DNA, Sunlight, and Skin Cancer

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Approximately one out of every 10 Americans will contract skin cancer during his or her lifetime, making skin cancer the most prevalent form of cancer in this country. Fortunately, skin cancer is rarely fatal because it is so readily detected and treated. The connection between sunlight and skin cancer comes from a number of important observations. First, skin cancer occurs most frequently on portions of the body normally exposed to sunlight and most frequently in fair-skinned people who lack the light-absorbing pigment melanin. Second, it is known that exposure of DNA to sunlight leads to the formation of photoproducts, otherwise known as photolesions. Third, people with the DNA repair disorder *xeroderma pigmentosum* have an approximately thousandfold higher chance of contracting skin cancer, thereby linking the failure to repair DNA damage induced by sunlight with skin cancer (Fig. 1). Presumably, misrepaired DNA damage results in mutations, and it is mutations in genes coding for proteins involved in the regulation of replication and cell differentiation that lead to the cancerous state. Other mutations lead to cell death or benign mutants and tumors.

Recently, it has been shown that the incidence of malignant melanoma and squamous cell carcinoma, the most serious forms of skin cancer, have been steadily increasing over the past 25 years (*1*) leading some to predict that by the year 2000, one out of 90 Americans will contract malignant melanoma. The origin of this increase is unknown but may be due in part to increased exposure to sunlight, through the popular pastime of sun tanning, coupled with the decrease in the amount of stratospheric ozone, which absorbs the wavelengths in sunlight most damaging to DNA. (Its absorption spectrum is almost identical with that of DNA!) The risk of contracting skin cancer can be sizably reduced, however, by limiting one's direct exposure to sunlight or by the use of sunscreens.

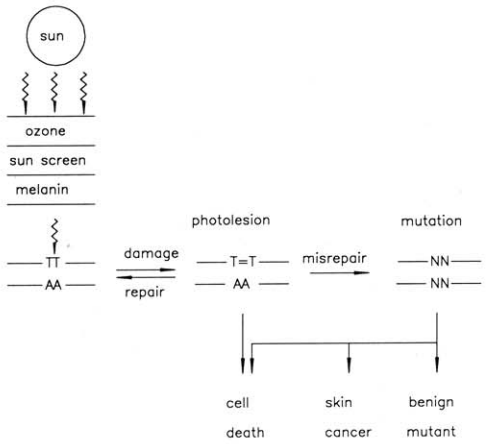

Figure 1. General pathways linking failure to repair DNA damage induced by sunlight with cell death, mutation and cancer. T = T represents any one of the several photoproducts of a TpT site shown in Figure 5.

DNA Photoproduct Structure–Activity Relationships

Though the structures of many DNA photoproducts are known, their lethality, mutagenicity, and mutation spectra are not. Almost all studies in the area of light-induced mutagenesis have relied on directly photolyzed, high molecular weight DNA for study. Directly photolyzed DNA contains a multitude of photoproducts at a multitude of sites, making elucidation of precise photoproduct structure–activity relationships almost impossible. The lack of progress in deriving structure–activity relationships can therefore be directly attributed to a lack of pure, well-characterized, photoproduct-containing DNA for study.

Our approach to obtaining precise photoproduct structure–activity relationships is diagrammed in Figure 2 and is based on the ability to prepare and study DNA containing site-specific photoproducts. The basic elements of this approach were pioneered by John Essigmann of the Massachusetts Institute of Technology for the study of the mutagenic effects of O6-methylation of guanine (*2*). The key intermediate used for the preparation of the wide variety of photoproduct-containing substrates is an oligonucleotide containing a site-specific photoproduct. The most general and versatile method for the preparation of such an oligonucleotide uses a photoproduct "building block" that is compatible with standard automated DNA synthesis. Such a method allows the site-specific incorporation of a photoproduct into any sequence, any number of times, and insures that the photoproduct-containing oligonucleotide will be obtained in both high yield and purity. Once in hand, the photoproduct-containing oligonucleotides can be annealed to complementary strands to form mini-B DNA duplexes for high resolution structural studies by NMR and X-ray crystallography. The oligonucleotides can also be incorporated via DNA ligase into polymers for bending and other biophysical stud-

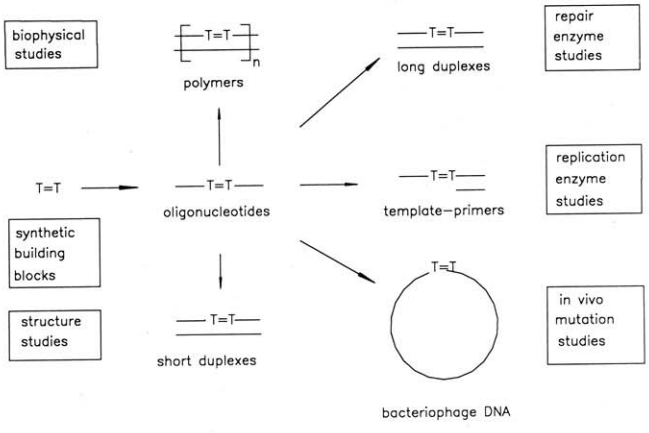

Figure 2. Our combined, chemical, physical, and biological approach to unraveling the structure–activity relationships in sunlight-induced skin cancer. T = T represents any one of the several of photoproducts of a TpT site shown in Figure 5.

