tions by using a set of tester strains that carry different types of mutations—one strain with a transition, one with a frameshift mutation, and so forth.

Over a period of several years during which they tested hundreds of different chemicals, Ames and his colleagues observed a greater than 90 percent correlation between the mutagenicity and the carcinogenicity of the substances tested. Initially, they found several potent carcinogens to be nonmutagenic to the tester strains. Subsequently, they discovered that many of these carcinogens are metabolized to strongly mutagenic derivatives in eukaryotic cells. Thus, Ames and his associates added a rat liver microsomal fraction to their assay systems in an attempt to detect the mutagenicity of metabolic derivatives of the substances being tested. Coupling of the rat liver microsomal activation system to the microbial mutagenicity tests expanded the utility of the system considerably. For example, nitrates are not themselves mutagenic or carcinogenic. However, in eukaryotic cells, nitrates are converted to nitrosamines, which are highly mutagenic and carcinogenic. Ames's mutagenicity tests demonstrated the presence of frameshift mutagens in several components of chemically fractionated cigarette smoke condensates. In some cases, activation by the liver microsomal preparation was required for mutagenicity; in other cases, activation was not required. The Ames test provides a rapid, inexpensive, and sensitive procedure for testing the mutagenicity of chemicals. Since mutagenic chemicals are also carcinogens, the Ames test can be used to prevent the widespread use of mutagenic and carcinogenic chemicals.

Key Point: **By using *Salmonella* tester strains that carry various types of mutations in genes encoding histidine biosynthetic enzymes, Bruce Ames and coworkers developed an inexpensive and sensitive method for detecting the mutagenicity of chemicals.**

DNA REPAIR MECHANISMS

The multiplicity of repair mechanisms that have evolved in organisms ranging from bacteria to humans emphatically documents the importance of keeping mutation, both somatic and germ-line, at a tolerable level. For example, *E. coli* cells possess at least five distinct mechanisms for the repair of defects in DNA: (1) light-dependent repair or photoreactivation, (2) excision repair, (3) mismatch repair, (4) postreplication repair, and (5) the error-prone repair system. Moreover, there are at least two different types of excision repair, and the excision repair pathways can be initiated by several different enzymes, each acting on a specific kind of damage in DNA. Mammals seem to possess all of the repair mechanisms found in *E. coli* except photoreactivation. Because most mammalian cells do not have access to light, photoreactivation would be of relatively little value to them. Humans and other mammals undoubtedly possess some DNA repair mechanisms that are not present in bacteria, but many details of these repair processes require further documentation.

The importance of DNA repair pathways to human health is clear. Inherited disorders such as xeroderma pigmentosum, which was discussed at the beginning of this chapter, vividly document the serious consequences of defects in DNA repair. We discuss these inherited disorders in a subsequent section of this chapter.

Light-Dependent Repair

Light-dependent repair or **photoreactivation** of DNA in bacteria is carried out by a light-activated enzyme called **DNA photolyase.** When DNA is exposed to ultraviolet light, thymine dimers are produced by covalent cross-linkages between adjacent thymine residues (see Figure 14.23). DNA photolyase binds to thymine dimers in DNA and uses light energy to cleave the covalent cross-links (Figure 14.26). Photolyase will bind to thymine dimers in DNA in the dark, but it cannot catalyze cleavage of the bonds joining the thymine moieties without energy derived from visible light, specifically light within the blue region of the spectrum. Photolyase also splits cytosine dimers and cytosine-thymine dimers. Thus, when ultraviolet light is used to induce mutations in bacteria, the irradiated cells are grown in the dark for a few generations to maximize the mutation frequency.

Excision Repair

Excision repair of damaged DNA involves at least three steps. In step 1, a DNA repair endonuclease or endonuclease-containing enzyme complex recognizes, binds to, and excises the damaged base or bases in DNA. In step 2, a DNA polymerase fills in the gap by using the undamaged complementary strand of DNA as template. In step 3, the enzyme DNA ligase seals the break left by DNA polymerase to complete the repair process. There are two major types of excision repair: **base excision repair** systems remove abnormal or chemically modified bases from DNA, whereas **nucleotide excision repair** pathways remove larger defects like thymine dimers. Both excision pathways are operative in the dark, and both occur by very similar mechanisms in *E. coli* and humans.

