High Frequency and Error-prone DNA Recombination in Ataxia Telangiectasia Cell Lines*

(Received for publication, July 18, 1995, and in revised form, December 18, 1995)

Chen-Mei Luo, Wei Tang, Kristin L. Mekeel, Jeffrey S. DeFrank, P. Rani Anné, and Simon N. Powell‡

From the Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114

The only specific DNA repair defect found in ataxia telangiectasia (A-T)1 is mis-repair of cleaved DNA. In this report we measured DNA recombination, given its role in DNA repair and genetic instability. Using plasmids containing selectable reporter genes, we found a higher frequency of both chromosomal recombination (>100 times) and extra-chromosomal recombination (27 times) in SV40-transformed A-T cell lines compared with an SV40-transformed normal fibroblast cell line. Southern analysis of single A-T colonies exhibiting post-integration recombination revealed that 24/27 had undergone aberrant rearrangements; recombination in normal fibroblast colonies was achieved by gene conversion in 8/11 clones and 10/11 clones showed unchanged copies of the plasmid. Using co-transfection of two integrating plasmids, each containing a separate deletion in the xgprt reporter gene, the 27 times difference in extra-chromosomal recombination was found when the plasmids were cleaved at a distance from the reporter gene. When the plasmids were cleaved within the reporter gene, the co-transfection frequency was reduced in A-T, but was increased in normal cells. We conclude that A-T cell lines have not only a high frequency chromosomal and extra-chromosomal recombination, but also exhibit error-prone recombination of cleaved DNA.

Ataxia telangiectasia (A-T)1 is an autosomal recessive disorder characterized by progressive cerebellar ataxia, skin changes, endocrine disorders, immune defects, and high risk of cancer. A-T cell lines have abnormalities in DNA damage processing including hypersensitivity to ionizing radiation (1) and radiation-resistant DNA synthesis (2, 3). The A-T gene function appears to be central to DNA damage processing, cell cycle checkpoints, and genomic stability; interest in determining the function of the gene comes from many disciplines.

Following localization of the A-T gene to 850 kb on the long arm of chromosome 11 (4, 5), it now appears to have been cloned (6). The surprising result is that one gene is mutated in all the complementation groups, which makes the original observations of complementation groups difficult to rationalize. The cloned gene of Schizosaccharomyces pombe, rad3, encodes a phatidylinositol 3-kinase and the rad 3 gene of has either an altered 3-kinase activity or lack of function of this gene achieves the pleiotropic phenotype remains unsolved.

Progress in understanding this complex syndrome has been restricted to extensive definition of the phenotype (7). One of the main puzzling aspects of the A-T response to ionizing radiation is the lack of a gross defect in DNA double-strand break repair. Recently it has been suggested that all or most of the ionizing radiation sensitivity of A-T is due to early triggering of programmed cell death by otherwise non-lethal DNA damage (8). Another view of the A-T gene function is a lack of damage surveillance, which may help explain both radiosensitivity and lack of cell-cycle arrest in response to ionizing radiation. The role of the A-T gene in the damage signaling pathway was suggested by the lack of or delayed rise in p53 following ionizing radiation (9–12). However, the model proposed by both Meyn (8) and Thacker (7) are incongruous: if the damage signaling pathway is at fault, how is apoptosis triggered so readily? Signaling of damage appears sufficient to stimulate known response markers such as poly(ADP)-ribose (13).

Although A-T cells have no gross defect in closing double-strand breaks, they have been shown to have a high frequency of double-strand break mis-repair (14–16) using plasmid reactivation. A high rate of spontaneous intra-chromosomal, but not extra-chromosomal, recombination has also been described using a plasmid recombination substrate (17). Our previous observations had suggested that mis-repair may be linked to recombination, and a working hypothesis was that double-strand breaks from ionizing radiation trigger recombination, which in A-T is error prone. Thus, although strand breaks are closed readily, residual damage is present which leads to mitotic cell death or apoptosis. The cell-cycle arrest response to ionizing radiation may be more complex than first described, and was recently shown to be cell-cycle phase dependent (18). The A-T gene may highlight a difference in the signal pathway of cell-cycle arrest and apoptosis.

In this report we transfected plasmids, designed to evaluate DNA recombination/repair events, into A-T and normal cell lines. Specifically, we measured chromosomal and extra-chromosomal recombination; whether cleavage of the recombination substrates affected the outcome; and the type of recombination events seen in A-T compared with normal cells.

