



# BEYOND THE SKIN

## WHY WE NEED TO LOOK BEYOND THE SKIN WHEN UNDERSTANDING MANY PHOTSENSITIVE DISORDERS

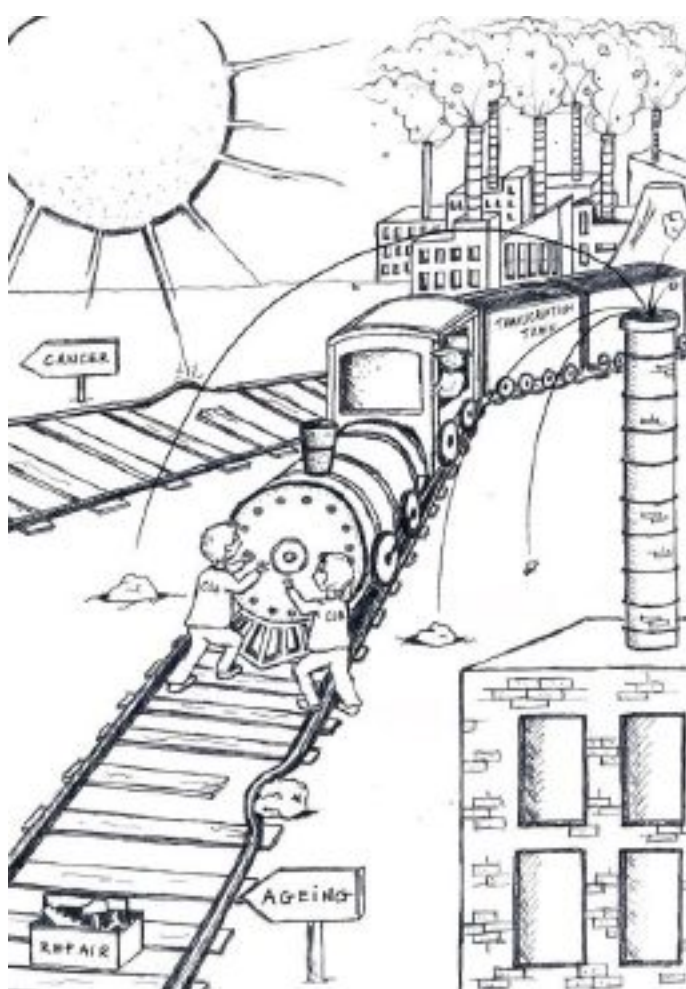
*Arjan F. Theil, Nicole S. Verkaik, and Wim Vermeulen from the Department of Molecular Genetics, Erasmus University Centre, Rotterdam, explain why skin is only part of the puzzle, and why we need to look beyond the epidermis to understand the full extent of photosensitive disorders*

Xeroderma pigmentosum (XP) is a very rare inherited disease that is characterised by extreme sensitivity to sunlight, abnormal skin pigmentation, and a terrible increased risk for skin cancer, especially in sun-exposed areas.

UV-light present in sunlight damages DNA, the carrier of genetic information. XP patients cannot remove UV-light induced DNA damage from skin cells as they have an inborn defect in one of the many enzymes required for repairing UV-damaged DNA.

This inability causes many skin cells to die or to lose control over cell duplication, eventually resulting in XP-specific skin and skin cancer. Early diagnosis of XP by expert dermatologists, a rigorous protection programme to shield the skin from solar radiation, and regular check-ups for suspect, early-stage skin tumours allow a reasonable skin cancer-free prospect. This prognosis is, however, unfortunately only valid for developed societies.

Sadly, a small group of XP patients also suffer from progressive neurologic decline. For long, scientists have struggled to understand why.