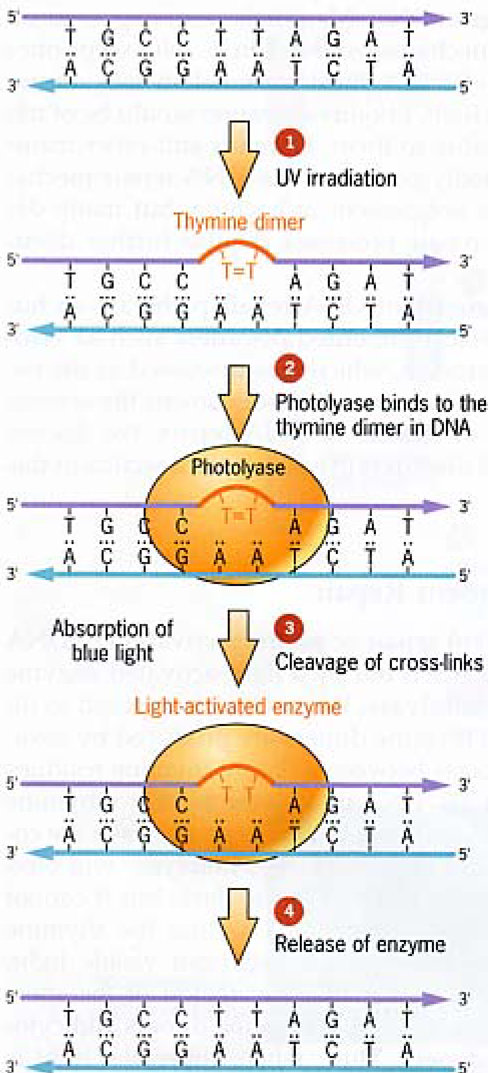

Figure 14.26 Cleavage of thymine dimer cross-links by light-activated photolyase. The arrows indicate the opposite polarity of the complementary strands of DNA.

Base excision repair (Figure 14.27) can be initiated by any of a group of enzymes called DNA glycosylases that recognize abnormal bases in DNA. Each glycosylase recognizes a specific type of altered base, such as deaminated bases, oxidized bases, and so on. The glycosylases cleave the glycosidic bond between the abnormal base and 2-deoxyribose, creating apurinic or apyrimidinic sites (AP sites) with missing bases. These AP sites are recognized by AP endonucleases, which act together with phosphodiesterases to excise the sugar-phosphate groups at sites where no base is present. DNA polymerase then replaces the missing nucleotide according to the specifications of the complementary strand, and DNA ligase seals the nick.

Nucleotide excision repair removes larger lesions like thymine dimers and bases with bulky side-groups from DNA. In nucleotide excision repair, a unique excision nuclease activity produces cuts on either side of the damaged nucleotide(s) and excises an oligonucleotide containing the damaged base(s). This nuclease is called an **excinuclease** to distinguish it from the endonucleases and exonucleases that play other roles in DNA metabolism.

The *E. coli* nucleotide excision repair pathway is shown in Figure 14.28. In *E. coli*, excinuclease activity requires the products of three genes, *uvrA*, *uvrB*, and

