# Supporting Information

## Verzijlbergen et al. 10.1073/pnas.0911164107

### SI Materials and Methods

**Yeast Strains and Plasmids.** All *Saccharomyces cerevisiae* strains used in this study are derived from S288C strains BY4705, BY4727, BY4741, and BY4742 (1) and are listed in Table S1. Plasmids pRS400, pFvL99, and pFvL100 were used for gene replacements by KanMX4, NatMX4, and HpHMX4, respectively (2, 3). To generate pFvL99 and pFvL100, the PacI-BsmI KanMX4 insert of pRS400 was replaced by a PacI-BsmI fragment of pAG25 or pAG32 (4), respectively. The drug resistance cassettes were amplified using the standard pRS primers. RITE cassettes were constructed by restriction enzyme-based cloning of PCR fragments in a modular fashion to generate the following basic construct: NotI-spacer-LoxP-KpnI-Tag1-Spe1-stop-ADH1term-BamHI-HygroMX-XbaI-LoxP-SalI-Tag2-BsrGI-stop. The following modules were used: spacer, GGTGGATCTGGTGGATCT; LoxP, ATAACTTCGTATAATGTATGCTATACGAAGTTATCA; HA, TACCCATACGATGTTCCTGACTATGCG; T7, ATGGCAAGCATGACTGGTGGACAGCAAATGGGT; HphMX, 1.8-kb fragment amplified from pFvL100 AGATTGTACTGAGAGTGCAC..... CGGTGTGAAATACCGCACAG; ADH1 terminator, amplified from pFA6a-3HA-KanMX (5) CTTCTAAATAAGCGA..... GGGATAACAGGGTAA. The encoded short peptide spacer sequence (GGSGGS) was found to be required for viability of strains carrying tagged histones. The 34-bp LoxP DNA sequence is part of the coding region (resulting in the peptide sequence ITSYNVCYTKLS) and is present in front of the epitope tags both before and after the switch. RITE cassettes were PCR amplified and targeted to the 3′ end of the endogenous genes by homologous recombination to tag the C terminus and ensure regulation by the endogenous promoter. The hormone-dependent Cre-EBD was described previously (6). We used a derivative of this construct (Cre-EBD78) that is constitutively expressed and contains several mutations to make the recombinase more tightly dependent on β-estradiol (7). A *TDH3* promoter fragment, Cre-EBD78, and a *CYC1* terminator sequence were cloned into pRS303 (1) to generate pTW040, which was linearized with Eco47III or MluI to integrate the construct at the *HIS3* locus or the *CYC1* locus, respectively.

**Galactose Induction.** To perform a galactose induction the cells were grown to saturation in YP containing 3% raffinose. Because the cells cycle a little slower in raffinose than in glucose, twice the amount of cells was grown for the same amount of time as previously. To release the cells into the cell cycle, one-half of the culture was resuspended in YPD and the other half in YP + 1% raffinose + 2% galactose. Both media contained 0.5 ng/μL of α-factor. To determine the speed of release in either medium, the cells were grown identically to previously, but released into media containing 15 μg/mL Nocodazole. Cells were harvested every hour for FACS analysis of DNA content.

**Polyclonal Antibody Production.** A polyclonal antibody was obtained by immunizing rabbits using the peptide GGSGGSITSYNVC*YTKLS against the spacer and LoxP sequence (the asterisk indicates the cysteine present as a sulfhydryl necessary for conjugation). For immunization 2 mg of the peptide was covalently conjugated to Imject Mariculture Keyhole Limpet Hemocyanin (mcKLH) (Pierce) as a carrier protein. The concentration of the conjugated hapten was determined using Bradford (Bio-Rad). Per immunization 100 μg in 1 mL PBS was injected. Each rabbit received three boosts with 1-month intervals; two rabbits were immunized. Each antibody was tested for specificity using a WT, an HA, and a T7 tagged strain.

**Southern Blotting.** For Southern blotting $5 \times 10^8$ cells were spun and frozen at −80°C. A histone H3 (HHT2)-specific probe was made by PCR amplification using the primers HHT2_HindIII_for GAATCTTTCTGTGACGCTTGG and HHT2_HindIII_rev GGGGAAGAACAGTTGGAAGG, resulting in a 650-bp amplicon covering the region 576,144–576,794. When used on genomic DNA that was digested using the HindIII enzyme, the three bands recognized are specific for before the switch (3000 bp), after the switch (931 bp), or as an internal control (1538 bp). Radioactive Southern blotting was performed using 50 μCi of $^{32}$P-dCTP; incubation was done overnight at 65°C.

