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Mini review

The NPR/Hal family of protein kinases in yeasts: biological role, phylogeny and regulation under environmental challenges

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ABSTRACT

Protein phosphorylation is the most common and versatile post-translational modification occurring in eukaryotes. In yeast, protein phosphorylation is fundamental for maintaining cell growth and adapting to sudden changes in environmental conditions by regulating cellular processes and activating signal transduction pathways. Protein kinases catalyze the reversible addition of phosphate groups to target proteins, thereby regulating their activity. In *Saccharomyces cerevisiae*, kinases are classified into six major groups based on structural and functional similarities. The NPR/Hal family of kinases comprises nine fungal-specific kinases that, due to lack of similarity with the remaining kinases, were classified to the "Other" group. These kinases are primarily implicated in regulating fundamental cellular processes such as maintaining ion homeostasis and controlling nutrient transporters' concentration at the plasma membrane. Despite their biological relevance, these kinases remain poorly characterized and explored. This review provides an overview of the information available regarding each of the kinases from the NPR/Hal family, including their known biological functions, mechanisms of regulation, and integration in signaling pathways in *S. cerevisiae*. Information gathered for non-*Saccharomyces* species of biotechnological or clinical relevance is also included.

Contents

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1. Introduction

Phosphorylation of proteins is one of the most well-studied post-translational modifications (PTMs), indispensable for the regulation of several cellular processes and response to stimuli in eukaryotes [1,2]. Protein kinases and phosphatases control the phosphorylation state of a protein; thus, a protein controlled by its phosphorylation state will have its activity dependent on the activity of the regulating kinases and phosphatases [3]. Protein kinases predictably phosphorylate about one-third of all the proteins in humans, flies and yeasts [4]. In eukaryotic cells, kinases catalyze the transfer of the terminal phosphate of ATP to serine, threonine or tyrosin aminoacyl residues, which protein phosphatases can reverse. The specific recognition of substrates by kinases is made through their active site [5]. Some kinases are highly specific – only modulate the phosphorylation of a few select substrates – while others may have a broad spectrum of protein targets. Protein phosphorylation/dephosphorylation is an extremely efficient and dynamic mechanism of control in protein activity and signaling pathways due to its rapid and reversible nature that does not require synthesis or degradation of proteins [5,6]. This regulatory mechanism allows alterations in protein stability, location, and activity, including modifications in catalytic function, often through structural rearrangements that can induce alterations in interacting partners or subcellular localization [5]. The analysis of the regulation of cellular processes by phosphorylation is complex. It includes the identification of the phosphoproteins and respective phosphorylation sites, which is not straightforward; the identification of the effects phosphorylation has on biological processes, the protein kinases and phosphatases involved in phosphorylation regulation, and the environmental conditions and mechanisms leading to the activation of the involved kinases and phosphatases [7,8]. The currently proposed kinase classification systems are based on sequence conservation, phylogeny analysis of the catalytic domains, presence of accessory domains, and similarity in their modes of regulation [9–11]. The classification of the eukaryotic protein kinase superfamily comprises nine groups of "conventional" protein kinases (ePKs) and four groups of "atypical" protein kinases (aPKs), which are proteins with kinase activity but do not share clear sequence similarity with ePKs [9]. In the budding yeast *Saccharomyces cerevisiae*, kinases are classified into six ePKs groups: the AGC group; the CAMK group (calmodulin-regulated kinases); the CKI group (casein kinases); the GMGC group (cyclin-dependent kinases, mitogen-activated protein kinases, glycogen synthase kinases and CDK-like kinases); the STE group (including protein kinases involved in MAP kinase cascades); and the Other kinases group (kinases that could not be easily classified into one of the other groups due to lack of similarity) [9,12]. The atypical kinases in *S. cerevisiae* include the PIKK group (phosphatidyl inositol 3' kinase-related kinases); the PDHK group (pyruvate dehydrogenase kinases); and the RIO group (named after "right open reading frame") [9,12]. Originally considered as part of the "Other" kinases group, the NPR/Hal family includes nine fungal-specific kinases primarily associated with the regulation of plasma membrane transporters: Hal4 (Sat4), Hal5, Hrk1, Kkq8, Npr1, Prr2, Ptk1, Ptk2, and Rtk1 [12]. More recently, these kinases have been assigned as part of the CAMK group – based on the automatic classification of syntenic homologues from *Ashbya gossypii* and *S. cerevisiae* [9] – or even classified as "Snf1-related" – based on a re-analysis using full-length primary sequences (instead of only the catalytic domains) [13]. The NPR/Hal kinases play important roles in signaling pathways associated with the yeast response to nutrient availability and environmental stress but are often overlooked in the scientific literature. The objective of this review article was to update, integrate and consolidate the information available to date regarding the NPR/Hal family of kinases in *S. cerevisiae* and, when available, in other yeast species of biotechnological or clinical relevance. These protein kinases' biological roles and regulation in diverse environmental conditions are reviewed, and data from genome-wide analyses are explored.

