LETTERS

Antitumour drugs impede DNA uncoiling by topoisomerase I

Daniel A. Koster[1], Komaraiah Palle[2], Elisa S. M. Bot[1], Mary-Ann Bjornsti[2] & Nynke H. Dekker[1]

Increasing the ability of chemotherapeutic drugs to kill cancer cells is often hampered by a limited understanding of their mechanism of action. Camptothecins, such as topotecan, induce cell death by poisoning DNA topoisomerase I, an enzyme capable of removing DNA supercoils[1–4]. Topotecan is thought to stabilize a covalent topoisomerase–DNA complex[5–7], rendering it an obstacle to DNA replication forks[2,3,8,9]. Here we use single-molecule nanomanipulation to monitor the dynamics of human topoisomerase I in the presence of topotecan. This allowed us to detect the binding and unbinding of an individual topotecan molecule in real time and to quantify the drug-induced trapping of topoisomerase on DNA. Unexpectedly, our findings also show that topotecan significantly hinders topoisomerase-mediated DNA uncoiling, with a more pronounced effect on the removal of positive (overwound) versus negative supercoils. *In vivo* experiments in the budding yeast verified the resulting prediction that positive supercoils would accumulate during transcription and replication as a consequence of camptothecin poisoning of topoisomerase I. Positive supercoils, however, were not induced by drug treatment of cells expressing a catalytically active, camptothecin-resistant topoisomerase I mutant. This combination of single-molecule and *in vivo* data suggests a cytotoxic mechanism for camptothecins, in which the accumulation of positive supercoils ahead of the replication machinery induces potentially lethal DNA lesions.

DNA topoisomerases resolve topological problems by means of transient DNA strand breakage and religation[1,4,10]. However, drug-stabilized topoisomerase–DNA complexes may also induce potentially lethal DNA damage. Indeed, eukaryotic DNA topoisomerase IB (TopIB) is the cellular target of the camptothecin class of chemotherapeutics[2,3,11]. The camptothecin analogues topotecan and irinotecan have significant activity against adult and paediatric solid tumours, and have gained US Food and Drug Administration approval for the treatment of ovarian and small-cell lung cancer[12–14]. A detailed understanding of topoisomerase–drug interactions is critical for optimal clinical development of these chemotherapeutics. However, the dynamic interactions underlying this poisoning and their biological ramifications remain largely unknown.

TopIB removes DNA supercoils by first forming a clamp around duplex DNA[15,16]. The active-site tyrosine acts as a nucleophile to cleave a single DNA strand, forming a transient DNA–(3′-phosphotyrosyl)-enzyme 'covalent complex' and a free 5′-OH DNA end. Torsional energy within the DNA then drives uncoiling about the intact DNA strand. Following the removal of a random number of supercoils, a ligation reaction restores the DNA backbone[17,18]. Topotecan intercalates into the nick generated by TopIB, thereby preventing religation and trapping TopIB on the DNA[5–7,19]. During S phase, these reversible ternary topotecan–TopIB–DNA complexes are converted into cytotoxic DNA lesions that cause cell death[2,3,8,9]. Because DNA replication is required for topotecan-induced cell lethality[8,9], it has been proposed

