High-temporal-resolution view of transcription and chromatin states across distinct metabolic states in budding yeast

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Under continuous, glucose-limited conditions, budding yeast exhibit robust metabolic cycles associated with major oscillations of gene expression. How such fluctuations are linked to changes in chromatin status is not well understood. Here we examine the correlated genome-wide transcription and chromatin states across the yeast metabolic cycle at unprecedented temporal resolution, revealing a ‘just-in-time supply chain’ by which components from specific cellular processes such as ribosome biogenesis become available in a highly coordinated manner. We identify distinct chromatin and splicing patterns associated with different gene categories and determine the relative timing of chromatin modifications relative to maximal transcription. There is an unexpected variation in the chromatin modification and expression relationship, with histone acetylation peaks occurring with varying timing and ‘sharpness’ relative to RNA expression both within and between cycle phases. Chromatin-modifier occupancy reveals subtly distinct spatial and temporal patterns compared to those of the modifications themselves.

Chromatin has fundamental roles in DNA-related processes, including transcription, replication, recombination and repair. For example, most histone acetylations and certain methylations (for example, K4- or K36-trimethylated histone H3 (H3K4me3 or H3K36me3, respectively)) are correlated with active transcription, whereas deacetylation and other methylations are correlated with repression. The pervasiveness of biological dynamics suggests that temporal interrogation of chromatin function is warranted. Compensatory deacetylation and other methylations are correlated with active transcription, whereas transcription-factor binding and RNA expression9–11, and they support a dynamic role for histones (and perhaps other proteins). Studies on acetyl CoA, a temperature-sensitive mutant, and examination of bulk acetylation upon depletion of acetyl CoA in a temperature-sensitive mutant. Examination of chromatin state and RNA expression level at single genes upon activation has revealed many dynamic changes, such as the sequential appearance of K123-ubiquitinated histone H2B (H2BK123ub) and H3K4me3 at GAL1 during galactose induction. Genome-wide ChIP-seq studies have revealed temporally dynamic chromatin patterns in circadian rhythm, heart development, yeast meiosis and other dynamic processes. These patterns have been correlated with transcription-factor binding and RNA expression9–11, and they support a generally dynamic role for chromatin in gene regulation. However, to our knowledge, no prior studies have had sufficient temporal resolution to dissect the specific roles of individual histone modifications during distinct steps in transcription in vivo, and the scope of genes regulated in these paradigms on a fast time scale has been limited.

Here we exploit a uniquely informative dynamic system, the yeast metabolic cycle (YMC), to examine the regulation of >3,000 genes in Saccharomyces cerevisiae. Under continuous glucose-limited conditions, yeast exhibits a ~4- to 5-h respiratory cycle with >3,000 transcripts and dozens of metabolites oscillating at the same pace. Although nearly all cycling genes share the same period of oscillation, the YMC can be broadly divided into three phases on the basis of the defining expression profile: OX (oxidative), RB (reductive building) and RC (reductive charging). The overall logic of the YMC is well defined: growth genes, such as ribosomal and amino acid biosynthesis genes are activated in OX phase; mitochondria and cell-cycle genes are expressed in RB; and genes responding to starvation, stress and survival are elevated in RC. These divergent expression profiles imply that the dynamic interaction between chromatin modifications and transcription across distinct biological processes can be queried in a single system. Additionally, acetyl CoA, a dynamically oscillating metabolite, is a critical metabolic signal driving the YMC via acetylation of histones (and perhaps other proteins). Studies on acetyl CoA, S-adenosylmethionine and other metabolites directly involved in histone modification have indicated that chromatin could have key roles in coordinating metabolism and gene expression2,15. Given such prevalent orchestration of expression and metabolites in the YMC, investigating the dynamic patterns of chromatin state could be useful for gaining a systems-level understanding of the YMC and beyond, such as how cells respond to a wide variety of nutrient and other biological stimuli through chromatin modifications. Discoveries made during distinct steps in transcription in vivo, and the scope of genes regulated in these paradigms on a fast time scale has been limited.

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in the YMC could also extend the understanding of other cycling systems, such as circadian clocks.

We set out to do time-course chromatin immunoprecipitation and sequencing (ChIP-seq) of histone modifications and modifiers in the YMC. We reveal the genome-wide landscape of seven histone modifications across the YMC and compare it to a high-resolution RNA sequencing (RNA-seq) profile and dynamic localization of three critical chromatin modifiers: Gcn5p, Esa1p and Set1p. High sampling frequency allowed definition of clear-cut subphases of gene expression in each phase, such as the sequential activation of ribosome-biogenesis (ribi) and ribosomal protein (RP) genes during the growth phase. We also defined seven distinct chromatin patterns associated with genes of different functional classes, revealing the diversity of histone modification pathways during transcription. We observed that binding of certain chromatin modifiers does not always correlate with the appearance of the corresponding histone modifications, thus suggesting sophisticated temporal regulation. Inspired by the pervasiveness of the changes on the H3 and H4 N termini during the YMC, we evaluated mutants in H3 and H4 modificlysines and showed that only certain multilysine mutants abolish the YMC, thus implying high system redundancy. Together, these data provide a comprehensive and high-temporal-resolution view of the dynamics of transcription and chromatin states. As such, they reveal how transcription and chromatin modifications are coordinated for different classes of genes during distinct phases of the life of a yeast cell.

RESULTS
Gene expression patterns in the YMC

Microarray-based transcriptome analysis previously revealed three superclusters of genes defined by expression pattern in the YMC12. Because of the short duration of the OX growth phase (~0.5 h), the previous sampling strategy used (evenly spaced every ~25 min and spread over three cycles) may have missed important transient regulatory events. To improve temporal resolution, we collected 16 samples across one cycle for RNA-seq by increasing the density of time points taken in the OX phase (Fig. 1a). We observed the same three superclusters of genes, results in excellent agreement with the prior data (Supplementary Fig. 1a and Supplementary Table 1). We also identified an additional ~500 genes with a clear expression peak in the OX phase from the ‘nonperiodic’ genes defined previously (Supplementary Fig. 1b and Supplementary Table 1). Gene Ontology (GO) analysis indicated enrichment of ribosome-biogenesis genes. Many showed hints of periodic expression in the microarray data set but evaded detection by the periodicity algorithm, owing to less frequent sampling.

Thanks to the high temporal resolution, we resolved several temporal subclusters associated with distinct biological functions within previously defined OX, RB and RC superclusters (Fig. 1b–d). We subdivided OX-phase genes into OX1, defining an earlier and less frequent sampling.

