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Fowlpox Virus Encodes a Novel DNA Repair Enzyme, CPD-Photolyase, That Restores Infectivity of UV Light-Damaged Virus

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Fowlpox virus (FPV), a pathogen of poultry, can persist in desiccated scabs shed from infected hosts. Although the mechanisms which ensure virus survival are unknown, it is likely that some type of remedial action against environmentally induced damage is required. In this regard, we have identified an open reading frame (ORF) coding for a putative class II cyclobutane pyrimidine dimer (CPD)-photolyase in the genome of FPV. This enzyme repairs the UV light-induced formation of CPDs in DNA by using blue light as an energy source and thus could enhance the viability of FPV during its exposure to sunlight. Based on transcriptional analyses, the photolyase gene was found to be expressed late during the FPV replicative cycle. That the resultant protein retained DNA repair activity was demonstrated by the ability of the corresponding FPV ORF to complement functionally a photolyase-deficient Escherichia coli strain. Interestingly, insertional inactivation of the FPV photolyase gene did not impair the replication of such a genetically altered virus in cultured cells. However, greater sensitivity of this mutant than of the parental virus to UV light irradiation was evident when both were subsequently photoreactivated in the absence of host participation. Therefore, FPV appears to incorporate its photolyase into mature virions where the enzyme can promote their survival in the environment. Although expression of a homologous protein has been predicted for some chordopoxviruses, this report is the first to demonstrate that a poxvirus can utilize light to repair damage to its genome.

Among the environmental hazards encountered by living organisms is exposure to UV light, which can damage DNA. This induced formation of potentially lethal cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts can be reversed by a photoreactivation process (17) dependent on the presence of blue light (300 to 500 nm) (23, 38). Such repairs are performed by enzymes (photolyases) which bind to the dimers, capture the energy from photons via associated chromophores, and then use electron transfer to split the dimers and restore the DNA to its original form (39). Photolyases maintain specificity for only one type of potential substrates and are designated as such (39, 47). The CPD-photolyases can be further separated into class I and class II on the basis of divergent primary structures, the former being more closely related to the (6-4) photolyases (24). Since representatives of all three groups of repair enzymes have been found in each of the three domains of life (24), there is no correlation between evolutionary status and type of photolyase.

For most DNA-containing viruses, the absence of associated photolyase genes precludes their ability to utilize sunlight for repairing UV light-induced genomic damage. Therefore, any DNA repair dependent on photoreactivation is conditional on successful infection of a host which can provide the necessary enzyme. Indeed, it is well documented that through this process the viability of UV-irradiated viruses can be restored (19). Moreover, the photoreactive ability of marine bacteria has been implicated as the reason for high concentrations of infectious phage in surface water despite the unmitigated exposure to solar radiation (50). Although some viruses such as bacteriophage T4 (34) and possibly the Chlorella virus PBCV-1 (30) encode enzymes which are involved in the excision repair of pyrimidine dimers resulting from UV light irradiation, these proteins would be active only when the virus is internalized in a bacterium and an alga, respectively. In contrast, at least some poxviruses may have circumvented the direct requirement for host intervention. Determination of the nucleotide sequences of the complete genomes of the Melanoplus sanguinipes entomopox virus (2), the leporipoxviruses myxoma virus (11) and Shope fibroma virus (52), and the avipoxvirus fowlpox virus (3) has identified a putative class II CPD-photolyase gene in the DNA of each. Since the encoded enzyme utilizes photons in lieu of physiologically renewed compounds as an energy source and does not require a supply of nucleotides for restoration of damaged DNA, the potential for autonomous genomic repair by extracellular pox virions exists.

In view of the complexity of poxvirus genomes, it is not surprising that these highly successful pathogens (10) have acquired the ability to protect themselves from the perils of their immediate surroundings. Clearly, their production of proteins which mimic host immunoregulators can be construed as being directed toward the provision of a habitat favorable for replication (28). Likewise, their mass accumulation into inclusion bodies may offer some resiliency to environmental insults (8). Moreover, the capture and retention of a gene encoding a light-activated DNA repair enzyme could be indicative of genetic plasticity which would enable poxviruses to adjust to an otherwise hostile environment.

