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## Regulation of Nucleocytoplasmic Transport in Skeletal Muscle

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### Abstract

Proper skeletal muscle function is dependent on spatial and temporal control of gene expression in multinucleated myofibers. In addition, satellite cells, which are tissue-specific stem cells that contribute critically to repair and maintenance of skeletal muscle, are also required for normal muscle physiology. Gene expression in both myofibers and satellite cells is dependent upon nuclear proteins that require facilitated nuclear transport. A unique challenge for myofibers is controlling the transcriptional activity of hundreds of nuclei in a common cytoplasm yet achieving nuclear selectivity in transcription at specific locations such as neuromuscular synapses and myotendinous junctions. Nucleocytoplasmic transport of macromolecular cargoes is regulated by a complex interplay among various components of the nuclear transport machinery, namely nuclear pore complexes, nuclear envelope proteins, and various soluble transport receptors. The focus of this review is to highlight what is known about the nuclear transport machinery and its regulation in skeletal muscle and to consider the unique challenges that multinucleated muscle cells as well as satellite cells encounter in regulating nucleocytoplasmic transport during cell differentiation and tissue adaptation. Understanding how regulated nucleocytoplasmic transport controls gene expression in skeletal muscle may lead to further insights into the mechanisms contributing to muscle growth and maintenance throughout the lifespan of an individual.

### 1. Introduction

Skeletal muscle is a very plastic tissue that readily undergoes changes in mass and function in response to aging, injury, and disease. Such changes in muscle can impact breathing, locomotion, and metabolism and affect motility and lifespan. Proper skeletal muscle function is dependent on spatial and temporal control of gene expression mediated by proteins, such as transcription factors, that require facilitated transport to enter the nuclei. The subcellular localization of these regulatory proteins must be tightly controlled because altered import or export could result in aberrant muscle function.

Proper muscle function is dependent on myofibers, which are multinucleated cells containing many hundreds of nuclei distributed along the length of the cell in a common cytoplasm. Alongside each myofiber are adult muscle stem cells, called satellite cells, that

lay beneath the basal lamina surrounding each myofiber. These satellite cells are normally quiescent but in response to muscle damage they are activated to begin proliferating and undergo differentiation and eventual fusion with each other or existing myofibers to repair muscles in a process called myogenesis. Myogenesis can be modeled *in vitro* by culturing myoblasts, the progeny of satellite cells, and inducing them to differentiate into multinucleated myotubes by changes in culture media. Myogenesis both *in vivo* and *in vitro* requires the coordinate activation and repression of many genes. Numerous nuclear proteins are required for proper gene expression and the nuclear repertoire of these proteins is very different along the myogenic continuum of quiescent satellite cells to mature postmitotic myofibers. How satellite cells differentially regulate nucleocytoplasmic transport of these nuclear proteins critical for regulating gene expression during quiescence, activation, and differentiation is unknown. In addition, how a myofiber with hundreds of nuclei coordinates and regulates nucleocytoplasmic transport is not clear.

## 2. Nuclear Envelope

The nuclear envelope of eukaryotic cells provides separation of the genetic material and transcriptional machinery within the nucleus from the translational machinery in the cytoplasm enhancing regulation of gene expression. The nuclear envelope is comprised of two lipid membrane bilayers, the outer nuclear membrane which is contiguous with the endoplasmic reticulum and the inner nuclear membrane which faces the nucleoplasm (Hetzer and Wentz, 2009). The inner nuclear membrane contains integral transmembrane proteins that interact with lamins within a nuclear lamina meshwork of intermediate-type V filaments that lines the inner nuclear membrane. The nuclear lamina contributes to nuclear envelope stability and provides a platform for proteins involved in chromatin anchoring, DNA replication, and gene transcription (Kind and van Steensel, 2010).

Several studies have revealed a critical role for inner nuclear envelope proteins in regulating the expression of muscle-specific genes during muscle differentiation (Datta *et al.*, 2009; Huber *et al.*, 2009; Liu *et al.*, 2009; Ostlund *et al.*, 2009). For example, loss of function or mutation of lamins or lamin-associated inner nuclear membrane proteins can result in tissue-specific diseases which are referred to as nuclear envelopopathies (Holaska, 2008; Mattout *et al.*, 2006). These diseases encompass a wide range of clinical phenotypes with different envelopopathies affecting different tissues including muscle. Mutations in the nuclear envelope transmembrane protein emerin are associated with Emery-Dreifuss muscular dystrophy (Manilal *et al.*, 1996), while mutations in lamin A/C lead to two muscular dystrophies, Emery-Dreifuss muscular dystrophy and limb-girdle muscular dystrophy 1B (Muchir *et al.*, 2000). Emerin sequesters  $\beta$ -catenin and Lim domain protein, LMO7, at the inner nuclear periphery to regulate their participation in gene transcription; therefore, disease-causing mutations in emerin may disrupt access of these proteins to the transcriptional machinery (Holaska *et al.*, 2006; Markiewicz *et al.*, 2006). In addition, nuclear envelope transmembrane proteins, termed NETs, have been identified, a subset of which are hypothesized to have skeletal muscle-specific roles since they are highly expressed in skeletal muscle tissue compared with other mouse tissues (Chen *et al.*, 2006; Schirmer *et al.*, 2003). Specific roles for NETs appear to exist in signaling pathways during muscle differentiation (Datta *et al.*, 2009; Huber *et al.*, 2009; Liu *et al.*, 2009). For example, during

differentiation of C2C12 cells, a mouse muscle cell line (Yaffe and Saxel, 1977), depletion of NET25 led to elevated mitogen-activated kinase (MAPK) signaling which delayed myogenesis (Huber *et al.*, 2009). In contrast, depletion of NET39 accelerated myogenesis through diminished mammalian target of rapamycin (mTOR) signaling and increased insulin-like growth factor 2 (IGF-2) production (Liu *et al.*, 2009). Together, these studies expose a crucial role for nuclear envelope proteins in regulating gene expression during muscle differentiation. Mutations in nuclear envelope transmembrane proteins may alter signaling at the nuclear envelope and may contribute to the altered gene expression observed in laminopathies affecting skeletal muscle. Further studies will likely uncover functional roles for other nuclear envelope proteins in regulating skeletal muscle gene expression.

### 3. Nuclear Pore Complexes

The nuclear envelope is perforated by nuclear pore complexes (NPCs) which fuse the outer nuclear membrane and inner nuclear membrane together to create channels for nucleocytoplasmic transport (Fig. 10.1; Lim and Fahrenkrog, 2006). NPCs are multiprotein suprastructures (~50 MDa) which provide channels for the nucleocytoplasmic exchange of ions and macromolecules (Alber *et al.*, 2007). While smaller ions and molecules can diffuse through the NPC, molecules larger than ~40 kDa require a targeting signal and a soluble transport receptor to mediate transport through the NPC (Freitas and Cunha, 2009; Rabut *et al.*, 2004; Weis, 2003). The NPC is comprised of ~30 types of nucleoporins or Nups, many of which are present in multiple copies, consistent with the eightfold symmetry of the NPC (Frenkiel-Krispin *et al.*, 2010). Based on the current model of the NPC, Nups are categorized as scaffold, transmembrane, or peripheral Nups (Fig. 10.1; Fernandez-Martinez and Rout, 2009). Scaffold Nups, also termed core Nups, provide structure to the NPC core by forming a cage-like scaffold, while transmembrane Nups located at the nuclear envelope–NPC interface, function in NPC biogenesis and nuclear envelope anchoring (Strambio-De-Castillia *et al.*, 2010). Peripheral Nups within the NPC function in cargo transport, chromatin anchoring, and gene transcription. Peripheral Nups lining the pore channel contain phenylalanine–glycine (FG) repeats that extend into the channel to function in NPC permeability and mediate facilitated transport of macromolecules (Strambio-De-Castillia *et al.*, 2010). The physical mechanics of NPC permeability and transport are still unclear with several models proposing varying arrangements of FG-Nups during transport; however, the functional role of individual FG-Nups in mediating transport and regulating different transport pathways has been well established (Walde and Kehlenbach, 2010).

