Global nucleosome distribution and the regulation of transcription in yeast

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Abstract

Recent studies show that active regulatory regions of the yeast genome have a lower density of nucleosomes than other regions, and that there is an inverse correlation between nucleosome density and the transcription rate of a gene. This may be the result of transcription factors displacing nucleosomes.

It has been known for nearly three decades that there is a relationship between the chromatin structure of a gene and its transcriptional status. This relationship was first identified when nuclease-hypersensitive sites were observed to appear at the 5′ end of genes upon activation of transcription [1,2]. Later, the transcription-dependent changes in the chromatin of a gene came to be understood better through examination of the chromatin structures of individual genes - such as *PHO5*, *GAL1* and *GAL10* - under active and inactive conditions [3-7]. These studies found that the nucleosomes - the basic repeating units of chromatin, each consisting of a histone octamer encircled by about 146 base-pairs of DNA - are modified, unfolded or lost at the promoters of genes upon activation of transcription. It remains unclear, however, whether such remodeling or loss of nucleosomes is a general feature of eukaryotic gene regulation. Recently, two papers have analyzed the nucleosome distribution throughout the yeast genome. The authors of the new studies [8,9] propose that the genome-wide distribution of nucleosomes is heterogeneous and that this pattern may be involved in, or result from, the regulation of gene expression.

Nucleosomes are depleted from regulatory regions of the yeast genome

A recent study by Lee *et al.* [8] analyzes nucleosome distribution over the entire yeast genome, and another by Bernstein *et al.* [8] investigates nucleosome occupancy specifically at yeast promoters [9]. Both groups used a combination of chromatin immunoprecipitation and microarray analysis: after cross-linking proteins to DNA, different sets of antibodies were used to immunoprecipitate histones from yeast cell extracts, thereby enriching for DNA that is bound to histones. Lee *et al.* [8] performed comparative hybridization of the enriched DNA and total genomic DNA on microarrays in order to determine the relative histone occupancy at the entire genome, whereas Bernstein *et al.* [9] used the same approach with only intergenic DNA in order to determine histone occupancy in intergenic regions. Although it should be kept in mind that the degree of histone cross-linking may not always accurately reflect the presence or absence of histones, these studies do make a compelling argument for differences in nucleosome density across the genome.

One of the recent studies [8] found that the distribution of histones is heterogeneous over the genome, such that intergenic regions appear to have a sparser distribution of nucleosomes than the open reading frames (ORFs). Furthermore, the regulatory regions, such as promoters, have even fewer nucleosomes than other intergenic regions. Importantly, there is an inverse correlation between nucleosome occupancy at a promoter region and the transcription rate of the gene downstream of the promoter: the upstream regions of active genes have a lower density of nucleosomes than those

of less-transcribed genes. Interestingly, the transcription rate also affects nucleosome occupancy within the ORFs. ORFs that are transcribed at rates of more than about 30 mRNAs per hour have a lower density of nucleosomes than ORFs overall. This is an important observation because it suggests not only that nucleosomes are transiently dissociated from DNA during the elongation phase of transcription but also that they are not fully replaced within at the coding regions of heavily transcribed genes after each RNA polymerase passes along [10-12].

The heterogeneous distribution of nucleosomes over the genome is consistent with an earlier study showing that one can physically fractionate regions transcribed by RNA polymerase II from other regions in the genome [13]. This fractionation is done by cross-linking chromatin to DNA *in vivo* and then separating the aqueous phase from the organic phase in phenol:chloroform extractions. Free DNA segregates into the aqueous phase and DNA bound to proteins remains in the organic phase. This results in differential segregation of intergenic regions of the genome into the aqueous phase. Nagy *et al.* [13] proposed that this may be a result of different efficiency of chromatin cross-linking along the genome and that these differences in efficiency might be mediated through differentially modified histone tails. The heterogeneous distribution of nucleosomes suggests, however, that the regulatory regions are simply depleted of nucleosomes, and other proteins bound at these regions may not cross-link to DNA as efficiently as histones. In either case, the physical fractionation of yeast chromatin suggests that the chromatin is organized differently between coding and noncoding regions of the genome, and the heterogeneous distribution of nucleosomes may be part of this organization.

