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Review Article

Nuclear import of histones

Natalia Elisa Bernardes and ⓘ **Yuh Min Chook**

Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, TX, U.S.A.

Correspondence: Yuh Min Chook (yuhmin.chook@utsouthwestern.edu)

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The transport of histones from the cytoplasm to the nucleus of the cell, through the nuclear membrane, is a cellular process that regulates the supply of new histones in the nucleus and is key for DNA replication and transcription. Nuclear import of histones is mediated by proteins of the karyopherin family of nuclear transport receptors. Karyopherins recognize their cargos through linear motifs known as nuclear localization/ export sequences or through folded domains in the cargos. Karyopherins interact with nucleoporins, proteins that form the nuclear pore complex, to promote the translocation of their cargos into the nucleus. When binding to histones, karyopherins not only function as nuclear import receptors but also as chaperones, protecting histones from non-specific interactions in the cytoplasm, in the nuclear pore and possibly in the nucleus. Studies have also suggested that karyopherins might participate in histones deposition into nucleosomes. In this review we describe structural and biochemical studies from the last two decades on how karyopherins recognize and transport the core histone proteins H3, H4, H2A and H2B and the linker histone H1 from the cytoplasm to the nucleus, which karyopherin is the major nuclear import receptor for each of these histones, the oligomeric state of histones during nuclear import and the roles of post-translational modifications, histone-chaperones and RanGTP in regulating these nuclear import pathways.

Introduction

The compartmentalization of the cell into nucleus and cytoplasm is a major feature of all eukaryotic cells. The nuclear envelope separates DNA in the nucleus from protein synthesis process, which occurs in the cytoplasm. The separation then necessitates controlled and efficient passage of thousands of different macromolecules across the nuclear envelope through the nuclear pore complex (NPC). For most macromolecules, this process occurs in an energy-dependent manner, mediated by proteins of the Karyopherin-β (Kap) family. Kaps that transport proteins into the nucleus are also known as importins (Table 1). Several importins bind many of their protein cargos by recognizing linear sequence motifs known as nuclear localization signals (NLSs) that are found in their cargos. A variety of linear NLS motifs can be found in different nuclear import cargo proteins. Several classes of NLSs have been defined at this time, each recognized by a cognate Kap (Table 1). For example, the Importin-α/Importin-β (Impα/Impβ) heterodimer recognizes classical-NLSs (cNLSs) that are rich in lysines and arginines, Karyopherin-β2 (Kapβ2; also known as Transportin-1 or TNPO1) binds to the longer and completely distinct proline-tyrosine- or PY-NLSs and Importin-5 (Imp5; also known as Karyopherin-β3 or Importin-β3) recognizes the short isoleucine-lysine- or IK-NLSs [1–3]. Other Kaps, such as Importin-4 (Imp4), bind diverse sequences in their cargo and the consensus motifs or rules that define the NLS that represent these sequences have not been identified yet [4]. Some importins such as Imp4, Imp5, Importin-7 (Imp7), Importin-9 (Imp9), Transportin-SR and Importin-13 (Imp13) also bind folded domains rather than, or in addition to, linear NLS elements in their cargos [5–7]. Kaps also bind phenylalanine-glycine or FG repeat nucleoporins to translocate cargos across the NPC (review [8,9]). Once in the nucleus, the small GTPase Ran regulates cargo release. RanGTP is highly concentrated in the nucleus, while RanGDP is highly concentrated in the cytoplasm. RanGTP binds with high affinity to importins in a competitive manner with the binding of cargos, causing cargos to be released [8].

Table 1. Nuclear import receptors in the Karyopherin family of proteins

Human importins (abbreviations/alternative names)	*S. cerevisiae* importins (alternative names)	NLS
Importin-α/Importin-β (Impα/Impβ/Kapα/Kapβ1)	Kap60/Kap95	Classical-NLS
Karyopherin-β2 (Kapβ2/Transportin-1)	Kap104	PY-NLS
Importin-4 (Imp4/RanBP4)	Kap123	ND#
Importin-5 (Imp5/Kapβ3, Impβ3, RanBP5)	Kap121 (Pse1)	IK-NLS
Importin-7 (Imp7/RanBP7)	Kap119 (Nmd5)	ND
Importin-8 (Imp8/RanBP8)	Kap108 (Sxm1)	ND
Importin-9 (Imp9/RanBP9)	Kap114 (Kapβ)	ND
Importin-11 (Imp11/RanBP11)	Kap120	ND
Transportin-3 (TNPO3/Trn-SR, Imp12)	Kap122 (Pdr6)	RS-NLS
Importin-13* (Imp13/Kap13, RanBP13)		ND
Exportin-4* (Exp4)		ND

#ND: not determined; * bidirectional transport receptor.

DNA is organized into condensed and non-condensed states in different stages of the cell cycle. Chromatin is the condensed form of DNA and its basic unit, the nucleosome core, is formed by ∼147 bp DNA wrapping around a histone octamer, which consists of two histone H3–H4 dimers in the center that are flanked by two histone H2A–H2B dimers (Figure 1A) [10–12]. The four core histone proteins, H3, H4, H2A and H2B, have low sequence homology but are highly similar in their secondary and tertiary structures [13]. The majority (∼72%) of each of the four histone proteins form the core histone fold domain while most of the remaining portions (∼17–44 residues) comprise the intrinsically disordered N-terminal tails, which contain post-translational modification sites and are important in maintaining nucleosome stability (Figure 1B,C) [10,14]. Histones H2A also contain a ∼30 residue long C-terminal tail.

In addition to the core histones, chromatin also contains linker histones. One H1 linker histone protein binds to a ∼ 10 bp stretch of linker DNA at both the entry and exit of a nucleosome core. The bound H1 histones mediate interactions of adjacent nucleosome cores to form the chromatosome (Figure 1A), which may help the folding into higher order structures of chromatin [15]. H1 linker histones differ structurally from core histones as they contain a tripartite structure formed by a central globular domain, which is distinct from the histone fold, flanked by a short (20–35 residues) N-terminal and a long (∼100 residues) C-terminal tail (Figure 1B,C) [16,17].

Histones are highly basic proteins. During the S phase of the cell cycle, new histones, which are synthesized in the cytoplasm, are actively incorporated into newly replicated DNA in the nucleus. The transport of these newly synthesized histones from the cytoplasm into the nucleus thus becomes one of the major processes in the cell. Although core and linker histones are small proteins, ranging from 11 to 22 kDa as monomers, they do not diffuse into the nucleus but are actively transported by importins across the NPC (Figure 2A–C) [18–20]. Pull-down binding assays with immobilized histones reported in the 1990s and early 2000s showed that core and linker histones bind to several importins for transport into the nucleus, but each histone seems to have its preferred importer [20,21].

