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Further Characterizations of Bleomycin-Sensitive (*blm*) Mutants of *Saccharomyces cerevisiae* with Implications for a Radiomimetic Model

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Direct selection for 12 mutations (*blm*) conferring hypersensitivities to lethal effects of bleomycins in *Saccharomyces cerevisiae* resulted in mutants exhibiting cross-hypersensitivity to ionizing radiation and hydrogen peroxide. Remaining mutations did not confer cross-hypersensitivity to radiation. All *blm* mutations were recessive, except codominant *blm3-1*, and were assigned to seven complementation groups.

In order to improve our understanding of radiomimetic damage to cells and how cells respond to and reverse such damage, we isolated a large number of mutants (*blm* [17]) of *Saccharomyces cerevisiae* on the basis of their hypersensitivities to lethal effects of the bleomycin family of low-molecular-weight chemical congeners (M_r approximately 1,500 to 1,600). Objectives of the current study were to determine for 12 *blm* mutants the inheritance patterns of the mutant genes, genetic complementation among the mutants, hypersensitivities conferred by the *blm* mutations to ionizing and UV radiation and hydrogen peroxide, and temperature sensitivities. A common property of these agents is the generation of hydroxyl free radicals, which mediate DNA breaks (for examples, see references 1–3, 6–8, 10–15, 23, 24, 27–31).

Meiotic segregants for this study were constructed by conventional techniques of crossing, sporulation, and dissection (22, 26). Culturing, media preparation, ionizing and UV irradiation, and quantitative survival assays were carried out as previously described (17–20, 22).

The range of sensitivities conferred by the *blm* mutations and the importance of each defective gene on survival are illustrated in Fig. 1. Survival of the parental strain (Fig. 1A) decreased approximately linearly from 100 to 16% ± 0.38% in a dose range where survival of each mutant strain (Fig. 1B through L) decreased to 0.05 to 0.0001%. Elevated sensitivities to lethal effects of the chemical congeners segregated 2:2 in complete tetrads derived from 3 to 15 independent heterozygous diploids constructed for each mutant, indicating single-gene or Mendelian inheritance.

With one exception, all mutations behaved as recessive mutations. Hypersensitivities to lethal effects of bleomycin were not exhibited in heterozygous diploid strains. Fifteen heterozygous diploids containing the mutation from the X1-30 mutant exhibited sensitivities to bleomycin between the sensitivities of parental strains, indicating codominant expression of the mutation.

