The latest version is at http://www.jbc.org/cgi/doi/10.1074/jbc.R114.573675

Spore photoproduct lyase: the known, the controversial, and the unknown

Linlin Yang & Lei Li

Department of Chemistry and Chemical Biology, Indiana University-Purdue University Indianapolis (IUPUI), 402 N Blackford Street, Indianapolis, Indiana, 46202
Department of Biochemistry and Molecular Biology, Indiana University School of Medicine (IUSM), 635 Barnhill Drive, Indianapolis, Indiana 46202

To whom correspondence should be addressed: Lei Li, IUPUI, Indianapolis, Indiana, USA. Tel: (317) 278-2202; E-mail: lilei@iupui.edu

Abstract: Spore photoproduct lyase (SPL) repairs 5-thyminyl-5,6-dihydrothymine, a thymine dimer which is also called the spore photoproduct (SP), in germinating endospores. SPL is a radical *S*-adenosylmethionine (SAM) enzyme, utilizing the 5′-deoxyadenosyl radical generated by SAM reductive cleavage reaction to revert SP to two thymine residues. Here we review the current progress in SPL mechanistic studies. Protein radicals are known to be involved in SPL catalysis; however, how these radicals are quenched to close the catalytic cycle is under debate.

Introduction:

UV light induces intra-strand crosslinking reactions in DNA at bipyrimidine sites, which are mutagenic as they alter the DNA structure, inhibit polymerases, and arrest replication (1). Among the four DNA nucleobases, thymine (T) is the most sensitive to UV irradiation followed by cytosine (C) (1). In typical cells after photochemical excitation, a T residue dimerizes with an adjacent T or C, generating either the cyclobutane pyrimidine dimers (CPDs) or the pyrimidine (6-4) pyrimidone photoproducts (6-4PPs) as the major photo-lesions (Figure 1). In contrast, in bacterial endospores, the dominant DNA photoproduct is 5-thyminyl-5,6-dihydrothymine, a unique thymine dimer which is also called the spore photoproduct or SP (2-4).

Formation of SP *in vivo* is largely determined by the unique spore DNA conformation. The spore genomic DNA is saturated by a group of DNA binding proteins named small acid soluble proteins (SASPs), as they are readily soluble in 0.5 M acetic acid (5). The SASP-DNA interaction, coupled with other factors such as the low spore hydration level, changes the DNA from B-like to A-like conformation (6-9), which subsequently alters the outcome of the thymine photoreaction, making SP the dominant DNA photoproduct (3,4,6,9-11). SPs accumulate in dormant spores and are repaired rapidly when spores start germinating. Unrepaired SPs prove lethal to the resulting vegetative cells (12,13).

The germinating spores utilize two major pathways to repair SP: the general nucleotide excision repair pathway (NER) (14) and a spore-specific DNA repair system mediated by the spore photoproduct lyase (SPL) (15-18). The RecA-mediated pathway may also be involved in DNA repair, albeit to a less extent (2,19,20). Blocking either major pathway only slightly affects spores' UV sensitivity. Spores become highly sensitive to UV irradiation only when both pathways are interrupted (16,21). The NER pathway also repairs other thymine dimers such as CPDs and 6-4PPs; while SPL is specific toward SP (22-24). Both SPL and NER proteins are synthesized during sporulation and packaged in spores. The NER enzymes are expressed constitutively at a low level; while each spore contains 100~200 copies of SPL (25). However, the NER enzymes can be induced by DNA damages in germinating spores while SPL cannot. The

SPL is the major enzyme for repairing SPs, although the NER can at least partially substitute for SPL in terms of SP repair (11,16).

SPL has been long suggested to utilize a direct reversal strategy to repair SP. This conclusion was reached via a tritium labeling strategy where the radioactivity disappearing from tritiated SPs was fully recovered in thymines in NER-deficient germinating spores (18,26). SPL shares some sequence similarity with the DNA photolyase (19), which repairs CPDs and 6-4PPs under light and also utilizes a direct reversal strategy. These enzymes are structurally very different (27,28). Photolyase uses a flavin cofactor and a second chromophore to harvest light. The light energy is then passed to FADH, to trigger electron transfer to the lesion. SPL is light-independent (26); it uses the radical *S*-adenosylmethionine (SAM) chemistry to generate radical species for catalysis.

SPL mechanism - the known

SPL studies to date have established several important facts regarding the mechanism used by this intriguing DNA repair enzyme:

I. SPL repairs SP via radical SAM chemistry
Rebeil et al. found that SPL is an iron-sulfur enzyme, whose activity depends on reducing conditions and addition of SAM (25). They later established that the enzyme utilizes the 5′-deoxyadenosyl radical (5′-dA•) generated by SAM reductive cleavage to catalyze the SP reversal to two thymine residues (29) and proposed a SPL mechanism which is later proved to be largely correct. SPL is now known as a member of the radical SAM superfamily, which is defined by the characteristic CXXXCXXC motif (30), although other tri-cysteine motifs may also facilitate this radical chemistry (31-34). The three C residues in this motif binds to three irons in the [4Fe-4S] cluster; the fourth iron is coordinated by SAM (35). The cluster at its +1 oxidation state donates an electron to SAM to cleave its C5'-S bond, generating a 5′-dA•. This 5′-dA• catalyzes a number of highly diverse biochemical reactions in animals, plants and microorganisms, including steps in metabolism, DNA/RNA modification, and the biosyntheses of vitamins, coenzymes and many antibiotics (22,32-59).

II. SPL repairs the 5R-isomer of SP As shown in Figure 1, SP contains a chiral center at the C5 of 5′-T. Its chirality was predicted to be *R* by Kim et al. based on the right-handed DNA helical structure (60). This prediction was confirmed by Mantel et al. using NMR spectroscopy coupled with DFT calculations and dinucleotide SP TpT (61). Later, a dinucleotide SP structure containing a formacetal linker solved by Lin et al. further confirmed this conclusion (62). After incorporating dinucleoside 5*R*- and 5*S*-SP, which lack the phosphodiester linker between the two thymine residues, into a 12-mer oligonucleotide respectively via solid phase DNA synthesis, subsequent structural studies found that the 5*R*-SP fits in the topology of the right-handed helix well, while the 5*S*-SP results in a severe strand distortion (63). Jian et al. prepared the biologically relevant dinucleotide 5*R*-SP TpT phosphoramidite and incorporated SP TpT into an oligonucleotide. The latest structure in a 16-mer duplex oligonucleotide revealed that the 5*R*-SP TpT results in little structural distortion except widening the DNA minor groove by 2.5 Å (64). Such a small conformational change suggests that SP may be difficult to be recognized by SPL and the NER enzymes.

