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Intrinsic histone-DNA interactions are not the major determinant of nucleosome positions *in vivo*

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We assess the role of intrinsic histone-DNA interactions by mapping nucleosomes assembled *in vitro* on genomic DNA. Nucleosomes strongly prefer yeast DNA over *Escherichia coli* DNA, indicating that the yeast genome evolved to favor nucleosome formation. Many yeast promoter and terminator regions intrinsically disfavor nucleosome formation, and nucleosomes assembled *in vitro* show strong rotational positioning. Nucleosome arrays generated by the ACF assembly factor have fewer nucleosome-free regions, reduced rotational positioning and less translational positioning than obtained by intrinsic histone-DNA interactions. Notably, nucleosomes assembled *in vitro* have only a limited preference for specific translational positions and do not show the pattern observed *in vivo*. Our results argue against a genomic code for nucleosome positioning, and they suggest that the nucleosomal pattern in coding regions arises primarily from statistical positioning from a barrier near the promoter that involves some aspect of transcriptional initiation by RNA polymerase II.

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Eukaryotic genomes are packaged into regularly spaced arrays of nucleosomes, although the spacing between nucleosomes varies among species and cell types[1]. Despite this regularity, high-resolution, genome-wide analyses reveal a common nucleosomal pattern[2–8]. Nucleosomes are depleted at many (but not all) enhancer, promoter and terminator regions, and they typically occupy preferred positions within mRNA coding regions and just upstream of the promoter. In yeast, the −1 and +1 nucleosomes flanking the promoter are strongly positioned, and the degree of nucleosome positioning gradually decreases from the 5′ to the 3′ end of the coding region[6,8].

There are three distinct mechanisms for nucleosome depletion *in vivo*. First, specific DNA-binding activator proteins can generate nucleosome-depleted regions by recruiting ATP-dependent remodeling complexes and histone acetylases[9–11]; this mechanism is independent of transcriptional activity[12]. Second, the process of transcriptional elongation by RNA polymerase II (Pol II) involves cycles of histone eviction and reassembly, and continuous high levels of Pol II elongation reduce histone density within coding regions[13–15]. Third, certain DNA sequences, notably poly(dA:dT) tracts, intrinsically disfavor nucleosome formation *in vitro*, increase chromatin accessibility *in vivo* and are strongly overrepresented in nucleosome-free regions[16–18], indicating a role of intrinsic histone-DNA interactions.

The positioning of nucleosomes along the DNA is related to, but distinct from, the issue of nucleosome occupancy. Nucleosome occupancy or density reflects the average histone levels on a given region of DNA in a population of cells, but it does not address where an individual nucleosome is positioned with respect to a certain DNA sequence. Indeed, differently positioned nucleosomes within a given genomic region will all contribute to nucleosome density. Nucleosome positioning refers to two fundamental relationships between the histone octamer and the DNA wrapped around it. Rotational positioning defines the orientation of the DNA helix on the histone surface. Nucleosomes are rotationally positioned with a 10-bp helical periodicity, reflecting preferences for dinucleotides that face inwards or outwards with respect to the histones and optimize DNA bending[19–21]. The translational position of a nucleosome refers to the specific 146-bp sequence covered by the histone octamer, and it is often defined as the midpoint of this sequence. The degree of translational positioning can vary from perfect positioning, in which a nucleosome occupies a given 146-bp stretch in all DNA molecules in a population, to random positioning, in which nucleosomes occupy all possible genomic positions equally. *In vivo*, translational positioning is strongly influenced by relatively constant spacing between nucleosomes, which is presumably due to the action of nucleosome-remodeling complexes.

Nucleosomes can also be statistically positioned from a fixed barrier such as a DNA-binding protein[22]. Nucleosomes near the barrier are highly positioned, and the degree of positioning decreases in accordance with the distance from the barrier owing to variations in spacing between nucleosomes. A barrier model for statistical positioning can explain the location of nucleosomes in yeast genes[8], but the molecular nature of the barrier is unknown.

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Nucleosomes have intrinsic DNA sequence preferences[19,20,23], and many yeast promoter regions are nucleosome depleted because the DNA sequence intrinsically disfavors nucleosome formation[16,17]. More generally, it has been proposed that there is a nucleosome code in which the pattern of nucleosome positioning *in vivo* is determined primarily by genomic DNA sequence and hence can be predicted[16,24]. Here we use *Saccharomyces cerevisiae* and *E. coli* DNA to examine the role of intrinsic histone-DNA interactions for establishing the nucleosomal pattern *in vivo* on a genome-wide basis.

RESULTS

Assembly of nucleosome arrays on genomic DNA

The experimental design involves a comparison of nucleosome positions in samples prepared from yeast cells with those generated *in vitro* with purified histones and yeast genomic DNA, and it represents a genome-wide and high-resolution version of an approach we have used previously[17]. We assembled nucleosomes on a mixture of yeast and *E. coli* genomic DNAs either by salt dialysis or by ACF, an ATP-dependent chromatin assembly factor, using a 1:1 mass ratio of core histones to DNA to produce arrays of regularly spaced nucleosomes (**Fig. 1** and **Supplementary Methods**). Nucleosomes assembled by salt dialysis are formed solely by intrinsic histone-DNA interactions, and the linker regions between nucleosomes are short. In contrast, ACF assembles nucleosomes into regularly spaced arrays with ~20-bp linker regions between nucleosomes that are similar to those observed in yeast cells. The comparison between nucleosomes assembled by salt dialysis or ACF addresses the issue of how chromatin assembly factors and nucleosome spacing affects nucleosome positioning. We treated the assembled chromatin with micrococcal nuclease (MNase) under conditions in which mononucleosomes were the major product and linker regions were degraded. As a control, the same mixture of yeast and *E. coli* DNA was sonicated to generate DNA fragments of comparable size. Parallel DNA sequencing of the resulting samples generated 1–3 million uniquely mapped reads per sample, which corresponds to approximately 10-fold to 40-fold coverage of the genome.