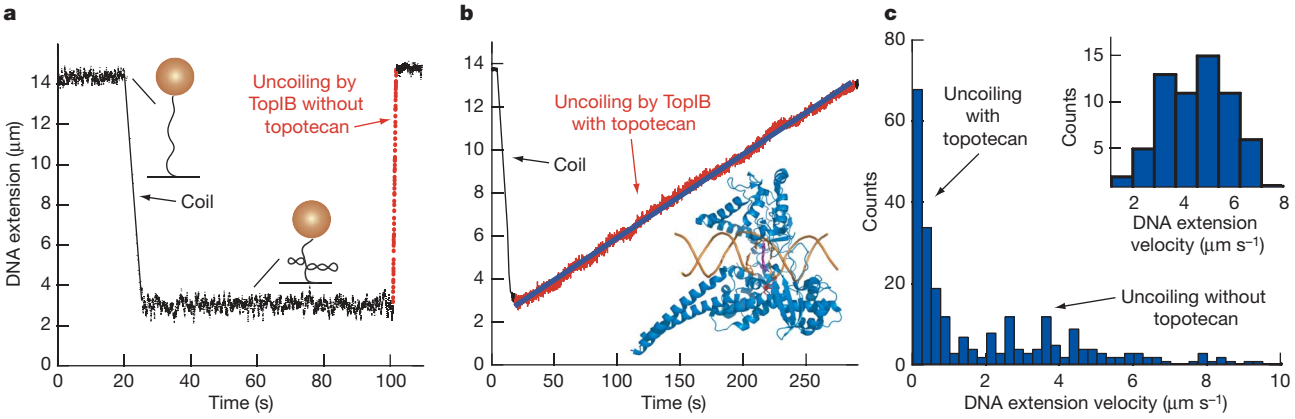

Figure 1 | Drug-dependent slow DNA uncoiling by human TopIB. **a**, In the absence of topotecan, uncoiling (red dots, at 100 s) proceeds rapidly. **b**, In the presence of topotecan, slow and continuous uncoiling of positive supercoils is observed. The uncoiling rate is constant, as expected in the constant torque regime (Supplementary Information IV). Inset, ribbon diagram of the carboxy terminal 70 kDa of human TopIB (blue) covalently linked to DNA (yellow) in the presence of topotecan (magenta). **c**, The distribution of uncoiling velocities in the presence of 5 μM topotecan ($n = 275$) and $F = 0.2$ pN shows contributions from topotecan-mediated slow uncoiling and topotecan-independent uncoiling (inset, measurement in the absence of topotecan ($n = 64$)). Topotecan-mediated uncoiling of positive supercoils proceeds ~20-fold more slowly than topotecan-independent uncoiling.

[1]Kavli Institute of Nanoscience, Faculty of Applied Sciences, Delft University of Technology, Lorentzweg 1, 2628 CJ Delft, The Netherlands. [2]Department of Molecular Pharmacology, St Jude Children's Research Hospital, 332 N. Lauderdale, Memphis, Tennessee 38105, USA.

that these lesions occur as a result of the covalent complex 'colliding' with an advancing replication fork[2,3]. Here we describe single-molecule experiments that test key predictions of the proposed mechanism of poisoning of TopIB, and reveal dynamic interactions between topotecan and TopIB. The consequences of these interactions are also observed in living cells, suggesting an alternative mechanism for cell death.

To examine the consequences of camptothecin binding on TopIB swivel function, we explored its real-time dynamics in the presence of topotecan in the context of a single molecule. Our experimental configuration, the magnetic tweezers, is described elsewhere[20] (Supplementary Information I). In a typical experiment the DNA is mechanically coiled in the presence of TopIB, introducing multiple plectonemes that reduce the DNA extension (Fig. 1a). We usually observe a plateau (for example, from ~25 to 100 s in Fig. 1a), which is indicative of DNA in a supercoiled and unnicked state. In the presence of human TopIB (encoded by the *TOP1* gene) but the absence of topotecan, the DNA is subsequently (at 100 s, shown in red) rapidly uncoiled by the enzyme. Plectoneme removal occurs either in a single enzymatic event or in multiple steps (Supplementary Information II). Surprisingly, on addition of topotecan, a very different signature is observed (Fig. 1b). First, topotecan-mediated uncoiling occurs slowly (red points) compared with uncoiling in the absence of drug. Slow uncoiling is observed immediately following mechanical coiling; this proceeds in a fashion that seems to be continuous, and can be fitted by a linear relation (blue line). Second, slow uncoiling continues for long periods of time. Finally, during the slow uncoiling, no plateaux are apparent, suggesting that religation does not take place (Supplementary Information III). Structural studies revealed that the ring-like structure of topotecan intercalates into a TopIB-generated nick and is stabilized by hydrogen bonds to TopIB and base-stacking interactions with adjacent bases[7,19]. Consistent with biochemical assays of camptothecin-induced covalent TopIB–DNA complexes[5,21], structural studies also show that topotecan locally deforms the DNA duplex and displaces the 5′-OH, presumably decreasing the probability of religation. It was also speculated that contacts of topotecan with TopIB and DNA ought to significantly hinder rotational motion[7,16,22]—a prediction borne out by our observations.

To quantify the reduction in uncoiling rate caused by topotecan binding, we examined the complete distribution of DNA extension velocities during enzymatic uncoiling (Fig. 1c). In the presence of topotecan, we identified two populations: one topotecan-mediated and one non-topotecan-mediated. The fraction of topotecan-mediated events increases with drug concentration (data not shown), but, even at the highest concentrations, a significant fraction of events remains non-topotecan-mediated. These findings are consistent with biochemical studies demonstrating that topotecan stabilizes a subset of TopIB–DNA complexes[2,3]. Non-topotecan-mediated events, unambiguously identified by experiments in the absence of topotecan (Fig. 1c, inset), take place, on average, at 4.1 μm s^{-1}. Topotecan-mediated events, however, take place, on average, at 0.2 μm s^{-1} (corresponding to a plectoneme relaxation rate of ~3 Hz; Supplementary Information IV), independent of drug concentration. We conclude that topotecan-mediated uncoiling by TopIB occurs roughly 20-fold slower than uncoiling by TopIB alone.

