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Recombinant Viral Vectors for Investigating DNA Damage Responses and Gene Therapy of Xeroderma Pigmentosum

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1. Introduction

1.1 The dark side of the sun

The genome of all living organisms is constantly threatened by a number of endogenous and exogenous DNA damaging agents. Such damage may disturb essential cellular processes, such as DNA replication and transcription, thereby resulting in double-strand breaks (referred to as 'replication fork collapse'), which can lead to chromosomal aberrations and/or cell death, ultimately contributing to mutagenesis, early aging and tumorigenesis (Ciccia & Elledge, 2010). One of the most important exogenous sources of DNA damage is the ultraviolet radiation (UV) component of sunlight, since it is responsible for a wide range of biological effects, including alteration in the structure of biologically essential molecules, such as proteins and nucleic acids. Indeed, UV is one of the most effective and carcinogenic exogenous agents that act on DNA, threatening the genome integrity and affecting normal life processes in different aquatic and terrestrial organisms, ranging from prokaryotes to mammals (Rastogi et al., 2010). In addition, UV is the major etiologic agent in the development of human skin cancers (Narayanan et al., 2010).

Sunlight is the primary UV source, whose spectrum is usually classified according to its wavelength in UVA (320-400 nm; lowest energy), UVB (280-320 nm) and UVC (200-280 nm; highest energy). Although these three UV bands are present in sunlight, the stratospheric ozone layer entirely blocks the UVC and most of UVB, thus the solar UV spectrum that reaches the Earth's ground is composed by UVA and some UVB, even though ozone layer depletion can cause changes in this spectral distribution (Kuluncsics et al., 1999).

The chemical nature and efficiency in the formation of DNA lesions greatly depend on the wavelength of the incident photons. Despite its lowest energy, UVA light can deeply penetrate into the cells, mostly damaging DNA by indirect effects caused by the generation of reactive oxygen species which may react with nitrogen bases, resulting in base alterations and breaks in the DNA molecule. On the other hand, UVB can be directly absorbed by DNA bases, producing two main types of DNA damage, the cyclobutane pyrimidine dimers (CPDs) and pyrimidine-pyrimidone-(6-4)-photoproducts (6-4PPs), both resulting from

covalent linkages between adjacent pyrimidines located on the same DNA strand, which leads to severe structural distortions in the DNA double helix. Interestingly, it has been recently demonstrated that UVA can also be directly absorbed by the DNA molecule, efficiently generating both CPDs and 6-4PPs (Schuch et al, 2009).

CPDs correspond to the formation of a four-member ring structure involving carbons C5 and C6 of both neighboring bases, whereas 6-4PPs are formed by a non-cyclic bond between C6 (of the 5'-end) and C4 (of the 3'-end) of the involved pyrimidines. Since those lesions induce strong distortions in the DNA molecule, they may lead to severe consequences to the cell if not properly removed, such as transcription arrest and replication blockage, thus disturbing cell metabolism, interfering with the cell cycle and, eventually, inducing cell death. DNA mutations can also result from misleading DNA processing. Long term consequences may include even more deleterious events, such as photoaging and cancer (Sinha & Häder, 2002; Narayanan et al., 2010; Rastogi et al., 2010).

1.2 DNA repair of UV lesions and related human syndromes

To ensure the maintenance of the genome integrity, several mechanisms that counteract DNA damage have emerged very early in evolution, including an intricate machinery of DNA repair, damage tolerance, and checkpoint pathways (Figure 1).

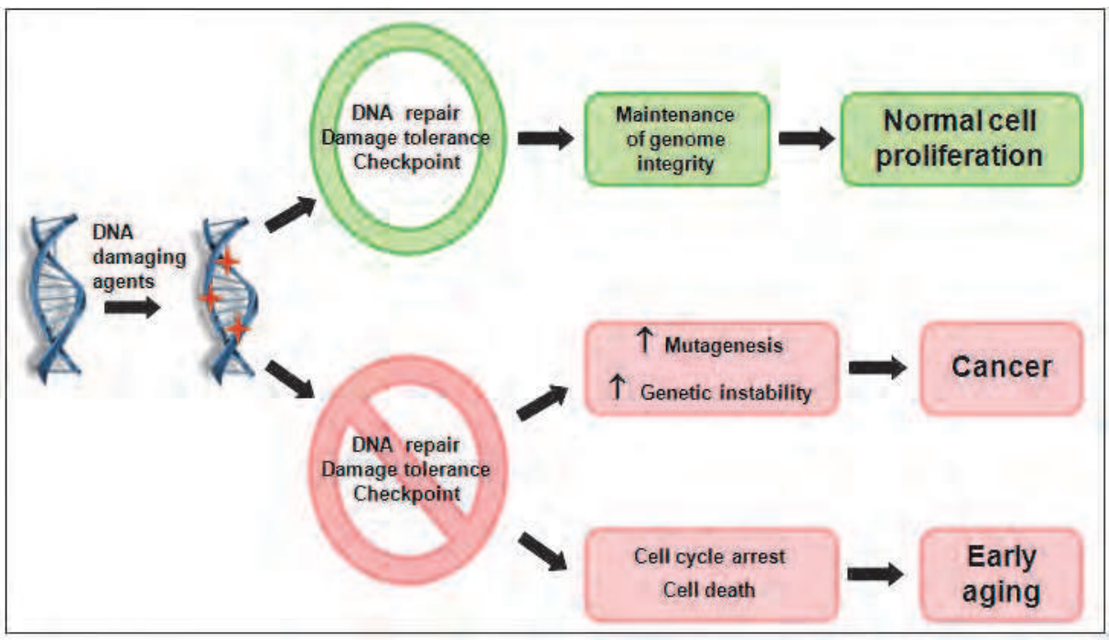

Fig. 1. Main consequences of DNA damage. DNA damage can be induced by a variety of endogenous and exogenous agents. Several mechanisms, including an intricate machinery of DNA repair, damage tolerance, and checkpoint pathways, counteract DNA damage, aiming for the maintenance of genome stability, and guaranteeing normal cell proliferation. When these mechanisms fail, errors in DNA replication and/or aberrant chromosomal segregations take place, increasing mutagenesis and genetic instability and contributing to a higher risk of cancer development. Alternatively, these damages may disturb the transcription and/or cause replication blockage, leading to cell death , thus contributing to early aging.

The nucleotide excision repair (NER) is one of the most versatile and flexible DNA repair systems, removing a wide range of structurally unrelated DNA double-helix distorting lesions, including UV photoproducts, bulky chemical adducts, DNA-intrastrand crosslinks, and some forms of oxidatively generated damage by orchestrating the concerted action of over 30 proteins, including the seven that are functionally impaired in xeroderma pigmentosum patients (XPA to XPG) (Costa et al., 2003; de Boer & Hoeijmakers, 2000). The NER pathway has been extensively studied at the molecular level in both prokaryotic and eukaryotic organisms. Depending on whether the damage is located in a transcriptionally active or inactive domain in the genome, its repair will be processed by one of two NER subpathways: global genome repair (GG-NER) or transcription-coupled repair (TC-NER). Indeed, while GG-NER is a random process, removing distorting lesions over the entire genome, TC-NER focus on those lesions which block RNA polymerases elongation, thus being highly specific and efficient (Fousteri & Mullenders, 2008; Hanawalt, 2002).

Briefly, the NER pathway involves a sequential cascade of events that starts with damage recognition, which defines the major difference between GG-NER and TC-NER. The latter is triggered upon blockage of RNA polymerase translocation at the DNA damage site, whereas GG-NER is evoked by specialized damage recognition factors, including the XPC-hHR23B heterodimer, and also XPE for certain lesions. The subsequent steps are carried out by a common set of NER factors that are shared by both subpathways and involve opening of the DNA helix around the lesion site by the concerted action of two helicases; dual incision of the damaged strand at both sides of the lesion by two endonucleases; removal of the damaged oligonucleotide (24-32 mer); gap filling of the excised patch using the undamaged strand as a template by the action of the replication machinery; and ligation of the new fragment to the chromatin by DNA ligase (Cleaver et al., 2009; Costa et al., 2003). Even though the core NER proteins that carry out damage recognition, excision, and repair reactions have been identified and extensively characterized, the regulatory pathways which govern the threshold levels of NER have not been fully elucidated (Liu et al., 2010). A schematic representation of this repair mechanism in humans is illustrated in Figure 2.

Several human autosomal recessive diseases are caused by dysfunction of the NER pathway, xeroderma pigmentosum (XP) being the prototype. Although this chapter will mainly focus on the XP syndrome, deficiencies in NER can also lead to other genetic diseases, such as trichothiodystrophy (TTD), Cockayne syndrome (CS), cerebro–oculo–facial–skeletal syndrome (COFS) and UV-sensitive syndrome (UVsS), all of which have photosensitivity as a common feature.

