Topoisomerase I regulates open chromatin and controls gene expression *in vivo*

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DNA topoisomerases regulate the topological state of the DNA double helix and are key enzymes in the processes of DNA replication, transcription and genome stability. Using the fission yeast model *Schizosaccharomyces pombe*, we investigate genome wide how DNA topoisomerases I and II affect chromatin dynamics and gene expression *in vivo*. We show that topoisomerase I activity is directly required for efficient nucleosome disassembly at gene promoter regions. Lack of topoisomerase activity results in increased nucleosome occupancy, perturbed histone modifications and reduced transcription from these promoters. Strong correlative evidence suggests that topoisomerase I cooperates with the ATP-dependent chromatin remodeller Hrp1 in nucleosome disassembly. Our study links topoisomerase activity to the maintenance of open chromatin and regulating transcription *in vivo*.

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Introduction

DNA topoisomerases solve the topological problems associated with the double helix structure of DNA. They enable essential processes such as DNA replication, transcription, recombination and chromatin remodelling by introducing temporary cleavage of DNA to relieve the supercoiling generated by these processes (Almouzni and Mechali, 1988; Mondal *et al.*, 2003). In the case of chromatin assembly, negative supercoils generally favour nucleosome formation, as one nucleosome absorbs one negative supercoil (Clark and Felsenfeld, 1991; Negri *et al.*, 1994; Hizume *et al.*, 2004).

To remove DNA helical tension, eukaryotic cells use two classes of DNA topoisomerases, which induce either single-strand (type I) or double-strand (type II) DNA breaks (Champoux, 2001; Wang, 2002). Although Top1 (type I) is

essential for early development in multicellular eukaryotes, it is not essential in yeast, likely because Top2 (type II) can substitute for Top1 function (Uemura and Yanagida, 1984; Champoux, 2001; Wang, 2002). One of the main cellular functions of Top1 is believed to be removal of transcription-generated helical stress (Champoux, 2001; Wang, 2002). Consistent with this notion, camptothecin (CPT) inhibition of Top1 has been reported to reduce tyrosine aminotransferase mRNA levels (Stewart and Schutz, 1987), and Top1 has been identified as a coactivator of transcription from a circular DNA template *in vitro* (Kretzschmar *et al.*, 1993; Merino *et al.*, 1993). Furthermore, simultaneous inactivation of Top1 and Top2 has been shown to reduce rRNA, and to a lesser extent mRNA synthesis (Schultz *et al.*, 1992; Collins *et al.*, 2001).

Although these earlier studies using cellular extracts, chromatin templates or the introduction of minichromosomes have implicated topoisomerases in chromatin structure and transcription, a holistic *in vivo* view of topoisomerase function in control of chromatin structure is currently missing. Here we show, using genome-wide techniques in *Schizosaccharomyces pombe*, that topoisomerase activity is essential for the regulation of transcription. We show evidence that this regulation, which occurs through the maintenance of open promoter-region chromatin, is primarily a function of Top1. Finally, we suggest a mechanism in which Top1 acts with the ATP-dependent chromatin remodeller Hrp1 to maintain this open chromatin by facilitating nucleosome disassembly.

Results and discussion

Top1 and Top2 mainly bind intergenic regions, where Top1 occupancy is linked to active transcription

We investigated the genome-wide localization of DNA topoisomerases in fission yeast by chromatin immunoprecipitation (ChIP) and hybridization to high-resolution tiling microarrays (ChIP-chip). ChIP was performed with functional (Supplementary Figure S2) c-Myc epitope tagged Top1 and Top2 proteins to obtain their binding profiles. Analysis reveals that Top1 binding is mainly enriched in the intergenic regions (IGRs) (Figure 1A). We observe a similar binding pattern for Top2, indicating that Top1 and Top2 in many cases act together to regulate DNA supercoiling in the cell. Indeed, a high proportion of Top1-bound IGRs (65%) also show Top2 binding (Figure 1B). Next, we investigated whether topoisomerase occupancy relates to gene transcription *in vivo*. We grouped the genes according to their expression levels and performed an average gene analysis of relative Top1 and Top2 occupancy (Figure 1C). Top1 mainly binds the gene promoter and in the 3' parts of transcribed regions (ORFs) and occupancy is positively correlated with mRNA levels. In contrast, Top2 binding to regions upstream and downstream of the ORF does not correlate with transcription. Rather, we notice reduced Top2 binding in highly transcribed ORFs. To confirm

