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Stimulated nuclear import by β-like importins

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Abstract

Classic nuclear shuttling is mediated by an importin-α · β heterodimer that binds to cargoes containing a nuclear localization signal, and shuttles most nuclear proteins immediately after their translation. Aside from this canonical mechanism, kariopheryn-βs or β-like importins operate by binding to non-canonical nuclear localization signals to mediate translocation without the assistance of importin-α. The mechanism by which these components operate is much less understood and is currently under investigation. Recently, several β-like importins have been implicated in the stimulated nuclear translocation of signaling proteins. Here, we propose that this group of importins might be responsible for the swift nuclear shuttling of many proteins following various stimuli.

Mechanisms of stimulated nuclear import

Intracellular signaling pathways transmit signals of various extracellular stimuli to their cytosolic and nuclear targets in order to induce biological responses, such as proliferation, differentiation, cell death and migration. When needed, the signals are transmitted from the cytoplasm to the nucleus via translocation of one or more components of each of the signaling pathways involved. Thus, after stimulation, a large number of signaling proteins are rapidly translocated to the nucleus to induce and regulate many nuclear processes. However, despite the importance of stimulated nuclear signaling, the mechanisms by which these components reach the nucleus upon stimulation have been elucidated only for a few signaling pathways.

Classic nuclear shuttling is mediated by an importin-α · β complex that binds to cargoes containing a nuclear localization signal (NLS), consisting of mono- or bi-partite clusters of basic amino acids [1-3]. This importin-α · β complex often acts as a housekeeping mechanism that shuttles most nuclear proteins immediately to the nucleus after their translation [4]. The relocalization of cargoes is followed by the dissociation of the proteins from the importins upon binding to RanGTP [5], which exports the importins back to the cytoplasm, while the cargo remains in the nucleus [6]. However, only a limited number of signaling proteins, such as NFκB [7] and ERK5 (extracellular signal-regulated kinase 5) [8-10], use this machinery for their stimulated nuclear shuttle. Aside from this canonical mechanism, importin-β [11] or similar karyopherins, termed β-like importins [12], operate by binding to non-canonical NLSs to mediate translocation without the assistance of importin-α. The mechanism by which these components operate is much less understood and is currently under investigation. Recently, several β-like importins have been implicated in the stimulated nuclear translocation of signaling proteins. Here, we propose that this group of importins might be responsible for the swift nuclear shuttling of many proteins following various stimuli.

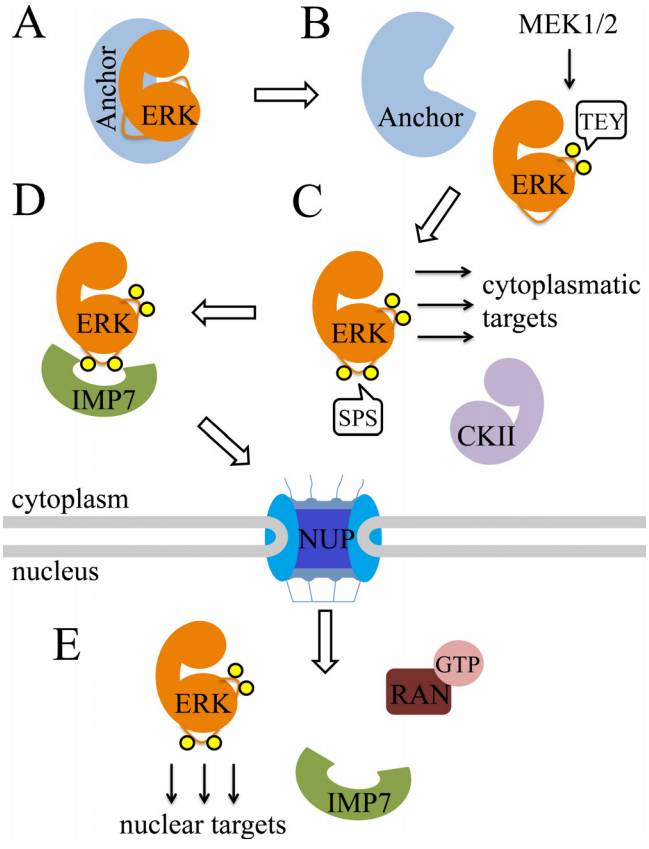
The mechanism of ERK1/2 translocation to the nucleus

