Published in final edited form as:
Biochim Biophys Acta. 2011 September ; 1813(9): 1654–1662. doi:10.1016/j.bbamcr.2011.01.013.

Karyopherins in nuclear transport of homeodomain proteins during development

Wenduo Ye[1,#], **Wenbo Lin**[1,#], **Alan M. Tartakoff**[2], and **Tao Tao**[1,*]

[1]Xiamen University School of Life Sciences, Xiamen, Fujian 361005, China

[2]Department of Pathology and Cell Biology Program, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106, USA

Abstract

Homeodomain proteins are crucial transcription factors for cell differentiation, cell proliferation and organ development. Interestingly, their homeodomain signature structure is important for both their DNA-binding and their nucleocytoplasmic trafficking. The accurate nucleocytoplasmic distribution of these proteins is essential for their functions. We summarize information on a) the roles of karyopherins for import and export of homeoproteins, b) the regulation of their nuclear transport during development, and c) the corresponding complexity of homeoprotein nucleocytoplasmic transport signals.

Keywords

Homeodomain; homeoprotein; nuclear transport; importin α; karyopherin β; NLS; NES; development; regulation

Nucleocytoplasmic transport of macromolecules is essential in eukaryotes to regulate gene expression, signal transduction and cell cycle progression [1-7]. Nuclear import and export are signal-dependent. Proteins bearing nuclear localization signals (NLSs) or nuclear export signals (NESs) are recognized by receptors that relocate them from the cytoplasm into the nucleus (or *vice versa*) *via* nuclear pore complexes (NPCs). The receptors are in both cases members of the karyopherin β superfamily and are referred to as karyopherins. Nuclear localization signals are categorized into classical NLSs (cNLS) and nonclassical NLSs (ncNLS). cNLSs are characterized by either monopartite (e.g. PKKKRRV from SV40 large T antigen) or bipartite (e.g. KRPAATKKAGQAKKKK from nucleoplasmin) stretches of basic amino acids [8]. Shared characteristics of ncNLSs have not been identified. The best-known ncNLSs are the M9 sequence from heterogeneous nuclear ribonucleoprotein (hnRNP) A1 and the importin-β-binding domain (IBB) of importin α (a protein that is not related to the karyopherin β superfamily) [9]. The best-characterized NES is the so-called “leucine-rich” NES (e.g. LxxLxL). Transport cargoes interact with members of karyopherin β superfamily either directly or as complexes with adaptor proteins such as importin αs. Ran, a small GTPase of the Ras superfamily controls transport due to its asymmetric

*Corresponding author. .
#These authors contributed equally to this work.

distribution across the nuclear envelope, with Ran-GTP being concentrated in the nucleus and Ran-GDP being concentrated in the cytoplasm. RanGTP binds the “Ran-binding domain” of karyopherin β superfamily members, thereby regulating their conformation – which governs their affinity for cargo. For nuclear import, RanGTP binding causes karyopherin βs to release their cargoes in the nucleus. For nuclear export, RanGTP stabilizes the interaction of exportins with cargo. These complexes are then translocated through the NPC and the absence of Ran-GTP in the cytoplasm leads to dissociation of the cargo-exportin complexes.

Machinery of nucleocytoplasmic transport

There are 14 members of karyopherin β superfamily in *S. cerevisiae* and 20 in man [10, 11]. Although their sequence similarities are quite low, their molecular weights range from 95-115 kD and they share the following structural features: an N-terminal Ran-binding domain, NPC-binding sites, and 18~21 HEAT repeats. Karyopherin βs can import cargoes bearing both cNLS and ncNLSs. When cargoes bear a cNLS, the cNLS is recognized by adaptor importin αs. Importin β1 then interacts with the IBB domain of importin α and carries the importin α/β-cargo complex through the NPC. Several karyopherin βs import cargoes by directly recognizing their ncNLSs.

Leucine-rich NESs are recognized by the exportin, Crm1, which is also a member of karyopherin β superfamily [1]. A recent crystal structure shows that the leucine-rich NES occupies a hydrophobic groove between the outer helices of Crm1 HEAT repeats 11 and 12 [12]. Crm1, exportin 5 and exportin T (Xpo-t) export microRNAs, tRNA and rRNPs [13-17]. Although most karyopherin βs appear to function in either import or export, interestingly, three karyopherin βs (Msn5p [18, 19], importin 13 [20-22] and exportin 4 [23]) can transport cargoes both into and out of the nucleus, suggesting that they have a flexible structure, and may - in fact - participate in yet-uncharacterized cyclic transport events.

Importin α (karyopherin α) adaptors contain an N-terminal IBB that binds karyopherin β and a structure comprising ten tandem armadillo repeats [8]. These repeats include cNLS-binding sites. There are six importin α’s in man and five α’s in mice [24, 25]. Each importin α is highly conserved among species [26]. Based on sequence comparisons, importin α’s can be subdivided into three subtypes, one including importin α1, one including importin α3 and α4, and one including importin α5, α6 and α7 [27, 28].

The nuclear pore complex consists of about 30 different nucleoporins (Nups). Structurally and functionally, there are three classes of Nups: “structural Nups” which contribute to overall NPC architecture; “pore membrane proteins“ (Poms), which include a transmembrane domain and could contribute to anchoring the NPC in the nuclear envelope; and “FG-Nups”, which include multiple phenylalanine-glycine (FG), GLFG or FxFG repeat motifs which are interspersed among sequences of varying polarity. The FG-rich domains of Nups are unstructured and are essential for maintaining the NPC permeability barrier [29]. During translocation, the surfaces of karyopherin βs are thought to engage in multiple low-affinity interactions with FG-repeats [30, 31].

Homeoproteins

Homeodomain proteins (homeoproteins) are master control transcription factors that are important for diverse functions in development [32]. Homeoproteins regulate axial patterning, segment or cell identity and proliferation by modulating expression patterns of target genes in a temporal, spatial, and tissue-specific manner. Their name derives from the original identification of proteins that bind *Drosophila* homeotic loci [33]. The sequence

similarity of homeodomains is 50-80% for a given sub-type and 20% among all homeodomain sequences [34]. The homeodomain itself has a 60 residue-long conserved DNA-binding motif (Figure 1). Activities of homeoproteins are regulated by post-translational phosphorylation [35-37] and sumoylation [38]. The subcellular distribution of homeoproteins is critical for their functions. In *Drosophila*, for example, Extradenticle (Exd) is necessary for proximal leg development, but is not required for distal leg development [39-41]. Accordingly, Exd concentrates in the nucleus in cells that will give rise to proximal leg segments, while it is cytoplasmic in cells with distal leg fates [39-43]. The behavior of Oct6 also exemplifies the same theme. Oct6 is mainly cytoplasmic in undifferentiated ES cells, but localizes increasingly to the nucleus during retinoic acid-induced differentiation, and becomes predominantly nuclear in differentiated neurons, where it is required for function [44].

The homeodomain

Each homeodomain has an N-terminal flexible arm, a short helix I (a.a.10-22), helix II (a.a. 28-38) linked to helix I by a short loop, and helix III (a.a. 42-59), forming a classical helix-turn-helix motif along with helix II (Figure 2) [32, 34, 45]. Homeodomain-DNA interactions have been identified by NMR and by X-ray crystallography [46-48]. The basic residues preceding the N-terminal arm support contact with the DNA minor groove, and the helix-turn-helix motif binds to the major groove of DNA. Helix III serves as the major helix for interaction with DNA and harbors several highly conserved amino acid residues [32, 49]. In helix III, interactions between the conserved Arg52, Arg53 and DNA have been found in all homeodomains studied to date [32, 48-53]. Interestingly, Arg52 and Arg53 are also mutational hot spots in homeoproteins [49, 54]. Most homeoproteins contain not only a conserved homeodomain but also other functionally-important domains. These domains are either found alone as a DNA-binding motif or in tandem with another module. For example, members of the Pax family have both a “paired domain” and a homeodomain. “**Paired domains**” contain two DNA binding subdomains named *PAI* and *RED*. Both sub-domains include a helix-turn-helix structure [55-57]. The “**POU-domain**” is derived from three mammalian genes, *PIT-l, OCT-l,* and *OCT-2* and the *C. elegans* gene *Unc-86*, which share a region of homology. The POU domain is a bipartite DNA-binding domain, consisting of two highly conserved regions tethered by a variable linker. The 75-amino acid N-terminal region is called the POU-specific domain and the carboxy-terminal 60-amino acid region is called the POU homeodomain [58]. The “**Cut domain**” contains three highly homologous regions of −70 amino acids, the Cut repeats. Cut repeats are specific DNA binding domains, and Cut repeat lll cooperates with the Cut homeodomain to bind DNA with high affinity [59]. The “**LIM domain**” is composed of two contiguous zinc finger domains, separated by a two residue-long hydrophobic linker. It functions as a modular protein-binding interface and is named after its initial characterization in Lin11, Isl-1 & Mec-3 [60].