NPCs within the nuclear envelope display variability in density and distribution between cell types and even within a single cell (Hetzer and Wentz, 2009). For example, NPC density differs between *Xenopus* oocytes (>50 NPCs/mm<sup>2</sup>) and C2C12 muscle cells (5 NPCs/mm<sup>2</sup>) by 10-fold (D'Angelo *et al.*, 2009; Hetzer and Wentz, 2009), meanwhile a 50% increase in NPC density was observed as mouse embryonic stem cells differentiated into cardiomyocytes (Perez-Terzic *et al.*, 2007). In addition, differences in NPC density across the nuclear envelope have been observed in *Saccharomyces cerevisiae* which suggests nuclear transport may be spatially regulated across the nuclear envelope (Winey *et al.*, 1997). NPC nucleoporin composition also varies between cell types where multiple Nups involved in nucleocytoplasmic transport display differential expression between tissues

(Hetzer and Wentze, 2009; Smitherman *et al.*, 2000; Tran and Wentze, 2006). In skeletal muscle, the transcripts for numerous Nups are upregulated during satellite cell activation suggesting that an increase in NPC biogenesis occurs in proliferating satellite cells (Fukada *et al.*, 2007; Pallafacchina *et al.*, 2010). Meanwhile, *Tetrahymena thermophila*, a binucleated ciliated protozoa expresses a subset of Nups that differentially localize to either the macronucleus or the micronucleus to regulate transport of cargoes involved in nucleus-specific functions (Malone *et al.*, 2008). Variations in NPC density, distribution, and nucleoporin composition between cell types or nuclei sharing a common cytoplasm, suggest that NPCs and nucleoporins are regulated to accommodate for ever changing demands on nuclear transport in both mono- and multinucleated cells.

In proliferating eukaryotic cells, new NPCs are formed during mitosis and interphase which allows for regular replacement of Nups (Doucet and Hetzer, 2010). Therefore, *de novo* assembly of NPCs would not be predicted to occur in postmitotic cells. Experiments examining the synthesis of scaffold and peripheral Nups in postmitotic C2C12 myotubes, revealed that some peripheral Nups, such as NUP153 and NUP50, are continuously synthesized, whereas scaffold Nups, such as NUP107 and NUP160, are transcriptionally downregulated in myotubes and are consequently not replaced (D'Angelo *et al.*, 2009). Accumulation or loss of damaged scaffold Nups in nondividing cells, such as muscle, could result in dysfunctional NPCs and loss of integrity of the nucleocytoplasmic barrier. Indeed, oxidized scaffold Nups in neuronal nuclei from aged rats are associated with leakage of cytoplasmic proteins into the nucleus (D'Angelo *et al.*, 2009). These studies have significant implications for the functional integrity of the NPC in quiescent satellite cells and postmitotic multinucleated muscle cells. Further studies are required to determine if Nups in NPCs become damaged in skeletal muscle and whether the nucleocytoplasmic barrier is altered and contributes to muscle dysfunction during disease and aging.

#### 4. Nuclear Import Pathways

Nuclear transport is a process whereby proteins or other macromolecules traverse the NPC either by directly interacting with peripheral FG-Nups within the NPC channel or by binding to import or export receptors that mediate transport through the NPC (Walde and Kehlenbach, 2010). The majority of nuclear transport receptors are karyopherin family members termed importins, transportins, or exportins which mediate transport across the NPC in an energy-dependent manner (Tran and Wentze, 2006; Weis, 2003). Transport receptors tend to be divided into importins, which bind a nuclear localization signal (NLS) within a cargo protein to target it into the nucleus and exportins, which bind a nuclear export sequence (NES) within a cargo protein to target it for export to the cytoplasm (Cook *et al.*, 2007; Kalderon *et al.*, 1984a,b; Kutay and Guttinger, 2005; Lange *et al.*, 2007). Karyopherin transport receptors fall within two families, the karyopherin alpha (KPNA) family and the karyopherin beta family (Wagstaff and Jans, 2009). Increasing evidence suggests that both these karyopherin families have critical roles in controlling the nuclear import and export of key proteins involved in genetic reprogramming and cell adaptation in a large number of cell types and tissues (Kohler *et al.*, 1999; Quensel *et al.*, 2004; Talcott and Moore, 2000; Yasuhara *et al.*, 2007). Below we describe the mechanisms of karyopherin-dependent

nuclear transport and detail what is currently known about these transport receptors and pathways in skeletal muscle.

#### 4.1. Classical nuclear import: Karyopherin alpha family

Classical nuclear import, which is the best characterized of the nuclear transport pathways, is an active process that depends on KPNA and beta family members as well as a classical nuclear localization signal sequence (cNLS) defined by a string of basic residues contained within a protein (Hodel *et al.*, 2001; Kalderon *et al.*, 1984b; Robbins *et al.*, 1991). In *Mus musculus*, 35–55% of nuclear proteins may depend on classical nuclear import for nuclear targeting as determined using a bioinformatics approach (Marfori *et al.*, 2010). KPNA recognize two types of cNLS, a monopartite signal comprised of a single string of basic amino acid residues or a bipartite signal containing two strings of basic variable residues flanking a 10–12 amino acid linker region (Kalderon *et al.*, 1984b; Robbins *et al.*, 1991). The prototypical sequence for the monopartite is the SV40 large-T antigen sequence, PKKKRKV, and for the bipartite, the nucleoplasmin sequence KRPAATKKAGQAKKKK (Hodel *et al.*, 2001; Kalderon *et al.*, 1984a; Robbins *et al.*, 1991). Classical nuclear protein import is mediated by a heterotrimeric complex of KPNA, which recognizes and binds the cNLS signal within a cargo protein, and karyopherin beta1 (KPNB1), which binds KPNA and interacts with FG-Nups within the NPC to mediate nuclear import (Fig 10.2; Matsuura *et al.*, 2003; Matsuura and Stewart, 2005). Once in the nucleus, a small GTPase, Ran-GTP, binds KPNB1, triggering disassembly of the trimeric complex and subsequent cargo release (Cook *et al.*, 2007). Disassembly of the import complex is also facilitated by CAS, the export receptor for KPNA and another member of the karyopherin family (Kutay *et al.*, 1997), and the nucleoporin, NPAP60L (Ogawa *et al.*, 2010). Upon cargo release, KPNA is recycled back to the cytoplasm by CAS, while KPNB1 is returned to the cytoplasm in complex with Ran-GTP (Hood and Silver, 1998; Kutay *et al.*, 1997). Thus, classical nuclear import cycles and directionality depend upon the GTPase, Ran, which facilitates assembly and disassembly of transport complexes (Cook *et al.*, 2007). Ran-GTP, but not Ran-GDP, triggers cargo release in the nucleus upon binding to KPNB1, therefore, the directionality of import is driven by the presence of Ran-GTP in the nucleus and Ran-GDP in the cytoplasm (Lonhienne *et al.*, 2009). Ran-GTP levels are maintained in the nucleus through the nuclear import of Ran-GDP (Ribbeck *et al.*, 1998) and conversion to Ran-GTP by the nuclear localized Ran guanine nucleotide exchange factor (Ran GEF; Cook *et al.*, 2007; Smith *et al.*, 1998). Meanwhile, in the cytoplasm, Ran-GTP is hydrolyzed to Ran-GDP by the Ran GTPase-activating protein (Ran GAP).

