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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.

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-

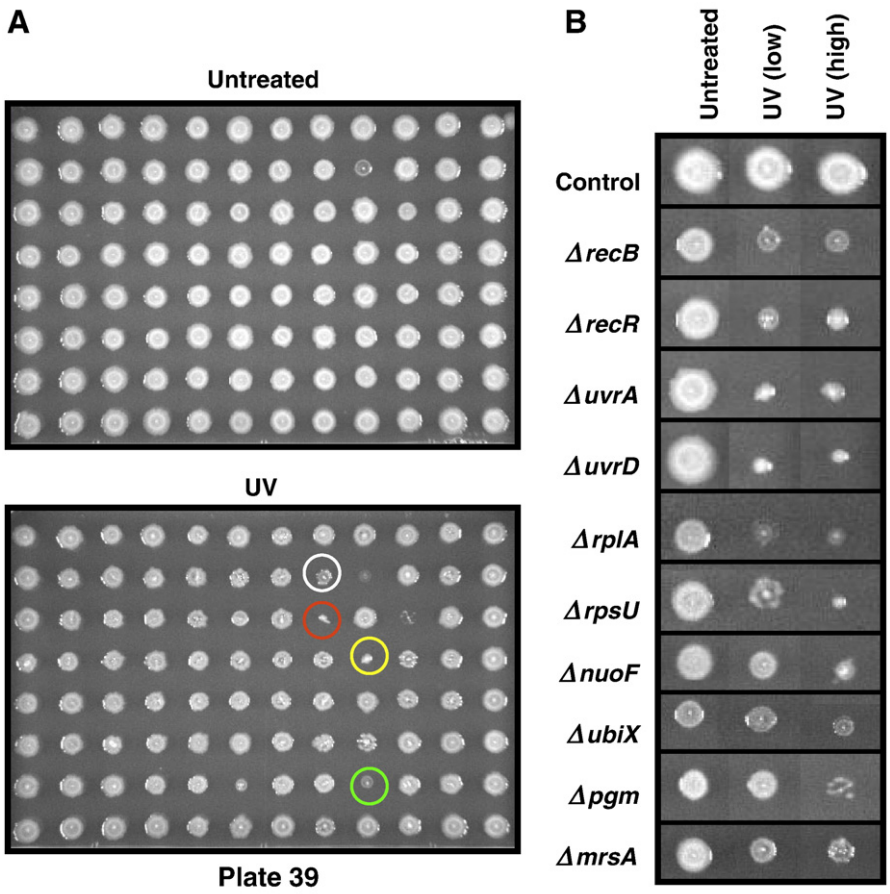

Fig. 1. Genomic phenotyping of *E. coli* mutants with UV. (A) 96 gene deletion mutants were spotted onto agar plates, left untreated or exposed to two doses of UV, incubated at 37 °C for 16 h and imaged. These doses were used because some cells were only sensitive to the higher dose, while others were sensitive to both conditions. White, red, yellow and green circles identify the UV-sensitive mutants *ΔruvA*, *ΔruvC*, *ΔuvrA* and *ΔholC*, respectively. (B) Images were taken from many different plates and recompiled to demonstrate that varying degrees of UV-sensitivity were observed in the screen. Examples of a colour change from white to grey are shown for *ΔrecB* and *ΔmrsA*.

exposure conditions. We used a cut-off of 3 to identify virtual mutants that were slightly sensitive to UV, as this category of mutants repeatedly displayed a UV-induced reduction in growth and/or a colour change (Fig. 1B).

Ultimately, we identified 303 *E. coli* virtual mutants as being sensitive to UV (Table 1) and observed a range of sensitivities from high (25 mutants scored from 10 to 16), medium (30 mutants scored from 7 to 9), low (90 mutants scored from 5 to 6), to slightly (157 mutants scored from 3 to 4). Mutants classified as being highly sensitive to UV included *uvrAΔ*, *uvrBΔ*, *uvrCΔ*, and *uvrDΔ*, all of whose corresponding proteins are components of NER. It is known that *uvrAΔ*, *uvrBΔ*, *uvrCΔ*, and *uvrDΔ* mutants are sensitive to UV [1,31] and their identification in our screen validated our methodology. Other mutants highly sensitive to UV included those deficient in the peptide chain release factor PrfB and the 50S ribosomal subunit protein RflA. These results suggest that a defect in ribosome assembly, ribosomal protein deficiency or corrupted protein synthesis disrupts cellular responses to damage. Mutants in the medium sensitivity category included *dnaGΔ*, *parCΔ*, and *ruvAΔ*, all of which encode activities associated with DNA synthesis and DNA repair. Again, these mutants highlight the importance of DNA repair and DNA metabolism after UV-damage and their presence in our list was expected. Other mutants that fall into the medium sensitive category included those cells deficient in the 30S ribosome binding factor (RbfA), ribonuclease RNase T and adenylate cyclase (CycA), highlighting the roles for protein, RNA and small molecule metabolism in response to UV-damage. Mutants in the low sensitivity category included those deficient in the NER activity Cho, the 30S ribosomal subunit protein (RpsT) and NADH ubiquinone oxidoreductase (NuoF). Our mutants classified as having low sensitivity further demonstrated that deficiencies in DNA repair and protein synthesis corrupt cellular viability after UV-exposure. In addition, the identification of NuoF in our UV-sensitive catalog introduced respiration and energy metabolism to the list of cellular process important after damage. The slightly sensitive list is also populated by mutants defective in DNA repair (UmuC), ribosome assembly (RpsO), protein synthesis (PoxA), RNA metabolism (Tgt) and aerobic respiration (NuoH), further highlighting the wide range of cellular processes involved in the response to UV-damage. [We note for the preceding paragraph all annotation information was obtained from EcoGene [32].]

2.2. Conserved biological process that modulate UV-toxicity identified by Functionome mapping

After we identified 303 *E. coli* proteins that modulate the toxicity of UV-damage and linked them to a number of cellular processes, we wanted to determine which of these responses were conserved across species. In most model organisms, DNA repair is essential for survival after UV-damage and we wanted to determine if any other biological processes showed a similar association. Previously, we had performed high throughput screens in *S. cerevisiae* and identified 288 proteins that modulate the toxicity of UV in this eukaryotic organism [24]. To identify conserved cellular responses to UV-damage, we developed a functional interactome approach to align our list of UV-toxicity

Table 1
Proteins corresponding to the 301 UV-sensitive mutants identified in *E. coli*.

Protein	Description	Sensitivity
ArtM	Arginine transporter subunit, membrane component of ABC superfamily	High
DedD	Affects Col V production	High
DnaT	DNA biosynthesis protein (primosomal protein I)	High
Fis	Global DNA-binding transcriptional dual regulator	High
FlgH	Flagellar protein of basal-body outer-membrane L ring	High
FtnA	Ferritin iron storage protein (cytoplasmic)	High
Mfd	Transcription-repair coupling factor	High
Pnp	Polynucleotide phosphorylase/polyadenylase	High
PrfB	Peptide chain release factor RF-2	High
PriA	Primosome factor n' (replication factor Y)	High
RecA	DNA strand exchange and recombination protein with protease and nuclease activity	High
RecC	Exonuclease V (RecBCD complex)	High
RecO	Gap repair protein	High
RecR	Gap repair protein	High
RuvC	Component of RuvABC resolvasome, endonuclease	High
UvrA	ATPase and DNA damage recognition protein of nucleotide excision repair excinuclease UvrABC	High
UvrB	Excinulease of nucleotide excision repair	High
UvrC	Excinuclease UvrABC, endonuclease subunit	High
UvrD	DNA-dependent ATPase I and helicase II	High
YohF	Predicted oxidoreductase with NAD(P)-binding Rossmann-fold domain	High
CyaA	Adenylate cyclase	Medium
DacD	D-alanyl-D-alanine carboxypeptidase (penicillin-binding protein 6b)	Medium
Dam	DNA adenine methylase	Medium
DnaG	DNA primase	Medium
DnaK	Chaperone Hsp70, co-chaperone with DnaJ	Medium
Hfq	HF-I, host factor for RNA phage Q beta replication	Medium
IntA	CP4-57 prophage; integrase	Medium
JW1227.5	Unknown function	Medium
JW5460	Predicted protein (pseudogene)	Medium
ParC	DNA topoisomerase IV, subunit A	Medium
PdxH	Pyridoxine 5′-phosphate oxidase	Medium
RbfA	30s ribosome binding factor	Medium
RecF	Gap repair protein	Medium
Rnt	Ribonuclease T (RNase T)	Medium
RplA	50S ribosomal subunit protein L1	Medium
RseA	Anti-sigma factor	Medium
RuvA	Component of RuvABC resolvasome, regulatory subunit	Medium
Uup	Fused predicted transporter subunits of ABC superfamily: ATP-binding components	Medium
YbaB	Conserved protein	Medium
YceB	Predicted lipoprotein	Medium
YciG	Unknown function	Medium
YdaE	Rac prophage; conserved protein	Medium
YddO	D-ala-D-ala transporter subunit, ATP-binding component of ABC superfamily	Medium
YdeH	Unknown function	Medium
YdiI	Unknown function	Medium
YehP	Unknown function	Medium
YeiC	Predicted kinase	Medium
YgfA	Predicted ligase	Medium
YibP	Protease with a role in cell division	Medium
YihX	Predicted hydrolase	Medium
YjeK	Predicted lysine aminomutase	Medium
YneF	Predicted diguanylate cyclase	Medium
YnjI	Predicted inner membrane protein	Medium
YohG	Unknown function	Medium
YphB	Conserved protein	Medium
Cho	Endonuclease of nucleotide excision repair	Low
CybB	Cytochrome b561	Low
CysA	Sulfate/thiosulfate transporter subunit, ATP-binding component of ABC superfamily	Low
DedA	Conserved inner membrane protein	Low
FecA	KpLE2 phage-like element; ferric citrate outer membrane transporter	Low
FecC	KpLE2 phage-like element; iron-dicitrate transporter subunit, membrane component of ABC superfamily	Low
FimC	Chaperone, periplasmic	Low
FumC	Fumarate hydratase (fumarase C),aerobic Class II	Low
GntP	Fructuronate transporter	Low
Gph	Phosphoglycolate phosphatase	Low
HolC	DNA polymerase III, chi subunit	Low
HolD	DNA polymerase III, psi subunit	Low
HtgA	Unknown function	Low
IlvC	Ketol-acid reductoisomerase, NAD(P)-binding	Low
IspZ	Predicted inner membrane protein	Low
JW0258	Predicted IS protein	Low
JW5411	Unknown function	Low
JW5766	Unknown function	Low
MtlA	Fused mannitol-specific PTS enzymes: IIA components , IIB components , IIC components	Low
NuoF	NADH:ubiquinone oxidoreductase, chain F	Low
OppD	Oligopeptide transporter subunit , ATP-binding component of ABC superfamily	Low
PriB	Primosomal protein N	Low
PrpB	2-methylisocitrate lyase	Low
RadA	Predicted repair protein	Low
RecB	Exonuclease V (RecBCD complex), beta subunit	Low
RpsT	30S ribosomal subunit protein S20	Low
SgcE	KpLE2 phage-like element; predicted epimerase	Low
UbiX	3-octaprenyl-4-hydroxybenzoate carboxy-lyase	Low
YbhD	Unknown function	Low
YcaM	Predicted transporter	Low
YceP	Cold shock gene	Low
YcfS	Conserved protein	Low
YeaH	Conserved protein	Low
YebG	Conserved protein regulated by LexA	Low
yeeL	Predicted protein, C-ter fragment (pseudogene)	Low
YfaO	Predicted NUDIX hydrolase	Low
YfbQ	Predicted aminotransferase	Low
YfdZ	Prediected aminotransferase, PLP-dependent	Low
YfiM	Predicted protein	Low
YgaY	Predicted transporter (pseudogene)	Low
YgfY	Conserved protein	Low
YnjE	Predicted thiosulfate sulfur transferase	Low
YodB	Predicted cytochrome	Low
DicB	Qin prophage; cell division inhibition protein	Slightly
FhiA	Flagellar system protein, promoterless fragment (pseudogene)	Slightly
FimH	Minor component of type 1 fimbriae	Slightly
FliD	Flagellar filament capping protein	Slightly
GroL	Cpn60 chaperonin GroEL, large subunit of GroESL	Slightly
HisB	Fused histidinol-phosphatase , imidazoleglycerol-phosphate dehydratase	Slightly
IhfB	Integration host factor (IHF), DNA-binding protein, beta subunit	Slightly
JW1277	Unknown function	Slightly
JW5474	Unknown function	Slightly
LuxS	S-ribosylhomocysteinase	Slightly
MinC	Cell division inhibitor	Slightly
NhaA	Sodium-proton antiporter	Slightly
QueA	S-adenosylmethionine:tRNA ribosyltransferase-isomerase	Slightly
Rof	Modulator of Rho-dependent transcription termination	Slightly
RrmJ	23S rRNA methyltransferase	Slightly
RspB	Predicted oxidoreductase, Zn-dependent and NAD(P)-binding	Slightly
SapA	Predicted antimicrobial peptide transporter subunit , periplasmic-binding component of ABC superfamily	Slightly
SotB	Predicted arabinose transporter	Slightly
SpeG	Spermidine N1-acetyltransferase	Slightly
SucB	Dihydrolipoyltranssuccinase	Slightly
ThrL	Thr operon leader peptide	Slightly
WbbL	Lipopolysaccharide biosynthesis protein, N-ter fragment (pseudogene)	Slightly
WecG	UDP-N-acetyl-D-mannosaminuronic acid transferase	Slightly
YadB	Glutamyl-Q tRNA(Asp) synthase	Slightly
YadM	Predicted fimbrial-like adhesin protein	Slightly
YagT	Predicted xanthine dehydrogenase, 2Fe-2S subunit	Slightly
YahN	Neutral amino-acid efflux system	Slightly
YbgI	Conserved metal-binding protein	Slightly
YcgR	Protein involved in flagellar function	Slightly
YchE	Predicted inner membrane protein	Slightly
YciQ	Unknown function	Slightly
YcjG	L-Ala-D/L-Glu epimerase	Slightly
YdbL	Conserved protein	Slightly
YdfA	Qin prophage; predicted protein	Slightly
YdhL	Conserved protein	Slightly
YdhZ	predicted protein	Slightly
YdiK	predicted inner membrane protein	Slightly

