The role of \textit{TDP1} from budding yeast in the repair of DNA damage

Chunyan Liu, Jeffrey J. Poulbot, Howard A. Nash*

Laboratory of Molecular Biology, National Institute of Mental Health, 9000 Rockville Pike, Bethesda, MD 20892-4034, USA

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Abstract

The \textit{TDP1} gene encodes a protein that can hydrolyze certain types of 3'-terminal phosphodiesters, but the relevance of these catalytic activities to gene function has not been previously tested. In this work we engineered a point mutation in \textit{TDP1} and present evidence that, as per design, it severely diminishes tyrosyl-DNA phosphodiesterase enzyme activity without affecting protein folding. The phenotypes of yeast strains that express this mutant show that the contribution of \textit{TDP1} to the repair of two kinds of damaged termini-induced, respectively, by camptothecin (CPT) and by bleomycin—strongly depends on enzyme activity. In routine assays of cell survival and growth the contribution of this activity is often overshadowed by other repair pathways. However, the value of \textit{TDP1} in the economy of the cell is highlighted by our discovery of several phenotypes that are evident even without deliberate inactivation of parallel pathways. These non-redundant mutant phenotypes include increased spontaneous mutation rate, transient accumulation of cells in a mid-anaphase checkpoint after exposure to camptothecin and, in cells that overexpress topoisomerase I (Top1), decreased survival of camptothecin-induced damage. The relationship between the role of \textit{TDP1} in \textit{Saccharomyces} and its role in metazoans is discussed.

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1. Introduction

Several years ago our laboratory reported that extracts of eukaryotic cells contain a novel phosphodiesterase activity, one that is capable of hydrolyzing a bond between tyrosine and the 3'-end of DNA [1]. In nature, the only known occurrence of this type of linkage is in the covalent complex that is transiently formed between DNA and eukaryotic topoisomerase I (Top1) [2]. Accordingly, we suggested that the enzyme responsible for the novel activity was involved in repair of aborted topoisomerase I intermediates, i.e., those that had failed to complete the catalytic cycle and had become converted to dead-end complexes. This prediction was at least partially borne out following the identification of a gene in \textit{Saccharomyces cerevisiae} that encodes an enzyme with tyrosyl-DNA phosphodiesterase activity [3]. When tested in a genetically sensitized background, null mutations of this gene (\textit{TDP1}) increased the lethal effectiveness of camptothecin (CPT), a drug that specifically blocks the completion of the Top1 catalytic cycle.

Interest in \textit{TDP1} has been growing [4,5]. In large measure this is due to that fact that the gene is highly conserved in eukaryotes, with identifiable orthologs in a wide variety of animals, including man [3]. As in all organisms surveyed, the human genome contains a single copy of \textit{TDP1} and this encodes an enzyme with an activity comparable to that of the yeast enzyme [6]. Messenger RNA for \textit{TDP1} is found in many human tissues and this expression appears to have functional significance since a human disease is associated with the inheritance of a mutation in the gene [7]. Despite many advances, including a spectacular series of studies that have established the structural basis for enzyme activity [8–10], many questions about the biological role of this gene remain. In our opinion, the most prominent of these is the degree to which repair by Tdp1 depends on its enzymatic activity. A complete dependence has been implicitly assumed in work by us and others, but studies of other repair proteins that display enzymatic activity have revealed that catalysis can be irrelevant to important biological functions [11–14]. The possibility that this might apply to \textit{TDP1} is raised by the familial disease associated with an alteration in the human ortholog of the gene. Although the SCAN1 mutation maps to the active site of the enzyme [7] and substantially lowers enzyme activity (H. Interthal, personal communication),
the affected patients suffer from neuronal degeneration but
do not show symptoms that are typically associated with in-
adeguate DNA repair. Despite such hints, in this work we
use genetic and pharmacological manipulations of Saccha-
romyces cerevisiae to refute the idea that enzymatic activity
is irrelevant to the repair function of TDP1. In order to gain
a better understanding of the contribution of this enzyme to
fitness, we have also sought conditions in which a tdp1 mu-
ant phenotype can be observed without recourse to sensi-
tizing mutations and/or exogenous DNA damaging agents.
The results of our search help to define its biological niche.

