ent continents proves the existence of a genetically determined form of this disease. Many precedents suggest that any gene that plays a role in a hereditary form of cancer is likely to be involved in a significant number of "sporadic" cases of the same tumor type, by virtue of somatic mutations, germ line alterations, or inherited alterations that are poorly penetrant (*15*). The current study lays the groundwork for further mapping, isolation, and characterization of the HNPCC gene on chromosome 2, and the examination of its role in colorectal and other tumors. If, as expected, this gene is involved in a significant fraction of familial CRC cases, then it is responsible for one of the most common forms of heritable disease yet identified in humans (*5*, *16*). The study of such a gene has obvious implications for public health. Most immediately, these results make presymptomatic diagnosis of susceptibility possible in HNPCC family members and will enable more effective surveillance programs for the early detection and treatment of cancer.

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11. For most microsatellite markers, the polymerase chain reaction (PCR) was done in the following final reaction conditions: 1 × PCR buffer [10 mM tris (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% bovine serum albumin]; 200 μM deoxyguanosine 5′-triphosphate, deoxyadenosine 5′-triphosphate, and deoxythymidine triphosphate; 2 μM deoxycytidine 5′-triphosphate (dCTP); 0.7 μCi of α-[^{32}P]dCTP (3000 Ci/mmol); 10 ng of each primer (1 to 5 primer pairs at a time); 30 ng of genomic DNA template; and 0.3 units of AmpliTaq polymerase (Perkin-Elmer–Cetus) in a volume of 10 μl. Twenty-seven cycles were performed at 94°C for 30 s, 55°C for 75 s, and 72°C for 15 s, and the samples were incubated at 72°C for 6 min after the last cycle. The PCR products were subjected to electrophoresis in 6% polyacrylamide gels containing 7.7 M urea. After electrophoresis, the gels were fixed in 10% acetic acid for 15 min, dried, and exposed to x-ray film.
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17. Phenotypes were coded as affected with an autosomal dominant mode of inheritance and four liability classes according to age at the time of observation (healthy) or at diagnosis. Penetrances for heterozygotes were set at 0.15 at age 30 years or below (liability class 1), 0.40 at age 31 to 45 years (liability class 2), 0.70 at age 46 to 60 years (liability class 3), and 0.90 at age 61 or over (liability class 4). Phenocopies were introduced, with frequencies set at 1, 3, and 5% in liability classes 2, 3, and 4, respectively. A model without phenocopies was also tested and was found to have only a minor effect on the results. The frequency of the HNPCC gene was set at 0.001 (but values of 0.01 and 0.0005 were also tested; see text). The allele frequencies of markers D2S119, D2S123, and D2S136 were calculated from 53 to 58 spouses of HNPCC family members.

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Clues to the Pathogenesis of Familial Colorectal Cancer

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A predisposition to colorectal cancer is shown to be linked to markers on chromosome 2 in some families. Molecular features of "familial" cancers were compared with those of sporadic colon cancers. Neither the familial nor sporadic cancers showed loss of heterozygosity for chromosome 2 markers, and the incidence of mutations in *KRAS, P53*, and *APC* was similar in the two groups of tumors. Most of the familial cancers, however, had widespread alterations in short repeated DNA sequences, suggesting that numerous replication errors had occurred during tumor development. Thirteen percent of sporadic cancers had identical abnormalities and these cancers shared biologic properties with the familial cases. These data suggest a mechanism for familial tumorigenesis different from that mediated by classic tumor suppressor genes.

Colorectal cancer (CRC) is one of the most common forms of neoplasia in industrial countries and its incidence is increasing (*1*). Familial forms of CRC account for up to 13% of all CRC cases (*2*). Except for the rare familial adenomatous polyposis (FAP) syndrome, the molecular basis of the cancers that arise in these families has not been previously studied but is of great interest for both scientific and practical reasons. Inherited forms of cancer can provide unique insights into the pathogenesis of specific tumor types that often extend to nonfamilial cases (*3*). In the studies reported here, we evaluated molecular aspects of tumorigenesis in families with hereditary nonpolyposis colorectal cancer (HNPCC). This study revealed surprising findings that are relevant not only to this familial form of cancer but also to individuals with no strong family history of the disease.

