Communication

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 269, No. 20, Issue of May 20, pp. 14367–14370, 1994
Printed in U.S.A.

Defective Mismatch Repair in Extracts of Colorectal and Endometrial Cancer Cell Lines Exhibiting Microsatellite Instability*

(Received for publication, March 11, 1994)

Asad Umar‡, Jayne C. Boyer‡, David C. Thomas‡, Dinh C. Nguyen‡, John I. Risinger§, Jeff Boyd§, Yurij Ionov¶, Manuel Perucho¶, and Thomas A. Kunkel‡‖

From the ‡Laboratory of Molecular Genetics and the §Gynecologic Pathobiology Section, Laboratory of Molecular Carcinogenesis, NIEHS, National Institutes of Health, Research Triangle Park, North Carolina 27709 and the ¶California Institute of Biological Research, La Jolla, California 92037

A replication error (RER⁺) phenotype, characterized by somatic instability in simple repeated sequences, is associated with several types of cancer. To determine if a defect in DNA replication fidelity or repair of replication errors might explain this instability, we compared both processes in cell-free extracts from RER⁺ endometrial and colorectal cancer cell lines to RER⁻ cell lines. SV40 origin-dependent replication of a microsatellite sequence is highly accurate in cell extracts regardless of their RER phenotype. However, extracts from RER⁺ cell lines are defective in mismatch repair, while extracts of RER⁻ cell lines are not. Lack of repair was observed when the signal (a nick) for strand-specific repair was either 3′ or 5′ to the mispair. One colorectal cancer cell line contained deletions in both alleles of the putative mismatch repair gene hMSH2, and one endometrial cancer cell line contained a 4-base pair duplication in one hMSH2 allele. No hMSH2 mutation was detected in the other allele or in the other five RER⁺ cell lines. Repair was readily detected when each of the defective extracts was mixed with a repair-proficient extract, demonstrating that no trans-acting inhibitor is present. Attempts to complement the repair deficiencies by mixing two different defective extracts identified three combinations that restored repair. The data suggest that: (i) defective repair is associated with colorectal and endometrial cancer and, by extrapolation, with other types of cancer; (ii) mutations in the hMSH2 gene, and possibly other genes, result in defective mismatch repair; (iii) the defect(s) in these lines likely involves pre-incision events or the excision step, but not the incision, polymerization, or ligation steps; and (iv) at least four functional complementation groups for mismatch repair may be involved in human cancer.

The eukaryotic genome contains many tandemly repeated short sequences. Deletion or addition of one or more repeat units of these microsatellites has been observed in a subset of sporadic colorectal cancer tumors and in tumors from patients with hereditary nonpolyposis colorectal carcinoma (HNPCC)[1] syndrome (1–6). Microsatellite instability has also been observed in tumors from a variety of other tissues (7–11) and is observed at alleles on several chromosomes. One explanation for this genome-wide mutator effect is reduced DNA replication fidelity; hence, the phenotype has been called RER⁺ (2), for Replication ERror. A second (but not mutually exclusive) possibility is that replication errors occurring at a normal rate are not corrected by post-replication mismatch repair. The latter is suggested by the fact that deletion and addition replication errors in repetitive DNA sequences are corrected in *Escherichia coli* (for review, see Ref. 12) and yeast (13) and in human cell extracts (14, 15). Additionally, the locus on chromosome 2p linked to HNPCC kindreds (16) encodes the human homolog (17, 18) of the *E. coli mutS* gene that is essential for mismatch repair, and both germline and somatic mutations in this gene, designated hMSH2, are found in HNPCC patients. Moreover, an extract of a RER⁺ colorectal cancer cell line is defective in mismatch repair *in vitro* (15).

Recently, microsatellite instability has been demonstrated in several human cell lines established from sporadic colorectal tumors (6). In the present study, we identify two sporadic endometrial tumor cell lines that are also RER⁺. These cell lines provide the opportunity to examine the biochemical defect(s) that might be responsible for microsatellite instability, using assays to measure the fidelity of DNA replication (19) and the efficiency of mismatch repair (14) in extracts of human cells. Here we have prepared extracts from RER⁺ and RER⁻ cell lines and determined error rates during replication of a homopolymeric microsatellite sequence and the efficiency of mismatch repair. We find no defect in replication fidelity, but an exact correlation between RER⁺ phenotype and defective repair. We then performed several studies in an attempt to define the nature of the repair defect(s).

EXPERIMENTAL PROCEDURES

Materials—The sources of materials are given in Refs. 14 and 19. Mismatched substrates, prepared as described (14), contained a nick in the minus strand at either position −264 (designated as the 3′ nick) or at position −276 (designated as the 5′ nick). Position +1 is the first transcribed base of the *lacZα* gene.

