Chemistry & Biology, Vol. 12, August, 2005, ©2005 Elsevier Ltd All rights reserved. DOI 10.1016/j.chembiol.2005.08.002

Indirect Detection of DNA Damage

In this issue of *Chemistry & Biology*, Naegeli and coworkers [1] show that the nucleotide excision repair system of mammalian cells detects bulky DNA adducts, not by recognition of the adduct per se, but by recognition of the undamaged partner strand in bulged form.

Human cells contain paternal and maternal copies of the three billion-rung genomic DNA ladder. Though tightly packaged in chromatin, genomic DNA suffers an astounding number of potentially mutagenic chemical insults each second. Certain DNA functional groups are susceptible to spontaneous hydrolysis reactions that alter the pairing potential of DNA bases [2, 3]. Damage also comes both from outside the organism (ionizing radiation, UV energy from sunlight, reactive chemicals) and from within (reactive oxygen species derived from mitochondrial oxidative metabolism).

DNA represents an ancient solution to the problem of preserving encoded information, and the cellular response to DNA damage appears equally ancient. Though thousands of kinds of chemical DNA damage are known, mammals employ just four major DNA repair systems [4]: end joining (to repair double-strand breaks), homologous recombination (to rescue damaged information using the second copy present near the replication fork), base excision repair (to replace damaged bases), and nucleotide excision repair (to repair bulky, helix-distorting lesions).

Mechanisms of DNA repair have been illuminated by studies examining model organisms and inherited human diseases [5]. Despite this progress, many details of DNA repair remain unclear. This situation is particularly true for the complex nucleotide excision repair (NER) process, which involves at least 25 different proteins. Seven of these NER proteins are encoded by genes that, when lost, give rise to an inherited human cancer disorder called xeroderma pigmentosum (XP) [6]. XP patients are characterized by extreme sensitivity to sunlight-induced cancers, suggesting that UV-induced cyclobutane pyrimidine dimers in DNA are important substrates for the NER system. The comparison of cell extracts from normal human cells and cells derived from XP patients has allowed both in vitro reconstitution of the first steps of DNA repair on model substrates, and the assessment of the roles of different XP gene products in the complex NER process (Figure 1) [4, 7].

A fascinating issue in the DNA repair field surrounds the precise initiating signal that is interpreted as DNA damage. Unlike the combinatorial chemistry of the immune system, which provides billions of antibodies to sense different possible "nonself" molecular surfaces, the vast number of different bulky DNA lesions must be detected and handled by a single generic NER system. What common feature of damaged DNA triggers the NER response?

Part of the answer may come from the process of transcription-coupled repair itself. Here NER factors are simply recruited to stalled RNA polymerases [8, 9]. However, cells also perform a general kind of genome surveillance called global genome repair that can direct NER proteins to bulky chemical lesions in the absence of transcription. It is the initial molecular recognition of these DNA adducts that is the focus of a series of studies from Naegeli and coworkers at the University

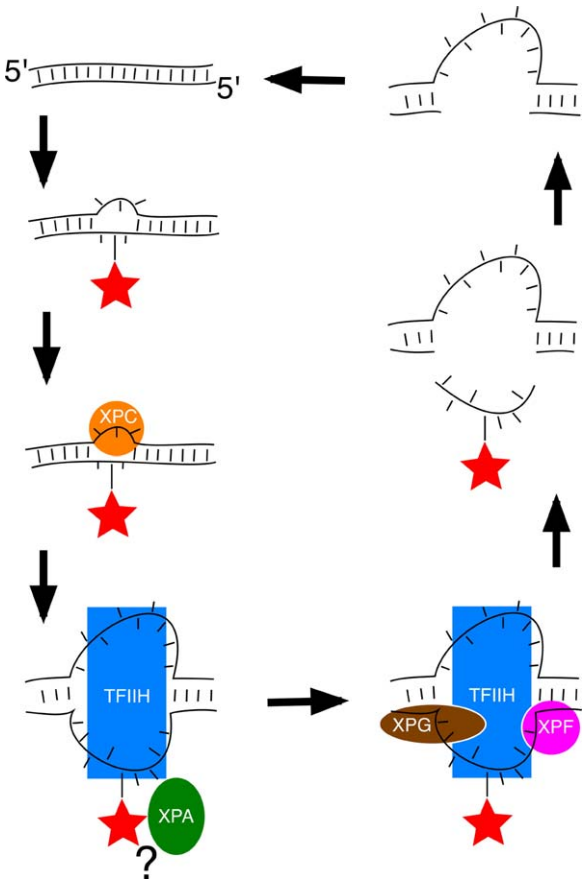

Figure 1. Simplified Nucleotide Excision Repair Cycle as It Might Occur during Global Genome Repair

XPC protein (orange) recognizes a distortion in the undamaged DNA strand near a lesion (red star). The TFIIH complex (blue) is recruited. XPA protein (green) may detect the actual adduct. XPF (violet) and XPG (brown) endonucleases cleave the damaged strand flanking the lesion. The damaged DNA segment is displaced during subsequent repair synthesis. Illustration adapted from [4].

