Role of Nucleotide Excision Repair Proteins in Oxidative DNA Damage Repair: an Updating

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Abstract—DNA repair is a crucial factor in maintaining a low steady-state level of oxidative DNA damage. Base excision repair (BER) has an important role in preventing the deleterious effects of oxidative DNA damage, but recent evidence points to the involvement of several repair pathways in this process. Oxidative damage may arise from endogenous and exogenous sources and may target nuclear and mitochondrial DNA as well as RNA and proteins. The importance of preventing mutations associated with oxidative damage is shown by a direct association between defects in BER (i.e. MYH DNA glycosylase) and colorectal cancer, but it is becoming increasingly evident that damage by highly reactive oxygen species plays also central roles in aging and neurodegeneration. Mutations in genes of the nucleotide excision repair (NER) pathway are associated with diseases, such as xeroderma pigmentosum and Cockayne syndrome, that involve increased skin cancer risk and/or developmental and neurological symptoms. In this review we will provide an updating of the current evidence on the involvement of NER factors in the control of oxidative DNA damage and will attempt to address the issue of whether this unexpected role may help unlock the difficult puzzle of the pathogenesis of these syndromes.

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In 1968 Jim Cleaver reported that a disorder characterized by high incidence of skin cancer upon sunlight exposure, xeroderma pigmentosum (XP), is caused by a defect in the repair of UV lesions. Since then all the eight genes that cause XP (XPA–XPG and XPV or variant) have been cloned. All of them, with the exception of XPV, work in different steps of the same biochemical pathway, the nucleotide excision repair (NER). The complex biochemistry of this pathway has been clarified by the joint effort of several groups. We know today that NER oper-
ates by two distinct pathways: global genome repair (GGR) that removes lesions from the genome overall and transcription-coupled repair (TCR) that repairs transcriptionally active domains. The step of damage recognition involves different factors in the two pathways. In GGR, XPC–HR23B/centrin-2 and XPE (UV-DDB) protein complexes, and in TCR the RNA polymerase II (RNAPII) stalled at a lesion on the transcribed strand, play a role in the recognition step. Transcription arrest is increased by CSA and CSB proteins that are required for ubiquitylation of the carboxy-terminal domain of RNAPII. The repair process follows then the same path involving the binding of the ten-component basal transcription factor H-II (TFIIH) via interaction with either XPC or the arrested transcription apparatus. Two helicases, XPB and XPD, initiate the opening around the lesion, and the DNA around the damaged site is cleaved by the XPG 3′ nuclease and the XPF-ERCC1 5′ nuclease. Once the damaged oligonucleotide is removed resynthesis occurs by proliferating cell nuclear antigen (PCNA), DNA polymerase δ, DNA polymerase κ, and DNA ligase.

The complex biochemistry of NER has been established by using UV-induced photoproducts as model lesions and similar chemically induced products that distort DNA are recognized and repaired by the same factors. However, the clinical heterogeneity in disorders with NER mutations opens the question of whether defects in this pathway are solely due to impaired repair of helix-distorting DNA lesions. XP patients with also defects in TCR (XP-A, XP-B, XP-D, and XP-G) present, besides increased skin cancer risk, accelerated neurodegeneration. Patients with Cockayne syndrome (CS) show also severe developmental and neurological symptoms but do not show skin cancer despite the presence of photosensitivity. Neuronal death might be due to accumulated endogenous damage, and indeed a growing body of evidence indicates that NER proteins participate in the processing of oxidative DNA lesions that are produced by the normal cell metabolism. The role of NER proteins in different pathways might explain the heterogeneity in disorders with NER mutations.

In this review we concentrate on four NER genes, two involved in DNA damage recognition, XPC and XPD, and two belonging to TCR, CS4 and CSB, that have been involved in the response to damage from endogenous sources. The role of XPG in the stimulation of oxidative DNA damage repair has been recently reviewed [1] and will be not covered in this review.

**XPC**

**Biochemical properties and protein structure.** The human XPC gene is located on chromosome 3 and encodes a basic protein of 940 amino acids [2] that functions, in concert with XPE, as a damage detector in the first step of GGR. XPC comprises at least four structural domains: a transglutaminase-homology domain (TGD) and three consecutive-hairpin domains (designated BHD-1, -2, and -3) (Fig. 1a). Against the conventional dogma that DNA lesions are recognized through direct contacts with modified nucleotides, XPC protein seems to distinguish between damaged DNA and the native double helix by sensing the single-stranded character of non-hydrogen-bonded bases in the undamaged strand [3]. This mode of action is confirmed by structural analysis of the yeast Rad4 homolog that identifies critical chains making contacts with extra-helical nucleotides [4]. In addition, XPC provides a landing platform for TFIIH [5] that, together with XPA and replication protein A (RPA), generates an open repair intermediate in which the DNA around the lesion is melted (over 25-30 nucleotides). XPC is polyubiquitinated by the UV-DDB–Cul4A–Roc1 complex upon DNA damage, a reversible process that does not result in its degradation, but rather increases its affinity for DNA, damaged or not [6]. The human XPC protein in vivo is a heterotrimeric complex including HR23B and centrin-2 proteins [7, 8]. HR23B seems to stabilize XPC, whereas centrin-2 is required to enhance the damage recognition function of XPC [9]. This complex binds to various types of helix-distorting lesions, thus triggering GGR and, unexpectedly, it also stimulates the repair of small base lesions.

The XPC–HR23B complex functionally interacts with 3-methyladenine DNA glycosylase [10] and thymine DNA glycosylase (TDG) [11] that initiate BER of alkylation and deamination products, respectively. XPC–HR23B stimulates TDG activity by promoting the release of TDG following the excision of mismatched T base. In the presence of apurinic/apyrimidinic endonuclease 1 (APE1), XPC–HR23B has an additive effect on TDG turnover without significantly inhibiting the subsequent action of APE. XPC–HR23B complex significantly stimulates also the activity of 8-oxoguanine DNA glycosylase (OGG1) in human cell extracts (Fig. 1b) as well as in a reconstituted repair reaction with purified proteins [12]. OGG1 is known to bind tightly the AP site generated by its glycosylase activity [13], and XPC–HR23B may be required to facilitate its release from the AP site, thereby freeing OGG1 to react with remaining sites. The question of whether XPC operates as an active displacement of the DNA glycosylase or competes at AP sites (it has been shown that XPC complex can bind specifically to AP sites [11]) waits to be clarified. A recent study [14] points to the importance of protein–protein interaction for the stimulation of DNA glycosylases by XPC for AP sites by showing that XPC stimulates the activities of sumoylated TDG and single-strand-specific monofunctional uracil-DNA glycosylases (SMUG1), both of which interact physically with XPC. XPC–HR23B recognizes also 5R-thymine glycol (Tg), formed by exposure to radiation and