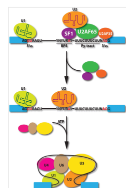

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Coupling between Nucleotide Excision Repair and Gene Expression

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Abstract

Gene expression and DNA repair are fundamental processes for life. During the last decade, accumulating experimental evidence point towards different modes of coupling between these processes. Here we discuss the molecular mechanisms by which RNAPII-dependent transcription affects repair by the Nucleotide Excision Repair system (NER) and how NER activity, through the generation of single stranded DNA intermediates and activation of the DNA damage response kinase ATR, drives gene expression in a genotoxic scenario. Since NER-dependent repair is compromised in Xeroderma Pigmentosum (XP) patients, and having in mind that these patients present a high degree of clinical heterogeneity, we speculate that some of the clinical features of XP patients can be explained by misregulation of gene expression.

Keywords: Gene expression; Nucleotide Excision Repair; Xeroderma Pigmentosum; UV light

Introduction

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Human beings have hundreds of different cell types expressing different set of genes and performing specific roles within the body. Differential gene expression among different cell types, or in a single cell type but in different contexts, is the key to accomplish the variety of responses and the morphological and behavioral complexity that humans display. Expression of a protein-coding gene requires not only transcription but also pre-mRNA processing and nuclear export. While transcription is directly catalyzed by RNA polymerase II (RNAPII), this enzyme also modulates pre-mRNA processing and mRNA export via a unique domain among DNA-dependent RNA polymerases: its repetitive carboxyl terminal domain (CTD). This domain, present in RNAPII largest subunit RPB1, comprises 52 tandemly repeated heptapeptides containing the consensus sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser. This repetitive sequence can be extensively modified by phosphorylation as well as other post-translational modifications such as acetylation, glycosylation, ubiquitylation, SUMOylation and methylation. Hence, different CTD states dictate the interaction with a plethora of proteins, which in turn, affect the generation of a translatable mRNA by controlling not only transcription but also pre-mRNA processing reactions such as capping, splicing and polyadenilation [1, 2]. In the past we demonstrated that in human cultured cells subjected to genotoxic stress triggered by UV exposure, the phosphorylation status of the CTD is altered, which in turn slows down transcriptional elongation rates affecting the expression of several genes, some of which are key for survival/apoptosis decisions [3].

DNA is the only biopolymer that is neither disposable nor recyclable and therefore must be repaired when damaged. Nucleotide Excision Repair (NER) is one of the most versatile DNA repair systems in human cells, dealing with lesions induced by chemicals, UV radiation and some forms of oxidative damage [4]. NER is initiated by two distinct DNA-damage-sensing mechanisms that use the same machinery to repair the damage: transcription coupled repair (TC-NER) and global genome repair (GG-NER). TC-NER detects and removes damage from the template strand of genes that are being transcribed, and depends on RNAPII elongation as well as on the Cockayne syndrome proteins CSA and CSB. Mutations in CSA or CSB are associated with cell death probably due to deficiencies in transcriptional re-start after damage [5]. GG-NER removes damage present elsewhere in the genome and depends on lesion recognition factors such as the UV-DDB complex, in which DDB2/XPE interacts with the lesion, and XPC. Once the damage is recognized by either TC-NER or GG-NER factors, both pathways converge at the recruitment of the general transcription factor TFIIH, which contains the helicases XPB and XPD and opens the DNA helix around the damage. Together with XPA, the TFIIH complex scans the DNA for helicase-blocking lesions therefore verifying the need for repair and hence favoring the recruitment of the endonucleases XPF and XPG to excise the damaged strand. The resulting single-stranded DNA (ssDNA) gap is filled by DNA synthesis and ligation [6]. The most conspicuous UV-induced DNA lesion are cyclobutane pyrimidine dimers (CPD) and, to a lesser extent, (6-4) pyrimidine-pyrimidone photoproducts ((6-4)PPs) which are, at least in placental mammals, mainly repaired by the NER system. Xeroderma pigmentosum (XP) is an autosomal recessive disease caused by a defective GG-NER system and characterized by extreme sun sensitivity and increased risk of skin cancer. There are 8 complementation groups in XP: XPA through XPG, plus a variant group XPV that results from the inactivation of the translesion DNA polymerase eta, which is able to bypass CPDs in an almost error-free manner [4].