Cross-hypersensitivities to radiation. A goal of the genetic

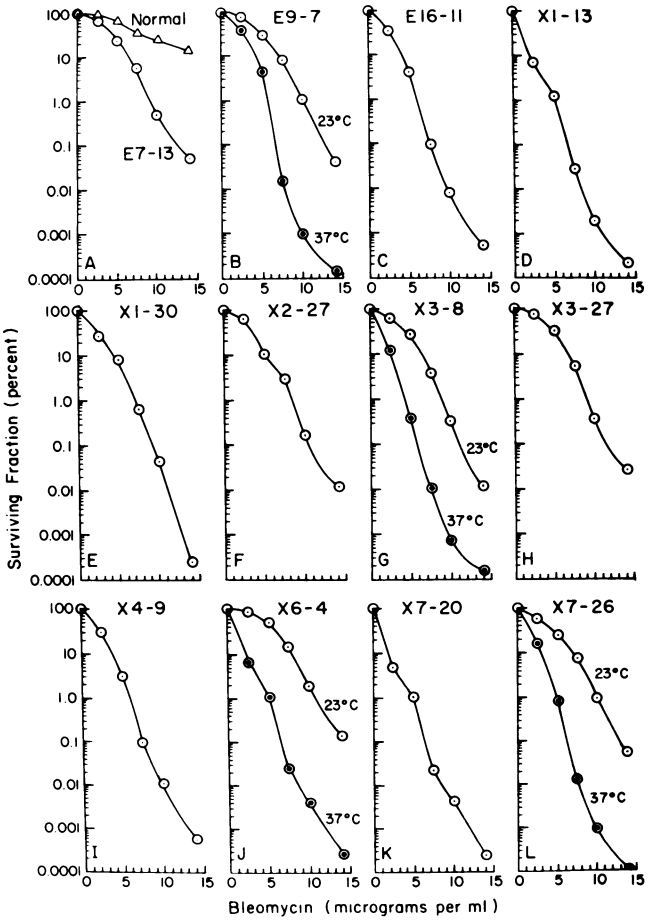

FIG. 1. Representative survival curves for parental (CM1069-40, *MATα*, *ade2-40*, *trp5-12*, *ilv1-92*, *cyc1-45* [17, 21]) and mutant strains. Cells were plated and grown on nonsynthetic complete medium (1% Bacto-Yeast extract, 2% Bacto-Peptone, 2% dextrose, and 0.08 mg of adenine sulfate per ml, solidified with 1 to 1.5% Noble agar or Bacto-Agar [YPAD]) containing 0 to 14 μg of bleomycin per ml. Survival at 23 and 37°C is compared for mutants exhibiting temperature-sensitive expression of bleomycin sensitivity, except for X1-13, whose survival at 37°C at high test concentrations was too low to be determined accurately. For all other mutants, survival at 23°C is illustrated. The X2-27, X3-27, X4-9, and X7-20 mutants exhibited slower, poor, or no growth in the absence of the chemical congeners at their restrictive growth temperature of 37°C.

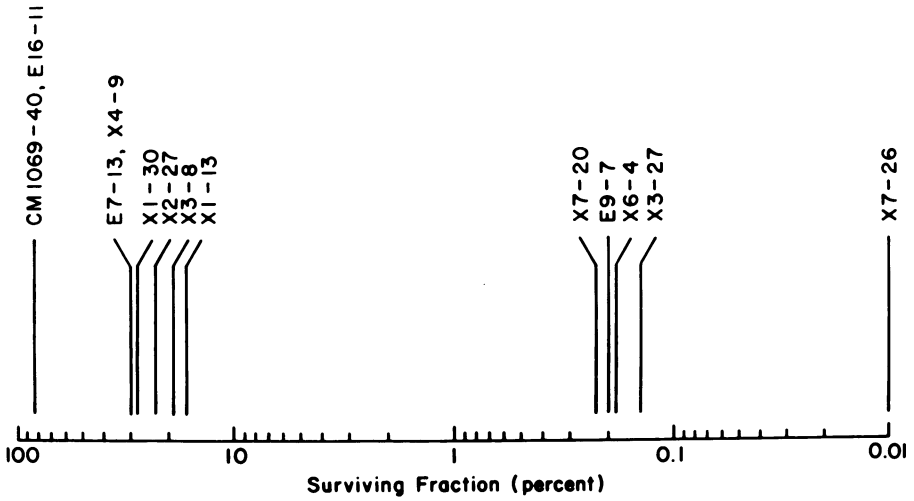

FIG. 2. Range of sensitivities to lethal effects of hydrogen peroxide. Mean values for survival at 30°C on YPAD medium containing 120 μg of hydrogen peroxide per ml were determined from quantitative survival curves (80 to 160 μg of hydrogen peroxide per ml; pH of medium was adjusted to 7.5 with 50 mM phosphate buffer).

pedigree analyses conducted for the current study was to determine whether single mutations conferred cross-hypersensitivities. The E7-13, E9-7, X1-13, X1-30, X2-27, X3-8, X3-27, X7-20, and X7-26 mutants manifested hypersensitivities to radiation. Hypersensitivities to bleomycin and ionizing radiation cosegregated in pedigrees, indicating that the hypersensitivities most likely were conferred by the same mutation. In contrast, hypersensitivities to UV light in X1-13, X1-30, X7-20, and X7-26 mutants were conferred by mutations in separate genes, and independent recessive mutations were confirmed in testcrosses and backcrosses. In mutants possessing slightly higher sensitivities to radiation than their isogenic parent, radiation sensitivity could not be attributed to monogenic control.

