We describe two ring finger proteins in the fission yeast \emph{Schizosaccharomyces pombe}, Rfp1 and Rfp2. We show that these function redundantly in DNA repair. \textit{Rfp1} was isolated as a Chk1-interacting protein in a two-hybrid screen, and is a close sequence relative to \textit{Rfp2}. Deletion of either gene does not cause a phenotype, but a double deletion (\textit{rfp1\textDelta rfpp2\textDelta}) shows poor viability and defects in cell cycle progression. These cells are also sensitive to DNA damaging agents, though maintain normal checkpoint signaling to Chk1. Rfp1 and Rfp2 are most closely related to human Rnf4, and we show that Rnf4 can functionally substitute for Rfp1 and/or Rfp2. The double mutants also show significantly increased levels of protein SUMOylation, and we identify an \textit{S. pombe} Ulp2/Smt4 homolog that, when overexpressed, reduces SUMO levels and suppresses the DNA damage sensitivity of \textit{rfp1\textDelta rfpp2\textDelta} cells.

The fidelity of cell cycle progression is paramount for tissue homeostasis, development and avoidance of disease. A major contributor to this fidelity are the plethora of responses that detect damage to DNA, ensure its repair, and activate checkpoints that modify the cell cycle to allow time for these events to occur. These responses are rapid, highly conserved, and frequently regulated by cascades of post-translational modifications that control the activities of repair proteins and signal transducers of the checkpoints.

The fission yeast \emph{Schizosaccharomyces pombe} has been an important model system for the dissection of the molecular controls over DNA damage responses (1,2). \textit{S. pombe} has a prominent G2 period in vegetative cell cycles that represents \~70\% of total cell cycle time (3). The DNA damage checkpoint functioning during this period signals to activate the Chk1 protein kinase, which in turn prevents entry into mitosis through modulating the Cdc2 regulators Wee1 and Cdc25 (4-6). Chk1 activation is controlled by reversible phosphorylation on S345 by Rad3 (7-9), an ATR homolog, though the mechanism by which this activation occurs is largely obscure. ATR homologs also phosphorylate a number of other proteins in this pathway (1,10).

In addition to phosphorylation cascades, other post-translational modifications regulate DNA damage response pathways. Among these are the covalent modification of proteins by ubiquitin and its related protein SUMO. In addition to directing the proteolysis of its targets, ubiquitination can directly influence responses to DNA damage. A well-documented example of this is through the post-replication repair proteins that control the mono- and poly-ubiquitination of proliferating cell nuclear antigen, which in turn enables the bypass of lesions during DNA replication (11-13).

SUMO modification of several DNA repair enzymes has also been described (14), though in most cases the molecular effects of such modification remains unclear. Control over protein SUMOylation is achieved by a balance of the activity of several E3 SUMO ligases, and the deconjugating activity of SUMO peptidases such as Ulp2/Smt4 in \textit{Saccharomyces cerevisiae}. SUMO is not essential in \textit{S. pombe}, though cells deleted for the SUMO gene (\textit{pmt3}) (15), or the E2 SUMO-conjugating enzyme gene \textit{hus5} (16), are sensitive to DNA damaging agents and show defects in chromosome segregation. \textit{S. cerevisiae} cells lacking Ulp2 also show defects in chromosome segregation and DNA damage responses (17-20), suggesting that a balanced control over protein SUMOylation and deSUMOylation is important in these responses.

Here we describe two novel genes in \textit{S. pombe}, \textit{rfp1} and \textit{rfp2}, which encode proteins with
Ring Finger domains, which are characteristic of E3 ubiquitin ligases. These proteins function redundantly in DNA repair; a double mutant is hypersensitive to different forms of DNA damage, though checkpoint signaling through to Chk1 activation is normal. We show that human Rnf4, an E3 ubiquitin ligase that has been implicated as a co-factor for several transcription factors (21-25), is a close sequence relative of Rfp1 and Rfp2, and can indeed functionally substitute for these proteins. We show that the control over protein SUMOylation is defective in cells lacking Rfp1 and Rfp2, and that indeed this is the cause of the DNA repair defect. These data raise the possibility that human Rnf4 may similarly function in the DNA damage response.

**EXPERIMENTAL PROCEDURES**

**Two-hybrid screening:** Chk1 was cloned onto the Gal4 DNA binding domain (pAS2) and transformed into the PJ69A strain of *S. cerevisiae* (26). An *S. pombe* cDNA, Gal4 activation domain fusion library in pact (a kind gift of Steve Elledge) was transformed into these cells, and putative interactors were screened for on SC medium lacking adenine. Plasmids were recovered into *E. coli*, sequenced and re-tested for bait dependence.

