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# Karyopherins and Nuclear actin transport

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Although compartmentalization of a eukaryotic cell into the nucleus and cytoplasm has benefits, it further necessitates transport between the two compartments. Active transport between these two compartments requires metabolic energy and transporter proteins. Nuclear transporter receptors belong to the karyopherin super family and aid in the movement of cargo into (importers) or out of the nucleus (exporters) or are bidirectional. Presence of actin in nucleus and its nuclear trafficking remains intriguing due to its lack of nuclear import or export signals. Despite the fact that importin-9 and exportin-6 have been linked to nuclear import and export of actin respectively, the mechanistic details about actin-karyopherin interaction are not known yet. In this review, we compared the structural details of already available karyopherins and provide the initial structural prediction for exportin-6. The WH2 domain from diverse actin binding proteins display a similar architecture of N-terminal  $\alpha$  helix followed by the LKKT(V) motif. A similar LKPS motif was identified near helix 14A of exportin-6, which might be the binding site for actin. Based on these predictions, we have postulated a mechanism of actin-karyopherin interaction.

## INTRODUCTION

Eukaryotic cells contain a nucleus and other organelles and each perform different functions. The entire cell can be divided into two major compartments, the nucleus and cytoplasm that comprises the other organelles. Particularly, DNA replication and transcription, RNA processing, and assembly of ribonucleoprotein particles (RNPs) occur in the nuclear compartment, whereas translation occurs in the cytoplasm. As the nucleus cannot synthesize the proteins needed for replication and transcription, nucleoproteins must be transported into the nucleus and tRNA, mRNA and ribosomes must be transported out of the nucleus. All nucleo-cytoplasmic exchange proceeds through nuclear pore complexes (NPCs) (Feldherr et al., 1984).

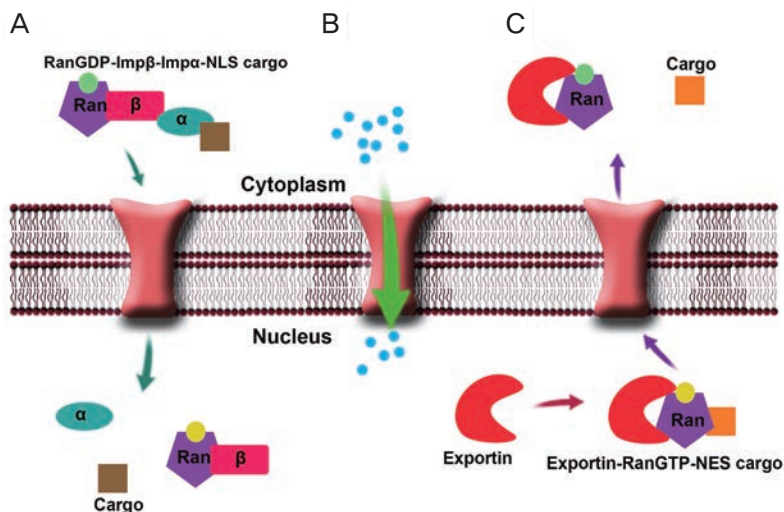
Nuclear pore complexes (NPCs) are the sites of macromolecule exchange between the two compartments (Figure 1). NPCs have a mass of about 125 megadaltons in higher eukaryotes and roughly contain about 30 different nucleoporin (Nup) proteins (Fabre and Hurt, 1994; Rout and Wente, 1994). The structure of the NPC consists of two main functional regions, such as the NPC central structure, which is embedded in the plane of the nuclear envelope, and the NPC peripheral structures, which extend the NPC towards the interior of the nucleus and the cytoplasm. The NPC functions as a molecular sieve to regulate bidirectional transport of macromolecules and small metabolites.

Nucleo-cytoplasmic exchange through the NPC proceeds by passive diffusion (Figure 1B) and facilitated translocation (Figure 1A and 1C). Ions and neutral proteins that do not bind to the NPC diffuse through the 45nm NPC tunnel (diameter 8-10nm). This passive diffusion is independent of GTP hydrolysis and the size of the transported molecules may range from freely permeable ions to molecules of 10nm diameter (Figure

1B). Passive diffusion of molecules appears to be driven by concentration gradient alone. (Gorlich and Kutay, 1999; Keminer and Peters, 1999) (Figure 1B). Whereas facilitated translocation accommodates the transport of highly selective larger objects, as it is receptor-mediated transport that requires metabolic energy such as GTP hydrolysis and is capable of transport against steep chemical activity gradients (Figure 1A and 1C) (Pante and Kann, 2002; Ribbeck and Gorlich, 2001).

Cargo translocation is often facilitated by soluble nuclear transport receptors (NTRs) that bind to the NPC and recognize nuclear localization signals (NLS) within the cargo (Gorlich, 1998; Pemberton et al., 1998). These receptors are called importins (Figure 1A) and those that export cargo out of the nucleus into the cytoplasm are called exportins (Figure 1C). Most of these receptors target a specific cargo to be transported, such as Imp $\alpha$  for the cellular apoptosis susceptibility (CAS) protein (Kutay et al., 1997), tRNA for exportin-t (Arts et al., 1998; Kutay et al., 1998), actin-profilin complexes for exportin-6 (Stuven et al., 2003), and p50RhoGAP for exportin-7 (Mingot et al., 2004). Other receptors export a wide variety of cargoes out of nucleus such as Leu-rich nuclear export signal (NES) containing proteins by CRM1 (Fornerod et al., 1997; Stade et al., 1997), and dsRNA binding proteins, pre-miRNAs and 60S pre-ribosomal subunits by exportin-5 (Bohnsack et al., 2002; Calado et al., 2002; Gwizdek et al., 2003). Some bidirectional receptors, such as importin-13, import Mago-Y14 and Ubc9, and export eIF1A (Mingot et al., 2001), exportin-4 imports Sox2 and SRY, and exports eIF5A (Guttler and Gorlich, 2011).