Nucleosomes preferentially form on yeast DNA

Although we were primarily interested in the comparison between nucleosomes assembled *in vitro* and *in vivo* on yeast genomic DNA, we included *E. coli* DNA in the samples to address whether yeast cells show evolutionary selection either for or against sequences that favor nucleosome formation. As *E. coli* does not have histones, we presumed that *E. coli* DNA sequences are evolutionarily neutral with respect to nucleosome formation, such that preferred nucleosome-forming sequences will occur by chance. Notably, when compared to the sonication control, nucleosomes assembled *in vitro* by salt dialysis are nine times more likely to form on yeast DNA than *E. coli* DNA (**Fig. 1**). A similar effect, albeit to a lesser extent (three-fold), is observed with ACF-assembled nucleosomes. These results strongly argue that the yeast genome has evolved to favor nucleosome formation.

Many promoters and terminators disfavor nucleosomes

For *in vivo* and *in vitro* samples, we generated a heat map of histone density at each nucleotide position and aligned these to promoters (**Fig. 2a–c**), terminators (**Fig. 2d–f**) and transcription factors (**Fig. 2g,h**) on a gene-by-gene basis. As observed *in vivo*, promoter and terminator regions are substantially nucleosome depleted (visualized as blue in **Fig. 2**) in comparison to coding regions in chromatin assembled by salt dialysis or ACF. However, only a subset of nucleosome-depleted promoters *in vivo* are also depleted in chromatin assembled *in vitro*, and histone depletion at promoters is markedly more pronounced (darker blue) *in vivo* than *in vitro*. These observations indicate that intrinsic histone-DNA interactions are important for generating nucleosome-depleted promoter regions *in vivo*, but other mechanisms (for example, activator-dependent recruitment of nucleosome-remodeling activities and perhaps transcriptional initiation) have an important (and perhaps greater) role at many promoters. Intrinsic histone-DNA interactions seem to be more important at terminator regions *in vivo*, because the correlation between *in vitro* and *in vivo* data is higher for terminators than for promoters (**Supplementary Fig. 1**).

Rotational positioning is intrinsically determined

To address the role of intrinsic histone-DNA interactions on rotational and translational positioning, we used an approach that did not involve histone density but, rather, treated each mapped read as an individual 146-bp nucleosome in the population. For chromatin assembled by salt dialysis, alignment of nucleosome 5′ ends reveals a marked 10-bp periodicity of AA/TT/AT dinucleotides both for yeast (**Fig. 3a**) and *E. coli* (**Fig. 3b**) DNA, indicating that intrinsic histone-DNA interactions have a major role in rotational positioning. Power spectrum analysis (a discrete Fourier transform that quantifies the amount versus the frequency component of the data) shows that this 10-bp periodicity is substantially lower in nucleosomes assembled *in vivo* (**Fig. 3c**), suggesting that nucleosome assembly or remodeling factors decrease the effect of rotational positioning. Our results are consistent with the presumption that rotational positioning reflects the requirement of DNA to bend around the histone octamer, with more bendable sequences in contact with the histones and less bendable sequences being solvent exposed. In this regard, the yeast genome shows preferential 10.2-bp periodicity of AA and TT dinucleotides, whereas the *E. coli* genome does not (**Fig. 3d**), and this probably contributes to the preferential assembly of nucleosomes on yeast DNA.

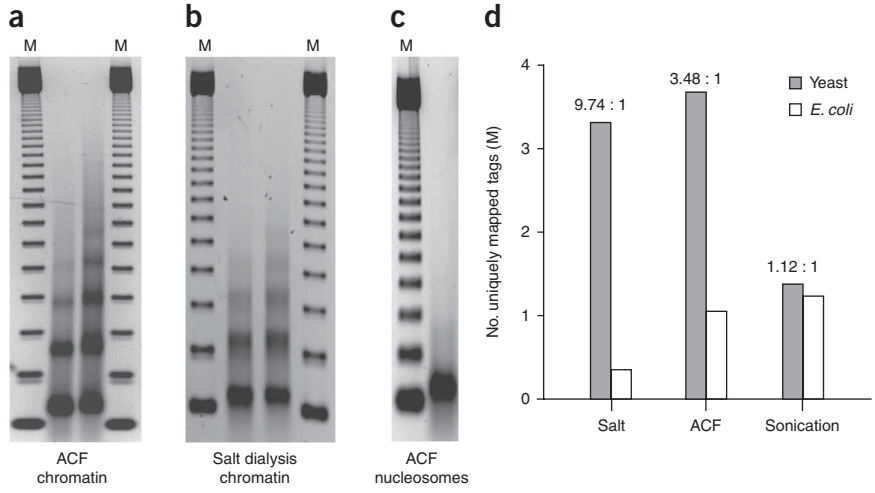

Figure 1 MNase digestion analysis of chromatin and generation of mononucleosomes. Chromatin assembled using ACF (**a**) or salt dialysis (**b**) was partially digested with MNase. (**c**) Mononucleosomes were generated by extensive digestion of chromatin with MNase. DNA size markers (M), 123-bp ladder (Invitrogen). (**d**) Number of sequence tags for yeast and *E. coli* from the indicated samples.