**Fission yeast methods:** All strains are derivates of 972h and 973h'. Standard methods and media for the propagation, transformation, mating and culturing of *S. pombe* were as described (27). Null alleles were constructed by Start-Stop codon replacement by *ura4* by homologous recombination. Gene targeting was confirmed by Southern blotting, and strains were backcrossed to wildtype before analysis. Microscopy was performed on cells fixed in 3.7% formaldehyde or 70% ethanol, and images captured on Nikon Eclipse 800 microscope with a Spot RT/SE camera. Immuno-detection of proteins in fixed cells was performed as previously described (28,29). Methods for UV-C and methyl methanesulfonate (MMS) survival assays were as previously described (30,31). Briefly, for UV-C, exponential cultures were plated in triplicate at densities of 100-10,000 cells/plate, and irradiated with UV-C using a Stratalinker (Stratagene). Colonies were allowed to form over 4 days at 30°C, and normalized to unirradiated controls. For MMS sensitivity, 10-fold serial dilutions were spotted onto plates containing a range of MMS concentrations, and colonies were allowed to form over 4 days at 30°C. Plasmid curing was achieved by selection relief for auxotrophic markers on YES medium, followed by re-selection of minimal medium. To avoid selection for extragenic slow-growth suppressors, all experiments with *rfp1Δ rfp2Δ* double mutants were performed with freshly made strains derived from non-parental di-types.

**Protein extraction and Western blotting:** Proteins were extracted from cell pellets disrupted with glass beads and a mini-bead beater. Native extracts for immunoprecipitations were performed as described (9). Denatured extracts for SUMO conjugates were made using 8M Urea buffer (5). Pmt3 (SUMO) was detected using an HA-tagged *pmt3* allele (15). Anti-alpha tubulin antibody B-5 (Sigma) was used as a loading control. Western transfer of SDS-PAGE gels to nitrocellulose was performed with 10mM CAPS, 10% Methanol. Immune complexes were detected with horseradish peroxidase conjugated secondary antibodies were detected with ECL reagent (GE Biosciences). Chk1 activity assays were performed in triplicate as described (32,33).

**Recombinant proteins and Chk1 kinase assays:** Recombinant GST-fusion proteins were expressed in BL-21 *E. coli* and purified on GSH-Sepharose as described (34). Phosphorylation of these proteins by Chk1 purified from *S. pombe* extracts was performed as described (8,32).

**RESULTS**

**Identification of Chk1 interacting proteins**

To search for Chk1-interacting proteins we performed a yeast two-hybrid screen using full length Chk1 fused to the Gal4 DNA binding domain as bait. Among several positive clones (table 1) were *rad24* and *ded1*, which have been previously described as Chk1-interactors by this assay (35,36). Among the unique clones was a novel 254 amino acid protein (SPAC19A8.10) with a C-terminal Ring finger domain, which we denoted Rfp1. Using N- and C-terminal Chk1
baits, Rfp1 was shown to interact with the C-terminal regulatory domain of Chk1 (Fig 1A). The interaction was confirmed by co-immunoprecipitation of Chk1 and Rfp1 expressed from the medium-strength attenuated nmt1 promoter (37) (Fig 1B). However, under endogenous expression conditions, no such interaction could be observed (data not shown). Thus, the interaction in the yeast two-hybrid assay may not be physiological, though we cannot rule a transient interaction that is difficult to detect.

**Rfp1 acts redundantly with Rfp2 in DNA repair**

As we could not detect interaction between endogenous Chk1 and Rfp1, we sought to uncover function for Rfp1 to gauge the physiological relevance of the yeast two-hybrid interaction. The chromosomal rfp1 locus was deleted and replaced by ura4. These cells grew normally, were fertile and showed wildtype sensitivities to DNA damaging agents. We conclude that rfp1 is, by itself, not essential for cell viability.