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Figure 1 Top1 and Top2 are mainly located at intergenic regions (IGRs). (A) ChIP-chip binding profiles for Top1 and Top2 for part of chromosome I in Hu1606 (*top1-myc*) and Hu1609 (*top2-myc*) at 30°C. The lower part of the figure shows open reading frames (ORFs) and their orientations. Top1 and Top2 binding in terms of relative enrichment are represented in log 2. ChIP was performed in biological duplicates. (B) Venn diagrams illustrating the similarity of Top1 and Top2 gene binding targets at 5' IGRs ($P = 6.03 \times 10^{-201}$) and at 3' IGRs ($P = 1.44 \times 10^{-53}$). Cutoff values of 1.25-fold enrichment were applied. *P*-values were generated using a hypergeometric probability test in the Genespring 8.0 software. (C) Average gene analysis for Top1 and Top2 binding in relation to RNA transcription levels in Hu0303 (WT) at 30°C. Number of genes (*n*) in each category is indicated in the figure. Error bars represent 95% confidence intervals.

these observations, we used a DNA replication-independent assay for induction of transcription of the *inv1+* gene (Supplementary Figure S1). We show that Top1 is indeed the main enzyme that is recruited when this gene is induced, suggesting that its enzymatic activity is intimately linked to transcription. These observations are consistent with earlier work in *Drosophila* showing Top1 localization to transcriptionally active regions (Fleischmann *et al*, 1984; Gilmour and Elgin, 1987). Taken together, the recruitment of Top1 on induction of transcription and the correlation between promoter-region Top1 occupancy and gene transcription level indicate that Top1 has a special function at active gene promoter regions.

Top1 activity controls histone density, influencing the regulation of gene transcription

Next, we addressed the possibility that Top1 affects transcription by modulating nucleosome assembly or disassembly.

In vitro studies have suggested that nucleosome assembly and physiological spacing of chromatin arrays are tightly coupled to DNA topology (Almouzni and Mechali, 1988; Blank and Becker, 1996; Levchenko *et al*, 2005). As Top2 can substitute for the enzymatic activity of Top1, we used the double mutant *top1Δtop2-191^{ts}* strain for all of the functional analysis. This strain is deleted for the *top1⁺* gene and was grown at 30°C, at which temperature Top2 activity is strongly reduced (Uemura and Yanagida, 1984). Genome-wide nucleosome occupancy was investigated by ChIP-chip using an antibody against the carboxy terminus of histone H3. We found that the nucleosome occupancy in wild-type (WT) cells is generally low in IGRs and high in ORFs (Figure 2A; showing the same genomic region as Figure 1A). Clear differences are observed between *top1Δtop2-191* and WT cells, mainly in IGRs, indicating that topoisomerase activity is required to control histone density. We next investigated whether the nucleosome density ratio of *top1Δtop2-191*

Figure 2 Topoisomerase activity is required to regulate nucleosome occupancy at promoter regions. **(A)** ChIP-chip binding profiles for histone H3 C terminal (H3Cter) for part of chromosome I in Hu0303 (WT) and Hu1773 (*top1Δtop2-191*) at 30°C. The second panel shows the ratio of H3Cter binding in mutant versus WT. The lower part of the figure shows open reading frames (ORFs) and their orientations. H3Cter binding in terms of relative enrichment is represented in log 2. ChIP was performed in biological duplicates. **(B)** Average gene analysis for the ratio of H3Cter binding in mutant versus WT relative to mRNA levels in Hu0303 (WT) at 30°C. The number of genes (*n*) in each category is indicated in the figure. Error bars represent 95% confidence intervals. **(C)** Moving average plot (window size = 150 genes, step size = 1 gene) of the RNA transcription level ratios in Hu1773 (*top1Δtop2-191*) versus Hu0303 (WT) plotted as a function of mRNA levels in WT at 30°C. AU, arbitrary units. **(D)** RNA pol II density. Error bars represent 95% confidence intervals. **(E)** Venn diagram illustrating the overlaps between the top 500 genes that display increased H3Cter binding and the top 500 genes that display upregulated ($P=6.82E-5$) or downregulated ($P=6.88E-16$) mRNA levels in mutant versus WT. The *P*-values were generated using a hypergeometric probability test using the Genespring 8.0 software.