Modes of coupling between Nucleotide Excision Repair and Gene Expression

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Given that transcription and DNA repair both involve intimate interactions with DNA, it is not surprising that these two processes can influence each other and, in fact, this interplay occurs at different levels (Figure 1):

i) Both processes share common factors: Many factors are shared between the two machineries, including the TFIIH complex that contains the helicases XPB and XPD [7], as well as XPC, XPA, XPF or XPG. Even in the absence of genotoxic stress, many of these factors are recruited to promoters of inducible genes[8] (Figure 1A) and, upon UV exposure, it has been proposed a competition of TFIIH between the repair and transcriptional machineries that might, in turn, regulate transcription [9]. For reviews about this topic refer to [10].

ii) Transcription works as a DNA damage sensor: An elongating RNAPII stalled in front of a lesion acts as a lesion sensor for TC-NER, favoring the preferential repair of actively transcribed genes (Figure 1B) [11]. The contribution of TC-NER to the overall NER activity varies among cell types, with low TC-NER activity in keratinocytes and high TC-NER activity in fibroblasts [12-14]. This fact is likely to have consequences at the gene expression level, as we have shown that modulation of Alternative Splicing (AS, the main process amplifying DNA information by generating multiple mRNA variants from a single gene) upon UV exposure is different when comparing fibroblasts with keratinocytes: UV-induced modulation of AS can be mimicked in fibroblasts by a simple transfection with an in vitro-irradiated plasmid but only if the plasmid carries a promoter sequence which actively drives transcription and thus serves as a TC-NER inducer [15]. On the contrary, when using keratinocytes as the model system, the presence of the promoter is dispensable to mimic the UV effect on AS. Having in mind that upon UV treatment, the inclusion level of a prototypical alternative exon cassette (E33 of the Fibronectin gene) is different between fibroblasts (higher rates of exon skipping) and keratinocytes (higher rates of exon inclusion), it is feasible that different repair modes exert different downstream signaling, hence affecting gene expression in different ways. In this sense, it has been reported that in fibroblasts, RNAPII stalling upon UV treatment modulates AS through an R-loop and ATM kinase (ataxia telangiectasia mutated) dependent mechanism [16]; while, on the other hand, we reported that in keratinocytes, ssDNA exposure and ATR kinase (ataxia telangiectasia mutated and Rad3 related), but not RNAPII stalling, are the key to control gene expression, as discussed in detail in the following section [15].

iii) DNA Repair as a driver for gene expression: Recent work from our laboratory has demonstrated that in human keratinocytes exposed to UV light, RNAPII is the target, rather than a damage sensor, of a signaling cascade initiated by repair, therefore adding a new layer of complexity in the coupling between DNA repair and gene expression (Figure 1C). We found that ssDNA exposed during NER-dependent repair of CPDs, the most abundant lesion upon UV exposure, activates the ATR kinase that indirectly favors the hyper-phosphorylation of RNAPII CTD [15]. Consequently, NER activity is controlling the phosphorylation status of RNAPII that, in turn, controls gene expression not only at the quantitative level (the amount of a given mRNA) but also at the qualitative level (different mRNA variants mainly obtained by AS). Photolyases are white-light-activated flavoenzymes, absent in placental mammals, that quickly and specifically revert the different lesions generated by UV irradiation. Using these enzymes as a tool, we have provided direct proof that UV-induced CPDs are sufficient to initiate the gene expression response in skin cells as photolyase-mediated removal of CPDs abolished the UV-induced RNAPII hyper-phosphorylation and therefore the modulation of gene expression. Moreover, the UV effect was enhanced by inhibition of gap-filling DNA synthesis, the last step in NER, supporting the role of ssDNA in the activation of a ATR-dependent signaling cascade

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controlling RNAPII phosphorylation. On the contrary, the UV effect on gene expression was reduced in the absence of DDB2/XPE, the main GG-NER sensor of CPDs, strongly suggesting that less recognition would generate less ssDNA intermediates and consequently a decreased UV effect on gene expression.

ATR activation in NER deficient cells

ATR was originally identified as a key factor controlling DNA replication in S phase and it was later shown to be activated throughout the cell cycle by ssDNA generated during NER [17-21]. Nevertheless, ATR activation modes, targets and affected pathways appear to be much more complex than previously anticipated. ATR has been shown to be activated by osmotic and mechanical stress [22] and even in the absence of DNA damage [23, 24]. Moreover, ATR functions are not restricted to the nuclei [25] and known ATR targets are also not restricted to nuclei nor to factors involved in the DNA damage response [26-28].

