RATE OF DNA REPAIR IN PROGERIC AND NORMAL HUMAN FIBROBLASTS*

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Summary - The rate of rejoining of γ-ray induced DNA strand breaks has been measured in diploid skin fibroblasts from normal subjects at early and middle passage through their in vitro lifespan and compared to the rate of rejoining observed in cells from 4 patients with symptoms of the Hutchinson-Gilford Progeria syndrome of precocious aging. The rate of rejoining observed in middle passage diploid cells was less than that in cells in very early passage. The rate of rejoining in progeric cells varied markedly among cells from different patients but was significantly less than the rate in normal diploid cells. The reason for this decreased rate of DNA strand rejoining in progeric cells is not clear, but it may be related to accelerated in vitro senescence which is observed in some progeric cell strains.

We have previously reported (1) that skin fibroblasts from a patient (KH) with some symptoms of the Hutchinson-Gilford Progeria syndrome of precocious aging showed a greatly reduced capacity to rejoin gamma-ray induced single strand DNA breaks. Regan and Setlow in the accompanying paper (2) have demonstrated the ability of three progeric lines including (KH) to rejoin radiation induced DNA strand breaks to levels which allow detection of DNA molecules in the range of 165-180S. In the present report we present our more recent results with four strains of progeric fibroblasts including the three studied by Regan and Setlow. The rate of rejoining of DNA strand breaks in these four strains have been compared to the rates observed in normal diploid cells in early and middle passage. The results indicate that although a finite repair capacity exists, the rates of DNA repair in the four progeric strains are significantly less ($p<.001$) than those in normal diploid cells.

MATERIALS AND METHODS

Diploid human skin fibroblast strains EX-24 and EX-25 were derived in this laboratory from a biopsy of an 8 week fetus taken at hysterotomy and from

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a percutaneous punch biopsy of the forearm of a normal adult male respectively; EX-17 fibroblasts were taken by split-thickness biopsy of skin from the lumbar region of a 9 year old male with classical, Hutchinson-Gilford progeria. Progeric strains KH, LD, and SJ were provided through the courtesy of Dr. Robert Hay and received in monolayer. Other data concerning all cell strains are tabulated in Table I. The cells were grown as monolayers in 8 oz. glass prescription bottles at 37°C in a humidified, 5% CO_2 atmosphere. Cell strains were maintained in Eagle's Minimal Essential Medium (MEM) supplemented with 1X non-essential amino acids, 900 mg/l glucose, 6.6 mg/l sodium pyruvate, 0.6 mg/l ferrous nitrate, 50 U/ml potassium penicillin, and 50 mcg/ml streptomycin sulfate. KH cells were supplemented with 15% heat-inactivated calf serum, but all other human diploid strains, normal and progeric, were supplemented with 15% fetal calf serum. Primary human amnion cells were established and maintained as described previously (3). All diploid cell strains were tested by two separate laboratories and found free of mycoplasma contamination.

The trypsinization step during repair experiments was avoided in all experiments described in this report by subculturing the cells onto small glass or plastic coverslips several days prior to irradiation. 24-72 hrs after the cells were plated onto the coverslips, radiolabeled thymidine (methyl-^{3}H-thymidine 0.25 µCi/ml, 6.7 Ci/mM; or 2-^{14}C-thymidine 0.5 µCi/ml, 53.7 Ci/mM, New England Nuclear Corp.) was added to the dishes containing the coverslips for 24 to 48 hrs; this labeling medium was removed and replaced by fresh medium 30 min prior to irradiation. The cells were irradiated at 4°C with 10 krads of ^{60}Co gamma radiation in an elapsed time of about 40 sec. Following irradiation the cells were either lysed immediately by inverting the coverslip containing 5,000-10,000 cells directly into the high alkali-salt lysing solution which was already layered on top of a linear 5-20% sucrose gradient, or the coverslips were allowed to incubate in serum-containing medium for varying intervals at 37°C before lysing. Lysis was carried out for 4.5 hours prior to centrifugation (5,000 RPM for 15 min followed by 36,000 RPM for 60 min in an SW 50.1 swinging bucket rotor). Other

details concerning sucrose gradients, centrifugation technique, and analysis of data were as previously described (1).

