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Review

Arresting transcription and sentencing the cell: The consequences of blocked transcription

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ABSTRACT

Bulky DNA adducts induced by agents like ultraviolet light, cisplatin and oxidative metabolism pose a block to elongation by RNA polymerase II (RNAPII). The arrested RNAPII can initiate the repair of transcription-blocking DNA lesions by transcription-coupled nucleotide excision repair (TC-NER) to permit efficient recovery of mRNA synthesis while widespread sustained transcription blocks lead to apoptosis. Therefore, RNAPII serves as a processive DNA damage sensor that identifies transcription-blocking DNA lesions. Cockayne syndrome (CS) is an autosomal recessive disorder characterized by a complex phenotype that includes clinical photosensitivity, progressive neurological degeneration and premature-aging. CS is associated with defects in TC-NER and the recovery of mRNA synthesis, making CS cells exquisitely sensitive to a variety of DNA damaging agents. These defects in the coupling of repair and transcription appear to underlie some of the complex clinical features of CS. Recent insight into the consequences of blocked transcription and their relationship to CS will be discussed.

1. Introduction

The human genome contains approximately 3 billion base pairs of DNA (Venter et al., 2001). Cell growth and division require the coordinated transcription of over 25 000 protein coding genes distributed throughout the genome (Venter et al., 2001). RNA polymerase II (RNAPII) is responsible for the production of messenger RNAs (mRNAs) and most microRNAs (miRNAs) that together transcriptionally and post-transcriptionally control protein expression (Cai et al., 2004; Lee et al., 2004; Wild and Cramer, 2012). The fact that cells are constantly exposed to both endogenous and exogenous sources of DNA damage poses a challenge for the coordinated regulation of gene expression because many RNAPII complexes will inevitably transcribe damaged DNA templates. The density of DNA lesions and their structural properties will influence the progression of RNAPII elongation complexes grossly affecting gene expression.

Ultraviolet (UV) light is one of the most prevalent environmental carcinogens. Cyclobutane pyrimidine dimers (CPD) and (6-4)-photoproducts (6-4PP) are the predominant lesions induced by UV light (Cadet et al., 2005). These helix distorting lesions pose a block to elongating RNAPII complexes with a single CPD or 6-4PP in the template strand of an active gene sufficient to block RNAPII (Francis and Rainbow, 1999; Mayne and Lehmann, 1982; Protic-Sabaji and Kraemer, 1985; Sauerbier and Hercules, 1978; Selby et al., 1997; Tornaletti et al., 1997). In contrast, these lesions positioned in the coding strand have very little impact on transcription (Hanawalt and Spivak, 2008). RNAPII complexes arrested at CPD and (6-4)PP lead to a dose-dependent decrease in the synthesis of nascent RNA but the stalled RNAPII can initiate the repair of transcription-blocking DNA lesions through a process called transcription-coupled nucleotide excision repair (TC-NER) (Hanawalt and Spivak, 2008; Leadon and Lawrence, 1991; Mellon et al., 1987; Sauerbier and Hercules, 1978). The arrested polymerase signals the assembly of a repair complex that ultimately leads to incisions on either side of the damaged nucleotides, release of the damaged oligonucleotide, resynthesis of the resulting single strand gap and ligation to yield an intact double stranded DNA (for review see Hanawalt and Spivak, 2008). Importantly, defects in the repair of transcription-blocking DNA damage are associated with several hereditary disorders, including Cockayne syndrome (CS).

2. Cockayne syndrome: a disease of DNA repair and transcription

2.1. Cockayne syndrome and related disorders

Cockayne syndrome (CS) is an autosomal recessive disorder characterized by a broad spectrum of clinical features including

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but not limited to photosensitivity (UV wavelengths), progressive neurological degeneration and premature-aging (Licht et al., 2003; Nance and Berry, 1992). Life-expectancy of CS patients is heterogeneous but averages approximately 12.5 years (Nance and Berry, 1992). Most CS cases (>60%) are caused to mutations in the excision repair cross complementing 6 gene (ERCC6) encoding CS group B protein (CSB) with most of the remaining cases resulting from mutations in ERCC8 encoding the CSA protein (Laugel et al., 2010). Fibroblasts derived from CS patients have a defect in TC-NER of UV-induced DNA damage (van Hoffen et al., 1993) indicating that the CSA and CSB proteins are required for this repair process. Importantly, there is also clinical and genetic overlap with UV-sensitive syndrome (UVsS) and xeroderma pigmentosum (XP) (Cleaver et al., 2009).

UVsS is an autosomal recessive disorder associated with clinical photosensitivity that can be also caused by specific mutations in either ERCC8 or ERCC6 but without the typical developmental symptoms associated with CS (Horibata et al., 2004; Nardo et al., 2009; Spivak, 2005). UVsS fibroblasts are also TC-NER deficient and they fail to recover RNA synthesis normally following UV-irradiation (Itoh et al., 1994; Spivak et al., 2002; Spivak, 2005). The similarity in TC-NER capacity of CS and UVsS fibroblasts but dissimilarity in clinical phenotype suggests that the TC-NER-defect alone cannot explain differences in the clinical presentation of these disorders.

