TRANSCRIPTION

Mitotic transcription and waves of gene reactivation during mitotic exit

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Although the genome is generally thought to be transcriptionally silent during mitosis, technical limitations have prevented sensitive mapping of transcription during mitosis and mitotic exit. Thus, the means by which the interphase expression pattern is transduced to daughter cells have been unclear. We used 5-ethynyluridine to pulse-label transcripts during mitosis and mitotic exit and found that many genes exhibit transcription during mitosis, as confirmed with fluorescein isothiocyanate–uridine 5′-triphosphate labeling, RNA fluorescence in situ hybridization, and quantitative reverse transcription polymerase chain reaction. The first round of transcription immediately after mitosis primarily activates genes involved in the growth and rebuilding of daughter cells, rather than cell type–specific functions. We propose that the cell's transcription pattern is largely retained at a low level through mitosis, whereas the amplitude of transcription observed in interphase is reestablished during mitotic exit.

During mitosis, chromatin condenses (*1*), gene regulatory machinery is largely evicted from chromatin (*2*–*4*), and transcription is thought to be silenced (*5*–*7*). Yet reactivation of a specific gene expression program is needed to maintain cell identity during exit from mitosis. Long-distance interactions across the genome are lost during mitosis (*8*), as is hypersensitivity at distal enhancers, but not at promoters (*9*). "Bookmarking" transcription factors remain bound in mitosis to a subset of their interphase sites (*10*–*15*). Knockdown of these factors during mitosis delays reactivation of target genes (*10*, *11*, *13*), although the proper transcriptome is eventually regenerated. Thus, the basis for identity maintenance during mitosis remains unclear, and the hierarchy by which genes are reactivated during mitotic exit is not understood.

Because of nuclear envelope breakdown in mitosis and hence the inability to isolate nuclei for direct labeling of transcripts (*16*), genome-wide studies during mitotic exit used RNA polymerase II (RNAP2) cross-linking to assess active transcription (*4*, *17*) and found a burst in RNAP2 binding to promoters ~60 to 90 min after release from mitotic arrest (*17*). However, the dynamic range of antibody-based methods is much less than from direct measurements of nascent transcription, and cross-linking artifactually causes protein exclusion from mitotic chromatin (*14*, *18*). Transcription elongation inhibition of prometaphase HeLa cells elicits paused RNAP2 at promoters, suggesting the presence of elongating enzyme, even though elongating RNAP2 was not detected directly (*19*). The study also mapped nonpolyadenylated, chromatin-associated RNAs from prometaphase cells, but it was unclear whether these RNAs were transcribed during mitosis or, as suggested by the authors, at the G2/M transition. A study of pulse-labeled transcripts in arrested MCF-7 human breast cancer cells used nuclear isolation for bromouridine-5′-triphosphate labeling and hence did not appear to be assessing mitotic cells (*20*).

To define the timing of transcription events during mitotic exit, we used the cell-permeable 5-ethynyluridine (EU) to pulse-label nascent transcripts (*21*) in intact HUH7 human hepatoma cells during nocodazole-induced mitotic arrest, mitotic exit, and in asynchronous cells. Arrested cells, enriched by mitotic shake-off, were highly pure (fig. S1, A to D) and reenter G_1 (fig. S1, E to K). Previously, we labeled transcripts with EU during mitotic exit in HUH7 cells and attached azide-fluorophore, discovering that bulk global transcription initiates approximately 80 min after nocodazole wash-out (*11*). On the basis of this assessment of global reactivation, we pulse-labeled transcripts at 0, 40, 80, 105, 165, and 300 min after nocodazole wash-out in HUH7 cells, but instead conjugated azide-biotin to the EU-RNA in order to measure the relative changes over time (Fig. 1A). The addition of biotin allowed us to use streptavidin beads to isolate EU-labeled transcripts from total RNA and generate cDNA libraries on the beads for sequencing (figs. S2, A to E, and S3A and table S1). For direct comparison of transcription in asynchronous versus mitotic cells, we designed and generated biotinylated RNAs to add as spike-in controls (fig. S2, C and F to H, and tables S2 to S4).

