was amplified in three overlapping fragments. Mutations detectable by this assay were found in 49% of sporadic colorectal tumors previously evaluated (S. M. Powell *et al.*, unpublished data; *11*).
14. S.-H. Li *et al.*, *Genomics*, in press.
15. H. T. Lynch *et al.*, *Dis. Colon Rectum* **20**, 661 (1977); J.-P. Mecklin and H. J. Järvinen, *ibid.* **29**, 160 (1986).
16. M. Kouri *et al.*, *Cancer* **65**, 1825 (1990).
17. All of the sporadic tumors evaluated here for RER were previously assessed for FAL [B. Vogelstein *et al.*, *Science* **244**, 207 (1989)]. Despite the frequent changes in dinucleotide and trinucleotide repeats in the RER$^+$ tumors, VNTR (variable number tandem repeat) alleles were not similarly affected. Over 30 VNTR polymorphisms distributed throughout the genome were assessed. Additionally, no single base pair changes resulting in altered site polymorphisms were identified in the RER$^+$ tumors when studied with more than 30 site polymorphic markers [*ibid.* and S. J. Baker *et al.*, *ibid.*, p. 217].
18. J. L. Weber and P. E. May, *Am. J. Hum. Genet.* **44**, 388 (1989); J. Weissenbach *et al.*, *Nature* **359**, 794 (1992).
19. M. A. Peinado, S. Malkhosyan, A. Velazquez, M. Perucho, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 10065 (1992); M. Perucho, paper presented at the International Symposium on Human Oncogenes, Madrid, Spain, 8 April 1992.
20. C. T. Caskey *et al.*, *Science* **256**, 784 (1992).
21. P. J. McKinnen, *Hum. Genet.* **175**, 197 (1987); J. E. Cleaver, *Nature* **218**, 652 (1968); J. German, D. Bloom, E. Passarge, *Clin. Genet.* **35**, 57 (1989).
22. J.-P. Mecklin and H. J. Järvinen, *Cancer* **68**, 1109 (1991); H. T. Lynch *et al.*, *Cancer Genet. Cytogenet.* **53**, 143 (1991).
23. DNA was prepared from cryostat sections of tumors after removal of contaminating normal tissue (*7*).
24. We thank the patients and their families who provided specimens for this study with informed consent, S. Booker for organizing patient accrual and clinical data, S. Lindh for collecting specimens, C. Wadelius for providing primers for microsatellite markers, and J. Ott for assistance with the HOMOG program. Supported by grants from the Sigrid Juselius Foundation, the Academy of Finland, the Finnish Cancer Society, the Duodecim Foundation, the Ida Montin Foundation, the Finnish-Norwegian Medical Foundation, Suomen Astra Oy, the Finnish Foundation for Gastroenterological Research, the Clayton Fund, the McAshan Fund, and NIH grants CA 47527 and CA 35494. B.V. is an American Cancer Society research professor. Part of this study was carried out at the Folkhälsan Institute of Genetics.

8 April 1993; accepted 15 April 1993

Microsatellite Instability in Cancer of the Proximal Colon

S. N. Thibodeau,* G. Bren, D. Schaid

Colorectal tumor DNA was examined for somatic instability at $(CA)_n$ repeats on human chromosomes 5q, 15q, 17p, and 18q. Differences between tumor and normal DNA were detected in 25 of the 90 (28 percent) tumors examined. This instability appeared as either a substantial change in repeat length (often heterogeneous in nature) or a minor change (typically two base pairs). Microsatellite instability was significantly correlated with the tumor's location in the proximal colon ($P = 0.003$), with increased patient survival ($P = 0.02$), and, inversely, with loss of heterozygosity for chromosomes 5q, 17p, and 18q. These data suggest that some colorectal cancers may arise through a mechanism that does not necessarily involve loss of heterozygosity.

Recent studies of colorectal cancer suggest that tumorigenesis proceeds through a series of genetic alterations involving both proto-oncogenes and tumor suppressor genes (*1*). The dominant-acting proto-oncogenes Ki-*ras* (*2*, *3*) and c-*myc* (*4*) appear to have important functions in this process. Evidence for the involvement of tumor suppressor genes comes from numerous studies, including those demonstrating chromosomal allelic loss of heterozygosity (LOH) (*3*, *5*–*7*). About 75% of colon cancers show LOH for chromosomes 17p and 18q, and about 50% show LOH for chromosome 5q. Candidate tumor suppressor genes on these chromosomes include *APC* (familial adenomatous polyposis locus) and *MCC* (mutated in colorectal carcinoma) on chromosome 5q (*8*), *P53* on chromosome 17p (*9*), and *DCC* (deleted in colorectal carcinoma) on chromosome 18q (*10*). Although LOH has been demonstrated in a majority of colorectal tumors, it is not a general characteristic of all colorectal tumors. Furthermore, a number of studies indicate that the absence of LOH correlates with tumor site (*5*, *11*); those tumors without LOH tend to occur in the proximal colon.

