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Spore Photoproduct: A Key to Bacterial Eternal Life

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1. Introduction

The cells of all living organisms are continuously exposed to a large variety of harmful agents that can induce either temporary or permanent deleterious effects. In response to these genotoxic stresses, only those cells endowed with efficient defense mechanisms have been able to overcome life's challenges and evolutionary demands and survive.

By directly targeting DNA nucleobases, solar UV radiation is a ubiquitous and particularly potent physical agent capable of altering the genome integrity. The most deleterious UV wavelengths are those in the 200−280 nm range (UV-C). Fortunately for humanity, although UV-C permeates space, it does not in fact reach the surface of the earth thanks to the presence of ozone and other protective layers high in the atmosphere. Nevertheless, there are important industrial uses for artificially produced UV-C, mostly exploiting its germicidal properties.

Though UV radiation-induced DNA damage (UV damage or photodamage) has had an indisputable evolutionary function, from a conservative standpoint the only cells able to transmit their genetic material unaltered are those capable of minimizing the frequency of the photodamage and/or those possessing accurate DNA repair pathways. The frequency of photodamage occurrence can be attenuated by extranuclear constituents. In addition, inaccurate damage repair can result from two distinct phenomena: either overwhelming photodamage or a deficient DNA damage repair system. Both these phenomena can be induced by the long-term UV exposure of DNA. Once the cell chromosomal material has been UV damaged, the cell cycle often undergoes dramatic modification. In humans, unrepaired photodamage can lead to mutation and

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Dominique Guillaume was born in France in 1959. He obtained his PharmD degree in 1983 and Ph.D. degree in 1986 from Université de Reims Champagne-Ardenne (URCA). He spent 1986−1988 as a postdoctoral fellow in the laboratory of Daniel H. Rich at UW-Madison and became an assistant professor in 1988 at the school of pharmacy, Université Paris V, France. He was a visiting faculty member in 1997 at Washington University in Saint Louis. In 1999, he became Full Professor of Medicinal Chemistry and moved to URCA in 2005, where his research field includes peptide and nucleic acid chemistry. He has authored, or coauthored, more than 80 publications.

Pascale Clivio studied Pharmaceutical Sciences at the University of Bourgogne in Dijon between 1980 and 1985. In 1989, she obtained her Ph.D. degree in Natural Product Chemistry at the University of Reims Champagne Ardenne (URCA) under the supervision of Professor Monique Zeches. In 1989, she got a permanent research position at CNRS (ICSN, Gif sur Yvette) to work with Dr. Jean-Louis Fourrey in the field of nucleic acids chemistry and photochemistry. In 1997, she received a one-year fellowship from the International Agency for Research on Cancer to work with Professor John-Stephen Taylor at the Washington University in Saint Louis, Missouri. She recently moved to the FRE CNRS 2715, URCA. Her current research interests include the study of conformational impact on DNA photochemistry, the chemistry of DNA repair, and the synthesis of DNA photoproducts for biological studies. Another area of research interest is the synthesis of fat nucleosides.

cancer; premature cell apoptosis is another frequent outcome. In the vegetal kingdom, another frequently observed consequence is growth reduction. The equilibrium of fragile terrestrial ecosystems can be strongly affected in the long term. Similarly, UV damage affecting fungi, bacteria, or phytoplankton can lead to changes in soil microbial communities, the biomass, and in aquatic ecosystems.

Bacterial spores represent a curious and fascinating lifeform. Their resistance to UV-C radiation is much higher than that of their corresponding vegetative forms.[1] In addition, bacterial spores conserve their capacity for germination for an extremely long time.[2,3] Hence, concerns regarding the efficiency of UV-C-assisted sterilization[4,5] and, consequently, the actual usefulness of UV-C in controlling the biological safety[6] of food and the subsequent minimization of the bioterrorism threat[7] have recently been addressed. The potentially elevated risk of bacterial planetary contamination through space exploration is also currently of great interest.[8,9]

Spores also have to face UV-A and -B radiation in order to survive. The deleterious effect of UV on spores is a consequence of either direct effects on their DNA components or the results of the formation of reactive oxygen species (ROS). Bacterial spore resistance to UV-A and -B is attributed mainly to the presence of melanin-like absorbing pigments in the spore outer layers. Bacterial spore resistance to UV-C radiation has been associated with the unique photochemical behavior of its DNA. In vegetative bacterial cells, UV-C radiation induces DNA damage principally through pyrimidine dimerization, which produces cyclobutane pyrimidine dimers (CPDs) and (6−4) photoproducts ((6−4) PPs). In contrast, in bacterial spores a single and totally different photoproduct, 5-(α-thyminyl)-5,6-dihydrothymine, is produced by UV-C irradiation. During the 5 years between the initial isolation of this photoproduct and its structural elucidation, it was simply called the "spore photoproduct" or "SP", and this name is still commonly used. Damage can be repaired during germination through the action of a specific enzyme named SP lyase. Formation of the spore photoproduct and its subsequent ability to undergo such efficient repair explains the low vulnerability of bacterial spores to UV-C exposure.

Particularly during the last 15 years,[10−17] numerous reviews have covered various aspects of the UV resistance of spores. Only the spore photoproduct itself has not been the subject of its own review. This is particularly regrettable in light of the pivotal involvement of this photoproduct in the strong UV-C resistance of bacterial spores and the importance of its chemistry in helping to decipher DNA-specific behaviors. Hence, this review will focus on the chemistry of the spore photoproduct from its formation to its repair and including its chemical synthesis.

2. Sporulation and Its Implications

In response to environmental conditions such as nutrient depletion, some microorganisms initiate a unique survival process called sporulation.[18−20] This process begins with the formation of a predivisional cell whose gene expression is governed by the Spo0A protein. This is followed by an asymmetric division. As a result, a large mother cell and smaller cell called a forespore are formed. Each has its own individual but interconnected gene expression program. The forespore, which will become the core of the mature spore, acquires a second membrane through engulfment by the mother cell. The space between the two membranes is later filled in by cell wall material to give the spore cortex. Finally, an outer shell of protein (the coat) is laid on the external surface of the developing spore to provide an additional protective layer. Meanwhile, during its maturation, the forespore undergoes two additional major transformations. Both of these are of the utmost importance for the protection of its DNA: (1) dehydration, due in part to the uptake of pyridine-2,6-dicarboxylic acid (dipicolinic acid, DPA) from the mother cell, and (2) compacting of its chromosomal material by a family of small, acid-soluble spore proteins (SASP). In the final step, the mature spore is released into the environment through lysis of the mother cell.

Spores represent a unique form of life since most of them possess only a single chromosome and display no apparent metabolism, a state defined as cryptobiotic. Spore dormancies of longer than 25 million years[3] and possibly even 250 million years[21,22] have been reported. Despite their apparent lack of life some spores play an essential ecological and environmental role.[23]

Not all bacteria genera possess the ability to sporulate. The spore-forming *Bacillus* (and to a lesser extent *Clostridium*) species are the most studied. Observations of this species have often been generalized and extended to other spore-forming genera. However, species with extreme UV resistance have been recently isolated.[24] This clearly supports the existence in the spores of some bacterial species with specific biochemical processes much more complex than previously anticipated.

Under appropriate circumstances, spore dormancy ends and the spore germinates[25] into a vegetative cell whose genome, despite the possible accumulation of DNA UV damage during dormancy, is preserved to an amazing extent relative to the genome of the mother cell.

3. Major UV-Induced DNA Photoproducts

UV radiation is arbitrarily divided in three wavelength domains: UV-C encompasses the 220−280 nm, UV-B the 280−320 nm, and UV-A the 320−380 nm range. DNA absorbs mainly in the UV-C and to a smaller extent in the UV-B range. It barely absorbs in the UV-A domain. Consequently, only UV-B and -C have any direct effect on DNA. However, UV-A radiation is capable of indirectly damaging DNA through the participation of a photosensitizer. In this review, we will focus only on DNA photoproducts formed by low-intensity continuous UV irradiation that mostly involves the first excited states of the nucleobases. This is in contrast to the high-intensity radiation delivered by lasers which produces nucleobase radical cations. Numerous reviews have already covered the photochemistry of DNA.[26−34] In this section, we will only discuss the major classes of DNA photoproducts. Further references can be found in the reviews mentioned above.

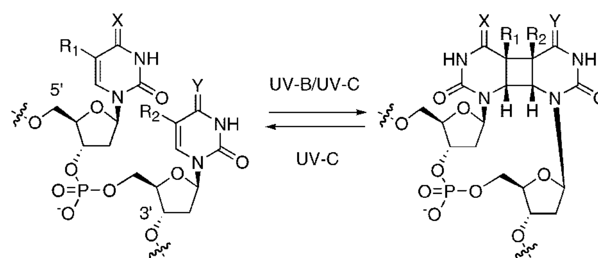
3.1. Direct UV Radiation Absorption by DNA Nucleobases

Both UV-C and -B photon absorption give rise essentially to cycloaddition and photohydration reactions. Pyrimidine nucleobases (thymine and cytosine) are the most sensitive to UV-B and -C radiation.

3.1.1. Cycloaddition Reactions

At the dipyrimidine sites (**1**−**4**) in DNA, cyclobutane pyrimidine dimers (CPDs) and (6−4) pyrimidine pyrimidone photoproducts ((6−4) PPs) are principally formed by direct photon absorption (Figure 1).

CPDs are the most abundant dipyrimidine photoproducts. They result from a [2 + 2] cycloaddition reaction between the C5−C6 double bonds of two adjacent pyrimidines (Figure 1). Depending on the nature of the C4 (O or NH) and C5 (CH_3 or H) pyrimidine substituent, four different PPs (**5**−**8**) are formed. These PPs consequently can have a cis-syn stereochemistry since pyrimidines in DNA adopt an anti glycosidic bond conformation. Cytosine-derived CPDs (**6**−**8**) are unstable and spontaneously deaminate at their C4 position, thus leading to secondary photoproducts belonging to the uracil-derived CPD family. Under UV-C radiation, CPDs can revert to their parent nucleobases.



1 (TT site)	X=Y=O ; R_1=R_2=CH_3	**5:** c,s T[CPD]T
2 (TC site)	X=O, Y= NH ; R_1= CH_3, R_2=H	**6:** c,s T[CPD]C
3 (CT site)	X= NH, Y= O ; R_1=H, R_2=CH_3	**7:** c,s C[CPD]T
4 (CC site)	X=Y=NH ; R_1= R_2=H	**8:** c,s C[CPD]C

Figure 1. UV-induced c,s CPDs in DNA.

(6−4) Pyrimidine pyrimidone photoproducts are the second most abundant PPs formed in DNA. They derive from a sequence-specific Paternó-Büchi reaction between the C5−C6 double bond of the pyrimidine on the 5′ side and the exocyclic double bond at the C4 atom of the pyrimidine on the 3′ side, i.e., the carbonyl of a thymine residue or the imine tautomeric form of a cytosine residue (Figure 2). The resulting four-membered ring intermediate (oxetane or azetidine) is unstable and undergoes a ring-opening reaction leading to (6−4) PPs (**9**−**12**) (Figure 2). Formation of the four-membered ring intermediate was ascertained using the photochemistry of thio-nucleobases that lead to a more stable thietane intermediate.[35] (6−4) PPs **11** and **12** involving a cytosine at their 5′ end can spontaneously deaminate, leading to secondary photoproducts.

After UV-A or -B absorption, (6−4) PPs can be converted into their corresponding Dewar (Dwr) valence isomer (**13**−**16**) (Figure 3). This latter adduct can revert to its (6−4) isomer under UV-C radiation.

Under prolonged UV-C exposure, the (6−4) pyrimidine pyrimidone motif can undergo a ring contraction reaction, leading to a 2-imidazolone (5−4) pyrimidone derivative.[36]

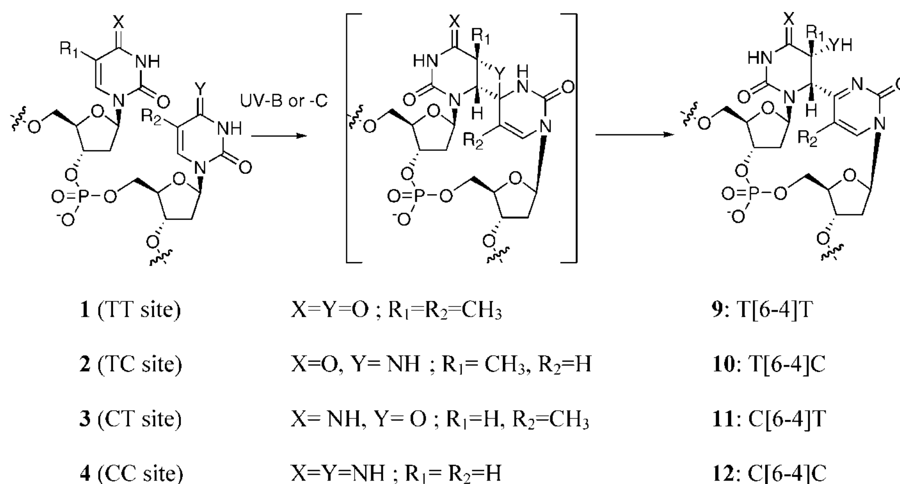


Figure 2. UV-induced (6−4) PPs (**9**−**12**) in DNA.