**Quantitative Immunoblotting.** For immunoblotting $5 \times 10^7$ cells were spun and washed once with cold 1× TE + 0.2 mM PMSF, and the pellet was frozen (3). Whole-cell extracts were obtained from $\approx 5 \times 10^7$ cells by the classical glass beads breakage method using 200 μL of glass beads and SUMEB (8) complemented with PMSF (1 mM), benzamidine (5 mM), pepstatin (1 μg/mL), leupeptin (1 μg/mL), and DTT (1 μM). The resulting lysate was separated onto a 16% polyacrylamide gel and blotted onto a 0.45-μm nitrocellulose membrane. Membranes were blocked with 2% Nutrilon (Nutricia) in PBS. Primary antibody incubations were performed overnight in Tris-buffered saline-Tween with 2% Nutrilon, anti-HA (12CA5), anti-T7 (Abcam, 1:1,000), and a polyclonal antibody obtained against the LoxP peptide (1:2,500). Secondary antibody incubations were performed for 45 min using LI-COR Odyssey IRDye 800CW (1:12.000). Immunoblots were subsequently scanned on a LI-COR Odyssey IR Imager (Biosciences), using the 800 channel. Signal intensities were determined using Odyssey LI-COR software version 3.0. Ratios of T7/HA were converted into %HA values by using a standard curve of samples with known amounts of H3-HA and H3-T7. These samples were generated by mixing cells expressing either only H3-HA (NKI4004) or only H3-T7 (NKI4009) in various ratios of cell numbers. Blots of the standard curve and the experimental samples were processed simultaneously.

**Reverse Transcription.** Ranking of genes based on estimated transcription frequencies was based on genomewide mRNA expression and stability data from Holstege et al. (9). Total yeast RNA was prepared from $5 \times 10^7$ cells of each of the indicated growth conditions, using the RNeasy kit (Qiagen) according to the manufacturer's protocol (10). RNA samples were treated with RNase free DNase (Qiagen), and cDNA was made by using Super-Script II reverse transcriptase (Invitrogen). To obtain an S-phase sample, cells were synchronized for 3 h in G1 using 0.5 ng/μL α-factor, released after two washes with YPD (containing 0.1 mg/mL ProNase E if the strain was *bar1Δ*), and isolated every 0.5 h. By FACS analysis it was determined that 0.5 h after release the maximum amount of cells were in S-phase.

**Chromatin Immunoprecipitation.** ChIP was performed as described previously (3, 10, 11). Approximately $1 \times 10^9$ cells were fixed with 1% formaldehyde for 15 min at room temperature. The formaldehyde was quenched with 125 mM glycine by shaking for 5 min at room temperature. Cells were washed once in cold TBS + 0.2 mM PMSF, and the pellet was frozen at −80°C.The chromatin was sheared using a bioruptor (Diagenode) for 6 min with 30-sec intervals at high power. The obtained fragments have an average size of 500 bp, as determined on a 2% TAE gel stained

with ethidium bromide and quantified using TINA software. The isolated chromatin of the equivalent of $5 \times 10^7$ cells was immunoprecipitated overnight at 4°C using magnetic Dynabeads (Invitrogen) that were previously incubated with antibody overnight at 4°C.

**Real-Time PCR.** ChIP DNA and cDNA were quantified in real-time PCR using the SYBR Green PCR Master Mix (Applied Biosystems) and the ABI PRISM 7500 as described previously (2, 10). An input sample was used to make a standard curve, which was then used to calculate the IP samples, all performed in the 7500 fast system software. As a measurement for exchange, the amount of DNA of the T7-IP was divided over the HA-IP. Primers used for qPCR are listed in Table S2.

**Staining Cells with N-Hydroxysuccinimide-Tetra-Ethylrhodamine (NHS-TER).** A 20% aqueous solution of an isomeric mixture of 5 (6)-carboxyrhodamine (Rhodamine WT) was obtained from Abbey Color. The free acid was precipitated with concentrated hydrochloric acid (two equivalents) as described (12). The precipitate was collected by centrifugation and resuspended in 1 M HCl. This procedure was repeated twice and the precipitate was frozen and freeze dried to remove residual traces of water. The free acid was converted in an active N-hydroxysuccinimide (NHS) ester by condensation with N-hydroxysuccinimide mediated by the agent di-isopropylcarbodiimide (DIC). This is a relatively simple and very economical procedure compared to other fluorescent labeling approaches. To stain yeast cells, cultures were washed twice with PBS. Cells were resuspended in PBS, and NHS-TER was added (0.8 mg NHS-TER per $10^8$ cells) and incubated at room temperature for 15 min. Cells were washed eight times with PBS and then resuspended in YPD medium. For each time point $10^7$ cells were fixed for FACS analysis or confocal microscopy. The samples were fixed with 4% formaldehyde in PBS for 10 min at room temperature and washed with water. Cells were briefly sonicated. For confocal microscopy the pellet was resuspended in 1 mL water and cells were stained with Hoechst (1 μg/mL) as a DNA stain. The pellet was resuspended in 50 μL water and 2 μL of this solution was mounted in Vectashield mounting medium on a concanavalin-A coated coverslip. Confocal analyses were performed using a Leica TCS SP2 confocal system, equipped with Diode 405 and 561 lasers. Images were taken using a 63× 1.4 objective. Emission windows 415–540 and 571–700 and Kalman averaging were used. For FACS analysis the cells were resuspended in 500 μL water.

