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Commentary

Plug-and-socket mechanisms in nutrient sensing by lysosomal amino acid transporters

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Unicellular organisms and epithelial cells from animals and plants must adapt to abrupt changes in their environment. Similarly, cells bathed in the protective *milieu intérieur* of multicellular organisms must monitor their intracellular nutrient and energy levels to regulate key cellular processes such as growth, proliferation, autophagy and transcriptional programs. To perform these adaptations, cells have evolved a capacity to sense nutrients and induce signaling cascades from nutrient sensors. Some sensors exist in soluble form within the cytosol while other sit in the plasma membrane and intracellular membranes (1). Early studies in yeast identified several nutrient sensors among membrane transporters or transporter-like proteins rather than bona fide receptors (2, 3), leading to the hybrid concept of 'transceptor': a transporter which has evolved to interact with signaling proteins in a substrate-dependent manner (4). In PNAS, Talaia et al. (5) characterize such transceptor interaction between a lysosomal amino acid transporter and a signaling complex implicated in two neurodegenerative diseases, amyotrophic lateral sclerosis and frontotemporal dementia. Their study unveils a plug-and-socket mechanism where a flexible protruding loop of the complex plugs into the central cavity of the transporter, suggesting a mechanism for the interplay between amino acid transport and amino acid signaling. Interestingly, this mechanism bears some resemblance with the interaction between another lysosomal amino acid transporter and a signaling complex intervening in the mechanistic target of rapamycin complex 1 (mTORC1) pathway elucidated in very recent studies (6, 7).

The transceptor concept is based on a key property of membrane transporters: to translocate small molecules or ions across biological membranes, these proteins must undergo structural changes that

alternately expose their substrate-binding site to one or the other side of the membrane (Fig 1A). This alternating-access mechanism allows translocating nutrients and waste products 'uphill' against their concentration gradient by coupling this translocation to the co-transport or exchange of an ion pumped by an ion-translocating ATPase. If a specific conformation of the transporter interacts with a signaling protein, and activates a signaling cascade, the transporter becomes a transceptor. The protein might eventually lose its transport activity during evolution while conserving its signaling function if it cannot visit all conformations of the alternating-access cycle.

The transceptor concept attracted attention since it was put forward (4) and several nutrient transceptors have been established or suggested in microorganisms, plants and animals. As the translocated nutrient or its driving ion(s) may have a downstream impact on signaling cascades (see (8) for amino acid-sensing by the yeast transporter Gap1), it is essential to demonstrate a physical interaction between the transporter and a component of the signaling cascade, and the dependence of this interaction on the conformational state of the transporter to establish a genuine transceptor mechanism.

The study of the lysosomal amino acid transporter PQ-loop repeat containing protein 2 (PQLC2) by Talaia et al. (5) and recent studies involving another lysosomal transporter (6, 7) unveiled such transceptor mechanisms in the membrane of an intracellular organelle, the lysosome.

Nutrient sensing by mTORC1 at the lysosomal surface

Lysosomes are membrane-bound hydrolytic organelles responsible for the degradation and recycling of cell and tissue components. They also constitute signaling hubs which integrate environmental and intracellular cues, including intracellular amino acids, to coordinate cell growth and metabolism. Amino acids trigger the recruitment of mTORC1 to the lysosomal surface, where it is activated by the small GTPase Ras homologue enriched in brain (Rheb) in response to energy status and growth factors (1, 9). Several amino acid sensors specific for leucine, arginine or S-adenosylmethionine are involved in this response (10). Among them, the amino acid transporter SNAT9, also known by its gene name *SLC38A9*, stands out by its localization in the lysosomal membrane and its ability to sense intralysosomal arginine. SNAT9 has a dual function in this membrane: it exports branched-chain and aromatic amino acids generated by lysosomal proteolysis to the cytosol (11) and it activates the heterodimeric Rag GTPase tethered to the lysosomal membrane by the Ragulator complex (12, 13). In its active state ($RagA/B^{GTP}$-$RagC/D^{GDP}$), the Rag GTPase in turn recruits mTORC1.

Arginine stimulates both transport and signaling activities of SNAT9 (11–13). The large, cytosolic N-terminal tail of SNAT9 had been implicated in the interaction with the Ragulator-Rag complex (12, 13), however, the arginine-sensing mechanism remained unclear. In a recent study (7), part of the N terminus was shown to form a β hairpin which plugs into the central cavity of SNAT9 in cytosol-open conformation and interacts with the arginine-binding pocket at the bottom of the cavity. Arginine competes with the β hairpin, thus releasing it from the central cavity of SNAT9 with two outcomes: the amino acid transport activity is upregulated because SNAT9 alternating-access movements are not hindered anymore, and the N-terminal tail can engage in other interactions (Fig. 1B). In another study (6), the N-terminal tail was

shown to associate with the inactive state (RagA/B^{GDP}-RagC/D^{GTP}) of the Ragulator-Rag complex by plugging into the cleft between the Rag nucleotide-binding domains. This displaces the folliculin-FNIP2 complex from the inactive Ragulator-Rag complex and activates its GTPase activating protein (GAP) activity towards RagC, thus promoting progression of the Rag heterodimer towards its active state to recruit mTORC1 (Fig. 1B).

