Nucleosomal organization and DNA base composition patterns

Alicia García ‡, Sara González ‡, and Francisco Antequera

Instituto de Biología Funcional y Genómica, Consejo Superior de Investigaciones Científicas (CSIC)/Universidad de Salamanca, Salamanca, Spain

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.

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. 1-5

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
different species. For example, mouse metaphase chromosomes are abnormally condensed in regions where long tracts of fission yeast DNA were introduced.\textsuperscript{12} Nucleosome positioning of Yeast Artificial Chromosomes (YACs) containing genomic DNA from \textit{Kluyveromyces lactis}, \textit{Kluyveromyces waltii} and \textit{Debaryomyces hansenii} differs from that of their original genomes when harbored by \textit{S. cerevisiae}, and do not adopt the endogenous \textit{S. cerevisiae} pattern either.\textsuperscript{13} This phenomenon is also observed between closely related species as \textit{Saccharomyces paradoxus} and \textit{S. cerevisiae},\textsuperscript{14} and even between orthologous genes like the \textit{ura4} gene in \textit{S. pombe}, \textit{Schizosaccharomyces octosporus} and \textit{Schizosaccharomyces japonicus}. The pattern of nucleosome positioning generated by the \textit{ura4} gene from \textit{S. octosporus} inserted into the \textit{S. pombe} genome is more similar to the endogenous profile than that of the \textit{S. japonicus ura4} gene, which correlates with \textit{S. japonicus} being more distantly related to \textit{S. pombe} than \textit{S. octosporus}.\textsuperscript{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 \textit{in vitro} and \textit{in vivo}.\textsuperscript{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 \textit{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.\textsuperscript{18-21} In fact, 72.5% of all NDRs in \textit{S. cerevisiae} include poly(dA:dT) elements 7 bp long (Fig. 1C). This situation is not universal, however, since in \textit{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\textsuperscript{1} (Fig. 1B). In contrast with the situation in \textit{S. cerevisiae}, only 18% of all NDRs include poly(dA:dT) elements 7 bp long in \textit{S. pombe} (Fig. 1C). In addition, high-resolution studies have shown that the A+T content oscillates in phase with the occupancy profile in \textit{S. pombe}, while in \textit{S. cerevisiae} it peaks at linker regions.\textsuperscript{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.\textsuperscript{9,26-28}

Many studies have addressed the potential of DNA sequences to form nucleosomes using \textit{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.\textsuperscript{31,32}

To explore the connection between the DNA sequence and nucleosome positioning \textit{in vivo}, we analyzed the distribution of the 4 nucleotides within 30,000 to 40,000 sequences of mononucleosomal DNA of \textit{S. cerevisiae} and 3 species of \textit{Schizosaccharomyces},\textsuperscript{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.\textsuperscript{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,\textsuperscript{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. cerevisiae*) 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 at 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. 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.\textsuperscript{15} Strikingly, the insertion of artificial sequences based on the nucleosomal signature found in the \textit{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 \textit{S. cerevisiae}. Conversely, the artificial sequence designed according to the \textit{S. cerevisiae} signature specified a strictly regular nucleosomal array in \textit{S. cerevisiae}, but failed to do so in \textit{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 \textit{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 \textit{ura4} gene of \textit{S. octosporus} and \textit{S. japonicus} based on the nucleosomal signatures of \textit{S. pombe} without modifying their native coding potential. Nucleosome mapping showed that the two designer versions were capable of positioning nucleosomes in \textit{S. pombe} with even a sharper profile than the endogenous \textit{ura4} ORF. Likewise, two customized versions of the bacterial gene \textit{kan}—a widely used marker for plasmids that confers resistance to geneticin—were used using the same strategy and generated regular nucleosomal arrays in \textit{S. pombe} and \textit{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,\textsuperscript{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.

\textbf{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.\textsuperscript{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.\textsuperscript{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. In this respect, the Sc2.0 international consortium is currently building a synthetic version of the \textit{S.cerevisiae} genome, with some chromosomes already completed.\textsuperscript{37,38} An important question currently being addressed is how these synthetic chromosomes are organized in the nucleus.\textsuperscript{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,\textsuperscript{40,41} DNA replication,\textsuperscript{42,43} and recombination\textsuperscript{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.

\textbf{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).

