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The Nuclear Transport Machinery Recognizes Nucleoplasmin–Histone Complexes

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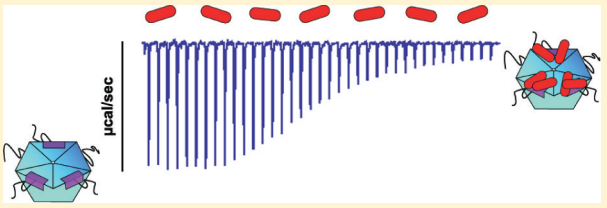
ABSTRACT: The nuclear transport of the chromatin remodeling protein nucleoplasmin and chromatin building histones is mediated by importins. Nucleoplasmin (NP) contains a classical bipartite nuclear localization signal (NLS) that is recognized by the importin α/β heterodimer, while histones present multiple NLS-like motifs that are recognized by importin β family members for nuclear targeting. To explore the possibility of a cotransport of histones and their chaperone NP to the nucleus, we have analyzed the assembly of complexes of NP/histones with importins by means of fluorescence anisotropy, centrifugation in sucrose gradients, and isothermal titration calorimetry. Data show that importin α ΔIBB (a truncated form of importin α lacking the autoinhibitory N-terminal domain) and histones (linker, H5, and nucleosomal core, H2AH2B) can simultaneously bind to NP. Analysis of the binding energetics reveals an enthalpy-driven formation of high affinity ternary, NP/Δα/H5 and NP/Δα/H2AH2B, complexes. We find that different amount of importin α molecules can be loaded on NP/histone complexes dependent on the histone type, linker or core, and the amount of bound histones. We further demonstrate that NP/H5 complexes can also incorporate importin α/β, thus forming quaternary NP/histones/α/β complexes that might represent a putative coimport pathway for nuclear import of histones and their chaperone protein NP, enhancing the histone import efficiency.



Nuclear import of the histone chaperone nucleoplasmin (NP) and nucleosomal core and linker histones is a necessary process for chromatin assembly and remodeling.[1] Both NP and histones are targeted to the nucleus by importins of the karyopherin family (Kaps), which act as nuclear transport receptors for many cargo proteins.[2]

Nucleoplasmin is one of the factors that modulate the chromatin condensation state, which in turn affects DNA accessibility and functions. It is responsible for the rapid decondensation of sperm chromatin upon fertilization, removing sperm specific basic proteins and replacing them with the somatic type histones.[3,4] This supply of nucleosomal H2AH2B histones enables the assembly of nucleosomes. NP is homopentameric, the N-terminal core domain being responsible for its oligomerization.[5] The flexible C-terminal tail of each monomer contains two short stretches of basic residues forming a nuclear localization sequence (NLS), which belongs to the "bipartite" class.[6]

The recognition of NLS by importin α, which forms a heterodimer with importin β, is one of the main steps of the "classical" nuclear import process.[7,8] The complex NLS protein/importin α/importin β is targeted to the nuclear pore complex and traverses it thanks to the interaction between importin β and certain nucleoporins.[2,9]

The core and linker histones participate in the two levels of chromatin structural organization and are highly abundant substrates that must be transported into the nucleus for the formation of new nucleosomes.[10] The different histone types are imported to the nucleus by multiple pathways and are recognized by different proteins of the β importin family.[11−16]

Import of core histones has been proposed to depend on several import sequence motifs that could be dependent on its globular domain structure.[17,18] The import of H3H4 core histones, which are first loaded onto DNA, is mediated in yeast by at least two karyopherins Kap123p and Kap121p.[13,19] H2AH2B histones, whose import depends on their dimerization,[14] have been shown to associate with Kap114p karyopherin, the interactions being specifically promoted by the yeast nucelosome assembly protein (Nap1),[18] which mediates formation of Kap114p−Nap1p−H2AH2B co-complex. Other Kaps may be also involved in the H2AH2B nuclear import. Linker histone, H1, is targeted to the nucleus *in vivo* by β/7 importin heterodimer.[11,14,15,20] Additionally, there are evidences pointing to redundancy in the histones transport mechanisms.[12,14]

On the other hand, the fact that at least some histones can bind importin α (and α/β) with high affinity (refs 11, 20, and 21 and our own results (see below)) and that fluorescein-labeled H2B can be transported by importin α/β[21] suggests that importin α/β could be yet another relevant import pathway for core and linker histones.

