Excision Repair in Mammalian Cells

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There are two types of structural anomalies that lead to mutation, a permanent change in DNA sequence. The first class involves normal bases in abnormal sequence context (mismatch, bulge, loop). The second class, which is referred to as DNA damage or DNA lesion, involves abnormal nucleotides (modified, fragmented, cross-linked) in normal sequence context. DNA lesions, in addition to causing mutations, also constitute replication and transcription blocks.

Both types of structural anomalies are rectified by a series of enzymatic reactions referred to by the general term DNA repair (1–5). The repair reactions employed for correcting mismatches and lesions are similar in principle. The incorrect or damaged base is removed either as a base (base excision) or as an (oligo)nucleotide (nucleotide excision), the single-stranded gap resulting from the excision reaction is filled in by a polymerase (repair synthesis), and the newly synthesized DNA is ligated. Hence, there are two basic assays for measuring repair (6): the "incision/excision assay" and the "repair synthesis assay."

Excision Repair

In base excision repair the mismatched or damaged base is cleaved off the deoxyribose by a DNA glycosylase, and the resulting apurinic/apyrimidinic (AP)1 deoxyribose is released by sequential actions of an AP lyase which cleaves 3' and an AP endonuclease which cleaves 5' to the AP site. The one-nucleotide gap is filled in and ligated (Fig. 1).

Nucleotide excision repair, conceptually, can be accomplished by two basic mechanisms. In one, a phosphodiester bond is hydrolyzed 5' or 3' to the mismatch (lesion), and then the incorrect base is removed by a 5' to 3' (or 3' to 5') exonuclease, which hydrolyzes DNA one nucleotide at a time starting at the nick and digesting past the lesion. This is the repair mode employed by both Escherichia coli and human general mismatch correction (repair) systems (1, 2). Thisendonuclease/exonuclease reaction pathway is not utilized for removing damaged bases from DNA. A possible explanation for this is that most base adducts eliminated from DNA by excision repair inhibit exonucleases. One way to circumvent this problem is to have an enzyme system that nicks the damaged strand on both sides of the lesion at some distance removed from the lesion. This second mechanism, indeed, is the excision repair mechanism found in all species investigated. As a matter of common practice, "excision repair" without further qualification means nucleotide excision repair of DNA damage, and hence it will be used as such in this review.

In excision repair, both procaryotes and eucaryotes hydrolyze the 3rd to 5th phosphodiester bond 3' to the lesion; on the 5' side the procaryotes hydrolyze the 8th (4) and the eucaryotes hydrolyze the 21st to 25th phosphodiester bond (7, 8). Thus procaryotes excise damage in 12–13-nucleotide oligomers and eucaryotes excise 27–29-nt-long fragments. This dual incision activity is referred to as excision nuclease (excinuclease). The single-stranded gap generated by either type of excision is filled in by DNA polymerases and sealed by ligase.

Genetics of Excision Repair

The excision repair genes (uvrA, uvrB, and uvrC) of E. coli show no homology to the human excision repair genes (1). In contrast, the sequences of excision repair genes in mammalian cells and yeast are highly homologous, and the enzymology of excision repair in these two systems is very similar (1, 5). Only mammalian excision repair will be covered in this review. Three human diseases are caused by a defect in excision repair (9): xeroderma pigmentosum, Cockayne's syndrome, and trichothiodystrophy.

Xeroderma pigmentosum patients suffer from photosensitivity, photodermatoses including skin cancers, and in some cases from neurological abnormalities. XP patients are defective in excision repair. Mutations in 7 genes, XPA through XPJ, cause XP. In addition, there is a group of patients with classic symptoms of XP but with normal excision repair. These are called XP variants (XP-V). Cells from XP-V patients are moderately sensitive to UV light but excise UV photoproducts at a normal rate and are defective in a biochemically ill defined phenomenon called postreplication repair (10).

Cockayne's syndrome patients suffer from growth failure, mental and neurological abnormalities, cataracts, dental caries, and photosensitivity and related dermatoses. Mutations in two groups of genes appear to cause Cockayne's syndrome. The CS-A and CS-B (ERCC-6) mutants exhibit classical CS symptoms without an increased rate of skin cancer. Cells from these patients have near normal UV sensitivity. A second group of patients manifest XP symptoms in addition to CS symptoms. Patients in this group have mutations in the XPB, XPD, or XPG genes.

Trichothiodystrophy (TTD) patients have ichthyosis and brittle hair and suffer from photosensitivity, skeletal abnormalities, and mental retardation. The patients may or may not have an increased rate of skin cancer. Mutations in three genes are associated with TTD. In the XP/TTD overlapping syndrome, the mutation is in either XPC or XPD. In classical TTD (TTD-A), the mutation is presumably in one of the other subunits of TFIH (1).

