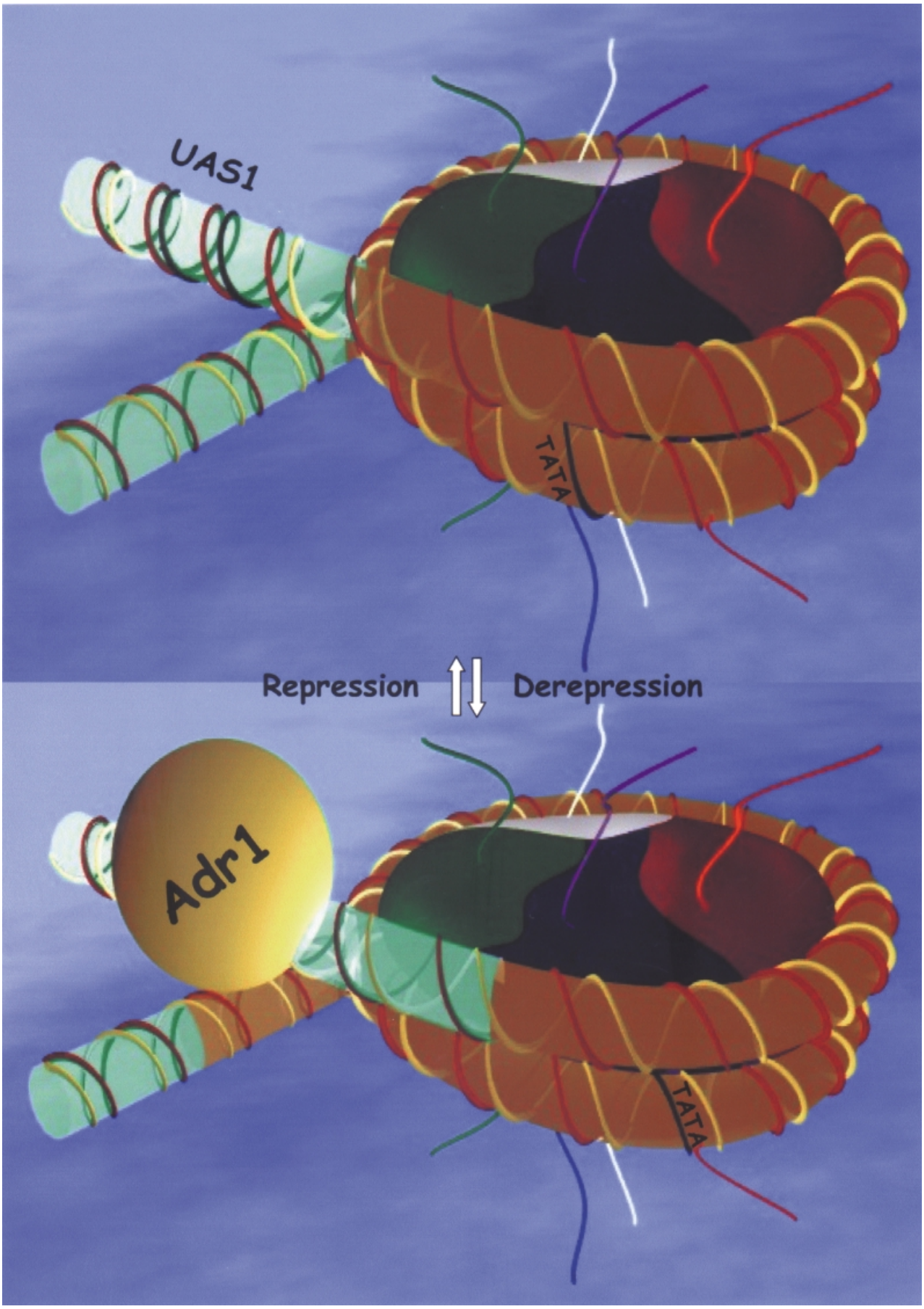

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Aspects of Nucleosomal Positional Flexibility and Fluidity

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Nucleosomes have been considered until recently to be stable and uniquely localized particles. We focus here on two properties of nucleosomes that are emerging as central attributes of their functions: mobility and multiplicity of localization. The biological relevance of these phenomena is based on the fact that chromatin functions depend on the relative stability of nucleosomes, on their covalent or conformational modifications, their dynamics, their localization, and the density of their distribution. In order to understand these complex behaviors both the structure of the nucleosome core particles and the informational rules governing their interaction with defined DNA sequences are here taken into consideration. The fact that nucleosomes solve the problem of how to locate a specific interaction site on a potentially infinite combination of sequences, with interactions recurring to a controlled level of informational ambiguity and stochasticity, is discussed. Nucleosomes have been shown to slide along DNA. This novel facet of their behavior and its implications in chromatin remodeling are reviewed.

KEYWORDS:

chromatin remodeling · DNA recognition · nucleic acids · nucleosome positioning · nucleosome structure

1. Introduction

Firstly, nucleosomes are topological objects. Removal of nucleosomes from closed DNA domains releases supercoiling,[1] thus making locally available the amount of torsional free energy that corresponds to one superhelical turn. A variation of the available free energy modifies the local DNA structure, thereby potentially exerting a profound effect on the interaction of DNA with proteins (as shown on a genome-wide scale by the modification of the activity of a large number of promoters caused by mutation or inhibition of DNA topoisomerase I—see ref. [2], and references cited therein). The understanding of the topology-related nucleosome behavior is still partial.

