Supplementary Figures

RNA polymerase II contributes to preventing transcription-mediated replication fork stalls

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Figure S1. Response of RNAPII mutants to DNA damage. (A) Viability of RNAPII mutants in the presence of genotoxic agents. (B) Genetic interactions between RNAPII mutations and homologous recombination mutations. (C) Viability of single and double mutants of rpb1-1 with mutations in genes involved in DNA related processes (* 0.002% MMS, ** 50 mM HU). Other details as in Fig. 1.
**Figure S2. Cell cycle progression in RNAPII mutants.** Cells were synchronized in G1 with α-factor and monitored at different times after release in control conditions and in the presence of 40mM HU.
Figure S3. *rad51Δ* partially suppresses replication defects of *rpb1-1*. (A) Cell cycle progression in WT, *rpb1-1*, *rad51Δ* and *rpb1-1 rad51Δ* in the presence of 40mM HU. (B) Replication fork progression through *SPF1* gene under replicative stress in the same strains. Details are as in Fig. 4.
**Figure S4.** Representative DNA fibers of DNA combing experiments in WT and *rpb1* mutants. BrdU incorporation is visualized in green.
Figure S5. *rpb1-1* mutant requires Rad53 to survive. (A) Tetrad analysis of different mutant combinations of *rpb1-1, rpb1-S751F, rad53Δ sml1Δ, rad53-21* and *rad53-1*. Δ indicates alive double/triple mutants, O indicates triple mutants that fail to grow. (B) HU sensitivity, assayed by 10-fold serial dilutions, of different mutant combinations of *rpb1* mutants and *rad53* mutants. C) FACS profiles of asynchronously growing cells.
Figure S6. Statistic analysis of length, G+C content and model-based expression levels of genes whose expression is altered in RNAPII mutants. Average length, G+C content and wild type expression levels of genes whose expression is affected in rpb1-1, rpb1-S751F and rpb2-10 compared to WT. Grey lines, genome averages. *, p < 0.001 (Mann-Whitney U test).
Figure S7. Change in gene expression of specific genes is not the cause of the genome instability observed in rpb1 mutants. (A) Recombination frequency measured with the pRS316LYΔNS system and Rad52 focus formation in WT, nat4Δ and htl1Δ strains. (B) HU or MMS sensitivity of RNAPII mutants upon IRC4 overexpression. Cells were transformed with the p425GPD:IRC4 plasmid, containing the IRC4 gene under the GAPDH constitutive promoter. (C) HU or MMS sensitivity of RNAPII mutants upon SUS1 overexpression. Cells were transformed with the pSR8 plasmid, containing the genomic sequence of the SUS1 ORF, and the pSR9 plasmid containing the SUS1 cDNA sequence. Overexpression of SUS1 occurs in the intron less version. (D) HU or MMS sensitivity of RNAPII mutants upon RNR3 overexpression. Strains were transformed with pBAD86, containing the RNR3 gene under the GAPDH constitutive promoter. Growth was tested in selective minimal medium with HU and MMS. o/e, overexpression.
Figure S 8. Gene expression in rpb1 mutants. (A) Retention of RNAPII in the actively transcribed SPF1 and PDC1 genes in RNAPII mutants. ChIP analysis of RNAPII in asynchronously growing WT, rpb1-1, rpb1-S751F, rpb2-10 and rpb9Δ cells at the SPF1 and PDC1 genes. The scheme of the gene and the PCR-amplified fragments are shown. Details are as in Fig. 7. (B) Northern analysis of the expression of the Tet::lacZ-URA3 (pCM184-LAUR) and LEU2 (pCM189-L2) fusion constructs and the endogenous SPF1 gene in WT and RNAPII mutant strains. RNA was isolated from mid-log phase cultures grown in SC-trp or YEPD rich medium respectively. As a 32P-labeled DNA probe, the 3-kb BamHI lacZ fragment, the Clal-EcoRV LEU2 internal fragment, an internal 1020-base pair SPF1 fragment and an internal 589-base pair 25S rDNA fragment obtained by PCR were used.
Figure S9. Genomic distribution of Rrm3 accumulation. Plot of each yeast chromosome with the signal log₂ ratio values for the significant ChIP hits. X-axis shows chromosomal coordinates in kb. Positions of centromeres are indicated as open circles.
Figure S10. Comparison of Rrm3 distribution profiles in WT and \textit{rpb1-1} cells. (A) Statistic analysis of average length, G+C content and wild-type model-based expression level of genes recruiting Rrm3. Grey lines indicate the genome average values. *, \( p < 0.001 \) (Mann-Whitney U test). (B) Composite profile of Rrm3 occupancy across the average ORF plotted as Rrm3 percentage of ChIP hits per segment. The average signal log\(_2\) ratio for Rrm3 hits mapping on each segment is plotted. Other details are as Fig. 8.
**Figure S11. Rrm3 enrichment correlates with gene expression.** (A) Correlation between Rrm3 binding and gene expression levels in genes with significant Rrm3 binding in WT and rpbi-1 cells. (B) Correlation between significant Rrm3 binding and gene expression levels in genes up or down regulated in rpbi-1 mutant respect to WT. A linear regression line and the Pearson’s R correlation coefficient are shown in each graph.