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Cross-species Functionome analysis identifies proteins associated with DNA repair, translation and aerobic respiration as conserved modulators of UV-toxicity

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ABSTRACT

Cellular responses to DNA damage can prevent mutations and death. In this study, we have used high throughput screens and developed a comparative genomic approach, termed Functionome mapping, to discover conserved responses to UVC-damage. Functionome mapping uses gene ontology (GO) information to link proteins with similar biological functions from different organisms, and we have used it to compare 303, 311 and 288 UVC-toxicity modulating proteins from *Escherichia coli*, *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*, respectively. We have demonstrated that all three organisms use DNA repair, translation and aerobic respiration associated processes to modulate the toxicity of UVC, with these last two categories highlighting the importance of ribosomal proteins and electron transport machinery. Our study has demonstrated that comparative genomic approaches can be used to identify conserved responses to damage, and suggest roles for translational machinery and components of energy metabolism in optimizing the DNA damage response.

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1. Introduction

Ultra-violet (UV) radiation is a major source of DNA damage and can cause the formation of cyclobutane-pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4PPs) [1]. Specifically, CPDs and 6-4PPs can inhibit the progress of RNA polymerases, resulting in transcriptional blocks that can promote cell death. CPDs and 6-4PPs can also block the action of replicative DNA polymerases, which can cause either cell death or promote translesion polymerase associated mutations [2], with the generation of UV-induced mutations dependent on the specific adduct. For example, while thymine–thymine lesions are often replicated correctly, lesions that contain cytosine frequently result in cytosine to thymine transition mutations [3]. These transition mutations are found at a high frequency in the p53 tumor suppressor gene in many skin cancers [4]; thus, the efficient removal of UV-induced lesions in DNA is critical for cancer prevention.

UV-induced DNA lesions can be removed by direct reversal and nucleotide excision repair (NER). Both mechanisms have been extensively characterized in *Escherichia coli* [1]. Direct reversal, or photo-reactivation, is facilitated by DNA photolyase enzymes using energy from light to split the CPD and 6-4 photoproduct lesions [5]. Photolyases are found in some bacteria and are closely related to the blue light sensing cryptochrome proteins. Although functionally absent in humans, photolyases are found in bacteria, Archaea and vertebrates [6]. UV photoproducts can also be removed from the genome through the action of proteins participating in nucleotide excision repair (NER), a process in which a short segment of the damaged DNA is removed and then re-synthesized. In *E. coli*, NER is initially facilitated by the binding of dimeric UvrA and UvrB to bulky DNA damage and the subsequent unwinding of the DNA and recruitment of UvrC to incise DNA 3′ and 5′ to the lesion [7,8]. Cho (UvrC homologue, ydjQ) can also make the 3′ incision downstream from the normal UvrC incision point, with Cho proposed to be an efficient 3′ endonuclease at some bulky lesions [8,9]. After 5′ and 3′ incisions are made around the lesion, the DNA helicase UvrD displaces the excised fragment and DNA polymerase I and DNA ligase fill and seal the gap, respectively [7].

NER is found in species ranging from bacteria to humans and its mechanism of action is highly conserved [1,10]. NER in eukaryotes involves the coordinated action of over 30 proteins and, similarly to *E. coli*, can occur in both a general and transcription dependent mode. In *Saccharomyces cerevisiae*, members of the Rad3 epistasis group participate in NER and, similarly to bacteria, these activities include proteins that recognize bulky lesions, those that incise the DNA 5′ and 3′ to the lesion and others that remove the DNA fragment containing

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the lesion [1]. In general, defects in genes belonging to the Rad3 epistasis group confer sensitivity to UV. Importantly, NER has been shown to operate in a global and transcription-coupled manner, with the latter coordinating DNA repair with the action of RNA polymerases [11,12]. NER defects in humans can lead to Xeroderma Pigmentosum (XP), Cockayne's Syndrome, and Trichothiodystrophy, all of which are associated with varying degrees of increased UV-sensitivity and in some cases, neurodegenerative conditions [1,13]. XP patients in particular demonstrate UV-induced genome instability and are diagnosed with skin cancer 50 years earlier than the general population [14].