Cell Lines and Extracts—Lines DLD1, HCT116, LoVo, LS180, TK6, and MT1 were single cell clones derived from original lines by plating limiting dilutions. Lines AN_3CA and HEC59 were grown from mass cultures. MT1 and TK6 cells (provided by P. Modrich, Duke University) were grown in RPMI 1640 medium containing 5% fetal bovine serum. Other lines were grown in Dulbecco's modified Eagle's medium/Ham's F-12 medium with 10% fetal bovine serum. Extracts were prepared as described (19).

DNA Replication Fidelity and Mismatch Repair Assays—Procedures for measuring DNA replication fidelity and mismatch repair have been described (19, 14). Repair reactions (25 μl) contained 30 mM Hepes (pH 7.8), 7 mM $MgCl_2$, 4 mM ATP, 200 μM each CTP, GTP, and UTP, 100 μM each dATP, dGTP, dTTP, and dCTP, 40 mM creatine phosphate, 100 mg/ml creatine phosphokinase, 15 mM sodium phosphate (pH 7.5), 1 fmol of the indicated heteroduplex DNA, and 50 μg of cell extract protein. (Some reactions were performed without addition of dNTPs, since we have demonstrated (14) that endogenous dNTP levels in extracts are

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‖ To whom correspondence should be addressed. Tel.: 919-541-2644; Fax: 919-541-7613.

[1] The abbreviations used are: HNPCC, hereditary nonpolyposis colorectal carcinoma; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism.

TABLE I
RER phenotypes of cell lines and replicative capacity of extracts

Cell line	Original ref.	Type of cell	RER phenotype[a]	Replication (% of HeLa)[b]
HeLa	23	Cervical carcinoma	−	100
SW480	24	Colorectal carcinoma	−	72
TK6[c]	25	Transformed lymphoblast	−	120
DLD1	26	Colorectal carcinoma	+	46
HCT116	27	Colorectal carcinoma	+	90
LoVo	28	Colorectal carcinoma	+	41
LS180	29	Colorectal carcinoma	+	63
AN_3CA	30	Endometrial carcinoma	+	87
HEC59	31	Endometrial carcinoma	+	34
MT1	32	Mutant derivative of TK6	+	190

[a] RER phenotypes were assigned as follows. For colorectal carcinoma cell lines, microsatellite instability was described quantitatively, via PCR amplification of several endogenous microsatellite alleles from a large number of independent single-cell clones (6). For the RER$^+$ lines, the microsatellite mutation rate per allele per cell generation varied from 5×10^{-4} to 10^{-2}, while the average rate for SW480 was $\leq 1.1 \times 10^{-4}$. The same PCR-based single cell clone approach was used for HeLa (a subclone designated D98), TK6, and MT1 cells. For HeLa cells, no mutations were observed when several microsatellite loci were examined in over 100 independent single cell clones (S. Casares and M. Perucho, unpublished results). For TK6 cells, no mutations were observed at the D1S158 allele (containing a $(CT)_{24}$ repeat) among 147 single cell clones grown for 13 (58 clones) or 33 (89 clones) generations. For MT1 cells, 12 mutant D1S158 alleles were observed among 108 single cell clones grown for 10 (77 clones, 5 mutants) or 17 (17 clones, 7 mutants) generations. For endometrial carcinoma cell lines, DNA was isolated from cells in mass culture. For AN_3CA cells, eight microsatellite markers were amplified by PCR. In contrast to the one or two expected parental alleles, multiple bands (up to 20) were observed at three loci. For HEC59 cells, nine markers were amplified; multiple bands were observed at two loci.

[b] In reactions containing 100 μM dNTPs and other components as described (19), the HeLa cell extract incorporated from 90 to 290 pmol of total nucleotide per reaction, depending on the experiment. The replicative capacity of other extracts is expressed relative to that of the HeLa extract in the same experiment.

[c] This cell line was renamed TK6 from the denomination H2B1 in Ref. 25, in order to reflect its tk$^{+/-}$ character.

sufficient to support repair.) After incubating at 37 °C for 15 min (or 60 min, where indicated), reactions were processed and introduced into *E. coli* NR9162 (*mutS*), which were plated to score plaque colors (19). Repair efficiency is expressed in percent as 100 × (1 − the ratio of the percentages of mixed bursts obtained from extract-treated and untreated samples).

