D, specifically conferred response to methyl methane sulfonate and 4-nitroquinoline-1-oxide, respectively.

INTRODUCTION

Activation of specific genes is a primary response of cells to damaged DNA. The physiological role of this response and its underlying mechanism are reasonably well understood in bacteria. However in eukaryotes these processes are not clearly defined. In yeast the genes activated by DNA-damaging agents include those involved in nucleotide excision repair, post-replication repair and double-stand break repair. Also induced by DNA-damaging agents are some of the genes which play a dual role in nucleic acid metabolism and DNA repair; for example, the genes encoding DNA ligase I, DNA polymerase I and ribonucleotide reductase (1). The activation of these genes is thought to be needed to fuel DNA synthesis during repair. The response to DNA-damaging agents in mammalian cells is complex and involves many genes and proteins in addition to those implicated in DNA repair and its related processes (2). The reason for such diverse responses is not clear but presumably reflects the requirement for coordination of regulated responses between cells in a multicellular organism.

The enzyme ribonucleotide reductase catalyzes the first reaction in dNTPs synthesis, the conversion of ribonucleotides into deoxyribonucleotides. Because of its essential role in DNA synthesis, this enzyme is expected to play an important part in the repair of damaged DNA. An increase in the number of chromosome aberrations was observed when irradiated human lymphoblastoid cells were incubated with inhibitors of ribonucleotide reductase (3–5). In addition, ribonucleotide reductase has been implicated in carcinogenesis. In the presence of activated oncogenes, overexpression of the small subunit of ribonucleotide reductase has been shown to affect the rates of tumor formation and metastasis in mice (6,7). For these reasons ribonucleotide reductase is a key target for drug design in cancer therapy (8). In bacteria, yeast and mammalian cells the ribonucleotide reductase genes are inducible by DNA-damaging agents (9). That induction of ribonucleotide reductase by DNA-damaging agents is observed in all species studied underscores the importance of this response. Despite its relevance in DNA repair and in tumorigenesis, the mechanism mediating the response of the ribonucleotide reductase genes to DNA-damaging agents is not fully understood.

Many of the genes activated by DNA-damaging agents are also under cell-cycle control. Thus analysis of the effects of DNA-damaging agents in proliferating cells may be complicated by mechanisms that overlap the repair and growth processes. Growth and development are mutually exclusive events in *Dictyostelium discoideum*. Processes operating during growth are down-regulated during development. Hence the developmental phase of *Dictyostelium* offers a convenient system to study the effects of DNA-damaging agents on gene expression in the absence of cell growth. We have previously isolated the gene encoding the small subunit of ribonucleotide reductase in *Dictyostelium*, *rnrB* (10). Here we show that the expression of *rnrB* is inducible by DNA-damaging agents during growth and at different stages of development. We have also identified the *cis*-acting elements of *rnrB* involved in the response to DNA-damaging agents.

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MATERIALS AND METHODS

Growth, development and transformation of *Dictyostelium* cells

Cells of strain AX2 were grown axenically in HL5 medium (11). The cells were harvested at 2–4 × 10^6 cells/ml, washed with KKP buffer (20 mM KH_2PO_4/K_2HPO_4, pH 6.2) and developed in KKP buffer at 10^7 cells/ml with shaking, or on polycarbonate filters as described (12). *Dictyostelium* cells were transformed by calcium phosphate coprecipitation (13). Transformants were selected in HL5 medium containing 20 μg/ml of G418 (Gibco). Pools of at least 50 transformants were used for analysis to minimize the variation due to integration site and/or copy number (14).

Generation of deletions

The plasmid pDdrnrB/lacZ contains half of the coding region from *capA* (15), two-thirds of the coding region of *rnrB* fused in frame to *lacZ*, and the complete intergenic region located between them (10). Construction and sequencing of the 5' deletions were described previously (16). The constructs are referred to by the number of bases remaining between the site of the deletion and the A of the first ATG codon.

