Nucleosome depletion at yeast terminators is not intrinsic and can occur by a transcriptional mechanism linked to 3′-end formation

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Genome-wide mapping of nucleosomes generated by micrococcal nuclease (MNase) suggests that yeast promoter and terminator regions are very depleted of nucleosomes, predominantly because their DNA sequences intrinsically disfavor nucleosome formation. However, MNase has strong DNA sequence specificity that favors cleavage at promoters and terminators and accounts for some of the correlation between occupancy patterns of nucleosomes assembled in vivo and in vitro. Using an improved method for measuring nucleosome occupancy in vivo that does not involve MNase, we confirm that promoter regions are strongly depleted of nucleosomes, but find that terminator regions are much less depleted than expected. Unlike at promoter regions, nucleosome occupancy at terminators is strongly correlated with the orientation of and distance to adjacent genes. In addition, nucleosome occupancy at terminators is strongly affected by growth conditions, indicating that it is not primarily determined by intrinsic histone–DNA interactions. Rapid removal of RNA polymerase II (pol II) causes increased nucleosome occupancy at terminators, strongly suggesting a transcription-based mechanism of nucleosome depletion. However, the distinct behavior of terminator regions and their corresponding coding regions suggests that nucleosome depletion at terminators is not simply associated with passage of pol II, but rather involves a distinct mechanism linked to 3′-end formation.

nucleosome positioning | chromatin structure | genome organization

The basic unit of eukaryotic chromatin is the nucleosome, which consists of a histone octamer wrapped around 147 bp of DNA. In yeast, adjacent nucleosomes are connected by ~20 bp "linkers" such that most of the genome is packaged in regularly spaced nucleosome arrays. Such arrays are visualized by treatment of chromatin with micrococcal nuclease (MNase), which preferentially cleaves within linker regions to release mono-, di-, tri-, etc. nucleosomes, depending on the level of cleavage.

Despite the overall regularity of nucleosome arrays, genome-wide analyses of nucleosomal DNA generated by MNase cleavage of chromatin from yeast cells reveal that most (but not all) promoters and terminators are strongly depleted for nucleosomes in vivo (1–7). Nucleosomes assembled on the *PET56-HIS3-DED1* region in vitro with purified histones revealed that both promoter regions intrinsically disfavor nucleosome formation (2). On a genome-wide basis, the relative depletion of nucleosomes at promoter regions with respect to the corresponding coding regions is independent of transcriptional activity, suggesting that nucleosome depletion at most yeast promoters is caused primarily by intrinsically weak histone–DNA interactions (2), as opposed to histone eviction mediated by activators (8–10) or pol II elongation (1, 11, 12).

Genome-wide comparisons of in vitro and in vivo assembled nucleosomes provide direct evidence that intrinsic histone–DNA interactions are a major cause of nucleosome depletion at yeast promoter and terminator regions (13, 14). However,

histone depletion at promoters is more pronounced in vivo than in vitro, indicating that activator-dependent recruitment of nucleosome-remodeling activities (and perhaps transcriptional initiation) also make an important contribution to nucleosome depletion at promoters. In contrast, the correlation between in vitro and in vivo data is higher for terminators than promoters, leading to the suggestion that intrinsic histone–DNA interactions appear to be more important for nucleosome depletion at terminator regions in vivo (13, 14).

The conclusion that intrinsic histone–DNA interactions are important for nucleosome depletion at promoters and terminators is based on the similarities between in vivo and in vitro mononucleosome samples. We have been concerned that the correlation between in vivo and in vitro samples might be inflated by the strong DNA sequence specificity of MNase (15, 16) and by biases in DNA sequencing (17) that apply to all mononucleosome samples. To address the issue, we generate an MNase cleavage matrix for all possible trinucleotides based on cleavage events on purified yeast genomic DNA (18). Using this matrix to simulate the generation or destruction of nucleosome-sized fragments, we show that the level of MNase cleavage strongly affects the population of nucleosome-sized fragments. Moreover, at one MNase concentration tested experimentally, we observe a significant correlation (0.3) between nucleosome-sized fragments generated on purified DNA with previously described in vitro and in vivo nucleosomal samples. Thus, some of the apparent similarities between in vivo and in vitro samples are due to experimental artifacts, not a true reflection of chromatin structure.