2. Materials and methods

2.1. Strains and plasmids

The yeast strains used in this work are derivatives of aux-
otrophic versions of S. cerevisiae strain S288C [15] in which
particular genes of interest have been disrupted or replaced
with selectable markers. In addition to previously described
strains [16,17], for this work we generated apn1 apn2 top1
and apn1 apn2 top1 tdp1 derivatives. These were made by
simultaneous transformation of the appropriate parent with an
apn1::hisG-URA3-hisG cassette and a PCR-generated
apn2::MET15 fragment; construct formation was confirmed
by PCR. To overexpress the TOP1 gene, we replaced the in-
digenous TOP1 with a construct in which the gene is placed
under the control of a GAL1 inducible promoter [18]. To
facilitate drug accumulation [19,20], all strains carried a
KanMX substitution of the ERG6 gene.

Plasmids that can be induced to express TDP1 were con-
structed as follows. The ORF of the gene was PCR-amplified
(with simultaneous addition of restriction site linkers)
from a trimmed subclone of pL10-13 [3]. To generate
pHN1857, the amplicon was then digested with SacI and
ligated into SacI-cleaved pYES2 (Invitrogen, Carlsbad, CA), a
URA3-selectable plasmid in which the GAL1 promoter
is situated upstream of the insert. Plasmid pHN1932 was
derived from pHN1857 by replacing the CAT codon for His
182 with a GCT alanine codon, using the QuikChange II
Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA).
To judge the effect of the plasmids, cells bearing either
pHN1857 or pHN1932 were compared to those bearing the
YES2 vector plasmid alone.

2.2. Growth and testing of cells

Yeasts strains bearing a YES2 derivative were grown in
CSM-URA, a variant of complete synthetic medium that
omitted uracil (QBioGene, Carlsbad, CA). Other strains
were grown in yeast extract/peptone-medium [21] contain-
ing either 2% glucose or 2% glycerol or 1% raffinose plus
2% galactose (respectively, YPD, YPG, and YPRGal), as
indicated. For spot tests of growth, 3 µl each of a 10-, 100-
and 1000-fold serial dilution of freshly prepared saturated
cultures was applied to the surface of a CSM-URA agar
plate that contained the indicated concentration of a drug
(unless otherwise noted, obtained from Sigma). Plates were
incubated at 30°C and photographed after 3 or 4 days. Cell
killing following exposure to bleomycin was performed as
described [17]. Cell killing by gamma irradiation was
performed as follows. Cells were grown in liquid medium
to logarithmic-phase and 1 ml aliquots were exposed to
137 Cs in an irradiator (Gamma Cell 1000, Atomic En-
ergy of Canada Ltd) for the designated dose. The cells were
then diluted and spread on appropriate plates. Each strain
was assayed at least three times and survival was calculated
as the mean (±S.E.M.) of at least three experiments.

2.3. Western blotting and enzymatic assay

Strains bearing derivatives of the YES2 plasmid were
grown to saturation in CSM-URA with 2% galactose and
1% raffinose as the carbon source. Cells (10 ml aliquots)
were pelleted and lysed by bead-beating [1]. For Western
blotting, an aliquot (14.6 µg) of the resulting protein ex-
tract was loaded onto each lane of an acrylamide gel. Af-
after electrophoresis and transfer, Tdp1 was detected with
a 1:1000-fold dilution of rabbit polyclonal antibody raised
against a His-tagged full length protein that had been ex-
pressed in Escherichia coli from plasmid pHN1856 [3] and
purified on a His Bind Quick 900 cartridge (Novagen, Mal-
dison WI). The blot was visualized using peroxidase sub-
strate system (KPL, Gaithersburg, MD) and a 1:4000-fold dilution
of a peroxidase-conjugated donkey anti-rabbit IgG (Jack-
on ImmunoResearch, West Grove, PA). For determination
of tyrosyl-DNA phosphodiesterase activity, aliquots (1.8 µg)
of crude extract protein or 2.2 ng of His-tagged yeast Tdp1,
purified as above, was added to 12 µl reaction and assayed
as described [16].

