


SPOTLIGHT

# WW domains: A globular NLS recognized by importin- $\alpha$

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**In this issue, the discovery by Yang et al. (<https://doi.org/10.1083/jcb.202308013>) that folded WW domains of YAP1 and other proteins bind to Imp $\alpha$  introduces a new class of globular NLS, contrasting with the extensively studied linear NLS motifs. This finding underscores the versatility of importins in recognizing their cargo proteins.**

The transport of proteins between the cytoplasm and the nucleus is often a key step in the regulation of gene expression. This process is mostly orchestrated by nuclear transport receptors in the karyopherin (Kap) family. Kaps bind protein cargoes to translocate them through nuclear pore complexes (NPCs) in the nuclear envelope (1). Kaps that transport cargoes into the nucleus are termed importins (1). Four of the 10 importins in human cells have been reported to recognize specific linear motifs in their cargoes known as nuclear localization sequences (NLSs) (1). The most-studied importin system is the heterodimeric importin- $\alpha$  (Imp $\alpha$ )/importin- $\beta$  (Imp $\beta$ ) complex that binds highly basic NLSs (1) (Fig. 1 A). Most of these NLSs are grouped into a class named the classical-NLS (cNLS), which includes short motifs of 4–20 amino acids that bind to the Armadillo domain of the Imp $\alpha$  adaptor protein (1). Imp $\alpha$  in turn binds Imp $\beta$ , and the cargo-Imp $\alpha$ / $\beta$  complex can cross the permeability barrier of the NPC as Imp $\beta$  interacts with phenylalanine-glycine motifs in the NPC (1) (Fig. 1 A). In the nucleus, RanGTP binds Imp $\beta$  with high affinity, causing disassembly of the cargo-Imp $\alpha$ / $\beta$  complex to release the cargo (1) (Fig. 1 B).

Over the past three decades, researchers have identified and characterized hundreds of cNLS motifs that bind Imp $\alpha$  (1). A new study by Yang et al. shows that Imp $\alpha$  also binds multiple members of a class of folded

domains, the small globular WW domains (2). Their discovery is the first identification of a class of globular NLS that binds Imp $\alpha$ .

Yang et al. studied the WW domain containing the Hippo pathway transcription factor YAP1, which is involved in cell proliferation and survival (2). YAP1 is phosphorylated by the MST-LATS kinase cascade, which leads to its cytoplasmic localization through interactions with the protein 14-3-3 (3). Inactivation of the Hippo pathway and subsequent inhibition of YAP1 phosphorylation frees YAP1 from 14-3-3, allowing YAP1 to be imported into the nucleus where it triggers the transcription of genes important for cell development and contribute to oncogene expression (3).

YAP1 contains an N-terminal TEAD-binding domain followed by two tandem WW domains and a C-terminal transcriptional activation domain. YAP1 nuclear import has been studied by multiple groups, but the findings differed and the mechanism was uncertain (4–7). In dissecting the roles of the tandem WW domains of YAP1, Yang et al. uncovered an unexpected function of the WW domains as a globular NLS that not only binds to Imp $\alpha$  but does so in a surprising manner (2).

The ~40-amino acid WW domain is characterized by a twisted three anti-parallel strands  $\beta$ -sheet that presents two highly conserved tryptophan residues (8). These tryptophans confer domain stability and

form the ligand binding pocket that binds proline-rich motifs (PRMs) (8). The sequence characteristics of PRM ligands divide WW domains into four groups: the WW domains of YAP1 belong to the largest group, termed group I, which recognizes PRMs with proline-tyrosine (PY) motifs. Groups II–IV recognize proline-leucine, proline-arginine, and phospho-serine or phospho-threonine motifs, respectively (9). Proline residues of the PRMs bind in hydrophobic grooves formed by aromatic residues of the WW domains (9).

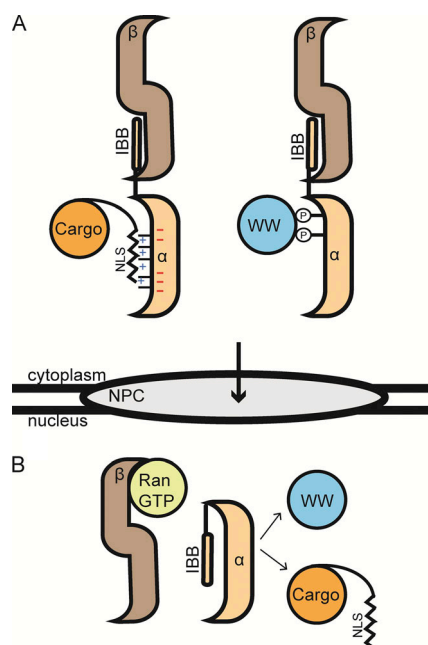
Yang et al. generated YAP1 mutants that either lacked both WW domains ( $\Delta$ WWs) or have their tryptophan residues mutated to alanines (YAP1-W4A) (2). Although these mutations did not affect YAP1 phosphorylation, they caused YAP1 to be retained in the cytoplasm and its transcriptional activity decreased (2). Because YAP1 transcriptional activation is controlled by its movement into the nucleus, the authors surmised that the YAP1 WW domains might contain an NLS. Indeed, GFP-YAP1  $\Delta$ WWs mutants failed to enter the nuclei of HeLa cells, whereas a chimera mutant, with tandem WW domains added to the N-terminus of YAP1  $\Delta$ WWs, rescued nuclear import (2). Moreover, each of the two WW domains promoted nuclear localization of GFP (2).