Soluble NTRs belong to the most conserved family of the karyopherins that play a central role in nucleo-cytoplasmic transport. Karyopherins are heteromeric molecules composed



**FIGURE 1 | Scheme of Nucleo-cytoplasmic transport. Nucleus and cytoplasm are separated by a lipid bilayered nuclear membrane.** The tunnel that connects the two compartments are called nucleoporins (Nups) marked as light brown. (A) Import: Importin-β/ importin-α complex in cytoplasm binds to the exposed nuclear localization signal (NLS) motif of cargo (brown square), passes through the nucleopore complex and becomes disassembled on binding with RanGTP in the nucleus. (B) Passive diffusion: Neutral proteins and ions (blue balls) can diffuse freely through Nups. (C) Export: Exporters (red) usually exist in an unbound free state in nucleus. It then binds to cargoes with the nuclear export signal. RanGTP enhances the interaction of exporter with cargo and facilitates nuclear export. The complex disassembles in cytoplasm. (green ball, GDP; yellow ball, GTP)

of karyopherin  $\alpha$  (Kapa) and karyopherin  $\beta$  (Kap $\beta$ ) that function together to transport molecules through the NPC. Kapa (also known as importin- $\alpha$ , Imp $\alpha$ ) is an adaptor protein that recognizes the first discovered or classical NLS, which is characterized by one or two stretches of basic residues. Kapa interacts with Kap $\beta$  (also known as importin- $\beta$ , Imp $\beta$ ) and these proteins form a transport pathway in which proteins containing a classical NLS are imported by Kapa-Kap $\beta$  heterodimer (Figure 1A) (Pemberton et al., 1998). Karyopherins are also critically involved in diverse cellular functions, such as gene expression, signal transduction, immune responses, oncogenesis, and viral propagation (Mohammed et al., 2005; Resa-Infante et al., 2014; Weis, 2003).

NTRs are RanGTP-binding proteins and respond to gradient between compartments by loading and unloading the cargo in the appropriate compartment. Importins recruit cargo at low Ran GTP levels in cytoplasm and release it upon RanGTP binding in the nucleus (Figure 1A) (Gorlich and Kutay, 1999). They return as RanGTP complexes into cytoplasm, where the Ran-bound GTP gets hydrolyzed, the complex dissociates and the importin goes for another round of substrate. Exportins function in exactly opposite manner. They recruit cargo at high RanGTP levels in the nucleus, forming ternary cargo-exportin-RanGTP complexes (Figure 1C) (Kutay et al., 1997). These complexes are transferred through NPC into the cytoplasm where GTP is hydrolyzed and the export complex gets disassembled. Just like importins, the cargo-free and Ran-free exportin can then re-enter the nucleus

and export another cargo molecule.