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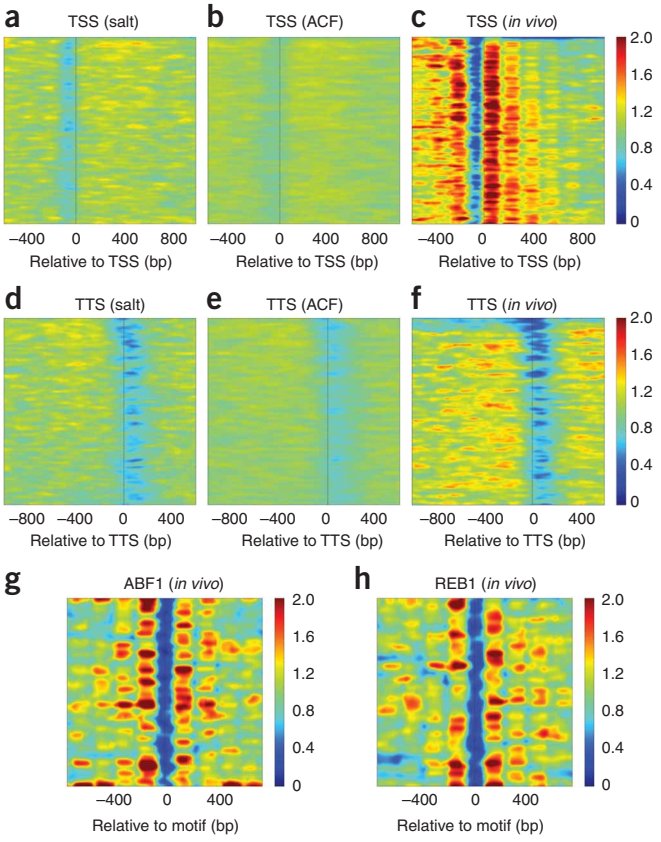

Figure 2 Nucleosome density profiles. (**a**–**c**) Nucleosome density profiles around transcriptional initiation sites (TSS) of 1,752 genes with isolated promoters (defined as having upstream regions of 1 kb that do not overlap with other genes) for chromatin assembled by salt dialysis, ACF or *in vivo* in YPD medium[16]. Each tag was extended to 146 bp, piled up and then normalized by average sequencing coverage. Nucleosome density for each gene is represented by a horizontal line, and genes are ranked from above to below by expression level. (**d**–**f**) Nucleosome density profiles of the indicated chromatin preparations around transcriptional termination sites (TTS) of 1,548 genes (ranked by expression level) with isolated terminator regions (defined as having downstream regions of 1 kb that do not overlap with other genes). (**g**–**h**) Nucleosome density profiles *in vivo* around the binding sites (ranked by genomic location) of transcription factors Abf1 and Reb1.

Translational positioning is primarily not intrinsic

We determined the degree of translational positioning by defining the central position of each nucleosome and counting the number of hits within 20-bp windows (to allow for the imprecision of MNase cleavage). In principle, if a nucleosome is perfectly positioned (that is, 100% of the DNA molecules contain a nucleosome center within the window), the expected number of hits is determined by the sequencing coverage, and we define this value as 1. Thus, the degree of nucleosome positioning is defined as the observed number of hits divided by the expected number for a perfectly positioned nucleosome. As expected, chromatin from yeast cells shows a great deal of translational positioning, with many nucleosomes being highly positioned (**Fig. 4a** and **Supplementary Fig. 2**). Nucleosomes assembled by either *in vitro* method show substantially less translational positioning than observed *in vivo*. Nevertheless, translational positioning of these *in vitro* assembled nucleosomes is above the level observed in randomly positioned nucleosomes.

To determine the contribution of intrinsic histone-DNA interactions for the nucleosome positioning pattern *in vivo*, we defined the peak 20-bp window for the +1, +2 (and so on) nucleosomes on an individual gene basis using *in vivo* nucleosome data[16]. We then determined the number of nucleosomes within each of these defined windows in the various nucleosome preparations (**Fig. 4b–d**). For nucleosomes generated by salt dialysis or ACF, the extent of nucleosome positioning was above that expected from randomly positioned nucleosomes (**Supplementary Fig. 3**). However, the degree of translational positioning was far below that observed with an independent sample of nucleosomes from yeast cells (compare **Fig. 4b,c** with **4d**). These observations indicate that intrinsic histone-DNA interactions make only a modest contribution to the *in vivo* pattern of translational positioning, and we estimate that they account for ~20% of the *in vivo* positions (**Supplementary Fig. 3**). More notably, unlike the situation *in vivo* (**Fig. 4d**), this modest contribution of intrinsic positioning is not appreciably affected by the location of the positioned nucleosome with respect to the mRNA initiation site (that is, nucleosomes defined as +1 through +10), indicating that other features underlie this positioning pattern.