BLAST searching revealed that Rfp1 was most similar to mammalian Rnf4 proteins, and to a 205 amino acid fission yeast ring finger containing protein (SPAC343.18), which we denoted Rfp2 (Fig 2A). The rfp2 locus was also deleted and replaced by ura4. These cells also grew normally, were fertile and showed wildtype sensitivities to DNA damaging agents. We made rfp1Δrfp2Δ double mutants by tetrad dissection, and found that double mutant colonies we severely growth inhibited (Fig 2B), forming small colonies that were prone to selection for slow growth suppressors. *S. pombe* grows by apical extension of cell tips, and a delay to cell cycle progression manifests as cells dividing at an increased cell length. Microscopic analysis showed the rfp1Δrfp2Δ double mutants were delayed in cell cycle progression, dividing at 23.2±6.5 μm compared to wildtype, which divided at 13.8±0.2 μm (Fig 2D). By immunofluorescence, HA-tagged Rfp1 was localized to the nucleus (Fig 2C). We have not been able to detect Rfp2 by western blotting or immunofluorescence, even when expressed from the strongest nmt1 promoter, and we could not detect an interaction between Chk1 and Rfp2 by two-hybrid assays (data not shown).

The poor growth and cell cycle delay of the *rfp1Δrfp2Δ* double mutants is reminiscent of strains deleted for genes involved in homologous recombination (HR). We therefore assayed DNA damage sensitivities of *rfp1Δrfp2Δ* double mutants and controls (Fig 3). *rfp1Δrfp2Δ* double mutants were indeed sensitive to UV-C irradiation (Fig 3A) and chronic exposure to the alkylating agent methyl methanesulphonate (MMS) (Fig 3B).

Both of these agents induce a Chk1-dependent DNA damage checkpoint and, at the doses tested, require HR for their repair in *S. pombe*. Inappropiate entry into mitosis with damaged DNA due to a Chk1-dependent checkpoint defect in *S. pombe* results in the bisection of the nucleus by the medial septum, known as the “cut” phenotype. Conversely, imposition of a Chk1-dependent checkpoint delay in response to DNA damage results in elongated cells, as while the nuclear cell cycle is delayed by thus checkpoint, cell growth continues. UV-C irradiated or MMS-treated *rfp1Δrfp2Δ* double mutants elongated further compared to untreated cells (Fig 3C), showing an intact checkpoint response emanating from signaling downstream of Chk1. This was confirmed biochemically via the phosphorylation and activation of Chk1 (Fig 3D), showing that signaling upstream of Chk1 to activate Chk1 activity was also intact. These data suggest that the *rfp1Δrfp2Δ* double mutants are defective in DNA repair, and this is most likely due to an HR defect. However, the poor viability of the *rfp1Δrfp2Δ* double mutants and of HR mutants such as *rhp51Δ* and *rad22Δ* (encoding homologs of Rad51 and Rad52), together with the high rate of spontaneous slow growth suppressors, precluded epistasis to confirm this.

We conclude that Rfp1 and Rfp2 act redundantly in DNA repair, and the poor viability of the *rfp1Δrfp2Δ* double mutant is due to the inability to process spontaneous lesions, as seen in *S. pombe* HR mutants. We do not see a defect in Chk1 activation, nor do we see a checkpoint defect downstream of Chk1 signaling as the *rfp1Δrfp2Δ* double mutant was proficient in a DNA damage induced cell cycle arrest.

We cannot rule out that Chk1 acts upstream of Rfp1 and/or Rfp2 to affect DNA repair, but several observations are not consistent with this. Firstly, the radiation sensitivity of *S. pombe* cells lacking Chk1 is restored by an imposed cell cycle delay using a conditional mutation in the Cdc25 phosphatase required for...
Cdc2 activation (38,39); that is, given the time to repair, chk1Δ cells are fully capable of doing so. This protocol does not rescue the radiation sensitivity of mutants defective in DNA repair (30,40). Moreover, HR mutants, like the rfp1Δrfp2Δ double mutant, show poor cell viability, chromosome instability and poor spore viability, and none of these phenotypes are seen in chk1Δ cells. In S. cerevisiae, direct assays of DNA gap repair, which is via HR, have shown wildtype repair efficiencies in chk1Δ cells (41). This is in keeping with their lack of radiation sensitivity (42), though these cells do show a higher frequency of crossovers for unknown reasons (41). Thus, Chk1 function following DNA damage is primarily to delay the cell cycle to allow time for DNA repair, rather than being directly required for DNA repair itself.