2.4. Nuclear morphology

Cells were grown in YPD to mid-log phase and then
exposed to CPT (100 µg/ml). At various times thereafter,
aliquots (1 ml) were removed, centrifuged, and resuspended
in 1 ml of 70% ethanol. After rocking at room temp for 1 h,
an aliquot (100 µl) of the cells was pelleted and washed
once in water. The resulting pellet was resuspended in 25 µl
of phosphate-buffered saline containing 0.1% Tween 20 and
2.5 µg/ml DAPI. After rocking for 30 min in the dark, the
cells were examined with an epifluorescence microscope.
Each of ~200 cells was scored for the presence and size of
a bud as well as the distribution of DAPI-stained material.
Comparison with a published overview [22] permitted assi-
gment of the vast majority of cells to a position in the cell
cycle. The proportion of such cells arrested at G2/M or mid-
anaphase was measured from three separate populations. In
several cases duplicate measurements were made on dif-
frent samples from the same population but, in every case,
wild-type and tdp1 mutant cells were analyzed in parallel.
2.5. Spontaneous mutagenesis rate

Cultures that had grown to saturation in YPD were diluted to \(~100\) cells/ml. A series of 15 aliquots (1.1 ml each) were then grown to saturation and, following suitable dilution, assayed for total colony-forming units on YPD plates and for canavanine-resistant cells on selective plates [21]. The best estimate of the rate at which mutations in the \textit{CAPI} gene arose per generation in each strain was determined from these values by the method of the median [23]. This estimate was independently determined several times for each strain; significance of differences among these rates between strains was evaluated by rank-sum tests [24].

3. Results and discussion

3.1. Enzymatic activity is critical for the contribution of \textit{TDPI} to repair of Top1 damage

Previous studies to assess the in vivo function of \textit{TDPI} in yeast [3,16,17,25] relied largely on deletion mutations. Such mutations remove not only enzymatic activity but any other contribution that the protein might make, such as helping in the construction of a repair complex. The one existing point mutation in yeast \textit{TDPI} changes a residue (P220) that lies far from the active site [9] (Stephen White, personal communication); the absence of enzymatic activity in this mutant [16] presumably reflects improper protein folding. To see the influence of a more subtle change, we used information gained from biochemical and structural studies of the human ortholog [6,8] to engineer a point mutation, H182A, in the active site of the yeast enzyme. The mutant gene was cloned into a plasmid downstream of the \textit{GALI} promoter; when induced, its product accumulates in yeast cells to levels that nearly match those of cells bearing plasmids with the wild-type gene product (Fig. 1A). But, as expected from its position in the active site, the mutation greatly reduces catalysis by tyrosyl-DNA phosphodiesterase (Fig. 1B).

To assess the effect of this mutation on repair, we introduced the \textit{TDPI} plasmid set into \textit{tdpi} \Delta strains that were also mutated in alternative pathways for repair of CPT-induced damage [3,17]. To limit expression of the plasmid-borne gene to the levels similar to those found in cells with an indigenous copy of \textit{TDPI}, the transformants are grown in 2% glucose. Under these conditions, in two different sensitized genetic backgrounds, strains bearing the wild-type \textit{TDPI} construct were clearly more resistant to CPT than strains