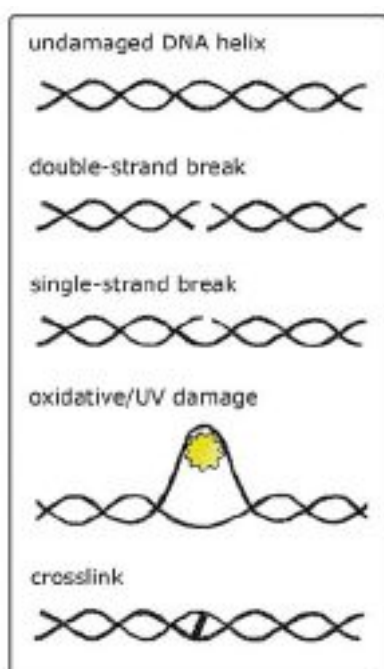


Why do photosensitive patients develop ageing related neurological problems, in which tissue is affected that could not have been damaged by UV as sunlight does not penetrate through skin? In this article we attempt to explain the current scientific view on this apparent mystery.

### The information is in the genes

DNA (deoxyribonucleic acid) is the molecule that contains the genetic code and is present in all cells of our body. It contains all the instructions for life and what defines us as a human being. Half of our DNA is inherited from our mother, the other half from our father and that's why children look like their parents.

DNA is a long chain of four different building blocks called nucleotides (A, C, G, and T).



Two of these nucleotide chains (DNA-strands) spiral around each other to form the so-called DNA double helix (**Figure 1**), the actual structure in which DNA is stored in our cells to allow proper reading of the instructions and transmission of the genetic information to daughter cells.

**Figure 1**

The order (sequence) by which these nucleotides are organized defines the genetic code. The most important regions within our DNA are called genes, which contain all information on how to make proteins. Proteins are the actual 'constructors' and 'actors' in the cell, as they, e.g. catalyse reactions (enzymes) to let us digest food, provide structure and strength to cells, transport oxygen, and make our heart beat.

Complex protein machineries are involved to 'translate' the genetic code into functional proteins, which require two steps. First, a process called transcription copies a gene's DNA into a DNA look-alike, messenger RNA (mRNA). Next, in the translation process this mRNA is used to make a specific protein. Transcription is carried out by the RNA polymerase enzyme that travels along the DNA rails during which it copies one strand into mRNA.

We have about 20,000 different genes within our DNA, encoding for 20,000 different proteins. Every cell in our body harbours the exact same genetic information. However, not every cell requires the same set of proteins. For example, the haemoglobin protein that is required to transport oxygen through our veins is only produced in red blood cells. In other words, cells can only perform their specific cell-function when genes can be switched 'on' (expressed) or 'off' (not expressed). To ensure that the correct set of proteins is produced at the right time, place and number, vital for cell function, a well-ordered control of transcription is required. Any interference in this process can have serious consequences for the cell and may even result in disturbed development, manifested as e.g. congenital malformations or microcephaly.

### Copy, paste

Another key function of DNA is to faithfully transmit the genetic information to the next generation and to properly copy its nucleotide sequence into each of our cells during growth from the fertilised egg into our full body plan made of trillions of cells. This huge cellular expansion is achieved by multiple cell duplications. When a cell divides, each daughter cell receives one identical copy of the original DNA. To achieve this, the entire DNA needs to be duplicated into two new identical copies prior to cell-division. This process is called 'replication' and is driven by DNA polymerase enzymes.

Although cell proliferation is essential for growth and renewal, this process should be tightly

controlled to avoid unrestrained expansion. Losing control over cell proliferation is one of the main causes of cancer.

The human body is a complex arrangement of many different cell types and include both mitotic and post-mitotic cells. Mitotic cells retain the capacity to divide and comprise the major renewable tissues and organs such as certain parts of the skin, intestines, or blood cells. A large part of our body is made of post-mitotic cells that lost the capacity to divide, such as brain cells (neurons), retinal photoreceptors (components of the eye) or heart cells (cardiac cells). In some cases cells (neurons) already stop dividing immediately after birth.