Internal deletions were constructed using two different PCR products of the *rnrB* promoter. The 5' primer for both products was 5'-TTACTAGTGAAATACCTGCACCTCC-3' (underlined base corresponds to a mismatch in the primer to its complementary sequence). This primer is located from base –1779 to base –1755 with respect to the A of the first ATG of *rnrB*. It contains an added *Spe*I site to allow cloning in the *Xba*I site of the deletion constructs. The sequences of the two 3' primers are as follows: box B primer (Fig. 5): 5'-TTGAATTCAAAATAC-ACACACATTCCCG-3', and box C primer (Fig. 5): 5'-TT-GAATTCATGATGGAATCACCGTTCC-3'. The engineered *Eco*RI sites in these primers, as shown by the underlined bases, were used for cloning in the deletion constructs. Polymerase chain reactions were performed with Expand™ (Boehringer Mannheim) according to the manufacturer's instructions, using an annealing temperature of 55°C. The internal deletions are designated –XΔ–Y, where X and Y indicate the nucleotides 5' and 3' from the deleted regions, respectively. All internal deletions retain the *Eco*RI site from the polylinker of the vector. Deletions –429Δ–340, –429Δ–280 and –429Δ–212 were constructed using the PCR product generated with the 3' box C primer, digested with *Spe*I and *Kpn*I, and inserted into the *Xba*I and *Kpn*I sites of the 5' deletion constructs Δ–340, Δ–280 and Δ–212, respectively. Deletions –444Δ–311, –444Δ–280 and –444Δ–212 were obtained by inserting the *Spe*I/*Xba*I fragment of the above PCR product into the *Xba*I site of Δ–311, Δ–280 and Δ–212, respectively. Deletions –359Δ–280 and –359Δ–212 were produced with the same PCR fragment digested with *Spe*I and *Eco*RI and cloned into the *Xba*I and *Eco*RI sites of constructs Δ–280 and Δ–212, respectively. Finally, deletion –292Δ–212 was constructed with the PCR product obtained with the 3' box B primer, digested with *Spe*I and *Eco*RI, and inserted into the *Xba*I and *Eco*RI sites of the deletion construct Δ–212.

Drug treatments and cell survival

For treatment of vegetative cells, stock solutions of the drugs were added directly to growing cells in HL5 medium. For treatment with chemical agents during early development, the cells were developed in suspensions of KKP for 4 h prior to the addition of drug solutions. Cells irradiated with UV and cells treated with genotoxic agents during late development were developed on filters saturated with KKP at 10^6 cells/cm^2. For treatment with chemical agents the filters were placed for 1 h on pads of blotting paper that had been saturated with KKP containing drugs at the specified concentrations. Irradiation with UV was performed using an UV cross-linker (Stratalinker 1800, Stratagene). The UV source was calibrated with uridylic acid as a chemical actinometer (17) correcting for absorption by the solution (18). Methyl methane sulfonate (MMS), 4-nitroquinoline-1-oxide (4NQO) and cycloheximide were purchased from Sigma. Hydroxyurea was obtained from ICN.

Following treatment with genotoxic agents, the cells were diluted in KKP. Aliquots of the various dilutions were spread together with *Enterobacter aerogenes* on SM plates (19). Survivors were scored by counting the number of plaques on the SM plates.

RNA preparation and hybridization

Cells were collected by centrifugation and washed once with cold KKP buffer. The cell pellets were frozen on dry ice and kept at –70°C until the RNA was extracted. Total RNA was isolated according to the protocol by Franke *et al.* (20) in microfuge tubes using 2 × 10^7 cells.

For northern blot analysis, 10 μg of RNA were mixed with ethidium bromide and resolved on formaldehyde gels as described (21). After electrophoresis, the gels were visualized under a UV illuminator to ensure even loading. Nucleic acids were transferred onto Nytran membranes (Schleicher & Schuell) in 10× SSC and cross-linked using a UV cross-linker. Radioactive probes were generated by random priming following the manufacturer's protocol (Pharmacia) with [α-^{32}P]-dCTP (ICN). The *rnrB* probe was the *Eco*RI–*Dra*I fragment of the *rnrB* coding sequence, a region not present in the reporter construct (10). Hybridizations were conducted according to standard protocols in Denhardt's hybridization solution with 50% formamide (22). Hybridizations and stringency washes were performed as follows: for the *rnrB* and pB47 probes, the blots were hybridized at 40°C overnight and washed twice for 30 min in 1× SSC, 0.1% SDS at 65°C; for the *lacZ* and the *capA* probes, hybridization temperature was 45°C and the washes were done in 0.1× SSC, 0.1% SDS at 65°C. Blots were exposed to Kodak X-Omat films with intensifying screens. For each experiment, the same blot was hybridized with different probes. Between each hybridization, the probe was stripped from the membrane by incubating twice for 15 min in a boiling solution of 0.1× SSPE and 0.5% SDS.