In this article, we will review the available knowledge on nuclear import processes for the H2A–H2B dimer, the H3–H4 dimer and the H1 linker histone. We will discuss the preferential importin partner for each histone, how different histone domains interact with importins, the role of post-translational modifications in the nuclear transport of histones H3–H4 and how interactions with histone chaperones and Ran GTP modulates histones recognition by importins and their release in the nucleus.

Nuclear import of core histones H3 and H4
Histones H3 and H4 in the cytoplasm, from synthesis to nuclear import

The transport of core histones H3 and H4 from the cytoplasm to the nucleus has been studied for more than two decades. Early works used nuclear import assays and binding experiments to show that many of the ten
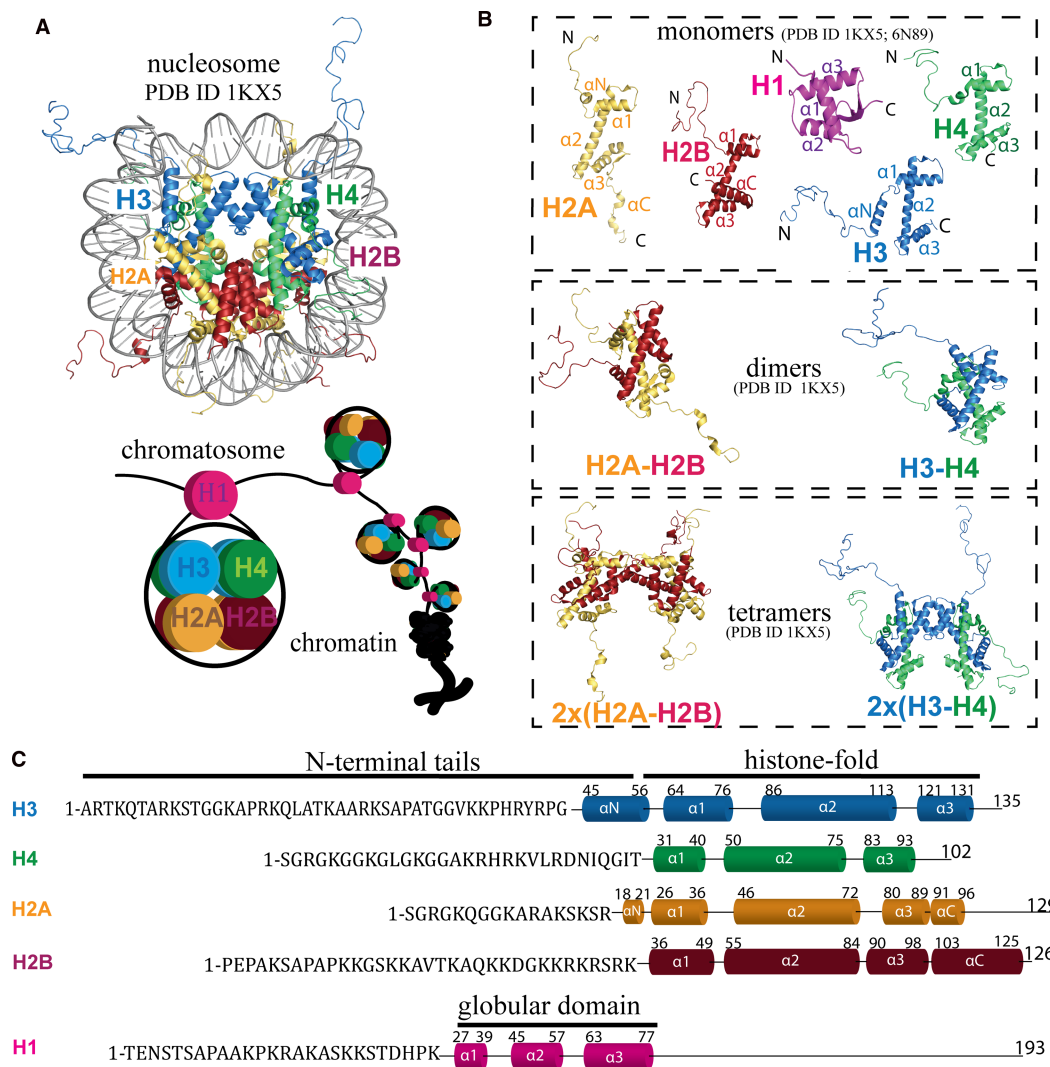
Figure 1. Structures of the nucleosome and histones.

(**A**) Top: a structure of the nucleosome (cartoon, PDB ID: 1KX5) with H3 (blue), H4 (green), H2A (yellow), H2B (red) and DNA (grey). Bottom: schematic of a chromatosome with one linker histone H1 (pink) binding to both DNA-ends that emerges from the nucleosome core, facilitating formation of higher-order chromatin structures. (**B**) Cartoon representation of the structures of core histones and linker histone H1 (PDB ID 1KX5 and 6N89). Individual histone polypeptides are shown in the same colors as in (**A**)). Top panel show single polypeptide chains of the histone proteins, middle panel show the H3–H4 and H2A–H2B heterodimers as found in the nucleosome and the bottom panel show the H3–H4 and H2A–H2B tetramers as found in the nucleosome. (**C**) Domain organization of canonical human core histones H3, H4, H2A, H2B and linker histone H1. Sequences of the N-terminal tails and secondary structures of the histone-fold domains are shown.

importins can bind to the N-terminal tails of histones H3 and H4 [20,27–29]. Histones lacking their N-terminal tails show reductions in nuclear localization, suggesting that they play a role in nuclear import [29]. However, other studies show that the histones can still localize to the nucleus without their N-terminal tails as yeast strains expressing H3 or H4 with no N-terminal tails are still viable despite having some growth defects [30].