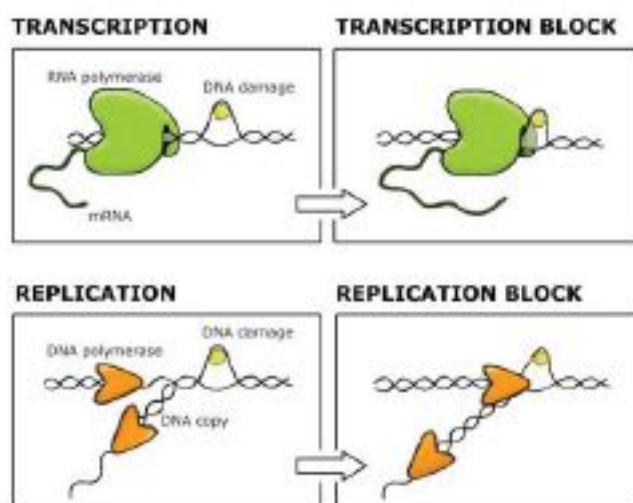
Preservation of neuronal function can thus only occur through maintenance and repair rather than via disposal of malfunctioning cells. The difficult task for each cell is thus to keep the genetic code of genes in shape to allow proper gene transcription to fulfill its specific function. On top of that, it is also important for mitotic cells to keep their entire DNA sequence (genome) intact.

## Genes at risk

DNA replication occurs in all living organisms and is the basis for biological inheritance. Replication must therefore be very accurate, as one mistake can permanently change (mutate) the genetic code in the progeny which may alter gene function. In the worst case scenario, when such a gene mutation occurs in a cell proliferation-controlling gene, the cell may become a cancer cell.

To maintain the DNA sequence is however very challenging, because our DNA is under constant attack by internal and environmental stressors that damage DNA. Paradoxically, even essential ingredients for life, such as water, oxygen, and light can damage DNA. Through the cell's own metabolism, (the combustion of nutrients in the cellular power plants (mitochondria)), unwanted by-products are generated, collectively called

Reactive Oxygen Species (ROS), which are highly reactive to DNA. DNA damage by ROS, which is one of the main internal sources of DNA damage, is thus unavoidable. DNA's structure is further endangered by several environmental sources, such as chemicals present in food and cigarette smoke, X-rays and ultra violet-light (UV, present in sunlight). It has been estimated that each cell of our body is confronted with up to 100,000 DNA lesions a day. DNA damage disturbs and may even fully block the vital DNA processes, of transcription and replication. **(Figure 2).**



**Figure 2**

## DNA rescue teams

Multiple DNA repair pathways, each focusing on specific (classes) of lesions **(Figure 1)**, constantly remove DNA damage to allow proper cell functioning. Nucleotide excision repair (NER) is one such repair process, specialised in the removal of lesions that distort the DNA helix, such as UV-induced DNA damage. NER is a complex process involving dozens of proteins (enzymes) and comprises four consecutive steps. In the simplified NER scheme **(Figure 3)** only the most important and clinically relevant (i.e. implicated in photosensitive disorders) proteins are depicted: (1) DNA damage detection; (2) DNA unwinding; (3) excision by dual incision in the damaged DNA strand; and (4) filling in the resulting DNA gap.



