Appropriate Partners Make Good Matches

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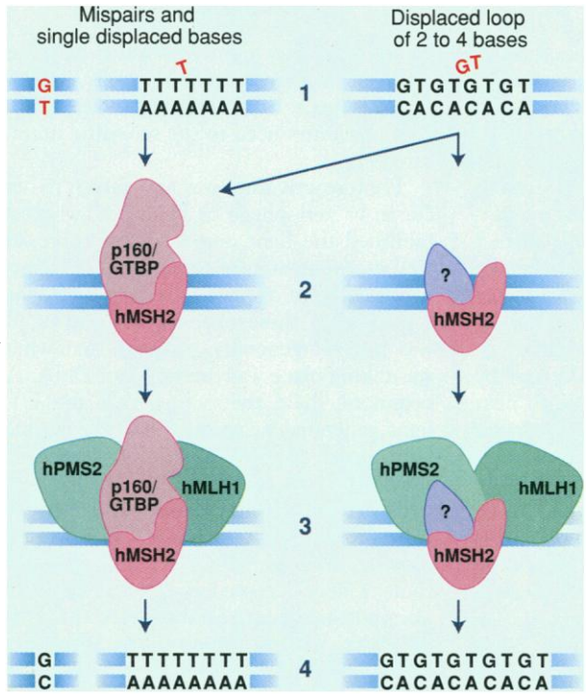
Six months ago, the extended family of DNA repair enzymes was honored as *Science*'s Molecules of the Year. Like heroines in a Victorian novel, the human mismatch repair siblings are finding appropriate partners and making good matches. Their job is to correct the mistakes left by DNA polymerase during replication. When they fail to do this efficiently, the cell is at risk of accumulating inactivating mutations in genes that restrain its division; defective mismatch repair is increasingly associated with human cancer. The human repair proteins have bacterial homologs, indicating that the overall correction strategy is conserved. As more details emerge, however, human mismatch repair appears rather more complicated than its bacterial counterpart. The identification of a new mismatch recognition protein (*1*, *2*) and a phenotype associated with its inactivation (*3*), reported in this week's issue of *Science*, has revealed unsuspected subtleties in the human pathway.

Defective mismatch repair underlies the genome instability in the familial colon tumors of hereditary nonpolyposis colorectal cancer (HNPCC) (*4*). Germline mutations in HNPCC families affect homologs of the bacterial mismatch repair proteins MutS and MutL. Mutations in microsatellites, reiterated mono- and dinucleotide elements scattered throughout the genome, accumulate in tumors in which both copies of one of these genes are inactivated. The extreme polymorphism of these repetitive elements represent the footprints of defective mismatch correction, because the polymorphism arises from slipped or mispaired replication intermediates that are normally reversed by mismatch repair. The mutator phenotype and microsatellite instability of cells from affected tumors arise as a result of the accumulation of such replication errors.

The first gene implicated in microsatellite instability in HNPCC families was hMSH2 (*5*, *6*), a homolog of the *mutS* gene from *Escherichia coli*. MutS protein binds to DNA mismatches and recruits the other repair proteins (*7*). Isolated hMSH2 protein does indeed have a mismatch binding activity (*8*), but its preferences are inconsistent with a simple role in mismatch repair. The discrepancies have been resolved by the new observations that hMSH2 does not act alone in the recognition step. In human cells, mismatches are recognized by a heterodimer, hMutSα, comprising hMSH2 and the newly discovered member of the extensive family of MutS homologs, named GTBP by Jiricny's group (*1*) and p160 by Modrich's group (*2*). Biochemical and genetic data indicate that inactivation of one or the other partner of the heterodimer confers different phenotypes.