**FACS Analysis of DNA Content and Cell Doubling.** The DNA content was measured using SYTOX Green in flow cytometry as described previously (2, 13), and detection was done using a 530/30 filter. For FACS analysis of DNA content $1 \times 10^7$ cells were spun briefly at maximum speed, resuspended in 1 mL of 70% ethanol, and kept at −20°C. NHS-TER stained cells were detected using a 585/42 filter of the FACS calibur (Becton-Dickinson). For each measurement 100,000 cells were counted. Analysis was performed using FCS express 2. To determine the percentage of stained cells (mother) versus unlabeled cells (daughter), NHS-TER stained cells were harvested at indicated time points. Additionally, cells were also counted using a count chamber and a wide field microscope. The signal in channel FL2 was divided into two regions on the basis of a 100% labeled control and an unlabeled control, and these regions were applied to all samples. The percentage of labeled cells ($L$) was used to calculate the number of population doublings (Dp) by $L = 100 \times 0.5^{Dp}$.

**Microscopy.** A total of $5 \times 10^6$ cells were pelleted, washed once with water, and fixed with 4% formaldehyde for 10 min at room temperature. Cells were then washed with water and nuclei were stained with Hoechst 33342 (Invitrogen, 1 μg/mL) for 15 min at room temperature. Cells were then washed and resuspended in 100 μL water. Resuspended cells were mixed with 1 vol Vectashield mounting solution (Vector Laboratories) and mounted onto ConA-coated coverslips. The images were made using a Leica AOBS LSCM (Leica Microsystems), using a 405-, a 488-, and a 563-nm laser to visualize Hoechst, GFP, and mRFP, respectively. Images were analyzed using customized Cell Profiler (open-source cell image analysis software). For each time point, four different micrographs, each of them containing ≈100 yeast cells, were quantified using the pipeline described below.

**Confocal Microscopy Pipeline.** The images were made using a Leica AOBS LSCM (Leica Microsystems) equipped with a HCX PL APO lbd.bl 63×/NA 1.4 oil corrected objective lense (Leica). The acquisition software used was Leica LCS. Cells were imaged using a 405-, a 488-, and a 563-nm laser to visualize Hoechst, GFP, and mRFP, respectively.

**Pixel Size: 1**

***Pipeline:***

LoadImages
Combine
IdentifyPrimAutomatic
IdentifySecondary
IdentifyTertiarySubregion
MeasureObjectIntensity
MeasureObjectIntensity
MaskImage
IdentifyPrimAutomatic
MeasureObjectIntensity
Relate

**Module 1: LoadImages Revision—2**

How do you want to load these files? Text-Exact match
Type the text that one type of image has in common (for TEXT options) or their position in each group (for ORDER option): ch02
What do you want to call these images within CellProfiler? OrigBlue
Type the text that one type of image has in common (for TEXT options) or their position in each group (for ORDER option). Type "Do not use" to ignore: ch00
What do you want to call these images within CellProfiler? (Type "Do not use" to ignore) OrigRed
Type the text that one type of image has in common (for TEXT options) or their position in each group (for ORDER option): ch01
What do you want to call these images within CellProfiler? OrigGreen
Type the text that one type of image has in common (for TEXT options) or their position in each group (for ORDER option): Do not use
What do you want to call these images within CellProfiler? Do not use
If using ORDER, how many images are there in each group (i.e., each field of view)? 3
What type of files are you loading? Individual images
Analyze all subfolders within the selected folder? No
Enter the path name to the folder where the images to be loaded are located. Type period (.) for default image folder. .
Note: If the movies contain more than just one image type (e.g., brightfield, fluorescent, field-of-view), add the GroupMovieFrames module. .

**Module 2: Combine Revision—3**

What did you call the first image to be combined? OrigRed
What did you call the second image to be combined? OrigGreen
What did you call the third image to be combined? Do not use
What do you want to call the combined image? RedGreen
Enter the weight you want to give the first image 1
Enter the weight you want to give the second image 1
Enter the weight you want to give the third image 1

**Module 3: IdentifyPrimAutomatic Revision—12**

What did you call the images you want to process? OrigBlue
What do you want to call the objects identified by this module? Nuclei
Typical diameter of objects, in pixel units (Min, Max): 8, 20
Discard objects outside the diameter range? Yes
Try to merge too small objects with nearby larger objects? No
Discard objects touching the border of the image? No
Select an automatic thresholding method or enter an absolute threshold in the range [0, 1]. To choose a binary image, select "Other" and type its name. Choosing "All" will use the Otsu Global method to calculate a single threshold for the entire image group. The other methods calculate a threshold for each image individually. "Set interactively" will allow you to manually adjust the threshold during the first cycle to determine what will work well. Otsu Adaptive
Threshold correction factor 0.05
Lower and upper bounds on threshold, in the range [0, 1] 0.2, 1
For MoG thresholding, what is the approximate fraction of image covered by objects? 10 Method to distinguish clumped objects (see help for details): Intensity
Method to draw dividing lines between clumped objects (see help for details): Intensity
Size of smoothing filter, in pixel units (if you are distinguishing between clumped objects). Enter 0 for low-resolution images with small objects (~<5-pixel diameter) to prevent any image smoothing. Automatic
Suppress local maxima within this distance, (a positive integer, in pixel units) (if you are distinguishing between clumped objects) Automatic
Speed up by using lower-resolution image to find local maxima? (if you are distinguishing between clumped objects) Yes
Enter the following information, separated by commas, if you would like to use the Laplacian of Gaussian method for identifying objects instead of using the above settings: Size of neighborhood (height, width), Sigma, Minimum Area, Size for Wiener Filter (height, width), Threshold Do not use
What do you want to call the outlines of the identified objects (optional)? Do not use
Do you want to fill holes in identified objects? Yes
Do you want to run in test mode where each method for distinguishing clumped objects is compared? No