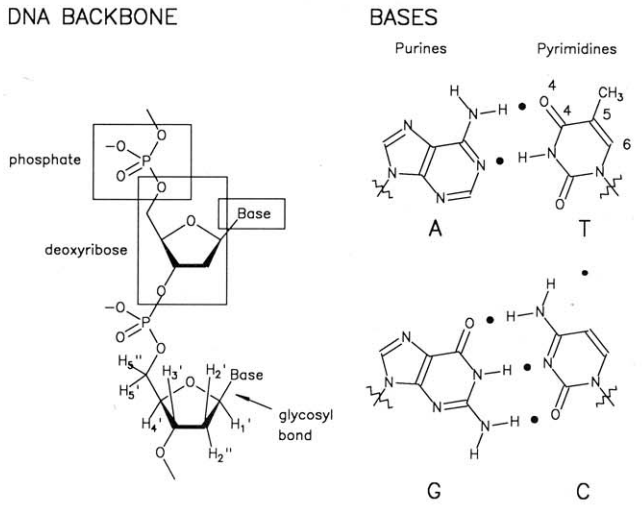

Figure 3. The basic structural subunits of DNA. Solid dots indicate hydrogen bonds.

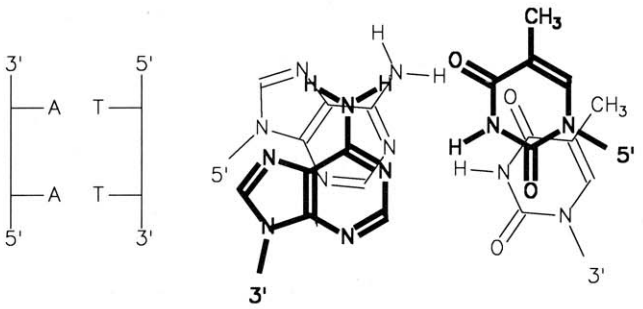

Figure 4. Conformation of the bases of a d(TpT)·d(ApA) site in B DNA as viewed down the helix axis, emphasizing the primarily intrastrand nature of the π-stacking interactions. The base pair on top is in bold.

ies, long duplexes for in vitro repair studies, template-primers for in vitro replication enzyme studies, and bacteriophage or viral DNA for in vivo mutation studies.

DNA Photochemistry

The photochemistry of DNA can be readily understood by considering the structure and conformation of B DNA and the wavelengths present in sunlight. DNA is a linear polymer of four nucleotide monophosphates, dAMP, dCMP, dGMP, and dTMP. Each nucleotide is composed of a base, which encodes information in a pattern of hydrogen bond donors and acceptors, and a sugar phosphate unit, which serves to link the bases together in a given sequence (Fig. 3). Sunlight at sea level is composed of wavelengths >290 nm, which just barely overlap the long wavelength absorption tail of the bases in DNA. In the simplest view, a base absorbs a photon and becomes excited to its first excited singlet state and then rapidly intersystem crosses to its first excited triplet state, which is lowest in energy for thymine. As a result, initial excitation energy becomes localized at a thymine by rapid energy transfer along the helix, and the excited thymine then either undergoes reaction with an adjacent base or relaxes to the ground state.

The structure and stereochemistry of the primary, and hence any secondary, photoproducts can be readily understood by consideration of the structure and conformation of the DNA from which they arise. In B DNA, an excited thymine is restricted to reacting with the base in the same chain which is either directly above or directly below. Most reactions occur at dipyrimidine sequences and can be best illustrated for a TpT site (Fig. 4). The major photochemical reactions at a TpT site involve [2 + 2] reactions between the C5,C6 double bond of the thymine on the 5′ side and either the C5,C6 double bond or the C4,O4 carbonyl double bond of the thymine on the 3′ side (Fig. 5). The first reaction leads to a cis–syn cyclobutane dimer, in which cis refers to the relative stereochemistry of the two methyl groups and syn to the relative orientation of the two C4 carbonyl groups. The second reaction leads to an intermediate oxetane, which is thermally unstable and rapidly decomposes to give what is known as the (6–4) product, so named because of the bond formed between C6 of the 5′-pyrimidine ring and C4 of the 3′-pyrimidinone ring. Cis–syn cyclobutane dimers are the major photoproducts of DNA and predominate at TpT sites, whereas (6–4) products are produced at about one-tenth the rate of cis–syn dimers and predominate at TpdC sites.

Though the cis–syn and (6–4) products are the major photoproducts of dipyrimidine sequences, minor amounts of a stereochemical isomer of the cis–syn product, the trans–syn product, are also produced. The trans–syn product of TpT results from photodimerization of two thymines in which one thymine is rotated by 180° about the N1–C4 axis relative to the other. Such a product could form in a section of B DNA in which the glycosyl bond of the 5′-T is in the syn, rather than the normal anti, conformation. Alternatively, the trans–syn product could form in regions of single-stranded DNA that are free to adopt many conformations or possibly at a junction between different helical conformations of DNA, such as B and Z DNA. Though the trans–syn product is a relatively minor product and may thus not appear worthy of great consideration, it is important to keep in mind that the mutagenicity of an agent may often be due to a minor, but highly mutagenic, product.

