Rous-Whipple Award Lecture

Nucleotide Excision Repair and Cancer Predisposition

A Journey from Man to Yeast to Mice

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"I do not know what I may appear to the world, but to myself I seem to have been only like a boy playing on the sea-shore, and diverting myself in now and then finding a smoother pebble or a prettier shell than ordinary, whilst the great ocean of truth lay all undiscovered before me."

Isaac Newton

The genomes of all living cells are constant targets for DNA damage.¹ Alterations of the bases in DNA can arise spontaneously as a result of errors during DNA replication, or due to alterations in the chemistry of the DNA bases. Such damage can also result from exposure of cells to a host of reactive environmental physical or chemical agents, such as ultraviolet (UV) radiation or a plethora of mutagenic and carcinogenic agents.¹ All free-living life forms have evolved multiple distinct responses to DNA damage and other insults to the function of the genome that can be collectively considered as biological responses to DNA damage (Figure 1). Signals that are initiated by various types of DNA damage are transduced through a number of complex damage checkpoint pathways, resulting in arrested cell cycle progression at various stages. This allows for increased time for repair of the DNA damage before continuing progression through the cycle. When cells are faced with arrested DNA replication due to the presence of base damage or DNA strand breaks, altered forms of DNA synthesis occur that essentially tolerate the presence of the base damage without its physical removal. The altered DNA synthesis can be either error-free or error-prone. In the latter instance mutations are generated. Finally, one of the primary ways in which living cells respond to DNA damage involves a series of biochemical reactions that are collectively referred to as DNA repair.¹ My own interest in this group of biochemical pathways began over 30 years ago as a postdoctoral fellow in the Department of Biochemistry at Case Western Reserve University, and was

strongly reinforced when I encountered James E. Cleaver's description of defective DNA repair in cells from individuals suffering from the skin cancer-prone hereditary disease xeroderma pigmentosum (XP).²

Xeroderma Pigmentosum: A Hereditary Defect in DNA Repair

The phenomenon of excision repair of DNA damage associated with exposure to ultraviolet (UV) radiation was discovered in bacteria by Richard Setlow and his colleagues in the mid-1960s.³ Nothing was known about excision repair in mammalian cells. At about this time Robert Painter and his associates developed a simple autoradiographic technique for monitoring DNA synthesis in mammalian cells to study the effects of ionizing radiation on DNA replication. To their surprise, they observed that when cells were exposed to UV radiation, low levels of DNA synthesis were also observed in cells that were not actively engaged in DNA replication, ie, cells that were outside the S phase of the cell cycle (Figure 2). In light of the discovery of excision repair in bacteria, Painter and his colleagues speculated that the low levels of DNA synthesis in UV-irradiated mammalian cells might reflect repair synthesis specifically associated with excision repair.⁴ Shortly after this observation, Cleaver joined Painter's laboratory. To validate Painter's experiments with an appropriate negative control as Setlow and his colleagues had done by using an excision repair-defective mutant strain of *Escherichia coli*, Cleaver was preparing to mutagenize mammalian cells to generate such a mutant, when he encountered a description in the San Francisco *Chronicle* of the high incidence of skin cancer

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CELLULAR RESPONSES TO DNA DAMAGE

Figure 1. When cells suffer DNA damage they undergo various responses. These include cell cycle checkpoint activation and/or apoptosis, various modes of DNA repair, various modes of DNA damage tolerance, and the transcriptional activation of multiple genes, some of which are involved in stress responses.

in XP patients exposed to sunlight. He reasoned, correctly, that XP individuals might be skin cancer-prone because of a defect in the putative excision repair mode observed by autoradiography in normal cells. If so, XP cells would validate the autoradiographic demonstration of excision repair in normal human cells. This experiment led to the demonstration of defective excision repair in XP cells in culture.²

Dirk Bootsma and his collaborators in The Netherlands took the autoradiographic assay an important step further. By fusing cells from different XP individuals, they were able to monitor phenotypic complementation of defective repair synthesis in heterodikaryons, suggesting that the genetic defect in DNA repair in each of the two complementing cells was in different genetic complementation groups required for excision repair.⁵ An extensive somatic cell genetic analysis using XP patients from different families established the existence of multiple genetic complementation groups for XP that were designated XP-A → XP-G.¹ These observations, in turn, suggested that multiple genes are required for the particular

mode of excision repair demonstrated in normal human cells and that mutational inactivation of any one of these genes results in XP.

