Influence Of Deubiquitinating Enzymes On Mutagenesis In Saccharomyces Cerevisiae

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Abstract

In recent years, it became clear that the mutagenic effect of base alterations in a DNA template is dependent on bypass synthesis, carried out by one or more translesion polymerases. A critical role for proliferating cell nuclear antigen (PCNA) and its ubiquitination following DNA damage has been established. Among Saccharomyces cerevisiae deletion mutants in which UV mutagenesis is compromised, we identified and characterized a mutant of the ubiquitin recycling protein Doa4. Similar cases may be represented by Doa1, previously described by others, as well as Bro1 and Ubi4. We discuss overall altered ubiquitin levels or failure to deubiquitinate specific target proteins as likely explanations. This study is of relevance for understanding and possibly modifying the mutagenic effect of DNA-damaging agents in environmental toxicology, cancer treatment and cancer prevention.

INTRODUCTION

The cellular responses to various kinds of DNA damage have by now been elucidated to a significant degree in pro- and eukaryotes (Friedberg et al., 2006). However, concepts explaining one of the most severe consequences of DNA damaging treatments – the enhancement of mutagenesis – have emerged only during the last few years. With the discovery of translesion polymerases, however, the frequently postulated replicative bypass of altered bases of reduced coding capacity gained a mechanistic foundation. Data from the eukaryotic model budding yeast (Saccharomyces cerevisiae) were essential for this progress and the basic mechanisms appear to be evolutionarily conserved.

Pathways leading to mutation are best understood for DNA damage by UV-C radiation, resulting in pyrimidine dimers. The prototype of an error-prone polymerase is polymerase eta, the complex of proteins Rev3 and Rev7 (Nelson et al., 1996b). This polymerase is responsible for most induced and spontaneous mutagenic events. Of equal importance is Rev1, a deoxycytidyl transferase with an independent structural role in mutagenesis, possibly as a platform for several bypass polymerases (Acharya et al., 2005; Acharya et al., 2007; Acharya et al., 2006; Guo et al., 2006a; Nelson et al., 1996a).

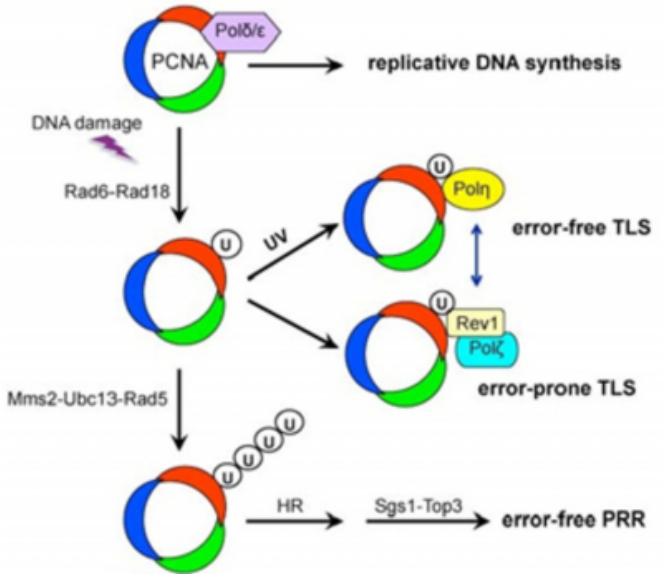
In contrast to this error-prone bypass, a mostly error-free bypass of the most frequent UV photoproducts, dipyrimidine cyclobutane-type dimers is catalyzed by polymerase eta (Johnson et al., 1999; Yu et al., 2001). Consequently, the enzyme's action lowers overall mutability since it competes with error-prone bypass mechanisms. A second major error-free tolerance pathway of different mechanism is the Rad5 pathway that appears to involve template switching (Li et al., 2002; Zhang and Lawrence, 2005).

