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DNA Repair Mechanisms and Mutagenesis

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I. INTRODUCTION

All living cells are constantly exposed to chemical and physical agents that have the ability to alter the primary structure of DNA. Such alterations, if not corrected, would result in mutations. While many of these mutations would be neutral (i.e., no changes in the amino acid sequences of peptides) or would be insignificant (no involvement of regulatory regions for the DNA and RNA or in the case of proteins no alteration of active sites), the accumulation of significant mutations has the potential to increase the genetic diversity of a species. Such genetic diversity is an essential component of evolution and the ability of species to survive in changing environments. However, there does come a critical point in the accumulation of mutations (genetic load) at which time the species can no longer exist (Dobshansky, 1950). Thus it would seem obvious that living systems must maintain mechanisms for the repairing of DNA damage. It would also seem obvious that these same systems must balance the removal of DNA damage with the accumulation of a finite number of mutations. In this chapter we discuss the diversity, as well as the exciting intricacy, of the DNA repair systems found in the paradigm *Escherichia coli*. In addition we consider the dramatic changes that have occurred within the last 10 years to our understanding of the processes of DNA repair and mutagenesis. Many of these changes have been brought about by the information gained through the various genome projects.

Beginning to understand the processes associated with DNA repair and mutagenesis requires visiting the debate over whether mutations arise spontaneously or are directed by environmental conditions—Darwin versus Lamarck. In 1942 Luria and Delbrück seemed to answer this question following the publication of their fluctuation tests (Luria and Delbrück, 1943). By combining statistics with

an elegant investigation of mutation numbers, these pioneers demonstrated that under their laboratory conditions bacterial mutations arose spontaneously during growth. While these and related results (Lederberg and Lederberg, 1952; Newcomb, 1949) clearly supported the view that mutations are nondirected and arise spontaneously, the debate has never really ended. The last 10 years has seen a dramatic increase in the interest shown in "directed" and stress-induced mutagenesis (Wright, 2000). While this is not a new concept, the very mention of naturally occurring "directed" mutagenesis invokes the passions associated with Lamarck's views on the inheritance of acquired characteristics. In all fairness, Lamarck should also be remembered for having articulated the need for a gradual evolution from the simplest species to the most complex. Evolutionists (Dobshansky, 1950) and mathematicians have consistently questioned the probability that evolution could have proceeded as rapidly as demonstrated had true random mutagenesis been the only factor in providing genetic diversity (Wright, 2000). The validity of these questions is attested to, since today we know of the impact that transposons and transpositions can have on diversity and on the evolutionary process (Labrador and Corces, 1997; see Whittle and Salyers ch. 17). Furthermore there are data that strongly support the existence of stress-related "directed" mutagenesis mechanisms (Wright, 2000). However, it is important to note that in all of these cases there is no evidence found to support the Lamarckian concept of the inheritance of acquired characteristics.

Consistently spontaneous mutations were thought to arise almost exclusively as a consequence of growth (either errors in replication, unrepaired DNA damage or as a result of errors during the process of repairing damaged DNA). As described in the chapter by Frishein, this volume, prokaryotic DNA replication is the primary responsibility of the replicating complex. This complex of DNA polymerases and accessory proteins perform the normal semiconservative replication with a great deal of accuracy (Friedberg et al., 2000; Friedberg et al., 1995; Ohashi et al., 2000). Without the involvement of any factors contributed by the bacteria, the potential error frequency associated with the pairing of bases would be between 1 to 10% per nucleotide. However, the actual mutation frequency for newly replicated *E. coli* DNA is six to nine orders of magnitude less frequent than the prediction based solely on energetics. At least three to six orders of magnitude of this enhanced fidelity is due to inherent properties associated with the replication machinery including the 3′ to 5′ exonuclease function that has editing or proofreading activity. Further reduction in the replication errors occur as a result of the functioning a protein systems involved in mismatch correction (described below).

Recently a family of error-prone polymerases that lack the 3′ to 5′ exonuclease editing function have been identified in eubacteria, archaea, and eukaryotes (Friedberg et al., 2000; Gerlach et al., 1999). In *E. coli* these designated DNA polymerases IV (DinB) and V (UmuD′C) have been associated with translesion processing of DNA (replication past a noninstructional lesion) and consequently with the potential generation of mutations. In eukaryotes, homologs of these polymerases have been associated with human diseases including cancer and potentially with the functioning of the diversity associated with the immune system. The existence of these polymerases and their stress-related regulation has spawned an intensive re-investigation into the nature of the mutagenesis process(es).

In 1988 John Cairns and his collaborators published a controversial and exciting article that forced rethinking about how spontaneous mutations might arise when cells are under a stress-induced selection (Cairns et al., 1988). Although there were some problems with this first report (Prival and Cebula 1996), Cairns and Foster (1991) confirmed that mutations arise in nondividing or stationary phase bacteria when the cells are subjected to nonlethal selective pressure

such as nutrient-limited environments. The authors termed the accumulation of these types of mutants as adaptive mutagenesis. Since this report, additional data have accumulated supporting the existence of mutations generated while cells are in stationary phases (Bridges, 1998; Cairns and Foster, 1991; Hall, 1997) (Foster, 1998) (Rosenberg et al., 1995). While most of the research has involved the *E. coli* model system, similar observations have been made for other prokaryotes (Kasak et al., 1997) as well as for eukaryotic organisms (Steele and Jinks-Robertson, 1992). Regardless of the organism utilized, these types of mutations (called either *adaptive-* or *stationary-phase induced*) and the processes that generate them are of real interest because of their implications to evolution and the generation of diversity across the domains of life.

In the frameshift-reversion assay system that has been studied in *E. coli*, stationary-phase or adaptive mutations can be distinguished from normal growth-dependent spontaneous mutations. Specifically, the mutations generated in stationary-phase cells require a functional homologous recombination system (see chapter by Levene and Huffman, this volume), F' transfer functions (see chapter by Porter, this volume), and a component(s) of the SOS system (see below). Genetic evidence suggests that DNA polymerase III and DNA polymerase IV are responsible for the synthesis errors that lead to these mutations (Foster 1999; McKenzie et al 2000). The mechanism(s) responsible for this stationary-phase mutagenesis have not yet been delineated. However, studies have suggested that in a starving or "stressed" culture a small subpopulation of the cells seem to have an overall increased mutation frequency (Hall, 1990; Bridges, 1997; Foster, 1998; Torkelson et al., 1997; Lombardo et al., 1999). Theoretically bacteria may differentiate a hypermutable subpopulation when cells are under stressed conditions, and these hypermutable cells generate mutations randomly. The existence of a hypermutable subpopulation(s) responsible for generating genetic diversity in a stressed population raises fascinating questions concerning the nature of the molecular mechanisms that control this process as well as the potential involvement of quorum sensing systems (see chapter by Fuqua and Parsek) and prokaryotic differentiation and development regulons (see chapters by Moran, Streips, Hartzell and Ream, in this volume). Significantly in the *Bacillus subtilis* model prokaryotic system a subpopulation has already been characterized that enhances diversity through differentiation and the development of natural competence (see chapter by Streips-Transformation, in this volume) and the induction of DNA repair systems (Bol and Yasbin, 1991; Cheo et al., 1993; Yasbin et al., 1992).