In previous studies we characterized the binding interactions of NP with nucleosomal and linker type histones[22] and with

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importin α and importin α/β heterodimer[8] by isothermal titration calorimetry (ITC). We also presented low-resolution solution structures of NP/H5 and NP/H2AH2B complexes and NP/importin α and NP/importin α/β complexes, constructed by means of small-angle X-ray scattering (SAXS).[8,22] Both techniques, ITC and SAXS, revealed that NP pentamer can accommodate five ligands, either histones (H5 or H2AH2B) or importins (α or α/β heterodimer). The two histone types bind to NP with significantly different affinities and importantly in a negative-cooperativity mode, most pronounced for the linker histone type. No cooperativity was found in the association of NP with importins. We have also shown that both domains of NP are involved in the binding of H5 and H2AH2B histones,[22] the tail domain being particularly essential in the binding to H5 molecules, whereas only the tail, namely NLS, is involved in binding to importin α.[8] The different involvement of both domains of NP in the binding of histone and importin ligands determines a more compact shape of NP/histone complexes than that of NP/importin, characterized by an open structure and inherent flexibility.

We have characterized herein the interaction of importin α ΔIBB ($\Delta\alpha$, a truncated nonautoinhibited form) with NP loaded with histones, either H5 or H2AH2B. To analyze the assembly of these complexes and to assess how the presence of histones bound to NP can influence recognition by importin α, we also characterized the interaction of importin α with both types of histones. We found that NP, linker and core histones, and importin associate and form co-complexes. All binding events are enthalpically driven and characterized by nanomolar affinity of importin to NP/histone complexes. We have obtained further evidence that physiologically more relevant, quaternary NP/histones/α/β complexes may form.

If importin α/β can bind to NP/histones complexes, both nuclear proteins could be co-transported, an energy-saving possibility that has been proposed for other histone chaperones such as Nap1p,[18,23] NAP-2,[24] and NASP.[25] Indeed, the role of histone chaperones has been proposed to go beyond chromatin remodeling events, i.e., possibly shielding the highly charged histones and escorting them all the way from the cytoplasm where they are synthesized to the nucleus.[1,26]

EXPERIMENTAL PROCEDURES

Proteins Production. NP (*Xenopus* NPM2 sequence), both wild-type and mutant with substitutions of Lys residues 167 and 168 for Asn (NP_{mut}), generated by PCR-based mutagenesis, were overexpressed in *E. coli* and purified as previously described for wild-type NP.[27] Importin α (*Xenopus laevis* α1 sequence) and importin β (human) clones in pQE60 and pQE70, respectively, were kind gifts from Dr. Görlich laboratory.[28,29] Truncated importin α (66−517 fragment, referred to as "importin α ΔIBB" or "$\Delta\alpha$" throughout the text) was subcloned in pET30a (Novagen).[8] Overexpression in *E. coli* and purification of importins were done as previously described.[8] Histones H2AH2B and H5 were purified from chicken erythrocytes following a protocol described elsewhere.[22] Protein concentration was determined with the BCA colorimetric assay (Pierce) and aminoacid analysis.

Sedimentation in Sucrose Gradients. Mixtures of NP (3 μM) and different amounts of ligands (histones and importins) were prepared adding first the histones to NP, incubating 10 min on ice, then adding importin, and incubating for an additional 10 min. Samples were loaded on top of 5−25% sucrose gradients in buffer 25 mM Tris/HCl pH 7.4, 100 mM NaCl, 2 mM DTT and ultracentrifuged at 100000*g* (in rotor SW60Ti) for 21 h at 4 °C. Fractions were collected from the bottom of the gradient inserting a pipet and aspirating 0.3 mL aliquots and were subsequently analyzed by A_{230} and SDS-PAGE.

