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Navigating the Nucleotide Excision Repair Threshold

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Abstract

Nucleotide excision repair (NER) is the primary DNA repair pathway that removes helix-distorting DNA strand damage induced by ultraviolet light (UV) irradiation or chemical carcinogens to ensure genome integrity. While the core NER proteins that carry out damage recognition, excision and repair reactions have been identified and extensively characterized, and the NER pathway has been reconstituted *in vitro*, the regulatory pathways that govern the threshold levels of NER have not been fully elucidated. This mini-review focuses on recently discovered transcriptional and post-translational mechanisms that specify the capacity of NER, and suggests the potential implications of modulating NER activity in cancer prevention and therapeutic intervention.

Introduction

The genome of living organisms is under constant threat of DNA damage from endogenous and exogenous sources. Proper cellular responses to DNA damage, including suspension of the cell cycle and activation of specific DNA repair mechanisms, are essential to maintain genomic integrity. Among the DNA repair pathways, nucleotide excision repair is one of the most versatile DNA repair systems as it can eliminate a wide range of helix-distorting DNA lesions caused by UV irradiation and chemical mutagens (E.C.Friedberg, 2006). In humans, genetic defects in NER components result in xeroderma pigmentosum (XP), an autosomal recessive disorder characterized by photosensitivity and predisposition to skin cancer (Friedberg, 2001; Hoeijmakers, 2001). There are seven NER-deficient genetic complementation groups for XP (XP-A through XP-G), and the specific roles of the corresponding proteins in NER have been extensively investigated and reviewed (D. Bootsma, 2001).

There are two sub-pathways of NER: global genome NER (GG-NER) identifies and repairs lesions throughout the genome (Fig. 1), while transcription-coupled NER (TC-NER) removes damage from the transcribed strand of active genes (Hanawalt, 2002). The multi-step process of NER starts with DNA damage recognition, which marks the major difference between the two sub-pathways. TC-NER is triggered upon blockage of RNA polymerase II translocation at a DNA damage site (van den Boom et al., 2004), whereas GG-NER is evoked by specialized damage recognition factors, including the UV-damaged DNA binding proteins (UV-DDB) (Chu and Chang, 1988; Tang et al., 2000), the complex of XPA and replication protein A (RPA) (Asahina et al., 1994; Li et al., 1995), and the XPC-RAD23B heterodimer (Reardon et al., 1996). Recent studies suggest that the XPC-RAD23B complex plays a principle role in damage recognition (Sugasawa et al., 1998; Volker et al., 2001), but its actions are dependent upon functional UV-DDB in processing certain lesions such as UV-induced photoproducts on chromatin (Fitch et al., 2003; Moser et al., 2005; Nishi et al.,

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2009; Wang et al., 2004). The XPA-RPA and TFIIH complexes are then recruited to damage sites and verify the authenticity of the DNA lesions as bona fide NER substrates (Sugasawa et al., 2009).

The subsequent steps are carried out by a common set of NER factors that are shared by both sub-pathways and involve the unwinding of the DNA duplex around the lesion site by the TFIIH complex (Araujo et al., 2001; Evans et al., 1997; Schaeffer et al., 1994; Schaeffer et al., 1993; Weeda et al., 1990), followed by dual incision of the lesion-bearing DNA strand by two structure-specific endonucleases, ERCC1-XPF and XPG. Incisions are made 5' and 3' to the lesion, resulting in the release of a 24-32 oligonucleotide containing the damaged DNA (Matsunaga et al., 1995; O'Donovan et al., 1994; Sijbers et al., 1996). The single-stranded gap is subsequently filled by DNA polymerase δ/ϵ in the presence of proliferating cell nuclear antigen (PCNA) and replication factor C, and the nick left behind is sealed by DNA ligase I (Araujo et al., 2000; Moser et al., 2007; Shivji et al., 1995) (Fig. 1). The NER process is accomplished through the sequential actions of over 30 proteins, and regulation of these NER factors establishes the NER threshold.