II. DNA DAMAGES

Each time DNA is synthesized (see chapter by Firshein, this volume), either following semiconservative chromosome replication or following repair-replication, there is the possibility that mispairing of bases will occur. The rate of mispairing can be significantly affected by cellular metabolism, chemical alterations of the bases, and by the presence of base analogues (Friedberg et al., 1995). A transient rearrangement of bonding among the bases—this process is called a *tautomeric shift*—can occur during normal cellular metabolism. Such a rearrangement results in the production of a structural isomer of a base. These tautomers will enhance mispairing. For instance, guanine and thymine can shift from their normal keto form to an enol form. When either is in its enol form, these bases will now be able to bond to each other rather than their normal bonding partners, cytosine and adenine, respectively. Similarly, when either cytosine or adenine shift from their amino form to the imino tautomer they can now bind with each other. Following the next round of replication, the improper bonding caused by the tautomeric shifts will result in the fixation of mutations in at least one of the newly replicated DNA strands.

The exocyclic amino groups that can be found on some of the bases in DNA can be lost spontaneously in reactions that are dependent on temperature and pH. This deamination process results in cytosine, adenine, and guanine being converted to uracil, hypoxanthine, and xanthine, respectively. The products of some of these deaminations can give rise to mutations due to incorrect pairing following DNA replication (Friedberg et al., 1995). The significance of this problem is demonstrated by the existence of repair systems specific for the removal of these deamination products from DNA (described below). One deamination product that potentially represents a very serious problem is the conversion of 5-methylcytosine (a common modification) to thymine. While the other deamination products mentioned are not normally found in DNA, thymine is a natural component and its recognition as being in an incorrect location is certainly not straightforward.

In addition to deaminations, environmental and metabolic factors result in the loss of purines and pyrimidines from the DNA (apurinic and apyrimidinic sites) as well as nonenzymatic methylations of bases within the DNA. Again, these chemical changes can result in mispairing during DNA replication.

Because of its charged nature, components of the DNA are subjected to attack by reactive oxygen species. Such interactions represent a major source of spontaneous damage to DNA. Normal by-products of oxidative metabolism as well environmental factors such as ionizing radiation, near-UV light (UVA), and heat. (Friedberg et al., 1995) generate a variety of these reactive oxygen species. These species include peroxides, as well as superoxide and hydroxyl radicals that all can react with DNA directly or indirectly (Balasubramanian et al., 1998; Imlay and Linn, 1988) to produce strand breaks as well as altered bases such as 8-Oxo-7,8-dihydrodeoxyguanine (8-oxoG). This particular altered base often pairs with adenine instead of cytosine resulting in a GC to TA transversion following replication and will be discussed in a subsequent section (Friedberg et al., 1995).

Substantial evidence has been presented that hydrogen peroxide and the superoxide radicals do not react directly with DNA. Instead, the primary source of DNA damage caused by the presence of these reactive species seems to be the result of the generation of hydroxyl radicals (*OH) through Fenton-like reactions (Imlay and Linn, 1988). With respect to strand breaks and base loss, it is known that the hydroxyl radicals can abstract protons from the deoxyribose of the DNA (Balasubramanian et al., 1998).

Classically the study of DNA damage and repair systems has primarily involved the effects of ultraviolet (UV) radiation (Friedberg et al., 1995). From a research view, UV can be easily administered to cells under defined conditions. From an evolutionary view, living systems have been continually exposed to UV from the very beginning of life on the planet. From a public health view, UV and its effects are important with respect to human disease (especially cancer; Setlow 1978) as well as the maintenance of our ecosystem (Pienitz and Vincent, 2000).

The wavelengths that comprise the ultraviolet spectrum have been divided into three bands: UVA (400–320 nm), UVB (320–290 nm) and UVC (290–100 nm). While most of the early laboratory work involved UVC, it is actually UVA and UVB that constitute the majority of solar radiation that reaches the surface of the planet since wavelengths below 320 nm do not penetrate well the atomospheric ozone layer (Friedberg et al., 1995). Nevertheless, all of the research performed using UV radiation has been instrumental in our understanding of a myriad of processes including DNA repair, replication, recombination, mutagenesis, cancer biology, and cell cycling.

Following exposure to UV, the bases in the DNA strongly absorb photons that energize and lead to rearrangements of the chemical bonds. The first type of UV damage that was extensively studied was the pyrimidine dimer. In this damage product, the rings

of two adjacent pyrimidines fuse. A cyclobutane ring is formed when the 5-carbon atoms and the 6-carbon atoms of adjacent pyrimidines join. Another type of dimer results when the 6-carbon of one pyrimidine is joined to the 4-carbon of an adjacent pyrimidine. This photoproduct is referred to as a 6–4 lesion or the pyrimidine-pyrimidone (6–4) photoproduct. Additional photoproducts are found less frequently in DNA following UV irradiation or only under special conditions. These products include 5, 6-dihydroxydihydrothymine (thymine glycol; Demple and Linn, 1982); the spore photoproduct (5′-thyminyl-5,6-dihydrothymine) (Varghese, 1970) and pyrimidine hydrates (Fisher and Johns, 1976).

While only a sampling of the types of DNA damage and base changes have been presented, the diversity of the sample highlights the importance and need of living cells to protect and repair their genetic material. While this point should have been obvious, interest in this important area of research did not really assume high priority until almost the start of the 1950s. One of the milestones was a report by Dr. Evelyn Witkin in 1947. Essentially Dr. Witkin (1947) observed that a mutant of *E. coli* could be shown to have decreased resistance to DNA-damaging agents. The conclusion that could be drawn from this result was that the bacterium had genetic information that determined how sensitive it was to the killing effects of DNA-damaging agents. Thus there must be a DNA repair mechanism(s). For the past 50 years there has been an extensive delineation of the mechanisms responsible for maintaining the integrity of DNA.

III. PHOTOREACTIVATION

The first DNA repair mechanism discovered was photoreactivation (Dulbecco, 1949; Kelner, 1949). Photoreactivation reduces the deleterious effects of UV irradiation (200–300 nm) by means of a light-dependent process in which the cis-syn cyclobutyl pyrimidine dimers are enzymatically monomerized (Setlow et al., 1965; Wulff and Rupert, 1962). This process (Fig. 1) involves a single enzyme, a DNA photolyase. In *E. coli*, this photolyase is a flavoprotein that functions by a two-step mechanism (Sancar et al., 1985). Initially DNA photolyase binds to pyrimidine dimers in a light-independent reaction. Upon subsequent exposure to light of wavelengths greater than 300 nm, the enzyme cleaves the dimer and dissociates from the substrate, leaving the original primary structure of the DNA.

DNA photolyase activity has been detected in a wide variety of microorganisms, plants, and animals (Rupert, 1975). The exceptions to this near-universal distribution are in naturally competent eubacteria (Campbell and Yasbin, 1979), and in animals higher on the evolutionary tree than marsupials (Friedberg et al., 1995). Since many or the organisms harboring a DNA photolyase never, or very rarely, come in contact with the necessary wavelengths of light, it remains a question as to why this genetic information would have been maintained through evolution of these organisms. It has been suggested that these enzymes might have additional functions. For instance, it has been demonstrated that the presence of a functional photoreactivation gene in strains of *E. coli* that are deficient in recombination (*recA*) decreases the sensitivity of these strains to UV irradiation even without any exposure to photoreactivating light (Yamamoto et al., 1984). Furthermore the *E. coli* photolyase, under nonphotoreactivating conditions, stimulates in vitro both the rate and cutting by the excision nuclease (see below) of UV-irradiated DNA (Sancar et al., 1984).

