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Transcriptional and post-transcriptional regulation of nucleotide excision repair genes in human cells

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ABSTRACT

Nucleotide excision repair (NER) removes DNA helix-distorting lesions induced by UV light and various chemotherapeutic agents such as cisplatin. These lesions efficiently block the elongation of transcription and need to be rapidly removed by transcription-coupled NER (TC-NER) to avoid the induction of apoptosis. Twenty-nine genes have been classified to code for proteins participating in nucleotide excision repair (NER) in human cells. Here we explored the transcriptional and post-transcriptional regulation of these NER genes across 13 human cell lines using Bru-seq and BruChase-seq, respectively. Many NER genes are relatively large in size and therefore will be easily inactivated by UV-induced transcription-blocking lesions. Furthermore, many of these genes produce transcripts that are rather unstable. Thus, these genes are expected to rapidly lose expression leading to a diminished function of NER. One such gene is *ERCC6* that codes for the CSB protein critical for TC-NER. Due to its large gene size and high RNA turnover rate, the *ERCC6* gene may act as dosimeter of DNA damage so that at high levels of damage, *ERCC6* RNA levels would be diminished leading to the loss of CSB expression, inhibition of TC-NER and the promotion of cell death.

1. Introduction

Ultraviolet (UV) light is a strong mutagen and has promoted natural selection among organisms throughout evolutionary time. Nucleotide excision repair (NER) evolved to safeguard the DNA from the deleterious effects of UV light and can be found in all species of life from bacteria and plants to mammals [1]. NER also protects cells from cyclopurines formed by endogenous reactive oxygen species (ROS) and cancer cells use NER to repair damage induced by certain chemotherapeutic agents such as cisplatin. A better understanding of the regulation of expression of NER genes could aid in predicting the sensitivity of cells to UV light and chemotherapeutic agents and may promote the development of new therapeutic regiments.

Global genomic NER (GG-NER) deals with lesions in the whole genome. The lesion recognition complex in GG-NER, consisting of XPC, RAD23A, RAD23B, CETN2, DDB1 and DDB2, acts by recognizing the DNA lesions in chromatin and recruits the core NER complex to sites of damage (Fig. 1) [1–3]. A specialized damage recognition step of NER has evolved to aid in the removal of bulky lesions that block transcription elongation [4]. This sub-pathway of NERis called transcription-coupled NER (TC-NER) where Cockayne's syndrome factors A (CSA) and B (CSB), XAB2 and UVSSA factors orchestrate the recruitment of the NER core complexes to sites of transcription-stalling lesions. Following damage recognition, which is the rate-limiting step, the pre-incision complex consisting of XPA and RPA verifies the presence of the lesion followed by the DNA unwinding by the TFIIH complex. The damaged strand is then incised by the incision enzymes XPG, ERCC1 and XPF, DNA polymerases re-synthesize the DNA in the excised gap and DNA ligase 1 seals the newly synthesized strand with existing strand (Fig. 1).

Mutations in core components of NER leads to the human disorders xeroderma pigmentosum and trichothiodystrophy [5] while defects in the factors responsible for TC-NER give rise to the Cockayne's and UV-sensitive syndromes [6]. Polymorphisms in NER genes have been linked to reduced repair capacity and cancer

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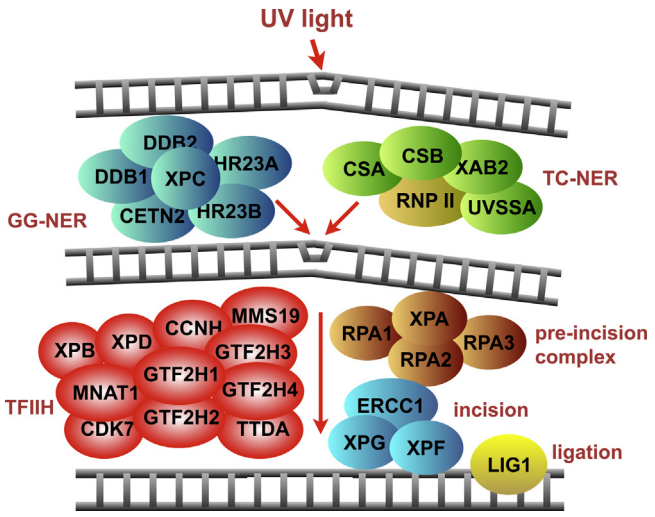

Fig. 1. The 29 gene products of NER that were examined in this study and their roles in NER.

predisposition [7]. Furthermore, inactivating somatic mutations of the NER genes ERCC2, ERCC3, ERCC4, ERRC5, XPA, XPC and DDB2 promote cancer and therefore these genes are known as cancer predisposition genes [8]. Many studies have also found a correlation between the expression level of DNA repair genes in cancer cells and their sensitivity to cisplatin [7]. Interestingly, a low level of expression or a defect in the GG-NER factors XPC and DDB2 does not sensitize cells to cisplatin or UV light while reduced expression or defects in the TC-NER factors CSA and CSB results in a marked sensitivity [9,10].