Sensitivities to hydrogen peroxide. The mutants exhibited a range of sensitivities to lethal effects of hydrogen peroxide (Fig. 2). The X7-26 mutant was extremely sensitive. The E9-7, X3-27, X6-4, and X7-20 mutants composed a somewhat less sensitive group. The survival curves of a third group, E7-13, X1-13, X1-30, X2-27, X3-8, and X4-9, had distinct shoulders, but the mutants were more sensitive than parental or related normal strains. The resistance of E16-11 and parental or related normal strains was equivalent.

All mutants possessing hypersensitivities to ionizing radiation (E7-13, E9-7, X1-13, X1-30, X2-27, X3-8, X3-27, and X7-20) exhibited elevated sensitivities to killing by hydrogen peroxide and fell into two of the three sensitivity groups (Fig. 2). These results broaden the radiomimetic model in *S.*

TABLE 1. Summary of complementation tests[a]

Mutant	E16-11	X6-4	X3-8	E9-7	X2-27	X3-27	X4-9	X1-30	X7-26	X1-13	X7-20	E7-13
E7-13	R^2	R^5	R^1	R^1	R^3	R^1	R^1	R^2/S^1	R^1	R^3	R^2	S^1
X7-20	R^7	R^2	R^3	R^2	R^6	R^3	R^2	R^5	R^3	R^3	S^2	
X1-13	R^4	R^3	R^2	R^2	R^3	R^2	$+-^2$	R^4	R^3	S^1		
X7-26	R^4	R^7	R^1	R^1	R^2	R^1	$+-^1$	R^2	S^1			
X1-30	R^3	R^5	R^2/S^2	S^2	R^4/S^2	R^1/S^1	R^2	S^2				
X4-9	R^2	R^2	R^2	R^2	R^2	R^1	S^1					
X3-27	S^5	S^6	R^2	R^2	R^3/S^1	S^2						
X2-27	S^5	S^5	R^3/S^1	R^1/S^1	S^1							
E9-7	$+-^2$	$+-^1$	S^1	S^1								
X3-8	$+-^2/S^2$	S^2	S^1									
X6-4	S^9	S^3										
E16-11	S^{11}											

[a] Pairwise crosses of mutants and mutant segregants were made. Mutant segregants were derived from heterozygous diploids after one to seven backcrosses to normal haploid strains. Mating was observed microscopically. Diploids were subcloned, and sporulation was checked. Hypersensitivities to bleomycin were determined by measuring survival on YPAD medium containing 0, 1, 2.5, 5, 7.5, 10, 15, and 20 μg of bleomycin per ml. Hypersensitivities to ionizing radiation were measured after 10, 20, 30, 40, 50, 75, and 100 krad of irradiation. If a diploid strain possessed significantly elevated sensitivity in comparison to the respective heterozygous diploids and sensitivities similar to those of the two respective homozygous diploids, the two mutations were determined to be noncomplementing. If survival assays on independent diploids differed, both results are tabulated. R, Diploid exhibited resistance to killing by bleomycin characteristic of normal diploid strains of the same or similar genetic backgrounds. S, Diploid exhibited significantly higher sensitivity than that characteristic of normal diploid strains of the same or similar backgrounds. Superscripts, numbers of independent crosses constructed with the original isolates or segregants bearing the mutant genes. +−, Weak complementation. The E16-11 mutation did not fully complement the X2-27, X3-8, X3-27, and X6-4 mutations. The X6-4 mutation did not complement the X2-27, X3-8, X3-27, and E16-11 mutations. The X2-27 mutation did not complement E16-11 or X6-4, but three of four crosses involving the X3-8 or X3-27 mutations and one of two crosses involving the E9-7 mutation exhibited complementation. The X3-27 mutation was not complemented by E16-11 or X6-4 but exhibited complementation in crosses to X3-8 and E9-7 and in three of four crosses to X2-27. Full complementation was not exhibited in some crosses of the codominant X1-30 mutant to some of the mutants in the first complementation group or in crosses of X1-13 and X7-26 to X4-9.