HNPCC is defined by the presence of three or more family members with CRC in at least two successive generations, with at

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least one affected member having been diagnosed at less than 50 years of age (*4*). Stimulated by the finding that chromosome 2 markers were closely linked to cancer predisposition in two large kindreds with HNPCC (*5*), we studied an additional 14 smaller kindreds (see Fig. 1 for pedigrees of two of these families). Linkage analyses were performed with marker D2S123, which is closely linked to HNPCC (*5*). In these analyses, individuals with CRC, with other HNPCC-associated cancers (*4*), or with colorectal adenomas were classified as "affected," as outlined previously (*5*). The results (Table 1) reflect the limited amount of linkage information that can be derived from these families in spite of the highly informative nature of the D2S123 marker (heterozygosity of 0.78, calculated from 58 unrelated spouses in these 16 families). The difficulty arises because affected members of these pedigrees are often deceased (and therefore unavailable for analysis) and because most living members without evident cancer are too young to be reliably classified as unaffected. Despite these problems, formal exclusion of linkage (lod score < -2.0) was obtained in families F11, B1, and B2, whereas the remaining 11 smaller families displayed varying degrees of positive and negative lod scores. The HOMOG program was used to calculate conditional probabilities (P_{cond}) of linkage in each family (Table 1) and revealed clear evidence of heterogeneity ($P = 0.0005$ by χ^2). This result suggested that several families with positive lod scores are likely to be linked to the HNPCC locus on chromosome 2 and that an equal number of others might be unlinked.

We next examined genes that have been demonstrated to undergo somatic mutations in sporadic CRCs. We reasoned that if the pathogenesis of familial CRC differed from that of the more common sporadic forms, then genes previously implicated in the latter may not be involved. For this investigation, we studied 18 tumors derived from 18 patients from 15 different HNPCC kindreds. Linkage analysis revealed that CRC was probably linked to chromosome 2 in one of these kindreds and probably unlinked in another. The other kindreds were uninformative or not studied for linkage. We first studied the *KRAS* oncogene, mutations of which occur relatively early in the development of sporadic colorectal tumors but do not generally initiate tumorigenesis. Mutations at codons 12 or 13 of *KRAS* were identified in 61% of the tumors, a percentage somewhat higher than that found in sporadic cases (~40%) [(*6–8*); Table 2]. Similarly, alterations in the tumor suppressor gene *P53*, which generally occur late in tumorigenesis [often at the transition between benign and malignant states (*9*)], were found in approximately the same proportion of familial and sporadic cancers when evaluated immunohistochemically (64 and 61%, respectively) [(*10*); Table 2]. We also evaluated *APC*, a candidate tumor suppressor gene. Mutations in *APC* are thought to initiate most sporadic colorectal tumors (*11*), and germ line mutations of *APC* are found in FAP, a syndrome that predisposes to CRC (*12*). FAP is distinguished from HNPCC by its association with the development of numerous benign colorectal polyps in addition to CRC. In contrast, HNPCC is generally associated with no phenotypic manifestations other than a high incidence of cancer. One intriguing possibility was that mutations in an HNPCC gene could substitute for *APC* in tumorigenesis and that tumors would progress along either an "APC" or "HNPCC" pathway. This possibility was ruled out by our observation that 57% of the familial cancers had mutations in *APC* when screened by methods that revealed mutations in 49% of sporadic cancers [(*13*); Table 2].

Because other genes responsible for cancer predisposition undergo allelic loss in tumors (*3*, *9*), we searched for loss of heterozygosity at the D2S123 locus by studying DNA extracted from tumor tissues and comparing it with matched normal DNA from the same individuals. Contrary to expectation, this locus was not deleted in any of the 14 HNPCC tumors and was deleted in only one of the 46 sporadic tumors examined. However, a remarkable and unexpected pattern of changes was observed in the majority of the HNPCC-