The biologically relevant SP stereoisomer has thus been established. Some earlier studies suggested that SPL recognizes and repairs the 5*S*-isomer (65,66), which has been corrected (67). In a recent SPL structure solved by Benjdia et al., the enzyme contains a dinucleoside 5*R*-SP, further confirming that the 5*R*-isomer is the SPL substrate (68).

III. SPL abstracts the H_{6proR} atom from SP

The first mechanistic insight of the SPL reaction was provided by Mehl and Begley from a clever small molecule model study using a bipyrimidine complex (69). Their data suggest that SP repair is initiated by H-abstraction at C6 followed by *β*-scission at the methylene bridge and back H-atom transfer. Cheek et al. utilized tritium to label the C6 of thymine and generated SPs via photoreaction under the assumption that the tritium will remain at C6 in the formed SP (70). Analysis of SP repair by SPL revealed that tritium entered the catalytic cycle, agreeing with Mehl's proposal.

Analysis of the SP structure shows that the C6 has two H-atoms and is pro-chiral. To reveal which H-atom is abstracted during SPL catalysis, Lin et al. utilized deuterium-labeled dinucleotide TpTs to generate two SP species via photoreaction, and proved that an H-atom from the 3′-CH_3 is transferred to the H_{6proS} of SP (71). Using SP with either the H_{6proR} or the H_{6proS} position labeled by a deuterium, Yang et al. proved that it is the H_{6proR} that is abstracted by the 5′-dA• in SP repair (Figure 2) (72). These results provide the chemical basis for the previous labeling studies which tritiated the –CH_3 moiety of thymine (Figure 3) (18,26). The tritium label was transferred to the H_{6proS} in photo-chemically produced SP and retained in DNA after the SPL reaction. Thus, no radioactivity loss in DNA was observed, leading to the hypothesis that SPL directly reverts SP. If the researchers chose to label thymine C6, the label at the resulting H_{6proR} of SP would leak into media after SP repair (72), potentially complicating the SPL mechanistic elucidation.

IV. A conserved cysteine as the intrinsic H-atom donor The β-elimination reaction induced by the SP C6 radical generates a thymine allylic radical, which was suggested to abstract an H-atom from the methyl group of 5′-dA (70). However, although no role was implied for the conserved cysteine141, spores carrying the C141A mutation are sensitive to UV irradiation (73). An later *in vitro* study by Chandor-Proust et al. found that the thymine allylic radical was quenched by the dithionite supplemented as a reductant to form a T-SO_2^- adduct (74), leading to a hypothesis that C141 may be the H-atom donor. Yang et al. found that the H-donor is able to exchange protons with the aqueous solution (72). They showed that C141 is solvent accessible via the iodoacetamide labeling assay; their enzyme kinetics data further indicate that the C141 in *Bs* SPL is likely the intrinsic H-donor to the thymine radical (Figure 2) (75). The conclusion is supported by the parallel SPL structural studies by Benjdia et al. using *Geobacillus thermodenitrificans (Gt)* SPL containing a dinucleoside SP and a SAM bound to the Fe-S cluster (Figure 4) (68). The conserved cysteine was implied to have no structural role. It is close to the methylene bridge of SP, supporting the assumption that it is the intrinsic H-donor.

V. Involvement of tyrosines Besides this cysteine, two conserved tyrosine residues, Y97 and Y99 in *Bs* SPL, may also be involved in enzyme catalysis. As shown in Figure 4, both tyrosines are close to SAM, suggesting that they can interact with the 5′-dA•/5′-dA pair. Y98(*Gt*), equivalent to Y99(*Bs*), is located between the conserved cysteine and SAM in the SPL structure (68), implying that it can involve in the radical propagation process (Figure 2). The Y98F(*Gt*) mutant was found to reduce the SPL activity by ~ 4-fold (76), which is consistent with the 6-fold reduction by the Y99F(*Bs*) mutant found in a separate study (77). Y96(*Gt*) seems to play a structural role to immobilize SAM at the SPL active site. However, the Y97F(*Bs*) mutation, which still enables SAM binding with the aromatic ring in phenylalanine, results in a 3-fold reduction of enzyme activity (77). Mutating both tyrosine residues almost completely abolishes the SPL activity (77). Such results, coupled with the drastically altered kinetic isotope effects between the

wildtype SPL and the Y/F mutants, indicate that the Y97(*Bs*) may also be involved in the radical transfer process, to facilitate the H-abstraction in putative SAM regeneration (77). Although the possibility that these tyrosines play structural roles to maintain the protein H-bonding network cannot be ruled out, they are more likely to be involved in the SPL radical transfer network as discussed above.

SPL mechanism - the controversial

To accommodate these results, an SPL mechanism that utilizes four hydrogen atom transfer (HAT) steps has been proposed (Figure 2). The first two HAT steps are well established; the other two steps and the question of whether SAM is regenerated are under debate:

Early tritium-labeling studies suggested that SAM is recycled (70). Cheek et al. found that ^{3}H at the C6 of SP incorporated into plasmid DNA was transferred to SAM (70). In a complementary experiment using [5′-^{3}H]-labeled SAM, it was found that ^{3}H was in the repaired thymine residues of the plasmid DNA (78). These observations supported the assumption that the thymine allylic radical directly abstracts an H-atom from the $–CH_3$ group of 5′-dA before SAM is regenerated (70,78). However, as cysteine-141(*Bs*) is now established as the H-donor, these labeling results need to be re-interpreted. The tritium transfer, especially that from SAM to thymine, may be due to reversible SAM cleavage and the subsequent H-abstraction steps, as observed in radical SAM enzymes BtrN (79) and DesII (50). This possibility needs to be tested in the future.

Studies using SP-containing pUC18 plasmid as substrate for SPL reported that one molecule of SAM catalyzed > 500 turnovers with a turnover number of 12.4 min^{-1} (70,78), indicating that SAM is used in a truly catalytic manner. However, other studies using smaller but chemically better-defined substrates have found much lower turnover numbers (72,75-77,80). Most other *in vitro* SPL studies utilized dinucleotide or dinucleoside SP as substrate and found that the ratio between 5′-dA generated and SP repaired ranges between 1 ~ 2, suggesting that SAM is partially regenerated. SPL activity using dinucleotide SP TpT as the substrate was reported to be ~ 0.3 min^{-1} by different groups (72,75,77,80), suggesting that the enzymes used were similarly active. Unpublished results from our laboratory however found SPL to repair SP in a 20-nucleotide duplex prepared via DNA synthesis (81) at only 0.08 ± 0.01 min^{-1}. This implies that the use of dinucleotide substrates is not the reason for the much slower kinetics of SPL compared with the reported values for the enzyme reacting with plasmid DNA (70,78).

