SUMO unloads the Kap114 cab

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Nucleocytoplasmic transport is an essential mechanism in all eukaryotic cells, for which the basic mechanisms seemed well understood. Transport receptors of the importin β /karyopherin family recognize, translocate and discharge cargo. Key to directed transport is the GTPase Ran, which determines compartment-specific interactions between receptors and their cargo. In this issue of *The EMBO Journal*, Rothenbusch *et al* (2012) now add a new energy-dependent event to this basic pathway by providing direct evidence that the posttranslational modification of the yeast import receptor Kap114 with small ubiquitin-related modifier (SUMO) is indispensable for its correct function. An exciting model emerges in which Kap114 sumoylation regulates Ran-dependent cargo release, and thereby acts as a mechanism for intranuclear targeting of the import cargo.

Nucleocytoplasmic transport of macromolecules is usually mediated by soluble receptors of the importin β /karyopherin family (Görlach and Kutay, 1999; Stewart, 2007). Specific receptors recognize different classes of cargoes and differ with respect to their specific transport direction. Cargo transport through nuclear pore complexes (NPCs) requires compartment-specific loading and unloading, and is accomplished with the help of the GTPase Ran and its regulators, the cytoplasmic Ran GTPase-activating protein and the nuclear Ran guanine nucleotide exchange factor; import receptors bind their respective cargo in the cytoplasm where RanGTP is low, traverse the NPC and dissociate from their cargo in the nucleus upon RanGTP binding. Export receptors bind cargo only in combination with RanGTP and dissociate from their cargo upon RanGTP hydrolysis in the cytoplasm.

While passage through the NPC is very rapid, the assembly of receptor/cargo complexes is a rate-limiting step prone to regulation, for example, by cargo modifications that reveal or mask transport signals. Disassembly of import complexes by RanGTP binding and of export complexes by RanGTP hydrolysis is generally assumed to happen rapidly, and cargo-specific regulation of this event is not yet known. However, *in vitro* studies with specific import and export receptors and their respective cargoes revealed that these disassembly mechanisms are not always efficient; additional interactions and/or modifications seem to be required *in vivo* (e.g., Pemberton *et al*, 1999; Greiner *et al*, 2004).

One posttranslational modification with extensive links to nucleocytoplasmic transport is sumoylation (Geiss-Friedlander

and Melchior, 2007; Palancade and Doye, 2008). Attachment of SUMO requires ATP and an enzymatic cascade that involves an E1-activating enzyme, an E2-conjugating enzyme and one of several known E3 ligases (e.g., Siz/PIAS proteins or the vertebrate-specific nucleoporin RanBP2/Nup358). A small family of SUMO-specific isopeptidases can revert the

Figure 1 Sumoylation regulates transport receptor/cargo complex disassembly. Disruption of nuclear import receptor/cargo interaction by RanGTP is a terminal step in nuclear protein import. However, *in vitro* studies indicate that Ran interaction does not always suffice to disrupt complexes. In this *EMBO J.* issue, Rothenbusch *et al* (2012) report that the efficient release of cargo from the importin Kap114 in yeast requires SUMO modification of Kap114 in addition to its interaction with RanGTP. Interfering with either sumoylating or desumoylating activity in yeast cells results in nuclear accumulation of Kap114 and cytoplasmic accumulation of its cargoes. Regulation of transport receptor/cargo complex dissociation by reversible sumoylation may represent a new concept in regulated protein interaction during nucleocytoplasmic transport and mitosis.

modification. The SUMO pathway is essential in most eukaryotes including *S. cerevisiae*, and participates, for example, in cell cycle regulation, signal transduction and DNA damage. The idea that SUMO contributes to nucleocytoplasmic transport exists ever since the discovery of RanGAP1 as the first SUMO target 15 years ago. The observation that the vertebrate nucleoporin RanBP2 has E3 ligase activity strengthened this idea. While these findings were specific to vertebrates, the enrichment of a SUMO isopeptidase (Ulp1/Senp2) at NPCs is conserved throughout the eukaryotic evolution, including yeast. A functional link between the SUMO machinery and nucleocytoplasmic transport in yeast was revealed by the finding that the import of proteins with a classical nuclear localization sequence is impaired in strains deficient for the SUMO E1 enzyme subunit Uba2 or the SUMO isopeptidase Ulp1 (Stade *et al.*, 2002). Inactivation of these enzymes caused accumulation of the import receptor adaptor importin α (S.c. Srp1) in the nucleus, but the underlying mechanisms remained unclear.

