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Repair of Topoisomerase I-Mediated DNA Damage

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Abstract

Topoisomerase I (Top1) is an abundant and essential enzyme. Top1 is the selective target of camptothecins, which are effective anticancer agents. Top1-DNA cleavage complexes can also be trapped by various endogenous and exogenous DNA lesions including mismatches, abasic sites and carcinogenic adducts. Tyrosyl-DNA phosphodiesterase (Tdp1) is one of the repair enzymes for Top1-DNA covalent complexes. Tdp1 forms a multiprotein complex that includes poly(ADP) ribose polymerase (PARP). PARP-deficient cells are hypersensitive to camptothecins and functionally deficient for Tdp1. We will review recent developments in several pathways involved in the repair of Top1 cleavage complexes and the role of Chk1 and Chk2 checkpoint kinases in the cellular responses to Top1 inhibitors. The genes conferring camptothecin hypersensitivity are compiled for humans, budding yeast and fission yeast.

A. Introduction: Mammalian Topoisomerase Families, Top1 Functions and Catalytic Mechanisms

Seven topoisomerase genes are encoded in the human nuclear genome [1]. The enzymes (abbreviated Topo or Top) have been numbered in the order of their discovery except for the most recent enzyme, mitochondrial topoisomerase I (Top1mt) [2,3]. Vertebrate cells contain two Top1 (Top1 for the nuclear genome and Top1mt for the mitochondrial genome), two Top2 (Top2 α and β) and two Top3 (Top3 α and β). The seventh topoisomerase is Spo11, whose expression is restricted to germ cells. Top3 α forms heterodimers with BLM (the gene product deficient in Bloom syndrome) and is functionally related to the resolution of post-replicative hemicatenanes and recombination intermediates [4,5]. Top1 proteins belong to the family of the tyrosine recombinases (which includes λ -integrase, Flip and Cre recombinases), and Top2 is related to bacterial gyrase and Topo IV, which are the targets of quinolone antibiotics.

Topoisomerases and tyrosine recombinases nick and religate DNA by forming a covalent enzyme-DNA intermediate between an enzyme catalytic tyrosine residue and the end of the broken DNA (Fig. 1). These covalent intermediates are generally referred to as “cleavage (or cleavable) complexes” (Fig. 2). Topoisomerases have also been classified in two groups depending whether they cleave and religate one strand (type I) or both strands (type II) of the DNA duplex. Type I enzymes include Top1 (nuclear), Top1mt, Top3 α and β and type II enzymes include Top2 α and β and Spo11.

Top1 is essential in vertebrates and flies but not in yeast. Knocking out the *TOP1* gene results in early embryonic lethality in mouse [6] and fly [7]. By contrast, yeast survives in the absence

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of *TOP1* [8]. Top1 is expressed constitutively throughout the cell cycle [9] and is concentrated in the nucleolus [10,11]. Its main function is to relieve both positive and negative DNA supercoiling generated by transcription and replication, and possibly DNA repair and chromatin remodeling [1,12–14]. The mechanistic similarities between Top1 and other tyrosine recombinases suggest that Top1 may also play a role in DNA recombinations [15, 16]. The Top1 recombinase activity has been proposed for the replication of vaccinia [17] and hepadnaviruses [16]. Top1 probably contributes also to RNA splicing by phosphorylating SR proteins [18,19].

Top1 relaxes DNA supercoiling in the absence of energy cofactor by nicking the DNA and allowing rotation of the broken strand around the Top1-bound DNA strand (Fig. 3B – curved arrow). Crystal structures of Top1 [20–22] show the enzyme encircling the DNA tightly like a clamp (Fig. 3D), which accounts for the fact that Top1 controls the processive relaxation of supercoiled DNA [20,23–25]. Once the DNA is relaxed, Top1 religates the breaks by reversing its covalent binding. Religation requires the DNA end 5'-hydroxyl-group to be aligned with the tyrosine-DNA phosphodiester bond. Under normal condition, the cleavage intermediates (Figs. 2A and 3B) are transient and religation is favored over cleavage.

B. Induction and stabilization of Top1 cleavage complexes by camptothecin and anticancer drugs and by carcinogens and endogenous DNA lesions

The normally transient Top1 cleavage complexes can be converted into potential DNA lesions. Stabilization of the cleavage complexes generally results from misalignment of the 5'-hydroxyl-DNA end. Misalignments can be generated by drugs bound at the interface of the enzyme and broken DNA [26,27] and by alterations of the DNA substrate (Table 1 and Fig. 4A).

Camptothecins and non-camptothecin Top1 inhibitors trap Top1 cleavage complexes by binding at the enzyme-DNA interface (Fig. 3D–E) [22, 28–30]. Hence Top1 inhibitors represent a paradigm for “interfacial inhibitors” [26, 27]. Interfacial inhibition has recently been shown to account for the molecular mechanism of inhibition of many natural products that block specific conformational states of macromolecular complexes. Aphidicolin and Top2 inhibitors have also been proposed to follow the interfacial inhibition paradigm [26, 27].

Two camptothecin derivatives are used in cancer therapy: hycamtin (Topotecan[®]) and CPT-11 (Irinotecan; Camptosar[®]) [31]. CPT-11 is an inactive prodrug. It needs to be converted to its active metabolite SN-38. Hence, it is preferable to use SN-38, topotecan, or camptothecin for pharmacological studies. Additional camptothecin derivatives are in preclinical and clinical development [32].

Two key pharmacological properties of camptothecins need to be stressed. First, camptothecins bind reversibly to the Top1 cleavage complexes. Under pharmacological conditions, a rapid equilibrium is established between the ternary drug-enzyme-DNA complex and the dissociated complex. Hence, once camptothecins are diluted out and removed from cell culture, the cleavage complexes reverse rapidly [33]. The equilibrium can be shifted toward religation by increasing the temperature to 65°C and the salt concentration (≥ 0.35 M NaCl) in biochemical reactions. Salt-reversal is commonly used to study the “on” and “off” rates of Top1 cleavage complexes under various conditions [for recent example see [34]]. A second key pharmacological feature of camptothecins is the trapping of only a subset of the existing Top1 cleavage complexes, i.e. those with a guanine at the 5'-end of the break (+1 position – see Fig. 3) [35,36]. Indenoisoquinolines (Fig. 3F) on the other hand tend to stabilize those cleavage complexes with a cytosine at the 3'-end of the breaks (–1 position) [34]. This sequence selectivity explains why camptothecins are relatively poor Top1 catalytic inhibitors as a

fraction of the Top1 cleavage complexes (those not bearing a guanine +1) are immune to the drugs. It also explains why camptothecins only reveal a subset of the Top1 sites and should not be used alone to map all the Top1 cleavage complexes in a given DNA or chromatin segment.

Top1 cleavage complexes can be trapped by endogenous and frequent DNA lesions including abasic sites, mismatches, oxidized bases, nicks and carcinogenic DNA adducts [37] (Table 1 and references therein). For instance, DNA modifications such as those associated with oxidative damage [thousands per cell per day [38]] can produce Top1 cleavage complexes [39]. By contrast to camptothecins and other Top1 inhibitory drugs, these DNA modifications can produce irreversible cleavage complexes when the 5'-end of the DNA is irreversibly misaligned as in the case of abasic sites [40] or DNA breaks [41] (Table 1, Fig. 4A). Finally, we recently found the formation of Top1 cleavage complexes during apoptosis [42–45], which we have been explained by the trapping of Top1 by chromatin modifications (primarily due to reactive oxygen species).

C. Conversion of Top1 cleavage complexes into DNA damage

Cleavage complexes induced by DNA modifications that induce pronounced DNA structural alterations (abasic sites, mismatches, breaks) can be irreversible (Fig. 4A, Table 1). Such irreversible cleavage complexes have been referred to as “suicide complexes” [46,47]. They constitute composite DNA lesions associating disruption of the DNA backbone (break on one or both strands) in association with a large protein covalently bound to the 3'-end of the broken DNA (Top1 is a 100 kDa protein). Reversible cleavage complexes can also produce irreversible cleavage complexes after processing by DNA and RNA polymerases (Fig. 4B and C). Thus, both DNA and RNA synthesis convert reversible cleavage complexes into DNA lesions. The relative contribution of DNA replication and transcription depends on the camptothecin concentration and the cell type. In highly proliferative cancer cells, replication-induced DNA damage contributes to most of the cytotoxicity at low camptothecin doses, whereas transcription-induced DNA damage contributes to the cytotoxicity of high doses of camptothecin [48,49]. In non-dividing cells (neurons and lymphocytes) transcription-induced damage can kill cells at pharmacological concentrations [50,51].

Camptothecin-induced Top1 cleavage complexes can readily be converted into replication double-strand breaks (Rep-DSB) (Fig. 4B) as demonstrated by: i) analyses of the broken ends by ligation-mediated PCR [52] showing the extension of the leading strand up to last nucleotide [leading to blunt-ended DSB by “replication run-off” [52]], and ii) rapid phosphorylation of histone H2AX (referred to as γ -H2AX) [53], which is a hallmark for double-strand breaks [54,55]. Inhibition of DNA synthesis occurs within minutes following camptothecin treatment. It is intense ($\geq 80\%$) and persists for several hours following drug removal [56]. At least two mechanisms lead to DNA synthesis inhibition: i) direct block of replication forks that have collided with the Top1 cleavage complexes (Fig. 4B), and ii) indirect replication arrest by S-phase checkpoint activation. The checkpoint implication is consistent with the fact that the checkpoint abrogator 7-hydroxystaurosporine (UCN-01) prevents inhibition of DNA synthesis by camptothecins [56–58]. The lethality of the replication double-strand breaks stems from the fact that when DNA synthesis is inhibited by aphidicolin, a specific inhibitor of replicative DNA polymerases [59], cells become immune to camptothecin in spite of their ability to form reversible cleavage complexes [48,49]. Similarly, aphidicolin prevents the formation of γ -H2AX foci in camptothecin-treated cells [53].

Camptothecin is a potent inhibitor of both nucleoplasmic (mRNA) and nucleolar (rRNA) transcription [60–62]. Although the overall level of transcripts decreases rapidly following Top1 inhibition, specific genes are differentially affected. For example, camptothecin causes

a strong holdback of the endogenous *c-myc* gene at the P2 promoter, whereas it produces minimal effect on an episomal *c-myc* gene or on the basal transcription of the *Hsp70* and *Gapdh* genes [63]. Camptothecin also enhances the expression of a large number of genes including *c-fos* [63–66]. Transcription inhibition is primarily due to transcription elongation blocks by trapped Top1 cleavage complexes (Fig. 4C), which is a high probability event considering that Top1 is associated with transcription complexes [12]. Camptothecin has little effect on transcription initiation [11,67]. It has been proposed that the elongating RNA polymerase collides with trapped Top1 cleavage complexes on the transcribed strand, resulting in the conversion of reversible Top1 cleavage complexes into irreversible strand breaks (Fig. 4C) [68,69]. Inhibition of Top1 catalytic activity by camptothecins might also inhibit transcription by producing an accumulation of positive supercoils upstream from the elongating RNA polymerase [63,70] and by compacting chromatin domains [70–72]. The transcriptional effects of camptothecins could also be related to functions of Top1 besides its DNA nicking-closing activity. Top1 regulates transcription initiation by interacting with TATA binding proteins [73,74], and phosphorylates/activates RNA splicing factors from the SR family [19].