The current concept of bypass pathway choice is based upon PCNA and its modifications by ubiquitin (Ub) and SUMO (Fig. 1). Monoubiquitination of PCNA (at K164 in yeast) is found after methylmethane sulfonate (MMS) or UV treatment (Hoege et al., 2002; Kannouche et al., 2004) and a requirement of ubiquitinated PCNA for UV mutagenesis has indeed been demonstrated (Stelter and Ulrich, 2003; Zhang et al., 2006). The Rad6-Rad18 complex can bind to single-stranded DNA (Bailly et al., 1994; Bailly et al., 1997) which emerges as a consequence of blocked replication. The Ub conjugating activity of Rad6 catalyzes monoubiquitination of PCNA at K164 which increases its affinity for bypass polymerases (Guo et al., 2006b; Kannouche et al., 2004). Monoubiquitination of PCNA attracts error-prone polymerases mediating a mutagenic bypass, presumably through the ubiquitin-binding domain of Rev1. On the other hand, the signal for the error-free bypass by template-switching is subsequent K63-linked polyubiquitination, resulting from the action of the Ubc13-Mms2-Rad5

complex, with the Ubc13-Mms2 heterodimer functioning as the ubiquitin-conjugating (E2) enzyme in conjunction with the Rad5 (E3) ubiquitin ligase (Hoege et al., 2002; Ulrich and Jentsch, 2000).

Figure 1

Fig. 1: Ubiquitination of PCNA and translesion synthesis. From (Zhang et al., 2011), reproduced with permission. See text for details



Given the importance of Ub for translesion synthesis, it is perhaps surprising that the impact of cellular Ub concentration on mutagenesis has not been studied in detail. For example, whereas a deletion of the polyubiquitin gene UBI4 (encoding a protein of five consecutive Ub as the major Ub source in yeast) is not lethal, altered stress resistance is detectable (Finley et al., 1987). However, an analysis of spontaneous or induced mutability has never been reported for this mutant.

Posttranslational protein modification by Ub and specifically by K48-linked polyUb has been traditionally linked with promoting degradation through the 26S proteasome. Ub is a long lived-protein and, consequently, deubiquitination enzymes (DUBs) that recycle Ub from proteins marked for degradation play an important role in Ub homoeostasis. DUBs are also needed to reverse ubiquitination from target proteins whose activity or localization was altered by Ub and from those that were accidentally ubiquitinated. This is a topic of considerable complexity, given the identification of more than 100 DUBs in humans (Amerik and Hochstrasser, 2004; Reyes-Turcu et al., 2009).

Our interest in exploring the relationship between DUBs and induced mutagenesis stems from the isolation of a mutant of Doa4, a DUB important for Ub homoeostasis, in a screen for mutants defective in UV mutagenesis (Gong and Siede, 2009). Doa1 may represent a similar case, studied by other investigators (Lis and Romesberg, 2006). Here, we present a characterization and discussion of the role of Doa4 in DNA-damage-induced mutagenesis.

MATERIAL AND METHODS

STRAINS

The initially used strains were all derivatives of BY4741 (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0) and part of the systematic yeast gene deletion collection, purchased from Open Biosystems. Using PCR-mediated transplacement (Rothstein, 1989) and Li-acetate transformation (Gietz and Schiestl, 1995), selected deletions were transferred into strain Y300 (MATa ade2-1 ura3-1 trp1-1 his3-11,15 leu2-3,112 can1-100), originally obtained from Dr. Stephen Elledge.

MUTAGEN TESTING

Cells were grown to logarithmic phase for 16 h or to saturation for 48 h in YPD (1% yeast extract, 2% peptone, 2% dextrose) at 30°C. For UV radiation assays, cells were washed and resuspended in sterile water, appropriate dilutions were plated on synthetic media plates with or without canavanine or tryptophane (Amberg et al., 2005) and irradiated (germicidal UV-C). For MMS assays, cells were incubated in YPD at a titer of 2x107 cells/ml for 1 h at 30°C, washed and plated. Colonies were counted after 3-5 days of incubation. Fractions of surviving cells and mutants per surviving cells were calculated.