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peaked ~5 min later. We confirmed the subtle differences between ribi and RP genes by reverse transcription followed by quantitative real-time PCR (RT-qPCR) (Supplementary Fig. 2f). This observation suggested precise temporal control of the energetically demanding process of ribosome biogenesis. Ribosomal assembly factors and RNA-processing factors were expressed just before ribosomal proteins and translational factors, thus providing a possible example of just-in-time orchestration of a critical process in the YMC leading to increased translational capacity. We also observed three temporal subclusters of RB and RC genes (Fig. 1c,d and Supplementary Fig. 1c). Cell-cycle and mitochondrial genes were readily distinguishable, although the two processes both occur under similar metabolic states. Collectively, the RNA-seq results not only recapitulated known gene expression patterns but also displayed elaborate and fine-grained temporal control of coordinated sequential processes.

Accumulation of precursor mRNAs in the OX phase

Besides the temporal compartmentalization of gene expression, RNA-seq analysis also revealed an unexpected pattern in intron sequence accumulation. As discussed previously, RP genes are activated in the OX phase, peaking at time point (t) 5. Surprisingly, we observed a dynamic intron signal in several intron-containing RP genes consistently peaking at t4 (Fig. 2a). Several other intron-containing OX genes showed similar patterns (Fig. 2a).

We evaluated RNA-seq reads across exon and intron regions separately for all intron-containing genes, displayed intron and full-length signals in a heat map and identified six clusters (Fig. 2b and Supplementary Table 2). Among them, only intron 1 showed temporal enrichment of intron signals in the OX phase. 89 of the 103 genes in intron 1 are RP genes. Both the intron signals and the ratio of intron/exon signals peaked at t4 (Fig. 2b and Supplementary Fig. 2a).
During RNA splicing, intron 5' ends are cleaved and join to the branch point in the intron, to form a lariat. To distinguish intron accumulation as lariats or precursor mRNA (pre-mRNA), we examined exon-exon and intron-exon junction reads of the 89 RP genes. As expected, exon-exon junction reads were highest at t5, in agreement with the timing of exon signals (Fig. 2c). However, 5' intron junction reads, representing the pre-mRNA form, peaked at t4 (Fig. 2d). Additionally, we examined individual RP genes and discovered that the majority of the RP genes exhibited the same pattern (Supplementary Fig. 2b–d). RT-qPCR of four RP genes showed that the peaks of

**Figure 3** Dynamic chromatin states across the YMC. (a) Oscillation of oxygen in one YMC. Cycling cells were collected at 16 intentionally uneven time points over one cycle for ChIP-seq. (b,c) Temporal relationship between RNA level and histone modifications at the RMT2 locus. (b) RNA level of RMT2 from normalized RNA-seq data. (c) CisGenome browser views of ChIP-seq of H3K9ac, H3K14ac, H4K5ac, H3K56ac, H3K4me3, H3K36me3, H4K16ac and H3 at RMT2. 16 tracks represent 16 time points from top to bottom consecutively. RMT2 is encoded on the Crick strand, labeled by the red arrows pointing to the left.
introns were consistently ahead of the peaks of exon signals by a mean time of 7.3 min ($P = 0.0286$ by two-sided Wilcoxon rank-sum test) (Supplementary Fig. 2f).

Interestingly, t4 marks a strikingly reproducible inflection point in the O$_2$ curve of unknown functional importance occurring near mid-OX (Fig. 1a). These data suggested exquisite temporal control of RP-gene splicing—RP-gene appearance to be transcribed at t4, but the corresponding mature spliced RP-gene transcripts do not emerge until 5–7 min later at t5. Moreover, prior studies demonstrated accumulation of RP pre-mRNAs under stress conditions in yeast$^{16,17}$. This delay in RP-gene mRNA splicing in the YMC suggests that yeast might suffer a certain level of amino acid or other stresses during early OX (i.e., t4). Alternatively, RP mRNAs may be spliced only on cue, after proper expression of CK2 components at t4, t5 (Supplementary Fig. 2e).

**Dynamics of histone modifications across the YMC**

Given the transcriptome dynamics and close dependence of transcription on chromatin modification, we next investigated chromatin states across the YMC. We collected a similar set of 16 samples across one cycle for ChIP-seq (Fig. 3a) and assessed seven histone modifications, including H3K4me3, H3K36me3 and acetylated K9, K14 and K56 on H3 and K5 and K16 on histone H4 (H3K9ac, H3K14ac, H3K56ac, H4K5ac and H4K16ac, respectively) and H3 as control. Most of these are associated with active transcription$^{3,18}$. H3K56ac also functions in DNA replication and repair$^{19}$, and H4K16ac is involved in silencing maintenance and lifespan$^{20–22}$. We computationally aligned the two time series by performing ChIP-seq for H3K9ac on both sets of samples used for RNA- and ChIP-seq (Online Methods).

We determined the spatial and temporal signals at the RMT2 locus (Fig. 3c), which encodes an arginine methyltransferase for ribosomal protein Rpl12 (ref. 23). Its expression peaked in mid-OX phase (Fig. 3b). Mapping of ChIP-seq signals across RMT2 revealed a typical spatial pattern$^5$ in which H3K9ac, H3K14ac, H4K5ac and H3K4me3 signals peaked in the 5′ end, whereas the H3K36me3 signal was located in the coding region (Fig. 3c). Although all positively correlated with transcription, the kinetics and relative timing were quite distinct at this locus. The H3K9ac signal was dynamic, with a transient peak at t4. Signals for K14-acetylated H3 (H3K14ac) and H4K5ac also increased in OX phase with temporally broader peaks than for H3K9ac. Instead, the H3K4me3 signal was much more stable than these acetylation marks, with clear-cut peaks across all 16 time points. It started to increase only in late OX at a modest level and peaked in early RB, after acetylation peaks. No other modifications were obviously dynamic at RMT2.

Expanding the examination to broader regions revealed another unexpected difference between modifications. Most H3K9ac peaks were in OX phase, despite the presence of nearby RB- and RC-phase genes, consistently with a previous study showing that H3K9ac appeared preferentially at OX growth genes (Supplementary Fig. 3). RC and stress-response genes were less dependent on H3K9ac (ref. 14). Instead, we found H3K14ac, H4K5ac and H3K4me3 peaks at most genes indiscriminately. Strikingly, H3K9ac peaks seemingly appeared and disappeared, completely coupled with the transcription of neighboring genes, whereas H3K14ac, H4K5ac and H3K4me3 displayed only an adjustment of amplitude. The data suggest that H3K9ac is a very active modification that regulates mainly OX growth genes, possibly by directly sensing metabolic stimuli. Others, like H3K14ac, H4K5ac and H3K4me3, have a more general role in transcription regulation (further examples in Supplementary Fig. 4).

