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# Genome-Organizing Factors Top2 and Hmo1 Prevent Chromosome Fragility at Sites of S phase Transcription

Rodrigo Bermejo,[1] Thelma Capra,[1] Victor Gonzalez-Huici,[1] Daniele Fachinetti,[1] Andrea Cocito,[1] Gioacchino Natoli,[3] Yuki Katou,[4] Hiroshi Mori,[4] Ken Kurokawa,[4] Katsuhiko Shirahige,[4] and Marco Foiani[1,2,*]

[1]Fondazione IFOM Istituto FIRC di Oncologia Moleculare (IFOM-IEO Campus)

[2]DSBB-Università degli Studi di Milano

[3]Department of Experimental Oncology, European Institute of Oncology (IFOM–IEO Campus)
Via Adamello 16, 20139 Milan, Italy

[4]Laboratory of Genome Structure and Function, Division for Gene Research, Center for Biological Resources and Informatics, Tokyo Institute of Technology, B-65 4259 Nagatsuta, Midori-ku, Yokohama City, Kanagawa 226-8501, Japan

*Correspondence: marco.foiani@ifom-ieo-campus.it

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## SUMMARY

Specialized topoisomerases solve the topological constraints arising when replication forks encounter transcription. We have investigated the contribution of Top2 in S phase transcription. Specifically in S phase, Top2 binds intergenic regions close to transcribed genes. The Top2-bound loci exhibit low nucleosome density and accumulate γH2A when Top2 is defective. These intergenic loci associate with the HMG protein Hmo1 throughout the cell cycle and are refractory to the histone variant Htz1. In *top2* mutants, Hmo1 is deleterious and accumulates at pericentromeric regions in G2/M. Our data indicate that Top2 is dispensable for transcription and that Hmo1 and Top2 bind in the proximity of genes transcribed in S phase suppressing chromosome fragility at the M-G1 transition. We propose that an Hmo1-dependent epigenetic signature together with Top2 mediate an S phase architectural pathway to preserve genome integrity.

## INTRODUCTION

Genome stability during chromosome replication can be challenged by drugs affecting fork progression, intra-S DNA damage, oncogene activation, and transcription (Aguilera and Gomez-Gonzalez, 2008; Branzei and Foiani, 2008; Di Micco et al., 2006; Prado and Aguilera, 2005). The mechanisms and pathways preserving the integrity of replicating chromosomes have been widely studied (Branzei and Foiani, 2008; Cha and Kleckner, 2002; Myung and Kolodner, 2002; Schmidt and Kolodner, 2006; Smith et al., 2005). Certain chromosomal loci are fragile (Casper et al., 2002; Lemoine et al., 2005), but the physiological and pathological transitions occurring at these fragile sites are still unclear. Prokaryotic genomes have evolved to avoid transcription-replication clashing by placing coding sequences at the leading strands of replication forks (Rocha, 2004). In eukaryotes specialized networks deal with transcription-replication interference when collisions occur. Those regions experiencing clashes between replication and transcription often slow down the forks and trigger recombination events (Deshpande and Newlon, 1996; Prado and Aguilera, 2005). Dedicated *r*eplication *f*ork *b*arriers (RFB) block fork advance opposite to RNA polymerase I-mediated transcription, thus allowing those forks progressing codirectionally with transcription to complete replication of the rDNA locus (Brewer and Fangman, 1988; Torres et al., 2004). When forks clash with transcribed units that are codirectional with fork movement, the replisome can utilize the 3′ end of the RNA species for repriming a DNA chain downstream (Pomerantz and O'Donnell, 2008). Specialized DNA helicases assist fork progression when replication clashes with RNA polymerase II- and III-dependent transcription (Ivessa et al., 2003; Prado and Aguilera, 2005; Scholes et al., 2001).

We identified a subpopulation of DNA topoisomerase II (Top2) localizing to intergenic regions in S phase. The S phase specificity of those Top2 clusters prompted us to suggest that Top2 alleviates the topological problems generated on transcribed regions during replication (Bermejo et al., 2007). Top2 is a type II topoisomerase that catalyzes the passage of two independent segments of DNA through another (Champoux, 2001; Wang, 2002) and is implicated in higher-order chromatin organization (Gasser et al., 1986; Li et al., 1999; Varga-Weisz et al., 1997). Intrachromosomal looping may facilitate transcription initiation through the establishment of productive interactions between distant regulatory elements, transcription factors, and chromatin-remodeling complexes (Schneider and Grosschedl, 2007). Gene looping, taking place between initiator and terminator regions, has been proposed to facilitate polymerase recycling and to increase transcription rates (Ansari and Hampsey, 2005; O'Sullivan et al., 2004).

Chromosomal architecture also regulates replication dynamics. Cells experiencing replication stress enhance replicon