RESULTS

The key data that initiated this project originated from a screen of the haploid S. cerevisiae deletion mutant collection for mutants that reduce the probability of UV-induced mutations (Gong and Siede, 2009). We used the well-studied system of forward mutation to canavanine resistance which selects for defects in arginine permease, thus preventing the uptake of the toxic arginine analog canavanine. Not surprisingly, deletion mutants of many genes known to be required for induced mutagenesis such as REV3 were reisolated. We also isolated mutants of genes not previously known to influence UV mutagenesis including DOA4 which encodes a DUB (Gong and Siede, 2009). The defect in UV-induced forward mutation to canavanine resistance conferred by a deletion of DOA4 was quantified with the original deletion strain (Table 1, BY4741 as genetic background).

After testing a cross-section of yeast DUB deletions in quantitative single-dose assays, it was suggested that this phenotype is shared with mutants of Bro1 (a Doa4 interacting protein), Doa1 and Ubi4 (polyubiquitin) but not with other DUB deletions tested (Table 1); others rather seem to increase mutability. Interestingly, decreased mutability appears to be accompanied by decreased UV resistance of colony formation – primarily in case of doa1Δ and less so for doa4Δ, bro1Δ, or ubi4Δ.

Figure 2

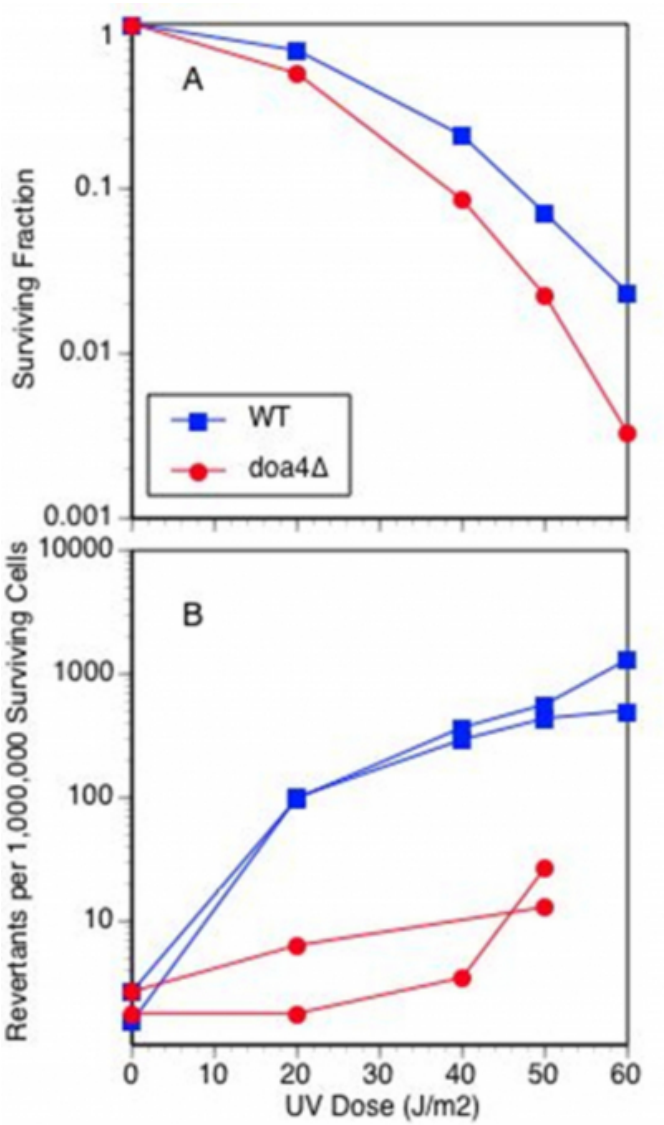
Table 1

Strain (Genotype)	% Survival (30 J/m² UV)	UV-Induced Can^r mutants per 10^6 surviving cells
WT	24	74
doa1Δ	7	6
doa4Δ	19	<3
ubp1Δ	26	170
ubp2Δ	51	48
ubp5Δ	51	130
ubp6Δ	40	222
ubp8Δ	40	130
ubp12Δ	43	145
bro1Δ	35	<2
ubi4	37	2

To prove the general validity of our observation, we transferred the DOA4 deletion to an unrelated strain (Y300) and tested a mutational system that is very different from canavanine forward mutation (trp1-1 reversion). A partial defect in UV mutagenesis was indeed confirmed over a range of doses. The influence was similar in saturated cultures (Fig. 2) and in logarithmic-phase cultures (Fig. 3).

