Prolonged Inhibition by X-Rays of DNA Synthesis in Cells Obtained by Transformation of Primary Rat Embryo Fibroblasts with Oncogenes H-ras and v-myc

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ABSTRACT

Transfection of primary rat embryo fibroblasts with the H-ras oncogene plus the cooperating oncogene v-myc results in the development of foci of morphologically altered tumorigenic cells. We examined radiation (X-rays) induced inhibition of DNA synthesis in cell lines derived from such transformed clones and compared the results to those obtained with the nontransformed parental cells, rat embryo fibroblasts, as well as with cells immortalized either spontaneously, or after transfection with nuclear oncogenes (v-myc, E1A). Inhibition by X-rays of DNA synthesis was higher and persisted for longer periods of time in the H-ras plus v-myc-transformed cell lines as compared to their nontransformed counterparts. When the rate of DNA synthesis was measured as a function of dose 3 h after irradiation, biphasic curves were observed in all cell lines tested with a radiation sensitive and a radiation resistant component, known to correspond to inhibition of replicon initiation and chain elongation, respectively. A substantially larger inhibition of DNA synthesis was observed between 0 and 30 Gy in H-ras plus v-myc-transformed cell lines, as compared to their nontransformed counterparts, presumably caused by sustained inhibition of replicon initiation. Hypersensitive DNA synthesis to X-rays was also observed in a transformed cell line obtained by transfection of rat embryo fibroblasts with H-ras in cooperation with the oncogene E1A, but normosensitive DNA synthesis in a rare transformant obtained by transfection with H-ras alone. These results suggest a direct or indirect involvement of the oncogene H-ras in cooperation with the oncogene v-myc (or other nuclear oncogenes such as E1A) in the control of DNA synthesis in irradiated cells. This control of DNA synthesis may be mediated via a trans-acting mechanism that involves the production of a diffusible factor in response to the radiation insult, or, by a cis-acting mechanism that directly affects the replication machinery. Circumstantial evidence for possible involvement of oncogenes of the ras and myc families in DNA synthesis support this hypothesis. There was an inverse correlation between sensitivity to radiation-induced killing and prolonged inhibition by radiation of DNA synthesis, with radioresistant cell lines displaying longer inhibition of DNA synthesis. However, inhibition by radiation of DNA synthesis was similar in normal human fibroblasts (W138) and cells derived from a radiation-resistant human carcinoma cell line (SQ-20B) suspected to carry an abnormal c-raf-1 oncogene. In addition, inhibition of DNA synthesis in SQ20B cells was similar to that of SCC61 cells, a radiation-sensitive cell line derived from a carcinoma of the tongue.

INTRODUCTION

There is evidence associating expression of certain types of oncogenes in cells of various origins with resistance to ionizing radiation. Chang et al. (1) reported that normal fibroblasts from skin biopsies of patients with the Li-Fraumeni syndrome show overexpression of the myc and activation of the raf oncogene and display at the same time increased resistance to ionizing radiation. Kasid et al. (2, 3) demonstrated that a cell line developed from a clinically radioresistant human squamous cell carcinoma is also characterized by an activation of the c-raf-1 oncogene. FitzGerald et al. (4) observed increased radioreistance in NIH3T3 cells containing the human N-ras gene. Increased radioreistance in NIH3T3 cells transfected with various forms of the ras oncogene has also been reported by Sklar (5). These findings correlate diverse genetic alterations with modifications in the intrinsic radiosensitivity of a cell and may be helpful in the elucidation of the basic genetic processes that determine cell radiosensitivity.