MATERIALS AND METHODS

Cell Lines and Tissue Culture—The cell lines, AT25F (GM00670) and AT5BIVA (GM5849A), are SV40-transformed fibroblast cell lines from patients with ataxia telangiectasia. NF (GM00637F) is an SV40-transformed normal human fibroblast cell line. All cells were grown in Dulbecco’s modification of Eagle’s medium with 10% (v/v) fetal bovine serum and containing penicillin (100 units/ml) and streptomycin (100 μg/ml). Cell cultures were incubated in 5% CO₂ at 37 °C. The cells were grown in monolayer culture and were detached when required using
trypsin (0.05%) and versene (0.02%).

Vectors—The construction of pTPSN has been described (19). In brief, it is a hybrid sequence and contains three antibiotic resistance genes (Fig. 1A), a neomycin (neo) resistance gene used to select for stable transformants flanked by two mutant genes encoding hygromycin resistance (hyg). The mutations in the hygromycin genes were made by an insertion of a HindIII linker at a different site in each gene. The first insertion mutation was introduced into the hyg gene at the unique PvuI site and the second at a SacI site. Recombination between the two defective genes can lead to a functional hygromycin gene. The two mutant genes are distinguishable because the PvuI mutant hyg gene is recoverable as a 2.9-kb DraI fragment, whereas the SacI mutant hyg gene is recoverable as a 2.6-kb DraI fragment. The presence or absence of the HindIIII site within the DraI fragment will determine whether the gene is unchanged or has undergone gene conversion, respectively. Exchange between the two mutant genes, detected by Southern analysis, results in a 2.5-kb band on DraI digestion, and no HindIIII site. Using only HindIIII digestion for rapid screening of clones, unchanged plasmid produces a 6- and 4-kb band; gene conversion leads to a 10-kb band; and exchange produces an 8- or a 12-kb band.

The plasmids pΔ2, pΔ3 (20) were derived from pSV2gpt (21) and are shown in Fig. 1B. These plasmids contain the bacterial amp gene and the gpt gene with a deletion. The bacterial gpt gene with flanking SV40 promoter and processing sequences, confers resistance to mycophenolic acid. pΔ2 lacks the SV40 promoter and the first 120 base pairs of the gene coding sequence, whereas pΔ3 lacks the 3' end of the gpt gene. There are 460 base pairs between the deleted sequences, retained in both deletion plasmids, containing the single KpnI site. A single EcoRI restriction enzyme site is distant from the gpt coding region. The deletion plasmids do not have an intact gpt gene, but if pΔ2+pΔ3 are co-transfected into cells, reconstitution of the gpt gene can occur via inter-molecular recombination.

pTPSN Transfection and Isolation of G418-resistant Clones—Twenty microgram quantities of linear pTPSN were transfected into each flask containing 2 × 10^6 A-T or NF cells using the calcium phosphate co-precipitation method. pTPSN was linearized at its unique ClaI site before transfection to facilitate integration of the two mutant hygromycin genes. Following 4–6 h incubation with plasmid DNA, 48–60 h was allowed for growth and expression before harvesting and reseding three flasks with 10^4, 10^5, and 10^6 cells. Three 100-mm dishes were also seeded with 10^4 and 10^5 cells for the isolation of single cell derived clones. Initial selection used 0.5 mg/ml G418. After 14–21 days growth in G418, with medium changed every 7 days, visible and viable colonies were marked and counted.

Single G418-resistant (neo^R) colonies were isolated and amplified with and without G418. In parallel, the clones were tested for hygromycin sensitivity and only hygromycin-sensitive clones were used for further testing. The initial cell count at the time of establishing hygromycin sensitivity and the final cell count following amplification, prior to seeding, was calculated amplification of cells prior to seeding in hygromycin. When there were sufficient cells amplified from either single or multiple G418-resistant colonies, 10^4, 10^5, and 10^6 cells were seeded into T75 flasks in medium with or without G418, and 50 μg/ml hygromycin was added the next day.

After 14–21 days growth in hygromycin, visible colonies (hyg^R) were stained with methylene blue. The frequency of hygromycin resistance per viable cell was recorded. A minimum of three flasks per seeding density and two different seeding densities were used per data point. Single neo^R and hyg^R colonies were isolated and transferred from dishes to small wells and maintained with the same selective medium until
**DNA extraction.**

**pΔ2,pΔ3 Co-transfection—**The plasmids pSV2gpt, pΔ2, and pΔ3, where indicated, were cut at their KpnI or EcoRV sites within the gpt gene or the EcoRI site at a distance from the gpt gene, with 2 units of enzyme per μg of plasmid before transfection. Agarose gel electrophoresis was used to confirm cleavage. Ten micrometers of each of pΔ2 and pΔ3 or 20 μg of pSV2gpt were used per flask containing 2 × 10⁶ cells in each experiment. Transfections were achieved using calcium phosphate co-precipitation as previously, with selection using XHATM medium containing xanthine (10 μg/ml), hypoxanthine (13.6 μg/ml), amiprophorin (0.176 μg/ml), thymidine (3.87 μg/ml), and mycophenolic acid (10 μg/ml). After 14–21 days growth in XHATM, visible colonies were selected in hygromycin only. Transfection frequency represents the efficiency of recombination.