Control experiments were performed to exclude the possibility that topotecan binding to DNA, or non-specific TopIB–topotecan interactions could result in such slow uncoiling. First, topotecan binding did not affect the mechanical properties of DNA (Supplementary Information V). Second, slow uncoiling was not specific to topotecan, occurring also with camptothecin. However, it did not occur with Top1(Y723F), a catalytically inactive human TopIB

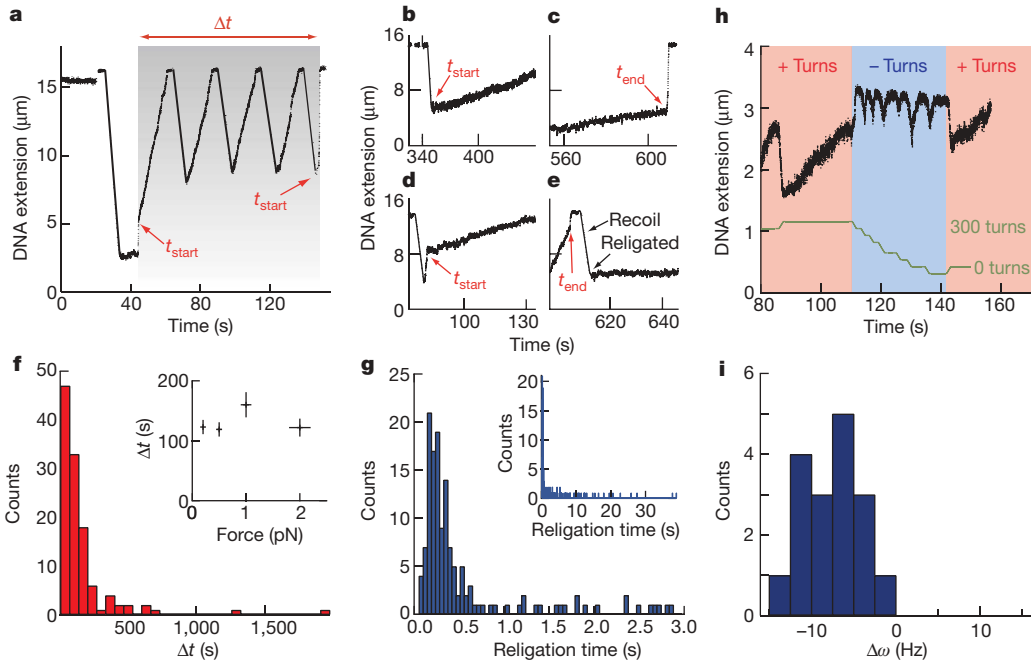

Figure 2 | The duration of slow removal and its uniqueness to the removal of positive supercoils. a, The duration Δt for a bound topotecan (grey area) is defined as the time period between t_{start} and t_{end} during which slow and constant uncoiling is observed. **b**, **c**, Typical illustration (70% of events, $n = 660$) of t_{start} (**b**) and t_{end} (**c**) events. **d**, t_{start} event in which nicking appears directly before the onset of slow uncoiling. **e**, Event suggestive of a topotecan molecule exiting at t_{end}, after which religation occurs (plateau onset at 610 s). **f**, Histogram of Δt for $F = 0.5$ pN, with mean of 121 ± 11 s ($n = 146$), which is not significantly force-dependent (inset). Means are numerical averages with the corresponding s.e.m. **g**, Distribution of TopIB religation times, with a most probable religation time of 0.3 s ($n = 132$) and a slowly decaying tail (inset), giving an average time of 4 s, far below Δt (121 s, as shown in **f**). **h**, The removal rate of positive (pink areas) and negative (blue areas) supercoils in the presence of topotecan. Removal of positive supercoils proceeds more slowly. Following the removal of negative supercoils, continued slow removal of positive supercoils is verified to ascertain that the topotecan molecule remained bound in the interim. The rotational position of the magnets, specifying the absolute number of turns that are mechanically injected into the DNA, is indicated in green. **i**, Histogram of the difference in uncoiling velocity, $\Delta\omega$, between positive and negative supercoils, in the presence of topotecan.