![Characterization of an active site mutant of yTDPI. A set of plasmids designed to express genes from the inducible \textit{GALI} promoter were introduced into yeast strains bearing a deletion of the indigenous copy of \textit{TDPI}. Individual members of this "\textit{TDPI} plasmid set" contained either wild-type \textit{TDPI} (wt), the H182A active site mutant allele (mut), or no insert (vector). (A) Western blots from lysates of galactose-induced cells bearing the indicated plasmid. The assignment of the band corresponding to Tdp1 in lanes 1–3 is confirmed by comigration with purified protein (lane 4). (B) Tyrosyl-DNA phosphodiesterase activity in crude extracts of galactose-induced cells bearing the indicated plasmid. The electrophoretic positions are shown for the 3'-tyrosine oligonucleotide substrate (Y), the 3'-phosphate product of Tdp1 hydrolysis (P), and the 3'-hydroxyl (OH) derivative that is formed from the 3'-phosphate by adventitious phosphatases. The reactions analysed in lanes 1, 2, and 3 used the standard amount (4.8 \(\mu\)g) of extract; those for lanes 3 and 4 used 10- and 100-fold less, respectively. In place of crude extract, the reactions analysed in lanes 6 and 7 contained purified yTDPI or enzyme dilution buffer, respectively. (C) Sensitivity to CPT of cells bearing the indicated plasmid. Each panel shows a series of 10-fold dilutions of saturated glucose-grown cultures spotted onto plates that were selective for the plasmid, contained 2% galactose as the carbon source, and had been doped with the indicated concentration (\(\mu\)g/ml) of CPT. (D) Effect of overexpression of mutant \textit{TDPI} on sensitivity to CPT. Cells with a deletion of the indigenous copy of \textit{TDPI} were transformed with the indicated plasmids and grown to saturation in selective medium under glucose repression. Each panel shows a series of 10-fold dilutions of these cultures spotted onto plates that were selective for the plasmid, contained 1% raffinose and 2% galactose as the carbon source, and were doped with the indicated concentration (\(\mu\)g/ml) of CPT. (E) Effect of simultaneous overexpression of wild-type \textit{TOP1} and mutant \textit{TDPI}. Cells in which the indigenous copy of \textit{TDPI} was deleted and the indigenous copy of \textit{TOP1} was replaced by a galactose-inducible overexpression construct [18] were transformed with the indicated plasmids and grown to saturation in selective medium under glucose repression. Each panel shows a series of 10-fold dilutions of these cultures spotted onto drug-free plates that were selective for the plasmid and contained either 2% glucose or 2% galactose (plus 1% raffinose) as the carbon source.}
bearing just the plasmid vector (Fig. 1C); the relative level of resistance approximated that provided by an intact indigenous copy of TDP1 ([17] and JIP, unpublished), indicating that sufficient expression levels were indeed achieved. Despite this, in both strain backgrounds tested, the construct with the active site mutation was more sensitive to CPT than the strain bearing wild-type TDP1 and was indistinguishable from the strain without TDP1 (Fig. 1C).

Taken at face value, the results of these spot tests imply that hydrolysis is a vital function of Tdp1. But this interpretation strongly depends on the assumption that the engineered mutation has disabled enzymatic activity without disrupting protein folding. The correctness of this assumption is supported by experiments in which the mutant form of TDP1 is overproduced. If the engineered amino acid change caused serious misfolding that rendered the protein completely inactive, its expression should have been inconsequential or, at worst, associated with non-specific changes in growth. Spot tests of growth on plates containing galactose indicate that, by itself, overexpression does not generally interfere with growth (Fig. 1D), even when the plates contain a DNA-damaging agent like MMS (not shown). But, overexpression of the mutant protein is deleterious when cells are exposed to CPT (Fig. 1D) and, in the absence of CPT, when cells are engineered to simultaneously overexpress Top1 (Fig. 1E). Both conditions are known to lead to an increased burden of tyrosyl-DNA phophodiesters [5,18], but we are not certain how overproduction of the mutant protein results in defective repair. An obvious possibility is sequestration of Top1 lesions but equally appealing is sequestration of an accessory protein that might be needed both for TDP1-dependent and for TDP1-independent repair pathways. In any case, the existence of a specific phenotype confirms the expectation that the active site mutant is folded correctly. Nevertheless, when expressed at normal levels, it is unable to complement the CPT hypersensitivity of a tdp1 knockout (Fig. 1C). Taken together, these two observations lead us to conclude that the principal contribution of Tdp1 to repair of Top1 damage is via its enzymatic activity.