Table 1 (*continued*)

Protein	Description	Sensitivity
YeeV	CP4-44 prophage; toxin of the YeeV-YeeU toxin-antitoxin system	Slightly
YegV	Predicted kinase	Slightly
YehK	Predicted protein	Slightly
YfdI	Unknown function	Slightly
YfhD	Predicted transglycosylase	Slightly
YgdB	Predicted protein	Slightly
YgfZ	Predicted folate-dependent regulatory protein	Slightly
YhbX	Predicted hydrolase, inner membrane	Slightly
YmgD	Unknown function	Slightly
YoaF	Conserved outer membrane protein	Slightly
YobD	Conserved inner membrane protein	Slightly
AcrR	DNA-binding transcriptional repressor	Slightly
AgaI	Galactosamine-6-phosphate isomerase	Slightly
AraF	L-arabinose transporter subunit , periplasmic-binding Component of ABC superfamily	Slightly
Asr	Acid shock-inducible periplasmic protein	Slightly
BtuE	Predicted glutathione peroxidase	Slightly
CycA	D-alanine/D-serine/glycine transporter	Slightly
DeaD	ATP-dependent RNA helicase	Slightly
EamA	Cysteine and O-acetyl-L-serine efflux system	Slightly
FruA	Fused fructose-specific PTS enzymes: IIBcomponent , IIC components	Slightly
FruB	Fused fructose-specific PTS enzymes: IIA component , HPr component	Slightly
HokD	Qin prophage; small toxic polypeptide	Slightly
HybA	Hydrogenase 2 4Fe-4S ferredoxin-type component	Slightly
IhfA	Integration host factor (IHF), DNA-binding protein, alpha subunit	Slightly
JW5386	Predicted protein	Slightly
JW5846	Predicted protein	Slightly
Kbl	Glycine C-acetyltransferase	Slightly
MglC	Methyl-galactoside transporter subunit , membrane component of ABC superfamily	Slightly
MiaA	Delta(2)-isopentenylpyrophosphate tRNA-adenosine transferase	Slightly
MotB	Protein that enables flagellar motor rotation	Slightly
NagA	N-acetylglucosamine-6-phosphate deacetylase	Slightly
OtsB	Trehalose-6-phosphate phosphatase, biosynthetic	Slightly
PotA	Polyamine transporter subunit , ATP-binding component of ABC superfamily	Slightly
PoxA	Predicted lysyl-tRNA synthetase	Slightly
PrpE	Predicted propionyl-CoA Synthetase with ATPase domain	Slightly
RhsA	rhsa element core protein RshA	Slightly
RpmF	50S ribosomal subunit protein L32	Slightly
RpsU	30S ribosomal subunit protein S21	Slightly
SsuB	Alkanesulfonate transporter subunit , ATP-binding component of ABC superfamily	Slightly
SufB	Complexed with SufC and SufD	Slightly
TfaR	Rac prophage; predicted tail fiber assembly protein	Slightly
TrpL	Trp operon leader peptide	Slightly
UbiF	2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinol oxygenase	Slightly
Upk	Undecaprenyl pyrophosphate phosphatase	Slightly
XylG	Fused D-xylose transporter subunits of ABC superfamily; ATP-binding components	Slightly
YagE	CP4-6 prophage; predicted lyase/synthase	Slightly
YbaL	Predicted transporter with NAD(P)-binding Rossmann-fold domain	Slightly
YbdF	Unknown function	Slightly
YbeQ	Unknown function	Slightly
YbfO	Unknown function	Slightly
YbgQ	Predicted outer membrane protein	Slightly
YbjS	Predicted NAD(P)H-binding oxidoreductase with NAD(P)-binding Rossmann-fold domain	Slightly
YcaQ	Unknown function	Slightly
YccA	Inner membrane protein	Slightly
YccS	Predicted inner membrane protein	Slightly
YcdL	Unknown function	Slightly
YceH	Unknown function	Slightly
YciW	Predicted oxidoreductase	Slightly
YcjO	Predicted sugar transporter subunit: membrane component of ABC superfamily	Slightly
YdaM	Predicted diguanylate cyclase, ggdef domain signalling protein	Slightly
YdbJ	Unknown function	Slightly
YddG	Predicted methyl viologen efflux pump	Slightly
YdhB	Predicted DNA-binding transcriptional regulator	Slightly
YdhQ	Unknown function	Slightly
YdiD	Acyl-CoA synthase	Slightly
YdjN	Predicted transporter	Slightly
yedS	Predicted protein, middle fragment (pseudogene)	Slightly
YeeJ	Adhesin	Slightly
YeiA	Predicted oxidoreductase	Slightly
YfbU	Unknown function	Slightly
YfcU	Predicted export usher protein	Slightly
YgcR	Predicted flavoprotein	Slightly
YhcP	P-hydroxybenzoic acid efflux system component	Slightly
YhdZ	Predicted amino-acid transporter subunit , ATP-binding component of ABC superfamily	Slightly
YliA	Glutathione transporter ATP-binding protein	Slightly
YmfA	Predicted inner membrane protein	Slightly
YmfE	e14 prophage; predicted inner membrane protein	Slightly
YmfO	e14 prophage; conserved protein	Slightly
YnbD	Predicted phosphatase, inner membrane protein	Slightly
YnhG	Unknown function	Slightly
YoaG	Unknown function	Slightly
BtuC	Vitamin B12 transporter subunit: membrane component of ABC superfamily	Slightly
BtuD	Vitamin B12 transporter subunit : ATP-binding component of ABC superfamily	Slightly
CinA	Unknown function	Slightly
CydB	Cytochrome d terminal oxidase, subunit II	Slightly
DcuB	C4-dicarboxylate antiporter	Slightly
GidA	Glucose-inhibited cell-division protein	Slightly
GrxA	Glutaredoxin 1, redox coenzyme for ribonucleotide reductase (RNR1a)	Slightly
GutQ	D-arabinose 5-phosphate isomerase	Slightly
HisG	ATP phosphoribosyltransferase	Slightly
HyfB	Hydrogenase 4, membrane subunit	Slightly
JW1421	Unknown function	Slightly
JW1640	Unknown function	Slightly
JW2679	Unknown function	Slightly
JW3017	Unknown function	Slightly
JW4246	KpLE2 phage-like element; predicted protein	Slightly
JW5029	Unknown function	Slightly
LdcC	Lysine decarboxylase 2, constitutive	Slightly
LeuC	3-isopropylmalate isomerase subunit, dehydratase component	Slightly
ManX	Fused mannose-specific PTS enzymes: IIA component , IIB component	Slightly
MenC	O-succinylbenzoyl-CoA synthase	Slightly
MrsA	Phosphoglucosamine mutase	Slightly
NhoA	N-hydroxyarylamine O-acetyltransferase	Slightly
NuoH	NADH:ubiquinone oxidoreductase, membrane subunit H	Slightly
NuoK	NADH:ubiquinone oxidoreductase, membrane subunit K	Slightly
PaaC	Predicted multicomponent oxygenase/reductase subunit for phenylacetic acid degradation	Slightly
PaaX	DNA-binding transcriptional repressor of phenylacetic acid degradation, aryl-CoA responsive	Slightly
PdxB	Erythronate-4-phosphate dehydrogenase	Slightly
Pgm	Phosphoglucomutase	Slightly
PpiB	Peptidyl-prolyl cis-trans isomerase B (rotamase B)	Slightly
Rmf	Ribosome modulation factor	Slightly
RpsO	30S ribosomal subunit protein S15	Slightly
Rtn	Conserved protein	Slightly
SanA	Predicted protein	Slightly
SapB	Predicted antimicrobial peptide transporter subunit , membrane component of ABC superfamily	Slightly
SfmF	Predicted fimbrial-like adhesin protein	Slightly
SirB2	Predicted transcriptional regulator	Slightly
SufD	Component of SufBCD complex	Slightly
Tgt	tRNA-guanine transglycosylase	Slightly
TruA	Pseudouridylate synthase I	Slightly
UgpA	Glycerol-3-phosphate transporter subunit , membrane component of ABC superfamily	Slightly
UmuC	DNA polymerase V, subunit C	Slightly
Usg	Predicted semialdehyde dehydrogenase	Slightly
VacJ	Predicted lipoprotein	Slightly
YagW	Predicted receptor	Slightly
YaiT	Predicted protein	Slightly
ycdN	Predicted protein, N-ter fragment (pseudogene)	Slightly
YcfF	Purine nucleoside phosphoramidase	Slightly