EU-RNA-sequencing (EU-RNA-seq) maps primary transcripts with high coverage (fig. S3B) because reads span introns and exons of annotated transcripts and are largely absent from intergenic regions (Fig. 1, B and C, and fig. S3C), with reproducibility (fig. S3D). The distribution of asynchronous FPKMs (fragments per kilobase of transcript model per million fragments mapped) (fig. S4A) and wide dynamic range helps distinguish genes that can reliably be detected (FPKM ≥ 19) (Fig. 1C and fig. S4B) from those that cannot (FPKM < 19) (fig. S4C). Reads from nonspecific RNA, not transcribed during the pulse ("NoEU"), primarily mapped to exons of highly abundant, stable mRNAs, such as for *ALB* (fig. S4D), and were removed from all samples without affecting asynchronous FPKMs as compared with microarray data (fig. S4E). We conclude that EU-RNA-seq is a robust and reliable method for mapping the nascent transcriptome.

With three spike-in replicates, we observed 8074 transcripts (3689 genes) (fig. S5A) consistently expressed in mitosis (fig. S5B and table S5). The mean decrement in expression was fivefold, with a much narrower range in expression compared with that in asynchronous cells (Fig. 1D). Of the mitotic transcripts, 97% are expressed above 5% of their asynchronous level (fig. S5C), and the different relative rank expression profiles (fig. S5D) indicate that the mitotic transcriptome is distinct from that in asynchronous cells. Furthermore, 3329 mitotically expressed genes are expressed higher in mitosis than can be attributed to the ~3% contaminating asynchronous cells, based on coalignment of the mitotic and asynchronous reads with those from 222 adult human liver RNA-seq studies (fig. S5E and table S6). Thus, the low-level transcription seen in the mitotic population cannot be explained by contaminating interphase cells.

We quantified fluorescein isothiocyanate–uridine 5′-triphosphate (FITC-UTP) incorporation in mitotic HUH7 cells with or without the transcriptional inhibitor α-amanitin, which has detected transcription at centromeres (*22*). Nascent RNA signals were evident across chromosome arms in metaphase spreads and were significantly reduced by the inhibitor (Fig. 1, E to V, and fig. S6, A and B). Chromosome arm transcription was also detected in BJ fibroblasts (fig. S6, C to U), further confirming that mitotic transcription is not limited to the centromere and occurs in nontransformed cells.

We used quantitative reverse transcription polymerase chain reaction to independently assess transcripts that were called to be mitotically expressed or not, using intron-directed primers. Primer sets were confirmed to detect nascent transcripts because treatment with triptolide, an RNAP2 inhibitor, diminished their signals but did not decrease signals for primer sets to glyceraldehyde-3-phosphate dehydrogenase mRNA (fig. S7A). All three mitotically expressed primary transcripts were detected in mitotic cells (fig. S7B) and were triptolide-sensitive (fig. S7C),

