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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.

Contents

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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) pyrimidone 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). 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 $64 \pm 2^{\circ}$) 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