(*continued on next page*)

Table 1 (*continued*)

Protein	Description	Sensitivity
YcgK	Unknown function	Slightly
YciF	Unknown function	Slightly
YciN	Unknown function	Slightly
YcjD	Unknown function	Slightly
YdaY	RAC prophage; predicted protein	Slightly
YddP	D-ala-D-ala transporter subunit , ATP-binding component of ABC superfamily	Slightly
YdeE	Predicted transporter	Slightly
YdeP	Predicted oxidoreductase	Slightly
YdfZ	Unknown function	Slightly
YdgD	Predicted peptidase	Slightly
YdjE	Predicted transporter	Slightly
YecN	Unknown function	Slightly
YeeY	Predicted DNA-binding transcriptional regulator	Slightly
YefI	Lipopolysaccharide biosynthesis protein	Slightly
YegN	Multidrug efflux system, subunit B	Slightly
YehL	Unknown function	Slightly
YehM	Unknown function	Slightly
YeiE	Predicted DNA-binding transcriptional regulator	Slightly
YejB	Predicted oligopeptide transporter subunit , membrane component of ABC superfamily	Slightly
YejO	Predicted autotransporter outer membrane protein	Slightly
yfaS	Predicted protein, C-ter fragment (pseudogene)	Slightly
YfbK	Conserved protein	Slightly
YfdQ	CPS-53 (KpLE1) prophage; predicted protein	Slightly
YfeH	Unknown function	Slightly
YfhQ	Predicted methyltransferase	Slightly
YgbI	Predicted DNA-binding transcriptional regulator	Slightly
YgcI	Unknown function	Slightly
YgdI	Unknown function	Slightly
YgfE	Protein that localizes to the cytokinetic ring	Slightly
YhhZ	Unknown function	Slightly
YjgK	Unknown function	Slightly
YjgM	Predicted acetyltransferase	Slightly
YkfC	CP4-6 prophage; conserved protein	Slightly
YmbA	Unknown function	Slightly
YmfL	e14 prophage; predicted DNA-binding transcriptional regulator	Slightly
YneC	Unknown function	Slightly
YneE	Unknown function	Slightly
YojI	Fused predicted multidrug transport subunits of ABC superfamily: membrane component , ATP-binding component	Slightly
YqcC	Unknown function	Slightly
YtfF	Predicted inner membrane protein	Slightly

modulating proteins from *E. coli* with those previously identified in *S. cerevisiae*. Matching toxicity modulating proteins from each organism using similarities in primary amino acid sequence is problematic in that sequence homology is limited between these prokaryotic and eukaryotic organisms. Conversely, functional classifications offered an avenue to perform a cross-species analysis of UV-toxicity modulating data. To compare functional categories associated with UV-toxicity modulating proteins, we took advantage of GO annotations. GO provides a controlled vocabulary describing a protein's biological process and the information has been methodically compiled by annotation teams [33]. Annotations reflect the biological function of each protein, and GO assignments are made using manual and automated approaches. In both cases, annotation assignments are based on an attributable source (literature, another database or computational study) and the annotation is detailed to indicate the type of evidence used to make each assignment. The controlled vocabulary allows for proteins from different organisms to be linked via an identical biological process and we exploited GO associations to generate a cross-species functional interactome that we call a Functionome. Functional associations have previously been used to group species-specific data and analyze high throughput data sets [22,34,35], but our cross-species Functionome expands this approach to efficiently compare data from different organisms. To compile the *E. coli–S. cerevisiae* Functionome, we identified 511 GO biological processes found in both organisms. Next, we associated 3120 *E. coli* and 4415 *S. cerevisiae* proteins with these GO identifiers. We note that not all *E. coli* and *S. cerevisiae* proteins are found in this Functionome, as some proteins are only associated with species-specific functional information. The 7535 proteins found in the Functionome averaged 2.4 functional interactions per protein, with a total of 18,250 functional interactions associated with the compiled structure (Fig. 2A).

After we compiled the Functionome, we mapped it with UV-toxicity modulating proteins from *E. coli* (171) and *S. cerevisiae* (236). Next, we computationally analyzed this mapped Functionome to identify GO-nodes significantly over-represented with UV-toxicity modulating proteins from both organisms. Significance was verified using two methodologies: random sampling of proteins in the Functionome and network randomizations. Using our computational approaches, we expected to identify the GO biological process of NER as a node that was significantly over-represented with UV-toxicity modulating proteins from both organisms. We specified that in order to be identified in our analysis at least two UV-toxicity modulating proteins from each organism must be associated with the GO biological process. This was done to prevent the identification of GO-nodes predominated by a single organism's UV-toxicity modulating proteins. As expected, our computational analysis identified NER as being over-represented with UV-toxicity modulating proteins from both *E. coli* and *S. cerevisiae*. NER was, in fact, one of the top scoring nodes ($P<0.0001$) in our Functionome analysis (Fig. 2B), helping to validate our methodology. Additionally, we identified 9 other GO biological processes that met our criteria (Fig. 2C and Table 2).

GO biological processes identified in our analysis included response to DNA damage stimulus, DNA repair, NER, DNA replication, DNA metabolic process, DNA recombination, translation, regulation of translation, tRNA aminoacylation for protein translation and aerobic respiration. In all, these categories spanned three general areas: DNA repair, protein synthesis and energy production. Because DNA repair is a conserved response to UV-damage [1], it was expected to be identified and was highlighted by a number of nodes. The identified DNA repair node serves as the namesake for this group and its inclusion reflects the fact that UV generates DNA lesions, that when left unrepaired can cause cell death. In addition, cells are known to initiate signal transduction cascades and activate cellular repair pathways after DNA damage [16]; thus, response to DNA damage stimulus was also a category that we expected to identify in our computational analysis. The repair of UV-induced DNA lesions can also occur by DNA recombination and as long tracks of DNA are replaced during recombination, our identification of DNA replication as an over-represented functional category was also expected. In all, the identification of these five DNA centric biological processes is consistent with published data and their identification further supports the validity of our computational analysis.

The surprising aspect of our Functionome study was the identification of protein synthesis and energy production categories, as these corresponding biological processes are not routinely associated with modulating UV toxicity. In fact, all of the identified biological processes associated with protein synthesis and energy metabolism were found to be more statistically significant than DNA recombination ($P<0.057$), a known and well studied response to DNA damage in both *E. coli* and *S. cerevisiae*. Biological processes involving protein synthesis were identified and included translation ($P<0.0278$), tRNA aminoacylation for protein translation ($P<0.0124$) and regulation of translation ($P<0.0455$). The identification of these three biological processes suggests that cells mount an evolutionarily conserved protein synthesis or ribosomal protein associated response to UV-damage. It is tempting to speculate that this protein synthesis based response is specific, as reports have indicated that regulation of translation initiation [36], tRNA modification status [37], tRNA cleavage [38] and tRNA mischarging [39] are cellular strategies used to respond to damage. It is also interesting to note that transcription was not identified in our GO analysis, which is