**Module 4: IdentifySecondary Revision—3.**

What did you call the primary objects you want to create secondary objects around? Nuclei
What do you want to call the objects identified by this module? Cells
Select the method to identify the secondary objects (Distance - B uses background; Distance - N does not): Propagation
What did you call the images to be used to find the edges of the secondary objects? For DISTANCE - N, this will not affect object identification, only the final display. RedGreen
Select an automatic thresholding method or enter an absolute threshold in the range [0, 1]. To choose a binary image, select "Other" and type its name. Choosing "All" will use the Otsu Global method to calculate a single threshold for the entire image group. The other methods calculate a threshold for each image individually. Set interactively will allow you to manually adjust the threshold during the first cycle to determine what will work well. Otsu Adaptive
Threshold correction factor 1.4
Lower and upper bounds on threshold, in the range [0, 1] 0, 0.1
For MoG thresholding, what is the approximate fraction of image covered by objects? 20 For DISTANCE, enter the number of pixels by which to expand the primary objects [Positive integer] 10
For PROPAGATION, enter the regularization factor (0 to infinity). Larger = distance, 0 = intensity 0.05
What do you want to call the outlines of the identified objects (optional)? Do not use
Do you want to run in test mode where each method for identifying secondary objects is compared? No

**Module 5: IdentifyTertiarySubregion Revision—1.**

What did you call the larger identified objects? Cells
What did you call the smaller identified objects? Nuclei
What do you want to call the new subregions? Cytoplasm
What do you want to call the outlines of the identified objects (optional)? Do not use

**Module 6: MeasureObjectIntensity Revision—2.**

What did you call the grayscale images you want to measure? OrigRed
What did you call the objects that you want to measure? Cells
Nuclei
Cytoplasm
Do not use
Do not use
Do not use

**Module 7: MeasureObjectIntensity Revision—2.**

What did you call the grayscale images you want to measure? OrigGreen
What did you call the objects that you want to measure? Cells
Nuclei
Cytoplasm
Do not use
Do not use
Do not use

**Module 8: MaskImage Revision—3.**

From which object would you like to make a mask? Cytoplasm
Which image do you want to mask? RedGreen
What do you want to call the masked image? MaskBlue
Do you want to invert the object mask? No

**Module 9: IdentifyPrimAutomatic Revision—12.**

What did you call the images you want to process? MaskBlue
What do you want to call the objects identified by this module? Aggregates
Typical diameter of objects, in pixel units (Min, Max): 3, 15
Discard objects outside the diameter range? Yes
Try to merge too small objects with nearby larger objects? No
Discard objects touching the border of the image? Yes
Select an automatic thresholding method or enter an absolute threshold in the range [0, 1]. To choose a binary image, select "Other" and type its name. Choosing "All" will use

the Otsu Global method to calculate a single threshold for the entire image group. The other methods calculate a threshold for each image individually. "Set interactively" will allow you to manually adjust the threshold during the first cycle to determine what will work well. Otsu Global
Threshold correction factor 1
Lower and upper bounds on threshold, in the range [0, 1] 0.35, 1
For MoG thresholding, what is the approximate fraction of image covered by objects? 0.01
Method to distinguish clumped objects (see help for details): Intensity
Method to draw dividing lines between clumped objects (see help for details): Intensity
Size of smoothing filter, in pixel units (if you are distinguishing between clumped objects). Enter 0 for low-resolution images with small objects (~<5-pixel diameter) to prevent any image smoothing. Automatic
Suppress local maxima within this distance (a positive integer, in pixel units) (if you are distinguishing between clumped objects), Automatic
Speed up by using lower-resolution image to find local maxima (if you are distinguishing between clumped objects)? Yes
Enter the following information, separated by commas, if you would like to use the Laplacian of Gaussian method for identifying objects instead of using the above settings: Size of neighborhood (height, width), Sigma, Minimum Area, Size for Wiener Filter (height, width),Threshold Do not use
What do you want to call the outlines of the identified objects (optional)? Do not use
Do you want to fill holes in identified objects? Yes
Do you want to run in test mode where each method for distinguishing clumped objects is compared? No

**Module 10: MeasureObjectIntensity Revision—2.**

What did you call the grayscale images you want to measure? OrigGreen
What did you call the objects that you want to measure? Aggregates
Do not use
Do not use
Do not use
Do not use
Do not use

**Module 11: Relate Revision—2.**

What objects are the children objects (subobjects)? Aggregates
What are the parent objects? Cytoplasm
What other object do you want to find distances to (must be one object per parent object, e.g., Nuclei)? None


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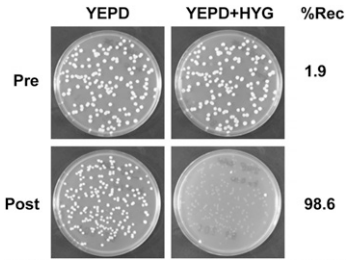


**Fig. S1.** The efficiency of Cre recombination in G0 cells determined by a plating assay. The efficiency of recombination in the cell population in G0 cells was determined by plating the yeast cells on nonselective media (YEPD) and subsequent replica plating to media containing Hygromycin (YEPD+HYG). The fraction of Hygromycin-sensitive colonies indicates the fraction of recombined/switched cells (%Rec) before (Pre) and after (Post) activation of Cre recombinase by addition of the hormone β-estradiol.