Chromatin immunoprecipitation using antibodies against the core domain of histone H3 is an MNase-independent method to measure histone occupancy on a genome-wide level (1, 2). However, these initial experiments were compromised by the relatively large size of the fragmented chromatin (300–500 bp) as compared to the size of nucleosome-depleted regions (100–200 bp). In previous work, we improved the chromatin immunoprecipitation method by using more extensive sonication to obtain much smaller DNA fragments (75–150 bp), thereby increasing the dynamic range and space resolution in measuring occupancy by histones and other DNA-binding proteins (19, 20). This method should also reveal unusually small RSC(remodels the structure of chromatin)/nucleosomes found at some yeast promoters (21), which would be missed with conventional MNase-generated mononucleosomes. Using this improved, MNase-independent method for measuring nucleosome occu-

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pancy on a genome-wide level, we confirm that promoter regions are strongly depleted of nucleosomes, but observe that terminator regions are much less depleted than expected. Moreover, the limited nucleosome depletion at terminators is affected by growth conditions and pol II transcription, indicating that it is not due to intrinsic histone–DNA interactions. Our results suggest that nucleosome depletion at terminators involves a transcription-based mechanism linked to some aspect of 3'-end formation.

Results

Sequence Preferences of MNase Affect Measurements of Nucleosome Occupancy and Account for Some of the Correlation Between Nucleosomes Assembled in Vivo and in Vitro. Previous genome-wide studies of nucleosome occupancy relied on mononucleosomal DNA generated by MNase, an enzyme that cleaves DNA with considerable sequence specificity. To address how MNase specificity might affect measurements of nucleosome occupancy, we generated genomic "occupancy-like" maps of ~10^6 sequenced nucleosome-sized (average size of 150 bp) fragments produced by digesting purified yeast DNA with MNase (18). These "occupancy" maps based solely on MNase sequence preferences were then compared to previously obtained nucleosome occupancy maps (13, 14) of mononucleosomal DNA obtained from yeast cells or from chromatin assembled in vitro with purified histones.

As expected from the sequence specificity of MNase, the occupancy-like map shows regions of apparent high and low occupancy. As a consequence, measurements of nucleosome occupancy based solely on MNase cleavage are problematic, because they do not account for the region-dependent variations in MNase cleavage efficiency. Furthermore, the correlation coefficients between occupancy-like maps of MNase-digested DNA with various nucleosomal samples generated in vivo (cells grown in glucose, ethanol, and galactose) or in vitro by salt dialysis or with ATP-dependent chromatin assembly factor are ~0.3 (Fig. 1A), indicating that some of the apparent nucleosomal pattern is due to MNase cleavage specificity. Most of this 0.3 correlation reflects MNase cleavage specificity as opposed to biases of DNA sequencing, because a sonicated chromatin sample (14) is much less correlated with nucleosomal samples (average $R^2 \sim 0.1$; Fig. 1A).

Detailed Analysis of MNase Cleavage Specificity and Computational Simulations of its Effect on Nucleosome Mapping. The DNA sequences of ~10^6 MNase cleavage events on purified DNA provide a rich and direct source of information about the sequence preferences of MNase. In accord with the fact that MNase cleavage of nucleosomal DNA generates 5' extended ends that are staggered by two nucleotides (22), the MNase specificity motif is shifted by two nucleotides from the cleavage site. The MNase specificity motif is biased to a TA dinucleotide, although AT, AA, and TT dinucleotides are also enriched (Fig. 1B). There is also a modest preference for an A or T residue immediately upstream of TA dinucleotide. Our results on purified DNA are similar to those obtained on nucleosomal DNA (23), indicating that the DNA bending induced by nucleosome formation has minimal effect on MNase sequence specificity.

To examine how MNase cleavage specificity might affect nucleosome mapping experiments, we calculated the relative frequency of forming nucleosome-sized DNA fragments centered at every position in the yeast genome (see *Materials and Methods*). The MNase cleavage scores vary over a 100-fold range, with a large subset of potential nucleosome-sized fragments being strongly underrepresented (Fig. 1C). Promoters and terminators have higher cleavage scores than the average genomic region (Fig. 1C). Thus, at limiting MNase concentrations that do not fully digest linker DNA, nucleosomes positioned to the same extent could be observed to very different degrees due to different MNase cleavage preferences of the linkers. Conversely, sequence preferences of MNase could lead to the appearance of positioned nucleosomes that are rarely present in chromatin.