IV. NUCLEOTIDE EXCISION REPAIR

Bulky, noncoding lesions that produce a block to DNA replication can be removed from damaged DNA through the action of a nucleotide excision repair (NER) system. A noncoding lesion constitutes some alteration of a nucleotide(s) contained within the DNA such that the replication machinery of the

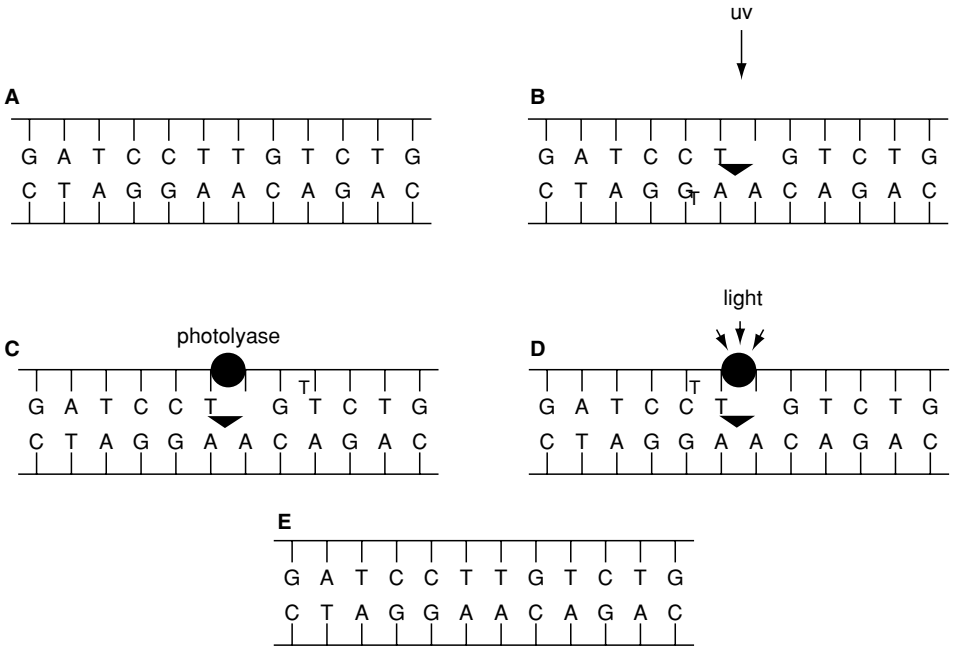

Fig. 1. Photoreactivation. Shown is a schematic of how the photolyase enzyme with the help of species-specific wavelengths of light enzymatically cleaves pyrimidine dimers and thus restores the integrity of the DNA. (**A**) A DNA sequence (**B**) That sequence following exposure to UV (approximately 254 nm). The triangle represents the pyrimidine dimer that was formed. (**C**) A molecule of photolyase recognizes the dimer, binds to it, and sits there until it is activated by specific wavelengths of light (**D**). Once activated, the dimer is cleaved and the DNA sequence is restored (**E**).

cell can no longer use this nucelotide as a template for normal base-pairing purposes. The general properties of NER include a five-step mechanism (Friedberg et al., 1995):

1. Recognition of the bulky lesion in the DNA.
2. Hydrolyzing a phosphodiester bond in the deoxyribose backbone on the 5′ side of the lesion.
3. Excising the lesion (along with a limited number of nucleotides on its 3′ side).
4. Filling in the resultant gap using the information from the complementary strand.
5. Closing the nicked DNA to generate intact strand (Schendel 1981).

The best characterized model system for NER involves the removal of the pyrimidine dimer by the *E. coli* UvrA, B, C exonuclease (Sancar and Rupp, 1983). The first three steps of the NER in *E. coli* are accomplished through the cooperative functioning of the UvrA, UvrB, and UvrC proteins (Fig. 2). These three proteins comprise the UvrABC endonuclease (Sancar and Rupp, 1983). Two copies of the UvrA protein, and one copy of the UvrB protein form a complex that binds to DNA even in the absence of damage. The complex moves along the DNA, apparently with the DNA wrapped around the A2-B complex (Verhoeven et al., 2001) until a helix distortion (bulky lesion) is identified. The complex will stop at the damage (in this case the pyrimidine dimer), the UvrA protein will exit and be replaced by the UvrC protein. The binding of the UvrC protein to the UvrB causes the UvrB to make a cut in the DNA usually 4 nucleotides 3′ of the damage. Then the UvrC protein cuts the DNA 7

nucleotides 5′ of the damaged base. Following the cuts in the DNA, the UvrD protein (a DNA helicase) removes the oligonucleotides that contain the damage, while DNA polymerase I resynthesizes the removed strand using the opposite strand as the template. Finally, the ligase reseals the newly synthesized strand.

It has been determined that low levels of the UvrA, B, C,and D proteins are found in normal cells. However, the levels of the UvrA, B, and D proteins are significantly enhanced following the introduction of certain types of DNA damage. The genes encoding these proteins have been shown to be part of the SOS regulon (see below).

Research into the NER systems in both prokaryotes and eukaryotes have shown that this type of repair process can be directed to specific regions of the chromosome(s). In particular, NER systems have evolved to treat specific regions of the chromosome(s) differently with respect to what genes should be repaired first or even repaired at all. The most dramatic example of this directed repair can be seen in higher eukaryotes where there is a repair bias for expressed genes as compared to the nonexpressed genes in each cell type (Friedberg et al., 1995). In prokaryotes there is also a mechanism that directs the NER system to preferentially function on transcribed regions (Selby and Sancar, 1994). A protein, the product of the *mfd* gene, called the *transcription coupling repair factor* (TRCF) causes the RNA polymerase to be