The stability of desiccated poxviruses has proven invaluable
for purposes such as the mass vaccination against smallpox of people in underdeveloped countries. However, this property can also be problematic when the virus is present in naturally formed scabs. For example, the avian virus FPV can persist in the dander shed from infected poultry and may later infect naïve birds, especially those in multiple-aged flocks, to produce an outbreak of fowlpox (48). Since the released virus is exposed to a variety of wavelengths of light, an FPV-encoded photolyase could contribute to the observed persistence. To address this possibility, we characterized a class II CPD-photolyase open reading frame (ORF) discovered during sequencing of the genome of an FPV field strain. In addition to demonstrating that the gene product was indeed an active enzyme, we also created a photolyase-deficient FPV and found that this mutant, unlike the parental virus, was unresponsive to photoreactivation.

MATERIALS AND METHODS

Virus and cells. An FPV isolate originating from a fowlpox outbreak in poultry at the University of Illinois (48) was used in this study. Initially this virus was propagated in the chorioallantoic membranes of developing chicken embryos obtained from a specific-pathogen-free flock. For virus purification, pock lesions on the eggs were collected. The infected allantoic fluid was clarified by centrifugation (500 g for 10 min). After additional grinding and clarification, virus was pelleted from the supernatant (53,000 g for 30 min). The suspended pellet was layered onto a discontinuous gradient consisting of layers of 14, 17, 20, 25, 30, and 40% sucrose in Tris-buffered saline (50 mM Tris [pH 8.0], 150 mM NaCl, 5 mM EDTA) and then centrifuged in a Beckman SW28 rotor at 4°C and 53,000 g. The virus pellet was resuspended in Tris-buffered saline and stored at −80°C.

Later, the FPV isolate was propagated in monolayers of the quail fibroblast cell line QT-35 as previously described (42). The virus was continuously exposed to cytosine arabinoside (40 μM/mL; Sigma, St. Louis, Mo.) starting at 1 h prior to virus infection. At 8 h postinfection, total RNA isolated from virus-infected cells in the presence and absence of cytosine arabinoside by using Trizol (Life Technologies) according to the manufacturer’s protocol.

RNA preparations were screened for the presence of FPV photolyase gene-specific transcripts by RT-PCR. Briefly, 1 μg of RNA and 0.05 μM reverse primer PRE were denatured at 70°C for 10 min and then placed on ice. First-strand cDNA synthesis using Superscript II reverse transcriptase (Life Technologies) then proceeded for 1 h at 42°C as instructed by the manufacturer and was terminated by incubation at 70°C for 15 min. PCR was performed in a 50-μl volume containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 200 μM each deoxynucleoside triphosphate, 0.2 μM primers 132.F and PRE, 0.25 U of Taq polymerase, and 5 μl of completed RT reaction mixture. Conditions for this PCR consisted of one cycle of denaturation at 94°C for 2 min, 35 cycles of denaturation at 92°C for 30 s, annealing at 45°C for 30 s, and extension at 72°C for 2 min, and a final cycle of elongation at 72°C for 10 min.

Overall, FPV thymidine kinase and A-type inclusion body protein gene RNAs were revesed transcribed as described above except that primer TK2 and 129.R2, respectively, replaced primer PRE. Likewise, thymidine kinase or A-type inclusion body protein gene transcript-specific primers, TK1 and TK2 or 129.F2 and 129.R2, respectively, were used in PCR. Although the composition of the PCR mixture remained constant, the conditions were altered to one cycle of denaturation at 94°C for 2 min, 35 cycles of denaturation at 92°C for 30 s, annealing at 45°C for 30 s, and extension at 72°C for 30 s, and a final cycle of elongation at 72°C for 5 min.

To evaluate amplification of transcripts reading through to the photolyase gene, RT was performed as described above except that (i) random hexamers (0.05 μM; Life Technologies) were used as primers and (ii) the reaction conditions were altered to include an initial primer annealing step at 25°C for 10 min. PCR using primers PT.F and PT.R was performed as described for amplification of the thymidine kinase and A-type inclusion body protein gene cDNAs.

Phenotypic complementation of photolyase-deficient E. coli. To generate an intact FPV photolyase gene flanked by EcoRI recognition sites, plasmid pPHF1 was used as the template in a PCR together with primers PFE and PRE. Amplification conditions were similar to those using the photolyase gene cDNA as the template except that the length of the initiation denaturation was reduced to 10 s and the number of repetitive cycles was decreased to 30. The EcoRI-digested amplicon was ligated into the corresponding site of plasmid pBAD 24 (21). In this plasmid there is a Shine-Dalgarno bacterial ribosome binding site positioned immediately upstream of its EcoRI recognition site. Further upstream is the E. coli arabinose promoter, which regulates expression of an inserted gene and whose activity is repressed in the presence of glucose and induced in the presence of arabinose.