Xeroderma pigmentosum (XP) is a rare human disorder transmitted in an autosomal recessive fashion characterized by severe UV light photosensitivity, pigmentary changes, premature skin aging and a greater than 1,000-fold increase incidence of skin and mucous membrane cancer, including squamous and basal cell carcinomas and melanomas, with a 30-year reduction in life span (Cleaver et al., 2009; Karalis et al., 2011; Narayanan et al., 2010). In addition to cutaneous features, patients often develop ocular abnormalities, including neoplasms which may cause blindness. For most patients, often referred to as classical XP, this syndrome is caused by an impaired GG-NER activity, with or without deficiencies in TC-NER, determined by mutations in one of seven NER genes (*XPA* to *XPG*). When TC-NER is also affected (mutations in *XPA*, *XPB*, *XPD* and *XPG* genes), accelerated neurodegeneration may also occur in a substantial number of patients, suggesting increased neuronal cell death due to accumulated endogenous damage (Gerstenblith et al., 2010; Hoeijmakers, 2009). The eighth complementation group corresponds to the XP-variant

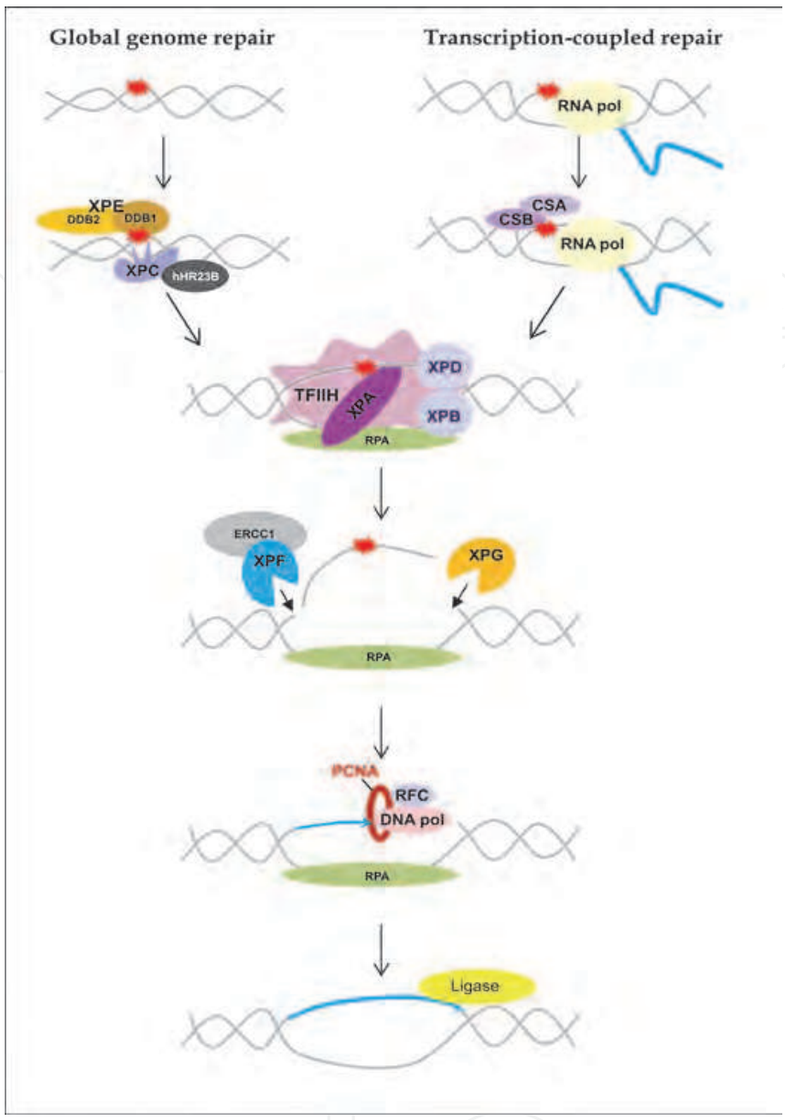

Fig. 2. Schematic representation of repair of DNA lesions by nucleotide excision repair (NER). Depending on where the DNA damage is located in the genome, it will be processed by one of the two NER subpathways: the global genome repair (GG-NER) or the transcription-coupled repair (TC-NER), that basically differ in the lesion recognition step. Lesions occurring randomly in the genome are recognized by the XPC-HR23B complex, with the participation of XPE (DDB1-DDB2) for certain lesions, both complexes are GG-NER-specific. On the other hand, lesions present in the transcribed strand of active genes that lead to the RNA polymerase arrest trigger the TC-NER subpathway, which involves the CSA and CSB proteins. The following steps are common to both subpathways. The DNA double helix around the lesion is opened by XPB and XPD (helicases belonging to the TFIIH complex) and the single strand region is stabilized by RPA, allowing damage verification by the XPA protein. The DNA around the damaged site is then cleaved by the XPF–ERCC1 and XPG endonucleases, excising an oligonucleotide of 24-32 mer, and this patch is resynthesized by the replication machinery using the undamaged strand as a template. Finally, the new fragment is sealed to the chromatin by the DNA ligase.

(XPV) patients, whose XP phenotype is related to mutations in the *POLH* gene, which encodes the translesion synthesis DNA polymerase eta responsible for the replication process on UV-irradiated DNA templates (Johnson et al., 1999; Masutani et al., 1999).
A list of NER genes, which are related to XP syndrome, with their specific functions is given in Table 1.

Gene	Protein	Protein size (A.A.)	Function	Pathway
XPA	XPA	273	Interacts with RPA and other NER proteins, stabilizing ssDNA regions and also facilitating the repair complex assembly.	GG-NER TC-NER
XPB	XPB	782	Belongs to TFIIH complex, working as a 3` → 5` helicase.	GG-NER TC-NER
XPC	XPC	940	Responsible for lesion recognition in GG-NER.	GG-NER
XPD	XPD	760	Belongs to the TFIIH complex, working as a 5` → 3` helicase.	GG-NER TC-NER
DDB2	XPE/p48 subunit	428	Forms a complex with XPE/p127 subunit, which is believed to facilitate the identification of lesions that are poorly recognized by XPC-hHR23B.	GG-NER
XPF	XPF	905	Found as a complex with ERCC1, which functions as an endonuclease 5′ of the lesion.	GG-NER TC-NER
XPG	XPG	1186	Functions as an endonuclease 3′ of the lesion.	GG-NER TC-NER

*GG-NER- global genome repair; TC-NER- transcription-coupled repair.

Table 1. List of NER genes related to xeroderma pigmentosum and their roles in human DNA repair.

The Cockayne syndrome (CS) is predominantly a developmental and neurological disorder, caused by mutations leading to a defective TC-NER, which prevents recovery from blocked transcription after DNA damage. CS patients are characterized by early growth and development cessation, severe and progressive neurodysfunction associated with demyelination, sensorineural hearing loss, cataracts, cachexia, and frailty (Weidenheim et al., 2009). Curiously, although severe photosensitivity is a common feature reported for most CS patients, it is not linked to an increased frequency of skin cancers, like it is in XP patients. Interestingly, specific mutations in one of three XP genes (*XPB*, *XPD* and *XPG*) may result in a clinical phenotype which reflects a combination of the traits associated with XP and CS (XP/CS patients). This observation indicates that simultaneous defects in GG-NER and TC-NER can cause mutagenesis and cancer in some tissues and accelerated cell death and premature aging in others (Hoeijmakers, 2009).

The hallmark of trichothiodystrophy (TTD) is sulfur-deficient brittle hair, caused by a greatly reduced content of cysteine-rich matrix proteins in the hair shafts. In severe cases, mental abilities are also affected. Abnormal characteristics at birth and pregnancy complications are also common features of TTD, which may imply a role for DNA repair genes in normal fetal development (Stefanini et al., 2010). As CS patients, TTD patients do not present a high incidence of skin cancers. Genetically, three genes were identified for this disease (*XPB*, *XPD* and *TTDA*), but most TTD patients exhibit mutations on the two alleles of the *XPD* gene (Itin et al., 2001).

Cerebro–oculo–facial–skeletal syndrome (COFS) is a disorder determined by mutations in *CSB*, *XPD*, *XPG* and *ERCC1* genes, leading to a defective TC-NER (Suzumura & Arisaka, 2010). It is characterized by congenital microcephaly, congenital cataracts and/or microphthalmia, arthrogryposis, severe developmental delay, an accentuated postnatal growth failure and facial dysmorphism.

Photosensitivity and freckling are the main features of patients with UV-sensitive syndrome (UVsS), but these patients have mild symptoms and no neurological or developmental abnormalities or skin tumors. Although other genes may be involved, mutations in the *CSB* gene were found in some of these patients, leading to defective TC-NER of UV damage (Horibata et al., 2004; Spivak, 2005).

Therefore, the general relationship between defects in NER genes and clinical disease phenotypes is complex, since mutations in several genes can cause the same phenotype, and different mutations in the same gene can cause different phenotypes (Kraemer et al., 2007).

Even though DNA repair malfunctions are autosomal recessive diseases and their incidence is therefore relatively low (~1/100,000), many of the individuals with DNA repair deficiencies die in early childhood since there is no effective treatment, only palliative care. Therefore, the search for a long-term treatment has been intense. Several strategies using recombinant viral vectors are being used in order to improve the resistance of cells from these patients to DNA damaging agents (Lima-Bessa et al., 2009; Menck et al., 2007). Also, the studies of DNA repair mechanisms have yielded a better understanding of specific cell processes which lead to human diseases such as cancer, neurodegeneration and aging (Hoeijmakers, 2009). This review will focus on the use of recombinant viral vectors for the purposes of investigating both the cellular responses to DNA damage and the perspectives of providing therapy for XP patients.