Recently, it was shown that in primary RE cells, H-ras confers radioreistance but that the effect of this oncogene, when transfected alone, is small. However, when H-ras is co-transfected with the cooperating oncogene v-myc, transformed clones are isolated that display a significant increase in radioreistance, as compared to nontransfected primary RE fibroblasts (5–9). These results suggest that cooperation of at least two oncogenes is required to confer radioreistance in cells taken directly from the animal. Cooperation between a myc and a ras oncogene has also been established for cell transformation (10–13), suggesting that, as with transformation, radiosensitivity also requires multiple alterations in the regulation of various cellular metabolic pathways. Identification of altered metabolic pathways and processes in myc plus ras transfected cells is of importance in oncogene research, since it provides information on the mechanism of action of these oncogenes in transformed cells, as well as on the action of the corresponding protooncogenes in nontransformed cells.

Although radioreistance associated with expression of oncogenes has been demonstrated in a number of cell lines, little is known about the mechanism that underlies the phenomenon. Radiation sensitivity, and, in direct analogy, radiation resistance, is a complex phenotype that can be produced by alterations in a number of probably independent cellular metabolic processes, each of which may be under different genetic control. Processes whose alteration is expected to affect radiation sensitivity include DNA repair and progression through the cycle; the latter process being further dissectable into alterations in G1 and G2 delays, as well as in alterations in radiation-induced inhibition of DNA synthesis. We have carried out experiments to evaluate the involvement of the above mentioned processes in the radiosensitive phenotype induced by transfection of RE fibroblasts with the oncogenes H-ras and v-myc. Because there is compelling evidence that the DNA dsb is the lesion responsible for lethality in cells exposed to ionizing radiation (see references in Ref. 14), induction and rejoining of this type of lesion was measured in a radioresistant cell line produced by transfection with the oncogenes H-ras plus v-myc (14). There was no difference either in the induction, or in the rate of rejoining, of DNA dsb between radioresistant and radiosensitive RE cell lines, suggesting that alterations in the induction or in the rejoining of DNA dsb is not a major determinant for H-ras

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The abbreviations used are: RE, rat embryo; dsb, double strand break; MR, MycRec; AT, ataxia telangiectasia; SIF, synthesis-inhibiting factor; REF, rat embryo fibroblasts.
plus v-myc-induced radioresistance (14). Study of cell cycle perturbations after irradiation, on the other hand, showed that radioresistant cell lines, produced by transfection with the oncogenes H-ras plus v-myc, undergo a significantly longer division delay compared to two immortalized radiosensitive cell lines that served as control (15). These results suggested a relationship between prolonged delays in the progression of irradiated cells through the cycle and increased resistance to radiation of cells transfected with oncogenes, and prompted experiments designed to investigate inhibition by radiation of DNA replication.

Here we report that the radioresistant phenotype observed in cell lines produced by transfection of RE fibroblasts with the oncogenes H-ras plus v-myc is associated with prolonged inhibition by radiation of DNA synthesis and show that this inhibition requires transfection with both oncogenes.

MATERIALS AND METHODS

Cell Culture. Table 1 gives a summary of the cell lines used in the experiments of the present study together with information regarding their origin, status (primary, immortalized, transformed), transfected oncogenes, radiation sensitivity, and references wherefrom the information given was extracted. All cell lines derived on rat embryo background were kindly provided by Dr. W. G. McKenna (University of Pennsylvania). SQ20B and SCC61 were kindly provided by Dr. J. B. Little (Harvard University), and W138 cells by Dr. P. Philipps (Medical College of Pennsylvania). Cell lines derived from primary rat embryo fibroblasts (including MycRec) were grown in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum, at 37°C in a humidified incubator in an atmosphere of 5% CO2 and 95% air. Cells were kept in a quasi continuous culture, by subculturing every second day at an initial density of 5–10 x 105 cells/flask (80-cm2 culture flask, 20 ml medium). SQ20B and SCC61 cells were grown under the same conditions in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 0.4 μg/ml hydrocortisone; they were subcultured once a week by splitting at a 1:10 and 1:4 ratio, respectively. W138 cells were used at population doublings between 25 and 35 and were grown in minimal essential medium supplemented with 10% fetal bovine serum. All media were supplemented with penicillin and streptomycin.

