

AN ABSTRACT OF THE THESIS OF

John C. Stenberg for the degree of Honors Baccalaureate of Science in Biochemistry and Biophysics and Honors Baccalaureate of Arts in International Studies presented May 31, 2006. Title: DNA UV Damage-related Control of Ribonucleotide Reductase; Possible Relationship to Mutagenesis and the Incidence of and Attitudes toward Skin Cancer in Several Western Nations.

Abstract Approved: _____
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Two strains of *Escherichia coli* were studied to determine the effect of constitutive expression of the SOS DNA damage repair response on the activity of ribonucleotide reductase. Activity of the reductase and cellular deoxynucleoside triphosphate (dNTP) pools were both determined before and after exposure of the cells to ultraviolet radiation. The experiments showed no significant difference in enzyme activity or cellular dNTP pool levels between normal *E. coli* and cells constitutively expressing the SOS DNA damage repair response. Although the SOS response and proportional accumulation of dNTPs both lead to DNA mutation, the occurrence of the latter after a damage event is not controlled or promoted by the first.

Cellular DNA damage due to ultraviolet light is of concern to humans because it can lead to skin cancer. In addition to laboratory research, the topic of skin cancer in the Western world was also examined. Data from cancer indices and journal articles were used to analyze the incidence of non-melanoma and malignant melanoma skin cancers. Studies analyzing the awareness of skin cancer amongst the populations of several Western nations were also discussed. The attitudes of people toward skin cancer and sun exposure were found not to correlate with statistical risk of developing the cancer.

Key Words:

Ribonucleotide (rNDP) reductase
Error-prone SOS repair response
Squamous / Basal cell carcinoma
Malignant melanoma

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DNA UV Damage-related Control of Ribonucleotide Reductase:

Possible Relationship to Mutagenesis

and

The Incidence of and Attitudes toward Skin Cancer in Several Western Nations

By

John C. Stenberg

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John C. Stenberg

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***DNA UV Damage-related Control of Ribonucleotide Reductase; Possible
Relationship to Mutagenesis***

Introduction

Ribonucleotide Reductase

With the exception of RNA viruses, deoxyribonucleic acid (DNA) is the genetic material in all living things. The sequence of its subunits directs the production of proteins, which then act to manage all metabolic processes of the cell and organism as a whole. DNA is composed of subunit nucleotides consisting of a sugar, 2-deoxyribose, a phosphate group, and one of four bases. DNA precursors, the four deoxyribonucleoside triphosphates (dNTPs) are called deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (dCTP), and deoxythymidine triphosphate (dTTP). The de novo precursor to each of the four deoxyribonucleoside triphosphates is its corresponding ribonucleoside diphosphate (rNDP).

Ribonucleotides are at least five times more abundant in the cell than deoxyribonucleotides. They also have multiple metabolic roles. Deoxyribonucleotides, by comparison, are thought only to serve the cell as precursors to DNA. As such, their metabolism is closely regulated. The process of deoxynucleotide synthesis for all four bases is linked to a single enzyme, ribonucleotide reductase.

Ribonucleotide reductase is a vital enzyme that catalyzes the reduction of rNDPs to dNDPs. The type of rNDP reductase used for this study is found in both *E. coli* and mammalian cells. It is a class I $\alpha_2\beta_2$ tetramer. The enzyme found in *E. coli* is further specified by its R1 protein consisting of two α chains weighing 87,000 Da each and the R2 protein consisting of two β chains weighing 43,000 Da each. The α chains contain

two allosteric control binding sites. The first is called an activity site and binds ATP and dATP. The second is called a specificity site and binds ATP, dATP, dGTP, and dTTP. The α subunits also contain a reduction-oxidation site binding glutaredoxin or thioredoxin, and a catalytic site binding any one of the four rNDPs. The β chains contain a tyrosine free radical that is part of the enzymatic reaction converting rNDPs to dNDPs and a dinuclear iron center that consists of an oxygen atom binding two ferric ions to stabilize the tyrosine radical.

As can be guessed, the regulation of rNDP reductase is controlled by the specific binding of rNTPs and dNTPs. This occurs at the R1 subunit of the protein. The binding of dATP at the activity site inhibits reduction of all four rNDPs. Conversely, the binding of ATP at the activity site functions to activate the reduction of particular rNDPs, depending upon what nucleotide is bound at the specificity site. This process ensures that reduction of each type of rNDP is closely matched so that the resultant pools of dNDPs are somewhat equal. After reduction, dADP, dCDP, and dGDP are phosphorylated by NDP kinases to dNTPs. dUDP, through a series of steps, is converted to dTMP by thymidylate synthase before phosphorylation. At this point, all four dNTPs are available for incorporation into DNA by synthesis or repair.

The purpose of the rNDP reductase regulatory process is thought to be related to mutation, since asymmetric pools have been shown to stimulate mutagenesis of DNA (Kunz *et al*, 1994). Such imbalances may not be the only dNTP pool-related inducer of mutation. Uncontrolled, but proportional synthesis of dNTPs has also been found to contribute to mutagenesis (Wheeler *et al*, 2005). This is of particular concern since mutagenesis in any form can lead to cancerous tumor formation.