2. The NPR/Hal family of kinases

The NPR/Hal family of kinases comprises nine fungal-specific kinases whose functions are mainly associated with the regulation of the stability of nutrient transporters at the plasma membrane and the maintenance of ion homeostasis [13–15]. Yeast adaptation to diverse and ever-changing environments relies on the proper sensing, transport and utilization of nutrients, as well as the efficient regulation of the intracellular levels of metabolites and ions [16]. Nutrient minerals, found as charged ions in the extracellular environment, are also essential to create and sustain electrochemical gradients across the plasma membrane to drive nutrient transport, protein structure and function, and activating signaling pathways [17]. Despite the importance of the NPR/Hal kinases in yeast cells' growth and development pathways, many of these kinases' regulating mechanisms, signaling pathways, and functions are largely unknown or poorly characterized. There are many modalities of kinase regulation: some kinases have constitutive activity (unregulated), while many are regulated in a complex manner, involving more than one regulation mechanism [18]. The most common regulation mechanism of kinase activity is the phosphorylation of its activation loop. The activation loop is a motif containing one or more conserved phosphorylatable residues that, upon phosphorylation, cause a conformational change within the kinase resulting in its activation [19]. Inspection of the activation loop of the NPR/Hal kinases reveals that they do not contain a conserved phosphorylatable residue, indicating that this mechanism of regulation is unlikely to occur. The regulation mechanism of these kinases is probably based on phosphorylation outside the activation loop, which can either activate or inactivate protein function. Indeed, Npr1, the most studied kinase from the family, is regulated in a complex manner involving inactivation through phosphorylation outside the activation loop, being dephosphorylation an activation mechanism [20,21]. Given the high conservation of the catalytic domains among the nine NPR/Hal kinases, their regulation mechanisms might be similar and likely suggest functional relationships [22]. Phylogenetic clustering of the NPR/Hal kinases protein sequences from *S. cerevisiae* is shown in Fig. 1, evidencing three major clusters: Ptk1 and Ptk2; Hal5, Hal4 and Kkq8; Npr1, Prr2, Hrk1 and Rtk1. Since most of the NPR/Hal kinases are functionally uncharacterized, a collection of phenotypes resulting from the deletion of each of these kinases in *S. cerevisiae* cells exposed to a wide variety of chemical compounds and environmental conditions is presented in Table 1.

As described below, members of each cluster tend to display similar functions or belong to the same signaling pathways. The following sections give a detailed description of the information gathered from the literature for each of the NPR/Hal kinases under analysis.

2.1. NPR1 (YNL183C) plays a pleiotropic role in the regulation of nutrient transporters

The Npr1 (nitrogen permease reactivator 1) protein kinase is the most well functionally characterized kinase from the NPR/Hal family of kinases. The predicted Npr1 consensus sequence (obtained with synthetic peptides) is (K/R)-X-X-S-(K/R) [25]. Npr1

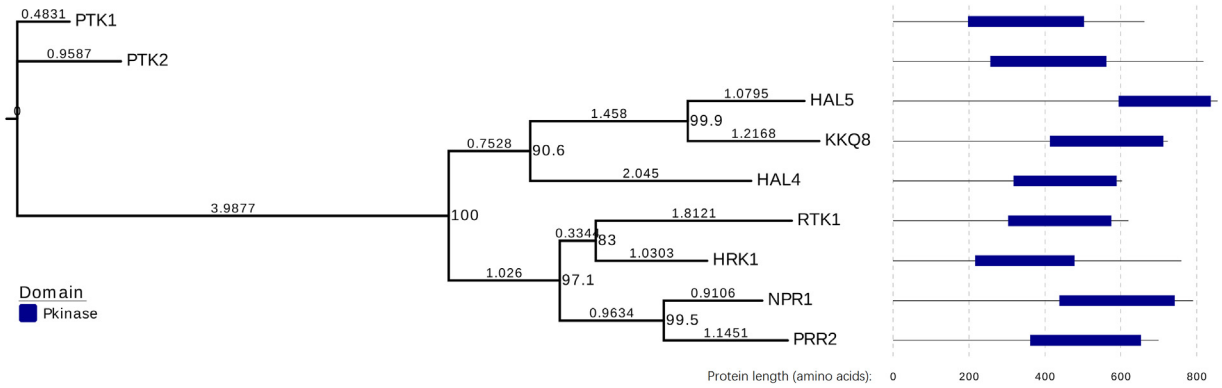

Fig. 1. NPR/Hal kinase family members phylogenetic clustering. A multiple sequence alignment of the Npr/Hal kinases complete amino acid sequences from *S. cerevisiae* S288c (retrieved from NCBI https://www.ncbi.nlm.nih.gov/) was performed using MAFFT [23], followed by phylogenetic inference by maximum likelihood using IQ-Tree [24]. Protein kinase domains (Pkinase) are colored blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

appears to have a requirement for a basic residue at the P-3 position and a substantial favoring for basic P + 1 residues, while a proline at the position P + 1 is disadvantageous [25]. Npr1 displays pleiotropic roles; however, it is best characterized and first described as a regulator of the sorting and stabilization of several amino acid and ammonium permeases at the plasma membrane [20,26–28]. The modulation of the activity of plasma membrane proteins is essential for proper yeast response to nutrient fluctuations. Yeast growth and proliferation are dependent on the availability of nitrogen sources. *S. cerevisiae* is able to grow in a variety of nitrogen sources and discriminates between preferred or non-preferred sources [29]. In the presence of preferred nitrogen sources (ammonia, glutamate, glutamine), yeast activate the nitrogen catabolite repression (NCR) pathway, resulting in the repression of the expression of genes responsible for the use of non-preferred sources (proline, urea, allantoin, gamma-aminobutyric acid (GABA)) [29]. The regulation of nitrogen metabolism is the result of the interplay of different complex regulatory pathways, which mainly include the Ssy1-Ptr3-Ssy5 (SPS) sensor system, the target of rapamycin (TOR) pathway, NCR, and the general amino acid control (GAAC) pathway (reviewed by [30]). The sensing of nitrogen sources is made through the SPS sensor system (extracellular amino acid sensing) and the TOR pathway (intracellular amino acid sensing) [30].

The Npr1 kinase is integrated into the TORC1-Sit4-Npr1 signaling pathway, which controls nutrient plasma membrane transporters' stability, trafficking and endocytosis. The phospho-regulation within this pathway is complex, and many aspects remain to be elucidated. The current model for the TORC1-Sit4-Npr1 pathway states that in the presence of preferred nitrogen sources, the TOR complex 1 (TORC1) is activated by the Pib2 and Gtr proteins and the Sit4 phosphatase is bound to Tap42, forming a complex, which impedes Sit4 from dephosphorylating Npr1 [31]. Therefore, Npr1 is found in its hyperphosphorylated state and presumed to be largely inactive. Under these conditions, the ammonium transport through Mep1 and Mep3 is inhibited by Par32 (Amu1), which is found dephosphorylated [32]. Contrastingly, under nitrogen limiting conditions or upon cells exposure to rapamycin, TORC1 is inactive, leading to the Ptc1- Tip41-mediated activation of Sit4 by dissociation from Tap42, effectively reducing the phosphorylation levels of Npr1 and rendering it active [21,33]. Activation of Npr1 results in the phosphorylation of α-arrestins (selective protein trafficking adaptors), such as Bul1/Bul2, Art1 and Aly2, causing their association with 14–3-3 proteins (in the case of Bul1/Bul2) or inhibiting the recruitment of the Rsp5 ubiquitin ligase (in the case of Art1), thereby impairing their endocytic function, and in turn leading to the stabilization of plasma membrane amino acid permeases (AAPs) [34–38]. Under these conditions, Npr1 further enhances the stabilization of the general AAP Gap1 through direct phosphorylation of the proteins Orm1 and Orm2 (mediators of sphingolipid homeostasis), which in turn promote the *de novo* synthesis of complex sphingolipids [39–41]. Npr1 also inactivates Par32 through phosphorylation, keeping the ammonium transporters Mep1 and Mep3 active, and directly phosphorylates the transporter Mep2, thereby leading to its activation [32,42]. The inhibition of Par32 activity leads to increased intracellular ammonium levels preventing the reactivation of TORC1 [43].