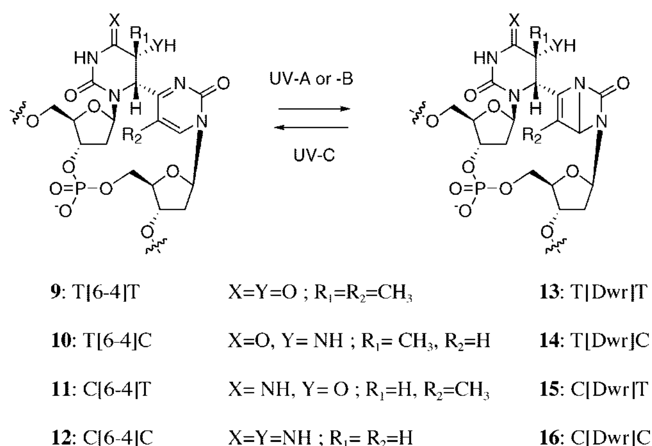


Figure 3. UV-induced Dewar PPs in DNA.

To a minor extent, purine nucleobases are also photoreactive. However, only adenine has been shown to give rise to cycloaddition reactions with adjacent nucleobases in DNA. Under UV-C irradiation the unstable azetidine **18** is generated at diadenine sites (**17**) by a cycloaddition reaction between the N7−C8 double bond of the 5′-end residue and the C5−C6 double bond of the 3′-end adenine residue (Figure 4). Azetidine **18** can then either spontaneously rearrange to afford **19** or undergo a hydration reaction, ultimately leading to **20** (Figure 4).

Sequence-specific photoaddition of adenine residues with vicinyl thymine residues can also occur. Thus far only the ring-expanded derivative **23** produced from the TA site **21** has been isolated. Derivative **23** results from ring opening of the cyclobutane adduct **22** generated by UV-C-induced cycloaddition between the C5−C6 double bond of the 5′-end thymine residue and the C5−C6 double bond of the 3′-end adenine residue (Figure 5). The X-ray structure of the TA adduct at the dinucleotide level is now available.[37]

3.1.2. Photohydration Reactions

Photohydration reactions usually involve pyrimidine bases. Hydration results in the saturation of the C5−C6 double bond and in the nonstereoselective substitution of the C6 atom by a hydroxyl group. Cytosine residues are the most prone to this kind of photoreaction. The resulting unstable cytosine hydrate derivative **24** can either revert through dehydration to the starting compound or deaminate to afford the uracil hydrate residue **25**. The latter dehydrates much less readily than **24** (Figure 6).[38,39]

The UV-C-induced hydration reaction of thymine and purine residues in DNA leading, respectively, to 5-hydroxy-5,6-dihydrothymine and 4,6-diamino-5-formamidopyrimidine or 2,6-diamino-4-hydroxy-5-formamidopyrimidine derivatives has been reported.[40] However, these results have been contested.[32]

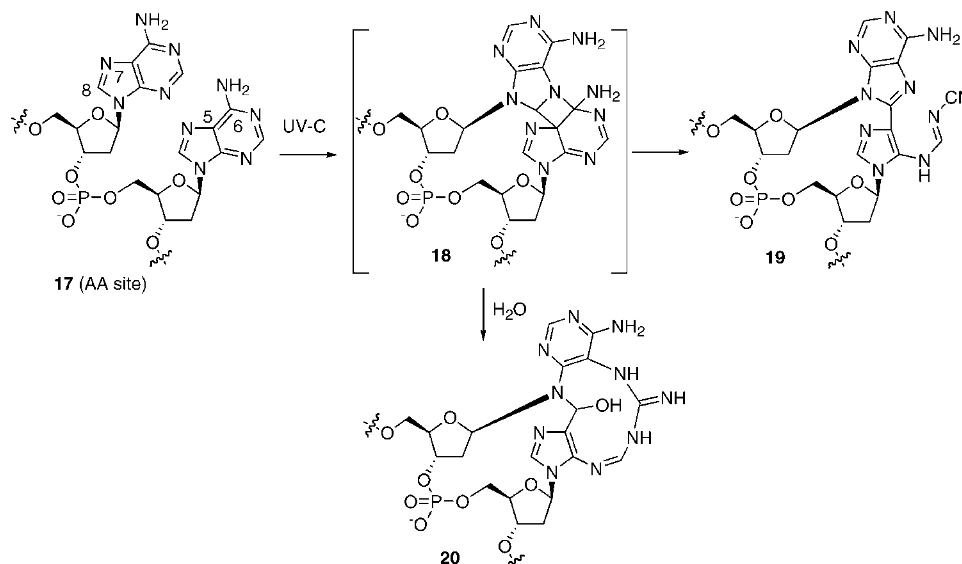


Figure 4. Formation of UV-induced AA PPs in DNA.

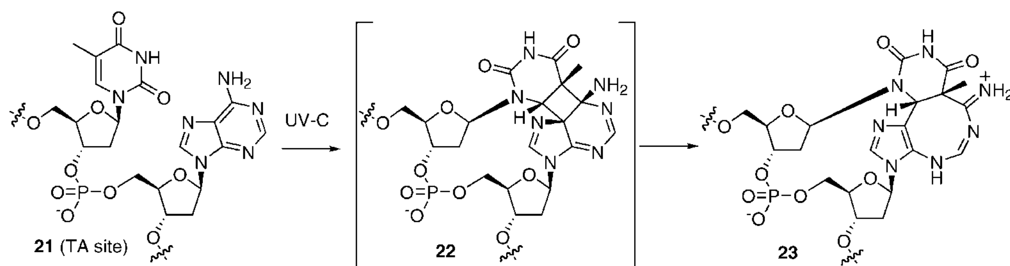


Figure 5. Formation of UV-induced TA photoproduct **23** in DNA.

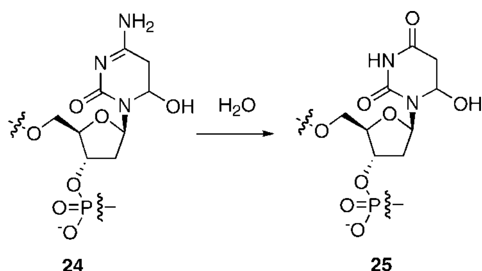


Figure 6. Hydrolytic deamination of cytosine hydrate derivative **24** in DNA.

In summary, pyrimidine as well as purine nucleobases are susceptible to UV radiation, and consequently, several types of photoproducts can be formed through radiation exposure of isolated DNA. However, in cellular DNA, CPD and (6−4) PPs are by far the most frequently produced adducts. Photohydrates, on the other hand, are generated in much smaller quantities. The formation of purine photoproducts in cellular DNA is yet to be confirmed.

3.2. UV-A-Induced Photosensitization

UV-A damage of DNA is an indirect effect arising from the action of so-far unidentified photosensitizers in cells. Three general pathways can lead to the formation of DNA photoproducts.

3.2.1. Energy Transfer from a Photosensitizer

Once excited by the UV-A radiation, the photosensitizer can directly transfer its energy to DNA, most likely via a triplet energy transfer mechanism. This type of reaction affects mainly dithymine sites producing as the major photoproduct c,s T[CPD]T (**5**). The cytosine-derived photoproducts c,s T[CPD]C (**6**) and c,s C[CPD]T (**7**) are generated in lower yields. It should be noted that this excitation process does not afford (6−4) PPs since they result from singlet-state excitation.

3.2.2. Type I Photosensitization

One-electron transfer from DNA nucleobases to the excited photosensitizer (Type-I photosensitization) can lead to nucleobase radical cations that can subsequently undergo either hydration or deprotonation. In DNA, guanine is the nucleobase most prone to one-electron oxidation. This can be directly attributed to the fact that guanine has the lowest ionization potential or indirectly because transfer through the DNA of a positive charge originating from another nucleobase radical cation can occur. Consequently, adenine and pyrimidine nucleobases are less susceptible to this photochemical process. Nevertheless, adenine and pyrimidine nucleosides can be targeted by a one-electron oxidation process.

3.2.2.1. Oxidized Purines. Hydration of the guanine radical cation **26** leads to the formation of the 8-hydroxy-7-yl-guanine oxidizing radical **27**. Upon reduction this in turn yields 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua, **28**) in DNA (Figure 7). Oxidation of **27** gives the 8-oxo-7,8-dihydroguanine derivative (8-oxoGua, **29**). Deprotonation of **26** leads to an oxazolone derivative **30** through a complex cascade of reactions.

3.2.2.2. Oxidized Pyrimidines. In DNA, the one-electron oxidation of pyrimidines is a minor pathway that ultimately leads to the formation of 5-(hydroxymethyl)uracil, 5-formyluracil, barbiturate, as well as hydantoin residues.

3.2.3. Type II Photosensitization

Finally, the excited photosensitizer can also generate singlet oxygen (type II photosensitization) that further reacts with nucleobases in the DNA to give oxidized products. Only guanine residues have been shown to be susceptible to this mechanism, producing the 8-oxoGua derivative (**29**) (Figure 8).

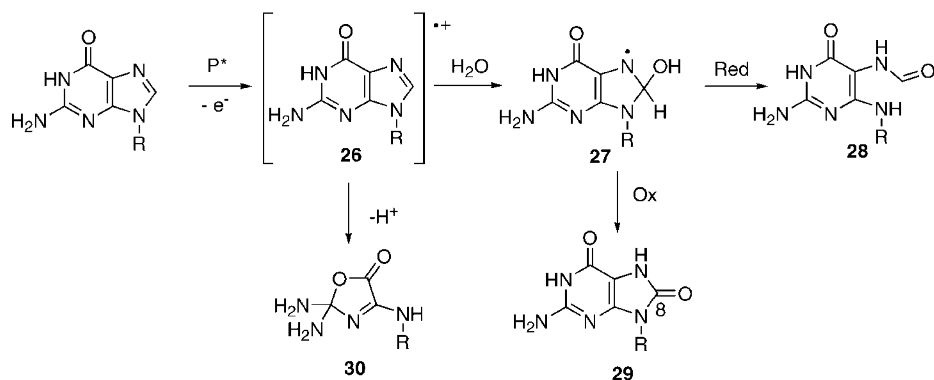


Figure 7. UV-A-induced guanine-oxidized PPs in DNA by type I photosensitization.

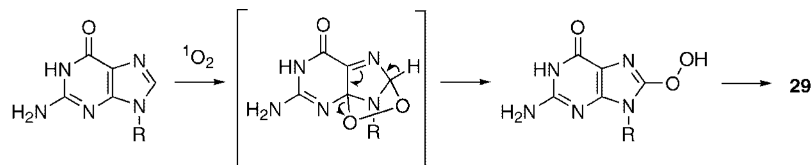


Figure 8. UV-A-induced 8-oxoGua formation in DNA by type II photosensitization.

3.3. DNA Damage Caused by As Yet Unidentified Mechanisms

3.3.1. Guanine Oxidation

Exposure of DNA to UV-B or -C radiation also results in the formation of some of the 8-oxoGua derivative **29**. The mechanism of its formation could involve either the generation of ROS or a one-electron oxidation. This kind of photoinduced oxidation reaction is, however, a minor process compared to the formation of CPDs in these wavelength domains.