Analysis of hMSH2 Gene—mRNA was isolated from each cell line, cDNA was synthesized, and the coding sequence of the hMSH2 gene was amplified by PCR to generate 13 fragments ranging from 212 to 307 base pairs. PCR primers (21-mers) had 5′-terminal nucleotide position numbers (18) as follows, listing the primer complemetary to the minus strand first: 1, 1–246; 2, 202–448; 3, 399–673; 4, 630–897; 5, 836–1114; 6, 1072–1350; 7, 1302–1586; 8, 1522–1805; 9, 1754–2059; 10, 2011–2082; 11, 2238–2544; 12, 2493–2731; 13, 2618–2829. SSCP and DNA sequencing were performed as described in Ref. 20. Southern blot analysis was performed as described in Ref. 21.

RESULTS

The RER phenotypes of the cell lines were established as described in the legend to Table I or in a previous study (6). HeLa cell extracts served as the control, since these accurately replicate repetitive DNA (22) and correct mismatches (14). SW480 is a colorectal carcinoma cell line having an RER$^-$ phenotype, while DLD1, HCT116, LoVo, and LS180 are all RER$^+$ colorectal carcinoma cell lines. AN_3CA and HEC59 are RER$^+$ endometrial carcinoma lines. TK6 is a human lymphoblastoid B-cell line, extracts of which have been shown to be mismatch repair-competent (33). Analysis of a microsatellite locus in 147 TK6 single cell clones showed no evidence of instability. The MT1 cell line, derived from TK6 cells, has been shown to be defective for repair of single-base mismatches (33). Examination of instability at one microsatellite locus in 108 single cell clones of MT1 cells (as described in the legend to Table I) revealed 12 mutant alleles, demonstrating that this microsatellite sequence is unstable in the MT1 cell line relative to the TK6 line.

Replication Fidelity Measurements—Extracts of human cells can replicate DNA substrates containing the replication origin for eukaryotic simian virus 40. To test whether extracts of RER$^+$ cells have lower replication fidelity than do extracts of RER$^-$ cells, we replicated M13mp2 DNA containing seven consecutive T·A base pairs in the *lacZ* α-complementation gene. The coding sequence is in the +1 reading frame, such that the M13 plaque phenotype on selective plates is colorless. Replication errors that restore the correct reading frame (*e.g.* −1, +2 etc.) are scored as blue plaques. Extracts of all 10 cell lines were active for T antigen-dependent replication of this DNA (Table I). We chose extracts of two RER$^-$ lines (HeLa and SW480) and two RER$^+$ lines (LS180, colorectal; AN_3CA, endometrial) for comparative determination of fidelity. As expected based on previous results (22), the HeLa extract generated replication products whose revertant frequency was not different from the unreplicated control, indicating that frameshift fidelity during replication was high. Similarly low reversion frequencies were observed for DNA replicated with the SW480, LS180 and AN_3CA extracts, in two independent experiments (not shown). Thus, replication of a homopolymeric sequence by extracts of RER$^+$ cells is not error-prone relative to extracts of RER$^-$ cells, within the limits of detection of the assay (10^{-5} errors/base pair/replication cycle). This does not exclude that replication infidelity might eventually be found using other reaction conditions, microsatellite sequences, or cell lines.

Mismatch Repair Measurements—Mismatch repair in human cells is similar to the methyl-directed mismatch repair system in *E. coli* (for review, see Ref. 12) but utilizes a nick to direct repair to one strand (14, 34). To measure the ability of extracts to repair heteroduplexes containing unpaired nucleotides in repetitive sequence motifs, we employed a M13 DNA substrate containing a covalently closed (+) strand and a (−) strand with a nick located several hundred base pairs away from an unpaired nucleotide in the *lacZ* α-complementation coding sequence. The two strands encode different plaque colors (*e.g.* blue *versus* colorless). If an unrepaired heteroduplex is introduced into an *E. coli* strain deficient in methyl-directed heteroduplex repair, plaques will have a mixed phenotype on selective plates, due to expression of both strands of the heteroduplex. However, repair occurring during incubation of the substrate in a repair-proficient human cell extract will reduce the percentage of mixed plaques and increase the ratio of the (+) strand phenotype relative to that of the (−) strand phenotype, because the nick directs repair to the (−) strand.

We previously showed strand-specific repair of frameshift heteroduplexes in a HeLa cell extract (14). A similar result is shown in Fig. 1*A*, where a HeLa extract efficiently repaired the nicked strand of a substrate containing one unpaired nucleotide in a run of A·T base pairs. Extracts of two other RER$^-$ lines (SW480 and TK6) also repaired the heteroduplex, whereas repair in extracts of the seven RER$^+$ lines was much less efficient. Thus, there is a correlation between the RER$^+$ phenotype and defective heteroduplex repair. That extracts from RER$^+$ cells are not generally inactive for all DNA transactions is indicated by the fact that all are competent for DNA replication (Table I). Extracts of RER$^+$ cells were also defective in repair of other heteroduplexes containing an unpaired nucleotide in either strand (not shown) or containing a G·G mispair (Fig. 1, *panels C* and *D*). Repair was observed in reactions containing any of the defective extracts plus an equal amount of HeLa extract (Fig. 1, *panel B*). Thus, the defective extracts do not contain a trans-acting inhibitor.