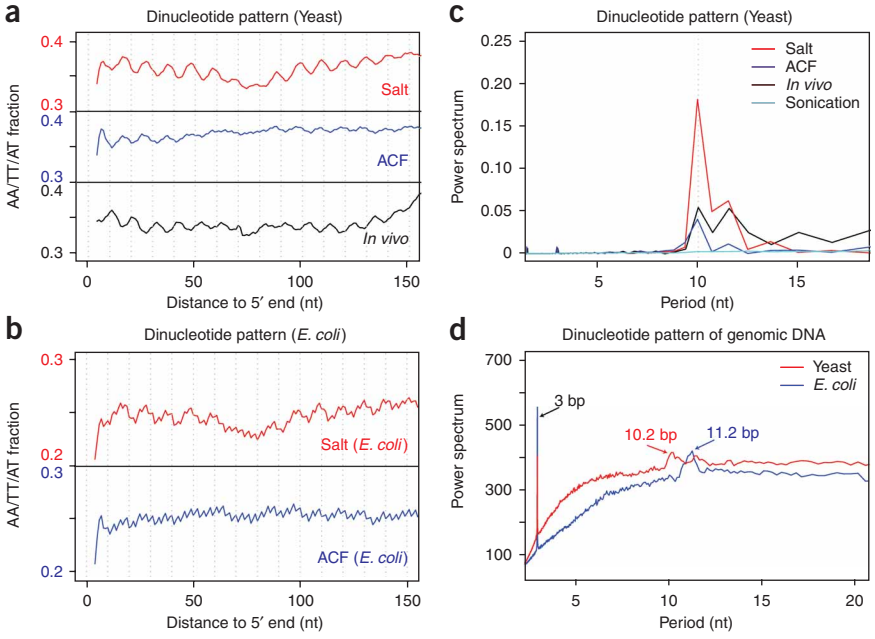

Figure 3 Rotational positioning. (**a**) Fraction of AA/TT/TA dinucleotides of yeast nucleosomes aligned by their 5′ ends from the indicated samples. (**b**) Fraction of AA/TT/TA dinucleotides of *E. coli* nucleosomes aligned by their 5′ ends. (**c**) Power spectrum analysis of dinucleotide periodicity. Discrete Fourier transform was performed on signals in the (+11 to +160) interval, and the signals were first subtracted from the mean value to remove the DC component. (**d**) Power spectrum of the AA/TT/AT pattern for yeast and *E. coli* genomic DNA. Each genome was split into 1,024-nt fragments. Discrete Fourier transform was then performed on each fragment and the results are presented as the average of all fragments.

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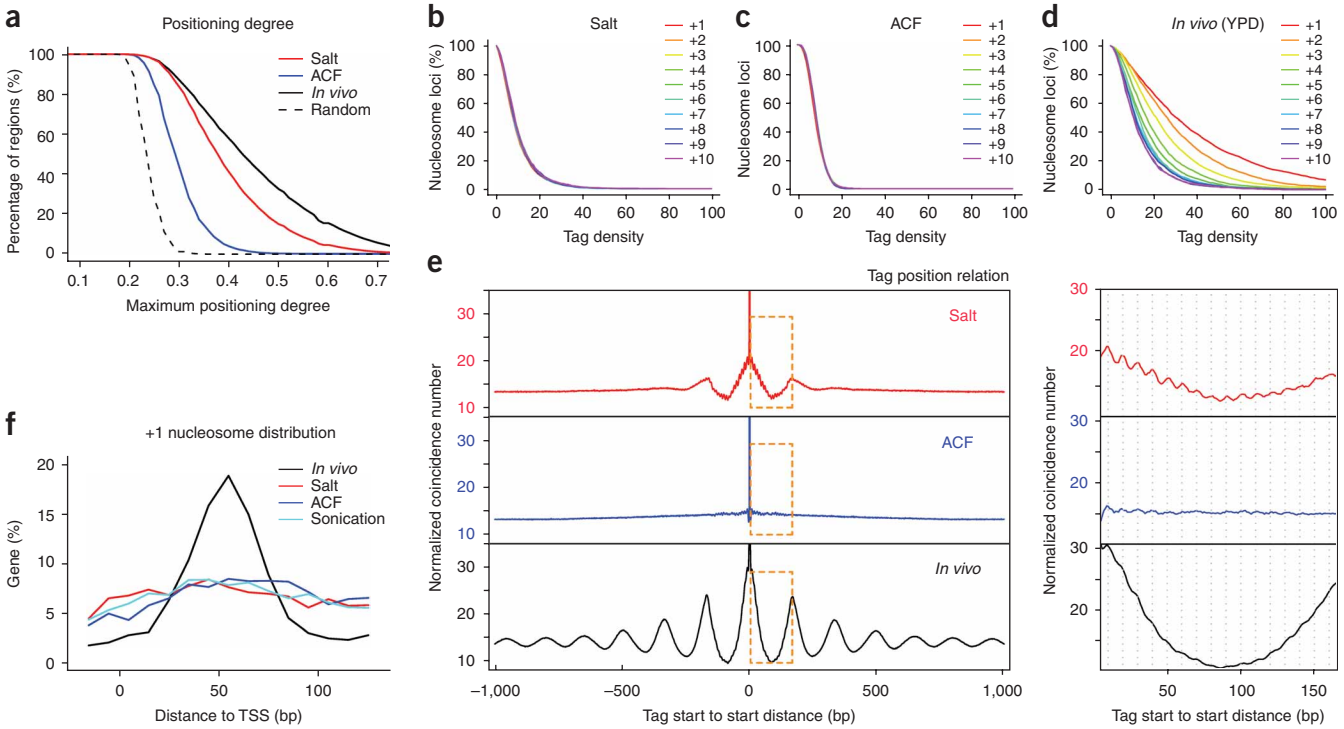