Not surprisingly, as both WW domains are small folded globular domains devoid of long loops, they appear to not contain any

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**Figure 1. Schematic of the nuclear import pathway mediated by the Imp $\alpha$ /Imp $\beta$  heterodimer. (A)** In the classical pathway, Imp $\alpha$  recognizes linear NLSs in their cargo in the cytoplasm (left), while WW domains are recognized through their folded domains by proline-rich motifs in Imp $\alpha$  (right). Imp $\beta$  binds to the importin  $\beta$ 1 binding (IBB) domain of Imp $\alpha$  and mediates the nuclear import of the complex through the NPC. **(B)** RanGTP binding to Imp $\beta$  promotes the release of the cargo in the nucleus.

recognizable cNLSs. However, WW domains have conserved tertiary structures, and Yang et al. showed that other WW domains, from the proteins WWP2, PIN1, FBP11, and PQBP1, also targeted GFP to the nucleus in a Ran-dependent fashion, suggesting importin-mediated nuclear import (2). Disrupting the WW domain conformation by mutating the tryptophan residues to alanine blocked its NLS function. Altogether, their results suggested that WW domains may act as globular NLSs that bind an importin to enter the nucleus (Fig. 1 A) (2).

Previous work had suggested that mammalian YAP1 may be imported into the nucleus either by importin-7 or the heterodimeric Imp $\alpha$ /Imp $\beta$  complex (7). When analyzing the amino acid sequences of these importins, Yang et al. found two kinds of PRMs with PPx(Y/F) and PxPP motifs, conserved within the IMP $\alpha$  family (2). Co-immunoprecipitation

confirmed that these IMP $\alpha$  motifs bind not only to the WW domains of YAP1 but also to four other WW domains encompassing the four different classes of WW domains (2). Binding assays showed that wild type YAP1 efficiently pulled down recombinant Imp $\alpha$  while YAP1 mutants did not. Transient knockdown of the Imp $\alpha$  interaction partner, Imp $\beta$ , significantly reduced YAP1 nuclear accumulation of YAP1, further supporting that Imp $\alpha$ / $\beta$  imports YAP1 (2).

The binding of globular domains of cargoes to their  $\beta$ -importin nuclear import receptors is not new. The many examples include the domains of SREBP-2 that bind directly to IMP $\beta$ , the histone-fold domains of the histone H2A-H2B and H3-H4 heterodimers that bind importin-9 and importin-4, respectively, the histone H1 domain that binds the importin-7/Imp $\beta$  heterodimer, the TBP domain that binds Kap114 (yeast homolog of importin-9), and the globular domains of Mago Y14 and UBC9 that bind importin-13 (1, 10). In all these cases, significant portions of the cargo domain surfaces interact with surfaces of the importin solenoids. Unlike linear NLS motifs defined by amino acid sequences, the determinants governing interactions between these folded entities are considerably more challenging to define. These interactions often involve multiple binding surfaces on both cargo and importin, each interface contributing differently to the total binding affinity of the importin-cargo complex. Consequently, understanding how a specific cargo's folded domain binds its importin does not necessarily allow us to predict whether another cargo with a similar domain will bind to the same importin. Even though these cargoes may share homologous folded domains, their surfaces could exhibit notable differences. Fortunately, the YAP1 WW domains bind linear sequences, PRMs in the Imp $\alpha$  loops, rather than the surfaces of the folded Imp $\alpha$  solenoid (2). This recognition mode is simply a ligand PRM binding to the WW domain and is thus generalizable to many if not all WW domains.

The work on YAP1 WW domains binding to Imp $\alpha$  has advanced our understanding of what an NLS is. Traditionally, distinct classes of NLSs such as cNLS, PY-NLS,

isoleucine-lysine NLS, and arginine-serine NLS are signal sequences that bind to different importins, directing nuclear localization of proteins that carry them (1). Nuclear import cargoes also use various folded protein domains to bind importins. Notably, the WW domain has emerged as a new category of a globular NLS that targets proteins into the nucleus. This research has also expanded our understanding of how Kaps recognize their cargoes. Elongated, non-globular importin solenoids have large surface areas that bind traditional NLS sequences and/or surfaces of globular cargo domains. The interaction observed between the WW domain globular NLS and the linear proline-rich motifs of Imp $\alpha$  represents an intriguing reversal of conventional mechanisms, offering exciting prospects for uncovering the diverse range of Kap-cargo interaction modes. We eagerly anticipate further discoveries and surprises in this dynamic field.

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