poly(A) addition sites, as occur in bovine prolactin mRNA (*26*).

Glyoxal-treated poly(A) RNA's from the tumor and from human pituitary glands were separated on an agarose gel, transferred to a nitrocellulose filter, and hybridized with ^{32}P-labeled plasmid pMS1. Two hybridizing species of about 1150 and 1300 bases were identified in the tumor poly(A) RNA, but only one species was observed in the pituitary poly(A) RNA (Fig. 2), even after different exposure times. It was not possible from the results of our experiments to determine whether the pituitary pro-OLMC mRNA corresponds to either of the two tumor species because of their similarity in size. These results are similar to those of Tsukada *et al.* (*13*) and demonstrate that another ACTH-producing thymic carcinoid tumor contains two proOLMC mRNA species. The additional tumor mRNA may be the product of a different proOLMC gene, or it may be a different product of the same gene because of incomplete or altered processing.

It is uncertain which of the two tumor proOLMC species we have cloned and what, if any, is the difference between it and the pituitary proOLMC mRNA. However, since the cloned sequence—including the 3′ nontranslated region, which is highly variable among species (*25, 27*)—is the same as the known genomic sequence, it is probable that the rest of this ectopic proOLMC mRNA is similar, if not identical, to the pituitary proOLMC mRNA.

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Formation of an Adenine-Thymine Photoadduct in the Deoxydinucleoside Monophosphate d(TpA) and in DNA

Abstract. *A photoadduct is formed between the adenine (A) and thymine (T) bases of the deoxydinucleoside monophosphate d(TpA) when it is irradiated at 254 nanometers in aqueous solution. Treatment of the photoadduct with acid converts it specifically into a fluorescent hydrolysis product, $C_7H_7N_3O$, incorporating the position-8 carbon of adenine and the methyl group of thymine. Isolation of the fluorescent hydrolysis product from acid hydrolyzates of oligo- and polydeoxyribonucleotides has shown that the photoadduct is formed by ultraviolet irradiation of d(pTpA), d(TpApT), d(TpApTpA), poly(dA-dT), and both single- and double-stranded DNA.*

The deleterious biological effects caused by exposure of living cells to ultraviolet (UV) light originate mainly from photochemical modifications to the cellular DNA molecules. Extensive studies have shown that the pyrimidine bases are the major targets of photochemical damage, and several photoproducts derived from thymine and cytosine have been identified in UV-irradiated DNA (*1–3*). The most abundant of these are pyrimidine photodimers, which can have lethal consequences (*2*), and bipyrimidine (6–4) photoadducts, which appear to act as mutagenic lesions (*4*). In contrast, no substantive evidence has hitherto been obtained for the formation of any photoproducts derived from adenine (*5*) or guanine when DNA is irradiated in vitro or in vivo. Consequently, it is generally assumed that the purines have comparatively little significance in photobiology (*1, 3*). We describe the formation of an adenine-thymine photoadduct in the deoxydinucleoside monophosphate d(TpA) (T, thymine; A, adenine) and present definitive evidence to show that it is also formed on UV irradiation of native and denatured DNA. To our knowledge, this represents the first reported occurrence of a photoreaction

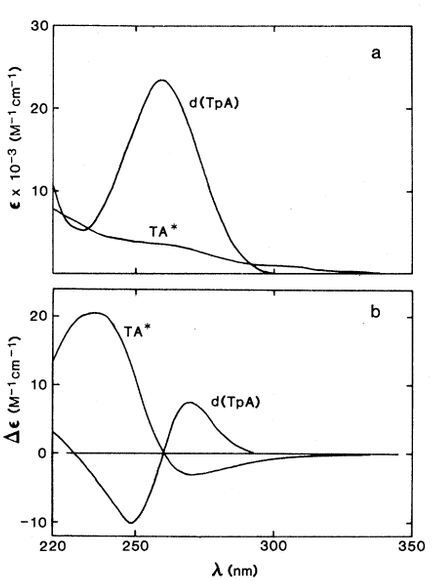

Fig. 1. Ultraviolet (a) and circular dichroism (b) spectra of d(TpA) and its photoproduct TA* at *p*H 7.0.