Figure 4 Intrinsic histone-DNA interactions do not account for the nucleosome positioning pattern *in vivo*. (**a**) Degree of translational positioning for each nucleotide position is determined by the number of nucleosome centers within a 20-bp window divided by the number of nucleosome centers within the corresponding 160-bp window. Then, for the indicated samples, the percentage of regions (*y* axis) above the maximal positioning degree within a 160-bp window (*x* axis; a value of 1 indicates a perfectly positioned nucleosome) is plotted. (**b**–**d**) Relationship between nucleosomal positions *in vivo* and *in vitro*. Predefined 20-bp windows for the centers of +1 through +10 nucleosomes were defined on a gene-by-gene basis in chromatin from cells grown in YP ethanol. For the indicated samples (*in vivo* represents cells grown in YPD and serves as a control for experimental variation), the percentage of nucleosomal loci (*y* axis) is plotted as a function of the number of tags (defined by the central position of each nucleosome) within the predefined windows (different colors indicate the +1 through +10 nucleosomes). (**e**) Tag position relationship. Start-to-start distances of tags in the same strand are shown, with the right plots being a blow-up of the boxed areas in the left plots. (**f**) The distribution of distance between +1 nucleosome center location and transcriptional start site (TSS), binned by 10 bp.

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ACF reduces effect of intrinsic histone-DNA interactions

We assessed how a chromatin assembly factor affects intrinsic nucleosome positioning by comparing the samples generated *in vitro* by ACF and salt dialysis. First, both the fraction of nucleosome-depleted promoters and the degree of histone depletion are lower in chromatin assembled by ACF as compared to chromatin assembled by salt dialysis (**Fig. 2**). Second, the 10-bp periodicity is substantially lower in nucleosomes assembled by ACF (**Fig. 3b**), indicating that nucleosome assembly decreases the effect of rotational positioning. Third, ACF reduces the degree of translational positioning beyond that which is due to intrinsic histone-DNA interactions (**Fig. 4a**). These observations indicate that ACF diminishes the effect of intrinsic histone-DNA interactions, presumably because nucleosome spacing constraints force nucleosomes to occupy positions that are not preferred solely on the basis of DNA sequence. These observations are consistent with the function of the Isw2 nucleosome-remodeling complex in yeast cells[25] and with the reduced rotational positioning *in vivo* (**Fig. 3b**). Furthermore, they suggest that nucleosome assembly factors that govern nucleosome spacing *in vivo* will diminish the effects of intrinsic histone-DNA interactions on histone density as well as rotational and translational positioning.

Statistical positioning is not intrinsically determined

In yeast cells, the +1 nucleosome is highly positioned, and the degree of positioning decreases progressively at more downstream positions within the coding region[8], a hallmark of statistical positioning. Thus, the mechanism by which the +1 nucleosome becomes highly positioned is the key to understanding the nucleosomal pattern *in vivo*. Our results in **Figures 2** and **4** demonstrate conclusively that the strong positioning of the +1 nucleosome is not due to intrinsic histone-DNA interactions. Furthermore, several observations indicate that nucleosome-depleted regions *per se* are insufficient to constitute the barrier needed for statistical positioning. First, statistical positioning occurs to a much lesser extent for nucleosomes upstream of nucleosome-free promoter regions (**Fig. 2c**), and hence it is largely directional. Second, statistical positioning is very limited in either direction from the nucleosome-depleted terminator regions (**Fig. 2f**). Third, strong statistical positioning is observed in both directions from Abf1 and Reb1 sites in promoter regions (**Fig. 2g,h**), indicating that these DNA-binding proteins (with associated factors) can serve as barrier elements for statistical positioning. Rap1 and (to a lesser extent) Mbp1 and Cbp1 also serve as barrier elements, but this is not the case for many other transcription factors (**Supplementary Fig. 4**). Last, the spacing between nucleosome ends (that is, tag position relationships) for the entire data set reveals strong statistical positioning *in vivo*, whereas this is limited in the salt-assembled chromatin and not detectable in ACF-assembled chromatin (**Fig. 4e**).

+1 Nucleosome linked to the transcriptional initiation site

The observation that statistical positioning occurs directionally from promoter regions strongly argues that the transcription initiation

process is crucial for establishing the strong translational position of the +1 nucleosome. This view is reinforced by the strong relationship between the position of the +1 nucleosome and the location of the mRNA initiation site (**Fig. 4f**). We therefore propose that, although intrinsically nucleosome-depleted regions facilitate assembly of the pre-initiation complex, an early step(s) in the transcription process (that is, those preceding extensive elongation) is the major determinant for a strongly positioned +1 nucleosome (see Discussion).

DISCUSSION

Evidence against the nucleosome code

Our results strongly argue against the idea of a nucleosome code in which nucleosome positions *in vivo* are determined primarily by DNA sequence[16,24]. Most notably, the *in vivo* pattern of statistical positioning (the strong positioning of the +1 and to a progressively lesser extent more downstream nucleosomes) is not observed *in vitro* and instead is linked to the process of transcriptional initiation. In addition, a nucleosome code does not explain why an *S. cerevisiae* genomic region shows different nucleosomal positions when present in *S. cerevisiae* or *Schizosaccharomyces pombe* cells[17], and it does not easily account for species- and cell type–specific differences in nucleosome spacing[1]. Our genome-wide analysis is consistent with the well-established idea that histones have strong DNA sequence preferences for nucleosome formation and rotational positioning[19–21] and that intrinsic histone-DNA interactions have an important role in generating nucleosome-depleted promoter regions[17,18]. However, the fact that histones have DNA sequence preferences for nucleosome formation is conceptually different from a nucleosome code that determines where nucleosomes are located *in vivo*. By analogy, DNA sequence motifs for a transcription factor do not constitute a code that is sufficient to specify where a protein binds *in vivo*[26,27].