In investigating the molecular genetics of colorectal cancer, we detected distinct genetic alterations in tumor DNA. These alterations appear to reflect amplification or deletion of DNA within interspersed repeat elements of the form $(CA)_n \cdot (GT)_n$ (*12*, *13*). Elements of this type (microsatellites) constitute one of the most abundant classes of repetitive DNA families in humans: ~50,000 to 100,000 $(CA)_n$ repeats are scattered throughout the human genome and many exhibit length polymorphisms. Although the rate of new mutations at these sites is slightly increased compared to that at other genomic sites, the overall rate is still quite low—on the order of 5×10^{-4} to 5×10^{-5} (*14*). In general, alleles at these sites are stably inherited from one generation to another and are now one of the most useful classes of DNA polymorphism for the purposes of linkage analysis (*15*).

We examined DNA from 90 colorectal tumors (*16*) for genetic alterations at four separate microsatellites localized to chromosome arms 5q (Mfd 27), 17p (Mfd 41), 18q (Mfd 26), and 15q (635/636). A marker on chromosome 15 was chosen because previous studies demonstrated a low frequency of allelic loss for this chromosome in colorectal cancer (6). Using polymerase chain reaction (PCR)–based assays, we detected differences between tumor and normal DNA banding patterns in 25 of the 90 (28%) tumors examined. The alterations were quite variable, ranging from a 2-bp change in some tumors to a larger change in length (perhaps the result of repeat expansion or deletion) in others (Fig. 1). For the

Table 1. Association of microsatellite alterations with LOH. Studies of LOH were performed as described (*20*). The DNA probes included 105-153A, πr227, C11P11, M4, and J0205H-C for chromosome 5q; YNZ22 and YNH37.3 for chromosome 17p; and HH64, DCC1.9, 15-65, and pert25 for chromosome 18q. Statistical comparisons of frequencies were calculated by Fisher's exact test (*21*). Although the total number of tumors is reported, synchronous tumors from single patients were omitted in the calculation of *P* values; the numbers in parentheses reflect the total when synchronous tumors are omitted.

Microsatellite	Genetic change	Number of tumors		*P*
		No LOH	LOH	
Mfd 27	None	34 (33)	37 (36)	–
Mfd 27	Type I	11 (8)	1	0.031
Mfd 41	None	17 (16)	56 (55)	–
Mfd 41	Type I	7 (6)	1 (0)	<0.0005
Mfd 26	None	15	53 (52)	–
Mfd 26	Type I	10 (8)	0	<0.0005

Molecular Genetics Laboratory, 970 Hilton Building, Mayo Clinic and Foundation, 200 First Street Southwest, Rochester, MN 55905.

*To whom correspondence should be addressed.