By this time it was also established that there are three biochemically distinct modes of excision repair in nature.^{1,6} These are designated as nucleotide excision repair (NER), a process required for the repair of bulky base adducts in DNA such as those resulting from exposure to UV radiation; base excision repair (BER), a totally distinct biochemical pathway required for the repair of many types of non-bulky base damage such as that which occurs spontaneously from reactive oxygen species in cells; and mismatch repair (MMR), a process required for the excision of mispaired bases in DNA.

XP individuals are specifically defective in NER.^{2,7} As a consequence their cells are highly sensitive to killing by UV radiation and to many chemical mutagens and carcinogens that result in bulky base damage in DNA¹ (Figure 3). The existence of multiple genetic complementation groups for the disease strongly suggested a profound biochemical complexity for NER in normal human cells. With the advent of the era of recombinant DNA technology, the marked difference in the sensitivity of normal and XP cells to UV radiation invited a gene cloning strategy based on screening the genome from normal human cells for phenotypic correction of UV radiation sensitivity. Several investigators embarked on this heroic experimental approach, despite the fact that in the decade of the 1970s the cloning of human genes by phenotypic complementation using human cells in culture was a formidable technical challenge.

The Switch to Yeast

Dissuaded by the technological complexities of human gene cloning at that time, my colleagues and I began to investigate the yeast *Saccharomyces cerevisiae* as a eukaryotic model for defective NER.⁸ Yeast mutant strains existed that, like human XP cells, were exceptionally sensitive to killing after exposure to UV radiation or selected

Figure 2. When cells in G1 or G2 of the cell cycle are exposed to UV radiation and incubated in the presence of [³H] thymidine, repair synthesis of DNA associated with nucleotide excision repair is reflected by autoradiographic labeling of the nuclei.

Figure 3. XP cells from genetic complementation group D (XP-D) are much more sensitive to killing by UV radiation than normal human cells.

chemicals. These were designated as *rad* mutants (for radiation-sensitive).¹ Multiple distinct *rad* mutants were known to exist, indicating a level of genetic complexity for NER in yeast comparable to that suggested by the multiple XP genetic complementation groups in human cells. In the late 1970s and early 1980s, we and others characterized the DNA repair phenotype of various yeast *rad* mutant strains and established that they are indeed defective in NER *in vivo*.^{9,10} Encouraged by these observations, we embarked on the molecular cloning of yeast genes by phenotypic complementation of UV radiation sensitivity, which was technically much more facile than cloning human genes (Figure 4). In short order we and others cloned and characterized a series of genes now designated *RAD1*, *RAD2*, *RAD3*, *RAD4*, *RAD7*, *RAD10*, *RAD14*, *RAD16*, and *RAD23*, all of which were shown to be indispensable for the process of NER in yeast.^{11,12}

An early, surprising observation in my laboratory was that the *RAD3* gene (but none of the other *RAD* genes tested) is essential for the viability of yeast cells in the absence of exposure to UV radiation or other DNA-damaging agents.¹³ This unexpected finding indicated that the *RAD3* gene product is required for some essential aspect of cellular metabolism in addition to NER. The nature of the essential function of *RAD3* was finally deciphered 10 years later when a collaboration with Roger Kornberg and his colleagues at Stanford University established that Rad3 protein is a subunit of the RNA polymerase II basal transcription factor TFIIF.¹⁴ This transcription factor is comprised of 9 subunits, and further studies demonstrated that in addition to Rad3 protein, six other subunits called Ssl1, Ssl2, Tfb1, Tfb2, Tfb3, and Tfb4 are indispensable for NER in yeast.¹⁵

The genetic and biochemical complexity of NER in yeast has now increased to at least 19 proteins (Table 1),

Figure 4. Yeast *rad3* mutants are highly sensitive to UV radiation. However, when such cells are transfected with plasmids containing the *RAD3* gene (*rad3/pRAD3*) UV radiation sensitivity is restored to that observed with wild-type cells (*RAD3*).

all of which are required for events that precede repair synthesis and DNA ligation. Overexpression of these genes in bacterial cells provided the starting material for the systematic purification and characterization of individual polypeptides required for NER. These studies have culminated in the identification of specific catalytic functions associated with particular polypeptides or polypeptide complexes. Additionally, highly conserved homologous genes have now been cloned from human cells and their polypeptide products have been purified (Table 1). Many of these genes have indeed turned out to be mutated in XP individuals. Reconstituted human and yeast systems that support NER *in vitro* have now been developed with recombinant proteins.^{16–19}