Secondly, nucleosomes are far from assuming uniquely defined localization and unmodifiable stability. We discuss here this second aspect of nucleosomal properties, focusing on two related phenomena: the multiple nature of their localization and their mobility.

Before that, we describe in the next four sections the structural properties of the system and the types of DNA information involved.

2. General Properties of the System

Histone octamers wrap DNA around themselves 1.7 times in a left-handed 'solenoid'. This local architecture is the consequence of a self-assembly process and it preferentially builds-up on DNA sequences endowed with defined properties.

Along the series of helical DNA repeats engaged in the binary DNA – nucleosome complex, sequence blocks exist which tend to partition along tandemly alternating conformational signals, locating themselves inwards and outwards relative to the nucleosomal surface in an orderly manner. The ordered distributions of phasing signals were defined in a series of pioneering studies (ref. [3] and references cited therein, reviewed in ref. [4]), which led to the general conclusion that the DNA sequences which allow preferential localization of nucleosomes are characterized by anisotropic flexibility (as defined in ref. [4]). The ordered and helically phased distribution of sequences that confer such flexibility favors nucleosome formation relative to bulk DNA, provided there is correspondence with the direction of DNA bending required on the nucleosomal surface. The overall conclusion of the studies that centered on the analysis of the relationship between histone octamers and the sequences on which they bind preferentially points to the relevance of the coherence between the sequence-determined anisotropic flexibility and the curvature of the DNA on the protein surface. These considerations have found a quantitative definition through the analysis of the free energies involved in complex formation on artificial nucleosome-positioning sequences.[5, 6] The energies involved differ as a function of the DNA sequence considered.

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Not taking into account extreme or exceptional instances, nor sequences which are purposely programed to involve higher or lower free energies of reconstitution, the range in which these energies are encompassed is rather limited.

The average reconstitution energy difference between a particularly efficient synthetic sequence taken as the reference and various mononucleosomal DNA sequences with a length of 250 base pairs (bp) is 2.85 Kcal mol^{-1}, while that observed for one of the most efficient natural sequences (the *Lytechinus variegatus* 5S RNA gene) is 1.25 Kcal mol^{-1}.[5] Thus, the difference between highly efficient and average sequences is only 1.6 Kcal mol^{-1} in natural systems. Similar values were obtained in the detailed analysis reported by Widom and co-workers for synthetic and/or selected sequences.[7, 8] Most remarkably, covalent modification of DNA results in a major alteration of the binding parameters.[9]

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3. The Structure of the Nucleosome Core Particle

The X-ray crystal structure of the nucleosome core particle (ncp) was determined at low resolution (7 Å) in 1984;[10] it revealed the left-handedness and defined the extent (1.65 turns) of the helical ramp. Distortions through kinks have been described.[11, 12]

The structure obtained at higher resolution (2.8 Å) revealed[13] that each histone in the complex consists of a structured three-helix domain (the 'histone-fold') and of two unstructured tails. The histone-folds assemble into the H2A – H2B and H3 – H4 heterodimers. The structural aspects of their further assembly to form the histone octamer have been known since the analyses by Arents and co-workers.[14, 15]

The structure solved at high resolution shows, in nearly atomic detail, how DNA is organized about the histone core. The salient features are:

- Each of the heterodimers binds about 30 bp of DNA.
- Of the overall sequence involved (146 bp), 121 bp complex with histone-fold domains.
- At the entry and exit sites of the DNA on the nucleosome the complex involves extensions of the H3 histone-fold.
- 14 contacts are observed between the DNA and the protein at $\approx$10 bp intervals. Arginine side chains penetrate all the 14 minor grooves that face the protein core in the contact points.
- Many contacts exist between the phosphate backbone of the DNA and the backbone of the protein; these contacts support the stability of the complex and allow an interaction to occur that is by its own nature chemically independent of sequence information.

The ubiquity of nucleosomes, a property that is closely related to their basic function as chromatin organizers and regulators, requires that their sites of interaction are unconstrained by local sequence information. Accommodation of the nucleosomes on different DNA sequence combinations is thus allowed, provided that the correct parameters of handedness, flexibility, and helical periodicity are respected. At the same time this conformation-based interaction mechanism elegantly solves the apparent paradox set by an essentially invariant protein (epigenetic covalent modifications apart) that is able to localize specific sites on an a priori potentially infinitely variant combination of sequences. Covalent modifications are thought not to be at the base of the localization mechanism but, rather, to mediate wide-spectrum regulatory patterns through subsequent acetylation,[16] methylation, ubiquitination, adenosine diphosphate (ADP) ribosylation, and phosphorylation of resident histones.

4. The DNA Rotational and Translational Information

The fact that the interaction of a single ncp with DNA is repeated 14 times and that it is, in principle, not sequence- but conformation-dependent has several important consequences.