ATR activation in NER deficient cells is controversial, with reports presenting enhanced or reduced ATR activation when repair is compromised. The best example of such a discrepancy was provided by Marini et al., who described that XPD mutant non-cycling fibroblasts obtained from patients with different clinical features phosphorylate Chk1, a well-established ATR substrate, at low or high levels depending on the XPD mutation [29]. This implies that apart from the common features of XP patients directly related to their impairment in lesion repair, as is the case for their increased risk of developing skin cancer, some other clinical features may involve downstream signaling. In line with this, Godon et al., showed that XPD mutant cells with high Chk1 phosphorylation levels also present high levels of ssDNA upon UV exposure [30], strongly implying that NER-dependent downstream signaling is ssDNA- and ATR-dependent.

NER dependent signaling: another layer of complexity to explain XP complex phenotypes

Considering what has been discussed so far, it is conceivable that defects in lesion recognition factors (DDB2/XPE and XPC) or in effector factors (XPA, XPB, XPD, XPG and XPF) could have, apart from the well documented inability for proper DNA repair, different levels of ssDNA exposure and hence ATR activation. This scenario would ultimately lead to diverse or even contrasting ATR-dependent cell responses, including not only classical ATR outputs but also the modulation of gene expression patterns. It is feasible that impairment in lesion recognition doesn't expose much ssDNA while the opposite might be true for defects in effector factors, or at least for some mutations, as discussed in more detail for XPD.

As it has been reviewed previously [31, 32], there is a high degree of clinical heterogeneity in different XP complementation groups, in particular when mutations affect effector factors (XPA, XPB, XPD, XPG and XPG) but not recognition factors (DDB2/XPE and XPC). It has been shown that mutations in DDB2/XPE and XPC lead to classical XP phenotype. However, mutations in effector factors lead, in some cases, to XP with neurological abnormalities or the combined features of

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XP with other repair disorders such as Cockayne syndrome (CS), Trichothiodystrophy (TTD), cerebral-ocular-facial-skeletal syndrome (COFS) and others [31, 32]. One possible argument could be that mutations in effector factors, but not in recognition factors, will also be detrimental for repair in actively transcribed genes with complex phenotypic outcomes. While this is of course possible, in human keratinocytes, where most of repair depends on GG-NER, differential gene expression responses were observed when inhibiting lesion recognition or lesion repair ([15] and unpublished results). In line with this observation, it is feasible that defects in effector factors could lead, in certain cases, to more ssDNA upon incomplete repair, and therefore to enhanced ATR-dependent signaling (Figure 1C). Having in mind that NER processing of a DNA lesion activates a signaling pathway that regulates gene expression, we speculate that some of the complex XP phenotypes are related to ssDNA exposure, ATR activation and finally RNAPII phosphorylation. It is expected that ATR promotes RNAPII CTD phosphorylation indirectly because there are no recognizable ATR target sequences in the CTD and, to date, the main Ser/Thr kinases shown to directly phosphorylate the CTD are CDK7, CDK9, CDK12, and CDK13 [2, 33].

Finally, there are some clinical features that are hard to understand only in terms of compromised DNA repair. For instance, whereas all XP patients show an increased risk of developing skin cancer, patients with defects in GG-NER recognition factors DDB2/XPE and XPC, plus the XPV group, don't develop blistering burns upon minimal sun exposure while the opposite is true for patients with defects in XPA, XPB, XPD and XPG [31]. In line with this, DDB2/XPE, XPC and XPV patients don't develop neurological abnormalities while, again, the opposite is true for XPA, XPB, XPD, XPG and XPF patients [31]. While it is clear that all of these patients have an increased risk of developing skin cancer, other puzzling clinical features can be grouped in terms of defects in recognition factors or in effector factors. These facts might be teaching us a lesson about altered ssDNA-dependent downstream signaling affecting gene expression. This new layer of coupling between repair and the control of RNAPII phosphorylation status should be taken into account when analyzing clinical features of XP patients.

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Figure legend

Figure 1: *Different modes of coupling between Nucleotide Excision Repair and Gene Expression*

A. *Common factors shared by transcription and DNA repair:* Apart from the well documented roles of TFIIH and its helicases subunits XPB and XPD, other NER factors such as XPC, XPA, XPF and XPG showed to be recruited to promoters of inducible genes in the absence of exogenous genotoxic attack.