3.3.2. Strand Break Formation

DNA strand breakage (single-strand breaks (SSBs) and double-strand breaks (DSBs)) is also observed after exposure to UV-A, -B, and -C radiation,[28,41−43] although it is a minor process compared to the formation of CPDs. Possibly, UV-A and -B irradiation triggers a complex oxidative process involving the production of the highly reactive hydroxyl radical by the mitochondria and type I photosensitization which ultimately leads to DNA strand breakage.[34,44] Strand breaks following UV-C irradiation would originate from a different mechanism involving the excited sugar phosphate backbone.[45]

4. UV-C-Induced DNA Photoproduct Formation in Bacteria

4.1. In Bacterial Vegetative Cells

In bacterial vegetative cell DNA, CPDs and (6−4) PPs are the major lesions formed as a result of UV-C irradiation (Figure 1).[26,27,46]

Not all of these PPs are generated with the same efficiency: the major adducts formed are the c,s T[CPD]T **5**, T[6−4]C **10**, and c,s T[CPD]C **6** and/or the c,s C[CPD]T **7**.[27,46] In *B. subtilis* vegetative cells, for a UV dose of 0.1 J/cm^2, c,s T[CPD]T **5** formation involves 5% of the total amount of thymine residues.[11]

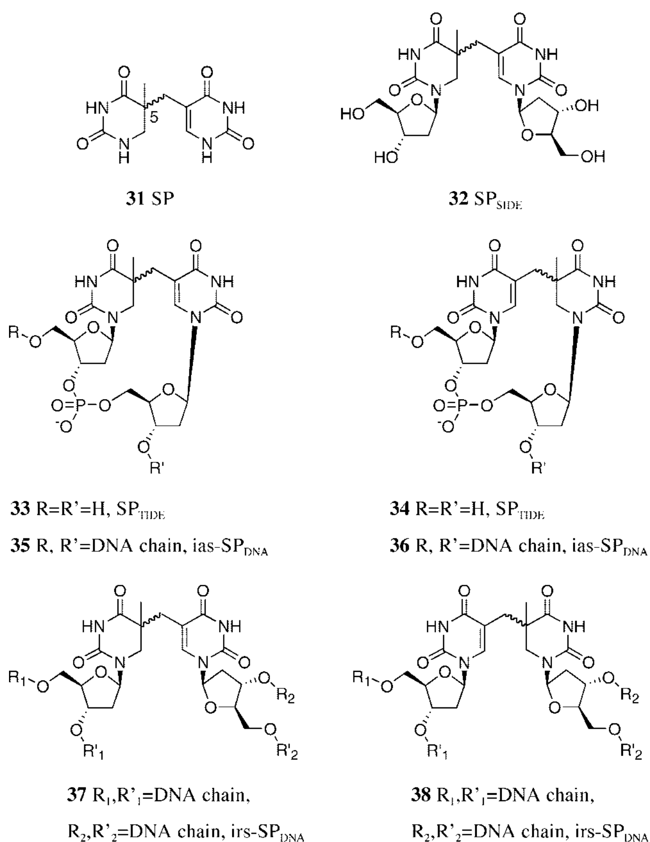
4.2. In Bacterial Spores

After UV-C irradiation only very small amounts of CPDs, (6−4) PPs, and single- and double-strand breaks are produced in spore DNA. Some indirect evidence derived from the slight UV sensitivity of nonhomologous end joining repair mutants has recently confirmed the minor formation of strand breaks induced in spore DNA.[47] For a UV dose of both 1[48] and 1.6 J/cm^2,[49] the cumulative effect of CPDs, (6−4) PPs, and single- and double-strand breaks represents less than 1% of all the DNA damage in the spores. Since this UV dose range far exceeds the minimum dose required to kill 90% of spores, the physiological effects of CPDs, (6−4) PPs, and single- and double-strand breaks are considered negligible.[49] The PP formed in spore DNA is almost exclusively the 5-(α-thyminyl)-5,6-dihydrothymine (or spore photoproduct (SP)).[50] It arises almost entirely from two adjacent thymidine residues on the same DNA strand. SP formation between thymidine residues from different DNA strands represents less than 1% of the intrastrand SP for a UV dose of 1 J/cm^2.[48]

In the DNA of *B. subtilis* spores, for a UV-C dose of 0.1 J/cm^2, spore photoproduct formation involves 7.5% of the total amount of thymine residues while c,s T[CPD]T (**5**) represents less than 0.2%.[11] Interestingly, when spores of *B. subtilis* are exposed to both UV-B and -A radiation, spore photoproduct formation still remains the major event even though production is much lower (a 10^3- and 10^6-fold decrease, respectively) compared to 254 nm irradiation.[49,51]

Strictly speaking, the term SP refers exclusively to the dipyrimidine nucleobase structure **31**. However, the term is frequently used rather loosely to refer to diastereomeric mixtures in which the N^1 atom of each nucleobase is substituted. In this review, the acronym SP will be used exclusively for the nucleobase dimer structure **31**. Acronyms SP_{SIDE} and SP_{TIDE} will be used for the dinucleoside analogue (**32**) and dinucleotide analogues (**33**, **34**) of SP, respectively.

The term ias-SP_{DNA} will be used for damaged oligodeoxynucleotides (ODNs) or DNA **35** and **36** containing at least one intrastrand SP-type lesion, and the term irs-SP_{DNA} will describe damaged ODNs or DNA **37** and **38** containing at least one interstrand SP-type lesion. If the intra- or internature of the spore photoproduct in DNA is not known, the acronym SP_{DNA} will simply be used.



4.3. Stereochemical Consideration of SP_{DNA} Formation

The chiral compound SP (**31**) was isolated in 1965 following the acid hydrolysis of SP_{DNA}.[50] Since the hydrolytic step resulted in the loss of the optically pure sugar moiety, no mention of the enantiomeric purity of the SP-5,6-dihydrothymine moiety C5 position was given.[52]

Four isomers of ias-SP_{DNA} (**35** and **36**, Figure 9) can be expected within the DNA strand depending on (1) the glycosidic bond conformation of the parent thymines at the time of the C5-CH_2 bond formation and (2) the 5′-end or 3′-end location of the thymine residue to be saturated. Theoretically, each ias-SP_{DNA} can result from two sets of adjacent thymidine conformers (Figure 9). The configuration of the 5,6-dihydrothymine residue C-5 atom is dictated by the syn/anti conformation of the glycosidic bond of its thymidine precursor, while the 5-α-thyminyl moiety can result from a syn or anti glycosidic bond conformation of the corresponding thymidine.

The formation of ias-SP_{DNA} within the double helix is likely to be regio- and stereospecific since SP_{TIDE} is observed as a single peak in the HPLC-MS/MS chromatogram of enzymatically digested SP_{DNA}.[53] In the A- or B-DNA double helix the glycosidic bond conformation of the nucleobases is in the anti domain. In the dry state, DNA adopts an A conformation. Thus, since SP_{TIDE} isolated from spore DNA and from UV-C-irradiated dry DNA display indistinguishable HPLC and mass fragmentation patterns,[48] the nucleobases in the DNA of spores are most likely in the anti glycosidic bond conformation domain. Therefore, only **35b** and **36a** can be formed. Recent experimental results using **32** as a model substrate for repair studies have identified the C5 *S* isomer of **32** as being diastereoselectively repaired by the SP lyase.[54,55] This result has led to the suggestion of a C5 *S* configuration in ias-SP_{DNA}, and consequently, the dihydrothymine moiety location should be at the 3′ end of the dinucleotide motif as in **36a** (Figure 9). However, extensive NMR studies carried out on SP_{TIDE} have recently demonstrated that the dihydrothymine moiety is located at the 5′ end of the dinucleotide motif and that its C5 configuration is *R*.[56] Therefore, **35b** is the correct structure for ias-SP_{DNA}. Repair experiments consistent with this result have also been reported.[57] Thus, ias-SP_{DNA} results from bond formation between the methyl group of the 3′-end thymine and the C5 atom of the 5′-end thymine residues.

4.4. Formation Mechanism of SP_{DNA}

Two mechanisms are currently envisioned to explain SP_{DNA} formation after UV-C irradiation. The methylene link formation could follow a reaction between two ‘close in space’ but independently UV-generated thymine-derived radicals: the 5-α-thyminyl radical (**37**) and the 5,6-dihydrothymin-5-yl radical (**38**) (Scheme 1, path A).[52] Indeed, evidence has been given for the possible formation by UV of these two different types of radicals from thymine.[58−60] Nevertheless, the simultaneous formation of these two

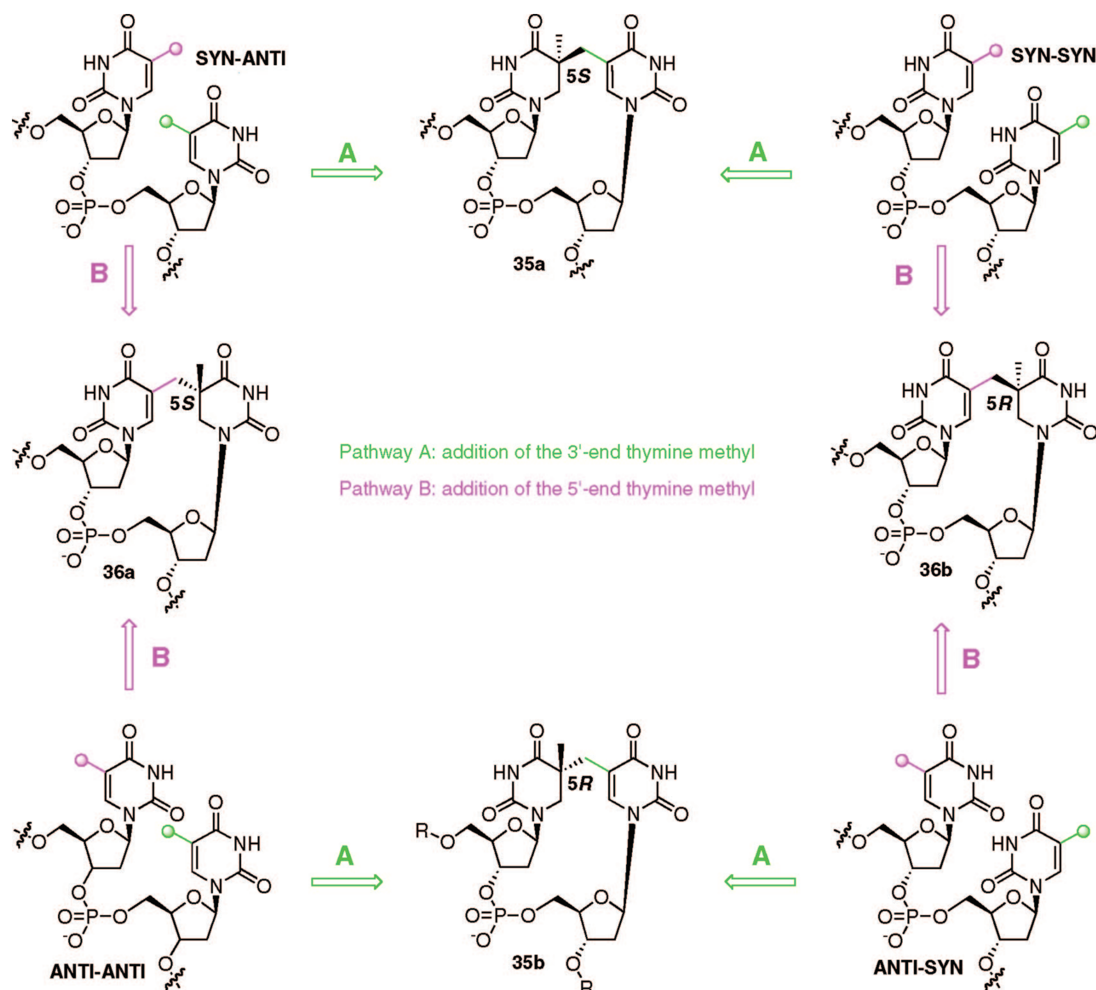
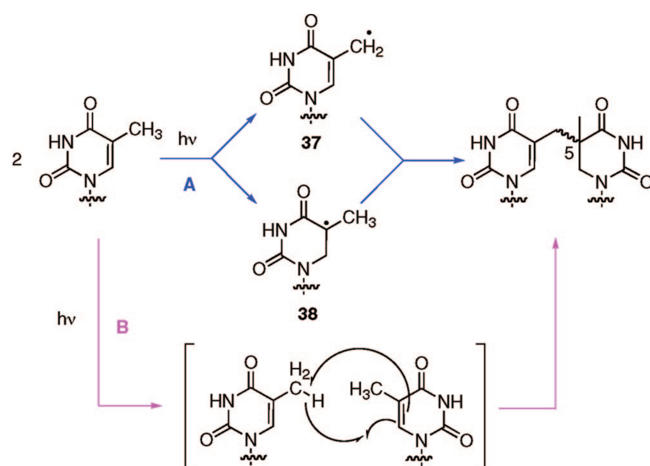


Figure 9. Possible glycosidic bond conformers at dithymine sites in DNA and induced structures of ias-SP_{DNA} isomers.

Scheme 1



different types of radicals at dithymine sites appears to be an event of low probability. In addition, neither the oxidative products derived from **37** and **38** or the dimerized adducts derived from two identical radicals have ever been isolated.

The second hypothesis was proposed after analyzing photolysis experiments using methyl-deuterated thymidine. These experiments suggested a concerted mechanism involving the methyl group of one thymidine and the double bond of the second thymidine (Scheme 1, path B).[26]

However, neither of these two hypotheses explains the respective role or difference in photoreactivities of the thymine residues (at either 5′ or 3′) in ias-SP_{DNA} formation. Nor is the influence of the pyrimidine C4 substituent fully explained, since no cytosine-derived SP-like PPs have ever been isolated either in vivo or in vitro even though in the latter case a large range of conditions has been studied.

Excited thymine residues can also be generated by photosensitization. Benzophenone and pyridopsoralens[61,62] as well as the physiologically relevant calcium dipicolinate[55,63,64] have all been identified as photosensitizers capable of performing this function.