Nucleosome occupancy at the promoters of individual genes is inversely proportional to their transcription rate

In order to understand further the relationship between nucleosome occupancy and the transcriptional status of a gene, Lee *et al.* [8] analyzed nucleosome occupancy over the entire genome after heat shock, a treatment that changes the transcription profile of the yeast genome considerably. When yeast cells are growing rapidly at an optimal temperature, some of the most active genes are those encoding ribosomal proteins, and these genes are also most repressed upon heat shock. Both studies [8,9] observed that the promoters of ribosomal protein genes are the most depleted of nucleosomes when cells are rapidly growing. When the cells are heat shocked, these genes are rapidly repressed and their nucleosome occupancy increases [8]. This suggests that nucleosome occupancy is either the cause or the result of the transcriptional status of a gene.

This raises an important question: what are the determinants of nucleosome occupancy, and how do they relate to

transcription? An attractive answer to this question might be that transcription factors replace nucleosomes at the promoters. One transcription factor that is known to target the promoters of ribosomal protein genes is Rap1p [14]. An unbiased search for sequence motifs at the promoters that are most depleted of nucleosomes during rapid growth also identified Rap1p-binding sites in ribosomal protein promoters [9]. What role, then, does Rap1p play in nucleosome occupancy after heat shock? It was previously shown that Rap1p can move or displace nucleosomes at the promoters of ribosomal protein genes [15], so one prediction is that the loss of nucleosomes may be a result of Rap1p binding at the promoters. This idea is supported by the results of an experiment showing that when the Rap1p-binding site is deleted at a number of ribosomal protein promoters, nucleosome occupancy increases at these promoters [9]. In contrast, after heat shock, although nucleosome occupancy at the promoters increases, Rap1p remains bound [8]. This observation is consistent with the result of another experiment: when the transcription of ribosomal proteins is repressed by rapamycin treatment and nucleosomes return to their promoters, Rap1p remains bound [9]. Together, these observations suggest that Rap1p binding alone is insufficient to keep nucleosomes off promoters, and it probably requires additional cofactors and/or chromatin-remodeling factors.

The determinants of global nucleosome distribution

Although the determinants of global nucleosome distribution are not known, transcription factors and the cofactors they recruit to the regulatory regions of the genes are strong candidates. Recent studies show that the relationship between transcription-factor binding and nucleosome occupancy is not simple. One reason for this complexity may be the presence of more than one binding site for transcription factors in a promoter region, such that a number of transcription activators and repressors will bind to their sites and influence the nucleosome occupancy of that region. Moreover, remodeling and displacement of nucleosomes often requires protein complexes to be targeted to promoters by specific transcription factors [16]. How these numerous proteins interact with the promoters and how transcriptional activators act synergistically has been an area of intense investigation. Although the promoter of each gene is unique, the possibility of a general rule that nucleosomes are displaced upon gene activation remains attractive. More importantly, the general depletion of nucleosomes from regulatory regions might be a fundamental property of genome organization in eukaryotes. A simplified model of this organization for one gene under different transcriptional states is shown in Figure 1.

As well as suggesting the general model shown in Figure 1, the recent studies identify a heterogeneous distribution of nucleosomes in the yeast genome [8,9]. These findings offer
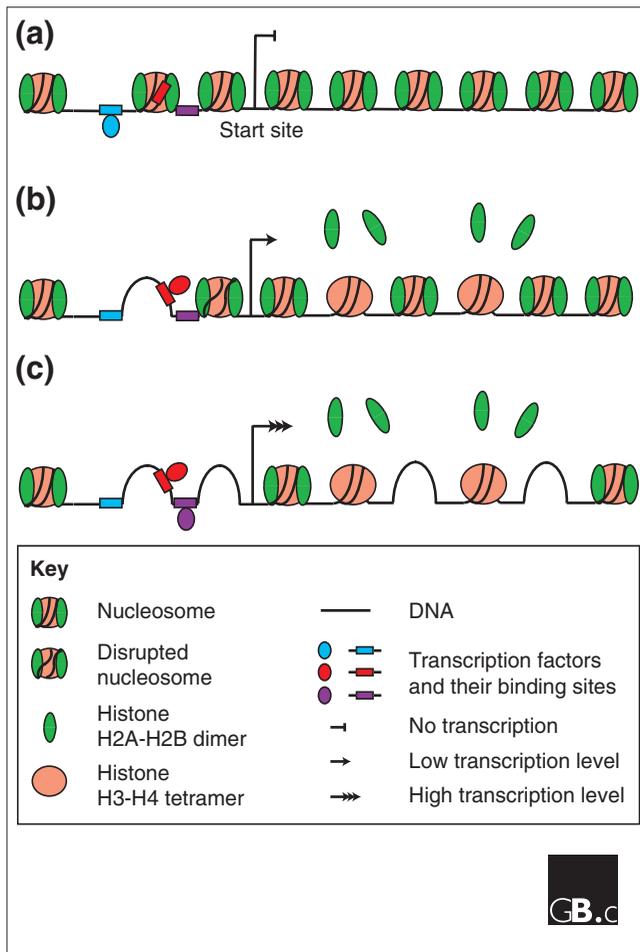
Figure 1
A model for the change in nucleosome occupancy in a typical yeast gene
in different transcriptional states. **(a)** When there is no transcription,
repressor proteins bind to their DNA-binding sites and maintain a
repressive chromatin configuration with nucleosomes all along the gene
and most of the promoter. **(b)** When activator proteins bind their DNA
elements, they promote changes in chromatin that disrupt or displace
nucleosomes from promoter regions, leading to transcription of the
gene. Subsequent transcript elongation through coding regions causes
the transient displacement of histones. **(c)** With higher levels of
transcription, nucleosomes become depleted from coding regions as well
as from the promoter.