B. *RNAPII as a DNA damage sensor:* In TC-NER a stalled RNAPII in front of a lesion serves as a DNA damage sensor favoring the recruitment of NER factors and hence the preferential repair of template strand in actively transcribed genes.

C. *DNA Repair as a driver for gene expression:* RNAPII is the target of a signaling cascade initiated by repair. ssDNA generated during repair activates ATR that in turn regulates RNAPII CTD phosphorylation status therefore affecting transcriptional properties, such as elongation rates, and hence gene expression patterns. Different mutations in NER effector factors could lead to different levels of ssDNA exposure and ATR activation.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed

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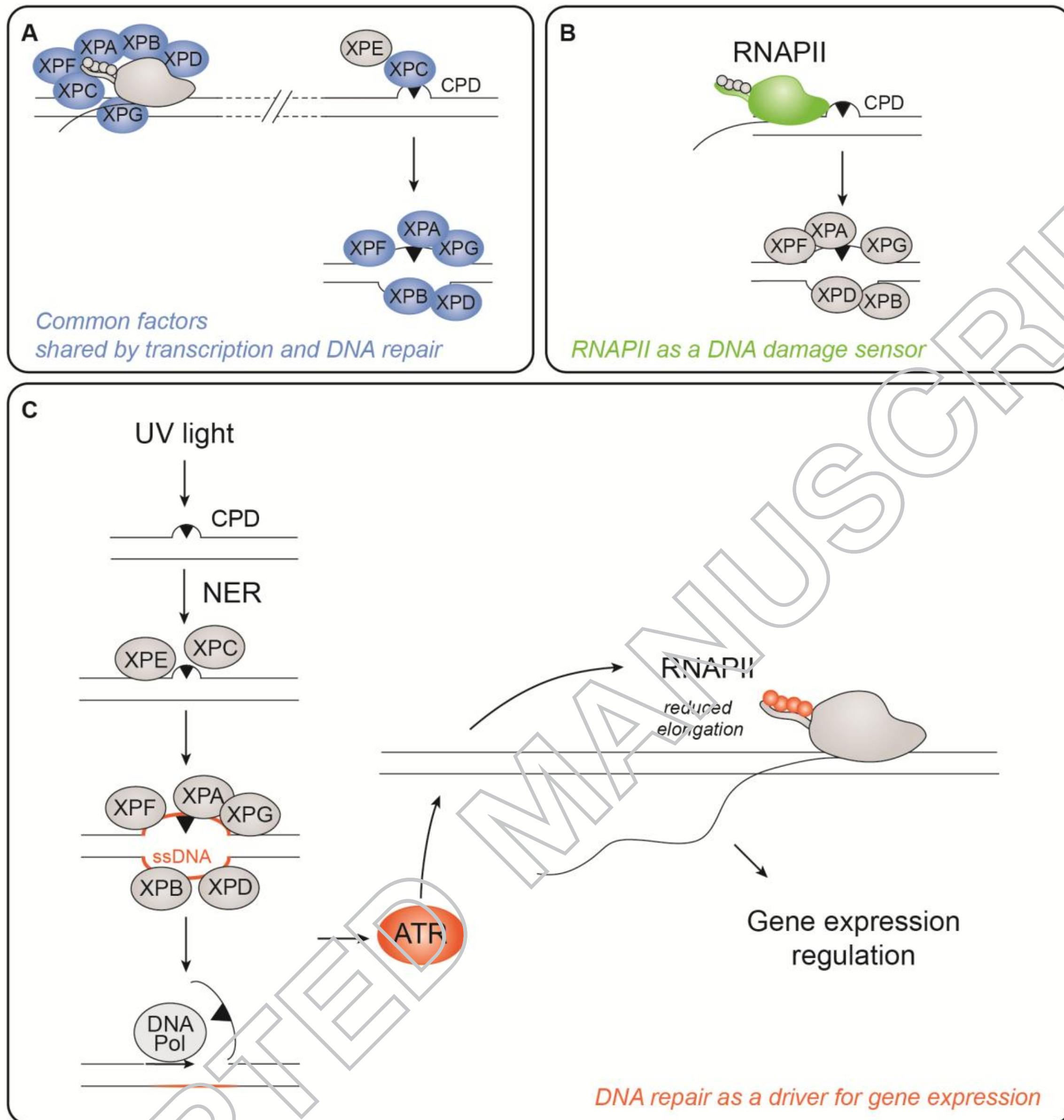

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