# Mechanisms of DNA Excision Repair

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DNA is constantly suffering damage, which ultimately causes 80 to 90 percent of human cancers (*1*). Usually, cells eliminate DNA lesions by molecular DNA repair, a process first described in 1949 by Kelner (*2*), who found that visible light protected microorganisms from the lethal effects of ultraviolet (UV) radiation. In 1958 Rupert and co-workers (*3*) showed that this phenomenon, called photoreactivation, was catalyzed by an enzyme, photoreactivating enzyme (DNA photolyase). Photolyase reverses the major UV-caused lesions in DNA, pyrimidine dimers, by converting light energy into chemical energy (*4*). Photolyase, however, is not essential for species survival, as many species including humans lack the enzyme (*5*).

In contrast, another DNA repair mechanism, excision repair, which was discovered in 1964 (*6*), is universal in the biological world; all free-living organisms including mycoplasmas rely on it (*7*). Although bacterial mutants defective in excision repair have been maintained in the laboratory for nearly 40 years, a species is unlikely to survive in its natural habitat without excision repair. What is excision repair? What does it recognize? How does it operate?

### The Basic Reaction

Excision repair relies on the redundant information in the duplex to remove a damaged base or nucleotide and replace it with a normal base by using the complementary strand as a template. In base excision repair the removal of the lesion occurs in two steps: First, the damaged base is released by a DNA glycosylase and then the abasic sugar (AP site) is excised by AP endonucleases. Base excision repair has a limited substrate range because the DNA glycosylases that initiate the repair process are in intimate contact with the lesion during catalysis. In nucleotide excision repair, an enzyme system hydrolyzes two phosphodiester bonds, one on either side of the lesion, to generate an oligonucleotide carrying the damage. The excised oligonucleotide is released from the duplex, and the resulting gap is then filled in and ligated to complete the repair reaction. The incision pattern and hence the size of the excised fragments are different in prokaryotes and eukaryotes (Fig. 1). Both prokaryotes and eukaryotes hydrolyze the 5th phosphodiester bond on the 3' side; on the 5' side, prokaryotes hydrolyze the 8th and eukaryotes hydrolyze the 24th phosphodiester bond. In general, the incision pattern is rather precise and as a consequence, depending on whether the lesion is a monoaddduct or a diadduct, the damage is removed in 12 to 13 nucleotide (nt) oligomers in prokaryotes (*8*) and in 27 to 29 nt oligomers in eukaryotes (*9*). This nuclease activity, which is unique to DNA repair, has been named excision nuclease (excinuclease) to clearly differentiate it from endonucleases and exonucleases that perform other functions in the cell (*8*).

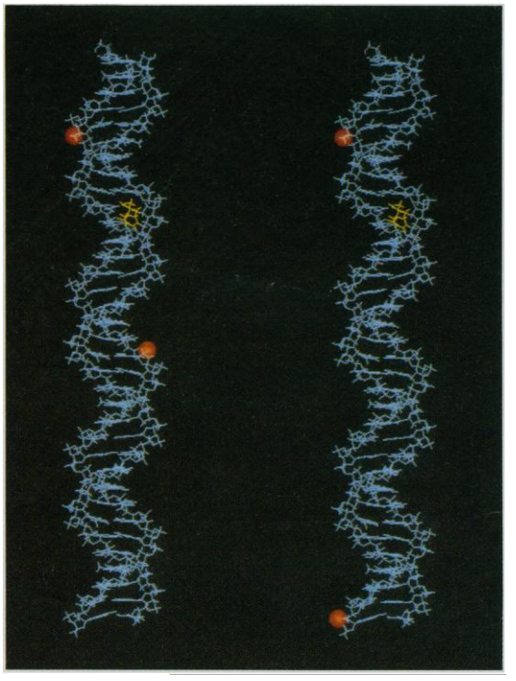

**Fig. 1. Incision patterns of *E. coli* (left) and human (right) excinuclease.** The lesion is a thymine dimer (yellow), and the incision sites are in orange. The dimer is above and the incision sites are below the plane of the paper in both cases. [Courtesy of J. E. Hearst]