Repair involves a mismatch-dependent excision reaction oc-

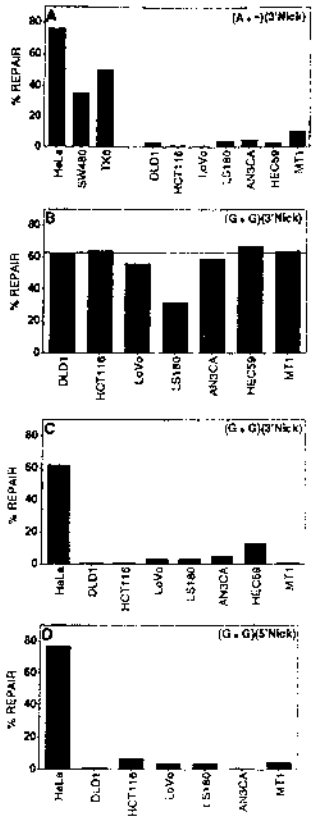


FIG. 1. **Repair efficiency in extracts.** *Panel A*, repair in RER⁻ *versus* RER⁺ cell extracts, using a frameshift heteroduplex at nucleotides 91–94, AAAA in (+) strand (blue), TTT in the (–) strand (colorless), 3′ nick at position –264. Reactions were incubated for 60 min. The results are based on counting several hundred to several thousand plaques per variable. Results representative of these experiments can be seen in Table II of Ref. 14. Since values between 0 and 10% repair represent experimental fluctuation (14) in this assay, we cannot conclude that repair is completely absent in extracts of RER⁺ cells, only that it is reduced. In 2 of 10 experiments, we observed values for repair of a frameshift heteroduplex in extracts of MT1 cells that are consistent with the possibility that some repair activity could be present. *Panel B*, repair in a mixture of RER⁺ extracts plus HeLa extract, using a heteroduplex containing a G·G mispair at position 88, blue (+) strand, light blue (–) strand, 3′ nick at position –264. The line in the graph represents the level of repair by a HeLa extract alone. Reactions were incubated for 15 min. In two other experiments combining HeLa + LS180 extracts, repair of a heteroduplex containing an unpaired nucleotide was comparable to that obtained with HeLa extract alone. *Panel C*, repair in RER⁻ *versus* RER⁺ cell extracts when the nick (position –264) is 3′ to the mispair, a G·G mispair at position 88. Reactions were incubated for 15 min. *Panel D*, repair in RER⁻ *versus* RER⁺ cell extracts when the nick (at position +276) is 5′ to the mispair, a G·G mispair at position 88. Reactions were incubated for 15 min, except for the LS180 and AN_3CA extract reactions, which were for 60 min.

curring on the incised strand that has bidirectional capability (35). Thus, in a circular substrate, excision of nucleotides via the shortest path between the nick and the mispair can occur from a nick either 3′ or 5′ to the mispair. To determine whether the RER⁺ extracts differed in their capacity for such bidirectional repair, we examined repair of a substrate containing a nick on either side of a G·G mispair. Whether the substrate contained a 3′ (Fig. 1, *panel C*) or 5′ (Fig. 1, *panel D*) nick, all seven RER⁺ cell lines are defective in repair of the mismatch.

Status of the hMSH2 Gene in RER⁺ Cell Lines—Mutations in the hMSH2 gene are found in tumors of HNPCC patients (17, 18) and HNPCC tumor cells usually exhibit an RER⁺ phenotype. We thus searched for mutations in this gene by reverse transcriptase-mediated PCR of mRNA followed by SSCP analysis. No differences in either allele were found between RER⁻ cell lines and five of the seven RER⁺ lines. However, in the RER⁺ endometrial cancer cell line HEC59, one allele appeared normal, while the other contained a mutation (data not shown). Sequence analysis of the cloned fragment showed that one allele was normal, while the other contained a duplication of the 4-base pair sequence TAAG (nucleotides 1442–1445, codon 482 from Ref. 18). With the colorectal cancer line LoVo, amplification of the coding sequence by PCR of cDNA yielded fragments for nucleotides 1522–2829 of the gene, but no bands for attempted amplification of nucleotides 1–1586. No bands were detected for either hMSH2 allele by Southern blot analysis (not shown) of genomic DNA using a randomly labeled probe for nucleotides 1072–1350. These data suggest that both alleles of the hMSH2 gene contain a deletion.