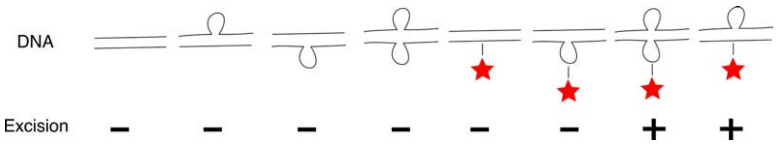

Figure 2. Schematic Summary of the Results of Buterin et al.

Excision of a bulky but nondistorting DNA lesion (red star) requires bulged unpairing in the undamaged DNA strand.

of Zürich. A fascinating new installment appears in this issue of *Chemistry & Biology* [1].

Previous work by others had established that NER proteins are assembled in a step-wise fashion at sites of bulky lesions [10] and that the early steps of NER can be observed when synthetic radiolabeled DNA molecules containing model adducts are incubated with whole-cell extracts [11–14]. Using such in vitro assays, NER is detected by the appearance of small internally radiolabeled excision products containing the chemical lesion after electrophoresis and autoradiography of the processed substrates.

This experimental approach creates an excellent opportunity for the combination of synthetic chemistry (preparation of site-specific bulky adducts on single-stranded DNA oligonucleotides), clever assembly of DNA substrates (~150 bp duplex DNA molecules created by enzymatic ligation of annealed duplexes with cohesive termini), and reconstituted repair (extracts from normal or XP cells). Naegeli and coworkers have previously applied this strategy to demonstrate surprising and intriguing results. They first showed that lesions such as a C4′ pivaloyl deoxyribose adduct (which, though bulky, does not distort the double-helical structure of DNA) is not recognized by the NER machinery unless positioned at a site of designed base unpairing [15]. In subsequent work, the group showed that the combination of a nondistorting lesion and a site of designed base unpairing could still trigger DNA repair when the two were separated by as much as 15 bp [16]. These results suggested that chemical lesions are detected during global genome repair only when they are found in the context of a region of stable single-stranded DNA. Cooperative recognition of the unpaired site by the XPA protein and the single-strand-specific protein RPA was suggested.

In the current work [1], the Naegeli group reports the surprising and counterintuitive discovery that NER components initiate repair of nondistorting chemical lesions only when the *undamaged* complementary DNA strand is made to adopt a bulged conformation due to designed base unpairing (Figure 2). On reflection, this proposal explains how a single NER system can detect a vast and diverse population of potential substrates: *the common feature shared by all of the lesions is local unpairing of the undamaged complementary strand*.

Buterin et al. [1] report well-reasoned and comprehensive experiments with an impressive array of chemical adducts and analogs to demonstrate the generality of this interesting result. The authors again begin with the clever use of the C4′ pivaloyl deoxyribose adduct in construction of ~150 bp internally radiolabeled duplex DNA substrates by ligation. They confirm that the lesion itself does not cue NER, except when base unpairing is detected in the undamaged complementary strand at, or flanking, the lesion. The study goes on to show that perturbation of the undamaged strand, either by a second lesion or by the presence of unnatural base analogs, inhibits repair. Using site-specific benzo[*a*]pyrene or acetylaminofluorene adducts, the authors generalize their result by showing that initiation of repair crucially depends upon at least one unpaired base on the undamaged DNA strand in the vicinity of these lesions. The XPC protein is proposed as a first sensor of unpaired DNA (even in the absence of a chemical adduct), although NER will not proceed to excision without subsequent detection of a bulky lesion. The present results are also consistent with the observations of others [17, 18].

Future studies will be required to understand what features of the unpaired and undamaged strand (exposed hydrogen bonding functions of the bases, hydrophobic character, flexibility, nonhelical conformation?) are recognized by XPC, and how these properties might be detected in the context of chromatin during global genome repair. Techniques to assemble chemically modified templates into plasmids for transient transfection into living cells may eventually be required. For the moment, the report of Buterin et al. provides a particularly fine illustration of chemical keys serving to unlock a biological mystery.

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Selected Reading

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