REVIEW

The Role of Cornichons in the Biogenesis and Functioning of Monovalent-Cation Transport Systems

Klára PAPOUŠKOVÁ[1], Karolína ČERNÁ[1], Viktorie RADOVÁ[1], Olga ZIMMERMANNOVÁ[1]

[1]Laboratory of Membrane Transport, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic

Received May 14, 2024
Accepted May 16, 2024

Summary

Monovalent-cation homeostasis, crucial for all living cells, is ensured by the activity of various types of ion transport systems located either in the plasma membrane or in the membranes of organelles. A key prerequisite for the functioning of ion-transporting proteins is their proper trafficking to the target membrane. The cornichon family of COPII cargo receptors is highly conserved in eukaryotic cells. By simultaneously binding their cargoes and a COPII-coat subunit, cornichons promote the incorporation of cargo proteins into the COPII vesicles and, consequently, the efficient trafficking of cargoes *via* the secretory pathway. In this review, we summarize current knowledge about cornichon proteins (CNIH/Erv14), with an emphasis on yeast and mammalian cornichons and their role in monovalent-cation homeostasis. *Saccharomyces cerevisiae* cornichon Erv14 serves as a cargo receptor of a large portion of plasma-membrane proteins, including several monovalent-cation transporters. By promoting the proper targeting of at least three housekeeping ion transport systems, Na^+, K^+/H^+ antiporter Nha1, K^+ importer Trk1 and K^+ channel Tok1, Erv14 appears to play a complex role in the maintenance of alkali-metal-cation homeostasis. Despite their connection to serious human diseases, the repertoire of identified cargoes of mammalian cornichons is much more limited. The majority of current information is about the structure and functioning of CNIH2 and CNIH3 as auxiliary subunits of AMPAR multi-protein complexes. Based on their unique properties and easy genetic manipulation, we propose yeast cells to be a useful tool for uncovering a broader spectrum of human cornichons´ cargoes.

Key words

Cornichon • Cation homeostasis • Transporters • Yeast • AMPAR

Corresponding author

O. Zimmermannová, Laboratory of Membrane Transport, Institute of Physiology of the Czech Academy of Sciences, Videnska 1083, 142 00 Prague 4 – Krč, Czech Republic. E-mail: olga.zimmermannova@fgu.cas.cz

Introduction

Cells need to incessantly adapt to gradual or sudden changes in cation concentrations in the extracellular milieu to maintain intracellular conditions that are suitable for their fitness (Fig. 1A). K^+, H^+, and Na^+ are the most important monovalent cations, required for all physiological processes [1]. From bacteria to mammals, cells need to maintain a stable cytosolic pH of 7.0 (the H^+ concentration) and accumulate a high concentration of K^+ (Fig. 1A), as it is essential for many physiological functions, including the regulation of cell volume and intracellular pH, and the maintenance of membrane potential [1]. Adequate K^+ content is also a pivotal signal for cell division and a prerequisite for resistance to various stresses [1,2]. A high intracellular concentration of Na^+ is generally toxic [1]. However, the electrochemical gradients of Na^+ and H^+ across the membranes are a driving force for secondary active transport systems. In mammals, Na^+ is the major cation in extracellular space, and both Na^+ and K^+ are also employed to generate membrane-potential changes in

A

Concentration (mM)	Sea water	Yeast	Plant cells	Animal cells	Blood plasma
K^+	≈ 10	300	200	100	4
Na^+	≈ 500	< 30	< 10	10	100-200

B

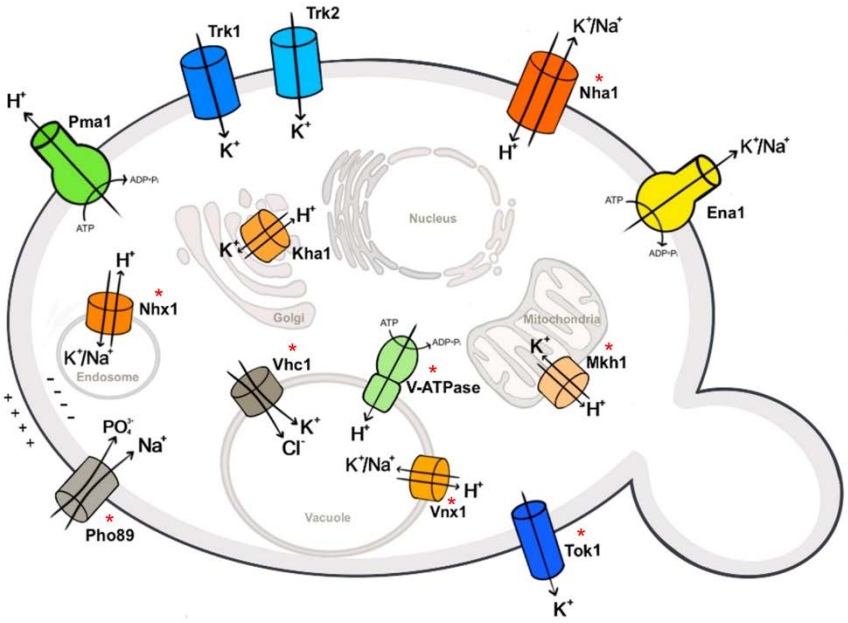

Fig. 1. Monovalent-cation homeostasis in eukaryotic cells. (**A**) Concentrations of K^+ and Na^+ in various representative organisms and extracellular environments. All cells accumulate K^+, keep low content of toxic Na^+, and preserve neutral cytosolic pH [1,11-13]. (**B**) An overview of transport systems for monovalent cations in *S. cerevisiae* (* transport systems with orthologues in humans [14]).

excitable cells, such as neurons. The intracellular concentrations of K^+, Na^+ and H^+ are strictly regulated *via* the activity of a series of membrane proteins – transporters and channels – mediating the fluxes of these cations across the membranes. In the model organism of eukaryotic cells, the yeast *Saccharomyces cerevisiae*, more than ten transport systems for monovalent cations have been characterized in the plasma membrane and in intracellular membranes (Fig. 1B, [3]). Some are fungi-specific, but many yeast transporters have orthologues in mammalian cells (Fig. 1B). Disturbances in H^+, Na^+ and/or K^+ concentrations result in many pathological conditions in humans, such as neurodegenerative diseases, metabolic disorders and malignant transformations [4]. Hence, the investigation of regulatory mechanisms that ensure cationic homeostasis is receiving increasing attention in biomedical sciences.

Cation transporters are complex membrane proteins with hydrophobic parts embedded in cellular membranes, and in many cases, large hydrophilic regions positioned on both sides of the membrane. The activity of transporters is tightly regulated at several levels, e.g., the level of their expression and/or post-translational modifications. Moreover, the correct function of each membrane protein depends on its effective delivery into the target membrane. In eukaryotic cells, approximately one-third of nascent polypeptides undergo maturation during proteosynthesis *via* their passing through the secretory pathway [5]. An important group of proteins that interact with maturing proteins and ensure their passage through the secretory pathway are cargo receptors that help to insert their interaction partners into COPII vesicles, which are exported from the endoplasmic reticulum (ER) to the Golgi apparatus (Fig. 2A). The goal of this overview is to summarize current findings about the structures and physiological roles of the cornichon family of cargo receptors, with the emphasis on *S. cerevisiae* Erv14 protein and its mammalian homologues CNIH1-4, and their particular importance for the biogenesis of monovalent-cation transporters.

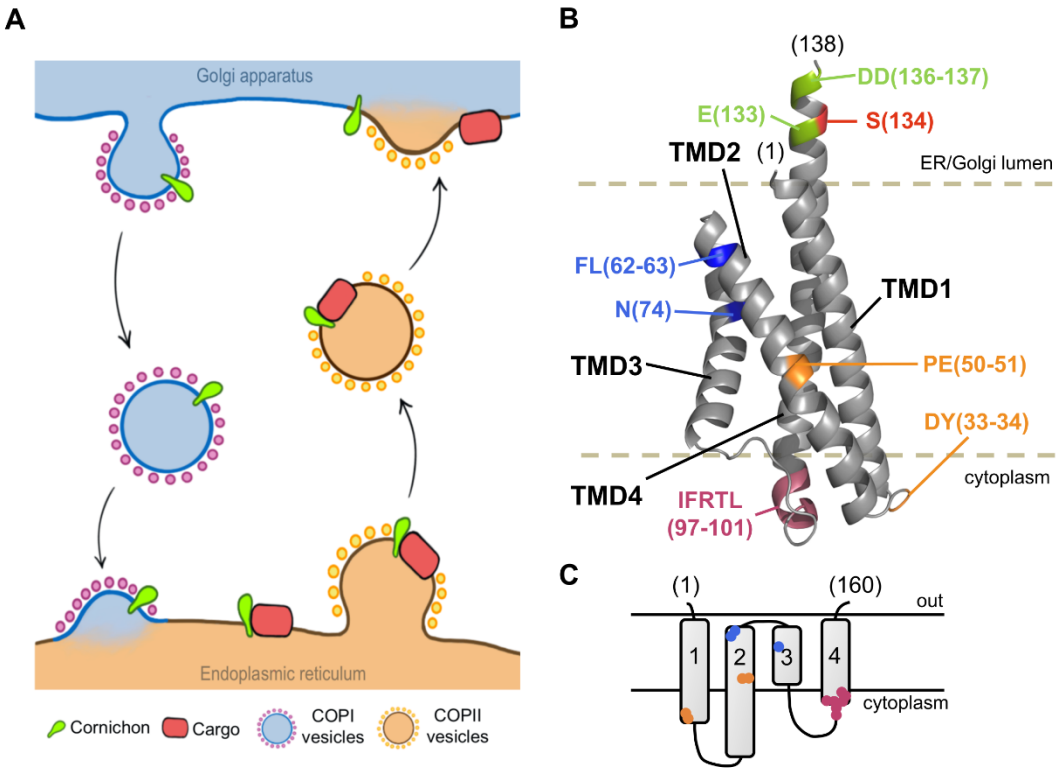

Fig. 2. Basic properties of cornichons. (**A**) Scheme of functioning of cornichons as COPII cargo receptors. (**B**) AlphaFold [23] structural model of Erv14. Regions important for the functioning of the protein (see the text) are depicted in different colors. (**C**) Scheme of topology of mammalian CNIH2/3 proteins. Positions of amino-acid residues homologous to Erv14 regions highlighted in (B) are depicted.