versus WT cells correlates with transcription (Figure 2B). The nucleosome density ratio for an average gene reveals a marked increase in nucleosome occupancy in the 5' IGRs, including the promoter regions of actively transcribed genes. We also observed a genome-wide effect of *top1Δtop2-191* on mRNA levels (Figure 2C) and on RNA pol II occupancy (Figure 2D). Surprisingly, both the RNA pol II ratio and the 'global expression profile' (i.e. a plot of the moving average of mutant versus WT mRNA ratio as a function of the WT mRNA expression levels) showed a striking difference between highly and lowly transcribed genes. The mRNA levels and RNA pol II occupancy of genes highly transcribed in WT tends to be reduced in *top1Δtop2-191*, whereas genes weakly or non-transcribed in WT show increased mRNA levels and RNA pol II in *top1Δtop2-191*. We then used hypergeometric probability tests to investigate how histone density changes related to changes in transcription in *top1Δtop2-191*. It is clear that the most significant effect is an increased H3 density in promoters of downregulated genes (Figure 2E). In the light of the unique function of Top1 at active gene promoters, this link between promoter-region nucleosome occupancy ratio and transcription in *top1Δtop2-191* indicates that Top1 activity is required to maintain a low-histone density in promoter regions and thereby allow for RNA pol II recruitment and high levels of transcription.

Topoisomerase I acts directly at promoter regions to stimulate nucleosome disassembly, histone acetylation and gene expression

For further analysis, we selected five target genes from the group of 107 most downregulated genes with increased H3

density at the gene promoter in *top1Δtop2-191* and Top1 binding based on the microarray data. We also included five control genes with relatively low-Top1 occupancy, for which H3 density at the promoter region was unaffected in *top1Δtop2-191* as judged by the microarray analysis. We first validated the five target genes by ChIP using Top1-myc and quantitative PCR (qPCR) analysis. The target genes showed between a 1.4- and 3.5-fold Top1 enrichment in the promoter regions, whereas the control genes showed less Top1 enrichment, ranging between 0.5- and 1.3-fold (Figure 3A). One target gene promoter (#4) was only slightly enriched compared with the highest control (#3). Next, we investigated mRNA levels for the target and control genes by reverse transcription followed by qPCR. It was clear that the genes were highly transcribed in WT cells as compared with the control genes. Four of the five target genes were found to be significantly downregulated in the *top1Δtop2-191* mutant (the slight downregulation of gene #2 was not statistically significant), whereas the control genes tended to be upregulated (Figure 3B). This slight upregulation of the control genes is consistent with the genome-wide trend of genes that are weakly expressed in WT to be upregulated in the *top1Δtop2-191* mutant (Figure 2C). Next, we performed H3Cter ChIP and qPCR and found that the H3 density was increased in gene promoter regions of all five target genes examined whereas the control genes were unaffected (Figure 3C). Acetylation of H3K9 is intimately linked to active transcription in fission yeast (Wiren *et al*, 2005). We tested H3K9 acetylation levels by ChIP and qPCR followed by normalizing to the H3Cter signal and found that, consistent with the downregulation of the genes, H3K9ac was strongly

Figure 3 Topoisomerase activity is required for nucleosome disassembly and maintenance of H3K9ac, supporting high levels of transcription. (A–E) Validation of five gene targets of topoisomerase activity and five control genes (selection based on microarray data) at 30°C. Target gene identities are the following: 1 = SPAC1F8.07c; 2 = SPAC4F8.07c; 3 = SPBC21c3.08c; 4 = SPBC1709.05; 5 = SPBPB7E8.01. Control gene identities are the following: 1 = SPAP14E8.04; 2 = SPAC222.06; 3 = SPBC11C11.02; 4 = SPAC1610.04; 5 = SPAC17A5.10. All data in this figure are represented as mean $\pm$ s.d. The ChIP DNA was analysed by qPCR. (A) ChIP relative enrichment of Top1 in Hu1606 (*top1-myc*). (B) mRNA levels in Hu0303 (WT) and Hu1773 (*top1Δtop2-191*). Note that the target and control genes are displayed with different y axis scales due to their differing transcription levels. (C) ChIP relative enrichment of histone H3 C terminal (H3Cter) in Hu0303 (WT) and Hu1773 (*top1Δtop2-191*). Note that the target and control genes are displayed with different y axis scales due to their differing H3 occupancy. (D) ChIP relative enrichment of H3K9 acetylation (H3K9Ac) normalized to H3Cter in Hu0303 (WT) and Hu1773 (*top1Δtop2-191*). (E) ChIP relative enrichment of catalytically active covalently bound Top1 after treatment of Hu1606 (*top1-myc*) with camptothecin (CPT) for 1 min. The IP was performed in triplicate and analysed by qPCR.

reduced at all five target gene promoters but not at the control gene promoters (Figure 3D).