Nucleocytoplasmic transport of homeoproteins

Homeodomains include functional NLSs and NESs

Interestingly, many mutations in the homeodomain not only reduce DNA binding but also impair the nuclear localization of homeoproteins, suggesting that the homeodomain overlaps with functional transport signals [49]. Moreover, the basic amino-acid clusters (BCs) at both ends of the homeodomain (Figure 2) structurally resemble classical NLSs and can function as NLSs when coupled to an irrelevant cytoplasmic protein [61, 62]. For the homeoproteins Chx10, Nkx2.5, Oct6, and Otx1, the N-terminal basic clusters (BC1) have been shown to function as a NLS [63-66]. For Pdx1, Pitx2 and Shox2, the C-terminal basic clusters (BC2) function as a NLS [62, 67, 68]. In Arx, Cart1, HB9, Nanog, Nkx2.2, and Pax6, both clusters (BC1/BC2) are required for NLS function [22, 61, 69-72]. As shown in Figure 3A, all the

basic amino-acid residues in BC1 can vary among homeoproteins, while several basic amino-acid residues in BC2 are highly conserved (Arg52 and Arg53) (Figure 3B). Indeed these arginine residues are required for both DNA binding and NLS function. Thus, homeoprotein Pitx2 localizes to the cytoplasm when Arg 53 is mutated to Pro [67]; Arx localizes to the cytoplasm when Arg 52 or Arg 53 is mutated [22, 73]; and Nkx2.2 localizes to the cytoplasm upon mutation of Arg 53 when BC1 is absent (unpublished observation) [69]. Studies of Pdx1 [68, 74], Pax6 [70] and Nanog [71] show that helix III of the homeodomain is also important for NLS function.

In addition to these basic motifs, some homeoproteins contain at least one additional NLS [22]. For two homeodomain proteins belonging to the Pax family, Pax5 and Pax8, the functional NLSs are located outside their homeodomains [75, 76]. In a rare case, import of homeoprotein Vsx1, a member of Vsx family, was reported to be mediated by its binding partner (Ubc9) and the Vsx1 homeodomain is dispensable for its nuclear localization [77]. By contrast, a functional NLS is found in the homeodomain of the PLC-HD protein, another member of the Vsx family [64].

Nuclear export of homeoproteins has been proposed to regulate their functions [78, 79]. Vax2 is a homeoprotein that ventralizes the vertebrate eye field by repressing transcription of *PAX6*. Thus, constitutively nuclear Vax2 in the chick optic vesicle results in constitutive repression of *PAX6*, resulting in the formation of an eyeless embryo [80]. Cytoplasmic retention of Exd, a homeoprotein of the PBC family which includes products of vertebrate *PBX1, PBX2, PBX3, Drosophila extradenticle* (*exd*) and *C.elegans ceh-20* [81], is critical for patterning the proximal–distal axes of appendages, and for the development of both the eye and antennae in *D. melanogaster* [82, 83].

NESs have been characterized in several homeoproteins. NESs can overlap with their homeodomains and are either leucine-rich NESs, e.g. in Engrailed and Oct6 [84, 85], or “divergent” leucine-rich NESs, e.g. in Prospero [86, 87] (Figure 3B). NESs are also found outside the homeodomain, e.g. in the “octapeptide” domain of CVC paired-like homeoproteins [64], in the tryptophan-rich region in Nanog [78] and in the PBC-B region of Exd [43, 88]. As expected, export of most of these homeoproteins is sensitive to leptomycin B (LMB), the inhibitor of Crm1-mediated export (Table).

Nuclear import of homeoproteins via their homeodomains is mediated by diverse karyopherin βs

Being transcription factors, homeoproteins must be in the nucleus at the correct time. Mislocalization can be catastrophic. For example, inhibition of Bcd (bicoid) import can result from mutation of the *Drosophila semushi* (*semi*) gene, which encodes an E2 enzyme that modifies the NLS of Bcd. Inhibition of Bcd import results in multiple defects in anterior segmentation of embryos [89].

NLSs within homeodomains can be recognized either by importin αs with importin β1 or directly by karyopherin βs. Import of Brn2, Oct3/4 and Oct6 in mouse ES cells provides examples of import *via* the classical import pathway [90]. Moreover, in fission yeast, homeoprotein Yox1p, which regulates transcription during G1/S [91], relocates to the cytoplasm when importin α/Srp1p is mutated [92], suggesting that the classical importin α/β pathway is involved. Additionally, several homeoproteins (e.g. Arx, Pax6) are recognized directly by karyopherin βs and cannot be imported by any importin α. Given the approximate constancy of structure of the homeodomain itself, the diversity of import pathways suggests that the structures of NLSs of homeodomains could be modulated by flanking sequences or post-translational modifications. The following is a summary of nuclear import of several key homeoproteins and their transport receptors (Table 1):

Arx—The aristaless-related homeobox protein (Arx) is a paired-like homeoprotein that is predominantly expressed in the brain [93]. It is important for the development of the forebrain, testis and pancreas [94]. Both basic amino-acid clusters (BC1, BC2) cooperate to form a functional NLS which is targeted by importin β1, importin 9 and importin 13, but not by importin αs [22, 73, 95]. Interestingly, importin β1 mainly interacts with BC2 but importin 13 prefers binding to BC1. Using *in vitro* nuclear import analysis, GST-pull downs and interfering small RNAs, importin β1 was found to play a major role in import of Arx. Arg53 (R382) in its homeodomain is a core amino acid for recognition by importin β1 [22]. Curiously, our unpublished observations show that *in vitro* expressed homeodomains from Arx can interact with importin αs. Thus, import of β-galactosidase-EGFP tagged with either BC1 or BC2 of the Arx homeodomain is inhibited by Bimax, a specific inhibitor of the importin α/β pathway [96]. Nevertheless, the subcellular distribution of wild type Arx is not affected by Bimax, showing that the classical import pathway is not the principal pathway for Arx import.

Caudal—Caudal regulates the anteroposterior body axis of *Drosophila* [97, 98]. The moleskin protein DIM7 (*Drosophila* homologue of importin-7) binds BC2. Moreover, RNA interference of moleskin inhibits Caudal nuclear localization, suggesting that moleskin mediates its import [99].

Nkx2.2—Nkx2.2 regulates development of multiple tissues [100, 101]. Both basic clusters of its homeodomain are functional NLSs, but each is inefficient for nuclear localization of Nkx2.2 [69]. We observe that, although intact Nkx2.2 binds importin α1, α3 and α5 in GST-pull down assays, nuclear import of wildtype Nkx2.2 is not affected by Bimax, suggesting that its import is normally mediated by nonclassical pathways. Multiple karyopherin βs such as importin β1, importin 4 and importin 13 interact with the homeodomain of Nkx2.2 and can import Nkx2.2 in *in vitro* nuclear import assays (unpublished observation).

Pax6—Pax6, encoded by *PAIRED BOX* gene 6, was the first paired-type homeoprotein to be identified [102, 103]. This highly-conserved vertebrate transcription factor is important for development of multiple tissues including the CNS, eyes and pancreas [104-109]. The NLS of the Pax6 homeodomain includes both BC1 and BC2 and is recognized by importin 13 [70]. Deletion of either cluster dramatically reduces the interaction between Pax6 and importin 13 as well as nuclear localization of Pax6 in *in vitro* nuclear import assays. Interestingly, neither importin α/β nor importin β1 imports Pax6 efficiently. Two other members of the Pax family, Pax3 and Crx, are also imported by importin 13 [70]. In *in vitro* assays, importin αs can directly interact with basic amino-acid clusters of exogenously expressed homeodomains of Pax proteins [75]. There is, however, no direct evidence that nuclear import of Pax proteins is mediated by importin α/β [75].

Pdx1—Pdx1 (Pancreatic duodenal homeobox-1) is essential for pancreatic development [110-112]. It rapidly accumulates in the nucleoplasm when cells are stimulated with glucose, insulin or sodium arsenite [113]. Importin β1 itself binds strongly to the Pdx1 homeodomain and microinjection of MIN6 cells with an antibody to importin β1 maintains Pdx1 in the cytoplasm [114], suggesting that nuclear import of Pdx1 is mediated mainly by importin β1.

PRH/Hex—PRH/Hex (proline-rich homeobox/hematopoietically expressed homeobox) plays an important role in early embryonic patterning and hematopoiesis and has been reported to act as both a tumor suppressor and as an oncoprotein [115]. Aberrant exclusion of PRH/Hex from the nucleus has been associated with thyroid and breast cancers and a subset of myeloid leukemia. Interestingly, nuclear localization of PRH is necessary for the

inhibition of eIF4E-dependent transformation [116]. Importin 7 (imp7) is a direct binding partner for PRH/Hex and the imp7-PRH complex dissociates in the presence of RanGTP, as expected for a nuclear import complex. Imp7 mediates the import of PRH/Hex in digitonin-permeabilized cells and *in vivo* depletion of imp7 dramatically reduces the accumulation of PRH/Hex in the nucleus [117].