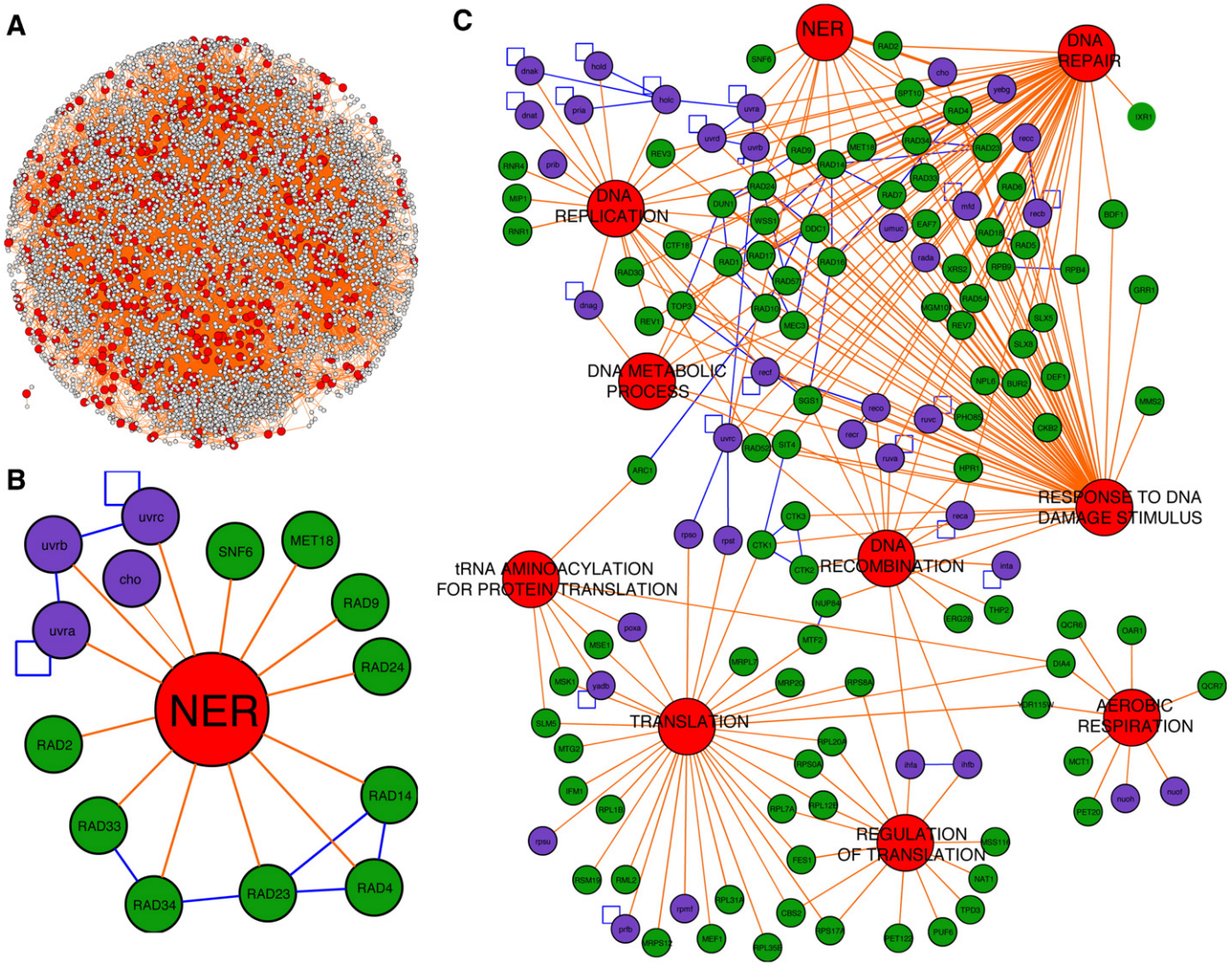

Fig. 2. Functional interactome mapping identified multi-species nodes over-represented with UV-toxicity modulating proteins. (A) The Functionome was compiled using GO identifiers for biological processes specific to 3120 *E. coli* and 4271 *S. cerevisiae* proteins (small grey spheres). A total of 511 GO identifiers (large red spheres) and 18,254 functional links (orange lines) were used to compile the functional interactome. (B) The Functionome was computationally analyzed to identify nodes over-represented with both *E. coli* (purple spheres, lower case protein names) and *S. cerevisiae* (green spheres, upper case protein names) UV-toxicity modulating proteins. One of the top scoring functional nodes was NER ($p<10^{-12}$). Blue lines represent protein-protein interactions. (C) All functional nodes that were over-represented ($p<0.06$) with UV-toxicity modulating proteins from both *E. coli* and *S. cerevisiae* were visualized using Cytoscape.

added support for a specific translational program. We note, though, that general protein synthesis will be important for producing enzymes that respond to DNA damage and for maintaining cellular homeostasis. Of note is the predominance of many ribosomal proteins (18) in the protein synthesis categories, suggesting the integrity of the ribosome or ribosomal protein-based responses is essential for repairing the damage.

Additionally, our Functionome analysis demonstrated that the node of aerobic respiration ($P<0.0164$) was over-represented with UV-toxicity modulating proteins. Associated *E. coli* proteins included NuoF and NuoH, part of the complex that shuttles electrons from NADH to quinones in the respiratory chain and acts to couple a redox reaction to proton translocation [32]. The *S. cerevisiae* proteins identified in the aerobic respiration node (Ydr115w, Qcr7, Qcr6, Dia4, Oar1, Mct1 and Pet20) include two components of ubiquinol cytochrome-c reductase complex involved in the electron transport chain (Qcr6 and Qcr7) and a protein required for respiratory growth (Pet20) [40]. Four of the *E. coli* and *S. cerevisiae* UV-toxicity modulating proteins linked to the aerobic respiration node ultimately promote ATP synthesis, suggesting that an intact metabolic response driving energy production is vital to cellular viability after UV-damage. This conclusion was further tested in *S. cerevisiae*. Ultimately our results suggest that cells with defective mitochondria (rho$^-$), and thus deficient in oxidative phosphorylation, would be sensitive to UVC. Using ethidium bromide induced rho$^-$ strains we have shown that these cells are in fact sensitive to UVC (Supplemental Figure S2), thus supporting our Functionome mapping conclusion.

2.3. Functionome results predict UV-sensitive themes for S. pombe mutants

Our Functionome analysis has highlighted three biological themes that modulate the toxicity of UV in both *E. coli* and *S. cerevisiae*. These results also lead us to predict that DNA repair, protein synthesis and energy production are important UV-toxicity modulating processes for cells from other species. To test this prediction, we performed a high throughput screen of the *S. pombe* gene deletion library to identify gene products that modulate the toxicity of UV. The *S. pombe* deletion set from Bioneer (Daejeon, Republic of Korea) contains mutant strains specific to 3006 genes [28]. Mutants were individually

Table 2
GO biological processes over-represented in both *E. coli* and *S. cerevisiae* UV-toxicity modulating proteins, as identified by Functionome mapping.

GO Functional category	*E. coli*		*Saccharomyces cerevisiae*		Combined	
	# UV sensitive	Genes	# UV sensitive	Genes	Total UV sensitive	P-value
Response to DNA damage stimulus	16	*cho*, *mfd*, *rada*, *reca*, *recb*, *recc*, *recf*, *reco*, *recr*, *ruva*, *ruvc*, *umuc*, *uvra*, *uvrb*, *uvrc*, *uvrd*, *yebg*	51	*RAD16*, *RAD18*, *SLX5*, *DUN1*, *NUP84*, *RAD57*, *HPR1*, *RAD9*, *RAD34*, *XRS2*, *RAD30*, *RAD23*, *SLX8*, *RAD4*, *RAD24*, *RAD6*, *RPB9*, *MMS2*, *RAD54*, *RAD2*, *WSS1*, *MET18*, *REV7*, *CTK2*, *RPB4*, *RAD7*, *GRR1*, *MGM101*, *DEF1*, *CTK1*, *RAD5*, *BUR2*, *TOP3*, *MEC3*, *BDF1*, *RAD33*, *RAD52*, *RAD10*, *CTK3*, *CTF18*, *NPL6*, *SGS1*, *RAD14*, *EAF7*, *CKB2*, *REV1*, *RAD17*, *RAD1*, *PHO85*, *REV3*, *DDC1*	67	1.00E−04
DNA repair	17	*cho*, *mfd*, *radA*, *recA*, *recB*, *recC*, *recF*, *recO*, *recR*, *ruvA*, *ruvC*, *umuC*, *uvrA*, *uvrB*, *uvrC*, *uvrD*,*lyebG*	42	*RAD16*, *RAD18*, *SLX5*, *SIT4*, *DUN1*, *RAD57*, *RAD9*, *RAD34*, *XRS2*, *RAD30*, *RAD23*, *SLX8*, *RAD4*, *RAD24*, *RAD6*, *RPB9*, *RAD54*, *RAD2*, *WSS1*, *MET18*, *REV7*, *SPT10*, *RPB4*, *RAD7*, *MGM101*, *IXR1*, *RAD5*, *TOP3*, *MEC3*, *BDF1*, *RAD33*, *RAD52*, *RAD10*, *CTF18*, *SGS1*, *RAD14*, *EAF7*, *REV1*, *RAD17*, *RAD1*, *REV3*, *DDC1*	59	1.00E−04
Nucleotide-excision repair	3	*uvrA*, *uvrB*, *uvrC*, *cho*	10	*RAD9*, *RAD34*, *RAD23*, *RAD4*, *RAD24*, *RAD2*, *SNF6*, *MET18*, *RAD33*, *RAD14*	13	1.00E−04
DNA replication	9	*dnaG*, *dnaK*, *dnaT*, *holC*, *holD*, *priA*, *priB*, *recF*, *uvrD*	14	*RAD9*, *RAD30*, *RNR1*, *RAD24*, *RNR4*, *WSS1*, *TOP3*, *RAD52*, *CTF18*, *SGS1*, *MIP1*, *REV1*, *RAD17*, *REV3*	23	1.00E−04
DNA metabolic process	3	*dnaG*, *recA*, *recR*	4	*RAD57*, *TOP3*, *MEC3*, *RAD1*	7	1.24E−02
tRNA aminoacylation for protein translation	2	*poxA*, *yadB*	5	*SLM5*, *ARC1*, *DIA4*, *MSK1*, *MSE1*	7	1.24E−02
Aerobic respiration	2	*nuoF*, *nuoH*	7	*YDR115W*, *QCR7*, *QCR6*, *DIA4*, *OAR1*, *MCT1*, *PET20*	9	1.64E−02
Translation	7	*poxA*, *prfB*, *rpmF*, *rpsO*, *rpsT*, *rpsU*, *yadB*	26	*RPS8A*, *FES1*, *SLM5*, *MTF2*, *RPL31A*, *RPL35B*, *YDR115W*, *CBS2*, *MRPL7*, *MRP20*, *RPL12B*, *RML2*, *RPL7A*, *RPL1B*, *RPS0A*, *DIA4*, *MTG2*, *CTK1*, *MEF1*, *RPS17A*, *RPL20A*, *MSK1*, *MRPS12*, *RSM19*, *IFM1*, *MSE1*	33	2.78E−02
Regulation of translation	2	*ihfA*, *ihfB*	13	*TPD3*, *RPS8A*, *FES1*, *NAT1*, *MSS116*, *CBS2*, *RPL12B*, *PUF6*, *PET122*, *RPL7A*, *RPS0A*, *RPS17A*, *RPL20A*	15	4.55E−02
DNA recombination	8	*ihfA*, *ihfB*, *intA*, *recA*, *recO*, *recR*, *ruvA*, *ruvC*	5	*HPR1*, *ERG28*, *THP2*, *RAD52*, *SGS1*	13	5.70E−02

spotted onto YES-agar plates using the 96-syringe Matrix Scientific Hydra. Upon drying, the cells were left untreated or exposed to increasing doses of UV (10, 15 and 20 J/m2) and then allowed to grow for 60 h (Fig. 3). UV exposure conditions were chosen based on the behavior of *rad17Δ* cells, as this DNA repair mutant was consistently identified as UV-sensitive in preliminary experiments. In total, 122 plates containing ~11,500 spotted cultures were assayed as described earlier and the resulting images of these plates were compiled into Supplemental Figure S3. We classified 310 *S. pombe* gene products as modulating the toxicity of UV (Table 3). In a similar fashion to our results in *E. coli* and *S. cerevisiae*, we identified a wide range of *S. pombe* cellular proteins that modulate the toxicity of UV. In all, we determined that 18 biological processes from *S. pombe* were over-represented with UV-toxicity modulating proteins (Table 4) and, as predicted by our Functionome study, the categories of DNA repair, protein synthesis and, to some extent, energy production were well represented. Specifically related to our Functionome prediction, we determined that *S. pombe* proteins associated with the GO biological processes of NER, DNA recombination and response to DNA damage stimulus were over-represented ($P<0.09$) in our list of UV-toxicity modulating proteins. It is worth noting that while NER proteins consistently modulate the toxicity of UV-damage in lower organisms, our observed trend may not be observed in higher eukaryotes, as the redundancy in DNA repair pathway and differences in cell metabolism may allow for compensatory responses. We also determined that the GO biological process of translation ($P<0.09$) was over-represented in our list of *S. pombe* UV-toxicity modulating proteins, supporting our prediction that components of the protein synthesis machinery play an important yet unknown role after UV-exposure. Significantly for both DNA repair and protein synthesis, we have observed exact identity in four GO terms (NER, DNA recombination, response to DNA damage stimulus and translation), as they were all over-represented with UV-toxicity modulating proteins from *E. coli*, *S. cerevisiae* and *S. pombe*. The identity in DNA repair-associated terms between *S. pombe*, *S. cerevisiae* and *E. coli* UV-toxicity modulating proteins was expected and provided validation of our methodology for comparing high throughput screening results. We note that in *S. pombe*, we only classified two GO annotated NER proteins as modulators of UV-toxicity (Rad8 and Uve1) and had a third fall just below our sensitivity cut off (Rad13). Analysis of the *S. pombe* library indicated that only 15 of the 28 mutants corresponding to NER proteins were represented and of these 15, we classified 5 as slow growers; thus, our search space was limited to 10 mutants. Our analysis suggests that in *S. pombe* NER is a vital cellular process under basal conditions.