The reason for these discrepancies is unclear, as the methodologies used by the various laboratories to analyze the products of the reaction do not differ that greatly. Collectively, it is safe to state that the *in vitro* data so far are insufficient to support a full SAM regeneration in SPL catalysis.

Despite the controversy, SAM regeneration is still the most reasonable route to close the catalytic cycle. As the conserved cysteine is known as the H-donor, a thiol radical will be generated on this residue. As a tyrosine (Y99 in *Bs* SPL) is next to the cysteine, its oxidation to a tyrosyl radical is possible. These radicals have to be reduced before the enzyme is ready for the next turnover. Considering the lack of other redox co-factors as revealed by the SPL structure (68), to abstract an H-atom from 5′-dA and subsequently regenerate SAM is still the most reasonable hypothesis, as shown by Figure 2.

Despite the attempts to trap and characterize the putative radical species involved in SPL catalysis during steady-state turnover, no radical species were observed by EPR spectroscopy (70,77). Based on the UV-visible difference spectrum after an overnight

reaction, Kneuttinger et al. reported the presence of a tyrosyl radical in SPL (28,76). However, the spectrum did not resemble the sharp-peak absorbance exhibited by a typical Y• (82-84), indicating that it may be due to the spectral difference between the Fe-S chromophores, rather than Y•. It is possible that the putative radical transfer pathway is tightly coupled like that in the class I ribonucleotide reductase (85,86), where its existence is not fully established until the transient tyrosyl radicals in the middle of the pathway were observed using unnatural tyrosines to fine-tune the redox potential and disturb the radical transfer process (87).

SPL mechanism - the unknown

The differing results from these *in vitro* studies question whether the current enzyme kinetic data truly reflect the SP repair *in vivo*. To clarify the controversy, some key questions need to be addressed in future SPL studies:

I. SPL efficiency in vivo It is found that >75% of SPs are repaired during the first hour of spore germination (11). However, the absolute quantity of repaired SPs is unclear. The *B. subtilis* genome has 4.2 million base pairs (88). As each spore may contain up to 200 copies of SPL (25), if 1% of total Ts are converted to SPs (6,89), each SPL has to repair ~ 80 SPs in 60 minutes in germinating spores. As the rate-limiting step for *in vivo* repair is likely to be damage recognition (90), the enzyme repair should be faster. However, the reported repair rate for dinucleotide SP TpT is only ~ 0.35 min^{-1} (24,72,80); the rate in duplex DNA may be even slower. Such slow rates cannot explain the fact that SPL plays a major role in SP repair in germinating spores. However, if only 0.1% of Ts are converted to SPs, the reported rates may be sufficient.

II. DNA conformation during in vivo repair

Besides the abnormally slow repair rate, the fact that the repair of dinucleotide SP TpT is 3~4 fold faster than that in a duplex DNA is also perplexing. SPL is found to bind 9 nucleotides (23) via electrostatic interaction between the negatively charged phosphates on DNA and the positively charged protein surface. Thus, its binding affinity toward an oligonucleotide should be much higher than that to SP TpT, which is expected to result in a faster SP repair in a duplex DNA. However, the opposite is observed. SPL is suggested to flip-out SP for repair (24); the structural study has identified a β-hairpin with an arginine and a tyrosine at the top to assist this lesion flipping process (68). Given that the SP TpT mimics the flipped-out stage, the slower repair rate implies that the SP in a duplex DNA may be difficult to be flipped out into the SPL active site. This hypothesis is supported by the observation that the hydrogen bonds between SP and the complementary adenines appear even strengthened in duplex DNA, as shown by an X-ray crystallographic study (64).

It is thus of interest to reveal how the SP-flipping process is achieved *in vivo*. One solution could be the involvement of other enzymes. As shown by the NER enzymes, UvrA recognizes the DNA damage, and UvrB bends the DNA before catalyzing the DNA hydrolysis reaction with UvrC (91,92). Given the minor DNA conformational change induced by SP (64), it is an intriguing possibility that SPL may have to team with other protein(s) for efficient damage recognition and repair. Alternatively, as the genomic DNA changes conformation from A- to B-form during spore germination (20), the DNA may adopt some transient conformation to facilitate the SP flip-out and repair by SPL.

III condition for SAM regeneration The lack of reactive DNA conformation may also explain why SAM regeneration cannot be observed in the *in vitro* studies so far. The reactive DNA conformer likely helps SP flip-out, accelerates the enzyme reaction, and induces the required protein conformation for SAM regeneration. Another possibility is that the appropriate experimental conditions for

SAM regeneration have not been adopted in *in vitro* studies. As shown by the investigations of lysine 2,3-aminomutase (93) and 7-carboxy-7-deazaguanine synthase (57), the two radical SAM enzymes known to use SAM catalytically, the *in vitro* experiments are sensitive to the reducing conditions adopted. Even though great care has been taken in SPL studies (72,75,77), we cannot completely exclude the possibility that the *in vitro* condition which potentially mimics the *in vivo* SP repair and enables SAM regeneration has not been identified.

Summary and perspective

After the past > 40 years of studies, the first several steps in SPL catalysis have been well-established. The remaining issues are mainly about how to close the catalytic cycle. To clarify the controversy in SPL catalysis, especially in a duplex DNA environment, the interaction between SPL and SP-containing oligonucleotide needs to be revealed. This will allow us to identify a biologically relevant system for mechanistic enzymology studies. In addition, the improved enzyme efficiency will enable us to trap and characterize the putative radical species via fast enzyme kinetic means and answer questions such as whether SAM is recycled, and whether the recycling requires the protein harbored radical transfer pathway.

Acknowledgement

The manuscript preparation was supported by the National Institute of Health under grant R00ES017177 and IUPUI startup fund. We thank the reviewers and Professor Neil Marsh at the University of Michigan for their insightful comments.