Biochemical analysis of cytosolic fractions of HeLa cells expressing Flag-H3.1 revealed the pathway by which histones H3.1 and H4 proceed from translation at the ribosomes to the first step of nuclear import in the cyto-plasm (Figure 2A) [31]. Following translation, H3 and H4 appear to be folded by the heat-shock protein HSC70 and then dimerized into the H3–H4 heterodimer with the assistance of the heat-shock protein HSP90 and the histone-chaperone NASP. The association of NASP with H4 recruits HAT-1 and RbAp46, which
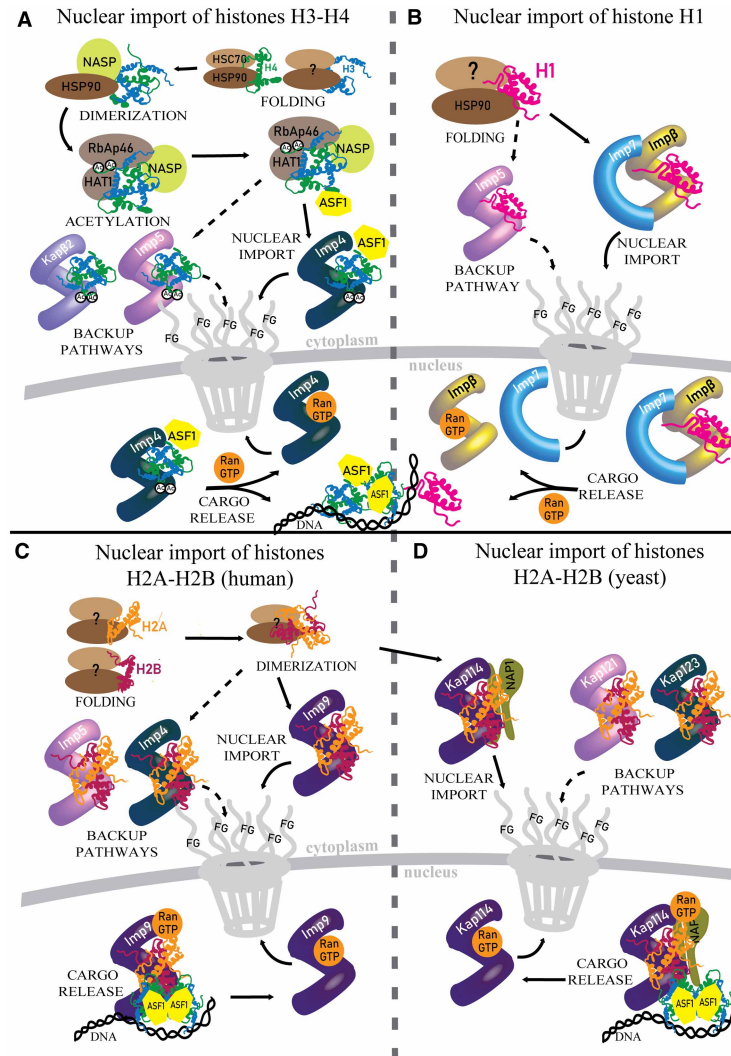
Figure 2. Nuclear import of histones.

(**A**) Schematic showing nuclear import of H3–H4: after translation, H3 (blue) and H4 (green) are folded by HSC70 and HSP90 and then dimerized by NASP and HSP90. NASP then recruits RBAP46/HAT1 to acetylate K5 and K12 residues in the H4 tail. The H3–H4 heterodimer then binds ASF1 and Imp4 for nuclear import, and also bind secondary nuclear importers Imp5 and Kapβ. Imp4•H3–H4•Asf1 is shown translocating through the NPC into the nucleus where RanGTP binds Imp4 to release H3–H4•Asf1 for nucleosome assembly. (**B**) Nuclear import of H1 (pink). Little is known about how H1 is processed from synthesis to nuclear import, but it is well established that the Impβ/Imp7 heterodimer imports H1. After translocation through the NPC, FG repeats of nucleoporins bind Imp7 to release Impβ, and RanGTP binds Impβ to release H1. (**C**) In human cells, H2A–H2B (yellow-red) are imported by Imp9, with Imp4 and Imp5 as secondary importers. In the nucleus, RanGTP binds to Imp9•H2A–H2B without releasing the histones. RanGTP•Imp9•H2A–H2B binds DNA and effectively deposits H2A–H2B into the assembling nucleosome. (**D**) Kap114 (yeast homolog of Imp9) imports H2A–H2B and NAP1. Kap114•H2A–H2B•Nap1 is insensitive to RanGTP, with H2A–H2B and NAP1 released only in the presence of DNA.

promotes histone H4 acetylation at lysine residues K5 and K12 [31,32]. Following acetylation at these two sites, H3–H4 associates with the histone-chaperone ASF1 and Imp4. Imp4 and its *S. cerevisiae* (*Sc*) homolog Kap123 are indeed the most abundant proteins that co-purify with histones H3 and H4. *Sc* strains with Kap123 deletion showed a decrease in the nuclear accumulation of expressed H3 and H4 tails [31,33,34]. Kap121 (*Sc* homolog of Imp5) also co-purified with H4, but with less abundance than Kap123, suggesting that Kap123 is the major nuclear import receptor of histones H3 and H4 and Kap121 is the secondary importer, in yeast.

Table 2. Atomic-resolution structures of Kap-histone complexes

Complex	PDB ID*	References
Kap123–H3(1–28)	5VE8	[22]
Kap123–H4(1–34)	5W0V	[22]
Imp4–H3(1–18)	5XBK	[23]
Kapβ2–H3(1–47)	5J3V	[24]
Imp9–H2A–H2B	6N1Z	[25]
Imp7/Impβ–H1	6N88	[26]

*Source: RCSB Protein Data Bank — http://www.rcsb.org.

Multiple studies have shown that H3 and H4 form H3–H4 dimers in the cytoplasm (Figure 1C) [31,35,36]. Kap123 binds to both H3 and H4, suggesting that the histones are most likely imported into the nucleus as the H3–H4 heterodimer [29]. Furthermore, after being released from importins in the nucleus, two H3–H4 dimers are assembled into one tetramer in the first step of nucleosome assembly [37,38]. Campos *et al.* also showed that the H3–H4 dimer binds both Imp4 and histone-chaperone Asf1 in the cytoplasm, suggesting that the histones, histone-chaperone and importin bind each other to form a nuclear import complex (Figure 2A) [31]. Asf1 is the most conserved histone-chaperone in eukaryotes, which binds the H3–H4 dimer and prevents formation of H3–H4 tetramers. Structures of Asf1 bound to H3–H4 showed Asf1 interacting with the histone fold domain at the same sites used for histone octamer/nucleosome formation [37,38].

Although many studies suggest that H3 and H4 are imported into the nucleus as heterodimers, a study using a tether-and-release system in living cells proposed that the histones are imported as monomers. H3 and H4, fused with EGFP followed by a tail-anchor, were captured on the cytosolic face of the outer mitochondrial membrane after translation. They were released into the cytoplasm only after cleavage of the tail-anchor by a site-specific viral protease, allowing the pathway of H3 and H4, from the cytoplasm to the nucleus and deposition onto DNA, to be followed in real time. This system showed interactions of Imp4 with both tethered H3 and H4 while Impβ interacted with only tethered H4 and not H3 [39], confirming Imp4 as the major importer of histones H3 and H4 but also suggesting Impβ as an alternative importer. When the tether-and-release system was observed by immunofluorescence, histones H3 and H4 were detected alone, without their binding partners, in the regions within the mitochondrial network, raising the hypothesis that histones H3 and H4 may exist as monomers in the cytoplasm. However, the possibility that H3 and H4 rapidly folds in a heterodimer after release from ribosomes in the cytosol is not excluded [39].