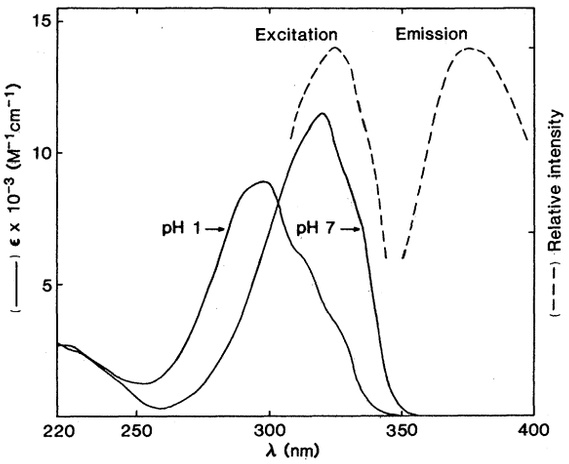

Fig. 2. Ultraviolet and fluorescence spectra of the fluorescent hydrolysis product, FHP, obtained from the d(TpA) photoproduct, TA*, on acid hydrolysis. (——) Ultraviolet spectra at *p*H 1 and *p*H 7.0. (------) Corrected fluorescence excitation and emission spectra at *p*H 7.0, recorded with a fluorescence spectrophotometer (Perkin-Elmer MPF-44B) with a differential corrected spectra attachment (DCSU-2). The excitation spectrum is based on the emission intensity at 375 nm. The excitation wavelength for the emission spectrum was 325 nm.

between a purine and a pyrimidine base in nucleic acids.

Irradiation of d(TpA) at 254 nm in neutral aqueous solution causes distinctive changes in its UV absorption owing to the formation of a photoproduct (TA*) with altered electrophoretic mobility (*6*). This sequence-specific photoreaction has a quantum yield of $\sim 7 \times 10^{-4}$ mole/E and does not occur with the sequence isomer d(ApT). We have isolated TA* by preparative paper chromatography (Whatman 3 MM; 1-propanol and water, 7;3; relative mobility, 0.26), purified it to electrophoretic and chromatographic homogeneity by elution with water from a column of Sephadex G-10, and investigated its physical and chemical properties.

The exact molecular mass of TA* was determined as 555.1462 from high-resolution mass spectra of its trimethylsilyl derivative ($M^+ = 915.3438$) and the corresponding $Si(CD_3)_3$ derivative (*7*). This value supports the composition $C_{20}H_{26}N_7O_{10}P$ (theoretical molecular mass, 555.1476), which is the same as that of d(TpA) and implies that TA* arises by an intramolecular rearrangement. The marked reduction in absorption intensity seen in the UV spectrum of TA* as compared with that of d(TpA) (Fig. 1) indicates that the conjugated chromophores of both the adenine and thymine bases have been radically altered. As is evident from their circular dichroism spectra (Fig. 1), the two compounds have quite different chiro-optical properties. Comparison of the 250 MHz proton magnetic resonance (^{1}H-NMR) spectra of d(TpA) and TA* in D_2O shows, in each case, a similar pattern of signals for the protons attached to the deoxyribose rings. However, the signals for the nonexchangeable base protons at δ 8.40, 8.14, and 7.34 in d(TpA) are replaced by singlets at δ 7.85, 7.29, and 5.03 in TA*. Furthermore, in the spectrum of TA*, the thymine methyl resonance at δ 1.58 is shifted 0.28 ppm to higher field than in d(TpA) and the splitting due to allylic coupling with H-6 is no longer discernible. The *pK* for protonation of TA*, determined from its electrophoretic mobility, is 5.5 compared with 3.5 for d(TpA). Taken together, the above findings imply that formation of TA* probably involves saturation of the 5,6 double bond of thymine by photoaddition across one of the double bonds in the six-membered ring of the adenine nucleus. Unlike pyrimidine photodimers, however, TA* is stable toward further irradiation at 254 nm.

The photoproduct TA* is unaffected by snake venom phosphodiesterase or spleen phosphodiesterase under conditions in which d(TpA) is completely digested by these enzymes. However, on treatment with 1*M* HCl at 100°C, for 4 hours, it is converted in ~ 50 percent yield into an intensely fluorescent hydrolysis product (FHP), and no trace of adenine or thymine is found in the hydrolyzate. Chromatographically and electrophoretically homogeneous FHP was isolated by preparative high-voltage paper electrophoresis at *p*H 2.5 (with a volatile ammonium formate buffer), followed by elution with water from a column of Sephadex G-10.