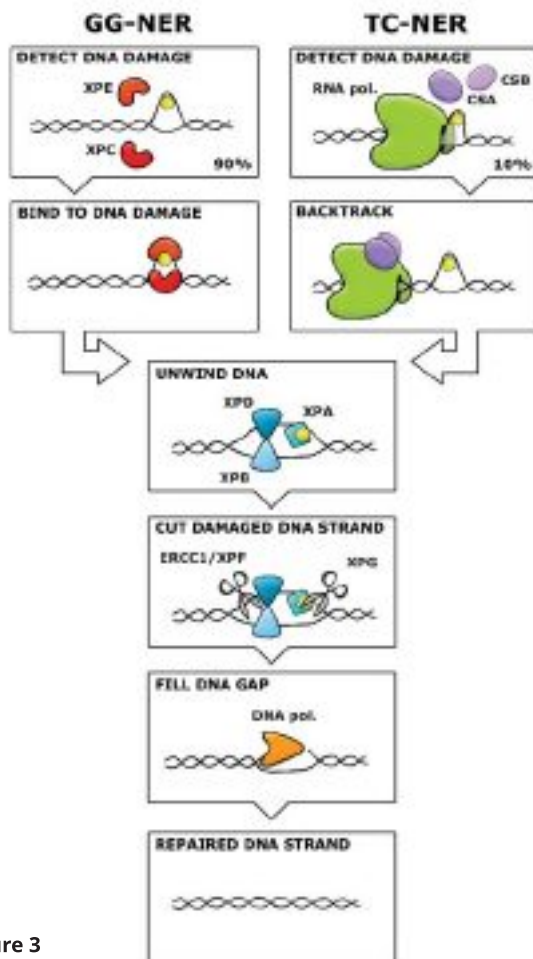


Figure 3

There are two modes of DNA damage: global genome NER (GG-NER) and transcription-coupled NER (TC-NER). GG-NER repairs lesions located anywhere in our DNA (global genome) and is responsible for the removal of the vast majority (>90%) of the lesions. This process is started by binding of the XPC and XPE proteins (defective in respectively XP groups C and E patients) to the DNA damage. Next, the XPD and XPB (XP groups D and B) unwind the double DNA helix around the lesion and, together with the XPA protein, verify the presence of the lesion.

In the third step, the proteins ERCC1-XPF and XPG (XP groups F and G) cut the DNA on both sides of the lesion, so a piece of the DNA strand that contains the damage will be removed. Finally, the resulting single-strand DNA gap is filled in by DNA polymerases.

TC-NER is a more specialised sub-pathway of NER that specifically repairs DNA lesions that

block the RNA polymerase trains when busy with producing mRNA. Different types of DNA damage, such as those induced by UV-light or by ROS can stop the RNA polymerase train (see cartoon 'model') during transcription. As such these DNA lesions will have a negative influence on cells' function. Unfortunately, RNA polymerases stuck at lesions cannot be repaired anymore by the normal DNA repair processes dealing with these lesions, as GG-NER (for UV-induced DNA damage) and base excision repair (BER, the common process to remove ROS-induced DNA damage, not further discussed here), cannot get access to those because the stalled RNA polymerase is in their way. Specific TC-NER enzymes, such as the CSA and CSB proteins (defective in respectively Cockayne syndrome group A and B patients) are required to displace or push back stalled RNA polymerase to allow repair.

### ***NER-deficient diseases: the XP and CS paradox***

The severe clinical consequences associated with inborn defects in NER, such as xeroderma pigmentosum (XP) and Cockayne syndrome (CS) dramatically underscore the vital importance of this DNA repair system. Both XP and CS patients are hyper-sensitive to sunlight, because DNA damage caused by UV-light cannot be repaired and the persistent lesions will severely hinder the vital DNA processes of replication (DNA duplication) and gene-transcription and cause cell malfunction or even cell death. But, why are both diseases so different if they are both based on defects in NER? This mystery is not easy to comprehend, which we will try to explain next.

UV-light only penetrates into a few cell layers of our skin, so UV-induced DNA damage will only occur in skin cells and only in the sun-exposed areas. The DNA repair process, NER, usually removes UV-induced DNA damage. When this repair system is not properly functioning due to mutations in one of the seven XP genes (XPA to XPG), a large number of cells will die and will

give rise to the typical sunburn, pigmentation (pigment freckles or lentigines) and aged-like skin. A bigger problem arises, however, when DNA damage is present in epithelial cells of our skin's epidermis (outer skin). These cells have the capacity to divide, so when UV-damage is present the DNA copy machine (DNA polymerase) has difficulty to pass the lesion.

To overcome this serious problem cells have alternative DNA polymerase enzymes that shortly take over the role of the normal ones. These alternative or so-called translesion (TLS) DNA polymerases have the capacity to copy DNA even in the presence of DNA damage. Simply said, these TLS polymerases may seem to solve the problem. However, their capacity to copy damaged DNA comes with a penalty and that is that the accuracy of copying is strongly reduced, with the consequence of making changes (mutations) in genes. When such a mutation occurs in genes controlling cell division, such an epithelial skin cell may become a cancer cell and thus explains why XP patients, unfortunately, develop skin cancer so easily from sunlight.