GTBP/p160 was first seen together with hMSH2 among proteins affinity-purified by binding to G-T mismatched DNA (*9*). Sequence analysis identified it as a new human MutS homolog and facilitated the cloning of its complementary DNA (cDNA). When GTBP/p160 or hMSH2 cDNAs are expressed and translated separately in vitro, the translation products are without detectable mismatch binding activity. Translation together yields a functional binding product. Antibodies against either hMSH2 or GTBP/p160 abolish mismatch binding by cell extracts. Both proteins are therefore required for mismatch binding. A direct demonstration that they form a heterodimer has been provided independently by Drummond *et al.* (*2*) They used purified protein fractions from correction-proficient cells to complement the in vitro mismatch repair defect of extracts of an *hMSH2* mutant colorectal carcinoma cell line. This approach identified an activity, designated hMutSα, that restored correction to the mutant cell extracts. hMutSα comprises hMSH2 associated with a protein (p160) of 160 kilodaltons. Peptide analysis indicates that p160 and GTBP are identical.

Other human colorectal carcinoma cell lines with mismatch repair defects provide indications of what the hMSH2:GTBP/p160 complex does. Purified hMutSα reverses two kinds of correction defect in cell extracts. Some extracts have a general defect and fail to rectify G-T mispairs, single displaced bases, or loops of two to four bases (see figure). Others have a more selective deficiency in which loops bigger than one base are corrected efficiently but G-T mismatches or single-base loops are not. Genetic analysis of hMSH2 and GTBP/p160 indicates that mutations in GTBP/p160 confer the selective defects, whereas hMSH2 mutations are associated with the more extreme repair deficiency. The molecules with one- or two-base loops used in the correction assay mimic the slipped or mispaired intermediates in the microsatellite mutations. The different correction defects are reflected accordingly in the cells' phenotypes (*3*). DNA from cells with defects in correction of all loops is unstable at both mono- and dinucleotide repeat microsatellites. If the extracts can repair two-base loops, instability in cellular DNA is confined to mononucleotide repeats. So although the two MutS homologs, hMSH2 and GTBP/p160, act as a dimer, inactivation of each partner has a different effect on genome stability. A simple explanation is that hMSH2 needs some help from GTBP/p160 to recognize more subtle mismatches—single-base mispairs and one-base loops—but can handle the larger aberrations itself (or



Differential repair of mismatched DNA. 1, Mispairs and displaced one- or two-base loops are generated during replication. 2, All mismatches require hMSH2 for repair. Single-base mispairs and slipped intermediates in single-base runs are recognized by the hMSH2:GTBP/p160 heterodimer, hMutSα. In the absence of p160/GTBP, slipped intermediates of two (or more) bases may be bound by hMSH2 either alone or possibly as a heterodimer with an unidentified second protein (?). 3, All mismatches require hMLH1 for repair (*2*). hMutLα is recruited by the hMSH2:DNA complexes. 4, The normal sequence is restored after removal of the mismatched DNA, resynthesis, and ligation.

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with an unidentified alternative partner). This idea accords with the rather surprising preference of purified hMSH2 for larger looped structures (*8*). If correct, it implies that the presence of GTBP/p160 may not be required for recruitment of the human MutL complex. This hypothesis is directly testable with purified protein components.

These new observations have some profound implications. Human mismatch repair defects are clearly not associated with a homogeneous phenotype. Put simply, the absence of microsatellite instability need not indicate a functional mismatch repair pathway. Equally important, "microsatellite instability" may not imply a substantial mutator phenotype. The human analog of MutL, hMutlα, is also a heterodimer (*10*). Are different phenotypes associated with defects in either of its subunits, hPMS2 and hMLH1? Although half of HNPCC families have hMSH2 mutations, germline GTBP/p160 mutations were not observed in 20 unassigned HNPCC kindreds (*3*). Are GTBP/p160 mutations poorly penetrant and not seen as familial cancer—or, as suggested, is haplo-insufficiency incompatible with normal development? These problems can be approached through use of animal models.

The complexity of human mismatch repair was foreshadowed by the identification of multiple homologs of the *E. coli* repertoire of repair proteins. One important consequence of this complexity is a high probability of dominant-negative effects as the number of proteins increases. Apparent dominant-negative effects have been observed in some HNPCC heterozygotes (*11*). There are likely to be many different phenotypes associated with mismatch correction defects. The new findings suggest a needed revision in the initial, perhaps naive, expectation that a massive mutator phenotype is an inevitable consequence of deficient mismatch repair.