KPNB1 is the sole member of the karyopherin beta family to participate in classical nuclear import with KPNA (Liu and Liu, 2007). KPNB1 and other karyopherin beta family members participate in nonclassical nuclear import which involves either direct NLS binding or the use of non-KPNA adaptor proteins. Modeling studies for cNLS import reveal that the addition of the KPNA adaptor to the transport cycle, as opposed to direct protein import by KPNB1 alone, reduces import efficiency (Riddick and Macara, 2007); however, the loss of import efficiency is offset by the addition of multiple KPNA adaptor paralogs that allows for additional points of control over the nuclear localization of cNLS proteins.

*Saccharomyces cerevisiae* contains a single, essential KPNA import receptor. Six KPNA paralogs are found in mouse: KPNA1, KPNA2, KPNA3, KPNA4, KPNA6, and KPNA7 (Hu *et al.*, 2010; Tsuji *et al.*, 1997). Seven KPNA paralogs exist in human with which the mouse homologues share 90% amino acid identity (Kelley *et al.*, 2010; Kohler *et al.*, 1997, 1999; Tsuji *et al.*, 1997). Confusion regarding KPNA protein nomenclature between species exists in the literature. While KPNA gene names between human and mouse are consistent, protein nomenclature using the terms importin alpha or importins does not match between these two species (Table 10.1). In this chapter, we refer to KPNA paralogs using the *KPNA/kpna* nomenclature, instead of importin alpha, to minimize confusion when discussing KPNA paralog function. KPNA paralogs in mouse and human are categorized into three subtypes based on percentage of amino acid identity. Mouse subtypes are Subtype S: KPNA1 and KPNA6; Subtype P: KPNA2; and Subtype Q: KPNA3 and KPNA4. Subtype members share 80–90% amino acid identity, whereas different subtypes share 40–50% amino acid identity (Tsuji *et al.*, 1997). All KPNA paralogs function as nuclear import receptors, but paralogs may differ in their cNLS binding affinities and/or specificities for cNLS proteins (Hodel *et al.*, 2001; Kohler *et al.*, 1999; Quensel *et al.*, 2004; Talcott and Moore, 2000; Timney *et al.*, 2006; Yasuhara *et al.*, 2007). For example, human regulator of chromosome condensation 1, RCC1, which is the Ran GEF, depends solely on KPNA4 to access to the nucleus, while other cNLS proteins, such as RNA Helicase A, may utilize multiple KPNA paralogs to access the nucleus, but may have preference for one paralog over another (Aratani *et al.*, 2006; Quensel *et al.*, 2004). *In silico* experiments suggest that the rate of nuclear import of a cNLS cargo is limited by the levels of KPNA and Ran (Riddick and Macara, 2005).

The steady-state levels of different KPNA paralogs can vary both among tissue types and within a single tissue during differentiation suggesting distinct roles for individual KPNA paralogs in importing key factors required for cell function and differentiation (Goldfarb *et al.*, 2004; Mason and Goldfarb, 2009; Okada *et al.*, 2008; Poon and Jans, 2005). For example, during mouse spermatogenesis, KPNA paralogs are expressed with unique cellular and temporal expression profiles at discrete stages of development (Hogarth *et al.*, 2006). In contrast, during neural differentiation of mouse embryonic stem cells *in vitro*, the steady-state levels of one KPNA paralog increase, while that of another paralog decrease, thereby allowing for differential nuclear import of transcription factors involved in maintaining either the undifferentiated or differentiated state (Yasuhara *et al.*, 2007). This KPNA paralog switching was proposed by the authors of this study as a general mechanism that enables cells to coordinate differentiation by controlling the subcellular localization of transcription factors. In support of the subtype switching model, KPNA steady-state profiles in multiple differentiating human and mouse cell types are characterized by increases in expression of one KPNA paralog with concomitant decreases in another paralog (Kamei *et al.*, 1999; Kohler *et al.*, 1997, 2002; Okada *et al.*, 2008); however, in skeletal muscle cells, an increase in the steady-state levels of all five *Kpnas* was observed during differentiation (Hall *et al.*, unpublished data). The increase in all *Kpnas* may suggest an overall increase in demand for nuclear import during skeletal muscle differentiation. These studies suggest that the role of *KPNAs* in cell differentiation may differ between cell types that express different cNLS-containing cargo proteins.

Additional evidence for the nonredundant roles of individual KPNA paralogs in cellular physiology stems from loss-of-function experiments in model organisms. *Kpna1* null *Drosophila melanogaster* developed normally but displayed defects in gametogenesis resulting in sterility in both males and females (Ratan *et al.*, 2008). Similarly, male and female sterility occurred in *Kpna2* null flies (Mason *et al.*, 2002). The sterility in females could be rescued only by *Kpna2* transgenes, whereas the sterility in males could be rescued by *Kpna1*, *Kpna2*, or *Kpna3* transgenes suggesting distinct requirements for KPNA2 in male and female gametogenesis (Mason *et al.*, 2002). In contrast, *Kpna3* null flies displayed defects throughout development, whereas late stages of development and photoreceptor development could only be rescued with *Kpna3* but not *Kpna1* or *Kpna2* transgenes (Mason *et al.*, 2003). RNAi experiments in *Caenorhabditis elegans* demonstrated that KPNA3 but not KPNA2 is required for oocyte development (Geles and Adam, 2001). Together, these results in genetic model organisms support the notion that KPNA paralogs have evolved distinct functions in different cell types during development.

Loss-of-function experiments *in vitro* also provide support for distinct roles of KPNA paralogs in controlling cell proliferation and differentiation. During neural differentiation of mouse embryonic stem cells, depletion of KPNA1 by RNAi-mediated knockdown resulted in accelerated neural differentiation, while loss of KPNA5 delayed differentiation (Yasuhara *et al.*, 2007). In primary mouse muscle cells, the nonredundant roles for individual paralogs were revealed by siRNA experiments in which KPNA1 knockdown increased myoblast proliferation but KPNA2 knockdown decreased proliferation (Hall *et al.*, unpublished data). In contrast, no proliferation defect was observed with KPNA4 knockdown. KPNAs import negative regulators of proliferation, such as Rb and p27Kip1 in other cell types (Hu *et al.*, 2005; Shin *et al.*, 2005), so results of these RNAi experiments could suggest that KPNA1 imports a negative regulator of proliferation during myogenesis. Contrary to these findings, in HeLa cells, RNAi-mediated knockdown of each KPNA, including KPNA1, resulted in a decrease in cell proliferation, which suggests that the role of individual KPNAs in cell proliferation may differ between cell types (Kohler *et al.*, 2002). Contrary to the results obtained *in vitro*, a recent study examining a *Kpna1* null mice revealed normal development of brain and other tissues, however, KPNA1 may have a different role in proliferation in the brain or compensation by other KPNAs may have occurred during development (Shmidt *et al.*, 2007). Further evidence for the nonredundant roles of KPNA paralogs in skeletal muscle comes from knockdown experiments where depletion of KPNA2, but not KPNA1 or KPNA4, resulted in reduced myotube size and decreased ability of muscle cells to migrate (Hall *et al.*, unpublished data). The small myotube phenotype observed may be due to the reduced import of multiple cNLS-containing proteins involved in regulating cell migration. Indeed, a similar migration defect was also observed upon loss of KPNA2 in a lung cancer cell line (Wang *et al.*, 2010). These studies suggest that myoblast proliferation and myotube growth rely on specific KPNA paralogs to regulate the nuclear import of key factors involved in proliferation and myotube growth during myogenesis.