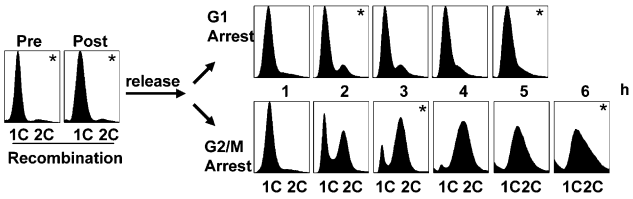


**Fig. S2.** Cell cycle progression and arrest monitored by flow cytometry. FACS analysis of DNA content to monitor release of starved cells from G0 (1C) into the G1 (1C) and G2/M (2C) blocks. Asterisks indicate the analyzed time points.

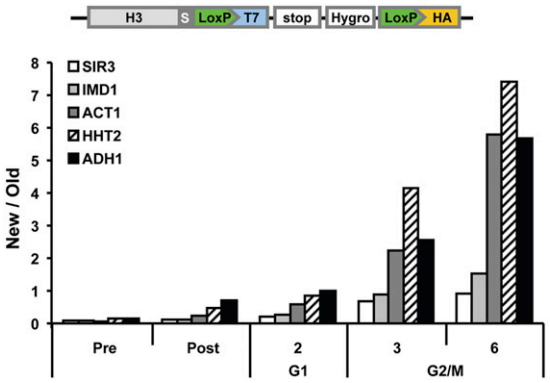


**Fig. S3.** Histone exchange is independent of the order of the RITE tags. Starved switched cells (see Fig. 3) containing a "swapped-tag" cassette that switches from T7 to HA (H3-T7→HA) were released into fresh media and arrested in G1 or G2/M. ChIP of T7 and HA was quantified by qPCR for the genes indicated. The 5-h G1 arrest time point was not analyzed because this BAR1 wild-type strain degrades α-factor and escapes from the arrest after 4 h.