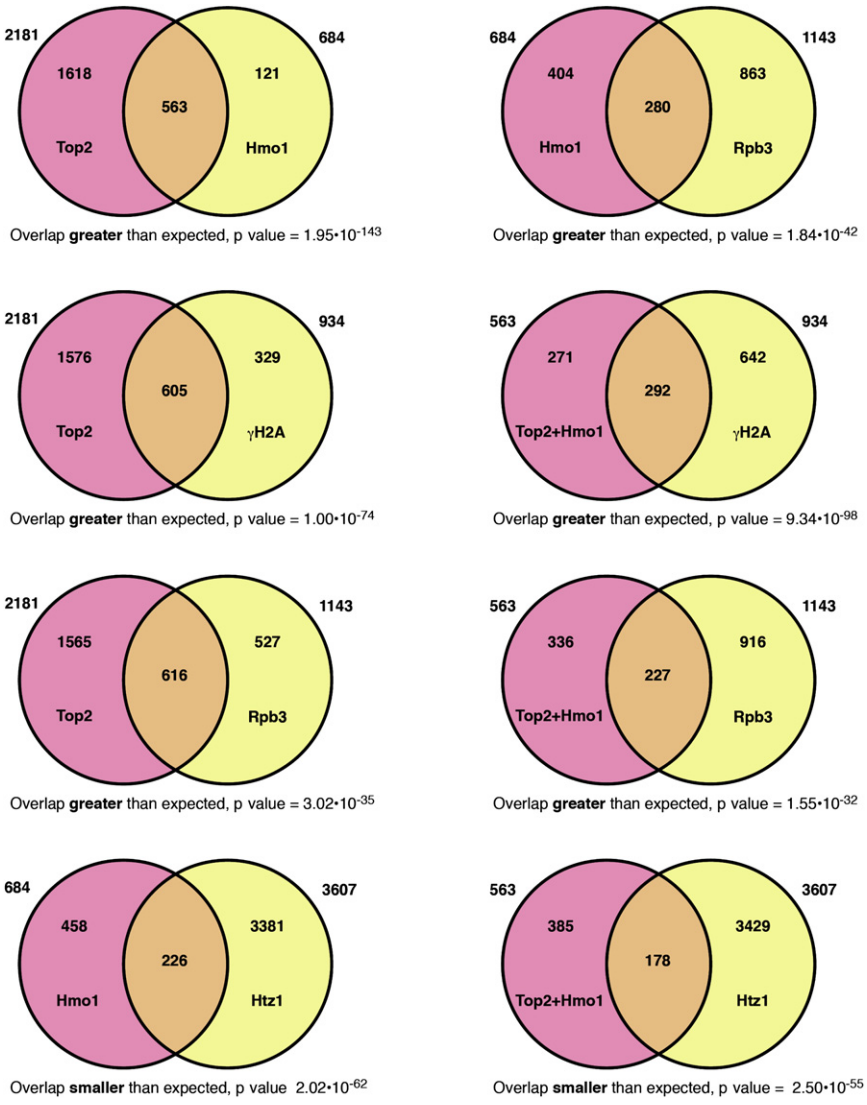


**Figure 1. Genome-wide Protein-Binding Correlations**

Overlap of candidate promoters bound by different targets of the experiments and assessment of their statistical significance. We considered as candidate promoters the 500 bp upstream each of the 5769 genes in *Saccharomyces* Genome Database (SGD), even where this region is overlapped by exons of other genes. When a promoter was overlapped by 30% of its size by a cluster, we considered the association significant. The absolute numbers shown in the diagrams are severely affected by the parameters used to define the clusters and thus should be considered relevant only to assess the statistical significance of the changes with respect to what would be the randomly expected. Lower- or upper-bound one-tailed exact fisher test p values are reported per each pair of targets; Top2+Hmo1 means genes bound by both.

firing by epigenetically priming higher-order chromosomal loops, and a DNA topoisomerase II mechanism has been involved in replicon resetting at mitosis through remodeling of chromosomal loops (Courbet et al., 2008; Lemaitre et al., 2005). Similarly, *h*igh *m*obility *g*roup (HMG) proteins bind DNA with low sequence specificity and act as chromatin architectural factors (Stros et al., 2007b; Thomas and Travers, 2001). They have affinity for DNA with a distorted conformation but can also induce changes in the structure of the DNA helix. HMG proteins have also been implicated in transcription regulation and maintenance of chromosomal integrity (Thomas, 2001; Sikdar et al., 2008).

The yeast HMGB protein Hmo1 modulates chromatin structure and transcription of certain RNA pol II transcribed genes through mechanisms that are still elusive (Berger et al., 2007; Hall et al., 2006; Kasahara et al., 2007; Lu et al., 1996). Hmo1 can bind four-way DNA junctions with high affinity and substitute for histones to organize rDNA transcribed units (Kamau et al., 2004; Merz et al., 2008). HMGB1, a human Hmo1-related protein, exhibits high affinity for distorted DNA structures like four-way junctions, cisplatin-modified DNA, or hemicatenated DNA loops (Bianchi et al., 1989; Gaillard and Strauss, 2000; Hughes et al., 1992). HMGB1 physically interacts with and stimulates TopoIIα activity on catenated DNA structures (Stros et al., 2007a).

Here we show that yeast Top2 binding at intergenic loci during replication correlates with a fraction of RNA polymerase II genes transcribed in S phase. The intergenic regions bound by Top2 accumulate γH2A when Top2 activity is attenuated. Hmo1 localizes at the Top2 intergenic regions even in G1 and G2/M, when Top2 is not present. *HMO1* ablation alleviates certain phenotypes of *top2* mutants and Hmo1 protein exhibits an abnormal chromosomal distribution when Top2 is not functional.

Our data unmask a chromosome architectural pathway, mediated by Top2 and Hmo1, that protects chromosome integrity likely by topologically coordinating DNA replication and transcription.

## RESULTS

### DNA Topoisomerase II Associates with Regions Transcribed by RNA pol II during S Phase

Top2 does not form obvious clusters in G1 and enriches at pericentromeric regions in G2/M (Figure S1 available online and data not shown). We performed a genome-wide computational analysis of the S phase Top2 and RNA polymerase II Rpb3 subunit clusters (Supplemental Statistical Analysis and Figure 1). Ninety-one percent of Top2 peaks close to mRNA-encoding genes (we will refer to these clusters as iTop2 [intergenic