Enhanced G₂ Chromatid Radiosensitivity, an Early Stage in the Neoplastic Transformation of Human Epidermal Keratinocytes in Culture

Raymond Gantt, Katherine K. Sanford, Ram Parshad, Floyd M. Price, Ward D. Peterson, Jr., and John S. Rhim

ABSTRACT

A deficiency in DNA repair, manifest as enhanced chromatid radiosensitivity during the G₂ phase of the cell cycle, together with a proliferative stimulus such as that provided by active oncogenes may be necessary and sufficient for the malignant neoplastic transformation of human keratinocytes in culture. Normal epidermal keratinocytes established as continuous cell lines by transfection with pSV3-neo or infection with adenovirus 12-SV40 hybrid virus developed enhanced G₂ chromatid radiosensitivity after 18 passages in culture. In contrast to cells from primary or secondary culture, these cells could be transformed to malignant neoplastic cells by infection with Kirsten murine sarcoma virus containing the Ki-ras oncogene or in one line by the chemical carcinogen, N-methyl-N'-nitro-N-nitrosoguanidine; both of these agents produced a marked proliferative response. Cytological heterogeneity and karyotypic instability characterized the cells during their progression to neoplasia. These results are interpreted in terms of a mechanism for neoplastic transformation.

INTRODUCTION

The slow multistep nature of cancer development in both in vivo and cell culture has been recognized for many years, and various aspects of "spontaneous" and induced carcinogenesis have been elucidated. However, much still remains unknown, particularly with respect to the mechanism of initiation and the force(s) that drives the progression from normal through pre-neoplastic to neoplastic state and ultimately to tumor cell heterogeneity (1). Studies of carcinogenesis in culture are frequently limited to comparisons of normal cells and their neoplastic derivatives which may have undergone secondary changes unrelated to primary events in carcinogenesis. Furthermore, human cells, in contrast to those of the mouse, rat, and hamster, have proved resistant to transformation in culture, although a few reports of such transformations have appeared in the literature.

The introduction of techniques for transfecting viral or cellular oncogenes into mammalian cells in culture has stimulated renewed interest in analysis of the steps leading to neoplasia. To this end, we have induced neoplastic transformation in human epidermal keratinocytes. This cell type seemed appropriate because of the relevance of epithelial cells to human cancers, most of which are carcinomas of epithelial cell origin. In two recent studies, neoplastic transformation, manifested as carcinoma formation in nude mice, was attained by infection of a primary culture with SV40 adenovirus 12 hybrid virus and subsequent infection with Ki-MSV (9) or treatment with the chemical carcinogen MNNG (2-4).

In the present study we have determined whether human epidermal keratinocytes, stimulated to continued proliferation, acquire enhanced G₂ chromatid radiosensitivity, and if so, how this cellular alteration is related to other steps in their progression to neoplasia. Enhanced G₂ chromatin radiosensitivity is manifested as a quantitative increase, relative to control cells, in frequency of chromatid breaks and gaps seen directly after irradiation during the G₂ period of the cell cycle with X-rays or fluorescent light; the effective wavelength of the latter is 405 nm in the visible range (5). This enhancement characterizes skin fibroblasts from genetically cancer-prone individuals (6-15) and all neoplastic cells examined to date (16-19). Previously, we showed that it has a genetic basis and results from a deficiency(ies) in DNA repair during G₂ (9, 20-22).

MATERIALS AND METHODS

Cells and Culture Procedures. Cultures of human epidermal keratinocytes were prepared from 3-day foreskin as described (23). Cells were grown in T-25 plastic flasks containing 4 ml NCTC 168 medium with 20% fetal bovine serum (FBS) (Flow Laboratories, McLean, VA), 5% Nu-Serum (Collaborative Research, Inc., Lexington, MA), and 5 µg/ml hydrocortisone (Behring Diagnostics, La Jolla, CA). Cultures were split 1:1 if the cells formed a confluent sheet slowly (2-7 weeks) and 1:2 if they reached confluence rapidly (1-2 weeks) or were postconfluent. In subculturing, cells were rinsed twice with EDTA (1:500; M. A. Bioproducts, Walkersville, MD) and incubated at 37°C with 0.5 ml trypsin (0.2% in EDTA, lyophilized 3 times; Worthington Biochemical Corp., Freehold, NJ) until the cell sheet detached. Medium was then added and the cells dispersed by pipet. Although antibodies were used for about 1 month on the primary culture, they were removed thereafter. Cells tested negative for Mycoplasma by direct staining and indirect immunofluorescence (24, 25) (Flow Laboratories).