Imp4 (or Kap123 in yeast) is clearly the major/primary importer of H3–H4, but other importins can also bind and import H3–H4. Pull-down assays and mass-spectrometry analysis showed Imp5 co-purifying with full length H3 and H4, consistent with the finding that homolog Kap121 is the secondary H3–H4 importer in yeast [29,39]. Impβ was also found to import H3–H4 into the nucleus, but less efficiently than Imp4 [39]. Several other importins also seem to bind the N-terminal tails of H3 and H4 [27]. These findings suggest significant redundancy in the nuclear import of H3–H4 (Figure 2A), which is not surprising given that core histones are essential for cell survival and development. It is unclear, at this time, if interactions between H3–H4 and the secondary/backup importins also involve Asf1.

Interactions of H3 and H4 with importins

The N-terminal tails of both H3 and H4 can bind Impβ, Kapβ2, Imp4, Imp5, Imp7 and Imp9, and all these complexes are dissociated by RanGTP [2,27]. Impβ, Kapβ2 and Imp4 bind to the basic region within residues 11–27 of H3, while Imp5, Imp7, Imp9 and Impα bind to two H3 tail segments, one spanning residues 11–27 and the other residues 35–40. Estimated affinities from pull-down titration data suggest that the H3 tail binds most tightly to Imp5 and Kapβ2 (K_Ds in the tens nM range) while Impα, Impβ, Imp4, Imp7 and Imp9 bind weaker, with K_Ds in the hundreds of nM range.

Several crystal structures are available for the H3 tail binding to importins (Table 2). The crystal structure of the H3 tail (residues 1–28) bound to the major H3–H4 importer Kap123 from *Kluyveromyces lactis* shows H3 residues 12–25 bound in an extended conformation to the C-terminal half of Kap123 (Figure 3A) [22,23]. Two
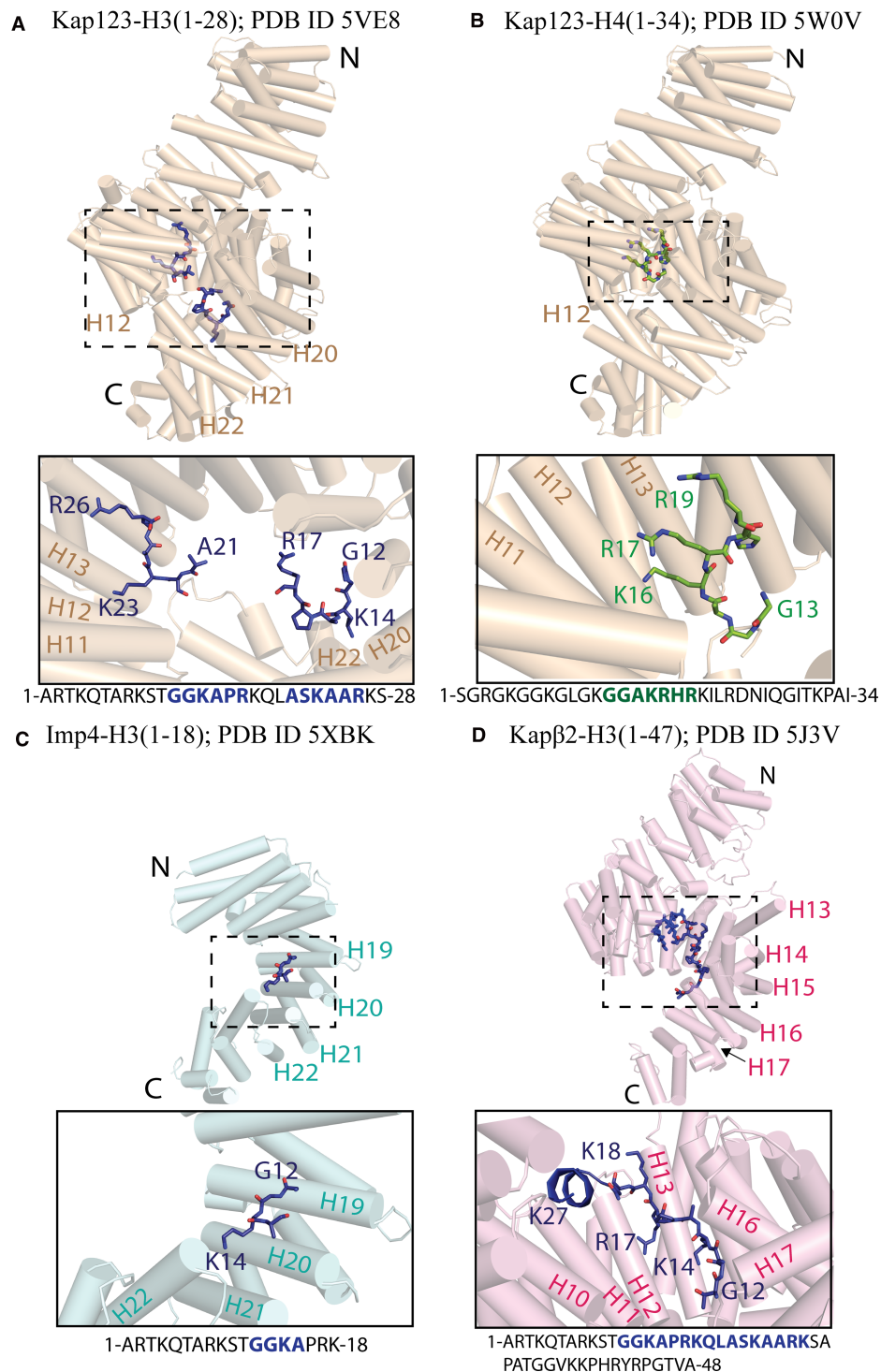
Figure 3. Structures of karyopherins bound to N-terminal tails of H3 and H4.

(**A**) Kap123 (tan, helices shown as cylinders and some HEAT repeats H11-H13 and H20-H22 labeled) bound to H3 tail (residues 1–28, dark blue sticks; PDB ID 5VE8). Only residues 12–17 and 21–26 of H3 were modeled. (**B**) Kap123 (tan) bound to H4 tail (residues 1–34, green sticks; PDB ID:5W0 V). Only residues 13–19 were modeled. (**C**) Imp4 (light green) bound to H3 tail (residues 12–15, dark blue sticks; PDB ID 5XBK). (**D**) Kapβ2 (light pink) bound to H3 tail (residue 1–47, dark blue sticks; PDB ID 5J3 V).