Classical nuclear import has been implicated in transporting cargoes across large distances or from specific sites in the cell to the nucleus. This phenomenon has been most extensively studied in neurons (Lai *et al.*, 2008; Mikenberg *et al.*, 2007; Thompson *et al.*, 2004), but



emerging evidence supports the presence of such spatial signaling in skeletal muscle. In rodent hippocampal neurons, classical nuclear import mediates the transport of cNLS cargoes from the synapse to the nucleus upon receptor activation (Thompson *et al.*, 2004). In skeletal muscle cells, KPNA mediates the nuclear import of myopodin, an actin bundling protein (Faul *et al.*, 2007), which has been shown to shuttle between the sarcomeric Z-disc and the nucleus in a differentiation and stress-dependent manner (Weins *et al.*, 2001). However, the role of myopodin nucleocytoplasmic shuttling during cell differentiation and stress is unclear. In contrast, a Z-disc-associated protein with known nuclear function is muscle limb protein (MLP), which acts as a mechanosensor in rat cardiomyocytes (Boateng *et al.*, 2009). Loss of cNLS-dependent import of MLP results in disarranged sarcomeres. Another cargo that undergoes similar shuttling is serum response factor (SRF), a transcription factor required for skeletal muscle growth that shuttles between the sarcomere and the nucleus (Li *et al.*, 2005). The nuclear import of SRF occurs via KPNA1/KPNB1 of the classical nuclear import pathway (McConville *et al.*, 2010). These findings suggest that nucleocytoplasmic import has a significant role in transmitting signals from sarcomeres to the nucleus in response to stimuli that induce muscle cell remodeling to adapt to cellular stress. Further studies should shed light on the role of classical nuclear import in overcoming the unique spatial challenges of signaling in a multinucleated muscle cell.

#### 4.2. Karyopherin beta family members mediate import and export

Karyopherin beta family members comprise the majority of nuclear transport receptors which includes karyopherin beta import receptors (importins or transportins) involved in protein import and exportins involved in protein export (Cook and Conti, 2010; Cook *et al.*, 2007). Similar to KPNA-dependent nuclear import, the karyopherin beta nuclear import pathway is dependent upon energy and the Ran gradient for directionality (Lonhienne *et al.*, 2009). Karyopherin beta import receptors bind directly to nonclassical NLSs in cargo proteins to mediate import (Fig. 10.2) or may use a non-KPNA adaptor protein for NLS cargo recognition (Cook *et al.*, 2007; Kutay and Guttinger, 2005; Lange *et al.*, 2007). Currently, only karyopherin beta2-dependent cargoes have defined NLSs termed PY-NLS, while cargoes depending on other karyopherin beta family members do not have recognizable amino acid sequences that comprise NLS motifs (Marfori *et al.*, 2010).

In total, 14 karyopherin beta import receptors exist in *S. cerevisiae*, while humans have over 19 karyopherin betas (Chook and Suel, 2010). Karyopherin beta family members display protein homology ranging from 15% to 20% and function in the import of distinct sets of proteins, RNAs, and Nups (Chook and Suel, 2010; Marfori *et al.*, 2010). KPNB1, the best characterized member of the karyopherin family, is one of four essential karyopherin beta family members in *S. cerevisiae* (Chook and Suel, 2010). In contrast to classical nuclear import, KPNB1-dependent nuclear import does not occur through a conserved receptor–cargo binding conformation which provides KPNB1 with the flexibility to bind a wide variety of cargoes containing unique classes of NLS signals (Fiserova *et al.*, 2009; Marfori *et al.*, 2010).

Several studies provide evidence that karyopherin beta family members have critical roles in regulating the cellular localization of different NLS proteins involved in myogenesis and

neuromuscular junction physiology (Giagtzoglou *et al.*, 2009; Higashi-Kovtun *et al.*, 2010; Mosca and Schwarz, 2010; van der Giessen and Gallouzi, 2007). In C2C12 myoblasts, the nuclear import of the RNA-binding protein HuR depends upon the karyopherin beta family member transportin-2 (van der Giessen and Gallouzi, 2007). HuR is a RNA-binding protein involved in regulating the stability of mRNA transcripts encoding MyoD and Myogenin which are myogenic transcription factors required for differentiation (Figuroa *et al.*, 2003; van der Giessen and Gallouzi, 2007). During differentiation, cleavage of HuR prevents its nuclear import by transportin-2 which results in the stabilization of MyoD and Myogenin mRNAs and enhancement of myogenesis (Mazroui *et al.*, 2008). Several studies using a *Drosophila* model system have uncovered roles for karyopherin beta family members in regulating the import of proteins involved in postsynaptic membrane development and neurotransmitter release at the neuromuscular junction (Giagtzoglou *et al.*, 2009; Higashi-Kovtun *et al.*, 2010; Mosca and Schwarz, 2010). Wingless signaling at the neuromuscular junction causes cleavage and release of the C terminus of Frizzled2 (Fz2-C), which is then imported into the nucleus by KPNA2/KPNB1, or by the karyopherin beta family member karyopherin-beta11 (Mosca and Schwarz, 2010). In *Drosophila* mutants lacking either KPNA2 or karyopherin-beta11, a reduction in the nuclear import of Fz2-C and defects in the postsynaptic membrane were observed suggesting that multiple transport pathways are required for membrane development at the neuromuscular junction. Another karyopherin beta involved in neuromuscular junction physiology is *Drosophila* karyopherin beta13 which controls neurotransmitter release and intracellular Ca<sup>2+</sup> levels at the neuromuscular junction (Giagtzoglou *et al.*, 2009). These data suggest that karyopherin beta family members regulate myogenesis, neuromuscular development and neurotransmitter release by importing a variety of proteins, including RNA-binding proteins and cell-surface receptor components.

Karyopherin beta family members also play roles in facilitated nuclear export of proteins to the cytoplasm (Cook and Conti, 2010; Wente and Rout, 2010). The best understood export pathway is the recognition of a classical nuclear export signal (NES) by the karyopherin beta, CRM1 (Fig. 10.2). The classical NES signal consists of a short string of hydrophobic leucine-rich residues, which are difficult to identify because they share sequence similarity with the hydrophobic cores of most proteins (Cook *et al.*, 2007). Prototypical sequences for an NES are the cyclin D NESs, RFLSLEPL, and TPTDVRDVI as well as the mitogen-activated protein kinase kinase (MAPKK) NES, LQKKLEEL (Kutay and Guttinger, 2005; Poon and Jans, 2005). Export receptors or exportins recognize and bind export cargoes while bound to the GTPase, Ran-GTP, in an obligate trimeric complex (Cook *et al.*, 2007; Kutay and Guttinger, 2005).

