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XPG: its products and biological roles

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Abstract

Xeroderma pigmentosum patients of the complementation group G are rare. One group of XP-G patients displays a rather mild and typical XP phenotype. Mutations in these patients interfere with the function of XPG in the nucleotide excision repair, where it has a structural role in the assembly of the preincision complex and a catalytic role in making the incision 3' to the damaged site in DNA. Another set of XP-G patient is much more severely affected, displaying combined symptoms of xeroderma pigmentosum and Cockayne syndrome, referred to as XP/CS complex. Although the molecular basis leading to the XP/CS complex has not yet been fully established, current evidence suggests that these patients suffer from a mild defect in transcription in addition to a repair defect. Here, the history of how the *XPG* gene was discovered, the biochemical properties of the XPG protein, and the molecular defects found in XP-G patients and mouse models are reviewed.

Introduction

Numerous important discoveries have resulted from studies related to xeroderma pigmentosum (XP) since the initial demonstration by Jim Cleaver that this disorder is caused by a cellular defect of repairing UV lesions in DNA.¹ Several complementation groups were discovered, the genes defective in XP cells were cloned, the biochemical basis for the nucleotide excision repair pathway, which is defective in XP cells, was uncovered, and links between XP, cancer and premature aging were explored.² One of the fascinating aspects of these studies has been that although all of the eight genes that cause XP (*XPA-XPG* and *XP-V* or variant) with the exception of *XP-V* work in the same biochemical pathway, many of the genes have their own characteristic mutant patient alleles that can result in unique phenotypic manifestations in addition to the XP symptoms of varying severity. Furthermore, each of the XP genes (except *XP-V*) codes for proteins with a unique set of biochemical activities that contributes to the overall NER pathway in a distinct way. In this chapter the unique properties of the *XPG* gene will be explored.³

Discovery and cloning of *XPG*

In the early 1970s the genetic heterogeneity of XP was established and XP patients were assigned to the complementation groups with an approach using cell fusion and complementation developed by Bootsma and colleagues.⁴ In 1979, the first patient was assigned to the complementation group G,⁵ marking the birth of XPG research. The first and second XP patient both exhibited some features not commonly seen in XP patients, such as bird-like faces, mental retardation and dental caries.^{6,7} We now know that those are features of Cockayne syndrome (CS) and that mutations in *XPG* can lead to both XP and combined XP/CS (see below).⁸

Just like all the XP patients and complementation groups have their unique properties, each one also has a unique story of how the corresponding gene was discovered and cloned. In the case of XPG, serendipity played an important role. While studying tRNA transcription, the

laboratory of Stuart Clarkson isolated cDNAs encoding frog and human homologs of the yeast *RAD2* gene (the *XPG* equivalent in *Saccharomyces cerevisiae*) and demonstrated that the human cDNA was able to complement the UV sensitivity of XP-G cells.⁹ XP-G cells were subsequently found to be equivalent to Chinese hamster ovary (CHO) cells from complementation group ERCC5,^{10–12} and the *XPG* gene was mapped at chromosome 13q32–33.^{13,14}

Biochemical properties of the XPG protein

Analysis of the primary sequence of XPG quickly revealed that it harbors two nuclease domains placing it in the Fen1 family of structure-specific endonucleases.^{9,15} Consistent with this observation XPG was shown to be a structure specific endonuclease that cleaves substrates with the polarity that would be required for the incision 3' to the lesion in NER.^{15,16} XPG cleaves a variety of substrates that contain a ss/dsDNA junction, including splayed arm and bubble substrates, which contain a 5' single-stranded overhang.^{17,18} At the primary sequence level, XPG shares two conserved regions, the N and I regions, with other nucleases (Figure 1). A first glance of the salient features of the active site of XPG was provided by crystal structures of the exonuclease domains of T4 RNase H and T5 5'-exonuclease.^{19,20} These studies showed a number of conserved acidic residues that coordinate metal ions. Sequence alignments of XPG with these structures revealed that several conserved acidic residues, including Glu77, Glu791 and Asp812, are poised to be part of the active site. Biochemical analysis confirmed a role of these residues in mediating XPG cleavage activity since mutation of these three residues to alanine abolishes the catalytic activity of the protein.^{21,22}