Although MNase preferentially cleaves linker DNA, it also cleaves DNA within a nucleosome. Based on MNase titration experiments (16), we estimate that nucleosomal DNA is ~25-fold more resistant to MNase cleavage than linker DNA on a single cut basis. MNase cleavage scores of 101 bp regions representing the centers of all possible nucleosomes (see *Materials and Methods*) vary almost exclusively over a 10-fold range, with most regions being within a threefold range, and promoter and terminator regions having high scores (Fig. 1D). Thus, at the relatively high MNase concentrations used to generate chromatin samples that predominantly contain mononucleosomes, promoter and terminator regions will be preferentially underrepresented simply due to MNase cleavage within nucleosomes. More generally, these observations demonstrate that the concentration of MNase has a differential effect on relative cleavage in both linker and nucleosomal regions, and that MNase specificity and concentration can significantly affect correlation coefficients between different chromatin samples.

Fig. 1. Effects of sequence preference of MNase on nucleosome calling. (*A*) Spearman's rank correlation coefficients of sonicated or MNase-digested DNA with nucleosomal samples generated in vivo (cells grown in glucose, ethanol, or galactose) (13) or in vitro (by salt dialysis or with ATP-dependent chromatin assembly factor) (14) or independently (indicated as in vitro) (13). (*B*) Motif overrepresented at ends of MNase-digested DNA. (*C*) Distribution of nucleosome-observation scores (cutting within both flanking 19-mers; plotted on $\log_2$ scale due to wide range of scores) based on MNase sequence preferences for all genomic positions. (*D*) Distribution of nucleosome-destruction scores (cleavage within the central 101 bp plotted on a linear scale) based on MNase sequence preferences for all genomic positions. Nucleosome-observation and nucleosome-destruction score distributions are indicated for transcription starts (red), transcription ends (blue), coding starts (purple), coding ends (orange), and overall (black).

Nucleosome Depletion is Less Pronounced at Terminator Regions than at Promoter Regions. Given the problems caused by MNase specificity, we used an MNase-independent method to quantitate nucleosome occupancy that involves immunoprecipitation of extensively sonicated chromatin (75–150 bp) with antibodies against the core domain of H3 (19). As the majority of genome is assembled into chromatin, we defined an occupancy value of 1.0 as a typical nucleosome-occupied region. Although histone occupancy is dramatically lower (>15-fold for the four promoters tested) in promoter regions than in coding sequences, histone depletion at terminator regions is significantly milder, with some terminator regions exhibiting little if any depletion (Fig. 2). Histone occupancy at terminator regions is more variable than at promoters, with a median H3 level ~50% of that in coding regions. These initial results suggest that nucleosome depletion is less pronounced at terminator regions than at promoter regions.
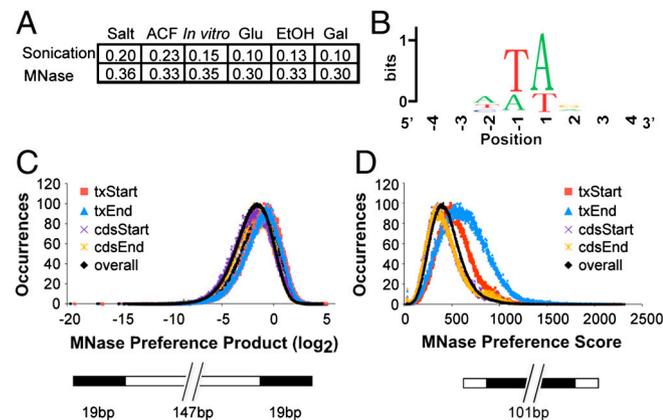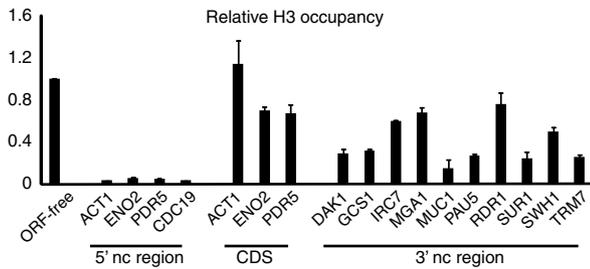
Fig. 2. Nucleosome occupancy of indicated promoter, terminator, and coding regions. H3 ChIP signals (mean ± SD for at least three independent experiments) are normalized to that of an ORF-free region on chromosome V.

We extended this analysis of H3 occupancy to the entire genome by using tiled microarrays. We compared nucleosome density at promoter regions and termination regions for all genes to published genome-wide experiments involving MNase digestion. In the MNase-based study, promoter and terminator regions exhibit very low (indicated by deep blue) and roughly comparable levels of nucleosome depletion (Fig. 3 *A* and *B*). In contrast, analysis of H3 occupancy on extensively sonicated chromatin shows that average histone occupancy at promoter regions is substantially lower than that at terminator regions (Fig. 3 *C* and *D*). In this analysis, nucleosome depletion appears to occur over a broader region, and positioned nucleosomes cannot be observed, but this pattern is expected from the heterogeneous fragment size and relative nonspecificity of breakage (i.e., not preferentially in linker regions) due to sonication (2, 19).