**Southern Blot Analysis of pTPSN Transfections—**The plasmid DNA content of the neoR and hygR or hygR clones was determined by South-ern blot analysis. DNA was isolated, using detergent lysis and phenol then chloroform extraction, and 10 μg of DNA were digested by restriction endonucleases. Probes were prepared by random priming of the 2.2-kb BamHI fragment of pTPSN containing the hygromycin gene and radiolabeling with deoxycytidine 5′-[α-32P]triphosphate (DuPont NEN).

**Results**

**Chromosomal Recombination—**Recombination between the two hyg genes seen following stable integration was measured in three single-cell derived neoR clones of AT5 and NF (see Table IA). All clones were shown to be sensitive to hygromycin at the time of cloning. The geometric mean recombination frequency following stable integration was 40.7 × 10⁻⁴ per cell generation in AT5 and 0.25 × 10⁻⁴ per cell generation in NF, 163 times more frequent in A-T cells (p < 0.001). Selection in

| Table I | A, rates of chromosomal recombination in single G418-resistant hygromycin-sensitive clones transfected with pTPSN and B, the effect of different selection media conditions upon the rate of chromosomal recombination in hygromycin-sensitive pTPSN clones |
|---------|---------------------------------------------------------------------------------|
| Cell line| Clone | Recombination frequency per cell generation (×10⁻⁴) |
|---------|-------|---------------------------------------------------|
| AT5     | C2    | 86.36                                             |
| AT5     | C6    | 10.66                                             |
| AT5     | C7    | 72.73                                             |
| Mean±   |       | 56.6 (40.7)                                       |
| AT2     | C1    | 2.15                                              |
| AT2     | C3    | 0.06                                              |
| AT2     | C6    | 0.23                                              |
| Mean±   |       | 0.48 (0.25)                                       |
| NF      | C1    | 0.25                                              |
| NF      | C3    | 0.06                                              |
| NF      | C6    | 0.23                                              |
| Mean±   |       | 0.14                                              |

**Table II**

| Cell line | Experiment No. | Transfection frequency (G418)^2 (×10⁻⁴) | Recombination frequency per cell generation (×10⁻⁴) |
|-----------|----------------|----------------------------------------|---------------------------------------------------|
| AT5       | 1              | 350                                    |                                                   |
| AT5       | 2              | 4.8                                    | 828                                               |
| AT5       | 4              | 17.6                                   | 186                                               |
| AT2       | 1              | 35                                 |                                                   |
| AT2       | 2              | 16.8                                   | 730                                               |
| AT2       | 4              | 2.0                                    | 60                                                |
| NF        | 2              | 10.1                                   | 3.3                                               |
| NF        | 3              | 0.15                                   | 0.73                                              |
| NF        | 4              | 1.1                                    | 15.0                                              |
| NF        | 5              | 0.44                                   | 2.21                                              |

**Table III**

| Cell line | Plasmid | Experiment No. | Transfection frequency (G418)^2 (×10⁻⁴) | Recombination frequency per cell generation (×10⁻⁴) |
|-----------|---------|----------------|----------------------------------------|---------------------------------------------------|
| AT5       | pSV2gpt | 2              | 5.1                                    | 64.9                                              |
|           |         | 3              | 9.0                                    | 55.0                                              |
|           |         | 4              | 0.6                                    | 5.5                                               |
| pΔ2,pΔ3   |         | 1              | 2.1                                    | 19.3                                              |
|           |         | 2              | 4.9                                    | 15.7                                              |
|           |         | 3              | 12.0                                   | 20.0                                              |
| NF        | pSV2gpt | 1              | 4.1                                    | 26.7                                              |
|           |         | 2              | 3.8                                    | 42.1                                              |
|           |         | 3              | 0.24                                   | 63.0                                              |
| pΔ2,pΔ3   |         | 1              | 1.9                                    | 34.0                                              |
|           |         | 2              | 1.8                                    | 1.5                                               |
|           |         | 3              | 2.6                                    | 0.19                                              |