### Substrates

In humans and in *Escherichia coli* the excinuclease is the sole enzyme system for removing bulky DNA adducts. These adducts include the carcinogenic cyclobutane pyrimidine dimers induced by UV radiation and the benzo[a]pyrene-guanine adducts caused by smoking, as well as the thymine-psoralen adducts and the guanine-cisplatin adducts caused by chemotherapeutic drugs. However, bulky lesions are not the sole substrate for excinuclease. The enzyme repairs many other lesions that do not distort the helix, including $O^6$-methylguanine and other methylated bases (*10*). A side effect of this wide substrate range is that the excinuclease even "excises" mismatched nucleotides from DNA (*10*). However, in contrast to the mismatch repair system (see the Perspective on page 1959 by Modrich), which has a built-in mechanism that enables it to differentiate the "right" strand from the "wrong" strand, the excinuclease excises the mismatched base from either strand and may actually cause mutation fixation rather than mutation avoidance. Indeed, the excinuclease "repairs everything," sometimes to the detriment of the cell. This is the price that the cell must pay for having an enzyme system that can remove an essentially infinite number of lesions that cannot otherwise be eliminated from DNA. Fortunately, the action of excinuclease on mismatches is rather inefficient compared to the true mismatch repair system, so that mutations caused by this side reaction do not significantly add to the mutation load of the cells.

### Mechanism

Excinuclease is an operational definition of repair activity, not an activity associated with a polypeptide or a complex of polypeptides. A system with such a wide substrate range cannot rely on a binary recognition mechanism in which the enzyme and substrate bind through complementary surfaces. Rather, the activity results from sequential and partly overlapping activities of several polypeptides that bind DNA and utilize the energy released from adenosine triphosphate (ATP) hydrolysis to deform it (kink and unwind), and eventually to excise the lesion by dual incisions. Even though the prokaryotic and eukaryotic excinucleases perform the same functions, the subunits comprising the excinucleases in the two systems do not share any homology whatsoever. Despite these differences and the large number of polypeptides required to carry out the excision reaction in humans, the excision mechanisms are remarkably similar (Fig. 2).

In *E. coli*, three proteins—UvrA, UvrB, and UvrC—are necessary and sufficient for the excinuclease activity, (A)BC excinuclease (*8*, *11*). UvrA is an adenosine triphosphatase, a damage recognition protein, and a molecular matchmaker (*12*); it makes an $A_2B_1$ complex with UvrB, binds to the

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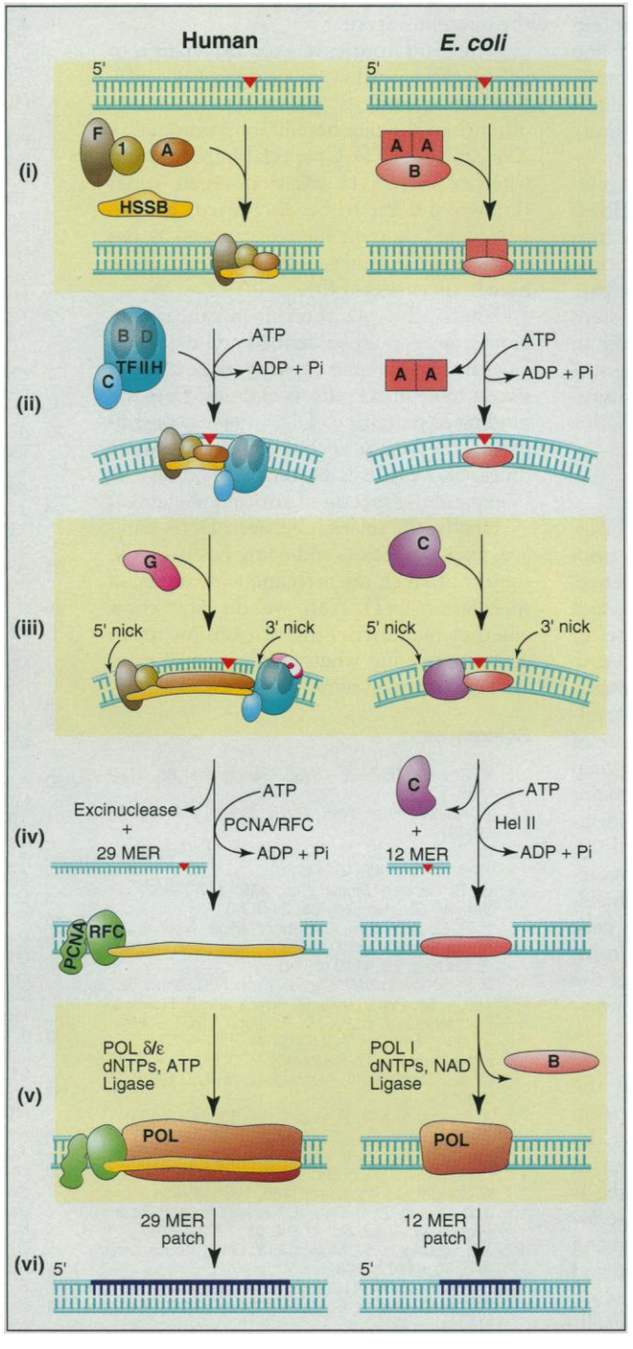
site of the lesion, unwinds and kinks the DNA, and causes a conformational change in UvrB that enables it to form a tight complex at the lesion site. UvrA dissociates from the UvrB-DNA complex, which is a specific binding target for UvrC. Upon binding of UvrC, UvrB makes the 3' incision that causes a conformational change in the complex, enabling UvrC to make the 5' incision (*13*). Helicase II (UvrD) releases the excised oligomer and UvrC. Then, DNA polymerase I displaces UvrB and fills in the excision gap, and the patch is ligated.