At the time we initiated our studies, the cis–syn, trans–syn, and (6–4) products were the only classes of dipyrimidine photoproducts known. The possible existence of another class of biologically relevant photolesions of dipyrimidine

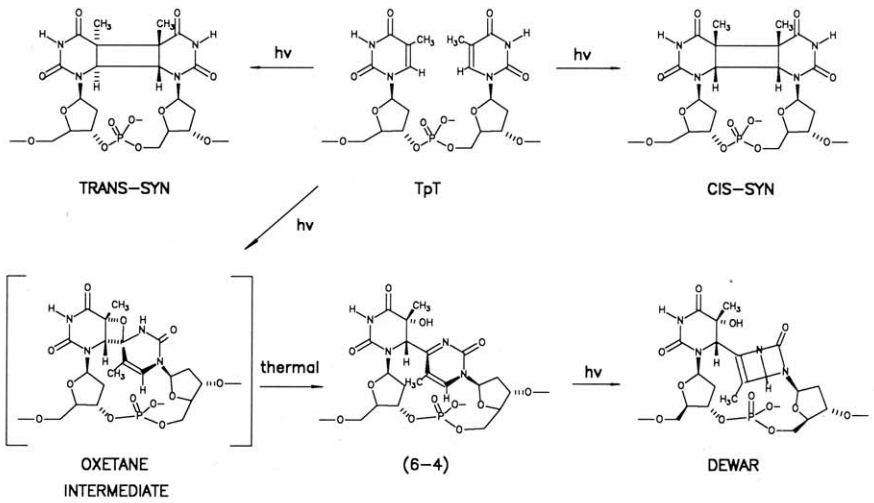

Figure 5. Reaction pathways available to a TpT unit exposed to sunlight (wavelengths >290 nm).

sites first became apparent from reports in the early 1970's of a curious type of photoreactivation (reversal) of the lethal effects of 254-nm light in certain bacteria. Type III photoreactivation, as it was called, was shown to be most efficient at 313 nm and to proceed through an unknown, though nonenzymatic, pathway that was correlated with the disappearance of (6–4) photoproducts. Interestingly, in 1964 Johns and co-workers had reported that the (6–4) photoproduct of TpT, known to them only as TpT4, could be quantitatively converted by 313-nm light to a new photoproduct, which they named TpT3.

Collectively, the results of the previous biological and photochemical studies suggested that the (6–4) products were being converted by 313-nm light to further, less lethal products, whose structures would be typified by that of TpT3. This prompted a determination of TpT3 structure, which was done by a combination of 1D and 2D NMR, IR, and FAB-MS (*3*, *4*). TpT3 was discovered to be the Dewar pyrimidinone valence isomer of the (6–4) product, and hence the name Dewar pyrimidinone, or more simply Dewar, was assigned to this class of photoproducts. The name Dewar comes from the name of the chemist who, in the late 1800's, proposed that benzene was such a valence isomer. Despite its apparent strain, the Dewar photoproduct is quite stable under physiological conditions, suggesting that it could persist for long times in vivo. Because the Dewar product is formed from the (6–4) product at wavelengths present in sunlight at sea level, it may well play a hitherto unrecognized, and possibly important, role in the mechanism of sunlight-induced skin cancer.

DNA Photoproduct Dimer Building Blocks

In order to determine the precise role that each photoproduct plays in mutagenesis there existed the need to develop building blocks for their site-specific incorporation into DNA by automated DNA synthesis. Initially the focus was on the design and synthesis of building blocks for the cis–syn and trans–syn thymine dimers because it was known that these classes of photoproducts could be prepared in high yield by triplet sensitized photolysis. These efforts led to the synthesis of building blocks for the cis–syn (*5*) and trans-syn (*6*) thymine dimers (Fig. 6). These building blocks are identical in structure to the corresponding dimers of TpT but have in addition: (1) a 3′-phosphoramidite group for coupling to the growing end of a DNA chain, (2) a methyl group for rendering the internucleotide phosphodiester neutral and the building block organic soluble, and (3) a 5′-DMT (dimethoxytrityl) group for preventing polymerization of the building block during the coupling step.

The sequence of steps used to incorporate the cis–syn and trans–syn thymine dimers into an oligonucleotide is illustrated in Figure 7 and is the same as that used in standard automated DNA synthesis machines. Automated DNA synthesis is based on sequential addition of suitably protected DNA building blocks to the 5′ terminus of a growing chain

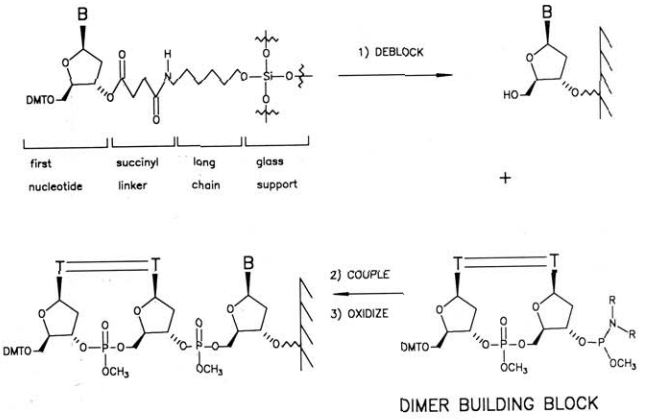

Figure 7. Basic sequence of steps used in automated solid-phase phosphoramidite-based DNA synthesis, illustrated for the addition of a photoproduct building block to a growing DNA chain.