2. Recombinant viral vectors as gene delivery tools

An ideal gene delivery tool should have the ability to transduce proliferating and fully differentiated cells with high efficiency; mediate high-level, prolonged and controlled transgene expression; have little toxicity (both at cellular and organism levels); elicit small immune responses *in vivo*; and be able to accommodate large DNA fragments for transgene transduction (Howarth et al., 2010). Unfortunately, there is no single tool that fulfills all these criteria.

Viruses have had million of years to improve their capacity to infect cells with the aid of evolutionary pressures. Researchers have been trying to take advantage of this ability creating recombinant viral vectors. In general, for that purpose, the viral genome is manipulated and sequences needed to form the infective virion are deleted, opening space to insert the transgene of interest.

Several viral vectors have been created and the most widely used are: adenovirus, retrovirus (including lentivirus) and adeno-associated virus. The main characteristics of these vectors are presented in Table 2.

Virus	Nucleic acid	Genome size (Kb)	Envelope	Virion size (nm)	Integration	Transgene size (Kb)	Immune response	Transgene expression
Adenovirus	dsDNA linear	36		90	episomal	8 - 25	***	days - months
Adeno-associated virus	ssDNA linear	4.7		25	site-specific	4.7 - 9	*	months-years
Retrovirus	ssRNA (homodimer)	7 - 12	*	100	random	<10	**	years
Lentivirus	ssRNA (homodimer)	9	*	100	random	10 - 16	**	years

Table 2. Main features of viruses currently used as recombinant vectors for gene delivery.

Searching for the perfect gene delivery tool, intense modifications have been added to the vectors' genomes, nucleocapsid and envelopes, always searching for less immunogenic vectors, with higher and more specific transduction properties. Currently, recombinant viruses are the vector of choice for research and clinical trials worldwide, but still only few phase II or III trials are being conducted (Atkinson & Chalmers, 2010). All viral vectors cited here have already been used in *in vitro*, *ex vivo* and *in vivo* experiments and in clinical trials.

2.1 Recombinant adenoviral vectors

Adenoviruses (Ad) are non-enveloped double-stranded DNA viruses with tropism for the respiratory and ocular tissues. The first generation recombinant vector can carry up to 8 Kbp of DNA, while the last generation, in which the viral DNA sequence is completely deleted (also named gutless), is able to efficiently transduce over 25 Kbp of DNA (Atkinson & Chalmers, 2010).

Despite the fact that the gutless vector needs the aid of helper viral proteins supplied *in trans*, adenoviral vectors are easily produced in high titers. Once the transgene has been delivered inside the nucleus it remains episomal, reducing the risk of tumorigenesis induced by insertional mutagenesis. On the other hand, the episomal DNA is not replicated and its segregation in mitosis leads to the eventual loss of the transgene in the daughter cells. Thus, the transgene expression is short-lived. A possible solution is to add a site-specific integration sequence next to the transgene, leading to a prolonged transgene expression (Atkinson & Chalmers, 2010). Another advantage of the adenoviral vectors is their ability to transduce post mitotic cells since the transgene is already delivered in its active form, as a double-stranded DNA. This property is of particular interest when aiming for gene therapy in neurons (Atkinson & Chalmers, 2010).

The biggest challenge for the use of adenoviral vectors *in vivo* is the immunological response it elicits. This strong response is not only due to the natural immunogenicity of its components, but also to pre-existing immunity caused by previous contact with at least one of the over 50 serotypes of human infecting adenovirus (Seregin & Amalfitano, 2009). Taking into consideration that these vectors are only capable of a transient expression of the transgene and that repeated dosage might be necessary, a strong immune response is very

undesirable. Possible alternatives to circumvent this issue are: manipulation of the viral capsid proteins and DNA, making them less immunogenic; the usage of a different serotype on each application; and the use of immunosuppressants (Atkinson & Chalmers, 2010; Seregin & Amalfitano, 2009).

The great importance of the immunological response against a gene therapy vector was brought to attention when, in 1999, a patient suffering from an ornithine transcarbamylase deficiency, died due to an unexpected inflammatory response reaction to the adenoviral vector used in a clinical trial (Edelstein et al., 2007). Still, adenoviral vectors are currently the most widely used viral vectors in clinical trials, accounting for approximately 24% of all vectors used in gene therapy clinical trials (Edelstein et al., 2007; Hall et al., 2010).

2.2 Recombinant adeno-associated viral (AAV) vectors

Adeno-associated viruses (AAV) are non enveloped, single-stranded DNA, with serotype-specific tropism viruses. To date, 12 serotypes have been identified in primate or human tissues (Schmidt et al., 2008) in a total of over 100 known serotypes (Wang et al., 2011). Their productive lytic infection depends on the presence of a helper virus, adeno or herpesvirus, that provide *in trans* the necessary genes for the AAV replication and virion production. In the absence of a helper virus, the AAV establishes its latent cycle integrating specifically in the 19q13.4 region of the human genome (Daya & Berns, 2008). The site-specific integration is mainly dependent on the virus internal terminal repeats (ITRs), the integration efficiency element (IEE) and Rep 68 and Rep 78 genes. In the 19q13.4 region, several muscle-related genes are present, including some responsible for actin organization. No significant side effects have been observed due to AAV genome integration in this chromosome region (Daya & Berns, 2008).

The onset of transgene expression delivered by an AAV vector is delayed, usually starting several days after the transduction, probably due to the time invested in the synthesis of the DNA second strand (Michelfelder & Trepel, 2009). Although late, the transgene expression is long lasting and there is a very low humoral response, mainly related to previous exposure to the viral antigens (Daya & Berns, 2008). Despite the small size of the AAV nucleocapsid and genome, it has been shown that transgenes up to 7.2 Kb can be delivered by AAV vectors, but the oversized genomes reduce at least 10 fold the transduction efficiency (Dong et al., 2010). Several strategies have been developed seeking to optimize the vector capacity, such as the *trans*-splicing vector. With the simultaneous usage of two AAV vectors, this technology takes advantage of the concatamers formed by the ITRs that can recombine to form the desired transgene inside the transduced cell. These trans-splicing vectors allow the final transgene to have up to 9 Kb (Daya & Berns, 2008).

Only recently adeno-associated viral vectors started being used in gene therapy research and account for less than 4% of all vectors used in gene therapy clinical trials (Edelstein et al., 2007; Hall et al., 2010). Although these vectors do not behave as the parental virus, since they do not integrate in the genome (due to the lack of the REP protein), gene expression can be very long and elicit low immunological responses, making AAV vectors promising in gene therapy investigations.

2.3 Recombinant retroviral vectors

The *Retroviridae* family is characterized by a single-stranded RNA genome which can only replicate inside the host cell with the aid of an RNA-dependent DNA polymerase, the reverse transcriptase. This enzyme transcribes the virus' RNA into a DNA sequence that the host cell machinery can transcribe and translate (Froelich et al., 2010).

Retroviral vectors are capable of transducing a wide range of cell types, are able to accommodate extensive changes in their genome, accept long transgenes, have low immunogenicity, can be produced in high titers, and promote a prolonged transgene expression due to their ability to integrate into the host cell genome (Froelich et al., 2010). On the other hand, most retroviral vectors can only transduce replicating cells since the transport of the transcribed viral DNA to the nucleus is mitosis-dependent. Additionally, there is always the risk of insertional mutagenesis due to the semi-random integration of the vector genome in the host cell's genome (Froelich et al., 2010). Nowadays, the most widely used retroviruses as gene therapy tools are the lentiviruses (LVs), such as the human immunodeficiency virus (HIV). These vectors have the same advantages as other retroviral vectors and are capable of transducing post mitotic cells. Moreover, the LVs tend not to integrate by transcription initiation sites, reducing the risk of insertional tumorigenesis (Froelich et al., 2010).

The retroviral vectors were the first vectors used in gene therapy clinical trials in 1989 (Edelstein et al., 2007, Rosenberg et al., 1990) and are extensively used in fundamental biological research, functional genomics and gene therapy (Mátrai et al., 2010). In 2004, 28% of the clinical trials involving viral vectors included retroviral vectors (Edelstein et al., 2007); in 2010 that number dropped to approximately 23% (Voigt et al., 2008). This drawback is due to the unfortunate events of the French severe combined immunodeficiency (SCID) trial in 2002, where two out of ten children died in consequence of a leukemia, which was related to the insertional mutagenesis of the retroviral vector used (Edelstein et al., 2007).

Since then, special attention has been paid to the safety of these vectors as many are known to derive from viruses that cause severe diseases, such as the acquired immunodeficiency syndrome (AIDS). Strategies are constantly developed to prevent the risk of insertional mutagenesis. For that purpose, in addition to the virions being replication-defective, generated by *trans*-complementation, several further manipulations of the viral genome were made. The development of a self-inactivating (SIN) vector (Iwakuma et al., 1999) prevents horizontal and vertical gene transfer and diminishes the probability of the production of a replicating virion or over-expression of a host cell oncogene (Edelstein et al., 2007).

3. Investigating DNA damage responses with adenoviral vectors in human cells

3.1 *In vitro* and *in vivo* adenoviral gene transduction for the correction of DNA repair defects

The knowledge of the molecular defects in XP cells was the starting point for understanding how human cells handle lesions in their genome. So far, different techniques have been used to study DNA repair mechanisms and reverse malfunctions in this essential system. One powerful tool employed in these studies has been the use of recombinant adenoviral vectors to transduce DNA repair genes directly into human skin cells, aiming to improve the knowledge of basic mechanisms that cells use to protect their genome.

