Association of Increased Spontaneous Mutation Rates with High Levels of Transcription in Yeast

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Complex processes such as transcription, replication, repair, and recombination require changes in chromatin structure and the interactions of numerous trans-acting factors with DNA sequences, raising the possibility that these processes may be interrelated. Here the effect of transcription on the rate of spontaneous mutation in the yeast *Saccharomyces cerevisiae* was examined. With the use of a *lys2* frameshift allele under the control of a highly inducible promoter, the rate of spontaneous reversion was shown to increase when the mutant gene was highly transcribed. Thus, transcriptionally active DNA and enhanced spontaneous mutation rates are associated in yeast.

The higher order structure of the compacted eukaryotic chromosome is not static, but provides a dynamic scaffold for various DNA functions such as transcription, replication, repair, and recombination. Only a fraction of the genome is transcriptionally active at any time in a given cell type, and this fraction has different structural features as compared to unexpressed genomic regions (*1*, *2*). Recent studies of induced DNA damage and repair have indicated that the efficiency of repair is related to the transcriptional state of the DNA (*3*–*5*). Specifically, the transcribed strand of an active gene is targeted for preferential repair (transcription-coupled repair), which leads to enhanced repair of transcribed genes relative to nontranscribed genes. Such strand-specific repair results from the stalling of RNA polymerase at a lesion, which effectively targets the repair machinery to the lesion. In addition to the association between transcription and repair, an association between the transcriptional state of DNA and the frequency of lesions or mutations induced by a variety of DNA-damaging agents has been reported (*6*–*9*). Although differential damage sensitivity and repair of active genes may contribute to heterogeneous mutation rates, there is no direct evidence that links spontaneous mutation rates to the transcriptional activity of DNA. The experiments reported here examine the relation between transcription and spontaneous mutation in the yeast *S. cerevisiae*.

For mutational studies, a chromosomal *lys2* frameshift allele was placed under transcriptional control of the highly inducible yeast *GAL1-10* promoter (*pGAL*) to yield the *pGAL-lys2ΔBgl* allele. Our previous studies with a *lys2ΔBgl* allele demonstrated that reversion events are predominantly, if not exclusively, intragenic (*10*). The yeast *GAL1-10* promoter is induced to high levels in the presence of galactose, the target of which is the negative regulatory protein Gal80p (*11*). Although *pGAL* activity is normally regulated by the presence or absence of the inducer galactose, we wanted to achieve comparable regulation without alteration of the growth medium. Therefore, we constructed isogenic strains that either contained ($Gal80^+$) or were missing ($Gal80^-$) the negative regulator of the *GAL* promoter. In the absence of galactose, our $Gal80^-$ strains produced approximately 50-fold more transcript from the *pGAL-lys2ΔBgl* allele than did $Gal80^+$ strains (*12*). $Gal80^+$ and $Gal80^-$ strains will henceforth be referred to as low- and high-transcription strains, respectively.

The accumulation of Lys^+ revertants in the low- and high-transcription strains was examined by plating nongalactose-grown cultures on lysine-deficient medium containing galactose. The newly appearing Lys^+ colonies derived from 18 independent cultures of each strain were counted daily, and the data are presented in Table 1. On the first day that Lys^+ colonies could be counted (day 3 in Table 1) there were several hundredfold more Lys^+ revertants derived from the high-transcription cultures than from the low-transcription cultures. On subsequent days, the numbers of newly appearing Lys^+ prototrophic colonies were similar for the low- and high-transcription cultures. Our previous studies showed that the earliest appearing prototrophs are revertants that arise during nonselective growth in liquid medium, whereas later appearing prototrophs correspond to reversion events