ORCID
Alicia García 10 http://orcid.org/0000-0003-0121-9437
Sara González 10 http://orcid.org/0000-0003-2710-075X

References
[1] Lantermann AB, Straub T, Stralfors A, Yuan GC, Ekwall K, Korber P. Schizosaccharomyces pombe genome-wide nucleosome mapping reveals positioning mechanisms distinct from those of Saccharomyces cerevisiae. Nat Struct Mol Biol 2010; 17:251-7; PMID:20118936; https://doi.org/10.1038/nsmb.1741
[2] Soriano I, Quintales L, Antequera F. Clustered regulatory elements at nucleosome-depleted regions punctuate a constant nucleosomal landscape in Schizosaccharomyces pombe. BMC Genomics 2013; 14:813; PMID:24256300; https://doi.org/10.1186/1471-2164-14-813
[3] Tsankov AM, Thompson DA, Socha A, Regev A, Rando OJ. The role of nucleosome positioning in the evolution of gene regulation. PLoS Biol 2010; 8; PMID:22885008; https://doi.org/10.1016/j.pmolcell.2010.07.003
[4] Grikopoulos T, Schofield P, Singh V, Pinksaya M, Mellor J, Smolle M, Workman JL, Barton GJ, Owen-Hughes T. A role for Snf2-related nucleosome-spacing enzymes in genome-wide nucleosome organization. Science 2011; 333:1758-60; PMID:21940898; https://doi.org/10.1126/science.1206097
[5] Pointner J, Persson J, Prasad P, Norman-Axelsson U, Stralfors A, Khrosjutina O, Krietenstein N, Peter Svensson J, Ekwall K, Korber P. CHD1 remodelers regulate nucleosome spacing in vitro and align nucleosomal arrays over gene coding regions in S. pombe. EMBO J 2012; 31:4388-403; PMID:23103765; https://doi.org/10.1002/emboj.2012.289
[6] Shim YS, Choi Y, Kang K, Cho K, Oh S, Lee J, Grewal SIS, Lee D. Hrp3 controls nucleosome positioning to suppress non-coding transcription in eu- and heterochromatin. EMBO J 2012; 31:4375-87; PMID:22990236; https://doi.org/10.1038/emboj.2012.267
[7] Yudkovsky N, Logie C, Hahn S, Peterson CL. Recruitment of the SWI/SNF chromatin remodeling complex by transcriptional activators. Genes Dev 1999; 13:2369-74; PMID:10500094; https://doi.org/10.1101/gad.13.18.2369
[8] Badis G, Chan ET, van Bakel H, Pena-Castillo L, Tillo D, Tsui K, Carlson CD, Gossett AJ, Hasinoff MJ, Warren CL, et al. A library of yeast transcription factor motifs reveals a widespread function for Rsc3 in targeting nucleosome exclusion at promoters. Mol Cell 2008; 32:878-87; PMID:19111667; https://doi.org/10.1016/j.molcel.2008.11.020
[9] Segal E, Fondufe-Mittendorf Y, Chen L, Ólafsson M, Field Y, Moore IK, Wang J-PZ, Widom J. A genomic code for nucleosome positioning. Nature 2006; 442:772-8; PMID:16862119; https://doi.org/10.1038/nature04979
[10] Kaplan N, Moore IK, Fondufe-Mittendorf Y, Gossett AJ, Tillo D, Field Y, Leproust EM, Hughes TR, Lieb JD, Widom J, et al. The DNA-encoded nucleosome organization of a eukaryotic genome. Nature 2009; 458:362-6; PMID:19092803; https://doi.org/10.1038/nature07667
[11] Kornberg RD, Stryer L. Statistical distributions of nucleosomes: Nonrandom locations by a stochastic mechanism. Nucleic Acids Res 1988; 16:6677-90; PMID:3399412; PMID:3399412; https://doi.org/10.1093/nar/16.14.6677
[12] McManus J, Perry P, Sumner AT, Wright DM, Thomson EJ, Allshire RC, Hastie ND, Bickmore WA. Unusual chromosome structure of fission yeast DNA in mouse cells. J Cell Sci 1994; 107:469-86; PMID:8006067; https://doi.org/10.1002/mol.110020.20
[13] Hughes AI, Jin Y, Rando OJ, Struhl K. A functional evolutionary approach to identify determinants of nucleosome positioning: A unifying model for establishing the genome-wide pattern. Mol Cell 2012; 48:5-15; PMID:22885008; https://doi.org/10.1016/j.pmolcell.2012.07.003
[14] Simpson RT, Stafford DW. Structural features of a phased nucleosome core particle. Proc Natl Acad Sci U S A 1983; 80:51-5; PMID:6572008; https://doi.org/10.1073/pnas.80.1.51
[15] González S, García A, Vázquez E, Serrano R, Sánchez M, Quintales L, Antequera F. Nucleosomal signatures impose nucleosome positioning in coding and noncoding sequences in the genome. Genome Res 2016; 26:1532-43; PMID:27662899; https://doi.org/10.1101/gr.207241.116
[16] Wilson AJ,ARCHAR WD, Bickmore WA. Unusual chromosome structure of fission yeast DNA in mouse cells. J Cell Sci 1994; 107:469-86; PMID:8006067; https://doi.org/10.1038/nature04979
[17] Segal E, Widom J. Poly(dA:dT), a ubiquitous promoter element that stimulates transcription via its intrinsic DNA structure. EMBO J 1995; 14:257-9; PMID:7781610
[18] Pennings S, Meersseman G, Bradbury EM. Mobility of positioned nucleosomes on 5 S rDNA. J Mol Biol 1991; 220:101-10; PMID:2067009; https://doi.org/10.1016/0022-2836(91)90384-I
[19] Iyer V, Struhl K. Poly(dAdT), a ubiquitous promoter element that stimulates transcription via its intrinsic DNA structure. EMBO J 1995; 14:257-9; PMID:7781610
[20] Zhang Z, Wippo CJ, Wal M, Ward E, Korber P, Pugh BF. A packing mechanism for nucleosome organization reconstructed across a eukaryotic genome. Science 2011; 332:977-80; PMID:21596991; https://doi.org/10.1126/science.1200508
[21] Raveh-sadka T, Levo M, Shabi U, Shany B, Keren L, Lotan-Pompan M, Zevei D, Sharon E, Weinberger A,
Segal E. Manipulating nucleosome disfavoring sequences allows fine-tune regulation of gene expression in yeast. Nat Genet 2012; 44:743-50; PMID:22634752; https://doi.org/10.1038/ng.2305