H3 tail lysine residues (K14 and K23) bind to two separate Kap123 pockets formed by HEAT repeats 20–22 and 11–13, respectively. A second structure of the H3 tail (residues 1–18) is also available bound to a C-terminal fragment of Imp4 (residues 668–1081 or HEAT repeats 16–23; Figure 3C). Here, only four H3 residues ^{12}GGKA^{15} could be observed binding in an extended conformation to the inner concave surface between HEATS 19–20. The binding of H3 tail residues ^{12}GGKA^{15} to both Imp4 and Kap123 appears to be similar, with the peptide binding to similar pockets formed by HEATS 19–22 of both Imp4 and Kap123. A third crystal structure of the H3 tail (residues 1–47) is available, bound to human Kapβ2, which is likely a backup importer for H3–H4 (Figure 3D). Here, residues 12–27 of H3 occupy the PY-NLS binding site of Kapβ2 even though the H3 sequence does not contain a proline-tyrosine dipeptide [22,24]. The N-terminal portion (residues 12–19) is extended and the C-terminal end (residues 20–27) is a short α-helix when bound to Kapβ2. However, the later (H3 residues 20–27) is in an extended conformation when bound to Kap123 and Imp4 [22,24]. In summary, the H3 tail peptide can adopt different conformations to bind with high affinity to several importins.

The H4 tail binds the same importins that binds the H3 tail. Impβ, Kapβ2, Imp4, Imp5, Imp7, Imp9, and Impα all bind the basic segment of H4 residues 5–20; Imp5 also binds to residues 29–32 [27]. Pull-down titration analysis suggests that the H4 tail binds importins ~10-fold weaker than H3 tail [27]. The crystal structure of a H4 tail peptide (residues 1–34) bound to Kap123 (Figure 3B) showed H4 residues 13–19 binding to only one of the two lysine binding-pockets (HEATS 11–13) of Kap123 that bind the H3 tail (Figure 3D) [22]. This observation is consistent with the weaker affinity of the H4 tail for importins [22,24]. The same binding site on Kap123 for both the H3 and H4 tails along with the higher affinity for the H3 tail suggest that Kap123 likely engages only the H3 tail at this site. When this happens, it is unclear if the H4 tail binds to a different site of Kap123.

Biochemical studies of several importins binding to the H3 and H4 tails, full-length H3–H4 dimer and H3–H4 bound to Asf1 suggested that Imp4 binding to the H3–H4•Asf1 complex makes the most likely nuclear import complex. In this complex, Imp4 binds not only the N-terminal tails of the histones, but the histone core and Asf1 [27]. This notion is supported by older studies of truncated-tails H3 and H4, which suggested that residues of the first α-helix at the junctions of the N-terminal tails with the histone-fold domains (residues 30–56 of H3 and 20–46 of H4) are also essential for nuclear import [39]. An atomic resolution structure of the Imp4•H3–H4•Asf1 complex is not yet available, but a structural model of Imp4 bound to H3–H4•Asf1 obtained through an integrative modeling approach using cross-linking mass-spectrometry, X-ray crystallography, negative-stain electron microscopy, SAXS and molecular modelling, showed the H3–H4 histone fold domain sandwiched between N and C-terminal regions of Imp4 and Asf1 located close to Imp4 [23]. Surface plasma resonance analysis also showed that Asf1 increases the dissociation rate of H3–H4 from Imp4, suggesting a potential role for Asf1 in facilitating the dissociation of the complex [23].

Post-translational modifications and nuclear import of histones H3 and H4

Many basic residues in N-terminal tails of H3 and H4 are also targets for post-translational modifications (PTMs), which regulate gene expression and epigenetic mechanisms [40]. Acetylation is one of the most studied type of PTM of histones. Since acetylation neutralizes positively charged amino acids in the histone tails, the PTM was suggested to affect nuclear import of histones, which likely involves interactions between basic histones and acidic karyopherins [30]. Newly synthesized H4 proteins are diacetylated at lysine residues K5 and K12 in the cytoplasm by the Hat1/RbAp46 complex, and this modification is well conserved from yeast to human [31,41]. In contrast, most PTMs of H3, including acetylation of lysine residues K14 and K18, were identified only in the nuclear fractions with low levels of K56 acetylation observed in cytosolic extracts [34]. Acetylation of H4 at K5 and K12 occurs prior to binding to Asf1 in the cytoplasm, but H3 acetylation happens mostly in the nucleus [31].

Mutations of K5 and K12 to arginine residues (mimicking deacetylation) in full-length H4 reduced binding with Imp4 and Asf1a/b [32]. Mutations of the same residues to glutamines (mimicking acetylation) improved nuclear import of full-length H3–H4 [42]. These findings suggest that acetylation of both H4 K5 and K12 facilitates binding to Imp4 and Asf1, and nuclear import of H3–H4. Surprisingly, another study indicates that acetylation of the same residues on a H4 tail peptide (residues 1–47) had no effect on importin-binding of the H4 tail [27]. It is not clear from these conflicting reports whether or how acetylation of H4 K5 and K12 influence importin-binding and nuclear import. Cross-linking/mass spectroscopy studies suggest there is little cross-linking between Imp4 and the H4 N-terminal tail, and residues K5 and K12 are not visible in the X-ray structure of

Kap123 bound to the H4_tail [23]. Further structural analysis of Imp4 bound to H3–H4 with acetylated H4 tail will be necessary to understand how acetylation may or may not affect the nuclear import of H3–H4.

The H3 tail is also acetylated. Acetylation-mimicking mutation of K14 of the H3 tail peptide (residues 1–28) decreased binding to importins and mislocalized the peptide to the cytoplasm of yeast cells [27,30]. However, K14 acetylation of H3 likely does not affect nuclear import of H3–H4 since it mostly occurs in the nucleus after translocation of the import complex through the NPC and possibly after release of histones from the nuclear import complex when the binding to Kap123/Imp4 is no longer relevant.

In summary, acetylation of H4 that occurs prior to the formation of the nuclear import complex may have a role in promoting the formation of the Imp4•H3–H4•Asf1 complex. On the other hand, acetylation of histones that occurs in the nucleus, such as most of the acetylation of H3 and acetylation of H4 beyond K5 and K12, may play a role in the release of H3–H4•Asf1 from the importin.