Regulation of nuclear transport of homeoproteins by multiple mechanisms

Nucleocytoplasmic transport of homeoproteins such as Exd, Otx1 and Pdx1 is regulated in development and this regulation is essential for their functions [82, 113, 118, 119]. The subcellular localization of homeoproteins depends on expression of specific karyopherins, on post-translational modifications of homeoproteins, and on interactions of homeoproteins with additional proteins (and perhaps DNA-binding sites).

Expression of importin αs/karyopherin βs [7, 10, 120-123]

Metazoans express multiple karyopherin αs that control cell differentiation [5] and development [5, 26, 90, 124-126]. Good examples are found during mammalian spermatogenesis [127] and in mouse ES cell differentiation into neurons, which is controlled by Brn2, Oct3/4, Oct6 and Sox2 [90]. Brn2, Oct6 and Sox2 are imported only by importin α3 and/or α5, not by importin α1. By contrast, import of Oct3/4 is mediated by importin α1. A switch in expression of importin α subtype in ES cells thus determines neuronal differentiation [90]: only importin α1 is expressed in undifferentiated ES cells, so Brn2, Oct6 and Sox2 remain in the cytoplasm, while Oct3/4 is in the nucleus. When cells start to differentiate, importin α1 is down-regulated and importin α3 and α5 are upregulated. Therefore, nuclear import of Oct3/4 is blocked but Brn2, Oct6, and Sox2 are imported. Nuclear Oct3/4 prevents differentiation, while nuclear Brn2, Oct6 and Sox2 promote neuronal differentiation.

Expression of karyopherin βs is also regulated [10, 122, 128] and is crucial for regulation of nucleocytoplasmic transport [7, 10, 121]. Although there is no direct evidence that they regulate the subcellular distribution of Arx, we observe that importin β1 and importin 13 import Arx through different mechanisms, suggesting that expression of different karyopherin βs could regulate Arx import and its functions in different contexts [22].

Phosphorylation

Phosphorylation regulates the subcellular localization of homeoproteins [80, 113, 129, 130]. One example is that of Vax2, a homeoprotein that as mentioned, ventralizes the vertebrate eye field by repressing transcription of *PAX6*. The subcellular localization of Vax2 is controlled by phosphorylation of serine 170. Wildtype Vax2 is in the nucleus but phosphorylation of S170 results in the exclusion of Vax2 from the nucleus. Exclusion likely reflects inactivation of its NLS – since expression of a nonphosphorylatable, constitutively nuclear Vax2 protein in the chick optic vesicle results in constitutive repression of *PAX6*, and leads to the formation of an eyeless embryo [80].

Exposure/concealment of transport motifs

The indirect masking of NLS/NES sequences of homeoproteins by association with other proteins can regulate their nucleocytoplasmic distribution [123, 131]. A good example is provided by *Drosophila* Exd, that is critical for embryogenesis [82, 83]. Exd localization correlates perfectly with the expression of a second homeoprotein, Homothorax (Hth) [42, 132, 133]. Both Hth and Exd are necessary for proximal leg development but are not required for distal leg development [39-41]. In the absence of Hth, Exd is cytoplasmic, but the co-expression of Hth causes it to localize to the nucleus. Exd has two NLSs in its

homeodomain and one NES in its PBC-B domain, a region of ~90 amino acids located between the PBC-A domain and the homeodomain. Hth binds Exd through its PBC-A domain. When Hth is present, the NES of Exd is masked and Exd is nuclear. When Hth is absent, the NES is bound by Crm1 and Exd is cytoplasmic [43, 79, 88, 133, 134]. Although Hth regulates the nuclear localization of Exd, the nuclear export of its mammalian homolog, Pbx, appears to be regulated by a fragment of murine nonmuscle myosin II heavy chain B, which conceals its NLSs [135]. Moreover, the N-terminal fragment of Pbx appears to interact with its homeodomain and mask its NLS. Thus, when Hth binds Pbx to release this interaction, the NLS of Pbx is exposed and Pbx is imported [136].

Conclusion

Given the diversity of structure and DNA-binding specificity of homeoproteins, it is not surprising that their nucleocytoplasmic transport is crucial in many biological contexts including cell differentiation, proliferation and tissue development. It is especially striking that their nuclear import is primarily mediated by NLSs that overlap the two basic clusters at the margins of homeodomains that bind DNA. Presumably, when they do bind DNA, these homeoproteins – like most import cargoes – will already have been efficiently released from their corresponding karyopherins. This functional overlap nevertheless could restrict sequence options at these sites. These options in turn could dictate the identity of the karyopherins that can be used.

A further possible spatial restriction that may limit interaction with transport factors is the tendency of homeoproteins to form homo- or heterodimers, as well as their interactions with additional proteins that modulate their transcriptional potency. An area for continued cell biological interest will be clarification of whether such protein-protein interactions occur prior to import. Especially when the cargo as well as the transport factors can be subject to post-translational modifications, the interplay between DNA-binding and nucleocytoplasmic transport – in light of the importance of proper nucleocytoplasmic distribution of homeoproteins for development – poses an intriguing challenge for coordinated evolution.

According to the homeoproteins under consideration, functional NLSs can be either in the N-terminal basic cluster (BC1/NLS1), in the C-terminal basic cluster (BC2/NLS2), or require both BC1 and BC2 (NLS3). Both BC1 and BC2 can be targeted by either importin αs or karyopherin βs, and both the classical and nonclassical pathways can mediate nuclear import of homeoproteins. When import of homeoproteins requires both BC1 and BC2, direct interaction with karyopherin βs is involved (Figure 4). Although it is too soon for conclusive comment, it is already seems surprising that one of the few “bidirectional” karyopherins, importin 13, imports several homeoproteins. At present, there is no evidence that importin 13 also participates in their export. For those homeoproteins that have been studied, export is mediated by leucine-rich NESs (that are recognized by Crm1), some of which also overlap with homeodomains.

The entire issue of whether homeoproteins can and do shuttle rapidly in and out of the nucleus is largely untouched. Closely linked to this uncertainty is the question of their stability. Some transcription factors turn over quickly within the nucleus; however, this possibility has not been addressed systematically for homeoproteins.

Given initial indications that the subcellular localization of homeoproteins can be regulated by phosphorylation, it will be of great interest to identify signals that adjust expression of karyopherins, covalently modify homeoproteins and coordinate the interactions of homeoproteins with additional proteins during development.

Acknowledgments

Research in the laboratory of Tao Tao is currently supported by grants from the National Foundation of Science of China (#30971669, #81071670), the Ministry of Science and Technology, China (#2006AA02A310), the Ministry of Education, China (#20090121110014) and the Department of Science & Technology, Fujian Province, China (#2009I0026). Research in the laboratory of Alan Tartakoff is supported by the National Institutes of Health.

References

[1]. Tartakoff AM, Tao T. Comparative and evolutionary aspects of macromolecular translocation across membranes. Int J Biochem Cell Biol. 2010; 42:214–229. [PubMed: 19643202]

[2]. Adam SA. The nuclear transport machinery in Caenorhabditis elegans: A central role in morphogenesis. Semin Cell Dev Biol. 2009; 20:576–581. [PubMed: 19577735]

[3]. Kelly SM, Corbett AH. Messenger RNA export from the nucleus: a series of molecular wardrobe changes. Traffic. 2009; 10:1199–1208. [PubMed: 19552647]

[4]. Jordan BA, Kreutz MR. Nucleocytoplasmic protein shuttling: the direct route in synapse-to-nucleus signaling. Trends Neurosci. 2009; 32:392–401. [PubMed: 19524307]

[5]. Yasuhara N, Oka M, Yoneda Y. The role of the nuclear transport system in cell differentiation. Semin Cell Dev Biol. 2009; 20:590–599. [PubMed: 19465141]

[6]. Itman C, Miyamoto Y, Young J, Jans DA, Loveland KL. Nucleocytoplasmic transport as a driver of mammalian gametogenesis. Semin Cell Dev Biol. 2009; 20:607–619. [PubMed: 19447185]

[7]. Terry LJ, Shows EB, Wente SR. Crossing the nuclear envelope: hierarchical regulation of nucleocytoplasmic transport. Science. 2007; 318:1412–1416. [PubMed: 18048681]

[8]. Lange A, Mills RE, Lange CJ, Stewart M, Devine SE, Corbett AH. Classical nuclear localization signals: definition, function, and interaction with importin alpha. J Biol Chem. 2007; 282:5101–5105. [PubMed: 17170104]

[9]. McLane LM, Corbett AH. Nuclear localization signals and human disease. IUBMB Life. 2009; 61:697–706. [PubMed: 19514019]

[10]. Quan Y, Ji ZL, Wang X, Tartakoff AM, Tao T. Evolutionary and transcriptional analysis of karyopherin beta superfamily proteins. Mol Cell Proteomics. 2008; 7:1254–1269. [PubMed: 18353765]

[11]. Gorlich D, Kutay U. Transport between the cell nucleus and the cytoplasm. Annu Rev Cell Dev Biol. 1999; 15:607–660. [PubMed: 10611974]