By contrast to replication [56], transcription inhibition recovers rapidly following camptothecin treatment [62,67,75]. Recovery of RNA synthesis depends both on degradation of Top1 and functional transcription-coupled nucleotide excision repair (TC-NER) [75]. Tumor cells that are deficient in Top1 degradation following camptothecin treatment, and Cockayne syndrome cells that are deficient in TC-NER are hypersensitive to camptothecin [76,77], suggesting the importance of transcription-coupled DNA repair for RNA-synthesis recovery and cell survival in response to Top1-mediated DNA damage.

D. Repair of Top1-associated DNA damage

The molecular mechanisms/pathways involved in the repair of Top1-associated DNA damage are better understood than for Top2. Because camptothecin can be readily used in yeast, multiple pathways have been uncovered. We will consider three main repair pathways: i) Reversal of the covalent Top1-DNA complexes by 5'-end religation, ii) Top1 excision by Tdp1, and iii) Top1 excision by endonucleases. In spite of an apparent redundancy, it remains to be determined which pathways are preferred or selective for the Top1-associated DNA damages represented in Figure 4.

I. Reversal of Top1-DNA covalent complexes by 5' end religation (Fig. 5A)

Top1-mediated DNA religation requires that the intact 5'-hydroxyl end be aligned with the 3'-end bonded to Top1 for nucleophilic attack of the tyrosyl-phosphoester bond (see Fig. 1B). Thus, this pathway/mechanism excludes the Top1 suicide complexes generated by DNA lesions affecting the 5'-end of the broken DNA (Fig. 4A and Table 1) unless they are repaired first. Top1 religation can be envisaged for the replication and transcription breaks (Fig. 4B and C) following regression (“pull-back”) of the replication or transcription complexes (Fig. 5A).

Replication fork regression could generate a “chicken foot” [see Fig. 6 in ref. [78]], which is topologically equivalent to a Holiday junction. RecQ helicases (the Bloom syndrome helicase BLM in mammals and Sgs1 in yeast) and Top3 (Top3 α in mammals) form helicase-Top3 complexes, which have been implicated in the regression of replication forks and their restart [4,79]. A plausible intermediate is the conversion of the “chicken foot” into a Double-Holiday junction catalyzed by Rad51, which can then be resolved by Top3 [80,81]. The role of the RecQ helicases in processing Top1-mediated DNA damage is demonstrated by the hypersensitivity of yeast Sgs1 and Rqh1 mutants (see Tables 3 and 4) and the hypersensitivity of Bloom syndrome cells to camptothecin [79,82] (see Table 2).

In the case of transcription, blocked RNA complexes might be displaced (“pull-back” mechanism) from the Top1 cleavage complexes without removal from the transcribed DNA. Transcription elongation restart can finish the incomplete mRNA. Rad26 (the yeast homologue of CSB) and TFIIS have been implicated in the backtracking of RNA polymerase II [83]. RNA polymerase II can also be degraded following camptothecin treatment but this process is limited to some cell types and delayed as compared to RNA-synthesis recovery [75].

Because Top1 is very effective in joining a 5'-hydroxyl end from a non-homologous substrate to the Top1 covalent complex, Top1 cleavage complexes might reverse by religation of a non-homologous end, which leads to DNA recombinations [84]. Camptothecin is indeed a potent inducer of sister chromatid exchanges and chromosomal abnormalities [85–87]. Top1-mediated 5'-end ligation with vaccinia Top1 is commonly used for cloning recombinant genomes (TOPO cloning kit; Invitrogen, Carlsbad, CA).

II. Top1 excision by Tyrosyl-DNA-phosphodiesterase (Tdp1) (Fig. 5B)

Tdp1 was discovered by Nash and coworkers [88] as the enzyme capable of hydrolyzing the covalent bond between the Top1 catalytic tyrosine and the 3'-end of the DNA [89]. Tdp1 generates a 3'-phosphate, which is further processed by a 3'-phosphatase, such as PNKP (hPNK) (Fig. 5B).

Tdp1 belongs to the phospholipase D superfamily [90] of phospholipid hydrolyzing enzymes. It is ubiquitous and highly conserved in eukaryotes (from yeast to humans – see Tables 2–4). Tdp1 is physiologically important since the homozygote mutation H493R causes spinocerebellar ataxia with axonal neuropathy (SCAN1) [91]. SCAN1 cells are hypersensitive to camptothecin [92–94] (Table 2) and ionizing radiation [95], but not to etoposide or bleomycin [94]. The budding yeast *TDP1* knock out is viable [88] and hypersensitive to high levels of Top1 cleavage complexes generated by overexpression of a toxic Top1 [96]. It is hypersensitive to camptothecin only when the checkpoint gene Rad9 is simultaneously inactivated [96] or when some endonuclease pathways (Rad1/Rad10 and Slx1/Slx4) are inactive [97–99] (Table 3). Because Tdp1 specifically processes 3'- but not 5'-tyrosyl-DNA complexes [88,89], Tdp1 cannot hydrolyze the cleavage complexes produced by other topoisomerases besides Top1 (see Fig. 2). However, Tdp1 function is probably not limited to the repair of Top1 cleavage complexes. Tdp1 can remove 3'-phosphoglycolate (Fig. 6D) generated by oxidative DNA damage, which suggests a broader role for Tdp1 in the maintenance of genomic stability [100].

Human Tdp1 acts as a monomer and crystal structures demonstrate the presence of two catalytic domains related by a pseudo-2-fold axis of symmetry [101–103]. Each domain contains 3 conserved HKN residues (H263, K266 and N283 and H493, K495 and N516 (equivalent to the “HKD motifs” of phospholipases) [101,102,104] forming a catalytic network with two water molecules critical for Tdp1 activity [90,91]. Tdp1 hydrolyzes the DNA phosphotyrosine bond in two consecutive SN2 reactions (Fig. 6B–C). In the first reaction, H263 of the first HKD motif releases the Top1 tyrosine from the phosphodiester by forming a transient covalent phosphoamide bond between the N ϵ 2 atom of the nucleophilic H263 and the 3'-end of the DNA (Fig. 6B). Mutating H263 totally abolishes Tdp1 catalytic activity [90]. In the second reaction, H493 from the second HKD motif catalyzes the nucleophilic attack of the phosphoamide bond by a water molecule. This regenerates Tdp1 and produces a 3'-phosphate DNA end [90] (Figs. 6C and 5B). The SCAN1 mutation H493R affects preferentially the second step of the reaction, which leads to an accumulation of Tdp1-DNA covalent intermediate and reduces Tdp1 catalytic activity \approx 25-fold [94,105]. Interthal, Champoux and coworkers have recently shown that wild-type Tdp1 can hydrolyze the phosphoamide Tdp1-DNA covalent intermediate (Fig. 6B) [105], and proposed that lack of symptoms in SCAN1 heterozygote carriers might be due to the release of these covalent intermediates by the coexisting wild-type Tdp1 (Fig. 6D). They

also proposed that the covalent Tdp1-DNA intermediates rather than deficient Tdp1 catalytic activity might be responsible for DNA damage leading to SCAN [105].

Both the structure of the DNA segment bound to Top1 [88,106] and the length of the Top1 polypeptide chain determine Tdp1's activity [106]. Optimum Tdp1 activity requires: 1/ a DNA segment consisting of at least a few nucleotides [106]; 2/ an exposed phosphotyrosyl bond at the Top1-DNA junction [a tyrosyl group linked to the 3'-end of a nick is a poor substrate [96], indicating that Tdp1 acts after the 5'-end of the broken DNA has been either digested or displaced to provide access to the 3'-phosphotyrosyl bond]; and 3/ a short Top1 polypeptide segment, as the effectiveness of Tdp1 decreases with the length of the Top1 polypeptide [106]. In fact, Top1 needs to be proteolyzed or denatured for efficient Tdp1 activity [89,105, 106]. Top1 ubiquitination and degradation have been observed following camptothecin treatment [107,108]. The Top1 degradation pathway appears selectively deficient in transformed cells, although not all transformed cells appear equally able to proteolyze Top1 following camptothecin treatment [77]. Such differences have been proposed to contribute to camptothecin resistance [77]. A recent structure of Tdp1 bound to a tyrosine-containing peptide demonstrate that both the DNA and the Top1 polypeptide need to adapt their structure to bind Tdp1 in the crystal structure [103]. The DNA binds in a narrow groove that fits a single-stranded substrate and the short Top1 polypeptide is folded differently from the native Top1 [103]. Recently, an alternative model has been presented for duplex DNA, which can also be processed effectively by Tdp1 [88,106,109].

The 3'-phosphate ends generated by Tdp1 need to be hydrolyzed to a 3'-hydroxyl for further processing by DNA polymerases and/or ligases (Fig. 5B). In budding yeast, this 3'-phosphatase activity is carried out by Tpp1 [110] and by the two functionally overlapping multifunctional apurinic (AP) endonucleases, Apn1 and Apn2 [97] (see Fig. 8A). Apn1 is the ortholog of *E. coli* endonuclease IV and represents the major yeast AP endonuclease. Apn2 (also called Eth1) belongs to the second family of AP endonuclease (the *E. coli* exonuclease III family), and is the ortholog of Ape1 in humans. Simultaneous inactivation of Tpp1, Apn1 and Apn2 is required to sensitize yeast to camptothecin [110], indicating the functional redundancy of the 3'-phosphatase pathways. Noticeably, the hypersensitivity of the *tpp1 apn1 apn2* triple mutant is rescued by inactivation of Tdp1 [111], which indicates that in the absence of Tdp1, budding yeast uses an alternative endonuclease pathway for removal of the Top1 covalent complexes (see Figs. 5C and 8A, and next section). The 3'-phosphatase orthologs of Tpp1 are Pnk1 in fission yeast [112] and PNKP (also referred to as hPNK) in humans [110,113,114] (see Tables 2-4). In addition to their 3'-phosphatase activity, Pnk1 [112] and PNKP/hPNK [113,114] possess 5'-kinase activity, which is missing for Tpp1. Stable down-regulation of human PNKP (hPNK) sensitizes to camptothecin (Table 2), ionizing- and UV-radiation, H₂O₂ and UV, and increases spontaneous mutation frequency [115].

In humans, Tdp1 and PNKP form a multiprotein complex with XRCC1, poly(ADP)ribose-polymerase (PARP), β -polymerase and ligase III [116,117] (Fig. 5B, bottom). This complex contains the critical elements for base excision repair. Mammalian cells deficient for XRCC1 or PARP are also hypersensitive to camptothecin (Fig. 7) (Table 1) [78,116,118]. The hypersensitivity of PARP^{-/-} cells to camptothecin (Fig. 7A) can be related to a functional defect in Tdp1 (Fig. 7B-C). It is not explained by abnormal levels of Tdp1 or Top1 proteins (Barcelo and Pommier, unpublished). Recently, a novel protein, aprataxin has been found to associate with the XRCC1 complex (Fig. 5B) [119]. Aprataxin is a 342 amino acid protein encoded by the APTX gene whose homozygote mutation produces AOA1 (Ataxia-oculomotor Apraxia; the most common autosomal recessive ataxia in Japan) [120,121]. AOA1 cells are hypersensitive to camptothecin [122] (Table 1).