For dot blot analysis, 10 μg of total RNA were treated with 0.3 U of RQ1 RNase-free DNase (Promega) for 30 min at 37°C. This suspension was mixed with 3 vol of denaturation solution (37% formaldehyde, 100% formamide and 20× SSC, in a 7:20:2 ratio), heated at 65°C for 15 min, and chilled on ice. Two volumes of 20× SSC were then added to the solution. The RNA samples were spotted in duplicate (5 μg per spot) onto Nytran membranes that had been washed with 10× SSC. The membrane was washed again with 10× SSC and finally the nucleic acids were cross-linked.

To determine the level of expression of the reporter transcript, dot blots were quantified using a PhosphorImager (BioRad GS-363) and the signal intensities were determined using Molecular Analyst™ software (BioRad). The fold-induction

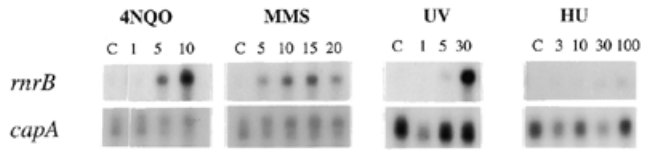

Figure 1. Effect of DNA-damaging agents on the accumulation of the *rnrB* transcript. AX2 cells were developed for 4 h and exposed for 1 h to 4NQO (1, 5 or 10 μg/ml), to MMS (5, 10, 15 or 20 mM) or to hydroxyurea (3, 10, 30 or 100 mM). For UV treatment 4 h cells were irradiated at 1, 5 or 30 J/m^2, then incubated on pads saturated with KKP for 1 h. Total cellular RNA was extracted, resolved by denaturing electrophoresis, and blotted onto membranes. Autoradiographs obtained from probing the same membrane for the *rnrB* gene and the *capA* gene are shown. C represents the untreated control.

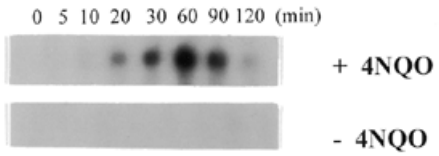

Figure 2. Time course of increase of *rnrB* transcript level in response to 4NQO. AX2 cells were developed for 4 h and exposed to 5 μg/ml of 4NQO. Total RNA was extracted from cells before the addition of the drug (0) and from cells that had been incubated for 5, 10, 20, 30, 60, 90, 120 and 180 min with the drug. Shown here is an autoradiograph of a northern blot probed for *rnrB* expression.

of *rnrB* was determined by dividing *rnrB* transcript level in treated cells by that of untreated cells. On average, induction for MMS and 4NQO was 7.5- and 15-fold, respectively. To compensate for variations among experiments, a correction factor was used to calculate the fold induction for the reporter gene activity. The correction factor was obtained by dividing the average induction level for *rnrB* by that of the observed induction level. Thus, if the observed induction for 4NQO was 30-fold, the correction factor would be 15/30 or 0.5. The fold-induction of the reporter transcript was calculated by dividing the level of *lacZ* message in treated cells by that of the untreated cells, then multiplying this value by the correction factor.