Fluorescence Anisotropy. For anisotropy binding experiments, NP was labeled with 5′ (and 6′)-carboxyfluorescein succinimidyl ester (Molecular Probes, Invitrogen) that attaches specifically to the protein N-terminus, basically following a described procedure.[30] NP (both labeled and not labeled) was separated from the free fluorescent probe by chromatography in a Superdex 200 HR column (GE Healthcare). Labeling yield was estimated to be 15% of the NP monomers. Titrations were made adding the different ligands onto 3 μM fluorescein labeled NP in buffer 25 mM Tris/HCl pH 7.4, 100 mM NaCl, 2 mM DTT, 10% glycerol. Steady-state fluorescence emission anisotropy parameter was measured at 20 °C using λ_{exc} 492 nm and λ_{em} 520 nm and slit widths of 4 nm.

Isothermal Titration Calorimetry. ITC measurements were performed in a VP-ITC microcalorimeter (MicroCal, Inc., Northampton, MA). Proteins were extensively dialyzed against buffer 25 mM Tris pH 7.5, 100 mM NaCl, 2 mM TCEP, 10% glycerol before titration. Successive injections (8 μL) of importin α ΔIBB (30−70 μM) were made into the calorimetric cell containing 1−1.2 μM NP or NP/histones complexes, with different degree of histone loading, i.e., NP/H5 1:1, 1:3, 1:4 and NP/H2AH2B 1:1, 1:2, and 1:3. For analysis of importin α/histone binding 25−30 μM importin α was injected into 1 μM H5 or H2AH2B in the calorimetric cell. Reverse titrations of 22−30 μM H5 or H2AH2B onto 1.5−3 μM importin α were also performed. Titration experiments (histones/NP and importin α/NP) performed in buffers with different ionization enthalpy (HEPES and PIPES) assert that protonation/deprotonation effects do not contribute to the observed enthalpy of binding. The binding isotherms, ΔH° vs molar ratio, were fitted assuming a model of identical independent binding sites, using MicroCal Origin software. The thermodynamic parameters stoichiometry n, binding constant K_a ($K_d = 1/K_a$), and enthalpy ΔH° are obtained from the fits of isotherms, and the Gibbs free energy and entropy of binding are determined from the expression $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ = -RT \ln K_a$.

RESULTS AND DISCUSSION

Formation of Ternary Complexes NP/Histones/Importin α. We have previously shown that importin α ΔIBB and histones form stable saturated complexes with NP[8,22] and therefore the anisotropy of fluorescein-labeled NP increases when titrated with either importin α ΔIBB or histones, reflecting binding to NP (Figure 1A). To explore the effects of the presence of histones on NP recognition by importin α, we followed the sequential binding of both types of ligands to NP by fluorescence anisotropy. In the presence of partly saturating amounts of histones (NP/H5 or NP/H2AH2B, molar ratio 1:3) the addition of importin causes a further increase in the fluorescence anisotropy of NP (Figure 1A). The same effect was observed in reverse titrations when histones were added to an NP-importin α saturated complex (data not shown). Therefore, loading of NP with histones does not impair association of importin with the NP/histones complex or vice versa, which strongly suggests that ternary complexes do form.

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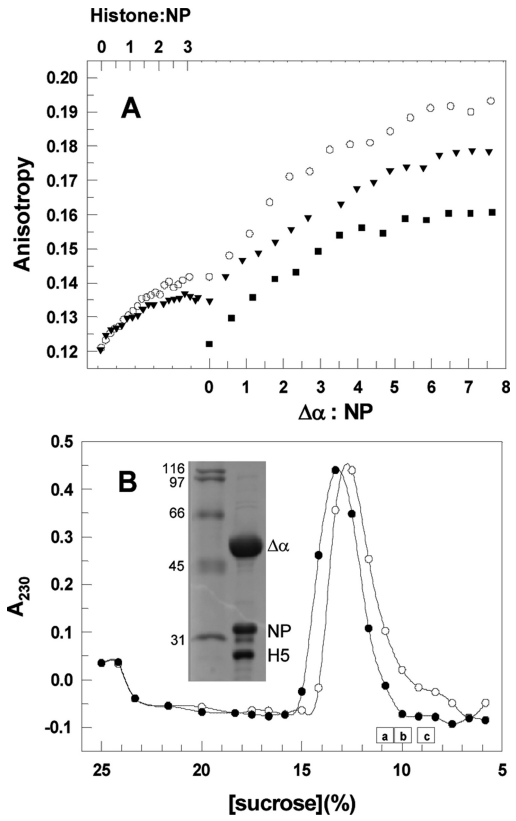

