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Alkali Metal Cation Transport and Homeostasis in Yeasts

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INTRODUCTION

The relevance of alkali metal cations to cell physiology, including their transport into and out of the cell, aroused scientists' interest as early as 100 years ago. It became obvious very soon that, whereas potassium is highly accumulated in different types of living cells and is indispensable for many physiological functions, sodium is toxic and at higher concentrations becomes lethal. Among other cell types, yeast cells were developed into an ideal model to study alkali metal cation transport and homeostasis more than 50 years ago, and the pioneering work in the field has already been brilliantly summarized (65, 235).

The reasons for using *Saccharomyces cerevisiae* as a model to

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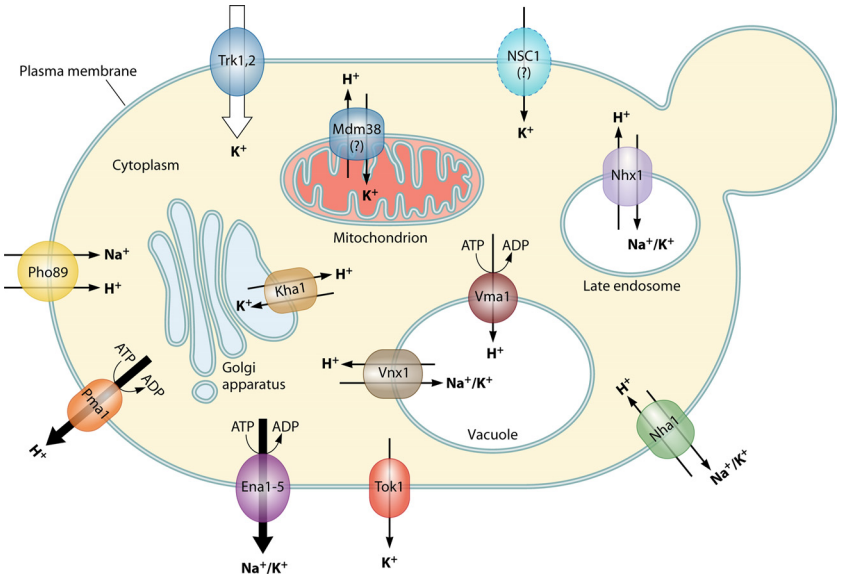

FIG. 1. The major plasma membrane and intracellular cation transporters in the yeast *S. cerevisiae*.

study alkali metal cation homeostasis in eukaryotic cells are the availability of the complete annotated genome sequence (the first one among eukaryotic cells) (95), a comprehensive *in silico* prediction of all transporters (190), and its fast growth and genetic tools to prepare mutants. Later, a comparative analysis of sequenced genomes from different yeast species showed that approximately 10% of a Hemiascomecete yeast genome corresponds to membrane transporters (56), and a detailed analysis of genes encoding putative transporters revealed that the systems ensuring efficient uptake and efflux of potassium and/or sodium have been highly conserved (56).

Although most yeast do not normally encounter high concentrations of sodium in their natural habitats, they may be confronted with situations in which larger amounts of toxic sodium than of requisite potassium cations are present in the environment. In these circumstances, cells spend a lot of energy accumulating sufficiently large amounts of intracellular K^+ and maintaining low cytosolic Na^+. Potassium is required for many physiological functions, such as regulation of cell volume and intracellular pH, maintenance of stable potential across the plasma membrane, compensation of negative charges in many macromolecules, protein synthesis, and enzyme activation. However, *S. cerevisiae* cells are able to grow in the presence of a broad range of external concentrations of K^+ (10 μM to 2.5 M) and Na^+ (<1.5 M), and some nonconventional halotolerant yeast species can grow in almost-saturated salt solutions. To maintain an optimum intracellular concentration of potassium ions and a stable and high intracellular K^+/Na^+ ratio, yeast cells employ three different approaches: (i) strict discrimination among alkali metal cations at the level of influx (transporters displaying higher affinity for potassium than for sodium [235]), (ii) efficient efflux of toxic or surplus cations from cells, and (iii) selective sequestration (compartmentalization) of cations in organelles. In yeast cells, transport systems exist at both the plasma and organellar membranes, which mediate alkali metal cation fluxes with different substrate specificities and using diverse mechanisms (e.g., primary active ATPases, secondary active symporters and antiporters, and passive channels) (Fig. 1). At least 10 different alkali metal cation-specific transporters in *S. cerevisiae* have been characterized so far (Table 1), and strong evidence exists indicating that at least a few additional transporters will be defined at the molecular level (see below).

Plasma membrane transporters serve to (i) provide cells with sufficient amounts of potassium; (ii) maintain the potassium homeostasis; (iii) eliminate toxic sodium (lithium) cations; (iv) preserve the membrane potential; (v) regulate the intracellular pH; (vi) keep a positive turgor inside the cell, which is necessary for plasma membrane/cell wall expansion and cell division, and (vii) cope with osmotic stress. There are six different well-characterized transporters at the plasma membrane. They comprise the potassium uptake systems Trk1 and Trk2, the potassium channel Tok1, the P_i-Na^+ symporter Pho89, and the efflux systems Ena Na^+-ATPases and Nha1 Na^+/H^+ antiporter. Besides these six specific alkali metal cation transporters, there is a cationic channel whose existence has been experimentally proven but whose gene has not been identified yet (NSC1) and several other (nonspecific) proteins/transporters marginally involved in potassium and/or sodium fluxes across the plasma membrane (e.g., Pmp3 and Qdr2).

Intracellular transporters have been identified and characterized only recently. They comprise mainly alkali metal cation/H^+ antiporters of vacuolar (Vnx1), endosomal (Nhx1), and Golgi apparatus (Kha1) membranes. Very efficient and physiologically crucial exchange of K^+ for H^+ by an antiport mechanism also exists across the mitochondrial membrane, but the transporter gene has not been identified yet. Similarly to the plasma membrane transporters, organellar systems serve to (i) regulate cytosolic cation homeostasis (e.g., detoxification of sodium by sequestration in the vacuole), (ii) maintain and regulate intraorganellar potassium homeostasis and pH, and (iii) modulate protein trafficking through the endosomal and/or secretory pathway.

TABLE 1. Alkali metal cation transporters in *S. cerevisiae*

Name	ORF	YETI no.[a]	Localization[b]	Transporter type	Length (aa)	No. of TMS	Substrate specificity[c]	Main function	Reference
Trk1	YJL129c	2.A.38.2.1	PM	Uniporter	1,235	12	**K^+**, Rb^+	K^+ uptake	82
Trk2	YKR050w	2.A.38.2.1	PM	Uniporter	889	12	**K^+**, Rb^+	K^+ uptake	128
Tok1	YJL093c	1.A.1.7.1	PM	Channel	691	10	**K^+**	K^+ efflux	116
Ena1	YDR040c	3.A.3.9.1	PM	ATPase	1,091	10	**Na^+**, **Li^+**, K^+, Rb^+	Detoxification	102
Nha1	YLR138w	2.A.36.4.1	PM	Antiporter	985	12	**K^+**, **Na^+**, Li^+, Rb^+	K^+ efflux	219
Pho89	YBR296c	2.A.20.2.2	PM	Symporter	574	10	**Na^+**, **P_i**	P_i uptake	156
NSC1	Not known		PM	Channel	Not known	Not known	Monovalent + bivalent cations	Not known	30
Kha1	YJL094c	2.A.37.4.1	GA	Antiporter	873	12	*K^+*, *Li^+*, *Na^+*	Detoxification	153
Nhx1	YDR456w	2.A.36.2.1	LE	Antiporter	633	8 (?)	**Na^+**, Rb^+, *K^+*, *Li^+*	Detoxification, vesicle trafficking	185
Vnx1	YNL321w	2.A.19.Y1.Z1	VAC	Antiporter	908	13	**Na^+**, **K^+**	Detoxification	37

[a] YETI, Yeast Transporter Information (55, 56), based on Transport Classification (TC) system (248).
[b] PM, plasma membrane; GA, Golgi apparatus; LE, late endosomes; VAC, vacuole.
[c] Bold, preferred substrate; italic, substrate predicted from phenotypic observations.

Role of H^+-ATPases in Alkali Metal Cation Homeostasis

Most of the proteins listed above mediate transport by an antiport mechanism, exchanging alkali metal cations for protons. Therefore, a proton motive force across membranes serves as a source of energy to pump alkali metal cations against their gradients. Nevertheless, in some cases (e.g., sudden alkalinization of cytosol at high pH_{out}), cells can use the outward gradient of potassium across the plasma membrane as a driving force to transport protons against their gradient into the cells (17). Under normal physiological conditions, the secondary active antiporters localized in the plasma and organellar membranes depend on the activity of primary active transporters that build up the necessary gradient of protons. In yeast cells, this function is fulfilled by the plasma membrane and vacuolar ATPases.

The plasma membrane H^+-ATPase, encoded by the *PMA1* gene, is the essential and main primary yeast transporter belonging to the widely distributed family of P_2-type ATPases (7). In yeast cells, it is the most abundant plasma membrane protein, which is very stable and consumes at least 20% of cellular ATP (177). Its electrogenic activity creates the electrochemical gradient of protons across the plasma membrane, which is in turn indispensable for all secondary active symporters and antiporters. The activity of Pma1 is tightly regulated according to the metabolic activity and physiological conditions of cells; e.g., external glucose is a powerful stimulus leading to a rapid and strong activation (254). Its activity is also positively regulated in response to decreased intracellular pH or increased potassium uptake (258). *S. cerevisiae* encodes a second plasma membrane H^+-ATPase, Pma2, which is 89% identical to Pma1 (252). Pma2 is able to export protons, although its enzymatic properties differ from those of Pma1 (267). The expression of *PMA2* under standard growth conditions is much lower than that of *PMA1*, which explains the nonessential nature of the former and its minor impact on cation homeostasis.

In contrast to the plasma membrane H^+-ATPase, the yeast vacuolar ATPase is not a single polypeptide but has a complex structure consisting of two main domains, peripheral V_1 and membrane-bound V_o. Each of the two domains is composed of several proteins, and the whole structure is related to the mitochondrial F_1F_o-ATP synthase (114, 115). This ATPase not only plays a crucial role in the acidification of the vacuolar lumen but is also indispensable for proper functioning of other organelles (251). In yeast cells, the analysis of its activity in organelles along the secretory pathway confirmed its importance but simultaneously suggested that this ATPase may exist in organelles in distinct forms and under selective regulation (250). It is very likely that its activity creates the gradient of protons across organellar membranes which, in turn, mediates the function of organellar alkali metal cation/H^+ antiporters. The plasma membrane and vacuolar H^+-ATPases most probably collaborate in maintaining cytosolic pH homeostasis, and there is evidence that they are functionally interdependent and coordinately regulated on multiple levels (158).

General Aspects of Alkali Metal Cation Homeostasis

The high potassium content in yeast cells corresponds to the steady state between simultaneous influx and efflux across the plasma membrane, and this continuous circulation is believed to be necessary for basic physiological functions (136, 198). The coordination of potassium uptake and efflux systems is necessary for the maintenance of the potential across the plasma membrane. Whereas the absence of potassium uptake systems leads to plasma membrane hyperpolarization (148), the deletion of efflux systems results in depolarization (122, 154).

In the presence of relatively low extracellular concentrations of sodium, the uptake of potassium via efficient transporters dominates the sodium influx. In K^+-starved cells, even though the concentration of external sodium is 100 times higher than that of K^+, the intracellular concentration of Na^+ remains relatively low (see below). When the extracellular Na^+/K^+ ratio exceeds approximately 700:1 (238), significant amounts of sodium enter, and as a consequence, equivalent amounts of K^+ must leave to maintain the electroneutrality inside the cells. The intracellular concentration of potassium cations in *S. cerevisiae* cells under normal growth conditions (i.e., in standard laboratory media which contain negligible amounts of sodium salts) ranges from 200 to 300 mM (depending on the strain and medium) and decreases with the influx of sodium. As soon as the intracellular concentration of Na^+ approaches that of K^+,

the growth of cells is inhibited, and with increasing Na^+ concentrations, cells die. The effect of sodium combines two components: first, the toxicity of sodium cations, inhibiting many enzymatic functions, and second, the considerable osmotic stress caused by the high concentrations of external sodium salts. The precise cellular targets for sodium (or lithium) toxicity are still poorly defined, although several likely candidates have been proposed (63, 161, 182). In any case, to survive under the high external sodium salt conditions, cells must react to the loss of water caused by the osmotic shock (as reviewed many times elsewhere; see, e.g., reference 108), as well as to the unfavorably low intracellular K^+/Na^+ ratio. Besides involvement of plasma membrane transporters, intracellular transporters, mainly Vnx1 and Nhx1 antiporters, play an important role in decreasing the cytosolic concentration of Na^+, as they help to sequester toxic sodium cations in the vacuole, where the intracellular supply of potassium is normally kept. Transport and storage of alkali metal cations in the vacuole serve not only to ensure optimal cytosolic potassium concentrations or to diminish the amount of toxic sodium in the cytosol but also to compensate for the negative charges of the vacuolar stock of polyphosphates (126).

CATION TRANSPORT AT THE PLASMA MEMBRANE LEVEL

Although for the sake of clarity we will classify plasma membrane cation transporters according to the most relevant cation transported (or at least the one originally identified), it is important to stress that in many cases they are not fully specific for a given cation (e.g., Trk transporters import both potassium and sodium).

Potassium Uptake

A historical introduction. The pioneering biochemical work by Conway and O'Malley (50) and Rothstein and Enns (240) during more than 2 decades established an important framework for the study of potassium transport in *S. cerevisiae*. Those authors showed that in yeast, K^+ was transported in exchange with H^+, and they proposed for the first time the existence of a plasma membrane carrier with higher affinity for K^+ than for other cations (49), the effect of external pH on the ability of yeast cells to discriminate between K^+ and Na^+ (10), and the competitive inhibition of potassium uptake by other alkali metal cations in yeast (11). Moreover, in that work, a model accounting for the inhibition kinetics was presented. That model was based on two cation-binding sites for which cations would compete: a carrier or transporting site and a second, nontransporting (modifier) site with a different array of affinities for cations. In the following years, several laboratories contributed to the characterization of potassium transport in *Saccharomyces* and in other yeasts (130, 132, 196, 204). A useful review, published by Borst-Pauwels, compiled the status of the field of energetics and kinetics of ion transport in yeast as of the late 1970s (32).