[12]. Dong X, Biswas A, Suel KE, Jackson LK, Martinez R, Gu H, Chook YM. Structural basis for leucine-rich nuclear export signal recognition by CRM1. Nature. 2009; 458:1136–1141. [PubMed: 19339969]

[13]. Castanotto D, Lingeman R, Riggs AD, Rossi JJ. CRM1 mediates nuclear-cytoplasmic shuttling of mature microRNAs. Proc Natl Acad Sci U S A. 2009; 106:21655–21659. [PubMed: 19955415]

[14]. Bussing I, Yang JS, Lai EC, Grosshans H. The nuclear export receptor XPO-1 supports primary miRNA processing in C. elegans and Drosophila. EMBO J. 2010

[15]. Okada C, Yamashita E, Lee SJ, Shibata S, Katahira J, Nakagawa A, Yoneda Y, Tsukihara T. A high-resolution structure of the pre-microRNA nuclear export machinery. Science. 2009; 326:1275–1279. [PubMed: 19965479]

[16]. Okamura K, Hagen JW, Duan H, Tyler DM, Lai EC. The mirtron pathway generates microRNA-class regulatory RNAs in Drosophila. Cell. 2007; 130:89–100. [PubMed: 17599402]

[17]. Bohnsack MT, Regener K, Schwappach B, Saffrich R, Paraskeva E, Hartmann E, Gorlich D. Exp5 exports eEF1A via tRNA from nuclei and synergizes with other transport pathways to confine translation to the cytoplasm. EMBO J. 2002; 21:6205–6215. [PubMed: 12426392]

[18]. Kaffman A, Rank NM, O'Neill EM, Huang LS, O'Shea EK. The receptor Msn5 exports the phosphorylated transcription factor Pho4 out of the nucleus. Nature. 1998; 396:482–486. [PubMed: 9853758]

[19]. Yoshida K, Blobel G. The karyopherin Kap142p/Msn5p mediates nuclear import and nuclear export of different cargo proteins. J Cell Biol. 2001; 152:729–740. [PubMed: 11266464]

[20]. Mingot JM, Kostka S, Kraft R, Hartmann E, Gorlich D. Importin 13: a novel mediator of nuclear import and export. EMBO J. 2001; 20:3685–3694. [PubMed: 11447110]

[21]. Tao T, Lan J, Lukacs GL, Hache RJ, Kaplan F. Importin 13 regulates nuclear import of the glucocorticoid receptor in airway epithelial cells. Am J Respir Cell Mol Biol. 2006; 35:668–680. [PubMed: 16809634]

[22]. Lin W, Ye W, Cai L, Meng X, Ke G, Huang C, Peng Z, Yu Y, Golden JA, Tartakoff AM, Tao T. The roles of multiple importins for nuclear import of murine aristaless-related homeobox protein. J Biol Chem. 2009; 284:20428–20439. [PubMed: 19494118]

[23]. Gontan C, Guttler T, Engelen E, Demmers J, Fornerod M, Grosveld FG, Tibboel D, Gorlich D, Poot RA, Rottier RJ. Exportin 4 mediates a novel nuclear import pathway for Sox family transcription factors. J Cell Biol. 2009; 185:27–34. [PubMed: 19349578]

[24]. Kohler M, Speck C, Christiansen M, Bischoff FR, Prehn S, Haller H, Gorlich D, Hartmann E. Evidence for distinct substrate specificities of importin alpha family members in nuclear protein import. Mol Cell Biol. 1999; 19:7782–7791. [PubMed: 10523667]

[25]. Kohler M, Ansieau S, Prehn S, Leutz A, Haller H, Hartmann E. Cloning of two novel human importin-alpha subunits and analysis of the expression pattern of the importin-alpha protein family. FEBS Lett. 1997; 417:104–108. [PubMed: 9395085]

[26]. Goldfarb DS, Corbett AH, Mason DA, Harreman MT, Adam SA. Importin alpha: a multipurpose nuclear-transport receptor. Trends Cell Biol. 2004; 14:505–514. [PubMed: 15350979]

[27]. Fagerlund R, Kinnunen L, Kohler M, Julkunen I, Melen K. NF-{kappa}B is transported into the nucleus by importin {alpha}3 and importin {alpha}4. J Biol Chem. 2005; 280:15942–15951. [PubMed: 15677444]

[28]. Mason DA, Stage DE, Goldfarb DS. Evolution of the metazoan-specific importin alpha gene family. J Mol Evol. 2009; 68:351–365. [PubMed: 19308634]

[29]. Denning DP, Patel SS, Uversky V, Fink AL, Rexach M. Disorder in the nuclear pore complex: the FG repeat regions of nucleoporins are natively unfolded. Proc Natl Acad Sci U S A. 2003; 100:2450–2455. [PubMed: 12604785]

[30]. Frey S, Gorlich D. A saturated FG-repeat hydrogel can reproduce the permeability properties of nuclear pore complexes. Cell. 2007; 130:512–523. [PubMed: 17693259]

[31]. Ribbeck K, Gorlich D. The permeability barrier of nuclear pore complexes appears to operate via hydrophobic exclusion. EMBO J. 2002; 21:2664–2671. [PubMed: 12032079]

[32]. Gehring WJ, Affolter M, Burglin T. Homeodomain proteins. Annu Rev Biochem. 1994; 63:487–526. [PubMed: 7979246]

[33]. McGinnis W, Levine MS, Hafen E, Kuroiwa A, Gehring WJ. A conserved DNA sequence in homoeotic genes of the Drosophila Antennapedia and bithorax complexes. Nature. 1984; 308:428–433. [PubMed: 6323992]

[34]. Banerjee-Basu S, Baxevanis AD. Molecular evolution of the homeodomain family of transcription factors. Nucleic Acids Res. 2001; 29:3258–3269. [PubMed: 11470884]

[35]. Seo JH, Jin YH, Jeong HM, Kim YJ, Jeong HG, Yeo CY, Lee KY. Calmodulin-dependent kinase II regulates Dlx5 during osteoblast differentiation. Biochem Biophys Res Commun. 2009; 384:100–104. [PubMed: 19393622]

[36]. Soufi A, Noy P, Buckle M, Sawasdichai A, Gaston K, Jayaraman PS. CK2 phosphorylation of the PRH/Hex homeodomain functions as a reversible switch for DNA binding. Nucleic Acids Res. 2009; 37:3288–3300. [PubMed: 19324893]

[37]. Janody F, Sturny R, Catala F, Desplan C, Dostatni N. Phosphorylation of bicoid on MAP-kinase sites: contribution to its interaction with the torso pathway. Development. 2000; 127:279–289. [PubMed: 10603346]

[38]. Shan SF, Wang LF, Zhai JW, Qin Y, Ouyang HF, Kong YY, Liu J, Wang Y, Xie YH. Modulation of transcriptional corepressor activity of prospero-related homeobox protein (Prox1) by SUMO modification. FEBS Lett. 2008; 582:3723–3728. [PubMed: 18948102]

[39]. Gonzalez-Crespo S, Morata G. Genetic evidence for the subdivision of the arthropod limb into coxopodite and telopodite. Development. 1996; 122:3921–3928. [PubMed: 9012512]

[40]. Casares F, Mann RS. Control of antennal versus leg development in Drosophila. Nature. 1998; 392:723–726. [PubMed: 9565034]

[41]. Gonzalez-Crespo S, Abu-Shaar M, Torres M, Martinez AC, Mann RS, Morata G. Antagonism between extradenticle function and Hedgehog signalling in the developing limb. Nature. 1998; 394:196–200. [PubMed: 9671305]

[42]. Rieckhof GE, Casares F, Ryoo HD, Abu-Shaar M, Mann RS. Nuclear translocation of extradenticle requires homothorax, which encodes an extradenticle-related homeodomain protein. Cell. 1997; 91:171–183. [PubMed: 9346235]

[43]. Abu-Shaar M, Ryoo HD, Mann RS. Control of the nuclear localization of Extradenticle by competing nuclear import and export signals. Genes Dev. 1999; 13:935–945. [PubMed: 10215621]

[44]. Ilia M, Bazigou E, Price J. Expression of the POU domain transcription factor, Oct-6, is attenuated in the adult mouse telencephalon, but increased by neurotoxic damage. Exp Neurol. 2003; 181:159–169. [PubMed: 12781989]

[45]. Qian YQ, Billeter M, Otting G, Muller M, Gehring WJ, Wuthrich K. The structure of the Antennapedia homeodomain determined by NMR spectroscopy in solution: comparison with prokaryotic repressors. Cell. 1989; 59:573–580. [PubMed: 2572329]

[46]. Genis C, Scone P, Kasahara H, Nam HJ. Crystallization and preliminary X-ray analysis of the NKX2.5 homeodomain in complex with DNA. Acta Crystallogr Sect F Struct Biol Cryst Commun. 2008; 64:1079–1082.