ERK1/2 are important signaling proteins that translocate to the nucleus upon stimulation. The rapid and robust activation of ERK1/2 allows the phosphorylation and modulation of the activity of more than 300 proteins, which are localized either in the cytoplasm or the nucleus [13-15]. These substrates are important for the induction and regulation of cellular processes, including proliferation, differentiation, and migration amongst

others [16-19]. The sub-cellular localization of ERK1/2 plays an important role in its regulation and physiological functioning [20,21]. Interestingly, it was shown that the nuclear accumulation of ERK1/2 is important primarily for the induction of proliferation [22,23], while other ERK-dependent processes are mostly regulated by cytosolic molecules [24].

ERK1/2 localization, as well as the mechanisms that govern it, has been elucidated over the past decades. In resting cells, all components of the ERK1/2 cascade are localized primarily in the cytoplasm due to their interaction with different anchoring proteins [25-28] (see Figure 1). Upon stimulation, MEK1/2 phosphorylates ERK1/2 in their TEY motif, thereby inducing a conformational change resulting in the activation of ERK1/2 and detachment from their anchors [28]. This detachment exposes ERK1/2 to an additional phosphorylation on two Ser residues (an SPS motif) within a nine amino acid sequence, termed nuclear translocation signal (NTS) [29]. This phosphorylation can be mediated by both stimulated and constitutively active protein kinases, including protein kinase CK2 and auto-phosphorylation by active ERK1/2 [30]. The phosphorylation of the SPS motif allows it to bind importin-7, which escorts ERK1/2 molecules to the nuclear pores, inducing nuclear sliding. Once in the nucleus, RanGTP dissociates importin-7 from ERK1/2, and consequently, induces their nuclear accumulation [29]. It was also shown that ERK1/2 may interact directly with the nuclear pores, and it is possible that these direct interactions are able to facilitate the ERK1/2 translocation [31]. In addition, this process may be regulated by calcium, as a reduction in intracellular calcium concentrations was shown to induce faster nuclear shuttling [32,33].

Figure 1. Schematic representation of the mechanism of stimulated ERK1/2 translocation to the nucleus



The following steps are illustrated: (A) Binding of ERK1/2 with anchor proteins in resting cells; (B) stimulation is followed by phosphorylation of the TEY motif of ERK1/2 by MEK1/2, and detachment of ERK1/2 from their anchors; (C) phosphorylation of ERK1/2 on its SPS motif by CKII. (D) Binding of phosphorylated ERK1/2 to importin-7 and nuclear sliding through the NUPs; (E) Dissociation of ERK1/2 from importin-7 by RanGTP, and nuclear accumulation of ERK1/2. For more details, see text.

Interestingly, these results in mammals were consistent with findings in *Drosophila* [34], where DIM-7 (the ortholog of importin-7) was identified as the carrier of D-ERK to the nucleus [35,36]. Once in the nucleus, ERK plays a critical role in the development of eyes and wings in *Drosophila* [37,38]. Moreover, while comparing the mechanism of nuclear translocation of components of the ERK cascade with other proteins, we established that the NTS might act as a specific stimulus-induced and importin-7-dependent nuclear translocation signal for some signaling proteins lacking an NLS. However, since many signaling proteins contain neither NLS nor NTS, it is possible that other ill-defined β-like importins participate in the stimulated translocation, using various non-canonical NLSs.