Next, we sought to determine average genome-wide chromatin patterns for various gene classes. First, we averaged the spatial signals of these modifications relative to the transcription start sites (TSSs) across the genome (Supplementary Fig. 5a). We calculated gene-specific signals on the basis of their localization and used a heat map to display the genome-wide interactive temporal patterns (Supplementary Fig. 5b and Supplementary Table 3). Generally, acetylation signals were more dynamic and less noisy than methylation signals, consistently with acetylation shifts reflecting the immediate-chromatin response under continuous glucose limitation. We observed fourteen defined clusters suggesting complex dynamic relationships among these modifications across the YMC.

Intriguingly, H3K56ac exhibited very dynamic but noticeably different temporal patterns compared to other histone modifications. By comparing the results to previous work$^{24}$, we observed clear cell cycle–related dynamics of H3K56ac (Fig. 4). H3K56ac was strongly elevated in early S phase; similarly, YMC signals peaked at t8, the middle of RB phase, coinciding with S-phase entry. Correspondingly, DNA-replication genes (RNA_RBI) were also expressed maximally at t8 (Fig. 1c). The expression level of H3K56 acetyltransferase RITT109 peaked at t8, whereas RNA levels of H3K56 deacetylase HST3 began to increase after t8 (Supplementary Fig. 1d). These data corroborated known involvement of H3K56ac in DNA replication in RB and supported the relationship between the cell cycle and YMC.

**Temporal association between transcription and chromatin**

An important function for histone modifications is transcriptional regulation$^2$. With this in mind, we examined the genome-wide temporal relationships between each chromatin modification and transcription. Given the correlation analysis (Online Methods) and previous studies$^3$, we focused on five modifications most clearly linked to transcription (Fig. 5). We discovered seven clusters of cycling genes (Fig. 5a and Supplementary Table 4). Are there different histone modification pathways associated with transcription of different gene clusters? To tackle this question, we estimated the relative timing of
Figure 5 Temporal combinatorial patterns of chromatin states and gene expression define functionally related genes. (a) Heat map showing seven clusters of cycling genes (combining Ox-, Rb- and RC-phase genes ordered by the time of peak gene expression. RNA, H3K9ac, H3K14ac, H4K5ac, H3K4me3 and H3K36me3 are included in this analysis, and each is displayed in a joint column, separated by white lines. The data are standardized to a mean of zero. Pie charts on the left indicate composition of Ox (magenta), Rb (green) and RC (blue) genes in each cluster. Enriched GO categories are listed on the right. (b,c) Estimation of time in YMC when RNA and histone modifications in RNA_H1 (b) and RNA_H2 (c) reach the maximum. The middle of the horizontal colored lines on the O2 curves represent the mean peak values of genes in each cluster, and the lengths represent s.d. for 464 and 516 genes in b and c, respectively. (d) Models of histone modification pathways during the transcription of aa and ribosomal genes. Purple ovals represent –1, +1 and subsequent open-reading-frame nucleosomes for the indicated gene. Hooked arrows represent transcription, and spots represent histone modifications.

Each modification peak relative to its corresponding RNA peak and examined GO enrichment on all clusters.

In clusters RNA_H1 and RNA_H2, the H3K9ac peak notably appeared just minutes before gene expression peak (9 and 6.8 min in clusters 1 and 2, respectively) whereas the H3K4me3 and H3K36me3 peaked 10–30 min after the peak of RNA (Fig. 5b,c). Surprisingly, H3K14ac and H4K5ac signals appeared concomitantly with peak gene expression in RNA_H2 (6.2 min of H3K14ac and 10.8 min of H4K5ac), but in RNA_H1 they were advanced by 1 h relative to RNA (48 min for H3K14ac and 75 min for H4K5ac). Aa genes were enriched in RNA_H1, whereas RNA_H2 contained mostly ribi and RP genes. Combining these data with the RNA-seq analysis (Fig. 1), we could define three distinct chromatin—transcriptional ‘program’ among Ox growth genes. Ribi and RP genes shared the same chromatin pattern distinct from that of aa genes. However, peak mRNA levels of RP genes and aa genes appeared later and lasted longer than those of the ribi genes. As discussed earlier, regulated splicing of RP genes might cause a delay of the peak level of mature RP mRNA (Fig. 2).

Among the Rb clusters, RNA_H3 contained primarily cell-cycle genes and exhibited similar chromatin patterns as in Ox clusters relative to RNA dynamics (Fig. 5a). In contrast, the genes encoding mitochondrial proteins (RNA_H4) behaved quite differently. Although they had a similar peak expression time, timing of the modification signals was out of phase. H3K14ac and H4K5ac increased in late RC, and H3K9ac increased in late RC through Ox. A motif sequence recognized by RNA-binding protein Puf3 was previously found in the 3′ untranslated regions (3′ UTRs) of nearly all mitochondrial ribosomal genes. This discrepancy between RNA level and chromatin state was consistent with the hypothesis that this gene class may be subject to post-transcriptional rather than transcriptional regulation.

The quantification of the relative timing of chromatin signals summarized different histone modification programs during transcription in yeast. In general, acetylation marks appear first and are followed by RNA peak signals and then by methylation marks, consistently with an overall initiation role for acetylation and an elongation role for methylation. Intriguingly, the fine-grained timing of different histone acetylation modifications could be a key factor to distinguish different transcription initiation mechanisms (Fig. 5d). In RNA_H1, H4K5ac appeared at first and was followed by H3K9ac and then RNA. This general sequence suggests that H4K5ac functions at an early stage of transcription initiation in these sets of genes, perhaps to ‘reset’ their activation, whereas H3K9ac, which peaks much more sharply, may have a critical role in triggering phase transitions and may regulate a later initiation step or a transition to elongation. In RNA_H2, H4K5ac and H3K9ac peaked together immediately before the RNA peak, thus suggesting the dispensability of a presetting stage during transcription of these genes.

The distinct patterns between chromatin and gene expression raised the question of whether specific transcription factors (TFs) are associated
analysis of ChIP-seq (MACS)-detected peaks. Signals of each modifier are plotted together with its corresponding modification normalized by H3. Chromatin modifiers and the corresponding modifications. ChIP signals were averaged from −1,000 to +1,000 bp from TSSs, derived from model-based states, TF-binding sites and post-transcriptional regulation.