5. Factors Influencing SP_{DNA} Formation in Spores

Any comprehensive description of SP_{DNA} formation must take into account its independent but complementary qualitative and quantitative aspects.

From a qualitative standpoint, only the ratio (expressed in percent) of the total number of spore photoproducts vs the total number of PPs is considered. Therefore, the qualitative aspect represents only the 'formation ability' of SP_{DNA} compared to the other PPs.

Conversely, the quantitative aspect represents the ability of the thymine residues to give rise to SP_{DNA}. To quantify SP_{DNA} formation, two methods are used. The first method requires the use of DNA which has either ^{3}H- or ^{14}C-labeled thymine residues. After acid hydrolysis of SP_{DNA} the results are expressed as the ratio (%) of the released SP versus the total number of thymine residues initially present in the DNA strand. The second method uses HPLC-mass spectrometry and standard solutions. It measures the amount of SP_{TIDE} (for ias-SP_{DNA}) and/or SP_{SIDE} (for irs-SP_{DNA}) released after enzymatic digestion of the SP_{DNA} obtained for a particular UV dose range. This amount is then normalized to 10^4 bases, and the final quantification, expressed as the number of PP per 10^4 bases per UV dose unit (J/cm^2), can be calculated using the initial linear part of the curve, giving the number of PPs per 10^4 bases as a function of the UV dose. It is important to note that the calculated values have no experimental significance when the dose unit lies outside the range of the linear part of the curve. Clearly, a comparison between the two methods is only possible if the sequence of the irradiated DNA is known and if the quantitative results expressed as percent of thymine have been calculated using the linear part of the curve reporting the number of PPs per 10^4 bases as a function of the UV dose. There are several limitations that need to be considered when comparing quantitative data since they depend on both the DNA concentration and the specific experimental setup used for irradiation. In addition, some early studies reported the amount of PPs formed for a single UV dose. However, since damage formation is not necessarily a linear function of the UV dose, this isolated information cannot be used to calculate the normalized amount of PPs formed per UV dose unit. Consequently, comparisons between quantitative studies are generally less than meaningful.

Irradiation of spores with the full spectrum of sunlight at the Earth's surface leads to the formation of SP_{DNA}.[51] However, quantitatively SP_{DNA} formation is much higher on UV-C radiation: radiation at 254 nm is 10^3 times more efficient for SP_{DNA} induction than that at 313 nm (UV-B) and 10^6 times more efficient than that at 365 nm (UV-A).[51] Maximum SP_{DNA} formation is reached near 260 nm.[65] Because 254 nm monochromatic UV radiation accurately reproduces the effects of UV-C on DNA, the 254 nm wavelength emitted by low-pressure mercury lamps, also called germicidal lamps, is the one most commonly used in laboratory studies.

Although in an early study no qualitative difference was observed in PP formation in the irradiated DNA spores, either dry or in an aqueous suspension,[65] large variations in the quantitative yield of PPs have been recently reported (Table 1).[63] When wild-type spores are irradiated in the dry state, PP formation appears to be dramatically lowered (15-fold) compared to irradiation in an aqueous suspension. Even though, as previously mentioned, experimental changes inherent in the different irradiation procedures may explain

Table 1. Quantitative (Quant) and Qualitative (Qual) Yields of Intrastrand Bipyrimidine Photoproducts in Spores and Mutants Exposed to UV-C Radiation in Aqueous or Dry State Conditions[a]

	ias-SP_{DNA} **35/36**		c,s T[CPD]T **5**		T[6−4]C **10**			
	Quant	Qual	Quant	Qual	Quant	Qual	total	refs
				spore[b]				
				wild type				
wet	365	99.7	0.3	0.08	ND		366.2	48
	252	99.6	0.69	0.27	0.10	0.04	253.1	63
dry	15.9	98.4	0.14	0.86	0.07		16.16	63
				α/β-type SASP ⊖				
wet	176	64.1	67	24.4	6	2.2	274.4	48
	108	37.3	78.4	27	71.2	24.6	289.8	63
dry	4.3	35.8	3.2	26.7	3.0	25	12.0	63
				Ca-DPA ⊖				
wet	23.3	71.2	5.3	16.2	0.82	2.5	32.7	63
dry	1.6	42.1	1.4	36.8	0.13	3.4	3.8	63
				α/β-type SASP ⊖				
				Ca-DPA ⊖				
wet	4.0	1.9	78.0	37.6	82.1	39.6	207.4	63
dry	0.31	8.9	1.2	34.3	1.3	37.1	3.5	63

[a] Expressed in lesions per 10^4 bases per J/cm^2. [b] Terms indexed ⊖ refer to the corresponding deficient spore.

these observed quantitative yield variations, it is nonetheless clear that the irradiation state has little or no influence on the respective distribution of PPs since both in the solid state and in suspension SP_{DNA} remains the main PP generated in spore DNA.

Since the aqueous environment has only a weak influence on SP_{DNA} formation, the impact of several other factors has been studied. Thus far, the most important factor is believed to be the association of spore DNA with specific spore proteins, namely, 'small acid-soluble spore proteins' (SASP).[19,66]

5.1. Small Acid-Soluble Spore Proteins

SASP are a group of spore proteins of low molecular weight (5−11 kDa, 60−75 residues). This group constitutes up to 10% of total spore proteins of dormant spores although it may comprise 20% of the spore core soluble proteins, a quantity sufficient to saturate the spore DNA. SASP, which are synthesized during sporulation, are rapidly degraded during germination and thereby provide the amino acids necessary for the bacterial protein synthesis.

The SASP group is composed of different types of proteins: for *B. subtilis* the two principal ones are termed α/β and γ. For other species, the corresponding SASP are named α/β- and γ-type SASP. Multiple α/β-type SASP exist; they all have a very similar amino acid sequence and a molecular weight of 5−7 kDa. α/β-Type SASP are monomers in solution. They are weak, nonspecific DNA-binding proteins and when bound to DNA form a dimer.[67] In the spore this binding induces a strong modification of the UV-C DNA photoreactivity. In addition, α/β-type SASP have a significant effect on gene expression during sporulation and germination.[68]

A single γ-type SASP (molecular weight around 8−11 kDa) exists, and it does not appear to influence the DNA photoreactivity. Its sole function appears to be supplying the necessary amino acids during the germination step.

In α/β-type SASP-less spore mutants, UV-C-induced SP_{DNA} formation is quantitatively reduced by 50% compared to wild-type spores.[69] Concomitantly, the formation of c,s T[CPD]T **5** undergoes a 40-fold quantitative increase.[69]

A subsequent and more accurate determination of the nature of the PPs formed by UV-C radiation together with their relative distribution has provided a better view of the impact of α/β-type SASP on spore DNA photoreactivity.[48,63] Even though α/β-type SASP have no significant influence on the global amount of PP formed (i.e., their quantitative impact is negligible), they still dramatically drive the photochemistry of spore DNA toward ias-SP_{DNA} formation (i.e., their qualitative impact is large). Indeed, in the presence of α/β-type SASP, ias-SP_{DNA} represents more than 99% of all the dipyrimidine PPs formed whereas in α/β-type SASP-less spore mutants ias-SP_{DNA} represents only 37−64% of the dipyrimidine PPs.[48,63] Interestingly, these studies also show that ias-SP_{DNA} formation is not fully prevented in α/β-type SASP-less spore mutants. Although α/β-type SASP-less spores still contain up to 15% of α/β-type SASP, this observation clearly indicates that, in vivo, factors other than SASP contribute to SP_{DNA} formation. The amount of PPs formed in experiments using α/β-type SASP-less spores is significantly reduced when the irradiation is performed in the dry state.[63]

Linking α/β-type SASP-modified DNA photoreactivity either to a DNA conformational change or to the consequence of an induced dehydrated state or to both has been proposed. However, this question is still a matter of debate, and the precise reason for the observed modified DNA photoreactivity is still far from being fully understood. In living cells, DNA adopts a B conformation. The hypothesis of a different DNA conformation in spores was made as early as 1965.[50] The early suggestion that spore DNA could adopt an A conformation was formulated 3 years later[70] and then supported by in vitro experiments (IR and CD).[71] Consequently, for several years spore DNA has been described as being in an A conformation, and this conformational switch has been held responsible for the specific photochemical behavior of spore DNA. However, electron microscopy studies have indicated that the base pair per helical turn in α/β-type SASP-bound DNA is not significantly different from that of vegetative cell DNA.[72] This has led to the proposal of an A-like conformation for spore DNA,[72,73] although more recent cryoelectron microscopy studies support a conformation close to the B form for spore DNA.[74]

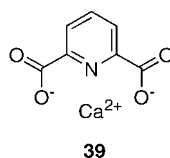
A detailed picture of the structural interactions occurring between α/β-type SASP and DNA has become recently available, nicely completing the few pieces of the puzzle that had been previously identified. α/β-Type SASP bind only to double-stranded (ds) DNA (or ds ODNs).[75] Although α/β-type SASP exhibit a random coil conformation in the absence of DNA,[76] upon binding to ds DNA, they become structured and hence α helical.[76] Crystallographic resolution of a α/β-type SASP bound to a 10 bp DNA duplex has revealed that the DNA helix adopts an A-like conformation with the base pair planes essentially parallel to each other and normal to the helix axis, an average value for the twist of 31.5°, and all sugar puckering in the C3′-endo conformation. However, because binding of α/β-type SASP to DNA widens the minor groove, the rise per base pair of SASP-bound DNA is now identical to that for B-DNA. Therefore, spore DNA adopts a "A-B-DNA" conformation.[67] Binding to DNA facilitates SASP dimerization, is cooperative, and follows both local conformational changes of ds DNA around the bound α/β-type SASP and also SASP−SASP interactions[76] mediated by the N-terminal amino acid residues.[77] Crystallographic observations have shown that when bound to DNA, each α/β-type SASP monomer comprises two long helical segments, one lying on the edge of the DNA minor groove and the other located in the minor groove and connected by a turn region.[67] The C-terminal residues are also implicated in DNA binding but so far only through unidentified interactions.[78] Both N and C termini are devoid of secondary structure.[67] α/β-Type SASP binding to ds DNA is nonsequence specific even though it is modulated by the ds DNA sequence. Binding of α/β-type SASP to DNA encompasses four[75,76] to six base pairs[79,67] and forms a helical coating around the DNA that greatly increases the DNA stiffness.[72] Cryoelectron microscopy experiments have revealed that these helical filaments (nucleoprotein helices) are tightly packed in a toroidal conformation by interdigitation of α/β-type SASP domains from adjacent helices.[74] Such assembly is stabilized by hydrophobic interactions and induces a substantial dehydration in the immediate vicinity of the DNA.[74,67] This is believed to modify its photochemistry. Indeed, lack of water is known to change the DNA reactivity, and for example, the dehydrated state of the spore core is most likely responsible for the α/β-type SASP protection of cytosine against hydrolytic deamination.[80]

The highly dehydrated state of α/β-type SASP-bound DNA is currently believed to be the major factor responsible

for specific spore DNA photochemistry. This hypothesis is in good agreement with the observations made for isolated DNA[81,82] (section 6.2.1). Nevertheless, the fundamental molecular and structural effects of the absence of water on the photoreactivity of DNA in spores remain unknown. An unidentified conformational change induced by dehydration or an unusual SASP-bound DNA rigidity cannot as yet be ruled out.

5.2. Dipicolinic Acid

Because SP_{DNA} is formed in α/β-type SASP-less mutants, α/β-type SASP is clearly not the only factor governing SP_{DNA} formation in spores. Among other factors influencing SP_{DNA} formation is dipicolinic acid, which is present in the spore as a Ca^{2+} chelate (**39**). Calcium dipicolinate constitutes 15% of the dry spore weight.[83] It strongly absorbs in the UV-C at 271 and 278 nm and to a lesser extent at 263 nm.[83,84]



During sporulation, DPA is synthesized in the mother cell by DPA synthetase. This enzyme is composed of the two subunits spoVFA and spoVFB, also called DpaA and DpaB.[85] DPA is then internalized into the spore core probably through proteins encoded by the *spoVA* operon and excreted in an early stage of spore germination.[86]

The role of Ca-DPA as a photosensitizer in spore SP_{DNA} formation has been demonstrated using DPA-less spore mutants.[87] In these mutants, depending on the experimental conditions, a 4−7-fold quantitative decrease of SP_{DNA} formation is observed (Table 1).[63] Additionally, although ias-SP_{DNA} remains the major PP produced (42−71%), its formation is less selective in these mutants than in wild-type spores. The concomitant formation of **5** is also observed (16−36%) (Table 1).[63]

The involvement of **39** as a photosensitizer in spores is in agreement with in vitro experiments performed with Ca-DPA.[55,63,87] The participation of Ca-DPA in SP_{DNA} formation may involve a selective triplet-state energy transfer from the UV-C-excited Ca-DPA to the thymine bases.[63] Such selective energy transfer could explain the exclusive involvement of dithymine in SP_{DNA} formation and consequently the absence of spore photoproducts involving cytosine residues.[48,63] A full understanding of the mechanism of the involvement of Ca-DPA remains elusive. Full clarification would first require a detailed examination of the excited-state properties of Ca-DPA.