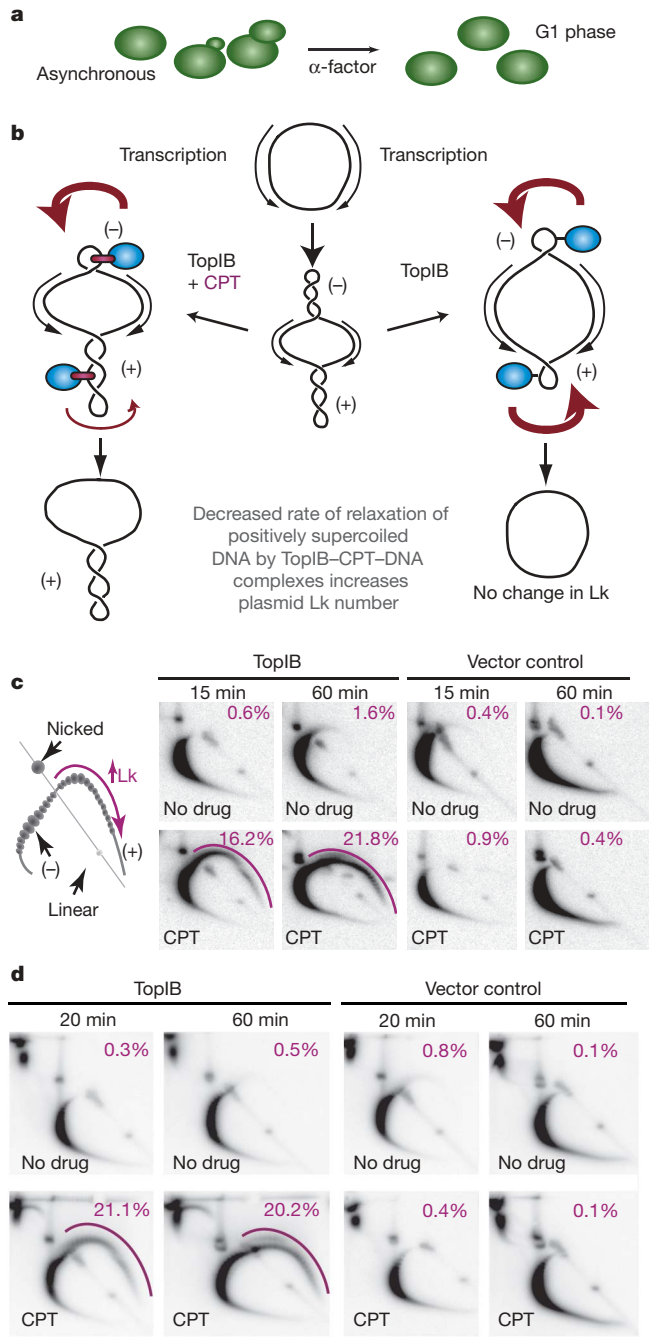

Figure 3 | Camptothecin poisoning of TopIB induces accumulation of positively supercoiled DNA in G1- and S-phase yeast cells. a, To eliminate the potential contribution of positive supercoils induced by replication forks, asynchronously growing haploid yeast cells are arrested in G1 phase with α-factor. **b**, Positive supercoils are generated ahead of advancing transcription bubbles, and compensatory negative supercoils are generated in their wake. Diverging transcription units bisect a plasmid into twin domains of positive and negative supercoils. If, in the presence of drug (left), positive supercoils are removed more slowly than negative supercoils, the plasmid should display an excess of positive supercoils. In the absence of drug (right), no asymmetry between the removal rates of positive supercoils and negative supercoils is observed (Supplementary Information VII) and no net change in linking number (Lk) is expected. **c**, Two-dimensional agarose gel analysis of the Lk distribution of 2 μm plasmid topoisomers isolated from G1-phase *top1*Δ, wild-type *TOP2* yeast cells, expressing plasmid-encoded human TopIB (TopIB) or vector control, and treated with camptothecin (CPT) or no drug for 15 or 60 min. Topoisomer resolution in two-dimensional agarose gels is discussed in Supplementary Information VI. The shift in topoisomer distribution is quantified by measuring the signal intensity of the portion of the arc to the right of the diagonal linear DNA (purple arc) relative to the amount of label detected across the entire arc (purple numbers). **d**, Topoisomer distribution of 2 μm plasmid in *top1*Δ, wild-type *TOP2* yeast cells released into S phase. Here, too, accumulation of positive supercoils is observed only when cells express TopIB and are treated with camptothecin.

protein, with vaccinia TopIB (which is insensitive to topotecan) or with a nick induced by a nicking enzyme (Supplementary Information V). As expected, these experiments show that catalytically active human TopIB is required to yield slow uncoiling, consistent with the *in vivo* activity and co-crystal structures of camptothecin analogues[7,19,23]. We thus conclude that slow uncoiling represents a clear signature for a topotecan molecule bound to the TopIB–DNA complex.