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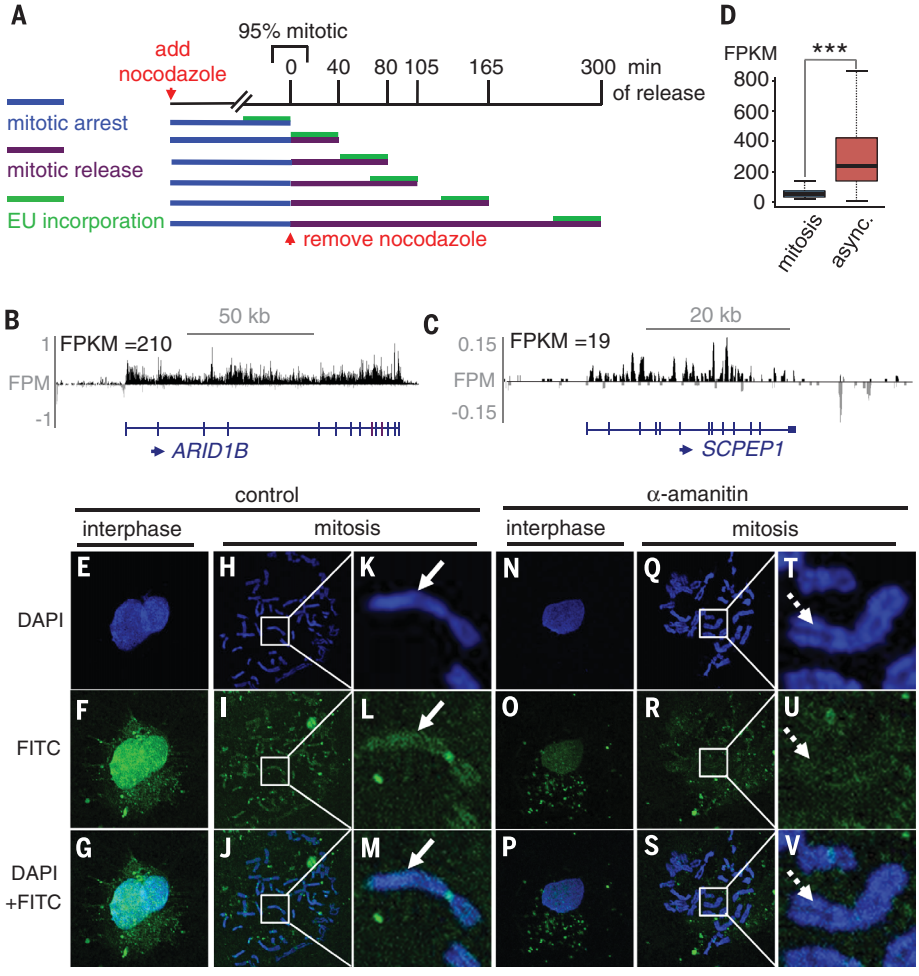

Fig. 1. EU-RNA-seq and direct FITC-UTP labeling reveal extensive transcription in mitosis. (**A**) Pulse-labeling during mitosis and mitotic exit. (**B**) Reads span exons and introns, not intergenic regions. *y* axis, fragments per million fragments mapped (FPM). (**C**) A representative transcript with an FPKM of 19. (**D**) FPKMs of mitotically expressed transcripts, in mitosis and in asynchronous cells. Bar, mean; whiskers, quartiles; $P < 0.001$, $n = 8074$ transcripts. (**E** to **G**) Interphase or (**H** to **M**) mitotic cells labeled with FITC-UTP; white boxes are magnified in (K) to (M). (**N** to **P**) Interphase or (**Q** to **V**) mitotic cells treated with α-amanitin and labeled with FITC-UTP; white boxes are magnified in (Q) to (S). Arrow, RNA signal; arrowhead, no RNA signal.

demonstrating the dependence of their expression on RNAP2.

To test for expression of mitotically expressed genes in naturally occurring mitotic cells, we performed RNA fluorescence in situ hybridization in asynchronous cells (Fig. 2, A to L). Exon and intron probes were used because colocalization, together with chromatin, is indicative of a transcriptional event. We detected a significant occurrence of primary transcripts in mitosis for all three genes tested (fig. S7D). We also found 789 transcripts (484 genes) that were higher in mitosis than in asynchronous cells (Fig. 2M and table S7). The genes were enriched for those involved in extracellular structure and transcription (Fig. 2N and table S8) and were not specific to G_2 or other nonmitotic phases (table S9) (*23*).

To assess whether there is a hierarchy of reactivation during mitotic exit, all transcripts expressed in asynchronous cells were parsed by the time at which their FPKM first increased 1.5-fold over that in mitosis (Fig. 3A, fig. S8A, and tables S10 to S15). The largest number of transcripts first increased at 80 min (fig. S8B and table S11) and were reactivated with the largest amplitude (fig. S9A), as seen previously (*11*, *17*). Of the transcripts first activated at 80 min in hepatoma cells, 55% maintained their transcription rate for the duration of mitotic exit (fig. S9B), similar to that seen in erythroblasts (fig. S9C) (*17*). Yet the sensitivity of our approach allowed for the identification of additional waves of reactivation after the initial burst (Fig. 3 and tables S12 to S14). EU-labeling affords the sensitivity to detect 927 transcripts that first increase at only 40 min (Fig. 3 and table S10), well before bulk transcription reactivation seen by EU-fluorophore labeling or RNAP2 antibody staining and chromatin immunoprecipitation (*11*, *17*).

