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Restricted expression of karyopherin alpha mRNA in the sea urchin suggests a role in neurogenesis

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ABSTRACT

Karyopherin alpha (KAP-α) proteins are critical for the transport of many molecules into the nucleus. In this study, we identified three members of the KAP-α family in the sea urchin *Lytechinus variegatus* and described the developmental expression of these proteins. Although many importins are assumed to have ubiquitous expression, we found that all three genes were differentially expressed. Both LvKPNA1/5/6 and LvKPNA3/4 accumulated at high levels during cleavage, exhibiting cyclic expression as cells divided. By the blastula and gastrula stages expression decreased, remaining highest in the vegetal plate and archenteron, and by the prism/pluteus stages expression was restricted to the oral surface and gut. Expression of a third KAP-α gene, LvKPNA2/7, was examined in embryos from the mesenchyme blastula to pluteus stages. LvKPNA2/7 mRNA is present in vegetal cells of the mesenchyme blastula and, during gastrulation, it is localized to the archenteron and appears in additional groups of ectodermal cells. Prism/pluteus stage embryos expressed LvKPNA2/7 in the gut and scattered distribution of transcripts in the ciliary band resembled expression patterns of neural cells. We hypothesize that LvKPNA2/7 maintains pluripotency in the neural precursors prior to activation of neural differentiation and believe that this study is an important first step in an effort to better understand the roles of importins during embryogenesis.

Nuclear transport is a process critical to the survival of any eukaryote. Even organisms as simple as yeasts produce proteins specialized to transfer molecules across the nuclear envelope. To do this, cargo often pass through a cylindrical aqueous channel called the nuclear pore complex (NPC). Highly conserved among eukaryotes (DeGrasse et al., 2009), the NPC is a massive macromolecular structure. For example, in yeast a single NPC is composed of 456 individual proteins that can be categorized into 30 distinct protein types called nucleoporins (Alber et al., 2007). Although molecules smaller than 40 kDa can freely diffuse through the NPC, translocation of larger macromolecules (e.g. RNA and proteins) requires active transport mediated by the karyopherins (Alber et al., 2007). Karyopherins attach to cargo and form low affinity but highly specific bonds with the nucleoporins. Repeated phenylalanine residues in the nucleoporins insert into crevices between alpha helices of the karyopherin, allowing the cargo–karyopherin complex to quickly transfer across the nuclear membrane (Aitchison and Rout, 2012).

Karyopherins are a diverse group of proteins and each is specialized to transport a specific cargo type. While some karyopherins (importins) typically transport cargo from the cytoplasm to the nucleus, others are most likely to transfer cargo from the nucleus to the cytoplasm (exportins) or to shuttle cargo in both directions (transportins). Further descriptions of the specific cargo of each karyopherin type are reviewed in Mosammaparast and Pemberton (2004) as well as Chook and Süel (2011).

To detect a specific cargo type, karyopherins bind to signal sequences in the cargo. During nuclear import, karyopherins bind to the nuclear localization signal (NLS). This signal sequence is often strongly hydrophilic, but can vary widely in composition, again allowing the karyopherins to functionally specialize in the types of cargo transported (Poon and Jans, 2005). Similarly, during nuclear export, cargo molecules bind to the karyopherin via a nuclear export signal (NES) (Görlich and Kutay, 1999). By preferentially masking the NLS and/or NES signals (e.g. by phosphorylation or binding of a heterologous protein to these sites), cells can regulate transport of cargo across the NPC (Kauffman et al., 1998; Li et al., 1998; reviewed in Poon and Jans, 2005).

There are two structurally distinct families of karyopherin proteins: the karyopherin alpha (KAP-α) family and the karyopherin beta (KAP-β) family. While many of the KAP-β importins interact

Abbreviations: ASW, artificial seawater; ESC, embryonic stem cell; IMP, importin; KAP-α, karyopherin alpha; KAP-β, karyopherin beta; KPNA, karyopherin alpha; NES, nuclear export signal; NLS, nuclear localization signal; NPC, nuclear pore complex; RanBP1, Ran binding protein 1; SAF, spindle assembly factor.