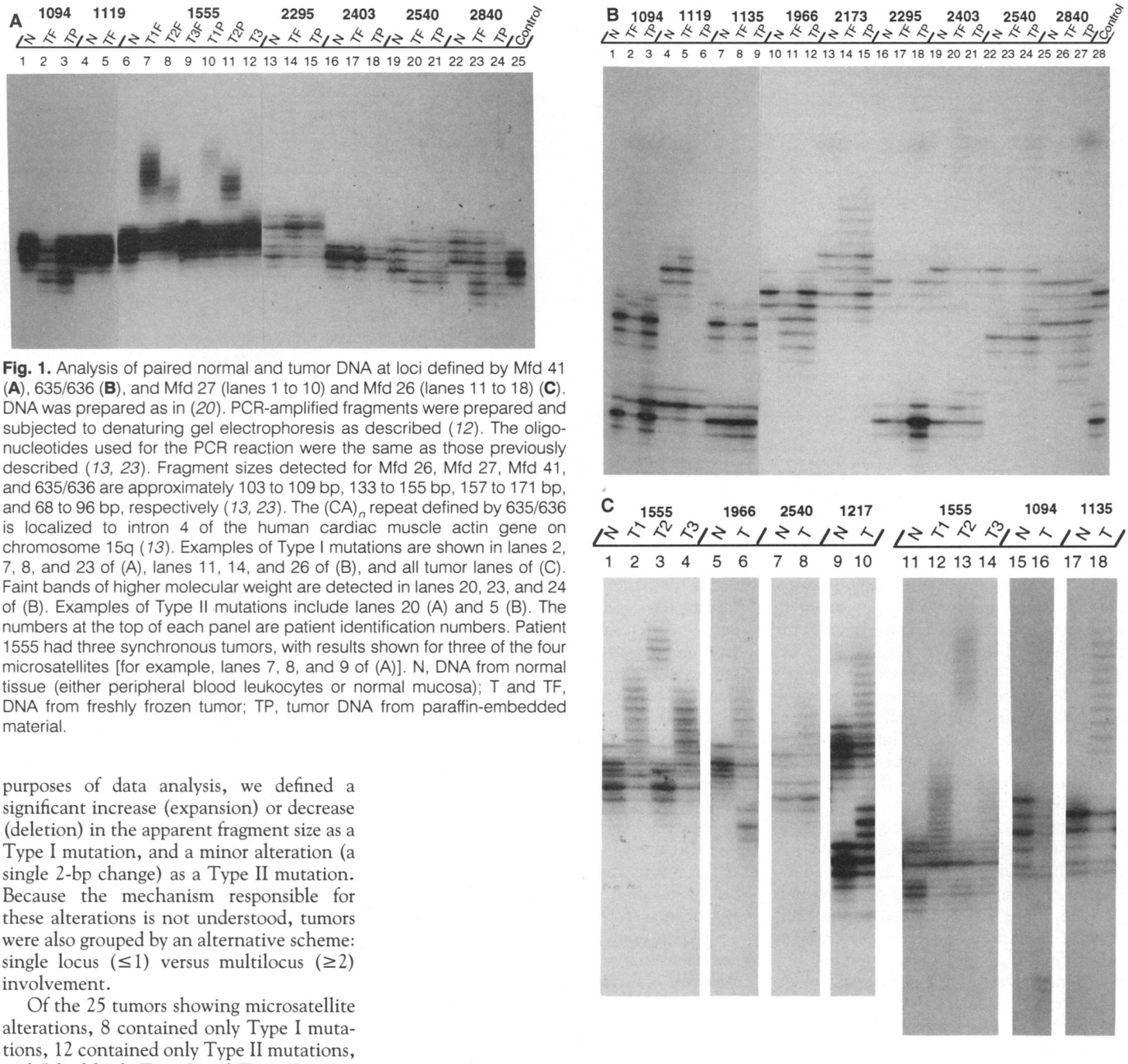

Fig. 1. Analysis of paired normal and tumor DNA at loci defined by Mfd 41 (**A**), 635/636 (**B**), and Mfd 27 (lanes 1 to 10) and Mfd 26 (lanes 11 to 18) (**C**). DNA was prepared as in (*20*). PCR-amplified fragments were prepared and subjected to denaturing gel electrophoresis as described (*12*). The oligonucleotides used for the PCR reaction were the same as those previously described (*13, 23*). Fragment sizes detected for Mfd 26, Mfd 27, Mfd 41, and 635/636 are approximately 103 to 109 bp, 133 to 155 bp, 157 to 171 bp, and 68 to 96 bp, respectively (*13, 23*). The $(CA)_n$ repeat defined by 635/636 is localized to intron 4 of the human cardiac muscle actin gene on chromosome 15q (*13*). Examples of Type I mutations are shown in lanes 2, 7, 8, and 23 of (A), lanes 11, 14, and 26 of (B), and all tumor lanes of (C). Faint bands of higher molecular weight are detected in lanes 20, 23, and 24 of (B). Examples of Type II mutations include lanes 20 (A) and 5 (B). The numbers at the top of each panel are patient identification numbers. Patient 1555 had three synchronous tumors, with results shown for three of the four microsatellites [for example, lanes 7, 8, and 9 of (A)]. N, DNA from normal tissue (either peripheral blood leukocytes or normal mucosa); T and TF, DNA from freshly frozen tumor; TP, tumor DNA from paraffin-embedded material.

purposes of data analysis, we defined a significant increase (expansion) or decrease (deletion) in the apparent fragment size as a Type I mutation, and a minor alteration (a single 2-bp change) as a Type II mutation. Because the mechanism responsible for these alterations is not understood, tumors were also grouped by an alternative scheme: single locus (≤1) versus multilocus (≥2) involvement.