A possible semiological ambiguity should be considered. The DNA local conformation descends from the properties of an invariant backbone and of a variable base sequence. The

information that DNA offers to the interaction is thus intrinsically based—whatever parameter is being considered—on the chemical and physical properties of a defined base-sequence-containing structure. When referring to the structure- or sequence-based properties of DNA, this very fact engenders interpretative ambiguities: sequence information always plays a role, even when only backbone properties are being considered. In the case of nucleosome localization, both the invariant and the variant information are of central importance.

It is well established that the positioning of the core particle on a DNA sequence has both rotational and translational components. The rotational positioning is defined by the orientation of the DNA sequence relative to the surface of the histone octamer and is dependent on its anisotropic flexibility.[17–19] The translational position of the core particle is defined by the position of the dyad relative to the DNA sequence. Pioneering analyses showed that the four central double-helical turns contain important determinants.[20, 21]

5. A Varying Helical Periodicity

Topological considerations and analyses led to the expectation that helical periodicity on the nucleosomal surface would be 10.2 bp,[22] different from the average 10.6 bp in solution. Substantial evidence from analyses by DNA sequencing,[23] photoinduced pyrimidine dimer formation,[24, 25] and cleavage by hydroxyl radicals[26] has actually shown that the average relative helical repeat of the bound DNA is 10.2 – 10.3 bp. This value has also been confirmed in the high-resolution analyses[13, 27] and on single-particle complexes.[28] Such a specific repeat value allows the minor and major grooves to align on the protein surface, thereby creating a structure deemed to have an important role in the interaction with other proteins[29] and to direct the histone tails (as pointed out in ref. [30]). The detailed study of the interactions of third-party proteins with DNA when engaged with nucleosomal surfaces is a field that is still only partially developed.

A sophisticated use of the structural information of DNA allows localization on a theoretically infinite number of sites and gives rise to potentially directional particles, as discussed below.

6. Indications of Asymmetries

The internal complex symmetry of the protein structure does not a priori entail that the overall structure should maintain complete symmetry upon interaction with DNA. The sequence used in the high-resolution structure reported[13] was palindromic, thus being endowed with an artificial convergent symmetry and leaving this problem open.

The polar nature of the natural DNA sequence onto which nucleosome cores normally form would actually suggest that symmetry is not maintained. Such an intrinsic tendency to asymmetry has been proposed[31] and supporting evidence based on sequence analyses was discussed. Structural asymmetry of the chromatosome (operationally defined as the particle produced during micrococcal digestion of chromatin yielding a DNA fragment of $\approx$166 bp[32–34]) was also reported[35] based on numerous data.

The analysis of the sequence dependence of translational positioning of core nucleosomes carried out on a set of synthetic, polar (as opposed to palindromically convergent) sequences has shown[28] that the translational position of a core particle is specified by sequence determinants additional to those specifying rotational positioning. The rotational settings on either side of the dyads of a core particle assembled on some of these sequences differed by $+2$ bp, which corresponds to an overall helical periodicity of $\approx$10.15 bp. The relevant finding of these analyses is that the average helical periodicity of the central two to four turns was 10.5 – 11 bp whilst that of the flanking DNA was close to 10 bp. This finding confirms, on a singly located nucleosomal particle, the early DNAse I digestion profiles of core particles,[36–38] which indicated that the number of base pairs between the cleavage maxima in the central three turns is 10.7 bp and in the flanking bound DNA is $\approx$10 bp.

The fact that the DNA immediately flanking the dyad in single-localization particles was also characterized by a more extensive susceptibility to cleavage by hydroxyl radicals[28] indicates the existence of a centrally located region with different structure and potentially different reactivity. The substantial symmetry in the vicinity of the dyad displayed by the crystal structure of the nucleosome core, while the linker histone binds asymmetrically in this region to select a single high-affinity site from potentially two equivalent sites, poses an apparent paradox (discussed in detail in ref. [39]). The observation that the helical repeat register changes by 2 bp in the vicinity of the dyad[28] reveals an asymmetry that could possibly[39] direct the binding of the linker histone to a single preferred site.

7. The Positioning Problem

The overall message conveyed by these studies is that the histone octamer, although being able to bind virtually all genomic sequences, occupies highly preferred positions on specific DNA sequences both in vitro and in vivo.[40–45] The positioning is achieved by using the information carried by both rotational and translational components. The fact that the interaction occurs on 14 helically phased alternately favorable/permissive interaction signals causes an internal ambiguity: in the absence of sequences that function as borders by providing strong repulsive determinants, the localization could take place almost isoenergetically on a set of positions that are shifted by one or more helical periods, both upstream and/or downstream relative to the most favorable set.

This systemic and intrinsic multiplicity could be counterbalanced by the energetics of the deformation observed in proximity of the central dyad (see above). The deformation could be favored by defined sequences located in correspondence with the central part of the protein complex. However, the DNA deformation observed at the dyad is possibly an a posteriori characteristic of all the core particles, independently of their position. That is, the dyad deformation is more a consequence and attribute of the final structure common to all ncps than a part of the nucleation process.

Asymmetries in the ncp at 2.5-Å resolution were observed in the analysis by Harp et al.[27] of a structure composed of native chicken histone octamer cores and a DNA palindrome. The interaction between these two-fold symmetric molecules results in an asymmetric structure owing to the binding of the DNA to the protein surface and to the packing of the particles in the crystal lattice. The observed asymmetries pertain both to the structure of the DNA as well as to the structure of the histones. The asymmetries in the DNA backbone are most noticeable between the second and third helical periods and the fifth and sixth helical periods of each hemi DNA moiety.

