copyright by
all rights reserved
Quintessenz

Characteristic and Import Mechanism of Protein Nuclear Translocation

Zi Yan SUN[1], Zhi Peng FAN[2,3]

Coordination and information exchange among the various organelles ensure the precise and orderly functioning of eukaryotic cells. Interaction between the cytoplasm and nucleoplasm is crucial for many physiological processes. Macromolecular protein transport into the nucleus requires assistance from the nuclear transport system. These proteins typically contain a nuclear localisation sequence that guides them to enter the nucleus. Understanding the mechanism of nuclear import of macromolecular proteins is important for comprehending cellular processes. Investigation of disease-related alterations can facilitate the development of novel therapeutic strategies and provide additional evidence for clinical trials. This review provides an overview of the proteins involved in nuclear transport and the mechanisms underlying macromolecular protein transport.
Keywords: *karyopherin, nuclear import, nuclear localisation sequence, nuclear pore complex, nucleocytoplasmic transport*
Chin J Dent Res 2024;27(1):39–46; doi: 10.3290/j.cjdr.b5136729

Eukaryotic cells are characterised by the presence of a complete nucleus enclosed by a double nuclear membrane and well-defined organelles that ensure the spatial segregation of nuclear DNA replication, gene transcription and protein translation. This structure confers a potent mechanism for regulating gene expression in eukaryotes.[1] After their synthesis in the cytoplasm, proteins are selectively and efficiently transported to distinct locations where they perform their physiological functions. Karyophilic proteins, including transcription factors, replication factors, DNA repair factors and cell-cycle regulators, require nuclear entry to execute their functions.[2] The nuclear envelope (NE), as a natural selective barrier, separates the nucleus from other organelles and the cytoplasm. The nuclear pore complex (NPC), located on the nuclear membrane, regulates molecular transport between the nucleus and cytoplasm. The transport mechanism used by the NPC depends on the size of the substrate transported: while small molecules diffuse passively through the NPC, larger proteins require active transport by the nuclear transport system (NTS) to enter the nucleus.[3] Karyophilic proteins typically possess a conserved amino acid sequence, known as the nuclear localisation sequence (NLS), that functions as a recognition site for protein nuclear import.[4]

Recent studies on the nuclear transport of NLS-containing proteins have been conducted in the field of oral and maxillofacial diseases.[5,6] PTHrP exerts its effects via intracrine/paracrine signalling or by entering the nucleus. Deletion of the PTHrP NLS leads to dental and mandibular dysplasia in mice and is associated with the p27 pathway.[5] IGFBP2 upregulates

1 Beijing Key Laboratory of Tooth Regeneration and Function Reconstruction, Capital Medical University School of Stomatology, Beijing, P.R. China.
2 Laboratory of Molecular Signaling and Stem Cells Therapy, Beijing Key Laboratory of Tooth Regeneration and Function Reconstruction, Capital Medical University School of Stomatology, Beijing, P.R. China.
3 Research Unit of Tooth Development and Regeneration, Chinese Academy of Medical Sciences, Beijing, P.R. China.

Corresponding author: Dr Zhi Peng FAN, Laboratory of Molecular Signaling and Stem Cells Therapy, Beijing Key Laboratory of Tooth Regeneration and Function Reconstruction, Capital Medical University School of Stomatology, No. 4 Tiantanxili, Dongcheng District, Beijing 100050, P.R. China. Tel: 86-10-57099114. Email: zpfan@ccmu.edu.cn

This work was supported by National Key Research and Development Program (2022YFA1104401), CAMS Innovation Fund for Medical Sciences (2019-I2M-5-031) and grants from Innovation Research Team Project of Beijing Stomatological Hospital, Capital Medical University (NO. CXTD202204).

copyright by all rights reserved Quintessence

ZEB1 expression in an NF-κB (p65)-dependent manner, thereby promoting epithelial-mesenchymal transition (EMT) in salivary adenoid cystic carcinoma. Mutations in the IGFBP2 NLS effectively prevent EMT and reduce lung and liver metastasis.[6] Based on these findings, NLS-mediated protein nuclear transport plays a crucial role in the occurrence and progression of oral and maxillofacial diseases via various proteases, cytokines and signalling pathways. The investigation of protein nuclear import processes offers potential targets for treating oral and maxillofacial diseases. The present review advances this by focusing on proteins involved in the NTS and elucidates the mechanisms underlying karyophilic protein transport.