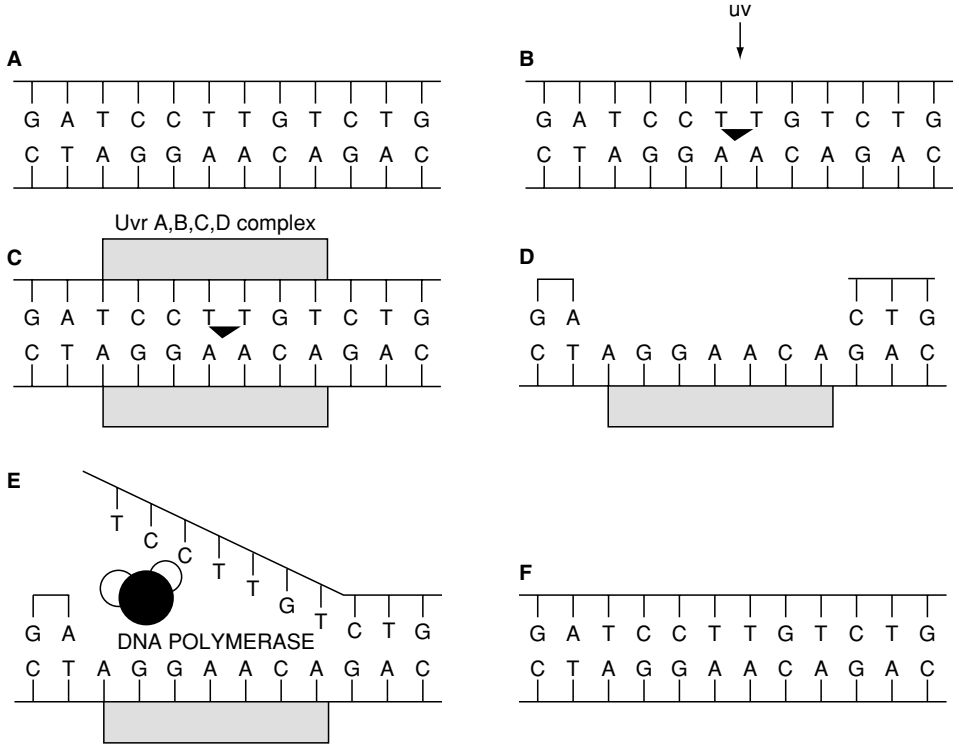

Fig. 2. Nucleotide excision repair. The DNA (**A**) has been exposed to the far UV (**B**) and the pyrimidine dimer has been formed. In (**C**) the UvrA,B,C complex has formed around the damaged DNA and with the help of UvrD this complex cuts the DNA and opens up the DNA (**D**) in order for the DNA polymerase (most often DNA polymerase I) to begin to resynthesize the damaged strand using the opposite strand as a template (**E** and **F**).

displaced when the transcription complex stalls at the site of DNA damage. Because of the damage the RNA polymerase cannot proceed. TRCF also binds to the UvrA protein. Following the TRCF mediated displacement of the RNA polymerase, the UvrA2B complex then binds to the DNA that contains the damage. This interaction accelerates the repair of actively transcribed regions of the genome. A phenomenon related to the functioning of the TRCF is the process called *mutation frequency decline*. This process is the rapid and irreversible decline in suppressor mutation frequency that occurs when the cells are kept in nongrowth media immediately following mutagenic treatment and requires the functioning of the TRCF or the *mfd* gene product.

V. BASE EXCISION REPAIR

Base excision repair (BER) is a second method by which bulky, noncoding lesions can be removed from DNA. In addition BER represents an efficient mechanism for the removal of many base alterations that are the result of metabolic factors (deaminations, alkylations, oxygen radicals, etc.). BER differs from NER in that damaged or incorrect bases are excised as free bases (Fig. 3) rather than nucleotides or oligonucleotides (Friedberg et al., 1995). Total removal of the DNA lesion requires a two-step process. First, BER involves the hydrolysis of the N-glycosylic bond that links the base to the deoxyribose-phosphate backbone of the DNA. This is performed by the action of a class of DNA repair enzymes called

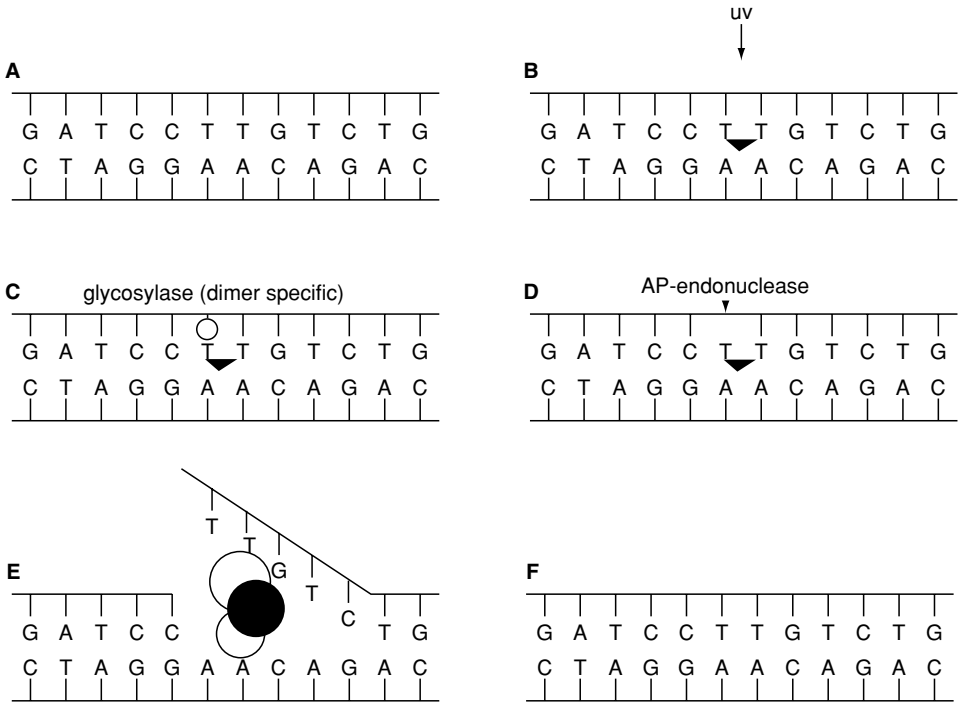

Fig. 3. Base excision repair. The pyrimidine dimer formed after exposure of the bacteria to UV is removed by the activities of a damage-specific glycosylase and AP-endonuclease(s). The DNA sequence (**A**) is exposed to UV and the dimer is formed (**B**). The damage-specific glycosylase recognizes the pyrimidine dimer, attaches to it, and cleaves one of the *N*-glycosylic bonds (**C**) and (**D**) In D, the apyrimidinic site is recognized by an AP-endonuclease, a cut is made in the DNA backbone, some bases are excised, and DNA polymerase resynthesizes the strand using the opposite strand as a template (**E** and **F**).

glycosylases. Once a damaged base, an incorrect base pair or an inappropriate base is recognized by a specific glycosylase, the N-glycosylic bond is cut, leaving an apurinic or apyrimidinic (AP) site in the DNA. The second step in BER in the removal of this AP site via the action of one or more nucleases. Sites of base loss in DNA are specifically recognized by enzymes known as AP endonucleases (Lindahl, 1979). Repair synthesis and ligation in BER proceed as discussed for NER.

Bacteria posses many different DNA glycosylases, and as stated above, each is specific fo a particular type of lesion in the DNA. Included in this group are glycosylases that recognize uracil, hydroxymethyl uracil, 5-methylcytosine, hypoxanthine, 3-methyladenine, 7-methylguanine, 3-methylguanine, and 8-hydroxyguanine (Friedberg et al., 1995). In addition glycosylases have been identified that recognize DNA containing 5,6-hydrated thymine moieties and DNA containing pyrimidine dimers.

As can be seen from this partial list, BER can function on altered bases as well as on bases that are not normally present in DNA (uracil, hypoxanthine, etc.). Another aspect of BER is the removal of bases involved in mispairing. Essentially there are glycosylases that recognize very specific types of mispairing events. Some interesting examples are the enzymes involved in handling the potential problems caused by the generation of 8-oxoG (described above). A DNA glycosylase has been identified that recognizes 8-hydroxyguanine residues in DNA as well as some imidiazole ring-opened forms. Subsequent evaluations determined that this glycosylase was product of the *mutM* gene. This loss of this gene had been shown to cause an increase in the GC to TA transversion rate and a decreased ability to handle the mutagenic effect of 8-oxoG. The biochemical analysis demonstrated that the MutM glycosylase also recognizes 8-oxoG residues in DNA. However, if all of the 8-oxoG residues are not removed by this mechanism, the glycosylase specified by the *mutY* gene functions to help reduce the potential problems caused by the presence of this mutagenic lesion in the DNA. Essentially the MutY glycosylase recognizes 8-oxoG-adenine mispairs in the DNA and removes the adenine. BER will now function to restore an 8-oxoG-cytosine pairing. This pairing could then be the substrate for the MutM glycosylase (to remove the 8-oxoG) (Friedberg et al., 1995).