One means by which the activity of rNDP reductase increases is related to ultraviolet light exposure. It has long been known that UV exposure to cells results in pyrimidine dimers, reduced cell survival, and mutagenesis (Hanawalt, 1979). It has also been shown that UV irradiation acts to selectively increase dNTP pool levels for both dATP and dTTP in *E. coli* (Das, 1983). The levels of dCTP and dGTP in that study were minimally affected. The mechanism by which UV irradiation acts to stimulate rNDP activity is discussed below.

DNA Repair and the SOS response

DNA differs from most biomolecules in that it is self-replicating. Whereas most molecules can be replaced once damaged, DNA must remain nearly identical to its synthesized form to function properly as storage and transmitter of all information in the cell. Although some base pair mutations are allowable, or even desirable for evolution, other mutations may alter or terminate the function of a protein for which it codes. Since errors in synthesis and environmental damage do occur, effective means to repair DNA are paramount.

When DNA is damaged, whether by faulty replication or by a chemical or environmental mutagen, multiple repair processes may be enacted to fix the damage. Among these processes are those that replace directly a damaged DNA, base such as photoreactivation and the use of alkyltransferases. Other processes act to replace a damaged segment of DNA, such as nucleotide excision repair (NER) by excinucleases or base excision repair by DNA-N-glycosylases. Damage to genes coding for proteins involved in NER is notable in that it has been shown to cause skin cancer. These repair

processes occur during and after replication, and are fairly accurate at correcting the damage. Additional repair processes may occur after replication. These include recombinational repair and the error-prone SOS response.

Recombinational repair occurs when a gap results opposite a thymine dimer during replication. In *E. coli*, DNA polymerase III cannot replicate DNA past such dimers, and the enzyme sits at the spot, continually inserting dAMP opposite the first thymine base and then removing it by way of 3' exonucleolytic activity.

Recombinational repair results from the activity of several enzymes that recombine the uninvolved parental strand with the damaged strand in a mechanism similar to homologous recombination. Although the dimer itself is not repaired in this process, excision repair can follow. Like the repair processes mentioned above, recombinational repair is fairly accurate.

The error-prone SOS response is different from the above processes in that, as its name implies, it is not very accurate. The SOS response is a series of metabolic events that occur after DNA damage and allow DNA polymerases IV and V to continue replication beyond a thymine dimer or other damage site without repair. Proteins involved in the SOS response are discussed in greater detail below. dNMPs are incorporated opposite the damage site in a highly inaccurate fashion. It is believed that the SOS response is activated only in cases of extreme DNA damage, when other repair processes become overwhelmed. Although SOS results in a considerable number of mutations, it is thought to be a survival method in cells that, due extensive DNA damage, would otherwise undergo cell death. Both recombinational repair and the SOS response require a protein called RecA.

RecA protein catalyzes strand pairing in both recombinational repair and homologous recombination. It wraps around a single-stranded DNA and transports it to a homologous sequence. In the case of bacterial recombinational repair, the homologous sequence is the parental strand on the opposite side of the replication bubble. During the SOS response, RecA acts in a very different way. Through association with other proteins, it participates in a process leading to low fidelity during DNA repair. Two of these proteins are the products of the *umuC* and *umuD* genes and form what is known as DNA polymerase V. Like *recA*, they are activated during cell damage. UmuD protein cleaves to form UmuD' and then combines with UmuC to form a trimer, UmuD'₂C. Upon association with RecA, this complex binds upstream from the damage site on DNA and allows the DNA polymerase to replicate beyond the damage. To prevent the error-prone SOS response from occurring when it is not necessary, a complex regulation system exists to control the expression of several aforementioned proteins.

recA, *umuC*, *umuD*, *dinB* (which codes for DNA polymerase IV), and many other DNA damage induced genes are regulated by the SOS regulon. At the heart of this system are a single gene, *lexA*, and its protein product, LexA. LexA acts as a repressor in the transcription of at least 40 different genes scattered about the *E. coli* chromosome. It also acts as a repressor for the operon corresponding to its own gene. During normal cell activities, LexA protein effectively stops transcription of all the genes in the SOS regulon except *recA* and its parent gene. When DNA damage occurs, RecA binds to single stranded DNA and in an unknown mechanism, acts as a protease, destroying the function of LexA protein and effectively ceasing the repression of SOS response genes. Many of the genes included in the SOS regulon have an unknown function. However, it is likely

that all act in some capacity to aid in recovery from DNA damage.

Ultraviolet Radiation-Induced Increases in rNDP Reductase Activity

As previously mentioned, an increase in dNTP pool levels has been observed in cells after exposure to ultraviolet light. In early 2000, Tanaka *et. al* proposed a mechanism for mammalian cells by which *p53*, a gene that prevents cell proliferation in damaged cells and that is discussed in the second part of this paper, induces the transcription of a previously unknown form of rNDP reductase. Two important conclusions resulted from their work. First, the p53 protein was found to stimulate transcription of a gene called *p53R2* when bound to an inducer. The product of this gene strongly resembles the small R2 subunit of rNDP reductase. R2 is the rate-limiting subunit of the reductase and under normal cell conditions only exists during the G1 and S phases of the cell cycle. Secondly, p53R2 protein is thought to associate with another protein resembling R1 in the nucleus of the cell. This differs from regular rNDP reductase, which is found only in the cytoplasm. Together, these conclusions form a model by which a nuclear form of rNDP reductase is able to synthesize deoxynucleotides for use in repair mechanisms in response to DNA damage.