whose 3′ terminus is covalently, but reversibly, linked to a solid support such as glass. Each cycle of addition involves three basic steps. In the first step, known as deblock, the dimethoxytrityl (DMT) group is removed from the 5′ terminus of the growing chain by dichloroacetic acid-catalyzed solvolysis. In the second step, known as couple, the DNA chain is elongated by tetrazole catalyzed phosphite ester bond formation between the 5′-hydroxyl and the phosphoramidite phosphorus. Typically, the phosphoramidite is used in a 10 to 100-fold excess to insure a high coupling yield. In the last step of the cycle, known as oxidize, the resulting phosphite triester is oxidized to a phosphate triester with iodine and water. For long sequences it is advantageous to prevent chains that failed to couple from undergoing coupling during subsequent cycles by blocking their 5′ ends with an acetyl group in a fourth step referred to as cap. Without capping, chains not elongated in one cycle might be elongated in a subsequent cycle, leading to oligonucleotides containing one less nucleotide than the desired product, making final purification difficult. After all of the nucleotides have been coupled, the product is first cleaved from the solid support with concentrated aqueous ammonia at room temperature. The phosphate and amino protecting groups are then removed by treatment with concentrated ammonia at 55 °C and the crude product purified by anion exchange chromatography.

NMR Studies on Mini B DNA Duplexes Containing Thymine Dimers

With the development of a method for synthesizing oligonucleotides containing site-specific cis–syn and trans–syn thymine dimers, it became possible to study their structure and properties. The most powerful approach available today for determining the three-dimensional structure of DNA in solution is to use interproton distance information derived from NMR experiments to constrain distance geometry and molecular dynamics calculations. In order to model best what happens to native DNA upon photo-product formation, a series of duplex DNA fragments were synthesized with and without a centrally located cis–syn or trans–syn thymine dimer. DNA fragments 10 nucleotides long (decamers) were chosen, as this was the length required to form a full turn of a B DNA helix. To this end, multimilligram amounts of the three duplex decamers shown below were prepared.

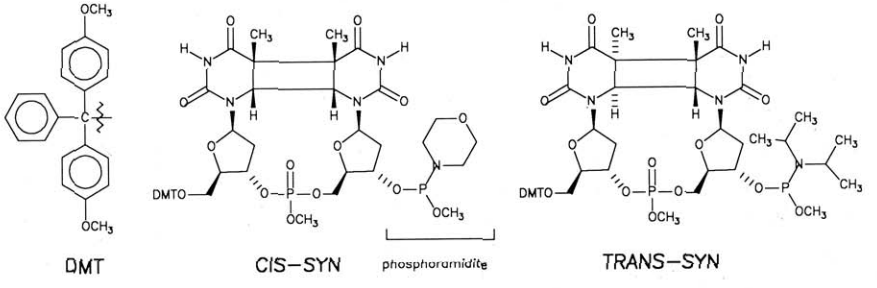

Figure 6. Cis–syn and trans–syn thymine dimer building blocks suitable for use in an automated solid-phase phosphoramidite-based DNA synthesis machine.

d(CGTATTATGC) d(CGTAT[c,s]TATGC)
d(GCATAATACG) d(GCATA——ATACG)

d(CGTAT[t,s]TATGC)
d(GCATA——ATACG)

The assignments of the H6/8, H5/CH_3, H1′, H2′, and H2″ proton NMR signals of each duplex (90 signals/duplex) were accomplished by use of standard sequential nuclear Overhauser enhancement (NOE) techniques (7). These assignment techniques are based on the fact that a proton can be induced to enhance measurably the signal intensity of protons within a 5-Å radius by way of the NOE effect. The assignment of proton signals is made by using the NOE effect to trace a path from proton signal to proton signal and by knowing what protons are expected to be within 5Å of each other in duplex DNA (Fig. 8). Two interesting observations emerged from comparison of the NMR data of the dimer-containing duplexes with that of the parent, undamaged, duplex. First, the trans–syn thymine dimer caused much larger changes in the proton chemical shifts than did the cis–syn thymine dimer, suggesting that the trans–syn dimer causes greater distortion of DNA structure. Second, many of the internucleotide NOE crosspeaks expected for a B DNA helix were not observed on the 5′ side of the trans–syn dimer, suggesting that the trans–syn dimer causes DNA to kink or dislocate at this site.

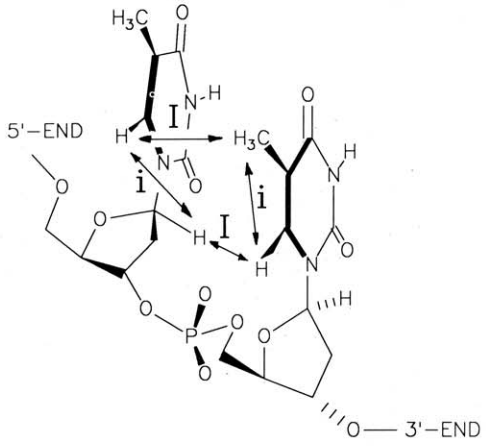

Figure 8. Selected NOE pathways expected for a TpT unit in a B conformation. *Intra*nucleotide NOE's are labeled **i** and *inter*nucleotide NOE's are labeled I.