We also tested whether Rfp1 was a substrate for Chk1 phosphorylation (Rfp2 protein expression could not be detected). When Chk1 is overexpressed, the phosphorylation of Wee1 results in a mobility shift assayed by Western blotting (5), but this was not the case for Rfp1 (Fig 3E). A consensus site for Chk1-mediated phosphorylation has been determine as ϕ-X-β-X-(S/T)*, where * indicates the phosphorylated residue, ϕ is a hydrophobic residue (M>I>L>V), β is a basic residue (R>K) and X is any amino acid (43). Rfp2 has no sequences related to this motif, but Rfp1 has one potential site (LTRSPS-22). We assayed the phosphorylation of recombinant Rfp1 in vitro, and of a mutant protein where S22 was substituted for Alanine. Neither protein was significantly phosphorylated, whereas a fragment of Wee1 containing phosphorylation sites at S104 and S117 (43) was efficiently phosphorylated (Fig 3F). It is possible that the recombinant Rfp1 is improperly folded, but the lack of in vitro phosphorylation is consistent with the lack of mobility shift in cells overexpressing Chk1. Thus, if Chk1 is regulating Rfp1, this effect is likely to be indirect.

**Human Rnf4 functionally rescues rfp1Δrfp2Δ**

BLAST searching identified the E3 ubiquitin ligase Rnf4 as the most related human sequence to Rfp1 and Rfp2. To test conservation of function, we cloned human Rnf4 onto the S. pombe nmt1 promoter, and expressed Rnf4 in wildtype and rfp1Δrfp2Δ double mutants. Figure 4 shows that RNF4 expression rescued the UV-C sensitivity (Fig 4A), MMS sensitivity (Fig 4B) and cell cycle delay phenotypes (Fig 4C) of rfp1Δrfp2Δ double mutants. To ensure this was indeed functional rescue and not the selection of suppressors (which are common with the rfp1Δrfp2Δ double mutant), we cured rfp1Δrfp2Δ double mutants of the nmt1-Rnf4 plasmid, and indeed recovered each phenotype (data not shown).

We conclude that Rnf4 is functionally rescuing the defects caused by deletion of either rfp1 and/or rfp2. Together with the sequence similarity, these data show that these proteins are therefore homologs, though no DNA repair function has been ascribed to Rnf4.

**rfp1Δrfp2Δ cells accumulate SUMOylated proteins**

Ring finger domains are characteristic of E3 ubiquitin ligases, and related domains are involved in the ligation of ubiquitin-like proteins such as SUMO. We assayed for Ubiquitin and SUMO-conjugated proteins in denatured extracts in rfp1Δ, rfp2Δ, and rfp1Δrfp2Δ double mutants by western blotting. We found that the rfp1Δrfp2Δ double mutants had vastly increased levels of SUMOylated proteins (Fig 5, compare lanes 2 (wildtype) and 5 (rfp1Δrfp2Δ)), though Ubiquitination was unaffected (data not shown). This was not evident in either of the single mutants, nor in UV-C irradiated or G2 arrested wildtype cells, which we tested given the cell cycle delay and DNA repair defects in these cells. Rfp1 and Rfp2 are therefore negative regulators of protein SUMOylation.

**DNA repair defects in rfp1Δrfp2Δ cells is due to deregulated SUMOylation**

We next wished to address whether the increase in SUMO conjugates in rfp1Δrfp2Δ double mutants was the cause of the DNA repair defects. Although SUMO is not essential in S. pombe, cells deleted for the SUMO gene (pmt3) are extremely sick, and it was not feasible to construct pmt3Δrfp1Δrfp2Δ triple mutants.

As an alternative approach to dampen SUMOylation in rfp1Δrfp2Δ double mutants we overproduced Ulp2 (SPAC17A5.07), which encodes a putative homolog of the S. cerevisiae SUMO deconjugating peptidase, Ulp2/Smt4. Expression of Ulp2 from the nmt1 promoter did
not affect wildtype cell viability, though did decrease the levels of SUMO conjugates in both wildtype and \textit{rfp1Δrfp2Δ} cells (Fig 6A). For the later, the reduction in SUMO conjugates also suppressed the sensitivity to UV-C irradiation (Fig 6B). We conclude that the DNA repair defects that manifest in the absence of Rfp1 and Rfp2 are due to the increased protein SUMOylation.

**DISCUSSION**

The co-ordination of DNA repair with cell cycle progression is essential to maintain genome integrity. Here we have described two new components of the DNA damage response in \textit{S. pombe}, Rfp1 and Rfp2. Cells tolerate the deletion of either gene without consequence, but the double deletion shows defects in cell cycle progression and is sensitive to DNA damaging agents. The synthetic nature of these phenotypes suggests that Rfp1 and Rfp2 have cellular functions that are redundant, or at least overlapping. Based on the normal activation of the DNA damage checkpoint in these cells, we conclude that the sensitivity is due to a DNA repair defect.