Figure 3

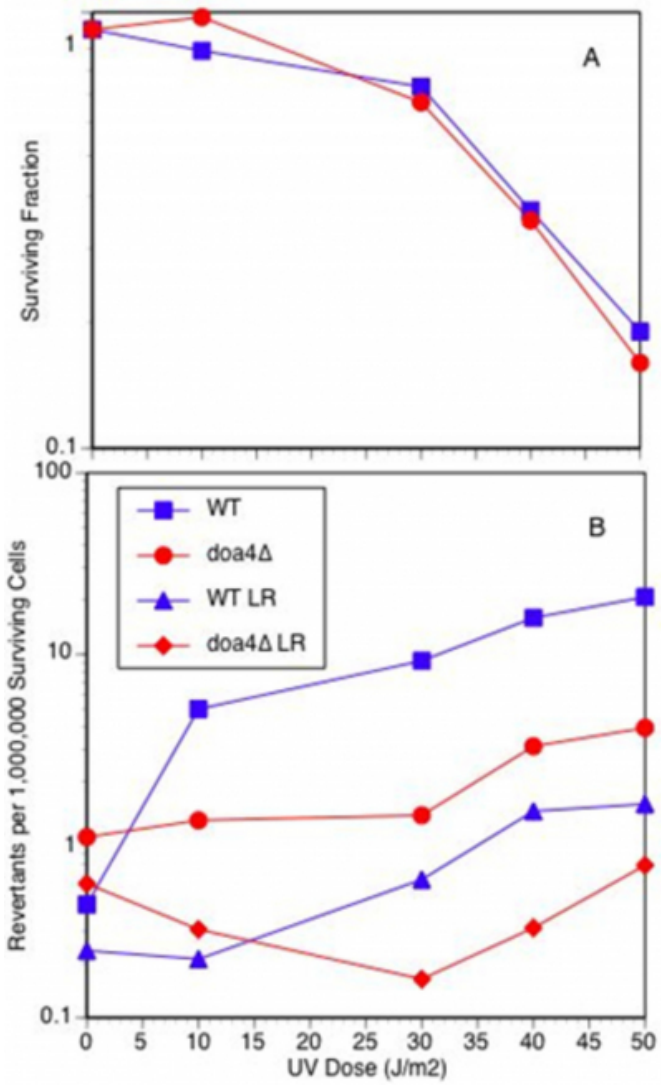
Fig. 2: UV survival (A) and mutation frequencies (reversion, two independent experiments, B) for WT and isogenic Δ. Cultures were grown to saturation (cultured for 48 h in YPD).



We noted a small fraction of distint fast growing mutants of the trp1-1 nonsense allele. We assume that these are certain locus-specific revertants among various kinds of suppressor mutants. If the frequencies of these revertants were plotted separately, a defect in mutagenesis in the doa4 deletion was still evident. Whereas the WT showed some mutant induction as a function of UV dose, the doa4 mutant shows frequencies that remain indistinguishable from the spontaneous background mutant frequency (Fig. 3).