The role of β-like importins in the nuclear shuttling of signaling proteins

Although importin-α·β complexes mediate the nuclear shuttling of a large number of proteins, it is now clear that other karyopherins are required for the translocation of the full repertoire of nuclear proteins. Such karyopherins were initially discovered as nucleoporin-binding proteins, and their homology with importin-β suggested a function in nuclear transport [39-43], which initiated their "importin" terminology [12] (see Table 1 for nomenclature). Subsequently, more dedicated studies identified at least 10 more β-like importins in mammals that share a sequence motif related to the Ran-binding site of importin-β, and can shuttle to the nucleus under various conditions [44,45]. The β-like importins known today share low overall sequence identity (10-20%), and have 19-20 helical HEAT repeats arranged into super-helical or ring-like structure [46]. Their molecular weights (90-150 kDa [46]), and

Table 1. List of β-like importins

Importin	Other terminology	Examples of signaling cargos (not always stimulated shuttle)
Importin-2	importin-β2, IPO2, KPNB2, MIP, MIP1, TNPO1, transportin, transportin 1, TRN, IMB2, Kapβ2, karyopherin-β2	c-Jun [50], NPM-ALK [57], hnRNP A1 [84,85] and several mRNA binding proteins [86], EWS [87], HuR [88], c-Fos [59,60], ribosomal proteins [89].
Importin-3	Importin-3, Imp3, transportin 2, FLJ1255, KPNB2B, TRN2, Karyopherin β-2b, IPO3, TNPO2, IMB2	HuR [88,90], hnRNP A1 [91].
Importin-4	Imp4, karyopherin-β4, Imp4b, FLJ23338, MGC131665, IPO4, IMB4, RanBP4, IMP4B	Vitamin D receptor [61], HIF1-α [51].
Importin-5	IPO5, IMB3, Pse1, Imp5, RANBP5, Kapβ3, KPNB3, MGC2068, FLJ43041, DKFZp686O1576, IMB3	c-Jun [50], p60TRP [92], RAG-2 [93], ribosomal proteins [89].
Importin-7	IPO7, Imp7, RANBP7, FLJ14581, MGC138673	ERK1/2 [29,35], MEK1 [29], SMAD3/4 [29,52], Egr1 [56], HIF1α [51], c-Jun [50], GR [54], Sox-2 [49], ribosomal proteins [89].
Importin-8	Imp8, IPO8, RANBP8, FLJ26580	SMAD1/3/4 [52], NPM-ALK [57], Ago2 [58], glucocorticoid receptor [54].
Importin-9	Imp9, FLJ10402, IPO9, RANBP9, KIAA1192, DKFZp761M1547	PR65 of PP2A [94], c-Jun [50], ARX [95], Sox-2 [49].
Importin-11	IPO11, Imp11, SLRN, RanBP11, KA120	UbcH6 [96], UB2E2 [96].
Importin-12	Imp12, importin-12, IPO12, MTR10A, TNPO3, transportin 3, transportin 3, transportin-SR, TRN-SR, TRN-SR2, TRNSR	MLF2 [97], RBM4 [97].
Importin-13	IPO13, Imp13, KAP13, RANBP13, LGL2, KIAA0724, Karyopherin 13	c-Jun [50], ARX [95], GR [98], Mago [99], Y14 [99].
Exportin-4	XPO4, Exp4, FLJ13046, KIAAA1721	Sox-2 and SRY [49].

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isoelectric points (pI = 4.0 – 5.9 [46,47]) vary. These importins mediate the translocation of proteins into the nucleus under varying conditions, including stimulation. Here, we describe the possible involvement of the β-like importins, as well as exportin-4, in the stimulated translocation of signaling proteins.

Among the β-like importins with the highest number of identified cargoes is importin-7, which seems to utilize several mechanisms and distinct NLSs to shuttle its distinct cargoes (Table 2). In some cases, usually in non-stimulated cells, importin-7 acts in a complex with importin-β [48,49], or in parallel to importin-2 [50], importin-4 [51], and importin-8 [52]. However, importin-7 also acts by itself, mainly in stimulated translocations.
Thus, importin-7 mediates the nuclear translocation of ERK1/2 described above, as well as MEK1 and SMAD3, by binding to the NTS sequences of these cargoes [29,53]. Moreover, it was reported that importin-7 is able to directly bind to a canonical NLS sequence in the glucocorticoid receptor to escort it to the nucleus upon hormonal stimulation [54]. In addition, importin-7 seems to shuttle other signaling proteins or transcription factors to the nucleus in an NTS- and canonical NLS-independent manner. Such molecules include the transcriptional regulators HIF1-α [51], c-Jun [50], several SMAD proteins [29,52,53], Sox-2 [49], HIV-1 [55], Egr-1 [56], and the oncogenic NPM-ALK [57]. However, it remains unclear whether the importin-7-mediated translocation of all these proteins is affected by stimulation, or is active merely in resting cells.