[47]. Wilson DS, Sheng G, Jun S, Desplan C. Conservation and diversification in homeodomain-DNA interactions: a comparative genetic analysis. Proc Natl Acad Sci U S A. 1996; 93:6886–6891. [PubMed: 8692913]

[48]. Gehring WJ, Qian YQ, Billeter M, Furukubo-Tokunaga K, Schier AF, Resendez-Perez D, Affolter M, Otting G, Wuthrich K. Homeodomain-DNA recognition. Cell. 1994; 78:211–223. [PubMed: 8044836]

[49]. Chi YI. Homeodomain revisited: a lesson from disease-causing mutations. Hum Genet. 2005; 116:433–444. [PubMed: 15726414]

[50]. Liu B, Kissinger CR, Pabo CO. Crystallization and preliminary X-ray diffraction studies of the engrailed homeodomain and of an engrailed homeodomain/DNA complex. Biochem Biophys Res Commun. 1990; 171:257–259. [PubMed: 1975495]

[51]. Clarke ND, Kissinger CR, Desjarlais J, Gilliland GL, Pabo CO. Structural studies of the engrailed homeodomain. Protein Sci. 1994; 3:1779–1787. [PubMed: 7849596]

[52]. Wolberger C. Homeodomain interactions. Curr Opin Struct Biol. 1996; 6:62–68. [PubMed: 8696974]

[53]. Billeter M. Homeodomain-type DNA recognition. Prog Biophys Mol Biol. 1996; 66:211–225. [PubMed: 9284451]

[54]. D'Elia AV, Tell G, Paron I, Pellizzari L, Lonigro R, Damante G. Missense mutations of human homeoboxes: A review. Hum Mutat. 2001; 18:361–374. [PubMed: 11668629]

[55]. Czerny T, Schaffner G, Busslinger M. DNA sequence recognition by Pax proteins: bipartite structure of the paired domain and its binding site. Genes Dev. 1993; 7:2048–2061. [PubMed: 8406007]

[56]. Noll M. Evolution and role of Pax genes. Curr Opin Genet Dev. 1993; 3:595–605. [PubMed: 8241771]

[57]. Miskiewicz P, Morrissey D, Lan Y, Raj L, Kessler S, Fujioka M, Goto T, Weir M. Both the paired domain and homeodomain are required for in vivo function of Drosophila Paired. Development. 1996; 122:2709–2718. [PubMed: 8787745]

[58]. Ryan AK, Rosenfeld MG. POU domain family values: flexibility, partnerships, and developmental codes. Genes Dev. 1997; 11:1207–1225. [PubMed: 9171367]

[59]. Coqueret O, Berube G, Nepveu A. The mammalian Cut homeodomain protein functions as a cell-cycle-dependent transcriptional repressor which downmodulates p21WAF1/CIP1/SDI1 in S phase. EMBO J. 1998; 17:4680–4694. [PubMed: 9707427]

[60]. Kadrmas JL, Beckerle MC. The LIM domain: from the cytoskeleton to the nucleus. Nat Rev Mol Cell Biol. 2004; 5:920–931. [PubMed: 15520811]

[61]. Furukawa K, Iioka T, Morishita M, Yamaguchi A, Shindo H, Namba H, Yamashita S, Tsukazaki T. Functional domains of paired-like homeoprotein Cart1 and the relationship between dimerization and transcription activity. Genes Cells. 2002; 7:1135–1147. [PubMed: 12390248]

[62]. Sabherwal N, Schneider KU, Blaschke RJ, Marchini A, Rappold G. Impairment of SHOX nuclear localization as a cause for Leri-Weill syndrome. J Cell Sci. 2004; 117:3041–3048. [PubMed: 15173321]

[63]. Kasahara H, Izumo S. Identification of the in vivo casein kinase II phosphorylation site within the homeodomain of the cardiac tisue-specifying homeobox gene product Csx/Nkx2.5. Mol Cell Biol. 1999; 19:526–536. [PubMed: 9858576]

[64]. Knauer SK, Carra G, Stauber RH. Nuclear export is evolutionarily conserved in CVC paired-like homeobox proteins and influences protein stability, transcriptional activation, and extracellular secretion. Mol Cell Biol. 2005; 25:2573–2582. [PubMed: 15767664]

[65]. Sock E, Enderich J, Rosenfeld MG, Wegner M. Identification of the nuclear localization signal of the POU domain protein Tst-1/Oct6. J Biol Chem. 1996; 271:17512–17518. [PubMed: 8663425]

[66]. Zhang YA, Okada A, Lew CH, McConnell SK. Regulated nuclear trafficking of the homeodomain protein otx1 in cortical neurons. Mol Cell Neurosci. 2002; 19:430–446. [PubMed: 11906214]

[67]. Kozlowski K, Walter MA. Variation in residual PITX2 activity underlies the phenotypic spectrum of anterior segment developmental disorders. Hum Mol Genet. 2000; 9:2131–2139. [PubMed: 10958652]

[68]. Hessabi B, Ziegler P, Schmidt I, Hessabi C, Walther R. The nuclear localization signal (NLS) of PDX-1 is part of the homeodomain and represents a novel type of NLS. Eur J Biochem. 1999; 263:170–177. [PubMed: 10429201]

[69]. Hessabi B, Schmidt I, Walther R. The homeodomain of Nkx2.2 carries two cooperatively acting nuclear localization signals. Biochem Biophys Res Commun. 2000; 270:695–700. [PubMed: 10772886]

[70]. Ploski JE, Shamsher MK, Radu A. Paired-type homeodomain transcription factors are imported into the nucleus by karyopherin 13. Mol Cell Biol. 2004; 24:4824–4834. [PubMed: 15143176]

[71]. Do HJ, Lim HY, Kim JH, Song H, Chung HM. An intact homeobox domain is required for complete nuclear localization of human Nanog. Biochem Biophys Res Commun. 2007; 353:770–775. [PubMed: 17196939]

[72]. Kosaka Y, Akimoto Y, Yokozawa K, Obinata A, Hirano H. Localization of HB9 homeodomain protein and characterization of its nuclear localization signal during chick embryonic skin development. Histochem Cell Biol. 2004; 122:237–247. [PubMed: 15338230]

[73]. Shoubridge C, Tan MH, Fullston T, Cloosterman D, Coman D, McGillivray G, Mancini GM, Kleefstra T, Gecz J. Mutations in the nuclear localization sequence of the Aristaless related homeobox; sequestration of mutant ARX with IPO13 disrupts normal subcellular distribution of the transcription factor and retards cell division. Pathogenetics. 2010; 3:1. [PubMed: 20148114]

[74]. Moede T, Leibiger B, Pour HG, Berggren P, Leibiger IB. Identification of a nuclear localization signal, RRMKWKK, in the homeodomain transcription factor PDX-1. FEBS Lett. 1999; 461:229–234. [PubMed: 10567702]

[75]. Kovac CR, Emelyanov A, Singh M, Ashouian N, Birshtein BK. BSAP (Pax5)-importin alpha 1 (Rch1) interaction identifies a nuclear localization sequence. J Biol Chem. 2000; 275:16752–16757. [PubMed: 10748034]

[76]. Poleev A, Okladnova O, Musti AM, Schneider S, Royer-Pokora B, Plachov D. Determination of functional domains of the human transcription factor PAX8 responsible for its nuclear localization and transactivating potential. Eur J Biochem. 1997; 247:860–869. [PubMed: 9288908]

[77]. Kurtzman AL, Schechter N. Ubc9 interacts with a nuclear localization signal and mediates nuclear localization of the paired-like homeobox protein Vsx-1 independent of SUMO-1 modification. Proc Natl Acad Sci U S A. 2001; 98:5602–5607. [PubMed: 11331779]

[78]. Chang DF, Tsai SC, Wang XC, Xia P, Senadheera D, Lutzko C. Molecular characterization of the human NANOG protein. Stem Cells. 2009; 27:812–821. [PubMed: 19350681]

[79]. Affolter M, Marty T, Vigano MA. Balancing import and export in development. Genes Dev. 1999; 13:913–915. [PubMed: 10215619]

[80]. Kim JW, Lemke G. Hedgehog-regulated localization of Vax2 controls eye development. Genes Dev. 2006; 20:2833–2847. [PubMed: 17043310]

[81]. Burglin TR. Analysis of TALE superclass homeobox genes (MEIS, PBC, KNOX, Iroquois, TGIF) reveals a novel domain conserved between plants and animals. Nucleic Acids Res. 1997; 25:4173–4180. [PubMed: 9336443]

[82]. Mann RS, Abu-Shaar M. Nuclear import of the homeodomain protein extradenticle in response to Wg and Dpp signalling. Nature. 1996; 383:630–633. [PubMed: 8857540]

[83]. Aspland SE, White RA. Nucleocytoplasmic localisation of extradenticle protein is spatially regulated throughout development in Drosophila. Development. 1997; 124:741–747. [PubMed: 9043089]

[84]. Maizel A, Bensaude O, Prochiantz A, Joliot A. A short region of its homeodomain is necessary for engrailed nuclear export and secretion. Development. 1999; 126:3183–3190. [PubMed: 10375508]

[85]. Baranek C, Sock E, Wegner M. The POU protein Oct-6 is a nucleocytoplasmic shuttling protein. Nucleic Acids Res. 2005; 33:6277–6286. [PubMed: 16260476]