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The Structure of Photolyase: Using Photon Energy for DNA Repair

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The major product of the illumination of DNA by the ultraviolet (UV) component of sunlight is the cyclobutane pyrimidine dimer. Pyrimidine dimers kill cells by blocking DNA replication and transcription, and on rare occasions when DNA polymerase is able to bypass the dimer, by causing a mutation at the site of the lesion. Cells protect themselves from these pyrimidine dimers by removing them through excision repair or through photoreactivation. Photoreactivation is the prevention of the deleterious effects of far-UV light (200 to 300 nm) by concurrent or subsequent exposure to near UV-visible light (300 to 500 nm). Examples of photoreactivation occur in all three kingdoms of life, but it is unpredictably missing in many species, including humans. This issue of *Science* reports a landmark for this field of DNA repair—determination of the crystal structure of photolyase, the enzyme that mediates photoreactivation (*1*).

It was already known in 1935 (*2*, *3*) that the lethal damage to bacteria caused by exposure to UV radiation was markedly reduced by maintaining the bacteria in a poor growth medium and exposing them to visible light. In the late 1940s, Albert Kelner generated mutant bacteria that made antibiotics, by irradiation with x-rays or with UV light. The survival of the UV-irradiated cells was initially highly variable because, as Kelner discovered, light from 350 to 500 nm was necessary. Photoreactivation was thus first introduced to the scientific literature (*4*).

Photoreactivation was also shown to occur in bacteriophage by Dulbecco, who established the light dose-response curve, as well as a temperature dependence, suggesting the active agent might be an enzyme (*5*). Despite these exciting discoveries, it was not yet generally accepted that what was taking place was the repair of DNA. A commonly held theory was that the UV light generated a "poison," and the visible light caused photoreactivation by acting on the poison.

When the Hershey-Chase experiment in 1952 proved that only phage DNA and no protein enters a phage-infected cell, it became widely accepted that photoreactivation must take place on the DNA itself (*6*). In 1956, Rupert, Goodgal, and Herriott showed that UV-irradiated *Haemophilus influenzae* DNA regained its ability to transform host cells only after photoreactivation in a cell-free extract of *Escherichia coli* (*7*). This experiment proved that an *E. coli* (protein) component was necessary to bring about photoreactivation and that the repair must be on DNA itself.

The nature of the chemical lesion on DNA became clear when Beukers, Ijlstra, and Berends in 1959 showed that UV irradiation of a frozen solution of thymine caused it to lose its 260-nm absorbance and that, upon melting and further irradiation, the 260-nm absorbance was restored (*8*). These same workers then proved that the photoproduct from the frozen solution was indeed the cyclobutane-type thymine dimer. Rupert (*9*) and R. Setlow and J. Setlow (*10*, *11*) subsequently showed that photoreactivation results in the reversal of pyrimidine dimers to the original pyrimidines in DNA.

Thus, by 1960 the gross mechanism of photoreactivation was understood to occur in three steps: (i) The photoreactivation enzyme (photolyase) bound to pyrimidine dimers in DNA in a light-independent step; (ii) the near UV-visible photon reversed the cyclobutane dimer to two pyrimidines; and (iii) photolyase then dissociated from the DNA, leaving it repaired.

The requirement of 350- to 500-nm light for photoreactivation introduces other mechanistic constraints. Because light of these wavelengths is not absorbed by pyrimidine dimers, by DNA, or by the usual amino acids contained in proteins, photoreactivation enzyme (photolyase) must contain chromophores capable of capturing photons of these energies and a mechanism for converting this electronic excitation into activation energy for cyclobutane reversal.

The photolyase gene was cloned by Sancar and Rupert in 1978 (*12*). In the intervening years, through the efforts of these researchers and others (*13*, *14*), the biochemistry and mechanism of action of this enzyme have become better understood, and the chromophores and cofactors have been isolated and chemically identified. Photolyase is an unusual enzyme in that it contains flavin-adenine dinucleotide in the form of $FADH^-$ as a cofactor. Although this molecule is a common cofactor in bio-

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