DNA Synthesis. For experiments, 1–5 x 105 cells were seeded in 60-mm tissue culture dishes (3 ml medium/dish) and were allowed to grow for 2 days in the presence of 0.37 kBq/ml [14C]thymidine and 2.5 mM cold thymidine. Cells were irradiated at room temperature and returned, after changing medium, to 37°C for various periods of time as required. For the experimental protocol, after this incubation period, [3H]thymidine at 37 kBq/ml was added for 30 or 60 min. Cells were trypsinized, loaded on glass microfiber filters (Whatman GF/A), washed twice with deionized water, and incubated for 20 h in 0.5 ml of 1 N NaOH at 60°C. Subsequently, filters were neutralized with HCl and scintillation fluid (Scintiverse, Fisher) was added. Samples were counted for [3H] and [14C] activity in a liquid scintillation counter (Packard TriCarb 2200CA), using a protocol that allows simultaneous counting for both isotopes and corrects for quenching and spillover. The rate of DNA synthesis was calculated as [3H] dpm/[14C] dpm for the various samples and is presented as percentage of the values obtained in sham-irradiated cultures at the corresponding times. In some experiments (Fig. 1) cells were not prelabeled with [3H]Thymidine; in this case, equal numbers of cells were loaded on the filters. The rate of DNA synthesis was calculated by the ratio between [3H] dpm in the various samples and [14C] dpm in samples obtained at various times for D = 0 Gy.

Flow Cytometry. The distribution of cells throughout the cell cycle was measured by flow cytometry. For this purpose, cells were stained by direct suspension in a solution containing 0.1 M Tris, 0.1 M NaCl, 5 mM MgCl2, 0.01% Triton X-100 and 2 μg/ml 4',6-diamidino-2-phenylindole. For the measurement an arc lamp flow cytometer was used (Partec, PAS II), interfaced to a computer, and equipped with the necessary software to calculate the fraction of cells in G1, S, and G2 + M phase (15).

Cell Survival. To measure radiosensitivity, RE cells (various cell lines) grown under the same conditions as in DNA synthesis experiments were exposed to graded doses of radiation, trypsinized, counted, and plated after the appropriate dilution in 60- or 100-mm tissue culture dishes aiming for 25–200 colonies/dish. This irradiation protocol is slightly different from the one previously used with these cells (7–9); therefore, results slightly different from those previously reported were obtained.

Irradiation. Cells were exposed to X-rays generated by a 250-kV therapeutic machine operating at 10 mA, with 2 mm Al filter, effective photon energy 70 keV. The dose rate at 25-cm irradiation distance was 8.4 Gy/min. Dosimetry was performed by using a Victoreen dosimeter.

RESULTS

The rate of DNA synthesis in MycRec, 3.7, and 2.8 cells exposed to 50 Gy X-rays, expressed as percentage of the rate measured in unirradiated controls, as a function of the postirradiation incubation time at 37°C is shown in Fig. 1. The period shown in the abscissa reflects the time between irradiation and addition of [3H]Thymidine and does not include the subsequent 30 min of incubation for label incorporation. In MR cells, exposure to radiation caused an immediate reduction in the rate of DNA synthesis to 65% of that of unirradiated controls. The rate of DNA synthesis decreased further during the subsequent incubation at 37°C and reached a minimum at 45% after 2 h. Some recovery was observed at later times and the rate of DNA synthesis approximated a plateau at 70–80% of the control levels between 3 and 6 h. Radiation exposure also inhibited DNA synthesis in 2.8 and 3.7 cells, albeit to a larger degree than in MR cells. The rate of DNA synthesis was at 45 and 55% of the control level immediately after irradiation in 2.8 and 3.7 cells, respectively. Postirradiation incubation at 37°C led to a further reduction in the rate of DNA synthesis; a plateau was reached after about 3 h. There was no evidence for recovery of the rate of DNA synthesis in 2.8 and 3.7 cells for up to a 6-h postirradiation incubation. A large difference in the degree of inhibition became thus apparent between 2.8 and 3.7 versus MR cells that reached maximum values between 3 and 6 h after irradiation. The difference is the combined result of a slightly larger initial inhibition of DNA synthesis and a lack of recovery in 2.8 and 3.7 versus MR cells. Based on the results of Fig. 1, all subsequent experiments were carried out with cells that were given [3H]Thymidine 3 h after irradiation.