The activity of Npr1 is regulated through phosphorylation in a complex manner [22]. Npr1 phosphorylation occurs in different degrees depending on the environmental conditions: in nitrogen limiting conditions, it is almost completely dephosphorylated; in rapamycin-induced TORC1 inhibition conditions, it displays intermediate phosphorylation; and in nitrogen-rich conditions, it is hyperphosphorylated by TORC1 [21,37]. Npr1 is also described to be autophosphorylated (at the residues Ser47, Ser257, and Ser357). However, this autophosphorylation seems to occur independently of the quality of the nitrogen source and has no regulatory effect [22,44]. Expansion of the aforementioned model extends Npr1 and Sit4 regulatory activities upon some targets (such as α-arrestins) even in conditions where TORC1 is active (where both Sit4 and Npr1 are presumably inactive) [37]. Npr1 and Sit4 presumably work as counterbalancing effectors of their targets' phosphorylation levels, while Sit4 negatively regulates the activity of Npr1. This observation is derived from the demonstrated ability of Npr1 to mediate the phosphorylation of selective targets (Mep2, Aly2) despite being in a hyperphosphorylated state in cells not expressing the Sit4 phosphatase [37,42]. While Npr1 is mainly inactive during TORC1 activation, Sit4 may dephosphorylate select α-arrestins, thereby stabilising specific AAPs at the plasma membrane and inducing the endocytosis of general AAPs. Furthermore, Npr1 can reduce TORC1 activity [45]. This negative regulation was recently found to be through the Npr1-mediated phosphorylation of Pib2 upon non-preferred nitrogen source supplementation, and even possibly under conditions where Npr1 activation is intermediate, creating a regulatory feedback loop [31,46].

The Npr1 kinase was also implicated in the transition to filamentous growth and suggested to have a role in the pheromone-response pathway in *S. cerevisiae* [47,48]. Contrarily to its paralogue *PRR2* (see Section 2.2), both overexpression and gene deletion of *NPR1* result in the exhibition of a filamentous growth phenotype [49–52]. The requirement of Npr1 for filamentous growth was shown to be exerted through the control of the ammo-

Table 1
Summary of NPR/Hal kinases deletion mutants phenotypes. Information collected from genetic screens based on *S. cerevisiae* deletion mutant cells for each of the NPR/Hal kinases exposed to diverse compounds and conditions. S is used when the deletion mutant strain displays sensitivity to the respective compound/condition compared to the parental strain, while R is used for resistance. Non-detected (ND) or non-tested (NT) phenotypes are also indicated.