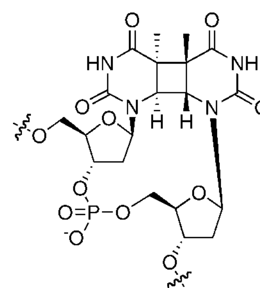
Interestingly, Ca-DPA also induces a decrease in the core hydration state.[88] This has led to the suggestion that **39** may also act indirectly and participate with α/β-type SASP in the highly dehydrated state of the spore core. In addition, Ca-DPA may bind, probably through intercalation to DNA. This could provide an additional hydration reduction mechanism in the immediate vicinity of the spore DNA.[89] The involvement of **39** in the conformational modification of spore DNA has also been raised. However, for the present at least this hypothesis is not strongly supported and the role of Ca-DPA is considered to be chiefly as a photosensitizer.[63]

In summary, among the chemical factors governing the spore DNA photoreactivity, α/β-type SASP and Ca-DPA appear to be the most critical. Their cumulative absence in spore mutants results in a dramatic decrease of SP_{DNA} formation and leads to spore DNA photochemical behavior qualitatively and quantitatively closely resembling that of vegetative cell DNA. When mutant spores lacking both α/β-type SASP and Ca-DPA are irradiated in the wet state, SP_{DNA} is formed in only a 2% yield of the total dimeric PPs, whereas c,s T[CPD]T (**5**) and T[6−4]C (**10**) are formed in yields of 38% and 40%, respectively (Table 1).[63] The decrease in the amount of PPs in the absence of both α/β-type SASP and DPA is even more pronounced when spore mutants are irradiated in the dry state (Table 1).[63]

5.3. Pressure/Hydration Level

Among the physical factors evaluated that are known to influence SP_{DNA} formation is that of pressure. This is because of the particular concerns regarding possible interplanetary contamination by spores.

Under the Earth's atmospheric pressure (101.3 kPa), the UV-C irradiation of spores leads almost exclusively to the formation of SP_{DNA}. Under an ultrahigh vacuum of 2×10^{-6} Pa with a 254 nm UV dose of 0.5 J/cm^2, SP_{DNA} remains the main photoadduct (69%) but c,s T[CPD]T (**5**) and t,s T[CPD]T (**40**) are also formed in qualitative yields of 21% and 10%, respectively.[65,90,91] Under ultrahigh vacuum, the quantitative formation of SP_{DNA} is reduced by ca. 30% compared to that under atmospheric conditions.[90] As observed in heat denaturation experiments, the partial DNA denaturation in the spore following the extreme dehydrated state induced by low pressure can explain the formation of t,s T[CPD]T **40**.[90,92] The photoreactivity of DNA in spores under a medium vacuum (1−2 Pa) is similar to that observed under ultrahigh vacuum.[91]



t,s T[CPD]T **40**

5.4. Temperature

Temperature is the other physical parameter identified as influencing SP_{DNA} formation at dithymine sites in spores. Because high temperature causes DNA denaturation, only low-temperature effects can be studied. Quantitatively, the optimum temperature for SP_{DNA} production is −80 °C.[93] At this temperature, SP_{DNA} formation is twice that observed at 22 °C and four times that observed at −196 °C for a UV-C dose of 0.02 J/cm^2. The combined effects of the humidity and temperature of the spore core on SP_{DNA} formation have not yet been explored.

5.5. Conclusions

Two physical (temperature and pressure) and three chemical (α/β-type SASP, water, and Ca-DPA) factors have been clearly identified as being particularly critical for SP_{DNA}

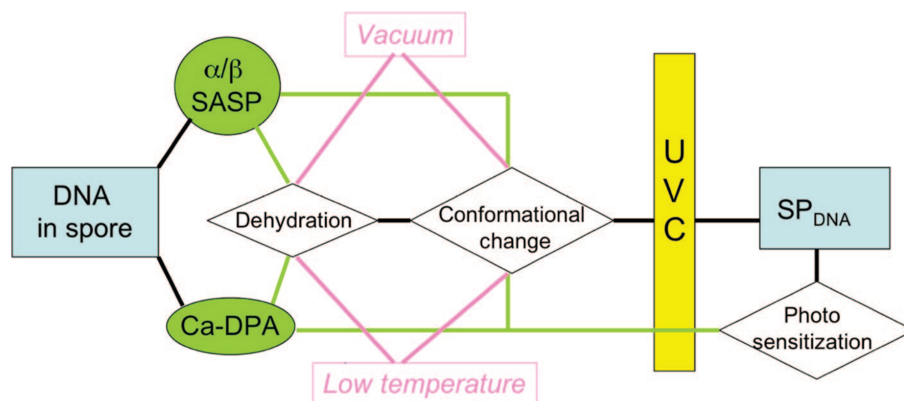


Figure 10. Representation of the influence of chemical (green) and physical (pink) factors on SP_{DNA} formation in bacterial spores.

formation. Although the exact elucidation at the molecular level of each step of the complex cascade of events linking these factors to SP_{DNA} formation remains, dehydration, whether intrinsic or induced, of the spore DNA environment appears to be the most critical factor governing SP_{DNA} formation. The different parameters identified so far are presented Figure 10.

6. Artificial Production of SP_{DNA}, SP_{TIDE}, and SP_{SIDE}

The intriguing photochemical properties of the DNA present in spores have encouraged (bio)chemists to explore the specificity of SP_{DNA} production and to define the conditions permitting its formation outside the spores. Oligonucleotide models containing a definite number of spore photoproducts at known locations are valuable tools in understanding the SP_{DNA} mechanism of formation for accurately studying its biological properties as well as for unraveling its repair processes.

6.1. UV Irradiation of Bacterial Vegetative Cells

Whereas it has been well established that the UV-C irradiation of bacterial vegetative cells at room temperature does not lead to SP_{DNA}, this latter lesion does form in the DNA of *E. coli* cells engineered to synthesize α/β-type SASP (3% of total thymine for a UV dose of 2.5 J/cm^2).[94] When *E. coli* cells are exposed to UV-C irradiation at −79 °C, SP_{DNA} is also produced (1% of the total thymine for a UV dose of 0.2 J/cm^2).[95] This result confirms that neither α/β-type SASP nor Ca-DNA is absolutely necessary for SP_{DNA} formation.

6.2. UV Irradiation of Isolated DNA

The formation of SP_{DNA} from isolated DNA has also been reported. The physical and chemical parameters that influence its formation in spores are also critical for its formation in isolated DNA.

6.2.1. Isolated DNA

In vitro, SP_{DNA} formation can be achieved by the UV-C irradiation of isolated DNA whose photochemical behavior depends on both the temperature and the level of hydration.

The photochemical behavior of plasmid and calf thymus DNA has been studied both in an aqueous environment and at room temperature. Under these conditions no SP_{DNA} is detected after UV-C irradiation. Qualitatively, the distribution of the PPs formed is c,s T[CPD]T **5** (40−42%), T[6−4]C **10** (20−33%), c,s T[CPD]C **6** (15−25%), and less than 7% each of T[6−4]T **9**, c,s C[CPD]T **7**, and c,s C[CPD]C **8** (Table 2).[48,96]

Table 2. Quantitative (Quant) and Qualitative (Qual) Yields of Intrastrand Bipyrimidine Photoproducts in Isolated DNA Exposed to UV-C Radiation under Aqueous Conditions or in the Dry State in Both the Presence or the Absence of α,β-Type SASP and Ca-DPA[a]

	ias-SP_{DNA} **35/36**		c,s T[CPD]T **5**		T[6−4]C **10**			
	Quant	Qual	Quant	Qual	Quant	Qual	total	refs
solution								
	ND		238	42.3	187	33.3	562	48
			29.7	40.4	15	20.4	73.5	96
dry								
	18	7.3	111	45.1	27	11	246.1	48
	3.19	10.6	9.72	32.3	10.1	33.5	30.13	53
	0.7	3.3	7.8	37.1	7.4	35.2	21	63
α/β-type SASP								
solution	25	23.6	49	46.2	2	1.9	106	48
dry	29	66.5	5	11.5	0.2	0.5	43.6	48
DPA								
dry	14.9	22.7	30.4	46.3	10.4	15.8	65.7	63

[a] Expressed in lesions per 10^4 bases per J/cm^2.

Table 3. Yields of SP_{DNA} and c,s T[CPD]T 5 in Isolated DNA Produced by UV-C Irradiation[a]

	SP_{DNA},	c,s T[CPD]T **5**,	dose, J/cm^2	refs
−100 °C	0.25	0.5	0.1	97
−99 °C	1.4	2 (+ U[CPD]T)	0.05	98
−100 °C W/EG	0.5	0.8	0.1	97
solution	0.7	4.3	1	100
dry	3.1	2.2	1	100
	0.25	ND	0.1	87
solution + α/β-type SASP	3.5	0.7	1	100
dry + α/β-type SASP	5	<0.5	1	100
	0.5	ND	0.1	87
dry + α/β-type SASP + Ca-DPA	3.4	ND	0.1	87

[a] Expressed as percent of the total thymine.

At low temperatures, the UV-C irradiation of a frozen aqueous solution of isolated *E. coli* DNA or *Haemophilus influenzae*-transforming DNA induces the formation of c,s T[CPD]T **5** and SP_{DNA} in an approximately 2:1 ratio (Table 3).[97,98] The optimum temperature for SP_{DNA} formation lies between −100 and −120 °C. Hence, *E. coli* DNA photochemical behavior is qualitatively similar both in

vivo and in vitro, and SP_{DNA} formation does not appear to be influenced by factors intrinsic to *E. coli* cells.

Concurrently with the discovery of SP_{DNA}, it was observed that dehydration promoted SP_{DNA} formation within isolated DNA.[50] In pUC19 plasmid DNA irradiated in the dry state, SP_{DNA} formation represents quantitatively 3.1% of the total thymine (Table 3).[94] Recent observations have allowed a more precise description of the effect of dehydration on SP_{DNA} formation. UV-C irradiation of isolated plasmid or calf thymus DNA in the dry state and at room temperature leads to ias-SP_{DNA} (**35** or **36**) formation (3−10% qualitative yield) together with c,s T[CPD]T **5** (32−45%) (Table 2).[48,53,63] pUC19 plasmid films obtained from air-dried 10 mM sodium phosphate buffer solution also afforded irs-SP_{DNA} (**37** or **38**), which represents 1% of the ias-SP_{DNA}.[48] In a dry film of calf thymus DNA prepared from a solution in deionized water, irs-SP_{DNA} and ias-SP_{DNA} have been obtained in equimolar amounts.[99] This difference in qualitative distribution has been attributed to a high salt to DNA ratio, a condition that promotes the A-DNA conformation, which in turn favors irs-SP_{DNA} formation.[48]

Promotion of SP_{DNA} formation from isolated DNA after dehydration is consistent with the pioneering observations made by Rahn and Hosszu,[81] who used DNA films to study the influence of the degree of humidity at 25 °C. Above 65% relative humidity, the photochemical behavior of DNA films is similar to that observed in solution and does not lead to the formation of SP_{DNA}. Below 65% relative humidity, SP_{DNA} formation occurs and is accompanied by a 2-fold quantitative reduction in the yield of c,s T[CPD]T (**5**). The maximum efficiency for SP_{DNA} formation is observed at a relative humidity of 40%.[81]

The influence of the isolated DNA water environment was also studied by adding varying amounts of a series of alcohols. At ethanol (EtOH) concentrations exceeding 60−70%, at room temperature, SP_{DNA} formation was detected. Optimum SP_{DNA} formation was observed for an EtOH concentration of 80%[82,99] in a similar quantitative yield to that for UV-C irradiation of heat-denatured DNA.[82] Such results are of the utmost importance in eliminating the DNA conformation as a determining factor for SP_{DNA} production.[82] Analysis of the SP_{DNA} structure revealed that UV-C irradiation of an 80% ethanolic solution of DNA furnished ias- and irs-SP_{DNA} (**35** or **36** and **37** or **38**, respectively) in a 9:1 ratio.[99]

The impact of the presence of alcohol and low temperature on SP_{DNA} formation has been studied using a 1:1 water/ethylene glycol solution. Compared to the yield obtained with a water solution irradiated at the same temperature, a 2-fold enhancement of the quantitative yield of SP_{DNA} was observed around −100 °C under UV-C irradiation.[97]

6.2.2. Isolated DNA Complexed with α/β-Type SASP

Because α/β-type SASP and Ca-DPA are known to influence SP_{DNA} formation within the spore, their influence in vitro has also been analyzed.