The trend that the GO term of translation was consistently identified in each organism's list of UV-toxicity modulating proteins (Fig. 4) further supports our conclusion that protein synthesis or ribosomal protein-based responses to UV-damage are universally important to many cell types. Detailed annotation information on 7 *E. coli*, 26 *S. cerevisiae* and 14 *S. pombe* proteins identified in the biological process of translation indicated that many are associated with the small (11) or large (13) ribosomal subunits. The ribosome is a complicated machine and in both prokaryotic and eukaryotic organisms, it is composed of ribonucleoproteins and divided into large and small subunits. We wanted to determine if there was any amino acid similarity between the individual proteins found associated with the translation node, and using the *S. pombe* gene database [41] we looked for orthologous proteins between the two yeasts. We determined that in the translation node, two sets of UV-toxicity modulating proteins from *S. pombe* and *S. cerevisiae* were orthologs and thus connected to each other: Rpl702–Rpl7A, and Rpl2002–Rpl20A. While the exact role for these large ribosomal proteins in preventing UV-toxicity is unknown, we can speculate that a protein synthesis based response to damage is corrupted in these cells. This response could include the use of specific ribosomal proteins to promote the translation of stress response genes, which is akin to a ribosomal code [42,43]. Other realistic possibilities exist, though, as ribosomal proteins have been demonstrated to perform auxiliary activities in stress signaling [44] and they may directly contribute to DNA repair or cell cycle in some fashion. It is worth noting that

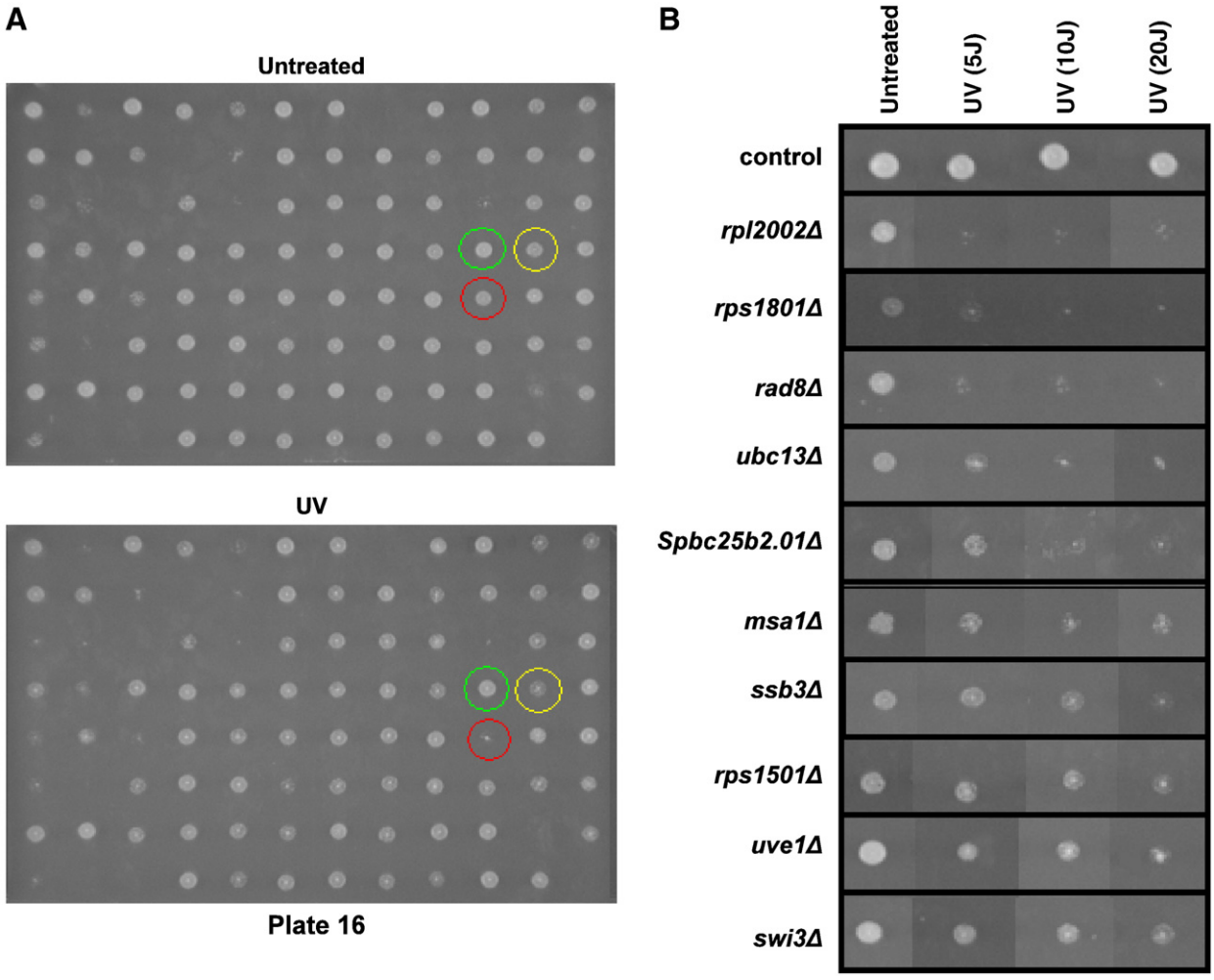

Fig. 3. Genomic phenotyping of *S. pombe* mutants with UV. (A) 93 gene deletion mutants were spotted onto agar plates, left untreated or exposed to UV, incubated at 30 °C for 60 h and imaged. Green, red and yellow circles identify *spbc21c3.02cΔ*, *ubc13Δ* and *msa1Δ*, respectively. (B) Images were taken from many different plates and recompiled to demonstrate that varying degrees of UV sensitivity were observed in the *S. pombe* screen.

ribosomal protein S3 has been shown to nick AP containing DNA, has affinity for abasic sites and 7,8-dihydro-8-oxoguanine DNA, and has been shown to localize to the nucleus in response to genotoxic stress [45,46]. Thus, there are already published biochemical and damage-response roles for ribosomal proteins in DNA repair. In addition, there is sufficient evidence that ribosomal proteins have extra-ribosomal functions [44]. Another possibility is that perturbations in ribosome assembly promote stress on their own and in conjunction with UV-damage, this may overwhelm the cellular stress response. The role of ribosomal proteins during stress is largely unexplored and the identified sets of orthologs (Rpl702–Rpl7A, and Rpl2002–Rpl20A) highlight starting points for focused studies to better understand the role of protein synthesis machinery after UV-exposure.

Many of the *S. cerevisiae* proteins (16 in total) belonging to the translation node play a role in mitochondrial protein synthesis. Defects in mitochondrial translation are associated with corrupted aerobic metabolism [40], which provides a link between the process of translation and energy production. This link is also demonstrated in our Functionome analysis as the dual annotation of specific proteins in both translation and energy metabolism based processes was observed (Fig. 2C). The biological process of aerobic respiration was specifically identified in our Functionome analysis of UV-toxicity modulating proteins from *E. coli* and *S. cerevisiae*. In *S. pombe*, we analyzed 8 of 25 mutants corresponding to proteins annotated with the aerobic respiration designation and none of these were sensitive to UV. In general though, the GO annotations for all *S. pombe* proteins used in this study are limited with ~1.3 annotations per protein as compared to ~2.7 (12130/4415) and ~2.0 (6120/3120) annotations per protein for *S. cerevisiae* and *E. coli*, respectively. In addition, the *S. pombe* deletion library only contained 32% of the mutants corresponding to the aerobic respiration category, suggesting that many are essential genes. Thus the small sample size (eight mutants) limited our search space. We did observe a hint of aerobic respiration in our list of *S. pombe* UV-toxicity modulating proteins, as the category of respiratory chain complex iv assembly was over-represented ($P<0.03$). Corresponding proteins included Oxa102, required for the insertion of integral membrane proteins into the mitochondrial inner membrane and essential for the activity and assembly of cytochrome c oxidase, and Sco1, a copper chaperone used to transport copper to the Cu(A) site on the cytochrome c oxidase subunit II [41]. While respiratory chain complex iv assembly is not an identical category to aerobic respiration, there is a connection via ATP formation. The precise reason for the identification of aerobic respiration in our analysis is unknown, but we speculate that ATP levels optimize stress responses. A deficiency in an energy-associated metabolite, NAD(+), has recently been implicated in the damage-induced death of DNA repair deficient cells [47], supporting a role for ATP synthesis in modulating DNA damage-induced toxicity. We also note that many of

Table 3
Proteins corresponding to the 310 UV-sensitive mutants identified in *S. pombe*.