Gis1p, Msn2/4p, Adr1p and Crz1p. Collectively, these results revealed Mbp1p, Fkh1/2p and Swi4p. Many TF motifs associated with the stress common cell cycle–related TF motifs including those for Xbp1p, related to factors involved in RP-gene expression, such as Stb3p, Sfp1p, revealed that binding sites for TFs involved in amino acid metabolism, such as Gcn4p, Bas1p, Chp1p and Leu3p, were enriched in RNA_H1, whereas in RNA_H2 we observed enrichment primarily of TF motifs related to factors involved in RP-gene expression, such as Sbh3p, Sfp1p, Rar1p, Pbf1p, Pbf2p, Dot6p and Tod6p. RNA_H3 contained several common cell cycle–related TF motifs including those for Xbp1p, Mbp1p, Fkh1/2p and Swi4p. Many TF motifs associated with the stress response were enriched in RNA_H6, a typical RC cluster, such as Gis1p, Msn2/4p, Adr1p and Crz1p. Collectively, these results revealed a complex, dynamic and interactive regulatory network of chromatin states, TF-binding sites and post-transcriptional regulation.

**Landscape of occupancy of chromatin-modifying enzymes in the YMC**

To further understand the dynamic patterns of histone modifications, we analyzed genome-wide occupancy of several chromatin-modifying enzymes in the YMC, specifically Gcn5p, Esa1p and Set1p. Gcn5p is the major H3-tail acetyltransferase, and Esa1p acetylates H4 tails, although other histone acetyltransferases also acetylate H3 and H4. Set1p is the catalytic subunit of the COMPASS complex H3 K4 methyltransferase.

To simultaneously evaluate all three enzymes, we constructed a Flag- and hemagglutinin (HA)-tagged strain, GCN5-Flag, SET1-HA₃, using an anti–native Esa1p antibody. This allowed ChIP-seq of Gcn5p, Esa1p and Set1p in one set of samples (Fig. 6a). First, we examined spatiotemporal patterns of these enzymes by displaying ChIP-seq signals at sample regions; temporal patterns were quite distinct (Fig. 6b). All three enzymes were recruited to RP gene RPS27A (YKL156W) in OX but with subtly distinct kinetics. We detected Gcn5p occupancy only at t5, corresponding to t4 in the 16-time-point ChIP-seq of histone modifications. However, Esa1p and Set1p signals preceded this peak, spanning mostly t2, t3, although Set1p occupancy lasted much longer, extending through early RB. We detected only Gcn5p and Set1p signals at GPM1 (RC) and SDH1 (RB) loci, in a manner correlated with RNA levels (Fig. 6b). Interestingly, the signal of Set1p appeared one or two time points (~5–20 min) before Gcn5p signal at GPM1, similarly to RPS27A.

Given the complex spatiotemporal relationships between the three enzymes, we sought to assess the interactive patterns and their relationship to corresponding histone modifications and gene expression from a genome-wide perspective. We found 2,369 Gcn5p peaks, 2,225 Esa1 peaks and 2,279 Set1p peaks, including 1,874, 1,810 and 1,629 with neighboring annotated genes (Supplementary Fig. 6a). Among these, ~1,750 genes were bound by at least two modifiers, and 1,035 genes were bound by all three modifiers, thus suggesting that these enzymes were not necessary for expression of every cycling gene. Spatial analysis
showed that Gcn5p and Esa1p were localized 200–300 bp upstream of the TSS, whereas Set1p was localized within the transcribed regions (Fig. 6c–e). This supported the well-accepted hypothesis that Gcn5p and Esa1p function in transcriptional initiation, whereas Set1p is associated with elongation25. Intriguingly, the locations of the modifiers did not overlap completely with corresponding histone modifications. Although Gcn5p and Esa1p located upstream of the TSS, H3K9ac and H4K5ac were shifted downstream. This suggested that Gcn5p and Esa1p can ‘reach across’ the TSS to act on the downstream nucleosome. The Set1p signal was slightly shifted downstream of the H3K4me3 peak, presumably because Set1p also produces mono- and dimethylation on H3 K4 during transcriptional elongation.

Next, we assessed temporal patterns of chromatin modifiers, focusing on annotated genes associated with all three modifiers. We defined four superclusters of temporal patterns by k-means clustering (Supplementary Fig. 6b and Supplementary Table 5). The results suggested that all three modifiers are functional in all three phases, and recruitment of modifiers is roughly in phase with corresponding gene expression.

However, chromatin modifiers, chromatin states and gene expression were not simply correlated. Here we focused on cell-growth genes, including ribi, RP and aa genes26. Ribi and RP genes individually exhibited one pattern, whereas aa genes showed three distinct patterns (Fig. 7a and Supplementary Table 5). Because RP genes showed the cleanest signal, we examined relative timing within this group (Fig. 7b). As described above, H3K9ac and H4K5ac appeared with the RNA peak, whereas H3K4me3 exhibited a delay. Surprisingly, Set1p was recruited ~10 min before the binding of Gcn5p and remained bound until dissociation of Gcn5p. This pattern was consistent at RPS27A, GPM1 (described above) and many other loci. We further confirmed the relative timing of histone modifications and modifiers at ribosome and aa genes by ChIP-qPCR in a similar YMC experiment using Gcn5-Flag3 and HA6-Set1 (Supplementary Fig. 6c–e). The temporal discrepancy between modifiers and modifications suggested that Set1p is recruited before transcription in a state that is nonfunctional, at least for trimethylation, until binding of Gcn5p occurs (Fig. 7c), whereas Esa1p is also recruited early but is immediately active.