At approximately the same time, Courcelle *et al.* (2001) conducted a microarray experiment using amplified DNA fragments from over 95 percent of open reading frames identified in *E. coli*. They found over 40 genes induced in a manner dependent upon LexA after exposure to ultraviolet light. They also showed that the transcriptional induction of genes coding for rNDP reductase after exposure to UV was independent of LexA protein.

The work reported in the following extends that of Courcelle et al. dNTP pool expansion and the SOS response have both been shown to increase mutation rates. The initial hypothesis was that increases in activity of rNDP reductase and corresponding proportional increases in dNTP pool sizes are independent of LexA and the error-prone SOS response. Although increased pools may enhance the SOS response, the occurrence of these two phenomena after DNA damage is unrelated.

Materials and Methods

Bacterial Strains

The two *E. coli* strains discussed in this study are described in Table 1.

Transcription of *lexA* was prevented in the second strain by transposon insertion.

Table 1

E. coli strains used

Strain	Relevant genotype	Use	Source
NR9338	<i>lexA</i> ⁺	Parent strain used for experiments on effects of <i>lexA</i> expression	Roel Schaaper National Institute of Environmental Health Sciences
NR9445	<i>lexA</i> ⁻	<i>lexA71</i> :: Tn5, transposon insertion into <i>lexA</i>	Same

Cell culture preparation

Bacteria were grown in LB medium to mid-log phase (A_{600} of approx. 0.65).

Cultures not treated with ultraviolet C radiation were placed on ice. Those treated with UVC radiation were pelleted by centrifugation for 10 min. at 3000 rpm and resuspended in 10ml M9 salts. UVC radiation was applied at $1.6 \text{ Jm}^{-2} \text{ s}^{-1}$ for 25 sec. to cell suspensions in a Petri dish on a mixing platform. After UV radiation, cells not allowed further incubation were placed on ice. The remaining cultures were pelleted as described above then resuspended in 10 ml LB. They were incubated for 40 min. at 37 degrees C and then placed on ice with the other samples in preparation for the following assays.

Assay for Ribonucleotide Reductase Activity

Ten-ml aliquots of each culture and for each treatment were pelleted by centrifugation for 10 minutes at 3000 rpm. The pellet was resuspended in 500 μ l HEPES buffer (0.1 M, pH 8) and transferred to a 1.5-ml microfuge tube. Cell suspensions were sonicated at 5 watts for 15 sec for a total of three times each. Suspensions were placed on ice between sonications to avoid excess heating. Following sonication, cells were centrifuged at 14,000 rpm for 10 min. The supernatant was transferred to a clean microfuge tube.

Reaction mixtures included 20 μ l enzyme prep (supernatant from above), HEPES (100 mM, pH 8.0), dithiothreitol (10 mM), adenosine-5'-(β,γ -imido) triphosphate (AMP-PNP) (4 mM), ferric chloride (20 μ M), nonradioactive CDP (50 μ M), magnesium acetate (2 mM), and [5- 3 H] cytidine 5'-diphosphate (3 H CDP) (0.29 μ l, 1 μ Ci/ μ l). Reactions were carried out for 60 minutes in a 37 degrees C water bath. A zero time sample was formed by adding 4.4 μ l of 10 M perchloric acid to the tube and placing it on ice. After completion of 60 min. reaction, an equal volume of perchloric acid was added to each tube and all were placed on ice for 15 minutes.

All samples were centrifuged for 2 min. at 14,000 rpm and the supernatant was transferred to a fresh tube. The samples were then placed in a boiling water bath for 20 min. 4 μ l of a UV marker solution containing 20 mM CMP, 20 mM dCMP, and 20 mM dUMP and 12 μ l 5 M KOH were added to the samples. The tubes were placed on ice for 15 min. The precipitate was then spun down by centrifugation for 10 min. at 14,000 rpm.

20 μ l of each sample was spotted on cellulose thin-layer chromatography (TLC) plates containing a fluorescence indicator. TLC running solution contained 110 ml 90%

ethanol, 40 ml saturated sodium tetraborate, 20 ml 5M ammonium acetate (pH 9.8), and 1 ml 0.25 M EDTA (pH 8.0). TLC was run overnight. Spots visualized under UV light corresponding to dCMP and dUMP were cut out of the TLC plate and placed in a scintillation vial with 1 ml 0.5 N HCl. After 30 min, 5 ml Ecolite scintillation fluid was added and the samples were counted using a Beckman LS 6500 liquid scintillation counter.

Assay for dNTP pool levels

Ten-ml aliquots of each culture were collected by rapid filtration through a 0.45- μ m filter. Filters were placed upside down in Petri dishes containing 5 ml ice cold 60% methanol and eluted for 1 hour at -20 degrees C. The eluant from each sample was transferred to a tube and the filter was washed twice with 1 ml cold methanol. Samples were placed in a boiling water bath for 5 min, centrifuged for 15 min. at 14,000 rpm, and the supernatant was transferred to fresh tubes. The samples were then dried overnight in a speed vac. After drying to completion, the samples were resuspended in 100 μ l ice cold sterile water and aliquots were made at dilutions of 5x and 25x.

dNTP pool assays were completed by Linda Wheeler and were subjected to the DNA polymerase-based assay for dNTPs as described by Diaz et al (2003).