In contrast to the wealth of information on the reaction mechanism of (A)BC excinuclease, the mechanistic details of human excinuclease (which requires the activity of at least 17 polypeptides) have become available only in the past 3 years, since the cloning of the human repair genes, CPA through XPG and ERCC1 (*14*). Equally important in this development has been the cloning, expression and purification, and characterization of *Saccharomyces cerevisiae* excision repair proteins—RAD1, 2, 3, 4, 10, 14, and 25—which are structural and functional homologs of the human excision repair proteins XPF, XPG, XPD, XPC, XPA, ERCC1, and XPB, respectively (*15*). The yeast and human proteins have been purified and characterized to varying degrees (*14*, *15*), and a functional repair system has been reconstituted in vitro with highly purified human proteins (*16*). XPA is a damage recognition protein (*17*); it binds to the XPF-ERCC1 heterodimer (*18*) and to the replication protein HSSB (RPA), which binds to the lesion site. XPB and XPD which have helicase activities (*19*) are the subunits of the general transcription factor TFIIH, which also contains six other polypeptides (*20*). The entire TFIIH is a repair factor (*21*) and is recruited to the damage site by XPA. The XPC and XPG proteins are either loosely associated with TFIIH or recruited to the complex through interaction with TFIIH (*16*). In the preincision complex the helix is presumably kinked and unwound by the TFIIH as in the prokaryotic excinuclease system. This conformational change enables the two proteins with nuclease activities, XPG (*22*) and XPF (*23*), to make the dual incisions. Work with model systems suggests that XPG makes the 3' incision (*24*) and XPF makes the 5' incision (*25*). After incision, at least a subset of the excinuclease subunits remains bound to DNA. Catalytic turnover of the enzyme is facilitated with proliferating cell nuclear antigen (PCNA) (and perhaps the RFC replication protein), which releases the excised oligomer and the excinuclease subunits (*26*). The excision gap is filled in by Polδ or Polε and ligated. The experimental data supporting this model are, in many instances, preliminary, in contrast to the extensively documented underpinnings of the excinuclease system of *E. coli*.




## Consequences of Repair Defects

*Escherichia coli* and yeast nucleotide excision repair mutants are sensitive to mutagenic and lethal effects of UV light and other genotoxic agents. In humans, three diseases are associated with defects in nucleotide excision repair (*27*): xeroderma pigmentosum (XP), Cockayne's syndrome (CS), and trichothiodystrophy (TTD). In fact, it was the landmark discovery of defective nucleotide excision repair in patients with XP (*28*) and the subsequent isolation of UV-sensitive mutants of rodent cell lines (*29*) that made the cloning of the human repair genes possible. The human nucleotide excision repair genes are therefore referred to by the XP or ERCC (excision repair cross complementing) designations. XP patients exhibit sunlight-induced photodermatoses, including skin cancers and neurological abnormalities. CS is caused by lack of coupling of transcription to repair, (*30*) and is characterized by growth and mental retardation and photosensitivity but not by an increased rate of skin cancer; CS is associated with mutations in the *CSB*/*ERCC6*, *XPD*, and *XPD* genes. TTD patients manifest brittle hair, mental retardation, and neuroskeletal anomalies. TTD is caused by mutations in *XPB*, *XPD*, and *XPG* genes and perhaps other subunits of TFIIH or TFIIH-associated excision repair subunits (*27*). Knockout mice with a defective *ERCC1* gene are runted at birth and die before weaning as a result of liver failure (*31*). No *ERCC1* mutations have been identified in humans, and hence this gene may perform an essential function other than nucleotide excision repair. Null mutants of *XPA*, *XPC*, and *XPG* exist (*14*), indicating that humans completely defective in nucleotide excision repair can sur-