Figure 1. Formation of NP/histones/Δα ternary complexes. (A) Binding of histones and then importin α ΔIBB to NP: fluorescein-labeled NP alone (■) or titrated with H5 (▼) or H2AH2B (○) up to a molar ratio NP:histones of 1:3 and then with Δα. (B) Complex formation detected by ultracentrifugation in sucrose gradient: Sedimentation profiles of a mixture of NP, H5, and Δα (full symbols) or NP_{mut}, H5, and Δα (empty symbols) at 1:2:5 molar ratio, as determined by UV absorbance of the different gradient fractions. The approximate positions of the control mixtures run in the same gradient are indicated with boxes: (a) NP/Δα; (b) NP/H5; (c) H5/Δα. Inset: SDS-PAGE of a fraction corresponding to the main peak of the wild-type complex.

To confirm the formation of NP/histones/importin α complexes, we also examined the separation of the different protein species and complexes in sucrose gradients. In a mixture of NP, H5, and Δα (molar ratio 1:2:5) the three proteins co-sediment as one discrete species (Figure 1B), as checked by gel electrophoresis of the main peak fractions (inset), reflecting that a ternary complex is formed. Similarly, the sedimentation profile of a mixture of NP, H2AH2B, and Δα (data not shown) also indicates the formation of a ternary complex. These results suggest that NP/histones/importin form discrete assemblies of defined composition.

Energetics of NP/Histones/Importin α Association (Isothermal Titration Calorimetry). We analyzed the binding of importin α ΔIBB to preformed complexes NP/H5 and NP/H2AH2B by ITC. Calorimetric titration curves of importin binding to NP loaded with different amounts of histones are compared to those of NP alone in Figure 2. Binding of Δα to the complex NP/H5 (1:1) is indistinguishable from that to NP alone (Table 1). However, binding to NP loaded with three and four H5 molecules proceeds with higher stoichiometries than in the absence of H5; i.e., six and eight importins bind per NP pentamer, respectively (Figure 2A and Table 1). This

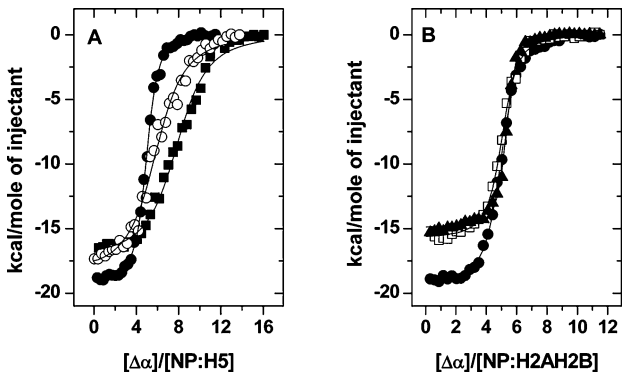

Figure 2. Binding of importin α to NP/histone complexes. Calorimetric titration of NP/histone complexes with importin α ΔIBB and fits of the experimental data to independent binding sites model (solid lines). (A) NP/H5 (molar ratios 1:3 (○); 1:4 (■)); (B) NP/H2AH2B (molar ratio 1:2 (□); 1:3 (▲)). Binding isotherm for Δα/NP (●) is shown for comparison.

indicates that at least part of the importin molecules should bind via the histone.

Different binding behavior is observed when NP is loaded with the core histone heterodimer H2AH2B. The complexes of NP/H2AH2B saturate with five molecules of importin α per NP pentamer, irrespective of the amount of H2AH2B in the complex (binding isotherms of Δα to NP/H2AH2B at molar ratios 1:2 and 1:3 are shown in Figure 2B). These data indicate a stoichiometric composition (Table 1) of the ternary complexes.

These "ternary titrations" are adequately fit using a model of independent binding sites but do not allow to assess whether importin binds to NP and/or to histones. However, NP partially loaded with histones offers two types of binding sites for importin - the interaction sites on histone molecules and the NLS of NP flexible tails. According to this, the titrations were also analyzed with a model considering two types of binding sites, the obtained parameters being very similar for both sites (data not shown). Probably these two binding sites are not distinguishable, as expected, since they have rather similar binding properties (Table 1), apparent affinity, and favorable binding enthalpy. Therefore, analysis of the ITC data shows that all binding events can be described applying a simple independent binding site model.