Expression of human Rnf4 fully rescued the phenotypes of the \textit{rfp1Δrfp2Δ} cells. Given that these phenotypes require the deletion of both genes, we cannot formally determine if Rnf4 is functioning as Rfp1, Rfp2, or a combination of the two. Rnf4 contains both DNA binding and E3 ubiquitin ligase activities (21,25), and has been shown to interact with several transcription factors that are not conserved in \textit{S. pombe}, including the androgen (24) and estrogen receptors (44). It is not known whether Rnf4 is required for DNA repair in human cells.

The DNA repair defects of \textit{rfp1Δrfp2Δ} double mutants were associated with a large increase in protein SUMOylation, and were rescued by the reduction of SUMO conjugates by Ulp2 overexpression. SUMO is known to modify a number of proteins involved in DNA repair (14), and it is likely that dysregulation of some if not all of these is contributing to the DNA damage sensitivity and repair defects of the \textit{rfp1Δrfp2Δ} double mutants. We note that the poor growth, nuclear abnormalities and DNA damage sensitivity of \textit{rfp1Δrfp2Δ} double mutants are similar to that of \textit{rad22Δ} cells (45,46). \textit{rad22} encodes the \textit{S. pombe} Rad52 homolog, which is required for all recombinational repair in \textit{S. pombe} (47), and is a target for SUMOylation (48).

As Rnf4 is known to be an E3 ubiquitin ligase (25), and Rfp1 and Rfp2 have conserved Ring finger domains, it is reasonable to conclude that these proteins are similarly E3 ligases. In this context, it is possible that Rfp1 and Rfp2 are regulating the levels of proteins involved in SUMOylation, or perhaps Rfp1 and Rfp2 are involved in directing the ubiquitination and degradation of SUMOylated proteins, and hence these accumulate in the \textit{rfp1Δrfp2Δ} double mutants. However, spontaneous slow growth suppressors of the \textit{rfp1Δrfp2Δ} double mutants arise at high frequency, and intercrosses between such suppressors indicated that many different loci are mutated in these suppressors. This observation suggests that the accumulation of SUMOylated proteins in \textit{rfp1Δrfp2Δ} double mutants are due to pleiotrophic effects, and whilst it is clear that this is the cause of the DNA repair defect, the mechanistic basis of this remains to be elucidated.

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FOOTNOTES

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FIGURE LEGENDS

Fig 1. Interaction between Chk1 and Rfp1. A) The indicated regions of Chk1 fused to the Gal4 DNA binding domain (BD) were co-expressed with Rfp1 fused to the Gal4 Activation domain (AD) in PJ69A S. cerevisiae in media lacking Tryptophan (T), Leucine (L) or Adenine (A). Interaction between Chk1 and Rfp1 is assayed by growth in the absence of Adenine. B) Myc-tagged Chk1 and HA-tagged Rfp1 expressed from the attenuated nmt1 promoter (pREP41) co-immunoprecipitate. Note that Chk1 co-migrates with IgG.

Fig 2. Rfp1 and Rfp2 encode two S. pombe RING-domain proteins related to human Rnf4. A) Alignment of S. pombe Rfp1 (SPAC19A8.10), Rfp2 (SPAC343.18) and human RNF4 (NP_002929). B) Tetrad dissection of an rfp1Δ x rfp2Δ cross. Double mutants are circled. Tetrad shown are either tetratype (TT) or non-parental ditype (NPD). C) HA-tagged Rfp1 is localized to the nucleus. D) DAPI-stained images of wildtype and rfp1::ura4 rfp2::ura4. Bar = 10µm.

Fig 3. Rfp1 and Rfp2 are required for DNA repair. A) The indicated strains, included both rfp1Δ rfp2Δ double mutants derived from a non-parental ditype, were assayed for UV-C survival. B) Survival of the indicated strains following chronic exposure to the alkylating agent MMS. C) DAPI-stained wildtype
and rfp1Δ rfp2Δ cells following treatment 100 J/m² UV-C or 0.01% MMS. Note that rfp1Δ rfp2Δ cells are uninucleate and elongated, demonstrating an intact DNA damage checkpoint. D) The indicated strains were assayed for Chk1 activity and activation by phosphorylation following 100 J/m² UV-C. E) Western blots of extracts from wildtype cells expressing HA-tagged Rfp1 and/or Myc-Tagged Chk1 from the nmt1 promoter. *non-specific band recognized by the anti-HA antibody (12CA5) F) The indicated GST fusion proteins were assayed for phosphorylation by Chk1. The top panel shows a coomassie stain of the substrate proteins, the bottom panel is an autoradiograph following phosphorylation by Chk1 in the presence of γ-[32]P-ATP. *C-terminally truncated GST-Rfp1 isoforms.