The first genes to increase have functions in lumen and envelope formation and translation (Fig. 4A and tables S16 and S17) (*24*, *25*). Therefore, genes that reconstitute basic cell structure and growth are prioritized immediately after mitosis, expanding on the ribosomal and metabolic

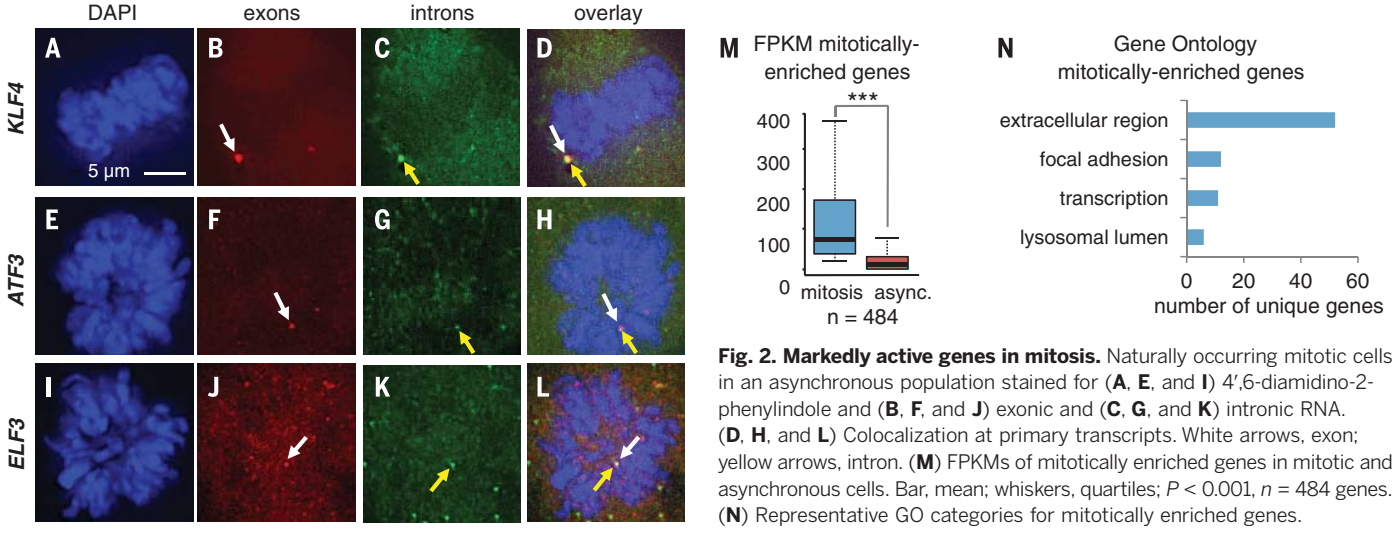

Fig. 2. Markedly active genes in mitosis. Naturally occurring mitotic cells in an asynchronous population stained for (**A**, **E**, and **I**) 4′,6-diamidino-2-phenylindole and (**B**, **F**, and **J**) exonic and (**C**, **G**, and **K**) intronic RNA. (**D**, **H**, and **L**) Colocalization at primary transcripts. White arrows, exon; yellow arrows, intron. (**M**) FPKMs of mitotically enriched genes in mitotic and asynchronous cells. Bar, mean; whiskers, quartiles; $P < 0.001$, $n = 484$ genes. (**N**) Representative GO categories for mitotically enriched genes.

genes seen elsewhere (*26*). The next wave of reactivation is enriched for adhesion genes, which is consistent with the epithelial nature of HUH7 cells (tables S18 and S19). Last, the last transcripts to increase are involved in cell cycle and DNA replication (tables S20 and S21), as the cells are preparing for S phase. To determine when liver-specific genes first increase, we analyzed the time at which the 149 liver-specific genes (*27*) expressed in HUH7 cells—a number similar to that seen in cultured hepatocytes (*28*)—first increases over mitosis. Although liver-specific genes are expressed throughout mitotic exit, most are reactivated at later stages (Fig. 4B and fig. S10A). Thus, HUH7 cells initially activate genes required for building daughter cells at the beginning of mitotic exit and then later activate cell type–specific genes.

