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XPC: Going where no DNA damage sensor has gone before

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Abstract

XPC has long been considered instrumental in DNA damage recognition during global genome nucleotide excision repair (GG-NER). While this recognition is crucial for organismal health and survival, as XPC's recognition of lesions stimulates global genomic repair, more recent lines of research have uncovered many new non-canonical pathways in which XPC plays a role, such as base excision repair (BER), chromatin remodeling, cell signaling, proteolytic degradation, and cellular viability. Since the first discovery of its yeast homolog, Rad4, the involvement of XPC in cellular regulation has expanded considerably. Indeed, our understanding appears to barely scratch the surface of the incredible potential influence of XPC on maintaining proper cellular function. Here, we first review the canonical role of XPC in lesion recognition and then explore the new world of XPC function.

Keywords

DNA repair; Nucleotide excision repair; XPC; Rad4; BER; GG-NER

1. Introduction

The wrath of UV and the search for damage - Rad4/XPC in nucleotide excision repair. When genomic DNA is affected by carcinogens or radiation, lesions can form which may compromise genomic integrity and greatly increase the chances for mutagenesis and diseases such as cancer [1, 2]. UV radiation typically induces bulky adduct lesions in the DNA, primarily pyrimidine (6-4) pyrimidine photoproducts (6-4PPs) and cyclobutane pyrimidine dimers (CPDs), and, if the lesions are not repaired properly, can result in a permanent mutation. Fortunately, cells have evolved a variety of repair pathways to remove the dangerous lesions. Though lacking the photolytic repair which lower organisms possess to repair UV-induced damage, humans primarily rely on nucleotide excision repair (NER).

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None

Global genome nucleotide excision repair (GG-NER), a sub-pathway NER, scans the genome for bulky DNA lesions and repairs them [3]. The yeast protein Rad4 (radiation sensitive) and the human ortholog XPC (xeroderma pigmentosum complementation group C), together with their accessory subunits, have been identified as the protein complexes principally involved in recognizing DNA lesions and then recruiting other repair proteins [4-7]. Thus, Rad4 and XPC serve as the initiators of GG-NER and, therefore, XPC can complement repair deficiency in certain cells from patients with xeroderma pigmentosum, a disease conferring hypersensitivity to UV radiation [6, 8, 9]. Consequently, XPC has been historically associated with repair of UV-induced DNA damage. Rad4 is found in complex with Rad23, and XPC exists *in vivo* in a heterotrimeric complex with centrin2 and Rad23 [10, 11]. When Rad4 or XPC bind to damaged DNA, the downstream NER pathway is triggered.

2. First contact: Rad4/XPC binding specificity

Studies have shown that XPC preferentially binds to damaged DNA, yet the type of the lesion does not affect binding efficiencies [12, 13]. Furthermore, these studies demonstrated that XPC binds to lesions that are not even repaired by GG-NER [13]. Appropriately considering XPC and Rad4 share most homology at their DNA binding domains, these two damage sensors bind DNA in the same topological manner [9, 14]. The homology of these domains allows for extrapolation of XPC binding properties based on the crystal structure of Rad4. It was shown by X-ray crystallography that Rad4 binds to DNA containing a CPD, yet Rad4 makes no contact with the lesion and binds downstream dsDNA [9]. Moreover, biochemical analyses have shown that XPC is a structure-specific (rather than damage-specific) DNA binding factor; XPC binds preferentially to lesion-induced junctions between double-stranded and single-stranded DNA [15] and not specifically to lesions themselves. Thus, it seems that Rad4/XPC does not directly recognize the lesion itself, but rather the accompanying helix distortion. In fact, the extent of the helical distortion affects XPC binding to DNA, as seen by XPC's low affinity for CPDs which induce minimal helical alteration and a higher affinity for 6-4PPs which induce more helical alteration [16-18]. Further, a recent study has shown that XPC-Rad23 has a higher affinity for damaged bubble DNA lesions (which mimic transcription bubbles and have a very large bending angle of 64±2°) in comparison to damaged duplex DNA. These studies demonstrate that XPC-Rad23's affinity for DNA correlates with the size of the DNA bend [19]. Footprinting experiments show that the strand-binding specificity of XPC affects its binding orientation and the efficient recruitment of subsequent unwinding and incision factors. Therefore, XPC can interact with DNA in one of two ways: 1) productive binding, in which XPC binds to the undamaged strand, thereby recruiting TFIIH and XPD to the 5’ side of the lesion on the damaged strand and causing 5’ to 3’ translocation and strand opening or 2) non-productive binding, in which XPC binds to the damaged strand and as a result is 3’ to the lesion, facing the opposite direction, and lesion extraction does not occur [15, 20]. Thus, ironically, the DNA damage recognition factor Rad4/XPC does not directly bind to the DNA lesion, and this paradox, in fact, fundamentally contributes to the broad substrate specificity of Rad4/XPC, allowing for GG-NER on its own to repair a variety of DNA damage-induced lesions.

