Figures and figure supplements

Pervasive transcription fine-tunes replication origin activity

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Figure 1. Metasite analysis of RNAPII occupancy and transcription termination at replication origins. (A) RNAPII PAR-CLIP metaprofile at replication origins. 228 confirmed ARSs were oriented according to the direction of the T-rich strand of their proposed ACSs (blue arrow) (Nieduszynski et al., 2006) and aligned at the 5' ends of the oriented ACSs (red dashed line). The median number of RNAPII reads (Schaughency et al., 2014) calculated...
Figure 1 continued

for each position is plotted. Transcription proceeding along the T-rich strand of the ACS is represented in blue and considered to be sense, while transcription on the opposite strand is plotted in red and considered to be antisense. (B). Distribution of poly(A)+RNA 3’-ends at genomic regions surrounding replication origins. Origins were oriented and anchored as in A. 3’-ends reads (Roy et al., 2016) of RNAs extracted from wild-type cells (WT, blue) or cells in which both Rrp6 and Dis3 were depleted from the nucleus (RRP6-DIS3-AA, transparent red) were plotted. At each position around the anchor, the presence or absence of an RNA 3’-end was scored independently of the read count. (C). Scheme of replication origins anchored at different ACS sequences. Left: sense polymerases transcribing upstream of primary ACSs (blue arrows) are colored in blue, while antisense polymerases transcribing upstream of secondary ACSs (orange arrows) are colored in red. Right: ARSs oriented according to antisense transcription were aligned at the 5’ ends of the primary ACSs (top, corresponds to red trace in D) or at the 5’ ends of the secondary ACSs (bottom, corresponds to black trace in D). (D). RNAPII PAR-CLIP metaprofile of antisense transcription aligned either to the 5’ ends of the primary (red) or the secondary (black) ACSs, as shown in (C). As in (A), the median number of RNAPII reads calculated for each position is plotted. (E). Distributions of RNA 3’-ends and RNAPII at genomic regions aligned at secondary ACSs. Origins were oriented and aligned as in (D). At each position around the anchor, presence or absence of an RNA 3’-end was scored independently of the read count (left y-axis). The distribution of RNAPII already shown in (C) is reported here for comparison (right y-axis).

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Figure 1—figure supplement 1. Measures on mapped secondary ACSs. (A) Average distribution of the distances between the main and the putative secondary ACS defined based on conformity to the consensus sequence defined in Coster and Diffley (2017) for every ARS. (B) Distribution of the average scores of main (blue) and putative secondary ACSs (red).

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**Figure 1—figure supplement 2.** Statistical analysis of pausing and termination signals. (A) Regions around the ACS used for the Box Plots represented in (B) and (C). (B) Distributions of RNAPII occupancy signal before and after the primary ACSs, as indicated. The 100 origins that had the highest levels of surrounding transcription were used for this analysis. (C) Same as in (B), but regions were aligned at the predicted secondary ACSs. (D) Statistical significance of the transcription termination peak at aligned primary ACSs. p-Values associated to the detection of the observed number of termination events were calculated under the H0 hypothesis that the frequency of termination events is equal in the whole alignment region (‘background termination’). The expected frequency of termination was estimated based on the frequency observed in a window of 100nt located 500nt upstream of the ACS. The negative Log of the corrected p-value is plotted on the y-axis. The red line represents the significance level (p=0.05). (E) Same as in (D), Figure 1—figure supplement 2 continued on next page
but origins were aligned at the 5’ ends of the predicted secondary ACSs. Note that in this case the significant termination peak is located downstream of the ACS and not immediately upstream, as in (D).
Figure 2. RNAPII occupancy at individual ARS detected by CRAC analysis. RNAPII occupancy at sites of roadblock detected upstream ARS305 (A), ARS413 (B), ARS431 (C) and ARS432.5 (or ARS453, (D)) by CRAC (Candelli et al., 2018). The pervasive transcriptional landscape at these ARSs is observed in wild-type cells (WT, blue) or cells bearing a mutant allele for an essential component of the CPF-CF transcription termination pathway (ma15-2, green) at permissive (25°C, dark colors) or non-permissive temperature (37°C, light colors). In the case of ARS305 (A), RNAPII occupancy is also shown in cells rapidly depleted for an essential component of the NNS transcription termination pathway through the use of an auxin-inducible degron tag (Nrd1-AID; (-) Auxin: no depletion, dark pink; (+) Auxin: depletion, light pink).