There is no pharmacological inhibitor of Tdp1 reported to date. Vanadate and tungstate act as phosphate mimetic in co-crystal structures and block Tdp1 activity at millimolar concentrations [102]. It would, however, be rational to develop Tdp1 inhibitors for cancer treatment in combination with camptothecins. The anticancer activity of Tdp1 inhibitors may prove dependent on the presence of cancer-related genetic abnormalities, since camptothecin hypersensitivity in Tdp1-defective yeast is conditional for deficiencies in the checkpoint (Rad9) [88,97,98]. A Rad9 defect in a Tdp1-deficient background confers marked sensitization to camptothecin [88], and it is tempting to speculate that Tdp1 is primarily required when the checkpoints are deficient as in the case of the yeast *RAD9* mutant. A second group of conditional genes (with respect to Tdp1 deficiencies) includes three sets of genes from the 3'-flap endonuclease pathway: Rad1/Rad10, Mre11/Rad50, and Mus81/Eme1 [88,97,98]. Mutation in each of these genes renders Tdp1-deficient cells highly sensitive to camptothecin (see Table 3). Hence, colon cancers, which are commonly mutated for Mre11 might be selectively sensitive to the combination of camptothecin with a Tdp1 inhibitor. The potent activity of Tdp1 against a number of artificial substrates (Fig. 6E) [105] can be used to design high throughput screens (our unpublished observations) [123,124], and it is likely that Tdp1 inhibitors will be reported in the near future.

III. Top1 excision by Endonucleases (Fig. 5C)

Studies in yeast demonstrate the existence of alternative pathways besides Tdp1 for removing the Top1 covalent complexes. Figure 8 summarizes the multiple endonuclease genetic pathways implicated in the repair of Top1 cleavage complexes [97–99].

Rad1/Rad10 and Slx1/Slx4 appear to function in parallel and redundant pathways with Tdp1 [97–99] (Fig. 8A). Rad1/Rad10 is the ortholog of the human endonuclease XPF/ERCC1, which cleaves DNA 3' from bulky adduct during nucleotide excision repair [125]. Like Tdp1, Rad1/Rad10 requires a single-stranded gap between the 3'-end to be processed and the 5'-end of the DNA (Fig. 8B) [126], suggesting that Tdp1 and Rad1/Rad10 share common substrates. *SLX1* and *SLX4* were originally identified as genes synthetically lethal with mutations in *SGS1* and *TOP3* [127]. The dimeric complex has strong endonuclease activity with a wide range of substrates [128], and Slx4/Slx1 appears to function as an alternative pathway in the absence of Tdp1 [99] (Fig. 8).

Mus81/Mms4 (the ortholog of budding yeast Mms4 is Eme1 in humans and fission yeast –see Tables 3 & 4) preferentially cleaves broken replication forks and requires the presence of duplex DNA near the 3'-end to be processed [see Fig. 8B and Fig. 4B in [78]] [126,129,130]. Mus81- and Rad50-deficient yeasts are highly sensitive to camptothecin (Tables 3 & 4) [98, 99,126,129,131].

The Mre11/Rad50/Xrs2 complex (MRX) (the human orthologs are Mre11/Rad50/Nbs1 [MRN]) preferentially cleaves gapped substrates (Fig. 8B) and hairpin structures [132]. MRX appears to function independently from the Tdp1 pathway, which is also the case for Mus81/Mms4 and Rad27 [97–99] (Fig. 8A). Sae1 is required for the endonuclease activity of MRX, and *sae2* deletion strains are among the strains most sensitive to camptothecin [99]. The MRN complex also possesses checkpoint functions, which probably contribute to cell survival in response to camptothecin [97,98].

Surprisingly, the 5'-flap endonuclease, Rad27 (the human ortholog is FEN-1) also contributes to the repair of Top1 covalent complexes (Fig. 8). Deletion of *RAD27* causes mild sensitivity to camptothecin (Table 3) [98,99,133]. The apparent discrepancy between the established 5'-flap endonuclease activity of Rad27 (FEN-1) and its role in Top1 repair can be explained by a recent study demonstrating that Rad27 in coordination with the Werner syndrome protein and replication protein A (RPA) possesses gap 5'-endonuclease activity (GEN activity) and

can process substrates that mimic stalled replication forks. Human FEN-1 is able to rescue the defect in resistance to camptothecin and UV in a yeast FEN-1 null mutant [133].

The recent yeast genome-wide screen to detect novel genes that are important for protection against growth inhibition and killing produced by camptothecin identified a large number of genes for further exploration [99]. SRS2 was among the most critical genes [98,99]. SRS2 is a DNA helicase that might contribute to the local unwinding (Fig. 8B) that would provide access to the endonucleases described above. A number of other genes involved in transcription, replication, ubiquitination and protein degradation await further studies (Table 3) [99,134–136]. However, extrapolating the results obtained in budding yeast to human cells is not straightforward. Cells from Mus81 knockout mice have recently been tested and failed to show significant hypersensitivity to camptothecin in spite of their hypersensitivity to DNA crosslinking agents (cisplatin and nitrogen mustards) [137]. Similarly, XPF cells do not demonstrate hypersensitivity to camptothecin (Pourquier and Pommier, unpublished). These apparent discrepancies may be related to the presence of additional repair pathways and different checkpoint controls in humans.

E. Checkpoint response to Top1-associated DNA damage

Cellular responses to Top1 poisons determine both tumor response and host toxicity. Efficient repair is probably coupled with checkpoint activation. Cell cycle arrest following checkpoint activation would have two beneficial consequences: 1/ it would give time for the repair of DNA damage; and 2/ it would prevent further replication-dependent DNA damage. Both the S-phase and the G2 checkpoints, as well as the p53/p21 pathways are activated by Top1-mediated DNA damage [57,138]. Because cell cycle checkpoints are also connected to the apoptosis machinery, it is likely that extensive DNA damage activates apoptosis by involving the same DNA damage sensors and checkpoints [42]. Thus, an exciting challenge is to elucidate the relationships between sensor proteins, checkpoints, DNA repair and apoptosis. Integration of these pathways should explain the cellular determinants of response to Top1 poisons. We will focus on the Chk1 and Chk2 pathways/responses elicited by Top1 poisons, and how defects in these pathways can sensitize tumors to Top1-mediated DNA damage. We will not review the roles of p53, c-Abl, and the stress kinase (JNK/SAPK) pathways, which have been detailed elsewhere [42,78,139].

I. Chk1 activation by camptothecin

Chk1 is an evolutionarily conserved kinase and essential member of the DNA damage checkpoint [140–142]. Deletion of Chk1 is embryonic lethal with massive apoptosis in stem cells [143,144]. Chk1 deletion is not lethal in budding and fission yeast or chicken somatic cells (DT40). However, these cells display an inability to recover from replication blockade and are hypersensitive to ionizing radiation [145–147]. In budding yeast, the upstream kinase Tel1 activates Chk1 in concert with the “9-1-1 PCNA-like” clamp proteins: Rad17, Mec3 and Rad24. Strains defective for the Tel1 or the 9-1-1 clamp proteins are hypersensitive to camptothecin (Table 3). In xenopus and humans, ATR (the Tel1 ortholog) with the Rad9-Rad1-Hus1 (“9-1-1”) complex regulates Chk1 via phosphorylation. The DNA-binding protein claspin also associates with Chk1 and is required for Chk1 activation [143,148–150]. Other kinases, including ATM [151,152], might also activate Chk1 by phosphorylation [152–154].

The first reports of Chk1 activation by camptothecin were obtained in the fission yeast [155]. Blocking DNA synthesis with a hydroxyurea pre-treatment blocked camptothecin-induced Chk1 phosphorylation/activation, and cells lacking functional Chk1 were hypersensitive to camptothecin [155–157]. Phosphorylation of Chk1 is rapid following exposure to camptothecin and reaches a plateau between 60 minutes and 3 hours [155]. Chk1 activation requires phosphorylation by Rad3 and is required for cell cycle arrest and interaction with

Rad24 and Rad25 (14-3-3 orthologs), which sequester (and functionally inactivate) Cdc25 [158–164]. In mammalian cells, camptothecin also activates Chk1, and Chk1 anti-sense oligonucleotides abolish the checkpoint response to camptothecin [165]. Chk1 activation requires phosphorylation by ATR on S345. G2/M arrest is mediated by phosphorylation of Cdc25C by Chk1 and subsequent inactivation of Cdc25C [161–164], and S-phase arrest is mediated by Chk1-mediated phosphorylation of Cdc25A and degradation of Cdc25A [166]. Chk1 can also phosphorylate Rad51 on T309. Rad51 is involved in homologous recombination and provides a link between Chk1 activation, DNA repair and survival after replicative stress [167].

Camptothecin also induces Chk1 degradation by the ubiquitin-proteasome pathway [168] via the E4 ligase complex containing cullins (Cul1 and Cul4A). Phosphorylation at S345 marks Chk1 for proteolytic degradation and may be indicative of a negative feedback mechanism that may promote the checkpoint termination. Chk1 degradation had been previously suggested to occur via a proteosomal pathway after exposure to geldanamycin, an Hsp90 binding agent that stimulate proteasome-mediated degradation of several other proteins [169]. Downregulation of Chk1 by siRNA potentiates the cytotoxicity of camptothecin in cancer cell lines [162,170]. Consistently, Chk1 inhibition by UCN-01 (7-hydroxystaurosporine) markedly potentiates the cytotoxicity of camptothecin via abrogation of the S-phase checkpoint [57]. Preincubation with UCN-01 prevents camptothecin-induced degradation of Cdc25A and cyclin E in a p53-dependent manner, which is consistent with an abrogation of S phase arrest [171]. In another study, concurrent treatment with camptothecins and UCN-01 resulted in S-phase checkpoint abrogation, increased phosphorylation of γ -H2AX (a marker of DNA double strand breaks) and cell killing independently of p53 [172].

II. Chk2 activation by camptothecin

Chk2 shares no sequence homology with Chk1 [for recent review see [173]]. Chk2 knockout mice are viable and are defective in p53 stabilization following DNA double-strand breaks [174,175]. Chk2 functions as a checkpoint and apoptotic kinase after being activated via phosphorylation primarily by ATM [141,176,177], and also by ATR [178], DNA-PK (via interactions with Ku70/80) [179], Polo-like kinases, Plk-1 and Plk-3, [180–184] and TTK/hMPS1 kinases [185]. Following initial phosphorylation on T68, Chk2 undergoes dimerization via the FHA domain of a second Chk2 molecule [186]. This interaction is followed by a cascade of autophosphorylation steps on T387/T383 and S516, which are required for full activation of Chk2 [reviewed in [187] and (<http://discover.nci.nih.gov/mim/>)].