The state of the art significantly changed during the second half of the 1980s, and several reports brought our understanding to the molecular level. While by that time it was already known that bacteria or plants could transport potassium through high-affinity processes (67, 68), no evidence for something similar in fungi was available. A first study, reported in 1981 (42), defining potassium requirements in *S. cerevisiae* provided the basis to propose, 3 years later, the existence of a dual mode for potassium transport (238). The functioning of high-affinity or low-affinity transport processes was shown to be dependent on the growth history of the cells. For instance, when cells were inoculated in a medium in which arginine was the only N source and neither ammonium nor sodium was present, they could grow at micromolar concentrations of external potassium and developed a transport system with high affinity for the cation (K_m in the micromolar range) (238). In addition, the isolation and characterization of the first yeast potassium transport mutant (229) laid the groundwork for the identification of the genes involved in K^+ transport (82, 128).

Trk1 and Trk2 high-affinity transporters. In a series of related papers, Gaber and coworkers reported the identification and characterization of the genes encoding the two main transporters involved in potassium uptake in *S. cerevisiae*: *TRK1* and *TRK2* (82, 127, 128).

TRK1 (YJL129c) was the first gene isolated and studied which encodes a K^+ transporter in eukaryotic nonanimal cells and it was cloned on the basis of its ability to suppress the potassium transport defect in *S. cerevisiae trk1* mutant cells. *TRK1* encodes a 180-kilodalton plasma membrane protein, 1,235 amino acids (aa) long, for which hydrophobicity analysis predicts 12 potential membrane-spanning domains (82). Significant amino acid sequence identity between Trk1 and the nucleotide-binding domains of a number of prokaryotic and eukaryotic proteins was observed, and later *TRK1* orthologs were identified in other yeasts, fungi, and higher plants (235). Like the H^+-ATPase Pma1, Trk1 is an integral plasma membrane protein localized to the "raft" domains (293), which are glycolipid-enriched microdomains of the plasma membrane postulated to form a platform for lipid and protein sorting and trafficking. The Trk1 protein contains four repeated motifs and resembles the structure of the KcsA channel of *Streptomyces lividans*, which is made up of four identical subunits. Therefore, it appears as a tetra-M1PM2 structure (where M corresponds to a hydrophobic segment and P to an α-helix that enters the membrane and connects the M segments) instead of standard 12-transmembrane-domain (TMS) structure. Haro and Rodríguez-Navarro have proposed a schematic model of the transporter and have identified residues which are important in the transport process (103).

TRK1 is nonessential in *S. cerevisiae*. Haploid cells that contain a null allele of *TRK1* (*trk1*Δ) rely on a remaining, less efficient transport activity for potassium uptake (82). While the wild-type *S. cerevisiae* strain grows at low-micromolar potassium concentrations and exhibits a transport process with high velocity and high affinity for K^+ and Rb^+ (V_{max} of 30 nmol/mg cells/min and K_ms of 0.024 and 0.08 mM, respectively) (238), *trk1* mutants show increased potassium requirements and defective K^+ (Rb^+) transport (82). These cells do not grow on 0.1 mM KCl, and the V_{max} of the high-affinity component of rubidium transport decreases from 30 nmol/mg/min in the wild type to 5 nmol/mg/min in the mutant. Whereas the role of Trk1 as the major high-affinity K^+ transporter is well documented, more recently an electrophysiological approach has revealed an addi-

tional function for Trk proteins, the efflux of chloride ions (133). A hypothetical structural model for Trk1 has been proposed, suggesting that chloride ions flow through a central pore formed by symmetric aggregation of four Trk1 monomers.

TRK2 (YKR050w) is the second *TRK* gene in *S. cerevisiae*. It encodes a shorter protein (889 amino acids) and is 55% identical to Trk1 in its overall sequence. It also contains 12 putative transmembrane domains (127, 128) arranged in four MPM segments, whose identity to those of Trk1 rises to 70 to 90%. The principal difference between Trk1 and Trk2 lies between the first and second MPM motifs; in Trk1 this loop is 642 aa long, while in Trk2 this intracellular segment is much shorter (326 aa). Interestingly, most *TRK* genes identified in other yeasts are more closely related to *TRK2* than to *TRK1*. It is worth noting that although the existence of a Trk1,2 system is often mentioned in the literature, this has to be taken just as a semantic notion, since there are no results supporting the possible existence of a hybrid multimeric complex or a physical interaction between the two Trk proteins.

The viability and the increased K^+ requirements of *trk1* mutants suggested the existence of an additional, functionally independent potassium transporter, whose function could be negligible in a wild-type strain. Therefore, the group of R. Gaber used *trk1*Δ cells to screen for mutants (named Kla^-) that required higher concentrations of K^+ in the medium to support growth. They isolated 38 independent mutants that contained a mutation in the same gene, *TRK2*. Double *trk1*Δ *trk2* mutants require up to 10-fold-higher concentrations of K^+ than *trk1*Δ *TRK2* cells for normal growth. K^+ and Rb^+ transport assays demonstrated that the mutant phenotype was due to defective K^+ uptake. Therefore, the primary function of *TRK2*, at least in *trk1* cells, was proposed to be K^+ influx (127). This has been subsequently confirmed by other laboratories (168, 228). It has been reported that strains deficient for both K^+ transporters (*trk1*Δ *trk2*) exhibit hypersensitivity to low extracellular pH that can be suppressed by high concentrations of K^+ but not Na^+. This additional phenotype is most probably a side effect derived from the fact that at acidic pH, the cells become depolarized and K^+ requirements are higher. This is probably the reason why either *TRK1* or *TRK2* can completely suppress both the K^+ transport defect and low-pH hypersensitivity of *trk1 trk2* cells (127, 168).

It is worth mentioning that the secondary function of *S. cerevisiae* Trk1, chloride efflux, can also be performed by Trk2, since patch clamp experiments using several mutant strains identified chloride currents via both Trk proteins (29, 133). It must be noted that *trk1 trk2* cells, while severely limited in their ability to take up K^+, are viable when the growth medium is supplemented with sufficient amounts of potassium, which reveals the existence of one or more additional, functionally independent systems able to transport potassium (see below).

High-affinity versus low-affinity potassium transport. The relationship between genes encoding K^+ transporters and transport modes has been somewhat controversial. We have mentioned that even before the isolation of the *TRK* genes, the existence of a dual mode for K^+ uptake showing low or high affinities for the cation was reported (238). Since the lack of Trk1 produced increased potassium requirements and impaired transport, this protein was proposed to be a high-affinity K^+ transporter, and, for the same reason, the orthologous *TRK2* gene was considered to encode the putative low-affinity system. This idea was reinforced by the modest contribution of Trk2 to total K^+ uptake in wild-type cells and by the low Trk2-mediated potassium uptake activity measured in *trk1 TRK2* strains (127, 278). However, this scenario was too simplistic, since it was shown that Trk2 may be a high/moderate-affinity K^+ transporter (168, 228) and, on the other hand, a residual potassium transport process with a K_m in the mM range was present in mutants lacking both the *TRK1* and *TRK2* genes (148).

The following summarizes our view about the relationship between *TRK* genes and the existence of different modes of potassium transport. In a wild-type strain, most if not all potassium influx is mediated by Trk1, which can work as a high- or low-affinity transporter according to the growth history and the K^+ status of the cell. Thus, wild-type cells grown without K^+ limitations would show the so-called low-affinity transport mode, but after starvation, they would display the high-affinity system. Trk2 would also transport potassium with high/moderate affinity, but the gene is very poorly expressed under standard conditions. Its activity, if any, would be masked in the presence of *TRK1*. Finally, in strains lacking the two transporters, an ectopic potassium transport process is still evident. This Trk-independent process shows very low affinity (K_m in the mM range), and the system(s) mediating transport has not been definitely established, although some clues about the nature of the transporters involved can be found when the activities of other transporters, such as the NSC1 channel or the Qdr2 drug exporter, are analyzed.

Low-affinity transport system. Using electrophysiological techniques, potassium currents were detected in *trk1 trk2* mutants, and the putative channel involved in that process was named NSC1 (nonspecific cation "channel"). Although the gene responsible has not been identified yet, this channel appears to be a good potential candidate to explain the "very-low-affinity" potassium uptake process in *trk1 trk2* mutants. Its activity is blocked by mM concentrations of calcium and other divalent metal ions. It is unblocked by decreasing the amount of extracellular free divalent metals to below approximately 10 μM and is independent of the other identified potassium transporters, Tok1, Trk1, and Trk2 (30). Analysis of this nonselective cation channel by whole-cell patch clamping (92, 234) suggested that this inwardly rectifying pathway could relieve the growth inhibition normally imposed on yeast by disruption of its potassium transporters, Trk1 and Trk2. Later on, it was proposed as the primary low-affinity uptake route for potassium ions in yeast. Factors which suppress NSC1-mediated inward currents and inhibit growth of *trk1 trk2* cells include high calcium, low extracellular pH, hygromycin B, and, to a lesser extent, tetraethylammonium. Growth of *trk1 trk2* cells is also inhibited by lithium and ammonium; however, these ions do not inhibit NSC1 but instead enter yeast cells via this channel. Growth inhibition by lithium ions is probably a toxic effect, whereas growth inhibition by ammonium ions probably results from competitive inhibition, i.e., replacement of intracellular potassium by entering ammonium (31).

The identification of the gene(s) encoding NSC1 is an urgent task that would clarify the final contribution of this

nonselective channel to the low-affinity ectopic transport process present in *trk1 trk2 S. cerevisiae* mutants.

The *QDR2* gene of *S. cerevisiae* encodes a putative plasma membrane drug/H^+ antiporter that confers resistance against quinidine, barban, bleomycin, and cisplatin. The presence of quinidine in the medium produces increased potassium requirements and defective Rb^+ (K^+) transport, and these effects are more dramatic in a *qdr2* strain than in the wild type. The growth differences registered between the parental strain and *qdr2* cells under K^+-limiting conditions, even in the absence of quinidine, suggested that the capacity to transport K^+ may be altered in this mutant. The lack of *QDR2* slightly reduces the K^+ content of cells compared with that in the parental strain. However, in quinidine-stressed cells, the values of internal K^+ measured in the *qdr2* mutant were about one-half of those determined in the wild-type strain. Results from Rb^+ transport assays indicated a defective uptake in the absence of *QDR2*. The direct involvement of Qdr2 in K^+ uptake is reinforced by the fact that increased Rb^+ uptake due to *QDR2* expression is independent of the Trk1 and Trk2 systems (272).

Gaber and coworkers have shown that mutations in sugar or amino acid transporters can partially suppress the increased K^+ requirements in *trk1 trk2* cells by increasing Rb^+ uptake (129, 290). Their hypothesis was that under certain conditions, sugar or amino acid carriers can accept and transport amounts of alkali metal cations that would improve growth at limiting external K^+ concentrations. Similarly, Navarre and Goffeau (188) reported the existence of Pmp3, a 55-amino-acid hydrophobic plasma membrane polypeptide that is conserved in yeast and plants. Yeast cells deleted for the *PMP3* gene showed increased plasma membrane potential and sensitivity to toxic cations, such as Na^+ and hygromycin B. This effect appeared to be unrelated to changes in Pma1 activity. Lack of Pmp3 also exacerbated the sensitivity of a mutant strain lacking the Ena1 and Nha1 proteins to Na^+ and suppressed the potassium dependency of a *trk1 trk2* strain. Interestingly, these effects were reversed by addition of 10 mM $CaCl_2$. The authors proposed that the absence of Pmp3 facilitates a Trk1,2-independent, nonspecific monovalent cation uptake through an increase in the membrane potential, although no further progress has been made in this direction.

In summary, the remaining ectopic potassium uptake process observed in *trk1 trk2* double mutants can be explained by the activity of one or, most probably, several processes that mediate potassium influx in a nonspecific manner. It is conceivable that the contribution of these transporters could vary depending on the growth conditions.

Mechanism of potassium transport. The mechanisms mediating potassium uptake in *S. cerevisiae* are still unresolved. Potassium is accumulated by yeast cells against a very wide range of concentration gradients. While intracellular potassium reaches concentrations of around 200 to 300 mM, extracellular potassium can be in the low mM or micromolar range. Therefore, yeast cells have to adapt to changing environmental conditions, and the mechanism of transport may not be the same under different growth conditions.

We have mentioned above that the plasma membrane ATPase extrudes H^+ and creates a membrane potential that draws potassium into the cell. Despite several decades of investigation, a reliable experimental determination of the membrane potential in *S. cerevisiae* has remained elusive. The quantitative results obtained using diverse probes (−100/−125 mV) seem to underestimate the real membrane potential. According to the Nernst's equation and assuming membrane potentials of close to −300 mV (reported for other organisms, such as *Neurospora crassa* [236]), the existence of a K^+ uniporter can explain the K^+i/K^+e gradients usually observed. However, under certain conditions, such as low potassium in combination with acidic external pH, where the cells are less hyperpolarized, uniport could not fulfill the cell's requirements, and an active process is likely to be required in *S. cerevisiae*. In this case, a K^+-ATPase or a symport mechanism could explain active K^+ uptake. The possible existence of a K^+-ATPase in *S. cerevisiae* has received little support during the last 2 decades, and furthermore, systems similar to the ACU (*a*lkali *c*ation *u*ptake) ATPases working in *Ustilago maydis* or *Pichia sorbitophila* are not present in *S. cerevisiae* (21). On the other hand, the functioning of a K^+-H^+ symporter has been supported by the proposal that two binding sites are present in the yeast K^+ transporter and by the existence of a K^+-H^+ symport in *Neurospora crassa* (241). However, the possibility that Trk systems work as a K^+-Na^+ symporter similar to the one described in plants (241) cannot be ruled out, and the fact that the HKT/TRK family of transporters is very specific for alkali metal cations in plants may be taken as an indication that a putative K^+-Na^+ transport mechanism may operate in *S. cerevisiae* (101).