In order to gain additional information about the effects of the thymine dimers on the structure and properties of DNA, the thermodynamic parameters for the helix ⇄ coil equilibrium (melting) of the three duplexes were determined by analysis of the temperature dependence of ^1H NMR and UV spectral data (7). Whereas the cis–syn dimer caused only a small decrease in the melting temperature and small changes in the thermodynamic parameters, the trans–syn dimer caused a dramatic decrease in the melting temperature and the magnitude of both ΔH and ΔS. These results can be readily understood by comparing the structures of the cis–syn and trans–syn thymine dimers to that of an undamaged TpT site (Fig. 9). The cis–syn dimer can be seen to retain the base-pairing properties of an undamaged TpT site and would therefore be expected to cause little disruption of the helix structure. Hence, only minor changes in the melting temperature and thermodynamic parameters were observed. The trans–syn dimer, on the other hand, has its 5′-thymine locked into a syn conformation and is therefore unable to base-pair with the pdA of the opposite strand to the same extent as a thymine in the normal anti conformation. In addition, having the 5′-thymine in the syn conformation thrusts the methyl group into the polar hydrogen bonding region where it is expected to cause sizable disruptions in base-pairing and π-stacking interactions and hence cause the observed drop in melting temperature and magnitude of ΔH.

DNA Polymerase Bypass of Thymine Dimers

The key step in mutagenesis is thought to involve errors introduced by a DNA polymerase when it synthesizes past a

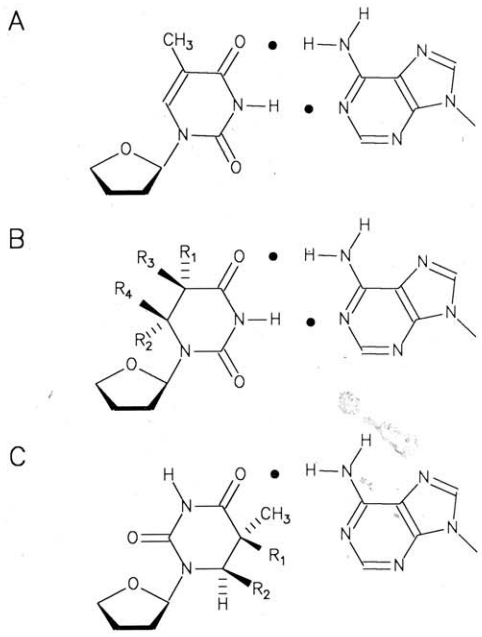

Figure 9. Comparison of the base-pairing interactions expected for (A) a normal TpT site, (B) the 5′-T of the cis–syn dimer of a TpT site [$R_1 = CH_3$, R_2 = H, $R_3 = R_4$ = 3′-T] and the 3′-T's of both the cis–syn and trans–syn thymine dimers [$R_1 = R_2$ = 5′-T, $R_3 = CH_3$, R_4 = H], and (C) the 5′-T of the trans–syn dimer of a TpT site [$R_1 = R_2$ = 3′-T].

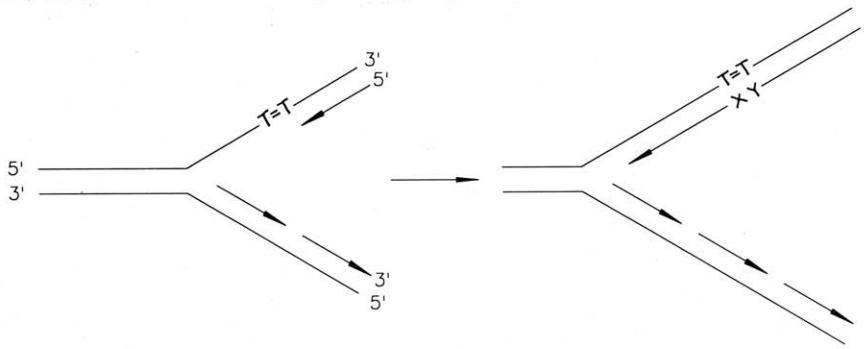

Figure 10. Schematic representation of the bypass of a photodimer in the leading strand of a replication fork. The arrows emphasize the fact that chain elongation can only take place in a 5′ → 3′ direction, and as a consequence simultaneous replication of the opposite, lagging strand can only take place in a discontinuous, piecewise fashion.

damage site during replication or repair (Fig. 10). In order to obtain structural and mechanistic insight into this process, we decided to investigate the action of polymerases on templates containing site-specific cis–syn and trans–syn thymine dimers. The polymerase we selected for the initial experiments was DNA polymerase I of the bacterium *Escherichia coli*, the first and most thoroughly studied polymerase known. Pol I is one of the three polymerases of *E. coli*, and its role is thought to involve filling in gaps resulting from the repair of DNA damage and from discontinuous replication. It has three basic enzymatic activities, the first and foremost of which is a reversible 5′ → 3′ polymerase activity that catalyzes the stepwise template-directed addition of nucleotides to the 3′ end of the primer (Fig. 11). The second is a 3′ → 5′ exonuclease activity that catalyzes the hydrolysis of the terminal nucleotide of the primer and is fastest when the terminal nucleotide is not complementary to the template (Fig. 12). This activity helps to correct any errors produced in the polymerization step, an activity often referred to as proofreading. The third activity is a 5′ → 3′ exonuclease activity whose function is to degrade the DNA ahead of the primer terminus. Recently, the structure of the Klenow fragment, a proteolytic fragment of Pol I that lacks the 5′ → 3′ exonuclease activity, has been determined by X-ray crystallography (Fig. 13) (*8*), making this enzyme particularly suitable for detailed structure–activity studies.