Nucleosome Depletion at Terminators but Not at Promoters Is Correlated with the Orientation of the Adjacent Gene. We classified promoters into two groups according to whether they are transcribed in tandem with or divergently from the upstream neighboring gene. The heat map in Fig. 4*A* expresses H3 occupancy for divergent (*Upper*) or tandem (*Lower*) genes ordered by distance from the upstream neighbor. For promoter regions, the extent of nucleosome depletion is unaffected by distance to or orientation of the upstream genes. At both tandem and divergently transcribed genes, there is a broader depletion zone at the promoter when the adjacent gene is farther away, suggesting that the neighboring coding region with its +1 nucleosome overrides the tendency of the promoter to be nucleosome free. Consistent with this observation, progressive shortening of the *HIS3-PET56* promoter region in either direction causes a progressive increase of nucleosome occupancy (2) and a progressive reduction of chromatin accessibility (24). When the same two groups of tandem or divergent promoters are ranked by transcription level instead of distance (Fig. 4*C*), nucleosome depletion is apparent across a broader region for highly transcribed genes (Fig. 4*C*, *Inset*).

In contrast to the situation at promoters, nucleosome depletion at terminators is highly correlated with the presence of an adjacent promoter of a gene transcribed in the same direction (Fig. 4*B*). However, if the terminator is adjacent to another terminator (convergently transcribed genes), nucleosome depletion is much more limited. Regardless of the orientation of the downstream neighbor, H3 depletion spans a wider region at terminators of the most highly transcribed genes (Fig. 4 *D* and *Inset*). Thus, nucleosome depletion at terminators is associated with adjacent promoters and transcriptional activity, arguing against a major role for intrinsic histone–DNA interactions.

Nucleosome Depletion at Terminator Regions is Largely Eliminated Under Conditions of Carbon Starvation. The above observations suggest that mechanisms of nucleosome depletion at terminator and promoter regions are different, and that the mechanism at terminators is not based on intrinsic properties of the DNA sequence. To test this idea, we compared histone occupancy in
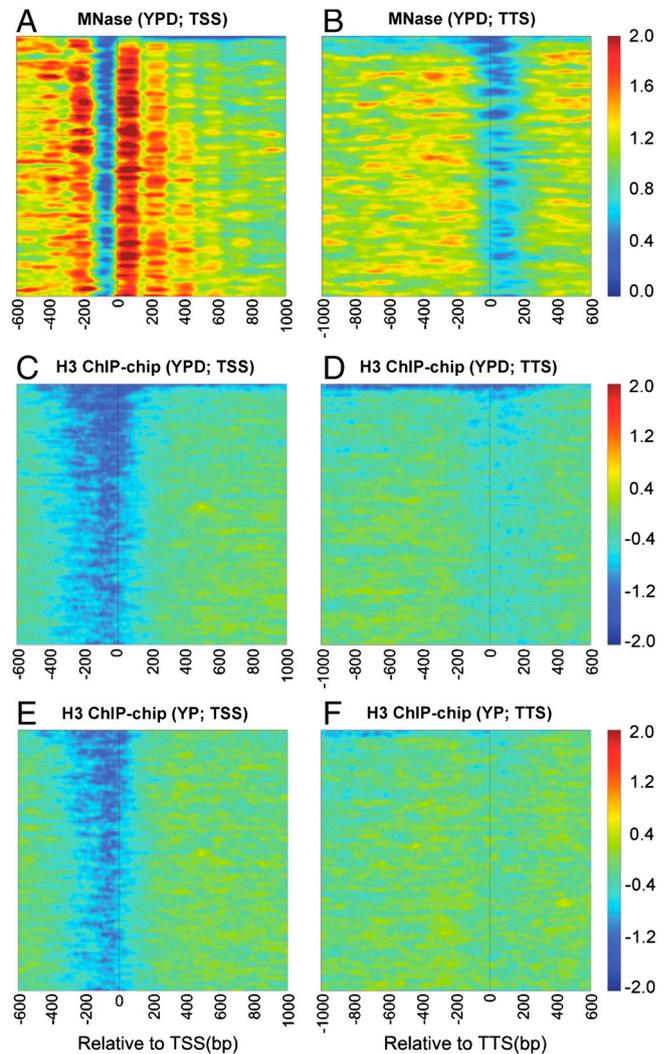