To clarify the assembly mode of the ternary NP/histones/importin α complexes, we made use of two approaches: first, we characterized the binding of importin α to histones, H5 and H2AH2B, and second, we studied the assembly of ternary complexes made with a mutant NP where NLS is abolished.

Although binding of importin to free histones in solution might differ from the recognition of histones associated with NP, characterization of binary importin/histones complexes would help to understand the more complicated ternary assemblies. Importin Δα shows exothermic high affinity binding interactions to both H5 and H2AH2B (Figure 3 and Table 1), suggesting specific recognition events. While two molecules of importin α ΔIBB associate with H5, only one binds to H2AH2B. Despite the different stoichiometry, the thermodynamic parameters are quite similar, the apparent binding affinity and the enthalpy are in the order of 9 and 28 nM and −20 and −17 kcal/mol for H2AH2B and H5, respectively (Table 1). This suggests that similar molecular interactions are involved in the complex formation of Δα with the binding motifs of the two histone types.

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Table 1. Thermodynamic Data for Importin Association with NP, NP/H5, and NP/H2AH2B

molar ratio NP:histones	n	K_d (nM)	$\Delta H°$ (kcal/mol)	$-T\Delta S°$ (kcal/mol)	$\Delta G°$ (kcal/mol)
			NP/Δα[a]		
	4.8 ± 0.4	54 ± 5.5	−18.6 ± 1.8	8.8 ± 1.6	−9.8 ± 0.2
			H5/Δα		
	2.2 ± 0.3	28 ± 9	−16.9 ± 2	6.8 ± 1.9	−10.1 ± 0.2
			NP/H5/Δα		
1:1	4.7 ± 0.2	42 ± 10	−18.1 ± 0.7	8.2 ± 0.5	−9.9 ± 0.1
1:3	6.3 ± 0.2	128 ± 89	−17.0 ± 1.2	7.8 ± 0.96	−9.2 ± 0.3
1:4	7.9 ± 0.1	266 ± 32	−17.2 ± 0.3	8.4 ± 0.2	−8.8 ± 0.1
			NP_{mut}/H5/Δα		
1:2	4.2 ± 0.2	199 ± 82	−13.3 ± 0.1	4.4 ± 1.1	−8.9 ± 1.2
1:3	5.9 ± 0.2	952 ± 18	−19.2 ± 0.9	11.1 ± 0.9	−8.1 ± 0.2
			H2H2B/Δα		
	1.1 ± 0.2	9.3 ± 6.7	−20.7 ± 3.6	10.9 ± 3	−9.8 ± 0.8
			NP/H2H2B/Δα		
1:1	4.9 ± 0.3	55 ± 7	−16.4 ± 0.4	6.7 ± 0.12	−9.7 ± 0.2
1:2	5.1 ± 0.5	29 ± 7	−15.8 ± 0.2	5.7 ± 0.3	−10.1 ± 0.1
1:3	4.8 ± 0.2	16 ± 4	−15.2 ± 0.2	4.7 ± 0.1	−10.5 ± 0.1
			NP_{mut}/H2AH2B/Δα		
1:2	1.8 ± 0.1	628 ± 106	−14.3 ± 0.1	5.9 ± 0.1	−8.5 ± 0.1
1:3	2.8 ± 0.2	719 ± 78	−18.6 ± 0.7	10.4 ± 0.4	−8.2 ± 0.1

[a]Data for NP/Δα are shown for comparison (ref 8).

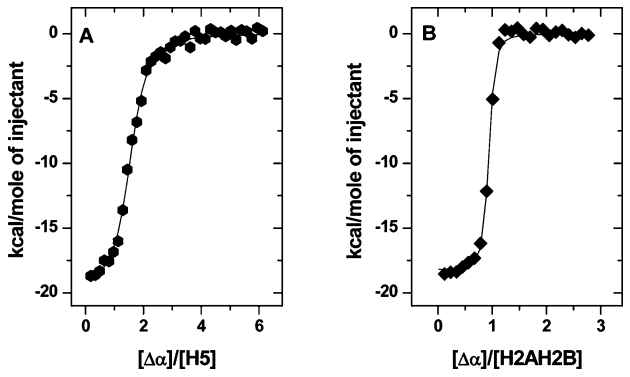

Figure 3. Importin/histones binding isotherms: (A) importin/H5 and (B) importin/H2AH2B.