The main function of NER is to recognize, excise, and repair DNA lesions without accidently repairing non-damaged sites, which could potentially induce mutation in the genome, rather than maintaining genomic integrity. Given that these distortions are scattered among an abundant sea of stable duplex DNA it is a daunting and almost impossible task for XPC to accurately find, recognize, and bind damaged DNA. Yet despite this crucial need for specificity in damage recognition, XPC has a generally low affinity for DNA, which increases by only ~100 fold when the DNA is damaged [12, 21]. Paradoxically, it is biologically advantageous for XPC to have a low affinity for DNA and poor recognition of damaged sites. Indeed, kinetic analysis of NER shows that the relatively low affinity of XPC for damaged DNA actually enhances XPC specificity for damaged DNA, due to kinetic proofreading mechanism acting through reversible unwinding of the DNA around a lesion [22]. If the interaction between repair proteins and DNA is not stable enough, the DNA can reanneal, preventing repair from occurring on a non-damaged DNA strand. Yet a balance must be struck. If the affinity of XPC for DNA was too high, the reversibility of its binding would be reduced, leaving repair proteins trapped in incomplete repair complexes; yet if it was too low, repair would be considerably slower. Thus, the strength of XPC's specificity lies in its rather weak affinity for damaged sites, mediating an appropriate balance between binding, binding reversibility, and repair speed.

However, DNA binding affinity alone cannot wholly account for the differentiation between damaged and undamaged DNA. In recent years, a two-stage model has been proposed to explain this differentiation [23]. In the first stage, two β hairpin domains of XPC (BHD1 and BHD2) act as sensors, rapidly testing the integrity of duplex DNA. In the second stage, when the first hairpins find a site that is not entirely stable, a third β hairpin domain (BHD3) is inserted, forming a more stable recognition complex. Thereafter, the damaged bases are flipped out, and the DNA becomes structurally disordered, melting and kinking by 42°. This indirect readout strategy depends on the unpaired bases oscillating in the undamaged strand and does not depend on the chemical nature of the damaged bases themselves. Yet while this model accounts for a basic level of discerning DNA stability, it does not fully address the XPC discernment between true damaged DNA and undamaged DNA. In fact, a very recent study further addressed this fundamental biological concern of how proteins can find their targets amongst other closely related molecules. By creating a crystal structure of Rad4 tethered to an undamaged strand of DNA and seeing that Rad4 flips non-damaged bases out as well, the authors proposed that Rad4 operates under a novel ‘kinetic gating’ mechanism [24]. This mechanism suggests that XPC's selectivity for damaged sites arises from the kinetic competition between how quickly Rad4 can flip the bases out and how long Rad4 remains at a given site, in addition to the previously mentioned binding affinity and hairpin sensing. The authors hypothesize that the opening rate for non-damaged DNA will be slower than that for damaged DNA and that the residence time of Rad4 at non-damaged sites will be shorter than that at damaged sites. In this way, Rad4 has a higher probability of opening damaged DNA instead of non-damaged DNA and minimizing time spent at non-damaged sites. The combination of these tree mechanisms – XPC binding affinity for damaged DNA, BHD sensing DNA duplex stability, and residence time at potential damage sites – allows for a more true and accurate XPC specificity. Despite this new information, it is still not known how XPC diffuses through the genome to find damaged sites. Perhaps a hopping

mechanism is used? Perhaps XPC slides along DNA until it finds distortions? Further studies will be needed to fill this gap in understanding.

3. Make it so: Post-translational modification of XPC

Many factors play a role in XPC production, stability, and activity. The transcriptional regulation of XPC by p53, on both basal and DNA-damage inducible levels [25], plays a role in ensuring that enough XPC is transcribed when needed. But as a complex multi-step process involving the coordination of roughly 30 proteins, NER requires tight regulation. This regulation is largely achieved by post-translational modifications (ubiquitination, sumoylation and possibly phosphorylation) that play key roles in modulating the activity of XPC.

3.1 Ubiquitination

Crucial for NER efficiency, XPC associates with the CUL4A-RING E3 ubiquitin ligase, a major complex involved in ubiquitination, complexed with the UV-damaged DNA-binding complex (UV-DDB). The association between XPC and DDB2, a subunit of UV-DDB, is particularly important. DDB2 helps to regulate the activity of both itself and XPC, and efficient recognition of CPDs requires DDB2 to compensate for the weak binding of XPC to these NER lesions. Studies using XP-E patient cells that lack functional DDB2 show no repair of the smaller CPDs and attenuated repair of the bulkier 6-4 PPs [26, 27]. Consistently, mice with deleted DDB2 display a drastically reduced repair of photolesions [28]. In addition to aiding in overall UV repair, DDB2 directly aids XPC function. UV-DDB was shown to recruit XPC to UV-induced damaged sites within the nucleus [29], and *in vitro* studies show that the DDB1-CUL4A^{DDB2} mediated ubiquitination of XPC after DNA binding stabilizes XPC and increases XPC affinity for damaged and undamaged DNA [30]. After XPC binding, DDB1-CUL4A^{DDB2} also auto-ubiquitinates DDB2, reducing DDB2 affinity for damaged DNA and sending DDB2 to the proteasome for degradation [30, 31]. In fact, when the expression of CUL4A is silenced in human cells, DDB2 is retained at damage sites, which prevents the loading of XPC and subsequent repair [32]. Thus, DDB2 is required during initial detection of CPDs (which XPC cannot efficiently recognize), yet critically, must be subsequently dispersed from the damaged site, allowing for proper XPC function. Further establishing the importance of XPC-DDB2 interplay, XPC has been shown to play a role in regulating DDB2 activity. Intriguingly, upon recruitment to sites of UV-induced photolesions, XPC has been very recently suggested to play a role in regulating stability of DDB2 by serving as a preferential target for DDB1-CUL4A^{DDB2} to ubiquitinate. By ubiquitinating XPC first, less DDB2 becomes poly-ubiquitinated and targeted for degradation, and is thus allowed to participate in several rounds of damage recognition [33].