Of the 25 tumors showing microsatellite alterations, 8 contained only Type I mutations, 12 contained only Type II mutations, and 5 had both Type I and Type II mutations. All 13 tumors with Type I mutations showed DNA alterations at multiple loci, 9 of them showing alterations at all four $(CA)_n$ repeats examined. On the other hand, tumors with only Type II mutations typically showed a change at only a single locus (10 of 12 tumors).

Similar genetic alterations were not detected in any of the paired normal tissue examined (either peripheral blood leukocytes or normal adjacent mucosa). A second source of normal tissue was available for 19 of the 87 patients (3 of which had previously demonstrated microsatellite alterations in the tumor specimen). Again, none of these normal tissues showed alterations at any of the loci tested. To further

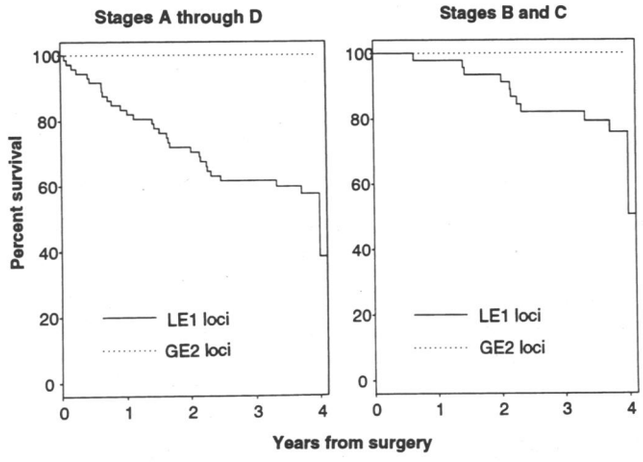

Fig. 2. Kaplan-Meier survival curves, showing the association of microsatellite alterations on patient survival (*24*). LE1, ≤1 involved microsatellite locus; GE2, ≥2 involved microsatellite loci.

Table 2. Association of microsatellite alterations with anatomic site of the tumor. Numbers in parentheses reflect the total when synchronous tumors are omitted. Abbreviations: C, caecum; AC, ascending colon; HF, hepatic flexure; TC, transverse colon; SF, splenic flexure; DC, descending colon; S, sigmoid; R, rectum; P, proximal colon; D, distal colon. Distal colon is defined as sites distal to and including the splenic flexure. *P* values were calculated as in Table 1.

Microsatellite	Genetic change	Number of tumors per tumor site										*P*
		C	AC	HF	TC	SF	DC	S	R	P	D	
Mfd 27	None	15	4	2	5	1	2	20 (19)	26 (25)	26	49 (47)	
Mfd 27	Type I	5 (4)	4 (3)	1	1 (0)	0	0	0	1	11 (8)	1	0.003
635	None	15	3	2	5	1	2	19 (18)	23 (22)	25	45 (43)	
635	Type I	3 (2)	4	0	1 (0)	0	0	0	1	8 (6)	1	0.018
Mfd 41	None	16	4	2	6 (5)	1	2	20 (19)	28	28 (27)	51 (50)	
Mfd 41	Type I	3 (2)	5 (4)	0	0	0	0	0	0	8 (6)	0	0.003
Mfd 26	None	15	4	1	5	1	2	18 (17)	27 (26)	25	48 (46)	
Mfd 26	Type I	4 (3)	4 (3)	1	1 (0)	0	0	0	1	10 (7)	1	0.006
	Change at ≤1 locus	15	3	1	5	1	2	20 (19)	26	24	49 (48)	
	Change at ≥2 loci	5 (4)	6 (5)	1	1 (0)	0	0	0	2	13 (10)	2	0.003

rule out the possibility of technical artifact or specimen contamination, we obtained paraffin-embedded tumor material for 29 patients and analyzed the DNA with two of the markers (635 and Mfd 41) for a total of 58 comparisons. When results from the paraffin-embedded tumors were compared with those obtained from the original tumor, only two equivocal and two discordant results were detected—both positive for paraffin-embedded tissue and negative for fresh frozen tissue. Furthermore, in all 12 specimens that demonstrated microsatellite alterations, the same alteration was found in DNA from both sources of tumor tissue (Fig. 1).