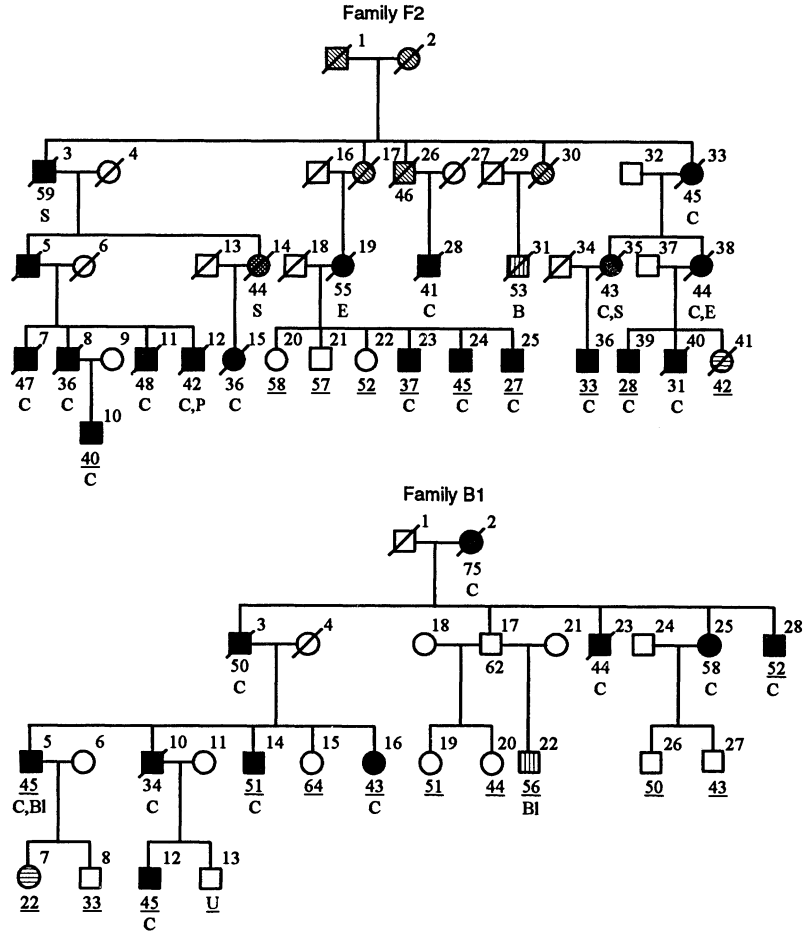

Fig. 1. Pedigrees of families F2 and B1. Linkage data shown in Table 1 indicate that the HNPCC phenotype is probably linked to marker D2S123 (and to flanking markers, not shown) in family F2, whereas family B1 shows exclusion of linkage at zero recombination. Numbers above the symbols are patient identifiers; numbers below the symbols indicate age at tumor diagnosis or age at last observation if unaffected; "U" indicates age unknown. If a blood sample was available, the age is underlined. DNA samples from most of the spouses were also available but are not indicated in the pedigrees. Letters signify the site of the tumor: C, colon or rectum; S, stomach; B, brain; Bl, urinary bladder; and P, pancreas. Alleles were omitted to protect confidentiality of family members. General symbols: squares, males; circles, females; all symbols with a diagonal, deceased. Open symbols, no neoplasm detected; solid, colorectal or endometrial carcinoma; dotted, other tumor of HNPCC spectrum; horizontal stripes, colorectal adenoma; diagonal stripes, diagnosis uncertain; and vertical stripes, tumor not typical of HNPCC.

derived tumors in this analysis. These changes consisted of shifts in the electrophoretic mobility of $(CA)_n$ dinucleotide repeat fragments, suggesting that replication errors (RER) had occurred in these sequences during tumor development (Fig. 2, A and B). Similar shifts were seen much more rarely in sporadic CRC (see below). To investigate whether the shifts were confined to marker D2S123, we searched for shifts with $(CA)_n$ markers D2S119 and D2S147, located approximately 10 centimorgans (cM) on either side of D2S123, as well as markers D10S197, D11S904, and D13S175, representing chromosomes 10, 11, and 13, respectively. All of these markers showed alterations in tumor DNA, with 43 to 71% of the tumors showing mobility shifts with each marker studied (examples in Fig. 2 and Table 3). To determine whether such alterations were confined to $(CA)_n$ dinucleotide repeats, we also studied CTG-B37, a $(CAG)_n$ trinucleotide repeat from the coding region of an unrelated gene on chromosome 12 (*14*). Mobility shifts were also observed frequently with this probe (Table 3). Overall, the alterations were distributed nonrandomly among the tumors analyzed: 11 of the 14 tumors had shifts in at least two of the seven markers studied and were categorized as RER^+; no shifts were observed in the other three tumors, categorized as RER^-.