Although the first transcripts to increase are among the shortest (fig. S10B), the longest early genes are still activated before the shortest late genes (fig. S10C). Gene ontology (GO) analysis of the longest genes to come up at 40 and 80 min reveals basic cell functions (fig. S10, C and D), as observed when considering all genes (Fig. 4A). Analysis of the shortest liver-specific genes indicates that they increase at later time points (fig. S10, E and F). Thus, the time of activation of gene classes relates to their function and not primarily to gene size.

We also assessed enhancer RNA (eRNA) dynamics as a surrogate for enhancer activity. We curated all intergenic human enhancers (*29*) for detectable eRNAs in asynchronous HUH7 cells and found them significantly down-regulated in mitosis (fig. S11A). The majority of eRNAs increased at the early time points during mitotic release (fig. S11B), as did genes (Fig. 3A). Curating for the enhancer subset within 100 kb of the nearest TSS, we found that eRNAs first increase around the same time as their putative targets (Fig. 4C). Therefore, enhancer and putative target gene reactivation appear concordant during mitotic exit.

We applied a sensitive approach to measuring the transcriptome during mitosis and mitotic exit. We found extensive residual transcription in mitosis and waves of transcription reactivation during exit. RNA polymerases have long been known to be stable in chromatin, persisting during salt-washes of nuclei that cause loss of transcription factors (*30*). Thus, a low level of transcribing RNAP2 could contribute to the inheritance of a cell's specific transcriptome pattern through mitosis. Because deoxyribonuclease hypersensitivity also persists at promoters in mitosis, whereas hypersensitivity at enhancers (*9*) and long-range interactions generally do not (*8*), we suggest that in mitosis, the promoter and its gene create rudimentary mitotic expression units (MEUs). MEUs retain residual activity and function along the general constraints of genes in yeast, which lack enhancers and long-range interactions. The MEU model posits that the transcription pattern is largely retained through mitosis by MEUs, whereas the amplitude of transcription observed in interphase is reestablished during mitotic exit.