In addition to the 9 genes identified by human diseases to be involved in excision repair, many rodent excision repair mutants have been isolated and characterized in order to define the entire set of excision repair genes (11, 12). The rodent mutants fall into 11 complementation groups, and the majority of these correspond to human XP and CS complementation groups as indicated. In fact, some of the human XP genes were cloned by virtue of complementing rodent mutant cell lines and hence are also referred to as excision repair cross complementing (ERCC) genes. Of these genes, XPF and ERCC6 through ERCC11 are not required for the basal excision reaction (13).

Structure and Function of Excision Repair Proteins

Table I summarizes some of the properties of excision repair proteins. Most of these proteins are in complexes in vivo, and hence the activity associated with a solitary protein in vitro may or may not be relevant to its function in excision repair. Human excision nuclease has been reconstructed in a defined system by mixing six highly purified polypeptides or polypeptide complexes (13).

XP A—This protein of 31 kDa has a zinc finger and is involved in damage recognition (14). It also interacts with several other components of excision repair and hence may function as a nucleation factor for excinuclease. XPA interacts through its N-terminal domain with the ERCC1-XPF heterodimer (6) to form a relatively stable complex (15, 16); it also binds to TFIH through its C-terminal domain (17). Finally, RPA (HSSB) binds to XPA and increases its specificity for damaged DNA (18). In addition to XPA,
Mechanism of Excision Repair

The three formal steps of excision repair are damage recognition, dual incision (excision), and repair synthesis and ligation.

Damage Recognition—Excision repair was first identified by the failure of UV-sensitive E. coli and human cells to remove thymine dimers from DNA. However, this repair system is not specific for UV damage as it excises all covalent DNA lesions tested (37–39). With regard to substrate recognition and preference, three interrelated questions must be addressed. Does the enzyme system recognize only DNA with damaged bases, how does the enzyme “know” which strand should be cut, and finally, what is the molecular basis for recognition?

First, damaged bases are not the sole substrate for the enzyme. Human excinuclease excises mismatched bases and 1–3-nt loops as well (38). However, in contrast to the true mismatch repair system, the excinuclease apparently has no way of discriminating the correct and incorrect strands and as a consequence excises the mismatch from either strand.

Regarding the problem of identifying the damaged strand from the undamaged one, the example of mismatch repair by excinuclease shows that the enzyme may not always be able to discriminate the damaged and undamaged strands. This point has not been investigated in detail. However, with thymine cyclobutane dimer there was no excision of the undamaged strand at a rate of 5% of the damaged strand (the detection limit of the assay). Thus, clearly with damage as opposed to mismatch, the enzyme has a mechanism of discriminating the right and wrong strands.3

This fact leads to the third question that was raised with regard to substrate recognition: what is the molecular basis of damage recognition? The simple answer at present is: we don’t know. The following facts are of relevance in searching for an answer for this question. (i) Although lesions that cause gross helical deformity are repaired, those that do not are also repaired, and there is no linear relationship between the specificity coefficient \( b_r \) of the excinuclease and the degree of helical deformity (39, 40). (ii) Recognition involves a protein complex that has a preference for damaged DNA. (XPA-RPA) a complex (TFIIH) with ATP-dependent local unwinding activity that is recruited to the damage site and, based on the precedent in E. coli, unwinds DNA and makes the ultimate damaged DNA-protein preincision complex. (iii) Recently, it has been found that three repair enzymes with rather narrow substrate specificity, namely DNA photolyase (pyrimidine dimers), uracil glycosylase (uracil in DNA), and exonuclease III (AP site), flip out the lesion from the duplex into a “hole” within the enzyme to bring the active site cofactor or residues in close contact with the target bonds (40–42). Whether the excinuclease system flips out the damaged nucleotide(s) or the entire excised fragment remains to be seen (43).

Dual Incision/Excision—The molecular details of mammalian excision repair are now known in considerable detail. Sixteen polypeptides are necessary and sufficient for excinuclease activity (13) as shown in Fig. 2.

The XPA-RPA complex binds to the damage site; then XPA recruits TFIIH, which makes a precincision complex in an ATP hydrolysis-dependent manner. XPC helps stabilize the precincision complex. The ATP-dependent unwinding of the DNA by TFIIH primes it for nuclelease attack by the two XP proteins known to have nuclease activity. XPG is recruited by TFIIH and incises on the 3' side (13, 44), and ERCC1-XPF, which is recruited by XPA, incises on the 5' (44) side of the damage. The dual incision is absolutely dependent on ATP hydrolysis (8).

The major sites of incision are relatively precise and are at the

3 C. H. Park and A. Sanzar, unpublished observation.

A. Kazantsev and A. Sanzar, unpublished observation.
The sites of incision are influenced by several factors, including the type of lesion (7, 8) and the sequence context (37, 39). The same incision pattern has been observed in in vivo in Xenopus eggs (8) and in cell-free extracts from Schizosaccharomyces pombe4 and is considered to be the universal incision pattern for eukaryotes (4).