Plasmid Transfection. Three plasmids, pSV3-gpt, pSV3-neo (American Type Culture Collection, Rockville, MD), and clone 4(E) pKi-MSV (supplied by Dr. S. Tronick of this laboratory, obtained originally from Dr. N. Tsuchoida) were used singly or in combination in 15 transfection experiments on secondary cultures. The plasmids pSV3-gpt and pSV3-neo contain the SV40 origin, early promoter region, polyadenylation sequences, and sequences coding for large and small tumor antigens (26, 27), whereas clone 4(E) pKi-MSV contains the ras oncogene from Kirsten SV (28).

Purified plasmid DNA (29) was transfected (20-30 µg/culture) into the cells by procedures described (30) as a calcium phosphate coprecipitate 24-48 h after a 1:3 split of a dense primary culture. In the last 4 of 15 experiments cells were treated for 2 min at 37°C with the insertion mixture (30 g polyethylene glycol, M, 6000, autoclave-sterilized for 20 min, and cooled to 45°C in 60 ml of medium without serum) essentially as described by Sutherland and Bennett (31). The cells were then rinsed three times with phosphate buffer saline, and 4 ml of growth medium containing 0.5 µl of the appropriate DNA-calcium phosphate suspension were added. After 24 h incubation the medium was removed, and the cells were rinsed with phosphate buffer saline and cultured in 4 ml of growth medium.

In all experiments control cells were treated with the calcium phosphate solutions, and in most experiments with calf thymus DNA (20-30 µg/culture) calcium phosphate suspension. In a double plasmid transfection, equal concentrations of plasmids were mixed, precipitated, and a total of 20-30 µg DNA were added per culture. Selective media for the pSV3 plasmids were not used since they inhibited growth of control cells, and our selection was for cells that continued to proliferate.
in culture showing enhanced or infinite life span. In the last 4 experiments we also plated cells in agarose (10^5 cells/T-25) by methods described (32) to select for anchorage-independent colonies.

Treatment with Carcinogenic Agents. A culture of the continuous pSV3 neo-transfected line (NCTC 11367) in the 20th passage was infected with Ki-MSV (BaEV), 10^4 plaque-forming units/ml, produced in human nonproducer cells (33) by superinfection with BaEV (34). This culture gave rise to line NCTC K11367. Another culture of NCTC 11367 in the 17th and again in the 21st passage was treated with 0.2 µg/ml MNNNG (Sigma) in sterile dimethyl sulfoxide, 0.5% final concentration (Baker-analyzed reagent grade; J. T. Baker Chemical Laboratory, Phillipsburg, NJ) to initiate a line designated NCTC M11367.

Assay for G2 Chromatid radiosensitivity. Cells to be assayed for chromosomal radiosensitivity were cultured for at least 2 weeks prior to test in Dulbecco's modification of Eagle's minimum essential medium with 10% fetal bovine serum. The assay procedures for irradiation by X-rays or fluorescent light have been described (9, 10). Briefly, 5 to 8 x 10^5 cells in 2 ml of medium were inoculated into Leighton tubes, each containing a 9 x 50-mm coverslip. After 48 h incubation at 37°C, cultures were irradiated with either 100 R or cool-white fluorescent light. After irradiation, cultures were incubated for approximately 30 min to allow mitotic cells to complete division, culture fluid was renewed and Colcemid was added (0.1 µg/ml) for 1 h or as indicated to accumulate metaphase cells. For chromosome analysis, the irradiated and control cells were processed in situ on coverslips. Analyses were made on randomized coded preparations; four cultures were used for each variable, and 100 metaphase cells were analyzed per variable except as indicated.

Aberrations scored as chromatid breaks showed distinct dislocation and misalignment of the broken segment. Aberrations scored as gaps showed a chromatid discontinuity longer than the chromatid width, but no displacement of the segment distal to the lesion.

Synchronization of cells was unnecessary since only metaphase cells were examined for chromatid damage and these were examined at 1.5 h after irradiation; we could, therefore, be assured that the cells were in G2 at the time of irradiation.

Cytological Procedure. For cytology, cells dispersed with 0.1% trypsin (3 x crystalline in 1:5000 Versene solution) were inoculated onto coverslips (9 x 50 mm; No. 1 thickness; Biejo Glass Co., Vineland, NJ) in Leighton tubes and incubated for 48 h before fixation in 95% ethanol. Coverslips were stained with Gill's modification of the Papanicolaou method (35) and mounted in Permount on glass slides. Photomicrographs were taken with Kodak Technical Pan 2415.