Characterizing the physiological functions of cargo receptors from yeasts and humans and their importance for the maintenance of monovalent-cation homeostasis is one of the research topics investigated in the Laboratory of Membrane Transport at the Institute of Physiology CAS. It has arisen from a fruitful collaboration with the Laboratory of Prof. Omar Pantoja from the Institute of Biotechnology at the National Autonomous University of Mexico [6-10], within which we also employed the yeast *S. cerevisiae* and its mutated strains to investigate the interaction between the cornichon CNIH1 and the Na^+ transporter HKT1;3 from *Oryza sativa* [7].

Cargo-receptor function and structure of cornichon proteins

The cornichon family of COPII cargo receptors is highly conserved in eukaryotic organisms. The first characterized member, *Drosophila* cornichon Cni, which was reported to be necessary for the proper delivery of the transforming growth factor α (TGFα) from the ER to the oocyte surface during *Drosophila* oogenesis, gave the name to the whole family of proteins [15,16]. The absence of either the TGFα Gurken (Grk) or cornichon Cni results in an altered shape of the fly´s eggs, which are elongated (Gurken means “cucumbers” in German) [16-18]. However, the most characterized cornichon is the *S. cerevisiae* Erv14 protein. It is a 14kDa, 138-amino-acid-residue-long protein that was originally found in isolated ER-derived vesicles [19]. Erv14 is predicted to be an integral membrane protein that localizes to the ER and Golgi apparatus [19]. By binding both the cargo protein and also a COPII coat subunit, Sec24 [20,21], Erv14 promotes the incorporation of its cargoes into COPII vesicles (Fig. 2A).

Previously, three transmembrane domains were predicted in Erv14 and, applying the “positive-inside rule”, the N-terminus of the protein was expected to be located in the cytoplasm and the C-terminus in the lumen of the ER/Golgi apparatus [21,22]. Although some experimental evidence has further supported this predicted topology of Erv14 [21], a more recent AlphaFold [23,24] model of Erv14 suggests a topology with four transmembrane domains and both termini

located in the lumen of the ER (Fig. 2B). This model is in full agreement with experimentally discovered structures of mammalian homologues, cornichon proteins CNIH2 and CNIH3, which act as auxiliary subunits of AMPA-type ionotropic glutamate receptors in the plasma membrane [25-28]. In both CNIH2 and CNIH3, cryo-electron microscopy revealed that the N- and C-termini are located in the extracellular space (Fig. 2C). The first transmembrane domain spans the membrane, and after a short loop in the cytoplasm, the second α-helical segment, which already starts in the cytoplasm, again enters the membrane bilayer. There, the amino-acid chain turns back, the third transmembrane domain crosses the lipid bilayer and finally, after a cytoplasmic loop, the fourth transmembrane domain once more fully spans the membrane (Fig. 2C). Thus, the majority of mammalian cornichons CNIH2 and CNIH3 seems to be buried in the membrane, and only small portions of the proteins are located in the cytoplasm [26,28].

Characteristics of *S. cerevisiae* cornichons

S. cerevisiae Erv14 helps its binding partners to be efficiently transported through the secretory pathway (Fig. 2A). When Erv14 is not present in cells, its cargoes are typically partially accumulated in the ER (Fig. 3, [10,19,29]). The first known cargoes of Erv14, such as Axl2, which is required for the axial budding pattern of haploid cells, or Sma2, which plays a role in the formation of prospore membrane during sporulation, were discovered based on phenotypes of cells lacking the *ERV14* gene [19,21,30]. Later, to study the cargo spectrum of individual yeast cargo receptors more systematically, Herzig and colleagues [29] developed a "PAIRS" (pairing analysis of cargo receptors) approach. The methodology was based on the crossing of strains with deletions of individual non-essential genes encoding cargo receptors, such as Erv14, with strains from the library of *S. cerevisiae* with GFP-tagged putative cargoes (proteins from post-ER compartments). The newly created libraries of strains lacking cargo receptors and simultaneously harboring GFP-tagged putative cargoes were then inspected using automated microscopy screening. The PAIRS approach revealed that Erv14 serves as a cargo receptor of a large proportion (18 of 57) of the tested plasma-membrane proteins [29]. So far, approx. 40 diverse cargoes whose ER exit is dependent on the presence of Erv14 have been identified. Erv14´s cargoes are proteins with various physiological functions; membrane transporters, including several monovalent-cation transporters, flippases or proteins involved in budding, sporulation and other processes belong among them (see below, [9,10,19,21,29,30]).

A paralogue of *ERV14*, *ERV15*, is also present in *S. cerevisiae* genome [19]. Erv15 is 142 amino-acid residues long and shares 63 % identity with Erv14. No cargo whose trafficking through the secretory pathway would depend on the presence of Erv15 was identified using the PAIRS method [29]. However, Erv15 could cooperate with Erv14 in supporting the proper trafficking of some cargoes, such as the ABC transporter Yor1 [20]. When the packaging of Yor1 to COPII vesicles was studied in *in vitro* vesicle-formation assays, Erv15 alone was insufficient to compensate for the packaging defects associated with the lack of Erv14. However, the deletion of both *ERV14* and *ERV15* in cells resulted in a more pronounced defect than in cells that only lacked Erv14 [20]. The lower importance of Erv15 for the trafficking of various cargoes compared to Erv14 might be connected to the differences in the expression levels of both proteins. In agreement, when *ERV15* was expressed under the control of *ERV14* promoter in *erv14Δ* and *erv14Δ erv15Δ* cells, the protein gained the ability to restore packaging of Yor1 to COPII vesicles [20].

The binding partners of Erv14 do not seem to share any amino-acid motif which would be responsible for their recognition by Erv14 [29]. However, since the transmembrane domains of integral membrane proteins residing in post-Golgi compartments are longer [31], it was suggested that Erv14 might recognize its cargoes based on the length of their membrane-spanning segments [29]. Indeed, a replacement of the only endogenous transmembrane domain of Mid2, a known Erv14 cargo, with a chain of 14-26 leucines (with two-amino-acid increments) showed that longer versions of Mid2 exit the ER more efficiently and in an Erv14-dependent manner. Thus, these findings supported the idea that the length of transmembrane domains is a determining factor for Erv14-dependent sorting [29].

Several amino-acid residues of Erv14 have been identified to be important for its proper functioning. The stretch of amino-acid residues 97-101 (IFRTL, Fig. 2B) was found to be necessary for the efficient packaging of Erv14 into COPII vesicles and for the binding of COPII-coat components *in vitro*. The mutations of amino-acid residues 97-IFRTL-101 to alanine residues also resulted in defects in axial bud selection, which is typical for *erv14Δ* cells [19,21]. Thus, amino acids 97-101 were

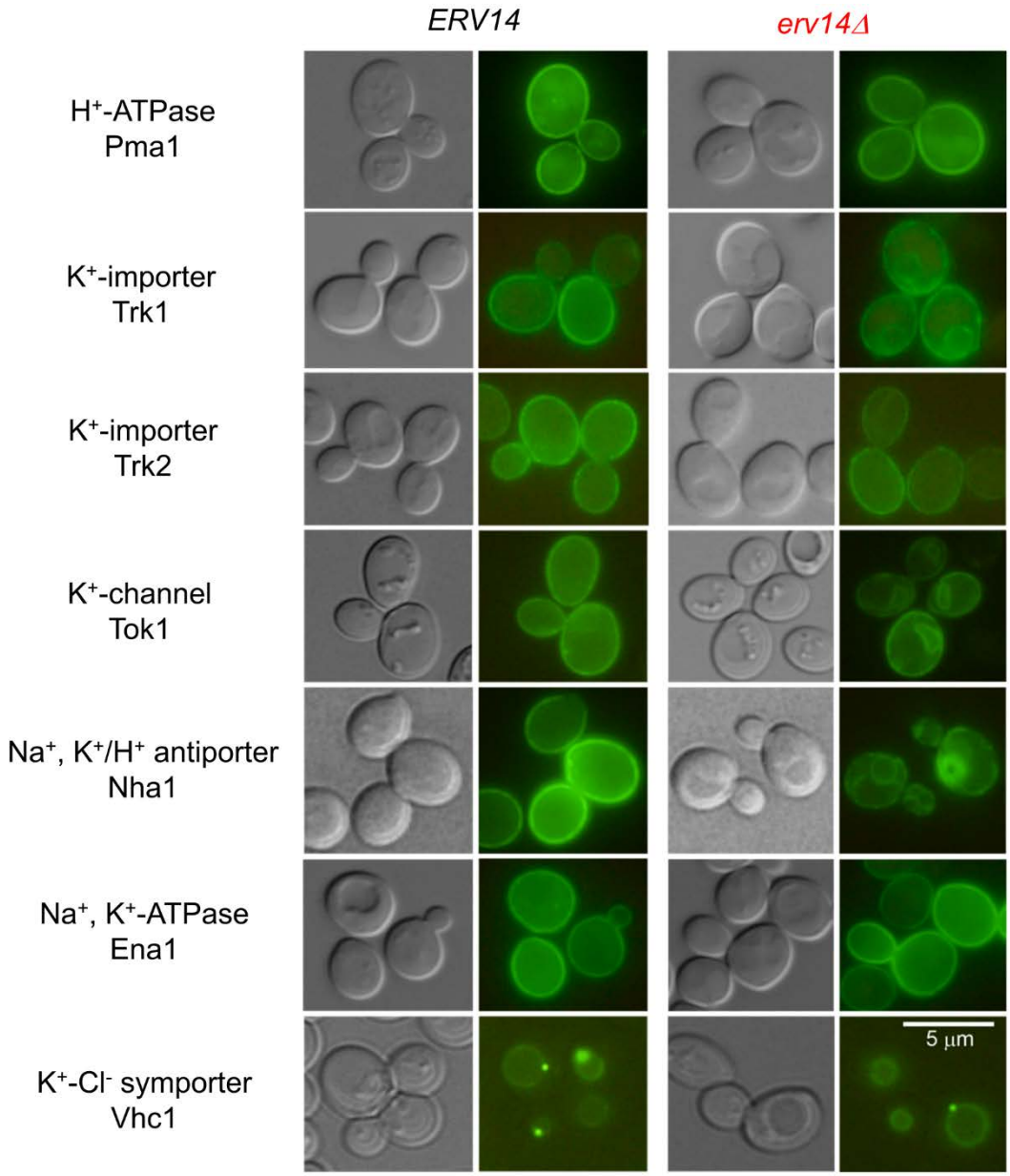

Fig. 3. Role of Erv14 in the targeting of various yeast cation transporters. Deletion of *ERV14* in *S. cerevisiae* results in retention of housekeeping K^+ transporters Trk1, Tok1 and Nha1 in endoplasmic reticulum. On the other hand, the localization of Pma1, Trk2, Ena1 and Vhc1 is not affected in *erv14Δ* cells. Nomarski (left) and fluorescence (right) micrographs of cells with or without Erv14 producing seven GFP-tagged membrane transporters as indicated. Cells were grown to the early exponential phase. The image was prepared using our results published in [9,10].