As a complementary approach we further generated a NP mutant, where Lys residues 167 and 168 of the second cluster of the NLS were substituted by Asn. It has been described that these mutations abolish NP import.[6] We checked by gel filtration and ITC that, as expected from its transport properties, the NP mutant does not bind importin α (data not shown). However, the complex NP_{mut}/H5 binds as many importin α molecules as the wild-type NP/H5 dependent on the number of bound histones (Table 1). Although this result may suggest that importin α binds preferably to H5 in the wild-type ternary complex, one should note that importin binding to NP/H5 (1:1) complex shows a stoichiometry of 5, which suggests that importin α can also occupy NP NLS site(s). Furthermore, we have observed in sedimentation experiments that NP_{mut} loaded with two molecules of H5 saturates with Δα forming a smaller complex as compared to wild-type NP (Figure 1B), in agreement with the notion that importin can bind to NP pentamer subunits as well. Therefore, at low histone load (1:1 and 1:2) importin α seems to bind to both types of binding sites, while at high histone load (e.g., 1:3 and 1:4) the possibility remains that importin binds only to H5. In these cases seemingly NP-bound H5 would accommodate as many importin α as free H5 (e.g., two molecules per H5).

These scenarios are illustrated in Figure 4, showing a repertoire of alternative configurations with Δα bound to histones and/or to NLSs of NP, depending on the histone load. On the basis of our previous results highlighting the contribution of NP tail domain in histone binding, especially in the case of H5,[22] we speculate that at low histone load NLS in NP monomers not involved in the histone binding might participate in the interaction with importin. On the other hand, at high histone load, the effect that the H5 negative cooperative binding possibly exert on NP[22] could account for the apparent preference that Δα displays for H5 biding sites over NLS NP tails, as deduced from the binding data with NP_{mut}. Hence, we hypothesize that the left most models for NP/H5 1:3 and 1:4 in Figure 4 are the most plausible configurations.

On the other hand, the complexes NP_{mut}/H2AH2B bind exactly as many importin molecules as the number of H2AH2B loaded on NP_{mut}, ca. 2 and 3 for NP_{mut}/H2AH2B (1:2) and (1:3) complexes, respectively (Figure 5 and Table 1). Considering the lower negative cooperative binding of H2AH2B to NP,[22] we suppose that of the five importins bound to the wild-type complexes, one binds to each H2AH2B dimer, the remaining ones occupying their canonical sites on NP (Figure 4, the left most configuration in the last raw of the scheme).

The apparent affinity of importin for NP/H5 complexes decreases at high histone load, while a minor increase in the affinity is found for NP/H2AH2B assembly, compared to NP alone (Table 1). This different behavior dependent on the type of histone bound might be due to significant differences in their binding interactions to NP: (i) stronger implication of NP tail domains in the association with H5, (ii) much lower binding affinity for H2AH2B, and (iii) more pronounced negative cooperativity for the linker histone, originated from allosteric conformational changes in NP upon histone binding.[22] Furthemore, since the analysis of the experimental data with

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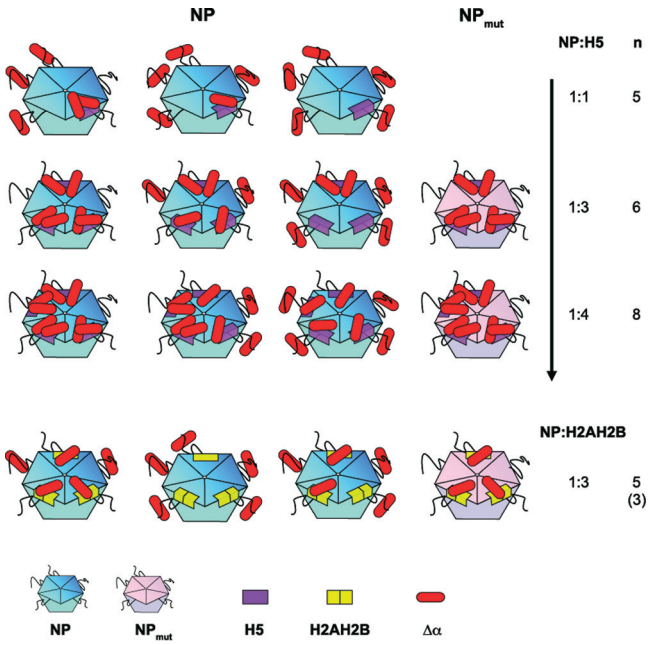