References

1. Cadet, J.; Vigny, P. In: *Bioorganic Photochemistry,Vol 1*: *Photochemistry and the nucleic acids*; Morrison, H., Ed.; Wiley: New York, **1990**; Vol. 1, p 1-272
2. Setlow, P. (2001) Resistance of spores of *Bacillus* species to ultraviolet light. *Environ. Mol. Mutagen.* **38**, 97-104
3. Desnous, C. l., Guillaume, D., and Clivio, P. (2010) Spore Photoproduct: A Key to Bacterial Eternal Life. *Chem. Rev.* **110**, 1213-1232
4. Li, L. (2012) Mechanistic studies of the radical SAM enzyme spore photoproduct lyase (SPL). *Biochim. Biophys. Acta - Proteins and Proteomics* **1824**, 1264-1277
5. Setlow, P. (1988) Small, acid-soluble spore proteins of *Bacillus* species: structure, synthesis, genetics, function, and degradation. *Annu. Rev. Microbiol.* **42**, 319-338
6. Nicholson, W. L., Setlow, B., and Setlow, P. (1991) Ultraviolet irradiation of DNA complexed with alpha/beta-type small, acid-soluble proteins from spores of *Bacillus* or *Clostridium* species makes spore photoproduct but not thymine dimers. *Proc. Natl. Acad. Sci. USA* **88**, 8288-8292
7. Nicholson, W. L., Setlow, B., and Setlow, P. (1990) Binding of DNA in vitro by a small, acid-soluble spore protein from *Bacillus subtilis* and the effect of this binding on DNA topology. *J. Bacteriol.* **172**, 6900-6906
8. Setlow, B., Hand, A. R., and Setlow, P. (1991) Synthesis of a *Bacillus subtilis* small, acid-soluble spore protein in *Escherichia coli* causes cell DNA to assume some characteristics of spore DNA. *J. Bacteriol.* **173**, 1642-1653
9. Mohr, S. C., Sokolov, N. V., He, C. M., and Setlow, P. (1991) Binding of small acid-soluble spore proteins from *Bacillus subtilis* changes the conformation of DNA from B to A. *Proc. Natl. Acad. Sci. USA* **88**, 77-81

10. Lee, K. S., Bumbaca, D., Kosman, J., Setlow, P., and Jedrzejas, M. J. (2008) Structure of a protein-DNA complex essential for DNA protection in spores of *Bacillus* species. *Proc. Natl. Acad. Sci. USA* **105**, 2806-2811
11. Moeller, R., Douki, T., Cadet, J., Stackebrandt, E., Nicholson, W. L., Rettberg, P., Reitz, G., and Horneck, G. (2007) UV-radiation-induced formation of DNA bipyrimidine photoproducts in *Bacillus subtilis* endospores and their repair during germination. *Int. Microbiol.* **10**, 39-46
12. Setlow, B., and Setlow, P. (1988) Decreased UV light resistance of spores of *Bacillus subtilis* strains deficient in pyrimidine dimer repair and small, acid-soluble spore proteins. *Appl. Environ. Microbiol.* **54**, 1275-1276
13. Setlow, P. (1995) Mechanisms for the Prevention of Damage to DNA in Spores of *Bacillus* Species. *Ann. Rev. Microbiol.* **49**, 29-54
14. Munakata, N. (1977) Mapping of the genes controlling excision repair of Pyrimidine photoproducts in *Bacillus subtilis*. *Mol. Gen. Genet.* **156**, 49-54
15. Fajardo-Cavazos, P., Salazar, C., and Nicholson, W. L. (1993) Molecular cloning and characterization of the *Bacillus subtilis* spore photoproduct lyase (spl) gene, which is involved in repair of UV radiation-induced DNA damage during spore germination. *J. Bacteriol.* **175**, 1735-1744
16. Munakata, N., and Rupert, C. S. (1972) Genetically Controlled Removal of "Spore Photoproduct" from Deoxyribonucleic Acid of Ultraviolet-Irradiated *Bacillus subtilis* Spores. *J. Bacteriol.* **111**, 192-198
17. Munakata, N., and Rupert, C. S. (1974) Dark repair of DNA containing “spore photoproduct” in Bacillus subtilis. *Mol. Gen. Genet.* **130**, 239-250
18. Wang, T.-C. V., and Rupert, C. S. (1977) Evidence for the monomerization of spore photoproduct to two thymines by the light-independent "spore repair" process in *Bacillus subtilis*. *Photochem. Photobiol.* **25**, 123-127
19. Nicholson, W. L., Munakata, N., Horneck, G., Melosh, H. J., and Setlow, P. (2000) Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiol. Mol. Biol. Rev.* **64**, 548-572
20. Nicholson, W., Schuerger, A., and Setlow, P. (2005) The solar UV environment and bacterial spore UV resistance: considerations for Earth-to-Mars transport by natural processes and human spaceflight. *Mutat. Res-Fund. Mol. M.* **571**, 249-264
21. Munakata, N. (1969) Genetic analysis of a mutant of *Bacillus subtilis* producing ultraviolet-sensitive spores. *Mol. Gen. Genet.* **104**, 258-263
22. Broderick, J. B., Duffus, B. R., Duschene, K. S., and Shepard, E. M. (2014) Radical S-Adenosylmethionine Enzymes. *Chem. Rev.,* **114**, 4229-4317
23. Slieman, T. A., Rebeil, R., and Nicholson, W. L. (2000) Spore photoproduct (SP) lyase from Bacillus subtilis specifically binds to and cleaves SP (5-thyminyl-5,6-dihydrothymine) but not cyclobutane pyrimidine dimers in UV-irradiated DNA. *J. Bacteriol.* **182**, 6412-6417
24. Chandor, A., Berteau, O., Douki, T., Gasparutto, D., Sanakis, Y., Ollagnier-De-Choudens, S., Atta, M., and Fontecave, M. (2006) Dinucleotide spore photoproduct, a minimal substrate of the DNA repair spore photoproduct lyase enzyme from *Bacillus subtilis*. *J. Biol. Chem.* **281**, 26922-26931
25. Rebeil, R., Sun, Y., Chooback, L., Pedraza-Reyes, M., Kinsland, C., Begley, T. P., and Nicholson, W. L. (1998) Spore photoproduct lyase from *Bacillus subtilis* spores is a

