Figure S1

A. Graph showing cell growth over time with Log, DS, Day 2, Day 3, and Day 7.

B. Western blot images of Q cells indicating H3K4me1, Actin, H3K4me2, Actin, H3K4me3, Actin, TBP, and Srb4.

C. Western blot images of Log and Q showing Rpb3-Myc, CTD-Ser5P, CTD-Ser2P, and Actin.

D. Western blot images of Log and Q showing TBP, Srb4, and Actin.

E. Western blot images of LogDS and NQ showing H3, H3K9/14ac, H4tetraAc, H4K16ac, H3K56ac, and Actin.

F. Bar graph showing levels normalized to H3 for Log, DS, Q, NQ, and 7Q.
Figure S2
Figure S3
### A

|            | Log   | Q    |
|------------|-------|------|
| All RNAs   | 6205  | 5105 |
| ORFs       | 5402  | 4347 |
| CUTs       | 189   | 120  |
| SUTs       | 184   | 210  |
| snRNAs     | 84    | 84   |

### B

**RNA**

| GO-Terms: Log cells only               |
|----------------------------------------|
| RNA processing                        |
| ribosomes                              |
| regulation of translation              |
| nuclear-cytoplasmic transport          |
| mitosis                                |
| budding/cell polarity                  |
| spindle                                |
| DNA synthesis                          |

| GO-Terms: Q cells only                 |
|----------------------------------------|
| response to heat                       |
| peroxisome                             |
| protein catabolism                     |
| glucose/hexose metabolism              |
| fatty acid oxidation                   |
| sporulation                            |

| GO-Terms: Common to Log & Q cells      |
|----------------------------------------|
| transposition/DNA integration          |
| protease activity/proteasome           |
| ion transport                          |
| DNA recombination                      |
| DNA polymerase activity/general nucleases |
| ribosome/translation                   |
| transcription                          |
Figure S5
**SUPPLEMENTAL FIGURE LEGENDS**

**Figure S1: Levels of histone methylation and acetylation, and preinitiation complex factors in quiescent cells.**

A. Change in cell number and O.D._600 during growth from log phase (Log) into stationary phase relative to the diauxic shift (DS), which was set as 1. B. TCA lysates were prepared from log phase cells and purified Q cells isolated 3, 5, and 7 days after culture inoculation. Western blots were probed with antibodies against H3K4me1, me2, and me3. C-D. TCA lysates were prepared from log cells and 7-day Q cells and probed with antibodies against (C) Rpb3-Myc and RNAP II CTD phosphorylated on Serine 5 (CTD-Ser5P) and Serine 2 (CTD-Ser2P), and (D) TBP and Mediator subunit, Srb4. (E) TCA lysates were prepared from cells in log phase (Log), at the diauxic shift (DS), and in purified populations of quiescent (Q) and non-quiescent (NQ) cells isolated 7 and 14 days after culture inoculation. Western blots were probed with antibodies against acetylated histones and histone H3. The H3 and actin blots are those shown in Fig. 1A, as the same lysates were used for both analyses. Actin served as a loading control in all blots. **F.** Band intensities of H3 PTMs in the western blots shown in Fig. 1A were quantitated using the program, Image Studio. The band intensity for each PTM was normalized to the intensity of histone H3 in the same cell sample, and further normalized to the PTM/H3 ratio in log cells, which was set as 1.

**Figure S2: Association of RNAP II, H3K4me3, H3K36me3, and H3K79me3 with genes in quiescent cells.**

A. Genome browser images of RNAP II (Rpb3-Myc), H3K4me3, H3K36me3, H3K79me3, and H3 ChIP-chip data in log and 7-day Q cells. B. Number of genes marked with RNAP II and the three H3 methylations in log and 7-day Q cells. C. Gene ontology (GO) categories for unique and commonly marked genes in log and Q cells that are associated with RNAP II, H3K4me3, H3K36me3, and H3K79me3.