Assay for Tumorigenicity. In each assay 10^5 cells were inoculated s.c. into 4 or 5 nude mice, NFR-nu/nu or NIH-nu/nu. These were examined for tumors over a 4-month period, and sections were taken for histological diagnosis.

Assay for SV40 T-Antigens. Extracts from cells that had been labeled with [35S]methionine for 4 h were subjected to indirect immunoprecipitation with either control hamster serum or hamster SV40 tumor antiserum. The cell extracts used were from either pSV3-neo-transfected human keratinocytes (NCTC 11367) or SV40-infected African green monkey kidney cells. The precipitates were analyzed on a 10% sodium dodecyl sulfate-polyacrylamide gel by methods described (36).

Analysis for ras Oncogene p21 Product. [35S]Methionine-labeled cell extracts from uninfected cells and Ki-MSV-infected cells were immunoprecipitated with p21 monoclonal antibody Y13-259 (37) and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (38).

RESULTS

Life Span of Transfected Cultures. In 15 experiments carried out over an 18-month period, 200 cultures were transfected, 115 with pSV3-gpt or -neo and 85 with both pSV3 and pKi-MSV; 101 cultures served as controls. Transfections which included pSV3 increased life span usually by 3 subcultures (1:2 splits); however, with one exception, epithelial cells ultimately ceased proliferation after ~6 subcultures (1:2 splits). Although an occasional colony developed in agarose, cells of these colonies failed to survive subculture. Transfection with the two plasmids (pSV3 + pKi-MSV) did not increase life span beyond that obtained with pSV3 alone. Only one of the 200 cell lines followed showed continuous apparently infinite life span. Cells of this line, designated NCTC 11367, maintained characteristics of epidermal keratinocytes, showing desmosomes and tonofilaments (Fig. 1). The line was subcultured weekly at a 1:4 split ratio and cryopreserved after 52 passages, without evidence of declining proliferation rate. It originated in one of the last 4 experiments in which the cells were transfected with pSV3-neo following polyethylene glycol treatment. This transfection procedure, however, proved inhibitory to growth in most control and treated cultures.

Thus, alteration to a continuous cell line with apparently infinite life span was a rare event under the transfection conditions used. Although we have no direct evidence for integration of the SV40 plasmid DNA, large and small SV40 tumor antigens were detected at passage 24 by indirect immunoprecipitation (Fig. 24). Another continuous line of epidermal keratinocytes, established previously in this laboratory, through infection with the hybrid virus adeno-12-SV40 also expressed SV40 tumor antigens (2, 3). This line, adeno-12-SV40 line (RHEK-1) (4), will be compared with line NCTC 11367 with respect to certain properties associated with neoplastic transformation.

Treatment with Carcinogenic Agents. Our repeated efforts over the years to induce in vitro neoplastic transformation of human epidermal keratinocytes by irradiation (X-rays, visible light), chemical carcinogens, [MNNG, benzo(a)pyrene, dimethylbenzanthracene] or both were unsuccessful (39), as

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were also efforts with Ki-MSV infection of primary and secondary cultures. The successful neoplastic transformation of the continuous line RHEK-1 with Ki-MSV containing the ras oncogene prompted us to apply a similar approach to cells of line NCTC 11367. Infection of the line at passage 20 with Ki-MSV (BaEV) readily produced foci of piled up rounded cells similar to those observed in RHEK-1 infected with Ki-MSV (2, 3) (Fig. 3, A and B). The cultures released focus-forming virus, proliferated rapidly, and showed increased expression of K-ras p21 protein confirming the presence of the K-ras oncogene (Fig. 2B).

The cells treated with MNNG, line M11367, did not show focus formation; however, in contrast to the untreated control line 11367, they did produce numerous colonies when plated in agarose at passage 37.

Cytopathology. Cytomorphological criteria of neoplastic transformation applicable to fixed and stained cell cultures have been described that correlate closely with the neoplastic characteristics of the cells as determined by their tumorigenicity in vivo (20, 40, 41). Papanicolaou-stained cultures of lines RHEK-1 and NCTC 11367 were examined before and after treatment with carcinogenic agents for cytological properties associated with cancer (42–44). Representative cells with descriptions of their properties are presented (Figs. 4 and 5).