Efflux of Potassium

The existence of potassium efflux processes in *S. cerevisiae* has been known for a long time. Nowadays, strong experimental evidence exists that at least three different transporters can contribute to the potassium efflux, depending on the physiological conditions. Two of them have been first identified as Na^+ efflux systems, but they can also mediate K^+ efflux (i.e., Ena1 and Nha1 [see below]). The third system is the only K^+-specific efflux system (Tok1) existing in *S. cerevisiae*.

Tok1 channel. Tok1 is the only outward-rectifying potassium channel present in the plasma membrane of yeast cells. Tok1 is 691 residues long (169), and its sequence conceptually corresponds to a channel protein with eight transmembrane domains and two P loops. The *TOK1* (YJL093c) open reading frame (ORF) (116) is also known as *YKC1* (296), *DUK1* (231), *YORK* (140), and *YPK1* (26).

The two Tok1 pore-forming P domains are structural entities common to potassium-selective ion channels (1). Each of the two pores forms a functional channel permeable to potassium, and the carboxyl tail would function to prevent inner gate closures. As deduced from its structure and demonstrated by electrophysiological experiments, the primary function of Tok1 is K^+ efflux. Gating of the channel is promoted by membrane depolarization; the channel is open at positive and very low negative membrane voltages (26, 72). Additionally, the activity of Tok1p is modulated by external K^+ (143, 275), both in yeast and when expressed in *Xenopus* oocytes (116, 231, 296).

Tok1 currents show a clear outward rectification above the equilibrium potential of K^+ (EK^+); i.e., when the membrane potential is more positive than the EK^+, the outward K^+ current increases disproportionately, indicating increased chan-

nel-open probability. This was observed in the K^+ current native to yeast (24, 26, 98, 231, 296) as well as in oocytes heterologously expressing *TOK1* (116, 140, 143). Deletion of *TOK1* abolishes the native yeast K^+ current observed in patch clamp experiments, whereas overexpression of *TOK1* enhances it (231, 296). Despite extensive efforts to find conditions that specifically affect the growth of *tok1* mutants, no clear growth-related phenotypes have been reported (231, 296). In addition, *tok1* mutants did not display changes in K^+ content, Rb^+ uptake, or K^+ efflux when the cations were measured by atomic absorption spectrophotometry, and the effect of the mutation was observed only by following an electrophysiological approach (25). Thus, although the biophysical properties of the Tok1 channel are well described, the physiological role of Tok1 is presently unclear (25). What we know is that Tok1 is activated by plasma membrane depolarization (26) and that K^+ accumulated in the cells is probably released in order to regenerate the membrane potential (24). In fact, the relationship between membrane potential regulation and Tok1 has been more recently confirmed by the observation that *tok1* mutants are depolarized and cells overexpressing *TOK1* are hyperpolarized (154).

Tok1 has been reported to be a molecular target of the yeast viral killer toxin K1 (1), which is thought to act on Tok1 from both sides of the plasma membrane (257). However, the physiological significance of the binding of K1 to Tok1 has been questioned (34). A secondary function has also been assigned to Tok1. It has been proposed that under certain conditions Tok1 could mediate some K^+ influx, but this possible passive influx would ordinarily be overshadowed by the strong uptake by Trk1 or even by Trk2. To maximize the chances of detecting this passive influx, a *trk1 trk2* strain overexpressing *TOK1* was examined; growth patterns and internal K^+ and membrane K^+ currents were studied, and evidence consistent with K^+ influx through Tok1 was found (72).

Sodium Uptake

There is no single, specific sodium transporter in the budding yeast *S. cerevisiae*, and this cation enters the cells significantly only if it is present at high external concentrations. This idea has been generally accepted since Conway and Duggan (49) and Armstrong and Rothstein (10) proposed the existence of a cation carrier transporting different alkali metal cations and was reinforced when it was proposed that Na^+ binds the activation site of the K^+ carrier, thus affecting Rb^+ and K^+ transport (32). Sodium competitively inhibits potassium uptake in wild-type cells, and therefore Trk1 must accept and transport Na^+ in addition to K^+, although with much lower affinity (229). *trk1 trk2* cells accumulate more Na^+ and less K^+ than wild-type cells, suggesting that the low-affinity and nonspecific transporters acting in these cells do not discriminate between K^+ and Na^+ (96). In this respect, it is important to point out that in the absence of calcium, the NSC1 channel mentioned above also transports sodium or lithium (30).

On the other hand, it was proposed in 1977 that phosphate uptake in *S. cerevisiae* was mediated by two mechanisms, with one of them being dependent on the presence of sodium (239). The same investigators reported that the mechanism involved two sites with affinity for sodium and that lithium, but not rubidium, also stimulated phosphate uptake. Subsequently, the *PHO84* and *PHO89* genes were found to encode two derepressible high-affinity phosphate cotransporters localized to the plasma membrane. Whereas Pho84 catalyzes a proton-coupled phosphate transport at acidic pH, Pho89 catalyzes a sodium-dependent phosphate uptake at alkaline pH (207). Northern analysis showed that *PHO89* is transcribed under conditions of P_i limitation, although it was shown later that it is also strongly induced by alkaline pH even when the cells are grown in medium with normal phosphate concentrations (256). Remarkably, induction of *PHO89* at high pH seems to be largely dependent on calcineurin, and the gene also transcriptionally responds to increased extracellular calcium (246, 280). Kinetic characterization of Pho89 showed that it has an apparent K_m for phosphate of 0.5 μM, has an optimum pH of 9.5, and is highly specific for Na^+; activation of P_i uptake is maximal at an Na^+ concentration of 25 mM (156). Pho89 represents the only Na^+-coupled secondary anion transport system so far identified in *S. cerevisiae*. As far as we know, no studies on sodium entry have been performed with the triple mutant lacking both *TRK* genes and *PHO89*. The results provided by those studies may be of interest to evaluate the actual relevance of this transporter in Na^+ uptake.

Sodium Efflux

To maintain a low cytosolic concentration of toxic sodium (or lithium) cations, *S. cerevisiae* cells use two types of efflux systems at their plasma membrane. As discussed below, while these systems complement each other in detoxification function, they differ in both transport mechanism and regulation of their expression and activity.

Ena ATPases. P-type ATPases couple the hydrolysis of ATP to the transport of cations against electrochemical gradients by forming a typical phosphoenzyme intermediate (44). The IID subtype, exemplified by the ENA system, corresponds to a subfamily of ATPases able to extrude Na^+, Li^+, or K^+ with different capacities (20). As discussed below, *S. cerevisiae* contains various copies of genes encoding identical or very similar Ena proteins (86, 102, 157, 288). On the basis of its sequence, *ENA1* was initially proposed to encode a putative calcium P-type ATPase (242), and the locus was named *PMR2* (for *p*lasma *m*embrane ATPase *r*elated), but in 1991 Rodriguez-Navarro and coworkers reported its capacity to extrude Na^+, Li^+, and probably K^+ (102), thus establishing its primary role in the cell. Therefore, *ENA1* (*e*xitus *na*tru) is currently the accepted gene name, whereas *PMR2* and *HOR6* are listed as aliases. Remarkably, in *S. cerevisiae* these genes are usually found in tandem repeats on chromosome IV. The number of copies varies from strain to strain. The FY1679 strain contains 3 *ENA* genes, *ENA1* (YDR040c), *ENA2* (YDR039c), and *ENA5* (YDR038c), encoding proteins of identical length (1,091 amino acids) and displaying very similar sequences (>97%), although *ENA1* is slightly more distant from *ENA2* and *-5*. Under standard growth conditions Ena1, -2, and -5 are present in the cell at low levels (around 600 molecules/cell) (91), although, as will be discussed below in detail, *ENA1* expression markedly increases in response to saline or alkaline pH stresses. Other strains, such as DBY746 and W303-1A, contain four copies of *ENA* (86, 237), while strains S288C,

A364A, and D273-10B contain five repeats and Σ1278b only one (288). A recent report has confirmed the existence of a single *ENA* gene in the CEN.PK113-7D strain (named *ENA6*) and found that the encoded protein shows a higher-than-normal degree of sequence divergence compared to those encoded in other genetic backgrounds. This difference may be responsible for the unusually high sensitivity of CEN.PK derivatives to Na^+ and Li^+ cations (54).

The ENA cluster (particularly *ENA1*), is a major determinant of sodium tolerance in *S. cerevisiae*. Deletion of the entire cluster results in strong sensitivity to sodium and lithium cations (86, 102, 213, 237, 288) and a significant growth defect at alkaline pH (102, 209), whereas expression of *ENA1* restores tolerance to these cations. Furthermore, expression of *S. cerevisiae ENA1* in other fungi, such as *Schizosaccharomyces pombe* (13), or in plant cells (184) increases tolerance to Na^+ and Li^+ and decreases the intracellular content of these cations. Although the molecular basis for cation transport has not been solved, it has been proposed that the slight divergence in structure between Ena1 and Ena2 may explain the observation of the former being more effective in extruding Na^+ cations and the latter more specific for Li^+ (288).

The amino acid sequence of Ena proteins leads to the prediction of 9 or 10 transmembrane domains, and their presence at the plasma membrane has been demonstrated for *S. cerevisiae* Ena1 and Ena2 (288) and for *Debaryomyces* (formerly *Schwanniomyces*) *occidentalis* (15). However, overexpressed *ENA1* is also present in intracellular membrane structures (22). The *SRO7*/*SOP* gene (homolog of the *Drosophila* Lgl tumor suppressor gene), which encodes a protein involved in exocytic docking and fusion of Golgi-derived vesicles with the plasma membrane, was initially identified as being required for normal salt tolerance (137). This requirement could be explained by its involvement in the possibly specific delivery of Ena1 to the plasma membrane (283). In contrast, whereas alteration of Ena1 function has been proposed as an explanation for the salt-sensitive phenotype of diverse *vps* mutants (141), Ena1 seems to be properly localized to the plasma membrane in all of them. Therefore, the precise mechanisms for this effect remain unclear.

Nha1 antiporter. Although plasma membrane Na^+/H^+ antiporters represent conserved transport systems existing in all types of organisms (so far identified in, e.g., archaea, bacteria, fungi, parasites, insects, plants, and mammals), their structures, substrate specificities, and probable cell functions have diverged during their evolution. While most microorganisms and plants use the inward gradient of protons created by the plasma membrane H^+-ATPase as a driving force to pump sodium cations out, animal cells usually consume the Na^+ gradient resulting from Na^+/K^+-ATPase activity in order to force the surplus protons out and regulate intracellular pH.

In *S. cerevisiae*, the Na^+/H^+ antiporter encoded by the *NHA1* gene was selected from a multicopy genomic library on the basis of its ability to improve the growth of a salt-sensitive strain (a derivative of Σ1278b) on a medium supplemented with a high concentration of NaCl (219). Sequencing revealed that the cloned DNA fragment was about 300 nucleotides (nt) shorter than the complete ORF (which is 2,958 nt long), and later studies showed that this truncation improved the transporter's capacity to mediate the Na^+ and Li^+ efflux (120). Further studies on Nha1 activity revealed potassium (and its analog rubidium) as additional substrates (17). These data represented the first evidence showing that a typical member of the Na^+/H^+ antiporter family displays specificity for multiple substrates and is directly involved in potassium efflux. The two yeast antiporters previously characterized, *S. pombe* sod2 and *Zygosaccharomyces rouxii* Sod2, were shown to be specific for sodium and lithium (see below). Construction of a mutant strain with deletions of genes encoding both the Na^+-ATPase and the Na^+/H^+ antiporter (*ena1-4*Δ *nha1*Δ) confirmed that both transport systems are necessary for tolerance to high external concentrations of salts and that they both mediate efflux of all four alkali metal cations. The plasmid-based overexpression of both *ENA1* and *NHA1* gene in the double-deleted strain showed complementary action in the maintenance of a high intracellular K^+/Na^+ ratio. The Nha1 antiporter is responsible for cell growth with high concentrations of KCl and NaCl at acidic external pH values, and the Ena1 ATPase is necessary at higher pH values (17).

In contrast to their complementary function, the expression of *NHA1* differs from the tightly regulated expression of *ENA1* (described below). *NHA1* transcription is constitutive and very low, and it does not seem to be induced by salts, pH changes, or osmotic shock, suggesting a housekeeping role for Nha1 in potassium homeostasis and intracellular pH (17). The involvement of Nha1 in the maintenance of intracellular pH was first shown by measurements of intracellular pH in cells lacking or overexpressing Nha1 (269) and was later confirmed by the observation of an internal pH increase in *nha1*Δ cells (36).

Besides its function in detoxification of Na^+ and Li^+ cations and its involvement in the maintenance of stable intracellular K^+ and pH levels, the participation of Nha1 in several other cell processes, e.g., regulation of cell cycle, cell volume, or membrane potential, was clearly demonstrated. Overexpression of *NHA1* results in a rescue of the G_1/S cell cycle blockage in cells with conditional *sit4 hal3* mutations, implying that Nha1 plays a role in some aspect of cell cycle regulation (262). Nha1 participates, together with other K^+ transporters (Tok1, Trk1, Trk2, and Ena1), in the maintenance of stable plasma membrane potential (122, 154), and it is involved in the rapid-rescue mechanism during the immediate cell response to osmotic or saline shock (119, 120) (see below). Nha1 also acts as a safety valve upon sudden alkalinization of the cytosol, when the outward gradient of potassium cations can serve as a driving force for proton influx (17, 120, 198). Nha1 is very stable, with a half-life longer than 6 h (H. Flegelová-Kučerová and H. Sychrová, unpublished results). Detailed analysis of the antiporter's activity in isolated secretory vesicles revealed the electrogenicity of its transport mechanism, i.e., the observed requirement for downhill transport of more than one proton for the extrusion of one sodium ion (195). Biochemical studies of Nha1 revealed that (i) it is most probably present in membranes as a dimer and the interaction of monomers is important for the activity (175), and (ii) it localizes predominantly to detergent-insoluble and ergosterol-rich membrane fractions. The interaction with lipid rafts probably occurs only once Nha1 reaches the plasma membrane, and for its stabilization, the presence of sphingolipids is necessary (172).