Fig. 3. Nucleosome occupancy profiles of promoter and terminator regions. Nucleosome occupancy around (*A*) transcriptional start sites (TSS) and (*B*) transcriptional termination sites (TTS) determined from MNase-generated mononucleosomes from cells grown in YPD (13, 14), (*C*) TSS and (*D*) TTS determined from H3 occupancy in YPD medium, and (*E*) TSS and (*F*) TTS determined from H3 occupancy in YP medium. Nucleosome occupancy (color coded with red being high and blue being low) for each gene is represented by a horizontal line, and genes are ranked from top to bottom by expression level.

normal rich medium [yeast extract/peptone/dextrose (YPD)] and during carbon starvation [yeast extract/peptone (YP)], a condition resulting in reduced pol II levels at most genes but increased pol II levels at stress-response genes (Fig. 5). Nucleosome depletion remains strong at promoter regions in YP medium (Fig. 3*E*), confirming previous conclusions that a great deal of nucleosome depletion at promoters is due to intrinsic histone–DNA interactions. In contrast, nucleosome depletion at terminators is largely eliminated in YP medium (Fig. 3*F*), indicating that much of the nucleosome depletion at terminators observed in YPD medium is not due to intrinsic histone–DNA interactions.

RNA Polymerase Dependency of Nucleosome Occupancy in Terminator Regions. To analyze directly the effect of pol II transcription on nucleosome occupancy, we employed the anchor-away system, which involves addition of rapamycin to cause rapid and stable translocation of an epitope-tagged nuclear protein to the cytoplasm (25), to deplete Rpb1, the largest pol II subunit. In the control strain, Rpb1 is untagged, and the addition of rapamycin has no effect on pol II occupancy or histone occupancy across the
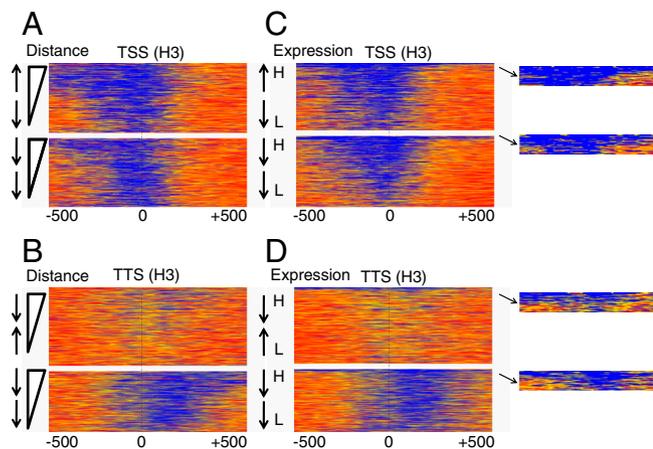
Fig. 4. Effect of neighboring gene orientation on nucleosome occupancy near promoter and terminator regions. (*A*) Heat map of nucleosome occupancy (red, high; blue, low) near the transcriptional start sites (TSS). Genes are classified as divergently or tandemly transcribed and ordered by the distance to the upstream gene from the ATG to the upstream ATG (divergently transcribed) or stop codon (tandemly transcribed). (*B*) Heat map of nucleosome occupancy near the transcriptional termination sites (TTS). Genes are ordered by distance to downstream genes from their stop codon to the downstream ATG (tandemly transcribed) or stop codon (convergently transcribed). (*C*) Similar to *A*, except genes ordered by transcriptional strength (35). Blowups are most highly expressed genes. (*D*) Similar to *B*, except genes are ordered by transcriptional strength (35).

PMA1 gene (Fig. 6 *A* and *B*). In contrast, in the anchor-away strain containing tagged Rpb1, the addition of rapamycin results in a rapid and dramatic drop in pol II levels across the entire *PMA1* coding region (Fig. 6*C*). Concomitantly, histone occupancy increases at and near the 3′ UTR, but there is no increase in histone occupancy near the promoter region (Fig. 6*D*). Similar results were obtained when another gene, *TEF1*, was examined (data not shown). More generally, depletion of Rpb1 causes a genome-wide increase in nucleosome occupancy at terminator regions, but the effect at promoter regions is minimal (Fig. 7). Thus, loss of pol II causes a specific increase in nucleosome occupancy at terminator regions, as opposed to promoter or coding regions.