Nuclear import of H3 and H4 variants

Mammals have eight H3 variants, while only one variant of histone H3 is known in *Sc* and two H3 variants in *Arabidopsis*, *Xenopus* and *Drosophila*. Most H3 variants share a high sequence similarity. For example, human H3.2 differs in only one amino acid from the canonical H3.1 and in five amino acids residues from H3.3. Testis-specific H3.T and H3.5 differs from canonical H3.1 in four and eight amino acids, respectively [43,44]. Early studies with the human H3.3 variant found it co-purifying with Imp4 and histone-chaperone Asf1 [35]. Considering the high sequence homology between H3.3 and H3.1, and between H3.2, H3.T and H3.5, it is not surprising that their nuclear import mechanisms may be conserved. A few H3 variants are less conserved. Primate-specific variants H3.X and H3.Y differ in 28 amino acids from the canonical H3.1. Centromere-specific variant CENP-A is only ∼50% homologous with other variants and shows no conservation in its N-terminal tail [45]. Further investigations are necessary to determine if nuclear import of H3.X, H3.Y, CENP-A is mediated by the same karyopherins as for variants that are more similar to the canonical H3.1.

In contrast with H3, only one H4 variant, H4G, is known in higher eukaryotes. H4G is 85% identical with the canonical H4 and its expression is elevated in breast cancer cells [46]. Several residues at the H4G N-terminal and C-terminal tails also differ from those in canonical H4, and mass-spectrometry experiments showed that Asf1 do not bind H4G [46], suggesting that the nuclear import complex for H4G may be different from that for canonical H4.

Nuclear import of core histones H2A and H2B
Histones H2A and H2B from synthesis to nuclear import

Compared with H3 and H4, a lot less is known about how H2A and H2B are processed in the cytoplasm, from translation to nuclear import (Figure 2C). There are also no studies that correlates the PTM of H2A–H2B to their nuclear import, and thus we will not discuss the topic of H2A–H2B PTMs here. The co-purification of H2A with H2B and the high salt concentration needed to separate the two proteins support the notion that they form a H2A–H2B heterodimer in the cytoplasm prior to import into the nucleus [47]. The histone-chaperone Nap1 also co-purifies with H2A and H2B from cytosolic and nuclear extracts of yeast and HeLa cells and forms a stable complex with histones under physiological conditions [47–49]. Nap1 is known to be involved in H2A–H2B deposition during nucleosome assembly together with histone-chaperones FACT and nucleoplasmin (review [50]).

Like H3 and H4, several importins can bind to full-length H2A or H2B. Pull-down studies from yeast and HeLa cells extracts along with *in vitro* binding studies with purified H2A and H2B have shown interactions with the Impα/β heterodimer, Impβ alone, Kapβ2, Kap114, Kap121 and Kap123 [28,47,51,52]. *Sc* Kap114 is the most abundant importin that co-purifies with H2A and H2B from yeast cytosol. Imp9, the human homolog of Kap114, co-purifies with H2A and H2B from HeLa cell extracts, suggesting that Kap114 and Imp9 are the major importers of H2A–H2B (Figure 2B) [47,53,54]. In the absence of Kap114, H2A and H2B interact with Kap121 (homolog of Imp5) and Kap123 (homolog of Imp4), suggesting that these two importins are secondary H2A–H2B importers [47]. Nap1 co-purifies with Kap114, H2A and H2B in both the nucleus and the cytoplasm, suggesting that the histone chaperone may play a role in H2A–H2B nuclear import [55]. Interestingly, the binding of Kap123 and Kap121 to the N-terminal tails of histones H2A (residues 1–46) and H2B (residues 1–52) is partially inhibited by the presence of Nap1, indicating that Nap1 might determine the specificity of H2A and H2B binding to Kap114 [55].

Interactions of H2A and H2B with importins

The N-terminal tails of H2A (residues 1–46) and H2B (residues 1–52) contain many basic residues. These NLS-like peptides could be imported into the nucleus and were found to bind Impβ, Kap114 and Kap121 [47,51]. However, deletions of H2A and H2B N-terminal tails did not abolish the nuclear import of H2A or H2B in yeast strains and did not affect the binding affinity with Imp9, suggesting the histone fold-domain of H2A–H2B is important for binding importins [25,56,57].

The structure of Imp9 bound to the H2A–H2B heterodimer showed only a few residues of the N-terminal tail of H2B binding to the importin, and removal of the H2B tail did not decrease the affinity of H2A–H2B for Imp9 (Figure 4A,C) [25]. Instead, the N- and C-terminal regions of Imp9 clamp the histone fold domain and shield H2A–H2B from promiscuous interactions in the cytoplasm, acting as a histone-chaperone (Figure 4A,B) [25]. At the N-terminus of Imp9, acid residues in the inner concave surface of Imp9 (HEAT repeats 2–5)
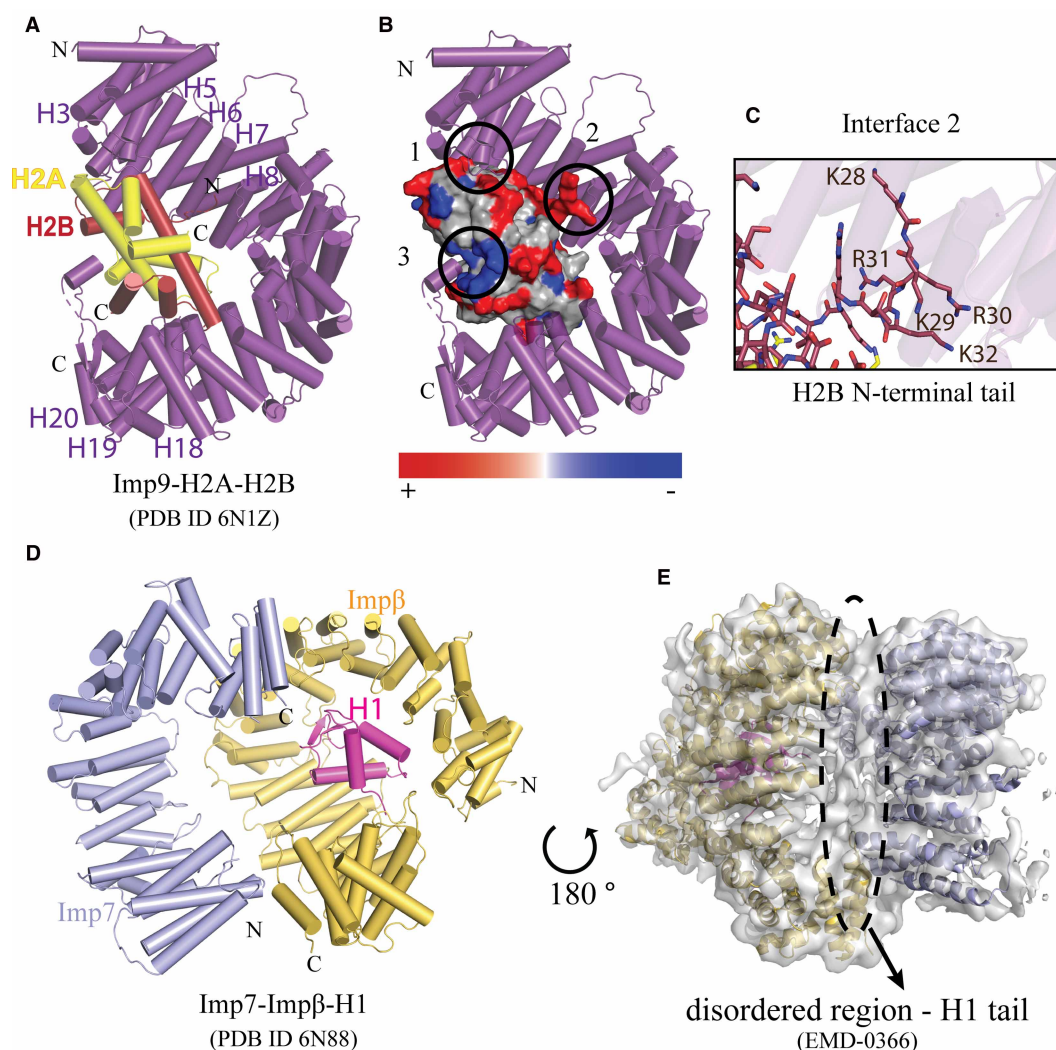