Because LOH is such a frequent event in colorectal cancer, we investigated its relation to the genetic alterations detected at these dinucleotide repeats. For this analysis, the presence or absence of microsatellite alterations was compared to the presence or absence of allelic loss for chromosomes 5q, 17p, and 18q, as determined by Southern (DNA) blot analysis. An inverse correlation was observed between Type I mutations and LOH for each of the three markers evaluated (Table 1). Of significance, 12 of the 14 tumors (80%) that did not demonstrate LOH for any of the three chromosomes tested exhibited microsatellite alterations at multiple loci.

We also explored the association of a number of clinical and pathologic parameters with microsatellite alterations. We did not detect correlations with the age or sex of the patients. However, we observed a significant correlation with tumor site for all four markers tested (Table 2). Tumors with microsatellite alterations occurred more frequently in the proximal colon. Overall, 12 of 13 (93%) tumors demonstrating Type I mutations or 13 of the 15 (87%) tumors demonstrating mutations at multiple loci arose in the proximal colon. However, not all proximal tumors showed microsatellite alterations. Such alterations occurred in 13 of the 37 (35%) proximal tumors we analyzed.

We then examined the association of microsatellite alterations with tumor stage and with patient survival. Although not statistically significant, a trend was detected between microsatellite alterations and Astler-Coller staging: Type I mutations or mutations at multiple loci were detected more frequently in patients with stage B tumors (Table 3). Univariate analysis of the 86 patients with stage A through D colorectal cancer revealed a correlation between alterations at multiple loci and overall survival ($P = 0.02$). An additional analysis of patients with stage B or C colorectal cancer revealed the same association, but because of the smaller number of patients ($n = 60$) this association did not reach statistical significance ($P = 0.15$) (Fig. 2).

In this study, the majority of tumors demonstrating somatic instability did so at multiple unrelated microsatellites, which suggests a common but nonspecific underlying mechanism. Such alterations, therefore, are very likely to be secondary events. Although somatic alterations of this specific type have not been previously described in cancer, probes directed toward "minisatellites" (in a Southern blot assay) have detected the loss and gain of DNA fragments in various malignancies, including colorectal cancer (*17*). The latter alterations may be similar in nature to those detected within microsatellites, but the majority more likely represent LOH. Conceivably, the tumors described here may contain mutations in a gene or genes that, when defective, promote genomic instability at numerous loci, especially those defined by microsatellites. Such mutations could nonspecifically alter the regulation of a wide spectrum of genes, thereby promoting tumor formation. A variety of genes have now been identified that contain microsatellites as an integral component of their structure (*18*), including *DCC* (*10*). Thus, the acquisition of a tumor phenotype may involve two independent pathways: (i) mutations in a variety of tumor suppressor genes followed by LOH or (ii) mutations within a specific group of genes with resultant instability at microsatellite-associated genes.

Although the abnormalities detected in this study are not likely, in themselves, to be causally involved in tumor initiation or progression, there is evidence to support the notion that instability at microsatellites can be involved in human disease. Heritable unstable DNA elements have recently

Table 3. Association of microsatellite (MS) alterations with the various Astler-Coller stages. All tumors were staged by the Astler-Coller modification of the Dukes' staging system (*22*). Of the 90 colorectal tumors, 5 were stage A, 38 were stage B1 or B2, 26 were stage C1 or C2, and 21 were stage D. Numbers in parentheses reflect the total when synchronous tumors are omitted. *P* values were calculated as in Table 1 and were determined to be not significant (>0.05).

MS	Genetic change	Number of tumors classified in Astler-Coller stages			
		A	B	C	D
Mfd 27	None	5	27	23 (21)	20
Mfd 27	Type I	0	9 (6)	2	1
635	None	5	25	20 (18)	20
635	Type I	0	6 (4)	2	1
Mfd 41	None	5	30 (29)	23 (22)	21
Mfd 41	Type I	0	6 (4)	2	0
Mfd 26	None	5	25	23 (21)	20
Mfd 26	Type I	0	9 (6)	1	1
	Change at ≤1 locus	5	26	22 (21)	20
	Change at ≥2 loci	0	11 (8)	3	

been identified as the basis of disease for four inherited disorders: fragile X syndrome, Kennedy's syndrome, myotonic dystrophy, and Huntington's disease (*19*). In each case, the mutation appears to be an expansion of a trinucleotide repeat that displays both somatic and germline instability. The fact that somatic instability has been observed suggests that similar events may be important in noninherited disorders, such as cancer. Finally, microsatellite instability, as assessed in this report, appears to be a good prognostic indicator. However, further studies on a larger patient population will be important to verify these initial observations and to determine if these DNA changes are independent prognostic indicators.