NTS

The NTS, which plays an important role in the nucleocytoplasmic translocation of macromolecular proteins, encompasses the NPC and nuclear transport receptors (NTRs).[7] The NPC is a large macromolecular assembly that spans the NE, forming a central transport channel with an outer diameter of ~1,200 Å, an inner diameter of ~425 Å and a height of ~800 Å. This structure regulates nucleocytoplasmic transport and mediates protein trafficking.[3,8] The NPC comprises > 30 nucleoporins (NUPs), which can be divided into three groups based on their functions: transmembrane proteins, which anchor the NPC to the NE; scaffold proteins, which constitute the inner and outer rings of the NPC; and phenylalanine-glycine (FG) NUPs (FG-NUPs), characterised by their abundant hydrophobic FG repeats. FG-NUPs, intrinsically disordered proteins that comprise approximately one-third of all NUPs, play an important role in precisely mediating cargo protein transport; during nucleocytoplasmic translocation, they exclude nonspecific macromolecular substances from the NPC's central transport channel, forming a dynamic selective barrier.[1,2,8-11]

Based on their structural and functional differences, NTRs can be separated into the Karyopherin α and β families.[3,4] Karyopherin α, also known as importin α, recognises and connects importin β with cargo proteins.[4,11,12] In mammalian cells, the importin α family comprises six members that are capable of recognising and binding cargo proteins; however, within the NPC, importin α lacks a shuttle function, and importin β is required to help translocate cargo proteins into the nucleus.[2,13,14] In eukaryotes, the karyopherin β family serves as a ubiquitous NTR; at least 20 distinct isoforms, responsible for most intracellular trafficking between the nucleus and cytoplasm, have been identified in human cells. The karyopherin β family includes the importin β proteins, which are composed of 11 members that function specifically as import receptors.[14] Most importin β members can bind directly to the NLS and carry it into the nucleus, where they then release the cargo protein.[14-16]

Importin α has three key domains, the first of which is the central domain, comprising ten Armadillo (ARM) repeats, each 42 to 43 amino acids in length. This domain is responsible for recognising the cargo protein NLS.[4,13,17] The cargo protein binds to two sites in the ARM repeat sequence: the major NLS binding site (ARM repeat sequences 2 to 4) and the minor NLS binding site (ARM repeat sequences 6 to 8).[13]

Second, at its N-terminus, importin α contains the importin β binding domain (IBB), which can bind to importin β1.[12] The IBB, containing an NLS-like sequence, can occupy the NLS binding site in the absence of importin β; this capability is thought to exert an autoinhibitory effect. This autoinhibition effectively prevents importin β from binding to an empty importin α, thus ensuring that nuclear transport is not blocked. The autoinhibitory domain competitively occupies the NLS binding site, facilitating the release of the NLS cargo upon transport to the nucleus.[16-18]

The third domain is the C-terminal domain of importin α, which binds to the nuclear export receptor (cellular apoptosis susceptibility protein [CAS]). CAS has a similar structure to importin β, facilitating importin export of α into the cytoplasm (Table 1).[2,17,19,20]

Importin β comprises tandem huntingtin, elongation factor 3, A subunit of PP2A, and TOR (HEAT) repeats arranged in a superhelix or ring-like structure to form binding sites for importin α, FG-NUPs and Ran-GTP.[15,21,22] By altering its own conformation, importin β can provide binding sites for protein-protein interactions (Table 1).[23]

In the karyophilic protein transport cycle, molecular recognition depends crucially on precise spatial and temporal regulation.[16] The binding and release of cargo proteins by the NTR is regulated by the small GTPase Ran. Upon binding to importin β, Ran-GTP triggers the dissociation of the cargo protein from the transport complex. Ran, characterised by an N-terminal globular domain and C-terminal extension structure, participates in various nuclear processes, including maintaining nuclear architecture, facilitating protein import, regulating mRNA processing and export, and controlling cell cycle progression.[8,24,25] Ran occurs as Ran-GDP and Ran-GTP on both sides of the nuclear membrane. The N-terminal globular domain of Ran binds to GTP and facilitates its hydrolysis. Its C-terminal extension structure features a unique acidic tail that binds to its

copyright all rights reserved Quintessenz

Table 1 Structural characterisation of NTRs.

NTR	Structural characterisation	Function
Importin α	Armadillo (ARM) repeats	Recognizes the cargo proteins NLS
	Importin β binding (IBB) domain	Acts as a competitive inhibitor to regulate cNLS-cargo binding by importin β1
	C-terminal domain of importin α	Binds to the export receptor CAS
Importin β	HEAT repeats	Forms binding sites with importin α, FG-NUPs, Ran-GTP

FG-NUP, phenylalanine-glycine (FG) nucleoporins (FG-NUPS); HEAT, huntingtin, elongation factor 3, A subunit of PP2A and TOR.

Table 2 Sequence features of cargo proteins with the NLS.

Category	Sequence features		Example
cNLS	Monopartite cNLS	K-K/R-X-K/R	PKKKRRV
	Bipartite cNLS	$(K/R)(K/R)X_{10-12}(K/R)_{3/5}$	KRPAATKKAGQAKKKK
ncNLS	PY-NLS	$R/K/H-X_{2-5}-P-Y$ or $R/K/H-X_{2-5}-P-\Phi$ motif	FGNYNNQSSNFGPMKGGNFGGRSSGPY RSGGNHRRNGRGGRGGYNRRNNGYHPY
	IK-NLS	K-V/I-X-K-X1-2-K /H/R	None
	RS-repeat NLS	None	None
Other types of NLS	Cryptic NLS	None	None
	Putative NLS	None	None

cNLS, classical NLS; ncNLS, nonclassical NLS; IK-NLS, isoleucine-lysine NLS; PY-NLS, proline-tyrosine NLS; RS-NLS, arginine-serine NLS.

