Nucleus

ISSN: 1949-1034 (Print) 1949-1042 (Online) Journal homepage: http://www.tandfonline.com/loi/kncl20

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To cite this article: Alicia García , Sara González & Francisco Antequera (2017): Nucleosomal organization and DNA base composition patterns, Nucleus, DOI: 10.1080/19491034.2017.1337611

To link to this article: http://dx.doi.org/10.1080/19491034.2017.1337611

Accepted author version posted online: 21 Jun 2017.
Published online: 21 Jun 2017.

NUCLEUS
2017, VOL. 0, NO. 0, 1–6
https://doi.org/10.1080/19491034.2017.1337611

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Nucleosomal organization and DNA base composition patterns

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ABSTRACT

Nucleosomes are the basic units of chromatin. They compact the genome inside the nucleus and regulate the access of proteins to DNA. In the yeast genome, most nucleosomes occupy well-defined positions, which are maintained under many different physiological situations and genetic backgrounds. Although several short sequence elements have been described that favor or reduce the affinity between histones and DNA, the extent to which the DNA sequence affects nucleosome positioning in the genomic context remains unclear. Recent analyses indicate that the base composition pattern of mononucleosomal DNA differs among species, and that the same sequence elements have a different impact on nucleosome positioning in different genomes despite the high level of phylogenetic conservation of histones. These studies have also shown that the DNA sequence contributes to nucleosome positioning to the point that it is possible to design synthetic DNA molecules capable of generating regular and species-specific nucleosomal patterns *in vivo*.

ARTICLE HISTORY
Received 20 April 2017
Revised 22 May 2017
Accepted 30 May 2017

KEYWORDS
base composition; nucleosome; chromatin; evolution; genome organization

Introduction

Eukaryotic genomes are packaged into chromatin to be confined within the nucleus. The basic units of chromatin are the nucleosomes, histones octamers wrapped by ~1.7 turns of DNA that accommodate 147 base pairs (bp). Genome-wide maps of several yeasts species have revealed that most nucleosomes occupy well-defined and stable positions along the chromosomes during the cell cycle and under different physiological conditions.[1-3]

Nucleosome positioning results from the combined contribution of chromatin remodelers, transcription factors, and the DNA sequence. Remodelers facilitate the sliding, eviction or exchange of nucleosomes using ATP hydrolysis, and the depletion of some of them, such as *hrp3* in *Schizosaccharomyces pombe* or *ISW1* and *CHD1* in *Saccharomyces cerevisiae,* can cause widespread alterations in nucleosomal organization.[4-6] Transcription factors can compete with histones for the access to specific locations in DNA to generate nucleosome depleted regions (NDRs).[2,7,8]

The contribution of the DNA sequence to nucleosome positioning *in vivo* remains unclear. Based on genome-wide *in vitro* chromatin assembly, it has been suggested that most nucleosomes are positioned according to a universal code specified by the DNA sequence.[9,10] In contrast to this proposal, the statistical positioning model proposes that regular nucleosomal arrays are passively propagated from physical barriers bound to DNA.[11] According to this model, the contribution of the DNA sequence would be to determine the location of such barriers, which could coincide with protein complexes bound to gene promoters or other DNA binding proteins. While both models may contribute to nucleosome positioning in specific genomic regions, neither of them can sufficiently account for the positioning pattern found *in vivo*.

Considering that histones are among the most conserved proteins in evolution, if nucleosome positioning was entirely independent of the underlying sequence, it would be expected that the insertion of exogenous DNA fragments into a host genome would adopt the endogenous nucleosomal pattern. Contrary to this expectation, there are many examples where the same DNA fragments are packed differently by

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Extra View to: González S, García A, Vázquez E, Serrano R, Sánchez M, Quintales L and Antequera F. Nucleosomal signatures impose nucleosome positioning in coding and non-coding sequences in the genome. Genome Research 2016, 26:1532–1543; https://doi.org/10.1101/gr.207241.116

different species. For example, mouse metaphase chromosomes are abnormally condensed in regions where long tracts of fission yeast DNA were introduced.[12] Nucleosome positioning of Yeast Artificial Chromosomes (YACs) containing genomic DNA from *Kluyveromyces lactis*, *Kluyveromyces waltii* and *Debaryomyces hansenii* differs from that of their original genomes when harbored by *S. cerevisiae*, and do not adopt the endogenous *S. cerevisiae* pattern either.[13] This phenomenon is also observed between closely related species as *Saccharomyces paradoxus* and *S. cerevisiae*,[14] and even between orthologous genes like the *ura4* gene in *S. pombe*, *Schizosaccharomyces octosporus* and *Schizosaccharomyces japonicus*. The pattern of nucleosome positioning generated by the *ura4* gene from *S. octosporus* inserted into the *S. pombe* genome is more similar to the endogenous profile than that of the *S. japonicus ura4* gene, which correlates with *S. japonicus* being more distantly related to *S. pombe* than *S. octosporus*.[15]