The observation that the asymmetric ncps assume only one of the two possible orientations in the crystal suggests that the two faces of the nucleosome are unique within the lattice. Asymmetry is seen[27] as a consequence of the dyad intersecting DNA base 73, which results in positioning 72 bp to the side of the dyad on one face and 73 bp on the other face. The structure of the individual histones in the ncp also deviates from symmetry in several positions, in spite of the overall twofold symmetry displayed by the histone octamer structure. The relevance of the conclusions drawn from crystallization studies as a whole for the in vivo properties of ncps remains to be established, due to the absence in the complexes of linker DNA and to the ensuing possible interference[7] with the physiological role of the N-terminal tails of H3 which emerge between the gyres of DNA.

The systemic and intrinsic multiplicity could also be counterbalanced by specific sequence elements acting as boundaries. Among the several sequences with this type of possible function, T-tracts have been frequently implicated. T-tracts are essentially straight and rigid,[46–48] thus providing the necessary inflexibility constraint. However, as pointed out,[49] folding of a T-tract DNA in nucleosomes can disrupt the T-tract structure, a fact indicating that the structural constraints in nucleosomes dominate over those in the T-tracts.[50, 51] In one of the multiple nucleosome systems described below (the *ADH2* promoter nucleosome -1), a 20 bp T-tract is incorporated into the upstream moiety of the nucleosomal DNA without apparent difficulties.

Nevertheless, the fact that most poly (dA – dT) sequences exist as rigid DNA structures in nucleosome-free yeast promoters in vivo is compelling and leaves the relationship of these sequences with nucleosome localization an open problem. Whatever their function is in respect to nucleosome-core localization and stabilization, a possible role of these sequences is supported by the observation that there are 1500 yeast genes whose promoters contain T-tracts >10 bp.[52]

Another exclusion mechanism could be provided by interspersion of short isotropically flexible sequence motifs within anisotropically flexible sequences. The analysis reported in ref. [53] concerning the selection of DNA sequences that bind less well to the histone octamer is quite relevant in this context. It was in addition reported[54] that interspersed CTG tracts reduce the curvature resulting from phased A-tracts. However, the observation that CTG repeats preferentially assemble into nucleosomes[55, 56] leaves the problem open in this case as well. The identification of nucleosome excluding sequences is difficult.

In summary, ncps are potentially able to complex, with low free energy discriminations, the vast majority of the genomic sequences according to an interaction mechanism that involves repetitive contacts and results in potentially asymmetrical structures. These considerations have lead us to hypothize that nucleosomes form on rigidly unique and unequivocal positions only in exceptional cases, with their localization occurring on quasi-isoenergetic multiple translational positions along the same rotational phase. Given that rotational positioning depends on the summation of multiple weak signals, these same signals would be expected to specify a family of rotationally related but translationally different and alternative positions, as is observed in vitro in several systems and in vivo notably in yeasts. Examining such positional variegation is the focus of the next section.

8. Multiple Positions

The picture described above fully applies to biological systems which, like many yeasts, are devoid of conventional linker histones whose stabilizing and regulatory functions are well established.[57–63] It also applies to in vitro reconstituted systems,[64–67] a fact showing that multiplicity is a widespread property in nucleosome formation.

Multiple nucleosome positioning with unique rotational setting were observed for the *Saccharomyces cerevisiae* 5S gene both in vitro and in vivo.[68] Distributed along the same rotational phase (defined as in Section 3.), the individual particles are alternative, in the obvious sense that they are present in a single translational position on a defined DNA molecule in vitro or in a defined individual cell in vivo. In this specific gene system the occupation of *all* the possible alternative translational positions along the gene was observed (although with different frequencies). The paramount relevance of the rotational information in determining the multiplicity of occupancy could be shown by inducing the change of nucleosome positions through the modification of the rotational phasing signals both in vitro[69] and in vivo.[70] In the *S. cerevisiae* 5S gene the positional multiplicity is the characterizing attribute of nucleosome localization. Multiplicity in *Xenopus laevis* oocyte and somatic 5S genes was also described.[71] A similar all-occupancy extreme behavior was observed in vitro when ncps were reconstituted on various DNA fragments containing a *Chamaecrista fasciculata* kinetoplast curved tract.[64] In this system, preferential deposition also occurred at multiple periodic positions, whose distribution revealed a unique rotational setting of DNA relative to the histone octamer surface, with an average periodicity of 10.26.