As with facilitated nuclear import, directionality of export is driven by the compartmentalization of Ran-GTP in the nucleus and Ran-GDP in the cytoplasm. At least six exportin genes are found in mouse and human (Okada *et al.*, 2008). These exportins facilitate the nuclear export of a variety of cargoes with some exportins displaying different specificity for individual cargoes. Similar to NLS-mediated import, NES-containing proteins may be exported by a single exportin or may utilize multiple exportins (Okada *et al.*, 2008). For example, the essential yeast exportin, CSE1, or CAS in vertebrates, has one export

cargo, KPNA of the classical nuclear import pathway (Cook *et al.*, 2007), while the best characterized exportin, CRM1(XPO1 or exportin-1), mediates export of at least 10 different classical NES-containing proteins (Cook *et al.*, 2007; Shen *et al.*, 2010; Wada *et al.*, 1998). Exportin family members also facilitate the export of tRNAs and pre-miRNAs (Cook *et al.*, 2007; Lund *et al.*, 2004). Together, these studies suggest exportins may have differing roles in the export of a wide variety of cargo proteins and RNAs required for proper cell function.

Nucleocytoplasmic shuttling via CRM1-dependent export and KPNA-dependent import coordinate the nuclear steady-state levels of proteins critical to muscle cell biology. For example, the nuclear localization of the transcription factor NF- $\kappa$ B is controlled by the subunit p65 which mediates both import and export of the complex through binding to KPNA/KPNB1 or CRM1, respectively (Micheli *et al.*, 2010; Zerfaoui *et al.*, 2010). The nuclear accumulation of p65 NF- $\kappa$ B suppresses MyoD transcription (Guttridge *et al.*, 2000) therefore modulation of nuclear import or export of p65 could control p65 NF- $\kappa$ B activity, MyoD expression, and ultimately myogenesis. The nuclear import and export of the forkhead box transcription factor FOXO3a is critical for skeletal muscle atrophy (Sandri *et al.*, 2004). In C2C12 cells, the nuclear import of Foxo3a was observed upon inhibition of the phosphatidylinositol 3-kinase, PI3K/Akt pathway, while nuclear export of FOXO3a was observed upon activation of the stress-activated protein kinase (SAPK) pathway (Clavel *et al.*, 2010). Control over the cellular localization of FOXO3a by two different signaling pathways may provide global control over atrophy by regulating the transcription of genes, such as *Atrogin-1*, that are involved in skeletal muscle atrophy (Clavel *et al.*, 2010). Control over the nucleocytoplasmic shuttling of cargo proteins by different transport pathways may provide global control over gene expression during myogenesis.

## 5. Identifying Classical Nuclear Import-Dependent Cargoes

Identifying the specific cargo proteins that are transported via various nucleocytoplasmic transport pathways is key for understanding the regulatory networks that govern cell function. Here we focus on how cNLS-dependent cargoes are identified since this pathway is the best characterized nuclear transport pathway; however, many of the challenges in cargo identification presented here apply also to other receptor-mediated transport pathways. In *Mus musculus*, 30–55% of nuclear proteins are predicted to depend on classical nuclear import (Marfori *et al.*, 2010). However, only a few proteins with key functional roles in muscle are known to contain a functional cNLS, such as NOTCH (Huenniger *et al.*, 2010) and NFATc2 (Okamura *et al.*, 2000). While bioinformatics approaches exist to identify classical nuclear import signal sequences within cargoes, these putative cargoes still require functional testing to ensure that such signals actually mediate transport via this pathway.

Putative cNLS motif sequences within proteins can be identified with prediction software (Cokol *et al.*, 2000; Horton *et al.*, 2007; Kosugi *et al.*, 2009; Nguyen Ba *et al.*, 2009). The consensus sequence for the monopartite cNLS has been characterized in both structural and thermodynamic studies where the first residue is a lysine followed by a second and fourth basic residue as follows: K(K/R)X(K/R) (Conti and Kuriyan, 2000; Fontes *et al.*, 2000; Hodel *et al.*, 2001). The consensus sequence for the bipartite cNLS has also been characterized as KRX10-12KRRK (Fontes *et al.*, 2003). Small deviations from the

consensus sequence may increase or decrease KPNA-cargo binding affinity, while large deviations likely result in failed import because KPNA-cargo binding is either too weak or too strong for efficient cargo import and release (Lange *et al.*, 2007). A drawback to cNLS prediction algorithms is that linear sequence is analyzed and these algorithms do not identify nonlinear synthetic cNLS signals created through intra- or interprotein interactions. For example, signal transducer and activator of transcription (STAT1) forms a homodimeric complex in which each dimer contributes basic residues to form a functional synthetic cNLS that is not detected by current cNLS algorithms (Fagerlund *et al.*, 2002). While cNLS prediction models identify consensus sequences, functional studies meeting several criteria must be performed before a cNLS is deemed functional (Lange *et al.*, 2007). A cNLS is functional if it is both necessary and sufficient for import of the cargo protein and import of the cargo depends upon the classical nuclear import machinery (Lange *et al.*, 2007).

An alternative approach to identifying cNLS-dependent cargoes is a candidate-based approach which involves transport receptor loss-of-function experiments, whereby a phenotype observed upon receptor depletion may offer hints to potential cargo. A candidate-based approach may prove difficult since the phenotypes observed during depletion of transport receptors are likely combinatorial due to the altered nuclear transport of many cargo proteins that are involved in regulating a large number of genes. Overall, identifying cargoes dependent upon classical nuclear import receptors will be critical to understanding the role of nucleocytoplasmic transport in regulating cell function and fate in skeletal muscle.

## 6. Remodeling of the Nuclear Transport Machinery

Alterations in global nucleocytoplasmic transport provide another layer of control over gene expression. Global changes in the efficiency or rate of nuclear transport can occur through alterations or remodeling of key components of the nuclear transport machinery. For example, altering the expression or localization of karyopherin transport receptors, Ran and/or Ran-associated proteins, or Nups results in changes in transport efficiency (Hodel *et al.*, 2001, 2006; Riddick and Macara, 2005; Timney *et al.*, 2006; Wagstaff and Jans, 2009). A muscle cell could alter nucleocytoplasmic transport to adjust for changes in demand for nuclear transport over a wide range of cellular conditions such as cell quiescence, proliferation, differentiation, stress, aging, and disease.