Histone-mutant analysis

Given the complex patterns of histone modifications and modifiers in YMC, we investigated the criticality of individual lysine-residue modifications. Gcn5p mediates entry into the growth phase, because a mutant lacking it does not exhibit metabolic cycles14. To broaden understanding of histone modifications in the YMC, we examined the dissolved oxygen (dO2)-oscillation phenotype of all histone H3 and H4 lysine mutants corresponding to residues at which acetylation or methylation have been reported. All single lysine-to-alanine or lysine-to-arginine mutants, at residues including K4, K9, K14, K18, K23, K27, K36, K56 and K79 on H3 and K5, K8, K12 and K16 on H4, exhibited normal cycles, thus suggesting that no single lysine modification on H3 or H4 is critical for the YMC (data not shown). Next, we combined lysine-to-arginine mutations in H3 or H4 tails, including H3K(9,14)R, H3K(9,14,18)R, H3K(9,14,18,23)R, H3K(9,14,18,23,27)R, H4K(5,8)R and H4K(5,8,12)R. Notably, we observed decreased growth on YPD with increasing numbers of lysine to arginine mutations, results consistent with published reports (Fig. 8a)27. As the number of lysine-to-arginine mutations increased, the amplitude of the dO2 oscillation decreased (Fig. 8b–i). Strikingly, H3K(9,14,18,23,27)R (H3-5KR) and H4K(5,8,12)R (H4-3KR) mutants abolished dO2 oscillation. These results supported the hypothesis that post-translational modifications such as acetylation of H3 and H4 tails are required for normal
YMC transcriptional profiling revealed H4K(5,8,12)R, H4K(5,8)R, H3K(9,14,18,23)R. WT, wild type. (b-i) $O_2$ curves of the indicated histone mutants.

cycling and support a role for acetyl CoA in establishing the YMC, although there is no essential role in the YMC for any individual lysine residue.

DISCUSSION

In this study, we described the dynamic landscape of transcription and chromatin modifications across distinct metabolic states in yeast. We uncovered distinct combinatorial patterns associated with different functional groups of genes, emphasizing high-resolution timing of expression of distinct groups of genes and relative timing of histone modifications and modifiers within each group.

A just-in-time supply chain?

Cellular processes are well organized spatiotemporally to maximize fitness especially in the face of limited nutrients. One interesting example is the sequential activation of promoters in bacterial aa-biosynthesis pathways. YMC transcriptional profiling revealed several examples of staged biosynthesis consistent with a just-in-time program. Ribi transcripts appeared just before those encoding RP and aa (OX-phase) genes (Fig. 1b), thus supporting a just-in-time supply chain that maximizes efficiency of ribosome biogenesis and translation. The short pulse of ribi genes may also play a critical part in helping them achieve an efficient ramping down of protein synthesis at the end of OX. The observation of transient RP-gene pre-mRNA accumulation suggested the possibility of elaborate control of splicing in regulation of ribosome biogenesis. Alternatively, this staging may reflect that ribi genes can be much larger and therefore take longer to translate than RP proteins, which are universally short. The translation of the longest ribi gene might require 3–4 min more than the longest RP gene, consistently with the relative timing of ribi and RP expression. It is likely that the sequential activation of ribi and RP leads to concurrent synthesis of all ribosome-biogenesis proteins. Likewise, cell cycle–related genes and mitochondrial genes, the two major groups that had an elevated RNA level in the RB phase, also exhibited subtle timing disparities (Fig. 1c), perhaps indicating two distinct YMC subpopulations: one that will enter the cell-division cycle and one that will not.

Collectively, the evidence suggested a complex and precise orchestration of macromolecular-biosynthesis processes in yeast during the YMC from a transcriptional perspective. It will be interesting to examine whether protein factors are also organized temporally.

Different histone modification programs at distinct genes

We defined seven different combinatorial clusters between histone modifications and gene expression, revealing several different regimes of chromatin states in transcription (Fig. 5a). Interestingly, different histone acetylation marks behaved distinctly in association with different groups of genes. H3K9ac, H3K14ac and H4K5ac all appeared coincidently with the peak of gene expression of ribosomal genes. However, H3K14ac and H4K5ac were shifted earlier at aa (OX phase, aa2) genes (1 h before the RNA peak). We hypothesized that H3K14ac and H4K5ac function in presetting the promoters, perhaps by chromatin remodeling. However, RP genes did not show evidence of this preactivation phase from the perspective of chromatin states, perhaps because they are permanently preactivated. RP genes could be immediate sensors of critical metabolic and environmental stimuli, for example, intracellular acetyl CoA levels. Conversely, aa2 genes appear to have a preparatory phase.

The dynamics of histone modifiers increases the complexity of chromatin states. In many cases, SET1p was recruited earlier than Gcn5p, in seeming contradiction to the order of appearance of H3K4me3 and H3K9ac (Fig. 7c). We hypothesize that SET1p may catalyze mono- or dimethylation in promoter regions to preset TSSs for activation. It is known that H3K4me1 accumulates at the MET16 promoter before induction in yeast. Additionally, H3K4me1 and H3K4me2 precede transcription in cardiac development and T-cell differentiation, thus suggesting that wiring of at least some chromatin-based programs is phylogenetically conserved. Roles of histone modifications in the YMC

Various histone modifications may function alone or in a combinatorial or cumulative manner. These modifications either change the charge status of histone tails or provide platforms to recruit transcriptional-regulatory complexes. The very different patterns between H3K9ac and H4K5ac suggested distinct functions during transcription. Histone-mutant analysis revealed functional redundancy within each histone tail. The H3-5KR quintuple and H4-3KR triple mutant each totally abolished the oscillation, partially
corroborating the evidence that the gcn5 strain does not support oscillation. Surprisingly however, the H3-5KA and H4-3KA mutants grew better than corresponding KR mutants and still exhibited O2-oscillation phenotypes (Supplementary Fig. 7), thus suggesting that charge status of H3 and H4 may have a fundamental role in the YMC. Maintaining the positive charges on these histone tails may ‘lock’ growth genes into a repressed state, resulting in inability to undergo the bursts of growth that are characteristic of the YMC and cells in general.

Similar chromatin dynamics between the YMC and circadian cycle
Remarkably, circadian rhythm in mammals and this yeast ultradian rhythm show several noteworthy similarities. Through systematic analysis of the transcriptome and chromatin state as a function of the circadian clock in mouse liver, Koike et al. revealed similar temporal patterns of histone modifications, although the genes under regulation are completely distinct and the time scales of the cycles are very different, results suggesting phylogenetically conserved roles of histone modifications during transcription. More intriguingly, beyond the oscillatory phenomenon of the transcriptome, metabolism and chromatin states in these two systems, the metabolism-epigenome-transcriptome loop is consistent in both systems, thus suggesting that the circular interactions among the three players actually sustain oscillations. Our findings on the dynamics of chromatin modifications and temporal associations between the epigenome and the transcriptome suggest an active metabolism-epigenome-transcriptome loop in the circadian cycle: temporal compartmentalization of cellular processes. Science 310, 1152–1158 (2005).