While XPG shares its catalytic core with other nucleases, notably Fen1, the flap endonuclease with roles in replication and other aspects of DNA metabolism,²³ it contains additional unique domains that are responsible for mediating its NER specific functions. The roughly 600 amino acids separating the N and I region in XPG are commonly referred to as the spacer region.⁹ This region is highly acidic and does not contain any known structural or functional motifs. Parts of the spacer region have been shown to interact with other proteins. The interaction of the N-terminal part of the spacer region with TFIIH has been shown to be important for the NER reaction.^{24–26} An interaction with RPA has also been reported, but the functional significance of this interaction remains to be established.²⁷ The 300 amino acids at the C-terminal end of the protein, beyond the I region, are also engaged in protein-protein interactions; this region contains interaction sites with TFIIH and CSB and a PIP-box motif that mediates an interaction with PCNA.^{24,28,29} A second interaction site with TFIIH is consistent with a strong functional interaction between XPG and TFIIH.³⁰ The functional significance of the interaction of XPG with CSB and PCNA has not yet been established. The C-terminus also contains two putative nuclear localization signals at residues 1051–1084 and 1169–1186.^{31,32}

The role of XPG in nucleotide excision repair

NER operates by two distinct pathways, global genome repair (GG-NER) and transcription-coupled repair (TC-NER) and XPG plays a key role in both of them. GG-NER is the better-understood pathway of the two and involves the removal of lesions from all sites of the genome, while TC-NER refers to the preferential repair of lesion from the transcribed strands of active genes.³³ GG-NER operates by the sequential assembly of all the proteins involved at sites of DNA damage.^{34–36} Accordingly, NER proteins diffuse freely through the cell and are recruited in a defined order to sites of DNA damage and are released again from NER complexes once the damage has been repaired. Current evidence suggests that the XPC/HR23B/centrin-2 protein complex is the initial damage recognition factor in GG-NER (Figure 2).^{35,37,38} XPC then recruits TFIIH to sites of UV lesions, and two helicase subunits XPB and XPD of TFIIH initiate the opening of the DNA around the lesion. XPA, RPA and XPG subsequently join the

complex to form a stable open structure and recruitment of ERCC1-XPF by XPA initiates the dual incision of the DNA 5' and 3' to the lesion by ERCC1-XPF and XPG, respectively. The replication machinery subsequently fills the gap and seals the nick.

XPG has a structural as well as a catalytic role in GG-NER. Upon arrival at the NER complex it forms a complex at damaged sites with TFIIH, XPA and RPA (Figure 2). This complex is stable enough to be subjected to band shift and footprinting assays.^{39,40} XPG appears to have none or at least very limited nuclease activity at this stage and the full catalytic activity of XPG is only revealed once ERCC1-XPF has made the 5' incision. While efficient 3' incision by XPG appears to require the presence and catalytic activity of ERCC1-XPF, the 5' incision by ERCC1-XPF is efficiently carried out in the presence of catalytically inactive XPG.^{21,22,40} One possibility therefore is that the 3' incision is triggered by a conformational change brought about in the NER complex by the incision activity of ERCC1-XPF. Consistent with this notion, XPG shows distinct requirements for binding and cleaving its DNA substrates.¹⁸

Protein-protein interactions are essential in mediating the various steps in NER. XPG interacts tightly with TFIIH and it is the interaction with TFIIH that recruits XPG to NER complexes at sites of UV damage.^{30,35,41} Although investigation of the intracellular behavior of XPG suggests that the protein has a mobility consistent with it being present in monomeric form,⁴¹ some studies have found a tight and perhaps constitutive association of XPG with TFIIH.^{42,50} It is currently believed that this constitutive interaction between XPG and TFIIH is important for the additional roles of the protein outside of NER (see below).

The role of XPG in TC-NER is less well understood. TC-NER is initiated by a RNA polymerase stalled at the site of the lesion.⁴³ TC-NER involves almost all of the GG-NER factors with the exception of XPC and additionally requires the CSA, CSB and XAB2 proteins. Current evidence suggests that the presence of CSB at a site of a stalled RNA polymerase is required for recruitment of the core NER factors and enabling the dual incision reaction.^{44,45} According to these studies, XPG is recruited as one of the core NER factors, playing a similar role as it does in GG-NER. Another study has suggested that XPG interacts directly with RNA polymerase II pointing to an earlier role of the protein in TC-NER in cooperation with the CSB protein.⁴⁶ The detailed mechanism of TC-NER and the role of XPG in this process are still not fully resolved and continue to be an active area of research.⁴⁷