Figure 4. Schematic representation of nucleoplasmin/histones/importin assemblies. Alternative configurations of NP/histones/$\Delta\alpha$ and NP$_{mut}$/histones/$\Delta\alpha$ complexes for NP loaded with linker H5 (1:1, 1:3, and 1:4 NP/H5) and nucleosomal core (1:3 NP:H2AH2B) histones are presented. The NP/histones ratios and the corresponding stoichiometries (*n*, the number of $\Delta\alpha$ per NP) of the ternary assemblies determined by ITC, are given on the right-hand side of the scheme. The left most configurations are the most probable ones considering the binding data with NP$_{mut}$. Note the difference in the binding stoichiometry of the ternary assemblies with H5 and H2AH2B. The stoichiometry of NP$_{mut}$/H2AH2B/$\Delta\alpha$ is shown in parentheses. Neither the spatial geometry of the assemblies nor the sizes of the binding partners are considered in the models. The assembly elements (nucleoplasmin, wild type, and mutant, pentamers; linker histone H5; nucleosomal histone H2AH2B heterodimer and importin $\Delta\alpha$) are shown at the bottom of the scheme.

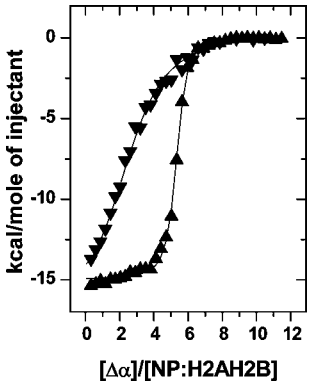

Figure 5. Comparison of importin α binding to NP$_{mut}$ and NP loaded with H2AH2B. Importin α ΔIBB binding to NP$_{mut}$/H2AH2B (▼) and to NP/H2AH2B (▲); NP(NP$_{mut}$)/H2AH2B molar ratio 1:3.

a model of independent binding sites yields an average of both binding constants, the lower K_d of $\Delta\alpha$ for NP/H2AH2B (Table 1) might result from the mixed binding mode, i.e., NLS of NP and H2AH2B binding motifs, which seems the most probable one for H2AH2B. On the other hand, a reduced affinity of importin to histones in the complexes with NP$_{mut}$ is observed (K_d of hundreds of nM, Table 1). Although point mutations in NLS of NP are not expected to alter histone binding ability of NP, which is indeed checked by anisotropy (data not shown), we cannot exclude that NLS abolishment might indirectly affect the histone binding, and consequently, recognition by importin.

Altogether, these results indicate that both types of ligands (importin α and histones, either H5 or H2AH2B) can bind simultaneously to NP, forming stable ternary complexes in which no ligand is displaced. Furthermore, the interaction between importin α and histones can occur, at least partly, on the complexes, allowing them particularly in the case of H5 to accommodate additional importin molecules than NP itself.

Formation of Quaternary Complexes NP/Histones/Importin α/Importin β. In case importin α can mediate co-transport of NP and histones to the nucleus, the assembled complexes should be able to bind importin β as well. To explore this possibility, we investigated whether quaternary complexes NP/histones/importin α/β can form. Of the two types of complexes, we focused on NP/H5/α/β given the rather complicated assemblies, with the heterodimer H2AH2B including five different proteins. Upon titration of the NP/H5 complex with the heterodimer, formed by full length importin α and importin β, we observed a stronger increase in the fluorescence anisotropy of labeled NP than that obtained when the complex is titrated with importin α ΔIBB (Figure 6A). This result is compatible with saturable formation of complexes containing the four types of proteins.