RESULTS

DNA sedimentation patterns from an experiment in which KH progeric fibroblasts and a normal diploid strain in middle passage (EX-25) were labeled, irradiated and centrifuged simultaneously are shown in Figure 1. As in all experiments described in this report, the rate of DNA strand rejoining was studied in cells which had not been exposed to trypsin or other unusual trauma (except for radiation from incorporated labeled thymidine and the acute test dose) for

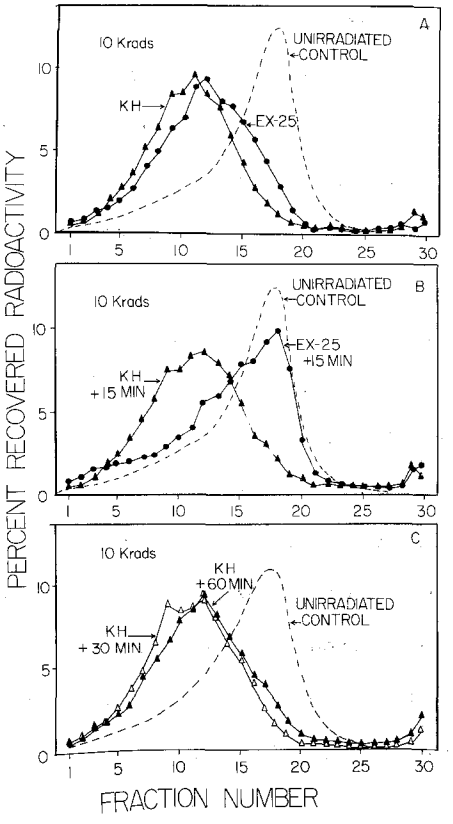

FIG. 1. DNA sedimentation profiles of normal diploid fibroblasts in mid-passage (EX-25) and progeric fibroblasts (KH) irradiated with 10 krads. (A) No repair incubation; (B) 15 min repair incubation; (C) 30 and 60 min repair incubation (KH only). Unirradiated control profiles are for EX-25 in panels A & B, and KH in panel C. Sedimentation left to right.

at least 48 hours prior to lysis on the gradient. The experiment in Figure 1 was performed blindly; that is, the individuals who performed the irradiation, sedimentation and data analyses knew neither the strain nor nature of the cells being tested. As can be seen in Figure 1B, nearly complete rejoining of the DNA strand breaks induced by the test radiation had occurred by 15 min in the normal diploid strain (EX-25), whereas cells from the progeric strain (KH) showed little evidence of rejoining by this time. After 30 and 60 min repair incubation (Figure 1C), the sedimentation profiles of the KH strain remained broad with only minimal evidence of repair, while the DNA profiles for the EX-25 strain were identical to those of unirradiated cells after 30 or 60 minutes of repair incubation.

The kinetics of DNA strand rejoining for two strains of normal human diploid fibroblasts at early passage, for three strains of normal human diploid cells at middle passage, and for primary human amnion cells 6 weeks after explant are shown in Figure 2A. The rejoining kinetics for cell strains derived from the four progeric patients are shown in Figure 2B. In these figures, the mean fraction number of the DNA sedimentation peak is plotted as a function of the time interval of repair incubation following acute irradiation (10 krads).

We have previously reported that the rejoining rate in diploid fibroblasts is influenced somewhat by the age of the culture (4). As is seen in Figure 2A, repair was always complete in middle passage cells (15-25 population doublings) by 15-20 min after irradiation. These repair times are more rapid than those we have previously reported following irradiation of cells in suspension (1). We have consistently found DNA strand rejoining to take place more rapidly following direct lysis of cells in monolayer from the growth surface after irradiation.