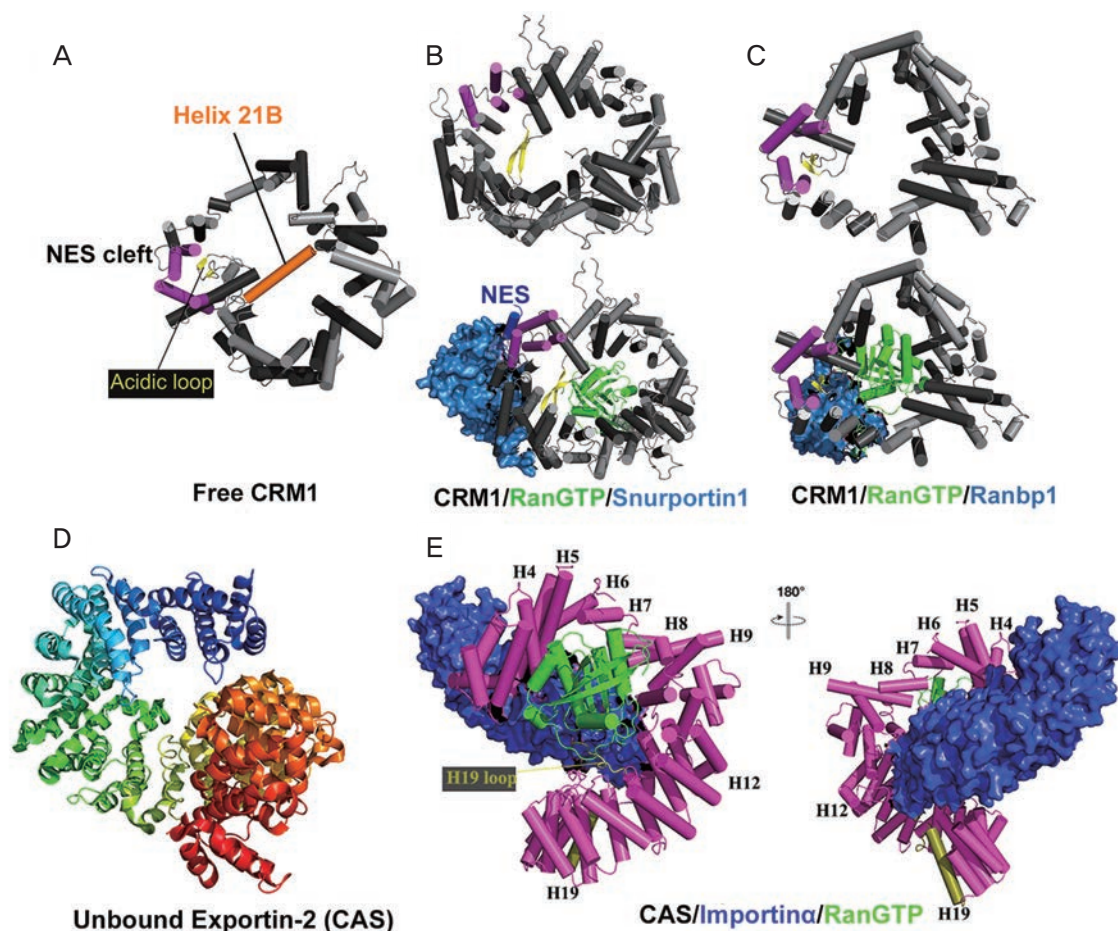
Nuclear transport receptors have variously been termed as karyopherins, importins, exportins and transportins. Exportin-6 (Exp-6), one of the exportins is highly conserved from amoeba to vertebrates. This is the only NTR for the export of nuclear actin established so far. Actin is imported into nucleus by importin-9 (Imp-9) and exported by Exp-6, with cofilin and profilin as cofactors respectively. Nuclear actin is dynamic by constantly moving in and out of the nucleus (Dopie et al., 2012). Accumulation of nuclear actin interferes with developmental programs and highly toxic at cellular level (Park et al., 2011). Only biochemical and cell biological data on actin nuclear trafficking are available. However, the mechanistic details of actin's interaction with karyopherin remain unresolved because of the lack of Exp-6 and Imp-9 structural details. The Exp-6 and Imp-9 structures must be elucidated to understand the underlying mechanism. Because the NTRs and passage through the NPC has been reviewed previously, this review will emphasize the significance of nuclear actin trafficking along with the structural aspects of several

known exportins to predict the structure of Exp-6.

## STRUCTURAL FEATURES OF EXPORTINS AND TRANSPORT MECHANISM

The features of the members of the same family must be understood to analyze the Exp-6 predicted model. In this section, we describe the CRM1 (exportin-1) and CAS (exportin-2) protein structures. Different structures may be assumed based on the different cargo types transported by exportins. However, Imp- $\beta$  like NTRs share a very similar architecture although they have only 15-20% sequence identity.

Exportin CRM-1 is a major nuclear exporter of a broad range of cargo macromolecules containing a leucine-rich NES and is essential in all organisms tested. Over the last decade, numerous crystal structures of CRM1 and CRM1 complexes have facilitated a detailed understanding of CRM1 action and inhibition. Human CRM1 contains 1,071 amino acid residues organized into 21 HEAT motifs. One HEAT repeat comprises two  $\alpha$ -helices (A and B) that are linked by a short intrarepeat loop (Figure 2A) (Dong et al., 2009; Monecke et al., 2009). These repeats are named after the proteins huntingtin, elongation factor 3, 'A' subunit of protein phosphatase A (PR65/A), and TOR1 lipid kinase, in which the motif was first identified (Andrade and Bork, 1995). Multiple protein binding sites are available along the inner concave surface, while the outer convex surface has HEAT domains, that interact with the Phe-Gly region of Nups during NPC passage



**FIGURE 2 | Structures of known karyopherins (CRM1 and CAS).** CRM1 helices are shown in dark grey cylinders. Light grey refers to A helices and thick grey refers to B helices. The hydrophobic cleft (HEATS 11 and 12; magenta) and the acidic loop (yellow) are indicated. RanGTP is depicted in green. Cargoes are depicted in sky blue for A, B and C. (A) Structure of free CRM1 (PDB: 4FGV) showing acidic loop, C terminal helix and NES cleft between helices 11 and 12. Note the location of C-terminal H21 and acidic loop in an unbound CRM1. The acidic loop is in contact with repeats of H10-H12, behind the NES binding groove. Also the groove is narrower in unbound state compared to bound CRM1. (B) Conformational change on binding to snurportin 1 and 19 RanGTP (PDB: 3GJX). Top shows the conformational change in CRM1 only, while hiding the rest of the complex. Position of acidic loop (yellow) before and after cargo binding has to be noted. See main text for more information. (C) CRM1 bound to Ranbp1 that disassembles the complex (3MIL). On binding with cargoes, the helix 21B is in stacked alignment with other helices. (D) Structure of CAS in unbound state (PDB: 1Z3H). Blue to red color denotes N- to C-terminal end. (E) Structure of CAS in complex with RanGTP and Importin- $\alpha$  (PDB: 1WA5). CAS is represented as magenta cylindrical helix. RanGTP is depicted in green and importin- $\alpha$  in blue. The surface of importin- $\alpha$  is shown. H19 loop is depicted in golden yellow. The N- and C-terminal of CAS protein brings RanGTP and importin- $\alpha$  in such a way that they are in contact with each other.