N-terminus when it is bound to GDP, thereby stabilising the structure of Ran-GDP.[15] Ran-GTP is hydrolysed to Ran-GDP by the RAN GTPase-activating protein (RanGAP) and RAN-binding protein-1 (RanBP-1), and can be converted back to Ran-GTP via interactions with the guanine nucleotide exchange factor for Ran (RanGEF). These key regulators exhibit a non-uniform distribution within cells, with RanGAP concentrated in the cytoplasm and RanGEF enriched in the nucleus, resulting in the accumulation of Ran-GTP in the nucleus and enrichment of Ran-GDP in the cytoplasm. The concentration gradient on either side of the NPC is important for directional nuclear transport.[2,15,22,24-26]

Molecular mechanism of protein nuclear transport

Karyophilic proteins require not only the NTS, but also the NLS. The latter can be located anywhere within a karyophilic protein; upon recognition and binding by NTRs, this complex is transported into the nucleus.[27]

Classification of NLSs

Since the discovery of the first NLS in the 1980s (the T-antigen of simian vacuolating virus 40, SV40 TAg), an NLS classification system has gradually been developed.[1,4,20,27] Based on their residue composition, NLSs are currently classified into one of three types: classical (cNLS), nonclassical (ncNLS) or other.[4]

Classical NLSs

Classical NLSs contain one (monopartite) or two (bipartite) clusters of basic amino acids rich in positively charged lysine and arginine. A monopartite cNLS comprises 4 to 8 amino acids and has a consensus sequence of K-K/R-X-K/R, where X can be any residue. A representative example is the SV40-TAg cNLS PKKKRRV. A bipartite cNLS comprises two clusters of basic amino acids separated by a 10 to 12 residue linker region; the consensus sequence is $(K/R)(K/R)X_{10-12}(K/R)_{3/5}$, where X_{10-12} is a linker of 10 to 12 residues and $(K/R)_{3/5}$ refers to three basic residues within five consecutive residues. The prototypical bipartite NLS is the Xenopus nucleoplasmin NLS (KRPAATKKAGQAKKKK) (Table 2).[2,4,20]

Nonclassical NLSs

For many proteins that lack distinct amino acid clustering, the structural characterisation of the NLS has not yet been clearly defined. Such NLSs are considered nonclassical. The most extensively researched ncNLSs, the proline-tyrosine (PY)-NLS, exhibits sequence diversity.[14] It comprises a loose N-terminal hydrophobic or basic motifs and a C-terminal $R/K/H-X_{2-5}-P-Y$ or $R/K/H-X_{2-5}-P-\Phi$ motif (where Φ refers to hydrophobic, H to histidine, P to proline and Y to tyrosine, and X2-5 is any sequence of 2-5 residues).[14,28,29] PY-NLSs can be divided into two subclasses, depending on the composition of their N-terminal motifs: hydrophobic PY (hPY)-NLSs,

copyright by all rights reserved Quintessence

containing ΦG/A/SΦΦ motifs (where Φ refers to a hydrophobic residue), and basic PY (bPY)-NLSs, which are enriched with basic residues.[14,29] hnRNPA1 and Hrp1 are representative PY-NLSs, with the sequences FGNYNNQSSNFGPMKGGNFGGRSSGPY and RSGGNHRRNGRGGRGGYNRRNNGYHPY, respectively.[4] Isoleucine-lysine (IK)-NLS, another ncNLS, has the consensus sequence K-V/I-X-K-X1-2-K /H/R. TNPO3-binding arginine-serine repeat NLS (RS-repeat NLS), is another type of ncNLS (Table 2).[29] Further research into these NLSs is required.

Other NLSs

Other specialised forms of NLS have been identified. These include cryptic NLSs, which occur in some proteins that do not bind to NTRs. However, upon specific signalling stimulation, these protein structures can undergo transformation and expose their NLS for entry into the nucleus.[4,30] Some NLSs predicted by software exhibit a characteristic NLS amino acid sequence composition (Table 2). Upon verification, some exhibited a nuclear-localisation function, whereas others did not and were therefore classified as putative NLSs.[4,31] This indicates that, despite the regularity of the NLS, there remain uncertainties that require investigation and investment.