Discussion

Nucleosome Depletion at Yeast Terminator Regions is Modest and Occurs by a Unique Transcription-Mediated Mechanism. Nucleosome mapping studies using MNase-based methods for generating mononucleosomes suggest that yeast promoter and terminator regions are strongly and comparably depleted for nucleosomes (1–7). Furthermore, the apparent nucleosome depletion at terminators in vivo is comparable to that occurring in chromatin assembled in vitro with purified histones and genomic DNA (13, 14). These observations have suggested that nucleosome depletion at terminators is determined primarily by intrinsic histone–DNA interactions (13, 14).

Here, using a method for measuring nucleosome occupancy that is not subject to the complications due to MNase sequence
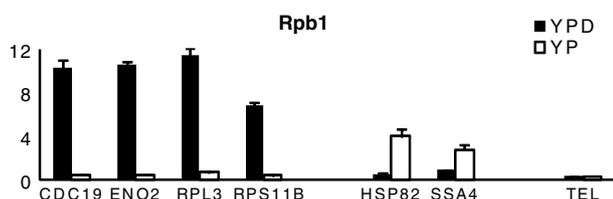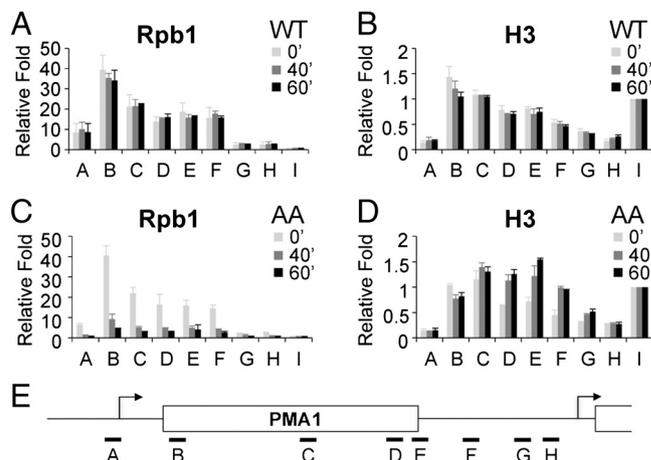

Fig. 6. Effect of depleting pol II on nucleosome occupancy at the *PMA1* gene. (*A*) Pol II (Rpb1) and (*B*) nucleosome (H3) occupancy in a wild-type (WT) strain upon addition of rapamycin for the indicated times. (*C*) Pol II and (*D*) nucleosome occupancy in an anchor-away (AA) strain upon addition of rapamycin. ChIP signals are normalized to that of an ORF-free region on chromosome V (I). (*E*) Schematic representation of positions of primer pairs.

specificity, we show that at many terminators, there is little, if any, nucleosome depletion. Even in cases where nucleosome depletion at terminators is observed, it is generally less pronounced than nucleosome depletion at promoters. Thus, although nucleosome depletion at terminators clearly exists, much of the drastic reduction in histone occupancy claimed in previous reports is an experimental artifact that likely arises from selective elimination of nucleosomes at terminators.

Several lines of evidence indicate that nucleosome depletion at terminators is not due primarily to intrinsic histone–DNA interactions. First, nucleosome depletion at terminators is strongly related to the presence of and distance from a nearby promoter region. This orientation dependence of nucleosome depletion is not observed at promoters, and it suggests that some nucleosome depletion at termination regions can be due to depletion at flanking promoter regions. Second, changes in growth conditions that are linked to overall lower levels of transcription largely eliminate nucleosome depletion at terminator regions, while having a much smaller effect on promoter regions. By definition, changes in nucleosome occupancy in response to environmental conditions cannot be due to intrinsic histone–DNA interactions. Third, depletion of pol II causes increased histone occupancy at terminators, but not at promoters or coding regions. This observation directly demonstrates that some nucleosome depletion at terminators is due to a pol II transcription-based mechanism.

Transcriptional elongation by pol II is associated with nucleosome eviction (1, 11, 12), and such eviction is critical for passage of pol II through nucleosomal DNA. In this regard, there is a relationship between the level of histone depletion at terminators and transcriptional activity. However, transcription-dependent depletion of nucleosomes at terminators cannot be explained simply by pol II elongation-mediated eviction of histones. His-

Fig. 5. Effect of carbon starvation on pol II occupancy. Pol II occupancy at the indicated genes in cells grown in rich medium (YPD) and under glucose starvation condition (YP).
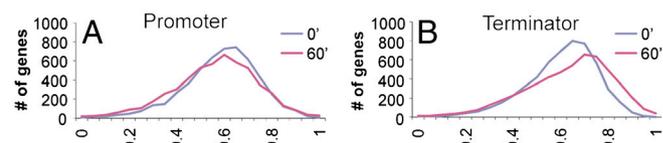

Fig. 7. Genome-wide analysis of nucleosome occupancy at promoter and terminator regions upon depletion of pol II. Distribution of relative MAT scores of promoter regions before (0′) and after (60′) rapamycin treatment in promoter (*A*) or terminator (*B*) regions in an anchor-away strain. MAT scores do not correspond directly to quantitative measurements of nucleosome occupancy.

tone eviction mediated by passage of elongating pol II should not discriminate between coding regions and terminator regions, yet such discrimination between terminators and coding regions is clearly observed.