The Nha1 antiporter is a typical membrane protein and is 985 amino acid residues long (Fig. 2). It has a very short

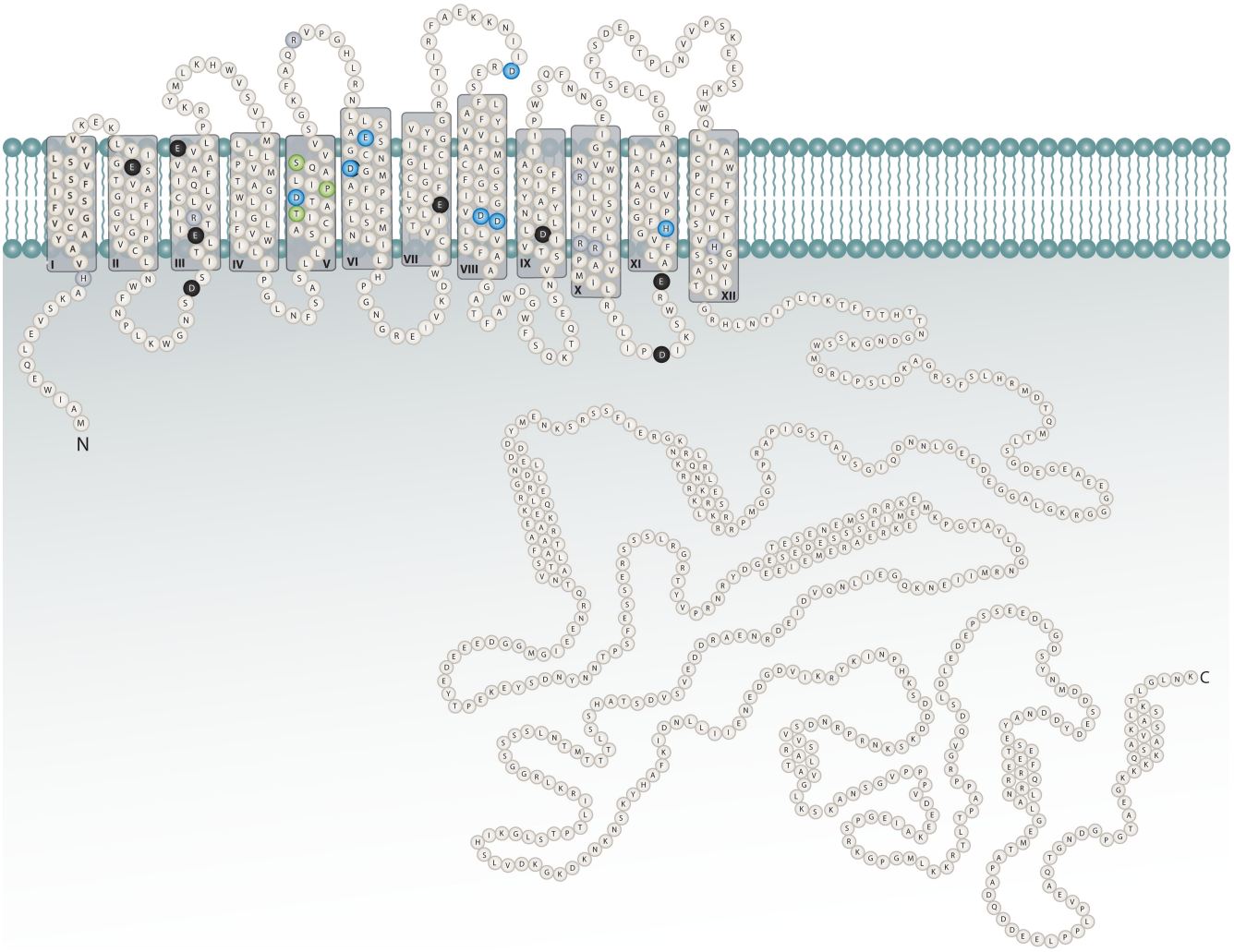

FIG. 2. Structure of the Nha1 Na^+, K^+/H^+ antiporter. Highly conserved charged (blue) and polar (green) residues important for function are highlighted in color; other highly conserved charged residues are shown in black (negatively charged) or gray (positively charged).

hydrophilic N terminus (only 12 amino acids) with a predicted cytosolic orientation, followed by probably 12 transmembrane hydrophobic segments and rather short connecting loops and terminated with a long terminal repeat hydrophilic C terminus. The membrane-spanning part of the protein, approximately 440 residues long, is highly conserved among yeast plasma membrane Na^+/H^+ antiporters (218), and it contains several, mainly charged, amino acid residues that have been shown to be important for alkali metal cation and/or proton translocation (Fig. 2). At least five highly conserved negatively charged amino acid residues and H367 have been identified as being crucial for the plasma membrane Na^+/H^+ antiporter function, first in *S. pombe* (62, 77, 287) and later in other yeast species. A tandem of aspartic residues (D266 and D267 in Fig. 2) in the eighth transmembrane segment was shown to be vital for the function of the *Z. rouxii* Sod2 antiporter as well (285), and mutations of these conserved residues to asparagine also abolished the sodium transport activity of *Candida albicans* Cnh1 and *S. cerevisiae* Nha1 (*Sc*Nha1) (262, 264). Interestingly, in the case of the *Sc*Nha1(D266N) mutant, the potassium efflux activity was not changed, and only the sodium export activity was abolished (195), whereas mutation of another aspartic residue (D241) affected K^+ extrusion (261). In addition, one proline (P146) and two polar residues (T142 and S151) in the fifth transmembrane domain were shown to be involved in the yeast plasma membrane Na^+/H^+ antiporter activity and/or substrate specificity (124, 125, 189).

Most of the known functions of Nha1 in cell physiology mentioned above are related to its very long hydrophilic C terminus, which represents approximately 56% of the total protein (Fig. 2). Though the overall level of identity of the C termini of the yeast plasma membrane Na^+/H^+ antiporters is much lower than that of the transmembrane domains (218), six conserved C-terminal regions (C1 to C6) have been identified (175). While the truncation of most of the C terminus (approximately last 510 amino acid residues) does not influence plasma membrane localization of *S. cerevisiae* Nha1 (120), the first conserved region, which is 16 residues long (aa 434 to 449) and overlaps the end of the 12th transmembrane segment and the beginning of the C terminus, is crucial both for proper

targeting and activity of the protein (173, 174). Some of the C-terminal regions (e.g., amino acid residues 920 to 930) have been shown to be important for the full transport capacity for Li^+ cations (120), and some (located mainly in two clusters, residues 869 to 876 and 918 to 927) were shown to be involved in the ability of Nha1 to suppress the G_1/S transition block observed in cells lacking the Sit4 and Hal3 proteins (261). Based on the analogy with mammalian NHE Na^+/H^+ exchangers of the same family (197), the long C terminus might be the target of the regulation of Nha1 activity and connect the Nha1 transport capacity with its physiological functions (see further).

Altogether, the main physiological function of Nha1 is probably housekeeping in *S. cerevisiae* and contributing to the essential continuous recycling of potassium cations across the plasma membrane. Its contribution to the detoxification of Na^+ and Li^+ cations is significant but not crucial. Nha1 seems to be more involved, via its potassium-transporting capacity, in intracellular potassium and pH homeostases and, consequently, in the rapid response to changes of external osmolarity, to cell volume adjustment, and to maintenance of plasma membrane potential. Probably in all these physiological functions, the Nha1 antiporter is linked to the activity of the Trk1 (and/or Trk2) potassium uptake system, as it has been shown that the deletion of *NHA1* affects the kinetics of high-affinity potassium uptake via Trk systems (16) and, vice versa, that deletion of *TRK1*,*2* results in a diminished potassium efflux upon potassium starvation (J. Zahrádka and H. Sychrová, unpublished results).

INTRACELLULAR CATION TRANSPORT

The concentration of alkali metal cations inside yeast cells is probably not homogeneous, and there is a presumed difference in the sodium and/or potassium amounts in the cytosol and in individual organelles. The physiological necessity of alkali metal cation transport across the organellar membranes is connected to the maintenance of potassium homeostasis and sodium detoxification in the cytosol as well as to the regulation of organellar pH and volume. So far, the antiport mechanism exchanging protons for potassium and/or sodium cations has been described for four *S. cerevisiae* organelles. Two of the antiporters involved, endosomal Nhx1 and Kha1 (Golgi apparatus) belong to the family of typical Na^+/H^+ exchangers, which also includes the plasma membrane-localized Nha1 (35, 218). In contrast, the vacuolar Vnx1 antiporter and the mitochondrial Mdm38 have different structures. The activities of Nhx1, Kha1, and Vnx1 antiporters are closely interconnected with the function of the vacuolar V-type H^+-ATPase, which creates the gradient of protons across membranes of these organelles (see above and reference 250).

Transport across the Inner Mitochondrial Membrane

Though the exchange of potassium and protons across the mitochondrial membranes had been predicted already by Peter Mitchell (171) and validated by direct measurements of proton and potassium fluxes in isolated mitochondria from many organisms, including yeast, the number, nature, and structure of the corresponding transporter(s) in yeast remain obscure. The *MDM38*/*MKH1*/YOL027c gene, whose product is localized in the inner mitochondrial membrane, has been shown to be indispensable for K^+/H^+ exchange. However, this gene is not likely to encode the transporter itself, as its product contains only one transmembrane hydrophobic segment. *MDM38*, which is well conserved among all eukaryotes, was isolated in a screen for genes involved in mitochondrial cation homeostasis (192). Mitochondria isolated from *mdm38* deletion mutants show highly reduced K^+/H^+ exchange, low membrane potential, high potassium content in the matrix and increased volume (80, 192). Detailed analysis of events following the downregulation of *MDM38* expression revealed that upon loss of K^+/H^+ antiport, mitochondrial swelling and reduction in membrane potential ensued, followed by fragmentation of mitochondrial reticulum, close association of mitochondria with vacuoles, and finally, uptake of mitochondrial material into the vacuoles (193). All these data confirm the important role of the active exchange of K^+ and H^+ in mitochondrial physiology, but nevertheless, the identification of the transporter molecule(s) remains open. Recently, two other proteins, Mrs7 and Ydl183, with a putative role in the mitochondrial K^+/H^+ exchange have been identified, and the existence of a multicomponent complex responsible for transport has been suggested (L. Zotova, M. Aleschko, G. Sponder, R. Baumgartner, S. Reipert, M. Prinz, R. J. Schweyen, and K. Nowikovsky, submitted for publication).

Vacuolar Transport

The vacuole is the place where cells sequester toxic sodium and store a substantial part of the intracellular potassium. A recent report (18) has proposed the existence of two types of fungi in regard to Na^+ tolerance: those tolerant to high Na^+ contents in the cytoplasm, which include *U. maydis*, and those intolerant to high Na^+ contents, which include the model yeast *S. cerevisiae*. However, in both types, vacuolar transport may play an important role in cation homeostasis. Exchange of sodium and potassium cations for protons across the vacuolar membranes was predicted a long time ago as the transport mechanism for accumulation of these cations in vacuoles. The existence of such a transport mechanism has been confirmed in experiments measuring the fluxes of cations and protons with the use of permeabilized cells, selective electrodes, and/or fluorescent probes (see, e.g., references 38, 107, and 159).

Vnx1 antiporter. For about 10 years, Nhx1 was believed to be the main antiporter involved in the transport of sodium and lithium cations to the vacuole, though its localization in the endosomal membranes and not in the membranes of vacuoles had been shown (186). This hypothesis was based mainly on the fact that the *S. cerevisiae* genome harbors only three genes (*NHA1*, *KHA1*, and *NHX1*) encoding typical members of the Na^+/H^+ antiporter family, with none of them being localized in the vacuolar membrane. Only recently, a gene encoding an alkali metal cation/H^+ antiporter has been identified and its product characterized (37). The *VNX1* gene (YNL321w) was identified using a reverse genetics approach (screen of the Euroscarf mutant collection), and, surprisingly, its sequence corresponds to a member of the Ca^{2+}/H^+ antiporter family, although with some atypical characteristics. Nevertheless, Vnx1 has been shown to reside in the vacuolar membrane and

not to mediate calcium uptake but to be indispensable for the vacuolar exchange of both sodium and potassium cations for protons (37). Besides the sequestration of alkali metal cations into the vacuolar lumen, Vnx1 also is likely to play a role in cytosolic ion homeostasis and pH_{in} regulation, similar to the case for the Nha1 and Nhx1 antiporters. Due to the unexpected nature of the transport mediated by Vnx1, additional studies to further validate its proposed cellular function seem to be necessary. Interestingly, a "residual" monovalent cation exchange activity could be detected in vacuolar fractions of *vnx1*Δ cells by using a slightly modified *in vitro* transport method. Disruption of potential candidate genes in *vnx1*Δ background strains permitted assignment of this "residual" activity to Vcx1 (O. Cagnac, personal communication), originally identified as a highly specific Ca^{2+}/H^+ vacuolar antiporter (53, 216) with possible affinities only for other divalent cations (208).

Endosomes and Golgi Apparatus

Kha1. Among the three yeast Na^+/H^+ antiporters, Kha1 (encoded by YJL094c) has the highest level of similarity to bacterial sodium or potassium/proton antiporters (218, 227). Although for some time it was believed to be a plasma membrane transporter (227), later it was shown to reside in the membrane of the Golgi apparatus (78, 153). Deletion of *KHA1* results in a growth defect in media with high pH or containing hygromycin B. There is no direct evidence regarding the substrate specificity of Kha1, but potassium is most probably its substrate, as the inability of the *kha1*Δ mutant to grow at higher pH can be suppressed by the addition of moderate concentrations of KCl (78, 153). Similar to the case for the other yeast intracellular alkali metal cation/H^+ antiporters, Kha1 is believed to be involved in the regulation of intraorganellar potassium and pH homeostasis and most probably acts in collaboration with the Gef1 anion channel, as high cellular levels of Kha1 are required for the functional expression of a mammalian ClC-2 channel in *S. cerevisiae* and for complementation of the *gef1*Δ phenotype (78).