(Terry and Wente, 2009). These tandem HEAT repeats define a ring-shaped solenoid that forms the hydrophobic core. A-helices of the HEAT repeats form the outer convex surface, whereas B helices form the inner surface that interacts with RanGTP (Figure 3A). The N-terminal region where Ran binds is called the CRIME domain (CRM1-importin- $\beta$ ). Another important region involved in RanGTP binding is the acidic loop or HEAT 9 loop, which is a stretch of 26 CRM1 residues forming a  $\beta$ -hairpin (Figure 2A). The NES binds to the outer surface of CRM1 and occupies a groove formed by the A helices of repeats 11 and 12 (Dong et al., 2009; Guttler et al., 2010; Monecke et al., 2009). The HEAT-9 loop contacts repeats of 10-12 of the B helices behind the NES-binding groove in an unbound state, and the NES-binding

groove is narrower than cargo bound structures (Figure 2B). The acidic loop adopts a seatbelt-like conformation when complexed with cargo or RanGTP, which locks Ran in a way that interacts with the CRM1 CRIME domain (Figure 2B). In contrast, during disassembly, when CRM1 binds with RanGTP and Ran binding protein RanBP1, the acidic loop changes its seat belt locked position and triggers release of RanGTP and the cargo (Figure 2C) (Dian et al., 2013; Dolker et al., 2013; Koyama and Matsuura, 2010; Monecke et al., 2009).

Another striking feature of this structure is the atypical arrangement of HEAT loop 21 (Figure 2A). The HEAT21 B-helix is in stacked arrangement with the other HEAT repeats when complexed with SPN1 and RanGTP or alone (Figure 2B and

2C). In contrast, the HEAT 21B-helix spans the central CRM1 channel and interacts close to the acidic loop interacting at the back side of the NES cleft in free CRM1 or when bound to SPN1 (Dian et al., 2013; Dong et al., 2009; Monecke et al., 2013; Saito and Matsuura, 2013). Moreover the orientation of the helix 21B impacts the distance between the N- and C-terminals of the HEAT repeats. The N- and C-terminals form a tight and intricate pattern in a parallel orientation when complexed (CRM1-RanGTP-SPN1), and such interactions are unavailable (Figure 2B) (Guttler et al., 2010; Monecke et al., 2009; Monecke et al., 2013; Saito and Matsuura, 2013).

CRM1 is unusual among karyopherins in that it has a cargo-binding site on its outer surface instead of the inner surface. However, this is a very important feature that allows CRM1 to carry a wide range of cargo sizes and shapes, including large cargoes such as ribosomal subunits. As exportin-6 has a single cargo target it may be slightly different from CRM1 in terms of cargo binding.

The CAS protein is also called exportin-2 and *cse1p* in *S.cerevisiae* and is fully dedicated to a single but highly abundant cargo type as it recycles importin- $\alpha$  to the cytoplasm for the next round of import (Figure 2D). The CAS protein is conserved and essential in all eukaryotes tested. The *S.Cerevisiae* RanGTP-CAS-Imp $\alpha$  complex is the first nuclear export complex whose structure has been elucidated (Matsuura and Stewart, 2004). Once the Imp $\alpha$ -Imp $\beta$ -cargo complex reaches the nucleus, it is disassembled when RanGTP binds Imp $\beta$  (Figure 1A). The CAS protein then selectively binds and exports the NLS-free Imp $\alpha$  thereby ensuring that only Imp $\alpha$  and not the imported cargo is exported.

The CAS protein holds both Ran and Imp $\alpha$  between its N- and C-terminal arches so Ran and Imp $\alpha$  are in contact (Figure 2E) (Matsuura and Stewart, 2004). The cooperative cargo and Ran binding is facilitated by a direct interaction between Ran's positively charged back and negative charged features of the cargo. Hence, formation of the complex is inhibited by deleting the Imp $\alpha$  C-terminal end (Herold et al., 1998). This export topology with Ran and Imp $\alpha$  stabilizes HEAT loop 19 and the complex cannot form in the absence of Ran or Imp $\alpha$  (Figure 2E). In addition, cargo loading requires a Ran-driven conformational change in the CAS protein. The CAS without a ligand is tightly closed with the N- and C-terminals bound together (Figure 2D). The structure changes to a horseshoe-like structure when RanGTP intercalates, which readily accommodates the cargo to be exported (Figure 2E) (Cook et al., 2005).

Same as CRM1 and CAS, almost all karyopherins undergo structural conformation change in order to pass through NPC. The known structures of unbound exportins underwent structural changes either at acidic loop in HEAT repeat for CRM1 or neighboring loop in C-terminal for CAS upon binding with RanGTP (Figure 2A-2C). The subsequent conformational changes result in aiding cargo binding, where CRM1 coils around RanGTP to hinder cargo entry and instead guide them to the

outer helices (Figure 2B and 2C). Unlike CRM1, most of other exportins carry their cargo inside the HEAT repeats between their C- and N-terminals. Exp-6 is reported to bind to actin part of the actin-profilin complex, suggesting that actin might undergo conformational change on binding with profilin to increase actin affinity to Exp-6 (Stuven et al., 2003). Similarly, it can be predicted that, upon binding to RanGTP, Exp6-Ran conformation might provide actin-profilin complex with proper site in HEAT repeats region for interaction. However, it is still unclear if RanGTP contacts the actin-profilin complex first or helps the complex in binding to Exp-6.