Experiments using first generation recombinant adenoviral vectors have been successfully employed in the transduction of both SV40-transformed and primary fibroblasts derived from XP-A, XP-C, XP-D and XP-V patients (Armelini et al., 2007). The expression of the respective functional proteins in all transduced defective cell populations was significantly increased, reaching levels even higher than seen for wild type cells (Armelini et al., 2005;

Lima-Bessa et al., 2006; Muotri et al., 2002). Moreover, different phenotypical analyses, including cell cycle, apoptosis and cell survival assays, have been carried out, all indicating that the protein expression mediated by the recombinant adenoviruses was clearly accompanied by the recovery of the DNA repair ability and increased resistance to UV radiation, thereby demonstrating functional correction of the XP phenotype. It is worth mentioning that, even though transgene expression mediated by adenoviruses is typically short-lived, sustainable high expression of XPA and XPC proteins with parallel increased UV-irradiation resistance was obtained even two months after cell transduction (Muotri et al., 2002).

For XP-A, XP-C and XP-D transduced cell lines, phenotypic analyses also involved assays aiming to investigate their ability to perform DNA repair after UV irradiation. This has been measured through determination of unscheduled DNA repair synthesis (UDS), which corresponds to the incorporation of [methyl-^{3}H] thymidine in cells that are not in S-phase, and is visualized by autoradiography as the presence of radioactive grains inside nuclei. Interestingly, UDS activity in all transduced deficient cell lines was restored to levels comparable to NER proficient cell lines, indicating those cells became able to efficiently remove UV lesions by restoring NER activity.

It is well known that UV radiation promotes DNA elongation delay as a result of replication blockage by UV photolesions (Cleaver et al., 1983), which can be easily seen by running pulse-chase experiments in alkaline sucrose gradients. Using this approach, it has been possible to show that XP-V transduced cells were able to elongate nascent DNA on UV-damaged DNA templates as efficiently as wild type cells (Lima-Bessa et al., 2006), once again demonstrating the great potential of recombinant adenoviruses in the transduction and expression of functional proteins.

One interesting conclusion came from the observation that even though *XPA*, *XPC* and *XPD* genes were over-expressed in all transduced cell lines when compared to NER proficient cells, this had no impact in the UV-resistance or NER capability, suggesting that neither of these proteins is limiting for NER in human cells. Another possible explanation is that once the NER pathway requires a coordinated action of several proteins, increasing only one of these proteins does not result in speeding up removal of the DNA lesions. Similarly, the excess of polη (*XPV*) mediated by adenoviral transduction has not affected cell survival nor elongation of replication products in UV-treated XP-A human cells, suggesting not only that polη is not a limiting factor for the efficient replication of the UV-damaged DNA in XP-A cells, but also demonstrating that the deleterious effects caused by the remaining DNA lesions in the genome cannot be mitigated by an efficient bypass mediated by polη.

However, the potential of such vectors is not restricted to *in vitro* assays. Indeed, another real perspective is their use to investigate the molecular mechanisms of DNA repair and their consequences *in vivo*, thus opening new avenues for a better understanding of cellular and physiologic responses to DNA damage. *In vivo* experiments may also help to establish the relationship between DNA repair, cancer and aging, as mice models for different DNA repair syndromes have been developed by different groups worldwide. Despite the extensive use of these models to broaden the understanding of several DNA repair related disorders, little work has been done *in vivo* testing gene therapy strategies for these diseases. Indeed, up to the present moment, only one study showed an efficient *in vivo* gene therapy protocol for complementation of the XP phenotype (Marchetto et al., 2004).

Exciting results by Marchetto and co-workers showed that the administration of subcutaneous injections of an adenoviral vector carrying the *XPA* human gene directly into

the dorsal region of XP-A knockout mice led to an extensive expression of the heterologous protein in different skin cells, including dermal fibroblasts, cells of the hair follicle and basal replicating keratinocytes, which are believed to be the starting point of most skin tumors. As a result, the repair capability of these transduced cells was restored, thus preventing UVB-induced deleterious skin effects, such as persistent scars, skin hyperkeratosis and, ultimately, avoiding the formation of squamous cell carcinomas (Marchetto et al., 2004).
Despite the promising results of this work, no others followed. Researchers are now aware of several possible limitations and complications of gene therapy after some unexpected severe events in clinical trials (Edelstein et al., 2007) and are spending more time improving gene targeting tools and techniques before risking *in vivo* approaches. In that sense, extreme progress has been made with experiments *in vitro,* as previously presented. A general panel showing the main uses of the recombinant adenoviral vectors carrying DNA repair genes is presented in Figure 3.

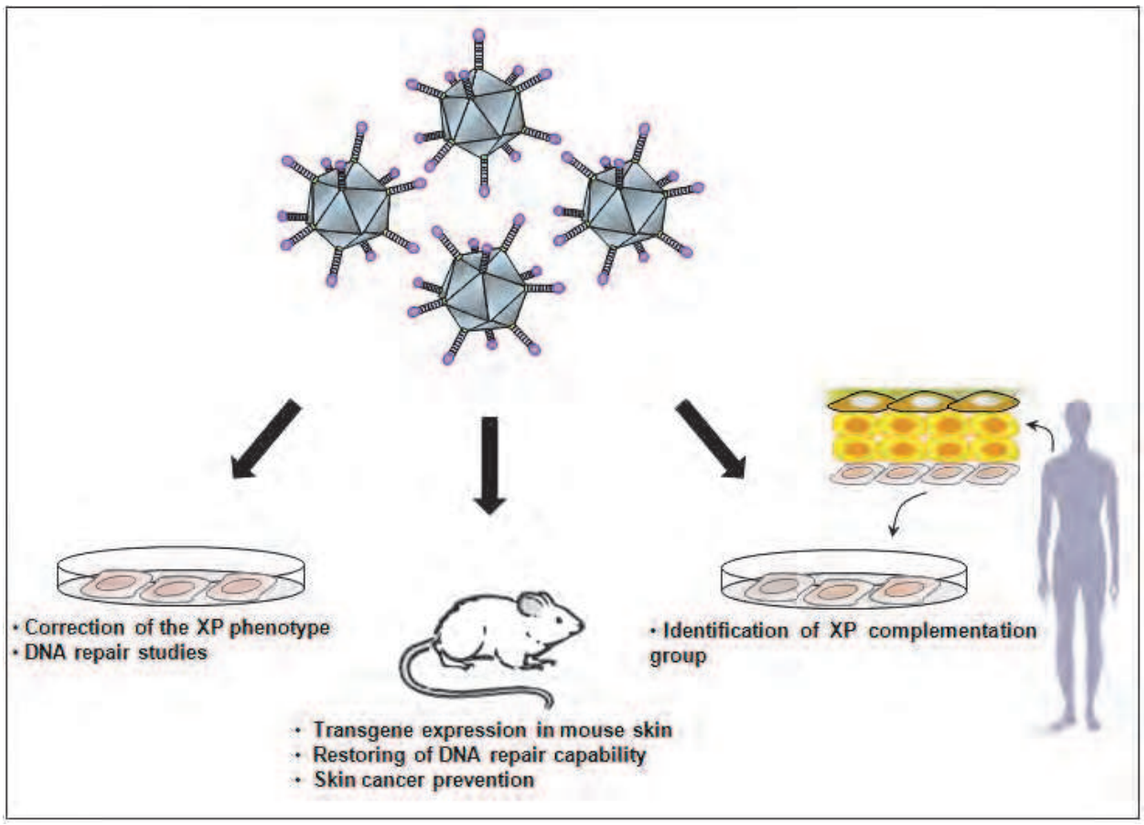

Fig. 3. DNA repair gene transduction by recombinant adenoviruses. Adenoviral vectors have been successfully employed to transduce human XP genes directly into established human cell lines (left), XP knockout mice skin (center), and fibroblasts from the skin of XP patients (right). Endpoints are indicated for each particular case.

Based on the successful complementation of the XP phenotype both *in vitro* and *in vivo*, adenoviral vectors could be proposed as an efficient tool for diagnosis and identification of XP patients' complementation groups. This hypothesis was recently tested and confirmed: with the use of adenoviruses carrying DNA repair genes, it has been possible to determine

the complementation group of three Brazilian XP patients, now characterized as XP-C patients. To that end, adenoviral transduced cells from these patients have been submitted to UV treatment and then analyzed by simple assays, such as cell survival and UDS (Leite et al., 2009). This diagnosis has been performed using the patients' skin fibroblasts but the potential use of adenoviral vectors for this purpose becomes even more exciting, considering that the adenoviral transduction could be held in cells present in the patients' blood, thus becoming a faster and less invasive technique. Besides scientific and epidemiological goals, the identification of the gene defect may help to predict clinical prognosis for the XP patients and guide appropriate genetic counseling for their families. Direct gene sequencing can be performed to identify the mutated genes, but as there are eight potential candidate genes for XP, functional complementation assays are still used for the genetic diagnosis of these patients.

