The epithelial cells of the small intestine migrate from the deep valleys of the crypts to the villous tips and, although rotavirus damage is most evident in the villous tips, IgA is secreted most efficiently from the crypt cells. Where do antibody and virus meet? We do not yet know. These new results challenge our traditional thinking about the mechanism of mucosal protection, the site of virus neutralization, and the key antigens to which protective antibodies are directed.

Rotavirus vaccines nearing licensure could provide an important new weapon to decrease diarrhea morbidity and mortality in both developed and developing countries. If these were administered together with the other routine childhood vaccines, they could rapidly reach nearly 80% of the world's 130 million newborns who are already covered by the expanded program for childhood immunizations (*14*). The impact—a decrease in diarrheal hospitalizations and deaths—could be measurable almost immediately.

In spite of this optimism, the vaccines nearest licensure do not prevent all episodes of severe disease in American children, so further improvements will likely be needed. Many new approaches to immunization—the use of viruslike particles, DNA vaccines, microencapsulated viruses, and other live strains for oral delivery—are all being pursued. Each approach will benefit from a more detailed understanding of the mechanisms of pathogenesis and immunity to disease. The issues raised in the two new papers will help redefine targets for the next generation of rotavirus vaccines and give pause to reconsider basic principles in the prevention of other enteric and respiratory infections where local immunity may be at play and where the mechanisms of pathogenesis remain unclear.

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No "End of History" for Photolyases

Aziz Sancar

After the fall of communism in 1989, Francis Fukuyama wrote a best seller titled *The End of History and the Last Man* (*1*). He argued that the absence of competing ideologies in the world would result in no more large-scale conflicts, the stuff of which history is made. Unfortunately, events since publication of the book have proven that humans are capable of conflict even in the absence of the Cold War. Hence, there is still history to be written.

A similar situation has occurred in the scientific subspecialty of photoreactivation research. Photoreactivation reverses the effect of ultraviolet (UV) light when the organism is either concomitantly or subsequently exposed to blue light. In DNA UV light induces two major types of damage—cyclobutane pyrimidine dimers (CPDs) and (6-4)photoproducts. In 1958 an enzyme was discovered that repaired UV-damaged DNA. Later work revealed that the enzyme, called photoreactivating enzyme or photolyase, binds to UV-damaged DNA and on absorbing a blue-light photon (of wavelength 350 to 450 nm) splits the cyclobutane ring of a CPD, restoring the bases to their native form (*2*). The gene for photolyase was later cloned from several organisms (*3*), and the enzyme was purified and extensively characterized (*4*).

The reaction mechanism of CPD photolyase has been elucidated in considerable detail (see figure). The enzyme is a 50- to 65-kD polypeptide with two chromophores. One of the chromophores [which can be either methenyltetrahydrofolate (MTHF) or deazariboflavin] is the photoantenna that absorbs the blue-light photon (step 1) and transfers excitation energy (step 2) to the active site cofactor, which is invariably two-electron–reduced flavin-adenine dinucleotide ($FADH^-$). Flavin in the excited state then donates an electron to the CPD (step 3), splitting the cyclobutane ring, and the electron is transferred back to flavin concomitant with the generation of the two canonical bases (step 4). The general outline of this mechanism was worked out in 1987 and was considered by some to be "the end of history" for photolyase. In the Keystone meetings of 1988 and 1995 on DNA repair there were no presentations on DNA photolyase. Similarly, for many repair scientists, the recent solution of the crystal structure of DNA photolyase from *Escherichia coli* (*5*) was considered an epilogue to a story completed long ago. However, as with sociopolitical history, events that have unfolded during the last 3 years have revealed that there are many more chapters to write on photolyase.