DISCUSSION

Using a nonparametric statistical analysis of the data in Figure 2, the significance of the differences in repair rate between early and middle passage normal diploid cells, between middle passage normal diploid cells and cells from

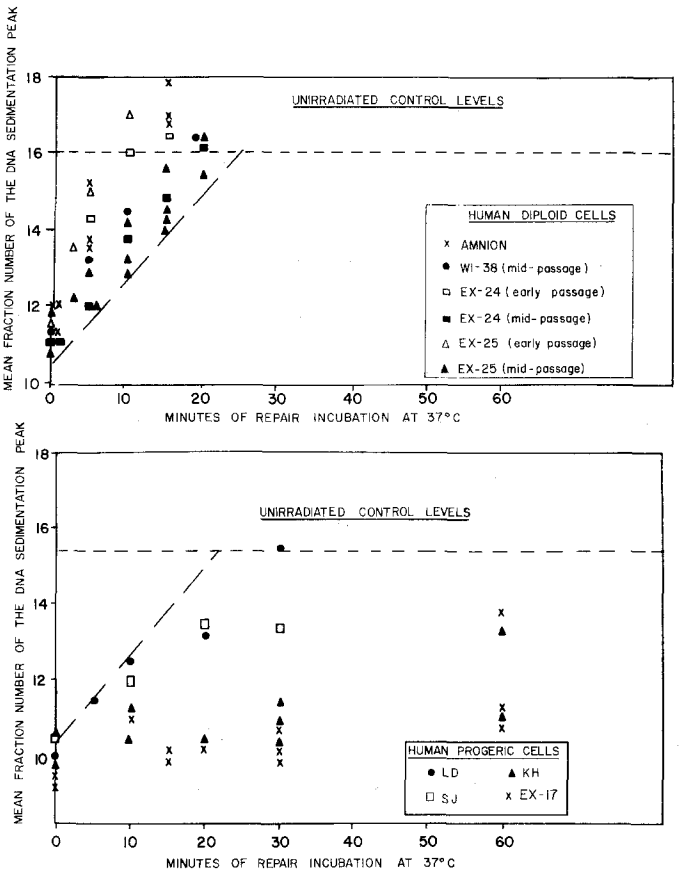

FIG. 2. Rate of repair of DNA strand breaks following irradiation with 10 krads in several normal human diploid cell strains (upper panel), and four progeric strains (lower panel). These cell strains are described in Table I.

progerics SJ and LD and between progerics SJ and LD and progerics EX-17 and KH is greater than .999 ($p<.001$).

The data in Table I and Figure 2 indicate a trend which appears attractive in its correlation of remaining growth potential and rate of DNA repair. Very young diploid cells (< 5 *in vitro* doublings) repaired their DNA to the 165S subunit somewhat more rapidly than did middle passage cells (15-25 *in vitro* doublings) (Figure 2A). The population doubling time of these cells was 18-22 hours which is in agreement with the data of others (5). The lowest rate of repair was observed in the KH strain which also showed the most limited growth potential in our hands. Furthermore, we have evidence from preliminary experiments (4) that the repair rate in normal human diploid skin fibroblasts

TABLE I

DNA Rejoining Rates and Cell Growth

Potential in Progeric, Normal Diploid and Amnion Cells

Cell	Origin of Cells	Growth Potential (total population doublings)	Culture age when experiments performed	Time required to rejoin DNA to fraction 14 (From Figure 2)
Early passage diploid				
EX-25	Biopsy of young normal male	50-55	< 5	5-7 min
EX-24	Biopsy of fetus	55-60	< 5	5-7 min
Middle passage diploid				
EX-25		50-55	15-25	7-15 min
EX-24		55-60	15-25	7-15 min
WI-38	Fetal lung	∿ 50	15-25	7-15 min
Primary amnion	Human amnion	< 5	∿ 3	7-10 min
Progeric				
SJ	20 yr old male Atypical progeria, diabetes; cachectic dwarfism	> 20	15-20	20-35 min
LD	Classical progeria	> 20	15-20	20-35 min
KH	12 yr old male Atypical progeria	∿ 11	8-10	> 60 min
EX-17	9 yr old male Classical progeria	∿ 18	8-15	> 60 min