Human kidney embryonic cells expressing anti-sense Chk2 display defective S-phase delay and enhanced cell killing in response to replication-mediated DNA damage induced camptothecin [188]. Chk2 siRNA experiments also demonstrate a role for Chk2 for cell survival after camptothecin [162]. However, the phenotype of Chk2-deficient cells may depend on the cell type. In cortical neurons, ATM deficiency, but not Chk2 deficiency, attenuates cell death and significantly inhibits the induction of p53 phosphorylation on S15 and p53 levels induced by camptothecin [189]. Further analyses in cells with fully functional replication machinery are warranted to determine the potential role of Chk2 in Top1-induced DNA replication stress. More specifically, the relative roles of ATR and ATM remain to be elucidated. Chk2 is rapidly phosphorylated by ATM in camptothecin-treated cells [190], and phosphorylated Chk2 forms nuclear foci that are associated with sites of DNA damage marked by 53BP1, γ -H2AX and NBS1 [191]. Camptothecin also reduces the amount of chromatin-associated Chk2, especially the active T68-phosphorylated forms of Chk2 [179]. Soluble Chk2 might be implicated in the transmission of signals to remote chromatin sites from the DNA damage.

Chk2 has a dual role on cell cycle checkpoints and apoptosis via phosphorylation of its downstream substrates including Cdc25A, Cdc25C, p53, BRCA1, E2F1, and PML [reviewed in [173]] (<http://discover.nci.nih.gov/mim/>). Chk2 provides an unexplored therapeutic target in cancer cells with inherent defects in G1 checkpoint function. By virtue of Chk2's role in both cell cycle checkpoint regulation and apoptosis, selective inhibition of Chk2 could improve the therapeutic index of DNA-damaging agents such as camptothecin [Reviewed in [173]]. This may be especially true in p53-deficient tumors where the p53-dependent apoptotic response is deficient. In normal tissues Chk2 may act as a pro-apoptotic effector, thus Chk2 inhibitors may protect normal tissues. However, no clinical agent currently presents selective Chk2 inhibition.

III. Chk1 vs. Chk2 activation by camptothecin and Top1-mediated DNA damage

As discussed above, Top1-induced DNA damage activates both the Chk1 and Chk2 kinases. However, the relative timing of such activations has not been described in detail. Understanding the kinetics of activation of each has the potential for presenting a better target for inhibition in combination with camptothecins. A recent study [192] reported that both Chk1 and Chk2 are rapidly activated following low dose exposure to camptothecin, suggesting concomitant activation of both the ATR-Chk1 and ATM-Chk2 pathways. Additionally, a deficiency of ATM kinase prevented the activation of Chk2, with no effects on Chk1 activity or the degradation of Cdc25A [192]. The authors proposed that the ATR-Chk1 pathway is sufficient, though not the only pathway, to induce checkpoint-mediated degradation of Cdc25A. One explanation for the ATM-independent analysis might be the cautionary finding of a highly activated ATR-Chk1 pathway in AT cells treated with ionizing radiation [193]. Recent reports identifying multiple phosphorylation sites on Chk1 and Chk2 and their unraveling functions also provide a rationale for further studies that are needed to detail the kinetics of Chk1 and Chk2 activation by camptothecin. Overall, the redundancy in activation of both Chk1 and Chk2 by camptothecin may be indicative of distinct functions for each kinase in collecting, sustaining and deploying the DNA damage signal to its various substrates for favorable use by the cell. Abrogation of these kinases, and consequently the cell cycle arrest checkpoint, thus presents novel opportunities for enhancing drug efficacy.

F. Conclusion and perspective

Studies performed in yeast have provided major insights in the DNA damage and repair of Top1 cleavage complexes. These insights include the demonstration that Top1 is the selective target of camptothecin, the discovery of Tdp1, the identification of genetic defects (in recombination pathways and endonucleases) that sensitize yeast to Top1-mediated DNA damage. It is clear that more lessons remain to be learned from yeast including the roles of chromatin structure, ubiquitin and protein modification pathways. However, not all yeast pathways can be translated to human cells even when the orthologs are present in both. For instance, the Tdp1 mutant in budding yeast is only sensitive to camptothecin if a checkpoint pathway (Rad9) or an endonuclease pathway (Rad1/Rad10) is also defective. By contrast, human cells deficient for Tdp1 (cells from SCAN1 patients) appear to be sensitive with this single alteration. Mus81-deficient yeast is highly sensitive to camptothecin whereas murine Mus81-knockout cells are not. These differences must be related to the "genetic background" of the cells.

The divergent phenotypes (and genotypes) of mammalian cells in culture provide therapeutic opportunities for cancer treatment. Programmed genetic and pharmacological deficiencies should have different consequences with respect to cellular response to Top1-mediated DNA damage depending on the genomic context of the cell. This challenge brings the opportunity to find out which genetic contexts provide selective sensitivity or resistance to Top1 inhibitors,

and the rationale for developing therapies tailored to the genetic deficiencies selective to particular tumors. It is in this context that one could foresee the use of inhibitors of Tdp1 and Chk1/2 in combination with Top1 inhibitors once it is established which tumor-specific deficiencies provide the greatest sensitization to Top1 inhibitors.

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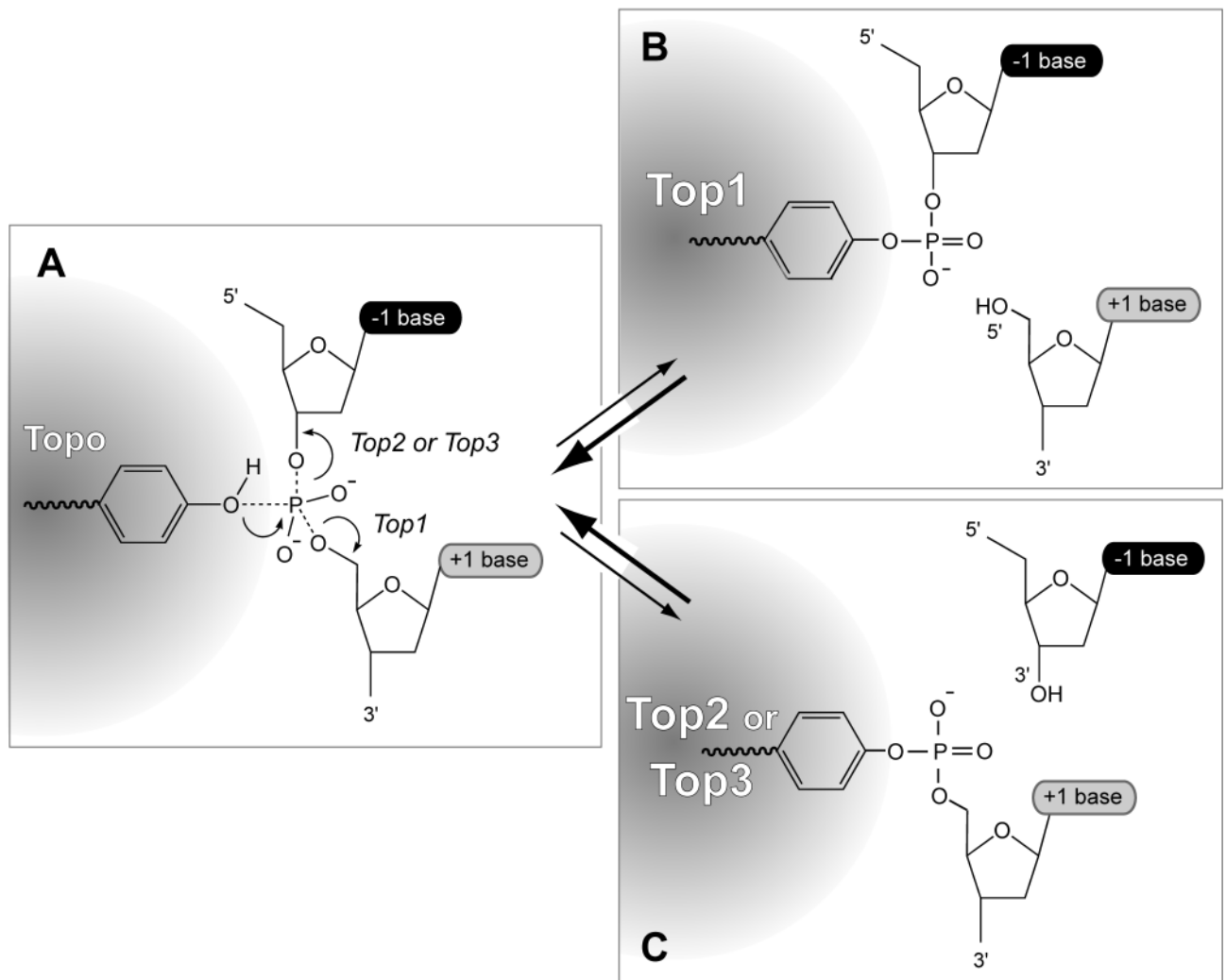


Fig. 1. Topoisomerase cleavage complexes

Topoisomerases (abbreviated *Topo* in panel A) utilize a catalytic tyrosine residue for nucleophilic attack and breakage of a DNA phosphoester bond. **A.** The polarity depends on the *Topo* (only human enzymes are considered here). **B.** Topoisomerases I (nuclear and mitochondrial *Top1*) form a covalent bond with the 3'-DNA end and generate a 5'-hydroxyl-end. This cleavage intermediate allow controlled rotation of the 5'-end around the intact DNA strand (see Fig. 3B). Under normal conditions, the reaction is reversible. Religation (back arrow from B \rightarrow A) is favored over cleavage and requires the alignment of the 5'-hydroxyl-end with the phosphoester tyrosyl-DNA bond for nucleophilic attack. **C.** All other human *Topo* enzymes (*Top2* and *Top3*) have an opposite polarity compared to *Top1* (see Fig. 2). They form covalent bonds with the 5'-end of the break and generate 3'-hydroxyl ends.