Cells of the continuous line 11367 transfected with pSV3-neo did not differ substantially in cytomorphology from untreated controls with the exception of some piling up associated with more active proliferation (Figs. 4A, 4B, 5A, and 5B). In contrast, RHEK-1 cells treated with the hybrid virus Ad-12-SV40 showed some pleomorphism, multinucleation, and other properties indicative of neoplasia; however, the majority of the cells appeared nonneoplastic (Fig. 5C and D). Both cell lines treated with Ki-MSV developed subtle alterations indicative of neoplasia (Figs. 4C, 4D, 5E, and 5F) and the line RHEK-1 treated with MNNG (MRHEK-1) showed extensive cytological features associated with clinical cancer (Fig. 5G and H). Line 11367 treated with MNNG resembled control cells when examined at the 15th passage, but at the 25th passage some areas of cells had altered (Fig. 4E and F). This alteration may be associated with their acquired capacity to grow in agarose observed at passage 37. Cells cultured from the tumor of K11367 cells showed cytological characteristics associated with clinical malignancy (Fig. 4G and H).

Tumorigenicity. Cells of NCTC line 11367 were injected at passage 20 into 4 nude mice and gave rise 2 months later to nonprogressive epidermal cysts at all inoculation sites; cells of M11367 were also nontumorigenic when assayed at passage 37. Cells of K11367 were injected at passage 25 into 5 nude mice and gave rise 1 month later to tumors diagnosed as poorly differentiated epidermoid carcinomas (Fig. 3Q). Cells of KRHEK-1 and MRHEK-1 also grew as carcinomas in nude mice as described (2–4). All tumors were cytogenetically identified as of human origin.
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Fig. 4. Representative cells from cultures of NCTC 11367 and derivatives photographed at low (x 93) and high (x 217) magnification. A, B, cells of 31st passage showing relatively uniform cell and nuclear size and shape, low nuclear:cytoplasmic ratio with well spread cytoplasm, evenly dispersed small chromatin granules, and rounded nucleoli; C, D, cells of K11367, 3 passages after Ki-MSV infection showing variation in cell size, altered chromatin pattern with some irregular distribution of granules, and variability in nucleolar size and shape; E, F, cells of M11367, 26 passages after treatment with MNNG, showing in certain areas increased nuclear:cytoplasmic ratio with retracted cytoplasm; however, cells have relatively uniform nuclear size and shape and small rounded nucleoli; G, H, cells cultured from tumor of K11367 cells showing variation in cell and nuclear size and shape, extremes in thickness and thinness of the chromatinic run (arrow), increased size of chromatin granules with irregular dispersion, and abnormally cleared parachromatin (arrowhead).

Cytogenetics. In contrast to line RHEK-1 which at passage 18 was still predominantly diploid (range, 44–50), line NCTC 11367 at passage 15 was heteroploid (range, 42–86) with only 32% of the cells diploid (2N = 46). At passage 25, this line was compared with line K11367 with respect to chromosomal aberrations. Both showed similar ploidy (distribution/100 metaphases 93–94 with 50–60 chromosomes and 6–7 with >100 chromosomes). Both lines had marker chromosomes revealed by Giemsa banding technique, 6 in line 11367 and 9 in line K11367; these appeared to have arisen by deletions or translocations. No abnormalities were detected in chromosome 12, containing the locus of cellular K-ras (45), but some differences were seen in X and chromosome 11, loci of cellular H-ras-2 and H-ras-1 genes (46) as well as in chromosome 22, locus of sis onc gene analogue (47).

Chromatid Radiosensitivity. The frequencies of chromatid breaks and gaps induced by X-irradiation (100 R) during G2 were initially low in both line NCTC 11367 and line RHEK-1 when assayed at passages 15 and 18, respectively (Fig. 6). By passages 28 and 25 the extent of radiation-induced chromatid damage had increased significantly. Thus, enhanced G2 chromatid radiosensitivity was acquired by both parental lines of epidermal keratinocytes. It was also acquired independently by lines KRHEK-1 and MRHEK-1 initiated from the parental line prior to the 18th passage; it may have been acquired independently by lines M11367 and K11367 initiated from the parental line at the 17th and 20th passages, respectively. Acquisition of enhanced G2 chromatid radiosensitivity, therefore, appeared to be a reproducible response of these proliferating cell populations.