Type of stress/drug or cellular component/process affected	Compound/Condition	Kinase							
		HAL5	HRK1	KKQ8	NPR1	PRR2	PTK1	PTK2	RTK1	SAT4
Actin	Latrunculin	S [60]	ND	S [60]	S [60]	ND	ND	ND	S [60]	S [60]
	Wiskostatin	S [60]	S [60]	ND	S [60]	ND	ND	S [60]	S [60]	S [60]
Alcohol stress	Ethanol	ND	ND	ND	ND	ND	ND	S [152,153]	ND	ND
Alkaline pH	pH 8.0	S [59,91]	ND	ND	ND	ND	ND	S [59,90,91]	ND	ND
Anti-bacterial	Acriflavinium Hydrochloride	ND	ND	ND	ND	S [60]	S [60]	ND	ND	ND
Anti-fungal	NaD1	ND	ND	ND	ND	ND	ND	R [154]	R [154]	ND
	Thiabendazole	ND	ND	ND	S [60]	ND	ND	ND	ND	S [60]
Anti-metabolite	5-Fluorouracil	S [60]	ND	ND	ND	S [60]	S [60]	S [60]	ND	S [60]
	Methotrexate	S [60]	ND	S [60]	ND	S [60]	ND	ND	ND	S [60]
Anti-neoplastic	1,3-Diallylurea	ND	S [60]	S [60]	ND	S [60]	ND	ND	ND	ND
	Actinomycin d	ND	ND	ND	S [59]	ND	ND	ND	ND	ND
	Amsacrine	S [60]	ND	ND	S [60]	ND	ND	ND	ND	S [60]
	Indirubin	ND	ND	ND	ND	ND	ND	S [60]	ND	ND
	Methoxsalen	ND	ND	S [60]	ND	ND	ND	ND	ND	ND
Anti-oxidant	Allyl disulfide	ND	ND	ND	S [60]	ND	ND	ND	S [60]	ND
	Allyl sulfide	ND	ND	ND	ND	ND	ND	ND	S [60]	ND
	Potassium disulfite	ND	ND	ND	ND	ND	ND	ND	S [60]	ND
Calcineurin function	FK506	ND	ND	ND	R [20]	ND	ND	ND	ND	S [60]
Cell cycle progression inhibition	Zymocin	ND	S [83]	ND	ND	ND	ND	R [83]	ND	ND
Cell wall	Calcofluor white	S [155]	ND	ND	ND	ND	ND	ND	ND	ND
	Chloroquine	ND	ND	ND	ND	ND	ND	ND	ND	S [156]
	HM-I (kiler toxin)	NT	ND	NT	NT	ND	NT	S [157]	ND	ND
	K28 (killer toxin)	NT	ND	NT	NT	ND	NT	S [157]	ND	ND
	KI (killer toxin)	NT	ND	NT	NT	ND	NT	S [157]	ND	ND
	Papulacandin	NT	ND	NT	NT	ND	NT	R [157]	ND	ND
DNA damaging	Bleomycin	S [60]	R [158]	ND	S [60]	ND	ND	R [87]	ND	S [60]
	Carboplatin	S [60]	ND	ND	ND	ND	ND	ND	ND	ND
	Chlorambucil	ND	ND	ND	S [60]	ND	ND	ND	ND	S [60]
	Cisplatin	S [60]	ND	ND	S [60]	ND	S [60]	ND	ND	S [60]
	Doxorubicin	S [59,159]	ND	ND	ND	ND	ND	ND	ND	ND
	Hydroxyurea	ND	ND	S [60]	S [60]	ND	ND	ND	S [60]	S [60]
	Melphalan	S [60]	ND	ND	ND	ND	ND	ND	ND	S [60]
	Mechlorethamine	S [60]	S [60]	ND	ND	S [60]	ND	ND	ND	S [60]
	Mitomycin c	ND	S [60]	ND	ND	ND	ND	ND	ND	ND
	MMS	S [160]	S [60]	ND	ND	ND	ND	ND	ND	ND
	Oxaliplatin	S [59,60]	ND	ND	ND	ND	ND	S [60]	ND	S [60]
	Streptozotocin	ND	ND	ND	ND	ND	ND	S [60]	ND	ND
Endoplasmatic reticulum	Dithiothreitol	ND	S [161]	R [161]	ND	ND	ND	ND	R [161]	ND
Fatty acid elongation	Cerulenin	ND	ND	ND	ND	ND	ND	ND	S [162]	ND
Ionophore	Calcium ionophore	ND	ND	ND	ND	S [162]	ND	ND	ND	ND
	Nigericin	ND	ND	ND	ND	ND	S [162]	ND	ND	ND
	Valinomycin	ND	ND	ND	S [162]	ND	S [162]	ND	ND	ND
Lipid modifying	Lovastatin	S [60]	S [60]	ND	S [60]	ND	ND	ND	S [60]	S [60]
Membrane biogenesis/ integrity	Amphotericin b	ND	ND	ND	S [60]	ND	ND	S [60,162]	ND	S [60]
	Clotrimazole	S [60]	ND	S [60]	ND	ND	ND	ND	ND	S [60]
	Miconazole	S [60]	ND	ND	ND	R [163]	ND	S [60]	ND	S [60]
	Nystatin	ND	S [60]	S [60]	S [60]	ND	ND	ND	ND	S [60]
Microtubules	Benomyl	ND	ND	ND	S [162]	ND	ND	ND	S [60]	ND
	Nocodazole	S [60]	ND	ND	ND	ND	ND	S [60]	ND	ND

(*continued on next page*)

Table 1 (*continued*)

Type of stress/drug or cellular component/process affected	Compound/Condition	Kinase								
		HAL5	**HRK1**	**KKQ8**	**NPR1**	**PRR2**	**PTK1**	**PTK2**	**RTK1**	**SAT4**
Multiple stresses	Desiccation	ND	ND	ND	ND	ND	ND	S [164]	ND	ND
	Synthetic must	S [165]	ND	ND	R [165]	ND	ND	ND	ND	ND
	WSH inhibitory compounds	ND	S [166]	ND	ND	ND	ND	S [166]	ND	ND
Nutrient limitation	Zinc deficiency	ND	ND	ND	S [167]	ND	ND	R [167]	ND	S [167]
Oxidative stress	Berberine chloride	S [60]	ND	ND	ND	ND	ND	ND	ND	S [60]
	Cadmium chloride	ND	S [60]	ND	ND	S [60]	ND	S [60]	S [60]	ND
	Cobalt chloride	S [60]	ND	ND	ND	ND	ND	ND	S [60]	S [60]
	Cobalt sulfate	R [168]	ND	ND	ND	ND	ND	ND	ND	R [168]
	Copper sulfate	S [60,168]	ND	ND	S [60]	ND	ND	ND	ND	S [60,168]
	Diamide	S [168]	S [161]	R [161]	ND	ND	S [169]/ R [161]	ND	ND	S [168]
	Ferric sulfate	S [168]	ND	ND	ND	ND	ND	ND	ND	S [168]
	Ferrous sulfate	R [168]	ND	ND	ND	ND	ND	ND	ND	R [168]
	Hydrogen peroxide	S [160]/ R [168]	S [60]	ND	ND	ND	ND	S [170,60]	S [60]	R [168]
	Linoleic acid 13-hydroperoxide	S [169]	ND	ND	ND	ND	ND	ND	ND	S [169]
	Menadione	ND	ND	ND	S [169]	ND	S [169]	ND	ND	ND
	Mpp+	S [60]	ND	ND	ND	ND	ND	ND	ND	S [60]
	Nickel sulfate	S [168]	ND	ND	ND	ND	ND	ND	ND	ND
	Nitric oxide	S [60]	ND	ND	ND	S [60]	ND	ND	ND	S [60]
	Paraquat	S [60,161]	ND	R [161]	R [161]	ND	R [161]	R [87,161]	ND	S [60,161]
	Potassium dichromate	S [60]	ND	ND	ND	ND	ND	ND	S [60]	S [60]
	Sodium arsenite	ND	S [60]	ND	S [60]	ND	ND	ND	S [60]	ND
	Sodium fluoride	ND	S [60]	ND	ND	ND	S [60]	S [60]	S [60]	ND
	Zinc sulfate	S [168]	ND	ND	ND	ND	ND	ND	ND	S [168]
Phosphatase inhibitor	Calyculin A	ND	ND	ND	ND	ND	ND	ND	S [60]	ND
	Cantharidin	S [60]	ND	ND	ND	ND	ND	S [60]	ND	S [60]
	Norcantharidin	ND	ND	ND	ND	ND	S [60]	S [60]	ND	S [60]
	Ptp2	S [60]	ND	ND	S [60]	ND	ND	S [60]	S [60]	S [60]
Phosphatidylinositol kinase signaling	Wortmannin	ND	S [162]	ND	ND	ND	ND	ND	ND	ND
PKC inhibitor	Staurosporine	ND	ND	ND	S [60]	ND	ND	ND	ND	ND
Pol II inhibitor (Chelator)	Phenantroline	ND	ND	ND	ND	ND	ND	ND	S [162]	ND
Proteasome	Aclacinomycin a	ND	ND	ND	ND	ND	ND	ND	ND	S [60]
	Canavanine	ND	ND	ND	R [171]	ND	ND	ND	ND	ND
Protracted fermentation	High-sugar medium	ND	S [172]	ND	ND	ND	ND	S [172]	ND	ND
Ribosome function	Neomycin sulfate	S [162]	S [162]	ND	ND	ND	ND	ND	ND	S [162]
Sphigolipid biosynthesis	Myriocin	ND	ND	ND	ND	ND	ND	S [60]	ND	ND
TOR signaling	Dieldrin	ND	R [173]	ND	S [173]	ND	ND	S [173]	ND	S [173]
	Rapamycin	S [60]	S [60]	ND	R [174,20]	ND	ND	ND	ND	S [60]
Toxic cation	Aluminium	R [168]	ND	ND	ND	ND	ND	R [81]	ND	R [168]
	Calcium chloride	S [168]	ND	ND	ND	ND	ND	S [157]	ND	ND
	Dysprosium	R [175]	S [175]	ND	ND	ND	ND	ND	ND	ND
	Erbium	ND	S [175]	ND	ND	ND	ND	ND	ND	ND
	Europium	R [175]	S [175]	R [175]	ND	R [175]	S [175]	R [175]	ND	R [175]
	Gadolinium	ND	S [175]	ND	ND	ND	ND	ND	ND	ND
	Hygromycin B	S [15,59,60, 155,82,162]	R [15,82,83]	ND	ND	ND	ND	R [15,14, 82–84]	R [15,82]	S [15,59, 60,82,162]
	Holmium	R [175]	ND	ND	R [175]	ND	ND	ND	ND	ND
	Lithium chloride	S [60,82]	R [82]	ND	S [60]	S [60]	ND	R [85,14, 86,82,84]	S [60]/ R [82]	S [60,82]