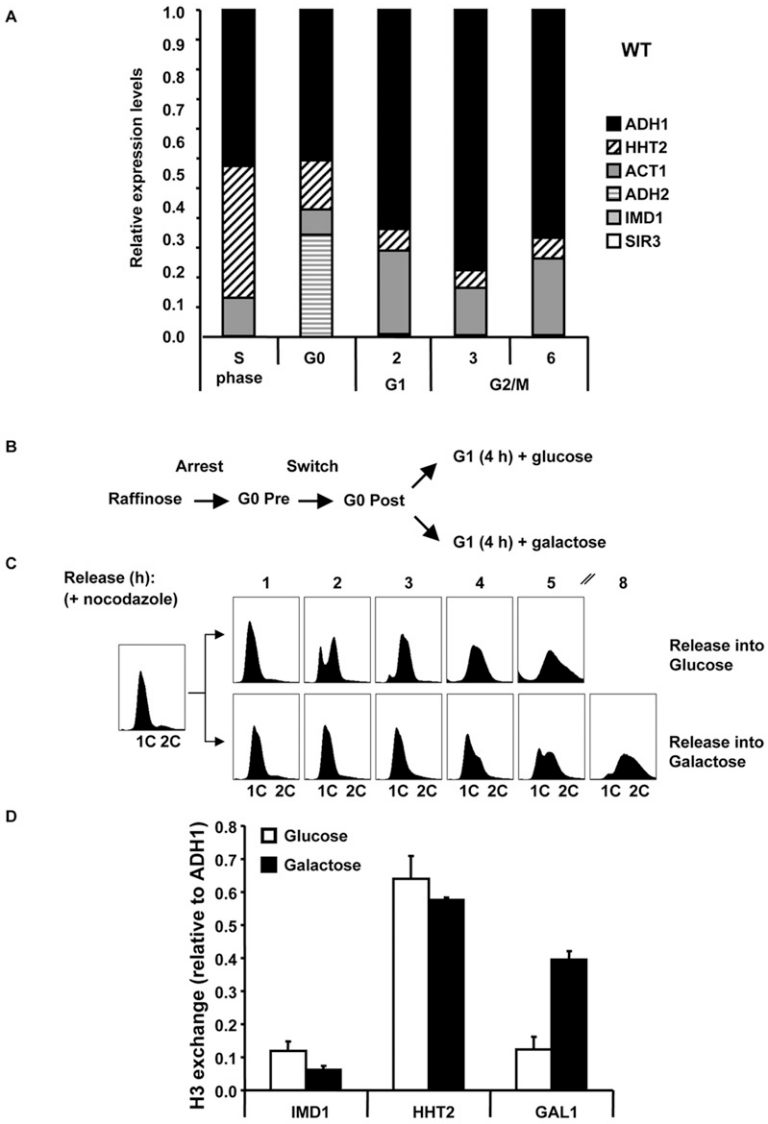


**Fig. S4.** Histone exchange correlates with mRNA expression levels. (*A*) To confirm that the RITE tags did not affect gene expression, mRNA was isolated from a wild-type strain (NKI2036) and relative expression levels were calculated at each indicated time. The relative expression levels are identical to those of the RITE-tagged strain (Fig. 4*B*). The relative mRNA expression patterns correlated very well with the observed histone exchange rates. For example, *ADH2* was active under low glucose conditions in starved cells and repressed in cells released in glucose-rich media. Indeed, *ADH2* belonged to the genes with high exchange in arrested cells or shortly after release and then dropped to the low exchange at later time points (Fig. 3 *C* and *D*). *ACT1*, which was induced in cells arrested by α-factor, specifically peaked late in the G1 arrest (Fig. 3*C*). Finally, relative exchange at *HHT2* was highest early in the G2/M arrest. Although *HHT2* expression was low in G2/M, cells at this time point have just exited S-phase, during which transcription of histone genes was induced (Fig. 3*D*). Therefore, the presence of new H3-T7 might be a mark of previous transcription events. (*B*) To analyze the causal effect of transcription on histone exchange, cells were starved and switched in medium containing raffinose and subsequently released into medium containing glucose or galactose, to repress or induce the *GAL1* gene, respectively. Cells were arrested in G1 for 4 h. (*C*) FACS analysis of cells starved in raffinose media and released in media containing nocodazole (G2/M arrest) and either glucose or galactose. Starved cells released in media with galactose reentered more slowly into the cell cycle and showed lower overall new histone expression at this time point. Therefore, subsequent exchange ratios were determined relative to *ADH1*. (*D*) Histone exchange (ChIP T7/HA) at *GAL1*, *HHT2*, and *IMD1*. Upon activation of *GAL1*, deposition of new H3-T7 was increased at the *GAL1* promoter, indicating that transcription enhanced histone exchange.