REFERENCES AND NOTES

1. E. R. Fearon and B. Vogelstein, *Cell* **61**, 759 (1990).
2. J. L. Bos *et al.*, *Nature* **327**, 293 (1987).
3. B. Vogelstein *et al.*, *N. Engl. J. Med.* **319**, 525 (1988).
4. K. Sikora *et al.*, *Cancer* **59**, 1289 (1987); M. D. Erisman, J. K. Scott, R. A. Watt, S. M. Astrin, *Oncogene* **2**, 367 (1988).
5. O. Delattre *et al.*, *Lancet* ii, 353 (1989).
6. B. Vogelstein *et al.*, *Science* **244**, 207 (1989).
7. M. Rees, S. E. A. Leigh, J. D. A. Delhanty, J. R. Jass, *Br. J. Cancer* **59**, 361 (1989); D. J. Law, *Science* **241**, 961 (1988); M. Okamoto *et al.*, *Nature* **331**, 273 (1988).
8. J. Groden *et al.*, *Cell* **66**, 589 (1991); K. W. Kinzler *et al.*, *Science* **253**, 661 (1991).
9. S. J. Baker *et al.*, *Science* **244**, 217 (1989).
10. E. R. Fearon *et al.*, *ibid.* **247**, 49 (1990).
11. S. E. Kern *et al.*, *J. Am. Med. Assoc.* **261**, 3099 (1989); G. J. A. Offerhaus *et al.*, *Gastroenterology* **102**, 1612 (1992).
12. J. L. Weber and P. E. May, *Am. J. Hum. Genet.* **44**, 388 (1989).
13. M. Litt and J. A. Luty, *ibid.*, p. 397.
14. C. M. Hearne, S. Ghosh, J. A. Todd, *Trends Genet.* **8**, 288 (1992).
15. J. Weissenbach *et al.*, *Nature* **359**, 794 (1992).
16. This study included 48 males and 39 females who had colorectal carcinomas resected at the Mayo Clinic between 1987 and 1988. The mean age of these patients at the time of surgery was 67.4 years (SD = 13.4 years; range = 25 to 91 years). Among the 87 patients were two with more than one tumor: one patient had two and another had three.
17. M. F. Fey, R. A. Wells, J. S. Wainscoat, S. L. Thein, *J. Clin. Invest.* **82**, 1532 (1988); Y. Matsumura and D. Tarin, *Cancer Res.* **52**, 2174 (1992); P. J. L. Lagoda, G. Seitz, J. T. Epplen, O. G. Issinger, *Hum. Genet.* **84**, 35 (1989); S. L. Thein *et al.*, *Br. J. Cancer* **55**, 353 (1987); E. M. Boltz *et al.*, *ibid.* **62**, 23 (1990).
18. R. I. Richards and G. R. Sutherland, *Cell* **70**, 709 (1992); G. J. Riggins *et al.*, *Nature Genet.* **2**, 186 (1992); S. N. Thibodeau, G. Bren, D. Schaid, unpublished observations.
19. F. Rousseau, D. Heitz, J. Mandel, *Hum. Mut.* **1**, 91 (1992); A. R. La Spada, E. M. Wilson, D. B. Lubahn, A. E. Harding, K. H. Fischbeck, *Nature* **352**, 77 (1991); C. Aslanidis *et al.*, *ibid.* **355**, 548 (1992); M. E. MacDonald *et al.*, *Cell* **72**, 971 (1993).
20. J. Cunningham *et al.*, *Cancer Res.* **52**, 1974 (1992).
21. C. R. Mehta and N. R. Patel, *J. Am. Stat. Assoc.* **78**, 427 (1983).
22. V. B. Astler and F. A. Coller, *Ann. Surg.* **139**, 846 (1954).
23. J. Weber, *Nucleic Acids Res.* **18**, 2201 (1990); *ibid.*, p. 4035; *ibid.*, p. 4640.
24. We ascertained the recurrence of cancer in the patients and their survival status by reviewing their charts and, in most instances, corresponding directly with the patients or their physicians. For patients who were still alive at their most recent follow-up (n = 48), the median follow-up time was 3.8 years from the date of surgery, with a range of 2.9 to 4.1 years. None of the patients were lost to follow-up. Statistical analyses were performed with the Statistical Analysis System (SAS Institute, Cary, NC). Overall survival was defined as the time from diagnosis until the date of death. End points were censored for patients dying of causes other than metastatic colon cancer. We compared distributions of survival times by using the log-rank statistic [N. Mantel, *Cancer Chemother. Rep.* **50**, 163 (1966)], and survival distribution curves were estimated by the method of Kaplan and Meier [F. L. Kaplan and P. Meier, *J. Am. Stat. Assoc.* **53**, 457 (1958)]. All P values are two-sided.