The prolonged inhibition of DNA synthesis observed in 3.7...
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Fig. 1. Rate of DNA synthesis, expressed as percentage of the values measured in unirradiated controls as a function of postirradiation (50 Gy) incubation time, in MR, 2.8, and 3.7 cells. Points, mean from 4 independent determinations, bars, SE. Error bars were omitted from 2.8 cells to reduce overlapping but were similar in size to those obtained with MR and 3.7 cells. The period shown in the abscissa reflects the time between irradiation and addition of [3H]thymidine and does not include the subsequent 30 min of incubation for label incorporation.

and 2.8 cells could, in principle, be attributed either to a reduction in the rate of DNA synthesis per cell, or, to a selective depletion of S-phase cells from the irradiated population as a result of perturbations in the progression through the cell cycle. To test this possibility, identically grown cultures of MR and 3.7 cells were exposed to X-rays and the distribution of cells through cycle was measured by flow cytometry, at various times thereafter. The results obtained are shown in Fig. 2. Plotted in the figure is the relative change in the fraction of cells in S, calculated by comparison to the nonirradiated control cells, after exposure to various doses of X-rays and measured 1.5 h, or, 3 h after irradiation. There was no change in the fraction of cells in S, within the experimental uncertainties, at all radiation doses examined in either cell line when measured 1.5 h after irradiation. At 3 h, however, an increase in the fraction of cells in S phase was observed, that was larger in 3.7 than in MR cells. These results suggest that the reduction in the rate of DNA synthesis observed in Fig. 1 is not due to a depletion of S-phase cells, and that the larger reduction in DNA synthesis in 3.7 is not due to a smaller percentage of increase in the fraction of S-phase cells (actually the opposite was observed).

Dose-response curves for inhibition by radiation of DNA synthesis in MR and 3.7 cells with radioactive label added 3 h after irradiation are shown in Fig. 3. The interval allowed for label incorporation in this and all subsequent experiments was 1 h. A biphasic response was observed in both cell lines, suggesting a radiation-sensitive and a radiation-resistant component. Similar experiments with other cell lines, in combination with velocity sedimentation measurements in alkaline sucrose gradients, have demonstrated that the radiation-sensitive component at low doses reflects inhibition by radiation of repilon initiation, whereas the radiation-resistant component, at high doses, reflects inhibition of DNA chain elongation (16, 17). In agreement with the results shown in Fig. 1, there was more inhibition of DNA synthesis in 3.7 than in MR cells for doses above 20 Gy. In 3.7 cells the steep component, at low doses, has a $D_0$ of $8 \pm 1$ Gy, and the shallow component, at high doses, $D_0$ of $118 \pm 5$ Gy. $D_0$ is the negative inverse slope value. It was not possible to determine accurately the steep component in MR cells due to the lack of measurements below 10 Gy where it was dominant, but the approximate values obtained were in the same range as for 3.7 cells. The shallow component reached in MR cells a $D_0$ of $82 \pm 5$ Gy. These values are similar to those reported for other cell lines (17) and suggest similar targets to radiation-induced inhibition of DNA synthesis for 3.7 and MR cells as well.

There was a large difference in the overall inhibition afforded by the initial, radiation-sensitive component, of DNA synthesis

Fig. 2. Relative change (compared to untreated controls) in S-phase cell fraction in MR and 3.7 cells exposed to various doses of X-rays as indicated, and measured for distribution through the cycle 1.5 h and 3 h later. Distribution of cells through the cycle was measured by flow cytometry.