The first unexpected and exciting discovery in the "New Age" history of photolyase was the detection of a photolyase specific for (6-4)photoproduct [(6-4)photolyase] in *Drosophila* cell-free extracts (*6*). The classic photolyase—now referred to as CPD photolyase—cannot repair (6-4)photoproducts. Furthermore, it was thought that photochemical (photoenzymatic) reversal of (6-4)photoproducts was very unlikely for the following reason: The formation of (6-4)photoproducts involves the transfer of the group at the C-4 position (–NH or –OH) of the 3′ base of the dinucleotide to the C-5 position of the 5′ base concomitant with the formation of the σ bond between the C-6 of the 5′ base and the C-4 of the 3′ base (see figure). Even if an enzyme breaks the σ bond joining the two adjacent pyrimidines (as occurs with CPD photolyase), the bases would not be restored to their original forms. However, Todo *et al.* (*6*) previously demonstrated that the (6-4)photolyase restores biological activity to UV-irradiated DNA, and Kim *et al.* (*7*) obtained direct evidence that the (6-4)photolyase converted this photoproduct to unmodified bases. Kim *et al.* (*7*) also proposed a chemical mechanism that involves an oxetane intermediate and an electron transfer reaction for repair by (6-4)photolyase. At the time there were no suspicions that the two enzymes are related, simply because CPD and the (6-4)photoproduct are structurally very dissimilar (*8*).

In contrast, two papers published shortly after the (6-4)photolyase paper revealed that

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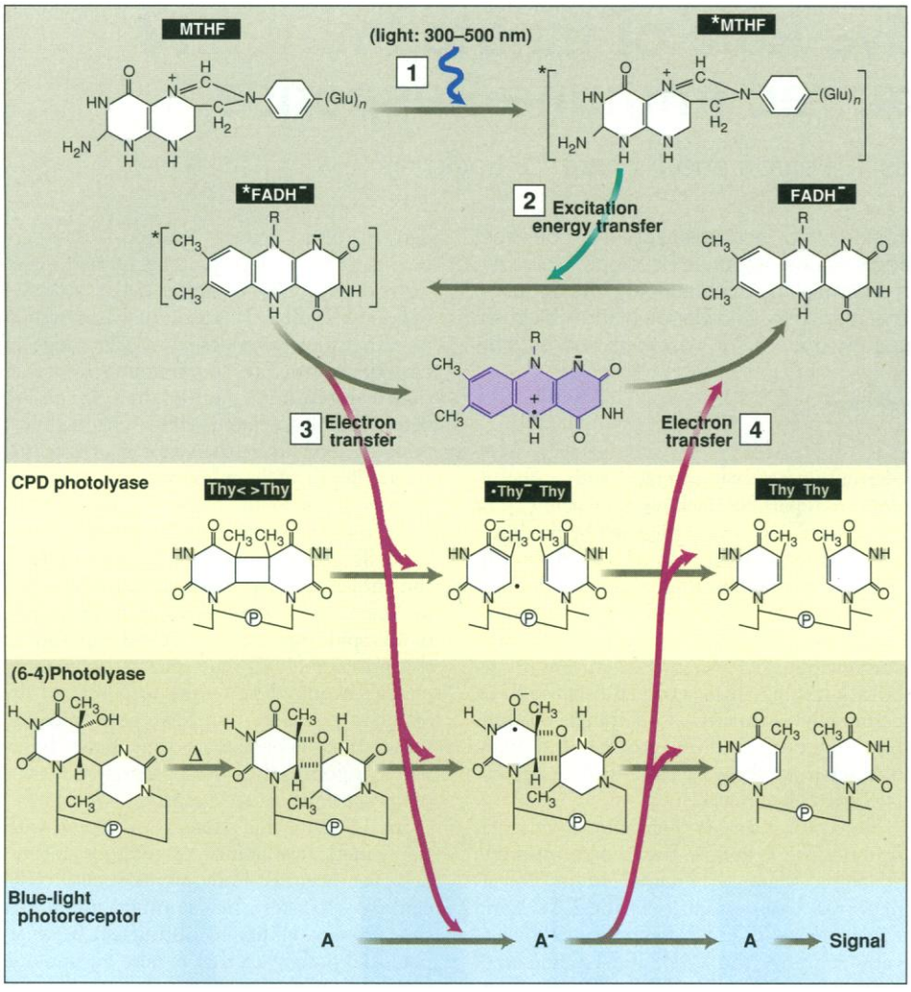