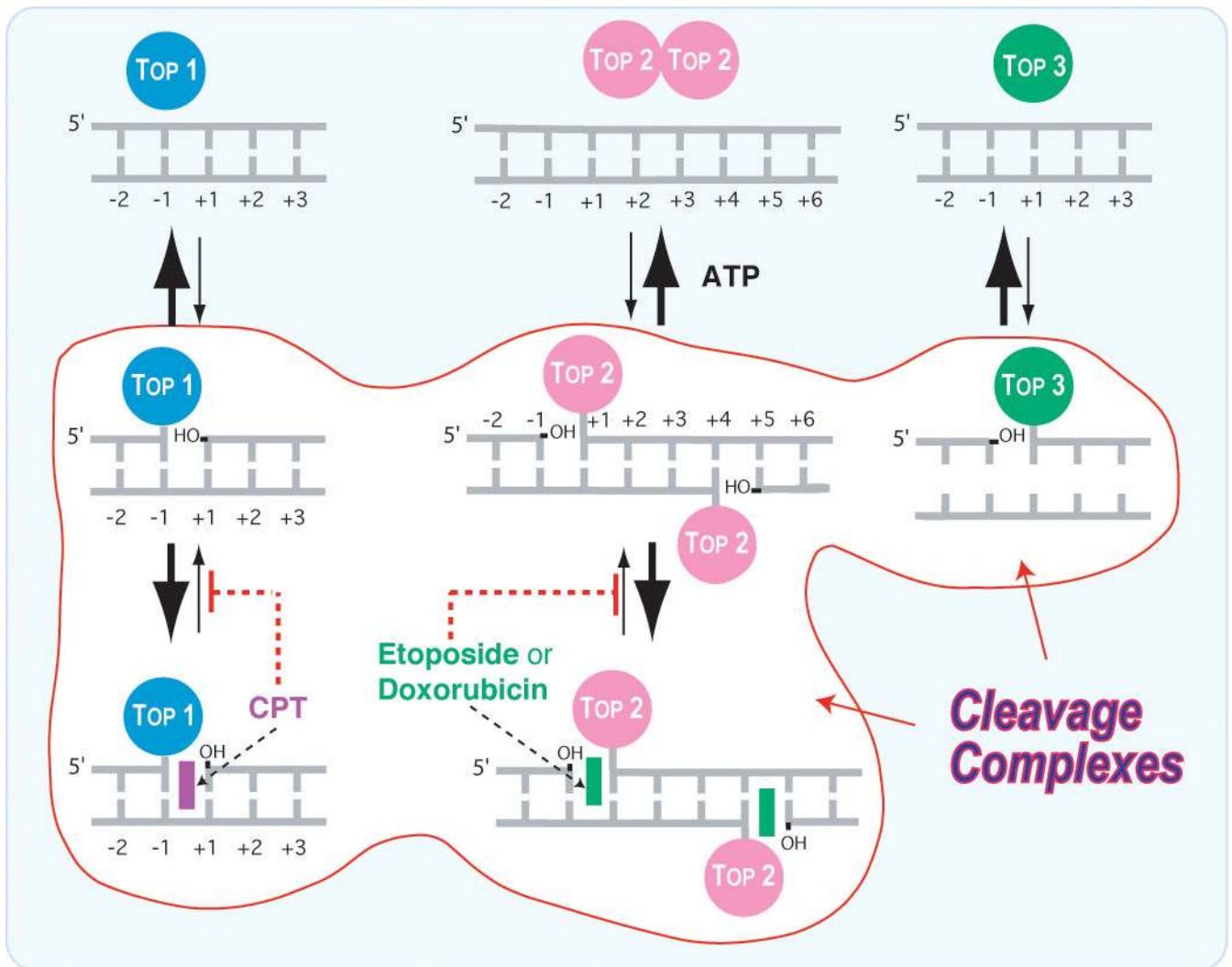


Fig. 2. Schematic architecture of the topoisomerase cleavage complexes

A. Topoisomerases I (Top1 nuclear and Top1mt) bind to double-stranded DNA and form covalent complexes at the 3'-end of the breaks. All other topoisomerases form covalent complexes at the 5'-end of the breaks. Top1 cleavage complexes are selectively stabilized by the natural alkaloid camptothecin (CPT). **B.** Topoisomerase II homodimers (Top2 α and Top2 β) bind to double-stranded DNA and form cleavage complexes with a canonical 4-base pair overhang. Top2 binds and hydrolyze ATP during catalysis. Top2 inhibitors stabilize the Top2 cleavage complexes and are potent anticancer drugs. **C.** Topoisomerases III (Top3 α and Top3 β) bind as monomers to non-canonical DNA structures (single-stranded DNA) [194] in association with a RecQ helicase (BLM in humans, Sgs-1 in budding yeast, Rhq1 in fission yeast). Top3 has been proposed to resolve double-holiday junctions arising from stalled replication forks (see Fig. 5A and corresponding text). Top3 inhibitors have not been reported.

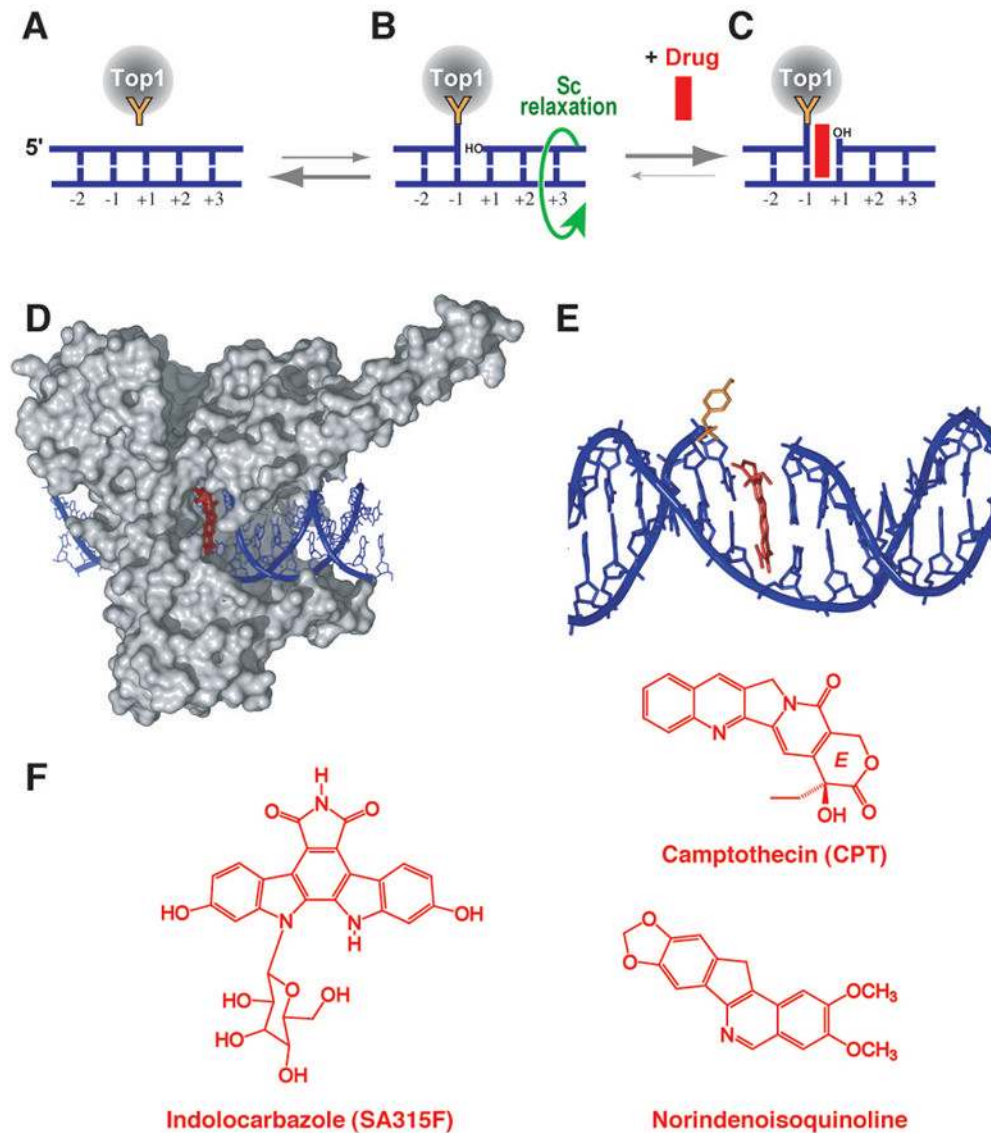


Fig. 3. Trapping of Top1 cleavage complexes by camptothecin and non-camptothecin inhibitors
A. Under physiological conditions, Top1 is associated with chromatin in non-covalent complexes. **B.** A small fraction of Top1 forms cleavage complexes that relax DNA supercoiling by controlled rotation of the cleaved strand around the intact strand (green curved arrow). **C.** Anticancer drugs such as those shown in panel F reversibly trap the Top1 cleavage complex by inhibiting religation. **D.** Crystal structure of camptothecin bound to the Top1-DNA cleavage complex [from [29]] showing “interfacial inhibition” [26,27] of the Top1 cleavage complex by camptothecin. Interfacial inhibition also applies to non-camptothecin Top1 inhibitors shown in panel F [29,30]. **E.** Same structure as in panel D. The Top1 has been removed except for the catalytic tyrosine (in orange). Camptothecin is shown intercalated between the base pairs flanking the Top1 cleavage site. **F.** Structures of three Top1 inhibitors.

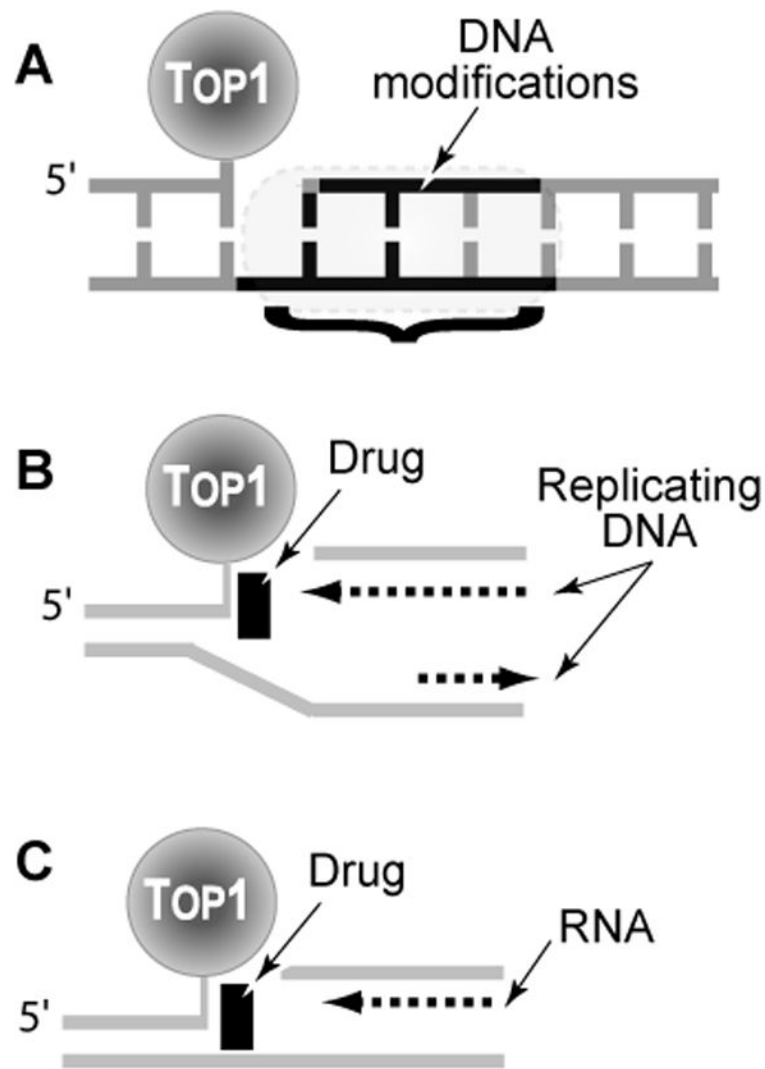


Fig. 4. Conversion of reversible Top1 cleavage complexes into DNA damage

A. Irreversible (“suicide”) Top1 cleavage complexes are produced when Top1 cleaves previously damaged DNA (DNA modifications that trap Top1 are detailed in Table 1). **B.** Top1 cleavage complexes can be converted to irreversible complexes upon replication fork collision when the Top1 cleavage complex is on the leading strand for DNA synthesis. The drug is shown as the initiating event for the collision. However, once the replication double-strand break (Rep-DSB) is formed, dissociation of the drug has no impact on the irreversible covalent complex. **C.** Conversion of Top1 cleavage complexes into irreversible covalent complexes by transcription complexes.

