Supplemental Experimental Procedures

Chromatin Immunoprecipitation

Cells were grown to an OD\textsubscript{600} of ~0.6 and fixed in 1% formaldehyde for 15 minutes. Cells were then lysed for 1 hour at 4°C in Lysis buffer (50mM HEPES pH 7.5, 150mM NaCl, 1mM EDTA, 1% Triton X-100, 0.1% Sodium Deoxycholate). Lysate was then sonicated using Biorupter (Diagenode) for 30 minutes, 30 seconds on/off, medium intensity. 2.5\(\mu\)l of \(\alpha\)-FLAG antibody (Sigma F3165) was added to 400\(\mu\)l of chromatin extract and incubated at 4°C overnight. 50\(\mu\)l of Protein G Dynabeads (Invitrogen 100-04D) was added incubated at 4°C for 2 hours then washed with Lysis buffer, twice in Lysis buffer with 500mM NaCl, TEL buffer (0.25M LiCl, 10mM Tris-HCl pH 8.0, 1mM EDTA, 1% NP-40, 1% Sodium Deoxycholate), then twice with 1x TE. Elutions were done by adding 200\(\mu\)l of Elution Buffer (1% SDS, 250mM NaCl, TE) at 65° with shaking, twice. Elutions were combined and treated with Proteinase K (Sigma) for 1 hour at 55°C. Crosslinking was then reversed and DNA was extracted. Quantitative PCR was performed and analyzed using iCycler iQ real-time PCR detection system (Bio-rad).

Immunoprecipitation and Western blotting analysis

Cells from a 220 ml culture grown to OD\textsubscript{600}=0.8 were pelleted and quickly frozen in liquid nitrogen. Pellets were suspended in 1 ml Lysis Buffer (50 mM Tris pH 7.5, 150 mM NaCl, 0.1% NP40, 1 mM DTT, 10% glycerol, and protease inhibitor tablet). For the Smc3 acetylation experiment, a cocktail of HDAC inhibitors (10 \(\mu\)M Trichostatin A, 50 mM nicotinamide, and 50 mM sodium butyrate) was contained. Then cells were lysed by adding glass beads followed by beadbeating for 60 sec 5 times with 2 min intervals on ice.
The supernatant was separated by centrifugation at 14K rpm at 4°C for 20 min. Immunoprecipitations were performed at 4°C using 30 µl α-HA affinity gel or α-Myc affinity gel (Sigma) overnight, followed by 5 washes with wash buffer (50 mM Tris pH 7.5, 150 mM NaCl, and 1% Triton X-100) and eluted with 2× SDS buffer (10 mM Tris pH 7.5, 1 mM EDTA, and 1% SDS). Eluates were loaded onto a 4-12% Bis-Tris gel for SDS-PAGE. Proteins were transferred from gels to a nitrocellulose membrane (Whatman), followed by blocking. Membranes were blotted with primary antibodies with α-HA (Roche, 3F10, #1867423), α-Myc (Covance, #MMS-150P), α-acetyl-lysine (CST, #9441) or α-GST (Covance, #MMS-112P). Detection was achieved with Amersham ECL Plus Western Blotting Detection System (GE Healthcare #RPN2132).

Transmission Electron microscopy

Cultures were grown in 200 ml YPD to OD600 ~0.8. Samples were frozen on the Leica EM-Pact at ~ 2050 bar, then transferred under liquid nitrogen into 2% Osmium Tetroxide/0.1% Uranyl Acetate/Acetone/2% Water and transferred to the Leica AFS2. The freeze substitution protocol started samples at -90° C for 72 hours, up 5° C an hour, then -20° for 12 hours, up 5° C hourly to 0 ° C for 5 hours. Samples were removed from the AFS2 and allowed to come to room temperature for 1 hour. Samples went through 3 changes of acetone over 1 hour and were then removed from the planchettes. Samples were stepwise embedded in acetone/Epon/Araldite mixtures to final 100% Epon/Araldite over several days as described previously [1]. Samples were cut into 80 nm section on a Leica UC6, stained with Uranyl acetate and Sato’s lead, and imaged on a FEI Technai Spirit.
**BrdU ChIP**

Exponentially growing cells were synchronized in G1 by α-factor. 5-bromo-2′deoxyuridine (BrdU) was added into the culture 20 minutes prior to release from G1 at a final concentration of 800 µg/mL. Cell pellets of the WT and eco1 strains were collected at 20 minutes and 40 minutes, respectively. α-BrdU ChIP was performed as described [2].

**Mass Spectrometry for proteomics**

TCA precipitated pellets were solubilized in Tris-HCl pH 8.5 and 8M urea; TCEP (Tris(2-Carboxylethyl)-Phosphine Hydrochloride, Pierce) and CAM (Chloroacetamide, Sigma) were added to a final concentration of 5 mM and 10 mM, respectively. Protein suspensions were digested overnight at 37°C using Endoproteinase Lys-C at 1:50 wt/wt (Roche). Samples were brought to a final concentration of 2M urea and 2 mM CaCl₂ before performing a second overnight digestion at 37°C using Trypsin (Promega) at 1:100 wt/wt. Formic acid (5% final) was added to stop the reactions. Samples were loaded on split-triple-phase fused-silica micro-capillary columns [3] and placed in-line with linear ion trap mass spectrometers (LTQ, ThermoScientific) coupled with quaternary Agilent 1100 or 1200 series HPLCs. A fully automated 10-step chromatography run (for a total of 20 hours) was carried out for each sample, as described in [4], enabling dynamic exclusion for 120 sec. The MS/MS datasets were searched using SEQUEST [5] against a database of 11990 sequences, consisting of 5819 S. cerevisiae non-redundant proteins, 177 usual contaminants (such as human keratins, IgGs, and proteolytic enzymes), and, to estimate false discovery rates, 5995 randomized amino acid sequences derived from each non-redundant protein entry. Peptide/spectrum
matches were sorted, selected and compared using DTASelect/CONTRAST [6]. Combining all runs, proteins had to be detected by at least 2 peptides, leading to FDRs at the protein and spectral levels of $1.14 \pm 0.27\%$ and $0.18 \pm 0.05\%$, respectively. To estimate relative protein levels, Normalized Spectral Abundance Factors (dNSAFs) were calculated for each detected protein, as described [7].

**Deep sequencing and data analysis**

Deep sequencing was performed on the Illumina HiSeq 2500 system. Sequencing libraries were made using the Nextera DNA Sample Preparation Kits (Illumina) as advised by the manufacturer. Illumina HiSeq single-end 50 bp sequence reads were aligned to the UCSC SaccCer3 genome using bowtie v0.12.9 and parameters (-k 1 -m1) which report only unique matches. Read counts were summarized on 1kb intervals across the genome using the GenomicRanges library from Bioconductor in R, and then normalized to counts per million. The log$_2$ ratio of normalized S to G1 phase cells was taken after adding 1 to the intervals from each data set to prevent division by zero errors. The data was then smoothed in R using loess with a span of 25kb and degree of 1. The final ratios were multiplied by 1.5 to aid visualization. Custom Perl scripts following the previous description [8, 9] were used to calculate this ratio. Early and late firing origins are determined according to previous publications [10, 11]. In order to measure completeness of replication, we divided the genome into 1 kb bins and scored replication for each bin by asking if replication reached a threshold of 0.05. These scores were summed to yield an indication of genome-wide replication.
Supplemental Reference