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Figure 3. Analysis of transcription termination at ARS305. (A) Scheme of the reporter system (Porrua et al., 2012) used to assess termination at ARS305. $P_{\text{TETOFF}}$: doxycycline-repressible promoter; $P_{\text{GAL}}$: GAL1 promoter. Termination of transcription at a candidate sequence (blue) allows growth on CANDELLI et al. eLife 2018;7:e40802. DOI: https://doi.org/10.7554/eLife.40802

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copper containing plates while readthrough transcription inhibits the GAL1 promoter and leads to copper sensitivity, as indicated. (B) Growth assay of yeasts bearing reporters containing a Reb1-dependent terminator, (Colin et al., 2014, used as a positive control), or ARS305 (lanes 1 and 3, respectively). Variants containing mutations in the Reb1 binding site (Reb1 BS ‘−’) or the ACS sequence are spotted for comparison (lanes 2 and 4, respectively). (C) Northern blot analysis of P_{TET} transcripts produced in wild-type and rpdΔ cells from reporters containing either a Reb1-binding site (Reb1 BS, lanes 1–2) or wild-type or mutant ARS305 sequences, as indicated (lanes 3–8). Transcripts terminated within ARS305 or at the CUP1 terminator are highlighted.

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Figure 3—figure supplement 1. ARS305 sequence confers mitotic maintenance to a centromeric plasmid when transcription is shut down. To assess the functionality of ARS305 in the reporter construct used for detecting transcription termination, we deleted the 2µ origin of the plasmid and transformed yeast in the presence or absence of doxycycline to control expression of the TET promoter. Transformants were only recovered in the absence of transcription, indicating that ARS305 is active but inactivated, as expected, when strong transcription runs through it. Candelli et al., Table 1.

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Figure 4. Role of ORC in the roadblock of RNAPII at origins. (A) Distribution of RNA 3'-ends at genomic regions aligned at ACS sequences recognized by ORC (ORC-ACS) as defined by Eaton et al. (2010) (i.e. defined based on the best match to the consensus associated to each ORC-ChIP peak).

Figure 4 continued on next page
Each origin was oriented according to the direction of the T-rich strand of its ORC-ACS and regions were aligned at the 5’ ends of the ORC-ACSs. As in 1B, RNA 3’-ends (Roy et al., 2016) were from transcripts expressed in wild-type cells (blue) or from cells depleted for exosome components (transparent red). At each position around the anchor, presence or absence of an RNA 3’-end was scored independently of the read count.

Distributions of RNA 3’-ends both on the sense (top) and the antisense (bottom) strands relative to the ORC-ACSs are plotted. (B) Same as in (A) except that genomic regions were aligned at ACS sequences not recognized by ORC (nr-ACS) as defined by Eaton et al. (2010) (i.e. defined as ACS motifs for which no ORC ChIP signal could be detected). (C) Quantification of the roadblock at individual ARSs. For each ARS, the snapshot includes the upstream gene representing the incoming transcription. The distribution of RNA polymerase II (dark blue) detected by CRAC (Candelli et al., 2018) at ARS404 (left) and ARS1004 (right) oriented according to the direction of their T-rich ACS strands is shown. The positions of the qPCR amplicons used for the RT-qPCR analyses in (D) are indicated. (D) RT-qPCR analysis of transcriptional readthrough at ARS404 and ARS1004. Wild-type, orc2-1, orc5-1 and cdc6-1 cells were cultured at permissive temperature and maintained at permissive (23°C, blue) or non-permissive (37°C, red) temperature for 3 hr. The level of readthrough transcription at ARS404 (left) or ARS1004 ACS (right) was estimated by the ratio of RT-qPCR signals after and before the ACS, as indicated. Data were corrected by measuring the efficiency of qPCR for each couple of primers in each reaction. Values represent the average of at least three independent experiments. Error bars represent standard deviation.

