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Mfd and UvrD proteins: essential mediators to the maintenance of life.

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Abstract: In our bodies, DNA is damaged for a variety of genotoxic agents including UV radiation in sunlight, and thus DNA-repair systems are fundamental to the maintenance of life. In human cells, this damage is removed exclusively by the nucleotide excision repair mechanism (NER). NER can be divided into two subpathways: global genomic NER (GG-NER or GGR) and transcription coupled NER (TC-NER or TCR). In transcription-coupled repair (TCR), NER occurs most rapidly in the template strand of actively transcribed genes. This work is focused in the use of eXcision repair-sequencing (XR-seq), an excision repair sequencing methodology to map the location of repair sites in different *Escherichia coli* (*E. coli*) strains. Using XR-seq, Adebali et al. have dissected the accurate role of two important excision repair proteins, Mfd and UvrD, confirming their role in repair of UV-induced damage. Genome-wide analysis of the transcribed strand/nontranscribed strand (TS/NTS) repair ratio demonstrated that, deletion of mfd globally shifts the distribution of TS/NTS ratios downward by a factor of about 2 on average for the most highly transcribed genes. These results indicate that Mfd-dependent TCR is widespread in the *E. coli* genome, whereas UvrD plays a role in excision repair by aiding the catalytic turnover of excision repair proteins.

Keywords: Transcription-coupled repair (TCR), *Escherichia coli* (*E. coli*), Nucleotide excision repair (NER), Mfd, UvrD, eXcision repair-sequencing (XR-seq).

Introduction

DNA damage is an important event in the initiation and progression of cancer. Lesions in DNA at the time of replication may be mutagenic. It has been shown that defects in this critical response to DNA damage underpin a wide array of human pathologies that include cancer predisposition, immune dysfunction, radiosensitivity, neurodegenerative disorders and aging, as well as cardiovascular diseases (1). All organisms have elaborate cellular responses to DNA-damaging agents, including both tolerance and repair mechanisms (2). The critical components of the cellular response to DNA damage include the repair pathways dedicated to correct damage or errors in DNA. Nucleotide excision repair (NER) is a versatile pathway that recognizes and removes a wide spectrum of DNA lesions (3). The basic strategy of this essential repair pathway is conserved from *E. coli* to humans, but the proteins are not conserved and there are some differences in the mechanistic details (4).

DNA repair is modulated by transcription and condensation into chromatin. Transcription stimulates excision repair in *E. coli* and humans in a protein dependent process called transcription-coupled repair (TCR) (5). TCR is a subpathway of nucleotide excision repair that acts specifically on lesions in the transcribed strand of expressed genes (TS) (or “template strand”) of a transcription unit. Transcription-coupled repair is relative to the non-transcribed strand (NTS) (or “coding strand”) of the genome (6, 7). Extending the analysis of the mechanism(s) underlying TCR, in previous works Selby and Sancar have used purified proteins to study the *E. coli* RNA polymerase (RNAP) role in repair *in vitro*. They observed, using cyclobutane pyrimidine dimer (CPD), that during TCR, the RNA polymerase elongation complex (EC) first encounters a lesion in the transcribed strand of a gene (7). If the RNAP remains arrested at the lesion, it prevents repair. Nevertheless, it has been shown that RNAP is an essential participant in TCR (7). In previous works, Selby and Sancar have also

shown that the Mfd protein, originally known as the transcription repair coupling factor (TRCF), when present, binds to the stalled RNAP–RNA–DNA ternary complex. The translocation activity of Mfd moves both proteins (RNAP and Mfd) toward the damage, which causes a collapse of the transcription bubble, leading to displacement of the stalled RNAP along with the nascent transcript from the site (8, 9). The interaction between Mfd and the arrested RNAP activates Mfd. Then, Mfd recruits UvrA, which attracts UvrB. Finally, the assembled UvrAB* complex initiates repair (10-14). As a result, the transcribed strand of the gene is repaired more rapidly than the non-transcribed strand or the genome overall. Therefore, the rate of excision repair of the TS is the rate-limiting step in excision repair due to the damage recognition step.

One of the remaining questions about TCR is exactly how the rate of repair is enhanced, considering that more enzymatic steps are involved in TCR than in global genomic repair (GGR). Biochemical and structural studies and single molecule assays have confirmed and refined the original model for the role of Mfd in TCR, suggesting that Mfd-catalyzed TCR is one of several pathways for TCR in *E. coli*. However, these studies were based largely on genetic data and indirect readouts for TCR (15). To resolve this issue, Jinchuan Hu and colleagues have developed a methodology called eXcision repair sequencing (XR-seq) (16). XR-seq is a genome-wide sequencing assay to map DNA excision repair, producing nucleotide-level resolution of repair for two types of ultraviolet light-induced damage, cyclobutane pyrimidine dimers and 6, 4-pyrimidine-pyrimidone photoproducts. When UV or some other agent damages the genome, the site is cut out in a fragment of DNA, which is usually then bound by the protein TFIIH. Using an antibody to bind that enzyme, Jinchuan Hu and colleagues have been able to isolate the fragment-protein complexes and pull them out of cells to sequence them (16).

Using XR-seq to detect and map the overall excision of damage from the entire *E. coli* genome, Abedali and colleagues have revealed the extent to which Mfd contributes to TCR in *E. coli* in strains with defined genetic mutations (17). To map the sites of DNA excision repair throughout the genome at nucleotide resolution, products have been analyzed by high-throughput sequencing after they were ligated to adaptors, immunoprecipitated again with anti-CPD antibody, photoreactivated, amplified by PCR, and gel-purified. The sequences of the excised oligomers were determined by next-generation sequencing (NGS) technology and aligned with the *E. coli* chromosome. Mapping the fragments back onto the genome then revealed where the DNA repair takes place (For a scheme of the methodology, reference 17, Figure 1).