Given the fact that ubiquitination of XPC plays a major role in NER, it is intriguing that XPC also associates with the ubiquitin receptor Rad23, a subunit in the NER recognition complex. Studies have shown that Rad23 plays a direct role in XPC lesion recognition activities by enhancing XPC binding to damaged DNA, and XPC binding to lesions is impaired in the absence of Rad23. However, given its quick dissociation from XPC after lesion binding, Rad23 does not appear to participate in downstream DNA repair [34].

Indeed, a proposed model suggests that after lesion recognition, Rad23 leaves, importantly exposing XPC's DNA binding residues, allowing XPC to bind damaged DNA in a stable manner as described above [9, 34]. It has also been suggested that Rad23 stabilizes XPC by protecting it from degradation, since some investigations demonstrate that ubiquitinated XPC does not undergo proteolytic degradation [30, 35]. Supporting this protective role, Rad4 in yeast is not stable without Rad23 [36], and mouse studies have shown that the disruption of the gene coding for the homolog of Rad23 compromises the XPC stability *in vivo*, leading to a reduced steady-state level of XPC [35]. Similarly, compromised XPC stability is reversed in Rad23-deficient mouse embryonic fibroblasts through reduced XPC ubiquitination by knockout of CUL4A (as seen by the increased half-lives of XPC) [37]. Further elucidating this protective role, studies in yeast found that Rad4 degradation is facilitated by a proteasome interacting DUB, Ubp3, which removes ubiquitin moieties from Rad4 to promote its entry into the proteasomal channel, and that the Rad4-Rad23 dimerization blocks Rad4 ubiquitination and protects Rad4 from Ubp3 promoted degradation [38].

As mentioned above, XPC becomes ubiquitinated when cells are irradiated with UV, yet ubiquitination of XPC does not lead to its proteasomal degradation but rather an increased affinity for DNA lesions [30]. Although it has not yet been clearly demonstrated, if ubiquitination marks XPC for degradation, then it is a biologically feasible and logical notion that XPC be prevented from this degradation when it is needed most, such as upon exposure to DNA damaging cellular stress; control through Rad23 levels would present a mechanism for determining XPC stability. However, despite very recent evidence showing that p97 (a chaperone that facilitates the remodeling of ubiquitinated proteins for proteasomal degradation or recycling [39, 40]) and XPC co-localize at sites of local UV irradiation and that p97 plays an integral role in the removal of XPC (and DDB2) from chromatin to prevent prolonged attachment [41, 42], XPC has not been yet shown to undergo proteasomal degradation upon ubiquitination. Indeed, it is not yet definitively known which types of ubiquitin moieties are attached to XPC or for which function these moieties target XPC. Further studies in this area would shed light onto the precise purpose of XPC ubiquitination.

3.2 Sumoylation

In addition to being ubiquitinated, XPC is also modified by small ubiquitin-like modifiers (SUMO) after UV irradiation. As sumoylation is highly similar to ubiquitination, it is not surprising that these pathways do not appear to operate in isolation but, rather, seem to incorporate each other in NER. Recently, the E3 ligase RNF111 was identified to ubiquitinate XPC, in addition to DDB1-CUL4A^{DDB2}. However, RNF111 specifically binds UV-induced sumoylated XPC and, as for a SUMO-targeted ubiquitin ligase (STUbL), thereby resulting in K63-linked chains on XPC. Although both CUL4A and RNF111 ubiquitinate XPC in response to UV, the two E3 ligases have opposite effects on recruitment of XPC to damaged DNA: a knockdown of CUL4A led to a decrease in XPC accumulation at locally UV-irradiated chromatin, while a knockdown of RNF111 led to an increase in XPC accumulation at these same sites. Yet, knockdowns of both ligases resulted in reduced DNA repair, and thus, both ligases are needed for proper NER function [43]. Fascinatingly, a

point mutation (K665A) at the site of XPC sumoylation prevents both UV-induced sumoylation and ubiquitination, supporting the role of the STUbL RNF111 in XPC modification and the importance of the interplay between ubiquitination and sumoylation [44]. Studies have also suggested that the sumoylation of XPC plays a role in its stabilization [45].