Protein	Description	UV sensitivity
Rad17	RFC related checkpoint protein Rad17	High
Spcc576.12c	Conserved eukaryotic protein	High
Rad9	Checkpoint clamp complex protein Rad9	High
Tpp1	Trehalose-6-phosphate phosphatase Tpp1	High
Apm1	AP-1 adaptor complex subunit Apm1	High
Zds1	zds family protein Zds1	High
Rhp18	Rad18 homolog Rhp18	High
Ubc13	Ubiquitin conjugating enzyme Ubc13	High
Spbc16g5.13	Sequence orphan	High
Sol1	SWI/SNF complex subunit Sol1	High
Spbc947.04	DIPSY family	High
Rad22	DNA repair protein Rad22	High
Spbc9b6.07	Nucleolar protein Nop52 family	High
Nmt1	No message in thiamine Nmt1	High
Spbc21c3.02c	Sds3-like family	High
Rip1	Ubiquinol-cytochrome-c reductase complex subunit 5	High
Spp27	RNA polymerase I upstream activation factor complex subunit Spp27	High
Crb2	DNA repair protein RAD9 homolog, Rhp9	High
Spac27d7.06	Electron transfer flavoprotein alpha subunit	High
Rad50	DNA repair protein Rad50	High
Spbc1539.02	Sequence orphan	High
Spcc794.10	UTP-glucose-1-phosphate uridylyltransferase	High
Kin1	Microtubule affinity-regulating kinase Kin1	High
Spac16c9.01c	carbohydrate kinase	High
Rpl2701	60S ribosomal protein L27	High
Spcp1e11.10	Ankyrin repeat protein, unknown biological role	High
Spbc16a3.10	membrane bound O-acyltransferase, MBOAT	High
Spbc29a10.16c	Cytochrome b5	High
Spbc11b10.07c	CDC50 domain protein	High
Spac4f10.16c	P-type ATPase	High
Spt6	Transcription elongation factor Spt6	High
Elf1	AAA family ATPase ELf1	High
Sty1	MAP kinase Sty1	High
Spac4a8.02c	Conserved protein (broad species distribution)	High
Spbc6b1.03c	Pal1 family protein	High
Cds1	Replication checkpoint kinase Cds1	High
Swi3	Replication fork protection complex subunit Swi3	High
Tom7	Mitochondrial TOM complex subunit Tom7	High
Mug42	Sequence orphan	High
Gar2	GAR family	High
Ras1	GTPase Ras1	High
Rpl2002	60S ribosomal protein L20	High
Rps1801	40S ribosomal protein S18	High
Spbc2a9.05c	DUF846 family protein	High
Fsv1	SNARE Fsv1	High
Mug183	Histone chaperone Rtt106-like	High
Spac1f5.03c	FAD-dependent oxidoreductase	High
Rhp55	RecA family ATPase Rhp55	Medium
Str1	Siderophore-iron transporter Str1	Medium
Ilv1	Acetolactate synthase catalytic subunit	Medium
Vps5	Retromer complex subunit Vps5	Medium
Alp14	Mad2-dependent spindle checkpoint component	Medium
Mto1	MT organizer Mto1	Medium
Mug80	Cyclin Clg1	Medium
Spac688.03c	Human AMMECR1 homolog	Medium
Mfm2	M-factor precursor Mfm2	Medium
Spbc17d11.08	WD repeat protein, human WDR68 family	Medium
Spac1486.01	Manganese superoxide dismutase (AF069292)	Medium
Spbc16d10.08c	Heat shock protein Hsp104	Medium
Spac869.04	Formamidase-like protein	Medium
Spbp35g2.14	RNA-binding protein	Medium
Mug136	Acetylglucosaminyltransferase	Medium
Pku80	Ku domain protein Pku80	Medium
Ctf1	mRNA cleavage and polyadenylation specificity factor complex subunit Ctf1	Medium
Cuf1	Cu metalloregulatory transcription factor Cuf1	Medium
Spac9e9.15	CIA30 family protein	Medium
Spbc1604.12	Sequence orphan	Medium
Spcc1020.05	Phosphoprotein phosphatase	Medium
Btf3	Nascent polypeptide-associated complex subunit	Medium
Spac22e12.18	Conserved fungal protein	Medium
Rpl803	60S ribosomal protein L8	Medium
Rtt109	RTT109 family histone lysine acetyltransferase Rtt109	Medium
Rad8	ubiquitin-protein ligase E3	Medium
Spac631.02	bromodomain protein	Medium
Spcc757.11c	membrane transporter	Medium
Dak1	dihydroxyacetone kinase Dak1	Medium
Spac589.10c	Ribomal-ubiquitin fusion protein Ubi5	Medium
Spbp23a10.02	Conserved fungal protein	Medium
Spac1851.02	1-acylglycerol-3-phosphate O-acyltransferase	Medium
Spac1705.02	Human 4F5S homolog	Medium
Spcc126.12	NGG1p interacting factor 3 family	Medium
Spbc3h7.07c	Phosphoserine phosphatase	Medium
Mug4	Sequence orphan	Medium
Cwf11	Complexed with Cdc5 protein Cwf11	Medium
Spcc1739.08c	Short chain dehydrogenase	Medium
Spcc736.13	Short chain dehydrogenase	Medium
Spbc2d10.11c	Nucleosome assembly protein Nap2	Medium
Rnc1	RNA-binding protein that suppresses calcineurin deletion Rnc1	Medium
Gms1	UDP-galactose transporter Gms1	Medium
Spcc663.06c	Short chain dehydrogenase	Medium
Spcc777.12c	Sequence orphan	Medium
Sir2	Sir2 family histone deacetylase Sir2	Medium
Wtf16	wtf element Wtf16	Medium
Spbc56f2.05c	Transcription factor	Medium
Mug96	Sequence orphan	Medium
Pnu1	Endodeoxyribonuclease Pnu1	Medium
Spac3a12.13c	Translation initiation factor eIF3 complex subunit	Medium
Spcc11e10.06c	RNA polymerase II elongator complex subunit Elp4	Medium
Spbc651.09c	RNA polymerase II associated Paf1 complex	Medium
Mpd2	GYF domain	Medium
Pex1	AAA family ATPase Pex1	Medium
Spbc418.02	NatA N-acetyltransferase complex subunit	Medium
Spac12g12.10	WD repeat protein, human WDR21 family	Medium
Chr2	Chitin synthase regulatory factor Chr2	Medium
Spcc794.03	Amino acid permease, unknown 13	Medium
Spcc1259.08	Conserved fungal protein	Medium
Spac22e12.03c	THIJ/PFPI family peptidase	Medium
Rps902	40S ribosomal protein S9	Medium
Ain1	Alpha-actinin	Medium
Spac20g4.01	CCR4-Not complex subunit Caf16	Medium
Did2	Vacuolar sorting protein did2	Medium
Spbc21d10.09c	Ubiquitin-protein ligase e3	Medium
Hem2	Porphobilinogen synthase Hem2	Medium
Rds1	Conserved fungal protein	Medium
Ish1	LEA domain protein	Medium
Sin1	Stress activated MAP kinase interacting protein Sin1	Medium
Spbc365.07c	TATA element modulatory factor homolog	Medium
Air1	TRAMP complex subunit	Medium
Spac15a10.07	Sequence orphan	Medium
Sgo2	Shugoshin Sgo2	Medium
Oxa102	Mitochondrial inner membrane translocase Oxa102	Low
Rpl702	60S ribosomal protein L7	Low
Rpl501	60S ribosomal protein L5	Low
Spac25g10.01	RNA-binding protein	Low
Spac1093.01	PPR repeat protein	Low
Mug182	YjeF family protein	Low
Mug24	RNA-binding protein	Low
Exo1	Exonuclease I Exo1	Low
Spcc24b10.12	CGI121 family protein	Low
Ctu1	ATP binding protein	Low
Mph1	Dual specificity protein kinase Mph1	Low
Spac31g5.07	Conserved fungal protein	Low
Rmt3	Type I ribosomal protein arginine N-methytransferase Rmt3	Low
Fta5	Sim4 and Mal2 associated (4 and 2 associated) protein 5	Low
Meu32	Sequence orphan	Low
Cwf21	Complexed with Cdc5 protein Cwf21	Low
Rps1602	40S ribosomal protein S16	Low
Spbc839.03c	Neddylation protein Dcn1	Low
Spac27e2.11c	Sequence orphan	Low
Vps1302	Chorein homolog	Low
Mcs4	two-component response regulator	Low
Gsk31	Serine/threonine protein kinase Gsk31	Low
Spbc543.10	GET complex subunit	Low
Spbc1709.14	Peptide N-glycanase	Low
Spbc1709.09	Mitochondrial translation termination factor	Low

Table 3 (*continued*)

Protein	Description	UV sensitivity
Spac11d3.14c	Oxoprolinase	Low
Spcc584.13	Amino acid permease, unknown 14	Low
Spbc2f12.03c	EST1 family protein	Low
Meu29	Sequence orphan	Low
Cys12	Cysteine synthase Cys12	Low
Yak3	Aldose reductase YakC	Low
Ctr5	Copper transporter complex subunit Ctr5	Low
Ssb3	DNA replication factor A subunit Ssb3	Low
Spbc1271.07c	N-acetyltransferase	Low
Spac17a5.08	COPII-coated vesicle component Erp2/3/4	Low
Cdd1	Cytidine deaminase Pcd1	Low
Arp42	SWI/SNF and RSC complex subunit Arp42	Low
Brl2	Ubiquitin-protein ligase E3	Low
Gaf1	Transcription factor Gaf1	Low
Arg4	Carbamoyl-phosphate synthase Arg4	Low
Spbc359.01	Amino acid permease, unknown 7	Low
Nse5	Smc5-6 complex non-SMC subunit Nse5	Low
Yam8	Calcium transport protein	Low
Mug165	Sequence orphan	Low
Spbc21c3.06	Sequence orphan	Low
Spac1f5.05c	Sequence orphan	Low
Spac29b12.08	Sequence orphan	Low
Rps1102	40S ribosomal protein S11	Low
Spac1687.08	Sequence orphan	Low
Omt2	4-alpha-hydroxytetrahydrobiopterin dehydratase	Low
Spcc320.14	Threo-3-hydroxyaspartate ammonia-lyase	Low
Spcc191.10	Sequence orphan	Low
Spbc18h10.16	Amino acid permease, unknown 9	Low
Spac212.02	Sequence orphan	Low
Spcc1322.10	conserved fungal protein	Low
Spac26a3.14c	DUF1748 family protein	Low
Spac27e2.01	Alpha-amylase homolog	Slightly
Ght7	Hexose transporter Ght7	Slightly
Rga9	RhoGAp, GTPase activator towards Rho/Rac/Cdc42-like small GTPases	Slightly
Thi5	Transcription factor Thi5	Slightly
Spbc3b8.06	Conserved fungal protein	Slightly
Coq2	Para-hydroxybenzoate–polyprenyltransferase Coq2	Slightly
Pof3	F-box protein Pof3	Slightly
Oca2	Serine/threonine protein kinase Oca2	Slightly
Lyn1	Sequence orphan	Slightly
Spac14c4.12c	SWIRM domain protein	Slightly
Kap1	Chromatin remodeling complex subunit Ngg1	Slightly
Ctu2	Conserved eukaryotic protein	Slightly
Spac22a12.17c	Short chain dehydrogenase	Slightly
Csn71	COP9/signalosome complex subunit 7a	Slightly
Spac644.13c	Rab GTPase binding	Slightly
Spbc543.08	Phosphoinositide biosynthesis protein	Slightly
Spbc31f10.02	Thioesterase superfamily protein	Slightly
Spcc1442.02	DUF1760 family protein	Slightly
Spbc25b2.01	Elongation factor 1 alpha related protein	Slightly
Spcc594.07c	Sequence orphan	Slightly
Bqt1	Bouquet formation protein Bqt1	Slightly
Spcc70.02c	Mitochondrial ATPase inhibitor	Slightly
Atp15	F0-ATPase epsilon subunit	Slightly
Spac9g1.05	Actin cortical patch component Aip1	Slightly
Pet127	Mitochondrial membrane protein Pet127	Slightly
Spac823.10c	Mitochondrial carrier with solute carrier repeats	Slightly
Nap1	Nucleosome assembly protein Nap1	Slightly
Nup124	Nucleoporin nup124	Slightly
Spac1687.07	Conserved fungal protein	Slightly
Pep3	Ubiquitin-protein ligase e3	Slightly
Smd3	Sm snRNP core protein Smd3	Slightly
Spbc776.16	Sequence orphan	Slightly
Spac1952.17c	GTPase activating protein	Slightly
Spac1f3.03	Lgl family protein	Slightly
Tcg1	Single-stranded telomeric binding protein Tgc1	Slightly
Spac1782.01	Proteasome component	Slightly
Spac959.06c	Sequence orphan	Slightly
Spac1687.19c	Queuine tRNA-ribosyltransferase	Slightly
Spbc25d12.06	RNA helicase	Slightly
Rex3	Exonuclease Rex3	Slightly
Spbc1685.05	Serine protease	Slightly
Hus1	Checkpoint clamp complex protein Hus1	Slightly
Spcc16a11.16c	ARM1 family	Slightly
Ats1	N-acetyltransferase Ats1	Slightly