Figure 4

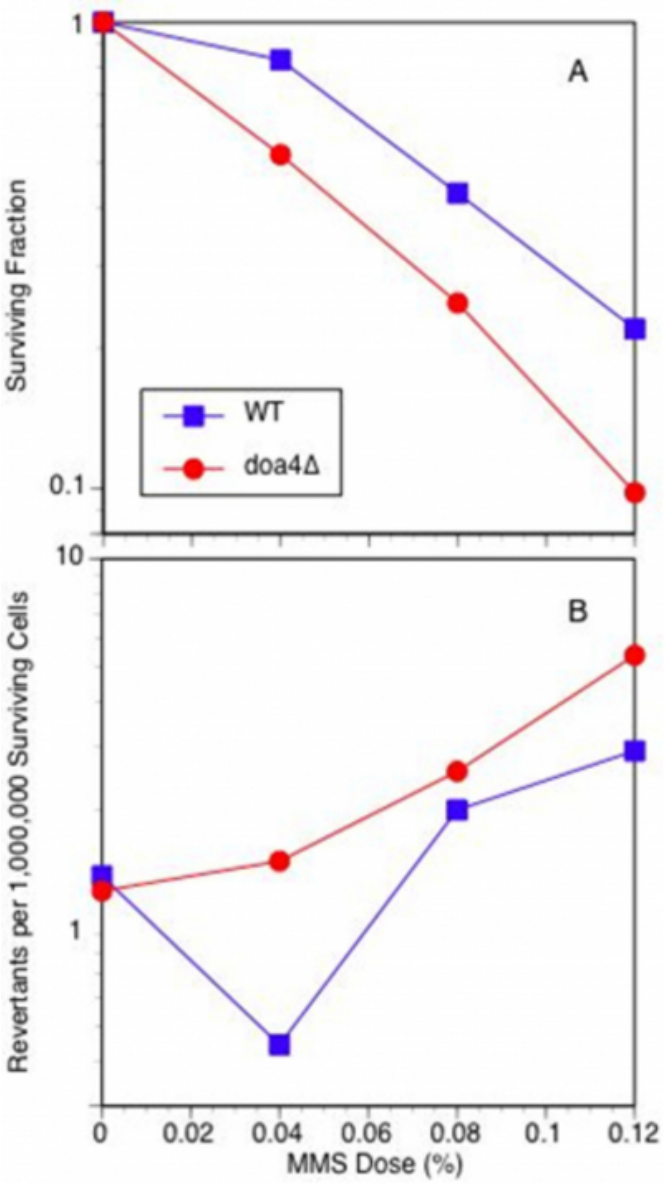
Fig. 3: UV survival (A) and mutation frequencies (reversion, B) for WT and isogenic ?. Logarithmic-phase cultures were used. In B, mutation frequencies of a distinct fraction of fast-growing mutants was plotted separately (putative locus-revertants, LR, triangles/diamonds).



However, this defect in mutagenesis appeared to be damage-specific. In response to the alkylating agent MMS, trp1-1 reversion was not compromised by the doa4 deletion (Fig. 4).

Figure 5

Fig. 4: Survival (A) and mutation frequencies (reversion, B) for WT and isogenic Δ. Cultures grown to logarithmic phase were treated with MMS.



DISCUSSION

We detected a partial defect in UV mutagenesis in deletion mutants of DOA4. DOA4 (= UBP4) encodes an ubiquitin isopeptidase. Initially, genetic and physical studies indicated an interaction of Doa4 with at least a subset of 26S proteasomes and suggested a role of Doa4 in detaching Ub from proteins destined for degradation by the proteasome (Papa et al., 1999; Papa and Hochstrasser, 1993). More recent data indicate that Doa4 acts at the cytoplasmic surface of the late-stage endosome together with the ESCRT-II complex and is required to recycle Ub from membrane proteins designated for the vacuole (Amerik et al., 2000;

Swaminathan et al., 1999).

Such a role of a DUB in DNA-damage responses is not without precedence. As described by Lis and Romesberg, Doa1 appears to represent a similar case since it is required for normal resistance to MMS and hydroxyurea (Lis and Romesberg, 2006) as well as UV (our data, Table 1). The protein is required for normal Ub levels (Ghislain et al., 1996), it binds Ub and prevents the accumulation of K-48 linked Ub trimers suggesting that Doa1 (or a co-purifying protein) acts as a Ub protease (Lis and Romesberg, 2006). MMS-induced histone H2B- and PCNA monoubiquitination are not detected in doa1 mutants (Lis and Romesberg, 2006). Especially the latter defect might result in a defect in induced mutagenesis (see Fig. 1). We have tested this hypothesis for UV radiation and found indeed a severe defect in induced mutability to canavanine resistance (Table 1 and data not shown).