3.3 Phosphorylation

XPC activity may also be regulated by phosphorylation as suggested by preliminary evidence. A study seeking to identify proteins phosphorylated at consensus sites recognized by ATM and ATR found residue S892 of XPC to be phosphorylated in response to DNA damage [46]. Consistently, the oncogenic phosphatase wild-type p53-induced phosphatase 1 (WIP1) was shown in an *in vitro* phosphatase assay to drastically dephosphorylate XPC Ser892, as a part of its role in dephosphorylating NER proteins and helping the cell return to a pre-stressed homeostatic state [47]. More studies are needed to fully understand how and why XPC is phosphorylated.

4. To seek out new life and new civilizations: Novel roles of XPC

XPC, along with DDB2, is responsible for sensing bulky DNA lesions and initiating GG-NER. However, because XPC binds to the undamaged DNA surrounding the lesion and thus can recognize a broad range of substrates as mentioned above, the idea that XPC recognizes lesions beyond those caused by UV irradiation is not a far extrapolation. Indeed, a number of reports implicate XPC in the recognition of lesions caused by damaging agents other than UV, such as cisplatin and benzo[*a*]pyrene [48, 49]. Hence, the ability of XPC to bind numerous structures not only expands its function within NER, but hints at the role of XPC being broader than expected, extending beyond the boundaries of lesion recognition and even of DNA repair. This expansion of XPC function is supported by a number of sources including explicit reports of alternative XPC function, studies using mice, analyses of single nucleotide polymorphisms linking mutations in XPC to disease risk, and unexplained clinical symptoms of XP patients.

Recent reports have extensively discussed XPC binding affinity for DNA lesions that are not repaired by NER [50]. Most recently, a group measured the binding affinities of XPC-Rad23 to three sets of stereoisomerically different bulky DNA adducts. No direct correlation was found between the strength of XPC-Rad23 binding and the susceptibility to NER cleavage of three adducts induced by polycyclic aromatic hydrocarbons [51]. A more high-throughput approach, measuring binding affinities of XPC-Rad23 across a variety of chemically unrelated lesions, produced similar results. The authors concluded that XPC could serve as a general sensor of DNA damage [13]. Moreover, both groups suggested that the binding of XPC to a lesion, the first step of the bipartite damage recognition mechanism for processing DNA lesions (lesion recognition by XPC-Rad23 occurs first, followed by lesion verification by XPD in TFIIH [20, 52]), does not necessarily confirm that the lesion will be repaired by NER. If indeed the later confirmation step avoids repair of those XPC-bound lesions not repaired by NER, the binding of XPC to these lesions could simply be a promiscuous side effect of binding all potential NER adducts. However, binding of XPC to certain lesions

could lead to yet another repair pathway. The authors of the high-throughput study phrase it best: “XPC may serve as a general sensor of DNA duplex instability and as an adapter molecule capable of initiating multiple repair pathways” [13].

4.1 Into darkness: New to XPC or new to NER?

While the repair capabilities of NER have and continue to be expanded, this expansion cannot be seen as a novel attribute of XPC in and of itself (regardless of XPC's essential contribution to the expanded activity). For example, reports discuss the NER response to DNA damage induced by AZT, a nucleoside that inhibits reverse transcriptase and is used in the treatment of HIV [53, 54]. The capability of NER to repair these lesions includes the idea, by necessity, that XPC, the core sensor of NER, senses the damaged DNA. How I do not understand this sentence ever, one cannot conclude that this repair is specifically a novel function of XPC since XPC recognition of AZT-induced DNA damage is completely dependent on XPC function in NER. While our discoveries of NER capabilities expand as more and more lesions are found to be repairable by this pathway, here we focus on those roles, or potential roles, of XPC which are independent of the ever-expanding NER pathway.

4.2 To explore strange new lesions: XPC in base excision repair (BER) and other repair pathways

The role of XPC in BER has emerged at the forefront of discussions on alternative functions of XPC in DNA repair. BER repairs alterations of single bases, often the result of oxidative damage, which generally do not introduce helical distortions unlike those lesions repaired by GG-NER [55] (see elsewhere in this issue). Demonstrating the essentiality of XPC in the repair of oxidatively-induced lesions, XPC-deficient cells show an increased mutation frequency when exposed to atmospheric conditions and XPC-deficient mice exhibit increased mutation frequencies after long-term exposure to pro-oxidants. By controlling for the involvement of NER (through use of XPA), this study also highlights the independence from NER of XPC function in BER [56]. For further discussion on the role of XPC in BER, please see the comprehensive review on oxidative damage and nucleotide excision repair by Melis et al. [57], which explores oxidative damage in XPC-deficient mice and cell lines and the association of XPC with BER proteins.

Along with the potential role of XPC in BER, there have been reports the participation of XPC in other DNA repair pathways. For instance, our group reported that XPC putatively interacts with a novel set of DNA damage and repair-related proteins, such as DNA damage-inducible transcript 3 (DDIT3) and polyhomeotic homolog 1 (PHC1) [58]. Interestingly, knockdown of XPC, but not of XPA, in HeLa cells leads to mild sensitivity to etoposide, a topoisomerase II inhibitor known to cause double-strand breaks (DSBs) [59]. Moreover, XPC is involved in repair of butadiene epoxide induced DNA lesions through an unidentified pathway [60]. Also implicating XPC in DSB repair is increased XPC transcription in response to ionizing radiation and to cyanotoxin cylindrospermopsin [61, 62]. Intriguingly, the majority of NER factors are not significantly affected by the cyanotoxin [63]. Given the alternative molecular mechanisms of XPC non-canonical function in NER, it is necessary to mention that the involvement of XPC in additional DNA repair pathways is not necessarily bound to lesion recognition. Perhaps XPC acts in these

pathways, similarly to in NER, by recruiting downstream factors or orchestrating modifications.