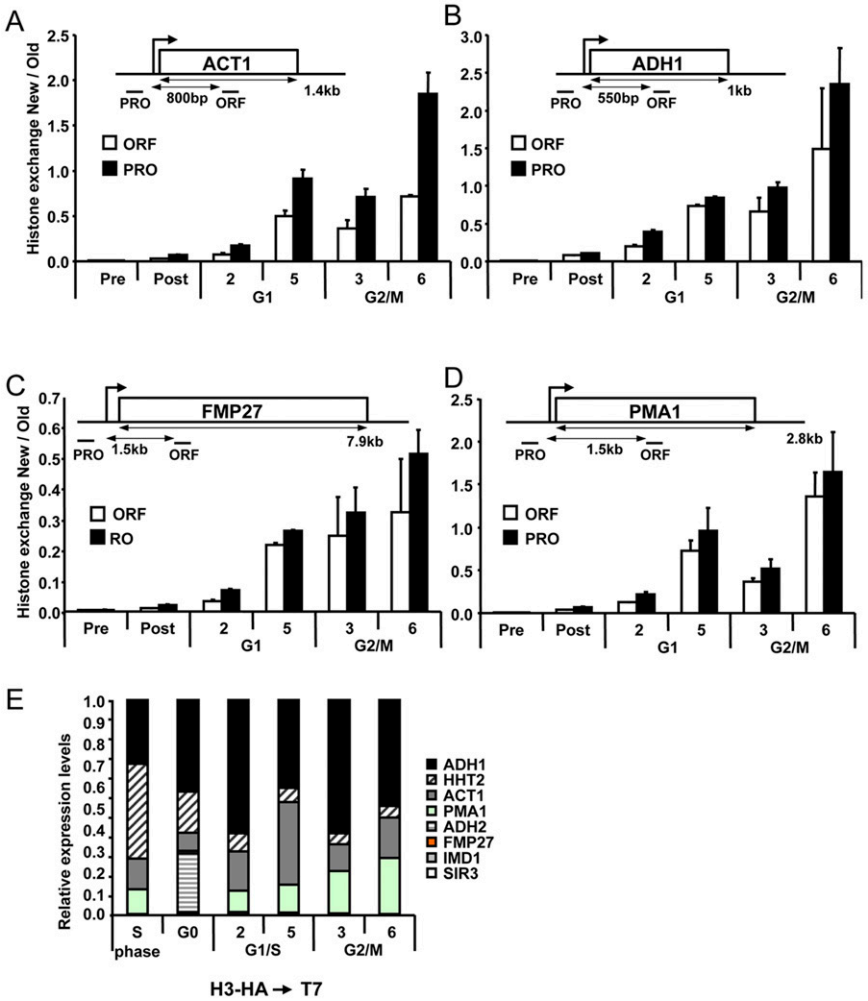


**Fig. S5.** Histone exchange occurs at promoters and coding sequences. (*A–D*) RITE-ChIP showed that new H3-T7 was readily incorporated in promoters by a transcription-coupled process. However, histone exchange at promoter regions, which represent less than a quarter of the yeast genome, was insufficient to explain the global deposition of new H3-T7 (~50% of the total H3 pool) observed by immunoblots (Fig. 3*B*). Therefore histone exchange in coding sequences was also determined. Histone exchange at promoters (PRO) was determined as in Fig. 3*C* and compared to exchange in coding sequences (ORF) of *ACT1* and *ADH1* and, in addition, of two long genes *FMP27* and *PMA1* to exclude effects of proximal promoter sequences. The location of the analyzed regions and the distance between promoter and ORF regions are indicated. (*E*) Relative mRNA expression levels in the H3-HA→T7 strains as shown in Fig. 4*B* but now including the expression of *PMA1* and *FMP27*. Ectopically expressed histones in yeast have been shown to be predominantly incorporated in promoter regions largely irrespective of the level of transcription, whereas the lower level of exchange in ORFs correlated with transcription rates (14–20). Using RITE we found by ChIP that exchange of endogenous histones in ORFs was nearly as high as in promoters and this notion was supported by the global turnover of bulk histones that we detected by immunoblot (Fig. 3*B*). In addition, the rate of exchange of endogenous histones in ORFs as well as promoters correlated with transcription levels. Thus, transcription-coupled replication-independent exchange of histones occurred in promoters and coding regions.

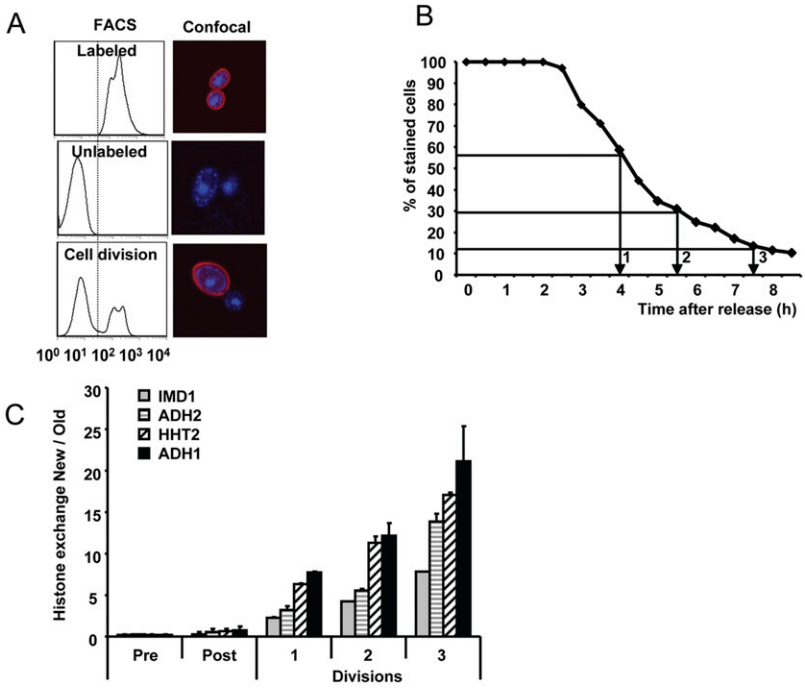


**Fig. S6.** Histone exchange in successive cell divisions. Starved switched H3-T7→HA cells were released into fresh media and harvested after one, two, and three rounds of cell division. To count cell divisions we developed a convenient and stable fluorescent cross-linker, NHS-TER, which labels the yeast cell wall. (*A*) Upon cell division, daughter cells or buds synthesize the cell wall de novo and emerge as unlabeled cells whereas the mother cells retain the label (21). Thus, the fraction of unlabeled cells, which can be quantitatively determined by FACS, is a measure of the number of cell doublings. (*B*) The cell wall of the starved cells was labeled with NHS-TER before induction of Cre recombinase to quantitatively determine the number of cells in the population that had undergone cell division. Mother cells retain the old cell wall and daughter cells make the cell wall de novo, as confirmed by confocal microscopy [*A* (*Right*): blue is Hoechst DNA staining]. The percentage of unlabeled cells identified by FACS [*A* (*Left*)] indicates the percentage of newborn daughter cells in the population and corresponds to the percentage of chromatin that is new. (*B*) Starved H3-T7→HA cells stained with NHS-TER before Cre induction were released after the switch into fresh media and samples were taken at the indicated time points to capture cells after one, two, and three cell divisions. The number of cell divisions in the population was confirmed by FACS. The percentage of cells that had undergone a cell division ($d$) relates to the percentage of unlabeled cells ($u$) by $100 \times u = d(100 + d)^{-1}$. (*C*) Histone replacement at promoters in samples described in *B* was determined by ChIP (HA/T7).