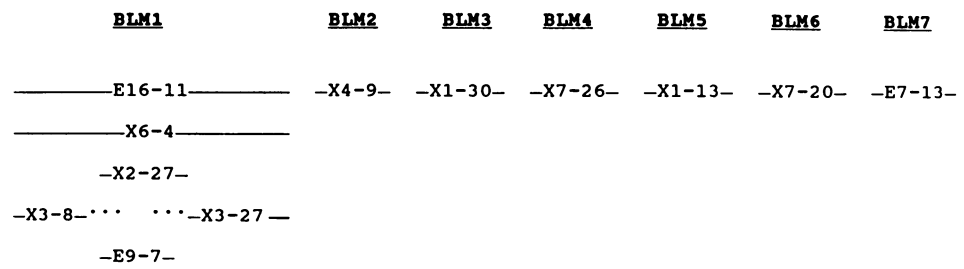

FIG. 3. A complementation map and assignment of gene numbers based on the results of complementation tests summarized in Table 1. The assignments to seven complementation groups appear to best fit the results of the complementation tests. The two short dotted lines express partial or full complementation in some crosses. Quantitative survival results from crosses involving the E9-7 mutation made it difficult to determine noncomplementation or complementation within the first complementation group. The E9-7 mutant did not exhibit complementation in one cross to the X2-27 mutant but exhibited varying amounts of complementation in some crosses to other mutants in the first complementation group. Allele numbers assigned to mutations in the *BLM1* complementation group are as follows: E16-11 (*blm1-1*), X6-4 (*blm1-2*), X2-27 (*blm1-3*), E9-7 (*blm1-4*), X3-27 (*blm1-5*), and X3-8 (*blm1-6*).

cerevisiae (16, 18) to include damage to cells by hydrogen peroxide and suggest that the mutants could sustain abnormally high levels of damage from hydroxyl-free radicals or respond abnormally to this damage.

Gene interaction leading to supersensitivity. All double-mutant segregants (hypersensitive to bleomycin and UV irradiation) in pedigrees involving the X7-26 mutant were significantly more sensitive than single mutants to lethal effects of bleomycins. These supersensitive double mutants also possessed the additional phenotype of growing poorly, particularly at temperatures above 30°C, in the absence of radiation or bleomycin. Homozygous diploid strains were also supersensitive, confirming that both genes were required for supersensitivity. This finding is somewhat surprising if bleomycin is strictly radiomimetic, rather than UV-like, and may reflect a long-patch mode of excision repair (25) recently described for human fibroblasts (4, 5).

Assignment of mutations to complementation groups. Complementation tests among the mutants are presented in Table 1. Six mutants appeared to fall into one complementation group (*BLM1*) and are at this time considered allelic on the basis of functional complementation tests in diploid strains (Table 1; Fig. 3). We have not ruled out the possibility that tightly linked genes are involved. Some of the *blm1* mutations manifested weak complementation in heteroallelic diploids. It is possible that the *BLM1* complementation group comprises more than one locus if, for example, the *BLM1* gene regulates more than one gene or gene product, interaction of more than one gene or gene product occurs (intergenic complementation), or different genes are involved in overlapping functions. Recombinant plasmids complementing the *blm1-1* mutation have been identified (24a), and their characterization should enable these alternative hypotheses to be tested. It is of interest that the four *blm1* mutations from E9-7, X2-27, X3-8, and X3-27 exhibited complementation in some crosses (Table 1; Fig. 3; unpublished results). Curiously, this group also exhibited hypersensitivities to lethal effects of ionizing radiation. The remaining two *blm1* mutations did not complement each other (Table 1; Fig. 3) and did not confer hypersensitivities to ionizing radiation.