Because X-irradiation produces a mitotic block ~1.5 h postirradiation, cells were also exposed to cool-white fluorescent light (8 W/m²) in order to follow the kinetics of chromatid damage postirradiation. Fluorescent light, like X-rays, produces chromatid damage through generation of intracellular hydrogen peroxide and free hydroxyl radicals (14) but does not block the cells in G2 (10). The frequencies of initial chromatid breaks and gaps in early and late-passage cells of NCTC 11367 were virtually identical directly after a 2-h exposure (Fig. 7); this finding indicates that the cells did not differ in susceptibility or
Fig. 5. Representative cells from cultures of RHEK-1 and derivatives and untreated epidermal keratinocytes photographed at low (× 93) and high (× 217) magnification. A, B, epidermal keratinocytes in 3rd passage showing low nuclearcytoplasmic ratio with spread abundant cytoplasm, small round nucleoli of similar number per nucleus, and relatively uniform nuclear size and shape; C, D, cells of 19th passage of RHEK-1 showing relatively uniform size and shape with rounded nucleoli of similar number per nucleus interspersed with large bizarre multinucleated cells; E, F, cells of 20th passage of KRHEK-1, 10 passages after treatment with Ki-MSV showing increased nuclearcytoplasmic ratio, coarse granular chromatin pattern, irregularly shaped nucleoli with occasional spicule formation (arrow), variable thickness of chromatinic run (arrowhead), and some abnormal parachromatin clearing; G, H, cells of 31st passage of MRHEK-1 showing extreme variations in cell size, multinucleation with variable size nuclei, irregularly shaped nucleoli, granular chromatin pattern, and variable thickness of chromatinic run.

their ability to cope with the H₂O₂ and OH generated intracellularly by radiation. These frequencies dropped precipitously in early-passage cells when sampled at intervals postirradiation. In the late-passage cells, the incidence of gaps declined more slowly, and the incidence of chromatid breaks remained at a high level for the 4-h postirradiation period. It may be noted that the extent of chromatid damage, both gaps and breaks, seen 1.5 h after a 2-h light exposure is considerably less than that seen 1.5 h after X-irradiation (Fig. 6) presumably because of the difference in intensity, dose, and quality of radiation and the concomitant repair during light exposure.

DISCUSSION

A G₂ Phase Deficiency in DNA Repair. The increased incidence of radiation-induced chromatid damage in the late-passage compared with the early-passage keratinocytes could result from (a) greater initial damage by irradiation, (b) a shorter G₂ period allowing less time for repair, or (c) an impaired capacity to repair the damage. The first two possibilities are eliminated by observations that the initial damage to the two cell populations was comparable and that even extending the repair period for 4 h postirradiation failed to produce significant repair of breaks by high-passage cells (Fig. 7). The third possibility, impaired capacity to repair the damage, presumably results from a deficiency(ies) in DNA repair during G₂ phase.

Interpretations of chromatid breaks and gaps in molecular terms have appeared in a number of cytogenetic publications since the late 1960s (8–10, 48–53). Evidence now supports the concept that each chromatid contains a single continuous DNA double strand (50, 51). A radiation-induced chromatid break with displacement of the broken segment thus represents at least one unrepaired DNA double-strand break. This may be produced directly by irradiation or indirectly from a single-
radiation-induced chromatid gaps represent unrepaired single-strand breaks (9, 10, 21, 49, 52). These could arise directly or indirectly if nucleotide excision repair is incomplete. In support of this concept, the incidence of chromatid gaps in normal but not G2 sensitive skin fibroblasts is significantly increased if an inhibitor (1-β-D-arabinofuranosylcytosine) of the polymerase step in excision repair is added to the cultures directly after irradiation during G2 (19, 21).

However, skin fibroblasts of xeroderma pigmentosum patients (Group A), virtually devoid of endonuclease activity, an early step in nucleotide excision repair, also fail to develop chromatid gaps after irradiation during G2 even in the presence of 1-β-D-arabinofuranosylcytosine (9, 21). Since other xeroderma pigmentosum complementation groups [C, E, variant (9)] which have significant endonuclease activity do show radiation-induced chromatid gaps, incision of DNA appears to be requisite for chromatid gap formation. Furthermore, the changes in frequency of chromatid gaps and breaks with time after irradiation with X-rays or fluorescent light are consistent with the sequence of events known to be associated with repair of DNA damage (10, 19).

Finally, by means of the alkaline elution technique, the kinetic difference between G2 susceptible and control cells in DNA strand break repair following a pulse label with radioactive thymidine corresponds to the kinetic difference in chromatid aberration incidence following irradiation (22). These results provide direct biochemical evidence in support of the cytogenetic findings implicating a DNA repair deficiency in G2 susceptible cells.