RESULTS

DNA-damaging agents stimulate the accumulation of *rnrB* transcript

We examined the effects of the UV-mimetic agent 4NQO, the alkylating agent MMS and UV irradiation on the expression of the *mrB* gene in *Dictyostelium.* As shown in Figure 1, the level of *rnrB* transcript increased when 4 h-developing cells were exposed to DNA-damaging agents. The increase in the accumulation of *rnrB* transcript in response to DNA-damaging agents was dose-dependent. The levels of induction elicited by 10 μg/ml of 4NQO, 15 mM of MMS and 30 J/m^2 UV-irradiation were ~15-, 7.5- and 15-fold, respectively. The survival rates for 5, 10, 15 and 20 mM of MMS were 99, 70, 30 and <1%, respectively. In the case of 4NQO the survival rates for 1, 5 and 10 μg/ml were 99, 50 and 2%, respectively. Over 95% of the cells survived a dose of 30 J/m^2 of UV irradiation. These values are consistent with the data published previously (23,24).

To ensure that the induction of *rnrB* by DNA-damaging agents is a specific event we investigated the accumulation of *capA* transcripts (25). The *capA* gene is located upstream of *rnrB* and is transcribed in the opposite orientation in relation to *rnrB* (10). Unlike *rnrB*, the *capA* gene is constitutively expressed during growth and throughout development (25). Figure 1 shows that the levels of the two *capA* transcripts, generated by alternative splicing of a retained intron (25), remained relatively unchanged in the presence of various DNA-damaging agents. The expression of the gene encoding calmodulin is also not affected by DNA-damaging agents (data not shown).

Hydroxyurea has little effect on *rnrB* expression

We tested the effect of the ribonucleotide reductase inhibitor hydroxyurea on the level of *rnrB* transcript. Figure 1 shows that the presence of hydroxyurea has only a very modest effect on the expression of *rnrB*. The expression of the *capA* gene was not affected by these treatments. The doses we used, ranging from 3 to 100 mM, are known to have dramatic effects on the growth of *Dictyostelium* cells (26).

Up-regulation of *rnrB* is rapid, transient and independent of protein synthesis

We determined the rate of accumulation of the *rnrB* transcript in response to DNA-damaging agents. Figure 2 shows the accumulation of *rnrB* transcript in the continuous presence of 5 μg/ml of 4NQO. The increase in *rnrB* transcript level was detectable as early as 10 min after the beginning of treatment. The up-regulation of the *rnrB* transcript level was transient, reaching the peak level of accumulation after 60 min of treatment.

The rapid response suggested that the up-regulation of the *rnrB* message by DNA-damaging agents involves pre-existing factors. To gain further support for this assertion we investigated the induction process in the presence of the protein synthesis inhibitor cycloheximide. Figure 3 shows the effects of 4NQO on gene expression in the presence of cycloheximide. Prior treatment with cycloheximide did not alter appreciably the 4NQO-stimulated increase in the accumulation of the *rnrB* transcript. We tested the effectiveness of the cycloheximide treatment by examining the expression of a gene we serendipitously found to be stimulated by protein synthesis inhibitors. Partial sequence analysis revealed that the clone pB47 encodes a homolog of seryl-tRNA synthase (unpublished data). Cycloheximide, but not 4NQO, stimulated an increased accumulation of the pB47 transcript. Also shown in Figure 3 are the levels of the *capA* transcripts, which remained relatively unaffected by either of the drug treatments. These results indicate that the induction of *rnrB* by DNA-damaging agents can take place in the presence of a protein synthesis inhibitor.

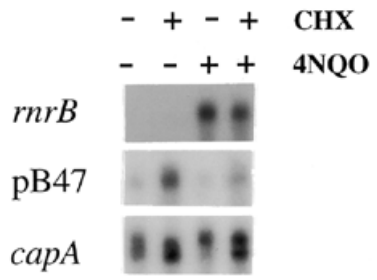

Figure 3. Effect of 4NQO and cycloheximide on the accumulation of the *rnrB* transcript. AX2 cells which had been developed for 4 h were exposed to either 500 µg/ml cycloheximide, 10 µg/ml 4NQO or to both for 1 h. When both drugs were given, cycloheximide was added 10 min before 4NQO. Shown here are autoradiographs of the same membrane probed with *rnrB*, pB47 and *capA*. + indicates the presence and – shows the absence of the drugs.