Mechanisms of nuclear transport

Nuclear transport of proteins with a CNLS

Importin α and β1 participate in the cNLS-mediated nuclear protein import.[4,12,13,32] The ARM domain of importin α initially recognises and binds to the cNLS of the cargo protein. Monopartite cNLSs bind to the major NLS-binding site, whereas bipartite cNLSs interact with both major and minor NLS binding sites.[16] The IBB domain of importin α then interacts with importin β1, forming a trimeric complex (cargo-importin α-importin β1) that localises to the NE.[16,20] Finally, the importin β1 in this complex binds to FG-NUPs, facilitating translocation of the trimeric complex into the nucleus.[16] During cargo-protein transport into the nucleus, nuclear RanGTP binds to importin β1, inducing conformational change and elongation of its superhelical structure. This leads to the dissociation between importin α and importin β1, releasing the IBB domain, which in turn competitively binds to importin α and promotes cargo-protein dissociation. NUPs such as Nup50 also facilitate the dissociation of the cargo proteins that remain in the nucleus.[8,16,18,33] Importin β1-RanGTP dimers are transported directly back to the cytoplasm, while importin α is exported from the nucleus with the assistance of RanGTP and the nuclear export receptor CAS. Aided by RanBP1, cytoplasmic RanGAP catalyses the conversion of RanGTP to RanGDP, causing the disassociation of the importin β1-RanGTP dimer and the CAS–RanGTP–importin α complex, freeing the importin β1 and importin α to participate in subsequent nuclear import cycles. RanGDP in the cytoplasm is transported back to the nucleus via its carrier nuclear transport factor-2 (NTF2), replenishing the nucleoplasmic RanGTP and maintaining the RanGTP-RanGDP concentration gradient (Fig 1).[13,16,34]

Nuclear transport of proteins with an ncNLS

Most of the ncNLS-mediated nuclear transport proteins are imported via importin β.[15,16] Many karyophilic proteins lack classical monopartite and bipartite NLSs, necessitating other modes of transport into the nucleus. The mechanism of nuclear entry of PY-NLS-carrying proteins has been well investigated. PY-NLS, the best characterised substrate of importin β2, is directly recognised by importin β2. Various PY-NLS-containing proteins, including hnRNPA1, hnRNP D, hnRNP M and HCC1, have been identified as importin β2 cargo proteins. Although the sequence identity of importin β2 and β1 is only 24%, the have almost the same structure: while their RanGTP-binding domains are highly similar, they differ substantially in their cargo-protein binding positions.[35]

Importin β2 can bind directly to cargo proteins in the absence of importin.[14,35] Two major NLS-binding sites have been identified within importin β2. The first, Site A, corresponds to H8-H13 and exhibits a high affinity for PY-NLS; it plays an indispensable role in recognition. The second, Site B, corresponds to H14–H20 and exhibits a lower affinity for PY-NLS.[36] The interaction between Site B and the NLS governs the overall binding affinity of importin β2.[36] PY-NLS has three important epitopes involved in importin β2 binding.[37] Epitope 1 corresponds to the N-terminal hydrophobic/basic motif, epitope 2 to the positively charged residue of the C-terminal R/K/H-X_{2-5}-P-Y motif, and epitope 3 to the proline–tyrosine dipeptide of the C-terminal R/K/ H-X_{2-5}-P-Y motif.[37] Site A of importin β2 interacts with epitopes 2 and 3, whereas Site B interacts with epitope 1.[35]

Initially, importin β2 binds to PY-NLS via its C-terminal arch: first, Site A binds to the C-terminal R/K/H-X2-5-P-Y motif, triggering structural rearrangement at H13–H14 and inducing Site B to bind to epitope 1. Importin β2 exhibits biochemical proper-