Authors' Results

The un-sticker: Mfd

This work has been largely focused on Mfd translocase, a protein known from prior work by Sancar and Selby, to have a special and an unusual mechanistic role in excision repair in bacteria (10). In order to decipher a more accurate role of Mfd protein during TCR, Adebali et al. have used XR-seq to map genome-wide repair, and thus genome-wide transcription-coupled repair in *E. coli*, using at the same time different mutants to analyze the contributions of candidate proteins to TCR. To date, TCR, as TS/NTS repair ratios, has been measured in a number of systems. Interestingly, TCR ratios from a large number of *in vitro* studies, which use different transcriptional units, usually have shown high ratio (above 4) of TS/NTS repair in the region studied (7, 10, 18). In contrast, Abedali et al. using eXcision repair-sequencing (XR-seq) method, have demonstrated that deletion of mfd globally shifts the distribution of TS/NTS ratios downward by a factor below 2 on average for the most highly transcribed genes. This result demonstrates that Mfd accounts for a much

higher rate of excision repair on strands of DNA that are being actively transcribed.
According to the authors, the high levels of TCR previously reported could be due to different factors; (1) for instance, in this study the ratio of thymine dimer sites (TT sites) in the template/coding strand have been corrected to reflect repairs per strand, which effectively lowers the TS/NTS repair ratio; (2) Abedali and colleagues claim that another possible source of bias in some of the previous studies is the use of simple transcription units and defined *in vitro* conditions that enable TCR experiments. Besides these factors, other uncharacterized factors, such as DNA binding proteins, or the use of strains with different genetic backgrounds, may also limit TCR and add variability to the measurements of transcription and repair. In this study, as the authors claimed, alterations to gene expression induced by UV were not taken into account in the transcription measurements.

The unwinder: UvrD.
In further experiments, the researchers have defined the role of the accessory excision repair protein in *E. coli*, UvrD. UvrD is a helicase with DNA-dependent ATPase activity that unwinds DNA duplexes with 3' to 5' polarity with respect to the bound strand and helps clear away each excised segment of damaged DNA (18). Using XR-seq on UV-damaged *E. coli* cells, the authors have found that without UvrD, excised DNA fragments remain stuck to chromosomal DNA. Analysis of nucleotide repair products from *E. coli* by excision assay performed in this work showed that, in *uvrd* mutant cells, the excised DNA fragments survive much longer by holding onto excision repair proteins and slowing down the overall rate of excision repair. This fact makes it hard for enzymes to remove the DNA fragments for cellular waste-disposal. As a consequence of the retention of the DNA fragments onto the DNA, Abedali et al. have demonstrated that the repair proteins that excised the strand tend to remain stuck to it. As a consequence of this, they are kept from moving on to excise other bits of damaged DNA. Abedali and colleagues have showed that UvrD's job is to unwind these damaged and discarded strands from chromosomal DNA, so that they can be disposed quickly and the associated repair proteins can go on to catalyze additional rounds of repair.

On the other hand, according to XR-seq results and RNA-seq patterns at individual gene resolution, the lack of UvrD slightly pushed the distribution of TS/NTS ratios to higher ratios. Putting all these results together, authors indicate that Mfd is the transcription repair-coupling factor whereas UvrD, plays a role in excision repair by aiding the catalytic turnover of excision repair proteins.

Model for the TCR in *E.coli* and Discussion.
The model for the nucleotide excision repair in *E. coli* controlled by Mfd and Uvr proteins proposed by the authors suggests: RNAP stalls at damage sites in the template strand, and the stalled complex recruits Mfd at a relatively fast rate. Mfd, due to its translocase activity, releases the nascent transcript and dissociates RNAP from the template. RNAP remains bound to the Mfd–DNA complex, in which Mfd assumes a conformation that recruits UvrA2B1 by binding to UvrA at about a 20- to 200-fold faster rate than the direct recruitment of UvrA2B1 to sites of damage (global repair). This recruitment is coupled with the loading of UvrB onto the transcription-blocking damage and release of RNAP, Mfd, and UvrA, which subsequently dissociate to component proteins. Then, UvrC binds to the UvrB–DNA complex and makes the dual incisions, which is followed by displacement of the excised oligomer and UvrB and UvrC from the repair site by the UvrD helicase.

In addition to clarifying the roles of Mfd and UvrD, this work generally heralds the use of the new XR-seq technique in mapping and studying excision repair processes. Nucleotide excision repair is the sole mechanism for removing bulky adducts from the human genome, including those formed by chemotherapeutic drugs such as cisplatin and oxaliplatin. Improving our understanding of DNA repair is beneficial, not

only for understanding carcinogenesis, but also for understanding the processes cancer cells use to cope with chemotherapy. Such information is expected to aid in improving currently used chemotherapy regimens. The use of XR-seq methodology has also brought information about the function of intergene sequences. XR-seq determines the exact location of sequences being repaired and indicates whether these are potentially important regulators. This novel method will allow uncovering repair characteristics and sequence preferences of treatment-induced DNA damage and as such might facilitate studies of the effects of mutational patterns on transcriptional activity on DNA repair in human tumor cells. This method should also prove useful in determining the effects of drugs like histone-modifying therapeutics or poly ADP ribose polymerase (PARP) inhibitors on nucleotide excision repair, and how they eventually interfere with radio-or chemosensitivity of tumor cells.

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