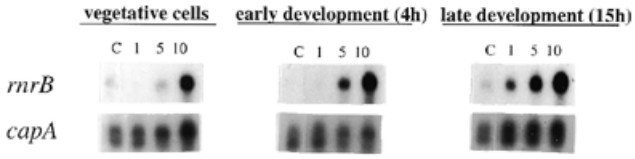

Figure 4. Regulation of *rnrB* by DNA-damaging agents during growth and development. Growing cells and cells which had been developed for different lengths of time were treated with 1, 5 or 10 µg/ml of 4NQO for 1 h at the times indicated. Total RNA was extracted and analyzed by northern blot. Autoradiographs of the same membrane probed with *rnrB* and *capA* are shown. C, untreated control.

Effect of DNA-damaging agents on *rnrB* is independent of developmental stage

When deprived of nutrient *Dictyostelium* cells embark upon a program of development and differentiation. A 24 h developmental program transforms the cells into fruiting bodies composed of two main cell types, spores and stalk cells. The rate of DNA synthesis decreases as the cells enter development. Interestingly there is another round of DNA synthesis shortly after the cells have undergone aggregation, at ~12 h of development (27–31). Coincident with the increase in the rate of DNA synthesis is the expression of *rnrB*. The level of *rnrB* transcript is moderate in growing cells, low in the first 10 h of development and high during late development (10). To evaluate the process of DNA damage induction under different physiological conditions we treated vegetatively growing cells, 4- and 15 h-developing cells with 4NQO. Figure 4 shows that at these three stages of the life cycle, the cells were capable of responding to 4NQO in the induction of *rnrB*. A similar effect was observed when the cells were treated with MMS or UV irradiation (data not shown). Moreover the level of induction was very similar for vegetative cells and for cells from different stages of development. At 10 µg/ml of 4NQO, the level of induction was ~15-fold for all three stages of the life cycle.

We also investigated the expression of the *capA* gene and found that the levels of the *capA* transcripts varied only slightly. The variations observed did not correlate with either the drug or the dosage used.

Identification of *cis*-acting elements controlling the DNA damage response

Similar to other *Dictyostelium* promoters, the 5' untranscribed region of the *rnrB* gene contains >85% A and T residues with clusters of G/C-rich sequences of ~15–20 bp in length. In several *Dictyostelium* promoters that have been analyzed previously, G/C boxes have been shown to be important for control of gene expression (32,33). The location of the four G/C-rich boxes that are closest to the *rnrB* transcription start site is indicated in Figure 5. We refer to these sequences as elements A, B, C and D, from the most proximal to the transcription start site to the most distal, respectively. Table 1 shows the sequence of each of these elements.

Table 1. Sequence of the four GC-rich boxes most proximal to the *rnrB* start site

Box	Position	Sequence
A	–237 to –222	5'-GGACCAAAATTGCGC-3'
B	–313 to –299	5'-CGGAATGTGTGTG-3'
C	–378 to –362	5'-GGAACGGTGATTCCATC-3'
D	–450 to –430	5'-TCTAGAATCGGAGTGGTACC-3'

We used promoter deletion analysis to define the *cis*-acting elements of the *rnrB* promoter involved in the response to DNA-damaging agents. A schematic representation of the constructs used in this study is shown in Figure 5. *Dictyostelium* cells carrying the deletion constructs were developed for 4 h and exposed for 1 h to either 10 µg/ml of 4NQO or 25 mM MMS. The fold-increase in *lacZ* transcript level for each construct in the presence of MMS or 4NQO is shown in Figure 5. Deletion of the upstream region of the *rnrB* promoter up to base –450 did not affect the up-regulation of the gene following treatment with 4NQO or MMS. In addition, cells bearing the internal deletion construct –444Δ–212, missing the four G/C-rich boxes A–D, failed to respond to either drug. Together these results suggest that the sequence between –444 and –212 contains the elements necessary for the DNA-damage induction of *rnrB*.