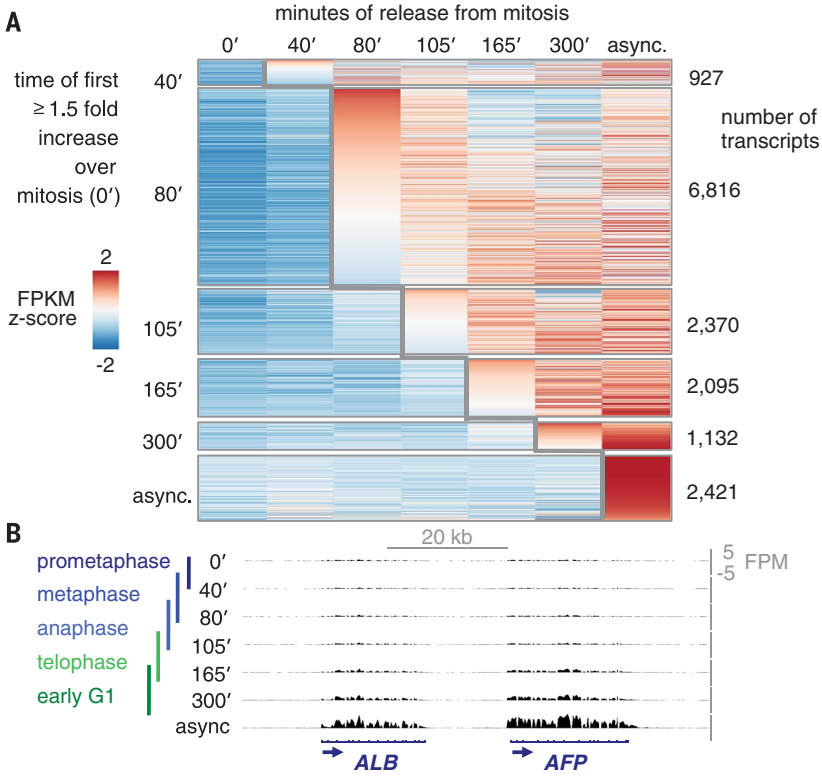

Fig. 3. Transcription reactivation during mitotic exit. (**A**) *z* scores of transcripts (rows) that first increase ≥1.5-fold over mitosis, rank ordered within each time point (columns). (**B**) Liver genes during mitotic exit. *y* axis, FPM. Colored bars to the left indicate mitotic or mitotic exit stages generally observed at each time point.

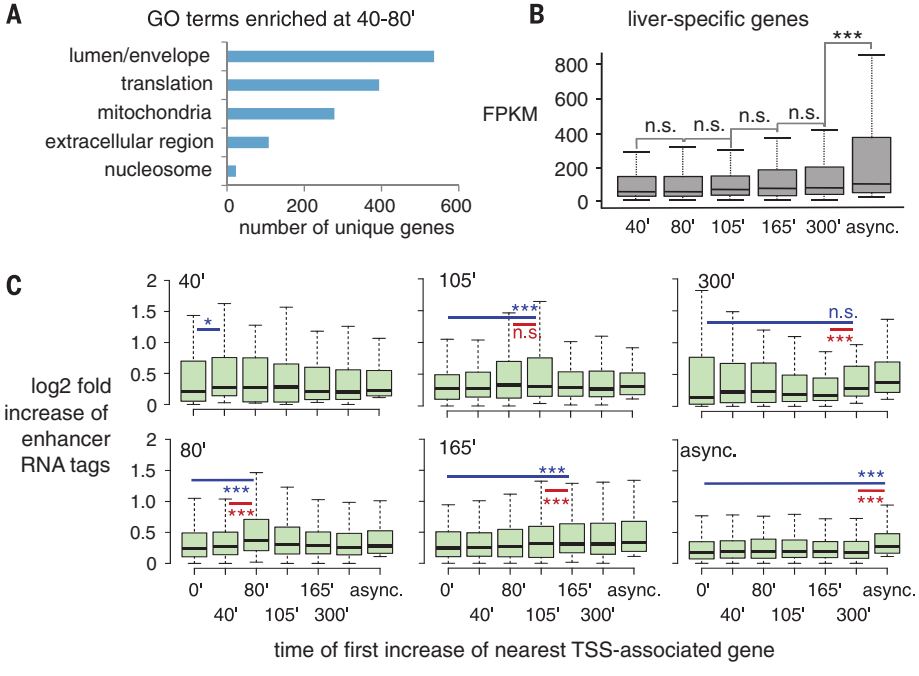

Fig. 4. Basic cell functions are prioritized over cell specificity during mitotic exit. (**A**) Representative GO categories for 40 to 80 min. (**B**) FPKMs of liver-specific genes. Bar, mean; whiskers, quartiles; $P < 0.001$ from 300 min to asynchronous; $n = 149$ liver-specific genes. (**C**) Increase in eRNAs (*29*) within 100 kb of transcription start site (TSS) during mitotic exit. Bar, mean; whiskers, quartiles; n.s., not significant; blue, comparison to mitosis; red, comparison to previous time point; $*P < 0.05$, $***P < 0.001$.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/358/6359/119/suppl/DC1
Materials and Methods
Figs. S1 to S11
Tables S1 to S24
References (*31*–*35*)

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Gene expression during mitosis

During mitosis, long-range interactions within chromosomes are lost, and many enhancers become inactive. It is generally thought that gene expression is silent at this time. However, transcription must be reactivated when cells reenter the cell cycle in order to maintain cell identity. Palozola *et al.* used a sensitive nascent RNA labeling and sequencing method to reveal low-level transcription of many genes in mitosis. Upon mitotic exit, the amplitude of gene expression was reestablished with basic cell functions prioritized over cell-specific genes. Thus, transcription itself may retain gene expression patterns through mitosis.

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