## NUCLEAR ACTIN

Actin is one among many proteins that are restricted to the nucleus, although its location has been controversial. Actin comprises the cytoplasmic cytoskeleton and was long believed to be located only in the cytoplasm. Studies emerging in the 1970s reported the presence of actin in isolated nuclei. However, these reports were generally dismissed as most likely reflecting cytoplasmic contamination. The perception started to change slowly in the 2000s when many studies on the functional roles of nuclear actin emerged, particularly after the development of imaging technology (Baarlink et al., 2013). Now it is believed that actin has a significant role in the nucleus and is highly dynamic.

Nuclear actin interacts with all three polymerases, engages in transcription (Hofmann et al., 2004; Hu et al., 2004; Philimonenko et al., 2004), binds to factors regulating pre-mRNA processing and export (Obrdlik et al., 2008; Percipalle et al., 2002), and regulates the localization and activity of myocardin-related transcription factor A, which is a co-activator of the serum response factor transcription factor (Vartiainen et al., 2007). The actin monomer reportedly binds to brahma-related gene 1 (BRG1), a subunit of the BRG1-associated factor (BAF) chromatin-remodeling complex from the SW1/SNF family. Actin is required for optimal BRG1 ATPase activity and the association between the BAF complex and chromatin (Zhao et al., 1998). The most fundamental biochemical property of actin is the generation of force either through polymerization or in conjugation with the motor protein myosin. These properties are used in the cytoplasm for cell migration or muscle contraction (Sweeney and Houdusse, 2010). Actin and nuclear myosins have been implicated in the movement of individual gene loci after transcriptional activation.

The homeostasis of actin, between the nucleus and cytoplasm is fine-tuned in response to the cell's needs, which proposed the idea that actin (>40 kDa) must be transported. The mechanisms of actin nucleo-cytoplasmic transport are very complex because of its size and lack of a classical NLS. However, many actin-binding proteins contain a NLS and are responsible for translocating actin into and out of the nucleus (Skarp and Vartiainen, 2010; Vartiainen, 2008). The active transport mechanism to import actin was deduced using RNAi experiments and the mediators of actin nuclear localization were identified to

be cofilin and importin-9 (Imp-9) (Dopie et al., 2012). Exportin-1 (CRM1) driven nuclear export was previously reported to keep actin within the cytoplasm (Wada et al., 1998). However, it was later shown that Exp-6 and not CRM1 is primarily responsible for exporting actin in humans, *X. laevis*, *D. rerio* and *Drosophila melanogaster* from the nucleus to the cytoplasm (Stuven et al., 2003).

Importin-9 transports cytoplasmic actin monomers into the nucleus and cofilin, an actin-binding protein, is necessary for this transport. Cofilin transfers actin to the nucleus using the

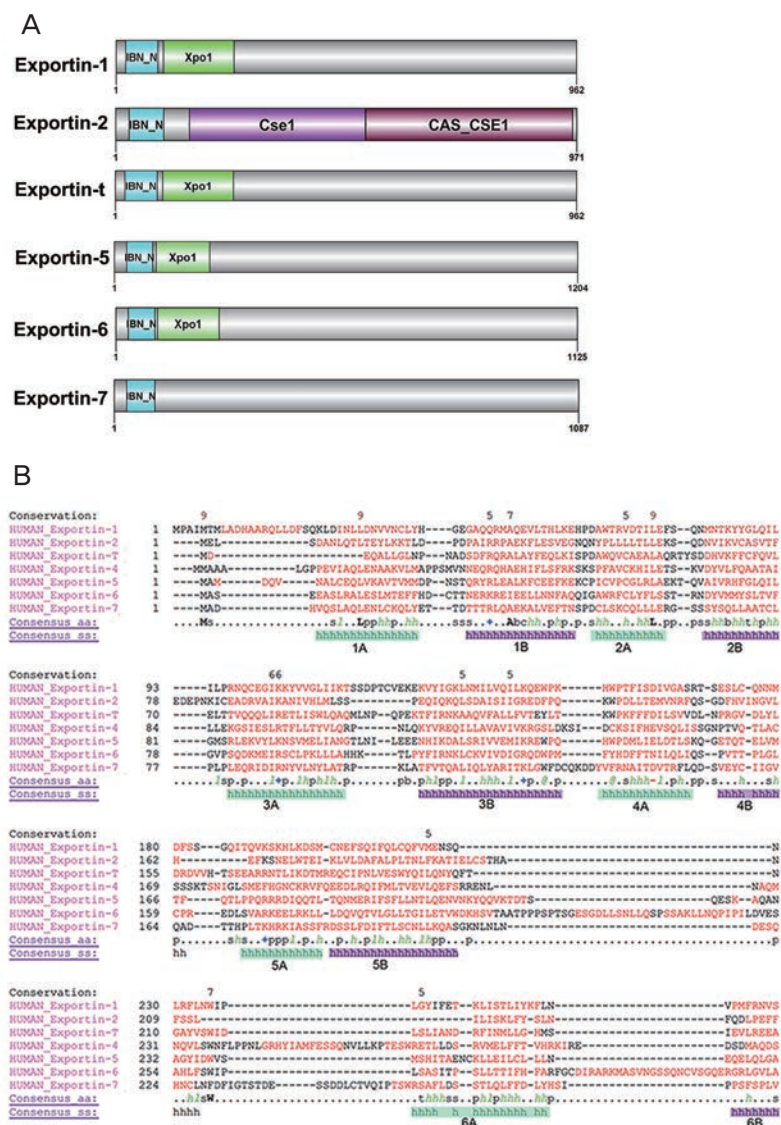
KKRKK basic amino acid motif, which is a sequence involved in accumulating the actin-cofilin filament in the nucleus in response to heatshock (Iida et al., 1992; Munsie et al., 2012). Only Exp-6 and RanGTP are responsible for exporting nuclear actin and profilin greatly facilitates recruitment of actin to Exp-6 and is regarded as a specific co-factor for actin export (Stuven et al., 2003). Nuclear actin dynamics study indicated that actin constantly shuttles between the nucleus and cytoplasm. Actin must be maintained at appropriate levels in the nucleus for maximal transcription (Dopie et al., 2012).