25. We thank K. Erwin for her excellent secretarial assistance. Supported in part by NIH grant CA-15083-18E8.1 and American Cancer Society grant ACS EDT-3.

3 March 1993; accepted 15 April 1993

Rapid Assessment of Drug Susceptibilities of *Mycobacterium tuberculosis* by Means of Luciferase Reporter Phages

William R. Jacobs, Jr.,* Raúl G. Barletta,† Rupa Udani, John Chan, Gary Kalkut, Gabriel Sosne, Tobias Kieser, Gary J. Sarkis, Graham F. Hatfull, Barry R. Bloom

Effective chemotherapy of tuberculosis requires rapid assessment of drug sensitivity because of the emergence of multidrug-resistant *Mycobacterium tuberculosis*. Drug susceptibility was assessed by a simple method based on the efficient production of photons by viable mycobacteria infected with specific reporter phages expressing the firefly luciferase gene. Light production was dependent on phage infection, expression of the luciferase gene, and the level of cellular adenosine triphosphate. Signals could be detected within minutes after infection of virulent *M. tuberculosis* with reporter phages. Culture of conventional strains with antituberculosis drugs, including isoniazid or rifampicin, resulted in extinction of light production. In contrast, light signals after luciferase reporter phage infection of drug-resistant strains continued to be produced. Luciferase reporter phages may help to reduce the time required for establishing antibiotic sensitivity of *M. tuberculosis* strains from weeks to days and to accelerate screening for new antituberculosis drugs.

Tuberculosis remains the largest cause of death in the world from a single infectious disease (*1*) and accounts for as much as 40% of deaths in human immunodeficiency virus (HIV)–coinfected individuals in some developing countries (*2*). Infection with conventional *M. tuberculosis* can effectively be cured with a combination of antituberculosis drugs. Ominously, multidrug-resistant tuberculosis (MDR-TB) strains have emerged in several countries, with case fatalities ranging from 40 to 60% in immunocompetent individuals and >80% in immunocompromised individuals (*3*). However, because *M. tuberculosis* has a doubling time of 20 to 24 hours, current methodology does not allow determination of drug susceptibility for 2 to 18 weeks (*4*, *5*), leaving patients, contacts, and health care workers at risk.

Firefly luciferase represents one of the most efficient available biological reporter molecules because it catalyzes the reaction of luciferin with adenosine triphosphate (ATP) to generate photons with a quantum yield of 0.85 photons per molecule of substrate reacted (*6*). Because of the availability of a variety of sensitive light-detection systems, luciferase has become the standard assay for measuring ATP (*7*). Since the molecular cloning of its cDNA (*8*), the firefly luciferase gene has been used directly as a molecular reporter in cells of a variety of animal, plant, and bacterial species (*9*). We reasoned that the expression of luciferase activity could serve as a sensitive in vivo measure of ATP in mycobacteria and thus allow us to rapidly test cellular viability of *M. tuberculosis* after its exposure to different antimycobacterial agents. The optimal use of the reporter gene could be realized if the luciferase activity could be

W. R. Jacobs, Jr., R. G. Barletta, R. Udani, J. Chan, G. Kalkut, G. Sosne, B. R. Bloom, Howard Hughes Medical Institute, Albert Einstein College of Medicine, Bronx, NY 10461.
T. Kieser, John Innes Institute and AFRC Institute of Plant Science Research, Norwich NR4 7UH, United Kingdom.
G. J. Sarkis and G. F. Hatfull, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15230.

*To whom correspondence should be addressed.
†Present address: Center for Biotechnology, Department of Veterinary and Biomedical Sciences, University of Nebraska, Lincoln, NE 68583.