Remaining mutants, X1-13, X1-30, X4-9, X7-20, X7-26, and E7-13, complemented each other as well as mutants in the first complementation group (Table 1; Fig. 3). The *blm6* and *blm7* mutations did not complement the X-ray or bleomycin sensitivities in repair-defective (for a review, see reference 9) *rad52* and *rad53* strains (unpublished results), respectively, and thus are considered allelic to *rad52* and *rad53*. Several sporulated diploids homozygous for the *blm6* mutation produced aborted asci. At present, we consider the six additional complementation groups to represent the identification of six additional genes. Fewer genes could be involved if any of these *blm* mutations are allelic and manifested interallelic complementation.

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REFERENCES

1. **Burger, R. M., J. Peisach, and S. B. Horwitz.** 1981. Activated bleomycin. A transient complex of drug, iron and oxygen that degrades DNA. J. Biol. Chem. **256**:11636–11644.
2. **D'Andrea, A. D., and W. A. Haseltine.** 1978. Sequence specific cleavage of DNA by antitumor antibiotics neocarzinostatin and bleomycin. Proc. Natl. Acad. Sci. USA **75**:3608–3612.
3. **Demple, B., A. Johnson, and D. Fung.** 1986. Exonuclease III and endonuclease IV remove 3′ blocks from DNA synthesis primers in H_2O_2-damaged *Escherichia coli*. Proc. Natl. Acad. Sci. USA **83**:7731–7735.
4. **DiGiuseppe, J. A., and S. L. Dresler.** 1989. Bleomycin-induced DNA repair synthesis in permeable human fibroblasts: mediation of long-patch and short-patch repair by distinct DNA polymerases. Biochemistry **28**:9515–9520.
5. **DiGiuseppe, J. A., D. J. Hunting, and S. L. Dresler.** 1990. Aphidicolin-sensitive DNA repair synthesis in human fibroblasts damaged with bleomycin is distinct from UV-induced repair. Carcinogenesis **11**:1021–1026.
6. **Floyel, R. A.** 1981. DNA ferrous iron catalyzed hydroxyl free radical formation from hydrogen peroxide. Biochem. Biophys. Res. Commun. **99**:1209–1215.
7. **Giloni, L., M. Takeshita, F. Johnson, C. Iden, and A. P. Grollman.** 1981. Bleomycin-induced strand-scission of DNA: mechanism of deoxyribose cleavage. J. Biol. Chem. **256**:8608–8615.
8. **Grollman, A. P., and M. Takeshita.** 1980. Interactions of bleo-