A limited multiplicity was observed in several nucleosomes located in vivo in the promoters of genes served by RNA polymerase II: the promoter nucleosomes of the *Hansenula polymorpha MOX* gene[72] and of the *S. cerevisiae TOP1*[73] and *ADH2*[74–77] genes (Figure 1). A similar behavior was observed in the *S. cerevisiae* ARS1 B-domain nucleosomes.[78] The multiplicity observed went from two – six alternative positions for each nucleosomal family, with the most consistently observed value being four. Only rarely were particles observed which localized in

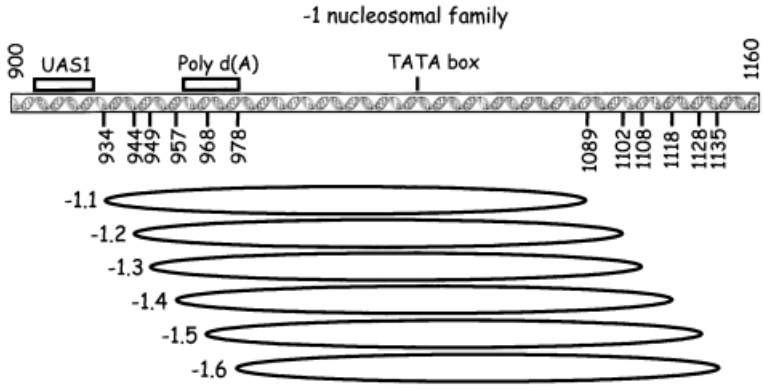

Figure 1. *The alternative localization of the nucleosome encompassing the relevant functional elements of the ADH2 promoter in* S. cerevisiae. *The promoter map is shown schematically in the top part. UAS1: Upstream activator sequence; Poly d(A): a dA – dT stretch that is 20-bp long. For the numbering system and other data on this promoter, see ref. [77]. This nucleosome (dubbed as − 1) encompasses the relevant elements of the ADH2 promoter and is alternatively present as individuals − 1.1, − 1.2, etc. (depicted by the ovals). The map positions of the borders of the individual nucleosomal particles are indicated. For experimental details, see ref. [77].*

a unique position, usually on neighboring functional chromosomal sites.[72, 78]

Multiplicity was analyzed by our group with a panel of different techniques, some of which are reviewed in ref. [79]. A variety of other technical approaches was used in the works reported below (see also ref. [80]), thus transforming into a safe fact the assumption that multiplicity is a characterizing property of nucleosomes both in vitro and in vivo. Concerning gene systems served by RNA polymerase II, multiple positioning was also observed on chicken and human globin genes,[81] in the *Drosophila HSP6* promoter,[82] in the MMTV LTR,[83] in the *CUP1* chromatin,[84] and in the active and inactive alleles of the *HPTR* promoter.[85]

A particularly interesting set of analyses was performed in the *S. cerevisiae URA3* gene.[86, 87] To characterize nucleosome structure and positioning in the chromosomal context, the chromatin structure of the whole *URA3* gene was studied in the genome and in a minichromosome by testing the accessibility of DNA to micrococcal nuclease and DNase I. While low-resolution mapping showed six regions with a positioned nucleosome, each region resolved in a complex pattern consistent with multiple overlapping positions. To investigate how intrinsic properties of nucleosomes modulate DNA accessibility in vivo, DNA repair in this gene by a photolyase was studied. Formation of DNA lesions (cyclobutane pyrimidine dimers, CPDs) and photolyase activity are precisely controlled by light. Preceding work by the same authors revealed that photolyase rapidly repairs nucleosome-free DNA, while repair of nucleosomes is severely inhibited. High-resolution data show slow repair in the center of nucleosomes and a gradual increase towards the periphery. This pattern was observed in all nucleosomes and demonstrates that dynamic properties facilitate DNA accessibility. Since the *URA3* nucleosomes can occupy alternative positions, the repair data are most consistent with nucleosome mobility that moves CPDs in linker DNA to where they are rapidly repaired. This study functionally couples nucleosome multiplicity and mobility. A partial compilation of the multiplicity-based architecture in promoters served by yeast RNA polymerase II is given in ref. [88].

Nucleosome multiplicity was also observed in the *S. cerevisiae* ribosomal genes, where the region of the ribosomal DNA defined as NTS2[89] has been shown to be arranged in positioned nucleosomes.[90] High-resolution mappings have shown that one nucleosome (lying about 340 bp from the transcriptional start of 35S RNA) of the array (represented by five positioned nucleosomes) occupies at least three major positions, as mapped by in vivo footprinting, definition of nucleosomal borders, and in vivo digestion with λ-exonuclease (G. Camilloni, personal communication).

Multiplicity in structural chromatin was observed in *Kluyveromyces lactis* centromeres[91] and in the CTCF-mediated insulator function of the *H19* imprinting control region.[92]

9. Nucleosome Dynamics

One of the questions raised by the existence of multiple localization of nucleosomes concerns the effects that a defined nucleosome position will have on gene expression when the DNA segment taken into consideration contains basic promoter elements such as the TATA box or the RNA initiation sites. If one considers a nucleosome located inside an open reading frame, it is expected that there will be no major differential effect on gene expression between a unique position and one differing by ten or twenty base pairs. But when a sequence like the TATA box or the binding site for a transcription factor is considered, then the exact location of the nucleosome particle can have profound effects on the transcription of the gene involved. In order to regulate the on/off state of a gene, the potentiality for a given octamer particle to occupy alternative positions becomes relevant.