**Table S1. Yeast strains**

| Strain | Relevant genotype | Reference |
|---|---|---|
| BY4705 | MATα ade2Δ::HisG his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 | (1) |
| BY4724 | MATa lys2Δ0 ura3Δ0 | (1) |
| BY4742 | MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 | (1) |
| NKI2036 | MATa his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0 Δhhf1-hht1::LEU2 | This study |
| NKI2148 | MATa his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0 Δhhf1-hht1::LEU2 HIS3::Ptdh3_CRE_EBD78 bar1Δ::HisG hht2::HHT2-LoxP-HA-HYG-LoxP-T7 | This study |
| NKI2158 | MATa his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0 Δhhf1-hht1::LEU2 CYC1term::Ptdh3_CRE_EBD78_HIS3 hht2::HHT2-LoxP-T7-HYG-LoxP-HA | This study |
| NKI4004 | MATa his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0 Δhhf1-hht1::LEU2 hht2::HHT2-LoxP-HA-HYG-LoxP-T7 | This study |
| NKI4009 | MATa his3Δ200 leu2Δ0lys2Δ0 met15Δ0 ura3Δ0 Δhhf1-hht1::LEU2 hht2::HHT2-LoxP-T7 | This study |
| Y7092 | MATα can1Δ::STE2pr-Sp_his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2 | (2) |
| NKI4101 | MATα can1Δ::STE2pr-Sp_his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2 pre3::PRE3-V5-loxP-HA-yEGFP-HYG-loxP-T7-mRFP | This study |
| NKI4103 | NKI4101 + lyp1Δ::NATMX-GPD_CRE_EBD78 | This study |
| NKI4104 | MATα can1Δ::STE2pr-Sp_his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2 lyp1Δ::NATMX-GPD_CRE_EBD78 pre3::PRE3-V5-loxP-T7-mRFP | This study |


1. Brachmann CB, et al. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: A useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 14:115–132.
2. Tong AH, Boone C (2006) Synthetic genetic array analysis in *Saccharomyces cerevisiae*. *Methods Mol Biol* 313:171–192.

**Table S2. qPCR primers**

| Gene name | Primer name | Primer sequence |
|---|---|---|
| ADH1 | ADH1 PRO ii fwd | CCGTTGTTGTCTCACCATATCC |
| ADH1 | ADH1 PRO ii rev | GTTTCGTGTGCTTCGAGATACC |
| ADH1 | ADH1 ORF II fwd | TAGGTTCTTTGGCTGTTCAATACG |
| ADH1 | ADH1 ORF II rev | CGGAAACGGAAACGTTGATGACACCG |
| HHT2 | HHT2_QFor1 | GTGCCAAACGACCACAGTTG |
| HHT2 | HHT2_QRev1 | GGGCGTGCCAATAGTTTCAC |
| HHT2 | HHT2_QFor2 | AAGCCCCAAGAAAACAATTAGC |
| HHT2 | HHT2_QRev2 | TGGCTTATATCTGTGAGGCTTCTTAA |
| ACT1 | ACT1_Qfor | CTCTTTTTATCTTCCTTTTTTTCCTCTCT |
| ACT1 | ACT1_Qrev | CGTGAAAAATCTAAAAGCTGATGTAGTAG |
| ACT1 | ACT1_QforORF | TCGTTCCAATTTACGCTGGTT |
| ACT1 | ACT1_QrevORF | CGGCCAAATCGATTCTCAA |
| ADH2 | ADH2 PRO ii fwd | AACACCGGGCATCTCCAAC |
| ADH2 | ADH2 PRO ii rev | AAGTCGCTACTGGCACTC |
| ADH2 | ADH2 ORF I fwd | ACACCCACGACGGTTCTTTC |
| ADH2 | ADH2 ORF I rev | CAAGATTGGCGCGACTTCAG |
| IMD1 | QFORimd1 | TTTCGTGGGCTAGTACATTTTACCT |
| IMD1 | QREVimd1 | TGATAAGAAAAGTAAGGCAAGGAATAGA |
| IMD1 | Qimd1for-2 | TTTGCAGGCTTCCCTGTCA |
| IMD1 | Qimd1rev-2 | TGATGGCACCCACCAACTTT |
| SIR3 | SIR3_Qfor | CGAAAACGCTATTCTTTCCAAAA |
| SIR3 | SIR3_Qrev | CCCCTGTAAGGAAGGTGATGAA |
| SIR3 | SIR3_ORF_Qfor1 | GACGGCCGAGAGAATTTTGTAT |
| SIR3 | SIR3_ORF_Qrev1 | CTTCAAGCCCACCATCATCA |
| PMA1 | PMA1_PRO_Qfor1 | TGGTGGGTACCGCTTATGCT |
| PMA1 | PMA1_PRO_Qrev1 | TGTTAGACGATAATGATAGGACATTTGA |
| PMA1 | PMA1_ORF_Qfor1 | AAATCTTGGGTGTTATGCCATGT |
| PMA1 | PMA1_ORF_Qrev1 | CCAAGTGTCTAGCTTCGCTAACAG |
| FMP27 | FMP27_PRO_Qfor1 | AGGGAGACATGAAAAAGGGTCTT |
| FMP27 | FMP27_PRO_Qrev1 | TCTCTGAGATGTCTAGGCCCTTTTA |
| FMP27 | FMP27_ORF_Qfor1 | TGGACAGCATTGCCATAGAAGA |
| FMP27 | FMP27_ORF_Qrev1 | TGTACTAATTCACTCAACATAACCATTGTT |
| NoORF | NoORF_Qfor | GGCTGTCAGAATATGGGGCCGTAGTA |
| NoORF | NoORF_Qrev | CACCCCGAAGCTGCTTTCACAATAC |