Fig. 3. Dose-response curves for inhibition by radiation of DNA synthesis in MR and 3.7 cells. [3H]Thymidine was added to the cells 3 h after irradiation. Points, mean from 6 independent determinations; bars, SE.
between 3.7 and MR cells. Whereas in 3.7 cells the initial steep component reduced synthesis to approximately 20% (values determined as the intercept between the y axis and a back extrapolation of the line defined by the shallow component) of the control levels, it inhibited synthesis to only approximately 80% of the control levels in MR cells. Assuming that this initial inhibition of DNA synthesis reflects inhibition of replicon initiation (17), it can be concluded that exposure to radiation induces a stronger and more persistent inhibition of replicon initiation in 3.7 cells than in MR cells. Since 3.7 cells have been obtained by transformation with the oncogenes H-ras and v-myc we hypothesized that the observed effect of DNA synthesis is associated, directly or indirectly, with the presence of these oncogenes.

To investigate whether this hypothesis is of more general validity, we examined the dose response for inhibition of DNA synthesis of another cell line also produced by transformation with the H-ras plus v-myc oncogenes, the 2.8 cells. The results obtained with these cells are shown in Fig. 4. The values obtained for the rate of DNA synthesis are essentially superimposable to those of 3.7 cells. The broken line shows the results obtained with 3.7 cells and has also been transferred from Fig. 3. Points, mean from 3 independent determinations, bars, SE.

We then examined whether prolonged inhibition by radiation of DNA synthesis can be induced by transfection and transformation with the H-ras oncogene alone. For this purpose, a rare transformant obtained by transfection with H-ras (line 4R) was examined for DNA synthesis radiosensitivity under the conditions described above. The results obtained are shown by the closed squares in Fig. 5. The dose response of 4R cells was similar to that of MR cells, suggesting that transformation with H-ras alone does not alter the response to radiation of DNA replication.

The above presented results provided evidence for cooperation of the oncogenes H-ras and v-myc in conferring the phenotype of prolonged inhibition of DNA synthesis in irradiated REF. Since results previously published on transformation suggest that in addition to myc, other nuclear oncogenes, such as the early region gene IA of adenovirus, can also cooperate with ras genes to induce full transformation of primary rat cells in culture (11), we examined the radiosensitivity of DNA synthesis in one immortalized cell line produced by transfection of RE fibroblasts with the oncogenes EIA, REFAdJ, as well as of a transformed, radioresistant cell line obtained by cotransfection of RE fibroblasts with the oncogenes H-ras plus EIA, AdEj. The results obtained with AdEj and REFAdJ cells are included in Figs. 4 and 5, respectively. It is evident that immortalized REFAdJ cells show a response similar to that of MR cells, the figure. Levels of inhibition of DNA synthesis similar to those observed in MR cells were also obtained with primary REF, with a cell line immortalized by transfection with the oncogene v-myc (MR4), and a spontaneously immortalized cell line (RC2) (see Table 1 for details). These results clearly indicate that the low sensitivity of DNA synthesis, as measured 3 h after irradiation, is not a peculiarity of MR cells and that it can also be observed in primary cells, as well as in cell lines immortalized by various means. The results of Fig. 5 further indicate that transfection with v-myc alone cannot produce phenomena similar to those observed in cells transfected with v-myc plus H-ras.
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whereas AdEj shows a response similar to that of 3.7 and 2.8 cells. Thus, prolonged inhibition by radiation of DNA synthesis can also be conferred by cooperation between the oncogenes H-ras and EIA.

All cell lines that displayed prolonged inhibition of DNA synthesis after exposure to ionizing radiation were previously reported to be radioresistant (7–9). We confirmed this property under the growth and irradiation conditions used in this study and the results obtained are shown in Fig. 6. 3.7 and AdEj cells were resistant to radiation as compared to MR cells and similar results were also obtained with 2.8 cells (data not shown to reduce congestion in the figure). 4R and REFAdl cells had a similar radiosensitivity as MR cells and similar results were also obtained with all other control cell lines used (again these results were not included in the figure to reduce congestion). Primary REF were more sensitive to radiation than either MR or 3.7 cells.