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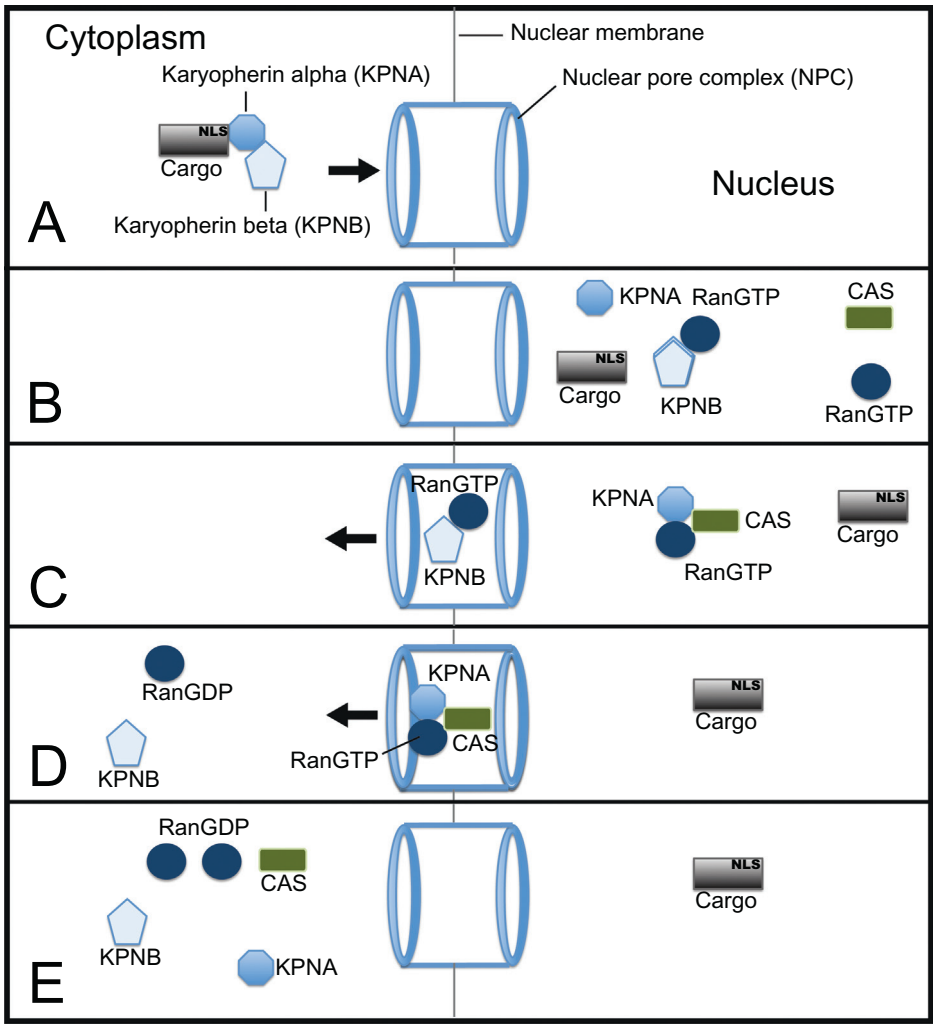

Fig. 1. Summary of molecular interactions in karyopherin alpha mediated transport. (A) Karyopherin alpha forms a ternary complex, binding to a karyopherin beta importin as well as the NLS site in the cargo molecule. (B) After the cargo is transported through the nuclear pore complex, RanGTP binds to KPNB, causing the KPNA and the cargo to dissociate from KPNB. (C) The RanGTP bound KPNB is transported through the NPC and both CAS and RanGTP bind to the KPNA. (D) RanGTP is converted to RanGDP and KPNB is released in the cytoplasm. Also, the KPNA/CAS/RanGTP complex moves through the NPC. (E) Ran GTP is converted to RanGDP and KPNA and CAS dissociate. CAS and RanGDP move back through the NPC and RanGDP is converted to RanGTP.

directly with the NPC to transport cargo into the nucleus, nuclear import of other cargo requires the assistance of an additional protein, a member of the KAP-α family (also known as the importin subunit α or importin-α family). In this process (reviewed by Pemberton and Paschal, 2005) KAP-α acts as an intermediary, binding to both the cargo molecule via the NLS and to the KAP-β molecule, KPNB1, to form a ternary complex (Fig. 1A). This complex then docks at the NPC and is transported through the central channel to the nucleus. Inside the nucleus, the small GTPase RanGTP binds to KPNB1, causing a conformational change in the molecule that allows it to dissociate from the complex (Fig. 1B). The KPNB1/RanGTP complex is then translocated through the NPC to the cytoplasm where it binds to Ran binding protein 1 (RanBP1) (Fig. 1C). This interaction with RanBP1 converts RanGTP into an intermediate that can be acted on by another cytoplasmic protein, RanGAP. When RanGAP encounters this intermediate, it stimulates GTPase activity by Ran (in RanGTP), causing conversion of RanGTP into RanGDP and resulting in the release of KPNB1 (Fig. 1D). In the nucleus, binding of Nup50/Npap60 to the KAP-α importin allows release of the cargo (Matsuura and Stewart, 2005). After this, the KAP-α importin is transported back to the cytoplasm by the exportin CAS (also known as XPO-2). CAS traverses the NPC with the KAP-α cargo by binding to RanGTP (Fig. 1C, D). Dissociation of the complex in the cytoplasm occurs in a manner similar to that described for KPNB1 (Fig. 1E).

When importins were first discovered, many investigators assumed that these gene products would be uniformly distributed throughout the organism. This, however, is not always true and subsequent studies have identified karyopherins that are both spatially and/or temporally restricted (Song and Wessel, 2007; Umegaki et al., 2007; Whiley et al., 2012; Yasuhara et al., 2007). Because each karyopherin specializes in the transport of particular cargo types, investigators have hypothesized that differential expression of these nuclear transport proteins could influence the availability of transcription factors in the nucleus and thereby affect cell differentiation (Okada et al., 2008; Poon and Jans, 2005; Yasuda et al., 2012). Studies examining functional roles of the KAP-α importins have supported this hypothesis and it is clear that the KAP-α family members are sequentially expressed in mammals during differentiation of sperm (Hogarth et al., 2006; Whiley et al., 2012), macrophages (Köhler et al., 2002), muscle (Hall et al., 2011) and neurons (Yasuhara et al., 2007, 2013).

The work of Yasuhara et al. (2007, 2013) provides some of the strongest support for this hypothesis. They showed that expression of the KAP-α subtypes switches from KPNA2 to KPNA1 during