Most of the genome is not actively transcribed at any given time. While GG-NER comprises the majority of NER activities between the two NER sub-pathways, TC-NER exhibits a more rapid repair rate on the actively transcribed DNA strand (Hanawalt, 2002). Another determinant of the NER capacity is the duration of cell cycle arrest upon DNA damage, which allows time for the repair to occur. Indeed, the duration of S phase entry delay triggered by the G1/S checkpoint is proportionately beneficial to the removal of DNA lesions (Grososky and Little, 1983; Konze-Thomas et al., 1982; Liu et al., 2009; Maher et al., 1979; Stone-Wolff and Rossman, 1982). Furthermore, prolonged cell cycle arrest caused by potent checkpoint activation leads to a dramatic change in the transcriptional program as well as post-translational modifications of NER factors, thereby resulting in more efficient removal of DNA lesions (Allen et al., 1994; Gasch et al., 2001; Sharma et al., 2007) (Bashkirov et al., 2000; Yao et al., 2003; Zhao and Rothstein, 2002). Cellular mechanisms regulating NER activities have been an area of intense investigation in recent years, and have been extensively reviewed (Adimoolam and Ford, 2003; Dip et al., 2004; Hanawalt, 2001; Maillard et al., 2008; Reardon and Sancar, 2005; Sugawara et al., 2009). Here, we will focus on recent findings of cellular pathways that modulate the NER threshold through regulation of rate-limiting NER factors.

Transcriptional regulation of NER

The biosynthesis of DNA damage sensors directly dictates the capacity of DNA lesion removal by NER. A critical threshold regulator of NER has been ascribed to the p53 tumor suppressor, which governs transcription and thus maintains basal-level expression of rate-limiting human DDB2 and XPC damage sensors under normal growth conditions (Adimoolam and Ford, 2003). p53-deficient cells contain reduced levels of DDB2 and XPC, and display diminished GG-NER activity, indicating a requirement for p53 in specifying the cellular capacity of GG-NER (Ford and Hanawalt, 1997) (Adimoolam and Ford, 2002; Hwang et al., 1999) (Smith et al., 1995). In contrast, p53 is dispensable for TC-NER, which employs a distinct damage recognition mechanism (Ford and Hanawalt, 1995; Prost et al., 1998; Zhu et al., 2000). The BRCA1 tumor suppressor also promotes transcription of *DDB2* in a p53-dependent manner following UV irradiation (Takimoto et al., 2002). Binding of p53 to the DDB2 promoter is enhanced by BRCA1, consistent with a cooperative effect of the two tumor suppressors in promoting repair of UV-induced CPDs (Navaraj et al., 2005).

In response to UV irradiation, p53 accumulates and, in turn, activates transcription of both DDB2 and XPC. Interestingly, there is at least a 16-hour delay in the transcriptional

upregulation and new synthesis of DDB2 and XPC (Adimoolam and Ford, 2002; Rapp-Otrin et al., 2002). The timing of p53-induced DDB2 synthesis occurs well after the instantaneous binding of pre-existing DDBs and XPC to DNA lesions and subsequent ubiquitination and proteasomal degradation of chromatin-bound DDBs, and parallels with the recovery phase of DDB2 and completion of NER. As such, p53-mediated transcriptional activation of DDB2 likely serves as a mechanism to replenish DDB2 consumed during damage recognition and following CUL4A-mediated degradation, rather than directly participating in the initial search for and identification of DNA lesions.

A third tumor suppressor that controls the DNA lesion recognition step of NER is the retinoblastoma (Rb) protein, which inhibits E2F1-mediated transcription (Prost et al., 2007). GG-NER is reduced in E2F1-deficient cells and enhanced in *Rb*^{-/-} *p107*^{-/-} *p130*^{-/-} MEFs, both of which are attributed to the role of E2F1 in the transcriptional activation of XPC and DDB2 (Lin et al., 2009). Indeed, E2F1 was found to bind directly to an E2F1-responsive element in the *XPC* promoter upon UV exposure, and augment *XPC* transcription. Collectively, it appears that three major tumor suppressors, p53, Rb, and BRCA1, play a role in maintaining genome integrity through regulation of the NER pathway in response to helix-distorting DNA damage.