Since the results obtained with 3.7, 2.8, and AdEj cells implicated a possible correlation between radiosensitivity to killing and prolonged inhibition by radiation of DNA synthesis (see Table 2 for a summary) we inquired whether these two phenotypes are associated with each other, independently of the presence of specific oncogenes. For this purpose, the dose response for the inhibition by radiation of DNA synthesis was measured in a radioresistant and a radiosensitive human tumor cell line (SQ20B and SCC61, respectively), under conditions similar to those used for rat cells, and the results were compared to those obtained with a nontransformed primary human embryo cell line (WI38). The results obtained are shown in Fig. 7 and indicate that there is no difference in the inhibition of DNA synthesis either between radiation-sensitive and radiation-resistant, or, between transformed and nontransformed cells. These results suggest that prolonged inhibition of DNA synthesis is not a necessary consequence of radioresistance to killing, and that the oncogenes H-ras and v-myc/EIA may contribute to the development of both phenotypes in RE cells.

DISCUSSION

Inhibition by radiation of DNA synthesis in eukaryotic cells was one of the earliest effects of radiation to be described. It is usually assumed to result from the production by radiation of damage in the DNA (17–19). The dose response for synthesis inhibition shows biphasic curves, suggesting the influence of at least two independent processes, one showing high and one showing low radiosensitivity; they were identified as replicon initiation and chain elongation, respectively. Target size considerations suggested that damage in the DNA is likely to simultaneously inhibit initiation of synthesis in a cluster of replicons rather than individual replicons. Replicon initiation inhibition in clusters is in agreement with the observation that clusters of synthesis units initiate synchronously but that different clusters initiate at different times during DNA synthesis (20). It was proposed that this inhibition may be afforded by alterations in DNA supercoiling, as a result of break accumulation, that prevent binding of the synthesis complexes in a DNA segment containing the cluster of replicons (17). An alternative interpretation has been recently offered based on studies of synthesis of episomal plasmids in mammalian cells (21, 22). These studies showed that synthesis can be inhibited over a range of doses...
that produce no detectable damage in the plasmid DNA, and suggested that radiation damage produces a trans-acting factor released by radiation and inhibiting DNA synthesis.

That DNA synthesis may be affected by intracellular factors and gene products was also indicated by experiments studying inhibition of DNA synthesis in cells from AT patients (17, 18). AT cells are highly radiation sensitive and display at the same time highly radioresistant DNA synthesis. It was shown that chain elongation is completely resistant to ionizing radiation in these cells and that the limited inhibition of DNA synthesis observed is due to inhibition of replicon initiation at the level of individual replicons rather than at the level of clusters of replicons. Since there is no evidence for differences in the structural organization of replicons and clusters in AT versus normal cells, it was suggested that the increased radioresistance of DNA synthesis is due to the lack of a factor, or the presence of a defective factor, which in normal cells inhibits replicon initiation (17). This may either be a constitutive factor that recognizes radiation-induced damage and inhibits DNA synthesis in cis (17), or a factor released, induced, or activated in response to damage in the DNA and acting in trans (21).

The results presented in the previous section further indicate a direct or indirect involvement of the oncogenes H-ras and v-myc in the recovery of DNA synthesis inhibition generally observed in eukaryotic cells after exposure to ionizing radiation. The most predominant effect in our experiments was a prolonged inhibition of DNA synthesis in H-ras- plus v-myc-transformed cell lines, as compared to their nontransformed counterparts. In the sample of cell lines examined, either of these genes, when transfected alone, was unable to induce measurable changes in the radiation response of DNA synthesis. Cooperation of the products of both oncogenes was required in order to induce measurable alterations in response. This result suggests that the mechanisms of inhibition of DNA synthesis is a complex process likely to involve activation of transduction pathways.