To address whether the effect of Top1 is a direct result of catalytic activity at those gene promoters, we first ruled out any indirect effects through global changes to chromatin structure or changes in transcription of chromatin regulators (Supplementary Figure S2; Supplementary Table 1). We then specifically trapped covalently bound and thus catalytically active Top1 on its DNA substrate with CPT. The DNA was purified by CsCl fractionation to enable selection of covalent DNA–protein complexes and removal of non-covalently bound proteins (Hartsuiker *et al*, 2009). We then subjected the fractions containing CPT trapped DNA-bound Top1 (Supplementary Figure S2) to formaldehyde cross-linking and ChIP against c-Myc epitope tagged Top1, and quantified the presence of catalytically active (CPT trapped) Top1 at the selected gene promoters (Figure 3E). It is clear that Top1 is strongly enriched in the immunoprecipitated DNA only on CPT treatment. Furthermore, four out of five gene promoters (all except #4) observed to be Top1 targets based on the microarray data were enriched for Top1 activity relative to the control promoters. Thus, these promoters are not only enriched for Top1 occupancy, but also for catalytic activity of Top1. This strongly suggests that Top1 catalytic activity is directly required for nucleosome disassembly at gene promoters, allowing for acetylation of H3K9 and supporting a high rate of transcription. The phenotypic changes in the *top1Δtop2-191* mutant observed at target gene #4 despite

the fact that Top1 occupancy and activity is low could be due to reduced Top2 activity in the promoter region, as Top2 is normally present at this gene promoter according to the microarray data (data not shown).

Topoisomerases cooperate with the chromatin remodeller Hrp1 to achieve nucleosome disassembly

Snf2 ATP-dependent chromatin remodelling activity generates superhelical torsion *in vitro* (Havas *et al*, 2000; Gavin *et al*, 2001). It is clearly established *in vitro* that the ySWI/SNF remodeller requires DNA topoisomerases to alleviate DNA topology constraints (Gavin *et al*, 2001). Supercoil relaxation by topoisomerase I has also been shown to be a prerequisite for remodeller activity in *Xenopus laevis* oocyte extracts (Almouzni and Mechali, 1988). In *S. pombe*, the Snf2 chromatin remodeller and Chd1 homolog Hrp1, together with the Nap1 histone chaperone, is required for nucleosome disassembly *in vivo* (Walfridsson *et al*, 2007).

Interestingly, our genome-wide analysis shows a good correlation between Top1 and Top2 binding determined in this study and Hrp1 binding data (Walfridsson *et al*, 2007) despite the fact that two different DNA microarray platforms were used (Figure 4A and B). Hypergeometric probability tests also show a highly significant overlap between Hrp1 and Top1/Top2 genomic targets (Figure 4C). These tests also indicate a shared function between these factors and Nap1, as regions where histone density is changed in *hrp1* mutants also strongly correlate with Top1, Top2 and Nap1

Figure 4 Hrp1, Top1 and Top2 share many targets in intergenic regions (IGRs). (**A, B**) Comparison of Top1 and Top2 in Hu1606 (*top1-myc*) and Hu1609 (*top2-myc*) with Hrp1 occupancy at 30°C (Eurogentec spotted microarray platform versus Affymetrix tiling arrays). The correlation coefficients are for Top1 versus Hrp1: $R = 0.747241868$; and for Top2 versus Hrp1: $R = 0.727174702$. (**C**) Venn diagram illustrating the overlap of Hrp1 with Top1 ($P = 1.04 \times 10^{-134}$) and Top2 ($P = 5.94 \times 10^{-131}$) occupancy at IGRs. Top1 and Top2 microarray data from the Affymetrix platform has been adapted to the Eurogentec platform using 50th percentile normalization to enable comparison with earlier data (Walfridsson *et al*, 2007). Cutoff values of two-fold relative enrichment have been applied for Top1 and Top2 binding target genes. Hrp1 binding target genes have been defined as the upper 0.9 percentile. P -values were generated using a hypergeometric probability test in the Genespring 8.0 software. (**D**) Hierarchical cluster analysis according to the Euclidian distance showing the correlation between increased nucleosome density in Hu1773 (*top1Δtop2-191*) or *hrp1Δ* with Top1, Top2 and Hrp1 binding at IGRs at 30°C. The clusters were generated using the TM4 software (MeV_4_2). (**E**) ChIP relative enrichment of histone H3 C terminal (H3Cter) at promoter regions of Hrp1 targets in Hu0303 (WT) and Hu1773 (*top1Δtop2-191*) at 30°C. ChIP was performed four times using biological duplicates and analysed by qPCR.