Cells have different TLS enzymes, one of which is DNA polymerase-eta that is specialised in DNA copying over UV-induced lesions. When the gene encoding for this enzyme is mutated, a not proper functioning DNA polymerase-eta is made, this will also lead to XP symptoms, as in the cases of patients suffering from the variant form of XP (XP-V). Although in XP-V cells NER is normally working, the lack of this specialised TLS causes more mutations to arise when cells attempt to duplicate their DNA which contains UV-light induced damage.

Since GG-NER is responsible for the removal of the vast majority of UV-induced DNA damage, it is expected that an inherited defect in only TC-NER, (responsible for the removal of the other 5%), would not cause so many mutations. This is exactly what is seen among CS patients, which is based on a defective TC-NER, as these do not develop skin cancer on sun-exposed skin, in striking contrast to XP.

### **The lesion-stalled transcription train problem**

Unfortunately CS comes with other severe problems, such as the gradual loss of neuronal function (hearing, vision and gait) and extremely fast ageing. Strikingly, most of the serious clinical problems arise from complications in tissue (mainly neuronal) that is not exposed to sunlight and – importantly – is made of post-mitotic or non-dividing cells. Recent studies from several laboratories worldwide have shown that ROS, made by cells' own metabolism, also produces DNA damage that stalls gene transcription, similarly as UV-light. In cells from CS patients, that lack the TC-NER vital proteins CSA or CSB, lesion-stopped RNA polymerase cannot be pushed back and DNA damage cannot be repaired.

This will result in the accumulation of lesion-stalled RNA polymerases in several cells, causing incorrect function of these cells, or even cell death. When this happens in tissue or organs that lost the capacity to divide, such as neurons in the brain, improperly functioning cells cannot be renewed from stem cells and the tissue will gradually deteriorate. It is thus thought that loss of neurologic functions, as seen with CS patients, is mainly caused by endogenously produced (e.g. ROS) DNA damage, also explaining that cells not reached by UV-light are affected by the CSA or CSB defects.

Some of the NER enzymes, such as XPB, XPD and XPG, are required for both GG-NER and TC-NER or even have an additional role in transcription. When these proteins are not properly functioning (as in XP groups B, D and G, respectively) both DNA repair and transcription can be affected. It is therefore thought that in the very unfortunate cases which have specific mutations in XPB, XPD or XPG combined symptoms of both XP and CS are seen. Sadly, protection from sun exposure, important to avoid skin problems and cancer,



DNA IS DAMAGED FROM  
INSIDE AND OUTSIDE THE BODY

SORRY,  
METABOLISM  
SUCKS!

MY UV-  
LIGHT IS  
KILLING!

THE TRANSCRIPTION TRAIN  
HITS THE DNA DAMAGE

CSA AND CSB ARE COMING TO THE RESCUE

**BOOM**

STOP! WE  
WILL PUSH YOU  
BACK, THE DNA  
NEEDS TO BE  
REPAIRED

THE DNA REPAIR CREW  
IS WORKING HARD

REPAIR

THE TRANSCRIPTION TRAIN  
CAN CONTINUE ITS JOURNEY

THANKS  
EVERY BODY!

SURVIVAL



will not prevent the devastating neurologic decline in these patients.

In several laboratories research programmes are running to fully understand the TC-NER mechanism and to develop strategies to diminish endogenous-produced DNA damage and to avoid transcription stress, with the ultimate goal to reduce the severe disease symptoms.

### About the authors

The authors are scientists at the Department of Molecular Genetics of the Erasmus University Medical Centre in Rotterdam, the Netherlands. Their main research focus is on trying to unravel the molecular mechanism of DNA repair systems and how these protect us against cancer and ageing.

The laboratory offers functional and genetic tests for XP, CS, and related diseases such as Trichothiodystrophy (TTD), Fanconi Anemia (FA), and Cerebro Oculo Facio Skeletal Syndrome (COFS) upon request.

[www.vermeulenlab.com/diagnostics](http://www.vermeulenlab.com/diagnostics)

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### Editors note

Arjan Theil and his team have very kindly created a `child friendly` version of this fascinating Clinical Spotlight with stunning comic graphics which we look forward to featuring in our first Rare Revolution Kids Edition in the Autumn. See previous page for a hint of what's to come.

Watch this space!



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