Table 1. Numbers of Lys^+ prototrophs appearing daily in low- and high-transcription strains. SJR297 and SRJ298 are the low-transcription ($Gal80^+$) and high-transcription ($Gal80^-$) strains, respectively, containing the *pGAL-lys2ΔBgl* allele (*45*). Both were derived from strain SJR195 (*MATα ade2-101$_{oc}$ his3Δ200 uraΔNco*) by transformation (*46*). Colonies excised from plates were used to inoculate 5 ml of YEP liquid medium (1% Bacto-yeast extract; 2% Bacto-peptone) containing 2% each of glycerol and ethanol as carbon sources. Cultures were grown to saturation for 2 days at 30°C on a roller drum. Cells were harvested by centrifugation, washed with 5 ml of H_2O, and resuspended in 1 ml of H_2O. The entire contents of each culture were plated in 100-μl aliquots on 10 selective plates. Selective medium was synthetic-complete medium (*47*) deficient in lysine and contained 2% each of galactose, glycerol, and ethanol instead of glucose as carbon sources. Appropriate dilutions were plated nonselectively on YPD medium (*47*) to determine the total number of viable cells per culture. Daily numbers represent the total number of newly arising Lys^+ colonies derived from each culture. Cultures 1 to 8 and cultures 9 to 18 were from independent experiments. The mutation rate (mutants per generation) for the $Gal80^+$ and $Gal80^-$ strains were calculated from day 3 data by the P_0 method (*48*) and the method of the median (*44*), respectively. ND, not determined.

Culture	Cells plated ($\times 10^7$)	Day 3*	Day 4	Day 5	Culture	Cells plated ($\times 10^7$)	Day 3*	Day 4	Day 5
Low-transcription strain ($Gal80^+$)					*High-transcription strain ($Gal80^-$)*				
1	90	3	19	10	1	101	179	35	19
2	97	1	16	32	2	76	261	51	18
3	101	1	17	36	3	83	150	35	19
4	122	1	10	18	4	101	295	41	16
5	76	0	16	22	5	115	171	23	20
6	116	1	11	18	6	101	259	37	23
7	72	1	17	19	7	61	230	41	15
8	101	0	10	15	8	112	197	41	22
9	148	0	14	17	9	115	232	38	19
10	101	2	12	22	10	112	210	34	24
11	131	1	24	25	11	121	315	38	11
12	100	0	13	25	12	132	441	52	25
13	111	1	17	26	13	94	215	56	17
14	73	0	21	27	14	78	211	23	13
15	88	0	16	14	15	94	223	16	8
16	103	1	19	19	16	80	484	44	14
17	117	0	24	23	17	135	244	34	4
18	105	1	21	22	18	84	284	20	14
Mean		ND	16.5	21.7	Mean		255.6	36.6	16.7
Variance		ND	18.9	40.4	Variance		7541.4	119.2	30.6

*Day 3 rate = 0.9×10^{-9} ($Gal80^+$) or 31.1×10^{-9} ($Gal80^-$).

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that occur after selective plating (*10*). Consistent with this interpretation, the number of revertants that appeared daily approximated a Poisson distribution with time (the variance approaches the mean). Because cells were plated selectively in the presence of galactose, transcription is high in the Gal80$^+$ as well as in the Gal80$^-$ strain after selective plating. Therefore, it is not surprising that the numbers of late-appearing prototrophs were similar in the two strains. The day 3 data presented in Table 1 were used to calculate the mutation rates during the nonselective growth period when there is a large difference in *pGAL-lys2ΔBgl* transcription in the high- and low-transcription strains. Day 3 data indicate a 35-fold increase in reversion rate when the *lys2ΔBgl* allele was transcribed at a high level (31.1×10^{-9} versus 0.9×10^{-9}).

Two trivial explanations could account for the difference in mutation rates as a function of the transcriptional state of the *pGAL-lys2ΔBgl* allele. (i) There could be a general increase in mutation rates (a mutator phenotype) in the high-transcription strain relative to the low-transcription strain. (ii) The time of prototrophic colony appearance could be influenced by the transcriptional state of the reverted allele, so that Lys$^+$ revertants that arise in the high-transcription background form colonies faster or more efficiently than those arising in the low-transcription background.