DNA samples from 46 sporadic CRCs and corresponding normal tissues were then evaluated with the same markers. The 46 sporadic tumors had a much lower incidence of shifts than the 14 HNPCC tumors ($P < 0.000001$ for all markers combined and $P < 0.01$ for each of the seven markers tested, by χ^2). Moreover, the tumors could be divided into two subsets. Six of the 46 tumors had shifts in at least two of the markers tested and, by analogy to the HNPCC tumors, were categorized as RER^+. The six corresponding patients ranged in age from 62 to 82 years (mean age, 75) and none had a positive family history meeting HNPCC criteria. As with the HNPCC tumors, there was a dramatic difference between the RER^+ and RER^- tumors with every marker assessed (Table 3). Some of the shifts in the RER^+ tumors resulted in alleles larger than those present in the normal tissue, and other shifts resulted in smaller alleles (Fig. 2, C and D).

These observations prompted us to examine whether the RER^+ sporadic tumors had biologic features in common with the tumors from HNPCC patients. The latter tumors often occur on the right side of the colon (*15*) and are frequently diploid or near diploid (*16*). The RER^+ sporadic tumors displayed both these features. All six of the RER^+ sporadic cancers occurred on the right side of the colon, whereas only 17 of 40 of the RER^- tumors were right-sided ($P < 0.01$, by χ^2). "Diploidy" was evaluated in a quantitative fashion by allelotype assays, where each chromosomal arm was assessed for loss with a panel of cloned probes detecting restriction fragment length polymorphisms on Southern (DNA) blots (*17*). The fraction of chromosomal arms that undergo allelic loss (FAL) is a molecular measure of chromosome imbalance.

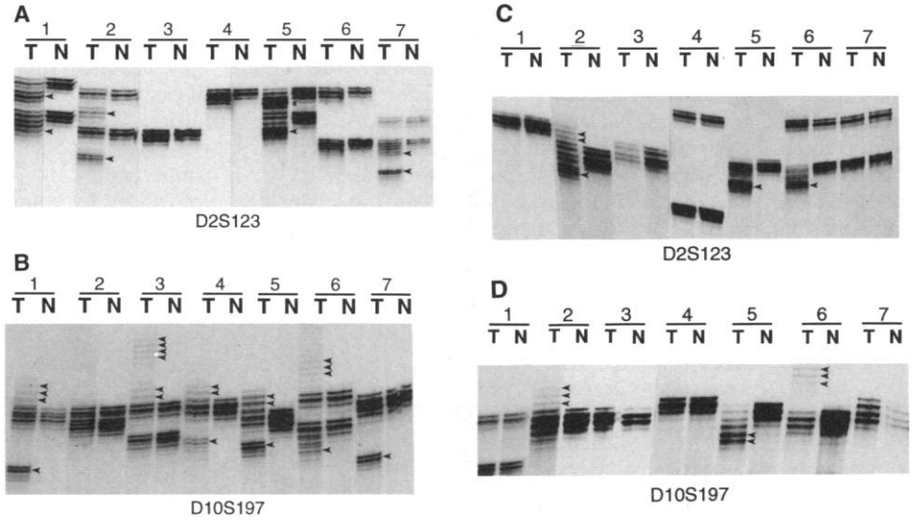

Fig. 2. (**A** and **B**) Dinucleotide repeat polymorphisms in normal and tumor tissue from HNPCC patients. The microsatellite markers D2S123 and D10S197 were used in PCR analysis (*5, 23*), and the products separated in 6% polyacrylamide gels. Patient numbers are shown above the lanes. In each case, the lane marked "T" contains DNA from a tumor and the lane marked "N" contains DNA from normal tissue of the same patient. Normal alleles are represented by a major band surrounded by one or two minor bands (*18*). All patterns shown were perfectly reproducible in replicate assays. Mixing experiments, in which tumor DNA samples were added to normal DNA samples from other patients, demonstrated that the deviations observed in the tumors reflected intrinsic changes in the template and were not due to interference with polymerase action. (**C** and **D**) Dinucleotide repeat polymorphisms in normal and tumor tissue from sporadic CRC patients, detected as in (A) and (B). Several of the alterations in tumor DNA are marked by arrowheads.