With respect to the 8-oxoG lesion, there is one additional type of repair that operates but is not part of the BER. The product of the *mutT* gene is a phosphatase that specifically degrades 8-oxodGTP to 8-oxodGMP. This action prevents the DNA polymerases from incorporating 8-oxoG into the DNA. Collectively the *mutT*, *mutY*, and *mutM* gene products function to reduce the mutagenic impact of 8-oxoG. (Michaels et al., 1992).

As mentioned earlier, BER functions via the combined mechanisms of damage-specific DNA glycosylases and AP endonucleases. AP endonucleases produce incisions in the duplex DNA by hydrolysis generally of the phosphodiester bond that is 5′ to the AP site. The result of this incision is the generation of a 5′ terminal deoxyribose-phosphate residue. These residues can be removed by the action of either exonucleases or DNA-deoxyribophosphodiesterases. In the former case there is a removal of tracts of nucleotides followed by DNA polymerase and ligase activity while in the later case, a single nucleotide gap is generated and that gap is replaced by DNA polymerase and ligase activities (Friedberg et al., 1995). In addition to the separate AP endonucleases that have been characterized, several of the glycosylases have associated AP-lyase activity that may or may not play important roles in the actual removal of DNA damage (Friedberg et al., 1995; Vasquez et al., 2000).

VI. MISMATCH EXCISION REPAIR

Classically *E. coli* mismatch excision repair (mismatch repair) was defined as a methyl-

directed postreplication repair system which eliminated replicative errors within newly synthesized DNA (Harfe and Jinks-Robertson, 2000; Modrich and Lahue, 1996). These replicative errors or mismatches are distinguished from the preexisting correct base in the parental strand due to the undermethylated state of the newly synthesized daughter strand. The repair of these mismatches involves localized excision and resynthesis of nucleotides at the site of the mismatch.

The methyl-directed mismatch repair system has been extensively characterized both genetically and biochemically. Mutations in the *dam*, *mutH*, *mutL, mutS*, and *uvrD* genes lead to increases in the spontaneous mutation frequencies between 10-and 1000-fold. This increase in the spontaneous mutation frequency is due to a deficiency in the mismatch repair system (Friedberg et al., 1995) (Modrich and Lahue, 1996). The product of the dam^+ gene is a DNA adenosine methylase that methylates the adenine in the site GATC (Herman and Modrich, 1982). The presence of hemimethylated DNA seems to trigger the enzymes involved in this repair system to search for mismatches. The hemimethylated state would tend to be more prevalent in the newly replicated DNA adjacent to the replication fork. This mechanism would imply that the mismatch would be corrected in favor the parental strand. This does in fact occur. As discussed later, this particular methyl-directed mismatch repair system is not as common as nonmethyl-directed mismatch repair systems.

As one would expect, strains carrying a mutant allele for the *dam* gene perform undirected mismatch excision repair (loss of preferential repair of newly replicated DNA strand). This undirected mismatch repair is the reason behind these strains having an increased mutation frequency or a so-called mutator phenotype. Also, as expected, *dam* mutants are more sensitive to agents that cause strand breaks either directly or as a result of attempted repair of the base damage inflicted by the agent. These mutants exhibit increased recombination frequency and an increased induction of prophage. In addition these mutations are in viable when strains also carry mutations in *recA*, *recB*, *recC*, *recJ*, *lexA*, or *polA* (Bale et al., 1979; Friedberg et al., 1995). These phenotypes can all be explained by the model for the methyl-directed mismatch repair system that has been advanced. Specifically, strains carrying a mutant *dam* gene would excise relatively long patches due to the undirected nature of the repair. This accumulation of long patches of single-stranded DNA would lead to an increase in the generation of double-strand breaks. These double-strand breaks would enhance an promote recombination, prophage induction and lethality (Section VII). As expected, suppressors of the *dam rec* double-mutation combinations have been found to be mutant alleles of *mutH*, *mutL*, and *mutS* (mutations that prevent the excision patches).

The *E. coli* mismatch repair system does not identify and correct all potential mismatches with equal efficiency (Radman and Wagner, 1986). In general, the extent of the repair depends on the type of mismatch as well as the neighboring nucleotide sequences. Specifically, transition mismatches (G-C to G-T and A-T to A-C) appear to be repaired more readily than are transversion mismatches. G-G and A-A mismatches seem to be repaired efficiently, while T-T, G-A, and C-T are repaired less efficiently. There seems to little repair of the C-C mismatch. Furthermore, increasing the G-C content in the neighboring nucleotide sequences enhances the probability that a given mismatch will be repaired.

Besides the mismatches listed above, the *E. coli* system can recognize and repair frameshift heteroduplexes. These types of heteroduplexes (the result of either additions or deletions to one strand of the heteroduplex) do not technically contain a mismatch. Rather, there is an extra, and therefore unpaired, base in one of the strands. Mismatch works equally well on both strands when the DNA is nonmethylated. In the presence of methylated DNA, the heteroduplex repair is

directed and would therefore seem to function in the region of the replication fork.

The mismatch repair system described above is the classic example of a type of repair process that has been designated long patch mismatch repair (LPMR). While the LPMR system in *E. coli* requires or is dependent on the activity of the *dam*$^+$ gene, in *Streptococcus pneumoniae* an LPMR directed by the *hex*$^+$ genes is independent of DNA methylation (Radman, 1988). This apparent paradox seems to be resolved by the observations that in *E. coli* the product of the *mutH*$^+$ gene nicks the nonmethylated GATC sequence and persistent nicks in heteroduplex DNA can effectively substitute for the functions of both the MutH protein and the nonmethylated GATC sequence (Radman, 1988). Thus LPMR is not necessarily dependent on a DNA methylation system (as is the case in *S. pneumoniae* and most other organisms) but instead could be directed against strands that have single-strand ends.

In the non-methyl-directed LPMR systems, homologues of MutS and MutL have been identified (Harfe and Jinks-Robertson, 2000). However, MutH homologues are not found in these systems. This again indicates that the MutH protein is specifically involved in the interaction with the methylated sequence. For the *E. coli* system, a complex of MutH, MutL, and MutS bind to the mispaired region, and then the DNA apparently forms an alpha loop (which requires ATPase activity). The excision of the mispaired base or region occurs once the unmethylated GATC site (or single-strand break) is reached. DNA helicase II (the product of the *uvrD* or *mutU* gene), DNA polymerase III, and ligase are required to complete the repair. It is interesting to note that this is one of the few cases in which DNA pol III is the preferred repair polymerase.