**Fig. 2. Processing of pTPSN to achieve hygromycin resistance.** The right-hand lanes show plasmid only marker lanes with digestion by either Hin III or Dra I or both; each pair shows 1 copy and 5 copies. When the plasmid remains unchanged, Hind III digestion results in 6- and 4-kb bands; Dra I digestion leads to a 2.9- and 2.6-kb bands; and digestion by both enzymes leads to 1.7-, 1.4-, and 1.2-kb bands. Three representative clones are shown: each clone has 3 lanes reflecting Hind III, Dra I, and Hind III + Dra I digestion. Gene conversion leads loss of the Hind III site within either copy of the hyg gene, resulting in the 2.9-kb or the 2.6-kb band being resistant to Hind III (AT5 and AT2 clones). Reciprocal exchange products in a 2.5-kb Dra fragment which is Hind III resistant (NF clone). Other rearrangements leading to hygromycin resistance can occur and result in bands of different size with the Hind III insertion mutation removed.

**Table IV**

| Transfection frequency (G418)^2 (×10⁻⁴) | Transfection frequency (G418)^2 (×10⁻⁴) |
|----------------------------------------|----------------------------------------|
| AT5                                    | 64.9                                   |
| AT5                                    | 55.0                                   |
| AT5                                    | 5.5                                    |
| pΔ2,pΔ3                                | 19.3                                   |
| pΔ2,pΔ3                                | 15.7                                   |
| pΔ2,pΔ3                                | 20.0                                   |
| NF                                     | 26.7                                   |
| NF                                     | 42.1                                   |
| NF                                     | 63.0                                   |
| NF                                     | 34.0                                   |
| NF                                     | 1.5                                    |
| NF                                     | 0.19                                   |

**RESULTS**

The mean is the arithmetic mean; the geometric mean is in parentheses.

Re-cloned from a single colony without G418.

Selection in hygromycin only.

Repeat measurement on same clone maintained in G418.
Recombination in Ataxia Telangiectasia

**TABLE IV**

| Cell | Clone | Gene conversion | Exchange | Unaltered | Other rearrangement | Copy No. |
|------|-------|-----------------|----------|-----------|--------------------|---------|
| AT5  | C54   | +               |          | +         |                    | 2       |
| AT5  | C6    | +               |          |           |                    | 6       |
| AT5  | C8    | +               |          | +         |                    | 5       |
| AT5  | C11   | +               |          |           |                    | 5       |
| AT5  | C1    | +               |          | +         |                    | 3       |
| AT5  | C52   | +               |          | +         |                    | 1       |
| AT5  | C53   | +               |          |           |                    | 4       |
| AT5  | C9    | +               |          |           |                    | 4       |
| AT5  | C50   | +               |          |           |                    | 6       |
| AT5  | C12   | +               |          | +         |                    | 8       |
| AT5  | C13   | +               |          | +         |                    | 8       |
| AT5  | C57   | +               |          |           |                    | 1       |
| AT5  | C58   | +               |          | +         |                    | 3       |
| AT5  | C4    | +               |          | +         |                    | 4       |
| AT5  | W6    | +               |          |           |                    | 3       |
| AT5  | W13   | +               |          | +         |                    | 1       |
| AT5  | C26   | +               |          |           |                    | 1       |
| AT5  | C22   | +               |          |           |                    | 1       |
| AT5  | C3    | +               |          |           |                    | 1       |
| AT5  | C5    | +               |          |           |                    | 1       |
| AT5  | W4    | +               |          | +         |                    | 6       |
| AT5  | W3    | +               |          | +         |                    | 1       |
| AT5  | W7    | +               |          | +         |                    | 1       |
| AT5  | C21   | +               |          | +         |                    | 1       |
| AT5  | W8    | +               |          | +         |                    | 1       |
| AT5  | W10   | +               |          | +         |                    | 1       |
| AT5  | C7    | +               |          | +         |                    | 15      |
| NF   | W4    | +               |          | +         |                    | 5       |
| NF   | C3    | +               |          | +         |                    | 2       |
| NF   | C1    | +               |          | +         |                    | 8       |
| NF   | C5    | +               |          | +         |                    | 8       |
| NF   | C8    | +               |          | +         |                    | 10      |
| NF   | C9    | +               |          | +         |                    | 8       |
| NF   | W5    | +               |          | +         |                    | 3       |
| NF   | W3    | +               |          | +         |                    | 5       |
| NF   | W2    | +               |          | +         |                    | 5       |
| NF   | C6    | +               |          |           |                    | 1       |
| NF   | C7    | +               |          |           |                    | 8       |

Hygromycin alone compared with selection in G418 and hygromycin did not alter the result (see Table I B; AT5-C7 = 100.2 and 94.2 × 10⁻⁴ per cell generation, respectively; NF-C6 = 0.31 and 0.23 × 10⁻⁴ per cell generation, respectively). It might be expected that maintaining G418 selection would preclude the mechanism of reciprocal exchange, since the neo gene is deleted by this mechanism. The relative proportion of reciprocal exchange recombination must be low because selection in hygromycin with or without G418 did not affect the recombination frequency. When the single-cell derived culture was re-cloned again, the recombination frequency was unchanged (AT5-C6 = 19.1; NF-C6 = 0.15; NF-C1 = 0.18 × 10⁻⁴ per cell generation) and selection in hygromycin with or without G418 did not alter the result.

