

## The Biology Behind

# Nucleotide Excision Repair, Oxidative Damage, DNA Sequence Polymorphisms, and Cancer Treatment

□ □ *Commentary on Gu et al., p. 1408*

**Stephanie Q. Hutsell and Aziz Sancar**

Department of Biochemistry and Biophysics, University of North Carolina School of Medicine, Chapel Hill, North Carolina

## INTRODUCTION

Can a single polymorphic site help tailor a cancer patient's chemotherapy regimen? The study by Zhao et al. (1) presented in this issue of *Clinical Cancer Research* suggests that in fact polymorphic sites in noncoding gene regions may determine how a patient will react to a given chemotherapy and the likelihood of cancer recurrence after treatment. The group focused their investigation on a common polymorphism termed xeroderma pigmentosum group A (−4) [XPA (−4)], whereby the fourth nucleotide before the ATG start codon is A in about half the population and G in about the other half (2, 3). The polymorphism is in the Kozak sequence and thus the A form of the allele may affect the translation efficiency of XPA resulting in reduced XPA level and reduced excision repair capacity. Indeed, this group previously reported that the A allele (XPA A variant in the authors' terminology) is associated with reduced excision repair capacity (4). In the current study, the authors report that the XPA G variant in which both alleles contain G at the fourth position before the initiation codon is associated with a higher rate of recurrence of superficial bladder cancer after Bacillus Calmette-Guerin (BCG) treatment. The authors report that during the follow-up period of 27.6 months from diagnosis, tumor recurred in 44% of patients with the AA genotype, 62% in those with the AG genotype, and 74% in those with the GG genotype. To explain these findings, the authors suggest that the XPA G variant exhibits increased DNA repair capacity by nucleotide excision repair. This allows cancer cells to bypass the apoptotic response induced by BCG treatment.

Nucleotide excision repair is the only human system that protects against the carcinogenic effects of sunlight by removing UV light-induced DNA adducts such as (6–4) photoproducts and cyclobutane thymine dimers (5–8). Along with light-induced lesions, the excision nuclease recognizes and excises a broad spectrum of bulky lesions such as benzo(a)pyrene, acetylaminofluorene, cisplatin, and psoralen DNA adducts (5). The mechanism of excision repair is highly conserved from *E. coli* to man and involves a damage recognition step, dual incisions bracketing the lesion, removal of the damage in the form of a 12- to 13-nucleotide-long oligomer in *E. coli* and a 24- to 32-

nucleotide-long oligomer in humans, repair synthesis to fill the resulting gap, and finally ligation of the repair patch.

Human nucleotide excision repair has been recently characterized at the biochemical level in considerable detail. Six repair factors, XPA, RPA, XPC, TFIIH, XPG, and XPF-ERCC1, are necessary and sufficient to remove damage from DNA. A current model for human nucleotide excision repair is as follows: XPA, RPA, and XPC locate the damage site and recruit the TFIIH transcription/repair factor, which contains six polypeptides including helicases XPB and XPD that unwind the DNA around the damage site; the XPG and XPF-ERCC1 subunits are responsible for the 3' and 5' dual incisions, respectively. Repair synthesis proteins replication factor C, proliferating cell nuclear antigen, and DNA polymerases  $\delta$  and  $\epsilon$  fill the gap, and in the final step, the repair patch is sealed by DNA ligase I (refs. 9, 10; Fig. 1). Mutations in any of the XP A-to-G coding sequences result in XP, a disease characterized by extreme light sensitivity and high incidence of skin cancer. Currently, all known XP cases contain mutations in the coding regions of one of the XP genes. It is conceivable that mutations in the noncoding or nontranscribed regions of the XP genes may cause slight alteration in gene expression so as not to give rise to an overt XP phenotype, but of significant magnitude that subtle repair defects may manifest.