Post-translational regulation of NER

Post-translational control of NER factors and DNA damage checkpoint regulators also determine the NER threshold. In particular, ubiquitination is one of several protein modifications that have drawn increasing attention in recent years. Rad23p in yeast and its human homolog hHR23B contain a N-terminal ubiquitin-like domain for proteasome binding and a C-terminal ubiquitin association (UBA) domain that is implicated in the delivery of ubiquitinated proteins for proteasome-mediated degradation (Watkins et al., 1993). Unexpectedly, the yeast XPC homolog Rad4p is degraded rapidly in the absence of Rad23, and association with Rad23 appears to protect against proteasome-mediated degradation of Rad4 (Lommel et al., 2002; Ng et al., 2003; Okuda et al., 2004), indicating an essential role for Rad23 in Rad4/XPC repair function (Batty et al., 2000; Sugawara et al., 1996). Mammals express two RAD23 homologues, HR23a and HR23b, which share structural similarity and redundant functions in NER (Ng et al., 2003). Simultaneous knockout of both murine *mHR23* genes results in markedly reduced XPC levels, leading to compromised NER after UV irradiation. Remarkably, increased Rad4 levels in Rad23-deficient cells restore efficient NER activity (Ortolan et al., 2004). Thus, mHR23 proteins play an important role in maintaining XPC stability via protection against proteasome-mediated degradation.

Post-translational modifications of proliferating cell nuclear antigen (PCNA) with mono- or poly-ubiquitin, or the ubiquitin-like protein SUMO contribute to the decision of which DNA repair or error bypass pathway is chosen to process DNA lesions during DNA replication (reviewed in (Ulrich, 2009)). In the absence of homologous recombination during the G1 phase of the haploid yeast *Saccharomyces cerevisiae* cell cycle, the repair of DNA interstrand cross-link damage requires both the NER pathway to cleave the lesioned strand and polymerase zeta ($\text{pol}\zeta$) to bypass the cross-linked oligonucleotides that remain at the incised intermediates. Ubiquitination of PCNA has been shown in the recruitment of $\text{pol}\zeta$ for translesion synthesis (Sarkar et al., 2006). In undamaged cells, the deubiquitinating enzyme USP44 prevents PCNA ubiquitination and recruitment of error-prone DNA polymerases, and is proposed as a safeguard mechanism against the mutagenic effects of these polymerases (Stegmeier et al., 2007).

Direct involvement of ubiquitin-dependent proteolysis in NER regulation was initially suggested by the detection of the physical association between DDB2 and CUL4A, a member of the cullin-RING family of ubiquitin ligases, and the finding that CUL4A controls DDB2 stability through ubiquitin-dependent proteolysis, thus restricting UV-DDB activity (Chen et al., 2001; Shiyonov et al., 1999). Using tandem affinity purification, Groisman and colleagues determined that DDB1 and DDB2 were integral components of the CUL4A ubiquitin ligase complex, and affect NER activity beyond their previously defined function in damaged DNA recognition (Groisman et al., 2003). Subsequent studies revealed that the CUL4A-Rbx1-DDB1-DDB2 ubiquitin ligase, presumably assembled on DNA damage sites, mediated both XPC ubiquitination to increase its affinity for DNA (Sugasawa et al., 2005), and histones H2A, H3 and H4 ubiquitination, which is believed to contribute to the alteration of local chromatin structure around the damage sites (Kapetanaki et al., 2006; Wang et al., 2006). DDB2 itself is also ubiquitinated by CUL4A (Sugasawa et al., 2005) (Chen et al., 2006), which likely occurs through an auto-ubiquitination mechanism frequently observed with other E3 ubiquitin ligases (Galan and Peter, 1999; Kao et al., 2000; Zhou and Howley, 1998). Ubiquitinated DDB2 has reduced affinity for damaged DNA and is targeted for proteasomal degradation. As such, DDB2 ubiquitination specifies the basal levels of DDB2 prior to DNA damage induction and directs the dynamic removal of DDB2 following the completion of damage recognition (Adimoolam and Ford, 2002; Chen et al., 2006; Chen et al., 2001; Nishi et al., 2009; Shiyonov et al., 1999). During TC-NER, CUL4A assembles an analogous ubiquitin ligase complex with Cockayne syndrome type A protein (CSA) and DDB1 to target Cockayne syndrome type B (CSB) for ubiquitination and degradation, which has been proposed as a means to terminate TC-NER and resume transcription elongation (Groisman et al., 2006).