We have revealed a network at very high temporal resolution of gene expression, chromatin state and chromatin modifiers across the YMC, as highly synchronized yeast cells transition between metabolic states akin to growth, division, and quiescence. Our study not only demonstrates how histone states relate to and direct the timing of gene expression but also provides examples of how these processes are coordinated in a dynamic fashion to achieve optimal growth, metabolic efficiency and, presumably, fitness. This yeast study may help illuminate the exquisite temporal control of various dynamic biological processes.

METHODS
Methods and any associated references are available in the online version of the paper.

Accession codes. Data of RNA-seq and ChIP-seq of histone modifications and modifiers have been deposited in the Gene Expression Omnibus database under accession code GSE52339.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS
Z.K., L.C., B.P.T. and J.D.B. designed experiments; Z.K. and L.C. collected ChIP-seq and RNA-seq data; Z.K., X.Z. and H.J. performed the analysis; Z.K. made histone mutants and analyzed growth and YMC phenotypes; Z.K. wrote the manuscript with help from all other authors; all authors reviewed the manuscript.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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ONLINE METHODS

Strains. Yeast strains and media used are described in Supplementary Note.

Plasmids and histone mutagenesis. Plasmids carrying histone mutations were obtained from the histone-mutant library described previously15: pJD233 and pJD234, each containing a hygMX selectable marker cassette, were used for making HHT1 and HHE1 mutants respectively. pZK8 was generated from pJD154 by replacing the URA3 cassette with a natMX cassette for mutagenesis on HHT2 and HHE2. Plasmids carrying multiple lysine mutations were generated by site directed–mutagenic fusion PCR and subcloning in the indicated backbones, pJD233, pJD234 and pZK8. Briefly, primers carrying the desired mutations were used to generate two PCR fragments containing the overlapping mutation regions. Fusion PCR was used to generate a single fragment, and the product was digested and ligated to the indicated backbones, pJD233, pJD234 and pZK8, and verified by DNA sequencing.

Metabolic cycles. Metabolic-cycle experiments were performed as previously described12 except for the timing of sampling for RNA-seq and ChIP-seq; samples were intentionally taken unevenly to more deeply sample the very rapidly changing OX phase and were taken less densely outside the OX phase. Fermentors were from New Brunswick Scientific (BioFlo 110 or BioFlo 3000). 10 ml overnight, saturated culture was inoculated to start each YMC run. Depending on the model, YMC runs were operated at an agitation speed of 400 r.p.m. (BioFlo 110) or 475 r.p.m. (BioFlo 3000), an aeration rate of 1 l/min, a temperature of 30 °C, and a pH of 3.4 in 1 l YM medium. Once the batch culture was saturated, it was subjected to at least 4 h of starvation. After starvation, fresh medium was added continuously at a dilution rate of ~0.09–0.1 h−1.

RT-qPCR. 2 OD BY5764 cells from the cycle were collected and flash frozen. RNA was extracted with the Qiagen RNeasy Mini kit (Qiagen, 74104) with the standard protocol. First-strand cDNA was synthesized with SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, 18080-051). Oligo(dT)20 primer was used for reverse transcription. Fast SYBR Green Master Mix (Applied Biosystems, 4385612) was used for real-time PCR, and experiments were done on the platform of StepOnePlus Real-Time PCR System (Applied Biosystems, 4385612). PCR primers are listed in Supplementary Table 6.

RNA-seq. RNA was extracted across 16 time points of one cycle at the indicated time points, and RNA-seq was performed. With this strategy, the shortest interval between two OX-phase time points is ~5 min, and across the entire cycle the samples were taken at intentionally uneven intervals. 1 ml BY5764 cells from the cycle (about 15 OD/ml) were collected and flash frozen. The pellet was resuspended in 200 µl RNA stat-60 (Tel-Test) and disrupted in a Mini-Beadbeater-16 (BioSpec), broken up three times with 100 µl glass beads in a 1.5 ml conical screw-cap tube (USA Scientific, 1415-8700, Ocala, FL). Supernatant was collected into a new microcentrifuge tube, and 800 µl RNA stat-60 was added to repeat bead-beating one more time. The supernatants were combined and incubated at room temperature for 10 min. 200 µl chloroform was then added, and the tube was vortexed for 15 s and left at room temperature for 10 min. The tubes were centrifuged at 4 °C, 16,000 r.c.f. for 15 min, and the supernatant was transferred to a new tube. 500 µl isopropanol was added and mixed by inversion of the tube several times. The mixture was incubated at room temperature for 10 min and centrifuged at 4 °C, 16,000 r.c.f. for 15 min. The pellet was washed with 70% ethanol twice, air dried and resuspended in 100 µl H2O. Library construction and sequencing were performed with the HiSeq platform supervised by the UTSW Microarray Core Facility. Briefly, samples were run on an Agilent 2100 Bioanalyzer to ensure that high-quality RNA was used. 4 µg total RNA was then prepared with the TruSeq RNA Sample Prep Kit (Illumina). mRNA was purified and fragmented before cDNA synthesis. cDNA was then end-repaired and A-tailed. After adaptor ligation, samples were PCR amplified and purified with AmpureXP beads (Agencourt, A63880), then validated again on the Agilent 2100 Bioanalyzer. Before being run on an Illumina HiSeq 2000, samples were quantified by qPCR. Primers within the adaptor sequence were: P1, 5′- AAT GAT ACG GGC ACC ACC GA-3′ and P2, 5′- CAA GCA GAA GAC GCC ATC CAGA-3′. Differential gene expression analysis was performed with TopHat and Cuffdiff according to a standard protocol36. Briefly, the TopHat parameters used were: –bowtie1 -i 40–genome-read-mismatches 4–no-coverage-search -G