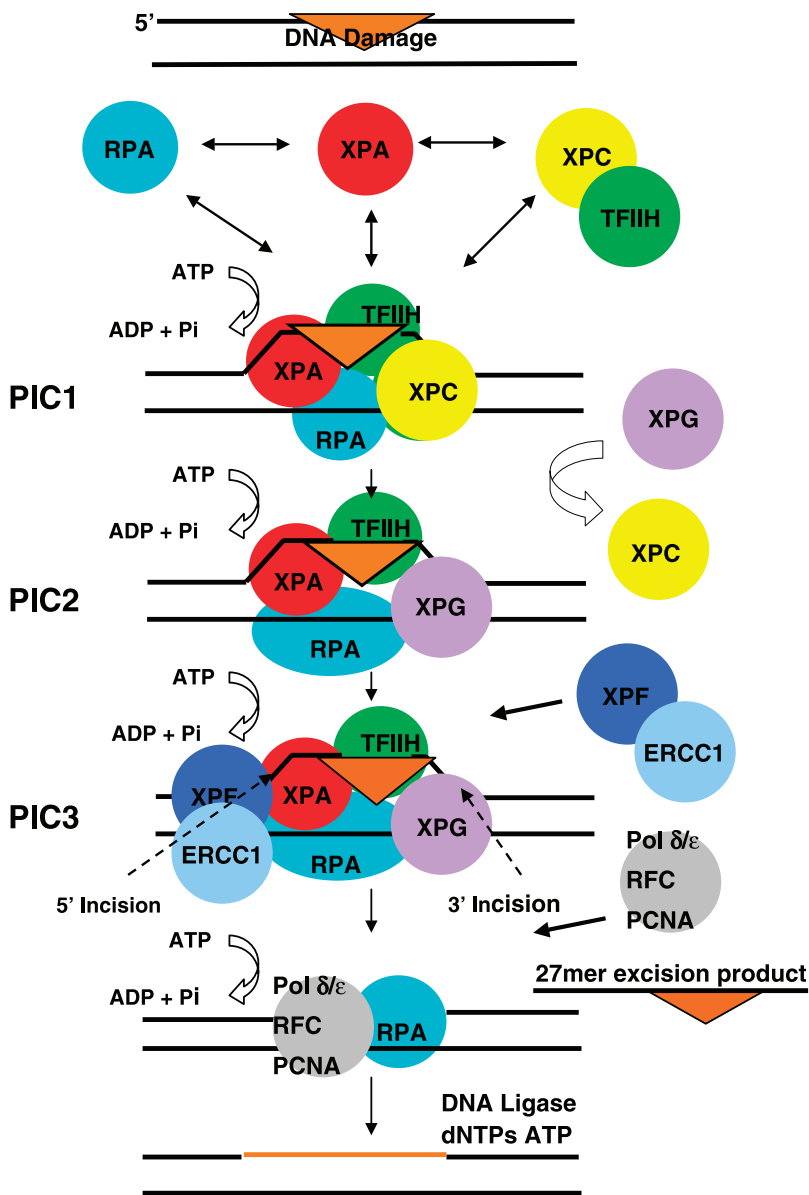
Even though nucleotide excision repair is generally thought of as a repair system for bulky adducts, it has been shown that it works with moderate efficiency on nonbulky DNA lesions, such as DNA bases altered by oxidative damage that are usually processed by base excision repair (11, 12). Because of this property, the nucleotide excision repair system plays a backup role for the base excision repair pathway in the removal of such DNA adducts. Reactive oxygen species are by-products of oxidative metabolism and are also produced in great quantities during inflammatory reactions. They induce numerous DNA lesions including thymine glycols, 8-oxoguanine, and cyclo-deoxyadenosine. Nucleotide excision repair is capable of removing all of these DNA lesions induced by reactive oxygen species (13, 14).

In the study by Zhao et al. (1), the authors propose a mechanism that involves the nucleotide excision repair pathway and its response to oxidative damage, to explain why the XPA G variant is less responsive to BCG treatment. The suggestion is that BCG therapy provokes an inflammatory cellular response, which subsequently results in the production of oxygen radicals and extensive oxidative damage to DNA followed by apoptosis. The XPA G variant was reported to have increased DNA repair capacity (4). This increase in DNA repair capacity may be responsible for removing a greater percentage of oxidative damage from DNA, preventing the apoptotic pathway, and permitting tumor cell survival, rendering BCG ineffective for patients homozygous for the XPA G variant.

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**Requests for reprints:** Aziz Sancar, Department of Biochemistry and Biophysics, University of North Carolina School of Medicine, Mary Ellen Jones Building, Campus Box #7260, Chapel Hill, NC 27599-7260. Phone: 919-962-0115; Fax: 919-966-2852; E-mail: Aziz\_Sancar@med.unc.edu.

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*Fig. 1* Model for nucleotide excision repair. Damage is recognized by RPA, XPA, and XPC in a cooperative way. TFIIH is recruited to form pre-incision complex 1 (PIC1). XPG displaces XPC from PIC1 to form PIC2. Finally, XPF-ERCC1 is recruited to form PIC3 in which XPG makes the 3' incision  $6 \pm 3$  nucleotides 3' from the damage site and XPF-ERCC1 makes the 5' incision  $20 \pm 5$  nucleotides 5' to the damaged bases releasing the damage in the form of a 27-nucleotide-long oligomer. Repair synthesis proteins replication factor C, proliferating cell nuclear antigen, and DNA polymerases  $\delta$  and  $\epsilon$  fill the gap. Repair patch is sealed by DNA ligase.

This is a reasonable speculation. However, there are certain caveats to this interpretation. The report that the XPA G variant allele shows increased DNA repair capacity requires verification by more than just the reporter gene assays reported previously (4). Currently, there is no evidence that the level of XPA protein is increased constitutively or in response to damage in XPA G variant patients. Furthermore, there is no evidence by independent repair assays that there is increased excision repair activity in cells with the XPA G variant. In addition, although it has been shown that human nucleotide excision repair excises 8-oxoguanine, thymine glycol, and perhaps other oxidative stress base lesions from DNA as efficiently as the light-induced cyclobutane thymine dimer (13), 8-oxoguanine and other oxidative stress lesions are repaired very efficiently by 8-oxoguanine DNA glycosylase and other glycosylases (15). Therefore, the magnitude of contribution of nucleotide excision repair in the removal of oxidative damage to

nucleotide bases remains to be determined. As a final caveat, previous studies (4, 16) have shown that the XPA G variant allele has a protective effect against the onset of lung cancer. Those reports predict that individuals with XPA G variant would have lower incidence of bladder cancer as well. Zhao et al. (1) did not study an unaffected sample of the population to determine if the XPA G variant allele shows a similar protective effect against superficial bladder cancer. If it does, this study reveals an interesting issue in the genetic consideration in carcinogenesis and cancer treatment: a mutation makes the carriers more resistant to cancer, but once they develop cancer, the same mutation may make them less responsive to treatment by agents that directly or indirectly damage DNA.

This is an interesting study, and in conclusion, the connection between the XPA G variant polymorphism and lack of response to BCG treatment could have significant clinical

applications. However, whether or not this polymorphism is relevant to nucleotide excision repair activity needs further investigation.

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