Table 1 (*continued*)

Type of stress/drug or cellular component/process affected	Compound/Condition	Kinase							
		HAL5	**HRK1**	**KKQ8**	**NPR1**	**PRR2**	**PTK1**	**PTK2**	**RTK1**	**SAT4**
	Lutetium	ND	S [175]	ND	S [175]	ND	ND	ND	ND	ND
	Manganese chloride	S [60]	ND	S [60]	S [60]	ND	ND	R [14]	S [60]	S [60]/ R [168]
	Mercury chloride	ND	ND	ND	S [60]	ND	ND	ND	ND	ND
	Putrescine	ND	ND	ND	ND	ND	ND	R [87]	ND	ND
	Spermidine	ND	ND	ND	ND	ND	ND	R [87]	ND	ND
	Spermine	S [15]	R [15]	ND	ND	ND	ND	R [15,87,85]	R [15]	S [15]
	Tetramethylammonium	S [15]	R [15]	ND	ND	ND	ND	R [15,14]	R [15]	S [15]
	Thulium	ND	ND	ND	ND	ND	R [175]	ND	S [175]	ND
	Zinc chloride	S [60]	ND	S [60]	S [60]	ND	ND	ND	S [60]	S [60]
Toxic cation/Osmotic stress	Sodium chloride	S [59,60,82, 153,91,161]	S [60,161]/ R [82]	S [60]	S [59,60, 153,91]	S [60]	ND	R [85,14, 86,82]	R [82]	S [59,60,82, 153,91,161]
Tyrosine kinase and HSP90 inhibitor	Radicicol	ND	ND	ND	ND	ND	S [162]	ND	ND	ND
Weak acid stress	Acetic acid	R [176]	S [97,96]	ND	R [176]	R [176]	R [176]	S [14,97]/ R [176]	ND	ND
	Citric acid	ND	ND	ND	ND	ND	ND	S [177]	ND	ND
	Formic acid	R [98]	S [98]	ND	ND	ND	ND	ND	ND	R [98]
	Mycophenolic acid	ND	S [60]	ND	S [60]	ND	ND	S [60]	S [60]	ND

nium transporter Mep2 activity [47,53]. In cells lacking *NPR1*, Mep2 localizes to the plasma membrane and is properly expressed; however, it is not able to transport ammonium [47]. Mep2 is an ammonium sensor essential for filamentous growth in conditions of low extracellular ammonium and independent of the available nitrogen source quality [47,54,55]. In the mating pheromone response case, Npr1 was shown to be dephosphorylated after pheromone treatment, or upon the deletion of *SAP155* (encoding a protein that forms a complex with the Sit4 phosphatase) [48]. In addition, Par32, also belonging to the TORC1-Sit4-Npr1 pathway, displays increased phosphorylation levels in this condition [48]. The TORC1-Sit4-Npr1 pathway is also linked to the regulation of intracellular potassium levels (see Section 2.5). Npr1 was shown to have low activity (is hyperphosphorylated) in potassium-limiting conditions or in *hal4*Δ*hal5*Δ mutant cells while acting as a multicopy suppressor of the *hal4*Δ*hal5*Δ phenotypes [56]. Inhibition of the Npr1 activity increases α-arrestins-mediated endocytosis of nutrient transporters in *hal4*Δ*hal5*Δ cells [56]. Moreover, the TORC1-responsive transcription factor Gln3, presumably regulated by Npr1, was reported to localize in the cytoplasm (thereby being inactive) in *hal4*Δ*hal5*Δ cells, whose intracellular potassium levels are low (favoring TORC1 activation and Npr1 inactivation) [57,58]. The regulation of the osmotic stress response is another process in which Npr1 is presumably involved through phosphorylation of Rho5, which is consistent with the salt stress sensitivity of *npr1*Δ deletion mutant cells (Table 1) [44,59–61]. Rho5 is a Rho-type GTPase implicated in the cell wall integrity signaling pathway and response to oxidative stress, which interacts with Ste50 leading to the activation of the osmotic stress-responsive HOG MAPK pathway [44].