suggested to play a role in the direct binding of Erv14 to COPII coat [21]. According to the most recent structural prediction of Erv14 and analogously to the topology of mammalian cornichons (Fig. 2), the IFRTL motif is supposed to be located on the cytosolic side of the membrane at the beginning of the fourth α-helix of Erv14, which is in full agreement with its proposed role in binding the COPII complex. The incorporation of Erv14 into ER-derived vesicles was also affected by mutations of the DYPE (DY33-34, PE50-51) site (Fig. 2B, [20]). These amino-acid residues are located in the cytoplasmic short loop connecting the first and second α-helix of Erv14 (DY33-34) and in the second transmembrane domain of the cargo receptor (PE50-51), according to the most recent model of Erv14 (Fig. 2B).

On the other hand, mutations of three amino-acid residues, F62, L63, and N74, which are predicted to be located in the second or third transmembrane domain of

Erv14, respectively (Fig. 2B), only affected *in vitro* packaging of the Erv14 substrate Yor1 into ER-derived vesicles, while the packaging of Erv14 itself remained unchanged [20]. These results suggested that the FLN motif might be involved in the binding of cargo proteins by Erv14. Indeed, mutations F62A and L63A impaired the binding of Erv14 to at least some of its cargoes, as observed by the yeast two-hybrid system and co-immunoprecipitation from microsomal membranes [20].

In the C-terminal parts of fungal and plant (but not animal) cornichons, a conserved acidic motif containing at least three aspartic or glutamic amino acids has been identified [8]. In *S. cerevisiae* Erv14, the motif is formed by amino-acid residues 133-137 (ESGDD, Fig. 2B). The mutations of the acidic amino-acid residues in the motif (E133A, D136A and D137A) did not influence the localization of Erv14 in the ER/Golgi apparatus. However, Erv14 with these mutations did not fully support the proper trafficking of at least some of its cargoes to the plasma membrane, and the yeast two-hybrid system, supported by co-immunoprecipitation assays, suggested that the C-terminal EDD motif is involved in the binding of cargo proteins by Erv14 [8]. S134 from the same Erv14´s C-terminal motif (Fig. 2B) was suggested to be phosphorylated [32]. The mutations of S134 to aspartate or to alanine (mimicking the phosphorylated or dephosphorylated state of Erv14, respectively) resulted in changes in the ER structure as observed by electron microscopy. While Erv14´s cargo proteins were properly localized to the plasma membrane in cells with Erv14-S134A, they were partially accumulated in the ER of cells with Erv14-S134D [32]. In contrast to the C-terminal acidic amino-acid residues, S134 does not seem to be involved in the interaction between Erv14 and its cargoes [32]. However, the phosphomimetic mutation S134D caused both an ER retention of Erv14 and also affected its packaging to COPII vesicles *in vitro*. Based on these results, it was suggested that a cycle of phosphorylation and dephosphorylation of serine at position 134 is important for the proper functioning of Erv14 as a COPII cargo receptor that ensures the exit of its cargoes from the ER [32].

Role of cornichons in monovalent-cation homeostasis of yeast and plant cells

Comparisons of several physiological parameters of *S. cerevisiae* cells with or without Erv14 revealed that the lack of Erv14 decreases cell tolerance to high NaCl, KCl and cationic-drug (hygromycin B, tetramethylammonium) concentrations, and also the ability of cells to cope with low-K^+ conditions. Moreover, the deletion of *ERV14* influences cell volume and results in a relative plasma-membrane hyperpolarization as well as in a lower intracellular pH level [9,10]. Taken together, these results suggested that Erv14 plays a complex role in the maintenance of monovalent-cation homeostasis in yeast cells, most likely *via* assisting several transporters in their trafficking through the secretory pathway.

The first alkali-metal-cation transporter whose proper plasma-membrane targeting was shown to be Erv14-dependent was the Na^+, K^+/H^+ antiporter Nha1 [9,29]. Nha1 is a house-keeping protein whose cation-export activity is driven by the electrochemical gradient of H^+, which is generated by the H^+-ATPase Pma1 (Fig. 1B). It mainly contributes to the tolerance of *S. cerevisiae* cells to high external concentrations of Na^+ and K^+ at low external pH levels [33,34]. In the absence of the Erv14 cargo receptor, Nha1 is partially stacked in the ER of cells (Fig. 3), and the consequent decreased Na^+ or K^+ efflux *via* Nha1 results in a lower ability of *erv14Δ* cells to cope with high amounts of NaCl and KCl in the environment [9,29]. The physical interaction of both proteins was proved by the yeast two-hybrid system as well as co-immunoprecipitation assays [9].

Nha1 is a large (985 amino-acid residues long) polytopic membrane protein with a short N-terminus, 13 predicted transmembrane domains, and a long hydrophilic highly disordered cytoplasmic C-terminus that forms more than half of the entire protein (Fig. 4, [23]). The majority of Nha1´s C-terminal portion is neither necessary for the antiporter´s cation-translocation function nor for its plasma-membrane localization. However, an increasing amount of evidence shows the importance of this large hydrophilic portion of the antiporter in the regulation of Nha1´s activity [35-38]. Surprisingly, a truncated version of Nha1 that is shortened to 472 amino-acid residues and that lacks the majority of its C-terminal part, Nha1-472 (Fig. 4), does not require Erv14 for its trafficking to the plasma membrane *via* the secretory pathway, though it still binds the cargo receptor [9].

In various yeast Nha1 homologues, the C-termini are the least conserved parts, both in terms of their sequence and length [35,39]. However, our recent broad bioinformatic analysis of fungal Nha1 homologues that estimated the evolutionary conservation of the

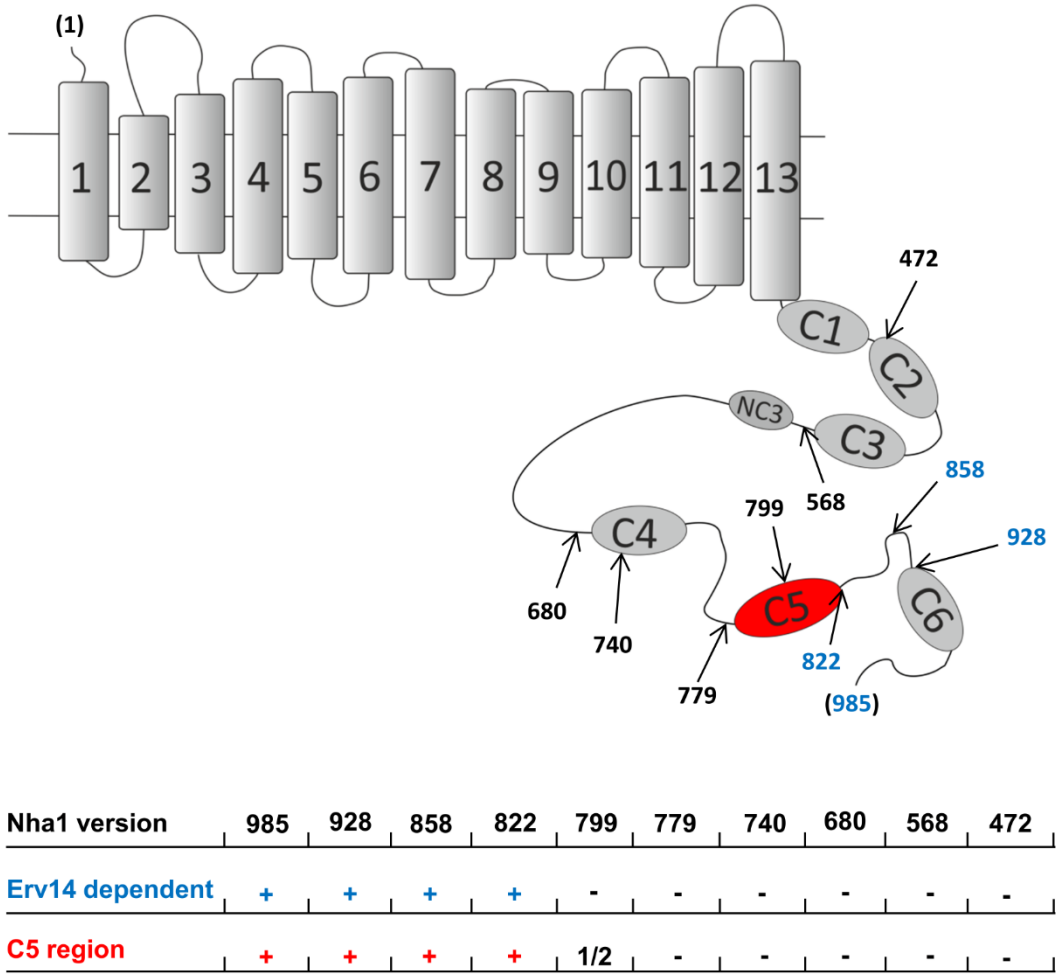

Fig. 4. Erv14 requirement of Na^+, K^+/H^+ antiporter Nha1. Only Nha1 versions that possess the C5 C-terminal conserved domain (in red) require Erv14 for their proper trafficking. Various C-terminally truncated versions of Nha1 were prepared, and their localization and functioning were studied in cells with or without Erv14. The arrows in the topological model of Nha1 point to the sites of C-terminal truncations, and the numbers correspond to the lengths of the Nha1 versions in amino-acid residues. The image was prepared using our results published in [6,9].

amino-acid positions of 286 sequences [6] specified the presence of seven approximately 15-30-amino-acid-residue-long conserved regions in the C-termini (C1-C6 described previously [35], and a new one, NC3, Fig. 4). The comparison of the localization and functioning of Nha1 versions gradually truncated from the end of the C-terminus in cells with or without Erv14 showed that only Nha1 versions that possess the C5 conserved region in their C-termini require Erv14 for their proper trafficking through the secretory pathway (Fig. 4, [6]). Thus, besides the long transmembrane domains [29], also a short hydrophilic portion of a cargo protein can underlie its need for Erv14´s assistance for its proper export from the ER.