3.2 Investigating UV-induced cell responses employing photolyases

Photoreactivation is a very efficient DNA repair mechanism, which specifically removes the two main UV photoproducts. Photoreactivation is carried out by flavoproteins known as photolyases. These enzymes recognize and specifically bind to UV lesions, thus reverting them back to the undamaged monomers, using a blue-light photon as energy source (Brettel & Byrdin, 2010; Sancar, 2008). Interestingly, photolyases demonstrate a great efficiency for discriminating the target lesion, either CPDs or 6-4PPs, and so far no photolyase has been shown to be able to repair both lesions. Thus, enzymes that repair CPDs are referred to as CPD-photolyases, while 6-4PP-photolyases specifically repair 6-4PPs (Müller & Carell, 2009). Both classes of photolyases are evolutionarily related, but functionally distinct (Lucas-Lledó & Lynch, 2009). Curiously, genes encoding genuine photolyases have been lost somehow in the course of the evolution of placental mammals, including humans. Instead, these organisms retain cryptochromes, photolyase-homologous proteins that participate in the maintenance of circadian rhythm, but that do not keep any residual activity related to DNA repair (Partch & Sancar, 2005).

Previous studies have confirmed that the CPD-photolyase is active when delivered to human cells, reducing mutagenesis (You et al., 2001), preventing UV-induced apoptosis (Chigançcas et al., 2000) and recovering RNA transcription driven by RNA polymerase II (Chigançcas et al., 2002). These successful studies have motivated the adenoviruses-mediated expression of the CPD-photolyase from the rat kangaroo *Potorous tridactylus* and the plant 6-4PP-photolyase from *Arabidopsis thaliana* in human cells aiming to discriminate the precise role of UV-induced cellular responses in both NER-deficient and NER-proficient human cells. Employing immunofluorescence, immunoblot and local UV experiments, it has been possible to see that these enzymes are not only very specific for their lesions, but are also really fast to find them, colocalizing with regions of damaged DNA and other DNA repair enzymes in less than two minutes (Chigançcas et al., 2004; Lima-Bessa et al., 2008).

Adenoviral-mediated photorepair of CPDs substantially prevented apoptosis in all UV-irradiated cell lines (both NER-deficient and NER-proficient cells), confirming the involvement of these lesions in cell death signaling, as previously reported. On the other hand, 6-4PP repair by the 6-4PP-photolyase decreased UV-induced apoptosis only in those cell lines deficient for both NER subpathways, causing minimal effect, if any, in NER-proficient cells, including those lacking polη. These results suggest that, when not efficiently repaired, 6-4PPs also have important biological consequences, triggering cell responses

leading to the activation of apoptotic cascades. Interestingly, in CS-A cells (TC-NER deficient), a substantial attenuation of apoptotic levels could be again detected when CPDs were removed from the genome by the means of CPD-photolyase, while no detectable effect was observed as a consequence of photorepair of 6-4PPs, indicating that CPD lesions are the major UV-induced DNA damage leading to cell death, also in cells that are only proficient in GG-NER, the main subpathway of NER responsible for the removal of 6-4PPs in humans (Lima-Bessa et al., 2008).

These results suggest that CPDs and 6-4PPs may play different roles in UV-induced apoptosis depending on the repair capacity of human cells. In GG-NER proficient cells, the harmful effects of UV light seem to be predominantly due to the prolonged remaining CPDs in the genome caused by their slow removal by NER, with the minor participation of 6-4PPs (Lima-Bessa et al., 2008). Indeed, it has been reported that about 80–90% of 6-4PPs are removed from the human genome in the first 4 hours following UV exposure, whereas 40–50% of CPDs still remain to be repaired 24 hours later, probably due to the higher affinity of the XPC/hHR23B complex for 6-4PPs (Kusumoto et al., 2001). Thus, the lack of noticeable effects on UV-induced apoptosis in NER-proficient cells after 6-4PPs photorepair may be simply due to their fast repair by GG-NER. On the other hand, as for CPDs, the remaining of 6-4PPs in the genome seems to cause major disturbances in cell metabolism that lead to cell death. A summary of these results is shown in Figure 4.

To further confirm the idea that the roles of CPDs and 6-4PPs in UV-killing are related to the cellular repair capacity, authors have expressed these photolyases in TTD1V1 cells, a particular TTD cell line with a slower kinetics of 6-4PPs repair, eliminating about 50% and 70% of 6-4PPs at 6 and 24 hours post-UV treatment, respectively. Once again, repair of both lesions by the respective photolyase notably reduced apoptosis in these cells, even though the 6-4PP photorepair was less effective than seen for NER-deficient cell lines (Lima-Bessa et al., 2008). These photolyases were also used to identify a defect in the recruitment of downstream NER factors on certain XPD/TTD mutated cells, slowing down the removal of UV-induced lesions. As this recruitment was recovered by treatment with the histone deacetylase inhibitor trichostatin A, the data indicated that this defect is partially related to the accessibility of DNA damage in closed chromatin regions (Chiganças et al., 2008).

Another interesting finding came from assays investigating the time-dependent kinetics of the apoptosis commitment after UV treatment. Transduced XP-A cells were UV-treated and photoreactivated (to allow photorepair of the respective UV lesions) at increasing periods of time. Surprisingly, the data suggests that the initial trigger event to cell death after UV irradiation is relatively delayed, since photorepair of CPDs or 6-4PPs was able to reduce apoptosis even when photoreactivation was performed up to 8 hours after UV irradiation. After that, photoreactivation did not prevent UV-killing in these cells, indicating a commitment by events that irreversibly lead to cell death. These results are also in agreement with the indications that fast removed lesions (such as 6-PPs) do not activate apoptosis in NER-proficient human cells (Lima-Bessa et al., 2008). The main implication of all these findings is the fact that skin carcinogenesis in XP patients may also have 6-4PP lesions as important players, suggesting that tumors from these individuals are not only quantitatively different from those of normal people, but may also have different causative lesions. Transduction of XP knockout mice with adenoviral vectors carrying photolyase genes may help to address this question.

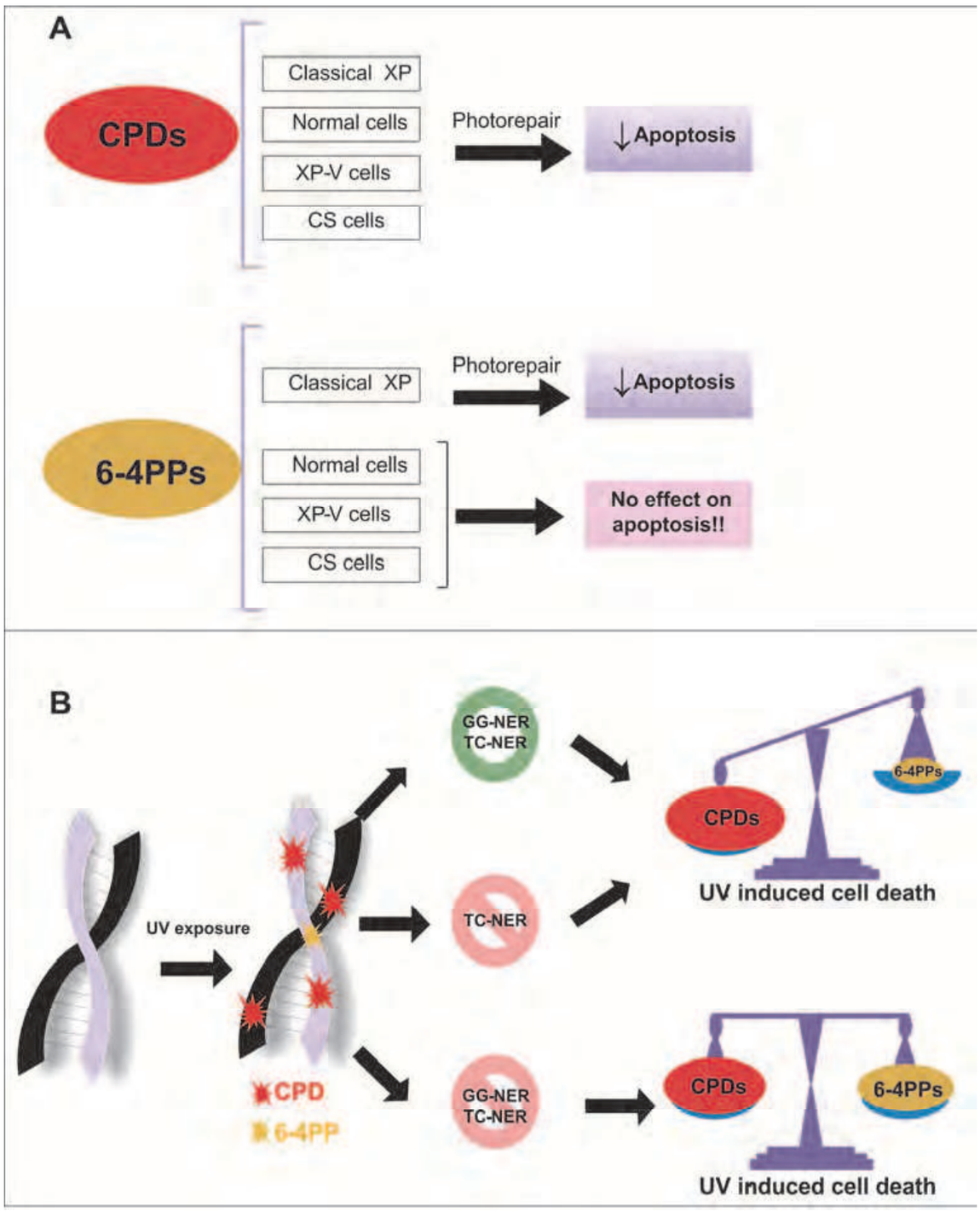

Fig. 4. Effects of photorepair of CPDs and 6-4PPs on UV-induced apoptosis. (A) Summary of the impact of the specific removal of CPDs and 6-4PPs by photorepair in human cell lines with different DNA repair capabilities. (B) Schematic representation of the main conclusions of the results shown in panel *A*. Those results clearly implicate that CPDs and 6-4PPs play different roles on UV-induced apoptosis depending on the cellular repair capacity.