The cis–syn and trans–syn thymine dimer-containing templates needed for the bypass studies were constructed by joining the same photoproduct-containing decamers used in the NMR studies to a 12-mer and 19-mer. The oligonucleotides were joined by the enzyme T4 DNA ligase in the presence of ATP and a complementary 34-mer that served to hold their ends in place (Fig. 14). The sequence of the template was made to be identical with that contained in the bacteriophage DNA that would be used for in vivo studies (vide infra). In this way, information derived from biophysical, enzymatic and in vivo experiments could all be directly compared. The ability of DNA Pol I and its Klenow fragment to extend a primer on the templates containing the thymine dimers was examined first, and the major extension products found were those terminating prior and opposite to the 3′-T of the dimers (Fig. 15) (*9*). Very significantly, how-

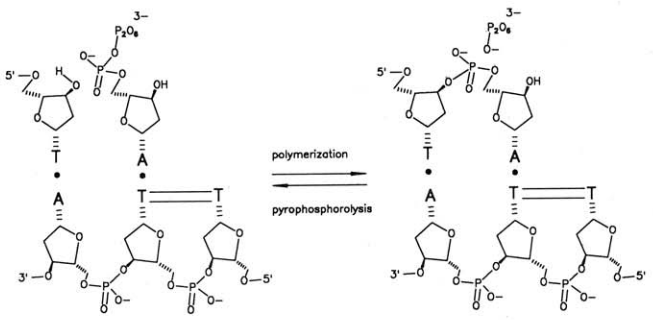

Figure 11. The reversible polymerization step catalyzed by DNA Pol I, illustrated for the incorporation of pdA opposite the 3′-T of the cis–syn thymine dimer.

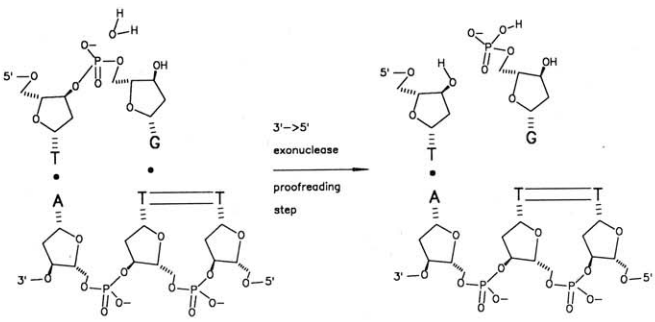

Figure 12. The proofreading step catalyzed by DNA Pol I, illustrated for a primer terminating in pdG opposite the 3′-T of the cis–syn thymine dimer.

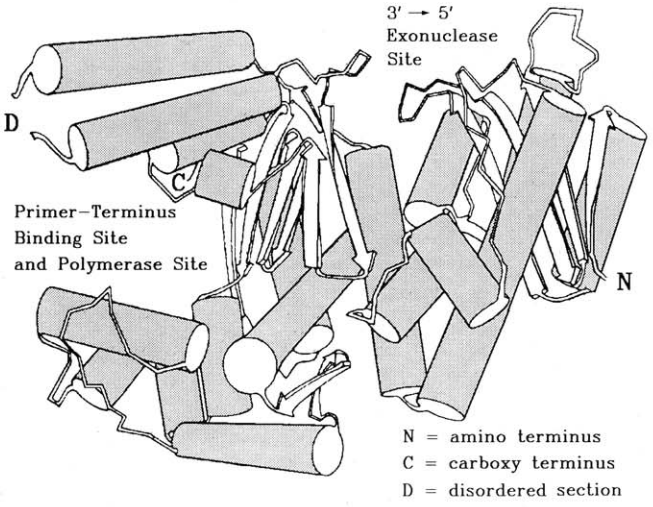

Figure 13. Schematic representation of the X-ray crystal structure of the Klenow fragment of DNA Pol I of *E. coli*. α-helical regions are symbolized by cylinders, and β-sheets by thick arrows. Regions of heterogeneous conformation are given by thick lines. Adapted from ref *8*.

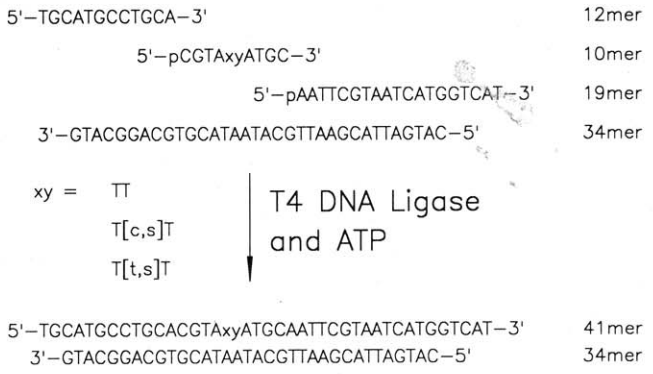

Figure 14. Construction of site-specific photodimer-containing templates for use in polymerase studies by enzymatic ligation of a photodimer-containing decamer to two other oligonucleotides utilizing a complementary 34-mer to hold the ends in place during ligation.

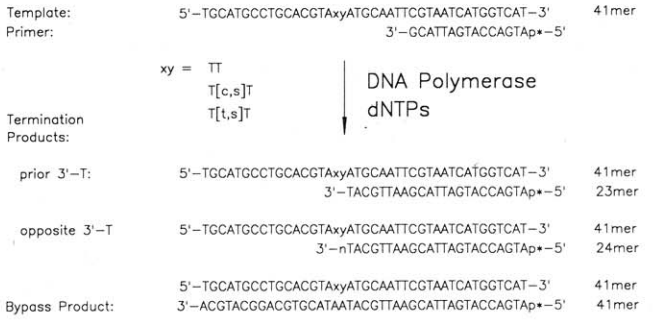

Figure 15. Products of primer-extension reactions catalyzed by DNA Pol I on the cis–syn and trans–syn thymine dimer-containing templates.