Nha1 was shown to form dimers, and this dimerization seems to be important for the antiporter´s cation-translocation activity [40]. The higher abundance of Nha1 dimers in both plasma and ER membranes of cells with Erv14 when compared to *erv14Δ* cells and also the lack of evidence of oligomerization of the truncated Erv14-independent Nha1-472 version suggested a possible importance of Erv14 for the formation of Nha1 dimers [9]. Whether the C-terminal region C5, whose presence determines the antiporter´s requirement of Erv14, plays a role in Nha1´s oligomerization, is not currently known.

In a systematic study addressing the role of Erv14 in the proper intracellular trafficking of various monovalent-cation transporting proteins, the localization of six transporters, namely of plasma-membrane H^+-ATPase Pma1, Na^+, K^+-efflux ATPase Ena1, K^+ importers Trk1 and Trk2, K^+ channel Tok1 and vacuolar K^+, Cl^- co-transporter Vhc1, was compared in

cells with or without Erv14 (Fig 3, [10]). In agreement with the decreased ability of *erv14Δ* cells to grow in the presence of low K^+, the main *S. cerevisiae* K^+ importer, Trk1 [41,42], was identified as a new cargo of Erv14. The lack of Erv14 results in a partial ER accumulation of Trk1 (Fig. 3), and consequently in a lower import of K^+ cations to cells. This phenomenon explains the inhibition of the growth of *erv14Δ* cells under conditions when K^+ is scarce [10]. In addition to Trk1, the evolutionarily highly conserved voltage-gated K^+ channel Tok1 was also found to be partially mislocalized in cells without Erv14 (Fig. 3). Tok1 is an outwardly rectifying channel that has been thoroughly described by electrophysiological methods [43-45]. The only known growth-related Tok1-specific phenotype is that *TOK1* overexpression improves the growth of a strain that lacks K^+ importers Trk1 and Trk2 under K^+-limiting conditions [46]. This positive effect of the overproduction of Tok1 on the growth of *trk1Δ trk2Δ* cells at low K^+ was diminished when Erv14 was not present in cells [10]. This finding provides evidence that Tok1´s role in K^+ supply is dependent on the presence of the Erv14 cargo receptor, which assists with the delivery of Tok1 to the plasma membrane. Besides the microscopy and physiological evidence of the influence of the lack of Erv14 on the localization and functioning of both plasma-membrane K^+ transporting proteins, the physical interaction between Trk1 or Tok1 and Erv14 was confirmed in protein-protein interaction assays [10]. However, the signal for Erv14 binding in the structures of Trk1 or Tok1 has not yet been elucidated.

The lack of Erv14´s requirement of the Trk2 K^+ importer (Fig. 3) was somewhat surprising, as Trk1 and Trk2 share a high degree of identity, especially in the transmembrane parts of the proteins. Thus, differences between so-far unidentified structural features of both K^+ importers may explain why the main yeast K^+ importer Trk1 requires Erv14 for its exit from the ER, while its paralogue, Trk2, is not an Erv14 cargo. In addition to Trk2, neither the main Na^+ and K^+ efflux system that is inducible at high-salt concentrations, ATPase Ena1, nor the vacuolar K^+-Cl^- cotransporter Vhc1, were identified as Erv14 binding partners (Fig. 3, [10]). Interestingly, the targeting of the essential H^+-ATPase Pma1 to the plasma membrane does not require Erv14 in vegetative yeast cells (Fig. 3, [10,30]). However, during sporulation, the proper trafficking of Pma1 from the ER to the prospore membrane is strongly dependent on the presence of Erv14 [30].

The number of genes encoding cornichon proteins in plants is variable (e.g., two *CNIH* genes in *O. sativa*, six in *Cucurbita moschata* or five in *Arabidopsis thaliana* [7,47,48]). Due to the presence of the C-terminal acidic motif that is involved in the binding of cargoes, plant cornichons seem to be structurally more related to fungal than to animal homologues [32]. Although much less is known about the functional importance of cornichons in plant cells, the role of these COPII cargo receptors in the proper trafficking of cation transporters seems to be conserved even in plants. Similarly to yeast Erv14, the rice (*O. sativa*) cornichon *Os*CNIH1 localizes to both the ER and Golgi apparatus. It was found to physically interact with *Os*HKT1;3, a Golgi-apparatus Na^+ transporter from the vascular tissue of roots and leaves [7,49]. Both proteins seem to bind to each other in the membranes of the ER. The ER/Golgi apparatus localization of CNIH1 is also preserved in pumpkin (*C. moschata*) [47]. The *CmCNIH1* gene is upregulated under salt-stress conditions. Moreover, the growth of seedlings of the pumpkin mutant *cmcnih1* is inhibited in the presence of salt stress, and the mutants also exhibit higher Na^+ and lower K^+ levels in shoots [47]. The key binding partner of *Cm*CNIH1 that seems to underlie the importance of the *Cm*CNIH1 cornichon in the ability of pumpkin to cope with the presence of salt stress is *Cm*HKT1;1, a Na^+-selective transporter, which can enhance plant salt tolerance [50]. In agreement with the role of *Cm*CNIH1 as a typical COPII cargo receptor, *Cm*CNIH1 contributes to the proper plasma-membrane localization of *Cm*HKT1;1, but its presence does not impact ion-transport activity/properties of the transporter [47]. Plant HKT transporters belong to the same family as yeast Trk proteins, and share a similar structure with them [51]. Thus, the involvement of plant cornichons and *S. cerevisiae* Erv14 in the ER exit of the same type of cation transporters suggests that the mechanisms of specific cargo recognition are at least partially conserved in both yeast and plant cornichons. Interestingly, *A. thaliana* cornichons were identified as cargo receptors that bind glutamate receptor-like (GLR) channels [48] that are involved in Ca^{2+} homeostasis. The GLR channels are related to mammalian ionotropic glutamate receptors, and even in mammalian cells, a type of these transporters, AMPA receptors, are binding partners of cornichon proteins (see below). These data demonstrate structural and functional conservation of the whole cornichon family.

Mammalian cornichon proteins and their role as auxiliary subunits of ionotropic AMPA receptors

Vertebrate genomes contain four *CNIH* genes encoding cornichon proteins [52]. The lengths of human cornichons, CNIH1-4, vary from 139-160 amino-acid residues (Fig. 5A). The multiple sequence alignments show the highest percentage identity between CNIH2 and CNIH3 (almost 82 %). Moreover, these two proteins share an additional unique sequence in the second transmembrane domain that is absent in CNIH1 or CNIH4 (Fig. 5A). CNIH4 appears to be quite distant from the other human cornichons (it shares approximately 32 to 37 % identity with CNIH1, -2 or -3) and at the same time it is the most similar (≈ 44 % identity) to the yeast Erv14 of all human CNIH proteins. Phylogenetic analyses of metazoan cornichons indicate that vertebrate CNIH1/2/3 and CNIH4 belong to different cornichon subfamilies; *CNIH1*, *-2* and *-3* genes probably appeared by duplication of an ancestor gene [52]. According to an analysis of evolutionary relationships among cornichons from various yeast, plant and animal species, vertebrate CNIH4 proteins belong to the same group of cornichons as *S. cerevisiae* Erv14 [47].