[86]. Bi X, Kajava AV, Jones T, Demidenko ZN, Mortin MA. The carboxy terminus of Prospero regulates its subcellular localization. Mol Cell Biol. 2003; 23:1014–1024. [PubMed: 12529405]

[87]. Demidenko Z, Badenhorst P, Jones T, Bi X, Mortin MA. Regulated nuclear export of the homeodomain transcription factor Prospero. Development. 2001; 128:1359–1367. [PubMed: 11262236]

[88]. Stevens KE, Mann RS. A balance between two nuclear localization sequences and a nuclear export sequence governs extradenticle subcellular localization. Genetics. 2007; 175:1625–1636. [PubMed: 17277370]

[89]. Epps JL, Tanda S. The Drosophila semushi mutation blocks nuclear import of bicoid during embryogenesis. Curr Biol. 1998; 8:1277–1280. [PubMed: 9822580]

[90]. Yasuhara N, Shibazaki N, Tanaka S, Nagai M, Kamikawa Y, Oe S, Asally M, Kamachi Y, Kondoh H, Yoneda Y. Triggering neural differentiation of ES cells by subtype switching of importin-alpha. Nat Cell Biol. 2007; 9:72–79. [PubMed: 17159997]

[91]. Aligianni S, Lackner DH, Klier S, Rustici G, Wilhelm BT, Marguerat S, Codlin S, Brazma A, de Bruin RA, Bahler J. The fission yeast homeodomain protein Yox1p binds to MBF and confines MBF-dependent cell-cycle transcription to G1-S via negative feedback. PLoS Genet. 2009; 5:e1000626. [PubMed: 19714215]

[92]. Pulliam KF, Fasken MB, McLane LM, Pulliam JV, Corbett AH. The classical nuclear localization signal receptor, importin-alpha, is required for efficient transition through the G1/S stage of the cell cycle in Saccharomyces cerevisiae. Genetics. 2009; 181:105–118. [PubMed: 18984568]

[93]. Bienvenu T, Poirier K, Friocourt G, Bahi N, Beaumont D, Fauchereau F, Jeema L. Ben, Zemni R, Vinet MC, Francis F, Couvert P, Gomot M, Moraine C, van Bokhoven H, Kalscheuer V, Frints S, Gecz J, Ohzaki K, Chaabouni H, Fryns JP, Desportes V, Beldjord C, Chelly J. ARX, a novel Prd-class-homeobox gene highly expressed in the telencephalon, is mutated in X-linked mental retardation. Hum Mol Genet. 2002; 11:981–991. [PubMed: 11971879]

[94]. Kitamura K, Yanazawa M, Sugiyama N, Miura H, Iizuka-Kogo A, Kusaka M, Omichi K, Suzuki R, Kato-Fukui Y, Kamiirisa K, Matsuo M, Kamijo S, Kasahara M, Yoshioka H, Ogata T, Fukuda T, Kondo I, Kato M, Dobyns WB, Yokoyama M, Morohashi K. Mutation of ARX causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. Nat Genet. 2002; 32:359–369. [PubMed: 12379852]

[95]. Shoubridge C, Cloosterman D, Parkinson-Lawerence E, Brooks D, Gecz J. Molecular pathology of expanded polyalanine tract mutations in the Aristaless-related homeobox gene. Genomics. 2007; 90:59–71. [PubMed: 17490853]

[96]. Kosugi S, Hasebe M, Entani T, Takayama S, Tomita M, Yanagawa H. Design of peptide inhibitors for the importin alpha/beta nuclear import pathway by activity-based profiling. Chem Biol. 2008; 15:940–949. [PubMed: 18804031]

[97]. Mlodzik M, Gehring WJ. Expression of the caudal gene in the germ line of Drosophila: formation of an RNA and protein gradient during early embryogenesis. Cell. 1987; 48:465–478. [PubMed: 2433048]

[98]. Moreno E, Morata G. Caudal is the Hox gene that specifies the most posterior Drosophile segment. Nature. 1999; 400:873–877. [PubMed: 10476966]

[99]. Han SH, Ryu JH, Oh CT, Nam KB, Nam HJ, Jang IH, Brey PT, Lee WJ. The moleskin gene product is essential for Caudal-mediated constitutive antifungal Drosomycin gene expression in Drosophila epithelia. Insect Mol Biol. 2004; 13:323–327. [PubMed: 15157233]

[100]. Doyle MJ, Loomis ZL, Sussel L. Nkx2.2-repressor activity is sufficient to specify alpha-cells and a small number of beta-cells in the pancreatic islet. Development. 2007; 134:515–523. [PubMed: 17202186]

[101]. Price M, Lazzaro D, Pohl T, Mattei MG, Ruther U, Olivo JC, Duboule D, Di Lauro R. Regional expression of the homeobox gene Nkx-2.2 in the developing mammalian forebrain. Neuron. 1992; 8:241–255. [PubMed: 1346742]

[102]. Walther C, Gruss P. Pax-6, a murine paired box gene, is expressed in the developing CNS. Development. 1991; 113:1435–1449. [PubMed: 1687460]

[103]. Ton CC, Hirvonen H, Miwa H, Weil MM, Monaghan P, Jordan T, van Heyningen V, Hastie ND, Meijers-Heijboer H, Drechsler M, et al. Positional cloning and characterization of a paired box- and homeobox-containing gene from the aniridia region. Cell. 1991; 67:1059–1074. [PubMed: 1684738]

[104]. Heller RS, Stoffers DA, Liu A, Schedl A, Crenshaw EB 3rd, Madsen OD, Serup P. The role of Brn4/Pou3f4 and Pax6 in forming the pancreatic glucagon cell identity. Dev Biol. 2004; 268:123–134. [PubMed: 15031110]

[105]. Ashery-Padan R, Zhou X, Marquardt T, Herrera P, Toube L, Berry A, Gruss P. Conditional inactivation of Pax6 in the pancreas causes early onset of diabetes. Dev Biol. 2004; 269:479–488. [PubMed: 15110714]

[106]. Hever AM, Williamson KA, van Heyningen V. Developmental malformations of the eye: the role of PAX6, SOX2 and OTX2. Clin Genet. 2006; 69:459–470. [PubMed: 16712695]

[107]. Osumi N, Shinohara H, Numayama-Tsuruta K, Maekawa M. Concise review: Pax6 transcription factor contributes to both embryonic and adult neurogenesis as a multifunctional regulator. Stem Cells. 2008; 26:1663–1672. [PubMed: 18467663]

[108]. Wen J, Hu Q, Li M, Wang S, Zhang L, Chen Y, Li L. Pax6 directly modulate Sox2 expression in the neural progenitor cells. Neuroreport. 2008; 19:413–417. [PubMed: 18287938]

[109]. Hanson I, Churchill A, Love J, Axton R, Moore T, Clarke M, Meire F, van Heyningen V. Missense mutations in the most ancient residues of the PAX6 paired domain underlie a spectrum of human congenital eye malformations. Hum Mol Genet. 1999; 8:165–172. [PubMed: 9931324]

[110]. Ohlsson H, Karlsson K, Edlund T. IPF1, a homeodomain-containing transactivator of the insulin gene. EMBO J. 1993; 12:4251–4259. [PubMed: 7901001]

[111]. Leonard J, Peers B, Johnson T, Ferreri K, Lee S, Montminy MR. Characterization of somatostatin transactivating factor-1, a novel homeobox factor that stimulates somatostatin expression in pancreatic islet cells. Mol Endocrinol. 1993; 7:1275–1283. [PubMed: 7505393]

[112]. Watada H, Kajimoto Y, Kaneto H, Matsuoka T, Fujitani Y, Miyazaki J, Yamasaki Y. Involvement of the homeodomain-containing transcription factor PDX-1 in islet amyloid polypeptide gene transcription. Biochem Biophys Res Commun. 1996; 229:746–751. [PubMed: 8954967]

[113]. Elrick LJ, Docherty K. Phosphorylation-dependent nucleocytoplasmic shuttling of pancreatic duodenal homeobox-1. Diabetes. 2001; 50:2244–2252. [PubMed: 11574405]

[114]. Guillemain G, Da Silva Xavier G, Rafiq I, Leturque A, Rutter GA. Importin beta1 mediates the glucose-stimulated nuclear import of pancreatic and duodenal homeobox-1 in pancreatic islet beta-cells (MIN6). Biochem J. 2004; 378:219–227. [PubMed: 14632628]

[115]. Soufi A, Jayaraman PS. PRH/Hex: an oligomeric transcription factor and multifunctional regulator of cell fate. Biochem J. 2008; 412:399–413. [PubMed: 18498250]

[116]. Topisirovic I, Culjkovic B, Cohen N, Perez JM, Skrabanek L, Borden KL. The proline-rich homeodomain protein, PRH, is a tissue-specific inhibitor of eIF4E-dependent cyclin D1 mRNA transport and growth. EMBO J. 2003; 22:689–703. [PubMed: 12554669]