Nucleosome relocation is, in fact, one of the most important aspects of the generally defined 'chromatin remodeling problem' that accompanies gene activation. Transcription in particular—but also other nuclear processes such as replication, recombination, and repair—requires that the DNA be accessible to sequence-specific transcription factors and RNA polymerase. Moreover, melting and reformation of the double helix throughout the length of the transcript is required: the chromatin structure interferes with all the steps necessary for transcription. Therefore, repression can be obtained by creating a stable inaccessible structure and activation can be achieved by a drastic chromatin reorganization.[93, 94]

In the last few years two general principles have emerged that explain the existence of convertible chromatin structures corresponding to distinct functional states.[95, 96] First, the histone N-terminal tails can be modulated through posttranslational modification (see Section 3.). The functional consequences of these modifications, which profoundly affect the recognition of nucleosomes by regulatory proteins and their higher-order folding, are currently the subject of numerous investiga-

tions.[97–102] Second, adenosine triphosphate (ATP) dependent nucleosome remodeling factors were recently characterized: they are capable of altering histone – DNA interactions such that nucleosomal DNA modifies its accessibility to regulatory proteins and to various components of the transcription machinery.[103–108]

Both histone-modifying enzymes and ATP-dependent remodeling factors are present in all eukaryotic cells as large protein complexes that can coordinate with each other and with the transcription machinery to create specific regulation (see ref. [109] and references cited therein). Various models have been proposed to account for the cooperative action of all these proteins.[109] Two specific examples point to the possibility that acetyltransferase complexes might stabilize the interaction of yeast SWI/SNF complexes with the template.[110, 111]

The emerging picture shows that nucleosomes are dynamic particles whose structure and/or location must be modified to allow many nuclear processes. In particular, a fluid state of chromatin in which the overall packaging of DNA is maintained but individual sequences are transiently exposed to interacting factors, can be established when histone octamers are relocated from an original and defined DNA site to a new previously inaccessible acceptor DNA position.

10. In Vitro Evidence of Nucleosome Repositioning

The most important biochemical evidence regarding the capability of ATP-dependent remodeling machines to relocate octamer particles either in cis (intramolecularly) or in trans (intermolecularly) is reported in Table 1. Yeast SWI/SNF and RSC complexes can both displace histones in trans.[112–115] Furthermore, the yeast SWI/SNF complex can reposition nucleosomes in an ATP-dependent reaction that favors the attachment of the histone octamer to an acceptor site on the same molecule of DNA.[116] This mechanism appears to be conserved in evolution since three complexes from *Drosophila melanogaster*, NURF,[117] CHRAC,[118] and ACF[119] can induce nucleosome sliding. In particular, CHRAC induces movements of intact histone octamers to neighboring DNA segments without facilitating their displacement in trans and with a specific directionality,[118] whereas NURF catalyzes the bidirectional redistribution of mononucleosomes.[117] The core of these three *D. melanogaster* complexes is constituted by the ISWI factor. Homologues of this factor have been characterized in *X. laevis* extracts[120] and in mouse cells.[121] In yeast, the two proteins more closely related to ISWI are Isw1 and Isw2: they both reside in complexes endowed with nucleosome remodeling and spacing activity.[122, 123] Interestingly, although they have not been shown to induce the change of translational positioning in vitro, a few reports indicate that in vivo they act by mobilizing nucleosomes into repressive positions (see below).[124–126]

Table 1. *Chromatin remodeling complexes showing in vitro nucleosome repositioning activity.*

In cis displacement		
Complex	Source	Reference[a]
SWI/SNF	*Saccharomyces cerevisiae*	[116]
NURF	*Drosophila melanogaster*	[117]
CHRAC	*Drosophila melanogaster*	[118]
ACF	*Drosophila melanogaster*	[119]
ACF	*Xenopus laevis*	[120]
Mi-2/NURD	*Drosophila melanogaster*	[128]
Mi-2/NURD	*Xenopus laevis*	[129]
NoRC	mouse	[131]
In trans displacement		
Complex	Source	Reference[a]
SWI/SNF	*Saccharomyces cerevisiae*	[112]
SWI/SNF	human	[113]
RSC	*Saccharomyces cerevisiae*	[114], [115]

[a] The reported references contain the first in vitro experimental evidence of nucleosome repositioning for each of the listed complexes.

Another important chromatin remodeling complex has been found in various biological systems and has been dubbed NRD (or NuRD or NURD). The interesting aspect of this complex is that it physically combines the two fundamental strategies for chromatin remodeling: covalent modification (deacetylation) and ATP-dependent remodeling.[127] The core subunit of this complex is the Mi-2 ATPase that, like ISWI, is an active enzyme able to disrupt histone – DNA interactions and to induce nucleosome sliding on DNA fragments.[128, 129]

Nevertheless, nucleosome repositioning cannot account for all the events underlying chromatin remodeling. One recent instance of a different molecular mechanism has been provided by the analysis of transcription by yeast RNA polymerase II when it occurs through nucleosome cores in vitro. The passage of the enzyme causes a quantitative loss of one H2A/H2B dimer without altering the location of the nucleosome.[130] A more detailed discussion regarding additional models is presented in ref. [104]. It should also be noted that temperature-induced histone octamer sliding can be commonly observed in in vitro reconstitutes. The original observations[65, 66] first revealed the close association of multiplicity and sliding phenomena.

The possible role of the HMGB proteins in the context of nucleosome mobility should also be mentioned: the biochemical and genetic connection between remodeling and NHP6A/B has been recently reported (refs. [131, 132]; for a comprehensive review, see ref. [133]).