As already indicated, a cardinal feature of NER is that it operates on structurally diverse types of bulky, helix-distorting base damage in DNA.^{1,11,12,16–19} A primary question is how a single biochemical mechanism is able to recognize so many types of base damage as substrates.²⁰ Some individual NER proteins have been shown to bind to damaged DNA with a higher affinity than they bind to native DNA,^{21–24} suggesting that they may be specifically required for the recognition of base damage. However, it is improbable that a single protein could recognize the many chemically distinctive types of base

Table 1. Yeast and Human Proteins Involved in Steps of Nucleotide Excision Repair that Precede Repair Synthesis and DNA Ligation

Yeast protein	Human homologue
Rad1	XPF
Rad10	ERCC1
Rad2	XPG
Rad4	XPC
Rad23	HHRAD23A, HHRAD23B
Rpa1	Rpa1
Rpa2	Rpa2
Rpa3	Rpa3
Rad3	XPD
Ssl1	p44
Ssl2	XPB
Tfb1	p62
Tfb2	p52
Tfb3	p44
Tfb4	p34
Rad7	?
Rad16	?
Abf1	?

damage that are known to comprise substrates for NER.²⁰ Studies in my laboratory have demonstrated co-fractionation of many of the individual polypeptides required for NER.^{25,26} These experiments are, in principle, open to several different interpretations. An explanation that I favor is that the NER proteins are organized in a large multiprotein complex, or repairosome (Figure 5), even in the absence of exposure of cells to DNA damage of the type that requires NER. This repairosome (or NER machine) may patrol the genome in a surveillance mode, and the recognition of appropriate substrates for NER may be a function of multiple interacting components of the entire complex rather than any single polypeptide. A definitive answer to the question of how cells recognize so many chemically distinct forms of base damage during NER still eludes us.

Regardless of whether the repairosome is preassembled in yeast cells or is only assembled in an ordered, step-wise fashion after cells experience bulky

Figure 5. The yeast nucleotide excision repairosome is comprised of at least 19 polypeptides, most of which are represented by highly conserved homologous proteins in human cells. The seven proteins represented in red are the subunits of the RNA polymerase II basal transcription factor TFIIF.

Figure 6. Schematic representation of nucleotide excision repair in yeast. **A:** Both the RNAPII transcription initiation complex (green) and the nucleosome (pink) assembled and bound at a site of base damage (triangle) include TFIIF (orange). The Rad3 and Ssl2 DNA helicases unwind a short stretch of DNA during both transcription initiation (left) and NER (right). **B:** In the case of NER, the unwinding of DNA generates junctions between double- and single-stranded DNA. These junctions are attacked by specific endonucleases such as the Rad2 junction-specific endonuclease cuts the damaged DNA strand 3' to the site of base damage and the Rad1/Rad10 endonuclease cuts the damaged strand 5' to this site. **C:** Following bimodal incision of the damaged strand, the Rad7 and Rad16 proteins somehow facilitate displacement of the resulting oligonucleotide fragment. DNA polymerase δ or ϵ , together with accessory proteins for DNA replication (RPA, RFC, and PCNA) fill in the gap generated by oligonucleotide excision. DNA ligation completes NER.

base damage, there is a consensus that the biochemical events that follow the recognition of base damage in DNA are catalyzed by this large multiprotein complex.^{27–29} Experimental evidence supports the notion that a very early event is the localized denaturation of the DNA duplex in the immediate vicinity of a site of base damage^{16–19,30} (Figure 6A). This denaturation is believed to be catalyzed by two of the TFIIF subunits, Rad3 (human XPD) and Ssl2 (human XPB) proteins, both of which are DNA helicases with opposite directionality^{31,32} (Figure 6A). A similar, if not identical function of the Rad3 (human XPD) and Ssl2 (human XPB) helicases is subserved during RNAP II transcription (Figure 6A). By this process, limited melting of DNA is achieved, which facilitates the initiation of mRNA synthesis and the clearance of the promoter region by the transcription apparatus.^{16–19} Though apparently co-opted for the process of NER, localized unwinding of DNA during this process serves an entirely different purpose. The products of the *RAD1* (human XPF) and *RAD10* (human ERCC1) genes function in a heterodimeric complex which specifically endonucleolytically cleaves the junction between single-stranded and duplex DNA in substrates in which the single-stranded DNA has 3' directionality³³ (Figure 6B). Correspondingly, the Rad2 (human XPG) protein is a DNA junction-specific endonuclease that cleaves be-