In the fungal pathogen *Candida albicans*, the transition from budding yeast morphology to filamentous growth is also induced in response to the low availability of nitrogen sources. *C. albicans* Npr1 (CaNpr1 (orf19.6232)) inactivation confers resistance to rapamycin, suggesting that this kinase activity, identically to *S. cerevisiae*, is controlled by TOR [62]. *C. albicans* has two ammonium permeases, CaMep1 and CaMep2. Similarly to *S. cerevisiae*, CaMep2, but not CaMep1, is required for filamentous growth induction [62,63]. The dependency on Npr1 of the ammonium permeases in *C. albicans* differs from *S. cerevisiae*. In *S. cerevisiae*, neither ammonium permeases (Mep1-3) can support growth in the absence of Npr1, whereas in *C. albicans* only CaMep2 transport activity appears to be significantly impaired in the absence of CaNpr1. Curiously, the dependence of CaMep2 on CaNpr1 is abolished when the cultivation temperature is increased to 37 °C, indicating that such temperature increase alone can induce a conformational change in CaMep2 permissive for transport [53,62]. In the nitrate-assimilatory yeast *Hansenula polymorpha*, the sole nitrate transporter Ynt1 activity is controlled by phosphorylation in an Npr1-dependent manner in conditions of nitrogen limitation [64]. Ynt1 phosphorylation mediated by the *H. polymorpha* Npr1 (HpNpr1) prevents its sorting to the vacuole. *HpNPR1* disruption, identically to *S. cerevisiae*, leads to reduced growth in ammonium medium [64].

2.2. PRR2 (YDL214C), an inhibitor of the pheromone-response pathway

The Prr2 (Pheromone Response Regulator 2) kinase was first identified as an inhibitor of pheromone-induced signaling in the *S. cerevisiae* mating pathway [65,66]. Overexpression of the *PRR2* kinase was shown to inhibit pheromone-dependent transcriptional induction [65]. Several mitogen-activated protein kinase (MAPK) signal transduction pathways have been characterized in *S. cerevisiae* [67]. The best described MAPK pathway modulates the mating of haploid cells. In haploid *S. cerevisiae* cells mating is induced by pheromone sensing, resulting in the fusion of two cells of

opposite mating types (reviewed in [68]). Most elements of the mating pheromone response are also required for filamentous growth, which is observed when *S. cerevisiae* cells grow on a semi-solid medium with limited nutrients. In haploid cells, this filamentous growth is often termed invasive growth, whereas, in diploids, it is termed pseudohyphal growth and is induced by the lack of a fermentable carbon source or by nitrogen limitation conditions [69]. The functional mechanism of Prr2 was inferred using a Prr2 kinase-inactive version (Prr2-KD) by demonstrating that Prr2-KD still led to signaling inhibition but in a less potent way than in its wild-type counterpart [65]. This observation suggested that Prr2 may act through two different mechanisms: enhanced phosphorylation (inhibition of a pathway element through phosphorylation) and competitive binding (binding to the regulatory subunit of a substrate protein, effectively reducing its phosphorylation and activity) [65]. Moreover, Prr2 was suggested to be a downstream effector of the Fus3 pheromone module (MAPK pathway), responsible for regulation of cell–cell fusion in response to pheromone signaling [65]. This conclusion was based on the inability of the *PRR2* overexpression or Prr2-KD to affect the pheromone-induced feedback phosphorylation of Ste7 [65]. Overexpression of *PRR2* inhibits transcriptional induction resultant from *STE12* overexpression, suggesting that Prr2 might act in conjunction with Ste12 through direct or indirect modulation of its activity [65]. Ste12 was shown to directly bind *PRR2*'s promotor in *S. cerevisiae* cells grown in synthetic low-ammonium dextrose (SLAD) medium (filamentous growth-inducing) [70]. The exact mechanism of action, as well as Prr2 targets and upstream regulators, remain elusive. Like *PRR2*, its paralogue *NPR1* (see Section 2.1) has also been implicated in the filamentous growth pathway [49–51,71,72]. *PRR2* and *NPR1* were identified as targets of the transcription factors Sut1, Sut2, Upc2, and Ecm22 [66,71,73]. The zinc cluster transcription factors Sut1, Sut2, Upc2 and Ecm22, initially implicated in the regulation of sterol uptake under anaerobic conditions, have key regulatory roles in filamentation and mating (reviewed in [74]). Briefly, in filamentous growth conditions, Ecm22 and Upc2 are both inducers, whereas Sut1 and Sut2 are inhibitors by partially repressing their targets in nutrient-replete conditions [71,73]. In nutrient-limiting conditions, Ste12 becomes active and consequently downregulates the expression of Sut1 and Sut2, resulting in the induction of Sut1/Sut2 targets, including *UPC2*, which in turn upregulates its targets [71]. In mating, Sut1 and Sut2 are positive regulators through inhibition of the expression of their targets, such as *PRR2* [66]. Ecm22 and Upc2 also seem to play a role in mating regulation through a mechanism independent of *PRR2* expression modulation [72]. Based on the gathered information, a model integrating Prr2 and Npr1 in the pheromone-response and filamentous growth signaling pathways was assembled and is depicted in Fig. 2.

Despite being an inhibitor of the pheromone-induced signaling pathway and playing a role in filamentation, the deletion of *PRR2* does not originate any phenotype in either condition; only *PRR2* overexpression does [65,71]. One of the possibilities is that the presence of *NPR1*, which displays similar expression patterns and overlapping functions with *PRR2* in the transition to filamentous growth, can compensate for the loss of *PRR2* [71,75]. Another possibility would be regulation by Prr2 of both the positive and negative components from the mating or filamentous growth signaling pathways [65].