References
1. Wu C, Wong YC, Elgin SC: **The chromatin structure of specific genes: II. Disruption of chromatin structure during gene activity.** *Cell* 1979, **16:**807-814.
2. Weintraub H, Groudine M: **Chromosomal subunits in active genes have an altered conformation.** *Science* 1976, **193:**848-856.
3. Reinke H, Horz W: **Histones are first hyperacetylated and then lose contact with the activated PHO5 promoter.** *Mol Cell* 2003, **11:**1599-1607.
4. Boeger H, Griesenbeck J, Strattan JS, Kornberg RD: **Nucleosomes unfold completely at a transcriptionally active promoter.** *Mol Cell* 2003, **11:**1587-1598.
5. Fedor MJ, Kornberg RD: **Upstream activation sequence-dependent alteration of chromatin structure and transcription activation of the yeast GAL1-GAL10 genes.** *Mol Cell Biol* 1989, **9:**1721-1732.
6. Boeger H, Griesenbeck J, Strattan JS, Kornberg RD: **Removal of promoter nucleosomes by disassembly rather than sliding *in vivo*.** *Mol Cell* 2004, **14:**667-673.
7. Lohr D: **Nucleosome transactions on the promoters of the yeast GAL and PHO genes.** *J Biol Chem* 1997, **272:**26795-26798.
8. Lee CK, Shibata Y, Rao B, Strahl BD, Lieb JD: **Evidence for nucleosome depletion at active regulatory regions genome-wide.** *Nat Genet* 2004, **36:**900-905.
9. Bernstein BE, Liu CL, Humphrey EL, Perlstein EO, Schreiber SL: **Global nucleosome occupancy in yeast.** *Genome Biol* 2004, **5:**R62.
10. Kaplan CD, Laprade L, Winston F: **Transcription elongation factors repress transcription initiation from cryptic sites.** *Science* 2003, **301:**1096-1099.
11. McKittrick E, Gafken PR, Ahmad K, Henikoff S: **Histone H3.3 is enriched in covalent modifications associated with active chromatin.** *Proc Natl Acad Sci USA* 2004, **101:**1525-1530.
12. Belotserkovskaya R, Oh S, Bondarenko VA, Orphanides G, Studitsky VM, Reinberg D: **FACT facilitates transcription-dependent nucleosome alteration.** *Science* 2003, **301:**1090-1093.
13. Nagy PL, Cleary ML, Brown PO, Lieb JD: **Genomewide demarcation of RNA polymerase II transcription units revealed by physical fractionation of chromatin.** *Proc Natl Acad Sci USA* 2003, **100:**6364-6369.
14. Lieb JD, Liu X, Botstein D, Brown PO: **Promoter-specific binding of Rap1 revealed by genome-wide maps of protein-DNA association.** *Nat Genet* 2001, **28:**327-334.
15. Yu L, Morse RH: **Chromatin opening and transactivator potentiation by RAP1 in *Saccharomyces cerevisiae*.** *Mol Cell Biol* 1999, **19:**5279-5288.
16. Owen-Hughes T, Utley RT, Cote J, Peterson CL, Workman JL: **Persistent site-specific remodeling of a nucleosome array by transient action of the SWI/SNF complex.** *Science* 1996, **273:**513-516.

an important factor that should be taken into account when
interpreting genome-wide experiments involving post-translationally modified nucleosomes. When examining the distribution of modified histones in the genome, one should
keep in mind that the histones are organized heterogeneously in the genome such that the regulatory regions
possess fewer nucleosomes. Thus, the apparent loss of a
histone modification may in fact represent the absence of
histones [3]. Overall, recent studies suggest a genome-wide
depletion of nucleosomes over regulatory regions that might
be a common feature of eukaryotic genomes.