In addition to LPMR, another type of mismatch correction system has been characterized in prokaryotes and eukaryotes by short spans of DNA being repair replicated (Coic et al., 2000; Lieb and Bhagwat, 1996; Lieb and Rehmat, 1995; Turner and Connolly, 2000). An example of such a short-patch repair system would by the one controlled by the *mutY* gene (Section IV). Another well-studied short-patch repair system is the one termed VSPMR (very short-patch mismatch repair; Radman, 1988). This system repair those G-T mismatches that apparently originate by the deamination of 5-methylcytosine to thymine in the sequence 5′-CC (A or T) GG-3′. The enzyme encoded by the *dcm* gene methylates the second C in the sequence. The repair of these G-T mismatches to the correct G-C pairing by VSPMR significantly reduces the mutation "hot spots" generated by the presence of 5-methylcytosine. In this *E. coli* system the products of the *dcm*$^+$ gene (cytosine methyl transferase) and the *mutS*$^+$, *mutL*$^+$, and *polA*$^+$ are essential. However, the products of the *mutH*$^+$ or *mutU*$^+$ are not required.

In the case of the *S. pneumoniae*, the VSPMR system acts on the sequence 5′-ATTAAT-3′, and the repair pattern involves the correction of G-A to G-C (Sicard et al., 1985, 2000) and seems to be involved in the efficiency of some markers during the transformation process (see chapter by Streips-Transformation, this volume).

VII. POSTREPLICATION REPAIR OR DAMAGE BYPASS

In *E. coli* treated with UV or other agents that cause the production of bulky, noncoding lesions in the DNA, damages that are not removed before being encountered by the replication machinery constitute a block to further DNA synthesis (Setlow et al., 1963). DNA replication can be resumed if the DNA polymerase dissociates from the DNA when it encounters a noncoding lesion and then initiates replication on the other side of the lesion (see chapter by Firshein, this volume). Such a mechanism was first proposed in 1968 (Howard-Flanders et al., 1968). This type of mechanism would result in gaps in the newly synthesized daughter strand, which subsequently become filled-in by some process. In support of this model it was observed that the

daughter strands are much smaller than the parental template following UV irradiation. This size of this newly synthesized DNA approximates the average interdimer distance in the template (Sedgwick, 1975). Upon continued incubation, daughter strands become longer until they eventually reach the same size as the parental strands.

The daughter-strand gaps are filled in by a recombination event (Ganesan, 1974). Hence this process has been called postreplication repair, daughter strand gap repair and recombination repair (Fig. 4). Regardless of the name that is applied, this type of mechanism exemplifies tolerance of DNA damage rather than a true repair process since the actual damage is night physically removed from the DNA. Rather, the damage is bypassed by this process.

The evidence for the involvement of recombinational events in this process is as follows: In UV-irradiated *E. coli*, newly synthesized DNA was found in both the daughter and parental strands, physically demonstrating that strand exchange had occurred. From these data it was estimated that one genetic exchange occurred per pyrimidine dimer. The reciprocal of these data was also observed in that dimers were found to be equally distributed be parental and progeny strands (Ganesan, 1974). In addition UV-irradiated DNA that has replicated is highly recombinogenic

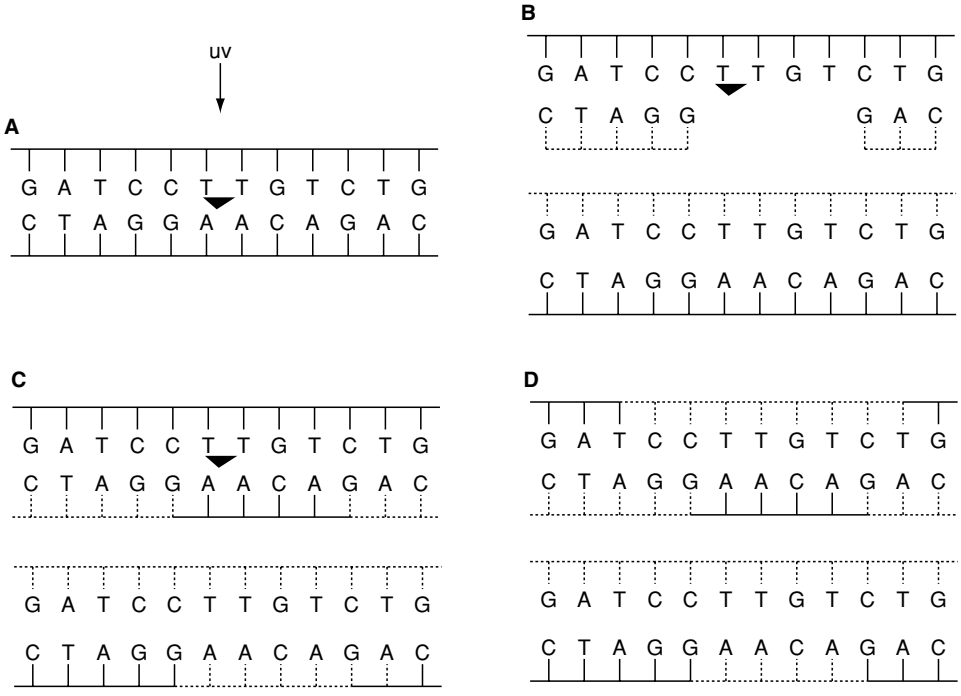

Fig. 4. Recombination or translesion bypass. This type of repair is actually a tolerance mechanism. Following the introduction of damage into the DNA. **A:** the DNA will now have problems being replicated. When the DNA is replicated, the two daughter strands cannot be completely finished. Opposite a site of damage in a parental strand there will be a gap in the daughter strand. **B:** As long as the gaps are not overlapping in the two daughter strands, recombination can be utilized remove the single-strand gaps. **C:** DNA from the other parental strand can be recombined into one of the daughter strands. This will now result in parental DNA being found in a daughter strand and newly synthesized DNA being found in the parental strand that was a donor of DNA to the gapped daughter strand. **D:** The damaged DNA (in this case a pyrimidine dimer) can be repaired and actually removed via any of the mechanisms previously discussed (photoreactivation, nucleotide excision repair, base excision repair).

(Howard-Flanders et al., 1968) and the presence of the photoproducts in the replicating DNA is responsible for the increase in recombination (Lin and Howard-Flanders, 1976). Finally, strains of *E. coli* carrying the *recA1* allele do not convert short, newly synthesized, DNA strands into high molecular weight DNA (Smith and Meun, 1970).

As mentioned above, the $recA^+$ gene product is required for postreplication repair. It is not too surprising that other genes known to be involved with general recombination events would also influence postreplication repair (see chapter by Levene and Huffman, this volume). In general, there is a strong correlation between the levels of recombination proficiency and UV resistance in various mutant strains. Specifically, mutations in *recA, recB,C,D, recE, recF, recJ, recN, recQ, ruvB,C,* and *ssb* reduce UV resistance in genetic backgrounds where they reduce recombination proficiency (Mahajan, 1988). Postreplication repair proceeds by two major $recA^+$ dependant processes. One pathway repairs most of the DNA daughter-strand gaps via the $recF^+$-mediated process, while the other repairs double-strand breaks produced by the cleavage of unrepaired gaps. This second pathway is dependent on the functions of the *recBCD* gene products (see chapter by Levene and Huffman, this volume). Finally, the repair of cross-links in the DNA presents a situation where both recombination (postreplication) repair and excision repair must function together. In this type of damage, adducts are covalently attached to both strands of the DNA. The UvrABC complex removes the adduct from one strand, producing a gap, while the opposite strand still contains the adduct. Recombinational repair would allow for the filling-in of the gap, permitting the adduct to now be removed from the opposite strand (Cole, 1973).