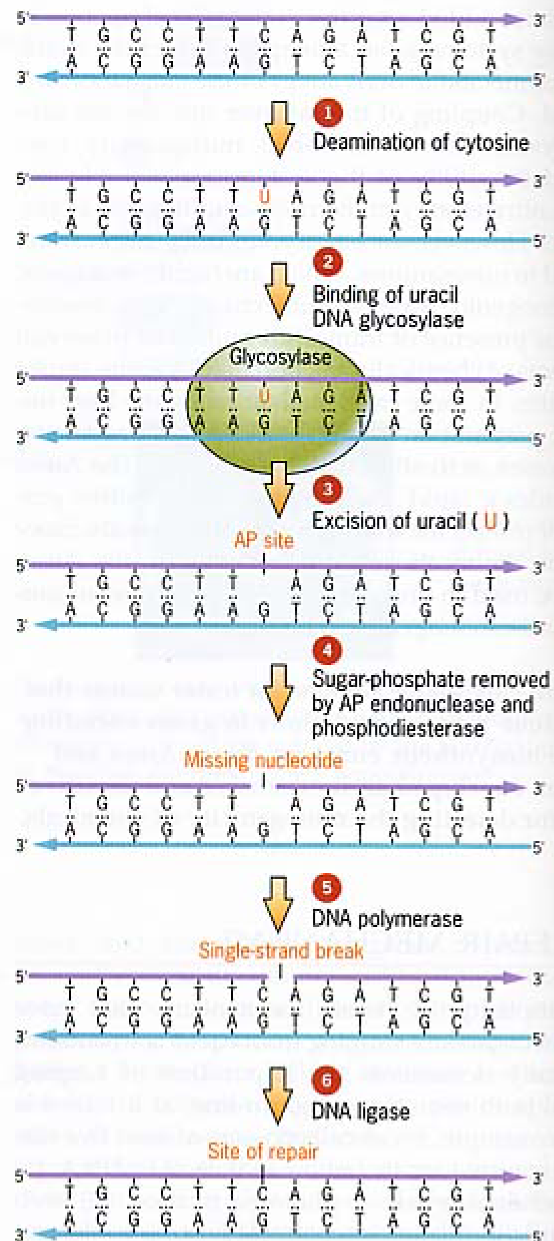

Figure 14.27 Repair of DNA by the base excision pathway. Base excision repair may be initiated by any one of several different DNA glycosylases. In the example shown, uracil DNA glycosylase starts the repair process.

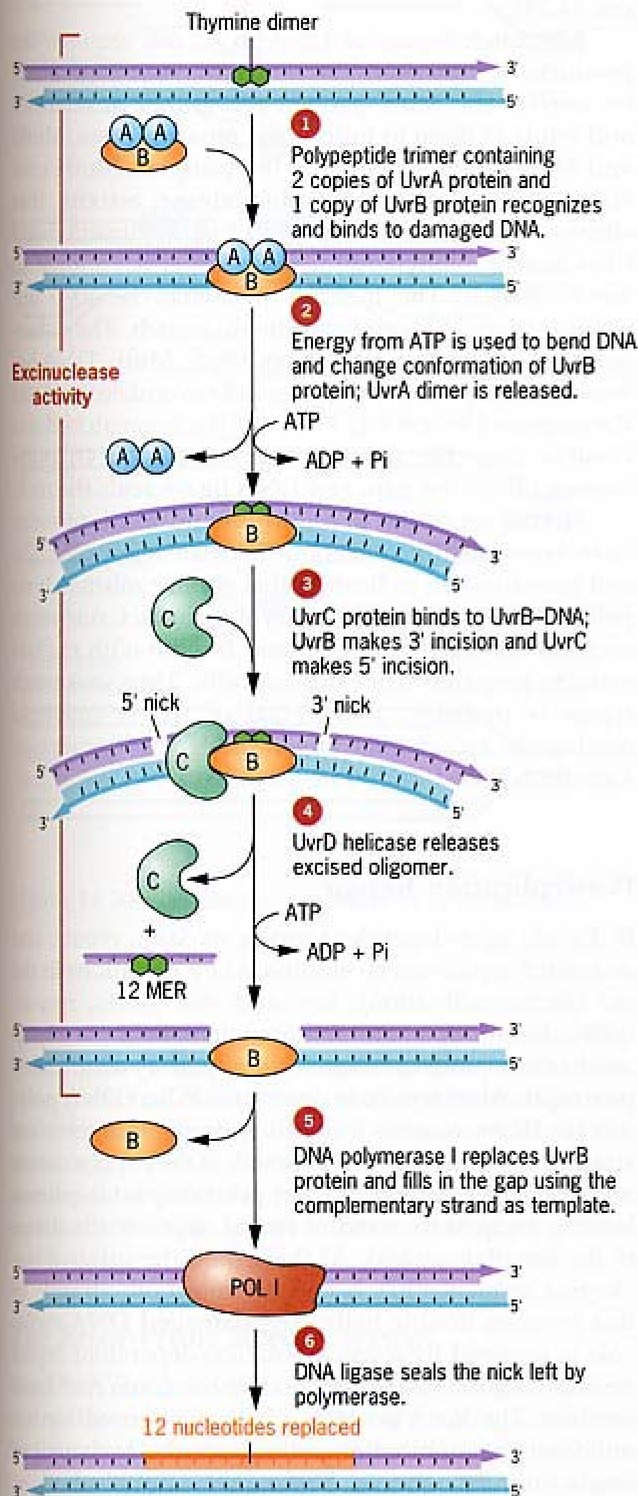

Figure 14.28 Repair of DNA by the nucleotide excision pathway in *E. coli*. The excinuclease (excision nuclease) activity requires the products of three genes—*UvrA*, *UvrB*, and *UvrC*. Nucleotide excision occurs by a similar pathway in humans, except that many more proteins are involved and a 29-nucleotide-long oligomer is excised.