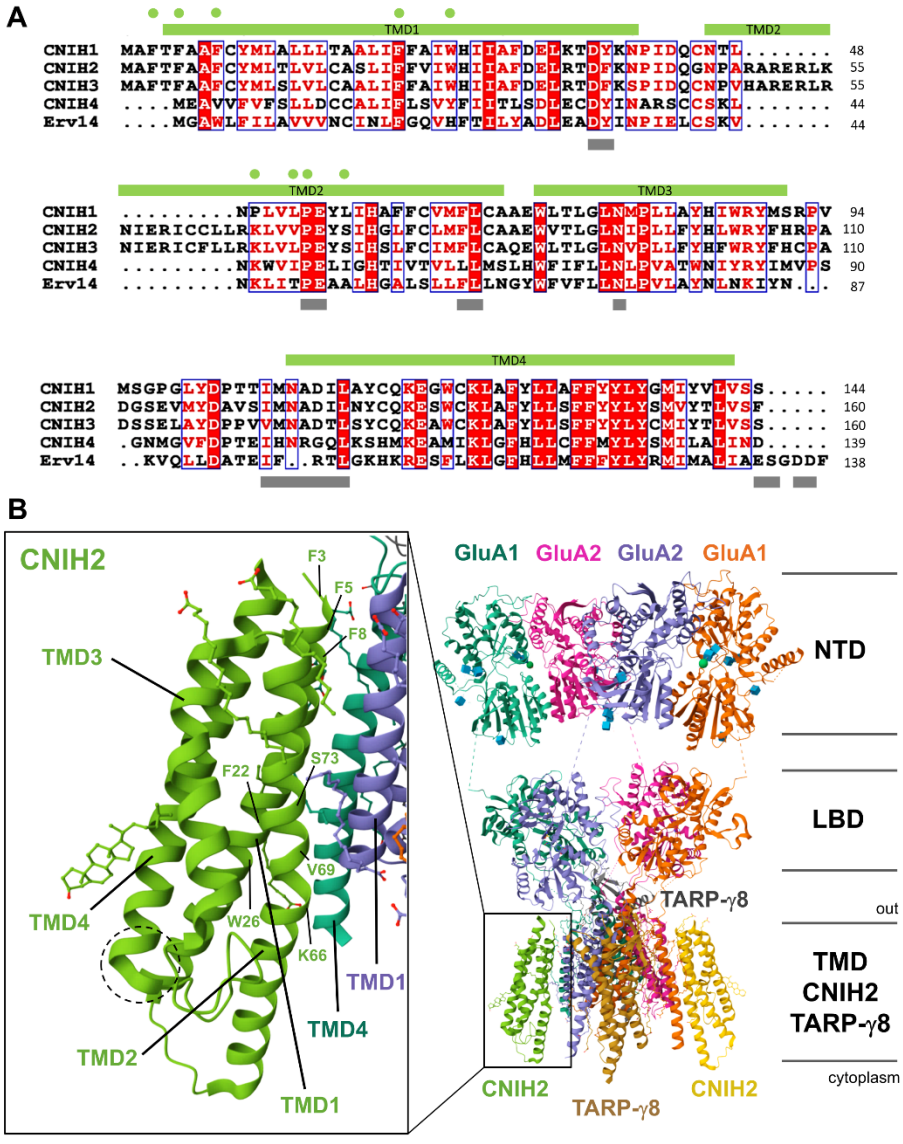

Fig. 5. Mammalian cornichon proteins. (**A**) Multiple sequence alignment of human cornichons and yeast Erv14 was created using ClustalO [70] and visualized with ESPript 3.0 tool [71]. Green lines highlight transmembrane domains of CNIH2, green dots depict amino-acid residues identified to be important for the functioning of CNIH2/3 as indicated in the text. Grey lines highlight functionally important motifs identified in Erv14 (see Fig. 2 and the text). (**B**) Structure of GluA1/A2 AMPA receptor in complex with pairs of CNIH2 and TARP-γ8 auxiliary subunits (PDB model 7OCA [28]). The proteins forming the AMPAR complex are visualized using different colors. NTD – N-terminal domains, LBD – ligand binding domains, TMD – transmembrane domains of GluA subunits. The second TARP-γ8 (in grey) is behind the complex. A detail of CNIH2 and the interaction interface with GluA subunits (left). Amino-acid residues that are in contact with GluAs are highlighted. The putative COPII-binding site (122-IMNADIL-128) is shown with a dashed-line oval.

The first experimental evidence of human cornichons was for the CNIH1 protein [53]. In HeLa cells, CNIH1 colocalized with both ER and Golgi apparatus markers, in agreement with its predicted function as a COPII cargo receptor. Analogous to the results obtained in *Drosophila*, CNIH1 was shown to be involved in the trafficking and also maturation of TGFα [53]. CNIH4 is known to be involved in the ER exit of G-protein coupled receptors [54]. However, the majority of current available information about mammalian cornichons concerns the proteins CNIH2 and CNIH3.

Proteomic and biochemical analyses of protein complexes from rat brains identified for the first time CNIH2 and CNIH3 as binding partners and new auxiliary subunits of pore-forming GluA subunits of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) glutamate-gated ionotropic receptors (AMPARs) [55]. AMPARs, which have characteristic fast kinetics, are concentrated at the postsynaptic membrane of excitatory synapses. In the central nervous system, AMPARs play a key role in excitatory synaptic transmission [56]. The core of AMPARs is formed of GluA1-4 subunits (Fig. 5B). They consist of a cytoplasmically-localized C-terminal domain, transmembrane domains (three membrane-spanning segments and a helix loop) that form the ion channel, a ligand-binding domain, and finally an N-terminal domain, which is the most distant from the membrane [57]. AMPARs are homo- or heterotetramers of GluA subunits. While the transmembrane-domain part of the complex is tetrameric, both ligand-binding domains and N-terminal domains form dimers [57]. The particular GluA composition of an AMPA receptor then determines its basic properties, including cation (Na^+, K^+, Ca^{2+}) permeation [58]. AMPARs additionally form multi-protein complexes with up to four various auxiliary subunits: transmembrane AMPAR regulatory proteins (TARPs), cornichons, or GSG1l (Fig. 5B). They are all transmembrane proteins that bind to GluA tetramers at two distinct pairs of sites. The variable composition of the entire multi-protein complex adds more functional and also spatiotemporal diversity to AMPARs [57,58].

As is typical for cornichon-family proteins, CNIH2 and CNIH3 enhance the plasma-membrane localization and also promote the tetramerization, intracellular trafficking and maturation of AMPAR GluA subunits [55,59-64]. Thus, both cornichons seem to work as ordinary COPII cargo receptors and to promote the ER exit of AMPARs. However, and very differently from what was found so far for yeast or plant cornichons, when CNIH2 or CNIH3 become auxiliary subunits of an AMPA receptor, they do not recycle back to the ER, but together with the entire AMPAR protein complex, they are delivered to the plasma membrane and become plasma-membrane proteins [60-62,65,66].

Recently, the first experimental evidence of a cornichon structure came from cryo-electron microscopy analysis of rat GluA2 AMPAR subunits in complex with mouse CNIH3 at a stoichiometry of 4:4 [26]. In the protein complex which was obtained upon heterologous expression in human embryonic kidney (HEK) cells the interaction interface is formed by the first and fourth transmembrane domains of adjacent GluA2 subunits and the first and second transmembrane domains of CNIH3. Three phenylalanines (F3, F5 and F8) from the N-terminal part of CNIH3 make contacts with the transmembrane domains of GluA2 subunits [26]. These amino-acid residues are conserved in CNIH1, 2 and 3 proteins, but absent in CNIH4 (Fig. 5A). Their importance for the interaction between GluA and cornichons was also confirmed in structural analyses of either rat GluA1/A2 AMPARs in complex with CNIH2 and TARP-γ8 after their production in HEK cells, or in native hippocampal mouse AMPARs in complex with the same auxiliary subunits [27,28]. In both studies, CNIH2 and TARP-γ8 proteins were present in AMPAR complexes in two diagonally located pairs with CNIH2 being in contact with the first transmembrane domain of GluA2 and the fourth transmembrane domain of GluA1 (Fig. 5B). In addition to the phenylalanine residues from the N-terminal part of CNIH2, which are located near the extracellular boundary of the TMD pore of the receptor (Fig. 5B), other amino-acid residues from the opposite side of the membrane were shown to make contacts with GluA subunits, namely K66, V69 and S73 located in the second transmembrane domain of CNIH2 and F22 together with W26 from the first transmembrane domain of the cornichon (Fig. 5B, [27,28]). Moreover, the analyses of the interface of CNIH2/3-AMPAR interaction also suggest the involvement of lipids in the formation of interactions between AMPARs and cornichons [26-28].

Being auxiliary subunits of AMPAR protein complexes, cornichons not only promote the trafficking of the complex to the cell surface, but there is also substantial evidence of the ability of CNIH2 and CNIH3 to modulate the gating of the receptor. Depending on the protein composition of the entire receptor complex, CNIH2 and CNIH3 were shown to influence AMPAR functioning by e.g. slowing its deactivation and

desensitization kinetics or blocking its TARP-γ8-mediated resensitization [25,55,63,65-68]. In early stages of ontogeny, there is an excess amount of AMPAR-free CNIH2/3 in rat brains. These cornichon molecules most probably function as typical COPII cargo receptors. However the amount of CNIH2/3 bound into AMPAR complexes increases with the progress of development. Thus the importance of cornichons as AMPAR auxiliary subunits seems to be higher later in the brain development [69]. Based on the topology of cornichons (Fig. 2C, 5B), their modulatory effects must be mediated by the intramembrane and cytoplasmic interactions between GluA and CNIH proteins. The activation of an AMPA receptor results in global structural changes to the whole protein complex, as shown by structural studies of the GluA1/A2 receptor in complex with CNIH2 and TARP-γ8 [28]. The two pairs of auxiliary subunits undergo counter-rotations, and the structural change to CNIH2 was suggested to stabilize the active state of the channel [28].

The ability of CNIH2 and CNIH3 to modulate AMPAR functioning seems to be dependent on the amino-acid region that is specific for these cornichons and that forms the cytoplasmically localized beginning of the second α-helix in these proteins (Figs 2C, 5). Using high-resolution mass spectrometry, all four human cornichons were identified as being interacting partners of GluA2 in the human brain [62]. Moreover, rat CNIH1 co-immunoprecipitates with rat GluA2 when both proteins are expressed in HEK cells [62]. However, although CNIH1 can physically bind the AMPAR complex, it is not efficient at modulating its gating properties [62,65]. Interestingly, insertion of the CNIH2/3 specific region into the sequence of CNIH1 results in a more pronounced modulatory efficiency of this cornichon [28]. On the other hand, the deletion of the entire CNIH2/3 specific region or especially amino-acid residues from the second half of this region resulted in CNIH3 with a reduced ability to both bind and modulate the functioning of the AMPA receptor [62]. The conserved amino-acid residue P70 in the second α-helical structure of CNIH2 appears to be connected to the structural flexibility of the CNIH2/3 specific region, which tilts toward the pore of the channel upon AMPAR activation [28].

Evolutionary conservation of the cornichon family´s functioning

Cornichon COPII cargo receptors are highly conserved in fungi, plants and animals. Several pieces of evidence also suggest that even the way in which they recognize their cargoes and other interaction partners might be conserved among cornichons from various groups of organisms: (i) *S. cerevisiae* Erv14 promotes the ER exit of rice cation transporter *Os*HKT1;3 in yeast cells, (ii) *O. sativa Os*CNIH1 and *A. thaliana At*CNIH1, -3 and -4 promote the proper trafficking of Erv14´s cargoes in yeast cells without Erv14, and (iii) human cornichon CNIH1 is able to rescue the non-axial budding phenotype of yeast *erv14Δ* cells [7,8,48,53].