Roles of XPG outside of nucleotide excision repair

The severe phenotype displayed by the XP/CS patient suggested early on that XPG might have important roles outside of NER. Despite the phenotypic connection with the CSA and CSB genes, a defect in TC-NER alone cannot account for this observation, as *XPG* alleles that lead exclusively to XP symptoms are also deficient in TC-NER. It has been suggested that CS does not only result in a defect in TC-NER, but also results in a mild transcription defect.⁴⁸ This transcription defect can of course not be absolute, as this would be incompatible with viability. Although a role for XPG in transcription has not yet been clearly demonstrated in mammals, data from *S. cerevisiae* studies support a role for Rad2 (the XPG homolog) in transcription.⁴⁹ Importantly, this transcription function is independent of the nuclease activity of XPG, as a nuclease-deficient variant of the protein containing E794A mutation (equivalent to E791A in human XPG) retains full transcriptional activity. By contrast, a truncated version of RAD2, lacking 200 C-terminal amino acids but with intact nuclease activity, did not support the transcriptional activity. The inability of the truncated Rad2 protein to support transcription correlates well with the XP/CS phenotype displayed by XPG patients that have truncated *XPG* alleles (see below). By contrast, *XPG* alleles that form full-length protein, but have nuclease deficiency display the normal XP phenotype. This observation provides strong evidence that a defect in RNA polymerase II transcription is indeed responsible for the XP/CS

phenotype. A very recent study has suggested that at least a fraction of XPG resides in a tight complex with TFIIH.⁵⁰ Interestingly, *XPG* alleles, with truncations in the C-terminus that lead to XP/CS phenotype, fail to associate with TFIIH and this lack of association leads to a dissociation of the CAK subunit from TFIIH. As a consequence, and in accordance with what had previously been observed for XPD alleles with C-terminal truncations with defective anchoring of the CAK subunit, phosphorylation and transactivation of nuclear receptors are reduced in cells from XP-G/CS patients. These findings provide further evidence for the notion that XP/CS patients suffer from some degree of impairment in transcription.

XPG has additional activities that are unique among the XP proteins that point to a role of the protein in the repair of oxidative damage. XPG has been shown to stimulate the activity of the DNA glycosylase NTH1, an enzyme involved in the base excision repair pathway that removes oxidized bases from DNA, independent of its nuclease activity.^{51,52} Although this observation suggests a possible role in the removal of oxidative DNA lesions in a NER-independent pathway, initial *in vivo* studies supporting this suggestion have remained unconfirmed.^{3,53,54} Thus although it is attractive to speculate that the nuclease-independent stimulation of the repair of oxidative lesions might contribute to the XP/CS phenotype, conclusive evidence to support this notion is currently lacking.

XP-G patients and their mutant alleles

XP-G patients are rare and only 14 XP cases have been assigned to this complementation group to date.^{25,55} XP-G patients can be subdivided into three categories that have been found to display typical XP symptoms (4 patients; XP124LO, XP125LO, XP65BE, XP31KO), XP with late onset CS symptoms (3 patients; XP3BR, XP2BI, XPCS1BD), or XP with severe CS symptoms (7 patients; XPCS1LV, XPCS2LV, XPCS1RO, XP82DC, XP96TA, XP20BE, XPCS4RO), respectively (Figure 3). In addition to the classical XP symptoms, this last group of patients exhibit developmental retardation, dwarfism, severe neurological abnormalities, bird-like faces and early death that are characteristic of CS. In 13 cases the molecular defects that account for the defects in the XPG genes and clinical symptoms have been characterized (Figure 3). Based on this analysis a clear distinction can be made in the nature of the mutations that lead to the XP and XP/CS phenotypes.⁵⁶

XP group G patients without CS

The patients of the XPG only group exhibit a mild XP phenotype with sensitivity to UV light, abnormal pigmentation, increased risk of skin cancer and no neurological abnormalities. All the patients of the XP class expressed at least one full-length allele with a point mutation or frame-shift induced sequence change. In the case of the XP124LO and XP125LO sibling patients, the only stably expressed allele contains an alanine to valine mutation at residue 792,^{56,57} immediately adjacent to the highly conserved glutamic acid 791, which is essential for catalysis. Although the A792V allele stably expresses full length XPG protein, this protein has severely reduced nuclease and NER activities.²² The mild XP phenotype of these two patients (no skin cancer at ~ 20 years of age) can be explained by the residual activity and UV resistance conferred by the A792A allele *in vivo*.

The full-length allele of patient XP65BE, containing a point mutation of Ala 874 to Thr, also resulted in the expression of full-length protein and conferred significant residual repair activity on XP-G/CS cells.⁵⁵ The patient accordingly exhibited a mild XP phenotype at age 14 without skin cancer, as proper care was taken to avoid exposure to sunlight (see chapter 16).