The expression of NER genes have been previously analyzed in cell lines using total cellular RNA, which reports on the steady-state level of RNA but does not distinguish between the contribution of synthesis and turnover of RNA to RNA homeostasis. In this study, we used Bru-seq and BruChase-seq [11,12] to specifically examine the rate of RNA synthesis and turnover of NER transcripts across 13 human cell lines. These techniques are based on the pulse-labeling of nascent RNA with bromouridine (Bru) followed by either immediate harvest (Bru-seq) or harvest after a 6-h chase in uridine (BruChase-seq). The Bru-labeled RNA is then isolated using anti-BrdU antibodies conjugated to magnetic beads, converted into a cDNA library and deep sequenced. Surprisingly, our results show that many critical NER genes produce fairly unstable transcripts that would be expected to quickly vanish following induction of transcription-blocking lesions by UV light and bulky chemical adducts – the very adducts NER was designed to remove.

2. Materials and methods

2.1. Cell lines

The cell lines used were HF1 (gift from Mary Davis, University of Michigan), HPNE, Panc1, MiaPaCa2, BxPC3, UM16, UM28, UM59 and HEK293 (gifts from Diane Simeone University of Michigan), HeLa, GM12878 and K562 (ATCC) and LN428 (gift from Rob Sobol, University of Pittsburgh). The media used were MEM with 10% FBS and antibiotics (HF1), DMEM with 10% FBS and Pen/Strep (Panc1, MiaPaCa2, UM16, UM28, UM59, HEK293), RPMI1640 with 10% FBS and antibiotics (BxPC3), F12K with 10% FBS and Pen/Strep (HeLa), RPMI1640 with 15% FBS (GM12878), IMDM with 10% FBS (K562), and MEMalpha with 10% FBS, antibiotics, gentamycin and puromycin (LN428). The HPNE cells were grown in 75% DMEM without glucose (Sigma D-5030) containing 2 mM L-glutamine (Sigma G7513), 1.5 g/L sodium bicarbonate (Sigma S 4019), 25% Medium M3 Base (Incell Corp, M300F-500) with the additives 10 ng/mL human recombinant EGF (BD Sciences 354052), 5.5 mM D-glucose (1 g/L) (Sigma G8644) and 750 ng/mL puromycin dihydrochloride (Invitrogen A11138-02).

2.2. Bru-seq and BruChase-seq

For detailed descriptions of these techniques, please see [11,12]. In short: the labeling of nascent RNA was performed for 30 min at 37 °C with 2 mM bromouridine in conditioned medium. For the BruChase-seq experiments, the bromouridine-containing medium was removed after 30-min labeling, the plates were rinsed twice in PBS and then conditioned medium containing 20 mM uridine was added. The cells were then incubated for 6 h at 37 °C. At the completion of the labeling +/− chase, the cells were lysed in TRIzol and the Bru-containing RNA was isolated using anti-BrdU antibodies conjugated to magnetic beads. The isolated RNA was converted into cDNA libraries using the Illumina True-seq library kit followed by deep sequencing to around 50 million single-end 50 nucleotide reads.

2.3. Analysis

Bru-seq and BruChase-seq gene expression was measured in transcripts per million reads (TPM) in order to compare gene expression across cell lines. The TPM of each gene can be thought of as the percent of expression associated with a gene in relation to the total expression of the genome [13]. The TPM formula was slightly modified and implemented as:

$$\frac{\text{transcript read count} \times 10^9}{\text{transcript length} \times \text{sum of all transcript densities}}$$

Where transcript densities are defined as:

$$\frac{\text{transcript read count}}{\text{transcript length}}$$

The natural logarithm of TPM measurements corresponding to the 29 NER genes was used in the analysis. When plotting Bru-seq and BruChase-seq data, genes with expression values equal to zero were represented in black. The stability measurement of each transcript is calculated by dividing the exonic reads at 6 h by the reads across the entirety of the gene at 0 h. Since it is impossible to calculate a stability value for a gene with no synthesis expression, we left such cases displayed as white boxes in Fig. 4B.

3. Results

The total, steady-state level of RNA in cells is a reflection of the equilibrium between synthesis and degradation of RNA. We previously showed that the expression of different human genes appear to have their unique ratios of RNA synthesis and degradation suggesting that both of these processes are coordinately regulated by cells to obtain defined, homeostatic, levels of RNA [12]. The particular settings of the ratios of synthesis and degradation of RNA from NER genes are not known. Since many of the adducts that NER has been designed to remove from DNA have the capacity to block the elongation of transcription, the ratio of synthesis and stability of a particular RNA may have a profound effect on the steady-state level of its expression following DNA insult. For example, a setting of high synthesis coupled with low stability would rapidly lead to the depletion of this RNA while a gene with the strategy of low synthesis and high RNA stability would fare much better at times of exposure to transcription-blocking DNA damage. Here we present the signatures of RNA synthesis and stability of 29 NER genes using Bru-seq and BruChase-seq across 13 human cell lines.