Base composition and nucleosomal patterns

It has long been known that different DNA sequence elements have different affinities to nucleosomes. For example, the 5S rRNA gene has a strong positioning potential *in vitro* and *in vivo*.[16,17] By contrast, poly (dA:dT) tracts are refractory to nucleosome formation due to difficulty in bending around the histone octamer. They colocalize with sites of lower nucleosomal occupancy in *S. cerevisiae* (Fig. 1A) and are overrepresented in NDRs at gene promoters (Fig. 1B), where they facilitate the access of transcription factors to their binding sites.[18-21] In fact, 72.5% of all NDRs in *S. cerevisiae* include poly(dA:dT) elements 7 bp long (Fig. 1C). This situation is not universal, however, since in *S. pombe* nucleosome occupancy is not reduced over the same elements (Fig. 1A) and they are not overrepresented at promoter NDRs relative to their average genomic distribution[1] (Fig. 1B). In contrast with the situation in *S. cerevisiae*, only 18% of all NDRs include poly(dA:dT) elements 7 bp long in *S. pombe* (Fig. 1C). In addition, high-resolution studies have shown that the A+T content oscillates in phase with the occupancy profile in *S. pombe*, while in *S. cerevisiae* it peaks at linker regions.[22-25] Despite the differences among species, several laboratories have reported a periodic 10 bp pattern in the distribution of AT- and GC-rich dinucleotides in aggregated profiles of mononucleosomal DNA. This feature favors the bendability of DNA around the histone core and has been described in different species.[9,26-28]

Many studies have addressed the potential of DNA sequences to form nucleosomes using *in vitro* chromatin assembly under controlled biochemical conditions (see for example refs. 28-30). These approaches have uncovered important properties of the DNA-histone interactions, but do not always mimic the nucleosomal patterns found in the genomic context.[31,32]

To explore the connection between the DNA sequence and nucleosome positioning *in vivo*, we analyzed the distribution of the 4 nucleotides within 30,000 to 40,000 sequences of mononucleosomal DNA of *S. cerevisiae* and 3 species of *Schizosaccharomyces*,[25] and found they followed well-defined patterns, showing a higher content of adenine at the 5' end and thymine at the 3' end of each strand of mononucleosomal DNA. This asymmetric distribution was also observed—although to a lesser extent—between cytosine and guanine and was present in transcribed and non-transcribed regions. We have called these patterns nucleosomal signatures and they are different among species. In the case of coding regions (ORFs), nucleosomal signatures determine a species-specific periodicity in the distribution of amino acids that establishes an unanticipated connection between the position of individual codons around the nucleosome and protein composition, which has important consequences for gene evolution.[25,33,34]

Nucleosomal signatures contain positioning information

These observations raised the question of whether nucleosomal signatures could represent a molecular footprint caused by the stable association between nucleosomes and the DNA molecule over evolutionary timescales,[33] or whether they could contribute to the positioning of nucleosomes in the genome.

To explore this possibility, we randomized the sequence of several mononucleosomal DNA regions and used them to replace the native sequences within the genome. It was observed that the regular wild-type nucleosomal pattern was severely disturbed in regions that coincided precisely with the modified sequences. The same result was obtained when the modification of the sequence was limited