tween single-stranded and duplex DNA in substrates in which the single-stranded DNA has 5' directionality³⁴ (Figure 6B). Cleavage of the single-stranded/double-stranded junctions generated at each end of the localized denaturation bubble will, if confined to the DNA strand carrying the damaged base, generate incisions (nicks) that flank the damaged base (Figure 6B). It is not known how cleavage of the opposite undamaged strand is avoided. A reasonable guess is that the topology of the repairosome-damaged DNA complex is such that the catalytic sites of the Rad1/Rad10 (human XPF/ERCC1) and Rad2 (human XPG) endonucleases are placed in immediate proximity to the duplex/single-stranded DNA junctions of only the damaged strand. Alternatively or additionally, the topological organization of the repairosome-damaged DNA complex may provide protection of the undamaged strand by single strand binding protein (SSB), which is known to be required for normal NER and is an integral component of the repairosome.

The decision to exploit yeast as a eukaryotic model for understanding the biochemistry of NER in humans proved to be a good one. Most of the NER proteins identified in yeast are highly conserved in the eukaryotic kingdom, including in humans, and independent studies in several laboratories have demonstrated that the essential features of damage-specific incision of DNA during NER are the same in yeast and humans.¹⁶⁻¹⁹

The dual function of TFIIH in both RNAP II transcription and NER raises the interesting evolutionary question of how this came about. A reasonable speculation is that an early eukaryotic form of the NER apparatus, which may now be extinct, did not use TFIIH and operated in a biochemically distinct mode, perhaps more akin to that known in *E. coli* and other prokaryotes.¹ However, a spurious binding affinity between this NER complex and one or more subunits of TFIIH promoted the sequestration of this complex from the RNAP II transcription machinery and resulted in reduced efficiency of transcription initiation. This phenotypic consequence was selected during evolution because reduced transcription in the presence of DNA damage afforded a significant protective mechanism against the mutagenic and/or lethal effects of genotoxic agents. In time this selection evolved to the permanent physical incorporation of TFIIH into the NER complex, and this in turn promoted a new mechanism for damage-specific incision of DNA, which is predicated on the DNA helicase function of TFIIH. Regardless of the accuracy of this speculation, the fact that TFIIH functions in both RNAP II transcription and NER affords potential regulation of each of these processes in the presence of the other. Indeed, work in my laboratory has directly demonstrated that RNAP II transcription of a yeast gene *in vitro* is inhibited in the presence of active NER.³⁵

The dual incisions that flank sites of base damage generate oligonucleotides ~30 nucleotides in length that are no longer covalently attached to DNA. The physical excision of these oligonucleotides requires further components of the yeast repairosome. Specifically, proteins called Rad7, Rad16, and Abf1 form a tight complex and are believed to act in concert to facilitate oligonucleotide fragment excision³⁶ (Figure 6C). The precise function of

the Rad7/Rad16/Abf1 repairosome subcomplex is not known. Whereas most of the proteins involved in NER in yeast are well conserved in humans, obvious homologues of the yeast *RAD7*, *RAD16*, and *ABF1* genes have not been detected in the human genome and are not present in the genome of *Drosophila melanogaster*, the sequence of which was recently completed. Hence, the mechanism by which damage-containing oligonucleotide fragments are excised may differ in lower and higher eukaryotes. Regardless, one anticipates that the process of oligonucleotide excision is coupled to repair synthesis of DNA in both yeast and human cells. In this way nucleotides can be replaced in the genome essentially concurrently with their removal during NER (Figure 6C) and the formal existence of large single-stranded gaps in the genome can be avoided. When repair synthesis is accomplished, DNA ligation restores the complete covalent integrity of the genome and the process of NER is completed.

This brief summary of the mechanism of NER completely has deliberately omitted consideration of the fact that in eukaryotic cells DNA is structurally organized into chromatin. The vexing question of how this intimate association of DNA with numerous histone and non-histone chromosomal proteins is disrupted and reconstituted to allow access of the NER machinery to sites of base damage remains a challenging issue.