occupancy (data not shown). Hierarchical clustering of gene promoters provides further support for this notion, as *top1Δtop2-191* H3 density affected promoters cluster with those affected in *hrp1Δ* (Figure 4D). To confirm these observations based on microarray correlations, we measured histone H3 density by ChIP and qPCR at six selected Hrp1 target promoters using WT and *top1Δtop2-191* cells. In all six cases, we observed a two- to three-fold increased histone H3 density also in *top1Δtop2-191* (Figure 4E). A mechanism describing how a SNF2 family chromatin remodeller could cooperate with Top1 to bring about nucleosome disassembly has already been established (Gavin *et al*, 2001). According to this model, Hrp1 remodelling activity generates supercoils that must subsequently be removed by Top1 to allow for nucleosome disassembly, as is the case for ySWI/SNF.

RNA Pol II accumulates on longer genes in the absence of topoisomerase activity

The twin-supercoiled domain model predicts a function for topoisomerases in relaxing supercoils to facilitate transcriptional elongation (Liu and Wang, 1987). We therefore set out to test whether our genome-wide data set could provide any support for this model in *S. pombe*. One would predict that

longer genes would be more affected, so we sorted genes into different length categories and plotted the average RNA pol II density across the coding regions (Figure 5C). We found that genes longer than 800 base pairs accumulate RNA pol II in coding regions in *top1Δtop2-191*. The longest genes show an increased RNA pol II occupancy relative to WT, followed by a decreased relative occupancy at the most 3' end of the ORF, suggesting a decrease in complete transcripts. Interestingly, we also observe an enrichment of Top2 in the 3'ORF of longer genes in particular (Figure 5B). Such a correlation of binding with gene length is not shared by Top1 (Figure 5A). This complements earlier *in vitro* work showing that transcription of long chromatin transcripts is repressed in the absence of a topoisomerase capable of relaxing positive supercoils (Mondal *et al*, 2003). The finding that Top2 tends to be localized to the coding region and Top1 to the relatively nucleosome-free promoter regions is also consistent with the finding that Top2 acts more efficiently than Top1 on nucleosomal DNA substrates (Salceda *et al*, 2006) and with the finding that long genes in *S. pombe* have a higher nucleosome density (Supplementary Figure S3). The increased RNA pol II density observed in *top1Δtop2-191* at long genes further supports the twin-supercoiling model in eukaryotes, as accumulating supercoils ahead of the

Figure 5 Open reading frames (ORFs) of long genes display Top2 enrichment and, in cells lacking topoisomerase activity, an accumulation of RNA polymerase II. (A) Average gene analysis according to gene length of ChIP-chip binding profile for Top1 in Hu1606 (*top1-myc*) at 30°C. (B) Average gene analysis according to gene length of ChIP-chip binding profile for Top2 in Hu1609 (*top2-myc*) at 30°C. (C) Average gene analysis according to gene length of the ratio of ChIP-chip binding profiles for RNA polymerase II in Hu1773 (*top1Dtop2-191*) versus Hu0303 (WT) at 30°C. RNA polymerase II, Top2 and Top1 occupancy in terms of relative enrichment is represented as a linear ratio. ChIP was performed in biological duplicates. Regions corresponding to ORFs common to all genes in each category are represented by a solid grey background, whereas regions corresponding to ORFs for a fraction of the genes are represented by grey stripes. Gene lengths and number of genes (*n*) in each category is indicated in the figure. Error bars represent 95% confidence intervals.

polymerase in the absence of topoisomerase activity should inhibit elongation. This function is likely to be more important at infrequently transcribed long genes as the polymerase have to travel a longer distance, allowing more supercoiling to accumulate, and as at highly transcribed short genes the high RNA pol II density may lead to positive supercoiling ahead of one polymerase being neutralized by

the negative supercoiling generated behind the polymerase in front.