Experimental evidence indicates that steady-state levels for different components of the nuclear transport machinery can vary during myogenesis. Microarray analyses suggest that satellite cell entry into the cell cycle is marked by global remodeling of the nuclear transport machinery (Fukada *et al.*, 2007; Pallafacchina *et al.*, 2010). A wide variety of mRNAs that encode components of the nuclear transport machinery, such as nucleoporins and various karyopherin transport receptors, were increased in proliferating satellite cells *in vivo* compared to quiescent satellite cells. This widespread upregulation of the nuclear transport machinery may be functionally required to allow for rapid changes in gene expression associated with the myogenic lineage progression of satellite cells. Nuclear pore composition can influence stem cell differentiation as evidenced from studies of NUP-133-deficient epiblast and embryonic stem cells in mice which differentiated inefficiently along

the neural lineage (Lupu *et al.*, 2008). Nuclear transport machinery remodeling has also been observed during muscle differentiation, however the extent and type of remodeling differs among muscle cell types. In mouse primary muscle cells, the steady-state levels of all five KPNA import receptors increased during skeletal muscle differentiation suggesting an increase in demand for nuclear import during differentiation (Hall *et al.*, unpublished data). The differentiation of mouse embryonic stem cells into a cardiac lineage resulted in the downregulation of karyopherins, exportins, Nups- and Ran-related proteins, while an increase in NPC density was observed along with the expansion of individual NPC diameter suggesting an increase in demand for nuclear transport during cardiac differentiation (Perez-Terzic *et al.*, 2007). In contrast, during the differentiation of C2C12s, the steady-state levels of Nup proteins and overall density of NPCs remained constant (D'Angelo *et al.*, 2009). Differences in nuclear transport machinery remodeling appear to be cell type-dependent and suggest that remodeling of the nuclear transport machinery is a key process that controls global nucleocytoplasmic transport during cellular differentiation. Characterizing the functional role of members of the nuclear transport machinery during skeletal muscle proliferation and differentiation will be essential to understanding the role of nucleocytoplasmic transport as a driver of muscle cell differentiation and function.

Remodeling of the nuclear transport machinery has also been observed during cellular response to chemical or mechanical stress in multiple cell types. Remodeling of the nuclear transport machinery to reduce or block transport during cellular stress may allow a cell to globally “pause” gene signaling pathways in order to redirect gene expression to respond to a particular cellular stress. Cellular stress, such as oxidative stress, can inhibit both import and export transport receptors and reduce the levels, localization and posttranslation modifications of several Nups involved in nuclear export (Crampton *et al.*, 2009; Kodiha *et al.*, 2008; Miyamoto *et al.*, 2004). In vascular smooth muscle cells (VSMCs), remodeling of the nuclear import machinery was observed during exposure to ceramide, a antiproliferative sphingolipid implicated in the final stage of atherosclerotic plaque formation. Ceramide treatment of cultured VSMCs resulted in reduced cell proliferation and inhibition of classical nuclear import due to mislocalization of KPNA and CAS to the cytoplasm (Faustino *et al.*, 2008). Nuclear transport machinery remodeling during mechanical stress was also observed during stretching of VSMCs which results in smooth muscle cell hyperplasia and hypertrophy (Richard *et al.*, 2007). Mechanical stretching of VSMCs *in vitro* resulted in an increase in nuclear import and the steady-state protein levels of Nups, along with alterations in MAPK signaling. Further studies are required to determine whether remodeling of the nuclear transport machinery also occurs with oxidative or mechanical stress in skeletal muscle cells and what role it may play in controlling the nuclear localization of critical proteins necessary for cellular responses to these physiologic perturbations.

Aging and disease are also associated with changes in the nuclear transport machinery. In myocardial microvascular endothelial cells and human fibroblasts, a reduction in expression of KPNA and therefore, classical nuclear import occurred with cellular aging (Ahluwalia *et al.*, 2010; Pujol *et al.*, 2002). Another study using HeLa cells expressing a mutant form of Lamin A responsible for the premature aging disease, Hutchinson Gilford progeria

syndrome, revealed a reduction in nuclear import efficiency along with alterations in the localization of NUP62, NUP153, and the exportin CRM1 (Busch *et al.*, 2009), suggesting that remodeling of the nuclear import machinery may have a role in disease pathology. In contrast, human cardiomyocytes from patients with heart failure, displayed an increase in karyopherin import receptors, exportins, Ran regulators, and Nups as well as differences in NPC configuration and morphology as compared to healthy cardiomyocytes (Cortes *et al.*, 2010) suggesting that remodeling may occur as a disease response. These findings suggest that changes in the nuclear transport machinery may occur either as result of aging and disease or may occur in response to disease to restore tissue function. Given the extensive loss of skeletal muscle mass that can occur with aging or disease, further studies are warranted to determine if remodeling of the nuclear transport machinery also occurs with age or disease in skeletal muscle and whether nucleocytoplasmic communication is impaired as a consequence.

## 7. Challenges in Studying Nucleocytoplasmic Transport in Multinucleated Cells

The basic mechanics for nucleocytoplasmic transport of proteins and RNA identified to date and described in this review have almost exclusively been examined in cells with a single nucleus. Skeletal muscle is the only permanent multinucleated cell type in the body and constitutes ~50% of body mass; yet, how these cells spatially and temporally regulate and coordinate nucleocytoplasmic transport among hundreds of nuclei is unknown.

Spatial and temporal regulation of nucleocytoplasmic transport between nuclei likely occurs within a single myofiber since transcriptional activity of specific gene loci can differ among nuclei within the same myofiber. Such differences in transcription could arise from specific regional requirements for cell function. Myonuclei at the neuromuscular junction express transcripts for subunits of the acetylcholine receptor at much higher levels than nonsynaptic nuclei leading to the accumulation of the acetylcholine receptor protein at the neuromuscular junction (Burden, 1993; Fontaine and Changeux, 1989; Sanes *et al.*, 1991; Simon *et al.*, 1992) and thereby facilitating coordinated neuronal activation of muscle contraction. In addition, the myonuclei located at the myotendinous junction in stretched myofibers express the transcript for sarcomeric myosin heavy chain at higher levels than other myonuclei (Dix and Eisenberg, 1990), thereby enhancing sarcomere addition and cell growth at the ends of myofibers in response to muscle stretching. Less clear are the reasons why myonuclei distributed along the length of a myofiber and at times even right next to each other exhibit differences in the transcription of both endogenous genes and transgenes (Newlands *et al.*, 1998). Transcriptional differences among nuclei also occur in myotubes *in vitro* (Berman *et al.*, 1990; Su *et al.*, 1995). The molecular mechanisms responsible for such nuclear diversity in transcriptional activity in skeletal muscle are unknown. Differences in transcriptional activity among nuclei in a common cytoplasm also occur in other cell types and organisms. For example, only a subset of nuclei in multinucleated mouse osteoclasts or human placental syncytiotrophoblasts is transcriptionally active (Ellery *et al.*, 2009; Youn *et al.*, 2010). In binucleated *Tetrahymena thermophila*, the macronucleus is transcriptionally active, whereas the micronucleus is transcriptionally inert (Karrer, 2000). Further, nuclei in the syncytial

blastoderm of *Drosophila* and the syncytial germ line of *C. elegans* are also transcriptionally distinct (Burden, 1993). The molecular mechanisms responsible for the transcriptional differences among nuclei in these other cell types and organisms are not fully elucidated.