Results and Discussion

Results of rNDP reductase and dNTP pool assays are consistent with the hypothesis that increases in activity of rNDP reductase and corresponding proportional increases in dNTP pool sizes are independent of LexA and the error-prone SOS response. This conclusion, along with that of Courcelle et al. (2001), shows that the LexA protein does not regulate ribonucleotide reductase activity at any level.

Results from rNDP reductase assays show that activity is the same or slightly lower after exposure to UV-C irradiation for both bacterial strains. After 40 minutes of incubation, or approximately one replication cycle, activity increases substantially. Increases from non-UV irradiated cultures are approximately 60 percent while increases from the UV irradiated but not further incubated cultures are approximately 100 percent. Figure 1 shows the specific activity of rNDP reductase for each of the three treatments. Slight decreases in activity after exposure to UV are attributed to cell loss, either through physical handling of the cells or the UV light itself.

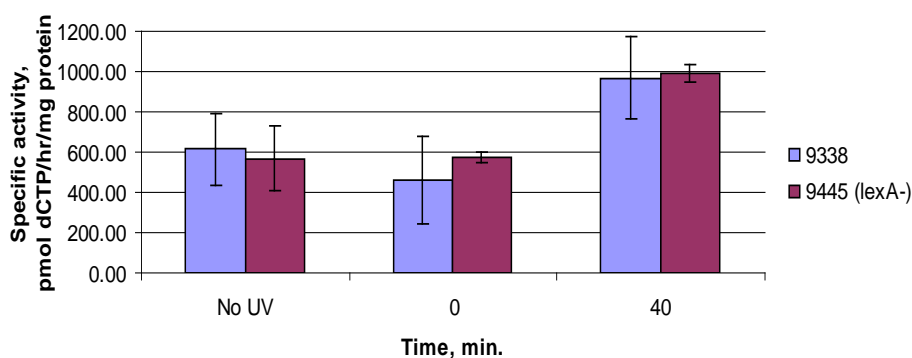


Figure 1. Effect of irradiation on ribonucleotide reductase activity. Specific activity per milligram protein of the reductase in NR9338 parental strain and NR9445 (lexA-) strain is shown. Bacterial cultures were grown to mid-log phase in LB and either immediately harvested, exposed to 40 Jm⁻² UV-C irradiation and harvested, or exposed to UV-C and allowed 40 min. of incubation before harvest. Results were averaged for three experiments. Standard deviation error bars indicate precision.

dNTP pool assays show increased accumulation of dATP, dCTP, and dTTP after exposure to UV irradiation followed by incubation. dGTP pools remained relatively stable before and after the treatment. Overall pool size was greatest for dTTP, followed by dCTP, dATP, and dGTP. The proportions are similar to those previously seen in *E. coli*. (Wheeler et al, 2005). In cultures not exposed to UV irradiation, dNTP pools for the *lexA* mutant were approximately twice the size of pools for the parental strain. After exposure, the levels are nearly equal, indicating that the parental strain had the greater fold increase in pools. There is not a statistically significant difference in UV irradiation-induced pool expansion in the two bacterial strains. Pool assay results for each nucleotide are displayed in figure 2.