Recombinein plasmid pPHF1, containing the correctly oriented photolyase gene, was used to transform the photolyase-deficient E. coli strain CSK 603 (reca1 wca64 phr-1; provided by the Escherichia coli Genetic Stock Center, Yale University). UV sensitivity and capacity of photoreactivation by the trans-
formed bacteria were assayed in the following manner. Individual colonies of both the parental bacterium and one harboring plasmid pHRI1 were separately grown to mid-log phase in Luria broth (LB) supplemented with 0.2% glucose. At this point, aliquots of the bacterial cultures were pelleted at 450 x g and 4°C for 10 min and resuspended to their original volume in LB containing either 0.2% arabinose or glucose. After incubation for 3 h at 37°C, each culture was diluted 100-fold in LB and then exposed to UV light (0.0, 0.1, 0.2, 0.3, and 0.4 J/m²) at 254 nm in a Spectrolite EF-16 shortwave UV lamp (Spectronics Corp., Westbury, N.Y.). Cultures were then diluted an additional 100-fold in LB, and equal aliquots were kept either in darkness or exposed to Plexiglas-filtered white light for 0 h at ambient conditions. The number of survivors in each situation was based on the CFU obtained after incubation of diluted samples on LB (with 100 µg of ampicillin/ml) agar plates in darkness at 16 h at 37°C.

Generation of photolyase-deficient FPV. The insertion vector pPHEL1 was generated by ligating the vaccinia virus P11 promoter-lacZ gene fusion, released from pVBX5 (41) by XbaI digestion, with Xbal-digested pSHPEP1. In pPHEL1, the lacZ gene is transcribed in the same direction as the photolyase gene and nearly bisects this FPV ORF. Plasmid pPHEL1 was maintained in E. coli DH5α and purified on an anion-exchange resin column (Qiagen, Valencia, Calif.) prior to use in transfection with FPV.

To produce recombinant virus, approximately 10° CT-35 cells in one well of a six-well cluster plate (Costar, Cambridge, Mass.) were infected with FPV at a multiplicity of infection of 5. At 6 h postinfection, the monolayer was overlaid with 2.5 ml of growth medium (42) lacking serum but containing 2 µg of pPHEL1 and 12 µl of Lipofectamine reagent (Life Technologies). After an additional 18 h, an equivalent amount of medium supplemented with 4% serum was added and the infection was allowed to proceed for 48 h more. At this time, cytopathic effect was nearly complete and the cells were harvested and stored at −80°C. Recombinant viruses were then identified and plaque purified six times on the basis of the ability to produce β-galactosidase (lacZ gene product) and thus hydrolyze the substrate Bluo-gal (Life Technologies) as previously described (41, 49). Purity and genotype of the recombinant virus was verified by using primers PRF and PRE to amplify the region flanking the Z gene insertion site in conjunction with Elongase enzyme (Life Technologies). In an attempt to detect any ORF sequence to the PIR database indicated that it represented the carboxyl half of a class II CPD-photolyase. In an attempt to obtain the intact gene, a radioactive probe representing the known portion was used in Southern hybridization with restriction endonuclease-digested FPV DNA. Partial sequencing (1.52 kb) of a identified 3.5-kb EcoRI-PvuII fragment enabled the remainder of the photolyase gene to be determined. The entire ORF plus its putative promoter was later found to be identical to that recently reported for an FPV (3) derived from a vaccine manufactured in the early 1960s (fowlpox challenge virus disclosure; Animal and Plant Health Inspection Service Center for Veterinary Biologics, Ames, Iowa). Interestingly, comparison to a cited nucleotide sequence derived from the genome of an FPV of unknown origin (36) revealed one discontinuous and two adjacent nucleotide mismatches. These alterations resulted in the nonconservative replacements of a glutamic acid and alanine moiety in the photolyase of the FPV field isolate by an alanine and valine, respectively.

The FPV-encoded photolyase is predicted to be 464 amino acids in length and to have a molecular mass of approximately 54.5 kDa, physical attributes similar to those of other photolyases. The primary structure of this virus protein is most similar (55% identity, 72% similarity) to that of the South American opossum photolyase (53) but is also well conserved (53 to 54% identity, 68 to 70% similarity) in other poxvirus-encoded photolyases. Regions of homology between these proteins are interspersed throughout the molecules and are especially prevalent in their carboxyl halves, where the class II DNA photolyase Prosite sequences (PS01083 and PS01084) are located (Fig. 1). As previously noted for the FPV enzyme (3), there is a conservative replacement of glutamic acid by aspartic acid at the third position of the first Prosite sequence. This substitution has also occurred in both leporipoxvirus photolyases. The only other deviation from the first Prosite sequence is at the ninth position of the insectpox virus protein, where there is a conservative replacement of arginine by lysine. As for the second consensus motif, the only divergence is at the last position, where a nonconservative and a semiconservative replacement of asparagine by lysine (leporipoxviruses) and serine (insectpox virus), respectively, is evident.