Our evidence indicates that the increase in the rate of Lys$^+$ prototroph formation observed in the high-transcription strain is specific to the highly transcribed *pGAL-lys2ΔBgl* allele. (i) Reversion rates of an uninducible *lys2ΔBgl* allele (*pLYS-lys2ΔBgl*; *lys2* sequences under control of the *LYS2* promoter) were measured in the same Gal80$^+$ and Gal80$^-$ backgrounds that were used in the *pGAL-lys2ΔBgl* reversion analyses (*13*). The reversion rates of the *pLYS-lys2ΔBgl* allele were similar in the Gal80$^+$ and Gal80$^-$ strains (1.9×10^{-9} and 2.8×10^{-9}, respectively). (ii) The forward mutation rates to canavanine resistance at the unrelated *CAN1* locus were measured in the Gal80$^+$ and Gal80$^-$ strains used for *pGAL-lys2ΔBgl* reversion studies. The forward mutation rates at *CAN1* were indistinguishable: 2.4×10^{-8} and 2.5×10^{-8} for the Gal80$^+$ and Gal80$^-$ strains, respectively (*14*). (iii) In addition to experiments done in the high-transcription Gal80$^-$ background, we were able to increase the reversion rate of the *pGAL-lys2ΔBgl* allele in the Gal80$^+$ strain 12-fold by growing this strain in the presence of the inducer galactose. The level of the *pGAL-lys2ΔBgl* transcript under these conditions was intermediate between that found in the high- and low-transcription strains (*12*), suggesting that there may be a proportionality between the rate of transcription and the mutation rate. On the basis of these analyses we conclude that Gal80$^-$ strains do not have a general mutator phenotype and that the reversion rate of the *pGAL-lys2ΔBgl* allele is specifically related to its transcriptional state.

A second trivial explanation for the different numbers of Lys$^+$ prototrophs that arise in the low- and high-transcription backgrounds is that the appearance of the Lys$^+$ colonies is related to the transcriptional state of the reverted allele. One could imagine that many of the revertants arising in the low-transcription strain were not detected because they failed to form colonies (low plating efficiency) or that they formed colonies slowly (phenotypic lag). To address these possibilities, we compared the growth properties of four independent Gal80$^-$Lys$^+$ revertants to those of four independent Gal80$^+$Lys$^+$ revertants in reconstruction experiments. After nonselective growth, approximately 100 cells of each revertant were plated selectively in the presence of a large excess (1×10^8) of Lys$^-$ background cells in order to approximate the cell density conditions of the reversion rate assays. We found that the growth rates of >95% of the Lys$^+$ cells of each revertant were such that colonies appeared by day 4 after selective plating. In addition, the colony sizes of the Gal80$^+$ and Gal80$^-$ revertants analyzed were indistinguishable, even on the earliest day of appearance. The Gal80$^+$Lys$^+$ revertants used in these reconstruction experiments, however, were those that appeared in the reversion rate assays. The possibility remained, therefore, that the relatively low numbers of Lys$^+$ revertants observed in the Gal80$^+$ strain resulted from the inability of many of the revertants to form colonies under conditions of low-level expression rather than from an inherently lower reversion rate. To test this possibility, four independent Lys$^+$ revertants isolated in the high-transcription, Gal80$^-$ strain were converted to Gal80$^+$ by transformation (*15*). Each Gal80$^-$/Ga80$^+$ pair was compared in reconstruction experiments identical to those described above. The conversion of a high-transcription, Gal80$^-$ lysine prototroph to a low-transcription, Gal80$^+$ prototroph affected neither the time nor the efficiency of colony formation.

Although the results described above relate to the reversion of a specific frameshift allele, we believe that the stimulatory effect of transcription on mutation is a general phenomenon because a similar relation exists between transcription and forward mutations to lysine auxotrophy. Strains that contain a wild-type *LYS2* gene fused either to the low-transcription, *LYS2* promoter or the high-transcription, *GAL1-10* promoter were constructed, and the rates of *lys2* mutations under different transcriptional conditions were estimated by plating cells on galactose medium containing α-aminoadipate, a compound that selects against Lys$^+$ cells (*16, 17*). Because the wild-type *LYS2* gene product must be diluted out in order for a mutant to form a colony on α-aminoadipate medium, there is likely to be a phenotypic lag in the expression of the Lys$^-$ phenotype. Selection with α-aminoadipate may, therefore, lead to an underestimation of forward mutation rates, especially when the *LYS2* gene is highly transcribed. Nevertheless, the forward mutation rate to lysine auxotrophy was increased 10-fold in the high-transcription strain relative to the low-transcription strain (1.7×10^{-6} versus 1.7×10^{-7}, respectively). This result not only supports the generality of an association between transcription and mutation, but also eliminates the possibility of an expression-associated artifact in the *lys2ΔBgl* reversion assays.