[22] Warnecke T, Batada NN, Hurst LD. The impact of the nucleosome code on protein-coding sequence evolution in yeast. PLoS Genet 2008; e10000250; PMID:18989456; https://doi.org/10.1371/journal.pgen.1000250

[23] Tilloy D, Hughes TR, G+C content dominates intrinsic nucleosome occupancy. BMC Bioinformatics 2009; 10:442; PMID:20028554; https://doi.org/10.1186/1471-2164-10-442

[24] Moyle-Heyrman G, Zaichuk T, Xi L, Zhang Q, Uhlenbeck OC, Holmgren R, Widom J, Wang J-P. Chemical map of Schizosaccharomyces pombe reveals species-specific features in nucleosome positioning. Proc Natl Acad Sci U S A 2013; 110:20158-63; PMID:24277842; https://doi.org/10.1073/pnas.1315809110

[25] Quintales L, Soriano I, Vazquez E, Segurado M, Antequera F. A species-specific nucleosomal signature defines a periodic distribution of amino acids in proteins. Open Biol 2015; 5:140218; PMID:25854683; https://doi.org/10.1098/rsob.140218

[26] Satchwell SC, Drew HR, Travers AA. Sequence periodicities in chicken nucleosome core DNA. J Mol Biol 1986; 191:659-75; PMID:3806678; PMID:3806678; https://doi.org/10.1016/0022-2836(86)90452-3

[27] Joshi-kshes I, Hosiad S, Pugh BF. Variety of genomic DNA patterns for nucleosome positioning. Genome Res 2011; 21:1863-71; PMID:21750105; https://doi.org/10.1101/gr.116228.110

[28] Lowary PT, Widom J. New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning. J Mol Biol 1998; 276:19-42; PMID:9514715; PMID:9514715; https://doi.org/10.1016/j.jmb.1997.1494

[29] Ngo TTM, Zhang Q, Zhou R, Yodh JG, Ha T. Asymmetric unwrapping of nucleosomes under tension directed by DNA local flexibility. Cell 2015; 160:1335-44; PMID:25768909; https://doi.org/10.1016/j.cell.2015.02.001