## EXPORTIN-6 AND ACTIN TRANSPORT

All Kar $\beta$  nuclear transport receptors recognize a wide range of substrates and are therefore highly diverse in sequence. Exp-6 was identified as a novel member of the family of Kar $\beta$  that mediated nuclear export of profilin-actin export (Stuven et al., 2003). The domain map of Exp-6 was compared with all other exportins (Figure 3A). IBN\_N and Xpo1 are the two conserved domains in almost all exportins. Importin $\beta$  N-terminal (IBN\_N) domain is a signature for all importin- $\beta$  family. The Nterminal CRM-1 like domain, Xpo1 corresponds to RanGTP binding in all of Kar $\beta$ . Structure based sequence alignment of Exp-6 with other exportins corresponding to RanGTP-binding region shows the presence of similar structural architecture in all exportins (Figure 3B).

Stuven *et al* in 2003 first reported about Exp-6 and provided the biochemical details of actin expulsion from nucleus. The binding assays performed by them showed that Exp-6 exports actin only as a complex with profilin. Exp-6 was able to export all three isoforms of profilin along with actin, which indicates that Exp-6 recognizes the actin component of the actin-profilin complex. When Exp-6 was depleted using RNA interference technique in *Drosophila* Schneider cells, aggregates of actin paracrystals was detected in the nucleus, which indicates that Exp-6 is essential for nuclear expulsion of actin (Stuven et al., 2003). In addition, overexpression of Exp-6 leads to lower levels of nuclear actin thereby increasing the size of oocyte nuclei rendering them fragile, indicating the need for a nuclear actin scaffold to maintain mechanical integrity of cells, as well as balance in nuclear actin levels (Bohnsack et al., 2006).

Knockdown of Exp-6 expression significantly



**FIGURE 3 | Comparison of Exportin-6 sequence and structure with other karyopherins.** (A) Domain map comparison. Domain map of all exportins were compared to find the conserved domains in exportin-6. IBN\_N and Xpo1 are conserved domains. (B) Structure based sequence alignment using PROMALS3D for all exportin sequences corresponding to the conserved domains shown in (A). All similar aminoacids that correspond to a similar secondary structure are colored red. 'h' with green shade represent 'A' HEAT helices and 'h' with purple shade represent 'B' HEAT helices.



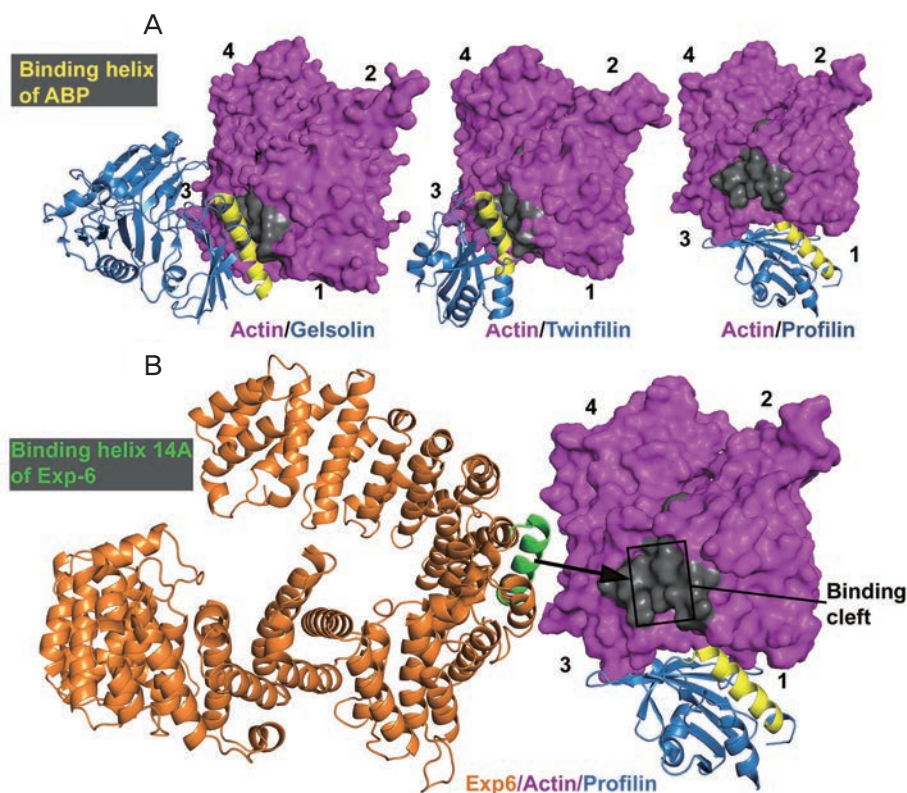
has similar architecture as that of the karyopherin family with a greater resemblance to CRM1 (Figure 4C). One interesting feature of the predicted structure is the absence of active loop of CRM1 that holds RanGTP. Although the actual structure might resemble the predicted model, the exact position for the actin-profilin complex binding to Exp-6 may not be the same.

