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Transcriptional consequences of XPA disruption in human cell lines

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ABSTRACT

Nucleotide excision repair (NER) in mammalian cells requires the xeroderma pigmentosum group A protein (XPA) as a core factor. Remarkably, XPA and other NER proteins have been detected by chromatin immunoprecipitation at some active promoters, and NER deficiency is reported to influence the activated transcription of selected genes. However, the global influence of XPA on transcription in human cells has not been determined. We analyzed the human transcriptome by RNA sequencing (RNA-Seq). We first confirmed that XPA is confined to the cell nucleus even in the absence of external DNA damage, in contrast to previous reports that XPA is normally resident in the cytoplasm and is imported following DNA damage. We then analyzed four genetically matched human cell line pairs deficient or proficient in XPA. Of the ~14,000 genes transcribed in each cell line, 325 genes (2%) had a significant XPA-dependent directional change in gene expression that was common to all four pairs (with a false discovery rate of 0.05). These genes were enriched in pathways for the maintenance of mitochondria. Only 27 common genes were different by more than 1.5-fold. The most significant hits were *AKR1C1* and *AKR1C2*, involved in steroid hormone metabolism. AKR1C2 protein was lower in all of the immortalized XPA-deficient cells. Retinoic acid treatment led to modest XPA-dependent activation of some genes with transcription-related functions. We conclude that XPA status does not globally influence human gene transcription. However, XPA significantly influences expression of a small subset of genes important for mitochondrial functions and steroid hormone metabolism. The results may help explain defects in neurological function and sterility in individuals with xeroderma pigmentosum.

1. Introduction

Nucleotide excision repair is the only pathway that mammalian cells have available for the removal of the major DNA lesions arising from ultraviolet (UV) radiation damage. NER also repairs helix-distorting lesions produced by reactive chemicals and other types of radiation. The NER process excises damage within a 24–32 nt oligonucleotide, followed by repair synthesis and ligation to complete repair. About 30 polypeptides are needed for the basal NER process [1–3]. The main pathway of global genomic NER involves distortion recognition by XPC-RAD23B, formation of a pre-incision damage recognition complex including TFIIH, XPA and RPA, and incision of the damaged strand on the 5′ and 3′ sides by the ERCC1-XPF and XPG nucleases respectively.

In eukaryotes, most of the components of the NER machinery have major additional biological functions that are essential for normal viability. This has profound consequences for cells or organisms with mutations in NER genes. The ten subunits of TFIIH form a core initiation factor for basal transcription of all mRNAs, for example, and XPG also has a transcription-related function [4–6]. ERCC1-XPF participates in some homologous recombination reactions and in crosslink repair [7]. Consequently, complete disruption of some NER components is incompatible with cellular survival or embryonic development (for example, TFIIH subunits or RPA), while perinatal lethality occurs following disruption of other components (ERCC1, XPF, XPG). Mutations that partially disable these factors can lead to severe diseases in human beings.

Complete disruption of two major NER factors is tolerated. One of these is XPC, part of a distortion recognition complex. XPC disruption causes xeroderma pigmentosum (XP) in humans, with a relatively less severe phenotype than other complementation groups because a transcription-coupled form of NER remains intact. Nevertheless, even XPC is reported to have an additional function as a component of a transcription complex for specific genes [8].

The other NER factor that can be completely inactivated without impairing cell viability is XPA. This is notable, as XPA is absolutely required for NER. It is a scaffold protein that contacts many of the other