**Figure S3: Gene association profiles of RNAP II and H3 PTMs in log and quiescent cells.**

A, B. Average enrichment of RNAP II on common genes, log genes, and Q genes in log and Q cells. Profiles were defined using the top 25% RNAP II associated common genes in log cells (A). and in Q cells (B). The 250 bp regions upstream of the TSS (-250) and downstream of the TES (+250) are indicated. Solid line, log-specific RNAP II enrichment; dashed line, Q specific RNAP II
enrichment; dotted line, RNAP II enrichment on common genes. C. Divergent IGRs bound by RNAP II were characterized for the presence of SUTs, CUTs, and RNAs associated with yeast transposons (Table S4). Prom represents IGRs that do not contain these transcripts and defines RNAP II bound upstream of ORFs at presumptive gene promoters. D-G. Average enrichment of RNAP II (D), H3K4me3 (E), H3K36me3 (F), and H3K79me3 (G) on genes associated with RNA only in log cells (solid line) or only in Q cells (dashed line). The top 20% of genes in log or Q cells associated with RNA TPMs >10 were analyzed.

Figure S4: Categorization of ORF RNAs in quiescent cells.
A. The number of transcripts present in log and Q cells were divided into 4 categories: ORF (open reading frame); CUT (cryptic unannotated transcript); SUT (stable unannotated transcript); and snRNA (small nuclear RNA). B. Gene ontology (GO) categories for unique and commonly marked genes in log and Q cells that are associated with ORF RNAs.

Figure S5. Correlation between RNAP II and H3 methylations on genes in log and Q cells.
Scatterplots showing the correlation between RNAP II (A), H3K4me3 (B), H3K36me3 (C), and H3K79me3 (D) signals on genes in log and Q cells. Red = genes enriched only in log cells; green = genes enriched only in Q cells; blue = genes enriched in both log and Q cells.

Figure S6: Transcript, RNAP II, and H3 methylation profiles of individual genes during the development of quiescence.
Log cells and purified Q cells isolated 3, 5, and 7 days after culture inoculation were analyzed for transcript levels and Rpb3-Myc, H3K4me3, H3K36me3, and H3K79me3 occupancy at (A) a log-only expressed gene, CLN3; (B) a Q-only expressed gene, SNZ1; and (C) a gene expressed in both cell types, OLE1. Transcript levels represent the change in RNA abundance relative to levels in log cells. Rpb3-Myc and H3 PTM occupancies were determined by ChIP and qPCR at the TATA, 5' ORF or 3' ORF regions of each gene. Relative occupancy represents the IP/Input at each position relative to IP/Input at TELV (Rpb3-Myc) or to H3 IP/Input (H3K4me3, H3K36me3, H3K79me3). The data represent the average with STD from 2 independent experiments.
Figure S7: RNAP II CTD phosphorylation profiles of individual genes during the development of quiescence.

A. RNAP II CTD Serine 5 and Serine 2 phosphorylation occupancy at the 5’ ORF (Ser5-P) or 3’ ORF (Ser2-P) of genes associated with RNA only in log cells, only in Q cells, and in both cell types (common). B. H3 occupancy at genes associated with RNA only in log cells (PMA1), only in Q cells (SNZ1), or in both cell types (BAP2). Relative occupancy represents the IP/Input ratio at each gene relative to the IP/Input ratio at TELV. The data represent the average with STD from 2 independent experiments. C. Boxplots showing the average enrichment of RNAP II and the 3 H3 PTMs on genes associated with RNA in both log and 7-day Q cells (common genes). D. Genome browser view of RNAP II, H3K4me3, H3K36me3, H3K79me3, and H3 occupancy on genes associated with RNA only in log cells (CLN3), only in 7-day Q cells (XBP1), and in both log and 7-day Q cells (BAP2).

SUPPLEMENTAL TABLE LEGENDS

Table S1: List of Saccharomyces cerevisiae strains.