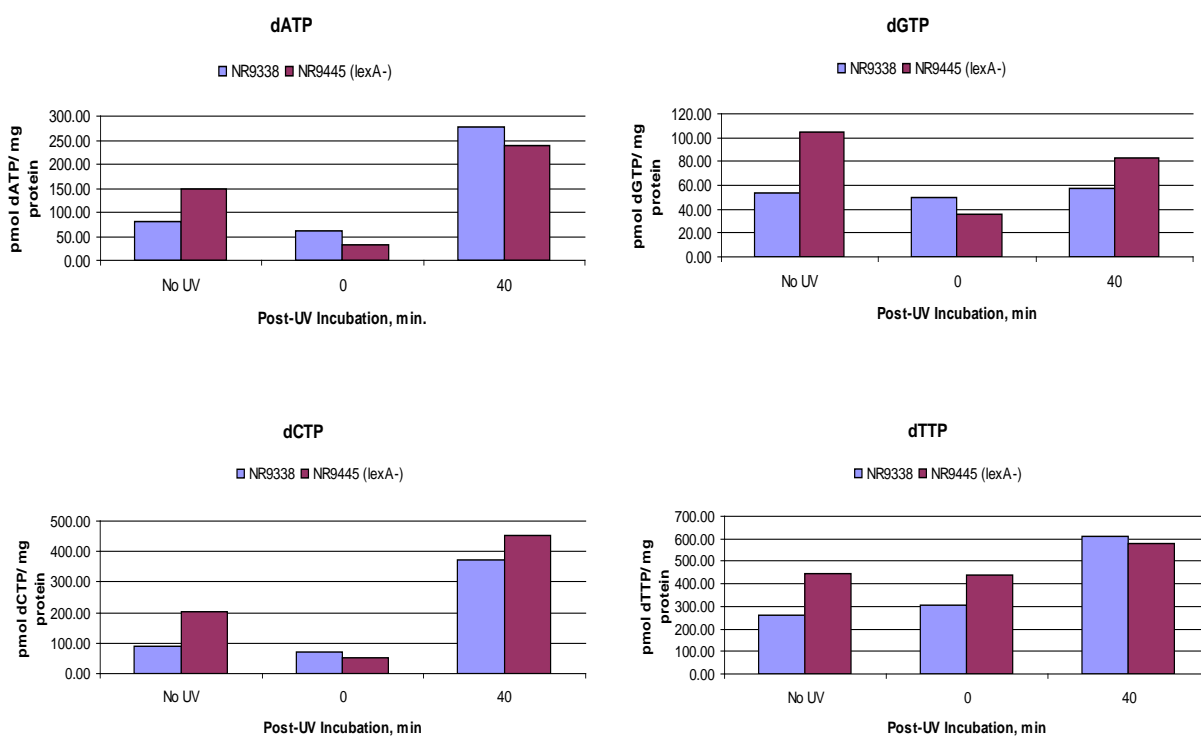


Figure 2. Effect of UV irradiation on dNTP pool sizes in picomoles per milligram protein for NR9338 parental strain and NR9445 (*lexA*⁻) strain. Bacterial cultures were grown to mid-log phase in LB and either immediately harvested, exposed to 40 Jm⁻² UV-C irradiation and harvested, or exposed to UVC and allowed 40 min. of incubation before harvest.

dNTP pools showed a decrease in size corresponding to the decrease in rNDP reductase activity mentioned above. The decreases in each assay, however, do not correspond to the same bacterial strain. In the reductase assay, the parental strain appears to lose more activity and therefore be more susceptible to cell death due to handling or UV irradiation than the mutant strain. The pool assays, on the other hand, show the mutant to be the more fragile strain. How the mutant strain appears to increase its rNDP reductase activity after exposure while simultaneously showing decreases in dNTP pool size is difficult to explain. It is possible that dNTPs are initially used up by repair processes at a greater rate than they are synthesized. In any case, this phenomenon was consistent in all experiments. Although interesting, this result does not suggest LexA control of the reductase. It does, however, result in a difference in the increases in activity seen between the bacterial strains that were incubated after UV exposure and those that were not.

Although final dNTP pool numbers are similar for the parental and *lexA* mutant strains, increases seen after a single replication cycle incubation are disproportionate. After UV irradiation, the mutant strain initially has smaller pools than the parental strain. However, since the final levels are nearly the same, the percent increase in the mutant strain is higher. A plot comparing the two strains is given in Figure 3. It should be noted that the percent increase from cultures not irradiated is nearly the same for each strain (not shown).

Percent increases are highest for dATP and dCTP. This differs from previous studies on the effect of UV irradiation on dNTP pool levels (Das, 1983). In that study, the greatest increase was seen for dTTP, with increases on the level of 3-fold to 8-fold.

Here, wild-type increases in dTTP were only 2-fold. The percent increase in dCTP was also unexpected. Das et al. reported wild-type dCTP increases of 2-3-fold. This study found dCTP increases to be the highest of all four nucleotides and on the order of 4-fold or higher. The percent increase for dGTP was low, as expected.

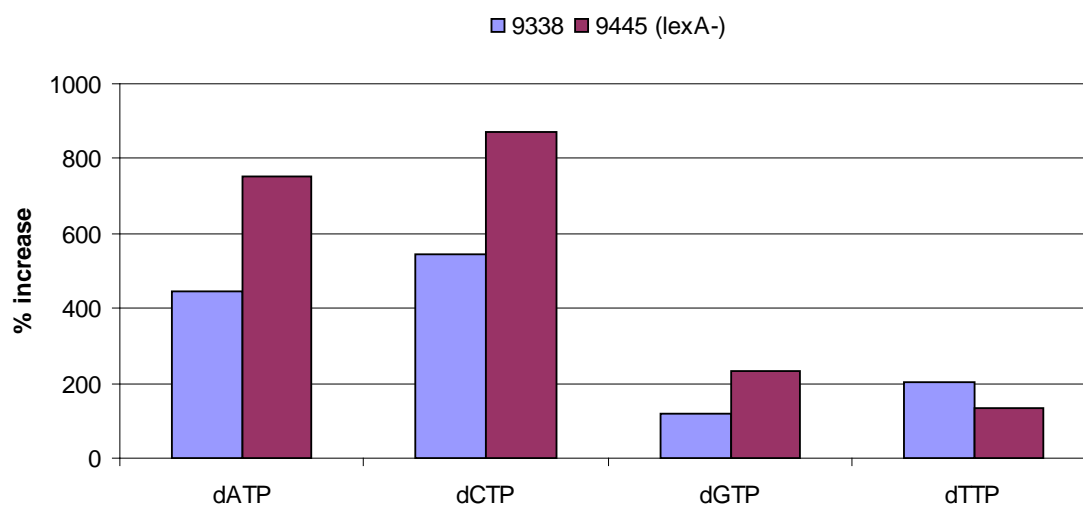


Figure 3. Post-UV percent increase in dNTP pool sizes for NR9338 parental strain and NR9445 (*lexA*⁻) strain after 40 min incubation. Bacterial cultures were grown to mid-log phase in LB, exposed to 40 Jm⁻² UV-C, and either immediately harvested, or allowed 40 min. of additional incubation before harvest.