Transfection frequencies for pTPSN and recombination frequencies for mixed colonies are shown in Table II. The transfection frequencies (neo° colonies per viable cell) in AT5(BIVA), AT2(SF), and NF were not significantly different. The geometric mean rate (± S.E.) of spontaneous chromosomal recombination observed directly following transfection (hyg° colonies per viable neo° cell) in AT5 was 378 (245–582) × 10⁻⁴ per cell generation, 126 times more frequent than NF, which was 2.99 (1.6–5.6) × 10⁻⁴ per cell generation (p < 0.001). The recombination rate for AT2 was 250 (119–525) × 10⁻⁴ per cell generation, 84 times more frequent than NF (p < 0.001). These data are not corrected for pTPSN copy number, which did not differ significantly and would not account for the differences between A-T and NF (see below and Table IV). Recombination frequencies were increased, 9.3 times in AT5 and 11.8 times in NF, compared with the recombination frequency of stable integrated sequences (Table I versus Table II). At first sight, this might imply that recombination occurring extra-chromosomally or with integration is not significantly different between A-T and normal cell lines.

Extra-Chromosomal, Inter-Molecular Recombination—The co-transfection frequency of pΔ2 and pΔ3 and the transfection frequency of pSV2gpt are shown in Table III. The relative transfection frequency of pΔ2 and pΔ3 compared with pSV2gpt gives a measure of the efficiency of recombination, which accounts for both frequency and accuracy of recombination. EcoRI cleaved the plasmids outside the gpt gene; conversely, KpnI cleaved within the gpt gene. The comparison between KpnI and EcoRI was to evaluate the impact of cleavage within the recombination substrate; in other words, to assess the impact of DNA double-strand breaks on DNA recombination. When the plasmids were cleaved at a distance from the gpt gene, the co-transfection frequency was 18.3 × 10⁻⁶ in AT5 and 0.68 × 10⁻⁶ in NF, a 27-fold increased extra-chromosomal recombination frequency in A-T cells. The pSV2gpt transfection frequency was similar (41.8 × 10⁻⁶ in AT5 and 43.9 × 10⁻⁶ in NF). Thus, the co-transfection frequency of pΔ2 and pΔ3 was 44% of the transfection frequency of linear pSV2gpt in AT5, but only 1.5% in NF. When the plasmids were cleaved within the gpt gene, the co-transfection frequency was 6.33 and 2.1 × 10⁻⁶ in AT5 and NF, respectively. The co-transfection frequency was reduced 3-fold in A-T but was increased 3-fold in NF, resulting in a final difference between the cells of only 3-fold. The extra-chromosomal recombination frequency can appear to be little
different between A-T and normal cells depending on the site of cleavage and the recombination substrate. A-T cells are adversely affected by cleavage within the recombination substrate, whereas NF cell recombination is enhanced. It is concluded that DNA cleavage promotes errors in recombination in A-T cells, and that extra-chromosomal recombination is more frequent in A-T cells.

pTPSN Plasmid Processing—Single-cell derived hyg<sup>+</sup> clones were analyzed to establish copy number and to evaluate the processing of transfected sequences for evidence of gene conversion, exchange, other rearrangement, or no sequence alteration. Clones demonstrating each type of processing are shown in Fig. 2. Analysis of 8 A-T and 9 NF G418-resistant clones showed the copy number to vary from 1 to 10. In the expected range for transformed fibroblast lines, with no difference between A-T and NF.