Table S2: Lists of genes marked with H3K4me3, H3K36me3, H3K79me3, and RNAP II in growing and quiescent cells. Genes marked only in growing cells (Log), only in quiescent cells (Q), or in both cell types (Common) were identified as described in SUPPLEMENTAL METHODS.

Table S3: Gene ontology (GO) categories for genes associated with Rpb3-Myc (RNAP II), H3K4me3, H3K36me, and H3K79me3 in growing and quiescent cells. The DAVID functional annotation tool was used to identify categories of genes enriched only in log cells, only in 7-day Q cells, or in both cell types (Common), P value ≥ 10^-4.

Table S4: Association of Rpb3-Myc with intergenic regions in growing and quiescent cells. The association of Rpb3-Myc (RNAP II) with divergent intergenic regions (IGRs) in growing (log) and 7-day quiescent cells (Q) was determined as described in SUPPLEMENTAL METHODS. The table contains columns with information about ChIP enriched regions (CHERS) that includes chromosome, start and end position of the IGR, and score. Additional columns contain information on the percent coverage of the IGR, GCR (gene coding region), or “other” region with the RNAP II CHER. The feature with the highest percentage of coverage is listed in the
“dominant feature” column, followed by the % coverage of the IGR with CUTs, SUTs, and RNAs, and peak RNAP II enrichment in log and Q cells.

Table S5: RNA-Seq data. Data were collected from 3 independent RNA-seq experiments conducted on total RNA isolated from growing (log) and 7-day quiescent cells (Q). The table contains a column with gene information followed by columns with conversions to TPM numbers of the merged values for the 3 log and Q replicates. The last column contains the log2 ratio of the merged log TPM values divided by the merged Q TPM values.

Table S6: Lists of genes associated with transcripts in growing and quiescent cells. Genes associated with RNA only in growing cells (Log), only in 7-day quiescent cells (Q), or in both cell types (Common) were identified as described in SUPPLEMENTAL METHODS.

Table S7: Gene ontology (GO) categories for genes associated with transcripts in growing and quiescent cells. The DAVID functional annotation tool was used to identify categories of genes associated with transcripts only in growing cells (log), only in 7-day quiescent cells (Q), or in both cell types (Common), with P values ≥ 10^{-4}.

SUPPLEMENTAL METHODS

CHIP-CHIP DATA PROCESSING

An adapted version of the Model-based Analysis of Tiling Arrays (MAT) algorithm for the R programming environment (rMAT) was used to normalize probe intensities (Droit et al., 2010). Each of the four ChIP DNAs (RNAP II, H3K36me3, H3K79me3 and H3K4me3) was normalized to input DNA, the resulting values were standardized, and biological replicates were averaged. Pearson correlation coefficients of the log2-transformed probe intensities were consistently above 0.9 between replicates. For each probe, the MAT scores were calculated as in Schulze et al. (2011) with a sliding window of 300 bp using a customized Perl script (available on GitHub, https://github.com/khokamp/Perl-scripts). The resulting scores were visualized across the genome using the JBrowse genome browser. ChIP enriched regions (CHERs) were computed using a customized Perl script (available on GitHub, https://github.com/khokamp/Perl-scripts). CHERs were computed using a 150 bp sliding window and were required to have a minimum average MAT score of 1.5.
Open reading frames (ORFs) were obtained from the *Saccharomyces* Genome Database (SGD) version R64.2.1. Only ORFs that were characterized as not ‘Dubious’ were used for the analysis. For RNAP II, H3K36me3 and H3K79me3, an ORF was defined as ChIP enriched when a CHER overlapped at least 50% of the ORF. For H3K4me3, the region around the transcription start site (TSS) (+200/-100 bp) was analyzed for ChIP enrichment. The overlap between CHERs and ORFs/TSS regions was computed using BEDTools (Quinlan and Hall, 2010). Using these criteria, ORFs that were ChIP enriched in either log phase or quiescent cells, but where ChIP binding was entirely absent in the other, were defined as log- or quiescent-specific, respectively. ORFs common to both log and quiescent cells showed ChIP enrichment in both cell populations (Table 1).