[117]. Ploski JE, Topisirovic I, Park KW, Borden KL, Radu A. A mechanism of nucleocytoplasmic trafficking for the homeodomain protein PRH. Mol Cell Biochem. 2009; 332:173–181. [PubMed: 19588232]

[118]. Ryoo HD, Mann RS. The control of trunk Hox specificity and activity by Extradenticle. Genes Dev. 1999; 13:1704–1716. [PubMed: 10398683]

[119]. Weimann JM, Zhang YA, Levin ME, Devine WP, Brulet P, McConnell SK. Cortical neurons require Otx1 for the refinement of exuberant axonal projections to subcortical targets. Neuron. 1999; 24:819–831. [PubMed: 10624946]

[120]. Wang H, Tao T, Tang J, Mao YH, Li W, Peng J, Tan G, Zhou YP, Zhong JX, Tseng SC, Kawakita T, Zhao YX, Liu ZG. Importin 13 serves as a potential marker for corneal epithelial progenitor cells. Stem Cells. 2009; 27:2516–2526. [PubMed: 19593795]

[121]. Jans DA. Nuclear transport in development and disease - The importance of importins. Semin Cell Dev Biol. 2009; 20:575. [PubMed: 19490949]

[122]. Loveland KL, Hogarth C, Szczepny A, Prabhu SM, Jans DA. Expression of nuclear transport importins beta 1 and beta 3 is regulated during rodent spermatogenesis. Biol Reprod. 2006; 74:67–74. [PubMed: 16192402]

[123]. Poon IK, Jans DA. Regulation of nuclear transport: central role in development and transformation? Traffic. 2005; 6:173–186. [PubMed: 15702986]

[124]. Geles KG, Johnson JJ, Jong S, Adam SA. A role for Caenorhabditis elegans importin IMA-2 in germ line and embryonic mitosis. Mol Biol Cell. 2002; 13:3138–3147. [PubMed: 12221121]

[125]. Geles KG, Adam SA. Germline and developmental roles of the nuclear transport factor importin alpha3 in C. elegans. Development. 2001; 128:1817–1830. [PubMed: 11311162]

[126]. Fang X, Chen T, Tran K, Parker CS. Developmental regulation of the heat shock response by nuclear transport factor karyopherin-alpha3. Development. 2001; 128:3349–3358. [PubMed: 11546751]

[127]. Hogarth CA, Calanni S, Jans DA, Loveland KL. Importin alpha mRNAs have distinct expression profiles during spermatogenesis. Dev Dyn. 2006; 235:253–262. [PubMed: 16261624]

[128]. Hogarth C, Itman C, Jans DA, Loveland KL. Regulated nucleocytoplasmic transport in spermatogenesis: a driver of cellular differentiation? Bioessays. 2005; 27:1011–1025. [PubMed: 16163727]

[129]. Kilstrup-Nielsen C, Alessio M, Zappavigna V. PBX1 nuclear export is regulated independently of PBX-MEINOX interaction by PKA phosphorylation of the PBC-B domain. EMBO J. 2003; 22:89–99. [PubMed: 12505987]

[130]. Boulanger J, Vezina A, Mongrain S, Boudreau F, Perreault N, Auclair BA, Laine J, Asselin C, Rivard N. Cdk2-dependent phosphorylation of homeobox transcription factor CDX2 regulates its nuclear translocation and proteasome-mediated degradation in human intestinal epithelial cells. J Biol Chem. 2005; 280:18095–18107. [PubMed: 15741163]

[131]. Tartakoff AM, Lichtenstein M, Nanduri J, Tsao HM. Review: dynamic stability of the interphase nucleus in health and disease. J Struct Biol. 2000; 129:144–158. [PubMed: 10806065]

[132]. Kurant E, Pai CY, Sharf R, Halachmi N, Sun YH, Salzberg A. Dorsotonals/homothorax, the Drosophila homologue of meis1, interacts with extradenticle in patterning of the embryonic PNS. Development. 1998; 125:1037–1048. [PubMed: 9463350]

[133]. Pai CY, Kuo TS, Jaw TJ, Kurant E, Chen CT, Bessarab DA, Salzberg A, Sun YH. The Homothorax homeoprotein activates the nuclear localization of another homeoprotein, extradenticle, and suppresses eye development in Drosophila. Genes Dev. 1998; 12:435–446. [PubMed: 9450936]

[134]. Berthelsen J, Kilstrup-Nielsen C, Blasi F, Mavilio F, Zappavigna V. The subcellular localization of PBX1 and EXD proteins depends on nuclear import and export signals and is modulated by association with PREP1 and HTH. Genes Dev. 1999; 13:946–953. [PubMed: 10215622]

[135]. Huang H, Paliouras M, Rambaldi I, Lasko P, Featherstone M. Nonmuscle myosin promotes cytoplasmic localization of PBX. Mol Cell Biol. 2003; 23:3636–3645. [PubMed: 12724421]

[136]. Saleh M, Huang H, Green NC, Featherstone MS. A conformational change in PBX1A is necessary for its nuclear localization. Exp Cell Res. 2000; 260:105–115. [PubMed: 11010815]

[137]. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994; 22:4673–4680. [PubMed: 7984417]

[138]. Gecz J, Cloosterman D, Partington M. ARX: a gene for all seasons. Curr Opin Genet Dev. 2006; 16:308–316. [PubMed: 16650978]

[139]. Friocourt G, Poirier K, Rakic S, Parnavelas JG, Chelly J. The role of ARX in cortical development. Eur J Neurosci. 2006; 23:869–876. [PubMed: 16519652]

[140]. Jaegle M, Ghazvini M, Mandemakers W, Piirsoo M, Driegen S, Levavasseur F, Raghoenath S, Grosveld F, Meijer D. The POU proteins Brn-2 and Oct-6 share important functions in Schwann cell development. Genes Dev. 2003; 17:1380–1391. [PubMed: 12782656]

[141]. James R, Erler T, Kazenwadel J. Structure of the murine homeobox gene cdx-2. Expression in embryonic and adult intestinal epithelium. J Biol Chem. 1994; 269:15229–15237. [PubMed: 7910823]

[142]. Shimozaki K, Nakashima K, Niwa H, Taga T. Involvement of Oct3/4 in the enhancement of neuronal differentiation of ES cells in neurogenesis-inducing cultures. Development. 2003; 130:2505–2512. [PubMed: 12702663]

[143]. Jaegle M, Meijer D. Role of Oct-6 in Schwann cell differentiation. Microsc Res Tech. 1998; 41:372–378. [PubMed: 9672420]

[144]. Chow RL, Volgyi B, Szilard RK, Ng D, McKerlie C, Bloomfield SA, Birch DG, McInnes RR. Control of late off-center cone bipolar cell differentiation and visual signaling by the homeobox gene Vsx1. Proc Natl Acad Sci U S A. 2004; 101:1754–1759. [PubMed: 14745032]

[145]. Ohtoshi A, Wang SW, Maeda H, Saszik SM, Frishman LJ, Klein WH, Behringer RR. Regulation of retinal cone bipolar cell differentiation and photopic vision by the CVC homeobox gene Vsx1. Curr Biol. 2004; 14:530–536. [PubMed: 15043821]

[146]. Doe CQ, Chu-LaGraff Q, Wright DM, Scott MP. The prospero gene specifies cell fates in the Drosophila central nervous system. Cell. 1991; 65:451–464. [PubMed: 1673362]

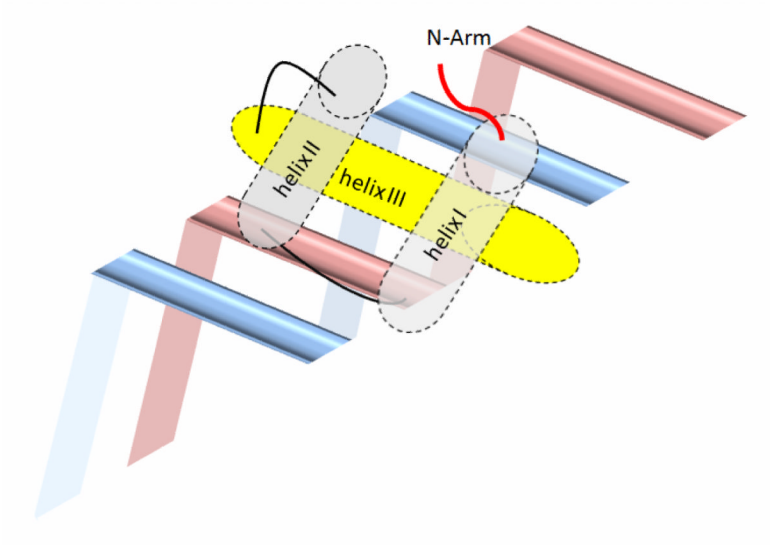

Figure 1. Cartoon of the DNA binding architecture of homeodomains

Helix III of the homeodomain binds the major groove of DNA, with helix I and II lying outside the double helix. Helix III, as a recognition helix, contains the C-terminal basic cluster that contacts both the phosphate backbone and specific bases. The N-terminal arm containing the N-terminal basic cluster lies in the minor groove and makes additional contacts.