The formation of α/β-type SASP-DNA complexes (5:1 wt/wt) leads to a moderate to strong quantitative reduction in the DNA UV-C reactivity.[48,100] However, examination of the respective distribution of the PPs has clearly established that the in vitro binding of DNA to α/β-type SASP dramatically modifies the photochemical specificity observed for spore DNA. Binding of α/β-type SASP to DNA induces a 3.5% quantitative formation of SP_{DNA} (versus 0.7% in the absence of α/β-type SASP) with respect to the total thymine (Table 3). A reverse trend is observed with c,s T[CPD]T (**5**) (4.3−0.7% of the total thymine) (Table 3).[100] If all the dipyrimidine PP formed is now considered, the SP_{DNA} yield of formation reaches 24% (SP_{DNA} is not formed by DNA UV-C irradiation in the absence of SASP) (Table 2).[48] Interestingly, α/β-type SASP binding to DNA also induces a decrease in the qualitative yield of formation of T[6−4]C (**10**) (from 33% to 6%). The respective qualitative distribution of the other photoproducts remains substantially unchanged. This indicates that the formation efficiency of all other PPs has been similarly altered.[48]

From a qualitative standpoint, however, the in vitro induction of SP_{DNA} formation following binding of α/β-type SASP to DNA is less efficient than in vivo. Here, SP_{DNA} represents 99% of the dipyrimidine PPs formed. α/β-Type SASP-DNA binding is consequently important for SP_{DNA} formation, but other factors are also clearly involved in vivo.

The UV-C irradiation of dry (i.e., films of) α/β-type SASP-DNA complexes yields principally SP_{DNA}. Under these conditions, SP_{DNA} represents 5% of the total thymine while c,s T[CPD]T **5** represents ca. 0.1% (Table 3).[100] SP_{DNA} represents 66% of the dimeric pyrimidine PPs versus 24% in the solution state (Table 2). Concomitantly, c,s T[CPD]T (**5**) formation strongly decreases (from 46% to 11% of the PP pool).[48] Interestingly, dehydration of the α/β-type SASP-DNA complex induces a quantitative 2.4-fold reduction of the PPs formed compared to the solution irradiation results. A reduction of the same order is observed between the amount of SP_{DNA} obtained through irradiation of isolated DNA in solution versus the amount of SP_{DNA} obtained by irradiation of isolated DNA in the dry state. The combined action of α/β-type SASP binding and dehydration leads to a quantitative 12.9-fold decrease in PP formation. Such a decrease suggests a cumulative effect of the dry state (2.3-fold) and α/β-type SASP binding (5.6-fold). Consequently, α/β-type SASP binding to DNA may quantitatively reduce ∼5-fold the formation of PPs in solution as well as in the dry state, and dehydration may reduce by 2-fold the formation of PPs in either free or α/β-type SASP-bound DNA. Again, it is important to keep in mind that the observed quantitative fluctuations could, in part, be the result of fluctuations induced by the experimental conditions.

6.2.3. Isolated DNA in the Presence of Ca-DPA

The influence of Ca-DPA (**39**) on the formation of PPs in isolated DNA both in solution and in the dry state has been studied recently using calf thymus DNA.[63] In aqueous solution, the presence of **39** induces a quantitative decrease in PP formation. This reduced DNA photoreactivity, attributed to UV-C radiation absorption by Ca-DPA, was not observed on the UV-C irradiation of dry films of DNA prepared in the presence of 1.5 mM Ca-DPA. Irradiation of such dry films induces a quantitative 3-fold increase of PPs, among which SP_{DNA}, c,s T[CPD]T (**5**), and T[6−4]C (**10**) qualitatively represent 23%, 46%, and 16%, respectively. These correspond to about an 8-fold increase, a 1.2-fold increase, and a 2-fold decrease compared to dry DNA films irradiated without Ca-DPA (Table 2). Such a PP distribution is fully in line with the proposed formation of SP_{DNA} and c,s T[CPD]T (**5**) via a DPA-mediated triplet energy transfer process and of a (6−4) PPs via a singlet-state mechanism.[63]

6.2.4. Isolated DNA in the Presence of α/β-Type SASP and Ca-DPA

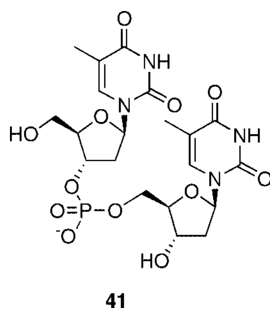
Irradiation of the pUC19 plasmid in the dry state with a UV dose of 0.1 J/cm^2 gives in the presence of α/β-type SASP and Ca-DPA an approximately 10-fold enhancement in SP_{DNA} formation from a α/β-type SASP/DNA dry complex and a 20-fold enhancement from dry DNA (Table 3).[87] Obviously, partial UV-C absorption by SASP and/or Ca-DPA can also occur, making an accurate quantitative comparison difficult.

6.3. UV-C Irradiation of Oligodeoxynucleotides

SP_{DNA} has also been generated by UV-C irradiation of ODNs in the single-stranded (ss) or double-stranded (ds) state. Irradiation at 280 nm of the dA:dT duplex in an equimolecular water/ethylene glycol solution at −196 °C leads to the formation of SP_{DNA} in a quantitative yield of 3% of the total thymine for a UV dose of 1.5×10^{-6} J/cm^2. However, under these conditions c,s T[CPD]T (**5**) is still the main PP formed with a yield 6.5% of the total thymine.[97]

An SP_{DNA} altered specifically at one site has been prepared using the 35-bp ds of 5′-CCCGGGATCCTCTAGAGT-TGACCTGCAGGCATGC-3′, a ds ODN that contains only one TT site. Whereas no SP_{DNA} was formed under irradiation of an aqueous solution of this ds ODN at 254 nm, irradiation at the same wavelength of a film prepared under 10% relative humidity resulted exclusively in SP_{DNA} formation, affecting 5% of the total thymine at a UV dose of 1.6 J/cm^2.[101] ias-SP_{DNA} has also recently been produced by UV-C irradiation in the presence of Ca-DPA of a film of the ss ODN 5′-GGTTGG-3′ obtained by lyophilization.[55]

It has been reported that dry films of the dinucleoside monophosphate of thymine (TpT, **41**) afford SP_{TIDE} (**33** or **34**) after UV irradiation in the presence of Ca-DPA or Na_2-DPA as photosensitizers.[64] However, no yield was reported. Without Ca-DPA, no (or only traces of) SP_{TIDE} is formed in dry films.[61,64] Analytical and enzymatic repair studies carried out on SP_{TIDE} (**33** or **34**) prepared by irradiation of TpT/Ca-DPA have shown that it is formed diastereoselectively. The configuration at the C5-5,6-dihydropyrimidine position is *R* as observed in ias-SP_{DNA} (**35** or **36**). The 5,6-dihydropyrimidine moiety is located at the 5′ end, and the configuration of its C5 position is *R* (5-*S* **33**).[56]



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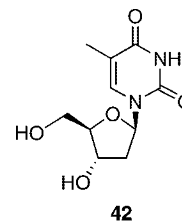
6.4. Irradiation of Thymidine

6.4.1. By Direct 254 nm Irradiation

Two different experimental conditions have been reported for the preparation of SP_{SIDE} **32** by the simple exposure of thymidine to 254 nm irradiation (**42**). The irradiation can be performed using either a frozen aqueous solution[102] or a thin solid film of **42**.[103]



42

6.4.1.1. In Water at −78 °C. The UV-C irradiation of frozen aqueous solutions of thymidine **42** yields SP_{SIDE} **32** in a qualitative yield from ca. 13%[63,102,103] to 38%[99] depending on the irradiation dose. CPD PPs are always the major photoproducts formed under these conditions, and the two C5-diastereomers of SP_{SIDE} **32** are produced in equal amounts.[99] Modification of either the aqueous solution ionic strength or pH qualitatively promotes SP_{SIDE} formation.[102] In a 1 M NaCl or in a 0.1 N NaOH aqueous solution, **32** is formed in 22% yield, compared to 14% in an aqueous solution.[102]

6.4.1.2. In the Dry State at Room Temperature. SP_{SIDE} **32** was isolated in a qualitative yield of 28% when a solid film of **42** (obtained by evaporation of a methanolic solution) was irradiated at 254 nm.[103] Using thymidine films prepared by lyophilization, Douki et al. reported a similar distribution yield and the equimolar formation of the two C5-diastereomers.[99] On the other hand, only one diastereomer, albeit of unknown configuration, was obtained in a 78% qualitative yield after the UV-C irradiation of thymidine films prepared from a thymidine ethanolic solution.[99]

6.4.2. By Photosensitization

The formation of SP_{SIDE} from a molecule as simple as thymidine (**42**) has stimulated the search for new preparative conditions. The UV-A (365 nm) irradiation of thymidine films prepared by the evaporation of a thymidine/pyridopsoralen methanolic solution leads to a diastereomeric mixture of SP_{SIDE} **32** in 3% quantitative yield (18% qualitative yield).[62] When benzophenone is used as the photosensitizer, the 350 nm irradiation of films of **42** (obtained by evaporation of an ethanolic solution) leads to only one SP_{SIDE} diastereomer of unknown C5-configuration.[61]

Ca-DPA (**39**) has also been used as a photosensitizer.[63] Addition of 1−9 mM of **39** to a frozen aqueous solution of thymidine **42** induces an increase in the qualitative SP_{SIDE} yields from 23% to 39%. However, the quantitative reaction yield decreases when the concentration of **39** increases.[63]

6.4.3. By γ-Irradiation, Heavy Ion, or Electron Bombardment

The formation of SP_{SIDE} (**32**) has also been observed when different radiation sources were used. Indeed, the γ-irradiation of frozen aqueous solutions of thymidine (**42**) at −78 °C leads to **32** formation in low yield.[104] Electronic or O^{7+} heavy ion bombardment of compressed thymidine pellets also leads to **32** in unreported yields.[105] However, these irradiation conditions also generate many radical reaction products.

7. Chemical Synthesis of SP and Derivatives

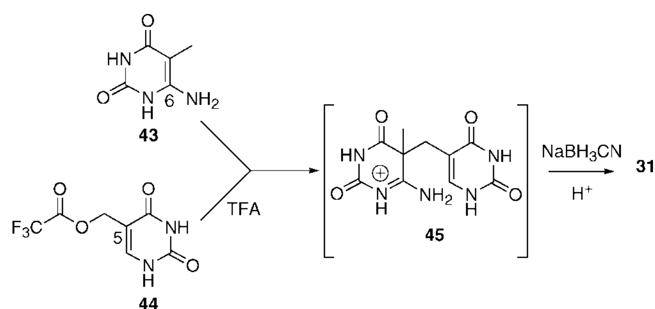
Work toward the chemical synthesis of SP_{SIDE} (**32**) is motivated by the need for precisely identified SP_{DNA} for use in mechanistic studies. The chemical synthesis of SP and its derivatives has naturally been seen as an alternative to the

irradiation method. Adequately functionalized SP derivatives can then be incorporated into ODNs at selected positions and in defined sequence contexts.

7.1. Synthesis of SP

Two strategies have been used for the chemical synthesis of SP (**31**). The first of these involves the bridging of two pyrimidine base derivatives; the second is building the 5,6-dihydropyrimidine skeleton onto a pre-existing pyrimidine base. The dihydrothymine C5−exocyclic methylene bond required in the first strategy has been created both under electrophilic conditions and by the reaction of an anion intermediate with an electrophilic center. Using the latter alternative, Bergstrom et al. successfully condensed 6-aminothymine **43** and 5-(trifluoroacetoxymethyl)uracil **44** to obtain the unstable 5,6-dihydro-6-imino-5-(α-thyminyl)-thymine **45** (Scheme 2). Reduction of **45** with $NaBH_3CN$ under acidic conditions allowed its conversion into **31,** which was isolated in an overall yield of 2.7%.[106]

Scheme 2



Taking advantage of the acidic character of the H5 atom of the 5,6-dihydrothymine moiety, the $C5-CH_2$ bond has also been formed using anion chemistry (Scheme 3). Accordingly, **31** was prepared in seven steps with an overall yield of 3%. In the presence of LDA, the N^1,N^3-diprotected derivative of 5,6-dihydrothymine **46** was transformed into an anion intermediate that subsequently reacted with the N^1,N^3-diprotected 5-formyluracil derivative **47**. Formation of the alcohol **48** resulted in a diastereomeric mixture formed in 37% yield. A two-step reduction of **48** via a bromide intermediate afforded the tetraprotected SP derivative **49** in 58% yield. Compound **49** was finally fully deprotected by treatment with CAN followed by TFA to afford **31** in 6% yield calculated from **46**.[107]

For the synthesis of **50**, the N^1,$N^{1'}$,N^3,$N^{3'}$-tetramethyl analogue of SP, the 5,6-dihydrothymine moiety was constructed (Scheme 4). Halogenation of 5-hydroxymethyluracil (**51**) afforded **52** in quantitative yield. It was then reacted with the diethyl methylmalonate anion to yield **53**. This was subsequently *N,N'*-dimethylated and then converted into the achiral barbiturate derivative **54** by treatment with urea under basic conditions. Dimethylation using MeI yielded **55**, and controlled reduction of one of the two amide carbonyl groups afforded the tetramethyl SP analogue **50**.[108]

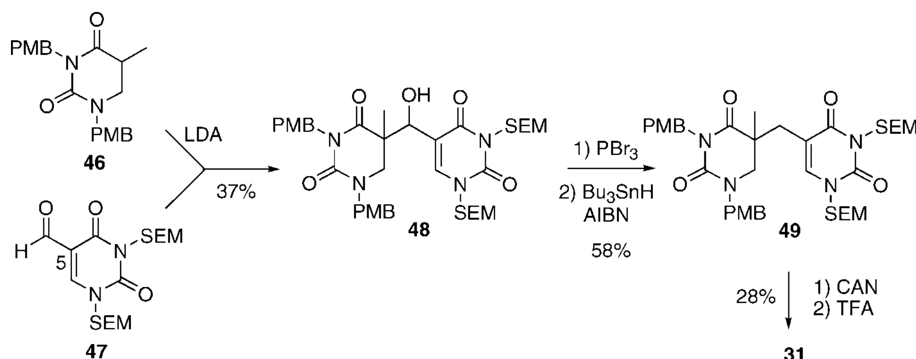
Although **50** is not a suitable starting compound for producing the necessary intermediates for the synthesis of SP_{DNA}, its successful synthesis validates this synthetic strategy.