XP is another rare autosomal recessive disorder characterized by clinical photosensitivity, changes in skin pigmentation but in contrast to both CS or UVsS, XP is associated with a 1000–2000 fold increase in the risk of UV-induced skin cancers (Cleaver et al., 2009; Kraemer, 1997; Moriwaki and Kraemer, 2001). Some mutations in the ERCC3, ERCC2 and ERCC5 genes encoding XP group B (XPB), XPD and XPG proteins give rise to a combined XP/CS disorder (Cleaver et al., 2009; Hoeijmakers, 2009). Unlike CS and UVsS, XP (including XP/CS) fibroblasts are not strictly associated with defective TC-NER. The non-transcribed strand and inactive regions of the genome are repaired through a genetically separable subpathway of NER termed global genomic NER (GG-NER) that differs in the rate limiting DNA lesion recognition step but proceeds through a common mechanism requiring XPB, XPD and XPG, as well as other proteins that are not associated with XP/CS (Hanawalt, 2002). As such, XP/CS fibroblasts have defects in both TC-NER and GG-NER (Hanawalt, 2002). Many XP patients (*i.e.* XP groups A and F) have NER defects comparable to XP/CS patients yet they do not have many of the clinical features associated with CS. Therefore, the full spectrum of developmental defects associated with CS is difficult to reconcile with TC-NER defects alone (de Boer et al., 2002; van der Pluijm et al., 2007). Importantly, CSA, CSB, XPB, XPD and XPG have all been linked to additional functions in transcription.

2.2. Cockayne syndrome and the recovery of RNA synthesis

CS and related syndromes (UVsS and XP/CS) are all associated with clinical photosensitivity and this is attributed to hypersensitivity to UV-induced apoptosis as a consequence of their TC-NER defect (Lagerwerf et al., 2011; Ljungman and Zhang, 1996; Ljungman and Lane, 2004; McKay et al., 1998, 2001a; Queille et al., 2001; van Hoffen et al., 1993; Venema et al., 1990). Specifically, fibroblasts derived from these patients are unable to preferentially repair the template strand of active genes by TC-NER and consequently, they fail to recover mRNA synthesis normally following UV exposure (Itoh et al., 1994, 1995; Itoh and Yamaizumi, 2000; Ljungman and Zhang, 1996; McKay et al., 2001a; Spivak et al.; van Hoffen et al., 1993; Venema et al., 1990). Failure to recover mRNA synthesis correlates with hypersensitivity to apoptosis following exposure to UV light, cisplatin and other agents that induce transcription-blocking DNA lesions (Andera and Wasylyk, 1997; Ljungman and Zhang, 1996; Ljungman et al., 1999; Ljungman, 2007; McKay et al., 1998, 2001a; Stubbert et al., 2010). Furthermore, non-genotoxic agents that inhibit transcription tend to be potent inducers of apoptosis (Andera and Wasylyk, 1997; Ljungman and Zhang, 1996; Ljungman et al., 1999; Ljungman, 2007). Taken together, CS, XP/CS and UVsS cells are all hypersensitive to apoptosis induced by a variety of DNA damaging agents due to their defect in TC-NER and failure to recover mRNA synthesis despite differences in their clinical features.

It has been recognized for many years that CS fibroblasts exhibit other defects in addition to TC-NER. For example, N-acetoxy-2-acetylaminofluorene (NA-AAF) induces bulky DNA lesions that, like CPD and 6-4PP, are repaired by both TC-NER and GG-NER (van Oosterwijk et al., 1996a). However, these DNA lesions are repaired so efficiently by GG-NER that there is no detectable defect in the repair of the transcribed strand of active genes in CS fibroblasts (van Oosterwijk et al., 1996b). Despite the removal of NA-AAF-induced lesions from the template strand of active genes in CS cells, they fail to recover mRNA synthesis normally and undergo apoptosis in response to lower concentrations of this drug (van Oosterwijk et al., 1996b, van Oosterwijk et al., 1998). Therefore, there can be discordance between repair of the transcribed strand and the recovery of mRNA synthesis and this implies that CS proteins have additional role(s) in transcription and/or the recovery of transcription following NA-AAF treatment (van Oosterwijk et al., 1996b).

2.3. Cockayne syndrome and transcription

More direct evidence of additional roles for CS proteins in transcription that are separable from its role in TC-NER has emerged. Extracts derived from CS-A, CS-B and XP-B (with XP/CS) cells exhibited reduced transcription of an 'undamaged' reporter gene, however reduced transcription was only observed when base damage was introduced during plasmid isolation (Dianov et al., 1997). Similarly, CSB protein can stimulate translesion RNA synthesis past some forms of oxidative DNA damage (Charlet-Berguerand et al., 2006). Intact and permeabilized CS-B fibroblasts have a reduced rate of RNA synthesis (Balajee et al., 1997). Collectively, these reports suggest that CS cells transcribe less efficiently from DNA containing oxidative DNA lesions. Their reported base excision repair defect likely compounds their transcription bypass deficit (Stevnsner et al., 2008).

CSA, CSB, XPB, XPD and XPG proteins have also been implicated in transcription by RNA polymerase I (RNAPI) (Bradsher et al., 2002). This latter effect appears to be associated with decreased initiation of RNAPI-dependent transcription, not elongation (Xie et al., 2012; Yuan et al., 2007). A defect in the initiation of transcription by RNAPII in UV-treated CS and XP/CS cells has also been reported (Proietti-De-Santis et al., 2006; Rockx et al., 2000; Velez-Cruz et al., 2012; Yamada et al., 2002). *In vivo* this leads to reduced expression of housekeeping genes (Proietti-De-Santis et al., 2006; Velez-Cruz et al., 2012). Taken together, CS and XP/CS fibroblasts appear to have one or more defects in transcription by RNAPI and RNAPII.

2.4. Mouse models of Cockayne syndrome

Mouse models of CS have been generated by gene targeting of the ERCC6 and ERCC8 genes (van der Horst et al., 1997, 2002). The CSB and CSA mice had very mild developmental and degenerative phenotypes despite their TC-NER defect so these mice did not model the disease well (van der Horst et al., 1997, 2002). Mice with combined CSA and CSB deficiency did not exhibit any further developmental, neurodegenerative or premature aging symptoms