### PROPOSED MECHANISM IN ACTIN-PROFILIN BINDING ON EXP-6

Actin dynamics are highly regulated in the cytoplasm by the presence of several actin-binding proteins (ABP). Several of these ABPs are localized inside the nucleus. As actin by itself does not have a NLS, it has been postulated that ABPs, which have a NLS, transport actin in and out of the nucleus. It has been demonstrated that actin does not accumulate in the nucleus when both cofilin and Exp-6 are silenced which agrees with the finding that actin cannot enter nucleus without a cofactor (Dopie et al., 2012). The Exp-6 binding site on actin could be discovered by identifying the strategy used by ABPs to bind actin.

Several ABPs, such as WASP, WAVE1, WIP, IRSp53 and T $\beta$ 4 use their WH2 domain to interact with actin. The WH2 domain from diverse ABPs display a similar architecture of an

N-terminal  $\alpha$  helix followed by a the LKKT(V) motif (Figure 4D). The interactions of the LKKT(V) domain are for the most part electrostatic in character, with positively charged residues of the WH2 domain facing negatively charged residues on the actin surface (Chereau et al., 2005; Dominguez, 2007; Lee and Dominguez, 2010). Exportin-6 has the similar motif (LKPS) near residue 765, which corresponds to helix14A. On comparing the same motif in all other exportins, LKPS motif was present only in exportin-6 (Figure 4E). The hydrophobic cleft in actin is the most important determinant of ABPs interaction with actin, despite the lack of sequence similarity between ABPs. Helical wheel diagrams for the A helices, show that most of the A helices have their hydrophobic groups facing inside to form the hydrophobic core, however, the 14A helix has hydrophobic residues facing outside (Figure 4F). This helix might bind to the hydrophobic cleft of the actin monomer. ABPs such as gelsolin, twinfilin, profilin and others present their N-terminal helix of their WH2 domain to bind at actin's binding cleft (Figure 5A). Although profilin binds to actin at the actin binding cleft, it occupies the back of the cleft allowing it to bind simultaneously with WH2-related sequences characterized by the presence of a short N-terminal helix as in the VASP's GAB domain (Figure 4G and

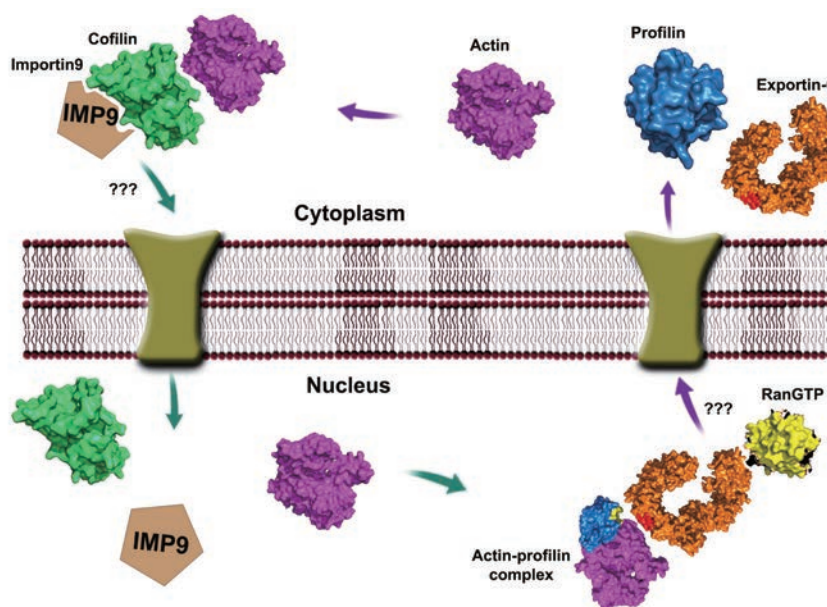


**FIGURE 5 | Predicted binding site of Exp-6's helix 14A to actin monomer.** Actin is shown in surface model (magenta) with 4 subdomains. ABPs (Gelsolin, Twinfilin, Profilin) are shown in blue ribbon with a particular helix in yellow. Hydrophobic cleft in actin is depicted in dark grey. (A) Structures of various ABPs bound to actin monomer. ABPs frequently present  $\alpha$  helix that binds to the hydrophobic cleft of the actin monomer. Note that profilin binds at the back of the cleft, whereas ABPs with WH2 domain (Gelsolin and Twinfilin) binds exactly on the hydrophobic cleft. (B) Arrow mark indicates the predicted site of actin's binding cleft (boxed) where helix 14A (Green) of Exp-6 binds.