Suggestions for Further Investigation

After exposure to ultraviolet light, ribonucleotide reductase shows a clear increase in activity. This is confirmed by increases in dNTP pools, the indirect product of the enzyme. The error-prone SOS response is currently the only known UV activated transcription inducing system. Since it has now been established that UV-induced increases in activity of rNDP reductase and corresponding proportional increases in dNTP pool sizes are independent of LexA and the SOS response, another UV activated system must exist.

The genes that code for ribonucleotide reductase, *nrdA* and *nrdB*, are not the only genes that show LexA independent induction following UV irradiation. Courcelle et al. found that *yeeF*, a gene that codes for an amino acid transport protein, also shows significant induction after UV irradiation. Additionally, minor upregulation was found for a number of genes involved in replication, purine and pyrimidine metabolism, and RNA metabolism. Clearly, LexA is not the only UV activated gene inducer in the cell. Until further studies illuminate one or more additional UV activated gene induction processes, the exact mechanism by which ribonucleotide reductase is induced by ultraviolet irradiation will remain unknown.

As discussed in the introduction, mutation rates increase in both cells that constitutively produce LexA and those in which ribonucleotide reductase is overexpressed due to plasmid insertion. Although the mechanisms by which each results in mutation is independent, it seems reasonable to assume that large accumulations of dNTP pools due to increased activity of ribonucleotide

reductase will further increase mutation rates in cells constitutively expressing for the SOS response. However, previous studies on non-irradiated cells show that mutation rates actually decrease in cells that both constitutively express *lexA* and overexpress ribonucleotide reductase (Wheeler et al, 2005). Studies on mutation rates for cells used in this study may help elucidate this result.

The Incidence of and Attitudes toward Skin Cancer in Several Western Nations

Background

The relationship between sun exposure and skin cancer is well documented and widely known. The biochemical basis for this relationship is, understandably, not as well recognized by the general public. The following part of this thesis will focus on the biochemical basis of ultraviolet radiation-induced skin damage and its incidence and treatment in several western nations. Although many people understand the danger, it will also be shown that preventive actions are commonly disregarded.

Ultraviolet radiation is defined as radiation with a wavelength between 100 and 400 nanometers (nm) in length. It is further divided into three groups, including ultraviolet A (315-400 nm), ultraviolet B (280-315 nm), and ultraviolet C (100-280 nm). Any one of the three groupings is capable of causing DNA damage. Ultraviolet A is known to cause the visible effects of skin aging. Ultraviolet B causes sunburn and immunosuppression (Gallagher, 2006). Both A and B are thought to cause cancers of the skin. Although it was used experimentally to damage *E. coli* DNA in the preceding research, ultraviolet C radiation emanating from the sun is almost entirely filtered out by the earth's atmosphere, and is not considered a risk to humans.

The mechanism of DNA damage and cancer development is well established for non-melanoma skin cancers. Ultraviolet radiation causes point mutations in DNA at nucleotide sequences where pyrimidines, cytosine or thymine, lie adjacent to one another (Ziegler). Approximately two thirds of these mutations involve a C to T substitution, and another ten percent involve a CC to TT double substitution. Such mutations are common to no other known carcinogens (Leffell, 2000). This unique type of damage is the likely

cause of all UV-induced skin cancers.

Cancer formation and growth in non-melanoma skin cancer is linked to p53, a tumor suppressor gene that is found to be mutated in over eighty percent of human tumors (Lozano, 2000). A mutation in p53 was first linked to colon cancer in 1992 (Goyette, 1992). It has since been found in basal cell carcinoma and over 90 percent of squamous cell carcinomas. To prove that a mutation in p53 does, in fact, lead to cancer growth, Ziegler et al. exposed two sets of mice to UV radiation. The first group expressed a normal p53 gene. The second lacked a functional p53 gene. After exposure, the normal mice showed sunburned cells undergoing the process of apoptosis, or programmed cell death. The second group did not reveal any sign of sunburn. p53 acted to kill UV damaged cells in the first group but not the second (Ziegler, 1994). Further studies have pin-pointed nine “hotspots” where mutations tend to occur in the p53 gene. It has been proposed that these sites occur in places that the cell is slow to repair after damage (Leffell, 1996). It is now well accepted that p53 protein, the product of the p53 gene, acts to arrest the cell cycle of damaged cells, thereby preventing them from proliferating.

What is unique to UV radiation as a carcinogen is that it acts as both a tumor initiator and tumor promoter. Following initiation of the previously described UV-induced process, the actual tumor develops from a single damaged cell. Ultraviolet light then further acts as a tumor promoter in an equally interesting manner. After the first exposure to sunlight, subsequent exposures continue to damage the cellular DNA. The majority of cells will not experience p53 damage, but due to damage in other regions of the genome, will undergo p53-dependent apoptosis. In these cells, proliferation is

suppressed via the usual mechanism. If a cell that lacks p53 function from a previous exposure exists amongst the apoptotic cells, however, it will not die. It will preferentially divide and expand into the void, thereby continuing tumor growth.