Highly relevant for any consideration of the 'collision' model is the lifetime of the covalent complex. Consequently, we asked over what timescale topotecan remains bound, and thus over what timescale TopIB remains covalently linked to the DNA. Figure 2a shows a time trace indicating the times at which topotecan enters (t_{start}) and exits (t_{end}) the covalent complex. The assignment of t_{start} occurs after a plateau is interrupted by a fast rise in DNA extension as a result of DNA cleavage by TopIB, followed by the appearance of slow uncoiling at t_{start}, which we attribute to a topotecan molecule entering a TopIB-linked nick. Figure 2b–e shows a collection of traces illustrating the assignment of t_{start} and t_{end}. The majority of events observed (~70%) resemble those in Fig. 2b, c. In Fig. 2b, at ~340 s, the magnets are rotated for a substantial number of turns on two occasions (the interruption of the DNA extension signal at 334 and 338 s signifies rotation), but no plectonemes are introduced. This situation corresponds to a nicked DNA resulting from the formation of the covalent complex. At t_{start} a topotecan molecule enters the covalent complex; at this stage, slow uncoiling is observed. Figure 2c shows the abrupt transition from slow to fast supercoil removal, prompting the designation of t_{end}. The bound time Δt is defined as $t_{end} - t_{start}$ and reflects the time supercoils are enzymatically removed at a constant slow rate (shaded box, Fig. 2a). Multiple mechanical recoiling events are often required to measure Δt (four shown in Fig. 2a). Figure 2f shows the distribution of Δt, which has a mean of 121 ± 11 s (n = 146) that is unchanged both within the practical force range of the technique (Fig. 2f, inset) and by a tenfold change in topotecan concentration (Supplementary Information III). The latter indicates that the lifetime is unlikely to be overestimated by topotecan unbinding/rebinding during mechanical recoiling or by the presence of occasional very short-lived plateaus (Supplementary Information III). We conclude that, in the presence of topotecan, TopIB remains trapped on the DNA for at least 121 s. This timescale is about 400 times longer than the religation time in the absence of topotecan (Supplementary Information VI), the distribution of which has a most probable value of ~0.3 s (Fig. 2g), with a tail that extends to longer times (Fig. 2g inset). This provides quantitative support for a critical aspect of the collision model, namely the topotecan-induced increase in the lifetime of the covalent complex.

We next focused on the enzymatic uncoiling rate of positive versus negative supercoils. In the absence of topotecan, no significant difference was detected (Supplementary Information VII). However, we observe a clear and unexpected difference in supercoil removal rate in the presence of topotecan. Figure 2h shows that the uncoiling of positive supercoils (pink regions) is significantly slower than the uncoiling of negative supercoils (blue region). This asymmetry in uncoiling rates is quantified in Fig. 2i as the differential in rates, denoted $\Delta\omega$ and defined as $|\omega_+| - |\omega_-|$, where $|\omega_+|$ is the uncoiling rate of positive supercoils and $|\omega_-|$ is the uncoiling rate of negative supercoils. We find a mean $\Delta\omega$ of –8 ± 5 Hz (n = 17). The relative difference, $\Delta\omega/|\omega_+|$, is –2.4 ± 2.2. The microscopic interactions responsible for the asymmetric rate of DNA uncoiling in the presence of topotecan were unforeseen by crystallographic studies, which only provide a static picture of crystalline TopIB.

To investigate the biological ramifications of the asymmetric hindrance of DNA uncoiling induced by topotecan, we asked if this bias was evident in drug-treated yeast cells. Our single-molecule observations led us to posit that positive supercoils would accumulate during cellular processes that induce DNA supercoiling, such as transcription[24,25]. Thus, we treat G1-phase-arrested *top1Δ TOP2* yeast cells (Fig. 3a) expressing low levels of plasmid-encoded human TopIB with camptothecin (Fig. 3b, left) or no drug (Fig. 3b, right). Arresting cells in G1 phase restricted the analysis to transcription-induced DNA supercoiling, and camptothecin was used to avoid drug transporter efflux of topotecan[11]. Two-dimensional gel electrophoresis (Supplementary Information VIII) resolves the distribution of purified 2 μm plasmid DNA topoisomers (Fig. 3c). In the absence of drug (Fig. 3c, upper row), an expected bias in the plasmid topoisomer distribution towards negative supercoils is observed, caused by DNA wrapping around the histone core in nucleosomes. However, camptothecin treatment of TopIB-expressing cells in G1 phase induces a remarkable skewing of the plasmid topoisomer distribution towards positive supercoiling (Fig. 3c, purple numbers quantify the skewing). No alteration in linking number was induced by camptothecin treatment of vector control cells that lack TopIB (Fig. 3c) or of cells expressing the catalytically inactive human Top1(Y723F) mutant (Supplementary Information IX); in both experiments the topoisomer distribution appears as in the case of no treatment. Combined with our single-molecule observations, these *in vivo* findings support a model in which the positive supercoils that accumulate ahead of the transcription bubble are removed less effectively by TopIB in the presence of drug than negative supercoils, which leads to a persistent overwinding of DNA in the absence of DNA replication in G1 phase.