| ChIP condition | Number Log | % Log | Number Q | % Q | Number Common | % Common | Number Unenriched | % Unenriched |
|----------------|------------|-------|----------|-----|---------------|----------|-------------------|--------------|
| RNAP II        | 1219       | 21    | 265      | 4.5 | 649           | 11.2     | 3664              | 63.2         |
| H3K4me3        | 664        | 11.4  | 240      | 4.1 | 3959          | 68.3     | 934               | 16.1         |
| H3K36me3       | 216        | 3.7   | 227      | 3.9 | 2358          | 40.7     | 2996              | 51.7         |
| H3K79me3       | 163        | 2.8   | 226      | 3.9 | 2040          | 35.2     | 3368              | 58.1         |

*Table 1.* Number of ORF regions with ChIP enrichment. An ORF was defined as ChIP-enriched if the ChIP enriched region (CHER) overlapping a given ORF had a minimum average MAT score of 1.5 and coverage of 50%. For H3K4me3, ChIP enrichment was measured in a region that encompassed +100 bp upstream and +200 bp downstream of the TSS, with 50% coverage.

Box plots, scatter plots and profile plots were all generated through the R statistical software (https://www.r-project.org/). For averaged gene profile plots the gene body and surrounding regions were organized into bins (40 for gene body, 20 each for upstream and downstream areas of 250 bp) to adjust for varying gene lengths. The Chromatra tool was utilized as described in Schulze et al. (2009).

**Definition of IGRs**

Intergenic regions (IGRs) that separate divergently transcribed ORFs were identified as described in Radonjic et al. (2005). Only ORFs with classification “verified” or “uncharacterized” were considered. GCRs represent ORFs. Any other regions in the genome not covered by IGRs or GCRS were classified as “other”. The percentage coverage of Rpb3-Myc in IGRs, GCRs, and “other” regions was calculated from the Rpb3-Myc CHERs. IGR regions bound by Rpb3-Myc were based on 50% coverage of IGR and 50% coverage of adjacent GCR.
**RNA-seq Data Processing**

Reads were aligned and then assigned to genes in the yeast genome using a Perl script (available on GitHub, https://github.com/khokamp/Perl-scripts). The Pearson correlation coefficients of the log2-transformed read counts for the three log or Q cell replicates were consistently above 0.96. Read counts were transformed into TPMs (Transcript Per Million) following the algorithm described in Li et al. (2010). TPM values from replicates were merged using the mean, and the ratios between log and Q cells were calculated as the difference on the log2 scale of the merged values. Density plots of the log2-transformed TPM values showed a bimodal distribution, and the minimum between the two peaks in log cells was used to determine a threshold separating expressed genes from background noise (Fig. 1). Transcripts with TPM ≥2 were used for analysis.

![Density plots of log2-transformed TPM values.](image)

**Figure 1:** Density plots of log2-transformed TPM values.

**Supplemental References**

Droit, A, Cheung, C, and Gottardo, R. 2010. rMAT – an R/Bioconductor package for analyzing ChIP-chip experiments. *Bioinformatics* 1; **26**:678-9.

Li B, Ruotti V, Stewart RM, Thomson JA, Dewey CN. 2010. RNA-Seq gene expression estimation with read mapping uncertainty. *Bioinformatics* **26**: 493-500.
Schulze JM, Jackson J, Nakanishi S, Gardner JM, Hentrich T, Haug J, Johnston M, Jaspersen SL, Kobor MS, Shilatifard A. 2009. Linking cell cycle to histone modifications: SBF and H2B monoubiquitination machinery and cell-cycle regulation of H3K79 dimethylation. *Mol Cell* 35: 626-641.