Reaction mechanisms of CPD photolyase, (6-4)photolyase, and the blue-light photoreceptor (cryptochrome). (**1**) A blue-light photon is absorbed by the MTHF photoantenna. (**2**) The excitation energy is transferred to the active site flavin. (**3**) The excited flavin donates an electron to a CPD, a (6-4)photoproduct, or an unknown downstream target. (**4**) Electronic rearrangement restores the DNA bases to normal, and the electron is transferred back to the ground-state flavin neutral radical. The nature of the electron acceptor (A) for the blue-light photoreceptor is not known.

the apoproteins of the blue-light photoreceptors (cryptochrome) of *Arabidopsis thaliana* (*9*) and mustard (*10*) are very similar to microbial CPD photolyase and hence are structurally related, although the photoreceptors function in signal transduction not in DNA repair. Indeed, the purified photoreceptors contained the folate and flavin chromophores characteristic of folate class photolyases (*11*). These findings, in turn, suggested a previously unknown mechanism of signal transduction, that is, photoinduced electron transfer that does not involve a net redox reaction.

And now, Todo *et al.* (*12*) in a landmark paper in this issue of *Science* show that the three systems, the CPD photolyase, the (6-4)photolyase, and the plant blue-light photoreceptors are evolutionarily and perhaps mechanistically related. They cloned and sequenced the (6-4)photolyase gene of *Drosophila* and found that the photolyase polypeptide exhibits sequence similarity to CPD photolyases of microbial origin and to the blue-light photoreceptors. Indeed, the (6-4)photolyase of *Drosophila* [which has both CPD photolyase and (6-4)photolyase] is more similar to blue-light photoreceptors than to *Drosophila* CPD photolyase and other animal photolyases.

How widespread is the (6-4)photolyase and how does it function? Todo *et al.* (*12*) have identified a human homolog with an astonishing 40% sequence identity to the *Drosophila* (6-4)photolyase. Whether this protein functions as a photolyase or as a photoreceptor remains to be seen. The presence of (6-4)photolyase in *Xenopus laevis* and rattlesnake suggests that the enzyme might be widespread (*13*). The sequence of the *Drosophila* (6-4)photolyase indicates that it contains folate and flavin (*12*), and the fluorescence properties of purified *Xenopus* photolyase are consistent with the presence of these two chromophores (*13*). If the chromophore is truly folate-flavin, the most likely mechanism (*7*) is as follows: The enzyme binds DNA and thermally converts the (6-4)photoproduct to its oxetane intermediate (see figure, Δ), which is known to be unstable. Upon absorption of a blue-light photon (step 1), the photoantenna molecule is excited and transfers electronic energy to the active site flavin (step 2), which donates an electron to the oxetane intermediate (step 3); the resulting radical collapses to the two canonical pyrimidines, with concomitant back electron transfer (step 4). Indeed, recent studies with model systems have provided experimental support for this model. Prakash and Falvey (*14*) demonstrated that the oxetane adduct of 1,3-dimethylthymine with benzaldehyde could be converted to 1,3-dimethylthymine and the benzaldehyde adduct by photoinduced electron transfer.

If this analysis sounds like another proclamation of the end of history for photolyase, it should not. The mechanism of the (6-4)photolyase and its distribution in the biological world remain to be elucidated. The role of the human (6-4)photolyase homolog will be of great interest to scientists in both the DNA repair and signal transduction fields and the downstream target of the blue-light photoreceptor, when identified, may open up a new way of thinking about signal transduction.

References and Notes

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15. On 8 July 1990, B. A. Horwitz from the Israel Institute of Technology wrote me a letter stating that "It seems possible that cryptochrome has flavin and pterin chromophores..." which "raises the possibility that cryptochromes might share homology with DNA photolyase" and offered to collaborate with our group to search for the cryptochrome genes by using the *E. coli* photolyase gene as a probe. I sent him the photolyase clone but did not take him up on his offer to collaborate. I thought it was a far-out idea.