The p53 dependence in squamous cell and basal cell carcinoma has not been seen with cutaneous malignant melanoma (Li et al, 1995). While the mechanism of melanoma development is not yet understood, its risks have repeatedly been linked to sun exposure (Fears, 2002). It is therefore reasonable to suggest that the growth of malignant melanomas may be initiated and promoted by ultraviolet radiation in a fashion similar to that of squamous and basal cell carcinomas. Although the exact tumor suppressor gene or oncogene in melanoma has yet to be discovered, the status of melanoma as the most dangerous form of skin cancer requires that it be discussed along with the other forms of skin cancer.

Basal cell carcinoma (BCC) is the most commonly occurring form of skin cancer worldwide. It is also the most common of all cancers in the Western world. It originates in the basal layer of skin cells, is almost always successfully treated with local surgery alone, and is rarely life-threatening. The risk factors for BCC include brief but intense sun exposure prior to adulthood and lifetime intermittent “beach exposure” according to studies done in Australia and southern Europe (Marks, 1993) (Rosso, 1996). Like all UV-induced skin cancers, risk also increases with fairness of skin color, proximity to the equator, family and personal history.

Squamous cell carcinoma (SCC) is the second most common form of UV-induced skin cancer. Although it accounts for only a fifth of all non-melanoma skin cancers, it has the ability to metastasize and is therefore more dangerous than BCC. Like BCC,

SCC is rarely life-threatening if diagnosed early and usually can be successfully treated with local surgery alone. Unlike BCC, risk factors for squamous cell carcinoma have been shown in southern Europe to include significant lifetime or occupational ultraviolet exposure (Rosso, 1996).

Cutaneous malignant melanoma (CMM) is the least common, yet most dangerous, form of skin cancer. As noted previously, CMM is not linked to damage to the p53 gene although it is convincingly associated with ultraviolet light exposure. Superficial CMMs are most often successfully treated with local surgery alone. Intermediate and thick CMM's require much more extensive surgery, and sometimes additional therapy as well. Metastatic melanomas are particularly lethal. Although they can be treated with chemotherapy and other treatments, success rates are very limited. Like BCC, malignant melanoma has been associated with childhood sun exposure in both Australia and the United States (Khat, 1992) (Mack and Floderus, 1991). Brief, intermittent sun exposure at all ages is also strongly correlated with CMM (Fears, 2002). Ten sunburns prior to age 18 are associated with a near 3-fold increase in risk as an adult.

Incidence of Skin Cancer: A Comparison of Several Western Nations

Skin cancer rates lower predictably from median latitude areas to higher latitude areas and from people with fair skin to those with dark skin. As a representative of Western nations, and because reliable information is readily available, the incidence rates in five countries will be discussed. These represent various subgroups of Western culture and all are European or predominantly descended from European ancestry. It should be noted that this group represents a particularly small cross-section of the world population, but those whose risk of developing skin cancer is the highest. The countries that will be discussed are Australia, Germany, Norway, Spain, and the United States.

Australians suffer from the highest incidence of skin cancer in the world. Their closest competitor in this regard is the United States, with approximately half the incidence of malignant melanoma. The majority of Australians are descendants of British or Irish immigrants and have fair skin. The geographic location of Australia presents its residents with much higher levels of UV radiation than is prevalent in the British Isles. Because of its national skin cancer epidemic, Australia is at the forefront in skin cancer research and maintains extensive public awareness campaigns. Although Australians are aware of skin cancer risks, the majority of them live near the ocean and cannot or choose not to avoid the sun. Data for all types of skin cancer in both temperate and tropical regions of Australia are given in Table 2.

Germany and Norway were selected to represent western European nations. Norwegians in general have fairer skin than Germans, but live at slightly higher latitude. Although both countries have temperate climates, their citizens often travel to warmer and sunnier climates on vacation. The frequency of travel to UV intense areas such as

the beaches of the Mediterranean or Caribbean may be a significant risk factor. Both countries have seen skin cancer rates rise with increased vacationing in recent years. One study in particular has shown a strong correlation between decreased airfares and skin cancer rates in Norway (Agredano, 2006). As with most European populations, tanned skin is commonly viewed as aesthetically pleasing and evidence of a healthy lifestyle. As seen from Table 2, the incidence of malignant melanoma is much higher in Norway than Germany. This suggests that fairness of skin may be a more important risk factor for this form of skin cancer than latitude or vacationing habits.

Table 2: Incidence of skin cancer in five western nations (per 100,000)

	Malignant Melanoma*	Basal Cell Carcinoma ⁺	Squamous Cell Carcinoma ⁺
Australia	21.4	955	321
Australia, Tropical	29.4	1626.4	1043.5
Germany (Saarland)	6	43.7	11.2
Norway	14.7	N/A	5.5
Spain	3	57.9	18.2
U.S.A., Seattle	11.5	N/A	N/A
U.S.A., Southwest	14.7	716.5	247.3

*Malignant Melanoma data from IARC

⁺Basal Cell and Squamous Cell data from Arranz et al. except Germany, from Stang et al.