3.2 The mechanism of TDP1 action in the repair of bleomycin-induced damage

In addition to hydrolyzing a tyrosyl-DNA phosphodiester, purified Tdp1 can cleave a 3'-terminal phosphoglycolate (PG) diester [26]. Stimulated by this observation, we previously tested the effect of a tdp1 mutation on sensitivity of yeast to the radiomimetic drug bleomycin, which generates DNA breaks that are almost exclusively terminated by 3'-PG residues [27]. There was no effect of removing TDP1 by itself [17]. But, when combined with mutations (apn1 and apn2) that sensitized yeast to bleomycin by removing prominent sources of PG hydrolysis, a tdp1 mutation led to decreased survival after exposure to the drug [17]. These experiments established a role for TDP1 in repair of bleomycin damage but left the mechanism in doubt. In particular, concerns about the relevance of the enzymatic activity of Tdp1 were heightened because a PG-terminated oligonucleotide is a much poorer substrate for the purified enzyme than a tyrosine-terminated substrate [17,26].

One way to maintain a unitary mechanism for Tdp1 is to invoke the possibility that the effect of the gene on bleomycin damage is not due to hydrolysis of PG residues but is the indirect consequence of Tdp1 action on Top1 that had become trapped at bleomycin induced lesions. To challenge this hypothesis, we studied cells that were deleted for TOP1. If Tdp1 worked exclusively by repair of covalent complexes, removing its gene should not influence sensitivity to bleomycin in this strain background. In fact, the opposite was observed: just as in strains with intact TOP1 [17], survival of clonogenic potential after treatment with bleomycin was depressed by deletion of TDP1 (Fig. 2A). The gene was also clearly beneficial after exposure to gamma irradiation, which also generates 3'-PG breaks [28]. Although we can not rule out more complicated possibilities, we suspect that the minimal effect of TDP1 on survival after exposure to peroxide (not shown) reflects the tendency of this agent to generate breaks that terminate in 3'-phosphate [29], a substrate on which purified Tdp1 has no detectable activity [16]. In any case, the results with bleomycin and gamma irradiation of TOP1-deleted strains show that Tdp1 can influence repair in a way other than hydrolysis of the tyrosyl-DNA bond.

Does this influence reflect a non-catalytic function for Tdp1? To test this possibility, we transformed an apn1 apn2 top1 tdp1 strain with the TDP1 plasmid set and examined growth on plates containing bleomycin. As expected from the survival experiments (Fig. 2A), compared to cells transformed with the plasmid vector alone, wild-type TDP1 increased the ability of this strain to grow in the presence of bleomycin (Fig. 2B). But, catalysis by Tdp1 appears to be essential for this effect since cells transformed with the mutated gene tolerated the drug no better than cells bearing the vector. Taken together, our results strongly suggest that, although the catalytic efficiency of purified yeast Tdp1 on the phosphoglycolate bond is low, this activity contributes significantly to repair of oxidative damage.