Our results suggest that there is a previously unknown transcription-based mechanism of nucleosome depletion that occurs at terminator regions. There are two classes of models, not mutually exclusive, to explain nucleosome depletion at terminator regions. In one model, a factor(s) acts specifically at terminator regions to mediate nucleosome depletion. Most likely, such a factor would function by recruiting a nucleosome-remodeling activity, although it could work by itself. In the alternative model, a factor(s) that mediates transcription-associated nucleosome deposition at coding sequences does not function at terminator regions. Candidates for such a protein are "elongation factors" that dissociate after pol II passes the polyadenylation signal (e.g., Paf and transcription export complexes), as opposed to elongation factors (e.g., Spt4/5, Spt6, FACT) that travel with elongating pol II throughout the entire mRNA coding region and immediate downstream regions (26).

Effect of Sequence Preference of MNase on Nucleosome Mapping Experiments.
MNase has been widely used to map nucleosome occupancy and positioning, based on its ability to cleave preferentially within linker regions. However, the selectivity of MNase cleavage of DNA within linkers vs. nucleosomes is only a factor of ~25, which is roughly comparable to the variation observed in producing nucleosome-sized fragments simply due to MNase sequence preferences. In addition to sequence-specific effects on generating nucleosome-sized fragments, there is also a 10-fold variation in cleavage among potential nucleosomes, and promoter and terminator nucleosomes are particularly sensitive. For these reasons, MNase specificity has a significant impact on the observed nucleosomal pattern, and our results provide a quantitative assessment of this impact.

Due to the effects of MNase specificity, the classical control of MNase cleavage on purified DNA for chromatin experiments on individual genes is useful for genome-wide experiments involving nucleosome mapping, even though it is rarely performed. However, this control is not ideal because, due to the absence of structural constraints imposed by nucleosomes, MNase digestion of purified DNA generates a different and broader size distribution of fragments, and because of sequence bias, the composition of nucleosome-sized fragments depends on the level of cleavage. Nevertheless, although a perfect control cannot be designed, it is important to consider that the observed results obtained with MNase-generated mononucleosomes are significantly complicated by MNase cleavage specificity, and hence that mononucleosomal data cannot be taken simply at face value.

Our MNase cleavage simulation predicts that the results of a nucleosome mapping experiment will depend on the level of MNase cleavage. At relatively low concentrations, nucleosomes with favored MNase cleavage sites in the linker regions will be preferentially obtained, and these are biased toward promoter and terminator regions. In contrast, high concentrations preferentially eliminate mononucleosomes that contain favored MNase cleavage sites within the region protected by histone octamers. This latter situation likely explains the artificially low level of nucleosomes at terminators observed in MNase experiments. In accord with our predictions, MNase titration experiments on chromatin samples reveals different populations of mononucleosomes, with promoter and terminator regions showing increased sensitivity to MNase (16). We suspect that this preferential sensitivity of promoter and terminator nucleosomes is due primarily to MNase preferences, not structural differences among nucleosomes. Lastly, the effect of MNase concentration on the population of mononucleosomes can influence correlation coefficients between pairs of nucleosomal samples, particularly samples prepared at different times, by different people, or in different laboratories.

Role of Intrinsic Histone–DNA Interactions on Nucleosome Location in Vivo.
Although chromatin structure in vivo is influenced by intrinsic histone–DNA interactions, the extent of this influence has been controversial. Although some reports argue that intrinsic histone–DNA interactions have a predominant role and represent a code for nucleosome positioning (4, 13, 27), others argue against a predominant role (14, 28–30). The best evidence for a predominant role has been based on a correlation coefficient of 0.74 for mononucleosomal samples prepared from yeast cells vs. those prepared in vitro (13). However, a similar comparison involving a different in-vitro-generated sample from a different laboratory revealed a lower correlation coefficient of 0.54 (14). This lower correlation coefficient might reflect differences between the level of MNase cleavage and/or conditions of in vitro nucleosome assembly. In addition, the 0.3 correlation coefficient between nucleosome-sized fragments generated by MNase cleavage of purified DNA and nucleosome samples generated in vivo or in vitro indicates that MNase cleavage specificity inflates the apparent similarity between chromatin samples. As such, correlation coefficients are not a straightforward measurement of the importance of intrinsic histone–DNA interactions on establishing chromatin structure in vivo.