It is important to distinguish between a general influence on bypassing DNA damage in a mutagenic fashion and effects restricted to this specific selection system (Gong and Siede, 2009; Lemontt, 1977; Lis et al., 2008). Preferably, mutation data need to be confirmed with mutational systems of different nature. Canavanine sensitivity is quite common in mutants of the Ub/proteasome system and has indeed been described for mutants of UBI4 (Reyes-Turcu et al., 2009). Canavanine sensitivity may limit mutability. For example, a defect in detoxification may increase canavanine toxicity and counteract the emergence of canavanine-resistant mutants. Such influences can indeed be rationalized in case of mutants involved in Ub metabolism.

Consequently, we constructed a DOA4 deletion in unrelated genetic background (strain Y300) and the defect in UV mutagenesis was verified in a different mutational system (trp1-1 reversion) (Fig. 2, 3).

Two hypotheses can be invoked to explain these observations. First, the DUB in question may be required for Ub recycling and the overall reduced Ub level may be detrimental for the mutagenic process, i.e. error-prone translesion synthesis. Certain proteins may need to be modified by mono- or K63-linked poly-ubiquitin or degraded following K48-linked polyubiquitination. Second, the DUB may be required for direct deubiquitination of a specific target protein(s) involved in mutagenesis.

The first hypothesis is preferred because of the characterized Doa4 function that has now been primarily implicated in recycling Ub from vacuolar membrane-bound proteins (Amerik et al., 2000; Swaminathan et al., 1999). A direct interaction with DNA repair/mutagenesis proteins appears therefore somewhat unlikely.

An obvious candidate for an indirect DUB target that influences mutability is PCNA. A defect in PCNA ubiquitination following MMS treatment has already been detected for doa1 (Lis and Romesberg, 2006) which we have shown to be defective in UV mutagenesis in this study.

However, three observations do not fit with this concept. First, UV sensitivity of the DUB mutants (Tab. 1) does not approach that of mutants of the Rad5/Mms2/Ubc13 complex, the E3/E2 enzymes required for polyubiquitination of PCNA and error-free damage tolerance (Fig. 1). If Ub homoeostasis is disturbed, it is not obvious why polyubiquitination may be less reduced than monoubiquitination on which it depends. Second, a significantly lower level of free ubiquitin is found in Doa4 mutant cells only when cells approach stationary-phase (Swaminathan et al., 1999). A defect in UV mutagenesis for doa4, however, was detected in both logarithmic phase and 48 h cultures (Fig. 2, 3). Third, ubiquitination of PCNA is found after UV as well as MMS treatment. A defect in induced mutagenesis, however, was not evident in MMS-treated cells (Fig. 4).

Future studies will address these discrepancies and may reveal other, relevant targets. For example, H2B represents another Ub modified protein that is important for DNA-damage transactions (Lis and Romesberg, 2006). Additional candidate targets that may need to be degraded through the proteasome pathway include the checkpoint clamp loader Rad24 (if it behaves similar to the human homolog) (Zhang et al., 2010).

In the future, it should also be explored why only certain DUBs and not others may have an influence on mutagenesis although their effect on free Ub levels might be quite comparable. The concept of Ub channeling to certain targets has been brought up in conjunction with the doa1 phenotype (Lis and Romesberg, 2006) but the mechanism remains unknown.

Protein degradation and Ub-metabolizing proteins were found to be conserved in evolution and thus, the suggested studies may very well be relevant for higher eukaryotes. Interestingly, Usp6 as a potential human ortholog of Doa4 is an oncogene critically important for the development of

aneurysmal bone cysts (Oliveira et al., 2006). The described chromosome translocations in this disease lead predominantly to the overexpression of Usp6.

Many additional implications can be listed. For example, manipulation of cellular Ub levels may be used to modify the outcome of cancer chemotherapy and to reduce mutagenic side effects.