11. In Vivo Evidence of Nucleosome Repositioning

In spite of the numerous examples of the changes of nucleosome translational positioning obtained in vitro, only a limited number of reports point to the relevance of this phenomenon in vivo. Initial evidence, based on low-resolution micrococcal nuclease analysis, established a correlation between transcriptional repression and nucleosome mobilization mediated by the Isw2 protein in *S. cerevisiae* cells.[124, 125] In another report, both Isw1 and Isw2 were shown to be responsible for changes in nucleosome positioning at various yeast promoters, independently of the transcriptional state.[126]

The involvement of sliding in gene activation has been demonstrated in a work showing that a nucleosome obstructing transcription from the IFN-β promoter slides in vivo in response

to virus infection.[134] The authors utilized HeLa cells that were first treated with formaldehyde, in order to fix the histone – DNA contacts, followed by micrococcal nuclease treatment of isolated nuclei. DNA extracted from the resulting mononucleosomes was annealed with radiolabeled primers, and this was followed by primer extension. In this case, the position of the relevant nucleosome in uninduced cells is unique: after virus induction, one additional position is observed. It is not known whether the same analysis, performed in the absence of formaldehyde, would have produced a pattern of multiple overlapping positions, as observed in almost all the systems analyzed at high resolution. The most important finding of this report has been obtained in vitro: recruitment of the SWI/SNF complex by the enhanceosome allows TBP binding which induces both DNA bending and the sliding of the nucleosome covering the TATA box.[134]

In a more recent work, nucleosome positioning was analyzed at high resolution at the *S. cerevisiae ADH2* promoter both in repressing and derepressing conditions.[77] In this system, nucleosome covering the TATA box consists of a family composed of six alternately rotationally phased particles, all of which encompass the TATA element. This distribution is normally present in high-glucose repressing conditions. When the cells are shifted to low-glucose medium (derepressing conditions) a change over the entire distribution of nucleosomes is observed. In particular, the frequency of the upstream particles decreases while the intensity of the downstream ones increases; this suggests a repositioning of the nucleosome containing the TATA box by a few nucleotides in the direction of transcription (Figure 2). Such repositioning does not occur in the absence of the *ADH2* transcriptional activator Adr1 or in the presence of its DNA binding domain alone. A construct consisting of the DNA binding domain plus a 43 amino acid residue peptide containing the Adr1 activation domain is sufficient to induce the same repositioning effect exerted by the full-length protein. Nucleosome repositioning occurs even when the catalytic activity of the RNA polymerase II is impaired, a fact suggesting that the Adr1 activation domain mediates the recruitment of some factor in order to correctly preset the relevant sequences for the subsequent transcription steps (Figure 3).

12. A Possible Function for Positional Multiplicity

We have described the structure- and the energy-related properties of nucleosome – DNA complexes that make the multiplicity of positioning an intrinsic and expected property. We have also reported numerous instances of this multiplicity both in vitro and, notably, in vivo. This property may be considered as an informational ambiguity to be amended (as higher eukaryotes have partly achieved by evolving additional structural control elements) or as an opportunity to be exploited in regulatory processes.

In higher eukaryotes, a fifth histone molecule binds from outside, stabilizes the localized ncps, and promotes higher-order chromatin organization.[135–141] In lower eukaryotes, which lack this stabilization mechanism, chromatin fibers do not condense in the same way for mitosis as in higher eukaryotes and have a larger freedom in folding/unfolding, dissociation/reassembly, and monodimensional sliding processes. The absence of the stabilizing histone and the multiplicity of alternative positions are possibly two aspects of the same phenomenon.

Independently of the differences in the organization between higher and lower eukaryotes, the basic principle that the structural and dynamic properties of chromatin affect DNA accessibility applies to both systems. As described above, multiplicity of nucleosome positions, sliding, and activation-related chromatin fluidity have been observed both in lower and higher eukaryotes.

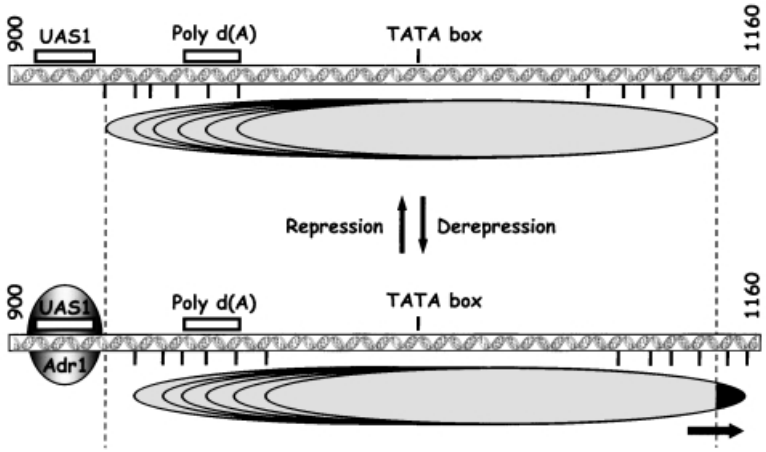

Figure 2. *The sliding of the ADH2 nucleosome −1 upon derepression. The model is based on experimental analyses reported in refs. [77] and [142]. Top line: the position of the components of the nucleosome −1 family relative to the UAS1 and TATA sequence in the repressed state. Lower line: upon binding of the regulatory protein Adr1 on the UAS1 sequence, the nucleosome slides downstream for an extent corresponding to one DNA turn (black tip) towards the RNA initiation site, thus changing its position relative to the TATA sequence. The cartoon shown in the frontispiece (p. 1172; kindly prepared by Marco Colasanti) is the three-dimensional representation of this model.*