The present findings indicate that the late-passage cells are deficient in DNA repair during G2 phase, particularly in repair of DNA double-strand breaks. The slow or incomplete repair of DNA double-strand breaks would leave broken ends available for chromatid interchanges and subsequent chromosomal translocations. Such translocations, as noted, were observed in the late-passage cells and could lead to activation of oncogenes.

Steps in Neoplastic Transformation. Three cellular alterations observed soon after transfection with SV40 transforming sequences may be interrelated and important for neoplastic development of epidermal keratinocytes in culture. These are: a shift from the diploid chromosome number; acquisition of infinite life span; and acquisition of the DNA repair deficiency(ies). A shift from the diploid number results from abnormalities in the sequence of events involving the genetic and chromosomal changes that occur in the SV40-infected human cells: it is mutagenic with induction of chromosome aberrations (55, 56); and it stimulates cellular DNA synthesis through the T-antigen (57, 58). This stimulation may account for the enhanced life span seen in the several cell lines of the present study. However, the acquisition of infinite life span, which behaves as a recessive trait in somatic cell hybrids (59), was a rare event and, therefore, seems more likely to represent a mutation of a gene or genes controlling growth. In support of this conclusion is the observation that SV40-infected human cells that continue to express T-antigen still exhibit a finite life span in culture (60).

The continued cycling of cells with an abnormal chromosome complement or the mutagenic effects of the SV40 genome may have produced the G2 deficiency in DNA repair. Its acquisition appears to be an early stage in carcinogenesis, possibly a prerequisite or initiating event as indicated by the following:

(a) it preceded or was associated with the three neoplastic transformations of this study; (b) repeated efforts to transform...
the cells prior to acquisition of infinite life span and the DNA repair deficiency were unsuccessful; (e) in previous studies it characterized skin fibroblasts from individuals with genetic disorders predisposing to a high risk of cancer and skin fibroblasts from individuals with a family history of neoplastic disease (6-9, 11-15); (d) it was observed in C3H mouse fibroblasts spontaneously transformed in culture (16), human fibroblasts transformed by chemical carcinogens (17, 18), and all 13 lines of human tumor cells examined, regardless of histopathology or tissue of origin (19). It has not been observed in early-passage C3H mouse or human cells or in skin fibroblasts from control individuals with one exception (a total of 41 to date, ranging in age from 1 to 96 years) (9, 13, 19); (e) it was observed in skin fibroblasts and/or lymphoblasts of BALB/cAn mice susceptible to plasmacytoma induction but not in cells of BALB/cJ, DBA/2, DBA/2 × BALB/cAn F1, AKR/N, C57BL/6N, or C3H mice resistant to plasmacytoma induction (61). The G2 DNA repair deficiency is thus associated not only with genetic susceptibility to cancer but also with neoplastic transformation. Furthermore, it can be acquired, as shown in the present study, by normal tissue cells as a new genetic trait (20).

Infection with Ki-MSV containing the K-ras oncogene and treatment of one line with the carcinogen MNNG as reported previously (4) produced proliferative foci and neoplastic transformation, manifested as carcinoma formation in nude mice. The Ki-MSV-transformed cells acquired an accelerated proliferation rate, presumably from the ras oncogene. Although the MNNG-treated line, which also had an accelerated proliferation rate, showed no evidence of ras activation, some other gene controlling proliferation may have been activated (4). The DNA repair deficiency in the presence of cell cycling and DNA damage may account for the chromosome instability, cell heterogeneity, and abnormal cytological features characterizing the neoplastic cells.

A Mechanism of Neoplastic Transformation. It has been proposed on theoretical grounds that the “first (initiating) phase of carcinogenesis involves the production of a clone of cells possessing an inherited alteration in the efficiency and/or fidelity of DNA repair. Such a clone would exhibit an elevated mutation rate thus increasing the probability of at least one of the clonal progeny undergoing the set of subsequent mutations necessary for the phenotypic expression of malignancy” (62). The G2 DNA repair deficiency may be this initiation step. A proliferative stimulus such as that supplied by an active oncogene may be the driving force in the progression to neoplasia. Since some tumor promoters such as the phorbol esters lead to generation of free oxygen radicals such as ‘OH, which in turn can damage DNA, the G2 DNA repair deficiency may be the initial mechanism through which promoters transform cells (63). Thus the G2 DNA repair deficiency, in the presence of exogenous or endogenous insults to chromosomal DNA, and cell proliferation stimulated by oncogene activation may be sufficient for the stepwise progression to malignancy in vivo and in culture.

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