Our central conclusion disagrees with that of a related study[16] that appeared after our work was completed and also involved large-scale sequencing of nucleosomes assembled on yeast genomic DNA by salt dialysis. However, our analysis of this independent data set (and a related analysis of this data; figure 4a of ref. 9) confirms, and indeed provides additional support for, the conclusion that the *in vivo* pattern of statistically positioned nucleosomes is not due to intrinsic histone-DNA interactions (**Supplementary Fig. 5**). The conflicting conclusions of these two studies are largely explained by the difference between the concepts of histone density and translational positioning. Although these concepts are related, histone density measures the average amount of histones on a given region of DNA in a population, and this cannot be used to determine translational positioning, which refers to the precise position of an individual nucleosome with regard to a given DNA sequence. Our central conclusion is based primarily on translational positioning analysis, whereas the major conclusion of the other study was based on the high correlation coefficient of histone densities between the *in vivo* and *in vitro* samples (see below) rather than on direct measurements of translational positioning. Thus, whereas low nucleosome density at many promoters and terminators *in vivo* is largely due to intrinsically weak histone-DNA interactions, the translational positions of where nucleosomes are actually located *in vivo* are not determined primarily by the underlying DNA sequence.

The correlation of histone densities between the *in vivo* and *in vitro* samples is lower in our experiments than observed in the independent data set (0.74 versus 0.54; **Supplementary Fig. 6**). However, correlation coefficients are strongly affected by methodological issues. Chromatin reconstitution in our study involved equal amounts of histones and DNA and the generation of regularly spaced nucleosome arrays. In contrast, reconstitution in the other study involved a histone:DNA mass ratio of 0.4, therefore resulting in isolated nucleosomes (~1 nucleosome per 400 bp) with most of the DNA not in the form of chromatin. The use of limiting histone concentrations is advantageous for measuring intrinsic affinities of different genomic regions, but it also represents an artificial competitive situation between DNA sequences that does not reflect the conditions *in vivo*, where histones are not limiting and most of the genomic DNA is nucleosomal.

Alternatively, the different correlation coefficients might arise from differences in the extent of micrococcal nuclease cleavage of the *in vitro*–generated samples used in the two studies (data from the same *in vivo* samples were used in both analyses). This can occur because the extent of enzymatic cleavage affects the ratio of cleavage within linker or mononucleosomal DNA, and the considerable sequence specificity of MNase[28] results in both differential cleavage of linker regions and differential cleavage of mononucleomes. In addition, correlation coefficients, and hence apparent similarities, of histone densities between *in vitro* and *in vivo* samples are likely to be inflated by the DNA sequence specificity of MNase and biases in DNA sequencing that apply to the analysis of all mononucleosomal samples. Indeed, when the contributions of MNase and sequencing bias are removed by measuring nucleosome affinities of a collection of 150-bp regions, correlation coefficients are substantially reduced[16].

These methodological issues do not affect our conclusion that intrinsic histone-DNA interactions are not the major determinant of nucleosome positioning *in vivo*. Indeed, our conclusions are robust to different data sets done under different experimental conditions by different laboratories.

Transcription-based mechanism for statistical positioning

The mechanism by which the +1 nucleosome becomes highly positioned is the key to understanding how statistical positioning within mRNA coding regions is achieved. By definition, strong positioning of the +1 nucleosome must be determined by specific DNA sequences. In contrast to the prediction from the nucleosome code hypothesis, we demonstrate that strong positioning of the +1 nucleosome is not due to intrinsic histone-DNA interactions and that an intrinsic nucleosome-depleted region is insufficient to strongly position a nucleosome. Instead, the position of the +1 nucleosome is highly related to the transcriptional initiation site, and it therefore depends on DNA sequences linked to the process of transcriptional initiation.

In support of a transcription-based mechanism, *Drosophila melanogaster* genes also show a highly preferential spacing between the transcriptional initiation site and the +1 nucleosome[4], although the spacing is larger. Notably, because the spacing between the pre-initiation complex and the mRNA initiation site is larger in yeast than in *D. melanogaster*[29], the distance between the pre-initiation complex and the +1 nucleosome is roughly similar in both species. This suggests the possibility that the location of the pre-initiation complex may be an important determinant for the location of the +1 nucleosome. However, it is also possible that the difference in spacing might be related to the fact that, unlike the case for yeast genes, many *D. melanogaster* genes have a paused RNA polymerase located downstream of the initiation site[30].

We propose the following transcription-based model for positioning the +1 nucleosome. First, as discussed previously[17,18], intrinsically nucleosome-depleted promoter regions facilitate association of the transcription machinery. Second, some component(s) of the transcriptional initiation machinery interacts with a nucleosome-remodeling complex and/or histones to position the +1 nucleosome. Third, once positioned, the +1 nucleosome might spatially constrain subsequent initiation events, thereby reinforcing the position of the +1 nucleosome.