4. Employing retroviral vectors for correcting XP phenotype

The first genetic analysis of XP patients was performed through somatic cell fusion followed by analysis of restoration of normal UDS. If somatic cell fusion complements XP genetic deficiency, it will then be positive for UDS activity. These experiments were able to identify the seven classical XP complementation groups and the variant group (Zeng et al., 1998). This implies that DNA repair deficiencies can, in fact, be corrected by the introduction of a normal copy of the affected gene, giving hope for the development of gene therapy protocols for XP patients. In fact, the introduction of a normal copy of the defective gene in XP cells can complement the DNA repair ability, as demonstrated by the delivery of conventional expression vectors, via calcium precipitation and microneedle injection (Mezzina et al., 1994).

In 1995, viral vectors were first used as gene delivery tools in DNA repair experiments (Carreau et al., 1995a). In this study, a LXPDSN retroviral vector carrying the wild-type XPD gene was capable of complementing primary fibroblasts of XPD patients with a long-term expression. A subsequent study showed that this complementation was gene-specific and that there was a long-term expression of the transgene (Quilliet et al, 1996). The use of retroviral vectors for DNA repair genes delivery was further validated in 1996 and 1997, when XP-A, XP-B, XP-C and TTD-D cells were also complemented with the aid of gene-specific retroviral vectors (Marionnet et al., 1996; Zeng et al., 1998).

The compilation of these results shows that the retroviral delivery of several DNA repair genes was able to specifically complement several deficiencies presented by XP, CS and TTD patients such as UDS, reduced catalase activity, UV-sensitivity, recovery of RNA synthesis, increased mutation frequency, stabilization of p53 (Dumaz et al., 1998) and deregulation of ICAM-1 (Ahrens et al., 1997).

Since XP patients already receive autologous graft transplants after massive skin tissue removal surgery (Atabay et al., 1991; Bell et al., 1983), most researches in the field of XP gene therapy focus on the three-dimensional skin reconstruction *in vitro*, using the patients' cells genetically corrected *ex vivo*. In this technique, the patients' fibroblasts and keratinocytes are cultured *in vitro* after a skin biopsy of a non-UV-irradiated area. Then, retroviral vectors are used to stably complement the genetic deficiency of these cells. Finally, the keratinocytes and the fibroblasts are used to three-dimensionally reconstruct the epidermis and dermis, respectively. This construct can then be used as a graft when the part of patient's damaged skin is removed in a necessary surgery. To that end, Arnaudeau-Bégard and co-workers managed to complement XP-C keratinocytes, recovering a wild-type phenotype and UV-resistance with the aid of a retroviral vector carrying a normal copy of the *XPC* gene (Arnaudeau-Bégard et al., 2003). Furthermore, Bergoglio and co-workers have also developed a selection method for genetically corrected keratinocytes that does not involve particles derived from microorganisms which could lead to immunological clearance of the transgene, using CD24 as an ectopic marker (Bergoglio et al., 2007).

In 2005, Bernerd and co-workers were able to reconstruct a three-dimensional skin model *in vitro* using fibroblasts and keratinocytes from a donor XP-C patient. With this model, they were able to see that the XP skin has peculiar characteristics: hypoplastic horny layers, decreased and delayed keratinocyte differentiation, epidermal invaginations, a generally altered proliferation control and fibroblasts with distinct morphology and orientation. Furthermore, the epidermal invaginations were proven to be related to alterations of both keratinocytes' and fibroblasts' functions and were characterized as epidermoid carcinoma-like structures (Bernerd et al., 2005). It is important to keep in mind that an XP skin biopsy

might give us further and more precise knowledge of the XP skin physiology, but this is a delicate procedure which requires the patients' agreement.

Since the use of common retroviral vectors in gene therapy can be dangerous due to semi-random insertional mutagenesis, researchers have developed several self-inactivating-lentiviral vectors carrying DNA repair genes. These vectors were shown to efficiently transduce primary and transformed fibroblasts, complementing in a gene-specific manner XP-A, XP-C and XP-D cells. Furthermore, the recovery of normal levels of UV-resistance in the transduced cells was shown to be persistent for at least 3 months (Marchetto et al., 2006). The reconstruction of a genetically corrected, three-dimensional XP skin followed by the implantation of the graft on a patient (Figure 5) is still an ongoing chore that has to be taken very cautiously, always prioritizing the patient's well-being.

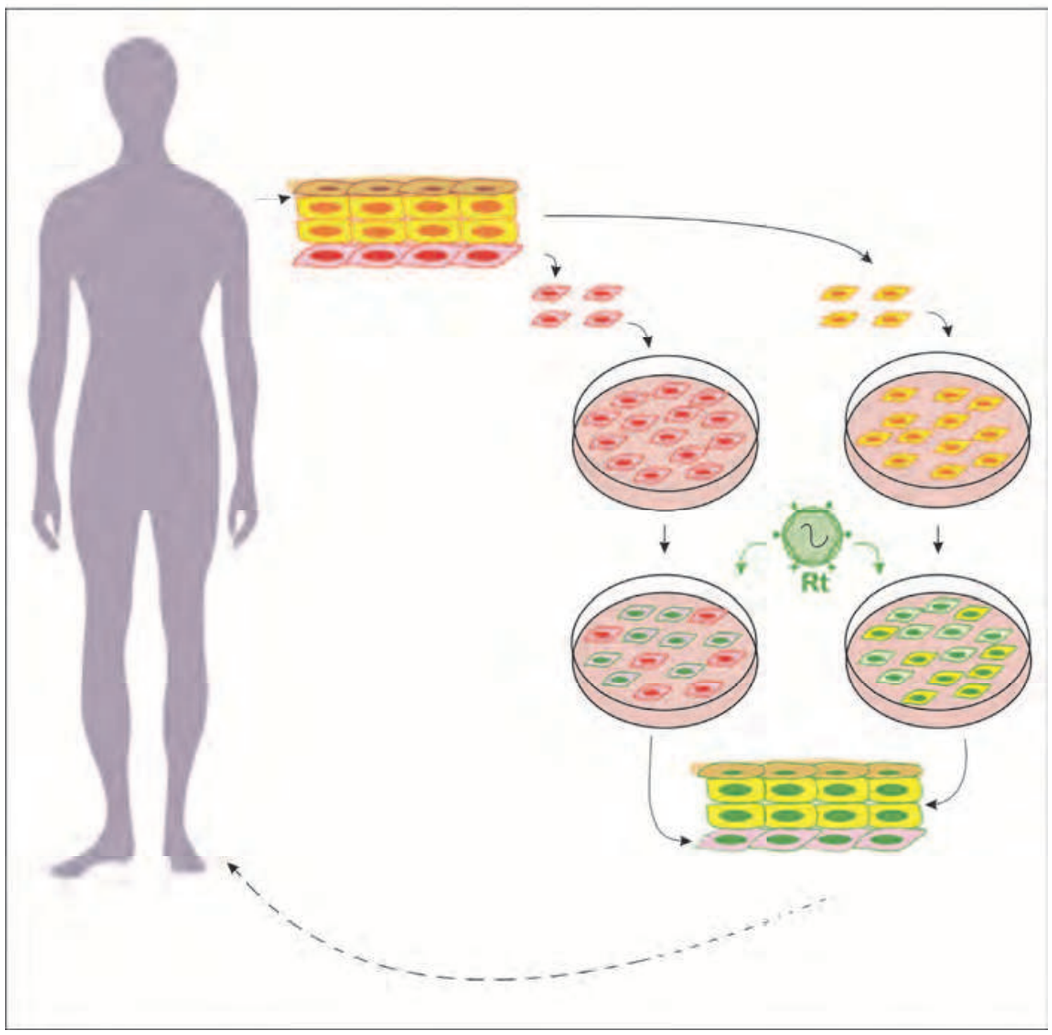

Fig. 5. Schematic representation of *ex vivo* gene therapy for XP patients using recombinant retrovirus (Rt). Skin-derived fibroblasts and keratinocytes from an XP patient are cultivated *in vitro*, and transduced with retroviral vector carrying the wild type *XP* cDNA. Transduced cells are then used to reconstruct the human skin *in vitro*, with a normal phenotype. Dashed line raises the possibility of engraftment of the reconstructed skin directly on XP patients.