4.3 The next generation: XPC's non-canonical roles in NER

The discussion of XPC function until this point has focused on the canonical role of XPC as a primary damage sensor within GG-NER and the implications of XPC function in other DNA repair pathways. Yet XPC boldly goes beyond lesion recognition and even beyond DNA repair, participating in newly explored pathways and functions. We first discuss these novel roles within the context of NER before entering the undiscovered country of novel functions outside of DNA repair. Figure 1 graphically represents the regulations and functions of XPC, both those known and those, which remain to be discovered.

4.3.1 NER-associated chromatin remodeling—Rad4/XPC's interplay with DNA in the NER pathway is not limited to lesion recognition, but is also involved in chromatin remodeling. In yeast, the SNF5 subunit of the chromatin remodeling complex SWI/SNF interacts with the Rad4-Rad23 complex and is involved in UV-induced chromatin rearrangement during NER [64]. In humans, XPC and hSNF5 (the human ortholog of SNF5) associate and, at sites of DNA damage, colocalize [65]. While the association between BRG1 (the ATPase catalytic subunit of SWI/SNF) and XPC was not demonstrated, our lab found that elevated levels of BRG1 associate with DDB2 in chromatin after UV irradiation, and, interestingly, knockdown of BRG1 affects the accumulation of XPC at CPD sites [66]. Our lab hypothesizes that BRG1 is recruited to damage sites by DDB2 to facilitate XPC damage binding in chromatin [66] though further studies are needed to clarify how exactly XPC and DDB2 interact with the SWI/SNF remodeling complex. On the other hand, other studies show that inactivation of hSNF5 does not affect the recruitment of XPC to the damage sites but affects recruitment and phosphorylation of the ATM checkpoint kinase. The authors propose that the SWI/SNF complex associates with XPC at damage sites, facilitating ATM access and thereby promoting downstream phosphorylation [65]. Further elucidating their proposed model, the authors found that ATR and ATM localize to UV damage sites through association with XPC, that XPC and DDB2 are upstream of the ATM and ATR pathways, and that these damage sensors are required for the damage specific recruitment and phosphorylation of ATM and ATR [67]. Additionally facilitating chromatin access of the NER proteins after UV irradiation, DDB2 has been implicated in the process of chromatin decondensation in a CUL4A ubiquitination-independent manner, thereby promoting recruitment of XPC to localized sites [68]. DDB2 is also found to associate with PARP1 (poly(ADP-ribose) polymerase-1) and this interaction is stimulated by UV irradiation on chromatin. PARP1-dependent PARylation of chromatin also mediates recruitment of the SWI/SNF chromatin remodeler ALC1 to UV-damaged DNA, thereby stimulating the recruitment of XPC [69, 70]. These studies highlight the role of chromatin remodeling in localizing NER proteins and checkpoint factors at sites of DNA damage. XPC appears to be crucial for maintaining cross-talk between these proteins and factors in chromatin, coordinating damage recognition and repair with checkpoint activation.

4.3.2 Coordination of NER and the cell cycle—XPC lies at the crossroads of stress-induced control of the cell cycle and cell cycle control of DNA repair. Upon suffering DNA

damage, cells pause their normal cell cycle activities in an attempt to allow time for repair of the damaged genetic information before replicating DNA in S phase. Reflecting the required coordination between NER and the cell cycle, XPC stability and NER activity are linked to cell cycle checkpoints. The cyclical nature of the cell cycle lends itself to the dynamic nature of XPC stabilization which shifts in parallel to the phosphorylation status of retinoblastoma protein (RB) [71]. The absence of phosphorylation of this tumor suppressor gene during the cell cycle prevents G_1/S transition and DNA replication. Studies conducted in p53 null cells (lacking p53 induced up-regulation of XPC and NER activity) found that the unphosphorylated form of retinoblastoma protein (RB) stabilizes XPC, greatly enhancing DNA repair. Consistently, overexpression of cyclin E, the cyclin which phosphorylates RB at the G_1/S transition and signals for entry into S phase, completely abolishes this RB-stabilized XPC and reduces DNA repair activity. The authors hypothesize that RB protects XPC from degradation and that cyclin E reverses this protection. Notably, since the general consensus in the field is that NER occurs in G_1 but not in S phase, the authors highlight the importance of allowing XPC function during G_1 phase and inactivating XPC during S phase [71]. Thus, the tight and careful regulation of XPC stability during the cell cycle is crucial in facilitating proper XPC activity and NER function.