3.3 Non-redundant roles for TDP1 in the repair of spontaneous and induced damage

In the work presented above, as in the past [3,16,17,25], substantially increased sensitivity to DNA damaging agents could only be observed when a tdp1 mutation was added to yeast strains that also carried mutations in other repair genes. This suggests that the pathway involving Tdp1 is only one of several alternative pathways for dealing with 3-damaged DNA, each of which has ample capacity to process DNA lesions. Consistent with this hypothesis is the result of an experiment designed to raise the level of CPT damage by overexpressing its target protein, Top1. Under these circumstances, we find that there is a substan-
As we have pointed out before [17], assays of colony-forming ability or growth are intrinsically slow, providing a long window of time during which alternative repair pathways may be induced and replace the one under study. We therefore sought to assess the acute response to Top1 damage. Specifically, wild-type and top1 mutant cells were fixed and DAPI stained during exposure to CPT and then examined by fluorescence microscopy for nuclear morphology. Within 2 h of exposure the majority of wild-type cells had suffered cell cycle arrest. As expected from a previous report [30], the predominant morphological form was a single nuclear mass located near the neck of a large bud, indicative of arrest at the G2/M boundary. In addition, with time there was an increasing proportion of cells in which the nucleus was elongated and stretched through the bud neck, indicative of arrest in mid-anaphase [22]. Although this checkpoint has not been reported before for CPT-treated yeast, it has been noted in cells expressing a toxic topoisomerase mutant [31]. Cells bearing a deletion of TDP1 showed the same overall pattern but there was a significant quantitative difference. At every time point tested, although the proportion of cells arrested at the G2/M boundary was virtually identical in the two strains, the proportion of mutant cells in mid-anaphase arrest was modestly but consistently higher than that for the wild-type strain (Fig. 3B). To test the significance of this trend, we made use of the fact that the data points were collected in pairs: in a single session a set of ~150 nuclei each from wild-type and mutant cells that had been exposed to CPT for the same time were scored by a single observer. Both by a paired t-test and by a Wilcoxon signed rank test [24], the difference in the proportion of cells in mid-anaphase from the 12 pairs of observations shown in Fig. 3B (encompassing three independent experiments) was highly significant (P < 0.01). Although survival curves (Fig. 3A) show that cells can recover from CPT damage without Top1, the altered arrest distribution show that mutant cells do not process checkpoint-inducing lesions with normal kinetics. The particular pattern observed suggests that TDP1 may be especially important for repair of Top1 damage that is revealed when sister chromatids undergo separation, although we can not rule out other possibilities. In any case, the mutant phenotype shows that, even when alternative pathways are available, cells rely on TDP1 for proper processing of some kinds of Top1 damage.

The work described above confirms and extends the conclusion that TDP1 is important for repair in cells induced to undergo high levels of Top1 damage. However, one must also ask whether TDP1 is conserved in the genome of eukaryotes only for its ability to repair extreme levels of damage or whether the gene also serves a non-redundant daily housekeeping function. When no DNA damage is artificially induced and no alternative pathways for repair are inactivated, we have found that yeast strains bearing a top1 null mutation are roughly equivalent to control strains in their ability to grow on a variety of media and at various temperatures, ability to mate and sporulate, and ability to enter...
and exit stationary phase (JJP and CL, unpublished observations). In addition, FACS analysis of cells in the midst of exponential growth reveals no significant difference in cell cycle distribution (JJP and HAN, unpublished observations). Consistent with our experience, it has been reported that the doubling time of *tdpl* mutants is indistinguishable from that of control strains [25]. It appears that more refined assays are needed to detect whatever contributions that *TDPL* may make to normal growth. Indeed, to our knowledge the only report of an unconditional effect of a *tdpl* mutation is a subtle alteration in nuclear structure and a modest defect in gene silencing [32]. To this short list we now add an effect of *TDPL* on spontaneous mutagenesis.

The spontaneous mutation rate at the *CAGL* locus (the average number of new mutations arising per generation during normal growth) was evaluated for a set of derivatives of strain S288C by the method of the median [23]. For each strain, this value was determined independently three or more times (Fig. 3C). We focused on selected pairs of strains and used a Mann-Whitney rank-sum test on the individual values [24] to evaluate whether there was a significant difference in mutation rate between them. This showed
that removal of TDP1 from a wild-type strain is associated with a significant \((P < 0.03)\) increase in mutation rate. The average increase, although small, is comparable or larger than that reported for other repair genes, e.g., RAD1, RAD6, APN1 [33, 34]. We have not determined the molecular change in the mutants but, since the CAN1 gene could be amplified by PCR in all 10 cases attempted (JJP, unpublished observations), the additional mutations that arise spontaneously in a tdp1 strain do not appear to be gross chromosomal rearrangements. Two candidates for the source of these additional mutations are Top1 lesions and oxidative damage, each of which can occur spontaneously as accidental events [18, 35]. In either case, removal of Tdp1 would presumably result in a shift to modes of repair that are more prone to error. We favor Top1 lesions as the culprit as a result of our comparison of spontaneous mutation rates of top1 and top1 tdp1 strains. We discovered that, when introduced into a wild-type strain, a top1 mutation by itself had a mild mutagenic effect \((P < 0.03)\) but there was no increase in mutagenesis rate when TDP1 was inactivated in this background \((P > 0.4)\). Although we can not rule out more complex reasons for the observed epistasis, the simplest explanation is that Tdp1 normally functions to repair spontaneous Top1 lesions. It would be of interest to know if the other unconditional defects that have been reported for tdp1 mutants [32] also reflect repair of spontaneous Top1 damage.