Since photolyase is apparently not produced by orthopoxviruses due to the apparent absence of the corresponding gene (20, 32, 44), it was of interest to determine the evolutionary relationship of the known poxvirus photolyases to each other and representatives from the three domains of life. A phylogenetic comparison of class II photolyases suggested that the FPV enzyme is most closely related to those of the leporipoxviruses (Shope fibroma virus and myxoma virus) (Fig. 2). Moreover, the inclusion of the insectpox virus (M. sanguinipes) counterpart within this clade is indicative of a common progenitor. Furthermore, the poxvirus photolyases appear to share a common ancestry with those of eukaryotes, and this group as a whole seems to have diverged from the eubacteria and the archaea bacteria as suggested by Kanai et al. (24) and also from plants.

**RESULTS**

**Identification of a photolyase gene in the FPV genome.** Based on sequence analysis of one of the terminal regions of an approximately 16-kb HindIII fragment of the genome of an FPV field strain isolated in the early 1970s (48), an incomplete ORF was detected. Comparison of its predicted amino acid sequence to the PIR database indicated that it represented the carboxyl half of a class II CPD-photolyase. In an attempt to obtain the intact gene, a radioactive probe representing the known portion was used in Southern hybridization with restriction endonuclease-digested FPV DNA. Partial sequencing (1.52 kb) of a identified 3.5-kb EcoRI-PvuII fragment enabled the remainder of the photolyase gene to be determined. The entire ORF plus its putative promoter was later found to be identical to that recently reported for an FPV (3) derived from a vaccine manufactured in the early 1960s (fowlpox challenge virus disclosure; Animal and Plant Health Inspection Service Center for Veterinary Biologics, Ames, Iowa). Interestingly, comparison to a cited nucleotide sequence derived from the genome of an FPV of unknown origin (36) revealed one discontinuous and two adjacent nucleotide mismatches. These alterations resulted in the nonconservative replacements of a glutamic acid and alanine moiety in the photolyase of the FPV field isolate by an alanine and valine, respectively.

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**Temporal expression of the FPV photolyase.** For predicting the temporal expression of poxvirus genes, consensus sequences representing portions of natural and/or mutated vaccinia virus early, intermediate, and late promoters have been described (5, 14, 15, 22, 37). Unfortunately, the region immediately upstream of and including the initiation codon of the FPV photolyase gene (ATATTGAATCTATATTTTGAATATAAAAACTG) does not conform to any of these. There is a somewhat A-rich stretch reminiscent of the early promoter consensus sequence (AAAGAGTAAAAAAAT) A, but its location would require transcription to initiate
within the promoter instead of at least the usual 10 to 20 nucleotides further downstream. Moreover, the only proximal early transcriptional termination signal (TTTTTNT) (54) is located within the FPV photolyase gene at a site 454 nucleotides upstream from its termination codon. Thus, it would appear that early production of photolyase is precluded, although a T5NT motif is present at unlikely sites within some apparently early and intermediate Shope fibroma virus genes (52). Since the consensus intermediate promoter AAANAA N_11–13TAAA and late promoter TAAAT(G) sequences are also absent, it is likely that the FPV photolyase is not strongly expressed.

To determine whether and presumably when the FPV photolyase gene is transcribed, RNA isolated from QT-35 cells infected with FPV in the presence and absence of cytosine arabinoside was subjected to RT-PCR. When primers capable of directing the specific amplification of a photolyase transcript were used, products were generated only with RNA produced...
observed when cDNA was substituted for the virus genome (Fig. 3B). This inadequacy could not be attributed to template preparation, as evidenced by successful amplification in the presence of the photolyase mRNA-specific primers. Therefore, authentic transcription of the FPV photolyase gene was being demonstrated.

FPV photolyase gene encodes a functionally active enzyme.