In yeast, mutation rates are influenced by the three genetically defined pathways for repairing DNA damage: the *RAD3* excision repair pathway, the *RAD6* error-prone repair pathway, and the *RAD52* recombination pathway (*18, 19*). We assessed the potential roles of genes in the *RAD6* and *RAD52* repair pathways on

Table 2. Effect of repair mutations on Lys$^+$ prototroph formation. The wild-type Gal80$^+$ and Gal80$^-$ strains are SJR297 and SJR298, respectively. All mutant strains were derived from SJR297 and SJR298 by transformation (*49*). The wild-type data are from Table 1. For the mutant strains, 5-ml cultures were grown and plated as described in Table 1. The entire contents of the cultures were plated selectively, with the exception of the *rad18* strains; only one-fifth of the cultures were plated for the *rad18* mutants. The rate determinations for the *rev3, rad6*, and *rad18* strains were derived with nine independent cultures for each strain; the rate determinations for the *rad52* strains were derived with five cultures for each strain. The rates are based on day 3 colony counts and were calculated by the P_o method (*48*) for the wild-type and *rev3* low-transcription strains, or the method of the median (*44*) for all other strains. Numbers in parentheses are rates normalized to the wild-type rate in the corresponding low- or high-transcription strain. The levels of *lys2ΔBgl* transcript in the high-transcription *rev3, rad6*, and *rad18* strains were the same as in the high-transcription wild-type strain (*12*).

Genotype	Rate of Lys$^+$ ($\times 10^{-9}$)	
	Low transcription (Gal80$^+$)	High transcription (Gal80$^-$)
Wild type	0.9 (1.0×)	31.1 (1.0×)
rev3	0.8 (0.9×)	9.6 (0.3×)
rad6	12.5 (14×)	51.0 (1.6×)
rad18	19.0 (21×)	101 (3.3×)
rad52	25.8 (29×)	167 (5.4×)

transcription-associated mutation by measuring the effects of mutations in representative genes on the reversion rate of the *lys2ΔBgl* allele (Table 2). The *REV3*, *RAD6*, and *RAD18* genes are in the error-prone *RAD6* pathway. Mutations in *REV3* confer an antimutator phenotype, with mutants exhibiting a reduction in both spontaneous and damage-induced mutation rates (*20–22*). DNA sequence analysis has revealed that Rev3p has homology to DNA polymerase, and it has been suggested that the protein may be involved in translesion synthesis on damaged DNA templates (*23*). The data in Table 2 demonstrate that *REV3* has a role in generating transcription-associated mutations; 70% of the *lys2ΔBgl* reversion events in the high-transcription strain were dependent on *REV3*. The *RAD6* and *RAD18* genes, while members of the so-called "error prone" repair pathway, also appear to be involved in a major, as yet undefined, error-free repair pathway (*24*). Mutations in *RAD6* or *RAD18* confer a spontaneous mutator phenotype (*20*), but reduce some types of induced mutagenesis (*22*, *25*, *26*). Rad6p has ubiquitin-conjugating activity that is essential for its role in repair (*27*), and Rad18p has been shown to bind to single-stranded DNA (*28*); these proteins can interact to form a complex (*28*). Similar to the increase reported for spontaneous mutations, the reversion rate of the *pGAL-lys2ΔBgl* allele was increased in the *rad6* and *rad18* mutant strains under conditions of both low- and high-transcription (Table 2). The *RAD52* gene is required for essentially all recombination in yeast (*29*) and has a role in mutation avoidance (*30*). In a *rad52* mutant strain, the reversion of the *lys2ΔBgl* allele was increased under both low- and high-transcription conditions (Table 2).