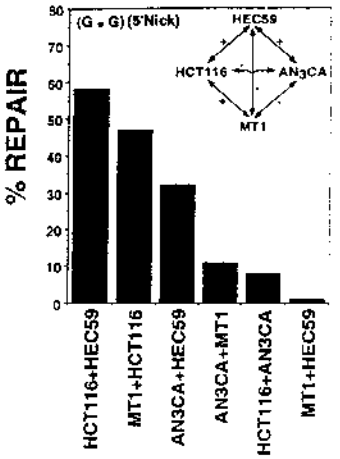


FIG. 2. **Complementation of repair by mixing two defective extracts.** The substrate contained a G·G mispair at position 88 and a 3′ nick at position –264. A repeat of this experiment using a substrate containing the same mispair and a 5′ nick at position 276 gave similar results.

Complementation of Repair Defect—Having identified seven repair-defective extracts and hMSH2 mutations in two cell lines, we attempted to complement the repair deficiencies by performing reactions with different pairwise combinations of defective extracts. No complementation was observed in reactions containing a LoVo extract plus either a HEC59, AN_3CA, HCT116, or MT1 extract. However, repair comparable to that catalyzed in a HeLa extract alone was observed when a HCT116 extract was combined with either a HEC59 or MT1 extract (Fig. 2). The combination of HEC59 and AN_3CA also yields detectable repair but at a lower efficiency. MT1 extracts were unable to complement HEC59 or AN_3CA extracts. This pattern, depicted in Fig. 2 (*inset*), suggests that at least four functional complementation groups may exist. These could be defined by different mutations in a gene encoding a multifunctional protein, by mutations in different genes, or by some combination of the two possibilities.

DISCUSSION

The data presented here provide a strong correlation between microsatellite instability *in vivo* and defective heteroduplex repair *in vitro*. Extracts of seven cell lines exhibiting microsatellite instability are less efficient for mismatch repair than are extracts of three RER⁻ cell lines. Given this correlation, defective mismatch repair may explain the RER⁺ phenotype now reported for tumors from a variety of tissues (1–11).

What is the nature of the repair defect(s) in the cell lines used in the present study? The occurrence of repair in reactions containing a HeLa cell extract and any of the seven extracts from RER⁺ cells (Fig. 1*B*) suggests that the defective extracts

do not contain a trans-acting inhibitor of heteroduplex repair. A similar result was reported for a cell line designated H6 (15), which is a cloned derivative of the sporadic colorectal line HCT116 used in this study. Defective repair of the substrates used here, which already contain a strand-specific incision, suggests that the defect is not in the incision step itself. Moreover, defects occurring after the excision step would not be detected because the assay used here scores repair by loss of a mixed plaque phenotype. Once excision beyond the mismatch has occurred, even if subsequent polymerization or ligation were defective, a substrate molecule would still be scored as repaired because such gapped molecules yield a pure plaque phenotype upon introduction into *E. coli* (36). Hence, the defects in these seven extracts are likely to be prior to incision or in the excision step itself.

An absence of the complete human homolog of the *E. coli* MutS protein, inferred from deletions in both hMSH2 alleles observed in the LoVo cell line, could affect events needed prior to incision or interactions required for excision. However, since repair of both mispaired and misaligned heteroduplexes is defective, LoVo and other RER$^+$ cell lines may have strongly elevated mutation rates, as has already been demonstrated for MT1 cells (32). Furthermore, we have not yet been able to complement repair in extracts of LoVo cells with extracts of the other six RER$^+$ cell extracts shown in Table I. Thus, deletion at the hMSH2 locus may not be the only mutation present in LoVo cells that is relevant to its RER$^+$ phenotype. Future studies with putative mutator cell lines should also bear in mind that these may accumulate secondary mutations that affect interpretations.