Clones derived from the AT and NF cell lines, which were resistant to hygromycin, are shown in Table IV, with a significant proportion of these clones shown in Fig. 3. The plasmid copy number in AT5 and NF was not different. AT2 clones had a single copy in 11/14 analyzed, which was lower than AT5 or NF and may explain why the recombination frequency was marginally lower in AT2 compared with AT5. However, differences in copy number cannot explain the differences in recombination frequency between A-T and NF. There was no detectable difference in the type of plasmid processing seen in clones containing a single copy of pTPSN compared with clones with multiple in tandem copies. For example, single copy status of clone C57 (AT5) was established by BamHI digest, which revealed a 2.2-kb band and one further larger band containing the other hygromycin gene (see Fig. 4A). Hygromycin sensitivity was confirmed by a failure to grow in selective media and cleavage of all DraI fragments by HindIII. Following the development of hygromycin resistance, the 2.6-kb DraI fragment has been lost, and a 3.1-kb DraI fragment developed. It is presumed that the combination of newly developed bands (3.1-kb DraI fragment; 1.9-kb DraI/HindIII fragment) accounts for hygromycin resistance, although the retention of a HindIII site in the DraI fragment is not usually found. A sequential analysis of AT5 clone C58, which contains 2 full copies of the plasmid and a further partial copy, is shown in Fig. 4B. Before selection with hygromycin, 2.9 and 2.6-kb DraI fragments are seen, which cleave with HindIII to the 1.7-, 1.4-, and 1.2-kb fragments. Additional DraI bands (5.4 kb and 3.1 kb) are seen which are both cleaved by HindIII. Following the development of hygromycin-resistance in a clone growing out of the previously donated cell culture, the following changes are apparent: the loss of the 2.9- and 2.6-kb bands, the development of a HindIII resistance in the 3.1-kb band, and the new development of HindIII-resistant 3.7- and 1.8-kb DraI bands. Thus, in both single copy and multiple (2-3) copy transfected clones, the development of post-integration aberrant rearrangements are a characteristic feature of A-T cells, not seen in normal cells. Hygromycin-resistant clones were obtained from multiple independent G418-resistant cultures to avoid the analyzed clones being siblings. The results showed remarkable consistency within cell type and difference between cell types; no effect of independent culture could be found.

Hygromycin-resistant NF clones retained at least one or more unaltered copies of the mutant hyg genes in 10/11 clones; 8/11 clones achieved hygromycin resistance via gene conversion of a single copy; 1/11 demonstrated an exchange event; 1/11 demonstrated an abnormal rearrangement and one exhibited hygromycin resistance without a clear etiology. By marked contrast, A-T clones retained unchanged sequences in 2/27 clones; 10/27 showed evidence of gene conversion; and 24/27 demonstrated complex rearrangements, in which hygromycin resistance was not a result of gene conversion or exchange in at least 17 of these 24 clones. The clone, C3, developed faint HindIII-resistant DraI bands, seen only on a later film, which suggested heterogeneity developing in the clone during the short period of amplification and presumably reflects the enhanced genetic instability of A-T cells. The clone W8 had no apparent hygromycin containing bands, despite keeping the cells in hygromycin until extracting DNA. Thawing the frozen cells from this clone revealed the cells to have re-developed hygromycin sensitivity. The suggestion is that intrinsic instability allowed the rapid development of the loss of hygromycin resistance genes. The intrinsic recombinational processes (and presumably repair) appear to be sequence altering in A-T and sequence conserving in normal cells. Rearrangements that fail to reconstitute a functional gene cannot be measured by these assays.
DISCUSSION

A-T cells show hypersensitivity to ionizing radiation which has been assumed to be due to a deficiency in DNA repair. In this paper, we have found A-T cells to have an abnormally high rate of recombination and to have abnormal, error-prone recombination in assays of chromosomal and extra-chromosomal recombination. The role of recombination in double-strand break repair and radiation sensitivity has been demonstrated recently in xrs cells (23) and scid cells (24, 25). Both cell types have reduced or absent V(D)J recombination and a failure to close DNA double-strand breaks. By contrast, A-T cells have normal double-strand break closure but also demonstrate frequent chromosome aberrations (5, 26). It is suggested that error-prone strand break processing may underlie the sensitivity to ionizing radiation in A-T.

Hyper-recombination—The only previous report of hyper-recombination in A-T cells emphasized that high frequency recombination was restricted to intra-chromosomal events, and that extra-chromosomal recombination was not different from normal. The data presented in this paper supports hyper-recombination in both chromosomal and extra-chromosomal sites. Transfection frequency did not differ between A-T and normal cells in this or other reports (14–17). However, when recombination between extra-chromosomal substrates was required to result in a stable integrated gene, we found a higher frequency of recombination in A-T. It is not clear how the relative frequency of recombination in this assay (pΔ2 and pΔ3) should compare with the relative frequency of intra-chromosomal recombination by pTPSN. The rate of recombination measured by the extra-chromosomal assay is limited by the overall transfection frequency, whereas the intra-chromosomal assay is restricted by only the rate of recovery of recombination events, determined by the plasmid, the extent of homology, the number of copies and possibly the site of integration.