Nuclear proteins are localized to some nuclei and not others within a myofiber which further suggests differential nuclear targeting occurs in skeletal muscle. One such protein is endonuclease G, which is a mitochondrial protein that can translocate to nuclei and induce DNA fragmentation and apoptosis independent of caspase. At the initiation of disuse muscle atrophy, endonuclease G translocates to a subset of myofiber nuclei (Dupont-Versteegden *et al.*, 2006) and may serve as a means to control the loss of myonuclei commonly observed in disuse atrophy without cell death. Other examples of such proteins are specific nuclear envelope proteins which are more highly concentrated in synaptic myonuclei compared to nonsynaptic nuclei. These include Syne-1, Syne-2, and nesprin-1 $\alpha$  which appear to participate in nuclear localization and/or anchoring (Apel *et al.*, 2000; Grady *et al.*, 2005; Puckelwartz *et al.*, 2010; Zhang *et al.*, 2007). Unequal nuclear localization of proteins is also observed in cultured myotubes. These include transcription factors with important roles in muscle differentiation and growth such as NFAT5 (O'Connor *et al.*, 2007), NFATc1 (Abbott *et al.*, 1998), and MyoD (Ferri *et al.*, 2009), the growth inhibitory protein, myostatin (Artaza *et al.*, 2002) as well as MYO18B (Salamon *et al.*, 2003), an unconventional myosin heavy chain with an unidentified role in muscle physiology. The mechanisms that govern differential protein targeting among neighboring myonuclei *in vivo* and *in vitro* are unknown but may be related to nucleus-specific transport mechanisms and merit further study.

Studies in *Tetrahymena* provide potential clues for how nucleus-specific transport mechanisms may be regulated in multinucleated myofibers. Certain nuclear proteins in *Tetrahymena* are selectively accumulated in either macro- or micro-nuclei (White *et al.*, 1989). Analyses of GFP-labeled KPNA proteins revealed that 9 of the 13 KPNA proteins localized exclusively to the micronucleus suggesting that nucleus-specific transport systems must exist (Malone *et al.*, 2008). Further studies demonstrated that the NPCs of macronuclei and micronuclei contain unique subsets of FG-containing nucleoporins which are responsible for this nuclear selectivity (Iwamoto *et al.*, 2009; Malone *et al.*, 2008). Interestingly, homologs of NUP98 contributed to nuclear selectivity: two NUP98 homologs localized exclusively to macronuclei, whereas the other two exclusively localized to micronuclei. Specific structural components of the NUP98 homologs were functionally required for the nuclear selectivity as shown by chimeric protein experiments (Iwamoto *et al.*, 2009). The NUP98 homologs that localized to the macronucleus contained amino acid repeats of GLFG, whereas homologs that localized to the micronucleus lacked GLFG and instead contained novel NIFN repeats. These results suggest that structural alterations of the NPC can contribute to nucleus-specific protein transport in a multinucleated cell. Such structural alterations may modulate the interaction of karyopherin transport receptors with specific components of the NPC and consequently alter nuclear accumulation of proteins. Immunofluorescence analyses of primary mouse myotubes *in vitro* reveal that the steady-state levels of KPNA2 differ among nuclei supporting the hypothesis that specific karyopherin transport receptors may also undergo selective nuclear targeting in skeletal muscle as in *Tetrahymena* (Hall *et al.*, unpublished data). Further studies are required to

define the contribution of NPC composition and karyopherin transport receptors to nuclear differences in transcription and protein content in skeletal muscle.

## 8. Summary

Nucleocytoplasmic transport plays a key regulatory role in cellular physiology. While much is known about facilitated nuclear transport in other cell types, the study of nucleocytoplasmic transport in skeletal muscle is still in its infancy. Multinucleated myofibers are faced with unique challenges compared to most other mammalian cell types in controlling the function of hundreds of nuclei in a common cytoplasm. Although a fair bit is known about nuclear envelope proteins in skeletal muscle because of their association with several muscular dystrophies, very little is known about the NPC or karyopherin transport receptors. Further knowledge about the nuclear transport machinery is needed in skeletal muscle to enhance our understanding of how gene expression is controlled in normal, aged, and diseased muscle as well as to provide insight into satellite cell biology.

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## Abbreviations

<b>cNLS</b>	classical nuclear localization signal
<b>NLS</b>	nuclear localization signal
<b>Kpna</b>	karyopherin alpha
<b>kpnb1</b>	karyopherin beta1
<b>NPC</b>	nuclear pore complex
<b>Nup</b>	nucleoporin