In most organisms studied to date, DNA repair pathways are activated after DNA damage and this activation usually coincides with activation of a global signaling program. For example, UV radiation has been shown to induce the SOS response in *E. coli*. The SOS response is regulated by the RecA protein, a single stranded DNA binding protein that accumulates at sites of DNA damage. RecA protein bound to DNA will promote cleavage of the repressor protein LexA [15]. LexA cleavage up-regulates the transcription of many genes important for cell survival after DNA damage, including those in the NER and recombination pathways [16,17]. DNA replication and repair are not the only cellular processes up-regulated by the SOS response. Other transcripts regulated as part of the SOS response include those whose corresponding proteins are involved in transcription (LexA, RpoD, and Fis), nucleoside metabolism (NrdA, NrdB, and GrxA), translation (ArgS, PrfB, and PrfC) and heat shock (YcaH, CorA, and GlvB). Similarly to prokaryotic cells, eukaryotic organisms have DNA damage response (DDR) pathways activated by DNA strand breaks. Recognition of DNA strand breaks is facilitated by protein-based damage detection resulting in signaling through the ataxia telangiectasia mutated (ATM) and ATM and Rad3-related (ATR) kinases [18–20]. In *S. cerevisiae*, the ATM and ATR homologues are named Mec1 and Tel1. Mec1-dependent transcriptional reprogramming occurs after DNA damage and includes hundreds of different transcripts corresponding to a wide range of cellular proteins. Transcripts regulated in a Mec1-dependent manner include those associated with the DDR, as well as transcripts belonging to the environmental stress response (ESR) [21]. The ESR is thought to protect the internal homeostasis of the cell and includes transcripts whose corresponding proteins participate in reactive oxygen species detoxification, protein folding and degradation, carbohydrate metabolism, ribosomal function and translational regulation. The regulation of a broad range of cellular functions after DNA damage is also conserved in humans. For example, ATM and ATR have been shown to phosphorylate over 700 downstream proteins including the DDR associated proteins Chk1, Chk2, p53, Brca1 and Cdc25 in addition to many other targets [22,23]. The other ATM/ATR targets belong to the cellular processes of nucleic acid metabolism, protein metabolism, cell cycle, signal transduction, cell structure and motility, protein traffic and oncogenesis. Thus, the regulation of many different cellular processes after DNA damage is a theme that is conserved from bacteria to lower and higher eukaryotes. In addition, the diversity of responses regulated by SOS, Mec1 and ATM/ATR signaling suggests that DNA repair is coordinated with other cellular processes.

In an effort to identify proteins and pathways that help cells respond to damaging agents, scientists have screened gene deletion libraries derived from different single celled organisms [24–28]. In these libraries, gene deletion is facilitated by targeted homologous recombination to replace a specific gene with a selection cassette. Gene deletion libraries are made when, in theory, all genes in a genome have been individually removed, and the resulting mutants have been arrayed into separate wells of a multi-well plate. In diploid cells, removal of one allele can result in haploinsufficiency or cell death in some cases [28]. In haploid cells, only 75–95% of the genes can be removed to yield a viable mutant, as many genes encode essential activities that when removed result in cell death. Gene deletion libraries have been made in *E. coli*, *S. cerevisiae*, and *Schizosaccharomyces pombe* [28–30], and all of these resources have been used in organized screens and have proven to be valuable tools for identifying gene products that modulate the toxicity of different DNA damaging agents. For example, after the *S. cerevisiae* gene deletion library was screened against UVC, we reported that in addition to DNA repair and cell cycle, a number of proteins associated with RNA and protein metabolism, aerobic respiration, and other functional categories were classified as toxicity-modulating [24]. This identification of a broad range of unexpected biological processes in functional screens supports the contention that either the corresponding proteins are linked to the DNA damage response in some way or that other metabolic pathways are coordinated with the repair of UV-induced DNA lesions. Other possibilities exist, though, and the identified UV-toxicity modulating proteins might just be experimental anomalies specific to the *S. cerevisiae* gene deletion library, as these cells have a distinct physiology.

In the following study, we have utilized comparative functional genomic approaches to identify similar biological processes that modulate the toxicity of UV in three different and evolutionarily distinct cell types: *E. coli*, *S. cerevisiae*, and *S. pombe*. Specifically, we have screened two different species-specific deletion libraries, *E. coli* and *S. pombe*, to identify 303 and 311 UV-toxicity modulating proteins, respectively. We have also computationally compared the UV-toxicity modulating proteins identified from *E. coli* and *S. pombe* to our previously reported list of 288 UV-toxicity modulating proteins from *S. cerevisiae* [24]. To do this, we have developed a functional interactome mapping approach to identify GO-specified biological processes that are significantly enriched for UV-toxicity modulating proteins from multiple organisms. We have demonstrated that multiple species use the biological processes associated with DNA repair, translation and aerobic respiration to modulate the toxicity of UV. In addition, we have demonstrated that our functional mapping approach is predictive and can be used to identify UV-toxicity modulating proteins in different cell types. Finally, at the mechanistic level, our results support the idea that cells use translational machinery and ATP levels to optimize DNA repair or coordinate DNA repair with other metabolic processes.

2. Results and discussion

2.1. 303 E. coli gene deletion mutants identified as sensitive to UV

The *E. coli* deletion set contains 8640 mutants specific for 3968 genes [29]. Mutants were individually spotted onto LB-agar plates using the 96-syringe Matrix Scientific Hydra. Upon drying, the cells were left untreated or exposed to two different doses of UV (10.0 and 12.5 J /m2) and then allowed to grow for 24 h (Fig. 1A). UV exposure conditions were chosen such that cells deficient in RecA and Cho were consistently identified as UV-sensitive in preliminary experiments. In total, 364 plates containing 34,944 spotted cultures were assayed as described earlier and the resulting images of these plates were compiled into Supplemental Figure S1. We analyzed our UV screen data using a similar methodology as previously reported [26]. First, a virtual mutant representing at least two isolates of each gene mutant was given a UV-toxicity modulating score, derived from the behavior of the corresponding mutants exposed to different doses of UV. For example, the Keio library contains 2 mutants representing *uvrD*, and the UV-toxicity modulating score describes the behavior of each of these *uvrD* mutants after exposure to two different doses of UV. For each exposure condition (low and high), mutants that demonstrated reduced growth were given a score of 4 to 2, depending on the severity of the growth defect (4 = high, 2 = low), and those displaying a color change were scored 1. In theory, the most sensitive virtual mutants scored 16 (4 + 4 + 4 + 4), because two corresponding isolates displayed severely reduced growth (score of 4) at both UV-

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