Nhx1 antiporter. Nhx1, encoded by YDR456w, was the first discovered yeast intracellular antiporter (185), and it is the best characterized. It was identified upon characterization of Na^+-tolerant phenotypes of the H^+-ATPase *pma1-α4* mutant (185), and though there were some reports indicating its localization in mitochondria (194), it has been repeatedly shown and confirmed that it is localized in the membranes of prevacuolar compartments, i.e., late endosomes (33, 186). Deletion of the *NHX1* gene results in an increased salt and low-pH_{out} sensitivity of cells, together with a drop in the cytosolic pH (36). Deletion of the *NHX1* gene in a background lacking the plasma membrane Nha1 and Ena1-4 transporters brings about an extreme sensitivity not only to Na^+ but also to K^+, Li^+, and Rb^+ cations (121, 225), which suggests the broad substrate specificity of this antiporter. Its ability to transport Rb^+ was confirmed in a transport assay (35). The Nhx1 antiporter, like the plasma membrane Nha1, has multiple functions in yeast cells. It contributes to the sequestration of toxic sodium and lithium cations, as well as surplus potassium, in the endosomes and vacuoles (225) and is involved in the early stage of cell adaptation to hyperosmotic shock (187). In addition, its activity contributes significantly to the maintenance of a stable intracellular pH, and its presence is required for proper protein trafficking among endosomes and other organelles (33, 36). Mutational analysis of Nhx1 revealed a direct connection between its function in the regulation of pH homeostasis and vesicle trafficking (178). Nhx1 homologs are conserved in both plant and mammalian cells (35), and its primary structure has a higher level of similarity with the NHE and NHA/SOS families of plasma membrane and intracellular Na^+/H^+ exchangers of mammalian and plant cells, respectively (see below). In fact, plasma membrane mammalian and plant antiporters are more closely related to *S. cerevisiae* Nhx1 than to Nha1 or Kha1 antiporters (218). Although the hydrophilic C-terminal part of Nhx1 is much shorter than that of Nha1 (175 versus 546 amino acid residues), it has important significance for proper functioning as well. Most probably it is N glycosylated (286), and it interacts with the Gyp6 GTPase-activating protein, which is proposed to be one of the coordinators of the vesicle trafficking between the Golgi apparatus and late endosomes (4), in accord with Nhx1's presumed role in intracellular vesicle trafficking. The *NHX1* gene is conserved in yeast species, but, as is the case for Kha1, only the Nhx1 homolog from *Debaryomyces hansenii* has been studied (see below).

The endosomal and Golgi apparatus antiporters Nhx1 and Kha1 are likely to have a similar function, but in different organelles. It is worth noting that although the deletion of both genes results in a decreased tolerance to hygromycin B, the overexpression of Kha1 does not suppress the hygromycin B sensitivity of *nhx1*Δ cells (153). On the other hand, deletion of the two genes affects the cell's ability to grow at limiting values of external pH: it leads to sensitivity to low (in the case of Nhx1) or high (for Kha1 absence) pH_{out}. Altogether, the available data suggest that Nhx1 and Kha1 antiporters, together with the vacuolar H^+-ATPase, are involved in intraorganellar pH and alkali metal cation balance, which in turn influences vesicle trafficking among the organelles of the secretory and endosomal pathways.

REGULATORY AND SIGNALING NETWORKS

Multiple signaling pathways are involved in the maintenance of cation homeostasis. A remarkable feature is that whereas in some cases (HOG [(*h*igh-*o*smolarity *g*lycerol] and calcineurin) a single pathway has, either simultaneously or sequentially, various relevant targets (e.g., HOG signaling on Nha1, Tok1, and *ENA1*), a given target can also integrate multiple signals that define its specific behavior (e.g., *ENA1* integrating HOG, calcineurin, and other signals). This network of functional interactions is depicted in Fig. 3.

Regulation of Pma1

Because of its role in modulating the osmotic and ionic balance as well as driving uptake of nutrients, the activity of the electrogenic Pma1 proton pump must be finely regulated. As described by Serrano in a seminal paper (254), glucose is an important signal for Pma1 regulation. This regulation occurs at two different levels: an initial rapid 5- to 10-fold increase in ATPase catalytic activity (254), affecting both K_m (which decreases) and V_{max} (which increases), is then followed by a

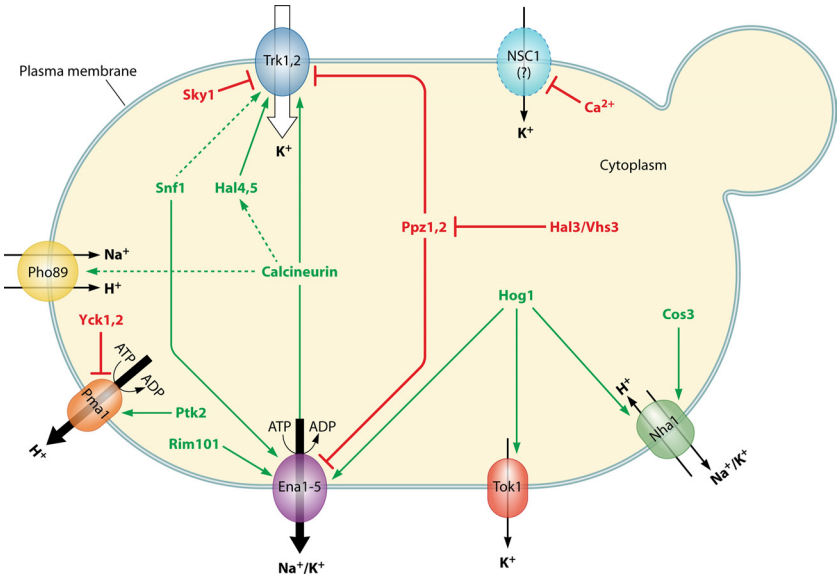

FIG. 3. The regulatory network for the plasma membrane alkali metal cation transporters. Discontinuous lines indicate interactions not fully documented.

second phase involving a slow and moderate increase in the expression of the gene in a process that involves the Rap1 and Gcr1 transcription factors (see reference 210 and references therein for early reports). Expression levels of *PMA1* (and also of *PMA2*) can be modulated in response to changing growth conditions, such as carbon sources, diauxic shift, or entry into stationary phase, and stress conditions (73, 230).

Early evidence suggested that short-term regulation of Pma1 activity by activators may involve protein phosphorylation at both Ser and Thr residues (46) targeting its inhibitory C-terminal tail, since mutations in this domain affecting Ser899 or Thr912 abolished the glucose-dependent K_m or V_{max} change, respectively (211). A role for Ptk2, a protein kinase belonging to the NPR/HAL5 family, has been proposed to activate Pma1 by phosphorylation of Ser899 (97). Ptk2 is localized to the plasma membrane, and *in vitro* phosphorylation of Pma1 Ser899 upon incubation with yeast membranes has been determined to be Ptk2 dependent. However, mass spectrometry analysis of Pma1 purified from plasma membranes of cells cultured in the presence or absence of glucose detected tandem phosphorylation of Ser911 and Thr912 in response to the sugar (138). In contrast, phosphorylation of Pma1 by the CK1 protein kinase, encoded by the *YCK1* and *YCK2* genes, at Ser507 results in decreased H^+ pumping activity (71). Therefore, it appears that site-specific phosphorylation of Pma1 may result in both down- and upregulation of its activity. Pma1 may also be posttranscriptionally regulated by additional inputs that are still poorly defined. For instance, Hrk1, a protein kinase related to the Npr1/Hal5 family, may also activate Pma1 (97). It has been recently proposed that the glucose-mediated increase in calcium concentrations may serve as a signal for Pma1 activation, in a Pkc1-dependent manner (205). An inhibitory interaction of Pma1 with acetylated tubulin has been reported. Addition of glucose to the cells disrupts the complex, leading to activation of the H^+-ATPase (43). In any case, the precise mechanisms controlling this essential plasma membrane transporter are still obscure.

Regulation of Trk1 and Trk2

Although *TRK1* seems to be less expressed at stationary phase (88), there is no experimental evidence for transcriptional regulation of *TRK1* under cation-related stresses. Several reports indicate that *TRK2* undergoes a rather complex transcriptional regulation; in this respect, it has been shown that *TRK2* expression is negatively regulated by the Rpd3/Sin3 histone deacetylase complex and by a URS1 element located within the promoter region (278, 279). Peña's group has also reported that Northern blot analysis showed no *TRK2* transcripts in a wild-type strain or in a *trk1*Δ mutant, strongly suggesting that in these strains *TRK2* was not expressed and that deletion of *SIN3* resulted in derepression of *TRK2* transcription (168). However, as for *TRK1* expression, *TRK2* expression is not altered by perturbations in cation homeostasis.

Despite the work of several laboratories, our understanding of the posttranscriptional regulation of Trk1 or Trk2 is still quite limited. Work by Serrano's laboratory identified the *HAL4*/*SAT4* and *HAL5* protein kinases as genes able to confer tolerance to toxic cations in a wild-type strain. The characterization of these genes revealed that they were partially redundant activators of potassium transport that acted by positively modulating Trk1 and Trk2 (180). This gave rise to the possibility that Trk systems may be directly controlled by phosphorylation events. Recent work has demonstrated that Hal4 and Hal5 may not be directly required for Trk1 activity, but they are required to stabilize the transporter at the plasma membrane under low-K^+ conditions, preventing its endocytosis and vacuolar degradation (206). The role of these kinases is not restricted to Trk1, and several nutrient transporters, such as Can1, Fur4, and Hxt1, were found to be destabilized in the *hal4*

hal5 mutant under low-K^+ conditions. The characterization of the molecular mechanism responsible for this mode of regulation will require the identification of the substrates of the Hal4 and Hal5 kinases.

The SR protein kinase Sky1 was isolated in a genetic screen for clones capable of restoring spermine sensitivity of spermine-tolerant mutants. Strains lacking *SKY1* were tolerant to toxic levels of spermine, while overexpression of *SKY1* in wild-type cells increased their sensitivity to this polyamine (69). In addition to spermine tolerance, *sky1*Δ cells were tolerant to lithium and sodium ions. The role of Sky1 in cation tolerance and potassium homeostasis is not clear. Some reports, on the basis of genetic analysis and Rb^+ uptake measurements, postulate that this kinase modulates the Trk1 and Trk2 transporters (79). In contrast, other authors support a Trk-independent role and favor the involvement of the Nha1 and Kha1 antiporters and of the Tok1 channel (70). Interestingly, it has been reported that the ability of overexpressed Sky1 to increase sensitivity to LiCl depends on the integrity of *PPZ1* but not on that of *ENA1* (69). Since Ppz1 has been reported to regulate *ENA1* expression and Trk activity (see below), this observation may support a regulatory role of Sky1 on the Trk uptake systems.

Further involvement of phosphorylation-dephosphorylation mechanisms in regulation of potassium uptake was derived from the identification of Hal3 as a modulator of both sodium and lithium efflux and potassium influx (74). Hal3 was identified as a negative regulator of Ppz1 (59), a Ser/Thr protein phosphatase known to influence salt tolerance (213) (see below). Yenush and coworkers demonstrated that the Hal3/Ppz1 pair is involved in the proper regulation of intracellular potassium concentrations and pH in a Trk-dependent manner (294). More recently, it has been shown that Trk1 is present in plasma membrane "rafts," where it physically interacts with Ppz1. Trk1 is phosphorylated *in vivo*, and its level of phosphorylation increases in *ppz1 ppz2* mutants. Protein stability and/or subcellular localization is not altered in the *ppz1 ppz2* mutant strain (293). The authors observed that both the interaction with and inhibition of Ppz1 by Hal3 are pH dependent, which led to the proposal that the Ppz1-Hal3 interaction could be a sensor of intracellular pH that modulates H^+ and K^+ homeostases through the regulation of Trk1 activity. It must be noted, however, that direct dephosphorylation of Trk1 by Ppz1 remains to be demonstrated. Similarly, an early report identified the calcium-activated protein phosphatase calcineurin as a necessary element for the transition of the Trk transporter system to the high-affinity potassium state induced by Na^+ stress, which helps to discriminate K^+ from Na^+ (167). The basis for this effect is still unknown, although calcineurin seems to be necessary for Trk1 activity even under basal conditions (C. Casado et al., submitted for publication).

Strains lacking *ARL1*, a gene encoding a conserved guanine nucleotide-binding protein, were found to be sensitive to several toxic cations, and these phenotypes were suppressed by K^+ (181). The *arl1* strain also displayed impaired Rb^+ uptake and exhibited normal K^+ and H^+ efflux. In spite of the recognized role of Arl1 in intracellular trafficking, loss of *ARL1* had no effect on the steady-state level or the localization of a tagged Trk1 version (181), as confirmed later by Perez-Valle and coworkers (206). Although from the reported data, it is likely that Arl1 controls Trk1 activity, its precise role is still poorly defined. The observation that localization of Trk1 seems unaffected upon *arl1* mutation makes it unlikely that Arl1 acts as a regulator of the Hal4/5 kinases, as was initially proposed (181).

The Snf1 stress-activated protein kinase has been proposed as an additional regulator of potassium influx via Trk1 and/or Trk2 (212). *snf1*Δ cells were found to be unable to fully activate K^+ import, and genetic analysis suggested that the weak kinase activity of the nonphosphorylated form of Snf1 activates high-affinity K^+ uptake in glucose-metabolizing yeast cells. However, this is most probably an indirect effect, perhaps mediated by the Sip4 transcriptional activator (212). A striking link between carbohydrate metabolism and potassium transport is highlighted by the observation that *TPS1*, a gene encoding trehalose-6-phosphate synthase and known to modulate glucose metabolism, activates Trk and reduces the sensitivity of yeast cells to many toxic cations independently of diverse Trk1 regulators (Hal4, Hal5, or calcineurin). Mutants defective in *PGM2* and *HXK2* exhibited phenotypes (reduced Trk1 activity and increased sensitivity to toxic cations) similar to those of *tps1* mutants. The authors observed a positive correlation between levels of glucose phosphates (Glc-1-P and Glc-6-P) caused by these mutations and Trk activity, leading to the proposal that these metabolites, directly or indirectly, activate potassium uptake via Trk systems (179). These findings would support the early observation that potassium uptake in yeast is activated by glucose and other fermentable sugars and that phosphorylation of the sugar was sufficient to trigger the activating pathway (5).