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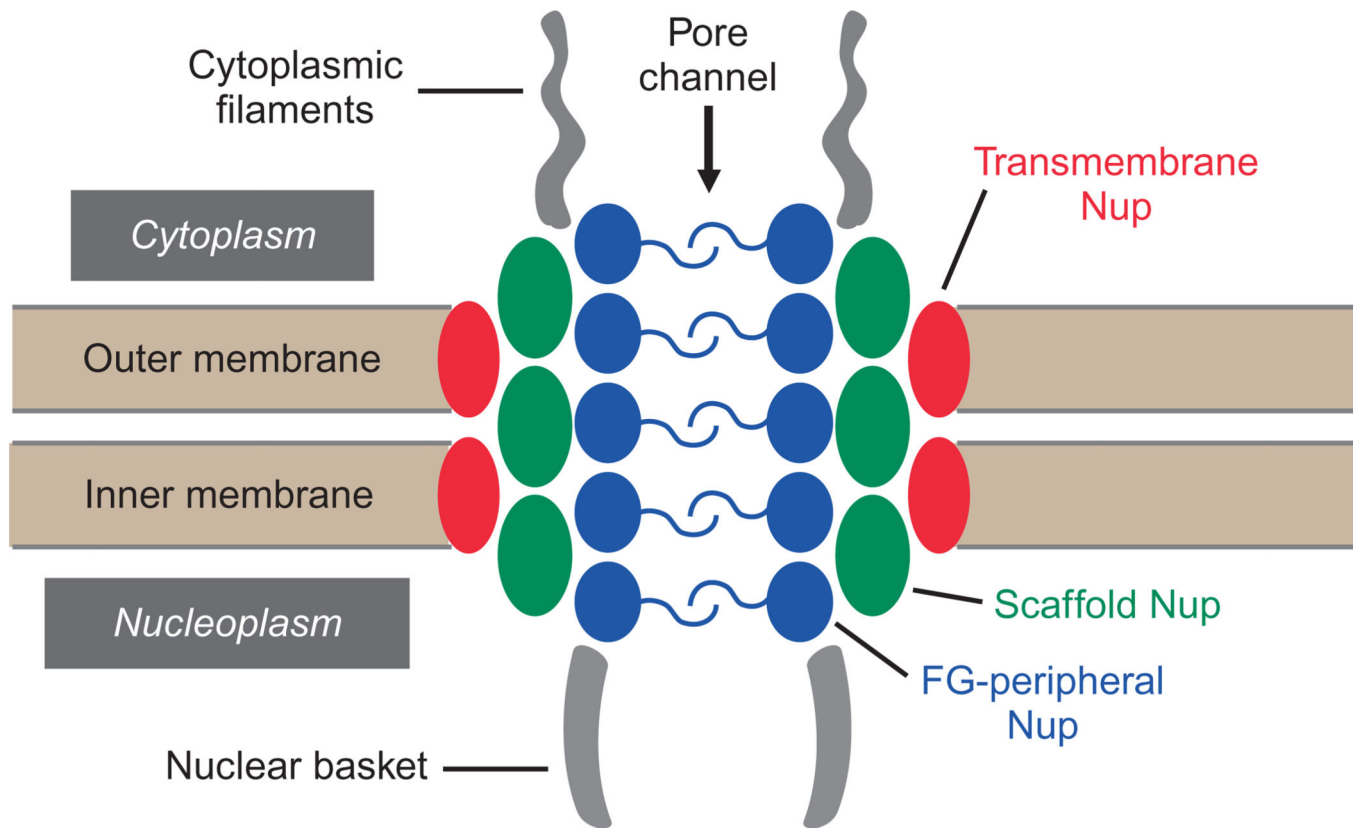
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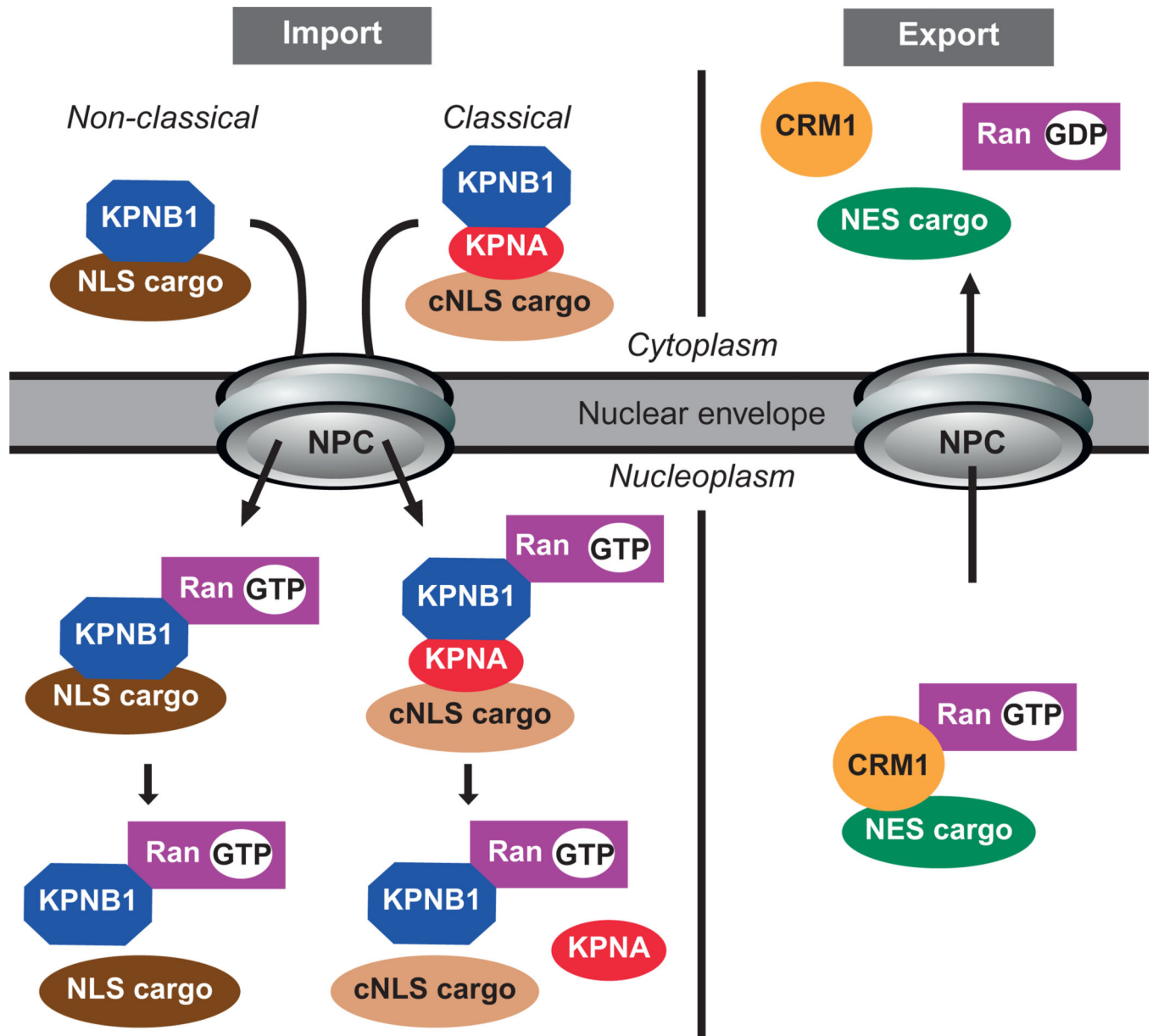
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**Figure 10.1.**

Schematic illustrating the relative location of various Nups within the NPC. The NPC resides within the nuclear envelope, a bilipid membrane comprised of an inner and outer nuclear membrane. The NPC has cytoplasmic filaments that extend into the cytoplasm and a nuclear basket that extends into the nucleoplasm. Peripheral Nups containing FG repeats line the pore channel to function in NPC permeability and the facilitated transport of macromolecules. Transmembrane Nups localize to the nuclear envelope–NPC interface, while scaffold Nups reside between transmembrane and peripheral Nups.



**Figure 10.2.**

Basic model of karyopherin-mediated nuclear import and export pathways. The nuclear import pathway involves the import receptors karyopherin alpha (KPNA) and/or KPNB1 which can recognize proteins containing a classical (cNLS) or nonclassical (NLS) nuclear localization signal, respectively. The nuclear import of a cNLS-containing cargo involves both KPNA and KPNB1 import receptors, since KPNA recognizes the cNLS motif in the cargo protein and then KPNB1 mediates translocation of the import complex through the NPC by interacting with FG-Nups within the NPC. Once in the nucleus, Ran-GTP binding to KPNB1 results in the dissociation of the import complex and cargo release into the nucleus. The nuclear export pathway consists of an obligate trimeric complex consisting of the exportin (CRM1 for classical NES-containing cargo), export cargo, and Ran-GTP. Translocation of the complex through the NPC is mediated by interaction between the

exportin and FG-Nups. Once in the cytoplasm, the hydrolysis of Ran-GTP to Ran-GDP results in the dissociation of the export complex and subsequent cargo release.

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Table 10.1

Karyopherin alpha paralogs in different organisms

<i>Homo sapiens</i>		<i>Mus musculus</i>		<i>Drosophila melanogaster</i>		<i>Saccharomyces cerevisiae</i>	
Subtype	Gene name	Importin designation	Gene name	Importin designation	Gene name	Importin designation	Importin designation
S	<i>KPNA1</i>	alpha5	<i>Kpna1</i>	alpha1	<i>Kap-alpha1</i>	<i>Srp1</i>	Kap60
	<i>KPNA5</i>	alpha6	-	-	-	-	-
	<i>KPNA6</i>	alpha7	<i>Kpna6</i>	alpha6	-	-	-
P	<i>KPNA2</i>	alpha1	<i>Kpna2</i>	alpha2	<i>Pen</i>	alpha2	-
	<i>KPNA7</i>	alpha7	<i>Kpna7<sup>a</sup></i>	alpha7 <sup>a</sup>	-	-	-
Q	<i>KPNA3</i>	alpha4	<i>Kpna3</i>	alpha3	-	-	-
	<i>KPNA4</i>	alpha3	<i>Kpna4</i>	alpha4	<i>Kap-alpha3</i>	alpha3	-

KPNA family members are categorized into three subtypes, S, P, and Q based on amino acid sequence homology.

*Saccharomyces cerevisiae* has a single karyopherin (SRP1), while *Homo sapiens*, *Mus musculus*, and *Drosophila melanogaster* each contain multiple karyopherin paralogs. The gene name and importin alpha designation are given for each species to clarify confusion regarding karyopherin/importin designations between species. A dash (-) indicates the absence of a karyopherin homologue in that species.

<sup>a</sup> Placement of recently discovered murine KPNA7 into subtype P is tentative.