The mutation in the XP-G patient XP31KO has not been established but this patient exhibited a very mild form of XP due a residual level of unscheduled DNA synthesis of about 25%.⁵⁸

6.2. Patients with severe XP and CS symptoms

All of the XP-G patients belonging to XP/CS complex class displayed very severe phenotype including severe developmental retardation, dwarfism, and neurological abnormalities in addition to the XP symptoms and suffered from early death (at or before six years of age).⁵⁵ Clarkson *et al* first reported in 1997 a common mutational pattern that distinguishes XP-G and XP-G/CS patients.⁵⁶ Analysis of the mutations in three XP-G/CS patients (XPCS1LV, XPCS2LV and XPCS1RO) revealed that each of them contained mutations that prevented the expression of full-length protein. The patient XPCS1LV expressed only one allele with a stop codon introduced by a single A deletion resulting in a protein of 659 amino acids in length. Patient XPCS2LV, who like XPCS1LV, is of Flemish origin, expressed the same allele and additionally a protein variant spanning the first 262 amino acids of the protein. The protein expressed in the cells of patient XPCS1RO (formerly known as 94RD27) is considerably longer. This patient is homozygous for an allele that contains a frameshift in the codon for amino acid 926 and expresses an XPG variant of 980 amino acids, of which the last 55 amino acids are unrelated to XPG.⁵⁷ Although this protein contains both the N- and I-region that make up the nuclease active site, it does not confer any UV resistance to XP-G cells and does not avert the symptoms of the XP/CS complex. This observation suggested that the C-terminal region of XPG plays an essential role in mediating functions of XPG within and outside of NER, while a localized defect in the nuclease activity only affects the NER specific functions. The first detailed neuropathological study of the XP/CS complex syndrome was carried out on XP-G patient XP20BE.^{59,60} This individual expresses two very short XPG proteins of 10 and 137 amino acids, respectively.^{59,61} These studies revealed that, consistent with the observed overt phenotype, the neuropathology is equivalent to that found in CS rather than the one found in severe XP patients with neurological abnormalities.

Two more recently characterized XP-G/CS patients, XP82DC, which expresses XPG proteins of 16 and 869 amino acids and XP96TA, which is homozygous for a mutation that leads to a frameshift in amino acid 308 resulting in a protein of 320 amino acids of which the last 12 amino acids are unrelated to XPG confirm the observations that truncation of the XPG protein results in an XP/CS phenotype.⁵⁵

The last XP-G/CS patient shows a different genetic make up. XPCS4RO is one of the most severe XP/CS patients known and she died at 11 months of age. One of her XPG alleles encoded a truncated protein of 175 amino acids. The other allele encoded a full-length protein with a single amino acid substitution of the highly conserved proline 72 to histidine.⁶² This allele does not confer any resistance to UV irradiation and the protein has been found to be highly unstable in overexpressed form, although the stability of this protein has not been assessed in cells of the patient directly. Based on these observation it is likely that the P72H mutation renders XPG completely unstable, resulting effectively in a null mutation.

XP-G Patients with late-onset CS symptoms

The XP2BI and XP3BR patients both express full length XPG protein from one allele.⁶³ XP2BI contains a mutation of Leu 858 to Pro, a conserved residue in the I region while XP3BR expresses two splice variants from one allele, one of which expresses full length protein but with an internal stretch of 44 amino acids (917–962) unrelated to XPG. Both of these XPG variants are completely deficient in nuclease activity and their expression does not confer any UV resistance to XP-G/CS cells. The XP2BI and XP3BR patients both suffered from XP symptoms and a mild, late onset version of CS, clearly distinct from the typical symptoms of the XP/CS complex.

Perhaps the most unusual patient at the molecular level is XPCS1DB. This patient is homozygous for deletion of a nucleotide in the splice acceptor site in the last nucleotide of

intron 6, which leads to a frameshift mutation in exon 7 resulting in the expression of a protein with 224 amino acids of XPG and 18 unrelated amino acids.²⁵ Based on the considerations discussed previously this would place him in the severe XP/CS category. However this patient displayed only mild late-onset CS symptoms with XP symptoms. Upon closer examination a second minor splice product was detected that results in the formation of a full-length protein with a deletion of amino acids 225–231. A cDNA expressing the XPG Δ 225–231 did not confer any UV resistance to XP-G/CS cells indicating that this allele was completely NER deficient. The corresponding protein had nuclease activity and the cause of the NER deficiency could be attributed to a defect in the interaction with TFIIH.²⁵ Interestingly the XPG Δ 225–231 protein was still recruited to NER complexes in living cells, but failed to mediate appropriate processing of substrates, presumably because it failed to stably associate with the NER complexes.