Saccharomyces cerevisiae Ensembl/EF2/Saccharomyces_cerevisiae/Ensembl/EF2/Annotation/Archives/archive-2012-03-09-08-22-49/Genes/genes.gtf
Saccharomyces cerevisiae Ensembl/EF2/Saccharomyces_cerevisiae/Ensembl/EF2/Sequence/BowtieIndex genome. A-means clustering and heat maps were produced with R. Three previously defined superclusters of genes were obtained from a previous study12. A subset of genes from those previously identified as noncycling showed elevated expression in the OX phase by hierarchical clustering with the dChip package37. Previously defined and newly identified OX-phase genes were combined. FPKM values were imported into R, and the list was intersected with the three superclusters. The values of each supercluster were then subject to k-means clustering with the function ‘kmeans’. Genes inside each subcluster were further ordered on the basis of the timing of peak expression. Ten clusters were calculated by k-means clustering and ordered by the timing of peak expression from visualization. Genes inside clusters were further ordered by weighted mean time of peak of expression. Weights were calculated as the percentage of RNA-seq reads at each time point over total reads across 16 time points. The weighted mean for each gene was calculated as the sum of weight × time at each time point. Intron-containing genes were defined from the SGD annotation file of 20100102 corresponding to the mapping index (http://download.yeastgenome.org/curations/chromosomal_feature/). To display RNA signals in the CisGenome Browser38, RNA-seq data were first mapped to sacCer2 by bowtie (-3 -5 3 -8 -best -saccer2). Results were imported into the Cis Genome browser package and were displayed in 20 bp windows in the browser. Signals at each window were first normalized by the total number of mappable reads across 16 time points and then displayed as the percentage of the maximum value of the 16 time points. Exon-exon–junction reads were directly called from the files of junctions.bed by TopHat. Intron-junction reads were identified with mappable reads of 39 bp (50 bp-3 bp-8 bp) from bowtie. Each end of a junction read should extend at least 4 bp away from the junction site. Junction reads spanning the 5′ and 3′ ends of 89 RP-gene introns at 16 time points were counted, and hierarchical clustering and heat maps were generated in R with the heatmap2 function with default parameters. Box plots were drawn with the box-plot function at every time point. The sums of the 16 time-point junction reads were calculated and displayed with the barplot function.

ChIP-seq. Chromatin immunoprecipitation was performed as described previously14. ~5 OD ZKY329 cycling cells per time point were collected for ChIP of single histone modifications, and ~50 OD ZKY428 cycling cells (GCN5-Flag–natMX, SET1-3HA–kanMX6) per time point were collected for ChIP of each chromatin-modifying enzyme. Antibodies used were: H3K9ac (Millipore 06-942), H3K14ac (Millipore 07-353), H3K36ac (Millipore 07-677), H3K5ac (Millipore 07-329), H3K16ac (Millipore 07-329), H3 (Millipore 05-928), H3K4me3 (Millipore CS200580), H3K36me3 (Abcam ab9050), Esa1 (Abcam ab4466), Flag M2 (Sigma, F1804), and HA 12CA5 (Roche, 11583816001). Validation is provided on the manufacturer’s websites. 3 µg primary antibody was used per ChIP experiment. Briefly, cells were first fixed in 1% formaldehyde at 25 °C for 15 min and quenched in 125 mM glycine at 25 °C for 10 min. Cells were pelleted and washed twice with buffer containing 100 mM NaCl, 10 mM Tris-Cl, pH 8.0, 1 mM EDTA, 1 mM PMSF, and 1 mM benzamidine-HCl before freezing. The frozen pellet was resuspended in 0.45 ml ChIP lysis buffer (50 mM HEPES-KOH, pH 7.5, 500 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% deoxycholate (DOC), 0.1% SDS, 1 mM PMSF, 10 µM leupeptin, 5 µM pepstatin A, and Roche protease-inhibitor cocktail) and lysed by bead-beating. Lysate from 50 OD cells was split into two tubes, each containing 280 µl lysis, and sonicated for 16 cycles (30 s on, 1 min off, high output) with a Bioruptor (Diagenode or Tosho Denki). The supernatant of the sonicated lysate was precleared. 50 µl lysis was saved as input. For ChIP with histone antibodies, 50 µl whole cell extract (WCE) was diluted 1:10 and used for each ChIP. For ChIP of histone-modifier proteins, 500 µl WCE and 3 µg antibody was used. After incubation overnight, 50 µl protein G magnetic beads (Invitrogen, 10003D) or protein A–Sepharose beads (GE Healthcare Life Sciences 17-5280-01) were resuspended in ChIP lysis buffer and added and incubated for 1.5 h at 4 °C. Beads were washed twice with ChIP lysis buffer, twice with DOC buffer (10 mM Tris-Cl, pH 8.0, 0.25 M LiCl, 0.5% deoxycholate, 0.5% NP-40, and 1 mM EDTA) and twice with TE, 125 µl of TES buffer (TE, pH 8.0, with 1% SDS, 150 mM NaCl, and 5 mM dithiothreitol) was added to resuspend the beads. Supernatant was collected after incubation at 65 °C for 10 min. A second round of elution was performed, and the eluates were combined. Reverse cross-linking...
was performed by incubation for 6 h at 65 °C. An equal volume of TE containing 1.25 mg/ml proteinase K and 0.4 mg/ml glycogen was added to the samples after reverse cross-linking, and samples were incubated for 2 h at 37 °C. Samples were extracted twice with an equal volume of phenol and once with 25:1 chloroform/isoamyl alcohol. DNA was precipitated in 0.1 volume 3.0 M sodium acetate, pH 5.3, and 2.5 volumes of 100% ice-cold ethanol at -20 °C overnight. Pellets were washed once with cold 70% ethanol and resuspended in 20 µl TE. Library construction and sequencing were performed according to the Illumina protocol. Briefly, DNA was end-repaired and A-tailed. Barcoded adaptors were ligated, and DNA was run on a 2% agarose gel. DNA fragments from 150 bp to 300 bp long were excised from the gel and used for PCR. PCR products were gel-extracted again and quantified on an Agilent Bioanalyzer. Sequencing was performed on an Illumina GXII or HiSeq 2000 or Solid. Raw reads were mapped to the reference genome (sacCer2) by bowtie, and peaks were visualized with the CisGenome Browser as described above.

Distribution of ChIP-seq signals relative to TSSs. Information on TSS locations was adopted from a previous study. TSSs of genes of interest were aligned at zero on the x axis, and ChIP-seq reads were counted for each 10-bp window from -1,000 bp to 1,000 bp relative to the closest TSS. Number of reads in each window were summed and plotted in R with the plot function.