Considering a) that informational ambiguity is an intrinsic property of a recognition mechanism not based on sequence specificity and b) that the multiple alternative rotationally phased nucleosome positions are quasi-isoenergetic, it is tempting to speculate that this informational ambiguity is used for regulatory purposes. At the same time it is difficult to conceive that an intrinsic systemic property of the genetic material has not found a use in regulatory functions through evolution.

Which would then be the possible advantage provided by informational ambiguity in a process that, like transcription, is best carried out in the quantitatively and kinetically most possibly controlled way? With the transcription machinery being highly complex, most of its components are present in single copy, both for the DNA and the protein elements, uniquely located, and kinetically precisely geared. Such mechanistic precision is necessary to ensure the correct and controlled performance of the transcription process. The multiplicity of nucleosome positions is a marked exception implying that each individual

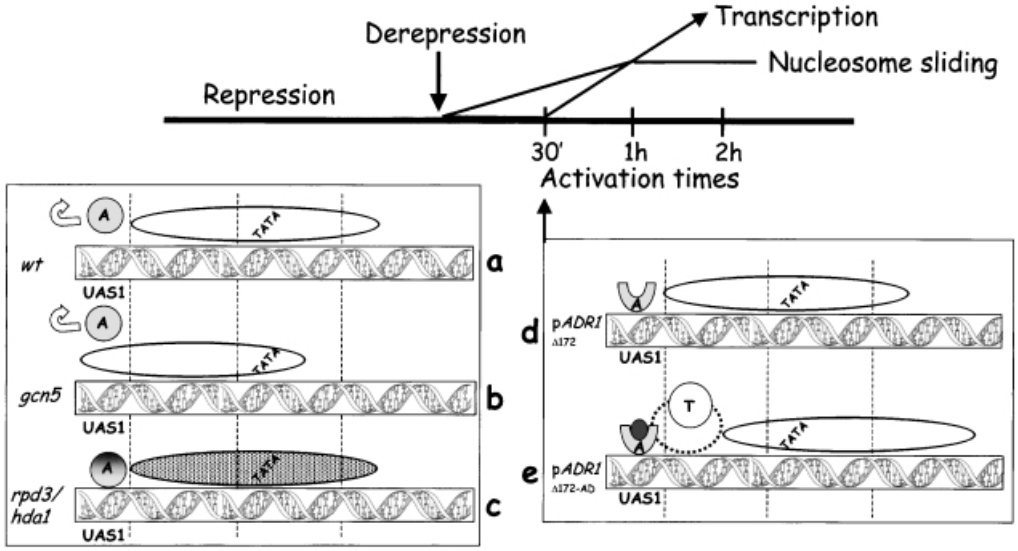

Figure 3. *Three different positions of the nucleosome occupying the ADH2 promoter. In the repressed condition nucleosome −1 (oval) occupies the position indicated in (a) and the regulatory protein Adr1 (circle 'A') does not bind to its cognate UAS1 site.*[77, 142] *In conditions of hypoacetylation (as in the* gcn5 *mutant) nucleosome −1 is located in a more upstream position, partially covering the UAS1 sequence (as depicted in (b); unpublished results). In hyperacetylation (as in the* rpd3/hda1 *double mutant) Adr1 binds and the nucleosome changes its conformation (stippled oval; c).*[77, 142] *Upon derepression, the nucleosome slides downstream only if the Adr1 binding domain (gray crescent) is complemented with its activation domain (dark gray sphere; as depicted in (e)). In these conditions TBP (circle 'T') binds and transcription takes place. The relative dynamics of transcription and sliding are reported at the top. In the absence of the Adr1 activation domain no sliding, no TBP binding, and no transcription occur. d) Data from ref. [142]. The alternative multiple positions of the components of this nucleosomal family (depicted in Figure 1) are not indicated for simplicity.*

cell may locate a functionally relevant nucleosome (that is, a regulatory nucleosome encompassing the TATA element) in one of the several possible different positions.

Given that the precise architecture of the transcription complex (and the ensuing onset of RNA polymerization) depends on the availability of the specific promoter DNA sequences involved, and that this in turn depends on the local interaction with a singly located nucleosomal particle, the distribution of the positions of the nucleosomes particles on the promoters is the starting point of the whole process. Being dictated by the very rules governing the DNA–nucleosome binary complex interaction, nucleosome multiple localizations are independent of the genetic background and are epigenetic in their nature.

Taking these considerations together, the possibility emerges that the genetic apparatus has transformed the handicap of informational ambiguity into a source of *variability*. Regulating the percentage of the initial, potentially productive positions and the kinetics of their sliding, yet another level of regulation can be obtained. The recent reports[77, 87, 134, 135] of in vivo regulated nucleosome dynamics open up the possibility of focusing on these fascinating phenomena.

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