The results presented here clearly demonstrate a stimulatory effect of high levels of transcription on spontaneous mutation rates in yeast. The earlier work of Korogodin *et al.* (*31*) suggested that the transcriptional state of a gene might influence its mutation rate in yeast. Whereas a similar effect of transcription on mutation has not been reported in other systems, those systems were not designed specifically to detect such a relation (*32*, *33*). Transcription has been shown to increase the efficiency of DNA repair in a number of systems (*3–5*) and to increase recombination rates in yeast (*34*). In the case of recombination, a transcription-associated relaxation of the highly compacted chromatin structure has been invoked, making transcriptionally active DNA more accessible to the recombination machinery. In relation to spontaneous mutation, the more open structure of transcriptionally active DNA may make it more accessible to endogenous or environmental DNA-damaging agents. Transcription-associated torsional stress (*34*) or the transient single-stranded nature of actively transcribed DNA (*35*) also may be factors in generating lesions and potentially targeting mutations to actively transcribed regions. The partial *REV3* dependence of transcription-associated mutation events implicates DNA damage and translesion synthesis as a source of many of these events. If DNA damage does play a role, however, a similar dependence of transcription-associated mutation on *RAD6* and *RAD18* might be expected; such a dependence was not observed.

Alterations in DNA structure may seem the most likely reason for an association between transcription and mutation, but other possibilities exist. Because cDNA-mediated recombination has been demonstrated in yeast (*36–38*), errors made during transcription could, in principle, be incorporated back into the DNA. The process of recombination itself also can generate mutations, which presumably result from errors made during recombination-associated DNA synthesis (*39*). The lack of dependence of transcription-associated mutation on the *RAD52* gene indicates that recombination does not play a major role in this process. In addition to recombination-based models, high levels of transcription may interfere with the fidelity of DNA replication or with the repair of replication errors. In relation to repair, transcription does interfere with at least some types of mismatch repair in *Escherichia coli* (*40*). Finally, since the blockage of transcription at lesions can trigger the DNA repair process, it is possible that the stalling of RNA polymerase at natural pause sites might similarly trigger "gratuitous" transcription-coupled repair (*41*). In highly transcribed genes, the resulting reiterative repair synthesis could be the source of additional mutations. Whatever the mechanism or mechanisms, the effect of transcription on mutational processes underscores the dynamic nature of the eukaryotic genome and has implications for genome stability and evolution.