**Fig 4. Human Rnf4 expression complements phenotypic defects of rfp1Δ rfp2Δ cells.** A) Wildtype (WT) or rfp1Δ rfp2Δ (R1ΔR2Δ) cells containing nmt1-driven Rnf4 or vector controls were assayed for UV-C sensitivity. B) The same cells in A) were assayed for survival of chronic MMS exposure. C) DAPI-stained images of exponential cultures of the indicated strains. Note that Rnf4 expression rescues the cellular elongation and nuclear defects of rfp1Δ rfp2Δ cells. Bar = 10µm.

**Fig 5. Accumulation of SUMO-conjugated proteins in rfp1Δ rfp2Δ cells.** Denatured (8M Urea) extracts were prepared from Wildtype cells alone (WT*), or wildtype (WT), rfp1Δ (R1Δ), rfp2Δ (R2Δ), and rfp1Δ rfp2Δ cells (1,2Δ) expressing HA-tagged Pmt3 (SUMO), and SUMO-conjugates assayed by anti-HA western blotting. Anti-alpha tubulin (tubulin) is used as a loading control. UV-C irradiated wildtype cells (UV) and cdc25-22 G2 arrested cells (G2) were included as controls.

**Fig 6. DNA damage sensitivity of rfp1Δ rfp2Δ cells is caused by excess SUMO conjugates.** A) Denatured (8M Urea) extracts were prepared from wildtype and rfp1Δ rfp2Δ cells (rfp1,2Δ), and from these strains overexpressing Ulp2 from the nmt1 promoter, which significantly reduced the levels of SUMO-conjugated proteins. Anti-alpha tubulin (tubulin) is used as a loading control. B) The UV-C sensitivity of the same strains in A) shows that reduction of SUMO conjugates by Ulp2 overexpression rescues the UV-C sensitivity of rfp1Δ rfp2Δ cells.
Table 1. Proteins identified by yeast two-hybrid interaction with *S. pombe* Chk1

| Gene         | # isolates | Null phenotype/Comment                                                                 |
|--------------|------------|----------------------------------------------------------------------------------------|
| *rad24*      | 3          | Null is defective in DNA Damage checkpoint; 14-3-3 protein                               |
| SPCC613.03   | 3          | Null has no phenotype; contains EF-hand domain                                           |
| *ded1*       | 2          | Essential general translation factor                                                    |
| *rfp1*       | 1          | See text; Ring finger domain                                                            |
| *cek1*       | 1          | Not essential, suppressor of *cut8*; protein kinase                                      |
| SPAC19A8.12  | 1          | Null is lethal; Nudix protein similar to *S. cerevisiae* DCP2                           |
A.

| Chk (BD) | Rfp1 (AD) |
|----------|-----------|
| 1        | -         |
| 2        | +         |
| 3        | +         |
| 4        | -         |
| 5        | +         |

B. + - + Chk1^myc
- + + Rfp1^HA

IP: myc IB: HA

Kosoy et al, Fig 1
A.

[Sequence lines with amino acid codes]

B.

[Images of cell arrangements with labels: TT, TT, NPD, TT]

C.

[Images of fluorescence staining: Rfp1-HA, DNA (DAPI)]

D.

[Images comparing wildtype and rfp1::ura4 rfp2::ura4]

Kosoy et al, Fig 2
A. % survival vs. (J/m² UV-C)

B. Control vs. 0.0075% MMS

C. Vector vs. Rnf4

Kosoy et al, Fig 4
Kosoy et al, Fig 5
SUMO Conjugates

wildtype

rfp1,2Δ

Ulp2 OE

kD

250

150

100

50

37

SUMO Conjugates

SUMO

Tubulin

B.

% survival

WT

WT, Ulp2 OE

rfp1,2Δ

rfp1,2Δ, Ulp2 OE

UV-C (J/m²)

WT

WT, Ulp2 OE

rfp1,2Δ

rfp1,2Δ, Ulp2 OE

Kosoy et al, Fig 6
Fission yeast RNF4 homologs are required for DNA repair
Ana Kosoy, Teresa M. Calonge, Emily A. Outwin and Matthew J. O’Connell

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