novel iron-sulfur DNA repair enzyme which shares features with proteins such as class III anaerobic ribonucleotide reductases and pyruvate-formate lyases. *J. Bacteriol.* **180**, 4879-4885
26. Donnellan, J. E., and Stafford, R. S. (1968) The Ultraviolet Photochemistry and Photobiology of Vegetative Cells and Spores of Bacillus megaterium. *Biophys. J.* **8**, 17-28
27. Benjdia, A. (2012) DNA photolyases and SP lyase: structure and mechanism of light-dependent and independent DNA lyases. *Curr. Opin. Struct. Biol.* **22**, 711-720
28. Kneuttinger, A. C., Kashiwazaki, G., Prill, S., Heil, K., Müller, M., and Carell, T. (2014) Formation and Direct Repair of UV-induced Dimeric DNA Pyrimidine Lesions. *Photochem. Photobiol.* **90**, 1-14
29. Rebeil, R., and Nicholson, W. L. (2001) The subunit structure and catalytic mechanism of the *Bacillus subtilis* DNA repair enzyme spore photoproduct lyase. *Proc. Natl. Acad. Sci. USA* **98**, 9038-9043
30. Sofia, H. J., Chen, G., Hetzler, B. G., Reyes-Spindola, J. F., and Miller, N. E. (2001) Radical SAM, a novel protein superfamily linking unresolved steps in familiar biosynthetic pathways with radical mechanisms: functional characterization using new analysis and information visualization methods. *Nucleic Acids Res.* **29**, 1097-1106
31. Marsh, E. N. G., Patterson, D. P., and Li, L. (2010) Adenosyl Radical: Reagent and Catalyst in Enzyme Reactions. *ChemBioChem* **11**, 604-621
32. Chatterjee, A., Li, Y., Zhang, Y., Grove, T. L., Lee, M., Krebs, C., Booker, S. J., Begley, T. P., and Ealick, S. E. (2008) Reconstitution of ThiC in thiamine pyrimidine biosynthesis expands the radical SAM superfamily. *Nat. Chem. Biol.* **4**, 758-765
33. Paraskevopoulou, C., Fairhurst, S. A., Lowe, D. J., Brick, P., and Onesti, S. (2006) The elongator subunit Elp3 contains a Fe_4S_4 cluster and binds *S*-adenosylmethionine. *Mol. Microbiol.* **59**, 795-806
34. Kamat, S. S., Williams, H. J., Dangott, L. J., Chakrabarti, M., and Raushel, F. M. (2013) The catalytic mechanism for aerobic formation of methane by bacteria. *Nature* **497**, 132-136
35. Walsby, C. J., Ortillo, D., Yang, J., Nnyepi, M. R., Broderick, W. E., Hoffman, B. M., and Broderick, J. B. (2005) Spectroscopic approaches to elucidating novel iron-sulfur chemistry in the "radical-SAM" protein superfamily. *Inorg. Chem.* **44**, 727-741
36. Henshaw, T. F., Cheek, J., and Broderick, J. B. (2000) The [4Fe-4S](1+) cluster of pyruvate formate-lyase activating enzyme generates the glycyl radical on pyruvate formate-lyase: EPR-detected single turnover. *J. Am. Chem. Soc.* **122**, 8331-8332
37. Vey, J. L., and Drennan, C. L. (2011) Structural Insights into Radical Generation by the Radical SAM Superfamily. *Chem. Rev.* **111**, 2487-2506
38. Frey, P., and Magnusson, O. (2003) *S*-Adenosylmethionine: A wolf in sheep's clothing, or a rich man's adenosylcobalamin? *Chem. Rev.* **103**, 2129-2148
39. Marsh, E., Patwardhan, A., and Huhta, M. (2004) *S*-Adenosylmethionine radical enzymes. *Bioorg. Chem.* **32**, 326-340
40. Decamps, L., Philmus, B., Benjdia, A., White, R., Begley, T. P., and Berteau, O. (2012) Biosynthesis of F0, precursor of the F420 cofactor, requires a unique two radical-SAM domain enzyme and tyrosine as substrate. *J. Am. Chem. Soc.* **134**, 18173-18176
41. Benjdia, A., Leprince, J. r. m., Sandström, C., Vaudry, H., and Berteau, O. (2009) Mechanistic Investigations of Anaerobic Sulfatase-Maturating Enzyme: Direct Cβ H-

Atom Abstraction Catalyzed by a Radical AdoMet Enzyme. *J. Am. Chem. Soc.* **131**, 8348-8349
42. Padovani, D., Thomas, F., Trautwein, A. X., Mulliez, E., and Fontecave, M. (2001) Activation of class III ribonucleotide reductase from *E-coli.* The electron transfer from the iron-sulfur center to S-adenosylmethionine. *Biochemistry* **40**, 6713-6719
43. Grove, T. L., Ahlum, J. H., Sharma, P., Krebs, C., and Booker, S. J. (2010) A Consensus Mechanism for Radical SAM-Dependent Dehydrogenation? BtrN Contains Two [4Fe-4S] Clusters. *Biochemistry* **49**, 3783-3785
44. Boal, A. K., Grove, T. L., McLaughlin, M. I., Yennawar, N. H., Booker, S. J., and Rosenzweig, A. C. (2011) Structural Basis for Methyl Transfer by a Radical SAM Enzyme. *Science* **332**, 1089-1092
45. Grove, T. L., Benner, J. S., Radle, M. I., Ahlum, J. H., Landgraf, B. J., Krebs, C., and Booker, S. J. (2011) A Radically Different Mechanism for *S*-Adenosylmethionine–Dependent Methyltransferases. *Science* **332**, 604-607
46. McCusker, K. P., Medzihradszky, K. F., Shiver, A. L., Nichols, R. J., Yan, F., Maltby, D. A., Gross, C. A., and Galonić Fujimori, D. (2012) Covalent Intermediate in the Catalytic Mechanism of the Radical *S*-Adenosyl-L-methionine Methyl Synthase RlmN Trapped by Mutagenesis. *J. Am. Chem. Soc.* **134**, 18074-18081
47. Fugate, C. J., Stich, T. A., Kim, E. G., Myers, W. K., Britt, R. D., and Jarrett, J. T. (2012) 9-Mercaptodethiobiotin Is Generated as a Ligand to the [2Fe–2S]+ Cluster during the Reaction Catalyzed by Biotin Synthase from *Escherichia coli*. *J. Am. Chem. Soc.* **134**, 9042-9045
48. Zhang, Q., Li, Y., Chen, D., Yu, Y., Duan, L., Shen, B., and Liu, W. (2011) Radical-mediated enzymatic carbon chain fragmentation-recombination. *Nat. Chem. Biol.* **7**, 154-160
49. Ruszczycky, M. W., Choi, S. H., and Liu, H. W. (2013) EPR-kinetic isotope effect study of the mechanism of radical-mediated dehydrogenation of an alcohol by the radical SAM enzyme DesII. *Proc. Natl. Acad. Sci. U S A* **110**, 2088-2093
50. Szu, P.-H., Ruszczycky, M. W., Choi, S.-h., Yan, F., and Liu, H.-w. (2009) Characterization and Mechanistic Studies of DesII: A Radical *S*-Adenosyl-*L*-methionine Enzyme Involved in the Biosynthesis of TDP-d-Desosamine. *J. Am. Chem. Soc.* **131**, 14030-14042
51. Ruszczycky, M. W., Choi, S.-h., Mansoorabadi, S. O., and Liu, H.-w. (2011) Mechanistic Studies of the Radical *S*-Adenosyl-L-methionine Enzyme DesII: EPR Characterization of a Radical Intermediate Generated During Its Catalyzed Dehydrogenation of TDP-d-Quinovose. *J. Am. Chem. Soc.* **133**, 7292-7295
52. Dowling, D. P., Bruender, N. A., Young, A. P., McCarty, R. M., Bandarian, V., and Drennan, C. L. (2014) Radical SAM enzyme QueE defines a new minimal core fold and metal-dependent mechanism. *Nat. Chem. Biol.* **10**, 106-112
53. Kuchenreuther, J. M., Myers, W. K., Stich, T. A., George, S. J., NejatyJahromy, Y., Swartz, J. R., and Britt, R. D. (2013) A Radical Intermediate in Tyrosine Scission to the CO and CN− Ligands of FeFe Hydrogenase. *Science* **342**, 472-475
54. Mehta, A. P., Abdelwahed, S. H., and Begley, T. P. (2013) Molybdopterin Biosynthesis: Trapping an Unusual Purine Ribose Adduct in the MoaA-Catalyzed Reaction. *J. Am. Chem. Soc.* **135**, 10883-10885