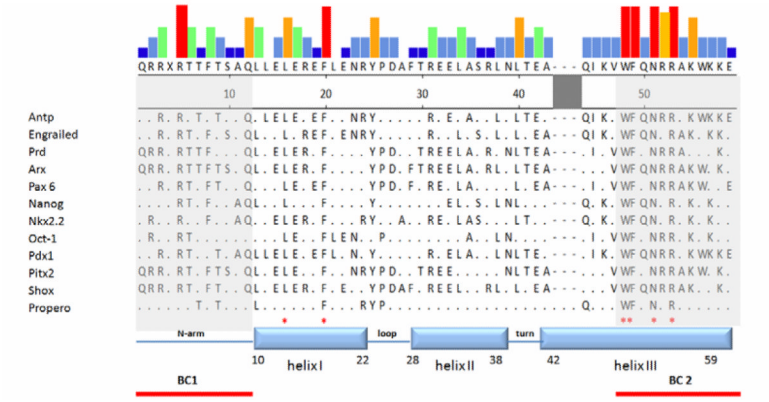

Figure 2. Sequences of homeodomains

Selected homeoproteins were aligned by the Clustal W program [137] embedded in MegAlign (DNASTAR, Inc). The colors of the top panel represent the frequency of given residues among sequences shown. The red column represents perfectly conserved residues for which there are no exceptions. The yellow and green columns indicate conserved residues with exceptions (frequency around 70%-90%). The grey and blue columns indicate residues that are much less well conserved among the homeoproteins (from high to low: red>yellow>green>grey>blue). Four amino acid residues in helix III and two amino acid residues in helix I indicated by '*' are conserved among all homeoproteins [32, 34, 49]. Note that two basic clusters are located at both ends of the homeodomain. The shaded region between helix II and helix III can form an extended loop for specific types of homeodomains.

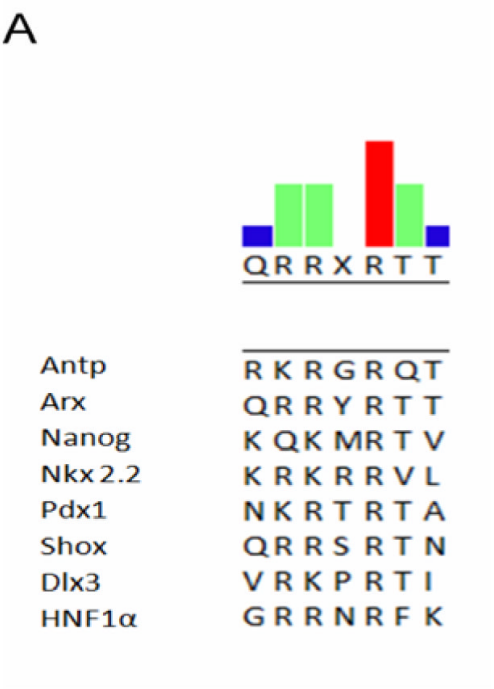

Figure 3A. Sequences of the N-terminal basic clusters

Selected homeoproteins were aligned by the Clustal W program. Note that amino acid R5 in this cluster is highly conserved. Mutation of this amino acid in HNF1α causes it to be sequestered in the cytoplasm [49].

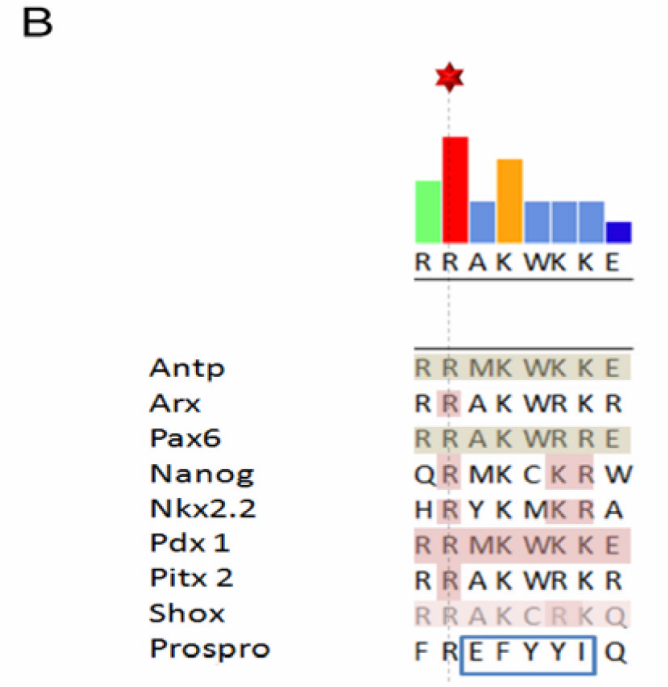

Figure 3B. Sequences of the C-terminal basic cluster

Selected homeoproteins were aligned by the Clustal W program. Note that the shaded region represents identified core residues for NLS function. The boxed region indicates a hydrophobic group of amino acids in the Prospro homeodomain which functions as a NES.

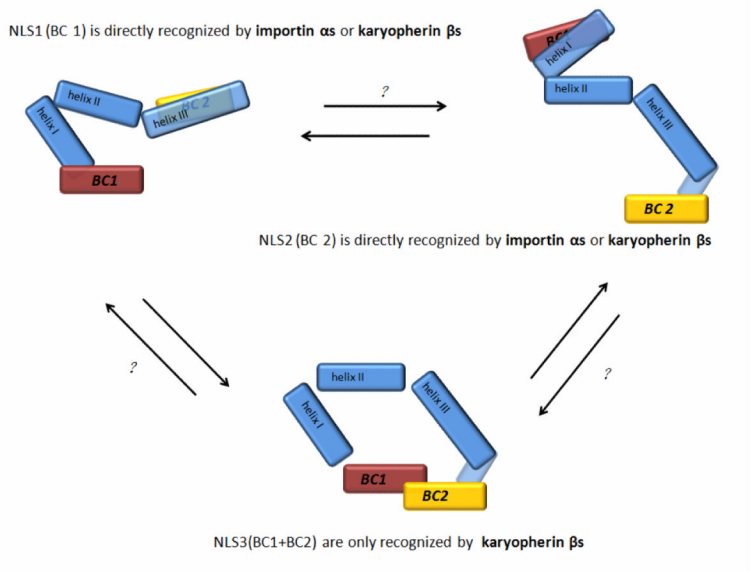

Figure 4. Model of nuclear import of homeoproteins mediated by the homeodomain

There are two basic amino-acid clusters (BC1 and BC2) at the ends of the homeodomain of homeoproteins. There are three forms of NLS found in the homeodomains: both BC1 and BC2 can function as an NLS independently (NLS1 or NLS2). BC1 can function as an NLS (NLS3) in conjunction with BC2. NLS1 and NLS2 can be recognized by either importin αs or karyopherin βs directly but NLS3 is only targeted by karyopherin βs. Therefore, nuclear import of homeoproteins can be mediated by both the classical and the nonclassical pathways. The homeodomain could use only a single NLS in certain conditions. As shown in the top left panel, if BC2 is structurally concealed, BC1/NLS1 could be functional. Alternatively as shown in the top right panel, if BC1 is structurally concealed, its BC2/NLS2 could function. When the homeodomain shows the NLS3 conformation (lower panel), only importin βs interact with NLS3 and nuclear import is mediated by a nonclassical pathway. The causes of possible interconversions among the three different NLS conformations are largely unknown (indicated by question marks).

Table 1

Summary of importin αs and karyopherin βs known to be responsible for nuclear transport of homeoproteins

Homeoprotein	Homeoprotein functions	Karyopherins/importins with a transport role	Sensitivity to LMB
Arx	early development of multiple tissues [94, 138, 139]	importin β1, 9, 13 [22, 73, 95] importin α3, α5 (unpublished observation)	
Brn2	differentiation of Schwann cells [140]	importin α5 [90]	
Caudal	anteroposterior body axis of *Drosophila* [97, 98]	Dim7 (importin 7) [99]	
Cdx2	pattern formation in the developing embryo [141]		Yes [130]
Exd (Pbx)	embryogenesis [41, 43, 82, 83]		Yes [134]
Hex	embryonic patterning [115]	importin 7 [117]	
Nkx 2.2	early development of multiple tissues [100, 101]	importin β1, 4, 7, 9, 13 (unpublished observation)	
Oct 3/4	differentiation of neuronal cells [142]	importin α1, α3, α5 [90]	
Oct6	differentiation of neuronal cells [143]	importin α3, α5 [90]	Yes [85, 90]
Pax6	development of CNS system and pancreas [104-109]	importin 13 [70]	
Pdx1	pancreatic cell-type maintaining [110-112]	importin β1 [114]	
PLC-HDP	ocular development [144, 145]		Yes [64]
prospero	regulation of cell fate [146]		Yes [86, 87]
Yox1p	regulating G1/S transition [91, 92]	importin α [92]	