4.3.3 Cell signaling in NER—Consistent with stimulating recruitment of ATR and ATM kinases and promoting downstream phosphorylation, XPC has been implicated in cell signaling. c-Jun N-terminal kinase (JNK) is activated in response to a variety of DNA damaging agents including UV irradiation and regulates numerous cellular activities including apoptosis. JNK activation is reduced after UV irradiation and cisplatin treatment in XPC defective human fibroblasts compared to primary human fibroblasts, and of note, XPA-defective human fibroblasts (cells deficient in another protein involved in NER) do not demonstrate this JNK activation [72, 73]. Therefore, while XPC affects pJNK activation after genotoxic stress that stimulates NER, this new XPC function in signal transduction is independent of the canonical role of NER.

4.4 The undiscovered country: XPC in non-DNA repair pathways

The unearthing of XPC's previously unconsidered DNA repair roles, which are so similar to its traditional and long-standing function, raises the question: How many more? How many more roles of XPC involvement exist yet has not been discovered? How many roles beyond the border of DNA repair? Indeed, XPC has been implicated in pathways completely outside the DNA damage response. Our group has published an XPC interactome resource discussing the putative XPC interactions in signal transduction, transcription regulation, and cellular metabolism [58]. More comprehensive analyses have implicated XPC in chromosomal stability, transcriptional regulation, proteasomal degradation, and cellular viability.

4.4.1 Chromosomal stability—XPC is involved in regulating genomic stability, furthering XPC's role as a protector of cellular health. Linking XPC to chromosomal stability, $Xpc^{-/-}$ primary mouse embryonic fibroblasts exhibit augmented characteristics of telomere instability compared to wild-type cells including an increased number of telomere sister chromatid exchanges, chromosome-type fusions, and multitelomeric signals (MTS).

While the already augmented levels of MTS in $Xpc^{-/-}$ cells increase further in response to several cellular stressors, hypoxic conditions in $Xpc^{-/-}$ cells actually lead to diminished levels of MTS, similar to the typical levels observed in $Xpc^{+/+}$ cells [74]. Although the authors argue that telomere fragility in $Xpc^{-/-}$ cells is linked to increased sensitivity to oxidative damage, sensitivity to conditions whose telomere fragility can be repaired in $Xpc^{+/+}$ cells yet not in $Xpc^{-/-}$ cells could be lauded as more indicative of XPC function. The authors attribute this XPC-dependent telomere instability to dysfunctional NER and the resulting accumulation of DNA damage in $Xpc^{-/-}$ cells. However, in light of the most recent research on XPC, a number of alternative pathways could cultivate XPC participation in telomere stability. Thus, additional studies are needed to clarify by which mechanism XPC is involved in telomere stability.

4.4.2 Transcriptional regulation—XPC has been implicated in transcriptional regulation of through different mechanisms [75-77]. As reviewed above, XPC interacts with chromatin remodeling factors to promote DNA accessibility for proteins involved in cell signaling, but also facilitates gene expression and silencing. Supporting a novel role for Rad4/XPC in transcriptional regulation as well as in chromatin remodeling, our group recently found a role for Rad4 in the regulation of heterochromatin and gene silencing in *Saccharomyces cerevisiae* [75]. Affecting transcription more directly, a recent paper shows that the Rad4-Rad23 nucleotide excision repair complex binds to the promoters of certain DNA damage response genes and inhibits their expression [78]. XPC can also regulate gene expression by binding to promoters. For example, XPC is recruited to the *RARβ2* promoter with and without UV irradiation [76]. Therefore, XPC binding to the promoter of the *RARβ2* gene is independent of NER. While it must be noted that additional excision repair proteins are recruited alongside XPC to the *RARβ2* promoter, XPC appears to be particularly essential to the transcriptional machinery. XPC initiates a sequential recruitment of NER factors, whereas knocked down XPC and mutated XPC (XP-C/R579st) recruit NER factors less efficiently to the *RARβ2* promoter. With the early requirement of XPC for transactivation of the *RARβ2* promoter, XPC has a clear and essential role in transcription. The distinguishability of the NER complex for repair with the NER complex for transcription highlights the independence from NER of the role of XPC in transcription. Indeed, the authors state that these results “may force us to question whether the role of XPC [and other NER proteins] would be first transcriptional and then upon genotoxic attack could also be required for elimination of DNA lesions”. Reasonably, the same authors advocate for additional studies to pinpoint the exact roles of the NER proteins in transcription [76]. XPC can also influence transcription as a coactivator independent from NER and other NER proteins in contrast to what is observed at the *RARβ2* promoter. In embryonic stem cells, Sox2, Oct4, and Nanog are essential transcription factors responsible for maintaining self-renewal and pluripotency [79]. In association with Oct4 and Sox2, XPC occupies the promoters of Nanog and Oct4. Further, knockdown of XPC leads to altered cell morphology, reduced ES cell proliferation rates, decreased expression of the mRNA of Nanog and other stem cell markers, and decreased reprogramming efficiency, clearly linking XPC to stem cell regulation [77, 80]. The emerging role of XPC as a direct regulator of transcription further adds to the complexity of the XPC/DNA interaction; XPC is essential for the actual stability

and function of DNA, whether through lesion recognition, coordinating chromatin remodeling, or controlling gene expression.