55. Kim, H. J., McCarty, R. M., Ogasawara, Y., Liu, Y.-n., Mansoorabadi, S. O., LeVieux, J., and Liu, H.-w. (2013) GenK-Catalyzed C-6′ Methylation in the Biosynthesis of Gentamicin: Isolation and Characterization of a Cobalamin-Dependent Radical SAM Enzyme. *J. Am. Chem. Soc.,* **135**, 8093-8096
56. Hover, B. M., Loksztejn, A., Ribeiro, A. A., and Yokoyama, K. (2013) Identification of a Cyclic Nucleotide as a Cryptic Intermediate in Molybdenum Cofactor Biosynthesis. *J. Am. Chem. Soc.* **135**, 7019-7032
57. McCarty, R. M., Krebs, C., and Bandarian, V. (2013) Spectroscopic, Steady-State Kinetic, and Mechanistic Characterization of the Radical SAM Enzyme QueE, Which Catalyzes a Complex Cyclization Reaction in the Biosynthesis of 7-Deazapurines. *Biochemistry* **52**, 188-198
58. Forouhar, F., Arragain, S., Atta, M., Gambarelli, S., Mouesca, J.-M., Hussain, M., Xiao, R., Kieffer-Jaquinod, S., Seetharaman, J., Acton, T. B., Montelione, G. T., Mulliez, E., Hunt, J. F., and Fontecave, M. (2013) Two Fe-S clusters catalyze sulfur insertion by radical-SAM methylthiotransferases. *Nat. Chem. Biol.* **9**, 333-338
59. Goldman, P. J., Grove, T. L., Booker, S. J., and Drennan, C. L. (2013) X-ray analysis of butirosin biosynthetic enzyme BtrN redefines structural motifs for AdoMet radical chemistry. *Proc. Natl. Acad. Sci. USA* **110**, 15949-15954
60. Kim, S. J., Lester, C., and Begley, T. P. (1995) Synthesis of the Dinucleotide Spore Photoproduct. *J. Org. Chem.* **60**, 6256-6257
61. Mantel, C., Chandor, A., Gasparutto, D., Douki, T., Atta, M., Fontecave, M., Bayle, P. A., Mouesca, J. M., and Bardet, M. (2008) Combined NMR and DFT studies for the absolute configuration elucidation of the spore photoproduct, a UV-induced DNA lesion. *J. Am. Chem. Soc.* **130**, 16978-16984
62. Lin, G., Chen, C.-H., Pink, M., Pu, J., and Li, L. (2011) Chemical Synthesis, Crystal Structure and Enzymatic Evaluation of a Dinucleotide Spore Photoproduct Analogue Containing a Formacetal Linker. *Chem. Eur. J.* **17**, 9658-9668
63. Heil, K., Kneuttinger, A. C., Schneider, S., Lischke, U., and Carell, T. (2011) Crystal Structures and Repair Studies Reveal the Identity and the Base-Pairing Properties of the UV-Induced Spore Photoproduct DNA Lesion. *Chem. Eur. J.* **17**, 9651 – 9657
64. Singh, I., Lian, Y., Li, L., and Georgiadis, M. M. (2014) The structure of an authentic spore photoproduct lesion in DNA suggests a basis for recognition. *Acta Crystallogr. D* **70**, 752-759
65. Friedel, M., Berteau, O., Pieck, J., Atta, M., Ollagnier-de-Choudens, S., Fontecave, M., and Carell, T. (2006) The spore photoproduct lyase repairs the 5*S*- and not the 5*R*-configured spore photoproduct DNA lesion. *Chem. Commun.*, 445-447
66. Friedel, M., Pieck, J., Klages, J., Dauth, C., Kessler, H., and Carell, T. (2006) Synthesis and stereochemical assignment of DNA spore photoproduct analogues. *Chem. Eur. J.* **12**, 6081-6094
67. Chandra, T., Silver, S. C., Zilinskas, E., Shepard, E. M., Broderick, W. E., and Broderick, J. B. (2009) Spore Photoproduct Lyase Catalyzes Specific Repair of the 5*R* but Not the 5*S* Spore Photoproduct. *J. Am. Chem. Soc.* **131**, 2420-2421
68. Benjdia, A., Heil, K., Barends, T. R. M., Carell, T., and Schlichting, I. (2012) Structural insights into recognition and repair of UV-DNA damage by Spore Photoproduct Lyase, a radical SAM enzyme. *Nucleic Acids Res.* **40**, 9308-9318