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13. The genomic *LYS2* allele in Gal80$^+$ and Gal80$^-$ strains (SJR195 and SJR282, respectively) was replaced with *lys2ΔBgl* by a two-step replacement procedure (*42*). Strains were transformed with Xho I–digested pSR125 (internal Eco RV fragment of the *lys2ΔBgl* allele cloned into the Klenow filled-in Hind III site of a pUC9 derivative containing a 1.2-kb *URA3* Bam HI fragment at the Bam HI site) and Ura$^+$ transformants were selected. Plasmid-loss events were identified on 5-fluoroorotic acid medium [5-FOA; J. D. Boeke, J. Trueheart, G. Natsoulis, G. R. Fink, *Methods Enzymol.* **154**, 164 (1987)] and Ura$^-$ segregants were screened for a Lys$^-$ phenotype. Presence of the *lys2ΔBgl* allele was confirmed by digestion of a polymerase chain reaction (PCR)–amplified *LYS2* genomic fragment with Cla I.
14. Independent cultures of the Gal80$^+$ and Gal80$^-$ strains (SJR297 and SJR298, respectively) were grown as described in Table 1. Forward mutation at *CAN1* to canavanine resistance was measured by plating cells on minimal medium deficient in arginine and supplemented with L-canavanine sulfate (45 μg/ml).
15. Four independent Gal80$^-$Lys$^+$ revertants were transformed to Gal80$^+$ by integration of plasmid pSR333 (3.1-kb *GAL80* Hind III fragment and 1.2-kb *URA3* Bam HI fragment in pGEM3). The plasmid was targeted to the *URA3* locus by digestion with Eco RV. Ura$^+$ transformants were screened for resistance to 2-deoxygalactose [T. Platt, *Mol. Cell. Biol.* **4**, 994 (1984)].
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17. Yeast strains SJR282 (*pLYS-LYS2 gal80::HIS3*) and SJR371 (*pGAL-LYS2 gal80::HIS3*) were used for the *LYS2* forward mutation analysis. Although both strains are Gal80$^-$, the *LYS2* gene is expressed at high levels only in SJR371. SJR371 was derived from SJR298 (*pGAL-lys2ΔBgl gal80::HIS3*) by transformation with a *LYS2*-internal Eco RV fragment from pDP6 (*43*), replacing the *lys2ΔBgl* mutation with wild-type sequences. Six independent cultures of each strain were grown as described in Table 1, and aliquots of cells were plated selectively on α-aminoadipate medium [R. S. Sikorski and J. D. Boeke, *Methods Enzymol.* **194**, 302 (1991)] containing 2% each of galactose, glycerol, and ethanol as carbon sources. Approximately 5×10^7 and 5×10^6 cells of SJR282 and SJR371, respectively, were spread on each selective plate. Because α-aminoadipate selection works best at high cell densities, a background lawn (8×10^7 cells) of a *LYS2/LYS2* diploid strain was added to the selective medium when plating SJR371 cultures. Lys$^-$ colonies were first evident 7 days after selective plating. Mutation rates were calculated by the method of the median (*44*) with day 7+8 cumulative colony counts.
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45. Plasmid pSR247, a pGEM2 derivative containing the *pGAL-lys2ΔBgl* allele and the yeast *URA3* gene, was constructed as follows. The unique Bgl II restriction site near the 5′ end of the *LYS2* gene on plasmid pDP6 (*43*) was filled-in with Klenow, adding 4 base pairs to the coding sequence and creating a Cla I site (plasmid pSR108). A 3-kb Nco I fragment containing the distal two-thirds of the *lys2ΔBgl* allele was deleted from pSR108 to give plasmid pSR227. A 1.5-kb Eco RV–Hind III fragment from pSR227 was subcloned into Hinc II-Hind III–digested pGEM2, yielding a plasmid (pSR232) containing a promoterless 5′ portion of the *lys2ΔBgl* allele. A 1-kb fragment derived from chromosomal sequences upstream of the *LYS2* locus (from plasmid pDCH6) (*43*) was subcloned into Eco RI–digested pSR232 yielding plasmid pSR233. A Pst I fragment containing the *GAL1-10* promoter region {the Bam HI–Eco RI promoter fragment of pBM150 [M. Johnston, R. W. Davis, *Mol. Cell. Biol.* **4**, 1440 (1984)] with added Pst I linkers} was then inserted at the unique Pst I site adjacent to the promoterless *lys2ΔBgl* fragment of pSR233 to give plasmid pSR239; the *lys2* sequences are transcribed from the *GAL10* promoter. Finally, a 1.2-kb *URA3* Hind III fragment was inserted at the unique Hind III site of pSR239 to give plasmid pSR247.
46. The *pGAL-lys2ΔBgl* allele was introduced by a two-step replacement procedure (*42*) in which pSR247 was targeted to integrate at the *LYS2* locus by digestion with Nru I. After selection of transformants on uracil-deficient medium, Ura$^-$ segregants were selected on 5-FOA and screened for a Lys$^-$ phenotype. The structure of the presumptive chromosomal *pGAL-lys2ΔBgl* allele was confirmed by Southern (DNA) blot analysis. Plasmid pSR244 contains a *gal80::HIS3* disruption allele and was constructed by replacing a Bgl II fragment within the *GAL80* coding sequence [T. E. Torchia, R. W. Hamilton, C. L. Cano, J. E. Hopper, *Mol. Cell. Biol.* **4**, 1521 (1984)] of plasmid pSR243 (3.1-kb *GAL80* Hind III fragment in pGEM3) with a 1.7-kb *HIS3* Bam HI fragment. One-step disruption (*42*) of *GAL80* was accomplished by transforming yeast with Nco I–Sma I–digested pSR244 and selecting His$^+$ transformants. The presence of the *gal80::HIS3* disruption in the resulting strain (SJR282) was confirmed by sensitivity of cells to 2-deoxygalactose and by Southern blot analysis.
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Correction of Radiation Sensitivity in Ataxia Telangiectasia Cells by a Truncated IκB-α