4. Concluding remarks

When the experiments reported in this paper are combined with the results of earlier work, an overall picture begins to emerge for the way in which the product of the TDP1 gene contributes to the fitness of budding yeast. First, enzymatic activity is essential for its contribution to DNA repair. Although this does not rule out its participation in multiprotein complexes, Tdp1 is thus not merely a conserved scaffold protein but relies on its capacity to hydrolyze phosphodiester bonds to improve growth in the presence of DNA damaging agents. Second, although hydrolysis of 3'-terminal tyrosyl-DNA phosphodiester accounts for the many effects of TDP1 on repair of Top1 damage, hydrolysis of 3'-terminal glycolate-DNA phosphodiester is also biologically relevant and contributes significantly to in vivo repair of oxidative damage. This suggests that accessory proteins might boost the much weaker phosphoglycolate cleavage that is observed in biochemical assays of purified Tdp1. Third, even when alternative pathways are left intact, TDP1 can have substantial effect on the cellular response to CPT. For example, in cells engineered to have high levels of Top1, enzyme action reduces the burden of lethal lesions by more than 50%. This non-redundant effect of TDP1 is much more impressive than the effects seen for long-term growth in the presence of CPT of strains with normal levels of Top1 [25] (CYL and JJP, unpublished observations). The comparison thus supports a previous suggestion [16] that the gene is particularly needed at high levels of damage. Under lower levels of CPT damage, a 50% increase in the proportion of cells that undergo arrest in mid-anaphase also indicate a role for TDP1 in the acute repair of Top1 lesions, a role that can not be efficiently fulfilled by alternative pathways. Finally, TDP1 is used by the cell in subtle ways during normal growth. Like the effect reported for gene silencing [32] and the effect on growth rate of rad1 mutants [25], there is a modest effect of a tdp1 knockout on mutation rate. Where tested, the phenotypes seen during normal growth depend on a functional TOP1 gene, implying that the enzyme is involved in repair of covalent complexes that occur as spontaneous accidents.

The view that emerges from the inventory of effects described above is a two-fold role for TDP1 in budding yeast. On the one hand, when the cell is confronted with a heavy burden of damage, the enzyme is involved in life or death decisions. On the other hand, at lower levels of damage, as judged by checkpoint responses, gene silencing assays, and mutation rate, it is important for the quality of the life of a cell. To what extent does the same hold true for higher eukaryotes? On the presumption that once Nature has invented something that works it tends to use it again and again, we expect that the enzymatic activity of the human ortholog of TDP1 is used to remove 3'-terminal blocking residues like tyrosine and phosphoglycolate. However, the relative importance of enzyme-dependent repair versus alternative pathways is likely to be species-specific. This is because each species has evolved to have its own balance of efficient repair modes and its own collection of interacting gene products. For example, it is well-known that yeast and animal cells emphasize different modes of double-strand break repair [36]. A more specific example comes from the recent finding that hTdp1 associates with XRCC1 [37], a scaffold for repair proteins in higher eukaryotes, but one for which there is no clear yeast ortholog. A final example of a species difference is the effect of overexpression of functional TDP1. It has been reported that this produces a significant resistance to CPT in animal cells [38] (F. Boege, personal communication) but in yeast we find that resistance is not increased and may even be decreased by overexpression (CYL, unpublished observations). Despite these caveats, we end by speculating that our discovery of a role for TDP1 in mutation avoidance during normal growth provides a plausible explanation for the death of motorneurons in patients that inherit a defective form of this gene [7]. According to this scenario, neurons die because they are long-lived cells and over a period of decades mutations generated by faulty DNA repair accumulate in them to a level that can not be tolerated.

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