**Fig. 2. Molecular mechanisms of nucleotide excision repair in humans and in *E. coli*.** (**i**) Initial damage recognition is ATP-independent. (**ii**) Distortion of the helix by molecular matchmakers to form the "ultimate recognition protein"–DNA complex and dissociation of the matchmaker. (**iii**) The binding of a nuclease subunit and the dual incision. The enzyme does not dissociate from the product after incision. (**iv**) Dissociation of excinuclease subunits and the excision product by auxiliary helicases. A gap free of protein never forms as an intermediate. (**v**) Replacement of excinuclease subunits by repair synthesis proteins. (**vi**) Gap filling and ligation.

vive, at least to early adulthood. As yet there is no evidence for malfunctioning of nucleotide excision repair in cancer aside from the rare XP syndrome.

### Coordinating Repair with Other Cellular Processes

Most DNA lesions block RNA and DNA polymerases and interfere with transcription and replication. The cell therefore has designed these three cellular phenomena to function in concert. Increased expression of the cell-cycle protein p21 inhibits replication by binding to PCNA, but reportedly does not inhibit repair (which is also PCNA-dependent) to the same extent (*32*). The DNA damage-induced protein Gadd45 may stimulate excision repair and inhibits cell replication (*33*). Furthermore, the replication protein HSSB, which binds to single-stranded DNA with high affinity and is essential for replication, is also required for the formation of the preincision complex (*16, 34*). This property of HSSB may couple repair to replication. Transcription and repair also cross paths at multiple points. Certain repair proteins participate in activation initiation or elongation steps of transcription and thus couple repair to transcription in more ways than one, ensuring cell survival by multiple mechanisms. (See the Perspective by Hanawalt in this issue on page 1957.)

### Repair and Chemotherapy

The majority of anticancer drugs cause DNA damage, which can be eliminated from DNA by nucleotide excision repair. Hence, the differential response of cancer cells and normal cells to lesions in DNA can be an important determinant of the therapeutic efficacy of a given drug. At present there is no evidence that increased nucleotide excision repair contributes to drug resistance of cancers, although certain high mobility group (HMG)–domain proteins bind to the major DNA adduct of cisplatin, the 1,2-intrastrand d(GpG) crosslink (*35*), and inhibit its repair in vitro by the human excinuclease (*36*). The amount and type of such "shielding" HMG-domain proteins in tumor cells could modulate the repair of cisplatin-induced lesions by excinuclease and affect the responsiveness of the cell to chemotherapy.

### Future Prospects

First, we will soon be able to elucidate the precise roles of each protein in the excision reaction. Similarly, the availability of defined systems for human excision repair, transcription, and replication will make it possible to understand the mechanistic aspects of transcription-repair coupling and the coupling of repair to replication and the cell cycle.

Second, in *E. coli* there are three well-defined cellular responses to DNA damage—the SOS response, the adaptive response to alkylating agents, and the adaptive responses to oxidative stress (*37*). Human cells also manifest apparently well-coordinated responses to DNA-damaging agents. There are at least two signal-transduction pathways for genotoxic stress in humans, one involving Ras (*38*) and one controlled by p53 (*39*). How these responses help the cell survive genotoxic stress is not completely understood. Clearly, the human response is different than the bacterial response reactions where the xenotoxic stress causes increased transcription of repair enzymes and of proteins that neutralize the offending agent.

The third frontier of excision repair is to define the contribution of defects in excision repair and its regulation to diseases other than the rare hereditary syndromes of XP, CS, and TTD. Of special importance is whether defects in excision repair genes (known and yet to be discovered) are involved in common human cancers, as has been shown for mismatch repair and some hereditary cancers (*40*).

Fourth, the role of repair in cancer chemotherapy should be defined and explored. Does increased repair capacity make cells resistant to cytotoxic effects of drugs? Does the binding of proteins to DNA lesions contribute to the tumor specificity of anticancer drugs, and can this be taken advantage of to expand the spectra of antitumor agents?

Finally, the relation between DNA damage, excision repair, and aging has been the subject of much research and a great deal of speculation (*41*). Yet, we do not know whether malfunctioning of excision repair causes aging and whether boosting of excision repair would retard the aging process.

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