The Return to Mammals: Mice as Model Organisms

The essential features of the biochemistry and molecular biology of NER in eukaryotes are now well understood. However, many questions concerning the relationship between defective NER and cancer predisposition in the human disease XP remain to be answered. XP is a rare disease; hence, the availability of human subjects for study is limited. Additionally, one is faced with the many limitations associated with all human experimentation and, not inconsequentially, the heterogeneity of genetic and other factors (such as age) that is intrinsic to human populations. Because (regrettably, from an experimental point of view) yeast do not develop cancer in the sense that we understand this disease in multicellular organisms, in the mid-1990s I sought a new experimental model that was free of these limitations, to begin to investigate the relationships between defective NER and cancer predisposition, and elected to join the new wave of investigators exploiting the many uses of genetically engineered mice.

Using conventional targeted gene replacement, my colleagues and I inactivated the mouse *Xpc* gene, the highly conserved (>90% amino acid identity) orthologue of the human *XPC* gene.³⁷⁻³⁹ Human patients from the XP-C genetic complementation group have the same primary clinical features of extreme photosensitivity and skin cancer predisposition observed in other XP individuals.^{1,40} However, at the molecular level they uniquely retain proficiency for NER of the transcribed strand of transcriptionally active genes, whereas other XP patients

Figure 7. When mice that are homozygously defective in the *Xpc* gene ($-/-$) are exposed to weekly doses of UVB radiation, they develop skin cancers on the shaved dorsal skin more quickly than *Xpc* wild-type ($+/+$) or heterozygous ($+/-$) littermates.

are totally defective in NER.¹ The only clinical phenotypic association that has been related to this difference is a reduced incidence of neurological dysfunction in XP group C patients compared to other XP individuals.⁴⁰

Like their human counterparts, *Xpc*^{-/-} mice are defective in NER of transcriptionally silent DNA and that of the nontranscribed strand of transcriptionally active genes, but retain the ability to repair UV radiation photoproducts in the transcribed strand of transcriptionally active genes.³⁷ The mice suffer a high predisposition to skin cancer after exposure to UVB radiation⁴¹ (Figure 7). By ~25 weeks after the onset of a regimen of daily exposure to UV light for 18 weeks, 100% of these animals develop cancers on the shaved dorsal skin (Figure 8). No skin cancers are observed in *Xpc*^{+/-} and *Xpc*^{+/+} littermates at this time (Figure 8). However, by about 70 weeks after initiation of the radiation protocol, *Xpc*^{+/-} animals are clearly more cancer-prone than wild-type controls⁴¹ (Figure 8). This result apparently reflects true haploinsufficiency at the *Xpc* locus, since no mutations have been detected in the remaining *Xpc* allele in a number of tumors examined.⁴¹ Extrapolating to humans, this observation raises the interesting and important possibility that individuals who are heterozygous for the XPC mutation may have an increased risk of skin cancer associated with sunlight exposure.

Because XP individuals are constitutionally defective in NER, one would expect that their predisposition to cancer

extends to any type of cancer that is etiologically associated with exposure to agents that cause DNA damage, which requires proficient NER. Comparisons of the incidence of cancers outside the skin in XP individuals are understandably anecdotal⁴² because of the very limited number of patients available for study and the early death of most XP individuals from the complications of skin

Figure 8. The kinetics of skin cancer induction in mice indicate that whereas irradiated *Xpc*^{-/-} animals develop skin cancers very quickly, in time heterozygous mutant ($+/-$) mice develop skin cancers more rapidly than wild-type ($+/+$) animals. Circles, *Xpc*^{-/-} mice; triangles, *Xpc*^{+/+} mice; squares, *Xpc*^{+/-} mice.

cancer. XP mice are therefore ideal subjects for such studies. Following an established protocol for acetylaminofluorene (AAF)-induced cancer in mice, we showed that *Xpc*^{-/-} animals are clearly more susceptible to benign and malignant tumors of the liver and lungs than heterozygous or wild-type mice⁴³ (Table 2). Hence, there is apparently no tissue specificity associated with the predisposition of XP patients to skin cancer (as has sometimes been argued in the literature⁴⁴), and such individuals should be appropriately counseled about the risks of exposure to all environmental carcinogens.