Regulation of Nha1

Expression of the *NHA1* gene seems to be rather stable and not affected by any form of stress. However, Nha1 is an important component of the early cellular response to sodium stress and is necessary for additional adaptive transcriptional responses. For instance, osmotic shock, as produced by high external NaCl concentrations, causes almost immediate dissociation of most proteins (transcription factors, TATA-binding protein [TBP], etc.) from chromatin, thus preventing the necessary adaptive transcriptional response (i.e., expression of *ENA1*). Upon osmotic shock, the Hog1 kinase is activated via the HOG signaling pathway and very rapidly phosphorylates Nha1 at its C-terminal cytosolic tail (presumably at T765 and T876). This phosphorylation event changes the activity of Nha1, by an unknown mechanism, and is proposed to be necessary for the reassociation of the transcriptional machinery (224). The same work also shows a participation (although less relevant) of the Tok1 channel in this early response. Therefore, Hog1 would behave as a key regulator for both pretranscriptional and transcriptional responses relevant for adaptation to cation stress. Later, it was shown that in response to osmotic shock mediated by sorbitol, the phosphorylation of Nha1 by Hog1 results in a transient inhibition of potassium efflux activity (123). This decrease in potassium loss via Nha1 helps cells to cope with the osmotic shock, and in the case of NaCl stress, fewer sodium cations enter the cells if less potassium is liberated, thus resulting in a detectable phenotype of increased sodium tolerance.

Overexpression of Sit4, a Ser/Thr phosphatase whose expression is induced by exposure to Li^+, Na^+, and K^+, causes increased K^+ and Rb^+ efflux upon salt stress (160). Further work identified *SAP155* as a high-copy-number suppressor of the hygromycin B-sensitive phenotype of the *arl1*Δ mutant (150). Sap155, together with Sap4, Sap185, and Sap190, have been previously identified as Sit4-interacting proteins (144). Overexpression of *SAP155* or deletion of *SAP185* decreased K^+ efflux, whereas overexpression of *SAP155* or deletion of *SAP155* had the opposite effect. In contrast, manipulation of Sap4 and Sap190 levels did not alter K^+ homeostasis (150). Therefore, *SAP155* and *SAP185* encode negative and positive modulators of K^+ efflux, respectively. These effects are mediated by Sit4 and require the presence of Nha1. Therefore, Sap155 and Sap185 could modulate the effect of Sit4 on Nha1 K^+ transport activity. Since Sit4 was initially identified as a regulator of the G_1-S transition (268), this functional link is consistent with the previous identification of Nha1 as a high-copy suppressor of the cell cycle blockage of a *sit4 hal3* mutant (262). However, no direct dephosphorylation of Nha1 by Sit4 has been demonstrated so far.

Though our knowledge on the posttranslational modification and activity regulation of Nha1p is still rather limited, it seems that regulatory events are concentrated in the C-terminal cytosolic tail of the protein (123, 261, 262) (Fig. 2). This notion would fit with the identification of Cos3 as a binding partner of Nha1p. Cos3 interacts with the first two conserved regions of the C terminus (C1 and C2), and its absence or overexpression results in decreased or increased salt tolerance, respectively, of *S. cerevisiae* cells, most probably as a consequence of Nha1 activity changes (174). The Nha1 C terminus also contains putative nuclear localization sequences (NLS) that can be functional, as the expression of the Nha1 C terminus as an independent protein targets it to the nucleus (123). Nevertheless, splitting off of the C terminus from the membrane segment and its subsequent trafficking to the nucleus has never been observed *in vivo*.

Regulation of Ena1

Whereas the expression of most structural components of the influx or efflux systems is essentially constitutive, expression of *ENA1* (but not that of other members of the cluster) is dramatically increased by exposure to high Na^+ or Li^+ concentrations or alkaline pH (86, 167, 213, 288). As first reported by Marquez and Serrano (155), exposure to salt or high pH causes activation of diverse signaling pathways that are integrated by the *ENA1* promoter. Remarkably, some of these pathways are also relevant for the control of other key components of cation homeostasis, as will be emphasized when appropriate.

One of the signaling pathways involved in the regulation of *ENA1* expression (calcium/calmodulin [see below]) was proposed in an early report to be also involved in posttranscriptional regulation of Ena1 activity (288), although no further progress has been made since then.

HOG osmoresponsive pathway. In addition to saline toxicity, exposure of yeast cells to relatively low extracellular concentrations of NaCl (~0.4 M) also provokes a situation of osmotic stress. Activation of the HOG (*h*igh-*o*smolarity *g*lycerol) pathway in response to high external osmolarity results in phosphorylation and activation of the Hog1 mitogen-activated protein (MAP) kinase (57). Hog1 promotes at least two important events for saline tolerance: control of Nha1 activity, as a fast response that decreases the intracellular amount of toxic cations (see above), and subsequent cooperation in the induction of *ENA1* expression. Lack of Hog1 largely attenuates the transcriptional response of *ENA1* to NaCl stress (155). Regulation of *ENA1* by Hog1 is accomplished through a downstream target of the kinase, the bZip transcription factor Sko1, which acts as a repressor by recruiting the general corepressor complex Ssn6-Tup1 to a cyclic AMP (cAMP) response element (CRE) site in the *ENA1* promoter (223). The Sko1-Ssn6-Tup1 repressor complex is disrupted upon phosphorylation by Hog1 of diverse residues at the Sko1 N-terminal region (222). In contrast, phosphorylation of Sko1 by cAMP-dependent protein kinase (PKA) increases its repressor activity (222). In addition to the Sko1-mediated mechanism, activated Hog1 recruits the specific Rpd3-Sin3 histone deacetylase complex to the promoter of *ENA1*, leading to histone deacetylation, entry of RNA polymerase II, and induction of the expression of the gene upon saline stress (60). In contrast to the relatively important role of Hog1-mediated signaling in the response to toxic concentrations of NaCl, exposure of yeast cells to relatively low concentrations of LiCl (50 to 100 mM) causes a growth defect that is not aggravated by deletion of *HOG1* (243) and triggers a significant increase in *ENA1* expression that is only slightly decreased by the deletion of *HOG1* (A. Ruiz, A. González and J. Ariño, unpublished results). Several lines of evidence (209, 256) suggest that the transcriptional response of *ENA1* to alkaline pH does not involve activation of the Hog1 kinase.

Calcineurin pathway. In *S. cerevisiae*, calcineurin is a heterodimer composed of one of two redundant catalytic subunit-encoding genes (*CNA1* and *CNA2*) plus a single regulatory subunit gene (*CNB1*). The relevance of calcineurin in ionic homeostasis is demonstrated by the sensitivity to Na^+, Li^+, or high pH of *cnb1* mutants or wild-type cells treated with the specific inhibitor FK506 (167, 183). Both the vacuole, through the Yvc1 channel (61), and the extracellular milieu, through the Mid1-Cch1 channel (163), have been proposed as the source for the burst of cytosolic calcium upon saline stress. Activation of calcineurin affects expression of a substantial number of genes (295). Gene activation occurs mainly through the dephosphorylation of the transcription factor Crz1/Tcn1/Hal8 (162, 166, 265), which then enters the nucleus and binds to specific sequences, known as CDRE, present in calcineurin-responsible promoters. *ENA1* has been known for a long time to be a transcriptional target of the calcineurin/Crz1 tandem, and the accumulation of sodium or lithium cations in calcineurin-deficient cells could be attributed, at least in part, to a decrease in the efflux capacity as a result of impaired induction of *ENA1* expression (106, 155, 167). Two Crz1-binding regions have been identified in the *ENA1* promoter at positions −813/−821 and −727/−719, with the downstream element being the most relevant for induction of the gene (165). The proposed antagonistic regulation of *ENA1* by the calcineurin and PKA pathways (106), supported by the observation that NaCl stress decreases intracellular levels of cAMP (155), can probably be explained by the fact that PKA can

phosphorylate Crz1, thus opposing the action of calcineurin (112). Approximately 40% of the total transcriptional response to high pH seems to be mediated by activation of calcineurin (209, 256), in this case as a result of almost immediate entry of external calcium into the cytosol through the Cch1-Mid1 calcium channel (280).

Rim101 pathway. Yeast cells lacking Rim101, a C_2H_2 zinc finger transcription factor that positively regulates the expression of genes involved in meiosis and sporulation (266), are sensitive to both high salt concentrations and high pH (135). *rim101* cells display decreased expression of *ENA1* upon alkaline (135, 256) or NaCl and LiCl (M. Platara and J. Ariño, unpublished results) stresses. Remarkably, the transcriptional role of Rim101 appears to be mediated by repression of other regulatory genes (134), thus acting itself as a repressor. In the case of *ENA1*, Rim101 would repress the expression of Nrg1, a repressor protein that negatively controls the expression of several glucose-repressed genes and invasive growth. It is worth noting that Nrg1 is also regulated by the Snf1 protein kinase (282), thus integrating two salt-activated signaling pathways (see below). Accordingly, the *nrg1* mutation confers tolerance to Na^+ and Li^+, suppresses the *rim101* growth defect at high pH (134), and leads to higher-than-normal *ENA1* levels even in the absence of stress (134, 281). Two putative binding sites for Nrg1 in the *ENA1* promoter were suggested (CCCTC and CCCCT), located at positions −725/−729 and −651/−647, respectively (134). While the upstream element is probably functional, the downstream one appears to be less important or ineffective (256). A third binding site at positions −561/−573 (AGACCCT) has been identified more recently as relevant for alkaline stress response (209). Taken together, these data suggest that Rim101 may control *ENA1* expression in response to alkaline stress through the Nrg1 repressor protein. In fact, the role of this pathway in the regulation of *ENA1* expression may be more relevant under alkaline stress than under saline stress, since lack of Rim101 significantly reduces *ENA1* expression at high pH but has little effect on the *ENA1* response to 0.4 M NaCl (209) or to 0.9 M NaCl or 0.2 M LiCl (M. Platara, A. Ruiz, and J. Ariño, unpublished results).

Regulation by nutrients. *ENA1* expression is higher on derepressing carbon sources, such as galactose or raffinose, than on glucose (2, 233). On the other hand, a strain with a mutation in the *SNF1* gene, encoding a protein kinase central to adaptation to glucose depletion, displays enhanced sensitivity to sodium or lithium, probably as a result of impaired induction of *ENA1* by high salt concentrations. The Snf1-mediated effect on *ENA1* expression was found to be independent of the HOG and calcineurin pathways (2). Glucose-mediated *ENA1* repression is based in the $URS_{MIG\text{-}ENA1}$ element at positions −533/−544, which serves as a recognition site for the Mig1/2-Ssn6-Tup1 complex (223). Snf1 activation by low glucose concentrations would then prevent Mig1/2-Ssn6-Tup1-mediated repression of *ENA1*. However, whereas Snf1 is phosphorylated and activated by saline stress (109, 164), this does not result in phosphorylation of Mig1 (164, 291), and Snf1 remains excluded from the nucleus (109). Instead, it has been proposed that Snf1 may mediate upregulation of *ENA1* through the Nrg1 repressor (291). A functional link between Gis4 (whose absence decreases saline tolerance, sodium and lithium efflux, and *ENA1* expression) and Snf1 has been proposed (292),

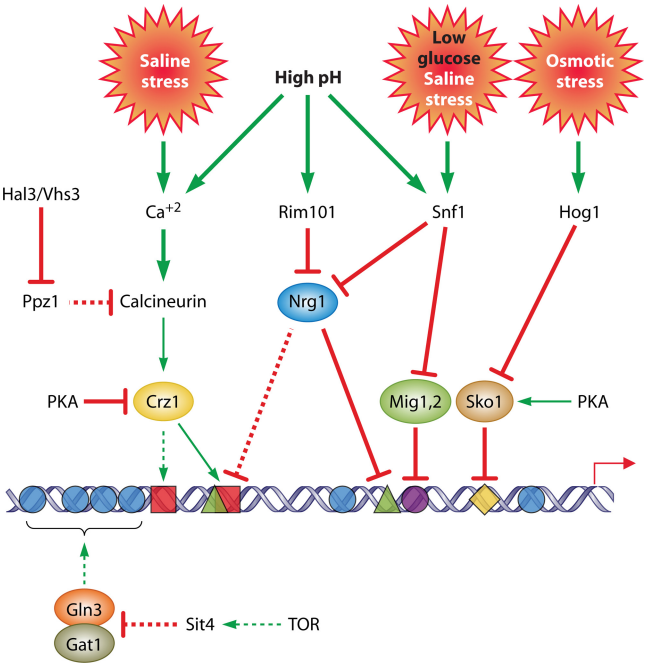

FIG. 4. Regulation of *ENA1* expression by saline and alkaline stress. Symbols denote known or predicted regulatory sites (○, GATA; ■, CDRE; △, NRG; ●, MIG; ◆, CRE). The promoter is not drawn to scale, but the relative positions of the regulatory sites are maintained. Discontinuous lines denote regulatory interactions still to be fully documented. See the text for details. (Adapted from reference 247a.)

although its nature is still unclear. Induction of *ENA1* by alkaline pH is markedly reduced in cells lacking *SNF1* (2, 209). In this case, Snf1 becomes enriched in the nucleus, similarly to what happens during carbon stress (109). Recent work (209) has demonstrated that activation of Snf1 by high pH increases expression of *ENA1* by releasing the repression mediated through the MIG element and by deactivating the Nrg1 repressor. Thus, the Nrg1 repressor would be under the control of two regulators, Snf1 and Rim101 (see above) (Fig. 4).

The TOR (*t*arget *o*f *r*apamycin) pathway plays an important role in regulating nutrient-responsive transcription (52). Tor kinases are inhibited by poor nitrogen sources or rapamycin treatment. Crespo and coworkers (52) demonstrated that treatment of yeast cells with rapamycin transiently increased *ENA1* expression and that this response required the presence of Gln3 and Gat1 (two Tor-regulated GATA factors) for the full effect. Similarly, induction triggered by exposure to 0.4 M NaCl was markedly reduced in these mutants. Moreover, *gln3* or *gat1* cells are sensitive to sodium, and the absence of the transcription factors eliminates the salt tolerance of *ure2* cells (51). These observations were in agreement with the early finding that mutations in *URE2* suppressed the sensitivity of calcineurin mutants to Na^+ or Li^+ cations and that this effect required the presence of the *ENA1* gene (289). Although at least six putative GATA motifs (GATAA and GATAAG) can be detected in the *ENA1* promoter (mostly in its upstream region), their functional role has not been tested so far. In fact, the regulation of *ENA1* by the TOR pathway is still controversial. For instance, whereas the Sit4 phosphatase (see above) plays a prominent role in Gln3 localization upon Tor signaling,

it has been reported that neither deletion nor overexpression of *SIT4* (160) affects basal or salt-induced *ENA1* mRNA levels (although these results were obtained using cells grown on galactose, which strongly affects the *ENA1* basal expression level [2, 233]). In addition, the observation that stressing cells with 1 M NaCl under conditions in which Gln3 is normally nuclear (i.e., nitrogen starvation) results in rapid relocation of Gln3 to the cytoplasm (270) is difficult to reconcile with a direct role of Gln3 in *ENA1* expression.