7.2. Synthesis of SP_{SIDE} and SP_{TIDE}

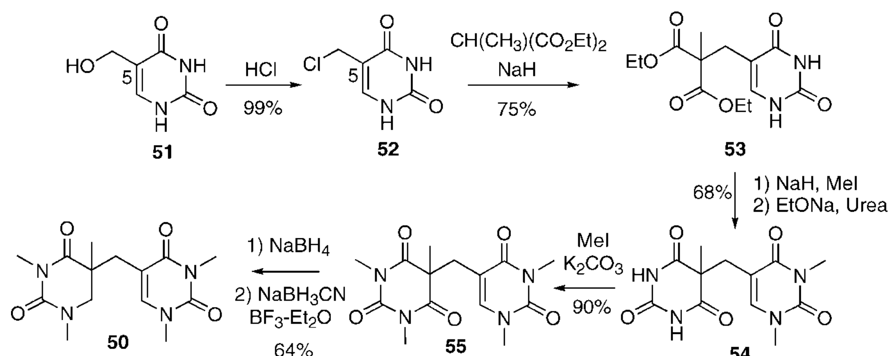
For the preparation of ODNs containing the spore photoproduct, direct access to SP_{SIDE} or SP_{TIDE} is more valuable.

SP_{TIDE} **33** has been prepared using the nucleophilic synthetic approach (Scheme 5). The N^3-protected 5,6-dihydrothymidine derivative **56** and the thymidine derivative **57** were independently prepared from thymidine **42** in 70−93% and 68−84% yield, respectively.[109,54,110] Using photobromination compound **57** was converted to its bromide analogue **58** in 53−60% yield.[109,54,110] This was then reacted with the anion (LDA) of **56** to afford the diastereomeric

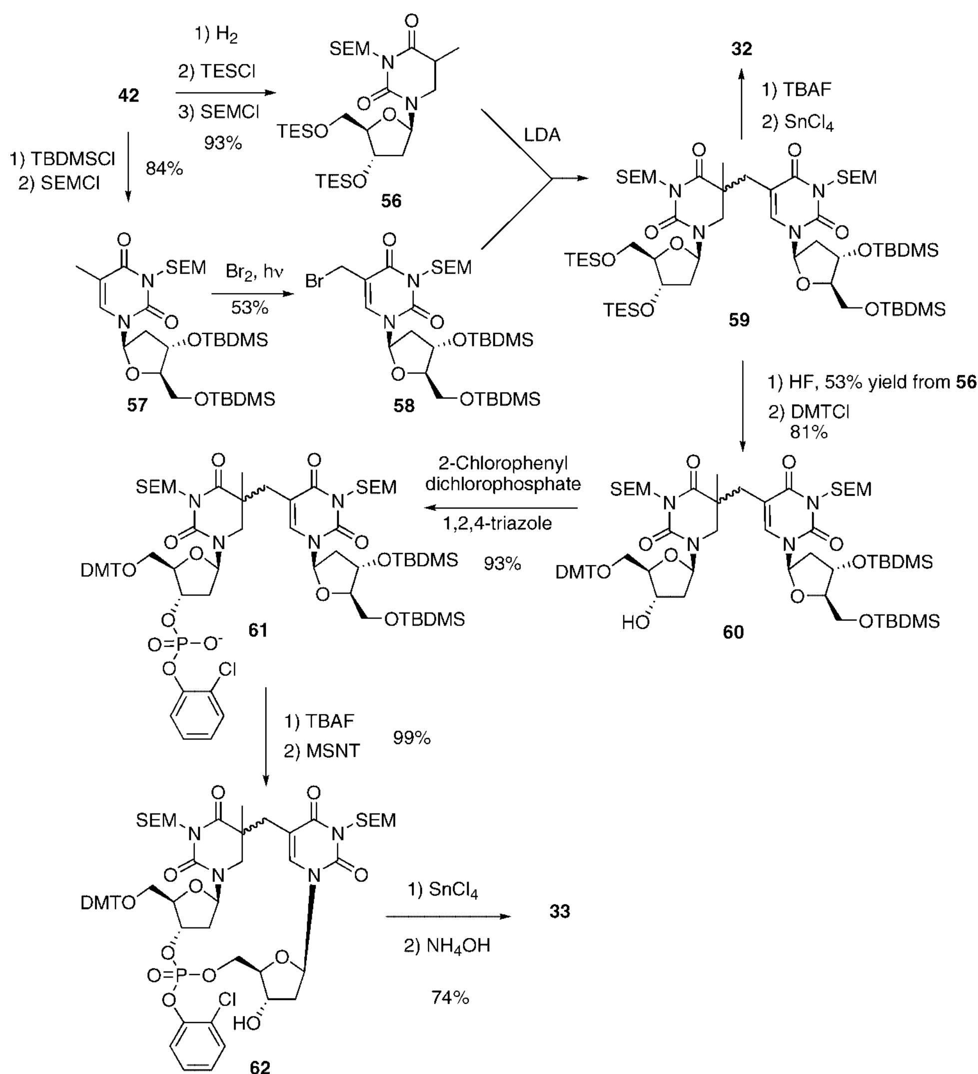
Scheme 3



Scheme 4



Scheme 5

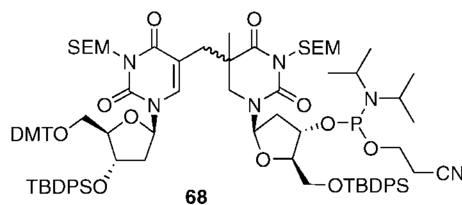


mixture **59**. A two-step deprotection of **59** using TBAF followed by treatment with $SnCl_4$ and an intermediate HPLC separation afforded the two SP_{SIDE} **32** diastereomers C5 *S* and C5 *R* separately in 8% each.[54,110] Their respective configuration was indirectly determined using an oct-4-enedioate rigid cyclic derivative.[110] From a slightly modified procedure, Chandra et al. were able to prepare **32** in ~50% yield from pure diastereomers **59**.[57] NMR analysis of each C5 diastereomer of **32**, their precursors, and a cyclic phosphotriester derivative allowed these authors to determine the configuration at C5 of **32**.[57] Their assignment is inverse to that previously reported by Friedel et al.[110]

Selective deprotection by HF in acetonitrile of the TES group of the "target 5′-end sugar residue" of **59** followed by primary alcohol protection by DMTC1 afforded **60** in 43% yield. Phosphorylation of the 3′ position gave **61** in 93% yield. This was followed by desilylation and cyclization to give the dinucleotide analogue **62** in 99% yield. The diastereomeric mixture was successfully separated using chromatography. Deprotection of the phosphate group of **62** led to the disappearance of the phosphorus chiral center, and finally, deprotection with $SnCl_4$ followed by NH_4OH resulted in the isolation of the C5 *R* and C5 *S* diastereomers **33** in 74% yield (Scheme 5).[109]

Protection of the N^3 atom of the 5,6-dihydrothymine moiety is not absolutely essential in order to prepare the SP_{SIDE} derivative (**67**). Indeed, deprotonation of **63** using *sec*-BuLi leads to a carbanion that can react with nucleoside aldehyde **64** to afford **65** as a diastereomeric mixture in 44% yield. The deoxygenation of **65** which is necessary to obtain the SP_{SIDE} analogue **67** was achieved with a 14% yield via the xanthate intermediate **66** using H_3PO_2 and AIBN as reducing agents (Scheme 6).[111]

Interestingly, SP_{SIDE} phosphoramidites **68** have been successfully incorporated into several oligonucleotides to investigate the properties of SP_{DNA} analogues.[112] Amazingly, the resulting SP_{DNA} analogues incorporate the 5,6-dihydrothymine moiety at the 3′ end of the dinucleoside motif.

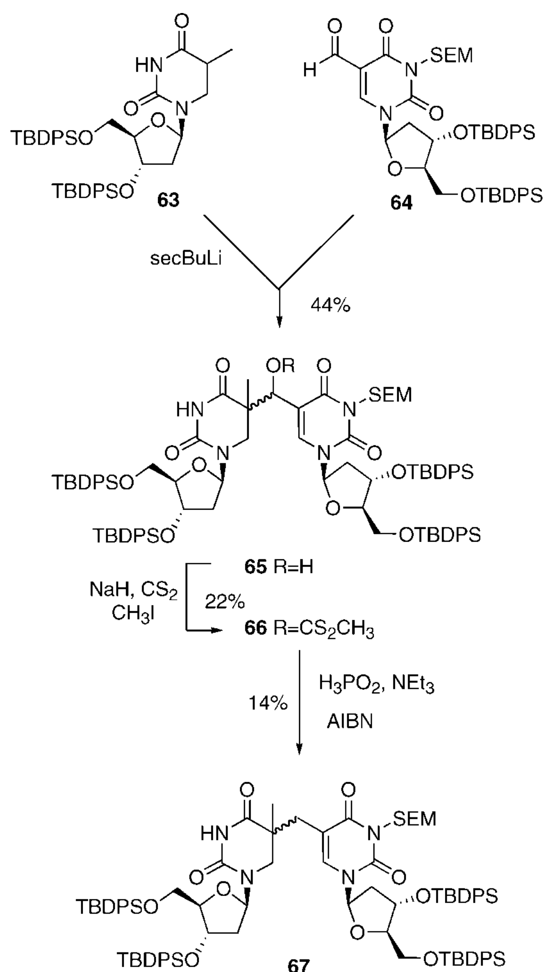


8. DNA Photoproduct Repair

8.1. Conventional DNA Photoproduct Repair

Persistent DNA photoproducts can preclude replication and transcription of DNA, thus leading essentially to cytotoxicity

Scheme 6



and ultimately cell death. Alternatively, low-fidelity replication and transcription of DNA, leading to heritable coding changes, can occur as a result of incorrect nucleotide incorporation opposite to the damaged nucleobases.[113−115] This phenomenon is nevertheless sometimes considered as a repair pathway and is referred to as mutagenic repair[116] or trans lesion synthesis.[117,118]

Stricto sensu repair of DNA photoproducts is achieved by at least five DNA repair pathways generally dependent on the type of PP. Repair of UV-induced DNA damage has been specifically reviewed,[116] while other broader reviews cover DNA repair in mammals,[119] humans,[117] or plants.[118,120] Because of the abundance of relevant literature, these repair modes will only be listed and only the most recent reviews cited.

8.1.1. Nucleotide Excision Repair (NER)[121,122]

Operating in both prokaryotes (including *Bacillus* species) and eukaryotes, NER displays a wide substrate specificity since it repairs dipyrimidine photoproducts and bulky DNA adducts induced by environmental mutagens and chemotherapeutic drugs. Although the number of proteins intervening in NER is different in prokaryotes and eukaryotes (4 versus at least 30, respectively), the general repair processes is identical for these two kingdoms: after recognition of the lesion, an ODN patch containing the photoproduct, which has about 10 nucleotides in prokaryotes and about 24−32 nucleotides in eukaryotes, is excised from the damaged DNA strand. The resulting gap is then filled by the action of a polymerase that uses the complementary strand as a template, and the resulting nick is sealed by a DNA ligase. Depending on the location of the photoproduct within the genome, i.e., whether it lies in a transcribed or nontranscribed gene, two distinct repair subpathways have been recognized. These subpathways, which differ in the lesion recognition step, are called transcription-coupled NER (TC-NER) and global genome NER (GG-NER).