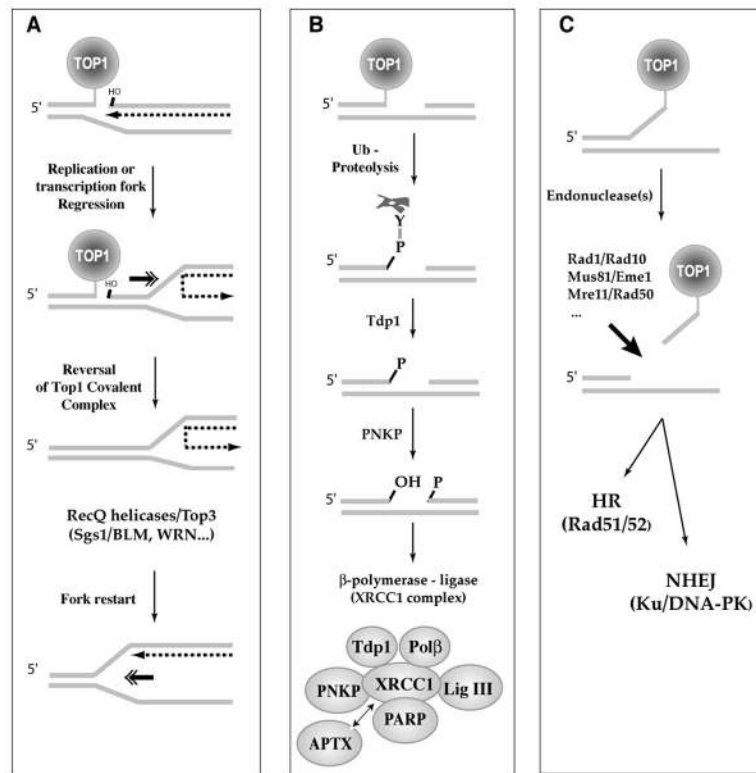


Fig. 5. Schematic representation of three main pathways for the repair of Top1 covalent complexes
A. 5'-end-religation requires realignment of the 5'-hydroxyl with the end of the broken DNA bonded to Top1. This would require a “pull-back” (“regression”) of the replication or transcription complexes (see Figs. 4B and 4C). Fork regression and restart require helicase activities (in particular RecQ helicases such as Sgs-1 in budding yeast and BLM or WRN in humans) in association with Top3. **B.** Top1 excision by Tdp1 requires prior proteolysis of Top1 [106] or denaturation of Top1 [105] to expose the phosphotyrosyl bond to be attacked (see Fig. 6A–C). Tdp1 generates a 3'-phosphate DNA end, which needs to be hydrolyzed by polynucleotide kinase phosphatase (PNKP = hPNK). PNKP also catalyzes the phosphorylation of the 5'-end of the DNA. Tdp1 and PNKP are part of the XRCC1 complex (shown at the bottom). **C.** Excision of the Top1-DNA covalent complex by 3'-endonucleases. Studies in budding yeast have implicated at least 3 endonuclease families. The human orthologs are listed: Rad1/Rad10, Mus81/Eme1 and Mre11/Rad50. The resulting DNA lesion is probably processed by homologous recombination initiated by the Rad51, Rad52 complexes and by non-homologous end joining (Ku-DNA-PK pathway).

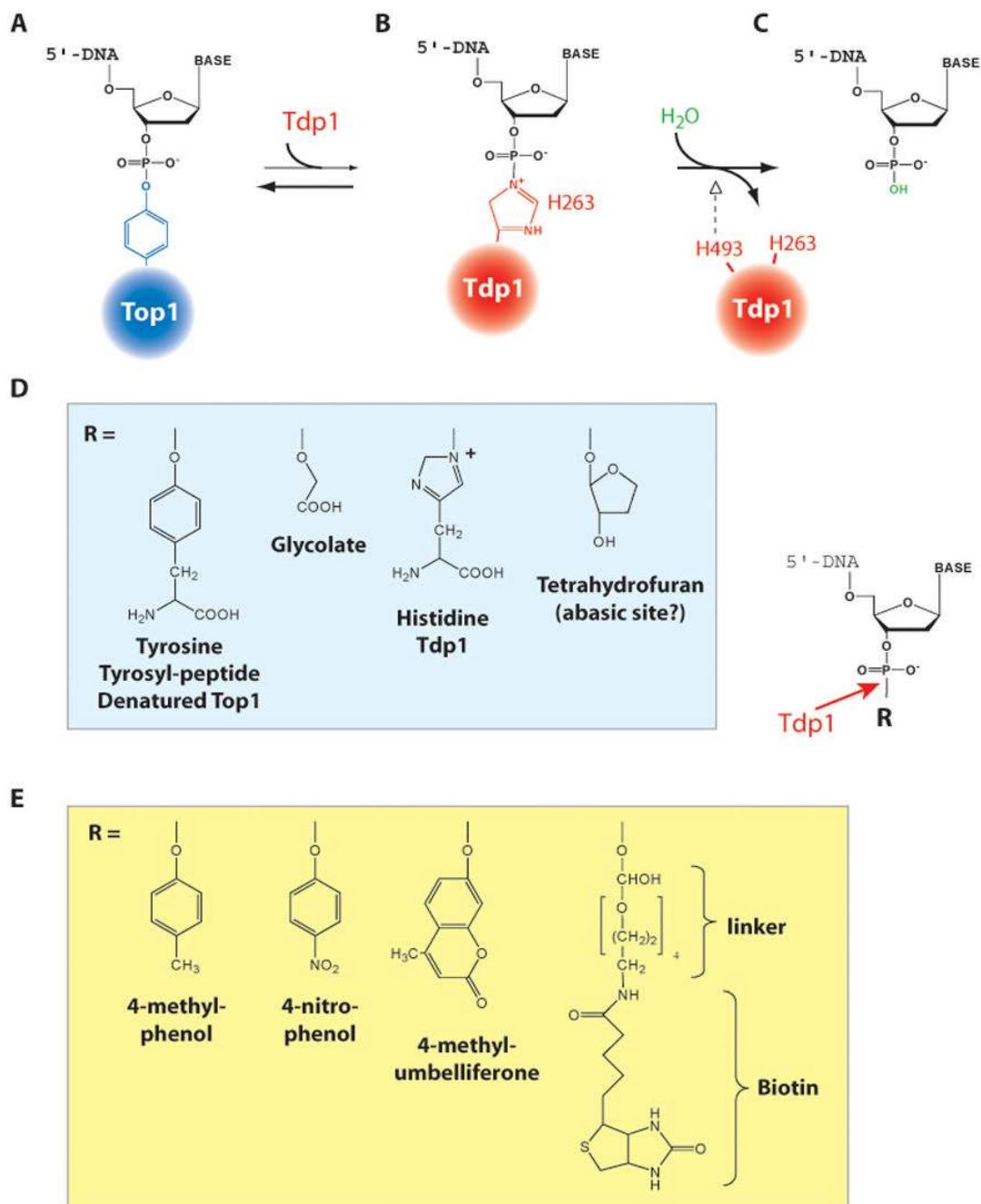


Fig. 6. Tyrosyl-DNA phosphodiesterase (Tdp1)-mediated reactions and substrates

A. Structure of the Top1-DNA covalent intermediate (see Fig. 1B). **B.** Tdp1 releases the Top1 by forming a covalent bond between its active histidine 263 and the DNA end. **C.** Histidine 493 from the second HKD motif of Tdp1 (and which is mutated H493R in SCAN1) promotes the hydrolysis of the Tdp1-DNA intermediate, and frees the 3'-phosphate DNA end. Tdp1 is regenerated for another catalytic cycle. **D.** Physiological substrates for Tdp1. **E.** Tdp1 substrates used for biochemical assays.

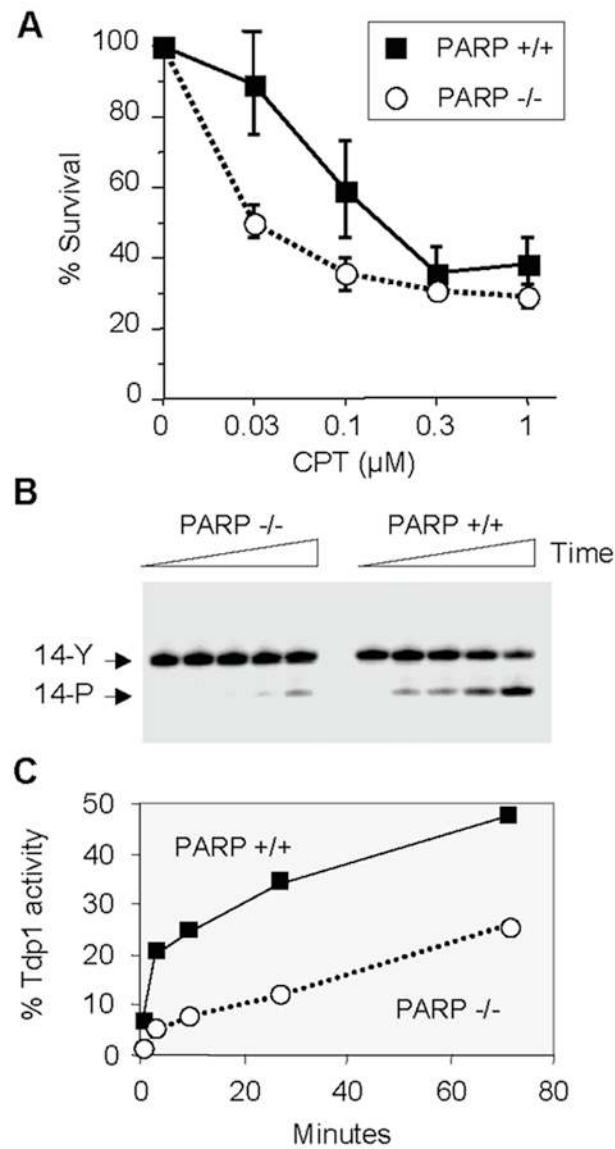


Fig. 7. Hypersensitivity of PARP-1^{-/-} cells to camptothecin and functional Tdp1 deficiency in PARP-1^{-/-} cells

A. Mouse fibroblasts were exposed to camptothecin (concentrations indicated on X axis) for 1 hour, washed, kept in culture for 4 days and counted. The curves represent the averages and standard deviations from five independent experiments. **B.** A 14-mer single-stranded oligonucleotide with a phosphotyrosine at the 3'-end (see Fig. 6) (14-Y) [106] was incubated with nuclear extracts obtained from PARP-1^{-/-} and PARP-1^{+/+} cells [195] in the presence of 50 mM EDTA and absence of MgCl₂ to eliminate PNKP activity, which requires MgCl₂. A representative experiment is shown. TDP1 activity was determined as a shift in band position (from 14-Y to 14-P). **C.** Quantitation of the results shown in panel B using ImageQuant (Molecular Dynamics, Sunnyvale, CA).