In this issue of The EMBO Journal, the group of Gabriel Schlenstedt reports that sumoylation regulates Kap114-mediated nuclear transport in yeast (Rothenbusch *et al.*, 2012). Kap114 (the homologue of mammalian importin-9) is a non-essential member of the importin β family and involved in import of, for example, the transcription factor Sua7 and the TATA-binding protein Tbp1. During a systematic screen of transport receptors for their potential to be sumoylated, Rothenbusch *et al.* (2012) identified Kap114 as a target for sumoylation *in vitro* and *in vivo*. The SUMO E3 ligase Mms21, which was previously only known to be involved in the maintenance of genome stability as part of the Smc5/6 complex, is required for *in vivo* modification. Sumoylation of Kap114 takes place on a single consensus sumoylation motif close to the cargo-binding region, but does not inhibit cargo binding. Importantly, cargo-bound Kap114 can also be sumoylated. Consistent with its function as shuttling receptor, Kap114 usually localizes both in the nucleus and the cytoplasm; however, manipulating its sumoylation status either positively or negatively resulted in a striking nuclear accumulation. This suggested that reversible sumoylation is required for Kap114 shuttling; in line with this, Sua7 and Tbp1 remained in the cytoplasm of Uba2-, Ulp2- and Mms21-deficient yeasts. A likely explanation for Kap114 accumulation in the nucleus was revealed when the authors tested whether

sumoylation may contribute to the disassembly of Kap114 import complexes. Indeed, SUMO modification of Kap114 stimulated RanGTP-mediated release of several cargoes from the import complex. In consequence, failure to sumoylate Kap114 can trap it in intranuclear cargo complexes. A major implication of these findings is that the Kap114/cargo complex is not disassembled immediately upon reaching the nucleus, but can only be released at an intranuclear site where the E3 ligase Mms21 resides. Kap114 sumoylation can therefore also be seen as an intranuclear targeting mechanism. Whether RanGTP binding precedes Kap114 sumoylation (Figure 1), or whether sumoylation precedes RanGTP binding (suggested in the model by Rothenbusch *et al.* (2012)) remains an open question. On the basis of the findings with isopeptidase-deficient yeast strains, Kap114 also needs to be desumoylated prior to nuclear export. This is intriguing, considering that sumoylation does not interfere with RanGTP binding. Possible explanations include SUMO-induced conformational changes of the receptor that preclude NPC interactions or SUMO-dependent nuclear retention.

Conceptually, the work by Schlenstedt and coworkers presents a truly exciting finding—it suggests an additional level of complexity in nucleocytoplasmic transport, in which transport complex disassembly is tightly controlled by an ATP-dependent posttranslational modification (Figure 1). It is very attractive to speculate that sumoylation-dependent dissociation is not limited to Kap114 and its cargoes in yeast, but is also used for other transport receptors and in other organisms. In light of this, it should be pointed out that several transport receptors were identified as candidates in screens for SUMO targets (e.g., Bruderer *et al.*, 2011). Last, but not the least, we would like to stress that tight control of nuclear transport receptor/cargo interactions is not only required in nucleocytoplasmic transport, but also contributes, for example, to mitotic spindle assembly, where spatially controlled release of spindle assembly factors from importin α/β complexes is required (Gruss *et al.*, 2001). Whether transport receptor sumoylation participates in mitotic events as well is one of the many exciting questions for future studies.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Bruderer R, Tatham MH, Plechanovova A, Matic I, Garg AK, Hay RT (2011) Purification and identification of endogenous polySUMO conjugates. *EMBO Rep* **12**: 142–148
- Geiss-Friedlander R, Melchior F (2007) Concepts in sumoylation: a decade on. *Nat Rev Mol Cell Biol* **8**: 947–956
- Görlach D, Kutay U (1999) Transport between the cell nucleus and the cytoplasm. *Annu Rev Cell Dev Biol* **15**: 607–660
- Greiner M, Caesar S, Schlenstedt G (2004) The histones H2A/H2B and H3/H4 are imported into the yeast nucleus by different mechanisms. *Eur J Cell Biol* **83**: 511–520
- Gruss OJ, Carazo-Salas RE, Schatz CA, Guarguaglini G, Kast J, Wilm M, Le Bot N, Vernos I, Karsenti E, Mattaj JW (2001) Ran induces spindle assembly by reversing the inhibitory effect of importin α on TPX2 activity. *Cell* **104**: 83–93
- Palancade B, Doye V (2008) Sumoylating and desumoylating enzymes at nuclear pores: underpinning their unexpected duties? *Trends Cell Biol* **18**: 174–183
- Pemberton LF, Rosenblum JS, Blobel G (1999) Nuclear import of the TATA-binding protein: mediation by the karyopherin Kap114p and a possible mechanism for intranuclear targeting. *J Cell Biol* **145**: 1407–1417
- Rothenbusch U, Sawatzki M, Chang Y, Caesar S, Schlenstedt G (2012) Sumoylation regulates Kap114-mediated nuclear transport. *EMBO J* **31**: 2461–2472
- Stade K, Vogel F, Schwienhorst I, Meusser B, Volkwein C, Nentwig B, Dohmen RJ, Sommer T (2002) A lack of SUMO conjugation affects cNLS-dependent nuclear protein import in yeast. *J Biol Chem* **277**: 49554–49561
- Stewart M (2007) Molecular mechanism of the nuclear protein import cycle. *Nat Rev Mol Cell Biol* **8**: 195–208