One of the unique opportunities afforded by the availability of increasing numbers of genetically engineered mice is the potential for crossing different mutant strains to generate strains with multiple mutations in relevant genes. Among numerous such experiments of this type, my colleagues and I generated all possible combinations of *Xpc* and *Trp53* (the mouse name for *p53*) heterozygous and homozygous double mutant mice. When *Xpc*^{-/-} mice missing one or both copies of the *Trp53* gene were exposed to UVB radiation, skin cancers appeared significantly earlier in a gene dosage-dependent manner.⁴⁵ Aside from its known functions in cell cycle checkpoint control and apoptosis, human *p53* protein has been directly implicated in NER.^{46,47} Hence, it is conceivable that the increased predisposition to skin cancer in *Trp53* mutant mice reflects inactivation of the residual proficiency of *Xpc* cells for repair of the transcribed strand of transcriptionally active genes. Alternatively, and more likely in my view, the increased predisposition to skin cancer in *Trp53* mutant mice reflects the additive effects of defective NER and defective *p53* function as a tumor suppressor gene.

Because the *Trp53*-null phenotype associated with loss of both alleles increases the rate of development of skin cancer more dramatically than the *Trp53*^{+/-} genotype, we reasoned that the later appearance of skin cancers in the latter genetic background reflects the time taken to inactivate the remaining *Trp53* allele due to a failure to repair UV radiation damage. We therefore sequenced the remaining *Trp53* allele in about 20 skin cancers in *Xpc*^{-/-} *Trp53*^{+/-} mice and confirmed mutations in >90% of the tumors.⁴⁸ Most unexpectedly, in addition to the mutational hot spots observed by us and others in the *Trp53* gene in wild-type animals, all of which are at dipyrimidine sites where major photoproducts (pyrimidine dimers and [6-4] photoproducts) are known to occur, we observed an unusually high frequency of mutations affecting the C residue in the sequence ACG, the coding trinucleotide of codon 122 of the *p53* open reading frame.⁴⁸ In this sequence C is flanked by purines; hence, pyrimidine dimers and [6-4] photoproducts cannot be generated. This C residue is believed to be methylated, as it is part of a CpG dinucleotide.

The observation of a UV radiation-dependent mutational hot spot in the *Trp53* gene raises several interesting questions. What is the nature of the putative photoproduct formed at this non-dipyrimidine CpG site? Does this represent a rare photoproduct that is normally repaired by the NER pathway, or conceivably by a novel repair pathway that requires the XPC protein but not other NER

proteins? In an effort to address the latter issue we examined the mutational spectrum in the single *Trp53* allele in skin cancers generated by UVB irradiation of *Xpa*^{-/-} *Trp53*^{+/-} mice. No mutations affecting codon 122 were detected in 11 skin cancers examined (D Nahari, LB Meira, and EC Friedberg, unpublished observations). In a twelfth tumor, an inactivating mutation was located elsewhere in the *Trp53* gene, and a single second mutation in the same tumor was found in codon 122 (D Nahari, LB Meira, and EC Friedberg, unpublished observations). These preliminary experiments suggest that the high frequency of mutations in codon 122 is indeed *Xpc* gene-specific. Whether this specificity relates to differences between *Xpc* and other XP mutant mice in the biology of neoplastic transformation associated with exposure to UVB radiation, or to differences in the repair of a rare photolesion in DNA, remains to be established and is the subject of ongoing studies.

Other observations suggest the possibility that XPC protein may be specifically required for the repair of a rare form of base damage associated with exposure to UVB radiation. The frequency of spontaneous mutations measured in the *Hprt* gene of lymphocytes increases dramatically (20- to 30-fold) as a function of age in *Xpc*^{-/-} mice, but not in *Xpa*^{-/-} mice (SWP Wijnhoven, HJM Kool, LHF Mullenders, AA van Zeeland, EC Friedberg, GTJ van der Horst, H van Steeg, H Vrieling, unpublished observations). These mutations presumably arise from unrepaired spontaneous base damage in DNA, such as that generated by reactive oxygen species in cells.

Trp53-null mice spontaneously develop various types of malignant tumors.⁴⁹ Initially these are predominantly testicular teratocarcinomas. However, lymphomas and other sarcomas are prominent later.⁴⁹ However, of relevance to the present discussion, when such mice are also genotypically *Xpc*^{-/-} the kinetics of the appearance of testicular (but not other) tumors is significantly increased.⁴³ This observation also suggests that some form of spontaneous base damage in the DNA, this time in testicular cells, requires XPC protein for efficient repair.

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