Quinlan, AR, Hall, IM. 2010. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* 26:841-842.
Table S1: Saccharomyces cerevisiae strains

| Strain | Genotype |
|--------|----------|
| FY406  | MATa, (hta1-htb1)Δ::LEU2, (hta2-htb2)Δ::TRP1, his3Δ200, leu2Δ1, trp1Δ, ura3-52, <pSAB6 (HTA1-HTB1::URA3)> <pZS145 (HTA1-Flag-HTB1::HIS3)> |
| YCH406 | MATa, (hta1-htb1)Δ::LEU2, (hta2-htb2)Δ::TRP1, his3Δ200, leu2Δ1, trp1Δ, ura3-52, RPB3-9Myc::hphNT1, <pSAB6 (HTA1-HTB1::URA3)> <pZS145 (HTA1-Flag-HTB1::HIS3)> |
| YCY1   | MATa, (hta1-htb1)Δ::LEU2, (hta2-htb2)Δ::TRP1, his3Δ200, leu2Δ1, trp1Δ, ura3-52, DOT1-9myc::KANMX, <pSAB6 (HTA1-HTB1::URA3)> <pZS145 (HTA1-Flag-HTB1::HIS3)> |
| YCY5   | MATa, (hta1-htb1)Δ::LEU2, (hta2-htb2)Δ::TRP1, his3Δ200, leu2Δ1, trp1Δ, ura3-52, SET1-9myc::KANMX, <pSAB6 (HTA1-HTB1::URA3)> <pZS145 (HTA1-Flag-HTB1::HIS3)> |
| YCY6   | MATa, (hta1-htb1)Δ::LEU2, (hta2-htb2)Δ::TRP1, his3Δ200, leu2Δ1, trp1Δ, ura3-52, SET2-9myc::KANMX, <pSAB6 (HTA1-HTB1::URA3)> <pZS145 (HTA1-Flag-HTB1::HIS3)> |
| YAF1   | MATa, hhf2-hht2::NAT, hta1-htb1::HPH, hht1-hhf1::KAN, hta2- htb2::NAT, ura3-52, trp1Δ2, leu2-3,-112, his3-11, ade2-1, can1-100; <pRS315 (HTA1-HTB1, HHT1-HHF1::LEU2)> |
| YAF3   | MATa, hhf2-hht2::NAT, hta1-htb1::HPH, hht1-hhf1::KAN, hta2- htb2::NAT, ura3-52, trp1Δ2, leu2-3,-112, his3-11, ade2-1, can1-100; <pRS315 (HTA1-HTB1, hht1-K4A-HHF1::LEU2)> |
| YAF9   | MATa, hhf2-hht2::NAT, hta1-htb1::HPH, hht1-hhf1::KAN, hta2- htb2::NAT, ura3-52, trp1Δ2, leu2-3,-112, his3-11, ade2-1, can1-100; <pRS315 (HTA1-HTB1, hht1-K79A-HHF1::LEU2)> |
| YAF11  | MATa, hhf2-hht2::NAT, hta1-htb1::HPH, hht1-hhf1::KAN, hta2- htb2::NAT, ura3-52, trp1Δ2, leu2-3,-112, his3-11, ade2-1, can1-100; <pRS315 (HTA1-HTB1, hht1-K123R, HHT1-HHF1::LEU2)> |
| YAF97  | MATa, hhf2-hht2::NAT, hta1-htb1::HPH, hht1-hhf1::KAN, hta2- htb2::NAT, ura3-52, trp1Δ2, leu2-3,-112, his3-11, ade2-1, can1-100; <pRS315 (HTA1-HTB1, hht1-K4A/K79A-HHF1::LEU2)> |
| YAF105 | MATa, hhf2-hht2::NAT, hta1-htb1::HPH, hht1-hhf1::KAN, hta2- htb2::NAT, ura3-52, trp1Δ2, leu2-3,-112, his3-11, ade2-1, can1-100; <pRS315 (HTA1-HTB1, hht1-K4A/K79A-HHF1::LEU2)> |