The site of cleavage in relation to the sequences required to recombine appears to be important. If the DNA termini are within or close to the recombination substrates, then the high frequency of recombination in A-T is offset by mis-repair of the recombining sequences. Previous studies have found up to 5-fold differences in recombination of extra-chromosomal plasmid DNA, which has been recorded as not significant (16, 27–30). The results seen with co-transfection of KpnI-cleaved plasmid could be due to extra-chromosomal rejoining between the 3' end of pΔ2 and the 5' end of pΔ3 rather than recombination. However, the same results are obtained when only one of the two plasmids is cleaved, or when pΔ2 is cleaved with EcoRV and pΔ3 is cleaved with KpnI, which precludes a simple re-ligation mechanism (data not shown). It is also concluded that A-T cells show recombination mediated mis-repair around the site of strand breakage.

Recombination: Before Integration, With Integration, or Post-integration?—The recombination frequency associated with integration of pTPSN was approximately 10 times higher than that for stable intra-chromosomal plasmid sequences. Selection
for hygromycin was only applied after G418 selection and not immediately following transfection. Integration enhances recombination by unknown mechanisms, perhaps by inducing strand breakage.

Recombination of vectors such as pΔ2/pΔ3 is generally thought to be extra-chromosomal, but also requires an integration step. Recombination may occur by events occurring extra-chromosomally, associated with integration, or intra-chromosomally. Our data with pΔ2/pΔ3 and the previously reported data cannot separate solely extra-chromosomal events from events associated with integration, even though these assays have been widely reported to reflect extra-chromosomal recombination. The assay of V(DJ) recombination evaluates entirely extra-chromosomal events, and A-T cells have been shown to make functional coding and signal joints (31). However, this assay does not measure frequency or fidelity of recombination separately, and may miss the error-prone tendency of A-T cells because the assay detects only functional rearrangements.

Error-prone Recombination—It has been suggested previously that A-T exhibits DNA mis-repair (32) which has been supported by plasmid-based repair assays (15, 16). Our previous work using integrating plasmid repair probes, investigating the rejoining of cleaved plasmid, had suggested that the rejoin fidelity was dependent on the process of integration rather than extra-chromosomal religation. When rejoin errors were made by A-T cells, they were frequently duplicated in multiple copies in a single clone, but not reproduced between clones. This suggested that mis-repair may be recombination dependent. Two separate observations in this paper have supported the hypothesis of error-prone recombination. First, the development of hygromycin resistance after pTPSN transfection was mediated by complex sequence rearrangements in A-T compared with the sequence conserving mechanisms seen in normal cells. Second, the introduction of DNA cleavage into the gpt gene of pΔ2 and pΔ3 led to a reduction in accurate recombination. First, the relationship of error-prone recombination to the function of the A-T gene remains unclear. The recombination was mediated by complex sequence rearrangements in A-T, but enhanced recombination in normal cells. Second, the introduction of DNA cleavage into the gpt gene of pΔ2 and pΔ3 led to a reduction in accurate recombination in A-T, but enhanced recombination in normal cells.

A-T Gene Function—The relationship of error-prone recombination to the function of the A-T gene is unclear. The recombination events described may be many steps downstream from the direct function of the gene. If each time a DNA double-strand break occurs, multiple sequence errors are introduced, radiation sensitivity may result. Error-prone, hyper-recombination is consistent with being cancer prone. The immune system sensitivity may result. Error-prone, hyper-recombination is consistent with being cancer prone. The immune system sensitivity may result. Error-prone, hyper-recombination is consistent with being cancer prone.

Acknowledgments—We thank Michael Liskay for use of the pTPSN plasmid, and John Thacker for the pΔ2/pΔ3 plasmids.