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Figure 5. Local pervasive transcription impacts origin competence and efficiency. Transcription levels were assessed in the first 100 nt of each ARS, starting at the 5’ end of the ACS, by adding RNAPII read counts (Schaughency et al., 2014) on both strands of the region. Origins were ranked based on transcription levels and the origins having the highest transcription levels (30/192, grey boxplots) were compared to the rest of the population (162/192, white boxplots). Origin metrics (licensing, 5A, and firing efficiency, 5B) for the two classes of origins were retrieved from Hawkins et al. (2013). Boxplots were generated with BoxPlotR (http://shiny.chemgrid.org/boxplotr/); center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range (IQR) from the 25th and 75th percentiles. Notches are 1.58*IQR/n^{1/2}. DOI: https://doi.org/10.7554/eLife.40802.011
Figure 6. Correlations between transcription and origin function. (A) Origins were first selected based on the levels of pervasive transcription to which they are exposed, calculated by adding RNAPII reads (Schaughency et al., 2014) over the 'A' (sense direction) or the 'C' (antisense direction) regions. For the selected ARSs, levels of pervasive transcription were then calculated over the 'B' region by summing RNAPII reads over the 'B_a' (sense direction) and the 'B_as' (antisense direction) regions, as indicated in the scheme. (B) Correlation between transcription over the ARS and origin competence. (C) Correlation between transcription over the ARS and origin efficiency. (D) Identification of two classes of origins, one that fires with high probability when licensing has occurred (high P_F|L, red dots) and the other that fires less efficiently once licensed (low P_FIL, black dots). (E) Correlation between P_FIL and transcription. The efficiency of firing at the post-licensing step correlates with the levels of pervasive transcription only for origins with low P_FIL (black dots). Origins that fire very efficiently once licensing occurred (P_F|L ≫ 1) are generally not sensitive to pervasive transcription (red dots). (F) Origins with a low P_FIL (black dots) have a firing time that correlates with pervasive transcription, while origins with high P_FIL (red dots) fire early independently of pervasive transcription levels.

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Figure 7. Asymmetry of origin sensitivity to pervasive transcription. (A) Top: pervasive transcriptional landscape detected by RNAPII CRAC (Candelli et al., 2018) at YLL026W (HSP104) and ARS1206 in wild-type cells, both on Watson (blue) and Crick (red) strands, at 25°C (dark colors) and 37°C (light colors).
colors). The 5’ ends and the sequences of the proposed primary ACS and the predicted secondary ACS for ARS1206 are shown. Bottom: schemes of the reporters containing the HSP104 gene and ARS1206 placed under the control of a doxycycline-repressible promoter (P_{TETOFF}). The position of the amplicon used for the qPCR in (B) is shown. pS and pAS differ for the orientation of ARS1206, with the primary (pS) or the secondary ACS (pAS) exposed to constitutive readthrough transcription from HSP104. The sequence and the organization of the relevant region are indicated on the right for each plasmid. The positions of the oligonucleotides used for RNaseH cleavage (black arrows) and of the probe used in (C) are also indicated. The sequences of the oligonucleotides is reported in Table 1, with the following correspondence: cleaving oligo ‘a’=DL163; Northern probe = DL164; cleaving oligo ‘b’ = DL473; cleaving oligo ‘c’ = DL3991; cleaving oligo ‘d’ = DL3994. (B). Quantification by RT-qPCR of the HSP104 mRNA levels expressed from pS or pAS in the presence or absence of 5 μg/mL doxycycline. The position of the qPCR amplicon is reported in (A). (C). Northern blot analysis of HSP104 transcripts extracted from wild-type cells and subjected to RNase H treatment before electrophoresis using oligonucleotides ‘a-d’ (positions shown in A). All RNAs were cleaved with oligonucleotide ‘a’ to decrease the size of the fragments analyzed and detect small differences in size. Cleavage with oligonucleotide ‘b’ (oligo-dT) (lanes 3, 4) allowed erasing length heterogeneity due to poly(A) tails. Oligonucleotides ‘c’ and ‘d’ were added in reactions run in lanes 1 and 6, respectively, to detect possible longer products that might originate from significant levels of transcription readthrough from HSP104, if the inversion of ARS1206 were to alter the transcription termination efficiency. Products of RNAse H degradation were run on a denaturing agarose gel and analyzed by Northern blot using a radiolabeled HSP104 probe (position shown in A). (D). Stability of plasmids depending on ARS1206 for replication as a function of ARS orientation. pS or pAS was transformed in wild-type cells and single transformants were grown and maintained in logarithmic phase in YPD for several generations. To assess the loss of the transformed plasmid, cells were retrieved at the indicated number of generations and serial dilutions spotted on YPD (left) or minimal media lacking uracile (right) for 2 or 3 days, respectively, at 30°C. (E). Mutation of ORC2 affects more severely the stability of pAS compared to pS. Transformation of pS and pAS in wild-type (ORC2, ‘−’) or mutant (orc2-1, ‘+’) cells. Pictures were taken after 5 days of incubation at permissive temperature (23°C). DOI: https://doi.org/10.7554/eLife.40802.013

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