Table 2. Importin-cargo interactions of β-like importins

Type of interaction	Example	Stimulated
Monomeric, direct canonical NLS-dependent cargo binding.	Importin-7 binds the canonical NLS in glucocorticoid receptor and escorts it to the nucleus upon stimulation [54].	Yes
Monomeric, direct non-canonical NLS-independent cargo binding.	Importin-7 binds to phosphorylated SPS domain of ERK1/2 upon stimulation [29].	Yes
Cargo binding in complex with importin-β. Usually via canonical NLS.	Heterodimers of importin-7 · β have been implicated in the nuclear accumulation of Sox-2 [49].	No
Cooperation with other β-like importins. Usually bind non-canonical NLSs.	Various β-like importins cooperate in mediating nuclear translocation of c-Jun [50] and SMAD1/2/3 [52,55]. No direct association between them was reported.	Yes

Although the information on other members of the family still lags behind that of importin-7, it seems that at least some of them play important roles in stimulated translocation as well. Accordingly, importin-8 was shown to induce the nuclear accumulation of Ago2 [58] and SMAD1/4 [52]. C-Jun was shown to be transported by importins 2, 5, 7, 9, and 13, that might be related, at least in part, to its stimulated nuclear accumulation [50]. Importin-2 shuttles c-Fos to the nucleus after translation [59] or upon stimulation [60], and importin-4 escorts

vitamin D receptor to the nucleus upon ligand stimulation [61]. Interestingly, exportin-4, which participates mainly in nuclear export [62], has been shown to function as an importin for Sox-2, in addition to importin-β·7 and importin-9 [49]. This makes exportin-4 a distant relative of the β-like importins (Table 1), although it is not clear whether it participates in stimulated translocations as well. In general, β-like importins are able to induce both stimulated and/or non-stimulated translocations, using at least 4 mechanisms: (i) monomeric, direct canonical NLS-dependent cargo binding; (ii) monomeric, direct non-canonical NLS-independent cargo binding; (iii) cargo binding in a complex with importin-β; and (iv) cooperation with other β-like importins (see more details in Table 2). Thus, as a group, β-like importins may play an important role in the stimulated translocation of signaling proteins and transcription factors.

Summary and future directions

There is increasing evidence that the translocation of signaling proteins into the nucleus is much more tightly regulated than it was thought just a few years ago. Aside from the NLS/importin-α·β machinery, other mechanisms, such as passive diffusion [63,64], active transport of homodimers [64-66], direct binding to nuclear pore machinery [31,67-70], escort to the nucleus by other NLS-containing proteins [71,72] and indirect aid by the canonical machinery [64], were initially proposed for several signaling proteins. However, some of these findings were not properly verified, and later were either disputed [73-75], or found to be cell-type specific [76]. Therefore, it is worthwhile to entertain the possibility that at least some of these alleged mechanisms are, in fact, part of the wider β-like importin-dependent networks.

In addition, since dysregulation of the signaling proteins described above is involved in diseases, such as cancer and autoimmunity, it would be interesting to study the potential therapeutic implications of inhibiting their nuclear translocation. Several attempts have been made to block canonical NLS/importin-α·β mediated nuclear translocation [77-80]. Since many proteins use this machinery to translocate to the nucleus, such inhibition might affect too many processes and may fail to develop into desired specific therapies. However, a more specific approach might be to target the non-canonical mechanism of translocation, which seems to act within a limited number of distinct proteins upon stimulation. In this direction, efforts were made to develop a blocking peptide for importin-2 [81,82]. This peptide is able to compete with natural substrates and is resistant to Ran-mediated release in the nucleus [83], therefore specifically inhibiting this process [81]. However, in order to develop strong inhibitors for a specific cargo/β-like importin complex, we need to extract precise information on the structural interaction, as well as the regulation of import. We will then be able to explore this mechanism as a new layer of therapeutic intervention.

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