uvrC (designated *uvr* for *UV* repair). A trimeric protein containing two UvrA polypeptides and one UvrB polypeptide recognizes the defect in DNA, binds to it, and uses energy from ATP to bend the DNA at the damaged site. The UvrA dimer is then released, and the UvrC protein binds to the UvrB-DNA complex. The UvrB protein cleaves the fifth phosphodiester bond from the damaged nucleotide(s) on the 3′ side, and the UvrC protein hydrolyzes the eighth phosphodiester linkage from the damage on the 5′ side. The *uvrD* gene product, DNA helicase II, releases the excised dodecamer. In the last two steps of the pathway, DNA polymerase I fills in the gap, and DNA ligase seals the remaining nick in the DNA molecule.

Nucleotide excision repair in humans occurs through a pathway similar to the one in *E. coli*, but it involves about four times as many proteins. In humans, the excinuclease activity requires at least 17 polypeptides. Protein XPA (for *x*eroderma *p*igmentosum protein *A*) recognizes and binds to the damaged nucleotide(s) in DNA. It then recruits the other proteins required for excinuclease activity. In humans, the excised oligomer is 29 nucleotides long rather than the 12-mer removed in *E. coli*. The gap is filled in by either DNA polymerase δ or ϵ in humans, and DNA ligase completes the job.

Mismatch Repair

In Chapter 11, we examined the mechanism by which the $3' \rightarrow 5'$ exonuclease activity built into DNA polymerases proofreads DNA strands during their synthesis, removing any mismatched nucleotides at the 3′ termini of growing strands. The **mismatch repair** pathway provides a backup to this replicative proofreading by correcting mismatched nucleotides remaining in DNA after replication. Mismatches often involve the normal four bases in DNA. For example, a T may be mispaired with a G. Because both T and G are normal components of DNA, mismatch repair systems need some way to determine whether the T or the G is the correct base at a given site. The repair system makes this distinction by identifying the template strand, which contains the original nucleotide sequence, and the newly synthesized strand, which contains the misincorporated base (the error). This distinction can be made based on the pattern of methylation in newly replicated DNA. In *E. coli*, the A in GATC sequences is methylated subsequent to its synthesis. Thus, an interval occurs during which the template strand is methylated and the newly synthesized strand is unmethylated. The mismatch repair system uses this difference in methylation state to excise the mismatched nucleotide in the nascent strand