Table 1. Statistical test of linkage heterogeneity (A-test as implemented in the HOMOG program) for 16 families analyzed for the anonymous DNA marker D2S123. The lod scores (Z) for each family at a recombination fraction (θ) of zero, the conditional probability of the family being linked (P_{cond}), and its approximate confidence limits are given where appropriate. The test is based on results of pairwise linkage analyses with low-stringency criteria (*5*) and with an HNPCC gene frequency set at 0.001. The P-value derived from the maximum likelihood estimate of the proportion of linked families (α) and the recombination value (θ) assuming heterogeneity of the linkage data are given in the footnote. The approximate 95% confidence limits for α and θ were 0.05–0.73 and 0.000–0.10, respectively.

Family	Number of affected individuals in kindred	Number of affected individuals analyzed	Mean age at diagnosis (years)	D2S123, Z at $\theta = 0$	P_{cond}	95% confidence limits
C*	22	13	39.7	6.39	1	
J*	23	15	40.5	1.45	0.9356	0.5918–0.9870
F2	21	7	41.2	0.59	0.6671	0.1667–0.9132
F3	9	5	47.3	0.83	0.7769	0.2580–0.9481
F6	7	4	44.9	−0.16	0.2628	0.0351–0.6823
F8	8	3	42.4	−0.95	0.0546	0.0059–0.4666
F10	9	5	46.0	−1.52	0.0153	0.0016–0.3452
F11	7	6	44.5	−2.28	0.0027	0.0003–0.2876
F39	4	3	41.8	−1.10	0.0393	0.0042–0.4839
F56	6	5	49.0	0.00		
F59	6	5	54.8	0.44	0.5866	0.1266–0.8816
B1	11	6	47.2	−2.17	0.0035	0.0004–0.1957
B2	12	4	56.5	−2.33	0.0024	0.0002–0.2223
B3	8	4	40.7	0.08	0.3825	0.0595–0.7647
B4	10	5	55.6	0.12	0.4044	0.0649–0.8245
B5	8	7	51.0	−1.80	0.0081	0.0008–0.1957

χ^2 heterogeneity = 11.013; $P = 0.0005$ at $\theta = 0$; $\alpha = 0.34$. *Studied in (*5*).

The FAL averaged 0.039 (SD = 0.034) in the six RER$^+$ tumors, sixfold lower than the 0.254 (SD = 0.142) value obtained for the 40 RER$^-$ cases ($P < 0.001$, by Student's t test).

Taken together, these studies suggest the presence of a gene closely linked to marker D2S123, mutations in which confer high susceptibility to CRC. Formal exclusion of linkage to the marker in three families and statistical tests indicate genetic heterogeneity. The nature of the mapped HNPCC gene is not known, but the studies described here provide important clues as to how it might act. Previously discovered tumor suppressor genes frequently are the targets of allelic loss in tumor tissues. If the remaining allele is mutant, the cell is left with no functional suppressor gene product, so tumor initiation or progression ensues. The absence of allelic loss of the D2S123-linked gene in HNPCC tumors therefore argues against its being a typical tumor suppressor gene (*3, 9*). The clonal genome-wide shift in microsatellite allele sizes observed in the majority of HNPCC tumors argues for a different mechanism generating susceptibility to tumor formation. Perucho and colleagues have previously observed consistent alterations in simple repeated sequences in a subset of sporadic CRC (*19*). Our results suggest that the tendency to form such alterations can be inherited and may be directly related to a defective gene on chromosome 2. Microsatellites occur, on average, once per 100,000 base pairs in genomic DNA (*18*); extrapolation of the results [(*19*); Table 3] would predict that the cancer cell genome in the RER$^+$ cases contains thousands of changes compared with the genome of normal cells from the same patients. Although many such changes may be silent, some may lead to qualitative or quantitative alterations in gene products. For example, trinucleotide repeats, often found in the coding regions of genes (*20*), are also affected in these tumors (CTG-B37 in Table 3).