Thus far, we have detected a mutation in only one of two hMSH2 alleles in the HEC59 line. Perhaps the other allele contains a mutation that is not detected by SSCP analysis of cDNA. Alternatively, HEC59 and the cell lines for which no hMSH2 mutation was found may contain mutations in other heteroduplex repair genes. Candidates are mutant human genes equivalent to the *E. coli mutL* gene, whose product is essential for mismatch repair. The gene encoded by the locus on chromosome 3 associated with HNPCC (4) could be one of these. The functional complementation observed between an extract of HEC59 cells, containing at least one known mutation in one allele of a heteroduplex repair gene, and extracts of either HCT116 or AN_3CA cells, suggests the latter two cell lines as candidates for different mutations. These could be different mutations in the hMSH2 that alter a separate but complementary function of this gene product, or they could be mutations in genes other than hMSH2. In *E. coli*, there is evidence for the participation of any of three different exonucleases in the excision step of mismatch repair, depending on the location of the nick relative to the mismatch (37). This is particularly interesting given the observation that extracts of all seven RER$^+$ cell lines are defective in repair of a G·G mismatch regardless of whether the substrate contains a 3′ (Fig. 1, *panel C*) or 5′ (Fig. 1, *panel D*) nick. A single defect in any extract could explain this bidirectional repair deficiency only if the defect were prior to excision or if it were in a protein controlling exonuclease activity in both directions, *e.g.* a DNA helicase or a bidirectional exonuclease.

Acknowledgments—We thank J. Carl Barrett and John D. Roberts for their comments on the manuscript.

Note Added in Proof—A single cell clone of HCT116 has no wild type *hMLH1* allele (38).