69. Mehl, R. A., and Begley, T. P. (1999) Mechanistic studies on the repair of a novel DNA photolesion: The spore photoproduct. *Org. Lett.* **1**, 1065-1066
70. Cheek, J., and Broderick, J. (2002) Direct H atom abstraction from spore photoproduct C-6 initiates DNA repair in the reaction catalyzed by spore photoproduct lyase: Evidence for a reversibly generated adenosyl radical intermediate. *J. Am. Chem. Soc.* **124**, 2860-2861
71. Lin, G., and Li, L. (2010) Elucidation of Spore-Photoproduct Formation by Isotope Labeling. *Angew. Chem. Int. Ed.* **49**, 9926-9929
72. Yang, L., Lin, G., Liu, D., Dria, K. J., Telser, J., and Li, L. (2011) Probing the Reaction Mechanism of Spore Photoproduct Lyase (SPL) via Diastereoselectively Labeled Dinucleotide SP TpT Substrates. *J. Am. Chem. Soc.* **133**, 10434-10447
73. Fajardo-Cavazos, P., Rebeil, R., and Nicholson, W. (2005) Essential cysteine residues in Bacillus subtilis spore photoproduct lyase identified by alanine scanning mutagenesis. *Curr. Microbiol.* **51**, 331-335
74. Chandor-Proust, A., Berteau, O., Douki, T., Gasparutto, D., Ollagnier-de-Choudens, S., Fontecave, M., and Atta, M. (2008) DNA repair and free radicals, new insights into the mechanism of spore photoproduct lyase revealed by single amino Acid substitution. *J. Biol. Chem.* **283**, 36361-36368
75. Yang, L., Lin, G., Nelson, R. S., Jian, Y., Telser, J., and Li, L. (2012) Mechanistic Studies of the Spore Photoproduct Lyase via a Single Cysteine Mutation. *Biochemistry* **51**, 7173-7188
76. Kneuttinger, A. C., Heil, K., Kashiwazaki, G., and Carell, T. (2013) The radical SAM enzyme spore photoproduct lyase employs a tyrosyl radical for DNA repair. *Chem. Commun.* **49**, 722-724
77. Yang, L., Nelson, R. S., Benjdia, A., Lin, G., Telser, J., Stoll, S., Schlichting, I., and Li, L. (2013) A Radical Transfer Pathway in Spore Photoproduct Lyase. *Biochemistry* **52**, 3041-3050
78. Buis, J., Cheek, J., Kalliri, E., and Broderick, J. (2006) Characterization of an active spore photoproduct lyase, a DNA repair enzyme in the radical *S*-adenosylmethionine superfamily. *J. Biol. Chem.* **281**, 25994-26003
79. Yokoyama, K., Numakura, M., Kudo, F., Ohmori, D., and Eguchi, T. (2007) Characterization and mechanistic study of a radical SAM dehydrogenase in the biosynthesis of butirosin. *J. Am. Chem. Soc.* **129**, 15147-15155
80. Silver, S., Chandra, T., Zilinskas, E., Ghose, S., Broderick, W., and Broderick, J. (2010) Complete stereospecific repair of a synthetic dinucleotide spore photoproduct by spore photoproduct lyase. *J. Biol. Inorg. Chem.* **15**, 943-955
81. Jian, Y., and Li, L. (2013) Chemical Syntheses of Oligodeoxyribonucleotides Containing Spore Photoproduct. *J. Org. Chem.* **78**, 3021-3029
82. Cotruvo, J. A., and Stubbe, J. (2011) *Escherichia coli* Class Ib Ribonucleotide Reductase Contains a Dimanganese(III)-Tyrosyl Radical Cofactor in Vivo. *Biochemistry* **50**, 1672-1681
83. Torrents, E., Sahlin, M., Biglino, D., Gräslund, A., and Sjöberg, B.-M. (2005) Efficient growth inhibition of *Bacillus anthracis* by knocking out the ribonucleotide reductase tyrosyl radical. *Proc. Natl. Acad. Sci. USA* **102**, 17946-17951

84. Bollinger, J. M., Jr., Edmondson, D. E., Huynh, B. H., Filley, J., Norton, J. R., and Stubbe, J. (1991) Mechanism of assembly of the tyrosyl radical-dinuclear iron cluster cofactor of ribonucleotide reductase. *Science* **253**, 292-298
85. Stubbe, J., and van der Donk, W. A. (1998) Protein Radicals in Enzyme Catalysis. *Chem. Rev.* **98**, 705-762
86. Stubbe, J., Nocera, D. G., Yee, C. S., and Chang, M. C. Y. (2003) Radical initiation in the class I ribonucleotide reductase: Long-range proton-coupled electron transfer? *Chem. Rev.* **103**, 2167-2201
87. Minnihan, E. C., Nocera, D. G., and Stubbe, J. (2013) Reversible, long-range radical transfer in *E. coli* class Ia ribonucleotide reductase. *Acc. Chem. Res.* **46**, 2524-2535
88. Kunst, F., Ogasawara, N., Moszer, I., Albertini, A. M., Alloni, G., Azevedo, V., Bertero, M. G., Bessieres, P., Bolotin, A., Borchert, S., Borriss, R., Boursier, L., Brans, A., Braun, M., Brignell, S. C., Bron, S., Brouillet, S., Bruschi, C. V., Caldwell, B., Capuano, V., Carter, N. M., Choi, S. K., Codani, J. J., Connerton, I. F., Danchin, A., and et al. (1997) The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*. *Nature* **390**, 249-256
89. Setlow, B., and Setlow, P. (1993) Dipicolinic Acid Greatly Enhances Production of Spore Photoproduct in Bacterial Spores upon UV Irradiation. *Appl. Environ. Microb.* **59**, 640-643
90. Siede, W., Kow, Y. W., and Doetsch, P. W. (2005) *DNA damage recognition*, Taylor & Francis, Boca Raton
91. Van Houten, B., Croteau, D. L., DellaVecchia, M. J., Wang, H., and Kisker, C. (2005) 'Close-fitting sleeves': DNA damage recognition by the UvrABC nuclease system. *Mutat. Res.-Fund. Mol. M.* **577**, 92-117
92. Croteau, D. L., DellaVecchia, M. J., Perera, L., and Van Houten, B. (2008) Cooperative damage recognition by UvrA and UvrB: identification of UvrA residues that mediate DNA binding. *DNA Repair* **7**, 392-404
93. Lieder, K. W., Booker, S., Ruzicka, F. J., Beinert, H., Reed, G. H., and Frey, P. A. (1998) S-Adenosylmethionine-dependent reduction of lysine 2,3-aminomutase and observation of the catalytically functional iron-sulfur centers by electron paramagnetic resonance. *Biochemistry* **37**, 2578-2585

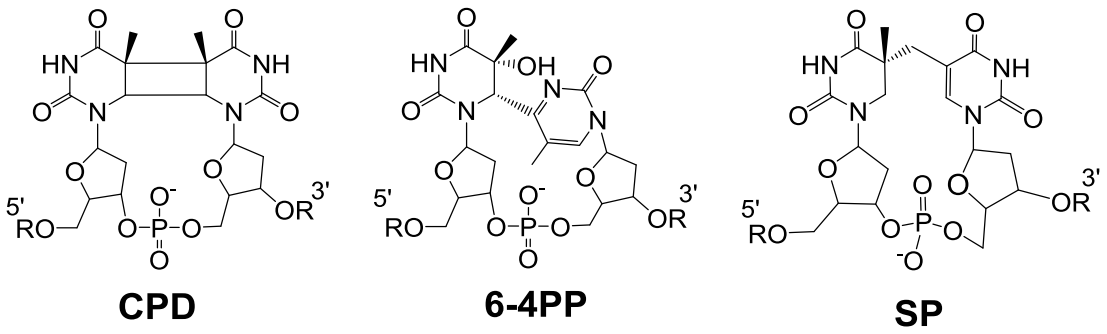

Figure 1. The chemical structures of naturally occurring thymine photo-dimers.