REFERENCES

1. Taylor, A. M., Hanrden, D. G., Arlett, C. F., Harcourt, S. A., Lehmann, A. R., Stevens, S., and Bridges, B. A. (1975) Nature 252, 427–429
2. Painter, R. B., and Young, B. R. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 7315–7317
3. Houldsworth, J., and Lavin, M. F. (1980) Nucleic Acids Res. 8, 3709–3720
4. Gatti, R. A., Lange, E., Rotman, E., Chen, X., Uhrhammer, N., Liang, T., Chiplunkar, S., Yang, L., Udar, N., Dandekar, S., Shekhahandi, S., Wang, Z., Yang, H. M., Polikow, J., Etaloff, M., tetelar, M., Sanal, O., Chesla, L., McConville, C., Taylor, M., Shiloh, Y., Porras, O., Borrose, A. L., Wegner, R. D., Curry, C., Gerken, S., Lange, K., and Concannon, P. (1994) Int. J. Radiat. Biol. 66, 557–562
5. Taylor, A. M., Byrd, P. J., McConville, C. M., and Thacker, S. (1994) Int. J. Radiat. Biol. 65, 65–72
6. Savitsky, K., Bar-Shira, A., Gilad, S., Rotman, G., Ziv, Y., Vanagaitaite, L., Tagle, D. A., Smith, S., Uziel, T., Slez, A., Ashkenazi, M., Peder, I., Frydman, M., Harsuk, R., Patanjali, S. R., Simmons, A., Clines, G., Sarbel, A., Gatti, R. A., Chesla, L., Sanal, O., Lavin, M. F., Jaspers, N. G. J., Taylor, A. M., Arlett, C. F., Miki, T., Weissman, S. M., Lovett, M., Collins, F. S., and Shiloh, Y. (1995) Science 268, 1749–1753
7. Thacker, J. (1994) Int. J. Radiat. Biol. 66, 587–596
8. Meyn, M. S., Strasfeld, L., and Allen, C. (1994) Int. J. Radiat. Biol. 66, 5141–5149
9. Kastan, M. B., Zhan, Q., de-Deiry, W. S., Carrier, F., Jackson, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B., and Fornace, A. J., Jr. (1992) Cell 71, 587–597
10. Nasrin, N., Kunhi, M., Eingesenner, M., de-Sedairy, S., and Hanna, M. (1994) Cancer Genet. Cytogenet. 77, 14–18
11. Knaha, K. K., and Lavin, M. F. (1993) Oncogene 8, 3307–3312
12. Lu, X., and Lane, D. P. (1993) Cell 75, 705–716
13. Edwards, M. J., and Taylor, A. M. (1993) Nature 365, 745–746
14. Debenham, P. G., Webb, M. B., Jones, N. J., and Cox, R. (1987). J. Cell Sci. Suppl. 6, 179–189
15. Powell, S., Whitaker, S., Peacock, J., and McMillan, T. (1993) Mutat. Res. 294, 9–20
16. Cox, R., Masson, W. K., Debenham, P. G., and Webb, M. B. (1984) Br. J. Cancer Suppl. 6, 67–72
17. Meyn, M. S. (1993) Science 260, 1227–1330
18. Beanish, H., and Lavin, M. F. (1994) Int. J. Radiat. Biol. 66, 175–184
19. Tsujimura, T., Maher, V. M., Godwin, A. R., Liskay, R. M., and Cormick, J. J. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 1566–1570
20. Hamilton, A. A., and Thacker, J. (1987) Mol. Cell. Biol. 7, 1409–1414
21. Mulligan, R. C., and Berg, P. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 2072–2076
22. Cole, J., Arlett, C. F., and Green, M. H. (1976) Mutat. Res. 41, 377–386
23. Taccicello, G. E., Rathburn, G., Oltz, E., and Starnato, T. J. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 3717–3720
24. Friedmann, K. A., Sun, J. R., Glacia, A. J., Tosto, L. M., and Brown, J. M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 1394–1397
25. Hendrickson, E. A., Qin, X. Q., Schatz, D. G., Oettinger, M., and Wieser, D. A. (1993) Mutat. Res. 294, 9–20
26. Taylor, A. M., Metcafe, J., Gats, C. K., and Bridges, B. A. (1975) Nature 260, 411–413
27. Dastgupta, U. B., and Summers, W. C. (1980) Mol. & Gen. Genet. 176, 617–623
28. Thacker, J. (1998) Mutat. Res. 220, 187–204
29. Timme, T. L., Wood, C. M., and Moses, R. E. (1989) Plasmid 22, 1–9
30. Wahls, W. P., and Moore, P. D. (1990) Somat. Cell Mol. Genet. 16, 321–329
31. Hecht, C. R., Arlett, C. F., and Lieber, M. R. (1993) J. Biol. Chem. 268, 20105–20109
32. Taylor, A. M. (1978) Mutat. Res. 50, 407–418
33. Lipkowitz, S., Garry, V. F., and Kirsch, I. R. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 5301–5305
High Frequency and Error-prone DNA Recombination in Ataxia Telangiectasia Cell Lines
Chen-Mei Luo, Wei Tang, Kristin L. Mekeel, Jeffrey S. DeFrank, P. Rani Anné and Simon N. Powell

J. Biol. Chem. 1996, 271:4497-4503.
doi: 10.1074/jbc.271.8.4497

Access the most updated version of this article at http://www.jbc.org/content/271/8/4497

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 33 references, 12 of which can be accessed free at http://www.jbc.org/content/271/8/4497.full.html#ref-list-1