REFERENCES

1. Ionov, Y., Peinado, M. A., Malkhosyan, S., Shibata, D., and Perucho, M. (1993) *Nature* **363,** 558–561
2. Aaltonen, L. A., Peltomäki, P., Leach, F. S., Sistonen, P., Pylkkänen, L., Mecklin, J.-P., Järvinen, H., Powell, S. M., Jen, J., Hamilton, S. R., Petersen, G. M., Kinzler, K. W., Vogelstein, B., and de la Chapelle, A. (1993) *Science* **260,** 812–816
3. Thibodeau, S. N., Bren, G., and Schaid, D. (1993) *Science* **260,** 816–819
4. Lindblom, A., Tannergard, P., Werelius, B., and Nordenskjöld, M. (1993) *Nat. Genet.* **5,** 279–782
5. Lothe, R. A., Peltomäki, P., Meling, M. E., Aaltonen, L. A., Nyström-Lahti, M., Pylkkänen, L., Heimdal, K., Andersen, T. I., Moller, P., Rognum, T. O., Fossa, S. D., Haldorsen, T., Langmark, F., Brøggr, A., de la Chapelle, A., and Borresen, A.-L. (1993) *Cancer Res.* **53,** 5849–5852
6. Shibata, D., Peinado, M. A., Ionov, Y., Malkhosyan, S., and Perucho, M. (1994) *Nat. Genet.* **6,** 278–281
7. Han, H.-J., Yanagisawa, A., Kato, Y., Park, J.-G., and Nakamura, Y. (1993) *Cancer Res.* **53,** 5087–5089
8. Risinger, J. I., Berchuck, A., Kohler, M. F., Watson, P., Lynch, H. T., and Boyd, J. (1993) *Cancer Res.* **53,** 5100–5103
9. Peltomäki, P., Lothe, R. A., Aaltonen, L. A., Pylkkänen, L., Nyström-Lahti, M., Seruca, R., David, L., Holm, R., Ryberg, D., Haugen, A., Brøgger, A., Borresen, A.-L., and de la Chapelle, A. (1993) *Cancer Res.* **53,** 5853–5855
10. Mironov, N. M., Aguelon, M. A.-M., Potapova, G. I., Omori, Y., Gorbunov, O. V., Klimenkov, A. A., and Yamasaki, H. (1994) *Cancer Res.* **54,** 41–44
11. Wooster, R., Cleton-Jansen, A.-M., Collins, N., Mangion, J., Cornelis, R. S., Cooper, C. S., Gusterson, B. A., Ponder, B. A. J., von Deimling, A., Wiestler, O. D., Cornelisse, C. J., Devilee, P., and Stratton, M. R. (1994) *Nat. Genet.* **6,** 152–156
12. Modrich, P. (1991) *Annu. Rev. Genet.* **25,** 229–253
13. Strand, M., Prolla, T. A., Liskay, R. M., and Petes, T. D. (1993) *Nature* **365,** 273–277
14. Thomas, D. C., Roberts, J. D., and Kunkel, T. A. (1991) *J. Biol. Chem.* **266,** 3744–3751
15. Parsons, R., Li, G.-M., Longley, M. J., Fang, W.-h., Papadopoulos, N., Jen, J., de la Chapelle, A., Kinzler, K. W., Vogelstein, B., and Modrich, P. (1993) *Cell* **75,** 1227–1236
16. Peltomäki, P., Aaltonen, L. A., Sistonen, P., Pylkkänen, L., Mecklin, J.-P., Järvinen, H., Green, J. S., Jass, J. R., Weber, J. L., Leach, F. S., Petersen, G. M., Hamilton, S. R., de la Chapelle, A., and Vogelstein, B. (1993) *Science* **260,** 810–812
17. Fishel, R., Lescoe, M., Rao, M., Copeland, N. G., Jenkins, N. A., Garber, J., Kane, M., and Kolodner, R. D. (1993) *Cell* **75,** 1027–1038
18. Leach, F. S., Nicolaides, N. C., Papadopoulos, N., Liu, B., Jen, J., Parsons, R., Peltomäki, P., Sistonen, P., Aaltonen, L. A., Nyström-Lahti, Guan, X.-Y., Zhang, J., Meltzer, P. S., Yu, J.-W., Kao, F.-T., Chen, D. J., Cerosaletti, K. M., Fournier, R. E. K., Todd, S., Lewis, T., Leach, R. J., Naylor, S. L., Weissenbach, J., Mecklin, J.-P., Järvinen, H., Petersen, G. M., Hamilton, S. R., Green, J., Jass, J., Watson, P., Lynch, H. T., Trent, J. M., de la Chapelle, A., Kinzler, K. W., and Vogelstein, B. (1993) *Cell* **75,** 1215–1225
19. Roberts, J. D., and Kunkel, T. A. (1993) *Methods Mol. Genet.* **2,** 295–313
20. Risinger, J. I., Berchuck, A., Kohler, M. F., and Boyd, J. (1994) *Nat Genet* **6,** in press
21. Boyd, J., and Risinger, J. I. (1991) *Mol. Carcinogen.* **4,** 189–195
22. Roberts, J. D., Nguyen, D., and Kunkel, T. A. (1993) *Biochemistry* **32,** 4083–4089
23. Puck, T. T., Marcus, P. I., and Cieciura, S. J. (1956) *J. Exp. Med.* **103,** 273–284
24. Leibovitz, A., Stinson, J. C., McCombs, W. B., McCoy, C. E., Mazur, K. C., and Mabry, N. D. (1976) *Cancer Res.* **36,** 4562–4569
25. Skopek, T. R., Liber, H. L., Penman, B. W., and Thilly, W. G. (1978) *Biochem. Biophys. Res. Commun.* **84,** 411–416
26. Dexter, D. L., Barbosa, J. A., and Calabresi, P. (1979) *Cancer Res.* **39,** 1020–1025
27. Brattain, M. G., Fine, W. D., Mahnaz-Khaled, F., Thompson, J., and Brattain, D. E. (1981) *Cancer Res.* **41,** 1751–1756
28. Drewinko, B., Romsdahl, M. M., Yang, L. Y., Ahearn, M. J., and Trujillo, J. M. (1976) *Cancer Res.* **36,** 467–475
29. Tom, B. H., Rutzky, L. P., Jakstys, M. M., Oyasu, R., Kaye, C. I., and Kahan, B. D. (1976) *In Vitro* **12,** 180–191
30. Dawe, C. J., Banfield, W. G., Morgan, W. D., Slatick, M. S., and Curth, H. O. (1964) *J. Natl. Cancer Inst.* **33,** 441–456
31. Morisawa, T. (1987) *J. Jpn. Soc. Clin. Cytol.* **26,** 433–442
32. Goldmacher, V. S., Cuzick, R. A., and Thilly, W. G. (1986) *J. Biol. Chem.* **261,** 12462–12471
33. Kat, A., Thilly, W. G., Fang, W.-H., Longley, M. J., Li, G.-M., and Modrich, P. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 6424–6428
34. Holmes, J., Clark, S., and Modrich, P. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 5837–5841
35. Fang, W.-h., and Modrich, P. (1993) *J. Biol. Chem.* **268,** 11838–11844
36. Kunkel, T. A. (1985) *J. Biol. Chem.* **260,** 5787–5796
37. Cooper, D. L., Lahue, R. S., and Modrich, P. (1993) *J. Biol. Chem.* **268,** 11823–11829
38. Papadopoulos, N., Nicholaides, N. C., Wei, Y.-F., Ruben, S. M., Carter, K. C., Rosen, C. A., Haseltine, W. A., Fleischmann, R. D., Fraser, C. M., Adams, M. D., Venter, J. C., Hamilton, S. R., Petersen, G. M., Watson, P., Lynch, H. T., Peltomäki, P., Mecklin, J.-P., de la Chapelle, A., Kinzler, K. W., and Vogelstein, B. (1994) *Science* **263,** 1625–1629