[30] Krietenstein N, Wal M, Watanabe S, Park B, Peterson CL, Pugh BF, Korber P. Genomic Nucleosome Organization Reconstituted with Pure Proteins. Cell 2016; 167:709-721; PMID:27768892; https://doi.org/10.1016/j.cell.2016.09.045

[31] Perales R, Zhang L, Bentley D. Histone occupancy in vivo at the 601 nucleosome binding element is determined by transcriptional history. Mol Cell Biol 2011; 31:3485-96; PMID:21690290; https://doi.org/10.1128/MCB.05599-11

[32] Zhang Y, Moqtaderi Z, Rattner BP, Euskirchen G, Snyder M, Kadonaga JT, Liu XS, Struhl K. Intrinsic histone-DNA interactions are not the major determinants of nucleosome positions in vivo. Nat Struct Mol Biol 2009; 16:1-7; PMID:19125164; https://doi.org/10.1038/nsmb.1636

[33] Washietl S, Machné R, Goldman N. Evolutionary footprints of nucleosome positions in yeast. Trends Genet 2008; 24:583-7; PMID:18951646; https://doi.org/10.1016/j.tig.2008.09.003

[34] Prendergast JGD, Semple CAM. Widespread signatures of recent selection linked to nucleosome positioning in the human lineage. Genome Res 2011; 21:1777-87; PMID:21903742; https://doi.org/10.1101/gr.122275.111

[35] Fath S, Bauer AP, Liss M, Spreiersbach A, Maertens B, Hahn P, Ludwig C, Schäfer F, Graf M, Wagner R. Multi-parameter RNA and codon optimization: A standardized tool to assess and enhance autologous mammalian gene expression. PLoS One 2011; 6:e17596; PMID:21408612; https://doi.org/10.1371/journal.pone.0017596

[36] Haimovich AD, Muir P, Isacys FJ. Genomes by design. Nat Rev Genet 2015; 16:501-16; PMID:26260262; https://doi.org/10.1038/nrg3956

[37] Annaluru N, Muller H, Mitchell LA, Ramalingam S, Stracquadanio G, Richardson SM, Dymond JS, Kuang Z, Scheiffele LZ, Cooper EM, et al. Total synthesis of a functional designer eukaryotic chromosome. Science 2014; 344:55-8; PMID:24674868; https://doi.org/10.1126/science.1249252

[38] Richardson SM, Mitchell LA, Stracquadanio G, Yang K, Dymond JS, DiCarlo JE, Lee D, Huang CLV, Chandrasegaran S, Cai Y, et al. Design of a synthetic yeast genome. Science 2017; 355:1040-4; PMID:28280199

[39] Mercy G, Mozziconacci J, Scolari VF, Yang K, Zhao G, Thierry A, Luo Y, Mitchell LA, Shen M, Shen Y, et al. 3D organization of synthetic and scrambled chromosomes. Science 2017; 355:eaa4597; PMID:28280150

[40] Bai L, Charvin G, Siggia ED, Cross FR. Nucleosome-depleted regions in cell-cycle-regulated promoters ensure reliable gene expression in every cell cycle. Dev Cell 2010; 18:544-55; PMID:20412770; https://doi.org/10.1016/j.devcel.2010.02.007

[41] Jimeno-González S, Ceballos-Chávez M, Reyes JC. A positioned +1 nucleosome enhances promoter-proximal pausing. Nucleic Acids Res 2015; 43:3068-78; PMID:25735750; https://doi.org/10.1093/nar/gkv149

[42] Eaton ML, Galani K, Kang S, Bell SP, MacAlpine DM. Conserved nucleosome positioning defines replication origins. Genes Dev 2010; 24:748-53; PMID:20351051; https://doi.org/10.1101/gad.1913210

[43] Soriano I, Morafraile EC, Vázquez E, Antequera F, Segurado M. Different nucleosomal architectures at early and late replicating origins in Saccharomyces cerevisiae. BMC Genomics 2014; 15:791; PMID:25218085; https://doi.org/10.1186/1471-2164-15-791

[44] de Castro E, Soriano I, Marín L, Serrano R, Quintales L, Antequera F. Nucleosomal organization of replication origins and meiotic recombination hotspots in fission yeast. EMBO J 2012; 31:124-27; PMID:21989386; https://doi.org/10.1038/emboj.2011.350