A 5' deletion construct missing all the sequence upstream of base –311 rendered the reporter construct unresponsive to 4NQO. Similarly, all constructs missing additional sequence were unable to respond to 4NQO. Therefore in this sequence context, the 94-bp region comprised between –405 and –311 and containing the G/C-rich box C is essential to confer 4NQO-induced DNA damage response. Results from experiments conducted with internal deletions constructs showed that the presence of box D could restore the response to 4NQO (compare constructs –444Δ–212 and –429Δ–212). This suggests that in addition to box C, box D also plays a role in the response to 4NQO. Consistent with the results from the 5'

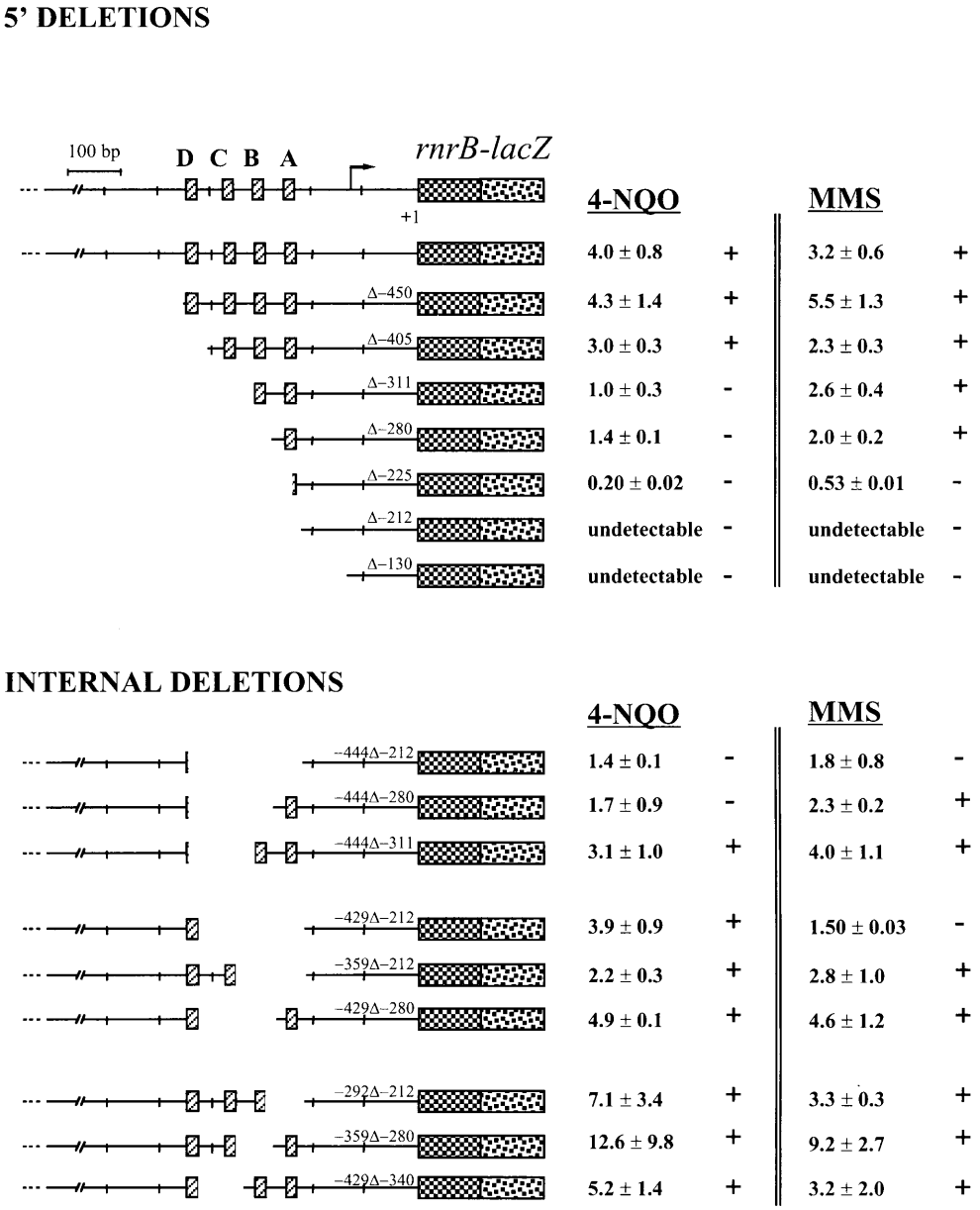

Figure 5. Transcriptional response directed by the deletion constructs in the presence of DNA-damaging agents. Cells were developed for 4 h and treated with 10 μg/ml of 4NQO or 25 mM of MMS for 1 h. Total RNA was extracted and analyzed by dot blot. The induction values correspond to the increase in the *lacZ* transcript level after correction for the endogenous *rnrB* induction as described in the Materials and Methods section. Each value is the average of two independent experiments ±standard deviation. A construct was judged damage-inducible (+) if the level of the *lacZ* transcript was at least 2-fold higher than that in untreated cells. Undetectable means that the signal for the *lacZ* transcript was too close to that of the background to be quantified accurately.

deletions an internal deletion construct bearing box A alone, construct –444Δ–280, was ineffective in promoting 4NQO response on the reporter construct. Construct –444Δ–311, containing both box A and box B, showed a response to 4NQO, which contradicted the results obtained with the 5' deletion constructs. Because this region was unable to confer response to 4NQO in the context of 5' deletion constructs, we concluded that boxes C and D are the major elements involved in the response to 4NQO.

When treated with MMS, the transcript encoded by the 5' deletion construct Δ–280 was up-regulated. The only G/C-rich element present in this construct is box A. Constructs with further truncation, Δ–225, Δ–212 and Δ–130, were unresponsive to MMS. Therefore it appears that box A is sufficient for the