2.3. PTK1 (YKL198C) and PTK2 (YJR059W) are regulators of polyamine uptake

The paralogues *PTK1* and *PTK2* were first identified through genetic screens as positive regulators of membrane polyamine transport in *S. cerevisiae* [76,77]. Polyamines (putrescine, spermidine and spermine) are polycations that interact with negatively charged molecules such as DNA, RNA and proteins. They are essential for maintaining cell growth, survival and macromolecular biosynthesis in yeasts [78]. In *S. cerevisiae*, the intracellular levels of polyamines are strictly regulated; low levels are insufficient to maintain cell growth, while high levels are cytotoxic [79]. The regulation of polyamine levels is exerted through biosynthesis, degradation and transport. In *S.cerevisiae*, the polyamine transport system comprises five genes, *TPO1-5*, encoding polyamine excretion proteins and five genes, *GAP1*, *AGP2*, *UGA4*, *DUR3*, and *SAM3*, encoding polyamine uptake proteins [80]. Ptk2 is described as a regulator of polyamines' uptake through phosphorylation of the polyamine transporter Dur3 [80]. Moreover, abrogation of *PTK2* expression leads to increased tolerance of the cells to toxic cations, such as lithium, sodium, manganese, aluminium, and Hygromycin B, in addition to polyamines (Table 1) [14,15,81–87]. Contrarily to *PTK2*, which is a crucial determinant of high-affinity polyamine uptake, *PTK1* is expressed at shallow levels, and only appears to affect low-affinity, low-capacity polyamine uptake [86]. It was suggested that the minor effects observed resulting from *PTK1* deletion on polyamine uptake might be masked by the activity of Ptk2 [86]. Indeed, there may be regulatory interplays between both encoding genes since the transcript levels of *PTK1* increased upon *PTK2* disruption [86]. Nevertheless, *PTK1* remains largely unexplored and uncharacterized.

The uptake of polyamines and several cations in *S. cerevisiae* is energy-dependent. The transmembrane proton gradient generated

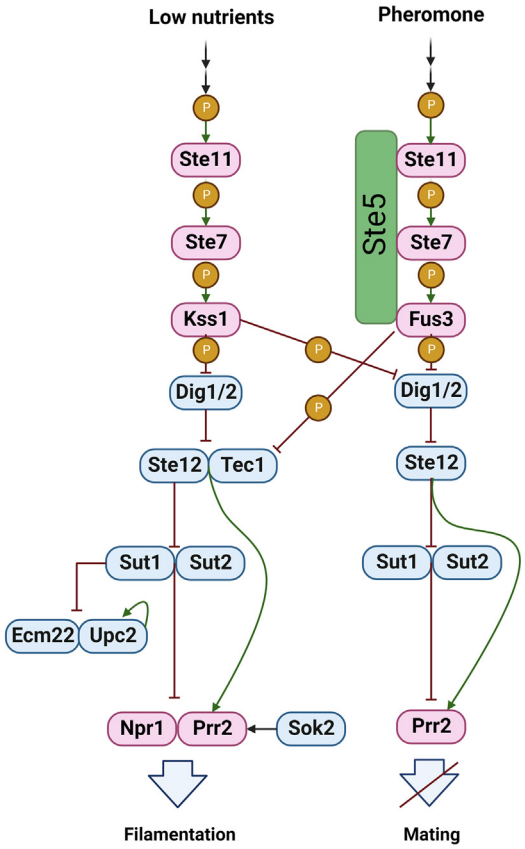

Fig. 2. Npr1 and Prr2 roles in the pheromone-response and filamentous growth pathways. Model depicting the functional integration of Prr2 and Npr1 into the pheromone-response and filamentous growth signaling pathways. Transcriptional and post-translational regulations are indicated by activating (green) or inhibitory (red) arrows. Kinases are highlighted in pink and transcription factors in blue. P designates phosphorylation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

at the plasma membrane by the proton plasma membrane ATPase (H^+-ATPase) Pma1 is essential for secondary transport of nutrients, regulation of intracellular pH, and uptake and extrusion of different ions, such as polyamines and toxic cations [88]. The activity of Pma1 is highly affected by *PTK2* expression; overexpression of *PTK2* significantly increases glucose-induced Pma1 activity, whereas its deletion significantly decreases Pma1 activity [14,84,85]. The modulation of Pma1 activity through Ptk2 indicates that polyamine and ion transport is affected by alterations in the plasma membrane electrochemical potential [85]. Ptk2 was shown to be localized at the plasma membrane and regulate Pma1 activity during glucose activation through phosphorylation of Pma1-Ser899, which causes a decrease in the H^+-ATPase K_m (or, in other words, an increase in affinity for ATP) [89]. This observation is based on the phenotype displayed by the *PTK2* deletion mutant strain (defective in H^+-ATPase activation through glucose-dependent K_m decrease) and *in vitro* and *in vivo* phosphorylation assays [14,82,89]. Altogether, Ptk2 appears to be involved in both direct (through Dun3) and indirect (through Pma1) regulation of polyamine transport.

In line with the aforementioned roles, Ptk2 has also been implicated in the adaptation to alkaline stress conditions, which are known to affect plasma membrane proton gradient [90]. Deletion of *PTK2* leads to high sensitivity towards alkaline conditions (pH 8.0) (Table 1) [59,60,91]. Adaptation and resistance to alkaline stress depend on Pma1 as a major regulator of plasma membrane potential and intracellular pH [90]. Alkaline stress induces the expression of *PTK2* [92], which is directly controlled by the transcription factor Pho4, responsible for the activation of the *PHO* genes in response to inorganic phosphate (Pi) starvation [90,93].

In the pathogenic yeast *C. albicans*, Ptk2 was described as a potential target of CaSky2 [94]. CaSky2 and CaSky1 are protein kinases homologous to the *S. cerevisiae* kinase Sky1, which is an essential factor in the regulation of polyamine transport, in addition to Ptk2, and a regulator of the Trk1/Trk2 potassium transport system [85,95]. CaSky1 presumably functions similarly to Sky1 since its deletion results in resistance to salt stress and toxic polyamine concentrations [94]. On the other hand, CaSky2 is functionally different from CaSky1, being described as playing a role in dipeptide utilization [94].