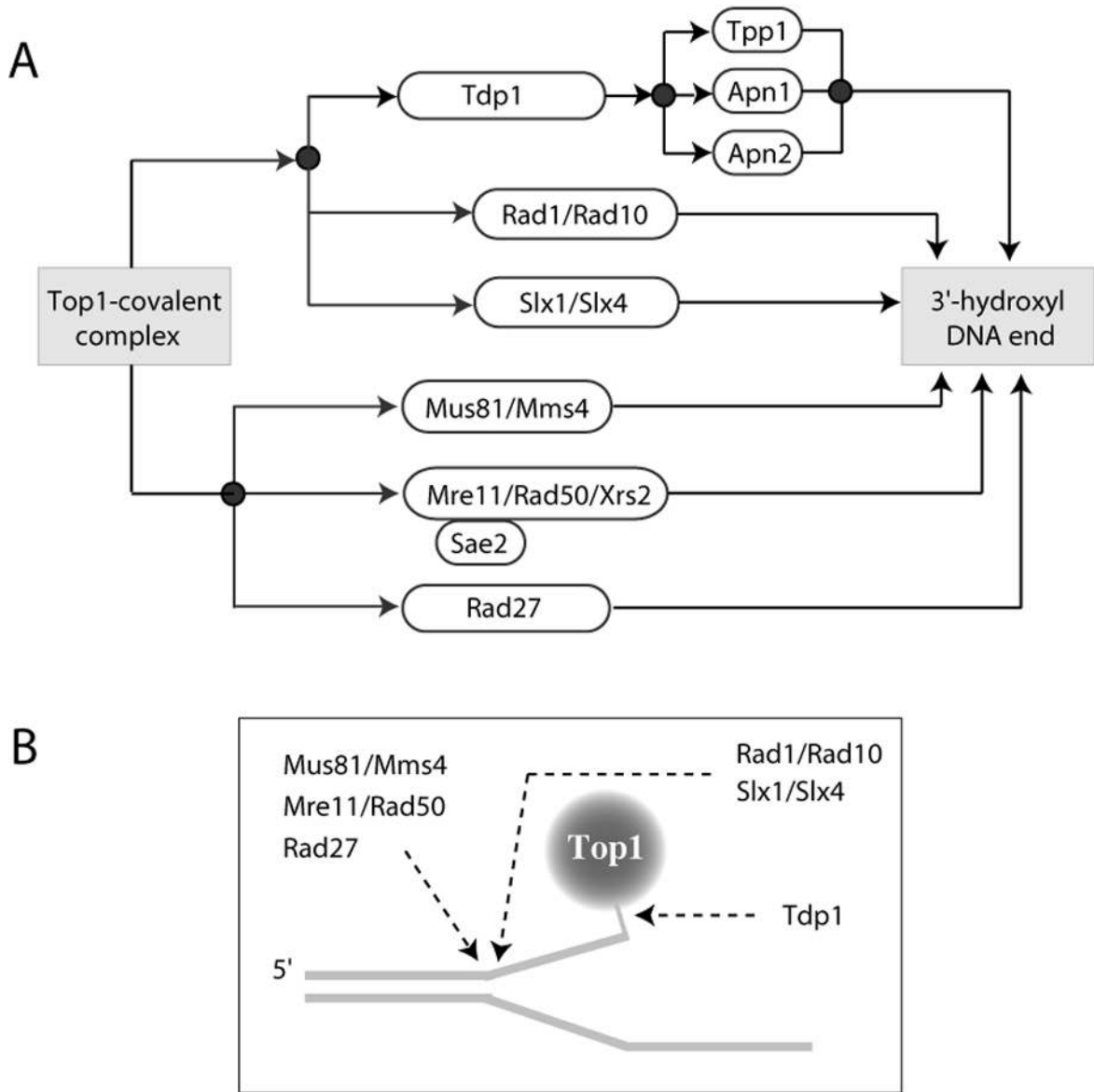


Fig. 8. Repair involved in the repair of Top1 covalent complexes in budding yeast

A. Schematic representation of the genetic pathways implicated in the removal of the Top1-DNA covalent complexes. Tdp1 appear to function in alternative pathways with Rad1/Rad10 and Slx1/Slx4. The other endonucleases (Mus81/Mms4; Mre11/Rad50/Xrs2 and Rad27) appear to function in parallel. Sae2 is a cofactor for the endonuclease activity of Mre11. Tpp1, Apn1 and Apn2 are 3'-phosphatases that remove the 3'-phosphate left after Tdp1 hydrolyzes the tyrosyl-DNA adduct (see Figs. 5B and 6C). **B.** Schematic representation of the sites of attack for Tdp1 and the endonucleases described in panel A. Srs2 helicase is also shown.

Table 1

Exogenous and endogenous factors producing Top1 cleavage complexes

Drugs ^a	Mechanism ^b	Rev ^c	Notes	Refs.
Camptothecins	T	r	Derived from the natural alkaloid	[196]
Indenoisoquinolines	T	r	Synthetic; in preclinical development	[32]
Indolocarbazoles (NB-506)	T	r	Semi-synthetic; in clinical development	[32]
Actinomycin D	T	r	Other effects: DNA, RNA polymerase	[196]
Hoechst minor groove	T	r	Other effects: DNA	[32]
Ecteinascidin 743 (Yondelis ^R)	T	r	Other effects: traps TC-NER complex	[32]
Cytosine Arabinoside	T	r	Other effects: blocks DNA synthesis	[197,198]
Gemcitabine	T	r	Other effects: blocks DNA synthesis	[199]
Endogenous DNA lesions				[37,44]
Single base mismatches	T	r	Polymerase & mismatch defects	[37,40]
Mismatched loops	T	ir	Mismatch deficiencies	[40]
Abasic sites	T	ir	AP sites; base excision repair	[40]
8-oxoguanosine	B	r	Free radicals	[21]
5-hydroxycytosine	?	r	Free radicals	[21]
Single-strand breaks	T	ir	Free radicals; base excision repair	[41]
Cytosine methylation	F+T	r	Physiological	[200]
Triple helix formation	F+T	r	?	[201]
Apoptotic chromatin fragmentation	B+T	ir	Appears ubiquitous during apoptosis	[42-45]
Exogenous DNA lesions				[37]
UV lesions	?	?	Dimers & 6,4-photoproducts	[202,203]
IR-induced DNA breaks	T	ir	Both single- & double-strand breaks	[41]
O ⁶ -methylguanine	T	r	Produced by alkylating drugs (MNNG)	[204]
O ⁶ -dA-benzo[a]pyrene adducts	T	r	Intercalated carcinogenic adducts	[205]
N ² -dG-benzo[a]pyrene adducts	F	ir	Minor groove carcinogenic adducts	[206,207]
N ² -dG-benzo[c]phenanthrene adducts	T	r	Intercalated carcinogenic adducts	[207]
N ⁶ -Ethenoadenine	T	r	Carcinogenic vinyl adduct	[208]
N ² -dG-ethyl adducts	T	r	Produced by acetaldehyde (alcohol)	[209]

^aFor detailed review on non-camptothecin inhibitors see [32].

^bMechanism for Top1 cleavage complex production: T: Trapping of the Top1 cleavage complexes (i.e.: inhibition of religation) (see Fig. 3B); B: enhancement of binding; F: enhancement of the forward (cleavage) reaction.

^cReversibility of the Top1 cleavage complexes: r: reversible; ir: irreversible.

Table 2
Genetic Alterations sensitizing mammalian cells to Top1 poisons

Genes	Functions	Refs.
APTX	Mutated in AOA1; Encodes aprataxin, which associate XRCC1 (Fig. 5B)	[210]
ATM ^a	Mutated in AT; Protein kinase from the PI3K family implicated in DSB response	[211–214]
ATR	Protein kinase from the PI3K family; Implicated in replication stress	[163]
BCL-2	Mutated in B-cell lymphoma; Apoptosis	[215]
BLM	Mutated in BS; Helicase from the RecQ family involved in genomic stability	[79,82]
BRCA1	Mutated in familial breast cancers; DNA damage response; TC-NER	[216]
BRCA2	Mutated in familial breast cancers; Rad51 loading; Homologous recombination	[217]
CSA/CSB	Mutated in CS; TCR/BER	[76]
Chk1	Checkpoint kinase phosphorylated/activated by PI3K (ATR)	[162,165]
Chk2	Checkpoint kinase phosphorylated/activated by PI3K (ATM etc...)	[162,218]
DNA-PKcs	Protein kinase from the PI3K family; Implicated in DSB response	[56,219,220]
FEN-1	Flap and gap endonuclease; processing of stalled replication forks	[133]
H2AX	Core histone; phosphorylated in response to DSB (γ -H2AX foci)	[53]
NBS1	Mutated in NBS; Scaffolding protein forming a complex with Mre11 and Rad50 (MRN complex); DSB repair and recombination pathways	[212,221]
PARP	BER (see Fig. 7)	[118,222]
PNKP/hPNK	Processing of DNA ends: 3'-DNA-phosphatase + 5'-DNA-kinase	[115]
Rad51C	One of the five Rad51 paralogs; Implicated in DNA strand exchange/homologous recombination	[223]
TDP1	Mutated in SCAN; Hydrolysis of 3'-phosphodiester (phosphotyrosyl and phosphoglycolate) and phosphamides (Tdp1 cleavage complex)	[93,94,224]
TP53	Mutated in Li-Fraumeni syndrome; encodes p53; Checkpoints; apoptosis	[225,226]
WRN	Mutated in Werner syndrome; RecQ helicase involved in genomic stability	[227–229]
XRCC1	BER; binds to Tdp1, PARP, β -polymerase, ligase III and aprataxin	[116,117,119,230–233]
XRCC2	One of the five Rad51 paralogs: Rad51B, Rad51C, Rad51D, XRCC2 & XRCC3; Implicated in DNA strand exchange/homologous recombination	[219,230,234]
XRCC3	One of the five Rad51 paralogs; Implicated in DNA strand exchange/homologous recombination	[167,219]

^aThe contribution of ATM has not been found consistently: ATM-siRNA cells are not hypersensitive to camptothecin [162] and we found that AT-complemented cells are not hypersensitive to camptothecin (our unpublished results).

Genes are in alphabetic order.

Abbreviations: AOA1: Ataxia-oculomotor apraxia 1; ATM: Ataxia Telangiectasia Mutant; ATR: Ataxia Telangiectasia and Rad3-related; BER: Base Excision Repair; BLM: Bloom syndrome (BS); CSA/CSB: Cockayne Syndrome (CS) complementation groups A and B; DNA-PKcs: DNA-dependent protein kinase catalytic subunit; DSB: DNA double-strand breaks; NBS: Nijmegen Breakage Syndrome; NER: nucleotide excision repair; PARP: poly (ADP-ribose) polymerase; PI3K: phosphatidylinositol 3 kinase; PNKP: polynucleotide kinase phosphatase; SCAN: Spino Cerebellar Ataxia Axonal Neuropathy; TCR: transcription-coupled repair; WRN: Werner syndrome.