copyright by all rights reserved Quintessence

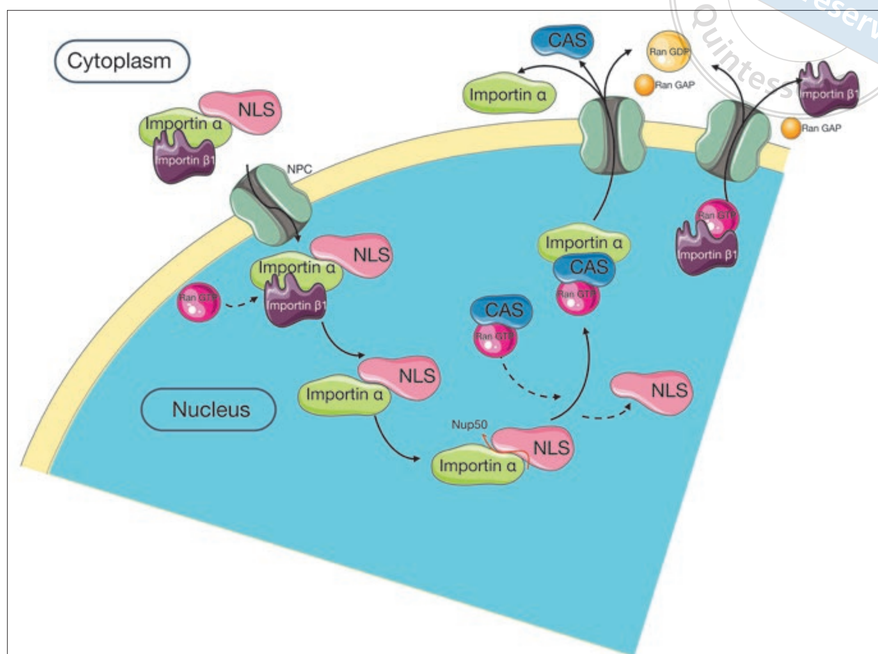

Fig 1 Nuclear transport guided by a classical NLS (cNLS). The cargo protein containing a cNLS binds to importin α, which is subsequently recognised and bound by importin β1 to form the cNLS cargo-importin α-importin β1 complex. After the complex enters the nucleus, RanGTP binds to importin β1, resulting in the dissociation of the complex. Upon dissociation, importin α exits the nucleus with the help of CAS and RanGTP, enters the cytoplasm, and is incorporated there. The importin β1-RanGTP complex is then returned to the cytoplasm for the next round of transportation.

ties that enable it to interact with FG-NUPs and pass through NPC-selective barriers, ultimately entering the nucleus.[35,36] The abundant RanGTP in the nucleus then binds to importin β2-transported cargo proteins, triggering the dissociation of the cargo protein. The complex first disassociates from Site B, and the cargo protein is completely released once Site A has disassociated.[36] Importin β2-RanGTP then moves towards and through the NPC. On the cytoplasmic surface of the NPC, RanGTP undergoes hydrolysis and dissociates from importin β2 upon binding to RanBP and RanGAP. The components are thus released into the cytoplasm for subsequent import cycles (Fig 2).[35]

IK-NLS and RS-NLS, other ncNLSs, are recognised and bound by Kap121 of the Karyopherin β family and by transportin 3, respectively[29]; however, these transport mechanisms require further study.

Therapeutic application of NLSs

Construction of a therapeutic system

Construction of a nuclear-targeted therapy system

Compared to conventional radiotherapy and chemotherapy, targeted therapy is a safer and more efficient form of cancer treatment, targeting pathogenic cellular and molecular targets precisely. As a result, there have been rapid advances in the development of drug-delivery systems.[38] Drug-delivery systems targeting the nucleus have become a research hotspot because the nucleus is the prime target for multiple cancer-targeting drugs. Barrabés et al[39] assembled a peptide NLS-BN3 drug-carrier system capable of nuclear-targeted delivery of metal-based chemotherapeutic drugs; the use of the NLS-BN3 peptide enhanced targeting efficiency towards gastrin-releasing peptide receptor (GRPR)-overexpressing tumour cells, resulting in increased nuclear accumulation and improved cytotoxic effectiveness.

Nanoparticle (NP)-based delivery systems can interact with biomolecules and access previously inaccessible parts of the body, thereby expanding the possibilities for disease detection and treatment.[40-42] Larger NPs (> 40 nm) require active NLS-assisted transport to pass through the NPC. The main components of NLS peptide nano-drug carrier systems include the NLS peptide; the basic nanomaterials skeleton; antitumour substances (chemotherapy drugs, nucleic acids and photosensitisers); and other substances, such as other drugs that can treat tumours.[43] For instance, Özçelik and Pratx[44] developed a targeted therapy system using arginylglycylaspartic acid (RGD) peptide–gold NPs modified with the NLS peptide (CGYGPKKKRKVGG); this modification resulted in an increased number of nuclear-targeting gold NPs, thus enhancing the radiosensitivity of A549 cells, inhibiting their proliferation, and inducing cell death.

