Supporting Information for:

Chromatin remodelers clear nucleosomes from energetically unfavorable sites to establish nucleosome-depleted regions at promoters

Denis Tolkunov ¹,⁴, Karl A. Zawadzki ²,³,⁴, Cara Singer ², Nils Elfving ², Alexandre V. Morozov ¹,⁵ and James R. Broach ²,⁵

¹ Department of Physics and Astronomy and BioMaPS Institute for Quantitative Biology
Rutgers University, Piscataway, NJ 08854

² Department of Molecular Biology
Princeton University, Princeton, NJ 08544

³ Current address:
Department of Developmental Biology
Stanford University School of Medicine, Stanford, CA 94305

⁴ both contributed equally to this work

⁵ Corresponding authors:
jbroach@princeton.edu morozov@physics.rutgers.edu
609-258-5981 732-445-1387
Supplementary Figures

**Figure S1**: Micrococcal digestion of nucleosomal DNA. Cultures of WT and \( asf1 \Delta \) strains were grown in SD+3% glycerol to mid-exponential phase, at which point glucose was added to 2% final concentration. Samples were removed at 0, 20, and 60 min following glucose addition treated with 4% formaldehyde, as described in Materials and Methods. Chromatin was prepared as described in Materials and Methods and digested with a titrated amount of micrococcal nuclease, after which the cross links were reversed with heat and samples fractionated on a 1.5% agarose gel. Photographs of EtBr stained gels are shown.
Figure S2: (A) Same as Figure 2B, for asf1 mutant grown in glucose vs. wild type grown in glucose. (B) Same as Figure 3B, for steady-state asf1 mutant and wild type in glucose. (C) Same as Figure 4B, for steady-state asf1 mutant and wild type in glucose. The p-values (computed using a two-tailed t-test) for the unsmoothed occupancy differences are $< 10^{-10}$ (B), 0.23 (C). The linear correlation coefficients for unsmoothed data are 0.21 (B), 0.02 (C).
**Figure S3**: *snf2* and *asf1* mutants contain excess nucleosomes over transcription termination sites (TTS). (A, upper panel) Nucleosome occupancy (quantified as the log-ratio of intensities from nucleosomal and control DNA hybridized to the tiling array) in the wild-type strain grown on glucose is subtracted from the corresponding occupancy in the *snf2* mutant strain; positive values indicate excess nucleosomes in the mutant relative to wild type. Nucleosome occupancy difference is shown 800 bp upstream and downstream of TTS (Nagalakshmi et al., 2008) for 5189 genes. The occupancy difference is clustered into two groups using K-means. The upper cluster shows the excess of nucleosomes in the *snf2* mutant. (B, upper panel) *asf1* mutant grown on glycerol vs. wild type grown on glycerol. (C, upper panel) *snf2* mutant 20 min. after downshift to glycerol vs. downshifted wild type. (D, upper panel) *asf1* mutant 20 min. after upshift to glucose vs. upshifted wild type. (A, B, C and D, lower panels) Average nucleosome occupancy in the two clusters for each mutant – wild-type combination. Solid lines correspond to the cluster with excess TTS nucleosomes in the mutant.
**Figure S4**: Histogram of steady-state differences in mRNA expression levels of ribosomal biogenesis (Ribi) and ribosomal protein (RP) genes in the *snf2* mutant relative to the wild type.
Figure S5: Genes were sorted into the two clusters shown in Figure 2 and the number and position of all glucose-related transcription factor binding motifs in the promoters of genes in each cluster were determined. The total fraction of transcription factors binding sites at each position, averaged over all the genes in each cluster, is plotted as a function of the distance from the transcription start site (TSS) in the -800 bp to +800 bp range. The histograms were smoothed with a 50-gene moving average. (A) Red solid line corresponds to the cluster with excess nucleosomes in the steady-state snf2 mutant grown in glucose. Black dashed line corresponds to the other cluster in which no difference was found between nucleosome occupancy in the snf2 mutant and the wild type. (B) Same as (A) but 20 min. after downshift to glycerol. (C) Red solid line corresponds to the cluster with excess nucleosomes in the steady-state asf1 mutant grown in glycerol. Black dashed line corresponds to the other cluster in which no difference was found between nucleosome occupancy in the asf1 mutant and the wild type. (D) Same as (C) but 20 min. after upshift to glucose.
**Figure S6:** (A) mRNA expression levels (log$_2$) in wild-type (wt), asf1Δ, and snf2Δ strains. Each row is an expression profile for a *S. cerevisiae* gene (red indicates induction and green represents repression with respect to the reference strain). Gene expression profiles were subjected to hierarchical clustering. The heat map columns are as follows: snf2Δ/wt at steady state in glucose; wt at 5, 20, and 60 min. after the glucose-to-glycerol downshift with respect to steady-state wt in glucose; snf2Δ at 5, 20, and 60 min. after the glucose-to-glycerol downshift with respect to snf2Δ at steady state in glucose; a biological replicate of wt at 20 and 60 min. after the glucose-to-glycerol downshift with respect to wt at steady state in glucose; a biological replicate of snf2Δ at 20 and 60 min. after the glucose-to-glycerol downshift with respect to snf2Δ at steady state in glucose.
glucose; wt at 5, 10, 15, and 20 min. after the glycerol-to-glucose upshift with respect to wt at steady state in glycerol; \textit{asf1}\Delta at 5, 10, 15, and 20 min. after the glycerol-to-glucose upshift with respect to \textit{asf1}\Delta at steady state in glycerol. (B) Ribosomal biogenesis (Ribi) gene repression upon glucose-to-glycerol downshift is more pronounced in the \textit{snf2} mutant compared to wild type. Shown are mRNA expression levels of three Ribi genes in the wild-type and the \textit{snf2} mutant mapped by quantitative PCR. mRNA expression (log$_2$) for each gene is plotted with respect to its expression at steady state in glucose.
Figure S7: Changes in nucleosome occlusion of transcription factor binding sites caused by glucose upshift and downshift. Nucleosome occupancy of regulatory motifs at steady-state is subtracted from the nucleosome occupancy 20 min. after the change in the carbon source. Nucleosome occupancy (quantified as log-intensity) was computed for a set of glucose-related transcription factor motifs from our previous studies (Zaman et al., 2009; Zawadzki et al., 2009). Shown are the average and the standard error of the nucleosome occupancy difference for each type of binding motif. The bar labeled “intergenic” indicates changes in nucleosome occupancy over entire promoters (defined as intergenic regions upstream of each TSS, between 100 bp and 800 bp in length), and the bar labeled “mean” refers to the net change in nucleosome occupancy.
over all types of binding sites. Mutants: grey bars, wild type: green bars. (A,D): all genes, (B,E): induced genes, (C,F): repressed genes. For the glycerol-to-glucose upshift (A,B,C) we consider genes to be induced or repressed if their expression changed at least 4-fold. For the glucose-to-glycerol downshift (D,E,F) we require at least a 2-fold change. Definitions of transcription factor motifs are listed in the legend of Figure 5.

References