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Cells from patients with ataxia telangiectasia (AT) are hypersensitive to ionizing radiation and are defective in the regulation of DNA synthesis. A complementary DNA that corrects the radiation sensitivity and DNA synthesis defects in fibroblasts from an AT group D patient was isolated by expression cloning and shown to encode a truncated form of IκB-α, an inhibitor of the nuclear factor kappa B (NF-κB) transcriptional activator. The parental AT fibroblasts expressed large amounts of the IκB-α transcript and showed constitutive activation of NF-κB. The AT fibroblasts transfected with the truncated IκB-α expressed normal amounts of the IκB-α transcript and showed regulated activation of NF-κB. These results suggest that aberrant regulation of NF-κB and IκB-α contribute to the cellular defect in AT.

Ataxia telangiectasia (AT) is a human autosomal recessive disease characterized by neurological, immunological, and radiobiological deficiencies. Four genetic complementation groups and two variants have been identified by heterokaryon analysis (*1*). Cells from patients with AT are hypersensitive to ionizing radiation and show aberrant regulation of DNA synthesis (*2*). To identify genes that contribute to the radiation sensitivity of AT cells, we used an Epstein-Barr virus (EBV)–based expression vector to screen a complementary DNA (cDNA) library for the ability to restore normal radiosensitivity to AT cells. When this vector (pCNCNot) is transfected into cells that produce the EBV nuclear antigen–1 (EBNA-1), the plasmids are maintained episomally and can be retrieved by Hirt DNA extraction methods (*3*, *4*).

SV40-immortalized fibroblasts from an AT group D patient (AT5BIVA) were first transfected with the p266CH2 plasmid (which carries the EBNA-1 gene), and a clonally derived cell line (ATCL2) was established to serve as the recipient for gene transfer experiments (*4*). A cDNA library from SQ-20B cells, a human squamous carcinoma cell line previously characterized as radiation-resistant, was constructed in the pCNCNot vector (*4*, *5*). We reasoned that these cells would express adequate quantities of the mRNAs associated with the radioresistant phenotype.

After cDNA transfection and drug selection, cells were pooled, expanded, and exposed to radiation selection with 8 gray (Gy) of ionizing radiation. Cells that survived the first radiation exposure were expanded and re-exposed. Several surviving colonies were then subcloned and cultured for further studies. Clonally derived cell lines ATCL2-8 and ATCL2-11 were isolated from independent, but procedurally similar transfection experiments.

Radiation sensitivity is defined by the steepness of the terminal slope of the survival curve when cells are exposed to graded doses of ionizing radiation. As determined from target theory analysis, the slope is proportional to $1/D_0$, where D_0 is the dose required to reduce cell survival to 37% (*6*). Values for D_0 range from 1.2 to 1.4 Gy for normal human fibroblasts and from 1.1 to 1.9 Gy for SV40-immortalized fibroblasts (*7*). The ATCL2-8 and ATCL2-11 cells were less sensitive to radiation (D_0 = 1.7 and 1.6 Gy, respectively) than were parental AT5BIVA cells (D_0 = 0.7 Gy) (Fig. 1).

Episomal DNAs were obtained by Hirt extractions, revealing seven candidate cDNAs in ATCL2-11 and one in ATCL2-8. The cDNAs ranged in size from 0.2 to 2 kb, and sequence analysis revealed that a 1-kb cDNA was common to both cell lines. The candidate 1-kb cDNA was then retransfected into ATCL2 cells, yielding

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