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References

r-0. Acharya N, Haracska L, Johnson RE, Unk I, Prakash S , Prakash L: Complex formation of yeast Rev1 and Rev7 proteins: a novel role for the polymerase-associated domain. Mol Cell. Biol.; 2005; 25: 9734-9740.
r-1. Acharya N, Haracska L, Prakash S , Prakash L: Complex formation of yeast Rev1 with DNA polymerase eta. Mol. Cell. Biol.; 2007; 27: 8401-8408.
r-2. Acharya N, Johnson RE, Prakash S , Prakash L: Complex formation with Rev1 enhances the proficiency of Saccharomyces cerevisiae DNA polymerase zeta for mismatch extension and for extension opposite from DNA lesions. Mol. Cell. Biol.; 2006; 26: 9555-9563.
r-3. Amberg DC, Burke DJ , Strathern JN (2005) Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual, 2005 Edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
r-4. Amerik AY , Hochstrasser M: Mechanism and function of deubiquitinating enzymes. Biochim Biophys Acta; 2004; 1695: 189-207.
r-5. Amerik AY, Nowak J, Swaminathan S , Hochstrasser M: The Doa4 deubiquitinating enzyme is functionally linked to the vacuolar protein-sorting and endocytic pathways. Mol Biol Cell; 2000; 11: 3365-3380.
r-6. Bailly V, Lamb J, Sung P, Prakash S , Prakash L: Specific complex formation between yeast RAD6 and RAD18 proteins: a potential mechanism for targeting RAD6 ubiquitin-conjugating activity to DNA damage sites. Genes Dev.; 1994; 8: 811-820.
r-7. Bailly V, Lauder S, Prakash S , Prakash L: Yeast DNA repair proteins Rad6 and Rad18 form a heterodimer that has ubiquitin conjugating, DNA binding, and ATP hydrolytic activities. J. Biol. Chem.; 1997; 272: 23360-23365.
r-8. Finley D, Özkaynak E , Varshavsky A: The yeast polyubiquitin gene is essential for resistance to high temperatures, starvation and other stresses. Cell; 1987; 48: 1035-1046.
r-9. Friedberg EC, Walker GC, Siede W, Wood RD, Schultz RA , Ellenberger T (2006) DNA Repair and Mutagenesis, 2nd Edition. American Society of Microbiology Press, Washington, D.C.
r-10. Ghislain M, Dohmen RJ, Levy F , Varshavsky A: Cdc48p interacts with Ufd3p, a WD repeat protein required for ubiquitin-mediated proteolysis in Saccharomyces cerevisiae. EMBO J; 1996; 15: 4884-4899.
r-11. Gietz RD , Schiestl RH: Transforming yeast with DNA. Methods Mol. Cell. Biol.; 1995; 5: 255-269.
r-12. Gong J , Siede W: SBF transcription factor complex positively regulates UV mutagenesis in Saccharomyces cerevisiae. Biochem. Biophys. Res. Commun.; 2009; 379: 1009-1014.
r-13. Guo C, Sonoda E, Tang T-S, Parker JL, Bielen AB, Takeda S, Ulrich HD , Friedberg EC: REV1 protein interacts with PCNA: significance of the REV1 BRCT domain in vitro and in vivo. Mol Cell; 2006a; 23: 265-271.
r-14. Guo C, Tang T-S, Bienko M, Parker JL, Bielen AB, Sonoda E, Takeda S, Ulrich HD, Dikic I , Friedberg EC: Ubiquitin-binding motifs in REV1 protein are required for its role in the tolerance of DNA damage. Mol. Cell. Biol.; 2006b; 26: 8892-8900.
r-15. Hoege C, Pfander B, Moldovan G-L, Pyrowolakis G , Jentsch S: RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature; 2002; 419: 135-141.
r-16. Johnson RE, Prakash S , Prakash L: Efficient bypass of a thymine-thymine dimer by yeast DNA polymerase, Poleta. Science; 1999; 283: 1001-1004.
r-17. Kannouche PL, Wing J , Lehmann AR: Interaction of human DNA polymerase with monoubiquitinated PCNA: a possible mechanism for the polymerase switch in response to DNA damage. Mol. Cell; 2004; 14: 491-500.
r-18. Lemontt J: Pathways of ultraviolet mutability in Saccharomyces cerevisiae. IV. The relation between canavanine toxicity and ultraviolet mutability to canavanine resistance. Mutat. Res.; 1977; 43: 339-355.
r-19. Li Z, Xiao W, McCormick JJ , Maher VM: Identification of a protein essential for a major pathway used by human cells to avoid UV-induced DNA damage. Proc. Natl. Acad. Sci. USA; 2002; 99: 4459-4464.
r-20. Lis ET, O'Neill BM, Gil-Lamaignere C, Chin JK , Romesberg FE: Identification of pathways controlling DNA damage induced mutation in Saccharomyces cerevisiae. DNA Repair; 2008; 7: 801-810.
r-21. Lis ET , Romesberg FE: Role of Doa1 in the Saccharomyces cerevisiae DNA damage response. Mol. Cell. Biol.; 2006; 26: 4122-4133.
r-22. Nelson JR, Lawrence CW , Hinkle DC: Deoxycytidyl transferase activity of the yeast REV1 protein. Nature; 1996a; 382: 729-731.
r-23. Nelson JR, Lawrence CW , Hinkle DC: Thymine-thymine dimer bypass by yeast DNA polymerase zeta. Science; 1996b; 272: 1646-1649.
r-24. Oliveira AM, Chou MM, Perez-Atayde AR , Rosenberg AE: Aneurysmal bone cyst: a neoplasm driven by upregulation of the USP6 oncogene. J Clin Oncol; 2006; 24: e1; author reply e2.
r-25. Papa FR, Amerik AY , Hochstrasser M: Interaction of the Doa4 deubiquitinating enzyme with the yeast 26S proteasome. Mol Biol Cell; 1999; 10: 741-756.
r-26. Papa FR , Hochstrasser M: The yeast DOA4 gene encodes a deubiquitinating enzyme related to a product of the human tre-2 oncogene. Nature; 1993; 366: 313-319.
r-27. Reyes-Turcu FE, Ventii KH , Wilkinson KD: Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. Annu Rev Biochem; 2009; 78: 363-397.
r-28. Rothstein R: Targeting, disruption, replacement, and allelic rescue: integrative DNA transformation in yeast. Meth. Enzymol.; 1989; 194: 281-301.
r-29. Stelter P , Ulrich HD: Control of spontaneous and damage-induced mutagenesis by SUMO and ubiquitin conjugation. Nature; 2003; 425: 188-191.
r-30. Swaminathan S, Amerik AY , Hochstrasser M: The Doa4 deubiquitinating enzyme is required for ubiquitin homeostasis in yeast. Mol. Biol .Cell; 1999; 10: 2583-2594.
r-31. Ulrich HD , Jentsch S: Two RING finger proteins mediate cooperation between ubiquitin-conjugating enzymes in DNA repair. EMBO J.; 2000; 19: 3388-3397.