Nutrient signaling by the lysosomal transporter PQLC2 to the C9orf72-SMCR8-WDR41 complex

Another complex formed by the C9orf72, SMCR8 and WDR41 (CSW) proteins responds to amino acid starvation at the lysosomal surface (14). This complex has received growing attention because a hexanucleotide repeat expansion in the *C9orf72* gene is a frequent genetic cause of amyotrophic lateral sclerosis and frontotemporal dementia. The *C9orf72*-mediated diseases are transmitted in an autosomal dominant manner and both gain of function (toxicity of RNA foci or dipeptide repeat proteins) and loss of function (C9orf72 haploinsufficiency) mechanisms have been implicated in pathogenesis (15). The C9orf72 and SMCR8 subunits share some homology with folliculin and FNIP2 while WDR41 is a β-propeller protein that associates with the C9orf72-SMCR8 heterodimer (14, 16, 17). Like folliculin, SMCR8 has a GAP activity directed, however, towards Arf and some Rab GTPases instead of Rags (6, 16).

Under amino acid starvation the CSW complex is recruited to lysosomes through an interaction between its WDR41 subunit and the lysosomal transporter PQLC2 (18). As PQLC2 transports cationic amino acids (19, 20) and the CSW complex selectively responds to cationic amino acid repletion (18), PQLC2 was a candidate transceptor of these amino acids. However, in contrast with established yeast transceptors and SNAT9, PQLC2 lacks prominent domains exposed to the cytosol.

How does PQLC2 transduce cationic amino acid binding to the CSW complex? To address this issue, Talaia et al (5) built 3D homology models of PQLC2 and WDR41 and performed protein-protein docking simulations to predict how the proteins interact. Interestingly, they identified a protruding loop of WDR41 that plugs into the central cavity of the PQLC2 model in cytosol-open conformation. This loop was not resolved in recent cryo-electron microscopy structures of the CSW complex (16, 17), suggesting it is flexible. Talaia et al (5) then mutated selective residues of the WDR41 loop and the PQLC2 cavity and analyzed the interaction between the mutant proteins in vitro and in live cells to test, and validate, their docking model. The WDR41 'plug' could be narrowed down to a 10-amino acid peptide necessary and sufficient for the interaction with the lysosomal transporter.

The CSW complex thus senses cationic amino acid levels by inserting a 10-amino acid plug into a specific conformation of PQLC2 (Fig 1C), thus linking the alternating-access cycle of the transporter to the dissociation of the complex.

A plug-and-socket mechanism where a short hairpin, either from the transporter itself (SNAT9) or from a signaling partner (WDR41), inserts into the central cavity of the transporter thus emerges as an efficient way to couple the transport and signaling activities of two lysosomal transceptors. The biological role of the CSW complex remains unclear and the significance of its regulation by amino acids is still unknown.

The mechanism unveiled by Talaia et al (5) provides useful tools to interrogate these roles and explore their potential relevance for the understanding and treatment of amyotrophic lateral sclerosis and frontotemporal dementia.

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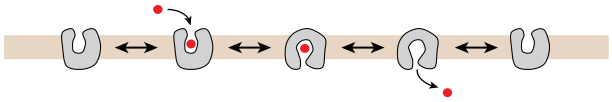
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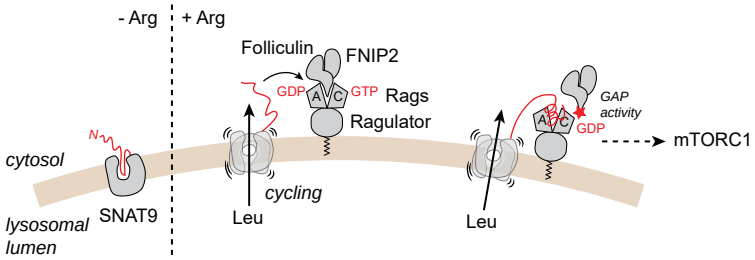
Figure legend.

Fig. 1: Nutrient sensing by lysosomal amino acid transceptors. (A) Alternating-access conformational cycle of a canonical transporter. (B) Arginine-sensing and signaling by the lysosomal amino acid transporter SNAT9/SLC38A9. (C) Cationic amino acid-sensing by the lysosomal transporter PQLC2. In both mechanisms (B and C), a short peptide from the transporter itself (SNAT9) or from its cognate signaling protein (WDR41) plugs into the central cavity of the transporter, thus linking the transport and signaling activities. See main text for mechanistic details of the schemes.

A nutrient transport



B nutrient signaling by SNAT9/SLC38A9



C nutrient signaling by PQLC2