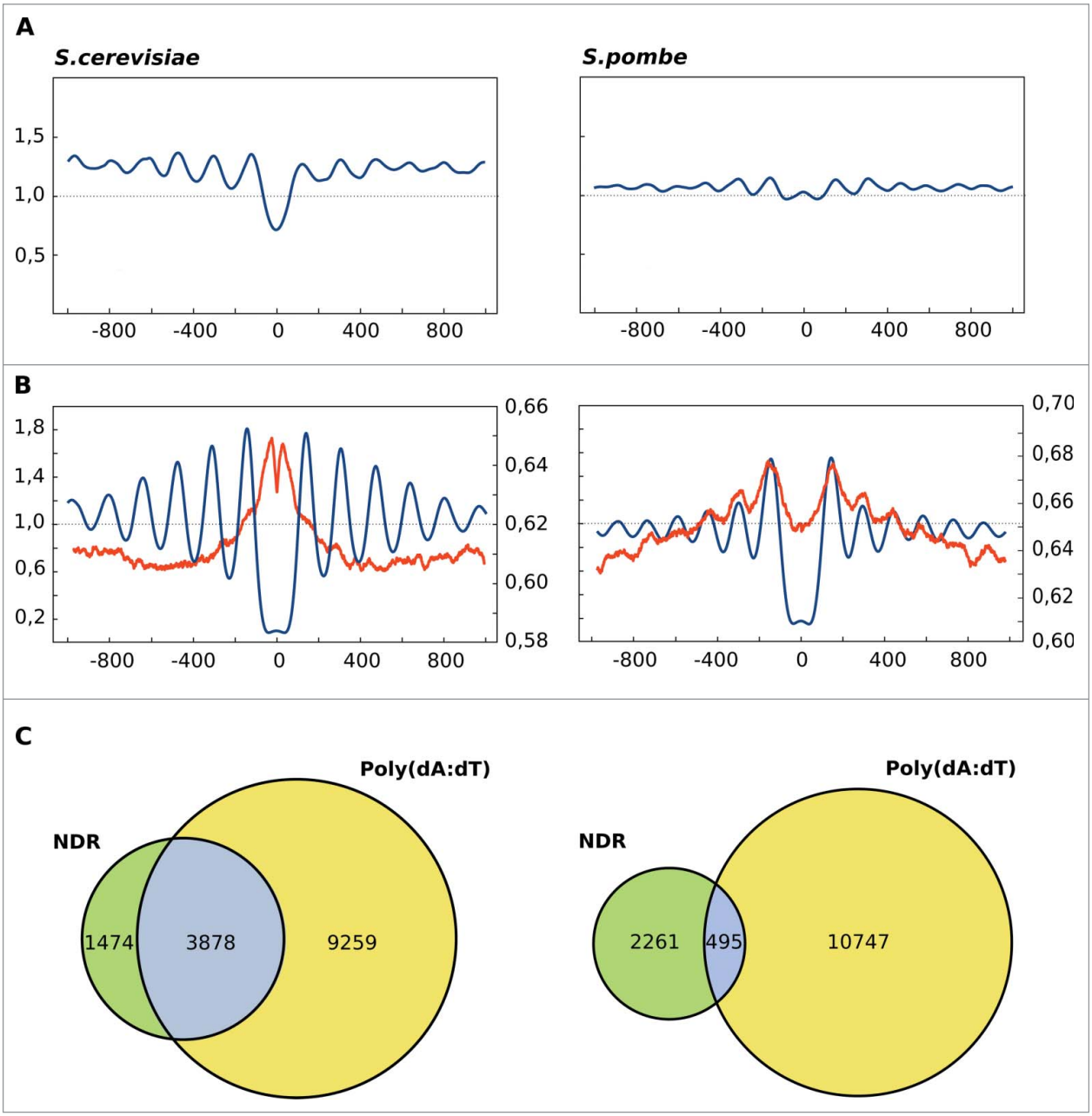

Figure 1. Base composition and nucleosomal organization in *S. cerevisiae* and *S. pombe*. (A) Aggregated profile of nucleosomal occupancy (blue, left y-axis scale) of genomic regions 2 kb long aligned to the central nucleotide of 13137 (*S. cerevisae*) and 11242 (*S. pombe*) poly (dA:dT) elements of 7 nucleotides present in their genomes. X-axis indicates nucleotide positions from the center of poly (dA:dT) elements. (B) Aggregated profile of nucleosomal occupancy (blue, left y-axis scale) of genomic regions 2 kb long aligned to the center of 5352 (*S. cerevisiae*) and 2756 (*S. pombe*) nucleosome depleted regions (NDR). NDRs were defined as regions spanning al least 90 nucleotides with a normalized sequence coverage lower than 0.3 relative to the genome average. The percentage of A+T (red, right y-axis scale) was calculated using a sliding window of 30 nucleotides and a step of 1 nucleotide. Profiles are symmetric from the site of alignment because genes are not oriented in the same direction. X-axis indicates nucleotide positions from the center of NDRs. The 2 peaks of A+T in the NDRs of *S. cerevisiae* probably correspond to the asymmetric localization of poly(dA:dT) elements in NDRs.[30] MNase-Seq data of nucleosomal occupancy for *S. cerevisiae* aw303–1a and *S. pombe* 972 h^- strains are taken from González et al. (2016).[15] (C) Overlap between NDRs and poly (dA:dT) elements in the 2 yeasts. 72.5% and 18% of NDRs colocalize with poly(dA:dT) elements of 7 nucleotides in *S. cerevisiae* and *S. pombe*, respectively.