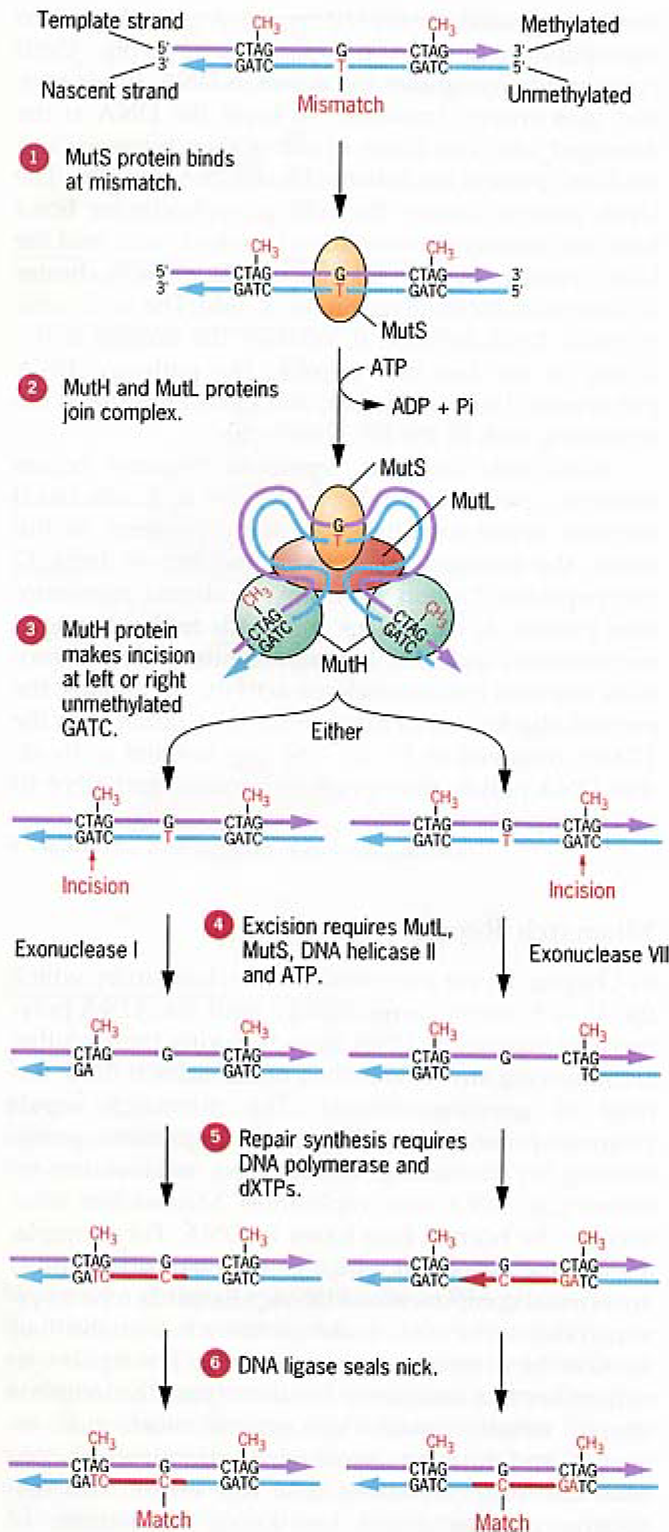

Figure 14.29 Mismatch repair of DNA in *E. coli*. The double helix has just replicated; the template strand is methylated, but the newly synthesized strand is still unmethylated. The MutH protein will cleave only unmethylated GATC sites, assuring that the original (template strand) sequence is restored.

and replace it with the correct nucleotide by using the methylated parental strand of DNA as template (Figure 14.29).

Mismatch repair of DNA in *E. coli* requires the products of four genes, *mutH*, *mutL*, *mutS*, and *mutU* (= *uvrD*). The MutS protein recognizes mismatches and binds to them to initiate the repair process. MutH and MutL proteins then join the complex. MutH contains a **GATC-specific endonuclease activity** that cleaves the unmethylated strand at hemimethylated (that is, half methylated) GATC sites either 5′ or 3′ to the mismatch. The incision sites may be 1000 nucleotide pairs or more from the mismatch. The subsequent excision process requires MutS, MutL, DNA helicase II (MutU), and an appropriate exonuclease. After the excision process has removed the mismatched nucleotide from the unmethylated strand, DNA polymerase fills in the gap, and DNA ligase seals the nick.

Homologs of the *E. coli* MutS and MutL proteins have been identified in both *Saccharomyces cerevisiae* and humans—an indication that similar mismatch repair pathways occur in eukaryotes. In fact, mismatch excision has been demonstrated *in vitro* with nuclear extracts prepared from human cells. Thus, mismatch repair is probably a universal or nearly universal mechanism for safeguarding the integrity of genetic information stored in double-stranded DNA.

Postreplication Repair

In *E. coli*, light-dependent repair, excision repair, and mismatch repair can be eliminated by mutations in the *phr* (*ph*oto*r*eactivation), *uvr*, and *mut* genes, respectively. In multiple mutants deficient in these repair mechanisms, still another DNA repair system, called **postreplication repair,** is operative. When DNA polymerase III encounters a thymine dimer in a template strand, its progress is blocked. DNA polymerase restarts DNA synthesis at some position past the dimer, leaving a gap in the nascent strand opposite the dimer in the template strand. At this point, the original nucleotide sequence has been lost from both strands of this progeny double helix. The damaged DNA molecule is repaired by a recombination-dependent repair process (Figure 14.30) mediated by the *E. coli recA* gene product. The RecA protein, which is required for homologous recombination, stimulates the exchange of single strands between homologous double helices. During postreplication repair, the RecA protein binds to the single strand of DNA at the gap and mediates pairing with the homologous segment of the sister double helix. The gap opposite the dimer is filled with the homologous DNA strand from the sister DNA molecule. The resulting gap in the sister double helix is