copyright by all rights reserved Quintessenz

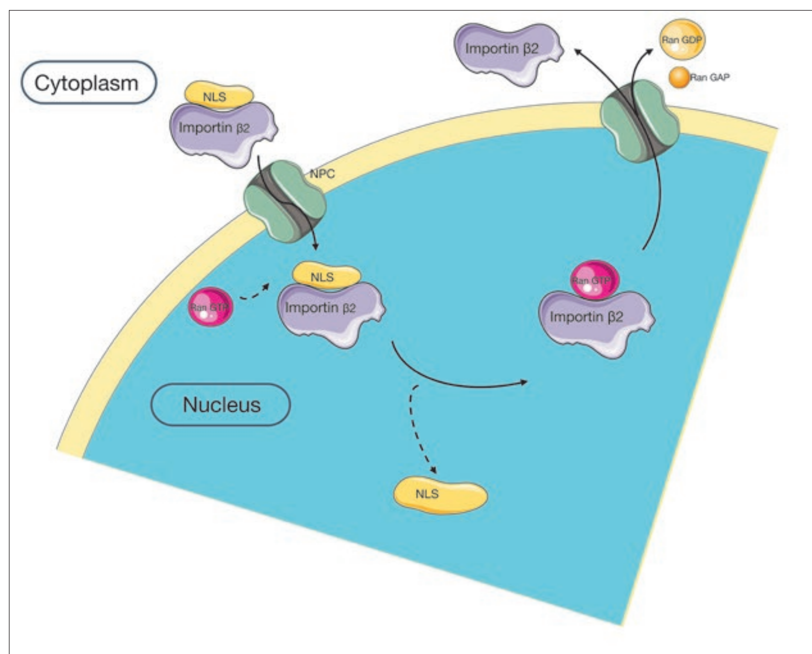

Fig 2 Nuclear transport guided by a proline-tyrosine NLS (PY-NLS). Importin β2 binds to the PY-NLS via its C-terminal arch, passes through selective nuclear pore complex (NPC) barriers and enters the nucleus. Once in the nucleus, RanGTP binds to the importin β2-cargo protein complex, triggering dissociation of the cargo protein. Subsequently, the importin β2-RanGTP complex approaches and moves through the NPC. On the cytoplasmic surface of the NPC, RanGTP undergoes hydrolysis and dissociates from importin β2 upon binding to RanBP and RanGAP.

Generating novel antimicrobial peptides

Since the 1940s, the emergence of antibiotic resistance has posed a worldwide challenge, necessitating the design of improved antimicrobial peptides.[45] The physicochemical parameters of cell-penetrating peptides and antimicrobial peptides overlap, resulting in shared functional characteristics between these peptide groups. Budagavi and Chugh[46] fused an NLS with a latarcin-derived peptide (LDP); the LDP-NLS effectively mitigated the cytotoxicity of LDP towards HeLa cells, demonstrated significantly enhanced cellular penetration of methicillin-resistant *Staphylococcus aureus* (MRSA) and successfully inhibited intracellular MRSA infection by entering HeLa cells.

Gene therapy

The nucleus serves as a regulatory centre for cellular genetics activity and metabolism, and many diseases are associated with disordered gene expression. Consequently, gene therapy has attracted significant attention for the treatment of both acquired and inherited diseases, rendering the nucleus a prominent target for gene therapy. Gene delivery vectors can be classed as viral or non-viral: while viral vectors are highly efficient, their application is limited by problems such as endogenous viral recombination, carcinogenesis and unexpected immune responses; and although non-viral gene vectors are safer, their nuclear delivery efficiency remains low.[20,47] The incorporation of NLS peptides into non-viral gene delivery systems can significantly enhance their nuclear translocation efficiency, thus augmenting their therapeutic efficacy via diverse systems.

Tarvirdipour et al[47] synthesised an NLS multi-compartment micelles (MCMs) nano-nonviral vector system integrating the minimal NLS (KRKR) into the hydrophilic domain of a peptide backbone carrying a synthetic DNA oligomer (antisense oligonucleotide [ASO]). In MCF-7 cancer cells, this NLS-mediated nuclear translocation machinery improved the nuclear incorporation efficiency of ASO, thus reducing the expression of BCL-2, which is closely associated with solid tumour growth.

Nuclear localisation probes

Alterations in nuclear metabolism

The nucleus is a metabolic centre in which the biochemical reactions primarily involve biosynthesis of metabolites that modulate gene expression.[48] Hydrogen peroxide, the foremost indicator of oxidative stress and a crucial mediator in signal transduction, is indispensable role in biological systems. Reactive oxygen species (ROS) generated by abnormal concentrations of hydrogen peroxide can damage cellular structures or biomolecules including proteins, liposomes and DNA. Such damage is closely associated with aging, Alzheimer's disease and cancer. As such, it is crucial to monitor the changes in hydrogen peroxide levels in cells and tissues.[49]

copyright
all rights reserved
Quintessence

Wen et al[49] developed a multifunctional ratiometric hydrogen peroxide fluorescent probe (NP1). Given the pivotal role of oxidative DNA damage in tumour initiation, NP1 contains a NLS peptide (VQRKRQKLMP-NH2) that acts as a transmembrane molecular carrier, facilitating nuclear delivery of the probe to detect DNA-proximal hydrogen peroxide. In HeLa cells, this peptide-based probe effectively localised in the nucleus and achieved quantitative detection of nuclear hydrogen peroxide.

Subcellular localisation of macromolecular substances

Monitoring dynamic changes in nuclear proteins in living cells and correlating them with changes in cellular behaviour could enhance understanding of the mechanisms and outcomes of cellular invasion behaviour.

For living cells, Sun et al[50] developed a Companion-Probe & Race (CPR) platform capable of real-time detection of nuclear proteins and simultaneous guiding and tracking of cell behaviours such as migration. The CPR platform comprises an intracellular probe region containing two peptide complexes for binding to target proteins, and each complex includes an NLS peptide to guide nuclear entry. This platform was used to validate the expression of the nuclear protein MDM2 and its impact on the migratory behavior of tumour cell lines and primary clinical cells.