To examine whether the FPV photolyase is functional, its activity in the photolyase-deficient E. coli strain CSR-603 (40) was indirectly assessed. For this purpose, the corresponding viral gene was placed under the transcriptional regulation of the bacterial arabinose promoter located in plasmid pBAD24 (21), and the resulting recombinant plasmid pPHE1 was introduced into the E. coli mutant. Expression of the foreign gene was induced or repressed by growth of the transformed bacterium in the presence of arabinose or glucose, respectively (21). The relative photoreactive capability of repairing UV light-induced lethal damage to the bacterial genome was then determined by comparing the viability curves of the transformed bacteria exposed to various doses of UV light and then either kept in darkness or placed in filtered white light (photoreactivated). A similar inactivation of the transformed bacterium grown in the presence of glucose was observed regardless of subsequent treatment and was comparable to that of the induced but not photoreactivated strain (Fig. 4). Likewise, placement of the nontransformed parent strain in white light failed to reverse the lethal effect of UV light exposure. In contrast, the induced and photoreactivated transformed bacterium exhibited an increased (up to approximately 1,000-fold) resistance to UV light inactivation. Thus, it appeared that the FPV
enzyme could phenotypically complement the photolyase-negative \textit{E. coli}.

**FPV photolyase is nonessential for virus replication in tissue culture.** To resolve the essentiality of virus-encoded photolyase for virus survival, elimination of this enzymatic activity was attempted. Rather than deleting the photolyase gene from the FPV genome, the intention was to render this ORF non-functional by insertion inactivation. In this procedure, a plasmid (pPHL1) containing a transcriptional marker unit consisting of the vaccinia virus P11 promoter fused to the \textit{E. coli} \textit{lacZ} gene and flanked by portions of the FPV photolyase gene was generated and transfected into FPV-infected cells. As a result of recombination between the homologous nucleotide sequences in the plasmid and replicating virus genome, transcription of the photolyase gene is disrupted due to insertion of the foreign DNA. Progeny from such a transfection can then be screened for the presence of recombinants based on their ability to express the inserted gene.

Using the above-mentioned protocol, putative photolyase-deficient FPV were identified as blue plaques due to hydrolysis of the supplied Blue-gal substrate by the \textit{lacZ} gene product \(\beta\)-galactosidase. One isolate was plaque purified to homogeneity (only blue plaques were obtained in two consecutive rounds of purification), and its recombinant status was determined by using PCR to amplify the region flanking the insertion site. As anticipated, only an amplicon whose size was indicative of the insertion event was obtained (data not shown). The generation of a pure population of FPV having an inactivated photolyase gene indicated that its protein product was not required for virus replication in tissue culture. Furthermore, comparison of the growth kinetics of the recombinant and parental viruses (Fig. 5) demonstrated that the mutagenic event did not alter the rate of virus replication in an established quail cell line.

**FPV contains a virus-encoded photolyase.** Although no phenotypic differences between the recombinant and parental FPV were detected with regard to interactions with the cell host, the two viruses did differ in the ability to use white light to reverse the lethal effects of previous exposure to UV light (Fig. 6). Whereas the UV light inactivation curve of either type of virus in the absence of photoreactivation was similar to that of the photoreactivated recombinant FPV, subsequent expo-
sue of the unaltered, parental virus to white light resulted in an approximately 100-fold increase in survivability. Presumably, the observed enhanced resistance of photoreactivated, parental virus to UV light inactivation can be attributed to an active, virus-encoded photolyase. Since the infectivity of cell-free virus was assayed, it is likely that this enzyme is an integral part of mature virions.

**DISCUSSION**

Although poxviruses are known to incorporate enzymes such as DNA-dependent RNA polymerase (7, 44), poly(A) polymerase (33), and mRNA guanylyltransferase and mRNA methyltransferase (6, 31) into virions, similar retention of a DNA repair enzyme had not been previously shown. The requirement by extracellular FPV for white light to overcome the otherwise lethal effects of exposure to UV light clearly indicates that this avipoxvirus contains an active photolyase. Moreover, based on the required expression of the FPV photolyase gene for phenotypic rescue of transformed photolyase-deficient bacteria and the increased UV light sensitivity of virions whose genomes contained an insertional inactivated photolyase gene, this enzyme appears to be virus encoded. Although the presence of a host-provided photolyase in mature virions cannot be excluded, its protective contribution would be minimal, as evidenced by the similarity of the UV light inactivation curves of photoreactivated recombinant and nonphotoreactivated parental virus. However, by analogy to better-characterized class II photolyses (26, 27, 53), the complete FPV enzyme should have at least flavine adenine dinucleotide (FAD) as an active-site cofactor. Since FPV cannot independently synthesize this chromophore, it and possibly another used as a photantenna (24) would be of host origin. In any case, the ability of photolyase to utilize photons instead of a renewable source of energy such as phosphorylated compounds for the cleavage of CPD dimers makes it ideal for functioning in an otherwise inert virion.