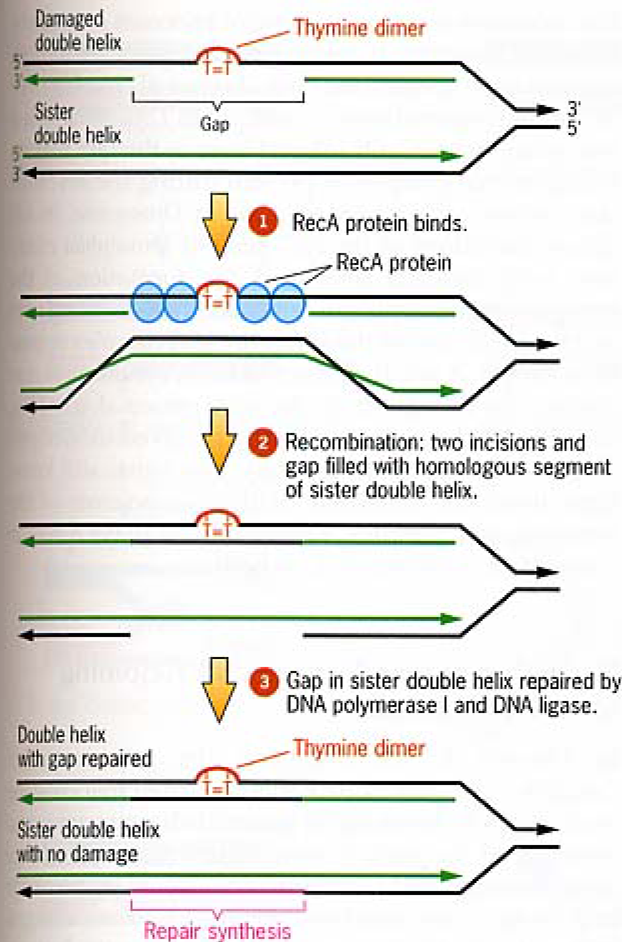

Figure 14.30 Postreplication repair of DNA in *E. coli*.

filled in by DNA polymerase, and the nick is sealed by DNA ligase. The thymine dimer remains in the template strand of the original progeny DNA molecule, but the complementary strand is now intact. If the thymine dimer is not removed by the nucleotide excision repair system, this postreplication repair must be repeated after each round of DNA replication.

The Error-Prone Repair System

The DNA repair systems described so far are quite accurate. However, when the DNA of *E. coli* cells is heavily damaged by mutagenic agents such as UV light, the cells take some drastic steps in their attempt to survive. They go through a so-called **SOS response**, during which a whole battery of DNA repair, recombination, and replication proteins are synthesized. Two of these proteins, encoded by the *umuC* and *umuD* (*UV mutable*) genes, encode proteins that allow DNA replication to proceed across damaged segments of template strands, even though the nucleotide sequences in the damaged region can't be accurately replicated. This **error-prone repair** system eliminates gaps in the newly synthesized strands opposite damaged nucleotides in the template strands but, in so doing, increases the frequency of replication errors. The SOS response appears to be a desperate attempt to escape the lethal effects of heavily damaged DNA. When the error-prone repair system is operative, mutation rates increase sharply.

Key Points: **Multiple DNA repair systems have evolved to safeguard the integrity of genetic information in living organisms. Each repair pathway corrects a specific type of damage in DNA.**

INHERITED HUMAN DISEASES WITH DEFECTS IN DNA REPAIR

As we discussed at the beginning of this chapter, individuals with xeroderma pigmentosum (XP) are extremely sensitive to sunlight. Exposure to sunlight results in a high frequency of skin cancer in XP patients. The cells of individuals with XP are deficient in the repair of UV-induced damage to DNA, such as thymine dimers. The XP syndrome can result from defects in any of at least nine different genes. The products of six of these genes, *XPA*, *XPB*, *XPC*, *XPD*, *XPF*, and *XPG*, are required for nucleotide excison repair. They have been purified and shown to be essential for excinuclease activity. Since excinuclease activity in humans requires at least 17 polypeptides, the list of *XP* genes will probably expand in the future. Two other human disorders, Cockayne syndrome and trichothiodystrophy, also result from defects in nucleotide excision repair. Individuals with Cockayne syndrome exhibit retarded growth and mental skills, but not increased rates of skin cancer. Patients with trichothiodystrophy have short stature, brittle hair, and scaly skin; they also have underdeveloped mental abilities. Individuals with either Cockayne syndrome or trichothiodystrophy are defective in a type of excision repair that is coupled to transcription. However, details of this transcription-coupled repair process are still being worked out.

In addition to the damage to skin cells, some individuals with XP develop neurological abnormalities, which appear to result from the premature death of nerve cells. This effect on the very long-lived nerve cells may have interesting implications with respect to the causes of aging. One theory is that aging results from the accumulation of somatic mutations. If so, a defective repair system would be expected to speed up the aging process, and this appears to be the case with the nerve cells of XP patients. However, at present,