The molecular formula of FHP was established as $C_7H_7N_3O$ by mass spectrometry of underivatized and trimethylsilylated material. The compound has distinctive UV absorption characteristics (Fig. 2). A maximum at 322 nm at *p*H 7 shifts to 297 nm at *p*H 1; there is minimal absorption in the region of 250 nm. Spectrophotometric titrations indicated that FHP has a basic *pK* of 2.9 and an acidic *pK* of 10.3. On excitation at 325 nm FHP displays intense fluorescence emission, with a peak at 375 nm (Fig. 2), which permits its fluorimetric detection at nanomolar concentrations. The ^{1}H-NMR spectrum of FHP in D_2O shows two signals for aromatic protons at δ 7.86 and 7.74 and a signal for a methyl group at δ 2.07. The latter exhibits weak allylic coupling ($J \sim 1$ Hz) with the signal at δ 7.74 indicating that the molecule contains a CH_3–C=C–H fragment derived from thymine. The signal at δ 7.86 disappears when FHP is heated in D_2O. This exchange is characteristic of a proton attached to the central carbon of an imidazole ring function in purines and other compounds (*8*). By preparing FHP from appropriately radiolabeled samples of poly(dA-dT) (see below) we have shown that it incorporates the C-8 of adenine and the methyl group from thymine but not the C-2 of thymine. The fact that atoms from both adenine and thymine are present in the FHP molecule confirms that these bases must be covalently linked in the primary photoproduct TA*. Although the structure of FHP cannot be defined with certainty, the properties of FHP—and mechanistic considerations—are consistent with its being 1-methyl-1-deazapurin-2-one.

Owing to its characteristic fluorescence, very small amounts of FHP can be visualized on paper strips illuminated with UV light. This provides a sensitive method for detecting and measuring the formation of TA* in UV-irradiated oligo- and polydeoxyribonucleotides. The irradiated sample is hydrolyzed with HCl and then subjected to high voltage paper electrophoresis at *p*H 2.5. Any FHP detected on the electrophoretogram is eluted from the paper with water and quantified by spectrophotometry or spectrofluorimetry. Quantum yields for the formation of TA* can then be estimated either on the basis of FHP produced or, in appropriate cases, from the change in absorbance of the irradiated sample with UV fluence.

In this way, we have demonstrated that TA* is formed with a quantum yield of $\sim 5 \times 10^{-4}$ mole/E upon UV irradiation of the oligodeoxyribonucleotides d(pTpA), d(TpApT), and d(TpApTpA). It is formed with approximately the same quantum yield in single-stranded poly(dA-dT), but this is reduced to $\sim 1 \times 10^{-4}$ in double-stranded poly(dA-dT) · poly(dA-dT) indicating that the photoreaction is quenched by base pairing. Most significantly, TA* is formed in both denatured and native calf thymus DNA with respective quantum yields of $\sim 5 \times 10^{-5}$ and $\sim 1 \times 10^{-5}$ mole/E. The identity of the recovered FHP was confirmed in each case from its spectroscopic properties and its mobility on thin-layer chromatography in three solvent systems. In separate experiments,

we have isolated radioactive FHP from acid hydrolyzates of UV-irradiated *Escherichia coli* DNA labeled with [methyl-^{3}H]thymine.

The overall yield of TA* in a particular DNA will depend on how frequently T-A doublets occur within its sequence. It is evident that TA* does not constitute a major photoproduct in UV-irradiated DNA, since the quantum yield for its formation is only 10^{-2} to 10^{-3} times the quantum yields quoted for pyrimidine photodimerization or the formation of bipyrimidine (6–4) photoadducts (*1*). However, its biological significance will be determined not only by its abundance but also by its susceptibility to cellular DNA repair mechanisms and the functional importance of the target sites. In the latter respect, it is noteworthy that the sequence T-A-T-A is a conserved feature of many prokaryotic and eukaryotic promoters (*9*). Both of the tandem base substitutions observed in a recent study (*10*) of the specificity of UV mutagenesis in the *lac* promoter of M13-*lac* hybrid phage DNA were found to occur at T-A sites. Because T-A is a self-complementary sequence, the photoproduct TA* could be formed at the same site on both strands of a DNA duplex. Such an event would cause a permanent loss of genetic information unless biological reversal of the photoreaction could be accomplished.