Figure 4. Structures of H2A–H2B and H1 nuclear import complexes.
(**A**) Imp9 (purple, helices shown as cylinders) bound to the H2A–H2B dimer (yellow-red, helices shown as cylinders; PDB ID: 6N1Z) (**B**) Charged residues at the interfaces of the Imp9•H2A–H2B complex (surface representation) with basic residues in red and acidic residues in blue. (**C**) 5 residues (28–32) in the N-terminal tail of H2B contact Imp9. (**D**) Imp7 (light blue, helices shown as cylinders) and Impβ (yellow) bound to linker histone H1 (magenta; PDB ID 6N88). (**E**) Cryo-EM map (gray) showing the density between Imp7 and Impβ, which cannot be modeled but possibly correspond to the disordered C-terminal tail of linker-histone H1 (EMD-0366).

interact with basic surfaces of the H2A–H2B core domain, the same basic residues that bind DNA in the nucleosome (Figure 4B). The C-terminal end of Imp9, with HEAT repeats 18–20 and a long loop between repeats 18–19, forms another interface with H2A–H2B. Here, basic residues of the Imp9 loop bind the acid patch of H2A–H2B (Figure 4B). No information is available for how Kap114 binds H2A–H2B, but given the homology between Kap114 and Imp9, it is very likely that Kap114 also wraps around the core domain of H2A–H2B.

Nap1, RanGTP and Imp9/Kap114 interactions regulates histones H2A–H2B nuclear import and deposition

Nap1 is a well-characterized histone-chaperone that is conserved in eukaryotes [48,58,59]. Although Nap1 binds to all five histones H3, H4, H2A, H2B and H1 to mediate nucleosome assembly in the nucleus, experiments with HeLa cells showed that Nap1 also binds to newly synthesized H2A and H2B in the cytoplasm [49]. Nap1 also co-purified with Kap114, H2A and H2B from yeast cytosol [60]. Biochemical analysis showed Nap1 binding directly to Kap114, and this interaction is compatible with the binding of H2A–H2B to Kap114. Nap1 appears to also be a cargo for Kap114 and has a binding site distinct from that for H2A–H2B [55].

A yeast strain depleted of Nap1 showed decreased nuclear accumulation of H2A and H2B, similar to what was observed in cells depleted of Kap114 [55]. Nap1 also seemed to increase the binding of Kap114 to the N-terminal tails of H2A and H2B [55]. However, given what we know now about how Imp9 binds mostly to the histone core, it is unclear if the N-terminal tails of H2A and H2B play a role in binding Kap114 [25].

A crystal structure of the complex Nap1•H2A–H2B shows one Nap1 dimer binding to one H2A–H2B heterodimer (Figure 2D) and how this complex might assemble in higher order oligomers [61]. Interestingly, the NLS of Nap1 (residues 290–295) in these oligomers remain accessible to bind importins, which might enhance the nuclear import of the Nap1•H2A–H2B complex [61]. A structure of Kap114 bound to both Nap1 and H2A–H2B will be necessary to understand how Kap114 imports both the histones and their histone chaperone, if and how Nap1 co-operates with Kap114 to deposit H2A–H2B onto nucleosomes.

Once inside the nucleus, the Kap114•H2A–H2B•Nap1 complex will encounter RanGTP. Importins generally release their cargos upon binding RanGTP inside the nucleus. However, the system of Kap114/Imp9 importing H2A–H2B is an exception. RanGTP does not seem to dissociate the Kap114•H2A–H2B•Nap1 complex; in fact, an assembly containing Kap114, H2A–H2B and Nap1 was found in the yeast nuclear extract and the complex is insensitive to RanGTP (Figure 2D) [55,60]. Similarly, the Imp9•H2A–H2B complex is also not dissociated by RanGTP. Instead, the GTPase binds the complex to form a stable a RanGTP•Imp9•H2A–H2B complex [25]. The inability of RanGTP to disassemble an importin-cargo complex though rarely seen was previously reported for the complex of Kap104 with mRNA-binding protein [62].

Nap1 is thought to assist deposition of H2A–H2B dimers onto an assembling nucleosome [60,61]. Curiously, the presence of Kap114 inhibits the chromatin assembly function of Nap1 in replication-independent chromatin assembly assays, but the presence of RanGTP restores the chromatin assembly function of Nap1 that was inhibited by Kap114 [60]. In the human system, Imp9 inhibits the interactions of H2A–H2B with DNA, but the RanGTP•Imp9•H2A–H2B complex effectively deposits H2A–H2B onto assembling nucleosomes [25]. Although RanGTP binds the Imp9•H2A–H2B and the Kap114•H2A–H2B•Nap1 complexes rather than dissociate them, the GTPase may modulate the importin–histone interactions to prime them for deposition onto nucleosomes.

Nuclear import of H2A and H2B variants

There are eight known H2A variants and thirteen H2B variants/isoforms in eukaryotes. Most of the H2B variants differ from the canonical by only few amino acids, which are mostly located in the histone-fold domain. The differences in the variants may affect the histone–histone interactions (see review [63]) but is not known if they may also affect karyopherin–histone interactions. Of the H2A variants, the H2A.Z variants family members have similar histone-fold domains but differ in their C-terminal tails [63]. Proteomics studies have found that H2A.Z and its yeast homologous Htz1 are major cargos of Imp9 and Kap114, respectively, suggesting conserved modes of nuclear import as canonical H2A–H2B [54,64]. A more extensive analysis combining biochemical experiments and sequence analysis of various H2A and H2B variants will be needed to understand how different variants are imported into the nucleus.