Repair Synthesis—In contrast to the excision reaction we know less about the details of the repair synthesis step. It is known that repair synthesis is PCNA-dependent and hence must be carried out by Polδ and Polε (45, 46), and, since PCNA is the polymerase clamp loaded onto template-primer by RFC replication factor, RFC may also be required. In a study with cell-free extract Polδ antibodies specifically inhibited repair synthesis (47), and in a highly purified in vitro system for repair synthesis it was found that Polε and even Klenow fragment of PolI performed repair synthesis pointing to the difficulty of assigning repair polymerase from in vitro reconstitution systems (48). Most likely, both Polδ and Polε participate in the repair synthesis step of excision repair (49).

Transcription-Repair Coupling
Transcribed sequences and in particular the template strand within a transcribed sequence are repaired at a higher rate than non-transcribed sequences (50). Cells from CS patients are defective in strand-specific repair (51). In E. coli, a transcription-repair coupling factor encoded by the mfd gene displaces stalled RNA polymerase and releases the stalled complex while recruiting the damage recognition complex of exincuclease (52). However, at present there is no in vitro system for transcription-repair coupling in mammalian cells. The CSB gene encodes a protein of 160 kDa, which contains the so-called helicase motifs and is likely to function in a manner analogous to the E. coli Mfd protein (50). Thus, a simple model for strand-specific repair based on behavior of CS-A and CS-B mutants and of the proteins is as follows.

RNA polymerase II stalled at a lesion is recognized by the CSA-CSB complex, which causes the polymerase to back off the lesion without disrupting the ternary complex. The CSA-CSB complex also recruits XPA and TFIIH to the lesion site and thus helps in the assembly of the exincuclease. The lesion is excised and the excision gap is filled in. The backed off RNA polymerase elongates the truncated transcript (53).

Regulation of Excision Repair
It appears that mammalian cells do not possess an SOS response like that in E. coli where DNA damage by bulky agents increases the transcription of excision repair genes (54). Similarly, damage-induced post-translational modification of repair proteins (55, 56) does not affect the activity of human exincuclease (57).

The connection between the p53 tumor suppressor protein and DNA repair has been the source of much speculation and debate because the p53 protein is stabilized by DNA damage and it is a transcriptional regulator. It has been reported that p53 protein binds to XBP (58) and RPA (59), both of which are essential for basal excision repair. However, p53(−/−) cells excise the two major UV photoproducts, pyrimidine dimers, and 6–4 photoproducts at the same rate as wild type cells and are equally resistant to UV

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4 J. C. Huang and A. Sancar, unpublished observation.

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Table I
The 16 polypeptides required for excision repair in mammalian cells

| Fraction | No. | Proteins (M)_r | Activity | Role in repair |
|----------|-----|---------------|----------|---------------|
| I. XPA   | 1   | XPA (p31)     | DNA binding | Damage recognition |
| II. RPA  | 2   | p70           | DNA binding | Damage recognition |
|          | 3   | p54           |           |               |
|          | 4   | p11           |           |               |
| III. TFIH| 5   | XPB/ERCC3 (p89) | (a) DNA-dependent ATPase | (a) Formation of incision complex |
|          | 6   | XPD/ERCC2 (p80) | (b) Local unwinding | (b) Transcription repair coupling |
|          | 7   | p62           | (c) Transcription factor |               |
|          | 8   | p44           | (d) Cdk-activating kinase |               |
|          | 9   | Cdk7 (p41)    |           |               |
|          | 10  | CyclH (p38)   |           |               |
|          | 11  | p34           |           |               |
| IV. XPC  | 12  | XPC (p125)    | DNA binding |               |
|          | 13  | HHR23B (p58)  |           |               |
| V. XPF   | 14  | XPF/ERCC4 (p112) | Nuclease | 5′-Incision |
|          | 15  | ERCC1 (p33)   |           |               |
| VI. XPG  | 16  | XPG/ERCC5 (p135) | Nuclease | 3′-Incision |

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Fig. 2. Model for mammalian excision repair. Step 1, ATP-independent damage recognition. Step 2, ATP-dependent formation of incision complex. It is quite likely that only a subset of the proteins shown is present in the actual incision complex. Some may act as molecular matchmakers and dissociate after formation of the incision complex. Step 3, dual incision. The two incisions occur in random order. Step 4, replacement of the excision proteins by repair synthesis proteins. Step 5, repair synthesis and ligation.
(60). Similarly, p53 protein at nearly micromolar concentrations has no effect on excision repair in a defined system. Finally, the report that the p53-induced Gadd45 protein stimulates excision repair has not been confirmed. Thus, existing data are consistent with the notion that p53 does not modulate excision repair either positively or negatively.

In contrast, the recent discovery that Cdk7 and cyclin H, which make up the Cdk-activating kinase, are constituents of TFIIH (62, 63) raises interesting possibilities regarding cell cycle regulation and DNA repair. Excision repair capability of the cell does not change during the cell cycle (64). However, replication and repair may be coordinated by differential effects of p21(Cip1/WAF1) on the replication and repair DNA synthesis (65). Future research is likely to uncover interesting interconnections between DNA repair, replication, cell cycle, and apoptosis.

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