Camptothecin toxicity is linked to on-going DNA replication in S phase[8,9], where positive supercoils accumulate ahead of the replication fork and are removed by TopIB. To study the effect of camptothecin treatment on TopIB activity during S phase, cells arrested in G1 phase were allowed to synchronously enter S phase by removal of α-factor from the culture medium, after which the experiment was performed as above (for cell cycle distribution and cell viability data, see Supplementary Information X). An analysis of 2 μm DNA topoisomers purified from S-phase cells (Fig. 3d) yields similar results to that observed in the G1-phase experiments: positive-supercoil accumulation occurs only in TopIB-expressing cells and only in the presence of camptothecin.

In G1- and S-phase, the accumulation of positive supercoils was slightly enhanced in yeast cells expressing a thermosensitive Top2 (Supplementary Information XI). Although Top2 is essential to maintain cell viability[26], these observations indicate that the positive supercoils induced by camptothecin poisoning of TopIB are not effectively resolved by Top2. Thus, camptothecin-induced accumulation of positive supercoils occurs in a background of other topoisomerases, is not confined to a single phase of the cell cycle, and may derive from the tracking of a variety of complexes along the DNA duplex, including the transcription and replication machinery.

Our single-molecule data provide a direct determination of the vastly increased lifetime of the covalent TopIB–DNA complex stabilized by topotecan. However, we also observed a second consequence of drug binding: topotecan hinders DNA uncoiling by TopIB such that negative supercoils are relaxed more readily than positive supercoils. These findings provide a compelling argument for an intriguing, previously unconsidered, mechanism of drug-induced cell death (Fig. 4a, left). We propose that positive supercoils generated ahead of the fork (which cannot be efficiently removed by drug-bound TopIB or wild-type levels of Top2) may hamper fork progression. This stalling of the replication machinery could result in fork collapse and the formation of potentially lethal DNA lesions that induce cell death.

Implicit in this model is that the ability of camptothecins to selectively hinder the uncoiling of positively supercoiled DNA by TopIB coincides with drug cytotoxicity. Therefore, if the accumulation of positive supercoils is predictive of cellular response to this class of chemotherapeutics, then cells expressing a catalytically active, yet camptothecin-resistant, TopIB mutant enzyme would fail to accumulate positively supercoiled plasmid DNA in the presence of camptothecin. To test this critical aspect of the model, *top1*Δ yeast cells were transformed with a plasmid expressing either wild-type human TopIB or the catalytically active, camptothecin-resistant mutant TOP1(G365C), which harbours a single amino acid substitution of Cys for Gly 365 (ref. 27). Figure 4b shows the 2 μm DNA topoisomer distribution from asynchronously growing cells containing wild-type TopIB (left) or Top1(G365C) (right). This mutant failed to induce positive supercoil accumulation in the presence of camptothecin, in full agreement with our proposed mechanism that positive supercoils are involved in TopIB-mediated and camptothecin-induced cell death. Thus, a single mutation in an otherwise identical cellular background provided a stringent test of the specificity of TopIB–camptothecin interactions in causing the accumulation of positive supercoils associated with camptothecin-induced cell death.

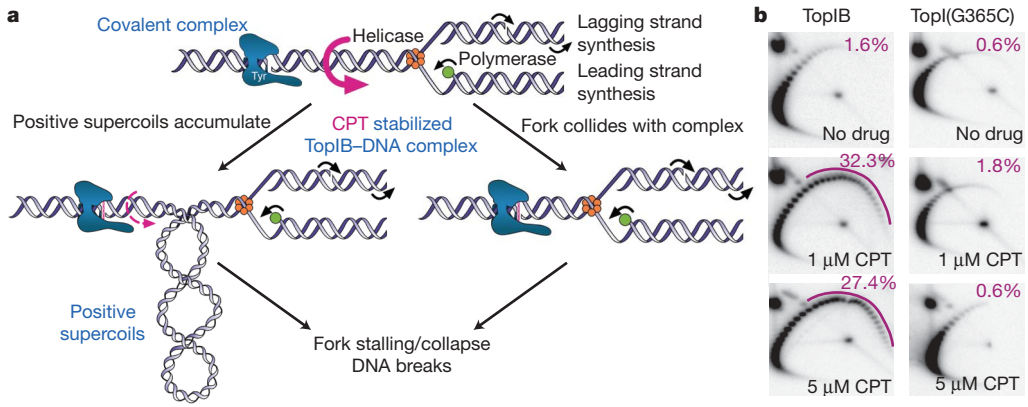

Figure 4 | Mechanism of camptothecin-induced toxicity derives from TopIB-dependent accumulation of positive supercoils. a, Representation of distinct mechanisms potentially underlying camptothecin-induced cell death. A (diagrammatically simplified) replication fork generates positive supercoils in the DNA, which are removed (magenta arrow) by TopIB (blue). In the presence of camptothecin, fork stalling and collapse have been predicted to result from the physical collision of the advancing replication complex with the drug-stabilized TopIB–DNA covalent complex (right). Our data suggest a second scenario, in which fork progression and integrity are indirectly impaired by unresolved positive supercoils (left). **b**, Analysis of 2 μm DNA topoisomer distribution in asynchronous cultures of *top1*Δ cells expressing either human wild-type TopIB or a catalytically active, camptothecin-resistant Top1(G365C) mutant. These were treated with indicated concentrations of camptothecin for 15 min. The accumulation of positive supercoils is only observed in drug-treated cells expressing wild-type TopIB.