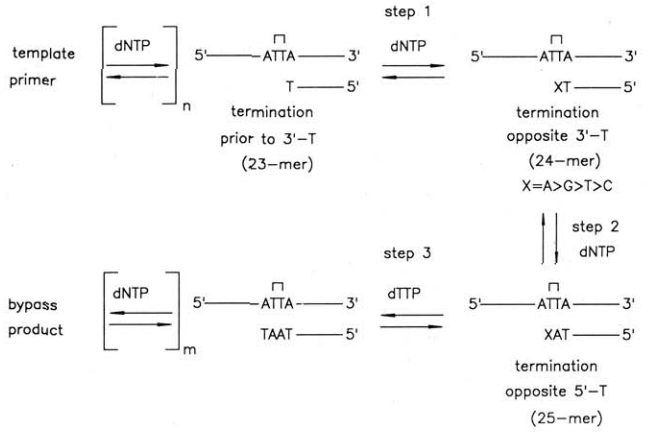

Figure 16. Critical steps involved in the bypass of the cis–syn thymine dimer by Pol I. All steps up to and after the dimer site are fast. Step 2 appears to be rate limiting at high dNTP and enzyme concentrations.

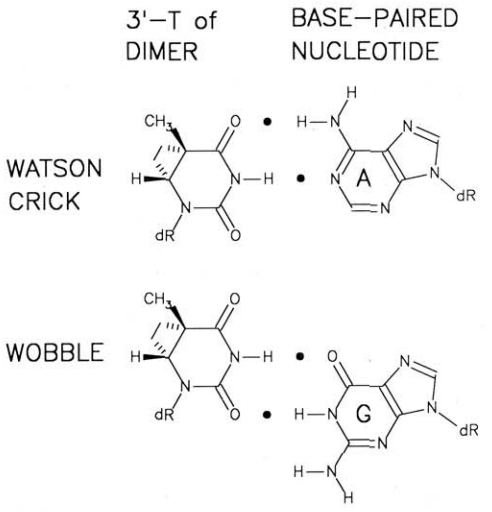

Figure 17. Base-pairing interactions between the 3′-T of the cis–syn thymine dimer and either pdA or pdG at a primer terminus, illustrating the displacement of the sugar ring (dR) of pdG relative to that of pdA.

ever, the discovery that a bypass product was also present in the extension reactions conducted on the cis–syn dimer-containing template clearly demonstrated that the cis–syn dimer was not an absolute block to replication by Pol I as had previously been thought. In comparison, the trans–syn thymine dimer could not be bypassed at all, a result easily explained by the syn orientation of the 5′-thymine that would be expected to prevent base-pairing to an incoming nucleotide triphosphate and thus block elongation (Fig. 9).

These results prompted the following question: was bypass of the cis–syn dimer mutagenic or nonmutagenic? In order to answer this question, the bypass product was isolated and sequenced, revealing that, within the limits of detection, only pdA's were inserted opposite the dimer site, making Pol I bypass of the cis–syn thymine dimer nonmutagenic. This result was in accord with the "A rule" in mutagenesis, which states that polymerases preferentially incorporate pdA's opposite noninstructional damage. Surprisingly, however, in addition to pdA, a substantial amount of pdG was incorporated opposite the 3′-T of the cis–syn dimer in the product terminating at this site (Fig. 16). In order to explain the almost exclusive incorporation of pdA's opposite the dimer in the bypass product, but not in the termination product, one needs a mechanism in addition to that of preferential incorporation.

One of the mechanisms that contributes to the fidelity of replication by Pol I is preferential elongation, a process whereby primers terminating in a correctly matched, i.e., Watson–Crick, base pair are elongated faster than those that are not (*10*). The same mechanism can also be used to explain the increased sequence specificity of the bypass product over that of the termination product, if the primer terminating in pdA opposite the dimer forms a better matched terminus than does the primer terminating in pdG. In such a case, the primer terminating in pdA would be elongated faster than the primer terminating in pdG, which would instead be degraded by the proofreading activity of Pol I (Fig. 16). The overall result of such a process would be a bypass product in which pdA's are almost exclusively incorporated opposite the dimer site. In support of this mechanism pdA can form a Watson–Crick-like base pair with the 3′-T of the cis–syn dimer, whereas pdG can only form a wobblelike base pair (which would explain why it could be incorporated in the first place) (Fig. 17). The 3′-hydroxyl of the terminal pdA is therefore expected to be in a suitable position for enzyme-catalyzed chain elongation, whereas that of the terminal pdG is not.