Table 3
Genetic alterations conferring hypersensitivity to Top1 poisoning in budding yeast:

Yeast <i>Saccharomyces Cerevisiae</i>				Humans		
Gene	Effect	Refs.	Function	Gene	Effect	Refs.
RAD52/51 homologous recombination (HR)						
RAD52 ^a	HS	[8,98,99,131,235–237]	Strand annealing	RAD52	?	
RAD51	HS	[98,99,131,236]	RecA homolog: strand invasion	RAD51C	HS	[223]
RAD55	HS	[98,99,131]	Strand annealing, exchange	XRCC2	HS	[219,230,234]
RAD57	HS	[98,99,131]	Strand annealing, exchange	XRCC3	HS	[219]
RAD54	HS	[98,99]	ATPase			
MMS1	S	[99,238]	Replication repair/epistatic Rad52			
RAD59	S	[98,99]	Rad52-related recombination			
MRX (MRN) 3'-nuclease/checkpoint (HR + NHEJ)						
SAE2	HS	[99]	Activates Mre11 endonuclease; meiotic and mitotic recombination			
MRE11	HS	[97–99,237]	MRX/N complex; endonuclease	MRE11	?	
RAD50	HS	[99,131,236]	MRX/N complex; scaffold	RAD50	?	
XRS2	HS	[131]	MRX/N complex; signaling	NBS1	HS	[212,221]
Mus81/Mms4 (Mus81/Eme1) 3'-Flap Endonuclease						
MUS81	S	[98,99,126,131]	3'-flap endonuclease with Mms4	MUS81	NS	[137]
MMS4	S	[98,99,126]	Partner for Mus81 endonuclease	EME1	?	
Tdp1-PNKP 3"-end processing						
TDP1	CS ^b	[97–99]	Tyrosyl-DNA phosphodiesterase	TDP1	?	[93,94,224]
TPP1	CS ^{b,c}	[98,110]	Polynucleotide 3'-phosphatase	PNKP ^b	HS	[115]
APN1	CS ^{b,c}	[97,98,236]	AP endonuclease (endo IV family)			
APN2	CS ^{b,c}	[97,98]	AP endonuclease (exo III family)	APE1	?	
Rad1/Rad10 (XPF/ERCC1) 3"-endonuclease						
RAD1	CS ^b	[97–99,236]	3'-flap endonuclease with Rad10	XPF	NS	(our observations)
RAD10	CS ^b	[97,98]	Partner for Rad1	ERCC1	NS	
Rad27 (FEN1) 5"-endonuclease						
RAD27	MS	[98,99,133]	5'-flap endonuclease	FEN1	HS	[133]
SLX4/SLX1	MS	[99]	Endonuclease with broad range of activities against various substrates	?		
Mismatch repair						
MMS1	S	[99]	Mismatch repair			
RecQ/Top3 helicases/topoisomerase						
SGS1	MS	[126,131]	Top3-associated helicase	WRN; BLM	HS	[82,227–229]
SRS2	S	[98,99]	Rad51-associated helicase		?	
TOP3	S	[98,126]	Replication/recombination topoisomerase	TOP3 α TOP3 β	?	
9-1-1("PCNA-like") Clamp						
DDC1	MS	[98]	Replication/Repair Clamp; "9-1-1"	RAD9	?	
RAD17	MS	[131,236,239]	Replication/Repair Clamp; "9-1-1"	RAD1	?	
MEC3	MS	[131]	Replication/Repair Clamp; "9-1-1"	HUS1	?	
RAD24	MS	[237]	Clamp loader for 9-1-1	RAD17	?	
Replication						
CDC45	S	[134]	Initiation of DNA replication	CDC45L	?	
POL32	MS	[131]	Small subunit for Pol δ	TEX14	?	
TRF4	S	[240]	DNA polymerase	POLS	?	
DPB11	S	[134]	Replication initiation/checkpoint	TOPBP1	?	
RAD6	MS	[131,236,241]	PRR ^d ; Ub conjug	RAD6A, B	?	
RAD18	S	[131,236]	PRR ^d ; loads Rad6	RAD18	?	
SLX4	MS	[99]	DNA replication	?		
CLB5	S	[99]	B type cyclin	Cyclin B	?	
Dec1	S	[242]	Sister chromatid cohesion	?		
Transcription						
HPR1	S	[131]	Transcription & recombination	MGC5350	?	
SFP1	S	[131]	Transcription factor	REQ	?	
CCR4	S	[131]	Transcription	KIAA1194	?	
BUR2	S	[131]	Cyclin partner for Bur1	Cyclin H	?	
RPB9	S	[131]	RNA polymerase subunit	POLR21	?	

<i>Yeast Saccharomyces Cerevisiae</i>				Humans		
Gene	Effect	Refs.	Function	Gene	Effect	Refs.
HTZ1	S	[99]	Regulation of transcription from Pol II promoter			
SPT21	S	[99]	Regulation of transcription from Pol II promoter			
PAT1	S	[99]	Controls mRNA decay			
LSM1	S	[99]	Controls mRNA decay			
HMO1	S	[99]	Involved in rDNA transcription			
MPH1	MS	[131,241]	RNA helicase	MPH1	?	
Sensor PI3K-related protein kinases						
MEC1	HS	[236,237]	PI3LK checkpoint sensor kinase	ATR	HS	[163]
DDC2	?		Partner for MEC1	ATRIP	?	
TEL1	S	[237]	PI3LK checkpoint sensor kinase	ATM	HS	[211–214]
			PI3LK checkpoint sensor kinase	DNA-PK	HS	[56,219]
Transducer protein kinases; BRCT proteins						
RAD53	MS	[236]	Checkpoint effector kinase	CHK2	S	[162,218]
RAD9	MS	[236,237]	Adaptor for checkpoint kinases	MDC1	?	
				BRCA1	HS	[216]
Chromatin						
HTA1/2	S	[237]	Histone H2A	H2AX	S	[53]
HHF1/2	S	[243]	Histone H4	H4	?	
GCN5	S	[244]	Histone H3 acetyltransferase	PCAF	?	
YNG2	S	[244]	Histone H4 acetyltransferase	ING1-5	?	
ESA1	S	[243]	Histone H4 acetyltransferase	MYST1/HAT	?	
ASF1	S	[98,245]	Chromatin assembly	ASF1B	?	
MCD1	S	[240]	Chromatin cohesion	RAD21	?	
CTF4	MS	[98]	Chromatid cohesion & segregation	AND-1	?	
TOF1	S	[99,242]	Chromatid cohesion	TIM-1	?	
CSM3	S	[99,242]	Meiotic chromosome segregation	TIPIN	?	
FUN30	S	[99]	DNA-dependent ATPase; chromosome stability			
Ubiquitin						
UBC9	S	[246]	Ubiquitin ligase	UBE2I	?	
DOA4	S	[246]	Ubiquitin hydrolase			
UBC4	S	[99]	Ubiquitin conjugation; stress response			
RTT101	S	[99]	Ubiquitin ligase; chromosome stability			
ULA1	S	[99]	RUB1-protein conjugation			
Others						
VAC14	S	[99]	Vacuole inheritance			
NUP60	S	[99]	Nucleocytoplasmic transport			
PPH3	S	[99]	Serine/threonine phosphatase			
PSY2	S	[99]	Unknown			
ILM1	S	[99]	Unknown			
CLB5	S	[99]	B type cyclin in G1/S transition			

Abbreviations for effects: HS, S, MS, and CS correspond to hypersensitivity, sensitivity, moderate sensitivity, and conditional sensitivity to camptothecin, respectively. NS: no hypersensitivity.

^aThe Rad52 epistasis group includes the RAD 50, 51, 52, 54, 55, 57, 59, MRE11 and XRS2 genes.

^bTdp1 deficiency results in HS only in the presence of Rad1/Rad10 deficiency [97,98]; conversely Rad1 deficiency does not confer hypersensitivity to CPT [126] unless the Tdp1-Apn1 pathway is defective [97]. Tpp1, Apn1+Apn2+Tpp1 need to be inactivated to confer full camptothecin hypersensitivity [111]; see Fig. 3A.

^cPNKP possesses both 3'-phosphatase and 5'-kinase activities, whereas the yeast ortholog, Tpp1 only possesses 3'-phosphatase activity. Neither Apn1, Apn2 or Tpp1 possess AP endonuclease activity) [111].

^dPRR: post-replication repair. Deficiency of Rhp6 or Rhp18 (YSP orthologs of Rad6 and Rad18) does not confer CPT hypersensitivity [247].

Table 4

Genetic alterations conferring hypersensitivity to Top1 poisoning in fission yeast:

Yeast <i>Saccharomyces Pombe</i>				Humans		
Gene	Effect	Refs.	Function	Gene	Effect	Refs.
Rhp54	HS	[129]	Homologous recombination (HR)	RAD52	?	
Rhp55	LS	[129]	Homologous recombination (HR)	XRCC2	HS	[219,230,234]
Rhp22A	LS	[129]	Homologous recombination (HR)	XRCC3	HS	[219]
Rhp51	S	[129]	RecA homolog; Rad52 epistasis G.	RAD51C	HS	[223]
rad22	HS	[248]	Rec A homolog; functions with Mus81			
Rad50	HS	[129]	MRX/N complex; scaffold	RAD50	?	
Mus81	S	[129]	3'-flap endonuclease with Eme1; meiotic recombination	MUS81	NS	[137]
Eme1	S	[129]	Partner for mus81 nuclease	MUS81	?	
RusA	RS ^a	[129]	HJ resolvase			
Pnk1	S ^b	[112]	Polynucleotide kinase phosphatase	PNKP	HS	[115]
Rqh1	MS	[98]	Top3-associated helicase	WRN BLM	HS HS	[227-229] [82]
Crb2	S	[249]	Adaptor for checkpoint kinases and checkpoint proteins	53BP1 BRCA1	? HS	 [216]
Chk1	S	[155,249,250]	Checkpoint effector kinase	CHK1	S	[162,165]
Swi1	HS	[251]	Mating-type switching	TIMELESS		

Abbreviations for effect: HS, S, MS, and CS correspond to hypersensitivity, sensitivity, moderate sensitivity, and conditional sensitivity to camptothecin, respectively. NS: no hypersensitivity.

^a *rusA* suppresses hypersensitivity of *Mus81/Eme1*⁻ but does not reverse sensitivity of *rqh1*⁻; *rusA* also suppresses the lethality of double mutants for *Mus81/Eme1 + rqh1* [129]. *RusA* expressed in budding yeast partially suppresses hypersensitivity to CPT in *Mms4*-deficient cells [126].

^b *Pnk1*- cells are hypersensitive to CPT in the absence of additional defects, indicating difference from budding (see [a]) and importance of this pathway in fission yeast, which like mammals possesses a gene that has both 3'-phosphatase and 5'-kinase activity [112].