4.4.3 Proteasomal degradation—While we have discussed at length the careful regulation of Rad4/XPC stability and activity, an interesting new line of studies have implicated Rad4/XPC in the regulation of stability and degradation of substrates belonging to different pathways [81-83]. For instance, evidence points to a regulatory role that Rad4/XPC plays in the turnover of ubiquitinated proteins. In *rad4*Δ cells, ubiquitinated substrates accumulate, suggesting that Rad4 regulates protein turnover at a post-ubiquitination step. Furthermore, immunofluorescent studies show that upon exposure to DNA damage, Rad4 heavily localizes in the nucleus, and this phenomenon is accompanied by the impaired degradation of its non-nuclear substrates. Importantly, these studies also found that Rad4 participates in the ubiquitin fusion degradation (UFD) pathway with Rad23-Ufd2-mediated degradation of ubiquitinated proteins. Similarly, XPC was found to bind to hUfd2 and to regulate the degradation of UFD substrates in humans, therefore suggesting that the Rad4/XPC regulation of proteolytic degradation is crucial enough to warrant evolutionary conservation. Rad4 is suggested to indirectly regulate proteolytic degradation by helping Rad23 maintain an open and active conformation, counteracting Rad23's preferred closed conformation (through interaction of its UBA and UBL domains) and promoting its association with substrates and/or the proteasome [81]. Moreover, the role of XPC in the regulation of proteolysis is supported by the compromise of p53 degradation and p53-proteasome association in XPC-deficient cells after UV irradiation. Further, XPC associates with MDM2, and MDM2-promoted p53 degradation is impaired in XPC-deficient cells. The authors propose that XPC serves as the link between p53 ubiquitination and Rad23-mediated proteasomal degradation [82]. Further supporting the role of XPC in Rad23-mediated p53 proteolysis, knockdown of XPC leads to decreased p53 stability and an increased interaction of p53 and Rad23 [83]. Thus, through regulating protein stability, XPC is primed for influence on diverse pathways.

4.4.4 Cellular viability—Considering that both XPC's canonical and non-canonical roles indirectly help support cellular health including through recognizing DNA damage and maintaining genome stability, it is not surprising that XPC has more direct links to cellular viability. In two recent studies, knockdown of XPC leads to metabolic alterations and the production of NOX1-dependent ROS (reactive oxygen species) [84, 85]. In $Xpc^{-/-}$ mice, XPC deficiency results in metabolic alterations such as an increase in senescence-associated beta-galactosidase (SA-β-gal) activity, ROS level, and NADPH oxidase (NOX) activity. Intriguingly, these phenotypes are not found in $Xpa^{-/-}$ mice. The authors attribute the phenotypic effects of XPC knockdown to the previously mentioned role of XPC in response to oxidative damage, further supporting the role of XPC in BER. Interestingly, the metabolic phenotypes of $Xpc^{-/-}$ mice mimic those of an aged mouse, as DNA damage and aging have long been linked [86]. Our group identified several metabolic proteins that could interact with XPC, including a phospholipase, a pyrophosphatase, a monooxygenase, and a decarboxylase [58], thereby establishing a potential link between XPC and cellular metabolism. Newer studies are also beginning to hint at a broader role for XPC in cell growth and proliferation; knockdown of XPC in A549 cells leads to decreased cell growth,

migration, and a faster growth rate in tumor xengorafts [87]. Compliments its proposed role in cell growth and proliferation, XPC regulates the DNA-damage-induced apoptosis, independent from the canonical XPC role in NER. The induction of apoptosis after DNA damage is an important mechanism to prevent mutation-prone cells from dividing and amplifying mutations in their prodigy. XPC-deficient cells apoptosize less frequently than their wild-type counterparts after UV irradiation and, intriguingly, after treatment with cisplatin, etoposide, and IR as well [88]. The influence of XPC on apoptosis after treatment with agents causing double-strand breaks indicates that the role of XPC in damage-induced apoptosis is not restricted to UV-induced or even NER-associated apoptosis. Further supporting the independence of this XPC role from NER, the knockdown of XPC in XP-A cells produces the same phenotype as in wild-type cells. XPC upregulates damage-induced apoptosis through direct binding of XPC to the *casp-2S* promoter leading to decreased expression of the antiapoptotic casp-2S (further supporting the previously discussed role of XPC in transcription regulation) [88]. XPC serving to both find DNA lesions for repair and help to kill the cell highlights XPC as a double safety net, serving as both the first and last defense of a cell in response to damage. Cellular metabolism or apoptosis are only singular parts of an interconnected web of pathways regulating cellular health. As we have enumerated in this review, XPC also plays essential roles in other pathways within this web, such as genomic stability and DNA damage. Thus, XPC appears to orchestrate the various pathways responsible for overall cell viability and represent the interconnectivity of biological pathways.