r-32. Yu S-L, Johnson RE, Prakash S , Prakash L: Requirement of DNA polymerase eta for error-free bypass of UV-induced CC and TT photoproducts. Mol. Cell. Biol.; 2001; 21: 185-188.
r-33. Zhang H, Gibbs PE , Lawrence CW: The Saccharomyces cerevisiae rev6-1 mutation, which inhibits both the lesion bypass and the recombination mode of DNA damage tolerance, is an allele of POL30, encoding proliferating cell nuclear antigen. Genetics; 2006; 173: 1983-1989.
r-34. Zhang H , Lawrence CW: The error-free component of the RAD6/RAD18 DNA damage tolerance pathway of budding yeast employs sister-strand recombination. Proc. Natl. Acad. Sci. USA; 2005; 102: 15954-15959.
r-35. Zhang L, Park CH, Wu J, Kim H, Liu W, Fujita T, Balasubramani M, Schreiber EM, Wang XF , Wan Y: Proteolysis of Rad17 by Cdh1/APC regulates checkpoint termination and recovery from genotoxic stress. EMBO J; 2010; 29: 1726-1737.
r-36. Zhang W, Qin Z, Zhang X , Xiao W: Roles of sequential ubiquitination of PCNA in DNA-damage tolerance. FEBS Lett; 2011.

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