VIII. TRANSLESION DNA SYNTHESIS

Postreplication repair illustrates DNA damage tolerance via a discontinuous mode of DNA synthesis. However, DNA damage tolerance could occur via a continuation of DNA synthesis, opposite a noncoding lesion, without gap formation. This is termed *translesion DNA synthesis* (Friedberg et al., 1995). While the former type of postreplication repair should be relatively error free (not produce mutations), translesion DNA synthesis should result in the production of errors or mutations.

Translesion DNA synthesis is one of a myriad of coordinately induced cellular responses observed in *E. coli* and collectively known as the SOS system or regulon (Little and Mount, 1982; Radman, 1974; Walker, 1987; Witkin, 1976). As part of this SOS system, translesion DNA synthesis usually has been called *error-prone* repair or inducible DNA repair. This nomenclature arose because an increased mutation frequencies was observed in *E. coli* populations induced for SOS functions following some type of DNA damage. However, as with postreplication repair, this "error-prone" repair was postulated to result in a dilution out of DNA lesions rather than a true repair of the DNA. Therefore it was suggested (Miller, 1982) that mutations should be thought of as occurring by replication across from altered bases rather than as a result of a true repair process. Hence the term *translesion DNA synthesis* arose.

A partial list of *E. coli* SOS responses is given in Table 1. These phenomena are coordinately induced in *E. coli* cells that have been exposed to UV radiation, chemicals that produce bulky lesions, or agents that arrest DNA synthesis (Walker, 1984; Witkin, 1976). Radman (1974) first formalized the SOS hypothesis by suggesting that DNA damage or the consequence of this damage initiates some sort of regulatory signal that simultaneously causes the derepression of a number of genes. He further speculated that this "danger" signal might be a temporary block in DNA replication. Expression of these SOS phenomena have traditionally been described as depending upon the functioning of the RecA and LexA proteins. However, some recent results in *E. coli* as

TABLE 1. Phenomena That Are Components of the SOS Regulon

Phenomenon	Description
Prophage induction	Resident prophage are induced to enter lytic cycle (i.e., λ)
W reactivation	Enhanced survival or irradiated phage
W mutagenesis	Enhanced mutation rate of W-reactivated phage
UV mutagenesis	Ability of UV to cause mutations
Filamentation	Bacteria grow as long filaments
Induction of Din genes	Genes that are DNA damage inducible such as *recA, lexA, himA, uvrA, B, dinA, B, D, F*
Cessation of respiration	Loss of active aerobic growth
Alleviation of restriction	Decrease in the effect of restriction enzymes
Stable DNA replication	New rounds of DNA replication begin

well as studies in other organisms indicate that the SOS system might involve more than one type of gene regulation (Humayun, 1998; Cheo et al., 1993; Yasbin et al., 1992). Despite this potential diversity in regulation, the general working model for the control of the primary SOS regulon is as shown in Figure 5 (Little and Mount, 1982; Walker, 1984; Witkin, 1976). Essentially, in the undamaged wild-type cell, the SOS regulon genes are repressed by the LexA protein. The products of these genes (including LexA and RecA) are synthesized at low constitutive levels (or not produced at all). An SOS-inducing signal is generated by DNA damage. There is convincing evidence that the major signal for this induction are the regions of single-stranded DNA that are generated when the molecular machinery attempts to replicate a damaged DNA template or when the normal process of DNA replication is blocked (Friedberg et al., 1995). RecA binds to these single-stranded regions, in the presence of nucleoside triphosphates, and allosterically converts (reversibly) to a form that has been called RecA*. LexA protein comes in contact with the RecA* nucleoprotein complex, resulting in the autoproteolysis of LexA at a specific Ala-Gly bond. In this sequence of events the RecA* functions as a coprotease and the proteolytic activity actually resides within the LexA protein itself. Following cleavage, the LexA protein can no longer function as a cellular repressor. In addition to LexA, the RecA* can cause similar activation of proteolytic activity in certain prophage repressors (i.e., λ) and the UmuD protein (discussed below). There have been recent reports that the activation of this proteolytic activity might involve interactions with polyamines (Kim and Oh, 2000). However, the complete nature of the activation process requires additional studies.

LexA has been shown to be the repressor of over 20 genes, including *recA*, *lexA*, *uvrB*, and *umuD,C*. It is possible that other cellular repressors in *E. coli* may exist that are sensitive to auto-proteolytic cleavage following activation RecA*. As mentioned above, RecA* also leads to the auto-proteolytic digestion of λ repressor and the UmuD protein. In any event the pools of LexA protein decrease very rapidly after inducing treatment (activation of RecA to RecA*), and the end result is the derepression of the SOS regulon and the expression of the SOS phenomena. This expression will continue as

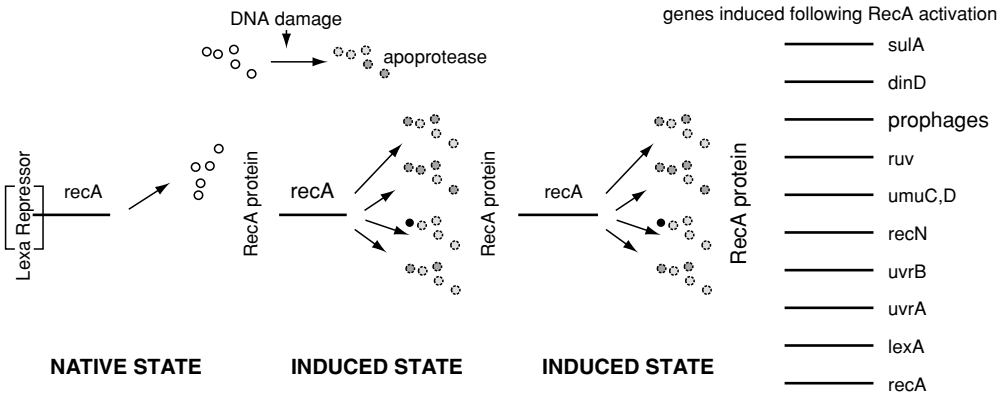

Fig. 5. Induction of the SOS system. The LexA protein is a repressor of at least 20 different genes on the *Escherichia coli* chromosome. This replicon can be induced following the introduction of certain types of damage into the bacterial DNA. Once the DNA has been damaged, a signal is produced that activates the RecA protein into becoming a apoprotease. This activated form of RecA causes LexA to cleave itself, thus inducing all of the genes under its control. Interestingly, *lexA* and *recA* are two of the genes under the control of the LexA repressor. Thus, when the system is induced, large quantities of RecA and LexA proteins are produced. The LexA protein is inactivated as long as there is an activated form of the RecA protein present. Once the damage has been removed, the signal no longer exists and then the RecA protein is no longer activated. When this occurs there is sufficient LexA present to shut down the SOS regulon. In addition to causing the LexA protein to cleave itself, the activated form of the RecA protein also causes the UvrD protein and many different types of prophage repressors to cleave themselves.

long as sufficient inducing signal persists. As the level of this signal subsides, less RecA* is available and the cellular concentration of the LexA protein will rise. Eventually the entire SOS regulon will again be repressed, and the cell will return to its normal, uninduced state. This return to steady-state level of LexA repressor following the removal of the inducing signal occurs rapidly.