As these examples demonstrate, the study of the various alleles found in XP-G patients has not only revealed insight into the molecular basis underlying the XP and XP/CS complex symptoms, but also into the mechanisms by which XPG exerts its roles in NER and other pathways.

Mouse models with XPG deficiency (see also chapter 17)

The consequences of XPG deficiency have also been studied in mouse models. These studies are in broad agreement with observations made in the analyses of the various alleles found in XP-G patients. A mouse in which the wild-type allele was exchanged for one that expresses XPG containing an E791A mutation that abolishes nuclease activity displayed the classical XP phenotype.⁶⁴ These mice displayed hypersensitivity to UV irradiation, but did not display any of the developmental defects characteristic of the XP/CS complex. By contrast, mice in which exon 3 corresponding to nucleotides 264 to 380 of XPG was deleted, lacked any functional XPG protein and displayed a phenotype much more reminiscent of XP-G/CS patients.⁶⁵ These mice showed severe growth retardation and died prematurely before weaning at about three weeks of age. Cells from these mice underwent premature replicative senescence and readily underwent transformation, suggestive of genetic instability. Like the E791A mutation, cells from the knock out mice were hypersensitive to UV irradiation.

The generation of additional XPG mutant mice provided insight into the nature of the mutations in the gene that cause XP and combined XP/CS phenotypes. Consistent with observations made in patients, mice in which the last 360 amino acids in the C-terminus of XPG were deleted displayed the expected symptoms associated with the XP/CS complex.^{66,67} By contrast deletion of only 183 C-terminal amino acids (exon 15) resulted only in partial UV sensitivity and none of the CS characteristics. However, when this 183 amino acid deletion in XPG was combined with a deletion in XPA, it resulted in CS-like phenotypes reminiscent of XPG/CS, indicating that this deletion has serious consequences only in an NER deficient background. This observation underscores the complexity of genotype/phenotype relationships and warrants further exploration.

Conclusions

Research of the XP group G patients has uncovered the genetic bases of why some patients display CS in addition to XP symptoms, while others do not. We now understand the role of the XPG protein in preventing XP symptoms as a structure-specific endonuclease in nucleotide excision repair in some detail. The mechanisms by which XPG prevents CS syndromes are less well understood, but there is increasing recent evidence that a XPG plays a role in transcription by interacting with TFIIH. To what extent a defect in the suggested role of XPG in the NER-independent repair of oxidative damage contributes to the CS phenotype is still in

need of further clarification. Without a doubt, current and future research efforts will clarify this and other questions regarding the function of XPG.

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Figure 1. Functional domains of the XPG protein

The N and I domain (blue) make up the active site of XPG and are separated by the 600 amino acid spacer region. The D1 and D2 boxes are conserved in higher eukaryotes. The PCNA binding motif and nuclear localization signals in the C-terminus are indicated in orange and green, respectively. Interaction regions with TFIIH (XPB, XPD), RPA and PCNA are indicated.

Figure 2. The role of XPG in nucleotide excision repair

In GG-NER, the XPC-HR23B protein is the initial damage recognition factor for helix distorting lesions. The DDB1/2 (XPE) proteins and associated factors play a role in recognizing lesions in the context of chromatin. XPC-HR23B recruits TFIIH to the site of the lesion and the two helicase subunits XPB and XPD open the DNA around the lesion. RPA, XPA and XPG are then recruited to assemble the "preincision" complex and verify the damage. XPG does not appear to be catalytically active at this stage. ERCC1-XPF finally joins the complex and the dual incision (5' by ERCC1-XPF and 3' by XPG) occurs. The resulting gap is filled in by the replication machinery and DNA ligase I seals the nick. TC-NER is less well characterized and is believed to be initiated by an RNA polymerase stalled at a damaged site. The CSB, CSA, XAB2, TFIIH and XPG proteins appear to play a role early in TC-NER, while the damage verification, dual incision and repair synthesis step are the same as in GG-NER.

Figure 3. Known alleles of XP-G patients

The characterized alleles from XPG patients are divided into those from patients without CS, late onset CS and severe CS symptoms. The wild type XPG sequence is shown for reference. The positions of point mutations, frameshift mutations (fs) and premature stop codons (ter) are indicated. Striped boxes indicate coding sequences that are foreign to XPG. See text for details.