METHODS

DNA constructs. Single-molecule experiments[28] used λ-phage DNA (48 kilobases (kb) or 16 μm contour length) or half-length λ-phage DNA (24 kb or 8 μm contour length). Measurements of the religation time were performed on shorter DNA (8 kb or 2.7 μm contour length) to decrease the magnitude of the brownian fluctuations of the bead and to increase the time resolution.

Enzyme and buffers. Full-length human TopIB and catalytically inactive TOP1(Y723F), each containing an amino-terminal Flag epitope, were partially purified from galactose-induced cultures of EKY3 *top1*Δ yeast cells[23]. To obtain homogenous protein preparations, TopIB fractions, bound to an anti-Flag M2 affinity gel and eluted with an excess of Flag peptide in Tris-buffered saline (50 mM Tris (pH 7.4), 150 mM KCl), were applied to a phosphocellulose column. Homogeneous TopIB was eluted in TEEG buffer (50 mM Tris (pH 7.4), 1 mM EDTA, 1 mM EGTA, 10% glycerol) plus 1.0 M KCl and protease inhibitors, diluted with 50% glycerol and stored at −20 °C. TopIB activity was assayed in plasmid DNA relaxation reactions and protein integrity was assessed in immunoblots[23].

Strains and plasmids. *Saccharomyces cerevisiae* strains EKY2 (MAT**a**, *top1*Δ) and EKY3 (MATα, *top1*Δ) have been described[29]. For constitutive expression of TopIB or TOP1(G365C), the epitope-tagged human *TOP1* sequences (*ehTOP1*) were excised from YCpGAL1–ehTOP1 (ref. 23) or YCpGAL1–top1(G365C) (ref. 27), and cloned under the yeast *TOP1* promoter in YCpScehTOP1•U or YCpScehtop1(G365C)•U. The empty vector YCpSc•U served as a control.

***In vivo* assays and two-dimensional gel electrophoresis.** Exponential cultures of MAT**a** cells, transformed with YCpScehTOP1•U or vector control, were arrested in G1 phase with α-factor, then either incubated with additional α-factor and 30 μM camptothecin or 0.25% DMSO (v/v) (no drug), or washed by filtration and released into S phase with or without camptothecin. Asynchronous cultures, incubated with 1 or 5 μM camptothecin for 15 min at 30 °C, were treated as above. To assess the 2 μm plasmid DNA topoisomer distribution, DNA isolated from cultures fixed with toluene/ethanol was resolved by two-dimensional gel electrophoresis and subjected to Southern blotting[29,30].

Received 6 January; accepted 15 May 2007.
Published online 24 June 2007.