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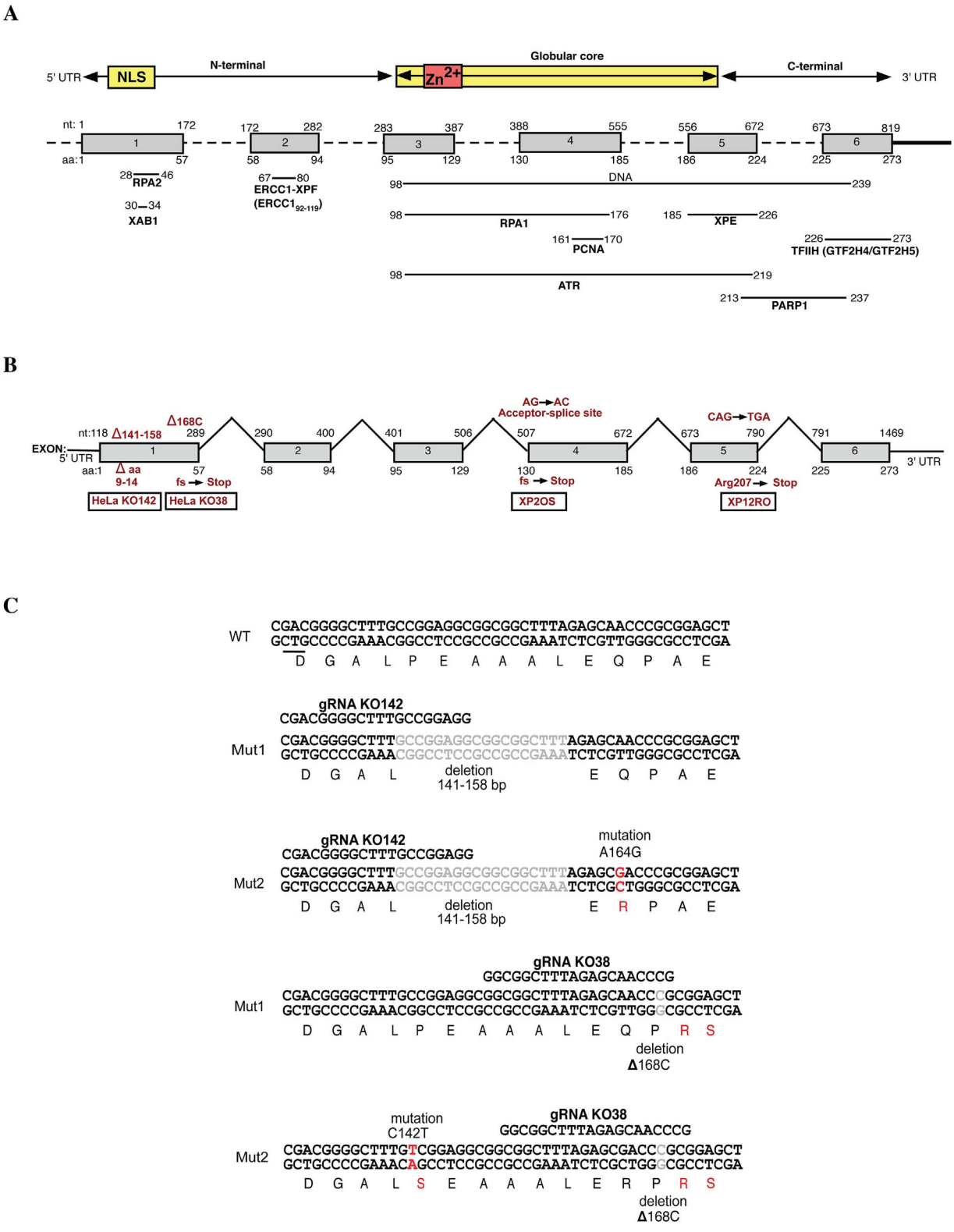

Fig. 1. Description of XPA-proficient and deficient cell lines used in the study.
A. Top, human XPA protein showing the location of the central globular core in relation to the six coding exons (gray). The locations of the nuclear localization signal (NLS) and zinc finger (Zn^{2+}) are shown. The bottom part of the panel shows mapped regions of XPA that interact with other proteins. B. The exon-intron structure of the human *XPA* gene showing the sites of the causative *XPA* mutation in each XPA-deficient cell line used here. C. Location of CRISPR-Cas9 mutations generated in the *XPA* gene of HeLa S3 cells. Two mutated HeLa cell lines were obtained using two guide RNAs (gRNA). KO142 had the indicated 18 bp deletion in both alleles, and an A164G mutation leading to a Q16R amino acid change in one allele. KO38 had a deletion of C168 (ΔC168) leading to early termination in both alleles and a C142T mutation leading to a P9S amino acid change in one allele.

NER protein components, and also binds to DNA [9–11] (Fig. 1A). XPA patients or mice without XPA function have no NER activity. Because each of the other NER factors has an identified additional function in cells, it is important to evaluate whether XPA also has a significant biological function other than NER. Broadly, elimination of XPA is compatible with mammalian development, growth, and cellular