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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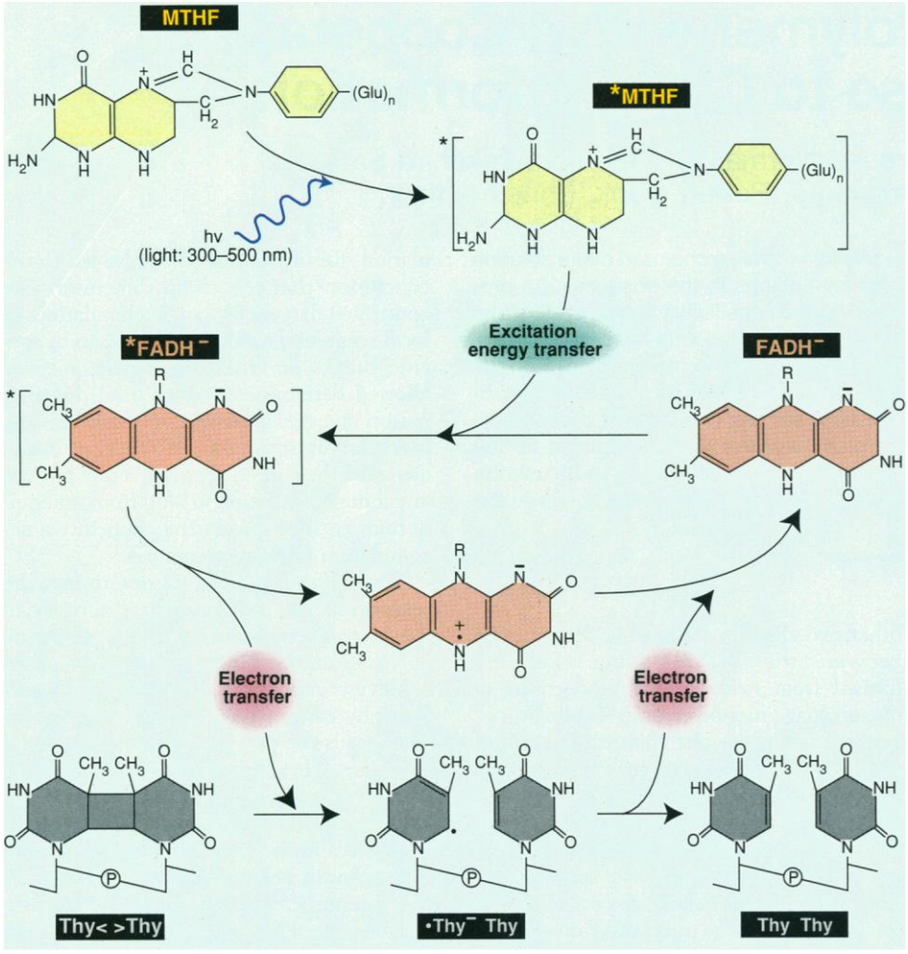

The mechanism of photoreactivation by photolyase.

chemical redox reactions, photolyase does not catalyze a redox reaction. In addition, the amino acid sequence of the enzyme does not contain the recognizable binding site common to other flavin-binding proteins. And, unlike flavin in these other proteins, the flavin in photolyase is permanently bound both in its ground and excited states. Furthermore, it transfers only a single electron to the pyrimidine dimer cyclobutane ring, and after reversal to the two 5-6 double bonds of the pyrimidines, accepts the electron back from the remaining pyrimidine radical.

The second cofactor, 5,10-methenyltetrahydrofolylpolyglutamate (MTHF), serves as the light-harvesting antenna (in some organisms the antenna is a deazaflavin). MTHF is an unusual folate structure that is also permanently bound in the photolyase, and the amino acid binding site for MTHF has no homology with other folate-binding proteins. Because folate is used as an antenna, both the ground and the excited states of MTHF must be bound tightly. The crystal structure of *E. coli* photolyase is reported in this issue of *Science* by Park, Kim, Sancar, and Deisenhofer (*1*). The geometry of the position of the antenna folate (MTHF) relative to the excitation acceptor flavin ($FADH^-$) suggests a new donor-acceptor pair for which detailed analysis of the energy transfer process can now be made. Similar crystallographic information is available for only one other system in which electronic energy transduction occurs, the integral membrane light-harvesting complex from a photosynthetic bacterium (*15*).

The bacterial photosynthetic system consists of light-harvesting complexes that collectively serve the role of the antenna folate (MTHF) in photolyase. The electronic energy is transferred to the bacteriochlorophyll special pair in the photosynthetic reaction center of the purple bacteria by the same basic mechanism as that incorporated in photolyase for the transfer of electronic energy from the folate (MTHF) to the flavin ($FADH^-$). The remaining steps that occur at the reaction center involve electron transfer. The final chemical step in the purple bacterial reaction center is the reduction of a quinone to a hydroquinone, using two independent single-electron reduction steps. The electron-transfer steps in the reaction center are formally equivalent to the catalytic step in photolyase which also uses a single-electron transfer to the cyclobutane ring of the pyrimidine dimer.

The crystal structure of the photolyase contains a pocket into which the pyrimidine dimer presumably inserts after being "flipped out" of the interior of the DNA helix. This mechanism may be similar to that of a methyltransferase and two uracil-DNA glycosylases for which the crystallographic structure has recently been reported (*16–18*).

There is one fascinating aspect of this study that should develop into a significant contribution to plant molecular biology. The blue light photoreceptor of plants, the essential light detector for the early development of plants from seed before plant pigmentation occurs, has amino acid sequence homology with the *E. coli* photolyase protein (*19*). Furthermore, the cofactors found in this blue light photoreceptor are FAD and MTHF (*20*). We must now assume that the crystal structure of *E. coli* photolyase will show similarity not only to other photolyases but also to the blue light photoreceptors of plants. This rather significant class of proteins is responsible not only for protecting biological organisms from the damaging effects of UV light (and the ozone hole), but also for providing an early developmental signal in green plants that uses a completely unknown and novel mechanism based on redox chemistry.

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