The NLS has also been applied in other subcellular localisation probes, such as APEX2-NLS, which detects the nuclear localisation of RNA. These probes offer a potent and versatile approach for exploring the spatial environment and function of macromolecules.[51]

Conclusion

NLS-mediated nuclear import of karyophilic proteins is crucial for maintaining cellular functionality. Abnormalities in NLSs can disrupt nuclear transport, resulting in aberrant protein localisation, which is closely associated with disease occurrence and progression. A comprehensive understanding of NLS-mediated protein nuclear-import mechanisms will provide valuable therapeutic strategies in the future.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Dr Zi Yan SUN contributed to the literature collection and drafting the manuscript; Prof Zhi Peng FAN supervised and revised the manuscript.

(Received Jun 29, 2023; accepted Nov 09, 2023)

References

1. Cautain B, Hill R, de Pedro N, Link W. Components and regulation of nuclear transport processes. FEBS J 2015;282:445–462.
2. Oka M, Yoneda Y. Importin α: Functions as a nuclear transport factor and beyond. Proc Jpn Acad Ser B Phys Biol Sci 2018;94:259–274.
3. Schmidt HB, Görlich D. Transport selectivity of nuclear pores, phase separation, and membraneless organelles. Trends Biochem Sci 2016;41:46–61.
4. Lu J, Wu T, Zhang B, et al. Types of nuclear localization signals and mechanisms of protein import into the nucleus. Cell Commun Signal 2021;19:60.
5. Sun W, Wu J, Huang L, et al. PTHrP nuclear localization and carboxyl terminus sequences modulate dental and mandibular development in part via the action of p27. Endocrinology 2016;157:1372–1384.
6. Yao X, Wang Y, Duan Y, et al. IGFBP2 promotes salivary adenoid cystic carcinoma metastasis by activating the NF-kappaB/ZEB1 signaling pathway. Cancer Lett 2018;432:38–46.
7. Beck M, Schirmacher P, Singer S. Alterations of the nuclear transport system in hepatocellular carcinoma - New basis for therapeutic strategies. J Hepatol 2017;67:1051–1061.
8. Lin DH, Hoelz A. The structure of the nuclear pore complex (an update). Annu Rev Biochem 2019;88:725–783.
9. Khan AU, Qu R, Ouyang J, Dai J. Role of nucleoporins and transport receptors in cell differentiation. Front Physiol 2020;11:239.
10. Nag N, Sasidharan S, Uversky VN, Saudagar P, Tripathi T. Phase separation of FG-nucleoporins in nuclear pore complexes. Biochim Biophys Acta Mol Cell Res 2022;1869:119205.
11. Matsuda A, Mofrad MRK. On the nuclear pore complex and its emerging role in cellular mechanotransduction. APL Bioeng 2022;6:011504.
12. Goldfarb DS, Corbett AH, Mason DA, Harreman MT, Adam SA. Importin alpha: A multipurpose nuclear-transport receptor. Trends Cell Biol 2004;14:505–514.
13. Miyamoto Y, Yamada K, Yoneda Y. Importin alpha: A key molecule in nuclear transport and non-transport functions. J Biochem 2016;160:69–75.
14. Soniat M, Chook YM. Nuclear localization signals for four distinct karyopherin-beta nuclear import systems. Biochem J 2015;468:353–362.
15. Chang CC, Hsia KC. More than a zip code: Global modulation of cellular function by nuclear localization signals. FEBS J 2021;288:5569–5585.
16. Stewart M. Molecular mechanism of the nuclear protein import cycle. Nat Rev Mol Cell Biol 2007;8:195–208.
17. Pumroy RA, Cingolani G. Diversification of importin-alpha isoforms in cellular trafficking and disease states. Biochem J 2015;466:13–28.
18. 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.