Although the rate of change in repeat sequences cannot be easily measured in tumor tissue, the data are consistent with the idea that genetic instability is a component of the familial cancer phenotype. There is ample precedent for this concept in tumor biology. For example, patients with xeroderma pigmentosum, ataxia telangiectasia, and Bloom's syndrome have a genetic instability (in some cases revealed by mutagens) that results in a predisposition to cancer (*21*). These syndromes are inherited as recessive diseases, but there is no reason that a dominant gene could not produce a similar result. A subtly altered replication factor, for example, would be a good candidate for the HNPCC gene. One might not expect allelic loss of such a gene because this event, coupled with mutation of the remaining allele, would be incompatible with cellular replication. This hypothesis is also consistent with the observation that cancer susceptibility in HNPCC kindreds is not confined to the colorectal epithelium, as affected individuals often develop other tumors (*22*). It is notable that in the HNPCC ovarian cancer studied here, six of seven microsatellite markers studied were altered (Table 3).

These results have straightforward practical implications. Because CRC is so common, it is difficult to know which families have a true familial predisposition and which represent chance clustering. In fact, we could not rule out the possibility that the apparent absence of chromosome 2 linkage in some families (Table 1) was not due to the admixture of sporadic cases. Although only half of the HNPCC kindreds provided some positive evidence for linkage, ten of thirteen families studied had RER$^+$ tumors, and these ten included one "unlinked" kindred (F11 in Table 1) and seven kindreds in which linkage analysis was impossible or equivocal. Moreover, 13% of "sporadic" CRC patients had the same RER abnormality in their tumors [(*19*); Table 3]. This proportion is in good agreement with previous estimates of familial CRC incidence (*2*), and the cancers from RER$^+$ patients shared biologic features with those from patients in clearly defined HNPCC kindreds. Thus, RER analysis might considerably augment standard linkage studies. Relatives of patients with RER$^+$ tumors may be at particularly high risk for developing cancers of the colon and other organs. Recognition of this risk, by the simple assays described here, could therefore have a substantial impact on cancer prevention strategies.

Table 2. Incidence of somatic mutations in *KRAS, P53,* and *APC* in HNPCC and sporadic tumors.

Tumor type	Number of tumors with mutations*		
	KRAS	*P53*	*APC*
HNPCC	11/18 (61%)	7/11 (64%)	8/14 (57%)
Sporadic	37/92 (40%)	81/132 (61%)	20/41 (49%)

**KRAS* mutations were analyzed as in (*6–8*), *P53* alterations as in (*10*), and *APC* mutations as in (*13*).

Table 3. Microsatellite alterations in HNPCC and sporadic tumors.

Tumor type*	Number of tumors	Percentage of tumors with alterations						
		D2S123	D2S147	D2S119	D11S904	D13S175	D10S197	CTG-B37
HNPCC								
RER$^+$	11†	91	55	55	55	64	91	55
RER$^-$	3‡	0	0	0	0	0	0	0
Total	14	71	43	43	43	50	71	43
Sporadic								
RER$^+$	6	67	83	83	67	100	83	67
RER$^-$	40	0	0	0	0	0	2	0
Total	46‡	9	11	11	9	13	13	9

*RER$^+$ tumors were defined as those with alterations in at least two of the indicated microsatellite markers. †Nine colorectal carcinomas, one colorectal adenoma, and one ovarian carcinoma. ‡All colorectal carcinomas.

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13. Mutations producing a translation termination signal between *APC* codons 686 to 2256 were evaluated by a PCR-based strategy. For this determination, DNA encoding amino acids 686 to 2256

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*).
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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].
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23. DNA was prepared from cryostat sections of tumors after removal of contaminating normal tissue (*7*).
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Microsatellite Instability in Cancer of the Proximal Colon

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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

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