up-regulation of *rnrB* by MMS. The results obtained using the strains bearing the internal deletion constructs are consistent with this idea. The internal deletion –444Δ–280 (with boxes B–D missing) responded to MMS whereas construct –444Δ–212 (missing boxes A–D) and –429Δ–212 (containing box D alone) did not. However the deletion construct -359Δ–212 containing boxes C and D was inducible by MMS. These results suggest that box C can confer induction by MMS. From these results we conclude that box A and box C are involved in the response to the alkylating agent MMS.

DISCUSSION

In mammalian cells and yeast, the level of expression of a large number of genes increases in response to DNA-damaging agents. Many of these responsive genes are normally required for repair and synthesis of DNA (1). In *Dictyostelium*, the genes encoding apurinic/apyrimidic endonuclease and the helicases *repB* and *repD* (*repB* and *repD* are homologs of the xeroderma pigmentosum *XPB* and *XPD* genes, respectively) have recently been shown to be up-regulated by DNA-damaging agents (34,35). We showed here that the transcript level of the small subunit of ribonucleotide reductase increased when *Dictyostelium* cells were treated with 4NQO, MMS or UV irradiation. These results suggest that, as in other organisms, DNA-damaging agents induce the expression of specific *Dictyostelium* genes involved in DNA repair and metabolism.

The mechanisms mediating the effects of DNA-damaging agents share common features. In yeast, the up-regulation of ribonucleotide reductase by DNA-damaging agents is mediated by pre-existing factors that do not require protein synthesis to become activated (36). The expression of the apurinic/apyrimidic endonuclease gene from *Dictyostelium* has also been shown to be DNA damage-inducible in a protein-synthesis independent fashion (34). Our results with the protein synthesis inhibitor cycloheximide strongly suggest that the up-regulation of the *rnrB* transcript upon treatment with DNA-damaging agents takes place via a similar mechanism. Furthermore, the demonstration by deletion analysis that specific promoter regions are necessary for the induction by DNA-damaging agents suggests that an increase in transcriptional rate of the *rnrB* gene is an important part of the response.

In *Dictyostelium*, the up-regulation of gene activity in response to DNA-damaging agents is transient. Besides *rnrB*, this has been shown to be the case for the apurinic/apyrimidic endonuclease gene as well as the *repB* and *repD* genes (34,35). The drop in transcript level after prolonged treatment observed for these genes is possibly caused by cell death and/or breakdown of the drugs. In yeast the induction of a number of DNA damage-responsive genes has also been shown to be transient, including *RNR2*, *POL1*, *RAD6*, *RAD7*, *RAD18*, *RAD23* and *RAD51* (36–42).