A model for the Top1-regulated maintenance of open chromatin at promoters

The twin-supercoiled domain model predicts the generation of negative supercoils behind and positive supercoils ahead

Figure 6 A model for how Top1 and Hrp1 may collaborate to maintain open chromatin at active gene promoters. The 5' end of an active gene is represented, along with proposed nucleosome occupancy and transcription levels, as affected by transcription and the concerted action of Top1 and Hrp1. The model is for genes with both Top1 and Hrp1 at the promoters (236 genes in total; Figure 4C). See main text for further discussion.

of a polymerase elongating through the DNA double helix, which must then be relieved by topoisomerase activity (Wang, 1985). In our model (Figure 6), the transcribing RNA polymerase leaves negative supercoils in its wake, which favour nucleosome incorporation. Increased promoter-region nucleosome density leads to decreased transcription if not prevented by the joint action of ATP-dependent chromatin remodellers and Top1. As described earlier (Gavin *et al*, 2001), Top1 is needed to relieve the supercoils generated by the remodeller Hrp1, thus permitting nucleosome release. In the case of the promoter of an active gene, Top1 would also be required to relax the supercoils generated by transcription, explaining the proportionate increase in promoter-region nucleosome occupancy with mRNA level in *top1Δtop2-191*. The maintenance by Top1 and Hrp1 of an open chromatin state at the promoter allows transcription to continue rather than shutting itself off.

Here, we show genome wide in *S. pombe* that Top1 regulates transcription by enabling nucleosome disassembly at gene promoters *in vivo*. We also present support for a mechanism by which the chromatin remodeller Hrp1 may work in concert with Top1 to achieve this effect. As nucleosome assembly and disassembly are associated with supercoiling changes in many experimental systems (Clark and Felsenfeld, 1991; Hizume *et al*, 2004), our findings suggest that Top1 contributes to the regulation of gene expression and the maintenance of open chromatin by controlling nucleosome disassembly at eukaryotic promoters. The Hrp1 homolog Chd1 was recently found to be required for the maintenance of mouse embryonic stem cell pluripotency (Gaspar-Maia *et al*, 2009). This elucidation of the nature of the link between topoisomerases and Hrp1 activity also provides insight into the function that topoisomerases may have in maintaining the open chromatin essential for cell fate plasticity.

Materials and methods

Construction of strains

Standard fission yeast genetic techniques and media were used according to Moreno *et al* (1991). The Hu1773 parental strain Fy8993 bearing the thermosensitive *top2-191* mutation was provided by M Yanagida (Kyoto University, Japan) (Uemura and Yanagida, 1984).

Replication-independent inducible transcription assay

Replication-independent topoisomerase binding, H3 occupancy and H3K9ac levels at transcriptionally induced and repressed *inv1* was assayed as described earlier (Choi *et al*, 2005) in cell-cycle arrested Hu1606, Hu1608 and Hu0303 at 30°C. RNA expression levels, checked by RT-PCR, confirmed *inv1* induction and repression.

Chromatin immunoprecipitation

Cultures of Hu0303, Hu1773, Hu1539 and Hu1789 were grown at 30°C and chromatin from triplicate cultures was isolated and subjected to ChIP as described in Durand-Dubief and Ekwall (2009) with the following details and alterations. A measure of 1 µl mouse 9E10 α-Myc (Sigma M4439), 1.5 µl αH3 (Ab1791), 3 µl αRNAPII (Ab5408) and 2 µl αH3K9Ac (Upstate #07-352) were used for IP. DNA was recovered using QIAquick PCR Purification columns (Qiagen) using Buffer PB.

Microarray hybridization and normalization

Immunoprecipitated chromatin from at least two independent ChIP experiments was hybridized to GeneChip[®] *S. pombe* Tiling 1.0FR Arrays (Affymetrix). ChIP DNA was amplified to 5 µg DNA as described earlier (Durand-Dubief and Ekwall, 2009) except that 5 mM dUTP was added to the second amplification. Fragmentation, labelling and hybridization to the Affymetrix GeneChip *S. pombe* Tiling 1.0FR was performed by the Affymetrix core facility (BEA) according to the standard protocols. Raw data were analysed with Tiling Analysis Software (TAS) v1.1 using quantile normalization plus scaling and run with a bandwidth of 150. Normalized data of each probe were assigned to *S. pombe* genome coordinates in TAS (September 2004 genome, Sanger). Data were visualized with Affymetrix Integrated Genome Browser. For RNA expression analysis, sense and antisense probes were separated in two channels and normalized separately using Affymetrix internal controls against genomic DNA fragmented with DNase I (Wilhelm *et al*, 2008). The resulting linear ratio was extracted for each probe.

position, defined as the centre (12th) base coordinate for each 25-nucleotide probe.