Temporally, production of photolyase appears to occur late during the FPV infectious cycle, at a time relegated primarily to the synthesis of structural proteins. However, the region located immediately upstream of the FPV photolyase gene lacks the canonical TAAAT(G) sequence characteristic of poxvirus late promoters (15). In reconciling this anomaly, it should be realized that this motif has been associated with strong regulatory elements (22, 37). Natural alterations such as replacement of the T at the +5 position with an A do not appear to eliminate functionality, as evidenced by the vaccinia virus A2L gene promoter (51). Likewise, the same deliberate manipulation of this nucleotide in a strong promoter reduced transcriptional activity only by 48% (15). Since this substitution in the vaccinia virus 11-kDa gene promoter rendered it inactive, other features such as the composition of the region upstream of the promoter influence the level of its activity (15). In this regard, the FPV photolyase gene promoter has a stretch of six T residues near the beginning of an 85% AT-rich 20-nucleotide sequence which is separated by a 6-bp spacer region from its presumed TAAAA transcriptional start site. Despite such favorable physical conditions (15), this FPV promoter is apparently very weak, as evidenced by its relative strength being approximately 1,000-fold less than that of the strong vaccinia virus 11-kDa gene promoter (V. Srinivasan and D. N. Tripathy, unpublished data). Conceptually, this finding is not surprising. A low level of photolyase synthesis would be expected since only a few copies of an enzyme, compared to the multitude of a structural protein, would be required per virion.

Interestingly, the poxvirus late promoter signature sequence is also absent at the corresponding site in the photolyase gene promoters of an entomopox virus and two leporipoxviruses. For one of the leporipoxviruses, myxoma virus, photolyase was predicted to be expressed early on the basis of some similarity between its gene promoter and the consensus poxvirus early promoter (11). No such homology with early or even intermediate transcriptional regulatory regions was observed for the FPV counterpart. Since the photolyase gene is probably conserved in all poxviruses infecting invertebrates and lower vertebrates, it is likely that its relative time of expression and the mode of action of its product have also remained unchanged. Therefore, one would expect photolyase to be produced during the morphogenesis of poxvirus particles into which this enzyme is incorporated.

Within the Chordopoxvirinae subfamily (poxviruses infecting vertebrates) of the Poxviridae family, the presence of a gene predicted to encode a photolyase has been detected only in the DNAs of members of the genera Avipoxirus (3) and Leporipoxvirus (11, 52). Such an ORF was not found in the sequenced genomes of the human-specific poxviruses variola virus (32, 44) and molluscum contagiosum virus (43). Thus, based on the absence of this DNA repair mechanism in placental mammals (12, 25, 29), photoreactivity has become vestigial, initially for the host and then for the pathogen. Since the responsible enzyme is active in the internal organs of opossums and chickens (12) and presumably other vertebrates, its evolutionary loss may be the inconsequential result of its replacement by other, possibly more efficient DNA repair enzymes which function independently of light activation. In this regard, the E. coli photolyase seems to be bifunctional in that it can stimulate the nucleotide excision repair system in the absence of light (35). Likewise, yeast photolyase also binds to DNA damaged by agents besides UV light but may inhibit repair by rendering the lesions inaccessible to the excision enzymes (18). In the case of FPV, as evidenced by the generation of recombinants unable to produce photolyase, any secondary function(s) of photolyase either is not necessary for virus replication or is provided by another virus protein or a host substitute. In view of the apparent requirement of direct transmission of the human poxviruses compared to the possibility of indirect routes for other poxviruses, the primary purpose of photolyase is most likely stabilization of virions during exposure to vectors and the environment. In that case, the ability of leporipoxviruses to infect rabbits, a mammal apparently lacking a photolyase gene (25), would not be compromised.

Clearly, the pathogenicity and persistence of the photolyase-deficient virus in its natural host, the chicken, needs to be evaluated. Although attenuated FPV is currently used in vaccination programs, its stability in the scabs and dander of immunized poultry, and possibly insect vectors, could pose a threat to immunologically naive chickens. Perhaps continual exposure of photolyase-deficient FPV to either natural sunlight or artificial incandescent light would cause its inactivation at a greater rate than occurs for vaccine strains. Such a prop-
erny could be of value in designing a new generation of FPV to be used for immunization of poultry and perhaps mammalian species.

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