More specifically, it has been emphasized that the intrinsic DNA sequence of yeast promoter and terminator regions is important for their apparent nucleosome depletion in vivo, and that these regions contribute greatly to the overall similarity between nucleosome samples generated in vitro and in vivo. Although our results strongly support the importance of intrinsic histone–DNA interactions for nucleosome depletion at promoters, they indicate that yeast terminator regions are much less depleted than originally thought and, more importantly, that this depletion is not primarily due to intrinsic histone–DNA interactions.

Materials and Methods

Yeast Strains and Growth Conditions. Yeast strains derived from BY4741 (31) and (for the anchor-away experiment) HHY168 (25) were grown in YPD medium. To generate carbon starvation conditions, YPD-grown cells were washed twice with sterile water, resuspended in YP medium, and then cultured for 6 h. For the anchor-away experiments, HHY168 and a derivative expressing an Rpb1-FRB fusion protein were grown in YPD and treated with rapamycin (final concentration of 1 μg/mL) for 40 or 60 min.

Computation of MNase Sequence Preferences. Based on the sequences at the ends of mononucleosomal-sized (average ~150 bp) fragments generated by MNase digestion of purified yeast DNA (18), we obtained an MNase cleavage frequency score for every possible trinucleotide. To determine the relative frequency of generating a nucleosome at any given position under limiting MNase concentrations, we assumed that a mononucleosomal-sized fragment could result from cleavage anywhere within the 19 bp (the size of a linker region) on each side of a 147-bp central region. We computed an MNase cleavage frequency score for every 19-mer in the yeast genome as the sum of each triplet score (1-nt moving window) across both strands. We then calculated an MNase preference score for every possible 147-mer in the genome as the product of the MNase frequency scores of its two flanking 19-mers. Conversely, to determine the effect of MNase cleavage within a nucleosome, which is relevant under conditions that generate primarily mononucleosomes, we assumed that cleavage anywhere within the central 101 bp of a mononucleosome-sized fragment would cause the fragment to be small enough for exclusion from the population. Using the same scoring system described above for 19-mer regions, we estimated the likelihood of MNase cleavage within every possible 101 bp DNA region in the yeast genome.

Chromatin Immunoprecipitation and Tiling Array Hybridization. Chromatin preparation and ChIP were performed using antibodies against histone H3 (ab1791 from Abcam) or Rpb1 (8WG16 from Covance) (19). For analysis of individual genomic regions by quantitative PCR, ChIP signals were normal-

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ized to an ORF-free region on chromosome V, and data are presented as mean ± SD from at least three independent experiments. For genome-wide analysis, input and immunoprecipitated DNA were amplified with the Whole Genome Transcription kit (Sigma) and purified using Qiagen columns. DNA (1–2 μg as determined by NanoDrop spectrophotometry) was treated with DNase I to obtain 50–100 bp fragments, then labeled with biotin-dideoxy-ATP by terminal transferase before hybridization to the Affymetrix GeneChip Yeast Genome 1.0 Array. Raw array data can be accessed at Gene Expression Omnibus (GSE23778).

Data Analysis. Genome data were downloaded from the Saccharomyces Genome Database (http://downloads.yeastgenome.org/ (Dec. 24, 2009). Transcriptional start sites and transcriptional termination sites (32, 33) information was used when available; otherwise, the beginning and end of the coding sequence were used. Nonoverlapping TSS were defined as those

for which the start of the ORF (ATG) is at least 1 bp away from any adjacent upstream gene (including ORFs, pseudogenes, small nucleolar RNAs, snRNAs, noncoding RNAs, tRNAs, long terminal repeats, and transposable element genes). Nonoverlapping TTS were defined as those for which the stop codon is at least 1 bp away from any adjacent downstream feature. Raw nucleosome mapping data of yeast chromatin digested with MNase (13) was downloaded and transformed with the supplied Perl program. ChIP-chip data were processed with MAT (34). For all datasets, average scores across the indicated window sizes were computed using programs written in Python 3. Data were visualized with HCE3.0 (http://www.cs.umd.edu/hcil/hce/hce3.html).

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