4.4.5 Hints of other potential XPC roles—The studies discussed up until this point sought to discover novel XPC functions as a primary objective. However, additional roles have been hinted at in sources where studying XPC function was not the primary focus of the study. While correlation between single nucleotide polymorphisms (SNPs) of the XPC gene and increased susceptibility to skin cancer could be explained cleanly by XPC function within GG-NER, reports linking XPC SNPs to susceptibility for non-skin cancers such as urinary system cancers [89], colorectal cancer [90] and lung and head and neck cancers[91] hints at XPC function outside GG-NER. Unexplained phenotypes in mice and humans could predict further roles for XPC. Studies in $Xpc^{-/-}$ mice have been an invaluable resource, and a recently published review nicely discusses the two XPC-deficient mouse models and their spontaneous and exposure-induced tumor phenotypes [92]. More functions can also be predicted from the phenotypes of xeroderma pigmentosum (XP) patients. XP is a skin disorder resulting from mutation in one of the eight complementation groups: XP-A, XP-B, XP-C, XP-D, XD-E, XP-F, XP-G, and XP-V. While all XP patients harbor similar phenotypes, there are variations across the complementation groups attributable to specific roles of their mutated gene products. All XP patients, regardless of which XP gene they lack, exhibit the typical UV sensitivity and predisposition to skin cancer [93]. However, the XP-C patients (those with missing or dysfunctional XPC), specifically can exhibit atypical symptoms such as autism, hypoglycinemia, and psychomotor delay [94, 95]. Therefore, unexplained clinical symptoms in XP-C patients can give more insight into the functions of XPC, further broadening XPC responsibility within the cell.

5. Concluding remarks and future directions: The final frontier

The role of XPC as a damage recognition repair factor has been well established, and a myriad of papers detail XPC regulation, modification, and complexity, influencing its molecular function during NER. The sheer volume of information regarding the NER-dependent role of XPC hints at the breadth of knowledge left to uncover, particularly now knowing that this role is actually broader and less NER-dependent than previously thought. We present here an overview of recent studies implicating XPC in non-canonical roles, including chromosomal stability, heterochromatin structure, proteasomal degradation, and transcriptional control of stem cells (though it is impossible to include all studies and manuscripts regarding XPC). This range of functions now begs the question: how does XPC know which repair pathway to instigate or which cell function to regulate? At least in part, answers could be found in the post-translational modifications of XPC, as much is still left unknown in terms of XPC ubiquitination, sumoylation, and other post-translational modifications. Specifically modified XPC residues and the characteristics of these modifications during these processes wait to be defined. Certainly, it would be fascinating if these diverse modifications could control which XPC function is “activated” and if combinations of these modifications were processed by XPC to determine different functional outputs in a variety of pathways (Fig. 1).

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Prologue

Captain's log - We would like to thank Dr. Michael Smerdon for his continued support of our career development. In addition to his numerous scientific contributions to the field of DNA repair, Mick is a great mentor. He will continue to be a great source of inspiration for all his students in the years to come.

Highlights

- A review of XPC's cellular functions is provided
- The most recent findings concerning XPC's involvement in non-canonical pathways are described

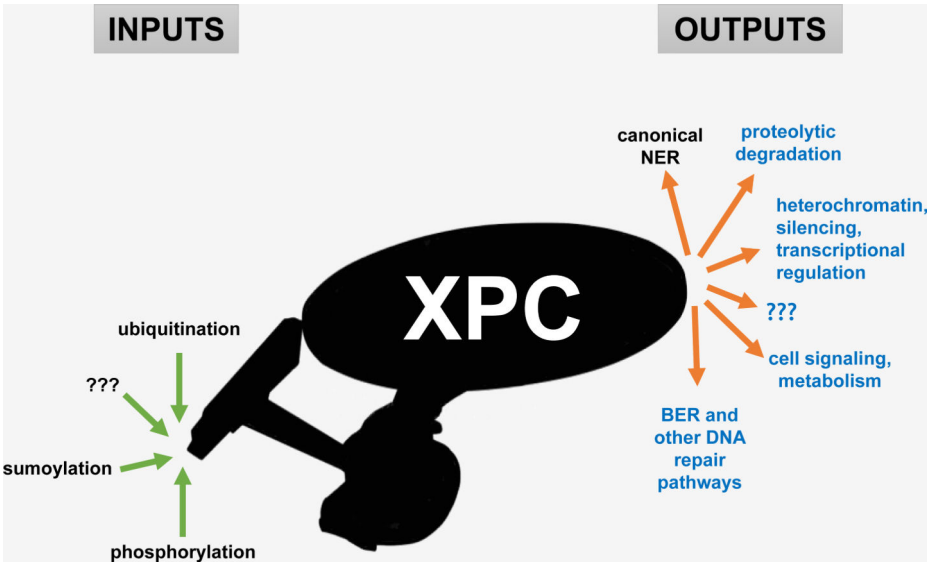

Fig. 1. The voyages of the starship XPC: XPC's expanding function

XPC figuratively represents a mathematical “black box”; different inputs are put into a function with an array of outputs being produced. In the context of XPC, different combinations of modifications or regulations result in different functions. In this way, XPC not only plays an integral role in the canonical NER pathway, but also in many novel roles in NER and other pathways (shown in blue). The question marks indicate regulations and functions that are likely still unknown and still to be explored.