Erv14´s amino-acid residues 97-IFRTL-101 are supposed to be involved in the binding of COPII-coat components [21]. Although at the level of primary structure, this motif is not fully conserved in mammalian cornichons (Fig. 5A, 122-IMNADIL-128 in CNIH2), at the structural level, it seems to be located at the cytoplasmic beginning of the fourth α-helical structure in both yeast and mammalian cornichons (Figs 2, 5). Moreover, the localization of this motif being on the opposite side of CNIH2/3 to that of the binding interface with AMPAR GluA subunits (Fig. 5B) is in agreement with the accessibility of the motif for interaction with COPII coat. The DYPE (DY33-34, PE50-51) site of Erv14 was also suggested to play a role in the incorporation of Erv14 into COPII vesicles [20]. While the first two amino-acid residues of this motif (DF37-38 in CNIH2/3) are located in proximity to the above-discussed putative COPII binding site in mammalian cornichon CNIH2, at the cytoplasmic end of the first transmembrane domain (Figs 2, 5), residues corresponding to the second part of the putative motif (PE70-71 in CNIH2/3) seem to have a different function in CNIH2. The conserved proline (P70, Fig. 5A) should be responsible for the structural flexibility of the specific CNIH2/3 region that is important for the gating modulation of the AMPAR channel [28]. Amino-acid residues F82 and L83 of CNIH2 and CNIH3, which correspond to the phenylalanine and leucine from the Erv14´s FLN site (FL62-63) that are likely involved in the binding of cargo proteins by Erv14 [20], are located in the second transmembrane domain of mammalian cornichons (Figs 2, 5). Interestingly, this transmembrane domain indeed forms the interaction interface between cornichons and AMPA receptors (Fig. 5B, [25-28]).

Although FL82-83 are not directly in contact with AMPAR GluA subunits, it might suggest that both yeast and mammalian cornichons involve similar regions in their interaction with cargoes.

Cornichons in medicine

The proper functioning of cornichon-family proteins is of high importance for human health. Abnormal vesicle-mediated transport plays a key role in cancer development [72]. Importantly, there is growing evidence of cornichons being prognostic markers and also putative therapeutic targets in various types of cancer. The gene encoding CNIH1, which plays a role in the trafficking and maturation of TGFα [53], was found to be highly expressed in lung-adenocarcinoma tissues, and knockdown of the *CNIH1* gene inhibited the growth and migration of cancer cells in *in vitro* experiments [73]. CNIH4, so far known as a cargo receptor of G-protein coupled receptors [54], has been associated with an increased risk and changes in the immune microenvironment in several types of cancer (e.g. colon cancer, head and squamous carcinoma, hepatocellular carcinoma, glioma, cervical, ovarian and gastric cancer) [74-81]. *In vitro*, the downregulation of *CNIH4* inhibited cell proliferation and migration and increased drug sensitivity in cancer cell lines [74,76,79,81]. The growth of glioma cells was also slower in mice after *CNIH4* silencing [79]. In cervical cancer cells, the CNIH4-mediated reduction in ferroptosis is connected to the expression of the gene encoding the cystine/glutamate transporter SLC7A11. However, the functional relationship between both genes/proteins remains to be elucidated [77].

As for neurological disorders, *CNIH1*, *-2* and *-3* were found to be significantly upregulated in the dorsolateral prefrontal cortex of patients with schizophrenia [82]. A *de novo* deletion of a 1 Mbp region that also contained the gene encoding CNIH2 has been associated with intellectual disability [83], and moreover, *CNIH4* was identified as the target gene of an Alzheimer-disease risk-associated CpG site [84]. The polymorphisms of the *CNIH3* gene were also implicated to be involved in the pathophysiology of opioid dependence, with some SNPs having a robust protective effect [85]. Last, but not least, AMPAR auxiliary subunits, including CNIH2 and CNIH3, may serve as potential therapeutic targets for controlling AMPAR functioning in individual regions of the brain [57].

Conclusions

As COPII cargo receptors, cornichons play a role in the proper targeting of a variety of proteins, including several cation transport systems. Monovalent-cation transporters of eukaryotic cells have a fundamental importance for the fitness of both single cells and multicellular organisms. In addition, their malfunctioning in humans results in many pathologies. Thus, understanding their functioning and biogenesis is relevant to a range of human occupations, such as biotechnology, agriculture or medicine. *S. cerevisiae* cornichon Erv14 assists with the proper trafficking of several monovalent-cation translocating proteins, and its role in the maintenance of ion homeostasis in yeast cells seems to be complex. However, our current knowledge of the cargo repertoire of human cornichons is much more limited, despite the fact that changes in their expression/functioning are connected to severe diseases. The only cornichons' cargoes involved in cation homeostasis of mammalian cells known so far are ionotropic AMPA receptors, whose gating is also modulated by direct interaction with CNIH proteins. Thus, there seems to be an urgent need to uncover cornichons´ so-far unknown interacting partners and at the same time new putative treatment targets. Taking into account the easy genetic manipulation and low-cost and fast cultivation of yeast cells together with the possibility of heterologous expression of functional mammalian ion transporters and cornichons ([53,86-89], our unpublished results), *S. cerevisiae* appears to be a useful tool not only to deepen our knowledge of the basic properties of cornichon-family proteins, but also to study the physical interactions of human CNIH proteins with their putative binding partners and thus to broaden the list of cornichons´ known cargoes. Such an approach has been already successfully used to uncover the interactions between fungal or plant cornichons and their binding partners [7,47,48,90].

Conflict of Interest

There is no conflict of interest.

Acknowledgements

We wish to thank Dr. Hana Sychrová for critical reading of the manuscript. This work was supported by a grant from the Czech Science Foundation [grant number GAČR 21-08985S].

References

1. Arino J, Ramos J, Sychrova H. Alkali metal cation transport and homeostasis in yeasts. Microbiol Mol Biol Rev 2010;74:95-120. https://doi.org/10.1128/MMBR.00042-09
2. Arino J, Ramos J, Sychrova H. Monovalent cation transporters at the plasma membrane in yeasts. Yeast 2019;36:177-193. https://doi.org/10.1002/yea.3355
3. Cyert MS, Philpott CC. Regulation of cation balance in *Saccharomyces cerevisiae*. Genetics 2013;193:677-713. https://doi.org/10.1534/genetics.112.147207
4. Pedersen SF, Counillon L. The SLC9A-C mammalian Na^+/H^+ exchanger family: molecules, mechanisms, and physiology. Physiol Rev 2019;99:2015-2113. https://doi.org/10.1152/physrev.00028.2018
5. Aviram N, Ast T, Costa EA, Arakel EC, Chuartzman SG, Jan CH, Hassdenteufel S, ET AL. The SND proteins constitute an alternative targeting route to the endoplasmic reticulum. Nature 2016;540:134-138. https://doi.org/10.1038/nature20169
6. Papouskova K, Moravcova M, Masrati G, Ben-Tal N, Sychrova H, Zimmermannova O. C5 conserved region of hydrophilic C-terminal part of *Saccharomyces cerevisiae* Nha1 antiporter determines its requirement of Erv14 COPII cargo receptor for plasma-membrane targeting. Mol Microbiol 2021;115:41-57. https://doi.org/10.1111/mmi.14595
7. Rosas-Santiago P, Lagunas-Gomez D, Barkla BJ, Vera-Estrella R, Lalonde S, Jones A, Frommer WB, ET AL. Identification of rice cornichon as a possible cargo receptor for the Golgi-localized sodium transporter *Os*HKT1;3. J Exp Bot 2015;66:2733-2748. https://doi.org/10.1093/jxb/erv069
8. Rosas-Santiago P, Lagunas-Gomez D, Yanez-Dominguez C, Vera-Estrella R, Zimmermannova O, Sychrova H, Pantoja O. Plant and yeast cornichon possess a conserved acidic motif required for correct targeting of plasma membrane cargos. Biochim Biophys Acta 2017;1864:1809-1818. https://doi.org/10.1016/j.bbamcr.2017.07.004
9. Rosas-Santiago P, Zimmermannova O, Vera-Estrella R, Sychrova H, Pantoja O. Erv14 cargo receptor participates in yeast salt tolerance via its interaction with the plasma-membrane Nha1 cation/proton antiporter. Biochim Biophys Acta 2016;1858:67-74. https://doi.org/10.1016/j.bbamem.2015.09.024
10. Zimmermannova O, Felcmanova K, Rosas-Santiago P, Papouskova K, Pantoja O, Sychrova H. Erv14 cargo receptor participates in regulation of plasma-membrane potential, intracellular pH and potassium homeostasis via its interaction with K+-specific transporters Trk1 and Tok1. Biochim Biophys Acta 2019;1866:1376-1388. https://doi.org/10.1016/j.bbamcr.2019.05.005
11. Casey JR, Grinstein S, Orlowski J. Sensors and regulators of intracellular pH. Nat Rev Mol Cell Biol 2010;11:50-61. https://doi.org/10.1038/nrm2820
12. Milo R, Phillips R. *Cell Biology by the Numbers*. Garland Science, New York, 2015, 400 p. https://doi.org/10.1201/9780429258770
13. Volkov V. Salinity tolerance in plants. Quantitative approach to ion transport starting from halophytes and stepping to genetic and protein engineering for manipulating ion fluxes. Front Plant Sci 2015;6:873. https://doi.org/10.3389/fpls.2015.00873
14. Wong ED, Miyasato SR, Aleksander S, Karra K, Nash RS, Skrzypek MS, Weng S, ET AL. *Saccharomyces* genome database update: server architecture, pan-genome nomenclature, and external resources. Genetics 2023;224:iyac191. https://doi.org/10.1093/genetics/iyac191
15. Bokel C, Dass S, Wilsch-Brauninger M, Roth S. *Drosophila* Cornichon acts as cargo receptor for ER export of the TGFalpha-like growth factor Gurken. Development 2006;133:459-470. https://doi.org/10.1242/dev.02219
16. Roth S, Neuman-Silberberg FS, Barcelo G, Schupbach T. Cornichon and the EGF receptor signaling process are necessary for both anterior-posterior and dorsal-ventral pattern formation in *Drosophila*. Cell 1995;81:967-978. https://doi.org/10.1016/0092-8674(95)90016-0
17. Berg CA. The *Drosophila* shell game: patterning genes and morphological change. Trends Genet 2005;21:346-355. https://doi.org/10.1016/j.tig.2005.04.010
18. Neuman-Silberberg FS, Schupbach T. The *Drosophila* dorsoventral patterning gene gurken produces a dorsally localized RNA and encodes a TGF alpha-like protein. Cell 1993;75:165-174. https://doi.org/10.1016/S0092-8674(05)80093-5