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An appealing feature of this model is that it can explain why statistical positioning occurs on genes that are poorly transcribed. In particular, Pol II is detected at nearly all yeast genes[31,32], and nucleosome replacement is inversely correlated with transcription[33]. Hence, it is likely that, once formed via an initiation event, a positioned +1 nucleosome will be relatively stable at genes whose transcription is initiated infrequently. In any event, whatever the precise molecular mechanism, the ultimate DNA sequence determinant for positioning the +1 nucleosome is an event linked to transcriptional initiation, not the intrinsic sequence preferences of histones to form nucleosomes.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/nsmb/.

Accession codes. Gene Expression Omnibus: DNA sequencing data has been deposited with accession number of GSE15188.

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

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AUTHOR CONTRIBUTIONS

Y.Z. performed all the bioinformatic analyses and contributed to writing of the manuscript; Z.M. prepared the genomic DNAs and the sonicated DNA sample, prepared the micrococcal nuclease–treated samples for DNA sequencing and contributed to the experimental design, data analysis and writing of the manuscript; B.P.R. assembled nucleosomes on genomic DNAs; J.T.K. contributed to experimental design and analysis of the nucleosome samples; G.E. sequenced the DNA samples with contributions from M.S.; X.S.L. contributed to the design and interpretation of the bioinformatic analyses and writing the manuscript; K.S. conceived of the project, contributed to the data analysis and wrote most of the manuscript.

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1. Kornberg, R.D. Structure of chromatin. *Annu. Rev. Biochem.* **46**, 931–954 (1977).
2. Yuan, G.-C. *et al.* Genome-scale identification of nucleosome positions in *S. cerevisiae*. *Science* **309**, 626–630 (2005).
3. Lee, W. *et al.* A high-resolution atlas of nucleosome occupancy in yeast. *Nat. Genet.* **39**, 1235–1244 (2007).
4. Mavrich, T.N. *et al.* Nucleosome organization in the *Drosophila* genome. *Nature* **453**, 358–362 (2008).
5. Schones, D.E. *et al.* Dynamic regulation of nucleosome positioning in the human genome. *Cell* **132**, 887–898 (2008).
6. Shivaswamy, S. *et al.* Dynamic remodeling of individual nucleosomes across a eukaryotic genome in response to transcriptional perturbation. *PLoS Biol.* **6**, e65 (2008).
7. Valouev, A. *et al.* A high-resolution, nucleosome position map of *C. elegans* reveals a lack of universal sequence-dictated positioning. *Genome Res.* **18**, 1051–1063 (2008).
8. Mavrich, T.N. *et al.* A barrier nucleosome model for statistical positioning of nucleosome throughout the yeast genome. *Genome Res.* **18**, 1073–1083 (2008).
9. Deckert, J. & Struhl, K. Histone acetylation at promoters is differentially affected by activators and repressors. *Mol. Cell. Biol.* **21**, 2726–2735 (2001).
10. Boeger, H. *et al.* Nucleosomes unfold completely at a transcriptionally active promoter. *Mol. Cell* **11**, 1587–1598 (2003).
11. Reinke, H. & Horz, W. Histones are first hyperacetylated and then lose contact with the activated *PHO5* promoter. *Mol. Cell* **11**, 1599–1607 (2003).
12. Schwabish, M.A. & Struhl, K. The Swi/Snf complex is important for histone eviction during transcriptional activation and RNA polymerase II elongation *in vivo*. *Mol. Cell. Biol.* **27**, 6987–6995 (2007).
13. Kristjuhan, A. & Svejstrup, J.Q. Evidence for distinct mechanisms facilitating transcript elongation through chromatin *in vivo*. *EMBO J.* **23**, 4243–4252 (2004).
14. Lee, C.K. *et al.* Evidence for nucleosome depletion at active regulatory regions genome-wide. *Nat. Genet.* **36**, 900–905 (2004).
15. Schwabish, M.A. & Struhl, K. Evidence for eviction and rapid deposition of histones upon transcriptional elongation by RNA polymerase II. *Mol. Cell. Biol.* **24**, 10111–10117 (2004).
16. Kaplan, N. *et al.* The DNA-encoded nucleosome organization of a eukaryotic genome. *Nature* **458**, 362–366 (2009).
17. Sekinger, E.A., Moqtaderi, Z. & Struhl, K. Intrinsic histone-DNA interactions and low nucleosome density are important for preferential accessibility of promoter regions in yeast. *Mol. Cell* **18**, 735–748 (2005).
18. Iyer, V. & Struhl, K. Poly(dA:dT), a ubiquitous promoter element that stimulates transcription via its intrinsic structure. *EMBO J.* **14**, 2570–2579 (1995).
19. Drew, H.R. & Travers, A.A. DNA bending and its relation to nucleosome positioning. *J. Mol. Biol.* **186**, 773–790 (1985).
20. Satchwell, S.C., Drew, H.R. & Travers, A.A. Sequence periodicities in chicken nucleosome core DNA. *J. Mol. Biol.* **191**, 659–675 (1986).
21. Widom, J. Role of DNA sequence in nucleosome stability and dynamics. *Q. Rev. Biophys.* **34**, 269–324 (2001).
22. Fedor, M.J., Lue, N.F. & Kornberg, R.D. Statistical positioning of nucleosomes by specific protein binding to an upstream activating sequence in yeast. *J. Mol. Biol.* **204**, 109–127 (1988).
23. Lowary, P.T. & Widom, J. New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning. *J. Mol. Biol.* **276**, 19–42 (1998).
24. Segal, E. *et al.* A genomic code for nucleosome positioning. *Nature* **442**, 772–778 (2006).
25. Whitehouse, I. & Tsukiyama, T. Antagonistic forces that position nucleosomes *in vivo*. *Nat. Struct. Mol. Biol.* **13**, 633–640 (2006).
26. Yang, A. *et al.* Relationships between p63 binding, DNA sequence, transcription activity, and biological function in human cells. *Mol. Cell* **24**, 593–602 (2006).
27. Liu, X. *et al.* Whole-genome comparison of Leu3 binding *in vitro* and *in vivo* reveals the importance of nucleosome occupancy in target site selection. *Genome Res.* **16**, 1517–1528 (2006).
28. Hörz, W. & Altenburger, W. Sequence specific cleavage of DNA by micrococcal nuclease. *Nucleic Acids Res.* **9**, 2643–2658 (1981).
29. Struhl, K. Yeast transcriptional regulatory mechanisms. *Annu. Rev. Genet.* **29**, 651–674 (1995).
30. Muse, G.W. *et al.* RNA polymerase is poised for activation across the genome. *Nat. Genet.* **39**, 1507–1511 (2007).
31. Steinmetz, E.J. *et al.* Genome-wide distribution of yeast RNA polymerase II and its control by Sen1 helicase. *Mol. Cell* **24**, 735–746 (2006).
32. Struhl, K. Transcriptional noise and the fidelity of initiation by RNA polymerase II. *Nat. Struct. Mol. Biol.* **14**, 103–105 (2007).
33. Dion, M.F. *et al.* Dynamics of replication-independent histone turnover in budding yeast. *Science* **315**, 1405–1408 (2007).