ChIP-seq signals at genes. ChIP signals of histone modifications at genes were evaluated by counting reads overlapping defined regions of every gene with the CisGenome package. Briefly, a window of -100 bp to +400 bp spanning the TSS was used for H3K9ac, H3K14ac, H3K36ac, H4K5ac and H3K4me3. The H3K36me3, H3K16ac and H4 signals were calculated from TSS to TES. Peaks of chromatin modifiers were called by the MACS peak-calling package with P value = 10⁻² (ref. 40) and annotated by the nearest TSS. ChIP values of chromatin modifiers were calculated similarly by counting reads in windows of -250 bp to +250 bp spanning the center of peaks or windows of -100 bp to +400 bp spanning the TSS. Ribosomal assembly (rbi) factors were described by Jorgensen et al. The ribosomal protein (RP gene) list is from the website http://ribosome.med.miyazaki-u.ac.jp/rgf?mode=orglist&org=Saccharomyces%20cerevisiae/. Amino acid–metabolism genes are from the GO term ‘cellular amino acid metabolic process’ of the SGD annotation file. ChIP values were normalized according to the total number of aligned reads. Time point 13 of H3K14ac and H4K5ac, time point 1 of H4K16ac and time point 5/7 of H3K36me3 show certain levels of fluctuation. We tried to delete these samples or average them with neighboring samples and observed no significant differences in the downstream analysis. We smoothed these samples on the basis of the continuity of time course experiments, by averaging the signals with neighboring samples for visualization. K-means clustering and generation of heat maps were performed in R as described above. Hierarchical clustering was performed with the dChip package.

Data preprocessing. For each sample, we normalized the observed value by the total number of aligned reads. For each modification, we then standardized the 16 observed values (log; of read counts plus 1) within each gene. We were primarily interested in the trend of change over time rather than the relative magnitude of expression among genes, so we standardized the 16 values within each gene; i.e., we subtracted the mean from the 16 values and then divided by the s.d. Then we pooled the data for all RNA-seq and ChIP-seq data sets together to get a data matrix, with each row representing a gene and each column a time point.

Notations. For a given marker, we denoted the matrixes as Y = [Yg] as processed gene expression levels, where g is the gene index, ranging from 1 to N, and j is the time point index, ranging from 1 to 16. We denoted t as observation times.

Correlation between chromatin modifications and gene expression. A gene-specific correlation coefficient was calculated between 16-time-point gene expression values and each histone modification value. A two-time-point shift was allowed to achieve the maximum correlation value. Basically, we calculated gene-specific correlation coefficients with time-shift points of -2, -1, 0, 1 and 2 and took the maximum correlation value as the output correlation coefficient. We note that some modifications are shifted forward or backward relative to the RNA level, and this could help get the accurate correlation value. A density distribution of correlation values was plotted across the whole genome or across subsets of interest (OX, RB, and RC). We observed a positive correlation in the distribution of H3K4me3, H3K36me3, H3K9ac, H3K14ac and H4K5ac with RNA level (data not shown). Interestingly, the OX-phase genes always showed the best correlation with expression, whereas RB-phase genes showed a relatively poor correlation. We also noticed that H4K16ac and total H3 exhibited a slight negative correlation, and H3K6ac showed no obvious correlation (data not shown).

Curve fitting. For each gene, we observed gene expression levels at 16 time points. To convert these discrete data into a continuous profile or function, we fit every set of 16 points into a smooth curve with a penalized B spline. We denoted function f_1(t) as fitted continuous profile from data [Yg_1, … , Yg_16] and [t_1, … , t_16]; f_2(t) is a linear combination of B-spline basis functions (definition in ref. 41). Estimation of the coefficient of each basis function was obtained by a penalized optimization problem.

Time alignment (batch effect removal). We assumed that the dynamics of gene expression levels changes according to a biologically specified clock or time system. Such time systems can be affected by unknown or uncontrolled fluctuations in experimental conditions such as minute variations in medium composition, temperature, etcetera. When data are generated in multiple batches, the results can be biased by the different time systems.

RNA-seq and ChIP-seq of histone modifications were generated in two batches. To minimize batch-to-batch variations, the histone modification H3K9ac, which is the most periodic, was independently measured in both batches. We used this information to align or ‘time warp’ the two batches into a single time system. We denoted f_1(t) and f_2(t) as the fitted gene profiles for two different batches. Theoretically, f_1(t) and f_2(t) should be the same under the identical time system. Thus we sought a mathematical transformation, s:

\[ s(t_1, ..., t_{16}) = (s(t_1), ..., s(t_{16})) \]

such that f_1(t) and f_2(t) were most alike for all genes. Mathematically, we needed to minimize E(f_1(f_1(t)), f_2(f_2(s(t)))) = \sum_{g=1}^{N} f_1(t_g) - f_2(s(t_g))^2 dt

We obtained the final result [s(t_1), ..., s(t_{16})] with a simulated annealing algorithm. As a result, the observation times of the raw RNA-seq data were replaced by [s(t_1), ..., s(t_{16})], and we assumed that the batch effect was minimized as much as possible given the current data.

Clustering genes. We clustered the genes with the processed gene expression levels. The k-means algorithm was used for its speed and robustness of dealing with large data. The k-means algorithm is affected by the choice of initial cluster centers. To minimize the dependency on initial cluster number, we repeated the k-means algorithm 50 times with different randomly chosen initial cluster centers and kept the result with the smallest total within-cluster sum of squares.

To choose the number of clusters, we tried different options ranging from 1 to 30. Finally we selected seven clusters because this yielded the best Bayesian information criterion (BIC) value: BIC is one of the most popular criteria for model selection. Similarly we selected 14 clusters of eight histone modifications in Supplementary Figure 5.

For each cluster of genes, we wanted to know when the expression profile reached its peak value. For each gene, we could find the peak (highest point) of the profile from the fitted smooth curve. The cluster peak location was defined as the average of peak locations for all genes in a cluster. Similarly we could calculate the s.d. of the cluster peak location.

GO analysis. Gene ontology analysis was performed by the hypergeometric test with the SGD annotation file at http://download.yeastgenome.org/curation/literature/go_slim_mapping.tab. P values were adjusted by the false discovery rate (FDR).

Enrichment analysis of motifs associated with clusters. We took advantage of three motif databases, TRANSFAC, JASPAR and UNIPROBE. We first identified the genomic coordinates of each motif on the basis of its weight matrix with the CisGenome package. Because we focused on transcriptional initiation, we filtered these binding sites by selecting sequences located between -600 bp and +400 bp of any TSS. Next, we examined genome-wide whether any motif sequence exists.
at the 5′ end of the gene. Subsequently, we calculated the percentage of genes with a certain motif sequence in all RNA_H clusters and performed the Wald test to determine whether the percentage in each cluster was significantly higher than the genome-wide percentage. We applied this procedure to all motifs in the three databases.

Analysis of H3K56ac. The analysis of H3K56ac in the cell cycle was performed according to a procedure similar to one described previously24. We adopted the same regions of nucleosomes in chromosome III and calculated H3K56ac signals at these nucleosomes as described above. We sorted nucleosomal H3K56ac signals by genomic location and fit our data with those of the previous study by generating heat maps in R.

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