19. Powers J, Barlowe C. Transport of Axl2p depends on Erv14p, an ER-vesicle protein related to the *Drosophila* cornichon gene product. J Cell Biol 1998;142:1209-1222. https://doi.org/10.1083/jcb.142.5.1209
20. Pagant S, Wu A, Edwards S, Diehl F, Miller EA. Sec24 is a coincidence detector that simultaneously binds two signals to drive ER export. Curr Biol 2015;25:403-412. https://doi.org/10.1016/j.cub.2014.11.070
21. Powers J, Barlowe C. Erv14p directs a transmembrane secretory protein into COPII-coated transport vesicles. Mol Biol Cell 2002;13:880-891. https://doi.org/10.1091/mbc.01-10-0499
22. von Heijne G, Gavel Y. Topogenic signals in integral membrane proteins. Eur J Biochem 1988;174:671-678. https://doi.org/10.1111/j.1432-1033.1988.tb14150.x
23. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, ET AL. Highly accurate protein structure prediction with AlphaFold. Nature 2021;596:583-589. https://doi.org/10.1038/s41586-021-03819-2
24. Varadi M, Anyango S, Deshpande M, Nair S, Natassia C, Yordanova G, Yuan D, ET AL. AlphaFold Protein Structure Database: massively expanding the structural coverage of protein-sequence space with high-accuracy models. Nucleic Acids Res 2022;50:D439-D444. https://doi.org/10.1093/nar/gkab1061
25. Gangwar SP, Yen LY, Yelshanskaya MV, Korman A, Jones DR, Sobolevsky AI. Modulation of GluA2-gamma5 synaptic complex desensitization, polyamine block and antiepileptic perampanel inhibition by auxiliary subunit cornichon-2. Nat Struct Mol Biol 2023;30:1481-1494. https://doi.org/10.1038/s41594-023-01080-x
26. Nakagawa T. Structures of the AMPA receptor in complex with its auxiliary subunit cornichon. Science 2019;366:1259-1263. https://doi.org/10.1126/science.aay2783
27. Yu J, Rao P, Clark S, Mitra J, Ha T, Gouaux E. Hippocampal AMPA receptor assemblies and mechanism of allosteric inhibition. Nature 2021;594:448-453. https://doi.org/10.1038/s41586-021-03540-0
28. Zhang D, Watson JF, Matthews PM, Cais O, Greger IH. Gating and modulation of a hetero-octameric AMPA glutamate receptor. Nature 2021;594:454-458. https://doi.org/10.1038/s41586-021-03613-0
29. Herzig Y, Sharpe HJ, Elbaz Y, Munro S, Schuldiner M. A systematic approach to pair secretory cargo receptors with their cargo suggests a mechanism for cargo selection by Erv14. PLoS Biol 2012;10:e1001329. https://doi.org/10.1371/journal.pbio.1001329
30. Nakanishi H, Suda Y, Neiman AM. Erv14 family cargo receptors are necessary for ER exit during sporulation in *Saccharomyces cerevisiae*. J Cell Sci 2007;120:908-916. https://doi.org/10.1242/jcs.03405
31. Sharpe HJ, Stevens TJ, Munro S. A comprehensive comparison of transmembrane domains reveals organelle-specific properties. Cell 2010;142:158-169. https://doi.org/10.1016/j.cell.2010.05.037
32. Lagunas-Gomez D, Yanez-Dominguez C, Zavala-Padilla G, Barlowe C, Pantoja O. The C-terminus of the cargo receptor Erv14 affects COPII vesicle formation and cargo delivery. J Cell Sci 2023;136:jcs260527. https://doi.org/10.1242/jcs.260527
33. Banuelos MA, Sychrova H, Bleykasten-Grosshans C, Souciet JL, Potier S. The Nha1 antiporter of *Saccharomyces cerevisiae* mediates sodium and potassium efflux. Microbiology 1998;144:2749-2758. https://doi.org/10.1099/00221287-144-10-2749
34. Prior C, Potier S, Souciet JL, Sychrova H. Characterization of the *NHA1* gene encoding a Na^+/H^+-antiporter of the yeast Saccharomyces cerevisiae. FEBS Lett 1996;387:89-93. https://doi.org/10.1016/0014-5793(96)00470-X
35. Kamauchi S, Mitsui K, Ujike S, Haga M, Nakamura N, Inoue H, Sakajo S, ET AL. Structurally and functionally conserved domains in the diverse hydrophilic carboxy-terminal halves of various yeast and fungal Na^+/H^+ antiporters (Nha1p). J Biochem 2002;131:821-831. https://doi.org/10.1093/oxfordjournals.jbchem.a003171
36. Kinclova O, Ramos J, Potier S, Sychrova H. Functional study of the *Saccharomyces cerevisiae* Nha1p C-terminus. Mol Microbiol 2001;40:656-668. https://doi.org/10.1046/j.1365-2958.2001.02412.x
37. Smidova A, Stankova K, Petrvalska O, Lazar J, Sychrova H, Obsil T, Zimmermannova O, Obsilova V. The activity of *Saccharomyces cerevisiae* Na^+, K^+/H^+ antiporter Nha1 is negatively regulated by 14-3-3 protein binding at serine 481. Biochim Biophys Acta 2019;1866:118534. https://doi.org/10.1016/j.bbamcr.2019.118534
38. Zimmermannova O, Velazquez D, Papouskova K, Prusa V, Radova V, Falson P, Sychrova H. The hydrophilic C-terminus of yeast plasma-membrane Na^+/H^+ antiporters impacts their ability to transport K^+. J Mol Biol 2024;436:168443. https://doi.org/10.1016/j.jmb.2024.168443

39. Pribylova L, Papouskova K, Zavrel M, Souciet JL, Sychrova H. Exploration of yeast alkali metal cation/H^+ antiporters: sequence and structure comparison. Folia Microbiol 2006;51:413-424. https://doi.org/10.1007/BF02931585
40. Mitsui K, Kamauchi S, Nakamura N, Inoue H, Kanazawa H. A conserved domain in the tail region of the *Saccharomyces cerevisiae* Na^+/H^+ antiporter (Nha1p) plays important roles in localization and salinity-resistant cell-growth. J Biochem 2004;135:139-148. https://doi.org/10.1093/jb/mvh016
41. Bertl A, Ramos J, Ludwig J, Lichtenberg-Frate H, Reid J, Bihler H, Calero F, ET AL. Characterization of potassium transport in wild-type and isogenic yeast strains carrying all combinations of *trk1*, *trk2* and *tok1* null mutations. Mol Microbiol 2003;47:767-780. https://doi.org/10.1046/j.1365-2958.2003.03335.x
42. Gaber RF, Styles CA, Fink GR. *TRK1* encodes a plasma membrane protein required for high-affinity potassium transport in *Saccharomyces cerevisiae*. Mol Cell Biol 1988;8:2848-2859. https://doi.org/10.1128/MCB.8.7.2848
43. Bertl A, Slayman CL, Gradmann D. Gating and conductance in an outward-rectifying K^+ channel from the plasma membrane of Saccharomyces cerevisiae. J Membr Biol 1993;132:183-199. https://doi.org/10.1007/BF00235737
44. Ketchum KA, Joiner WJ, Sellers AJ, Kaczmarek LK, Goldstein SA. A new family of outwardly rectifying potassium channel proteins with two pore domains in tandem. Nature 1995;376:690-695. https://doi.org/10.1038/376690a0
45. Lewis A, McCrossan ZA, Manville RW, Popa MO, Cuello LG, Goldstein SAN. TOK channels use the two gates in classical K^+ channels to achieve outward rectification. FASEB J 2020;34:8902-8919. https://doi.org/10.1096/fj.202000545R
46. Fairman C, Zhou X, Kung C. Potassium uptake through the TOK1 K^+ channel in the budding yeast. J Membr Biol 1999;168:149-157. https://doi.org/10.1007/s002329900505
47. Wei L, Liu L, Chen Z, Huang Y, Yang L, Wang P, Xue S, Bie Z. CmCNIH1 improves salt tolerance by influencing the trafficking of CmHKT1;1 in pumpkin. Plant J 2023;114:1353-1368. https://doi.org/10.1111/tpj.16197
48. Wudick MM, Portes MT, Michard E, Rosas-Santiago P, Lizzio MA, Nunes CO, Campos C, ET AL. CORNICHON sorting and regulation of GLR channels underlie pollen tube Ca^{2+} homeostasis. Science 2018;360:533-536. https://doi.org/10.1126/science.aar6464
49. Jabnoune M, Espeout S, Mieulet D, Fizames C, Verdeil JL, Conejero G, Rodriguez-Navarro A, ET AL. Diversity in expression patterns and functional properties in the rice HKT transporter family. Plant Physiol 2009;150:1955-1971. https://doi.org/10.1104/pp.109.138008
50. Sun J, Cao H, Cheng J, He X, Sohail H, Niu M, Huang Y, Bie Z. Pumpkin CmHKT1;1 controls shoot Na^+ accumulation via limiting Na^+ transport from rootstock to scion in grafted cucumber. Int J Mol Sci 2018;19:2648. https://doi.org/10.3390/ijms19092648
51. Rodriguez-Navarro A. Potassium transport in fungi and plants. Biochim Biophys Acta 2000;1469:1-30. https://doi.org/10.1016/S0304-4157(99)00013-1
52. Ramos-Vicente D, Bayes A. AMPA receptor auxiliary subunits emerged during early vertebrate evolution by neo/subfunctionalization of unrelated proteins. Open Biol 2020;10:200234. https://doi.org/10.1098/rsob.200234
53. Castro CP, Piscopo D, Nakagawa T, Derynck R. Cornichon regulates transport and secretion of TGFalpha-related proteins in metazoan cells. J Cell Sci 2007;120:2454-2466. https://doi.org/10.1242/jcs.004200
54. Sauvageau E, Rochdi MD, Oueslati M, Hamdan FF, Percherancier Y, Simpson JC, Pepperkok R, Bouvier M. CNIH4 interacts with newly synthesized GPCR and controls their export from the endoplasmic reticulum. Traffic 2014;15:383-400. https://doi.org/10.1111/tra.12148
55. Schwenk J, Harmel N, Zolles G, Bildl W, Kulik A, Heimrich B, Chisaka O, ET AL. Functional proteomics identify cornichon proteins as auxiliary subunits of AMPA receptors. Science 2009;323:1313-1319. https://doi.org/10.1126/science.1167852
56. Royo M, Escolano BA, Madrigal MP, Jurado S. AMPA receptor function in hypothalamic synapses. Front Synaptic Neurosci 2022;14:833449. https://doi.org/10.3389/fnsyn.2022.833449
57. Kamalova A, Nakagawa T. AMPA receptor structure and auxiliary subunits. J Physiol 2021;599:453-469. https://doi.org/10.1113/JP278701
58. Schwenk J, Fakler B. Building of AMPA-type glutamate receptors in the endoplasmic reticulum and its implication for excitatory neurotransmission. J Physiol 2021;599:2639-2653. https://doi.org/10.1113/JP279025