By ultracentrifugation in sucrose gradients, we could confirm, for H5, that complexes formed by the four proteins co-sediment, as checked by gel electrophoresis (Figure 6B). As confirmed by the control experiments, this putative quaternary complex behaves as a particle of a size bigger than any of the possible ternary mixtures; in particular, it is clearly larger than the corresponding NP/H5/α ternary complex.

It has been demonstrated[21] and we have also observed (data not shown) that histones can bind importin β, as it is expected since their import can be mediated by importin β itself or by transport receptors of the karyopherin β family. Additionally, the α/β heterodimer is also able to bind both types of histones (data not shown).[21] Therefore, so many different possible interactions exist that in practice the thermodynamical characterization of such complicated assemblies is precluded.

Interestingly, some histone chaperones such as Nap1p,[18] NAP2,[24] and NASP[25] have been reported to assist the import of histones to the nucleus. In the case of Nap1p it was shown that mutant yeast cells lacking this gene displayed a diminished accumulation of histone-NLS-GFP reporters in their nuclei, and remarkably, it has been recently shown that Nap1 forms a complex with the yeast histone heterodimer Htz1/H2B together with karyopherin Kap114.[23] In further support of a role of histone chaperones in the import of their substrates, Alekseev et al. suggested that transport of testis specific H1 is promoted by NASP.[25] Still, experimental evidence for co-transport of other protein pairs is otherwise scarce, and it is complicated to directly observe simultaneous import of two or more proteins.

The results presented in this study open an attractive possibility of a co-transport of histones and NP. It remains to be seen whether in cells, NP, and histones are indeed co-imported to the nucleus. This mechanism would be energetically

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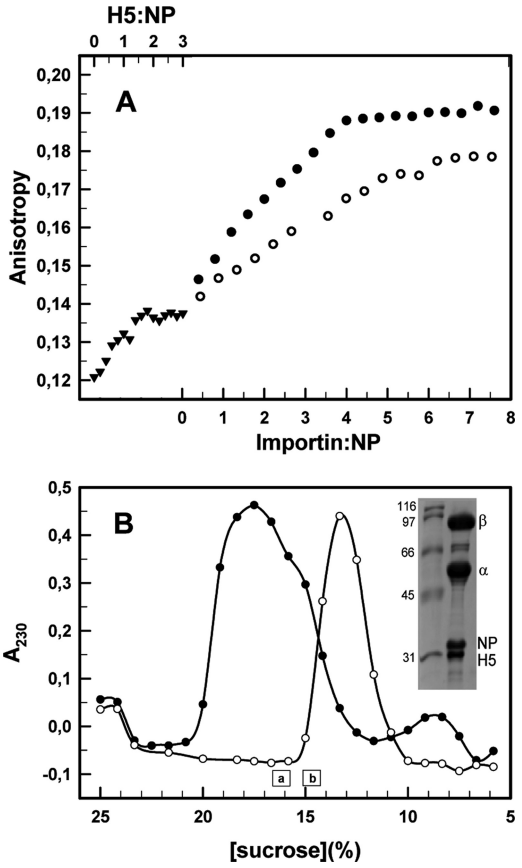

Figure 6. Quaternary complexes. (A) Anisotropy of fluorescently labeled NP titrated first with H5 (▼) and then with Δα (○) or the heterodimer α/β (●). (B) Sedimentation profiles of mixtures of NP, H5, importin α, and importin β (1:2:5:5 molar ratio) (full symbols) and NP, H5 and Δα at 1:2:5 molar ratio (empty symbols). The approximate positions of the control mixtures run in the same gradient are indicated with boxes: (a) NP/α/β, (b) H5/α/β. Inset: SDS-PAGE of a sample corresponding to the main peak of the quaternary complex.

advantageous and would guarantee the escort of histones, as it has been proposed.[1,31] The complexes we have described would support a co-transport pathway mediated by importin α/β.

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Notes

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ABBREVIATIONS

NP, nucleoplasmin; NP_{mut}, NP mutant with Lys residues 167 and 168 substituted for Asn; NLS, nuclear localization sequence; Δα, importin α ΔIBB (truncated importin α lacking the autoinhibitory N-terminal domain); ITC, isothermal titration calorimetry.

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