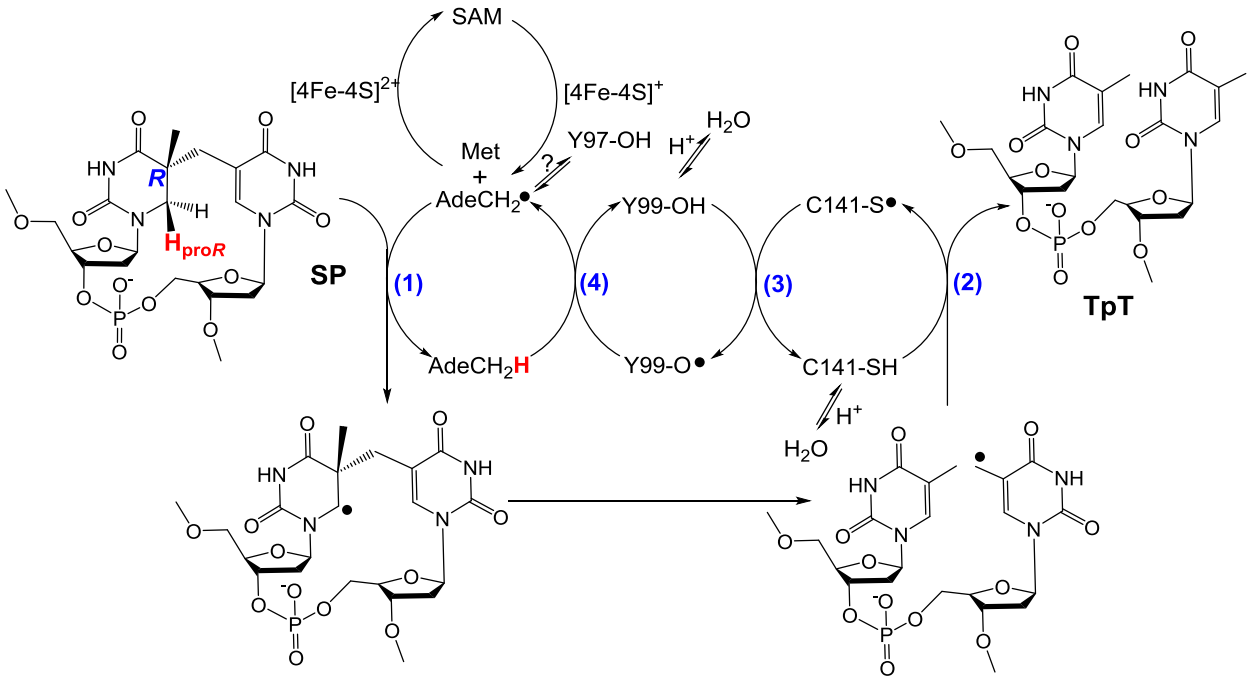

Figure 2. The hypothesized reaction mechanism for SPL (The residues are numbered according to the protein sequence in *B subtilis* SPL). This mechanism implies that SPL uses a minimum of four H atom transfer processes (labeled in blue numbers) in each catalytic cycle. The first two HAT processes are well-established and the last two HAT processes are under debate. SAM is shown to be regenerated at the end of the catalytic cycle, which is also controversial. (The figure is modified with permission from reference 77. Copyright (2013) American Chemical Society)

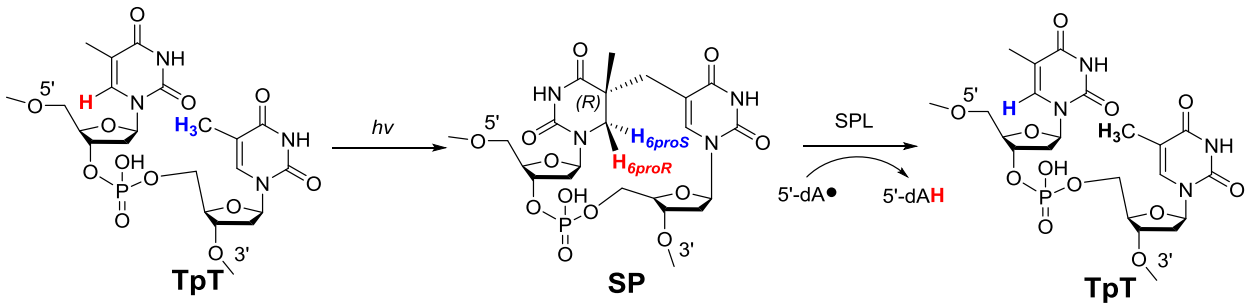

Figure 3. H-atom migration during the SP formation and repair by SPL. An H-atom migrates to the H_{6proS} position of the formed SP; while the H_{6proR} atom is abstracted to initiate the SP repair process. Therefore, the H6 atom of re-generated 5′-thymine after SPL repair is different from that before SP is formed. This observation provides the rationale for the previous tritium labeling experiments.

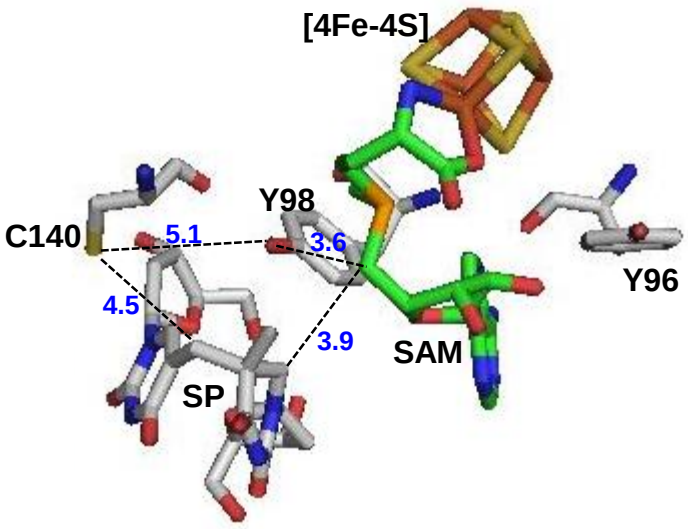

Figure 4. The active site of *Geobacillus thermodenitrificans (Gt)* SPL in complex with SP and SAM. $C140_{(Gt)}$, $Y96_{(Gt)}$ and $Y98_{(Gt)}$ equal to $C141_{(Bs)}$, $Y97_{(Bs)}$ and $Y99_{(Bs)}$ in *B. subtilis* SPL respectively. The distances (Å) between selected residues, SP and SAM are indicated by the blue numbers near the dashed lines (PDB code 4FHD).