to the substitution of the wild-type codons for their synonymous codons in the ORFs. This effect was not dependent on transcription since it was equally detectable in transcribed and non-transcribed regions, and suggested the existence of some type of information required for positioning that had been lost in the modified sequences.

To more directly test whether the nucleosomal signatures contributed to nucleosome positioning, we generated position-specific weight matrices to extract the information contained within them to design artificial non-coding sequences to evaluate their potential to position nucleosomes.[15] Strikingly, the insertion of artificial sequences based on the nucleosomal signature found in the *S. pombe* genome into its own genome led to the positioning of nucleosomes in the predicted positions. The same sequence, however, did not position nucleosomes in *S. cerevisiae*. Conversely, the artificial sequence designed according to the *S. cerevisiae* signature specified a strictly regular nucleosomal array in *S. cerevisiae*, but failed to do so in *S. pombe*.

The information present in nucleosomal signatures is degenerated and, in principle, would allow the design of thousands of different sequences with a similar positioning potential *in vivo*. This flexibility opened the possibility of incorporating this information into coding regions through the use of synonymous codons. Also, this would allow modifying heterologous ORFs to mimic the nucleosomal organization of the host genome to overcome the deficient positioning of exogenous sequences, as discussed above. We tested this possibility by re-designing the ORFs of the *ura4* gene of *S. octosporus* and *S. japonicus* based on the nucleosomal signatures of *S. pombe* without modifying their native coding potential. Nucleosome mapping showed that the two designer versions were capable of positioning nucleosomes in *S. pombe* with even a sharper profile than the endogenous *ura4* ORF. Likewise, two customized versions of the bacterial gene *kan*^{r}—a widely used marker for plasmids that confers resistance to geneticin—were used using the same strategy and generated regular nucleosomal arrays in *S. pombe* and *S. cerevisiae* that were not maintained when used interchangeably. These results indicated that nucleosomal signatures contain information that can direct nucleosome positioning in a species-specific manner,[15] and open the possibility of using them to engineer prokaryotic and eukaryotic genes to adopt the specific nucleosomal organization of the host organism. Their possible applications will be discussed below.

Possible applications of nucleosomal signatures

The DNA molecule contains multiple layers of information that have been extensively manipulated for biotechnological purposes. This includes optimization of genes to improve their heterologous expression through the incorporation of the codon bias of the host genome or the avoidance of cryptic splice-sites and some RNA secondary structures.[35] Our results show that nucleosomal signatures represent an additional layer of information that contributes to the species-specific organization of chromatin at its most basic level. In the case of eukaryotic hosts, the ability to reproduce the endogenous nucleosomal pattern could improve gene expression and to avoid undesired consequences of the lack of positioning such as antisense transcription and cryptic promoter formation.[13]

The information contained in nucleosomal signatures could also be of interest in synthetic biology and, more specifically, in the expanding field of genome design.[36] In this respect, the Sc2.0 international consortium is currently building a synthetic version of the *S.cerevisiae* genome, with some chromosomes already completed.[37,38] An important question currently being addressed is how these synthetic chromosomes are organized in the nucleus.[39] The close relationship between DNA sequence and nucleosome positioning discussed above suggests that small changes introduced in the sequence of synthetic chromosomes will probably have an impact on nucleosome positioning. Given that differences in nucleosome positioning or in their affinity for DNA have an effect on transcription,[40,41] DNA replication,[42,43] and recombination[44] it is possible that the feasibility of targeting nucleosomes to specific positions through the incorporation of nucleosomal signatures into DNA sequences could contribute to improve the functionality of designer chromosomes.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was funded by grant BFU2014–52143-P from the Spanish Ministerio de Economía y Competitividad (MINECO).

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