copyright by all rights reserved Quintessenz

19. Cook A, Fernandez E, Lindner D, Ebert J, Schlenstedt G, Conti E. The structure of the nuclear export receptor Cse1 in its cytosolic state reveals a closed conformation incompatible with cargo binding. Mol Cell 2005;18:355–367.
20. Kim YH, Han ME, Oh SO. The molecular mechanism for nuclear transport and its application. Anat Cell Biol 2017;50:77–85.
21. Xu D, Farmer A, Chook YM. Recognition of nuclear targeting signals by Karyopherin-beta proteins. Curr Opin Struct Biol 2010;20:782–790.
22. Yoshimura SH, Kumeta M, Takeyasu K. Structural mechanism of nuclear transport mediated by importin beta and flexible amphiphilic proteins. Structure 2014;22:1699–1710.
23. Fu X, Liang C, Li F, et al. The rules and functions of nucleocytoplasmic shuttling proteins. Int J Mol Sci 2018;19:1445.
24. Lonhienne TG, Forwood JK, Marfori M, Robin G, Kobe B, Carroll BJ. Importin-beta is a GDP-to-GTP exchange factor of Ran: Implications for the mechanism of nuclear import. J Biol Chem 2009;284:22549–22558.
25. Scheffzek K, Klebe C, Fritz-Wolf K, Kabsch W, Wittinghofer A. Crystal structure of the nuclear Ras-related protein Ran in its GDP-bound form. Nature 1995;374:378–381.
26. Avis JM, Clarke PR. Ran, a GTPase involved in nuclear processes: Its regulators and effectors. J Cell Sci 1996;109:2423–2427.
27. Christie M, Chang CW, Róna G, et al. Structural biology and regulation of protein import into the nucleus. J Mol Biol 2016;428:2060–2090.
28. Wang L, Li M, Cai M, Xing J, Wang S, Zheng C. A PY-nuclear localization signal is required for nuclear accumulation of HCMV UL79 protein. Med Microbiol Immunol 2012;201:381–387.
29. Wing CE, Fung HYJ, Chook YM. Karyopherin-mediated nucleocytoplasmic transport. Nat Rev Mol Cell Biol 2022;23:307–328.
30. Gu Y, Hinnerwisch J, Fredricks R, Kalepu S, Mishra RS, Singh N. Identification of cryptic nuclear localization signals in the prion protein. Neurobiol Dis 2003;12:133–149.
31. Mallet PL, Bachand F. A proline-tyrosine nuclear localization signal (PY-NLS) is required for the nuclear import of fission yeast PAB2, but not of human PABPN1. Traffic 2013;14:282–294.
32. Görlich D, Kutay U. Transport between the cell nucleus and the cytoplasm. Annu Rev Cell Dev Biol 1999;15:607–660.
33. Zachariae U, Grubmüller H. Importin-beta: Structural and dynamic determinants of a molecular spring. Structure 2008;16:906–915.
34. Stewart M. Function of the nuclear transport machinery in maintaining the distinctive compositions of the nucleus and cytoplasm. Int J Mol Sci 2022;23:2578.
35. Mboukou A, Rajendra V, Kleinova R, Tisné C, Jantsch MF, Barraud P. Transportin-1: A nuclear import receptor with moonlighting functions. Front Mol Biosci 2021;8:638149.
36. Imasaki T, Shimizu T, Hashimoto H, et al. Structural basis for substrate recognition and dissociation by human transportin 1. Mol Cell 2007;28:57–67.
37. Süel KE, Gu H, Chook YM. Modular organization and combinatorial energetics of proline-tyrosine nuclear localization signals. PLoS Biol 2008;6:e137.
38. Huang R, Zhou PK. DNA damage repair: Historical perspectives, mechanistic pathways and clinical translation for targeted cancer therapy. Signal Transduct Target Ther 2021;6:254.
39. Barrabés S, Ng-Choi I, Martínez MÁ, et al. A nucleus-directed bombesin derivative for targeted delivery of metallodrugs to cancer cells. J Inorg Biochem 2020;212:111214.
40. Tran VA, Thuan Le V, Doan VD, Vo GNL. Utilization of functionalized metal-organic framework nanoparticle as targeted drug delivery system for cancer therapy. Pharmaceutics 2023;15:931.
41. Doroudian M, Zanganeh S, Abbasgholinejad E, Donnelly SC. Nanomedicine in lung cancer immunotherapy. Front Bioeng Biotechnol 2023;11:1144653.
42. Herdiana Y, Wathoni N, Gozali D, Shamsuddin S, Muchtaridi M. Chitosan-based nano-smart drug delivery system in breast cancer therapy. Pharmaceutics 2023;15:879.
43. Li B, Shao H, Gao L, Li H, Sheng H, Zhu L. Nano-drug co-delivery system of natural active ingredients and chemotherapy drugs for cancer treatment: a review. Drug Deliv 2022;29:2130–2161.
44. Özçelik S, Pratx G. Nuclear-targeted gold nanoparticles enhance cancer cell radiosensitization. Nanotechnology 2020;31:415102.
45. Kumari A, Singh M, Sharma R, et al. Apoptin NLS2 homodimerization strategy for improved antibacterial activity and bio-stability. Amino Acids 2023;55:1405–1416.
46. Budagavi DP, Chugh A. Antibacterial properties of Latarcin 1 derived cell-penetrating peptides. Eur J Pharm Sci 2018;115:43–49.
47. Tarvirdipour S, Skowicki M, Schoenenberger CA, et al. A self-assembling peptidic platform to boost the cellular uptake and nuclear delivery of oligonucleotides. Biomater Sci 2022;10:4309–4323.
48. Ruiz-Rodado V, Lita A, Larion M. Advances in measuring cancer cell metabolism with subcellular resolution. Nat Methods 2022;19:1048–1063.
49. Wen Y, Xue F, Lan H, Li Z, Xiao S, Yi T. Multicolor imaging of hydrogen peroxide level in living and apoptotic cells by a single fluorescent probe. Biosens Bioelectron 2017;91:115–121.
50. Sun H, Dong Z, Zhang Q, et al. Companion-Probe & Race platform for interrogating nuclear protein and migration of living cells. Biosens Bioelectron 2022;210:114281.
51. Fazal FM, Han S, Parker KR, et al. Atlas of subcellular RNA localization revealed by APEX-Seq. Cell 2019;178:473–490.