The level of *rnrB* expression fluctuates during the life cycle of *Dictyostelium*. Regardless of the endogenous *rnrB* level, the same magnitude of induction by DNA-damaging agents was observed at different stages of development and during growth. This suggests that the factors involved in the response to DNA-damaging agents are present at all stages of the life cycle. It also implies that the mechanisms mediating the effects of DNA-damaging agents on *rnrB* operate independently from processes which regulate the expression of *rnrB* during growth and development. In support of this finding, the *repB* and *repD* genes have been shown to be induced by DNA-damaging agents at different stages of the *Dictyostelium* life cycle (35).

Hydroxyurea is a specific inhibitor of ribonucleotide reductase. It inactivates the tyrosyl free radical of the small subunit that is required for enzyme activity. Overexpression of the small subunit is sufficient to confer resistance to hydroxyurea in mammalian cell lines (43). The presence of hydroxyurea leads to the depletion of nucleotides, and consequently blocks DNA synthesis. The inhibition of DNA synthesis by hydroxyurea can be reversed by addition of nucleotides (reviewed in 44). Hydroxyurea causes an increase in *RNR2* transcript level in yeast (36,45–47). In response to hydroxyurea mammalian cells increase the rate of synthesis and the stability of both subunits of ribonucleotide reductase (48) but do not alter the levels of their transcripts (49). As in mammalian cells, hydroxyurea does not stimulate the accumulation of *rnrB* transcript in *Dictyostelium*. However, we do not know if hydroxyurea has an effect on the post-transcriptional regulation of ribonucleotide reductase in *Dictyostelium*.

To our knowledge, this is the first report that shows the involvement of different *cis*-regulatory elements in the response to different DNA-damaging agents. We observed that the *cis*-regulatory element box C was able to confer transcriptional response to both MMS and 4NQO. We also showed that box A mediated the response to MMS whereas box D promoted the response to 4NQO. The reason for the involvement of different *cis*-regulatory elements is not clear. These two drugs have different modes of action, MMS induces alkylation of DNA while 4NQO generates bulky adducts (1). The damage caused by these two drugs could activate a different, yet overlapping, set of transcription factors. Another possibility is that the damaged DNA is not the only cause of the response. MMS can generate alkylation damage to cellular components other than DNA damage, and 4NQO and UV are known to cause oxidative stress. These other types of damage could trigger signal transduction cascades that result in the expression of the *rnrB* gene in addition to the DNA damage signal.

The results obtained from promoter analysis are complex, especially those involving internal deletions. There are several reasons for some of the inconsistencies observed. Spacing between regulatory elements may influence the level of expression. For example, the internal deletion construct containing both box A and box B (–444Δ–311) was induced to a higher level than the construct containing only box A (–444Δ–280). But in the 5' deletions, the construct containing both box A and box B (Δ–311) exhibited a similar level of induction as the construct containing box A alone (Δ–280). Moreover, the transcript produced by the *lacZ* reporter is unstable (50,51; unpublished data). The latter observation may in part explain why the level of induction for *lacZ* is lower than that of the endogenous *rnrB* (Fig. 5).

Dictyostelium is highly resistant to radiation. Doses of UV light causing 90% killing range from 150 to 200 J/m^2 (23,52). In mammalian cells and yeast, a similar level of cell death is obtained with 30 J/m^2 (53–57). There is growing evidence that survival to genotoxic stresses in mammalian cells is determined by the ability to repair essential genes rather than to perform genome-wide repair (56–59). In *Dictyostelium*, there is indication of two different pathways for repairing UV-induced DNA lesions, one transcription-dependent and one transcription-

independent system (60,61). This might explain the unusual resistance of this organism to radiation. We show here that irradiation with UV strongly stimulates *rnrB* expression. A dose of 30 J/m^2 caused a 15-fold increase in the accumulation of *rnrB* transcript. In yeast, a similar dose elicits a 2–4-fold increase (36,45). Therefore, the high degree of resistance to UV in *Dictyostelium* cells might result from increased availability of dNTPs for repair. The high level of *rnrB* expression might enhance the repair capability of *Dictyostelium* cells to UV irradiation.

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