ONLINE METHODS

Assembly of nucleosomes *in vitro* and sequencing of nucleosomal DNA. We purified *S. cerevisiae* and *E. coli* genomic DNAs separately, lightly sonicated them to generate fragments 5–10 kb and then combined them in a 3:1 mass ratio. We assembled the resulting DNA mixture into chromatin either by salt dialysis or by using a purified system containing recombinant *D. melanogaster* NAP-1 and ACF as well as purified native histones from *D. melanogaster* embryos, as described[34]. We performed three independent chromatin assembly reactions on the same DNA mixture. We extensively digested chromatin with MNase to yield core particles, purified the resulting mononucleosomal DNA and then performed massively parallel sequencing on an Illumina Genome Analyzer. We aligned sequence tags to the *S. cerevisiae* (Stanford Genome Database April 2008 build) and *E. coli* K12 MG1655 (U00096) genomes, allowing 2 mismatches per mapped read. Depending on the sample, we generated 1–3 million uniquely mapped sequence tags. As a control, we sonicated the mixture of yeast and *E. coli* DNA to fragments of comparable size to the mononucleosomal DNA.

Nucleosome density profiles. We generated nucleosome density profiles by extending the 5′ end of each sequence tag 146 bp and then piling up all extended tags. We obtained heat maps of nucleosome density profiles (normalized to enrichment ratios) for 1,752 isolated promoters aligned by transcriptional start sites (TSSs), 1,548 isolated terminator regions aligned by termination sites (TTSs) and promoter regions aligned by sequence motifs of DNA-binding transcription factors. To avoid complications arising from genomic regions with multiple functions, we generated heat maps on genes with isolated promoter or terminator regions, which are defined as 1-kb regions upstream or downstream of a given gene that do not overlap with other genes.

Analysis of rotational positioning. We defined rotational positioning by the periodicity of AA/TT/AT dinucleotides in the mononucleosomal DNA. We determined dinucleotide periodicity and tag position relationships by aligning the 5′ ends of sequence tags as described[7]. For power spectrum analysis of the mononucleosomal samples, we performed discrete Fourier transform on the +11 to +160 interval of AA/TT/AT fraction pattern to measure the power of 10-bp periodicity. To apply power spectrum analysis to yeast and *E. coli* DNA, we converted these sequences to binary sequence according to whether AA/TT/AT was present at each dinucleotide position, split this binary sequence into 1024-bp fragments and then applied the Fourier transform.

Nucleosome positioning degree. The degree of translational positioning is defined as the number of nucleosome centers (the position 73 bp downstream from the 5′ end of the sequence tag) within 20-bp windows centered at each nucleotide position divided by the number of nucleosome centers with a 160-bp window centered at the same position. The maximum positioning degree within a 160-bp window is the positioning degree of most 'positioned' nucleosomes contained in this region; hence, the percentage of genomic regions with maximum positioning degree larger than a certain threshold is a global measurement of nucleosome positioning status. To compare the nucleosome positioning status among samples, we randomly sampled the same number of tags along with a control comprising the same number of random tags in mappable genomic regions.

Comparison of *in vitro* and *in vivo* nucleosomal patterns in genes. We defined the nucleosome patterns of 3,774 non-overlapping genes, on a gene by gene basis, as the peak 20-bp window for the +1 through +10 nucleosome centers based on chromatin from yeast cells grown in YP ethanol[16]. We first generated the average positions of the +1 to +10 nucleosomes for these 3,774 genes, and then for each gene we identified the peak 20-bp window within the 100-bp region defined by the average position. We then determined the number of nucleosome centers within these defined 20-bp windows in nucleosomes generated by salt dialysis, ACF or in cells grown in YPD medium.

34. Fyodorov, D.V. & Kadonaga, J.T. Chromatin assembly *in vitro* with purified recombinant ACF and NAP-1. *Methods Enzymol.* **371**, 499–515 (2003).

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