59. Certain N, Gan Q, Bennett J, Hsieh H, Wollmuth LP. Differential regulation of tetramerization of the AMPA receptor glutamate-gated ion channel by auxiliary subunits. J Biol Chem 2023;299:105227. https://doi.org/10.1016/j.jbc.2023.105227
60. Harmel N, Cokic B, Zolles G, Berkefeld H, Mauric V, Fakler B, Stein V, Klocker N. AMPA receptors commandeer an ancient cargo exporter for use as an auxiliary subunit for signaling. PLoS One 2012;7:e30681. https://doi.org/10.1371/journal.pone.0030681
61. Herring BE, Shi Y, Suh YH, Zheng CY, Blankenship SM, Roche KW, Nicoll RA. Cornichon proteins determine the subunit composition of synaptic AMPA receptors. Neuron 2013;77:1083-1096. https://doi.org/10.1016/j.neuron.2013.01.017
62. Shanks NF, Cais O, Maruo T, Savas JN, Zaika EI, Azumaya CM, Yates III JR, ET AL. Molecular dissection of the interaction between the AMPA receptor and cornichon homolog-3. J Neurosci 2014;34:12104-12120. https://doi.org/10.1523/JNEUROSCI.0595-14.2014
63. Shi Y, Suh YH, Milstein AD, Isozaki K, Schmid SM, Roche KW, Nicoll RA. Functional comparison of the effects of TARPs and cornichons on AMPA receptor trafficking and gating. Proc Natl Acad Sci U S A 2010;107:16315-16319. https://doi.org/10.1073/pnas.1011706107
64. Schwenk J, Boudkkazi S, Kocylowski MK, Brechet A, Zolles G, Bus T, Costa K, ET AL. An ER assembly line of AMPA-receptors controls excitatory neurotransmission and its plasticity. Neuron 2019;104:680-692e689. https://doi.org/10.1016/j.neuron.2019.08.033
65. Coombs ID, Soto D, Zonouzi M, Renzi M, Shelley C, Farrant M, Cull-Candy SG. Cornichons modify channel properties of recombinant and glial AMPA receptors. J Neurosci 2012;32:9796-9804. https://doi.org/10.1523/JNEUROSCI.0345-12.2012
66. Kato AS, Gill MB, Ho MT, Yu H, Tu Y, Siuda ER, Wang H, ET AL. Hippocampal AMPA receptor gating controlled by both TARP and cornichon proteins. Neuron 2010;68:1082-1096. https://doi.org/10.1016/j.neuron.2010.11.026
67. Boudkkazi S, Brechet A, Schwenk J, Fakler B. Cornichon2 dictates the time course of excitatory transmission at individual hippocampal synapses. Neuron 2014;82:848-858. https://doi.org/10.1016/j.neuron.2014.03.031
68. Hawken NM, Zaika EI, Nakagawa T. Engineering defined membrane-embedded elements of AMPA receptor induces opposing gating modulation by cornichon 3 and stargazin. J Physiol 2017;595:6517-6539. https://doi.org/10.1113/JP274897
69. Mauric V, Molders A, Harmel N, Heimrich B, Sergeeva OA, Klocker N. Ontogeny repeats the phylogenetic recruitment of the cargo exporter cornichon into AMPA receptor signaling complexes. Mol Cell Neurosci 2013;56:10-17. https://doi.org/10.1016/j.mcn.2013.02.001
70. Madeira F, Pearce M, Tivey ARN, Basutkar P, Lee J, Edbali O, Madhusoodanan N, ET AL. Search and sequence analysis tools services from EMBL-EBI in 2022. Nucleic Acids Res 2022;50:W276-W279. https://doi.org/10.1093/nar/gkac240
71. Robert X, Gouet P. Deciphering key features in protein structures with the new ENDscript server. Nucleic Acids Res 2014;42:W320-W324. https://doi.org/10.1093/nar/gku316
72. Aber R, Chan W, Mugisha S, Jerome-Majewska LA. Transmembrane emp24 domain proteins in development and disease. Genet Res 2019;101:e14. https://doi.org/10.1017/S0016672319000090
73. Qian C, Jiang Z, Zhou T, Wu T, Zhang Y, Huang J, Ouyang J, ET AL. Vesicle-mediated transport-related genes are prognostic predictors and are associated with tumor immunity in lung adenocarcinoma. Front Immunol 2022;13:1034992. https://doi.org/10.3389/fimmu.2022.1034992
74. Wang J, Wang S, Wang J, Huang J, Lu H, Pan B, Pan H, ET AL. Comprehensive analysis of clinical prognosis and biological significance of CNIH4 in cervical cancer. Cancer Med 2023;12:22381-22394. https://doi.org/10.1002/cam4.6734
75. Wang Z, Pan L, Guo D, Luo X, Tang J, Yang W, Zhang Y, ET AL. A novel five-gene signature predicts overall survival of patients with hepatocellular carcinoma. Cancer Med 2021;10:3808-3821. https://doi.org/10.1002/cam4.3900
76. Xiao F, Sun G, Zhu H, Guo Y, Xu F, Hu G, Huang K, Guo H. CNIH4: a novel biomarker connected with poor prognosis and cell proliferation in patients with lower-grade glioma. Am J Cancer Res 2023;13:2135-2154. https://doi.org/10.18632/aging.204821

77. Yang JY, Ke D, Li Y, Shi J, Wan SM, Wang AJ, Zhao MN, Gao H. CNIH4 governs cervical cancer progression through reducing ferroptosis. Chem Biol Interact 2023;384:110712. https://doi.org/10.1016/j.cbi.2023.110712
78. Mishra S, Bernal C, Silvano M, Anand S, Ruiz IAA. The protein secretion modulator TMED9 drives CNIH4/TGFalpha/GLI signaling opposing TMED3-WNT-TCF to promote colon cancer metastases. Oncogene 2019;38:5817-5837. https://doi.org/10.1038/s41388-019-0845-z
79. Fang Z, Kong F, Zeng J, Zhang Z, Wang Y, Wang Y, Duan J, ET AL. Integrated analysis based on vesicle trafficking-related genes identifying CNIH4 as a novel therapeutic target for glioma. Cancer Med 2023;12:12943-12959. https://doi.org/10.1002/cam4.5947
80. Kasavi C. Gene co-expression network analysis revealed novel biomarkers for ovarian cancer. Front Genet 2022;13:971845. https://doi.org/10.3389/fgene.2022.971845
81. Zhang H, Lin Y, Zhuang M, Zhu L, Dai Y, Lin M. Screening and identification of CNIH4 gene associated with cell proliferation in gastric cancer based on a large-scale CRISPR-Cas9 screening database DepMap. Gene 2023;850:146961. https://doi.org/10.1016/j.gene.2022.146961
82. Drummond JB, Simmons M, Haroutunian V, Meador-Woodruff JH. Upregulation of cornichon transcripts in the dorsolateral prefrontal cortex in schizophrenia. Neuroreport 2012;23:1031-1034. https://doi.org/10.1097/WNR.0b013e32835ad229
83. Floor K, Baroy T, Misceo D, Kanavin OJ, Fannemel M, Frengen E. A 1 Mb de novo deletion within 11q13.1q13.2 in a boy with mild intellectual disability and minor dysmorphic features. Eur J Med Genet 2012;55:695-699. https://doi.org/10.1016/j.ejmg.2012.08.002
84. Sun Y, Zhu J, Yang Y, Zhang Z, Zhong H, Zeng G, Zhou D, ET AL. Identification of candidate DNA methylation biomarkers related to Alzheimer's disease risk by integrating genome and blood methylome data. Transl Psychiatry 2023;13:387. https://doi.org/10.1038/s41398-023-02695-w
85. Nelson EC, Agrawal A, Heath AC, Bogdan R, Sherva R, Zhang B, Al-Hasani R, ET AL. Evidence of CNIH3 involvement in opioid dependence. Mol Psychiatry 2016;21:608-614. https://doi.org/10.1038/mp.2015.102
86. Flegelova H, Sychrova H. Mammalian NHE2 Na^+/H^+ exchanger mediates efflux of potassium upon heterologous expression in yeast. FEBS Lett 2005;579:4733-4738. https://doi.org/10.1016/j.febslet.2005.07.046
87. Kolacna L, Zimmermannova O, Hasenbrink G, Schwarzer S, Ludwig J, Lichtenberg-Frate H, Sychrova H. New phenotypes of functional expression of the mKir2.1 channel in potassium efflux-deficient Saccharomyces cerevisiae strains. Yeast 2005;22:1315-1323. https://doi.org/10.1002/yea.1333
88. Schwarzer S, Kolacna L, Lichtenberg-Frate H, Sychrova H, Ludwig J. Functional expression of the voltage-gated neuronal mammalian potassium channel rat ether à go-go1 in yeast. FEMS Yeast Res 2008;8:405-413. https://doi.org/10.1111/j.1567-1364.2007.00351.x
89. Velazquez D, Prusa V, Masrati G, Yariv E, Sychrova H, Ben-Tal N, Zimmermannova O. Allosteric links between the hydrophilic N-terminus and transmembrane core of human Na^+/H^+ antiporter NHA2. Protein Sci 2022;31:e4460. https://doi.org/10.1002/pro.4460
90. Zheng J, Yao L, Zeng X, Wang B, Pan L. ERV14 receptor impacts mycelial growth via its interactions with cell wall synthase and transporters in *Aspergillus niger*. Front Microbiol 2023;14:1128462. https://doi.org/10.3389/fmicb.2023.1128462