Given that multiple substrates targeted by CUL4A are directly or indirectly involved in NER reactions, the impact of CUL4A on the final outcome of NER is complex and difficult to reconcile with any single target or mechanism. The identification of p21 as a CUL4A substrate further complicates the direct delineation of specific pathways that regulates repair capacity, as p21 is involved both in G1/S DNA damage checkpoint control, which specifies the time allotted for NER to act upon damaged DNA, and the expression of NER components (Abbas et al., 2008; Kim et al., 2008; Nishitani et al., 2008). To address the overall impact of CUL4A on specifying the NER threshold, Liu et al. employed the genetic approach of gene targeting in mice to ask whether and how CUL4A deletion affects NER capacity and the response to UVB-induced skin carcinogenesis (Liu et al., 2009). Primary *Cul4a*^{-/-} MEF cells accumulate 2-4 fold higher steady-state levels of both DDB2 and XPC proteins, and display ~20% higher GG-NER activity for both CPDs and 6,4-PPs, indicating an unexpected role for CUL4A in restricting NER activity in normal cells. This is consistent with the observation by Alekseev et al. that enforced ectopic expression of DDB2 enhances NER and protects mice against skin carcinogenesis induced by chronic UV-B irradiation (Alekseev et al., 2005). Moreover, the duration of the G1/S DNA damage checkpoint was extended 4-6 hours in *Cul4a*^{-/-} MEFs as a result of increased p21 accumulation. Strikingly, skin-specific *Cul4a* knockout mice are hyper-resistant to UVB-induced squamous cell carcinomas. Collectively, restriction of the GG-NER threshold is decided by the net outcome of CUL4A-mediated ubiquitination events, and abrogation of CUL4A confers remarkably increased protection against UVB-induced skin tumorigenesis.

CUL4A ubiquitin ligase activity is regulated by a number of cellular pathways and mechanisms. Groisman et al. identified the physical association between CUL4A and the COP9 signalosome (CSN), a multimeric protein complex that is thought to reduce cullin-based E3 ligase activity through removal of the activating Nedd8-modification (Groisman et al., 2003). Inactivation of CSN subunits by RNAi led to deficiencies in unscheduled DNA synthesis, an indirect measurement of NER activity. On the other hand, Chen and colleagues

identified the c-Abl non-receptor tyrosine kinase as a potent activator of the CUL4A ubiquitin ligase, as it plays a critical role in the efficient ubiquitination of DDB2 by CUL4A under both normal conditions and upon UV irradiation to degrade chromatin-bound DDB2 (Chen et al., 2006). Notably, c-Abl exerts its stimulatory effect on CUL4A E3 ligase activity in a kinase-independent manner. In MEF cells deficient for the c-Abl family of kinases, CUL4A failed to degrade chromatin-bound DDBs following UV exposure. The resulting increase in the duration of damage recognition by DDBs correlates with dramatic enhancement of GG-NER activity, and is attributed to the lack of CUL4A activation by c-Abl.

Additional post-translational modifications regulate the NER threshold, as the stress-responsive p38 MAP kinase promotes DDB2 ubiquitination through serine phosphorylation following UV irradiation (Zhao et al., 2008). p38 also stimulates histone H3 acetylation, leading to chromatin relaxation, possibly to allow XPC and TFIIH access to the sites of DNA damage. Unlike c-Abl, the kinase activity of p38 MAPK is essential for efficient GG-NER. The precise mechanism by which p38 exerts its regulation on DDB2 and histone H3 remains to be determined.