mycin with DNA. Adv. Enzyme Regul. **18**:67–83.
9. **Haynes, R. H., and B. A. Kunz.** 1981. DNA repair and mutagenesis in yeast, p. 371–414. *In* J. Strathern, J. Broach, and E. W. Jones (ed.), The molecular biology of the yeast *Saccharomyces*. Life cycle and inheritance. Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.
10. **Henner, W. D., S. M. Grunberg, and W. A. Haseltine.** 1983. Enzyme action at 3′ termini of ionizing radiation-induced DNA strand breaks. J. Biol. Chem. **258**:15198–15205.
11. **Henner, W. D., L. O. Rodriguez, S. M. Hecht, and W. A. Haseltine.** 1983. Gamma-ray induced deoxyribonucleic acid strand breaks. J. Biol. Chem. **258**:711–713.
12. **Johnson, A. W., and B. Demple.** 1988. Yeast DNA diesterase for 3′-fragments of deoxyribose: purification and physical properties of a repair enzyme for oxidative DNA damage. J. Biol. Chem. **263**:18009–18016.
13. **Johnson, A. W., and B. Demple.** 1988. Yeast DNA 3′-repair diesterase is the major cellular apurinic/apyrimidinic endonuclease: substrate specificity and kinetics. J. Biol. Chem. **263**: 18017–18022.
14. **Keller, T. J., and N. J. Oppenheimer.** 1987. Enhanced bleomycin-mediated damage of DNA opposite charged nicks. A model for bleomycin-directed double strand scission of DNA. J. Biol. Chem. **262**:15144–15150.
15. **Meneghini, R., and M. E. Hoffman.** 1980. The damaging action of hydrogen peroxide on DNA of human fibroblasts is mediated by a nondialyzable compound. Biochim. Biophys. Acta **608**: 167–173.
16. **Moore, C. W.** 1978. Responses of radiation-sensitive mutants of *Saccharomyces cerevisiae* to lethal effects of bleomycin. Mutat. Res. **51**:165–180.
17. **Moore, C. W.** 1980. Isolation and partial characterization of mutants of *Saccharomyces cerevisiae* altered in sensitivities to lethal effects of bleomycins. J. Antibiot. **33**:1369–1375.
18. **Moore, C. W.** 1982. Control of *in vivo* (cellular) phleomycin sensitivity by nuclear genotype, growth phase, and metal ions. Cancer Res. **42**:929–933.
19. **Moore, C. W.** 1982. Ligase-deficient yeast cells exhibit defective DNA rejoining and enhanced gamma ray sensitivity. J. Bacteriol. **150**:1227–1233.
20. **Moore, C. W.** 1982. *cdc9* ligase-defective mutants of *Saccharomyces cerevisiae* exhibit lowered resistance to lethal effects of bleomycin. J. Bacteriol. **151**:1617–1620.
21. **Moore, C. W., and A. Schmick.** 1979. Genetic effects of impure and pure saccharin in yeast. Science **205**:1007–1010.
22. **Moore, C. W., and F. Sherman.** 1975. Role of DNA sequences in genetic recombination in the iso-1-cytochrome *c* gene of yeast. I. Discrepancies between physical and genetic distances determined by five mapping procedures. Genetics **79**:397–418.
23. **Murugesan, N., C. Xu, G. M. Ehrenfeld, H. Sugiyama, R. E. Kilkuskie, L. O. Rodriguez, L.-H. Chang, and S. M. Hecht.** 1985. Analysis of products formed during bleomycin-mediated DNA degradation. Biochemistry **24**:5735–5744.
24. **Oberley, L. W., and G. R. Buettner.** 1979. The production of hydroxyl radical by bleomycin and iron(II). FEBS Lett. **97**:47–49.

24a. **Pramanik, A., J. Mc.Koy, and C. W. Moore.** 1989. Meeting on Yeast Cell Biology, Cold Spring Harbor, N.Y., p. 159.

25. **Regan, J. D., and R. B. Setlow.** 1974. Two forms of repair in the DNA of human cells damaged by chemical carcinogens and mutagens. Cancer Res. **34**:3318–3325.
26. **Sherman, F., G. Fink, and J. B. Hicks.** 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Shiloh, Y., E. Tabor, and Y. Becker.** 1983. Abnormal response of ataxia-telangiectasia cells to agents that break the deoxyribose moiety of DNA via a targeted free radical mechanism. Carcinogenesis **4**:1317–1322.
28. **Sugiyama, H., C. Xu, N. Murugesan, and S. M. Hecht.** 1985. Structure of the alkali-labile product formed during iron(II)-bleomycin-mediated DNA strand scission. J. Am. Chem. Soc. **107**:4101–4105.
29. **Takeshita, M., P. Grollman, E. Ohtsubo, and H. Ohtsubo.** 1978. Interaction of bleomycin with DNA. Proc. Natl. Acad. Sci. USA **75**:5983–5987.
30. **Von Sonntag, C., U. Hagen, A. Schon-Bopp, and D. Schulte-Frohlinde.** 1981. Radiation-induced strand breaks in DNA: chemical and enzymatic analysis of end groups and mechanistic aspects. Adv. Radiat. Biol. **9**:109–142.
31. **Ward, J. F.** 1975. Molecular mechanisms of radiation-induced damage to nucleic acids. Adv. Radiat. Biol. **5**:181–239.