may be considerably reduced as the cultures become senescent. An exception to this pattern, however, is the primary human amnion cell strain. These cells, which are presumed to be in the terminal stages of proliferation *in vivo* and have a very limited growth potential *in vitro*, showed complete restitution of radiation induced DNA strand breaks by 10 min (Figure 2A), similar to early passage fibroblasts. These data would also argue against a simple correlation of the DNA repair rate with the proliferation rate, as the generation time of primary amnion cells is approximately 20 days (3). Amnion cells are of mixed ectodermal and somatic mesodermal origin, however, and may thus respond differently from diploid fibroblasts which are strictly of ectodermal origin.

Regan and Setlow (2), utilizing KH cells obtained from the same source as ours, have been able to observe faster and more complete rejoining of irradiated DNA than we have, and within a time interval comparable to that found in their normal diploid controls. In Figure 4 in the accompanying paper (2), for example, they show a return to the unirradiated profile in KH cells within 30 min after irradiation in suspension with normal cells. In some of our experiments the molecular size of the DNA of irradiated KH cells returned toward control levels, but only after long periods of repair incubation (60-120 minutes). On the other hand, with our techniques the return to the control profile in normal cells occurred within 10 - 20 minutes (Figures 1B and 2A). This repair rate is considerably faster than that observed by Regan and Setlow following irradiation and incubation on plates (Figures 1 and 2, accompanying paper); under these conditions complete repair appeared to take 60 to 85 min in both normal and progeric cells (2).

Other investigators have reported data which would tend to support the concept of an age associated decline in the rejoining capacity. Wheeler and Lett (6) have demonstrated DNA of molecular weights up to 300S in the neurons of young, normal beagles, while predominantly smaller DNA species were found in the neurons of beagles 10 - 13 years older. Price and Mackinoden (7), through their examination of fixed somatic tissue derived from young and old mice, demonstrated the accumulation of more DNA strand breaks in the older tissues. These data would be consistent with the hypothesis of a reduction in DNA strand rejoining capacity with age.

We consider the work which we report in this paper to represent preliminary investigations into a very complex but interesting problem. We believe, however, that there are statistically demonstrable differences in the rate of repair between the progeric fibroblasts and normal diploid cells. The biological meaning of these differences is not clear. Regan and Setlow (2) have shown that the repair mechanism in KH cells is particularly sensitive to trypsin treatment. We have evidence that it may also be sensitive to the incorporated radioactive label.

In two other progeric cell strains not described in this report, degraded DNA was always observed in unirradiated cells, presumably due to the radioactive label. Further investigation of these factors in aneuploid, normal diploid and progeric cells will be necessary to establish correlation between genetically defined cell lines and strains, their DNA repair rates, and the possible biological meaning of these differences.

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REFERENCES

1. Epstein, J., Williams, J. R., and Little, J. B. (1973) Proc. Nat. Acad. Sci. USA 70, 977.
2. Regan, J. D., and Setlow, R. B. (1974) Biochem. Biophys. Res. Com. Submitted with this manuscript.
3. Little, J. B. (1970) Radiation Res. 44, 674.
4. Epstein, J., Williams, J. R., and Little, J. B. (1973) Radiation Res. 55, 527.
5. Macieira-Coelho, A., Ponten, J., and Philipson, L. (1966) Exp. Cell Res. 42, 673.
6. Wheeler, K. T., and Lett, J. T. (1974) Proc. Nat. Acad. Sci. USA, in press.
7. Price, G. B., Modak, S. P., and Makinodan, T. (1971) Science 171, 917.