13. **Kartner, N., J. W. Manrahan, J. J. Jensen, A. L. Naismith, S. Sun, C. A. Ackerley, E. F. Reyes, L. C. Tsui, J. M. Rommens, C. E. Bear, and J. R. Riordan.** 1991. Expression of the cystic fibrosis gene in non-epithelial invertebrate cells produces a regulated anion conductance. Cell **54**:681–692.
14. **Kerem, B.-S., J. M. Rommens, J. A. Buchanan, D. Markiewicz, T. K. Cox, A. Chakravarti, M. Buchwald, and L. C. Tsui.** 1989. Identification of the cystic fibrosis gene: genetic analysis. Science **245**:1073–1080.
15. **Kerem, B.-S., J. Zielenski, D. Markiewicz, D. Bozon, E. Gazit, J. Yahaf, D. Kennedy, J. R. Riordan, F. S. Collins, J. M. Rommens, and L.-C. Tsui.** 1990. Identification of mutations in regions corresponding to the two putative nucleotide (ATP)-binding folds of the cystic fibrosis gene. Proc. Natl. Acad. Sci. USA **87**:8447–8451.
16. **Klausner, R. D., and R. Sitia.** 1990. Protein degradation in the endoplasmic reticulum. Cell **62**:611–614.
16a. **Kobayashi, K., and A. L. Beaudet.** Personal communication.
17. **Kobayashi, K., M. R. Knowles, R. C. Boucher, W. E. O'Brien, and A. L. Beaudet.** 1990. Benign missense variations in the cystic fibrosis gene. Am. J. Hum. Genet. **47**:611–615.
18. **Kunkel, T. M.** 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82**:488–492.
19. **Laemmli, U. K.** 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
20. **Lodish, H. F.** 1988. Transport of secretory and membrane glycoproteins from the rough endoplasmic reticulum to the Golgi. J. Biol. Chem. **263**:2107–2110.
21. **Mimura, C. S., S. R. Holbrook, and G. F.-L. Ames.** 1991. Structural model of the nucleotide-binding conserved component of periplasmic permeases. Proc. Natl. Acad. Sci. USA **88**:84–88.
22. **Osborne, L., R. Knight, G. Santis, and M. E. Hodson.** 1991. A non-conservative mutation in the second nucleotide binding fold of the cystic fibrosis transmembrane conductance regulator. Am. J. Hum. Genet. **48**:608–612.
23. **Pelham, H. R. B.** 1989. Control of protein exit from the endoplasmic reticulum. Annu. Rev. Cell Biol. **5**:1–23.
24. **Rich, D. P., M. P. Anderson, R. J. Gregory, S. H. Cheng, S. Paul, D. Jefferson, J. D. McCann, K. W. Klinger, A. E. Smith, and M. J. Welsh.** 1990. Expression of the cystic fibrosis transmembrane conductance regulator corrects defective chloride channel regulation in cystic fibrosis airway epithelial cells. Nature (London) **347**:358–363.
25. **Riordan, J. R., J. M. Rommens, B.-S. Kerem, B.-S., N. Alon, R. Rozmahel, Z. Grzelczack, J. Zielenski, S. Lok, N. Plavsic, J.-L. Chou, M. L. Drumm, M. C. Iannuzzi, F. S. Collins, and L.-C. Tsui.** 1989. Identification of the cystic fibrosis gene: cloning and characterization of the complementary DNA. Science **245**:1066–1073.
26. **Rommens, J. M., M. C. Iannuzzi, B.-S. Kerem, M. L. Drumm, G. Melmer, M. Dean, R. Rozmahel, J. L. Cole, D. Kennedy, N. Hidaka, M. Zsiga, M. Buchwald, J. R. Riordan, L.-C. Tsui, and F. S. Collins.** 1989. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science **245**:1059–1065.
27. **Rose, J. K., and R. W. Doms.** 1988. Regulation of protein export from the endoplasmic reticulum. Annu. Rev. Cell Biol. **4**:257–258.
28. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Thomas, P. J., P. Shenbagamurthi, X. Ysern, and P. L. Pedersen.** 1991. Cystic fibrosis conductance regulator; nucleotide binding to a synthetic peptide. Science **251**:555–557.
30. **Van Koppen, C. J., and N. M. Nathanson.** 1990. Site-directed mutagenesis of the m2 muscarinic acetylcholine receptor. J. Biol. Chem. **265**:20887–20892.
31. **Walker, J. E., M. Saraste, M. J. Runswick, and N. J. Gay.** 1982. Distantly related sequences in the α- and β-subunits of ATP synthase, myosin kinases, and other ATP requiring enzymes and a common nucleotide binding fold. EMBO J. **8**:945–951.
32. **Welsh, M. J.** 1990. Abnormal regulation of ion channels in cystic fibrosis epithelia. FASEB J. **4**:2718–2725.