Nuclear import of linker histones H1

Interactions of linker histones H1 from synthesis to nuclear import

Linker histones H1 have been shown to bind histones-chaperones HSP90, NASP and NAP1 in the nucleus and the cytoplasm but the chronology of these interactions is still unclear (Figure 2C) [65-67]. The distribution of NASP and HSP90 binding to linker histones H1 in both the nuclear and cytoplasmic compartments suggested a potential role for nuclear import of H1 [65]. Nuclear import assays also showed that NASP can translocate H1 histones into the nucleus in the absence of cytosolic factors [65]. However, the function of NASP in nuclear import is still unclear and NASP may be a nuclear import cofactor or chaperone rather than a nuclear import receptor of linker histone H1.

Binding studies in HeLa cell extracts found H1 variants (human $H1^0$ and thymus specific tH1) co-purifying with Imp7, Impβ, Impα and Imp5 [21]. Nuclear import assays using permeabilized HeLa cells in the presence/absence of Ran showed that efficient nuclear import of linker histones requires the presence of both Impβ and Imp7 (Figure 2C). Impα or Imp5 cannot import linker histones efficiently, but it cannot be excluded that Imp5 or Impα can work as secondary or backup nuclear-transport receptors for canonical linker histones or variants.

Interactions of H1 with importins

Either Impβ or Imp7 alone bind weakly to H1. Biochemical dissections found that Impβ binds the globular domain (residues 21–95) of H1 while both Impβ and Imp7 bind the C-terminal domain (residues 95–193) of H1 [21,68]. Truncated versions of H1 that binds Imp7 but not Impβ cannot be imported, suggesting the need for a heterodimeric complex of Impβ/Imp7 for efficient nuclear import [68].

A cryo-electron microscopy (cryo-EM) structure of the Imp7•Impβ•H1.0 complex shows how this nuclear import complex is held together (Figure 4D). Imp7 and Impβ heterodimerize as the disordered C-terminal tail of Imp7, which contains FxFG motifs, binds to the convex surface of Impβ. This interaction resembles that of Impβ binding to FG-nucleoporins. A cryo-EM map of Imp7•Impβ without histone H1 shows a weaker density for Imp7 and no density for the HEAT repeats that form the interfaces with Impβ and H1, suggesting the Imp7•Impβ heterodimer is flexible and the binding of Imp7 with Impβ is stabilized by interactions with histone H1 .The globular domain of H1 binds to the concave surface of Impβ and unmodeled density between Impβ and Imp7 suggests that the disordered C-terminal tail of H1 may form a contact surface between the two karyopherins (Figure 4E) [26]. The low-resolution density between Impβ and Imp7 suggests that the C-terminal tail of H1 is flexible even when bound to Imp7•Impβ. The Imp7•Impβ•H1.0 complex is sensitive to RanGTP as H1 binds to the RanGTP binding site of Impβ. It was proposed that the high concentration of FxFG motifs on the nuclear surface of NPC competes with Imp7 binding site in Impβ and together with RanGTP promotes H1.0 release into the nucleus [21,26].

Linker histones variants

Eleven linker histones variants have been identified in human and mice. These linker histones subtypes have conserved globular domains but have significant variations in their N and C-terminal tails [69]. We know from the structure of Imp7•Impβ•H1 that the globular domain of H1 binds Impβ [26], and therefore, this interaction interface should be preserved for interactions with many H1 variants. On the other hand, the binding of Imp7 to Impβ is mediated by the H1 C-terminal tail, which is more variable in H1 variants and could affect the Imp7–Impβ interaction [26]. Structural studies with H1 variants could help to understand if the binding of H1 variants would affect forming a complex with an Imp7–Impβ heterodimer.

Final considerations

Knowledge of how different histones are imported into the nucleus has grown substantially in the last decade with the availability of atomic-resolution structures of the karyopherins Imp9 and Imp7•Impβ binding to core histones H2A–H2B and to linker histone H1, respectively [25,26]. The revelation that H2A–H2B binds Imp9 predominantly through its histone fold-domain leads to the hypothesis that the histone fold domain is also key in the interaction of H3–H4 dimers with Imp4, although previous data suggests that H3–H4 tails are also important in this interaction [27,29,30]. An atomic-resolution structure of the Imp4•H3–H4 complex will help us understand the role of the histone core in nuclear import and if acetylation of N-terminal tails is important for assembling the nuclear import complex.

It is also relevant to investigate the role of histone-chaperones Asf1, Nap1 and the small GTPase Ran in Kap-histones complex assembly and release. In the case of Imp4/Kap123 binding to H3–H4, Asf1 appears to increase the affinity of the complex. The Imp4/Kap123•Asf1•H3–H4 complex also appears to be sensitive to RanGTP [27]. On the contrary, the Kap114•H2A–H2B•Nap1 complex and the Imp9•H2A–H2B complex are not dissociated by RanGTP [25,55]. Nap1 may increase the affinity of H2A–H2B binding to Kap114, but how is this complex released inside the nucleus? Besides, are Kap114 or Imp9 required for histone deposition in the cell nucleus?

Another aspect of histone nuclear import that requires further investigation is how different histones variants are imported. Although most variants have few amino acids that differ from canonical histones, others have more substantial differences that could affect the binding to karyopherins. It is possible that alternative nuclear import pathways exist to handle the variety of histones that are imported into the nucleus in different stages of cell cycle and development. Biochemical and structural studies with karyopherins and histones variants may shed some light on this hypothesis and helps to understand the traffic patterns of how histone subtypes are moved into the nucleus.

Perspectives

- Importance of the field: nuclear import of histones is essential to regulate the supply of new histones inside the nucleus during DNA replication and transcription.

- Current thinking: Imp4 and Kap123 are the major nuclear import receptors for histones H3–H4. Histones H2A–H2B are mostly imported by Imp9 and Kap114, and linker histone H1 is imported by the heterodimer Impβ/Imp7. However, other importins can serve as backup nuclear importers for these histones.

- Future directions: further investigation of the roles of the histone fold domain of H3–H4 in interactions with Imp4 and atomic resolution structures of Imp4/Kap123 bound to the Asf1–H3–H4 complex; the role of histone-chaperones Asf1, Nap1 and the small GTPase Ran in Kap-histones complex assembly and release; the roles of importins and Ran in histone deposition into the nucleosome.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Author Contributions

N.E.B. and Y.M.C. planned, discussed and wrote the manuscript.

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Abbreviations

cNLSs classical-NLSs
NLSs nuclear localization signals
NPC nuclear pore complex

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