Bacteriophage DNA Containing Site-Specific Thymine Dimers

All the studies described so far have been conducted under well-defined, in vitro conditions. Such studies lead to very precise structure–activity relationships, and many useful correlations and insights have begun to accumulate. However, the challenge now shifts to determining the lethality, mutagenicity, and mutation spectrum of a photoproduct in *vivo*. In order to do so, replicative form bacteriophage DNA's containing site-specific cis–syn (*11*) and trans–syn thymine dimers have been constructed. This has been accomplished

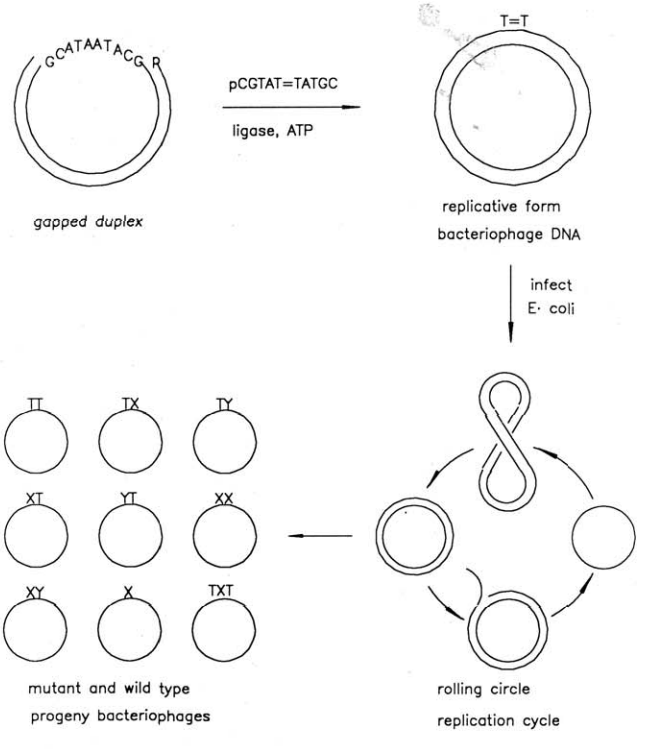

Figure 18. Construction and in vivo replication of bacteriophage DNA containing site-specific thymine dimers. By sequencing the progeny phage one can determine the mutation spectrum for an individual photoproduct. The observed mutation spectrum induced by light would be the sum of the mutation spectra for each photoproduct at each site weighted by its frequency of induction.

by a combined enzymatic and recombinant DNA approach similar to that pioneered by Essigmann for the study of O6-methyl guanine adducts (*2*). The key step in the construction was the ligation of the dimer-containing decamers used in the NMR studies to a gapped duplex bacteriophage DNA that had been engineered by recombinant DNA techniques to be complementary to the decamers (Fig. 18).

The lethality of the cis–syn and trans–syn thymine dimers will be determined by comparing the number of progeny produced when *E. coli* is infected with nondimer and dimer-containing bacteriophage DNA's. The mutagenicity will be determined by determining what fraction of the progeny are mutant, while the mutation spectrum will be determined by sequencing the individual mutants. A variety of repair and replication deficient hosts will be utilized in these studies to help identify the proteins and enzymes involved in mutagenesis and repair. By comparing the in vivo fates of the cis–syn and trans–syn dimer-containing bacteriophage DNA's with the structural and in vitro enzymatic data that have been obtained, the hope is to elucidate the fundamental structure–activity principles involved in light-induced mutagenesis in bacteria.

Conclusion

A general approach that combines chemical, physical, and biological techniques toward elucidating the structure–activity relationships in sunlight-induced mutagenesis in bacteria has been described. This approach should be readily extended to the study of DNA photoproduct structure–activity relationships in higher-organisms, including humankind. The ability to prepare pure, site-specific photoproduct-containing DNA should facilitate the isolation of previously unknown proteins and enzymes involved in the recognition, repair, and replication of DNA photodamage. By studying the action of human repair and replication systems on these uniquely damaged DNA's both in vitro and in vivo, there is hope for unraveling the molecular pathways that lead from sunlight to skin cancer. In the process, fundamental insights may be gained into the mechanisms by which DNA damage leads to mutation, cancer, and death. Such knowledge may ultimately lead to preventative measures against cancer and new criteria for the design of antivirival and anticancer drugs.

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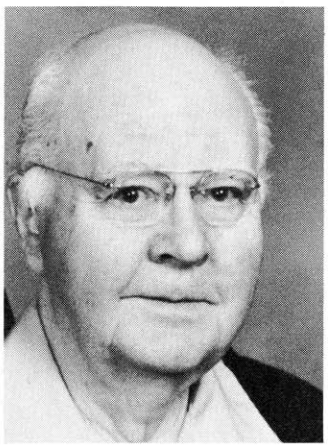
In Memoriam: R. T. Sanderson



Robert Thomas Sanderson, well-known among chemical educators for the approach he developed for teaching chemical bonding, died this past month at the age of 77. After graduation from Yale University in 1934, he was granted a PhD in chemistry by the University of Chicago in 1939 for his synthesis and study of the first complex hydride, aluminum borohydride, and his prediction of others. He then became chief chemist of the Western Geophysical Company and was a research chemist for Texaco for nine years. Eleven patents resulted from his industrial research. He next transferred to university teaching and research, spending a year and a half at the University of Florida, followed by 13 years at the University of Iowa and 15 years at Arizona State University. During these years he was a major contributor to chemical education, inventing unique atomic and molecular models and authoring five textbooks of general and inorganic chemistry. He was known for his work with the "principle of electronegativity equalization" and developed simple methods of calculating partial charges on combined atoms and, using these, polar bond energies in thousands of bonds. His approximately 150 publications include about a dozen books on chemistry, some of which have been translated into foreign languages. Following his retirement from active teaching in 1978, he continued research and writing in chemistry. In 1967 he received a CMA Award for Excellence in Teaching Chemistry, and in 1972 received the Distinguished Teacher Award of Arizona State University.

His marriage in 1939 to Bernice Shafer resulted in three children, and, after his first wife's death in 1977, he married Jean Menard with whom he made his retirement home in Fort Collins, Colorado. He joined the ACS in 1940.