Hal3/Ppz system. Ppz1 and Ppz2 are atypical Ser/Thr protein phosphatases related to the type 1 enzyme encoded in yeast by *GLC7* (48, 139, 214, 215; reviewed in reference 9. Mutants lacking Ppz1 display a marked tolerance to Na^+ and Li^+ (213). *ppz2* mutants display no phenotype, but deletion of *PPZ2* further enhances *ppz1* phenotypes. Single *ppz1* and double *ppz1 ppz2* mutants show increased *ENA1* mRNA levels even in the absence of stress and an enhanced response in salt-stressed cells. Therefore, Ppz1,2 control *ENA1* expression. *HAL3*/*SIS2* was identified almost simultaneously as a gene conferring halotolerance when overexpressed (74) and as high-copy-number suppressor of the growth defect of a Sit4 phosphatase mutant (64). Hal3 is necessary for full induction of *ENA1* upon saline stress. The role of Hal3 in salt tolerance and the cell cycle was clarified when de Nadal and coworkers discovered that Hal3 was a negative regulatory subunit of Ppz1 that acts by binding to its C-terminal catalytic moiety (47, 59). Hal3 is also able to bind and inhibit Ppz2 (247). Vhs3, a second regulatory subunit of Ppz1, was identified by means of a multicopy suppressor screen of the *hal3 sit4* synthetically lethal phenotype (245). Vhs3 also binds the C-terminal half of Ppz1 and inhibits its phosphatase activity *in vitro* with a potency similar to that of Hal3. However, Vhs3 has a less important role *in vivo* than Hal3, probably due to lower expression levels (245).

The precise mechanism for regulation of *ENA1* expression by the Ppz phosphatases is not completely solved. The higher-than-normal basal levels of *ENA1* mRNA in the *ppz1* single mutant seem to be fully caused by constitutive activation of the calcineurin/Crz1 pathway (247). In *ppz1 ppz2* mutants, however, additional regulatory mechanisms are activated, leading to a higher expression of *ENA1* even in the absence of stress. This may be explained on the basis of the negative control exerted by Ppz1,2 in regulating the Trk transporters, which has a substantial effect on saline tolerance and intracellular pH (294). It was shown that deregulated activity of Trk transporters in the *ppz1 ppz2* mutant causes intracellular alkalinization, which would further activate the *ENA1* promoter in a calcineurin-independent fashion (247).

A striking finding was the observation that the *hal3* and *vhs3* mutations resulted in a synthetically lethal phenotype that was independent of their role as Ppz1,2 inhibitors (245), suggesting an additional essential function for these proteins. This paradox has been solved very recently with the discovery that Hal3 and/or Vhs3 cooperate with the still-uncharacterized protein Ykl088w to constitute a heterotrimer responsible for the essential phosphopantothenoylcysteine decarboxylase activity in *S. cerevisiae* (244), a key step in coenzyme A (CoA) biosynthesis. Therefore, Hal3 and Vhs3 are moonlighting proteins. It is tempting to speculate on the evolutionary advantage gained by functionally linking the inhibition of an energy-consuming, stress-related ATPase such as the *ENA1* product and the generation of CoA.

Other possible regulatory inputs. Mutation or overexpression of a number of other genes has been shown to affect salt tolerance and, in some cases, *ENA1* expression. For instance, a link between *ENA1* expression and inositol metabolism has been established through the observation that *isc1* mutants, lacking inositol phosphosphingolipid phospholipase C activity, are sensitive to sodium or lithium and display decreased *ENA1* expression (27). Furthermore, overexpression of the genes encoding the inositol monophosphatases Imp1 and Imp2 conferred tolerance to both cations in an *ENA1*-dependent fashion (142), although without effect on the expression levels of the ATPase gene.

Mutation of genes encoding the regulatory subunits (*CKB1* or *CKB2*) of the conserved Ser/Thr protein kinase CK2 yields cells sensitive to high sodium or lithium concentrations (28, 58, 271). Tenney and Glover (271) proposed that lack of Ckb1 or Ckb2 decreased *ENA1* expression in cells stressed with 0.4 M NaCl. In contrast, de Nadal and coworkers (58) did not observe changes in *ENA1* promoter activity in *ckb1* cells challenged with higher concentrations of NaCl (0.75 M) for 60 min. The relevance of phosphorylation events in the regulation of *ENA1* expression is further stressed by the observation that *ptc1* cells (lacking one of the seven type 2C Ser/Thr protein phosphatases in *S. cerevisiae*) were sensitive to Li^+ (but not to Na^+) and that this phenotype was not additive to that of the *ena1-ena4* deletion (243). Both basal and lithium-induced expression levels of *ENA1* were decreased in the *ptc1* strain, and this effect was not attributable to the role of Ptc1 in Hog1 regulation (249). Several lines of evidence suggest that Ptc1 may be regulating the Hal3/Ppz system (and hence *ENA1* expression [see above]) through a still-unknown mechanism.

Other gene products, such as Psr1 and Psr2 (two similar membrane proteins with phosphatase activity [263]), Hal9 (a putative transcription factor containing a zinc finger that, when overexpressed, was able to confer increased tolerance to sodium or lithium ions [166]), or the nuclear phosphoprotein Lic4/Atc1 (related to cation homeostasis several years ago [105]) have been proposed to modulate saline tolerance by means of the control of *ENA1* expression, although no precise mechanism is known.

The *HAL1* gene plays an important role in maintaining a normal intracellular K^+/Na^+ ratio and confers salt tolerance when overexpressed in yeast cells. Mutants lacking *HAL1* are salt sensitive and display decreased *ENA1* expression, whereas overexpression of *HAL1* confers salt tolerance and increases expression of the ATPase gene (89, 233). Overexpression of *HAL1* also increased intracellular K^+ in a *TRK1*- and *TOK1*-independent fashion. This is probably caused by a reduced K^+ loss from the cells upon salt stress. The expression of *HAL1* itself is upregulated by osmotic stress via the Sko1 repressor and the Gcn4 activator, both competing for the CRE-like sequence present in the *HAL1* promoter (203). A dose-dependent effect of the *STD1* and *MTH1* genes on both *HAL1* and *ENA1* expression levels has been described (83).

Very recently, a role for Ref2 (a regulatory subunit of the Glc7 type 1 protein phosphatase) in cation homeostasis has been proposed (75). The *ref2* deletion mutant displays a marked decrease in lithium efflux, which can be explained by

the inability of these cells to fully induce the *ENA1* gene. The effect of lack of Ref2 was shown to be additive to that of blockage of the calcineurin pathway and might disrupt multiple mechanisms controlling *ENA1* expression. Remarkably, this role seems to be unrelated to the previously identified function of Ref2 as a component of the APT complex (75).

ALKALI METAL CATION TRANSPORTERS IN OTHER YEASTS: SIMILARITIES AND DIFFERENCES

Genes encoding putative alkali metal cation transporters (orthologous to those of *S. cerevisiae*) have been identified in the genomes of other yeast species. However, characterization of corresponding proteins has revealed extremely interesting exceptions and peculiarities that we summarize here, focusing mainly on some of the best known nonconventional yeasts.

K^+ Uptake Systems and Na^+ Efflux ATPases

Although the *S. pombe* genome contains two *TRK* genes (*Sptrk1*$^+$ and *Sptrk2*$^+$), similar to the case for *S. cerevisiae*, the situation concerning potassium uptake is far from identical in the two yeasts. *Sptrk1*$^+$ and *Sptrk2*$^+$ encode proteins of 833 and 880 amino acids, respectively, that are 34% identical to each other. Unlike in budding yeast, they seem to be equally important for the cell when it is growing under standard conditions. The presence of either of these genes is sufficient to allow growth at low K^+ and to transport the cation similarly to the manner in the wild-type strain. Northern analysis revealed that the two genes are expressed under different external conditions. Consequently, removal of both transporter genes is necessary to provoke increased potassium requirements for growth and impaired transport characteristics (39, 41). Only in the presence of some specific stress conditions, such as very low pH or salt concentration, does *Sp*Trk1 seem to contribute more efficiently than *Sp*Trk2 to the fitness of the fission yeast (40).

In the case of *D. hansenii*, most of the potassium and sodium transporters identified have been partially characterized by heterologous expression in *S. cerevisiae* mutants lacking the equivalent transporters, since molecular tools to manipulate and delete genes are not extensively developed for *Debaryomyces*. However, an interesting approach was followed by Prista and coworkers (221), who showed that transformation with genes from *D. hansenii* can improve the salt tolerance of a wild-type *S. cerevisiae* strain. Concerning K^+ transporters, the most important novelty compared to the situation in *S. cerevisiae* or *S. pombe* is the existence of a single *TRK* gene and an additional and completely different transporter encoded by *D. hansenii HAK1* (*DhHAK1*) (220).

Hak1 (*h*igh-*a*ffinity *K*$^+$ transporter) was first identified in *D. occidentalis*, and it was shown to have significant amino acid homology with the Kup gene product of *Escherichia coli* (12, 253). Subsequently, *HAK1*-orthologous genes have been found in many other organisms, such as the nonconventional yeasts *D. hansenii* (220) and *Hansenula polymorpha* (J. M. Siverio, personal communication), the micelial fungus *N. crassa* (104), the moss *Physcomitrella patens* (84), and higher plants (259, 276). When expressed heterologously in an *S. cerevisiae* mutant lacking its own K^+ transporters, *Dh*Hak1 improved growth under low-K^+ conditions, K^+ accumulation, and Rb^+ transport (220). Research with *N. crassa* and *D. occidentalis* has shown that Hak transporters work as K^+-H^+ symporters with a high concentrative capacity. When *D. occidentalis HAK1* was expressed in an *S. cerevisiae trk1 trk2* mutant, it was observed that it could deplete K^+ from the external medium to almost the same extent as seen with the wild-type strain of *D. occidentalis* (12).

For other yeasts, such as *C. albicans* or *Z. rouxii*, potassium transporters have not been characterized in detail yet. Database searches indicate that while *HAK*- and *TRK*-orthologous genes are present in *C. albicans*, *Z. rouxii* possesses only the Trk-type K^+ transporter. A recent paper reports that the primary function of Trk proteins, transport of K^+ ions, is quantitatively conserved between *C. albicans* and *S. cerevisiae*. However, the secondary function, chloride efflux channeling, is present but poorly conserved between the two species, being highly variant with respect to activation velocity, amplitude, flickering (channel-like) behavior, pH dependence, and inhibitor sensitivity (170).

D. hansenii is also endowed with Ena-type ATPases. While a variable number of *ENA* copies are present in *S. cerevisiae* genomes, only two *ENA* genes have been identified so far in *D. hansenii* (*DhENA1* and *DhENA2*). Heterologous expression of *DhENA* genes in an *S. cerevisiae* mutant indicated their function in sodium detoxification and extrusion (6). In addition, *ENA* orthologs are present in the genomes of *C. albicans*, *Candida dubliniensis* (66), and *Z. rouxii*.

With regard to cation efflux ATPases, additional differences between *S. cerevisiae* and *S. pombe* are also worth mentioning. Sequence comparisons indicate that *S. pombe* Cta3 belongs to the group of the ENA ATPases. However, when the function was studied, it was observed that Cta3 does not pump sodium. The *cta3*$^+$ gene product was initially proposed to work as an ATP-dependent calcium pump and not as an Na^+-ATPase (100). This notion is supported by the fact that Cta3 conserves several amino acids typical of Ca^{2+}-ATPases and is consistent with the functional absence of an Na^+-ATPase in *S. pombe*, where Na^+ tolerance and Na^+ efflux are dominated by the Na^+/H^+ antiporter Sod2 (99, 111). However, with the increasing number of P-ATPases isolated and studied, the phylogenetic isolation of Cta3 from other Ca^{2+}-ATPases became more evident, and doubts about its real function arose. Consistent with its capacity to increase the K^+ tolerance of the *S. cerevisiae ena1-4 nha1* mutant, Cta3 also induced a rapid K^+ efflux. Taken together, these results demonstrate that Cta3 is a K^+-ATPase (20).

Finally it is worth pointing out that a novel type of P-type ATPase, the ACU (*a*lkali *c*ation *u*ptake)-ATPases, mediating high-affinity K^+ or Na^+ uptake, has been identified in nonconventional yeasts such as *U. maydis* or *P. sorbitophila* (21).

Na^+/H^+ Antiporters

So far, the existence of plasma membrane Na^+/H^+ antiporters has been confirmed by functional characterization in 11 yeast species. According to the number of *ScNHA1* homologs in the plasma membrane and to the specialization of their function, these yeast species can be divided in two subgroups; one contains three members in which the original *NHA1* gene

has probably been duplicated and gained new functions, whereas in the larger subgroup, only one antiporter with broad substrate specificity and multiple physiological functions exists.