RNA extraction and reverse transcription

For RNA analysis, total RNA was extracted from mid-logarithmic phase cells using the hot-phenol method. For microarray analysis, RNA was treated according to the Affymetrix total RNA labelling protocol (<http://www.affymetrix.com>). For analysis by qPCR, RNA was reverse transcribed using random hexamers and the Invitrogen SuperScript II Reverse Transcriptase kit.

Microarray data analysis

Microarray data have been deposited in the ArrayExpress database (<http://www.ebi.ac.uk/microarray-as/ae/>) under the accession code E-MEXP-1850. Gene list comparisons and *P*-values were generated using Genespring 8.0 software.

Data sets from ChIP on chip experiments were used to map all coding genes onto an average gene as described in Buchanan *et al* (2009). Briefly, the analysed region was -800 to $+2800$ bp from the start codon of the gene with a 20-bp resolution. Values for each probe were attributed to the closest assigned position. In this way, a matrix was generated with 180 columns of 20 bp and a row for each gene. Next, each column was then averaged vertically for expression subgroup to create the average binding values. To estimate the variability of the average value at each position of the trend plots, error bars of 95% confidence interval were added. RNA polymerase II transcribed genes were assigned to five expression categories according to linear signal intensities (arbitrary units, AU) of detected sense RNA (very low (AU < 500), low (AU = 500–1000), medium (AU = 1000–4000), high (AU = 4000–8000) and very high (AU > 8000)). TM4 software (MeV_4_2) (Saeed *et al*, 2003) was used to perform hierarchical clustering using the Euclidian distance.

Semi-quantitative competitive PCR

Analysis by semi-quantitative competitive PCR was performed in duplicate or triplicate. Primers p53–p62 were used to determine Top1, Top2, histone H3 and RNA polymerase II occupancies throughout the *inv1* gene. ChIP enrichment was normalized against the p63/p64 ORF endogenous control and a non-immunoprecipitated sample.

Quantitative PCR

qPCR was performed in triplicate on samples from ChIP experiments, RNA extractions and the Top1 CPT trapping using an Applied Biosystems 7500 Fast Real-Time PCR System, associated Sequence Detection Software v.1.3.1 and Power SYBR[®] Green PCR

Master Mix (Applied Biosystems). Primers p1–p22 were used to determine total Top1, H3 and H3K9ac occupancy and Top1 trapping at 5' IGRs. cDNA quantities were determined using primers p23–p42. Primers p43–p52 were used to determine H3 occupancy at shared Hrp1 and topoisomerase targets.

Top1 CPT-trapping assay

An assay for detecting catalytically active Top1 bound to DNA was developed by combining the DNA-Linked Protein Detection Assay developed by Hartsuiker *et al* (2009) with immunoprecipitation of Top1–DNA complexes trapped using CPT. Hu1606 cells were grown to mid-exponential phase and treated for 0, 1 or 15 min with 50 μ M CPT at 30°C. The presence of covalently bound Top1 in the DNA fractions was verified by slot blot according to Hartsuiker *et al* (2009). Samples were cross-linked with 1% paraformaldehyde for 30 min at 4°C. Fixation was quenched with 0.125 mM glycine, and the samples were dialysed overnight against IP buffer (10 mM NaPi pH 7.0, 0.14 M NaCl, 0.05% Triton X-100). DNA and proteins were then precipitated and resuspended in IP buffer. DNA was sonicated using a Sonics Vibra cell, and triplicate samples (4 μ g DNA/200 μ l IP buffer) were immunoprecipitated as described above using 1 μ l (2 μ g) mouse 9E10 α -Myc (Sigma M4439).

MNase digestion

To monitor the overall chromatin structure integrity, chromatin from Hu0303 (WT) and Hu1773 (*top1 Δ top2-191*) was digested with micrococcal nuclease (MNase) according to Lantermann *et al* (2009) and visualized with ethidium bromide on an agarose gel.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (<http://www.embojournal.org>).

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Conflict of interest

The authors declare that they have no conflict of interest.

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