Details regarding the individual steps involved and the processes associated with the regulation of this hypothetical mechanism are not available at the present time. The results presented here identify potential candidate genes and suggest the involvement of a factor inducing persistent inhibition of replicon initiation. Henceforth, we will use the term synthesis-inhibiting factor to describe this factor. The hypersensitivity to radiation of replicon initiation in H-ras plus v-myc transfected cells may be interpreted as sustained inhibition of DNA synthesis resulting from the production of SIF. Production of SIF may be regulated by the products of the oncogenes ras and myc. The steps of this regulation process are not understood, and it is not known whether similar effects can also be induced by the corresponding protooncogenes. However, there is evidence implicating myc in DNA synthesis that is reviewed next.

Addition of antibodies against the human c-myc protein to nuclei isolated from several types of human cells reversibly inhibited DNA synthesis (23), and an SV40-based vector replicated poorly in human BJAB lymphoma cells but well in Burkitt lymphoma lines which have fused immunoglobulin and c-myc genes, and, thus, high c-myc expression (24). Also, plasmids possessing the SV40 ori sequences could replicate even in the absence of SV40 T-antigen in human HL-60 and Raji cells, which are expressing the c-myc gene at high levels (25), and synthesis of a plasmid containing mouse sequences which can autonomously replicate in mouse and human cells was inhibited by cotransfection with c-myc antibody (26); in the latter system c-myc was found to bind to the origin of replication. Furthermore, a c-myc antisense oligonucleotide inhibited in T-lymphocytes entry into S phase but not progress from G0 to G1 (27). Finally, it has been recently reported that an initiation site of DNA synthesis with transcriptional enhancer activity is present upstream of the c-myc gene (28). Thus, c-myc may be important in the regulation of the cell cycle and intimately involved in the process of DNA synthesis. Circumstantial evidence also associates ras genes with growth, and thus, indirectly, with DNA synthesis (29).

Based on these results, we hypothesize that damage in the DNA releases signals that activate cytoplasmic processes involving or influenced by the H-RAS protein, which in turn induce MYC-dependent nuclear processes that regulate transcription and/or activation of SIF. It is tempting to speculate that SIF, and the putative factor involved in the inhibition observed in DNA replication immediately after irradiation (see above), are identical. Oncogene-mediated alterations in the level or the control of transcription, the half-life of the message, or the translation of the sif gene could lead to prolonged presence of SIF and, thus, to the sustained inhibition of DNA synthesis observed. Experiments are in progress in our laboratory to examine this possibility.

The observation that all cell lines showing sustained inhibition of DNA synthesis were radioresistant to cell killing (see Table 2) suggests a correlation between the two phenotypes. It is possible that prolonged inhibition of DNA synthesis delays progression through the cycle of cells with excessively damaged DNA, allowing, thus repair and reducing fixation of radiation-induced damage. This explanation is analogous to the explanation given for the radiosensitivity of AT cells (16-18, 30, 31) based on the radioresistant phenotype of DNA synthesis. It was proposed that the genetic defect of AT cells leads to defective synthesis of a factor present in normal cells that inhibits DNA synthesis in damaged DNA. However, it has also been reported that radioresistant clones can be isolated from AT cells, following DNA-mediated gene transfer, that have normal radiosensitivity to cell killing but AT-like inhibition of DNA synthesis (31, 32). These results and the observation that there was no correlation between cell radiosensitivity and inhibition of DNA replication in the human cell lines examined, suggest that the correlation should be regarded as preliminary, and valid only for REF cells.

In summary, the results presented indicate that transfection of RE cells with the oncogenes H-ras and v-myc induces genetic alterations that affect the mechanism that controls DNA replication in irradiated cells. It is hypothesized that this occurs via the induction of transduction pathway(s) that transmit signals from damaged nuclear DNA to the cytoplasm and then back to the nucleus in order to modulate transcription or activation of key factors that inhibit DNA synthesis; H-ras and v-myc gene products may be components of this pathway(s).

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