Table 3 (*continued*)

Protein	Description	UV sensitivity
Spac17c9.15c	Sequence orphan	Slightly
Spcc306.02c	Rab GTPase binding	Slightly
Tsf1	Mitochondrial translation elongation factor EF-Ts Tsf1	Slightly
Spac31g5.15	Phosphatidylserine decarboxylase	Slightly
Spcc1919.07	Sequence orphan	Slightly
Spac13g7.09c	Sequence orphan	Slightly
Spac30d11.06c	DUF300 family protein	Slightly
But1	Neddylation pathway protein But1	Slightly
Rti1	Rad22 homolog Rti1	Slightly
Rrp16	rRNA processing protein Rrp16	Slightly
Med20	TATA-box related factor (TRF)	Slightly
Spbc21b10.03c	Ataxin-2 homolog	Slightly
Spbp22h7.05c	ATPase with bromodomain protein	Slightly
Gos1	SNARE Gos1	Slightly
Spcc1795.10c	Sed5 Vesicle Protein Svp26	Slightly
Spac9.02c	N-acetyltransferase	Slightly
Rpl1801	60S ribosomal protein L18	Slightly
Rep2	Transcriptional activator Rep2	Slightly
Spbc21c3.17c	Conserved fungal protein	Slightly
Spac8f11.02c	Diphthamide biosynthesis protein Dph3	Slightly
Sco1	Copper chaperone Sco1	Slightly
Spac3h8.07c	prefoldin subunit 3	Slightly
Spbc359.03c	Amino acid permease, unknown 8	Slightly
Spac4g8.03c	RNA-binding protein	Slightly
Dak2	Dihydroxyacetone kinase Dak2	Slightly
Ght1	Hexose transporter Ght1	Slightly
39722	Mitochondrial intermediate peptidase Oct1	Slightly
Spcc61.03	Conserved protein (broad species distribution)	Slightly
Spac13f5.03c	Glycerol dehydrogenase	Slightly
Spac227.06	Rab GTPase binding	Slightly
Rps1501	40S ribosomal protein S15	Slightly
Spac3g9.11c	Pyruvate decarboxylase	Slightly
Met16	Phosphoadenosine phosphosulfate reductase	Slightly
Spbc13e7.08c	RNA polymerase II associated Paf1 complex	Slightly
Spbc405.05	Sequence orphan	Slightly
Mug106	Sequence orphan	Slightly
Rsc1	RSC complex subunit Rsc1	Slightly
Hsp9	Heat shock protein Hsp9	Slightly
Spbc409.08	Spermine family transporter	Slightly
Dga1	Diacylglycerol O-acyltransferase	Slightly
Spbc106.13	Conserved eukaryotic protein	Slightly
Spac1952.03	Cysteine protease	Slightly
Spac29b12.11c	Human WW domain binding protein-2 ortholog	Slightly
Par1	Protein phosphatase regulatory subunit Par1	Slightly
Ppk4	Serine/threonine protein kinase Ppk4	Slightly
Srb11	Cyclin Srb11	Slightly
Ago1	Argonaute	Slightly
Vps3	GTPase regulator Vps3	Slightly
Spac3a11.04	Siepin homolog	Slightly
Cid12	Poly(A) polymerase Cid12	Slightly
Pep7	Prevacuole/endosomal FYVE tethering component Pep7	Slightly
Eaf1	RNA polymerase II transcription elongation factor SpEAF	Slightly
Spcc4b3.08	C-terminal domain kinase I (CTDK-I) gamma subunit	Slightly
Gyp1	GTPase activating protein Gyp1	Slightly
Spbc582.08	Alanine aminotransferase	Slightly
Sce3	Translation initiation factor eIF4B	Slightly
Cis4	Membrane transporter	Slightly
Spbc21c3.08c	Ornithine aminotransferase	Slightly
Uve1	Endonuclease Uve1	Slightly
Rrg1	Methyltransferase	Slightly
Tel1	ATM checkpoint kinase	Slightly
Spcc162.01c	RNA-binding protein	Slightly
Spbc29a10.07	Nucleoporin Pom152	Slightly
Spcc663.14c	Membrane transporter	Slightly
Spac10f6.11c	Kinase activator	Slightly
Spbc21h7.04	ATP-dependent RNA helicase Dbp7	Slightly
Apl1	AP-2 adaptor complex subunit Apl1	Slightly
But2	Neddylation pathway protein But2	Slightly
Wis2	Cyclophilin family peptidyl-prolyl cis-trans isomerase Wis2	Slightly
Spcc285.13c	Nucleoporin Nup60	Slightly
Str3	Siderophore-iron transporter Str3	Slightly

(*continued on next page*)

Table 3 (*continued*)

Protein	Description	UV sensitivity
Spap27g11.14c	Sequence orphan	Slightly
Spac23a1.14c	Cystathionine gamma-synthase	Slightly
Spac977.14c	Aldo/keto reductase, unknown biological role	Slightly
Spacunk4.15	2′,3′-cyclic-nucleotide 3′-phosphodiesterase	Slightly
Spac17c9.14	Pex19 protein family	Slightly
Spbc21d10.10	Bromodomain protein	Slightly
Spac31g5.18c	Ubiquitin family, human C1ORF55 related	Slightly
Spbc16h5.12c	Conserved fungal protein	Slightly
Spcc1840.04	Caspase	Slightly
Spcc1235.01	Sequence orphan	Slightly
Spbc27b12.14	Mitochondrial membrane protein complex assembly protein	Slightly
Spcc548.04	Ubiquitin family protein Urm1	Slightly
Spbc776.17	rRNA processing protein Rrp7	Slightly
Wsc1	Transmembrane receptor Wsc1	Slightly
Spcc736.07c	Cell polarity protein	Slightly
Spbp8b7.13	Conserved fungal protein	Slightly
Rif1	Telomere length regulator protein Rif1	Slightly
Rpl2301	60S ribosomal protein L23	Slightly
Mug63	TLDc domain protein 1	Slightly

the proteins that participate in DNA repair, recombination, and the DNA damage response are ATP-dependent enzymes and their activity could be affected in cells compromised for aerobic respiration. These enzymes include helicases and recombinases that manipulate DNA strands, as well as chromatin remodeling enzymes vital to transcriptional responses and DNA replication; as such deficiencies in ATP levels should thus impede their activity. While the exact reasons for our identification of proteins associated with aerobic respiration as modulating the toxicity of UV are speculative, our results highlight a potential area for future studies.

3. Conclusions

Cellular responses to DNA damage play an important role in dictating cellular outcomes, and model organisms continue to be an important tool for understanding stress response pathways. High throughput screens using the previously described model systems are fast and cost-efficient and when linked to computational analysis allowed for the identification of highly significant themes associated with UV-toxicity modulation. Our identification of over 600 new UV-toxicity modulating proteins in *E. coli* and *S. pombe* and their comparison to previously reported proteins from *S. cerevisiae* has further cemented a universal role for DNA repair after UV-exposure. In addition, our study has highlighted roles for protein synthesis machinery and aerobic respiration components after UV-exposure. Ultimately, we have demonstrated the feasibility of using comparative functional genomics approaches to identify highly conserved biological responses to UV-damage. Our methodology can be easily extended to other stress responses and has the potential to help identify novel damage-response themes and proteins in higher organisms.

4. Materials and methods

4.1. High throughput screening of E. coli gene-deletion mutants against UV

Luria Bertani broth (LB) (BP1426-3, Fisher Scientific, Waltham, MA) was used to culture *E. coli*. The library of *E. coli* gene deletion mutants was acquired from the Genome Analysis Project in Japan [29]. High throughput screening of the *E. coli* gene deletion library was performed as previously described [26]. This procedure is very similar to what we used to screen the *S. cerevisiae* gene deletion library [24]. Briefly, 96-well plates containing the gene deletion mutants were replicated into liquid medium (LB-kanamycin), grown for 16 h at 37 °C, diluted 10-fold into LB and 1 μl cell suspensions were then robotically (Matrix Hydra) spotted on LB-kanamycin agar plates. Plates were allowed to dry for 30 min and UV doses were applied using a Stratalinker (Stragene, Cedar Creek, Texas). Inoculated plates were incubated for 16 h at 37 °C and then imaged using an AlphaImager (Alpha Innotech Corporation, San Leandro, CA). Reduced growth for a specific gene-deletion mutant was identified relative to other mutants found on the 96-well plate and wild-type BW25113 cells, and was also relative to growth of the mutant on an untreated plate. To identify UV-toxicity modulating proteins, we linked sensitive mutants to their corresponding deleted gene and assumed the protein encoded by the deleted gene was responsible for the observed phenotype.

Table 4
GO biological processes over-represented in the 310 UV-toxicity modulating proteins from *S. pombe*.