8.1.2. Base Excision Repair (BER)[123−126]

As observed in NER, the BER pathway is also shared by prokaryotes and eukaryotes and involves the participation of several enzymes. Distinctively, BER processes nonbulky monomeric base lesions. Oxidized pyrimidine and purine photoproducts are two examples of damage commonly repaired by BER.

In brief, the first step in BER is the recognition and excision of the damaged base from the corresponding nucleoside residue through hydrolysis of its *N*-glycosidic bond by DNA glycosylases. A nick or gap is then generated by pathways using either monofunctional or bifunctional glycosylases. In the case of a nick, a nucleotide insertion/abasic site elimination process follows, whereas for a gap, a nucleotide insertion follows. Complete DNA repair is finally achieved by DNA ligases.

8.1.3. Photolyase-Induced Repair[127,128]

Photolyases are a group of enzymes that use visible light for repair and photoreactivate dipyrimidine photoproducts through an electron transfer mechanism. They have been identified in some prokaryotes and eukaryotes including fishes, plants, and aplacental mammals. This family of enzymes has not been identified in *B. subtilis*. DNA repair by photolyases is a single-enzyme process that requires two cofactors: a light-harvesting photoreceptor and $FADH^-$. It should be noted that unlike NER and BER, photolyase-induced repair results from interbase covalent bond breakage and not from an excision/synthesis process. Consequently, this type of repair should really be considered as a retro-damage pathway. CPD- and (6−4) PP-containing DNA is repaired by specific photolyases.

8.1.4. Repair of DNA Strand Breaks[129−136]

Repair of SSBs is controlled by mechanisms which although as yet unclear are likely to involve a subset of the repair enzymes also implicated in BER. Two distinct major pathways, homologous recombination (HR) and nonhomologous end joining (NHEJ), have been identified for the repair of DSBs. HR retrieves the coding information from a DNA molecule that shares extensive sequence homology with the damaged DNA and is therefore an accurate repair pathway. In contrast, NHEJ, whose occurrence in prokaryotes has been identified only recently, is more prone to errors since it rejoins the DNA ends independently of any sequence homology. In germinating spores, DSBs are principally repaired by NHEJ.[20,47]

8.1.5. Others[137,138]

N-Glycosylases specific to CPD and (6−4) PPs have been discovered in phages, eukaryotes, and prokaryotes including *Bacillus* species. These enzymes may be part of an alternative DNA excision repair pathway specific to UV-induced dipy-

rimidine PPs. The nick resulting from the glycosylase activity could possibly be further processed by the general BER pathway.

8.2. SP_{DNA} Repair

The nucleotide excision repair pathway (NER) and repair by the SP lyase enzyme are the two major distinct routes currently thought to be the main processes for the repair of SP_{DNA}.[139,140] Homologous recombination is a third albeit minor repair process.[141,142] Because NER is nonspecific and has already been the subject of numerous reviews (in the very similar *E. coli* system[121] and in prokaryotes[143]), we will focus only on the repair of SP_{DNA} by SP lyase.

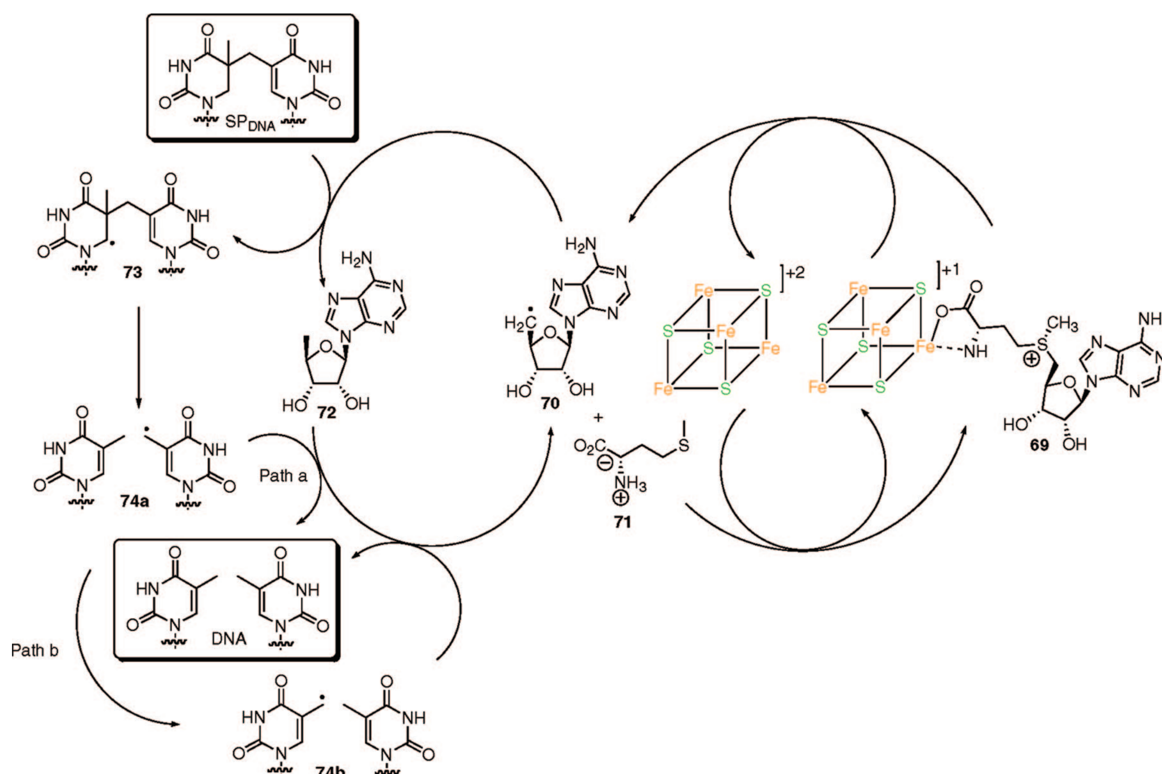
SP lyase is specific to the repair of SP_{DNA}.[101] SP lyase from only three origins has been studied so far. That from *B. subtilis* is currently the most characterized, while those from *Geobacillus stearothermophilus* and *Clostridium acetobutylicum* have been characterized more recently.

SP lyase was first cloned in 1993[144] and is encoded by the *splB* gene in *B. subtilis*[144] and *C. acetobutylicum*[145] and the *splG* gene in *G. stearothermophilus*.[55] SP lyase requires strictly anaerobic conditions and can function in *B. subtilis* as a monomer of ca. 43 kDa[146] or as a homodimer.[147] The homodimer appears to be the active form for *G. stearothermophilus* SP lyase.[55] The SP lyase is synthesized during sporulation[148] and is present in the developing spore. During spore germination, SP lyase specifically binds to SP_{DNA} and regiospecifically cleaves the methylene bridge of SP_{DNA}, returning it to the dithymine-containing DNA without excision of the SP lesion.[101,149] The binding of SP lyase to SP_{DNA} is sequence context independent but structure specific. It encompasses a 9 bp region surrounding the SP lesion and causes significant distortion of the DNA. This is presumably because the dinucleotide part of SP_{DNA} flips out from the interior of the helix.[101] The SP motif can also be repaired at the SP_{SIDE} and SP_{TIDE} level.[54,55,64,57]

SP lyase belongs to the radical *S*-adenosylmethionine (SAM) enzyme superfamily (for recent reviews, see refs 150−153). It uses an iron−sulfur cluster as a cofactor to provide a one-electron reduction of a second cofactor, *S*-adenosylmethionine (AdoMet, **69**), to yield a 5′-deoxyadenosyl radical, which in turn initiates an hydrogen-atom abstraction reaction.[154] The iron−sulfur cluster consists of four iron ions of variable oxidation states and four inorganic sulfide ions ([4Fe-4S]) arranged in a cubane-type structure. Three of the iron atoms are coordinated to three conserved cysteine residues of the $C_{91}X_3C_{95}X_2C_{98}$ motif of the SP lyase amino acid sequence. The fourth iron is crucial for the interaction between the cluster and **69** and is coordinated to the nitrogen and a carboxylate oxygen of the methionyl group of AdoMet, **69**.[145] There is one cluster per monomeric form.[55,146] The redox scheme involved is $[Fe_4S_4]^+ \overset{e^-}{\Leftrightarrow} [Fe_4S_4]^{2+}$: the reduced form is the active one, so that in vitro SP lyase needs anaerobic conditions for its activity. However, since *Bacillus* species are aerobes, this raises the question of the influence of oxygen on SP lyase activity in vivo.

The current knowledge concerning the repair mechanism of SP lyase is presented in Scheme 7. After binding to the reduced cluster, AdoMet **69** receives an electron and undergoes a reductive cleavage, leading to the 5′-deoxyadenosyl radical **70** and methionine (**71**). This radical then abstracts an hydrogen atom from the C6 position of the 5,6-dihydropyrimidine moiety of SP_{DNA} to yield 5′-deoxyadenosine **72**. The resulting SP_{DNA} C6 radical **73** undergoes a β-scission to regenerate the two parent thymines and 5′-deoxyadenosyl radical **70** after hydrogen abstraction from 5′-deoxyadenosine **72** (Scheme 7, path a). As a consequence of the formation of **73**, the energy barrier required to break the inter-pyrimidine C5−CH_2 bond is dramatically lowered to 6.2 kcal/mol.[155] For the final step, density functional theory calculations favor a two-step mechanism involving an

Scheme 7



interthymine hydrogen-atom transfer (Scheme 6, path b).[155] Then, the 5′-deoxyadenosyl radical **70** and methionine **71** recombine to regenerate SAM **69** with loss of an electron back to the iron−sulfur cluster: this completes the catalytic repair cycle. Indeed, the role of **69** as a catalytic cofactor rather than as a cosubstrate has recently been definitively addressed.[146] Support for this repair mechanism was initially provided by chemical model experiments[108] and confirmed by tritium label transfer experiments using either C6-tritiated SP_{DNA}[156] or 5′-tritiated S-AdoMet.[146] The catalytic role of **69** was inferred from three pieces of evidence: (1) the absence of 5′-deoxyadenosine **72** from the repair reaction, (2) the repair of a larger amount of SP than the amount of **69** present, and finally (3) the full incorporation of the tritium label of 5′-tritiated **69** into the repaired thymine residues.

In addition to the critical $C_{91}X_3C_{95}X_2C_{98}$ motif, SP lyase contains a fourth conserved cysteine residue at position 141. Even if this residue does not participate in the cluster formation, cys 141 appears to play an essential role because in mutants lacking this cysteine residue SP lyase activity is lost.[157] Cysteine 141 would be crucial for the conversion of the allylic radical intermediate **74** to the parent thymine by preventing its reaction with exogenous free radicals.[158]

9. Conclusions

The unique photoreactivity of spore DNA has been described in this review. The exact nature and influence of all the factors inducing this remarkable behavior are as yet unknown. Their discovery and role remains a true challenge for the scientific community.

Since spore photoproduct formation under the influence of UV-A and -B can be in part prevented by spore outer layer components, spore photoproduct formation under UV-C radiation and its subsequent efficient repair also raises the enigmatic question of the rationale for the conservation of this process in some *Bacillus* species and also of the preservation of some of its associated molecules (SASP, Ca-DPA). Whereas it can be easily understood that a UV-C protective system was necessary for prokaryote survival when UV-C was reaching the Earth's surface, UV-C has been filtered by high atmospheric layers for millions of years and no longer *naturally* reach the Earth's surface. Even if under sunlight (i.e., UV-B + UV-A) SP_{DNA} is formed in small amounts in bacterial spore DNA, such wavelengths lead to other photoproducts whose biological importance appears also critical and consequently whose repair is highly important for spore survival. Hence, SP_{DNA} formation and repair could be viewed as a complex but seldom used protective pathway whose genetic information has, however, been neatly preserved in the spore single chromosome. It can be safely assumed that because factors essential for SP_{DNA} formation such as α/β-type SASP and Ca-DPA are also used in sporulation and spore DNA heat protection, they have necessarily been preserved through evolution. Conservation of the complex and highly specific SP lyase activity necessary for the repair of SP_{DNA} is by far the more surprising and could even be considered an ancient process.

10. Abbreviations

AIBN	2,2′-azobisisobutyronitrile
BER	base excision repair
BuLi	butyllithium
CAN	cerium ammonium nitrate
DMT	dimethoxytrityl
DPA	dipicolinic acid
DSB	double-strand break
GG-NER	global genome nucleotide excision repair
HR	homologous recombination
LDA	lithium diisopropylamide
MSMT	1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole
NER	nucleotide excision repair
NHEJ	nonhomologous end joining
PMB	*p*-methoxybenzyl
ROS	reactive oxygen species
SASP	small, acid-soluble spore proteins
SEM	2-(trimethylsilyl)ethoxymethyl
SSB	single-strand break
TBAF	tetra-*n*-butylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
TC-NER	transcription-coupled nucleotide excision repair
TES	triethylsilyl
TFA	trifluoroacetic acid

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12. References

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