It is also important to keep in mind that these grafts do not include melanocytes, responsible for the very common melanomas in these patients (Khavari, 1998), and that the skin will only be genetically complemented in the areas that receive the grafts, all the other areas of the body will still be extremely photosensitive since no paracrine effect is known for DNA repair proteins and that immunological clearance or gene silencing by cellular methylation can always prohibit a long-term transgene expression (Magnaldo & Sarasin, 2002). Importantly, several XP complementation groups also present other relevant symptoms, such as neurodegeneration, which will not be improved by the skin grafts. For those patients, another kind of gene therapy might be more efficient, such as the development of genetically corrected stem cells (ESs) (Magnaldo & Sarasin, 2002) or induced pluripotent cells (iPSCs, see below (Alison, 2009). Unfortunately, there is still no reference on that kind of research for xeroderma pigmentosum.

5. Host cell reactivation (HCR) as a tool for DNA repair research

The host cell reactivation (HCR) technique was first described in human cells by Protic-Sabljic and co-workers in 1985 (Protic-Sabljic et al., 1985). In this first work, the technique consisted of transducing cells with a plasmid containing a putative cDNA with a selective gene into XP cells to look for a reversion of the UV sensitivity due to gene complementation, allowing identification of the genes responsible for that phenotype.
Other studies have refined the technique which is now widely used as an indirect measure of cellular DNA repair capacity. Mostly, a plasmid containing a reporter gene such as luciferase (LUC) or chloramphenicol acetyltransferse (CAT) is treated with a genotoxic agent such as UV radiation and introduced in the cell where DNA repair capacity is to be evaluated. If the cell is able to remove the lesions from the plasmid, the reporter gene will be expressed. Different DNA repair rates can be addresses by differences on the amount of gene reporter expression at a certain time (Merkle et al., 2004). A schematic representation of HCR is shown in Figure 6.

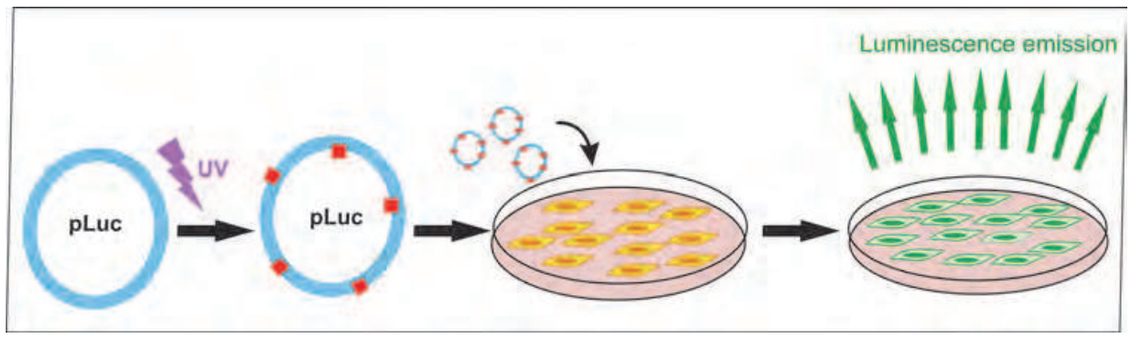

Fig. 6. Schematic representation of host cell reactivation (HCR) assay. A plasmid carrying a reporter gene (in this case, the luciferase gene) is UV-irradiated *in vitro* and then transfected into host cells. 48 hours later, the cellular DNA repair capacity is indirectly estimated by measurement of the reporter gene activity in the cellular extract.

In 1995, the HCR assay was further used to visualize the genetic complementation of mammalian expression vectors carrying the DNA repair genes *XPA*, *XPB*, *XPC*, *XPD* and *CSB*. In this study, plasmids containing LUC or CAT were UV irradiated and co-transfected with the plasmids containing each of the complementing genes of the DNA-repair deficient

cells. Again, only the cells with the correct complementation were capable of removing the DNA damage in the reporter gene, allowing the expression of that protein. This technique facilitates the identification of the complementation group of a given patient, being particularly useful in cases of CS, TTD and some XP patients such as XP-E that present a normal UDS after UV treatment (Carreau et al., 1995b).

Recent data using the HCR assay has shown that the CS proteins are essential for the reversion of oxidated lesions (Pitsikas et al., 2005; Spivak & Hanawalt, 2006; Leach & Rainbow, 2011) and evidence obtained with HCR suggests that, unlike what was previously shown with UDS assays, DNA repair capacity in fibroblasts does not decrease with aging (Merkle et al., 2004). This reduction may however be cell type-specific and DNA repair pathway-specific since blood cells repair capacity decreases approximately 0.6% per year of age (Moriwaki et al., 1996). This technique is still widely used and its great advantage is that the DNA plasmids or the viral vectors are treated in a controlled manner, not being subject to the cell's global response to the same treatment. Further technique improvements will surely allow HCR to be used in different assays such as *in vivo*, yielding a better knowledge of the DNA repair pathways and their interactions with other pathways and physiological events.

6. Other treatments for xeroderma pigmentosum

6.1 General care

There is no treatment that has been proven so far to be 100% effective in all XP cases. The only palliative measure that patients can rely on is complete sun avoidance. This includes not only avoiding going out even on cloudy days and covering all exposed body areas such as skin and eyes, but also using special artificial lights that emit no UV wavelengths (Kraemer, 2008). Premalignant lesions, such as actinic keratoses, and malignant lesions must be quickly treated with topical 5-fluoracil or liquid nitrogen, imiquimod cream, electrodesiccation and curettage, surgical excision or chemosurgery, as needed. When extensive areas are damaged and have to be surgically removed, skin grafts from sun unexposed areas of the same patient should be used. When eyes are affected, methylcellulose eye drops or contact lenses can help prevent trauma and corneal transplantations might be needed in extreme cases (Kraemer, 2008).

When caring for XP patients, it is very important to keep in mind that the total sun avoidance also prevents the production of vitamin D in the skin, so dietary supplementation might be needed. Furthermore, the DNA repair deficiencies which prevent the repair of photolesions may also make individuals sensitive to other mutagens such as cigarette smoke, so patients should be protected against these agents (Kraemer, 2008).

Aside from removal of local lesions and total sun avoidance, two other palliative treatments might help improving the XP patient's quality of life: topical use of T4 endonuclease (Yarosh et al., 2001) and oral intake of retinoids (Campbell & DiGiovanna, 2006).

6.2 Topical use of T4 endonuclease

In 1975, Tanaka and co-workers demonstrated that the bacteriophage T4 endonuclease V is capable of making an incision 5′ within a CPD lesion. The resulting DNA flap is recognized and removed by a 5′→3′ exonuclease, leaving a gap that is filled by a DNA polymerase, using the undamaged single strand as a template. A DNA ligase then joins the repaired fragment to the parental DNA (Tanaka et al., 1975).

In the 80's, Yarosh's laboratory discovered that the T4 endonuclease V could be delivered into cells using 200 nm liposomes as a delivery vehicle. The anionic liposomes not only protect the cationic enzyme inside, but also promote the escape from a clatrin-coated endosome after cellular intake by destabilizing the vesicle's membrane with an acid pH. By cleaving DNA at the site of UV-induced lesions, the enzyme reverses the DNA repair defect of XP cells (Yarosh, 2002). Further work by the same group also showed that these T4N5 liposomes in a 1% hydrogel lotion when applied in cultured human fibroblasts, mouse dorsal back or cultured human breast skin is capable of delivering the enzyme into cells in less than one hour, being almost entirely restricted to the epidermis (Ceccoli et al., 1989; Kibitel et al., 1991).

An inverse correlation was later shown between the T4N5 dose and the level of CPDs that remained in the epidermis. This curve reached a plateau (at 0.5 µg/ml), probably due to saturation of the cell machinery for further repairing the damage after the initial incision by the T4 enzyme (Yarosh et al., 1994). These studies also showed that even in the higher dose of T4N5 liposomes, only ~50% of the CPD lesions were removed but that was capable of reducing the mutagenesis rate by 99% in transformed fibroblasts and 30% in primary fibroblast cell culture. These numbers are probably not only related to the number of remaining lesions, but also to the smaller size of the repair patch filled by BER compared to that needed in NER (Yarosh, 2002; Cafardi & Elmets, 2008).

Finally, after two phase I clinical trials (Yarosh et al., 1996 as cited in Cafardi & Elmets, 2008) and three phase II clinical trials (Wolf et al., 2000 and Yarosh et al., 1996 as cited in Cafardi & Elmets, 2008), in 2001 the T4N5 liposomes were tested in XP patients. The patients were instructed to apply 4-5 ml of the lotion containing 1 mg/ml of endonuclease everyday for a year. Except for lesion removal when necessary, and daily use of sunscreens of 15 SPF or higher, no concomitant treatments were allowed. The treatment was shown to be efficient, reducing the rate of actinic keratoses and basal-cell carcinomas to 68% and 30% respectively in the placebo and treatment groups, reducing tumor promotion and progression. The treatment was also capable of reducing some immunosuppressant molecules, such as interleukin-10 (IL-10) and tumor necrosis factor-α (TNF- α). Unfortunately, the treatment was only effective for patients under 18 years-old. This might be because XP patients older than that already had too much DNA damage in their cells that could not be reversed (Yarosh et al., 2001). Despite the promising results, there are currently no topical DNA repair enzymes approved by the FDA. Clinical trials are still being conducted to analyze the application of T4N5 liposomes in other deficiencies and immunosuppressed patients (Cafardi & Elmets, 2008).