Chromatin structure is also regulated by high mobility group (HMG) proteins, and their binding to damaged DNA has diverse effects on NER (reviewed in (Reeves and Adair, 2005)). HMGN is thought to facilitate NER by loosening nucleosome packing, thus allowing NER proteins to bind the damaged DNA (Birger et al., 2003). In contrast, HMGA and HMGB have been reported to inhibit NER, possibly by blocking access of repair proteins to UV-induced lesions (Adair et al., 2005; Huang et al., 1994). More recently, however, HMGB was found to enhance NER by mediating histone acetylation following DNA damage, resulting in chromatin reorganization that is a prerequisite for NER to proceed (Lange et al., 2008). Further studies will clarify the role of these chromatin-binding proteins in DNA damage response.

Circadian regulation of XPA in NER

A recent finding by Kang et al. indicates a role for circadian control of the DNA damage sensor XPA (Kang et al., 2009). The Clock and Bmal1 transcription factors establish the circadian clock through an autoregulatory loop. Transcriptional activation of Cryptochrome (Cry) and Period (Per) genes by the Clock-Bmal1 complex results in the feedback inhibition of Clock-Bmal1, and thus represses further Cry and Per expression (reviewed in (Reppert and Weaver, 2002)). The delay between Cry and Per expression and their negative autoregulation allows for activation of their transcriptional targets in an oscillatory manner.

Transcription of the XPA gene is positively regulated by the Clock-Bmal1 complex in mammalian brain and liver. (Kang et al., 2009). Additionally, peak NER activity coincides with maximum XPA protein levels, indicating the importance of XPA in determining the NER capacity. It is noteworthy that circadian oscillation of XPA levels was not uniformly observed in all tissue types (e.g. absent in testis), and may contribute to a range of NER thresholds among different tissues. Coupled with circadian transcriptional regulation, XPA protein is subjected to constitutive ubiquitin-dependent degradation by the HERC2 ubiquitin ligase, independent of circadian oscillation, thus ensuring the prompt removal of XPA protein (Kang et al., 2009). This study revealed a novel function of XPA in setting the NER threshold according to the circadian clock. Further elucidation of the circadian regulation of NER has immediate implications for the administration of DNA damage-inducing chemotherapeutics, such as cisplatin, as therapeutic efficacy may be maximized when NER activity is lowest.

Conclusions and Perspectives

The extensive functional investigation of individual NER proteins and the successful *in vitro* reconstitution of NER reactions using recombinant proteins have established the framework of this major DNA repair pathway. Recent studies further revealed a host of cellular pathways that regulate NER capacity. While wild-type organisms are often perceived as best-equipped or adapted to respond to DNA damage, genetic and biochemical studies have led to the surprising realization that wild-type cells are restricted in their ability to launch the maximal NER response to assaults on the genome. Enforced expression of rate-limiting NER factors (e.g. DDB2) or disabling of inhibitory pathways (e.g. CUL4A) allows for elevation of the NER threshold and confers increased protection against carcinogenesis in mouse models (Alekseev et al., 2005; Liu et al., 2009). It is noteworthy that murine cells possess less robust NER activity than human cells. As such, the extent to which manipulating NER activities will benefit humans in DNA damage response remains to be established.

While enhancing NER confers increased protection against the accumulation of DNA lesions and maintains genome integrity, reducing the NER threshold may be beneficial for cancer patients undergoing chemotherapy to ensure the efficient action of DNA damage-inducing drugs. The newly identified circadian oscillation of XPA expression sheds light on an optimal time window for drug administration. In this regard, transient suppression of NER through pharmacological manipulation of core NER factors or regulatory pathways is anticipated to synergize with DNA damaging agents to optimize the chemotherapeutic outcome.

A wide range of transcriptional and post-translational regulatory mechanisms of NER factors has been revealed recently, providing attractive targets to adjust the NER threshold. Detailed mechanistic understanding of these regulatory pathways will be necessary to guide genetic and chemo-biological manipulations for research and disease intervention.

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Fig. 1. Regulatory mechanisms that establish the mammalian GG-NER threshold following UV exposure

The schematic diagrams depict both transcriptional and post-translational pathways that modulate GG-NER factors and threshold output of NER activity.