1. Champoux, J. J. DNA topoisomerases: structure, function, and mechanism. *Annu. Rev. Biochem.* **70,** 369–413 (2001).
2. Li, T. K. & Liu, L. F. Tumor cell death induced by topoisomerase-targeting drugs. *Annu. Rev. Pharmacol. Toxicol.* **41,** 53–77 (2001).
3. Pommier, Y. Topoisomerase I inhibitors: camptothecins and beyond. *Nature Rev. Cancer* **6,** 789–802 (2006).
4. Wang, J. C. Cellular roles of DNA topoisomerases: a molecular perspective. *Nature Rev. Mol. Cell Biol.* **3,** 430–440 (2002).
5. Hsiang, Y. H., Hertzberg, R., Hecht, S. & Liu, L. F. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J. Biol. Chem.* **260,** 14873–14878 (1985).
6. Porter, S. E. & Champoux, J. J. The basis for camptothecin enhancement of DNA breakage by eukaryotic topoisomerase I. *Nucleic Acids Res.* **17,** 8521–8532 (1989).
7. Staker, B. L. *et al.* The mechanism of topoisomerase I poisoning by a camptothecin analog. *Proc. Natl Acad. Sci. USA* **99,** 15387–15392 (2002).
8. Holm, C., Covey, J. M., Kerrigan, D. & Pommier, Y. Differential requirement of DNA replication for the cytotoxicity of DNA topoisomerase I and II inhibitors in Chinese hamster DC3F cells. *Cancer Res.* **49,** 6365–6368 (1989).
9. Hsiang, Y. H., Lihou, M. G. & Liu, L. F. Arrest of replication forks by drug-stabilized topoisomerase I–DNA cleavable complexes as a mechanism of cell killing by camptothecin. *Cancer Res.* **49,** 5077–5082 (1989).
10. Corbett, K. D. & Berger, J. M. Structure, molecular mechanisms, and evolutionary relationships in DNA topoisomerases. *Annu. Rev. Biophys. Biomol. Struct.* **33,** 95–118 (2004).
11. Bjornsti, M. A. Cancer therapeutics in yeast. *Cancer Cell* **2,** 267–273 (2002).
12. Minsky, B. D. Combined-modality therapy of rectal cancer with irinotecan-based regimens. *Oncology (Huntingt.)* **18,** 49–55 (2004).
13. Rodriguez-Galindo, C. *et al.* Clinical use of topoisomerase I inhibitors in anticancer treatment. *Med. Pediatr. Oncol.* **35,** 385–402 (2000).
14. Stewart, D. J. Topotecan in the first-line treatment of small cell lung cancer. *Oncologist* **9** (Suppl 6), 33–42 (2004).
15. Sekiguchi, J. & Shuman, S. Vaccinia topoisomerase binds circumferentially to DNA. *J. Biol. Chem.* **269,** 31731–31734 (1994).
16. Redinbo, M. R., Stewart, L., Kuhn, P., Champoux, J. J. & Hol, W. G. Crystal structures of human topoisomerase I in covalent and noncovalent complexes with DNA. *Science* **279,** 1504–1513 (1998).
17. Koster, D. A., Croquette, V., Dekker, C., Shuman, S. & Dekker, N. H. Friction and torque govern the relaxation of DNA supercoils by eukaryotic topoisomerase IB. *Nature* **434,** 671–674 (2005).
18. Stivers, J. T., Harris, T. K. & Mildvan, A. S. Vaccinia DNA topoisomerase I: evidence supporting a free rotation mechanism for DNA supercoil relaxation. *Biochemistry* **36,** 5212–5222 (1997).
19. Chrencik, J. E. *et al.* Mechanisms of camptothecin resistance by human topoisomerase I mutations. *J. Mol. Biol.* **339,** 773–784 (2004).
20. Strick, T., Allemand, J., Croquette, V. & Bensimon, D. Twisting and stretching single DNA molecules. *Prog. Biophys. Mol. Biol.* **74,** 115–140 (2000).
21. Champoux, J. J. & Aronoff, R. The effects of camptothecin on the reaction and the specificity of the wheat germ type I topoisomerase. *J. Biol. Chem.* **264,** 1010–1015 (1989).
22. Stewart, L., Ireton, G. C. & Champoux, J. J. A functional linker in human topoisomerase I is required for maximum sensitivity to camptothecin in a DNA relaxation assay. *J. Biol. Chem.* **274,** 32950–32960 (1999).
23. Woo, M. H., Vance, J. R., Marcos, A. R., Bailly, C. & Bjornsti, M. A. Active site mutations in DNA topoisomerase I distinguish the cytotoxic activities of camptothecin and the indolocarbazole, rebeccamycin. *J. Biol. Chem.* **277,** 3813–3822 (2002).
24. Giaever, G. N. & Wang, J. C. Supercoiling of intracellular DNA can occur in eukaryotic cells. *Cell* **55,** 849–856 (1988).
25. Liu, L. F. & Wang, J. C. Supercoiling of the DNA template during transcription. *Proc. Natl Acad. Sci. USA* **84,** 7024–7027 (1987).
26. Goto, T. & Wang, J. C. Yeast DNA topoisomerase II is encoded by a single-copy, essential gene. *Cell* **36,** 1073–1080 (1984).
27. Woo, M. H. *et al.* Locking the DNA topoisomerase I protein clamp inhibits DNA rotation and induces cell lethality. *Proc. Natl Acad. Sci. USA* **100,** 13767–13772 (2003).
28. Strick, T. R., Allemand, J. F., Bensimon, D., Bensimon, A. & Croquette, V. The elasticity of a single supercoiled DNA molecule. *Science* 271, 1835–1837 (1996).
29. Hann, C. *et al.* Increased camptothecin toxicity induced in mammalian cells expressing *Saccharomyces cerevisiae* DNA topoisomerase I. *J. Biol. Chem.* **273,** 8425–8433 (1998).
30. Megonigal, M. D., Fertala, J. & Bjornsti, M. A. Alterations in the catalytic activity of yeast DNA topoisomerase I result in cell cycle arrest and cell death. *J. Biol. Chem.* **272,** 12801–12808 (1997).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank K. Besteman and U. Keyser for discussions, S. Hage and Y.-H. Chien for DNA preparation, R. van Waardenburg for help with PyMol, and C. Dekker for critical reading and general support. We thank FOM, NWO, NIH and ALSAC for financial support.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to N.H.D. (n.h.dekker@tudelft.nl).