The country of Spain was selected as a model for southern European populations. Spanish people have darker skin than the other groups mentioned here. Although the Spanish climate and latitude provide a much more intense amount of ultraviolet light than

Norway, Germany, and many areas of the United States and Australia, Spanish skin cancer rates are dramatically lower. Still, skin cancer is seen as a national problem in Spain. According to Arranz et al (2004), 43,000 cases of basal cell carcinoma and 15,500 cases of squamous cell carcinoma will be diagnosed yearly in Spain.

The United States has the second highest incidence of skin cancer in the world. As in Australia, the majority of the U.S. population is composed primarily of individuals with European ancestry. The U.S. is somewhat unique in comparison with the other countries examined in that its population is distributed over a diverse range of climates. Predictably, those living in more temperate areas of the country have a higher incidence of skin cancer. As such, data are given for the Seattle area, where cloud cover is common for most of the year, and the Southwest, where it is not. As with the other countries referenced, skin cancer rates have increased coincidentally with increased skin tanning and the accessibility of vacation travel to warmer climates. It is estimated that the cost of treating non-melanoma skin cancer is 500 million dollars each year in the U.S. (Preston, 1992).

Attitudes toward Skin Cancer

Public knowledge of skin cancer and its association with exposure to solar ultraviolet radiation is generally well established. However, despite rising incidence of all types of skin cancer in the nations discussed above, protective measures are commonly disregarded. In a sense, sunlight may be regarded as the most common carcinogen in Western nations. The tanned skin that results from sun exposure is generally regarded as cosmetically appealing and is commonly associated with a healthy lifestyle and leisurely outdoor activities. Repeated mass media campaigns describing the dangers of UV radiation, while effective in educating the populace, have had minimal impact on their actual behavior in this regard.

A series of studies were conducted in the past five years to assess public awareness of basal cell carcinoma in Australia, Europe, and the United States. Americans and Australians were the most knowledgeable, with each having approximately fifty percent of respondents familiar with BCC. European nations varied in the number of respondents familiar with BCC, with Germans and Italians having between 20 and 30 percent, the Spanish having just less than 20 percent, and the French having less than 10 percent familiar (Halpern, 2005).

Interestingly, there appears to be little correlation between sensitivity to UV radiation and concern expressed about skin cancer. While 15 to 20 percent of French and German respondents reported having been burnt from sun exposure in the previous year, only 30 to 35 percent of them expressed concern about skin cancer. Meanwhile, while only about 5 percent of Italian and Spanish respondents reported a burn in the past year,

50 percent of Spaniards and over 65 percent of Italians expressed concern about skin cancer. (MacKie, 2006)

It follows with little surprise that sun exposure habits also do not relate to sensitivity to the sun. The study respondents from all nations strongly correlated skin cancer with exposure or overexposure to the sun. Each group had at least 80 percent of participants respond with agreement that sun exposure contributed to skin cancer. However, when asked about moderate tanning the results varied extensively. Australian and U.S surveys each had approximately 60 percent of respondents say that tanning was correlated with skin cancer. About 55 percent of Spanish respondents, 35 percent of French respondents, and 30 percent of Italian respondents saw a correlation. The Germans, arguably the fairest skinned European group in the survey, had only 15 percent of respondents say that tanning could be linked to skin cancer. (Halpern, 2005)

Skin cancer rates show no sign of declining, as the tanning habits of youth in all of the countries mentioned are as detrimental, if not worse, than what can be inferred from the statistics regarding their adult counterparts. Despite 80 percent of teens in the U.S. knowing that sun exposure as a child can lead to cancer later in life, 60 percent claim to have suffered a burn within the last year. Only 53 percent of teenage girls and 33 percent of teenage boys commonly wear sunscreen when outdoors for a long period of time (Dermatology nursing, 2005). Even when wearing sunscreen, young people are unlikely to avail themselves of the protection they need. A study of college age students in Europe revealed that the average person applies only one fifth of the amount of sunscreen that is used for determination of SPF (Autier, 2000). The overall notion implied by these studies is that sun exposure and even the occasional episode of burning

are not a health risk that is worth avoiding.

Despite the continuing increase in skin cancer rates and general nonchalance about the matter worldwide, some improvements have been made. Sunscreens have improved significantly and now include ultraviolet A protection. In Australia, where skin cancer rates remain the highest, government sponsored campaigns have been the most aggressive and successful. Campaigns in the late 1990s targeted at parents of children under the age of 12 proved to increase sun protective behavior during the campaigns, but not between campaigns (Smith, 2002). Most promisingly, a 1999 review of attitudes toward skin cancer in Australia claims that incidence rates among the younger generations have begun to level off. It further states that light tanning, or no tanning at all, has begun to replace the dark tans so popular in the past.

Conclusion

Skin cancer is a serious problem across much of the Western world. Incidence rates are at historical highs and will, by most estimates, continue to rise. Although knowledge of solar UV radiation as a carcinogen is high, perceived risk from spending time outdoors varies substantially from country to country. Indeed, countries whose populace is at a greater risk of developing skin cancer due to fair skin tone are, in some cases, the least concerned. Travel to sunny climates on vacation and the practice of tanning remains popular and tanned skin continues to appear both aesthetically pleasing and healthy to many Westerners. If progress is to be made in preventing skin cancer in future generations, aggressive and sustained mass media campaigns, as currently utilized in Australia, must be expeditiously implemented in both North America and Europe.

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