6.3 Oral use of retinoids

Despite interventions such as sunlight avoidance and tumor removal, most of the XP patients continue to develop a large number of skin cancers. These high-risk patients may suffer from field cancerization that may happen when a wide field of the epithelium has been exposed to the same genotoxic agent and adjacent but not contiguous areas present genetic and morphological alterations that may lead to a carcinogenesis process. As the whole skin area has been exposed to sunlight, inducing independent tumors with different growth rates, this hypothesis may explain why the patients have a 30% increase in the chances of having a second basal cell carcinoma (BCC) and then a 50% increase of a third BCC (Campbell & DiGiovanna, 2006).

In XP patients, the oral use of retinoids might be beneficial, regardless of the strong side effects. In chemoprevention the goal is to identify early biological events in the epithelium which may lead to a carcinogenesis process and intervene with chemicals which will help stop or reverse the process (Campbell & DiGiovanna, 2006). Retinoids, also known as vitamin A, are the most studied chemopreventive agent for skin cancers, upper aerodigestive tract and breast and cervical cancers. The exact mechanisms through which the retinoids are capable of reducing cancer incidence are still unclear, but it has been shown that they are capable of altering keratinocytes' growth, increasing their differentiation status, affecting their cell surface and immune modulation. Retinoids mediate gene transcription by binding to two families of nuclear receptors, the retinoid acid receptors (RARs) and the retinoid X receptors (RXRs). Retinoids have only a mild effect on existing tumors, but can suppress the development of new lesions (Campbell & DiGiovanna, 2006). In 1988, it was shown in a three year study that isotretinoin in a dose up to 2 mg/day/Kg was able to reduce skin cancers in XP patients by 63%. Unfortunately, in the year following the discontinuation of the treatment there was an increase of 8.5% of cancer incidence in those patients with reference to the two years of treatment (Kraemer et al., 1988).

Furthermore, the constant use of retinoids can have severe side effects ranging from inflammation in existing tumors, dry skin and mucosa and hair loss to pancreatitis, osteoporosis, hyperostosis and myalgia among others. The retinoids' toxicity is dose related and cumulative, but most of the side effects can be prevented with constant check-ups and use of local special moisturizers (Campbell & DiGiovanna, 2006). Indeed, several retinoids can be used as chemopreventives. The two most common are isotretinoin and acitretin, the first having a shorter half-life and being the drug of choice for women due to retinoids' theratogenic potential, especially in fetuses (Campbell & DiGiovanna, 2006).

6.4 Potential effects of DNA repair adjuvants

The use of DNA repair adjuvants and antioxidants may also help reducing skin cancer incidence in XP patients. Some known DNA repair adjuvants are selenium, aquosum extract of *Urcaria tomentosa* and Interleukin-12 (IL-12) (Emanuel & Scheinfeld, 2007).

Selenium seems to interact with Ref-1, activating p53, inducing the DNA repair branch of the p53 pathway, in a BRCA1-dependent manner, dealing mainly with oxidative stress (Fisher et al., 2006). On the other hand, it has been already reported that high levels of selenium can be mutagenic, carcinogenic and possibly teratogenic (Shamberger, 1985), probably due to non-specific sulfur substitution on proteins and consequent TC-NER activity decrease (Abul-Hassan et al., 2004). Thus, special attention should be taken regarding the dose of dietary selenium supplementation.

The aquosum extract of *Urcaria tomentosa* (cat's claw) seems to increase the removal of CPDs and reduce oxidative damage, either by an increase in base excision repair (BER) or by an antioxidant property, reducing erythema and blistering after UV. Despite several studies *in vitro* and *in vivo*, the precise mechanisms are still unknown (Emanuel & Scheinfeld, 2007).

Another interesting finding is that, besides IL-12 being a strong immunomodulatory molecule, able to prevent UV-induced immunosuppression through IL-10 inhibition (de Gruijl, 2008), it is also capable of increasing DNA repair by inducing NER, as shown by the RNA level increase in some NER molecules (Schwarz et al., 2005).

6.5 Gene therapy targeted approaches: The use of meganucleases for correcting XP-C cells

There are several techniques to specifically target, substitute, or correct a gene, diminishing the chances of insertional recombination, such as the use of recombinases, transposons, zinc-finger nucleases, endonucleases and meganucleases (Silva et al., 2011). Meganucleases can function as RNA maturases, facilitating the maturation of their own intron or as specific endonucleases that can recognize and cleave the exon-exon junction sequence wherein their intron resides, creating a specific double strand break (DSB), giving rise to the moniker "homing endonuclease". The meganuclease function is probably related to the current status of its lifecycle (Silva et al., 2011).

Meganucleases can be used as gene targeting tools in several ways. Ideally, they can provide a true reversion of the mutation, but the efficacy of correction is invertionally correlated to the distance of the initial DNA DSB. Alternatively, it can insert a functional gene upstream of the mutated one or in a safe location where it will not induce insertional mutagenesis. Also, meganucleases can be used for introducing specific mutations for research purposes such as understanding the role of a gene or of a specific point mutation. Furthermore, meganucleases capable of targeting viral sequences are being researched as antiviral agents (Silva et al., 2011).

Recently, the design of a specific I-CreI meganuclease targeting for the *XPC* gene was able to specifically target two *XPC* sequences, showing *in vitro* for the first time that extensive redesign of homing endonuclease can modify a specific chromosome region without lost of specificity or efficiency (Arnould et al., 2007). These results are very promising for the development of future gene therapy strategies for XP patients.

6.6 Induced Pluripotent Cells (iPSCs) as gene therapy agents

In 2006, the induction of pluripotent cells (iPSCs) by the expression of Oct3/4, Sox2, c-Myc and Klf4 in fibroblasts gave hope for a new gene therapy using pluripotent cells that would not elicit an immunological response in the patient, since his own cells would be used to induce the iPSCs and that would not be confronted by ethical issues like the use of embryonic stem cells (Takahashi & Yamanaka, 2006). Since then, this technology has been improved and iPSCs have been induced in a variety of cell types from different species. Also, iPSCs have been differentiated to several different cell types, from fibroblasts to neurons (Sidhu, 2011).

Fanconi Anemina (FA) is a DNA repair related disease, where mutations in one of fourteen genes lead to extreme sensitivity to interstrand crosslinking agents. Patients show progressive bone marrow failure, congenital developmental abnormalities and early onset of cancers, mostly acute myelogenous leukemia and squamous cell carcinomas. Bone marrow transplantation is a palliative treatment for the secondary leukemia but no cure is currently available for FA patients (Kitao &.Takata, 2011). In 2009, Raya and co-workers were able to use lentiviral vectors to genetically correct fibroblast and keratinocytes from patients with various FA complementation group deficiencies and then induce their dedifferentiation into pluripotent stem cells. Interestingly, uncorrected FA cells did not generate iPSCs, indicating a role for DNA repair in nuclear reprogramming. Thus, the generated iPSCs had normal FA genes and have the potential of being used for gene therapy of the donor patients, with no risk of inducing immunological rejection (Raya et al., 2009). Hopefully soon FA patients and others will be able to benefit from this technology as a safe gene therapy approach.

7. Concluding remarks

Recombinant viral vectors were developed more than thirty years ago, and they have provided extremely useful tools to understand cell metabolism. This chapter focuses on their use to understand cells' responses to DNA damage, especially UV-irradiated DNA repair-deficient cells. These vectors provide means to interfere in these responses, affecting DNA metabolism and revealing important aspects of the DNA repair mechanisms. The discovery of RNA interference mechanisms in human cells offer still more opportunities to modify cells' responses by silencing specific DNA repair genes. Several libraries of viral vectors for the expression of small double-stranded RNA molecules (shRNA) targeting human genes are commercially available, and are already being used for understanding gene function. The use of such vectors to make cells deficient in more than one DNA repair pathway, using cells deficient in XP genes as hosts, for example, may help us to reveal the intricate network of interactions between the different metabolic pathways that contribute to genome maintenance after damage induction (Moraes, et al., 2011; in press). Moreover, the progress that has been made towards gene therapy for xeroderma pigmentosum, using these recombinant viral vectors is also discussed. Although the results indicate a series of limitations, and it is clear that there is still a long way to go, they make researchers go forward, giving a gleam of hope to these patients and their families.

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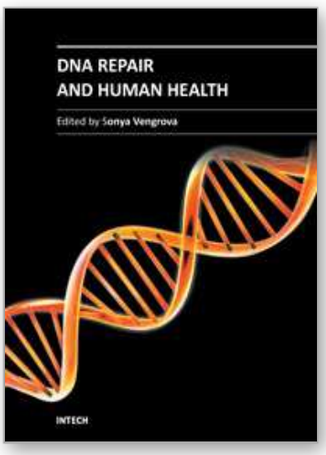

DNA Repair and Human Health

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Over the past decades, great advances have been made in understanding the cellular DNA repair pathways. At the same time, a wealth of descriptive knowledge of human diseases has been accumulated. Now, the basic research of the mechanisms of DNA repair is merging with clinical research, placing the action of the DNA repair pathways in the context of the whole organism. Such integrative approach enables understanding of the disease mechanisms and is invaluable in improving diagnostics and prevention, as well as designing better therapies. This book highlights the central role of DNA repair in human health and well-being. The reviews presented here, contain detailed descriptions of DNA repair pathways, as well as analysis of a large body of evidence addressing links between DNA damage repair and human health. They will be of interest to a broad audience, from molecular biologists working on DNA repair in any model system, to medical researchers.

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