This mechanism for regulation of the SOS response offers many opportunities for fine-tuning of the system. First, RecA* exhibits varied efficiencies in causing the auto-proteolysis of different proteins. For instance, the LexA protein is activated to auto-cleave itself more readily than is the λ repressor (Little and Mount, 1982). Therefore one would expect that the SOS regulon genes that are repressed by the LexA protein would be preferentially induced when compared to the induction of prophage λ. Second, the LexA protein has different binding efficiencies for the various operator regions of the SOS regulon genes. The *recA* operator binds LexA more strongly than do the operators of the *uvrB* or *lexA* genes (Brent and Ptashne, 1981). The binding strength of LexA protein is greatest for *dinD*, somewhat weaker for *umuD, C*, and weaker yet for *uvrA*, *dinA (polB)*, and *dinB (polIV)* (Kreuger et al., 1983). This indicates that the potential exists for intermediate induction of the SOS system and for the production of mutations (Walker 1984).

The operator regions of a number of SOS genes have been sequenced and protein protection experiments have resulted in the identification of DNA sequences that have been called SOS boxes or Lex boxes (Friedberg et al., 1995). The operator regions have similar base sequences, about 20 bp long, that are binding sites for the LexA protein. All the binding sites include inverted repeat sequences that contain as a minimum 5′CTG∼10N-CAG3′. The *lexA* gene has two nearly identical SOS boxes in its operator region, again adding another dimension of control.

Finally, the fact that the repression of the *lexA* gene is autoregulated (Friedberg et al.,

1995) markedly influences SOS induction. This autoregulation allows for the expression of only a subset of the SOS responses, depending on the strength of the inducing signal. It also guards against full induction of the system in response to a mildly damaging situation, since the LexA protein has a greater affinity for the *recA* operator than its own operator. In addition autoregulation of the production of the LexA protein allows for a speedy return to the repressed state, which is observed when the inducing signal subsides.

It has long been established that mutagenesis of the *E. coli* chromosome by UV, as well as certain chemicals such as methylmethanesulfonate (MMS) and 4-nitroquinoline-1-oxide (4NQO) is dependent on the *recA* and *lexA* gene products (Witkin, 1976). Therefore induced mutagenesis is one of the SOS responses. The mechanism for this mutagenesis has only recently begun to be elucidated. In *E. coli* three DNA polymerases have been shown to be under SOS regulation; Pol II, Pol IV, and Pol V (Wagner et al., 1999; O'Grady et al., 2000; Sutton et al., 2000). Pols IV (product of the *dinB* gene) and V (product of the *umuD, C* genes) belong to a superfamily of DNA polymerases that have been found in eubacteria, archaea, and eukaryotes (Friedberg et al., 2000; Gerlach et al., 1999). Pols IV and V are nonprocessive polymerases that can perform translesion bypass. In addition to their roles in translesion bypass, the UmuDC proteins have also been associated with prokaryotic cell cycle control (Sutton et al., 2001a, 2001b; Sutton and Walker, 2001), which represents another important survival aspect of the SOS system.

As mentioned above, variations on the SOS regulon have begun to be identified. In addition to regulation by a Lex-like cellular repressors, the SOS genes have been shown to be under the control of prokaryotic development and differentiation factors (Cheo et al., 1992, 1993; Lovett et al., 1989; McVeigh and Yasbin, 1996; Yasbin and Miehl-Lester, 1990). In addition the binding sites for the cellular repressors have shown divergence among the gram-negative bacteria and between the gram-positive and gram-negative kingdoms (Winterling et al., 1997). There is also a tremendous diversity of the types of genes that are grouped into SOS regulons in different organisms. These genes range from ones whose products are involved in DNA repair to genes that play essential roles in virulence, metabolism, growth, and development (Friedberg et al., 1995). Thus the SOS regulon developed early in evolution and has been conserved as well as modified to play important roles in the survival of species.

IX. ADAPTIVE RESPONSE

E. coli posses an inducible repair system that protects against the lethal and mutagenic effects of alkylation damage (Jeggo et al., 1977; Landini and Volkert, 2000; Samson and Cairns, 1977). This repair system has been termed the adaptive response, due to its particular mode of functioning. Specifically, *E. coli* cultures exposed to low levels of an alkylating agent such as N-methyl-N'-nitro-N-nitosoguanidine (MNNG) and then subsequently challenged by a much higher dose of this agent are able to withstand both the cytotoxic and mutagenic effects of such an exposure. Hence these cultures have "adapted" to the deleterious effects of MNNG.

The adaptive response is regulated by the product of the *ada* gene and also during stationary phase by rpoS-dependent gene expression (see Moran; Streips-Stress Shock, Landini and Volkert, 2000). This interesting protein has a molecular weight of 37,000 daltons and has at least three known functions. First, this protein is a positive regulatory element that is involved in the increased transcription of at least four genes (*ada, alkA*, *alkB*, and *aidB*). The *ada* and *alkB* genes are in an operon, while the other two known genes of this regulon are dispersed on the chromosome. The enzymatic function of alkB has yet to be clearly defined. However, it is known that bacteria deficient in this product are more sensitive to some alkylating agents and that this protein is needed to

remove certain damages (Landini and Volkert, 2000). The alkA gene encodes a glycosylase that repairs several alklyation caused lesions including N7-methylguanine, N3-methyl purines, and O2-methyl pyrimidines. The aidB gene is homologous to the mammalian isovaleryl coenzyme A dehydrogenase (IVD), and appears to have IVD activity to function and inactivate nitrosoguanidines or their reactive intermediates. However, its exact enzymatic activity has not been completely established.

In addition to its activity as a regulatory element, the Ada protein is a methyltransferase. Ada has two active methyl acceptor cysteine residues, Cys-69 and Cys-321, that are required for demethylation of damaged DNA (Friedberg et al., 1995). Both sites can be methylated but are utilized to repair different types of damages. The Cys-321 is the methyl acceptor site required for the removal of two very mutagenic lesions: methyl groups from either O6-methylguanine or O4-methylthymine. The Cys-69 is involved in the removal of methyl groups from the phosphomethyltriesters in the sugar-phosphate backbone. The Ada protein is not turned over following its acceptance of alkyl groups, and thus it can be classified as a "suicide" protein. Furthermore the transfer of a methyl group from the triester, rather than from the guanine or thymine, is responsible for causing the Ada protein to become a positive effector molecule for transcription. Importantly, the Ada protein can function as both a positive and negative effector of transcription (Saget and Walker, 1994) (Landini and Volkert, 2000).

X. UNIVERSALITY OF DNA REPAIR MECHANISMS

While *E. coli* has functioned as the principal model for investigations into DNA repair mechanisms, by no means is it a unique organism. The DNA repair systems identified in this paradigm have been discovered in most other organisms studied. While not all organisms may have all of the same systems identified in *E. coli*, it is clear that DNA repair systems are an important evolutionary advantage and as such they have been conserved in both prokaryotic and eukaryotic systems. This fact has been made even more evident by the results of the genome-sequencing efforts (Wood et al., 2001); Ronen and Glickman, 2001). Not only have the genes and the proteins discussed above been shown to be involved in survival and mutagenesis, but homologues of these proteins play essential roles in disease prevention, cell-cycle regulation as well as normal development and differentiation (Aquilina and Bignami, 2001; Khanna and Jackson, 2001; Modrich and Lahue, 1996; Sutton et al., 2001b). Clearly, the pioneering investigations into DNA repair mechanisms in *E. coli* and other prokaryotes have greatly enhanced our understanding of the ability of life systems to survive, adapt, and evolve.

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