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Gene Transfer by Polyoma-Like Particles Assembled in a Cell-Free System

Abstract. *Empty capsids of polyoma virus interact with DNA in a cell-free system to form polyoma-like particles (PLP). The DNA in these particles is protected from the action of pancreatic deoxyribonuclease. Transfer of genetic information by PLP has been accomplished by using a restriction fragment containing the transforming sequences of polyoma DNA as a model gene. Infection of rat F111 cells by PLP containing these sequences results in DNA-mediated cellular transformation. Gene transfer by PLP is 50 to 150 times more efficient than by the calcium phosphate precipitation method.*

Gene transfer studies are important in investigating fundamental questions of molecular biology, including the control of gene expression in mammalian cells. Genes have been introduced into cells by coprecipitation with calcium phosphate (*1, 2*), by microinjection (*3*), fusion (*4*), liposomes (*5*), the use of facilitating agents such as DEAE-dextran (*6*), and by electric pulses (*7*). Viral vectors, assembled intracellularly with the aid of coinfecting wild-type particles, have also been used (*8*). The most extensively applied method is calcium phosphate coprecipitation, but the efficiency of gene transfer by this method is close to the rate of spontaneous mutation (*9*), making it difficult in some cases to distinguish between revertant cells and cells that have acquired the exogenous genetic material.

The cell-free assembly of polyoma-like particles (PLP) from DNA and purified polyoma empty capsids has been described (*10, 11*). The DNA contained in these particles is approximately 1.2×10^6 daltons in size and is protected by the capsid from hydrolysis by pancreatic deoxyribonuclease. The particles are stable in solutions of high salt concentration. The formation of PLP is independent of the primary, secondary, or superhelical tertiary structure of the DNA substrate. Purified DNA, from a variety of cellular and viral sources, has been used in the preparation of PLP. A model for the mechanism by which empty capsids and DNA interact to form PLP has been proposed (*11*).

Whether PLP can transfer genetic information to cells has been investigated by using, as a model gene, a restriction fragment of polyoma DNA that has measurable biological activity. This fragment is approximately 1.2×10^6 daltons in size and extends clockwise from the Bcl I site to the Eco RI site on the conventional polyoma map (Fig. 1). It is the smallest of several restriction fragments that contain the genetic information necessary for the induction and maintenance of transformation (*12*) in rat cells (*13–15*). The rest of the polyoma genome, which includes the distal part of the early region and extends clockwise from the Eco RI site to the Bcl I site, has no oncogenic activity (*13*). These two fragments of DNA will be referred to as the Bcl I–Eco RI transforming fragment and the Eco RI–Bcl I nontransforming fragment of polyoma DNA, respectively.

Rat embryo F111 cells were infected with PLP containing either the Bcl I–Eco RI transforming fragment or, as a control, the Eco RI–Bcl I nontransforming fragment of polyoma DNA (Fig. 2). The production of dense foci was used as an indication of oncogenic transformation and, therefore, of gene transfer and expression. Dense foci were found after

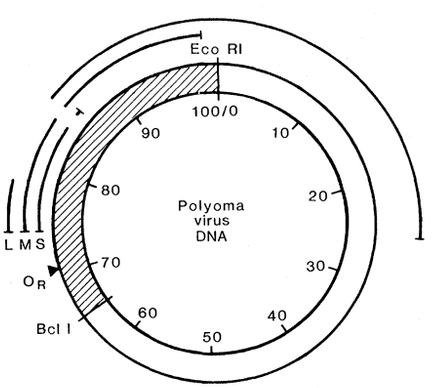

Fig. 1. Physical map of the polyoma virus genome divided into 100 units. The origin of DNA replication (O_R) and the coding locations of the early viral proteins small (*S*), middle (*M*), and large (*L*) tumor antigens are indicated. The fragment of DNA extending clockwise from the Bcl I site to the Eco RI site (shaded area) contains the coding sequences of the small and the middle tumor antigens and is sufficient for the induction of the transformed phenotype (*13*). The other fragment, which extends clockwise from the Eco RI site to the Bcl I site, has no oncogenic activity (*13*). These two fragments of DNA were generated by treating ^{3}H-labeled polyoma DNA with Bcl I and Eco RI (New England BioLabs) followed by electrophoretic separation on a 1.5 percent agarose gel (*11*). Each DNA fragment was purified from agarose with two rounds of KI density gradient centrifugation (*18*) or by electroelution into a dialysis bag.