5A) (Kovar *et al.*, 2006). Binding of profilin may enhance actin binding to Exp-6, which agrees with the biochemical results of Stuken *et al.* It is intriguing that VASP protein (>40 kDa), functioning as an actin filament elongation factor, was found in the nuclear actin export complex even with low ratio (Stuken *et al.*, 2003), suggesting the competition to the hydrophobic cleft of actin with Exp-6 helix 14A. It has been known that VASP contains two regions interacting actinprofilin complex, poly-Pro site and GAB (G-actin binding) domain, where both sites have each preference for loading and transition of the complex during actinpolymerization (Ferron *et al.*, 2007). Since, the actin tends to remain in monomeric form during nuclear actin export, the presence of VASP in the Exp-6 export complex may have a stronger affinity with poly-Pro site of VASP than GAB domain. Although elongation does happen in nucleus, VASP might just remain with actin-profilin complex without competing with Exp-6's actin binding site. However, it is still unclear why even trivial amount of VASP is accompanied with actin-profilin complex during export.

Based on our predictions thus far, Exp-6 like other karyopherins binds to actin-profilin complex in the nucleus. Ran induced structural changes on Exp-6 may push helix 14A harboring putative WH2-like motif outwards, which binds with actin's hydrophobic binding cleft (Figure 5B). In respect to the cases of other karyopherins, this structural modification enhances its binding towards actin-profilin complex. The C-terminal end might regulate the binding with actin-profilin complex by holding them. During the formation of ternary complex before exit, the actinprofilin complex may have two fates in the interaction with

Exp-6, including being tethered outside of the HEAT region or trapped inside of the Exp-6. With predictions from structural bio-informatics and sequence analysis, the binding mode of actinprofilin to Exp-6 may have a close resemblance to CRM1 than all other exportins mainly because the putative WH2-containing helix 14A is found exposed to outer surface layer of Exp-6 (Figure 4). This may suggest that the complex likely approaches outside of Exp-6 and expected to bind at helix 14A of Exp-6 in its outer surface. If the postulation is feasible, we may encounter a question of how the tethering of such big sized cargo could be stabilized. Stuken *et al.* reported that Exp-6 presented higher affinity to actin-profilin complex rather than actin alone, suggesting that the profilin binding to actin might enhance the affinity of actin-profilin complex to Exp-6. Similar case was found in the actin-profilin recruitment into VASP protein for elongation (Ferron *et al.*, 2007), in which profilin indeed increased the binding affinity of the complex to poly-Pro domain of VASP by 6-11 folds. Once bound, the HEAT helices provide structural flexibility for Exp-6-actin-profilin complex to pass through Phe-Gly rich NPC. After reaching nucleus, similar to other karyopherins, Ran-binding proteins might dissociate RanGDP from the complex, leading to the disassembly of actin-profilin complex from Exp-6 in cytoplasm. Exp-6 is likely to be recycled back to nucleus in a manner not known yet. Although the research in the import of actin-cofilin into nucleus by importin-9 is still in initial stage, the interaction of the complex with Imp-9 is not likely to be mediated through conventional actin-binding motif. Dopie *et al.*, (2012) reported that cofilin is responsible for the actin-cofilin cargo loading into Imp-9. Indeed,



**FIGURE 6 | Proposed actin nuclear trafficking scheme.** Surface structure of actin is shown in purple, profilin in blue, Exportin-6 in brown, RanGTP in yellow, cofilin in green and importin-9 (IMP9) in sand brown. Red mark on Exp-6 is the predicted site of interaction with yellow site of actin-profilin complex. Mechanistic details of actin nuclear trafficking are still vague due to the absence of structural details of both Imp-9 and Exp-6, which is marked with question mark. Future studies are needed to explain the interaction of these karyopherins with actin.

careful analysis to finding canonical WH2 motif inside the Imp-9 sequence failed.

## CONCLUSION AND PERSPECTIVES

Karyopherins are a family of proteins that are involved in nucleocytoplasmic transport. They have similar structural architecture although their sequence similarity is low (O'Reilly et al., 2011; Xu et al., 2010). Among several proteins within the nucleus the existence of actin and its nuclear trafficking remains intriguing. Actin is transported across the two compartments through Imp-9 and Exp-6. As actin does not have its own nuclear localization signal, it depends on ABPs for its transfer, such as cofilin for import and profilin for export (Dopie et al., 2012). In order to find the binding site of actin, the common LKKT(V) motif of ABP was located in Exp-6. This LKPS motif in Exp-6 was preceded by Helix 14A which had its hydrophobic residues outside. Helix14A is thus predicted to be the potential binding site of actin and this binding seems to be further enhanced with profilin. This prediction of structure and function for Exp-6 might aid in determining the interaction between actin and a karyopherin. A scheme of actin nuclear trafficking using predicted structural details of Exp-6 is shown in Figure 6. One more interesting question would be the pathway by which the Exp-6 in cytoplasm gets recycled back to nucleus. Furthermore, since proteins involved in the nuclear actin transport is tightly associated with diseases including cancer and angiogenesis-related disorders, the structural information of the transport in molecular level is urgently in need.

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## AUTHOR INFORMATION

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