Functional process	Total tested	# UV sensitive	Genes	P-value
Protein amino acid acetylation	5	3	*ATS1*, *SPBC1271.07C*, *SPBC418.02*	1.59E−04
Ribosome biogenesis	50	11	*GAR2*, *RPL501*, *RPS1102*, *RPS1602*, *RPS1801*, *RPS902*, *RRP16*, *SPBC776.17*, *RMT3*, *SPAC589.10C*, *TCG1*	6.87E−04
RNA processing	4	2	*PET127*, *REX3*	2.56E−03
Glycerophospholipid biosynthetic process	3	2	*SPAC1851.02*, *SPBC16A3.10*	2.56E−03
mRNA polyadenylation	4	2	*SPBC21B10.03C*, *CTF1*	3.47E−03
Protein homooligomerization	5	2	*RAD22*, *RTI1*	4.66E−03
Response to arsenic	6	2	*MCS4*, *STY1*	4.66E−03
Chromosome segregation	23	5	*AGO1*, *ALP14*, *CID12*, *SIR2*, *SPCC576.12C*	8.20E−03
Response to hydrogen peroxide	7	2	*MCS4*, *STY1*	1.39E−02
Cellular iron ion homeostasis	10	3	*STR1*, *STR3*, *CUF1*	2.28E−02
Copper ion transport	4	2	*CTR5*, *SCO1*	2.28E−02
Respiratory chain complex iv assembly	5	2	*OXA102*, *SCO1*	2.87E−02
DNA recombination[a]	6	2	*PNU1*, *SSB3*	4.46E−02
Response to DNA damage stimulus[a]	27	5	*NSE5*, *RAD8*, *RHP55*, *RTT109*, *SWI3*	6.68E−02
Nucleotide-excision repair[a]	10	2	*RAD8*, *UVE1*	8.08E−02
Intracellular protein transport	67	10	*APL1*, *APM1*, *SPAC4F10.16C*, *SPAC644.13C*, *VPS1302*, *VPS3*, *VPS5*, *FSV1*, *GOS1*, *SPAC17A5.08*	8.08E−02
Regulation of catalytic activity	59	9	*PAR1*, *RGA9*, *TSF1*, *ZDS1*, *SPAC1F3.03*, *SPAC227.06*, *SPAC644.13C*, *SPCC306.02C*, *VPS3*	9.68E−02
Translation[a]	116	14	*RPL1801*, *RPL2002*, *RPL2301*, *RPL2701*, *RPL702*, *RPL803*, *RPS1102*, *RPS1501*, *SPAC3A12.13C*, *RPL501*, *RPS1602*, *RPS1801*, *RPS902*, *SPBC25B2.01*	9.68E−02

[a] Indicates a biological process that was also identified in our Functionome analysis.

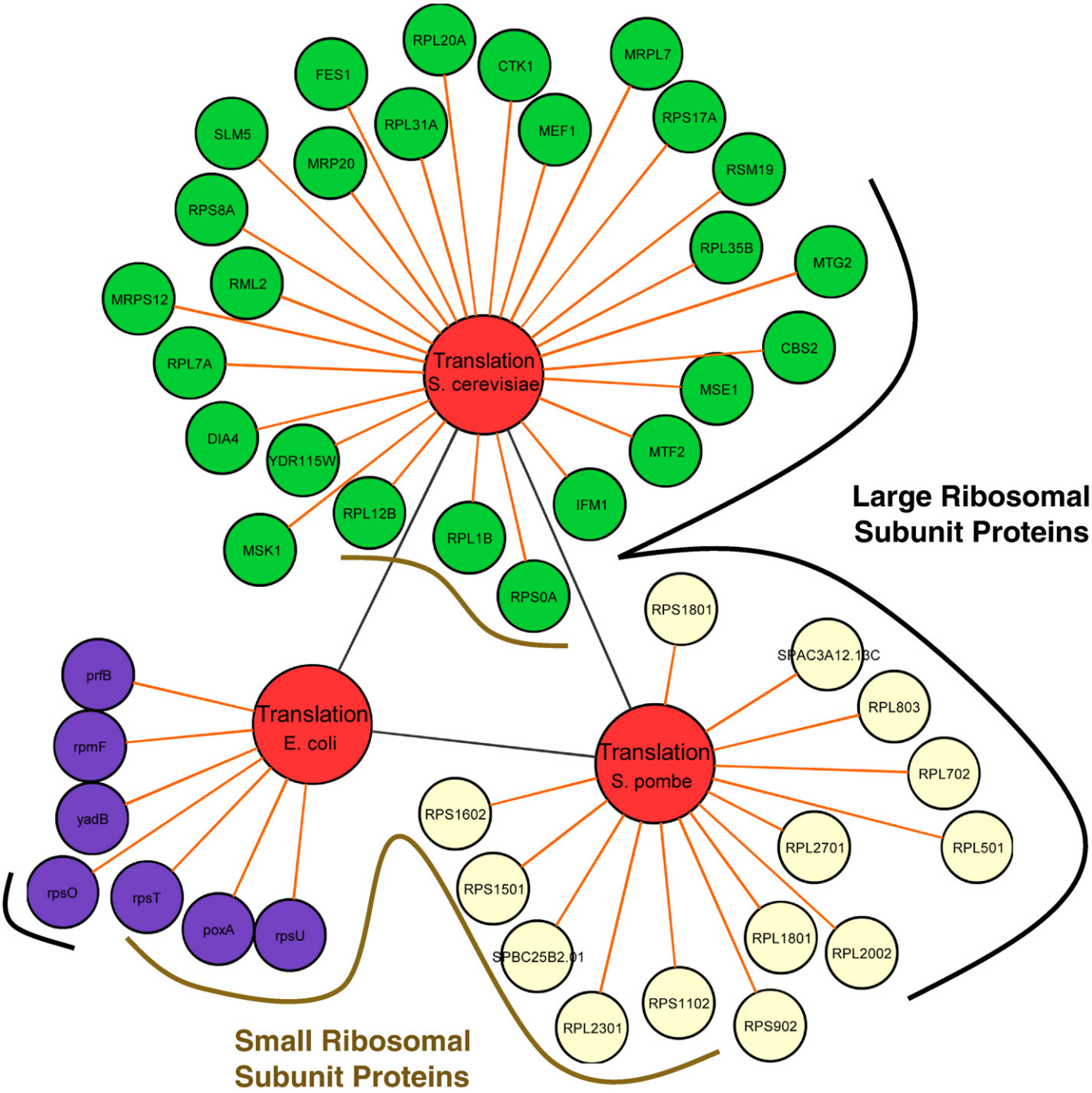

Fig. 4. Tri-species node of translation identified as a conserved biological process that modulated the toxicity of UV. UV-toxicity modulating proteins from *E. coli* (purple spheres, lower case protein names), *S. cerevisiae* (green spheres, upper case protein names) and *S. pombe* (yellow spheres, upper case protein names) were connected to their GO biological process of translation (orange line) and each protein's basic function was analyzed in a species-specific database (Ecogene, *S. pombe* GeneDB and SGD). Those proteins belonging to the large and small ribosomal subunit are underlined in black and brown, respectively.

4.2. Functional interactome construction, mapping and analysis

We constructed a Functionome by using gene ontology information for *E. coli* and *S. cerevisiae* proteins. Species-specific gene ontology information associated with biological process was downloaded from the Gene Ontology Project website (Feb 1, 2010, www.geneontology.org) [33]. We choose to use an intermediate level of biological process to limit redundancy in our search space and only used 511 biological processes common to both *E. coli* and *S. cerevisiae* (Supplemental Table 1). The data files were compiled so that each protein was linked to one or more biological processes and the compiled information was visualized as a functional interactome using the program Cytoscape [48]. Sensitivity data representing 171 *E. coli* and 236 *S. cerevisiae* UV-toxicity modulating proteins was mapped onto our functional interactome. Next, we identified GO-nodes that were significantly over-represented with UV-toxicity modulating proteins and contained at least two from *E. coli* and two from *S. cerevisiae*. GO-node significance was determined by mapping randomly sampled

proteins (171 from *E. coli* and 236 from *S. cerevisiae*) onto the Functionome and tracking occurrences in specific nodes over 200 iterations. Significant GO-nodes were first determined based on Z-score calculations and then using Normal curve approximations, similar as described [24,26]. The protein randomization method utilized a static network and random groups of proteins to determine significance. We also verified the significance of our UV-target lists by mapping our 171 and 236 UV-toxicity modulating proteins from *E. coli* and *S. cerevisiae* on 200 randomized Functionomes. This last method employed a static target list and randomly compiled functional interactions, and is similar to a method we have previously reported [49]. Once we identified GO-nodes that met our criteria, significance ($p<0.05$) and number of associated proteins ($=>4$), we utilized publically available protein–protein interaction information [50] to enhance our network visualizations. *S. cerevisiae* strains with mitochondrial mutations (rho^-) were generated as previously described [51] and tested for UVC sensitivity on plating a 10-fold dilution series of cells on YPD plates and treating with increasing doses of UVC.

4.3. High throughput screening of S. pombe gene-deletion mutants against UV

Yeast extract with supplements (YES) (Fisher Scientific, Waltham, MA) was used to culture *S. pombe*. The library of *S. pombe* gene deletion mutants was acquired from Bioneer Corporation (Daejeon, Republic of Korea). High throughput screening of the *S. pombe* gene deletion library was performed in a similar fashion to the methodology we have described for *E. coli* and *S. cerevisiae* [24,26]. Briefly, 96-well plates containing the gene deletion mutants were replicated into liquid medium (YES), grown for 24 h at 30 °C, and 1 μl cell suspensions were robotically (Matrix Hydra) spotted onto YES agar plates. Plates were allowed to dry for 30 min and UV doses were applied using a Stratalinker (Stragene, Cedar Creek, Texas) with 254 nm bulbs. Inoculated plates were incubated for 60 h at 30 °C and then imaged using an AlphaImager (Alpha Innotech Corporation, San Leandro, CA). Reduced growth for a specific gene-deletion mutant was identified relative to other mutants found on the 96-well plate and wild-type. This was done for both untreated and UV treated plates. Mutants were initially scored for their ability to grow on untreated plates, with 357 mutants displaying a general slow growth phenotype (Supplemental Table 2). UV-sensitive mutants were scored (4 to 1, high to slightly sensitive) based on their ability to grow normally on untreated plates and on their decreased growth after UV treatment. A cumulative UV-toxicity sensitivity score for each mutant was determined, representing the behavior of each mutant after three increasing UV doses over two biological replicates. In theory, the most sensitive mutant could score a 24 ((4 + 4 + 4) 2 replicates) and we set a minimum sensitivity score of 4. To identify UV-toxicity modulating proteins, we linked sensitive mutants to their corresponding deleted gene and assumed the protein encoded by the deleted gene was responsible for the observed phenotype. GO functional mapping using the 310 UV-toxicity modulating proteins and corresponding GO-annotations (Supplemental Table 3) was performed similar to as described earlier. We note that all GO assignments specific to each protein reported are included in Supplemental Table 4 and these tables can be imported into Cytoscape to visualize the Functionomes used in this study. In addition, all GO terms used in this study are reported in Supplemental Table 5.

Supplementary materials related to this article can be found online at doi:10.1016/j.ygeno.2010.12.005.

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