The identification of the very first gene encoding a yeast Na^+/H^+ antiporter, *sod2*, was based on the selection for increased tolerance to Li^+ in salt-sensitive *S. pombe* cells (111). The second member of the yeast Na^+/H^+ antiporter family characterized was Sod2 from the osmotolerant yeast *Z. rouxii* (284). In the *Z. rouxii* strain used (ATCC 42981), another highly similar gene, designated *SOD22*, was identified (110). Both *Z. rouxii* Sod2 (*Zr*Sod2) and *Zr*Sod22 were shown to enhance the NaCl tolerance of a salt-sensitive *S. cerevisiae* strain, but only *ZrSOD2* was confirmed to be transcribed in *Z. rouxii* cells (110, 284). In another, also highly salt-tolerant, *Z. rouxii* strain (CBS732), only one *SOD2*-like gene was identified. As it was highly similar to *ZrSOD2*, but contained an additional stretch of sequence unique for *ZrSOD22*, it was named *ZrSOD2-22* (119) Heterologous expression in an *S. cerevisiae* strain lacking its own alkali metal cation exporters revealed that all three *Z. rouxii* antiporters transport only sodium and lithium (110, 118, 119), similar to what was observed for the *S. pombe* Sod2 antiporter. This Li^+ and Na^+ specificity was in contrast to the case for the *S. cerevisiae* Nha1, which also transports potassium and its analog rubidium (see above), and thus presents the question of how these cells eliminate surplus potassium. The question was answered later by the characterization of two members of the Na^+/H^+ antiporter family in *Yarrowia lipolytica* (199). The *Y. lipolytica* genome encodes two putative plasma membrane Na^+/H^+ antiporters, and their heterologous expression in *S. cerevisiae* cells revealed that while the *Y. lipolytica* Nha1 (*Yl*Nha1) protein mainly increased cell tolerance to potassium, *Yl*Nha2 displayed a remarkable transport capacity for sodium. Thus, *Y. lipolytica* was the first example of a yeast species with two plasma membrane alkali metal cation/H^+ antiporters differing in their function in cell physiology, i.e., cation detoxification versus maintenance of stable intracellular potassium content. Findings with *Y. lipolytica* led to the reexamination of the *S. pombe* and *Z. rouxii* genomes and to the identification of second members of the NHA family of yeast antiporters in both species (201, 217). Their characterization revealed that *Sp*Sod22 and *Zr*Nha1 contribute significantly to potassium homeostasis. In the case of *Z. rouxii*, the difference between both Sod2-22 and Nha1 transporters in substrate preferences and physiological functions has been demonstrated directly by the characterization of *Z. rouxii* cells lacking or overexpressing the two antiporters (201, 217). In general, the sodium-specific antiporters in the three yeast species are shorter in the C-terminal hydrophilic parts than their potassium-transporting paralogs, and *Zr*Sod2-22 and *Yl*Nha2 proteins have extremely high capacities to export sodium cations (119, 200), much higher than those of *Sc*Nha1 or other yeast antiporters with broad substrate specificity.

A single plasma membrane antiporter with broad substrate specificity has been characterized in eight yeast species. Besides *S. cerevisiae*, it was identified in the osmotolerant *D. hansenii* (273), in *P. sorbitophila* (14), and in five members of the *Candida* family, i.e., *C. albicans*, *C. dubliniensis*, *C. parapsilosis*, *C. glabrata*, and *C. tropicalis* (113, 117, 131). All of these transporters have been characterized by heterologous expression in *S. cerevisiae* cells lacking the *NHA1* and *ENA1-4* genes, i.e., in cells unable to export alkali metal cations efficiently. Phenotypes of increased salt tolerance as well as direct measurements of cation efflux showed that the individual transporters, though having the same wide substrate specificity, differ in their capacity to transport cations; e.g., *C. parapsilosis* and *C. albicans* antiporters are the most efficient, whereas those of *C. dubliniensis* and *C. glabrata* are less effective.

ScKHA1 and *ScNHX1* homologs have been identified in the genomes of most yeast species (218), but only the Kha1 and Nhx1 antiporters of the osmotolerant yeast *D. hansenii* have been functionally characterized. Expression of *DhKHA1* in *S. cerevisiae* complemented *kha1*Δ phenotypes and, surprisingly, improved tolerance to Na^+ (87). Similarly, heterologous expression of the *DhNHX1* gene partially suppressed the alkali metal cation sensitivity of an *S. cerevisiae ena1-4 nha1 nhx1* mutant, and it led to the enhanced accumulation of Na^+ and K^+ cations in the vacuole (176).

CONSERVATION OF "YEAST" ALKALI METAL CATION TRANSPORTERS IN PLANTS

Most yeast alkali metal cation transporters have been conserved throughout evolution, and proteins similar to those reviewed in this work usually appear in higher plants. The only relevant exception is the *ENA* genes, which encode sodium pumps extremely important for salt tolerance in *S. cerevisiae* and other fungi but definitely absent in higher plants (85). Additionally, plant cells have developed other transporters that frequently carry out redundant functions, which makes the situation quite complex, especially if we consider that our knowledge on cation homeostasis in higher plants is not as detailed as it is for yeast (see references 235, 259, 276, and 277 for reviews). Below we briefly summarize the current knowledge concerning plasma membrane and intracellular alkali metal cation transporters in plants in comparison with what is known for *S. cerevisiae*.

K^+ Transporters and Na^+ Efflux ATPases

Regarding plasma membrane potassium transporters, the two different families identified in yeasts (Trk and Hak) are also present in plants (277). The HKT/TRK family, equivalent to the yeast Trk, contains a variety of members that can work in different ways, and, for example, it has been proposed that Hkt1 mediates a sodium uniport in roots (101). Also, many different genes belong to the *KUP*/*HAK* family, equivalent to the yeast Hak transporters. Different *HAK* genes may have different functions, depending, for example, on their location in the plant. The precise role of these genes in potassium transport is being determined, and it has been proposed that *HAK5* in *Arabidopsis thaliana* (93) or in *Solanum lycopersicum* (191) contributes to the high-affinity K^+ uptake by plant roots and work in a manner similar to that of yeast Hak proteins.

Concerning K^+ efflux, we noted above that the only outward-rectifying K^+ channel in the plasma membrane of *S. cerevisiae* is encoded by *TOK1*. From a sequence homology point of view, the TPK (KCO) plant K^+ channels belong to the same family. KCO channels display a hydrophobic core composed of either four TMS and two P domains (KCO-2P family) or two TMS domains and one P domain (KCO-1P family). In

Arabidopsis, the KCO-2P family has five members and the KCO-1P family has a single member. Their specific function is still unknown, although it has been recently shown that the *A. thaliana* Tpk1 (*At*Tpk1) mediates K^+-selective currents between cytoplasmic and vacuolar compartments and plays a role in K^+ homeostasis (94). Plant K^+ channels of the so-called Shaker family are good examples of additional channels that are not represented in yeast. Both inward K^+ channels (AKT1 and KAT1) and outward rectifiers (SKOR/GORK) have been described in plants (259, 277).

The Ena transporters, the main determinants of halotolerance in *S. cerevisiae* and probably present in most fungi (15, 18, 19), do not exist in flowering plants (85). Remarkably, two Na^+-ATPases in the moss *Physcomitrella patens* have been characterized (23) and at least one of them (*Pp*Ena1), appears to be functional as a sodium pump both in *S. cerevisiae* (23) and *in planta* (145). This observation has led to the proposal that Ena-like sodium ATPases were present in early land plants but that they have been lost during the evolution of bryophytes to flowering plants (23).

Alkali Metal Cation/Proton Antiporters

Our knowledge on the number and physiological functions of Na^+/H^+ antiporters in higher eukaryotes has dramatically increased during recent years, often boosted by the efficient expression of these proteins in *S. cerevisiae* mutant strains lacking their own transport systems. This phenotype complementation approach has been successfully used to characterize the localization and function of mammalian and (mainly) plant plasma membrane antiporters (76, 226) as well as the intracellular homologs of *Sc*Nhx1 (81, 90, 121, 225) and *Sc*Kha1 (152). However, much more research is needed to fully understand the role of alkali metal cation/H^+ antiporters in multicellular organisms because of the gene redundancy (e.g., 9 NHE isoforms in mammalian cells [202] or 40 genes encoding putative Na^+/H^+ antiporters in *A. thaliana* [161]) and the different expression levels and various functions according to tissue, organ, and phase of development (see, e.g., references 202 and 302).

Mineral nutrition and ion homeostasis are crucial to plant growth and development (146, 255). Moreover, maintenance of a high intracellular K^+/Na^+ ratio has been shown to be very important for plant growth in soils with increased salinity (147, 151, 202). Most of the plasma membrane and vacuolar antiporters characterized so far in different plants (263) belong to the *Sc*Nhx1 subfamily (151), although *Sc*Kha1 homologs (forming the CHX subfamily) have also been identified in plant chloroplast and endosomal membranes (45). Several members of the Nhx1 family have been shown to be crucial for plant salt tolerance. Sos1, the plasma membrane-localized plant homolog of *Sc*Nhx1, is a very important sodium extrusion system, and its overexpression can improve the plant's capacity to grow upon salt stress (8, 260). Sos1 is tightly regulated by the Sos2 protein kinase and the Sos3 Ca^{2+} sensor (149, 226). The general hypothesis is that the Ca^{2+} sensor, Sos3, interacts with and activates the Sos2 kinase. This Sos3-Sos2 complex activates the Na^+/H^+ antiporter activity of Sos1 and thereby reestablishes cellular ion homeostasis. However, the very interesting question regarding the molecular mechanism of how this phosphorylation event regulates transporter activity remains unsolved. Similarly, overexpression of the *AtNHX1* gene, encoding the vacuolar antiporter (8, 260), improves plant salt tolerance. Besides its role in Na^+ detoxification, Nhx1 from *Arabidopsis* vacuoles has been shown to transport K^+ with affinity similar to that of Na^+, and thus its role in K^+ homeostasis and consequently osmoregulation and internal pH regulation has been suggested (274).

PERSPECTIVES AND FUTURE CHALLENGES

Although our knowledge of alkali metal cation transport and homeostasis in yeast organelles has increased significantly during the last few years and certainly the major transport systems have been identified since the discovery of the Trk1 and Ena systems in the early 1990s, many questions still remain open. For instance, at the plasma membrane level, the residual low-affinity potassium transport system and the molecular nature of the NSC1 channel await identification. Similarly, the nature of the protein(s) involved, together with Mdm38, in the exchange of protons for potassium cations across the mitochondrial inner membrane remains to be solved, and our knowledge about how the endoplasmic reticulum and peroxisomes regulate their potassium content and pH homeostasis is still very poor. Therefore, although a detailed *in silico* analysis of the yeast "transportome" has identified and classified virtually all putative transporters of alkali metal cations (56, 190), the example of Vnx1 (classified by sequence comparison as a calcium/proton antiporter) and the presence of several ORFs with unknown function and weak homology to mammalian transporters whose substrates are probably alkali metal cations, along with the open questions mentioned above, suggest the existence of so-far-unidentified transporters for potassium and/or sodium in *S. cerevisiae* cells.

Our knowledge regarding the regulation of alkali metal cation transport is still quite fragmentary. Whereas we have a relatively good idea about how *ENA1* transcriptional regulation is controlled by saline and alkaline stress, no real advance has been made in understanding its possible posttranslational control. Similarly, the picture of the control of high-affinity K^+ uptake by posttranslational regulation of Trk1 and/or Trk2, which seems to involve diverse protein kinases and phosphatases, lacks molecular detail. The regulation of organelle transporters is currently a fully open field that deserves further investigation. However, it is of utmost importance to obtain a complete and integrated picture of how cells regulate alkali metal cation transport and content and how these parameters influence yeast biology. Therefore, in addition to advances in specific fields, a systems biology approach seems most desirable. Of course, this will probably require the development or improvement of current technologies, such as those that will permit the quantitative determination of actual membrane potential in *S. cerevisiae* or fast time-resolved measurements of cation fluxes across plasma and organellar membranes.

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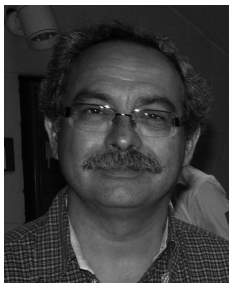
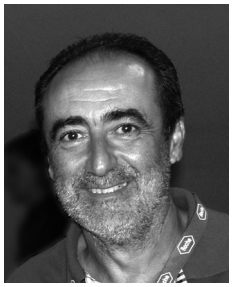


Joaquín Ariño was born in Barcelona (Spain) in 1957. He was awarded a Ph.D. degree in pharmacy at the Department of Biochemistry (School of Pharmacy, University of Barcelona) in 1986 and was a Fulbright postdoctoral fellow at the Department of Biochemistry, University of Massachusetts (1986 to 1987). In the early 1990s he was involved in the European *S. cerevisiae* sequencing project, and in 1993 became a professor of biochemistry at the Universitat Autònoma de Barcelona. His laboratory is interested in the control of cellular processes in yeast by phosphorylation-dephosphorylation mechanisms and is focused primarily on Ser/Thr protein phosphatases and stress responses (saline, alkaline pH, nutrients, etc.). He has published around 100 research papers or reviews in the fields of biochemistry, genetics, and microbiology. Together with the other authors, he participates in an international consortium (Translucent) that investigates cation homeostasis in yeast from a systems biology approach.



José Ramos was born in Córdoba (Spain) in 1957. He was awarded a Ph.D. degree in microbiology at the Department of Microbiology (University of Córdoba) in 1984. He was a visiting professor at the Department of Biochemistry, State University of New York (1987 to 1988) and at the Molecular and Cellular Biology Department, Catholic University of Leuven (1992). He had been a full professor in microbiology since 2006 (University of Córdoba). His research is focused on the study of ion homeostasis and abiotic stress responses in cell-walled eukaryotic organisms. He has published around 100 research papers in the fields of microbiology and biochemistry. They deal mostly with model yeasts such as *Saccharomyces cerevisiae* or nonconventional yeasts such as *Debaryomyces hansenii*. Together with the other authors, he participates in an international consortium (Translucent) that investigates cation homeostasis in yeast from a systems biology approach.

Hana Sychrová was born in Prague (Czechoslovakia) in 1959. She was awarded a Ph.D. degree in biochemistry at the Academy of Sciences in Prague in 1988 and a D.Sc. degree in molecular biology at the same institution in 2001. She spent almost 3 years as a postdoctoral fellow at the Luis Pasteur University in Strasbourg, France, and she became the head of the Department of Membrane Transport, Institute of Physiology AS CR, Prague, in 2006. Her laboratory is interested in characterization of membrane transport systems, in the use of yeast as a model organism to study transport processes in animal and plant cells, and in the study of specific properties of the nonconventional yeasts. She has published around 70 research papers in the fields of biochemistry, genetics, and microbiology. Together with the other authors, she participates in an international consortium (Translucent) that investigates cation homeostasis in yeast from a systems biology approach.