2.4. HRK1 (YOR267C), a determinant of tolerance to short-chain weak acids-induced stress

Hrk1 is a 759-residue polypeptide whose first biological role attributed was the activation of *S. cerevisiae* yeast plasma membrane H^+-ATPase (Pma1); however, this Hrk1-mediated activation occurs at a much lesser extent than the Ptk2-mediated activation (see Section 2.3) [14,84]. Phosphoproteomic analyses, including the Hrk1 kinase, indicate that it primarily regulates plasma membrane transporter proteins and proteins implicated in carbohydrate metabolism [75,96]. One of the most remarkable phenotypes associated with the *HRK1* gene is the conferred tolerance in *S. cerevisiae* to short-chain weak acids, such as acetic acid and formic acid; its deletion results in hypersensitivity to acetic acid or formic acid stress (Table 1) [97,98]. Moreover, *HRK1* expression is activated in yeast cells exposed to acetic acid stress [99].

Acetic acid is an important inhibitory compound present in lignocellulosic hydrolysates used as feedstock in advanced biorefineries and a byproduct of alcoholic fermentation. It is also a widely used preservative in foods and beverages. Knowledge of the mechanisms underlying yeast tolerance to this weak acid is therefore important to guide the development of robust industrial strains or preservation practices in the Food Industry (reviewed by [100]). In response to acetic acid stress, *HRK1* transcription is regulated by the transcription factor Haa1, the main player controlling the expression levels of 80% of the genes involved in the acetic acid response in *S. cerevisiae* [99]. The regulation of *HRK1* expression by Haa1 is yet to be demonstrated to be direct or indirect; however, based on the reported Haa1 binding motif (Haa1-responsive element (HRE)) [101], and making use of the YEASTRACT database [102], three HREs are found at the *HRK1* promoter.

The effect of *HRK1* expression in *S. cerevisiae* plasma membrane phosphoproteome profile was investigated during the early response of yeast cells suddenly exposed to acetic acid stress [96]. Hrk1 was shown to mediate the phosphorylation levels of 40% of membrane-associated acetic acid-responsive proteins [96]. One important mechanism of tolerance to weak acids is the remodelling of the cell wall, and plasma membrane [103–106]. Increasing the synthesis of sphingolipids has been proposed to enhance the tolerance to acetic acid in *S. cerevisiae* based on the observed increase in sphingolipids in this yeast species upon acetic acid stress [107]. In conditions of sphingolipid synthesis inhibition (30 min or 90 min exposure to myriocin), Hrk1 has significantly altered phosphorylation levels [108], suggesting a possible role of this kinase in sphingolipid regulatory mechanisms, even though no significant changes in the levels of sphingolipids could be observed in *hrk1*Δ mutant cells either in the absence or presence of acetic acid stress [96]. The lipid composition of *hrk1*Δ deletion mutant cells displayed increased levels of dihydroceramide in the absence or presence of acetic acid stress when compared to the parental strain and significantly decreased levels of phosphatidylinositol and phosphatidylcholine in *hrk1*Δ deletion mutant cells exposed to acetic acid stress [96]. Furthermore, the TORC2-mediated phosphorylation of Ypk1 and Ypk2 and Ypk1-mediated Orm1 were not perturbed in *hrk1*Δ mutant cells under acetic acid stress; indicating that Hrk1 is likely, not involved in the activation of TOR complex 2 (TORC2) or Ypk1 from the sphingolipid biosynthetic pathway under acetic acid stress conditions [103]. The expression levels of *HRK1* were also reported to increase significantly upon exposure to high temperatures [109,110]. In fact, Hsf1, a transcription factor described as the master regulator of heat shock response, binds to the *HRK1* promoter of yeast cells under basal conditions (30 °C) [111], following acute heat shock (30 °C to 39 °C for 5 min or 20 min) [111–113], or when chronically exposed to thermal stress (30 °C to 39 °C for 120 min) [112].

Curiously, *HRK1* contains a microsatellite locus that is commonly used for the estimation of levels of genetic variability within populations due to its high degree of polymorphism; YOR267C contains a poly CAA (encoding Gln) motif of variable length [114]. This motif is located outside the protein kinase domain in the region between residues 634 and 647 and is hypervariable among *S. cerevisiae* strains (additional information about microsatellites can be found in [115]).

In non-Saccharomyces yeasts, *HRK1* was also described as a determinant of tolerance to weak acid stress. In *Zygosaccharomyces bailii/parabailii*, two remarkably acetic acid-tolerant yeast species, the homologous *HRK1* gene – *ZbHRK1* (ZBIST_0481) – displays significantly lower mRNA levels in cells not expressing the *ZbHAA1* (*ZBIST_2620*) transcription factor [116]. ZbHaa1 is a functional homologue of ScHaa1 and is required for adaptive response and tolerance to both acetic acid and copper stresses [116,117] (re-

viewed by [100,118]). Unlike ZbHaa1, in *S. cerevisiae* ScHaa1 is not bifunctional and only controls the adaptive response to acetic acid, whereas the response to elevated copper concentrations is exerted through the transcription factor ScCup2 [116].

In the methylotrophic yeast species *Komagataella phaffii* (formerly *Pichia pastoris*), the *HRK1* orthologue *PpHRK1* (*PAS_chr3_1091*) was identified in a screening for kinases conferring resistance to acetic acid using a *K. phaffii* kinase deletion library [119]. Deletion of *PpHRK1* resulted in impairment of the cell growth upon exposure to acetic acid. On the other hand, its overexpression resulted in an improved acetate metabolism, a productivity improvement compared to the parental strain of 55% of acetyl-CoA-dependent 6-methylsalicylic acid (6-MSA) in a yeast culture with 30 mM acetate [119]. However, *PpHRK1* did not seem to be involved in the activation of PpPma1, and the molecular mechanism of Hrk1-mediated signal transduction in *K. phaffii* remains unclear [119].

In the pathogenic yeast *C. albicans*, deletion of *CaHRK1* (orf19.5408) results in increased resistance to LiCl and spermine, suggesting it might be a potential target of CaSky1 (see Section 2.3) [94]. A transcriptomic analysis study of the pathogenic yeast *Candida glabrata* during the early response to acetic acid stress revealed that the orthologue of *HRK1* in *C. glabrata*, CgHrk1 (*CAGL0C02893g*), displayed increased expression levels upon exposure to acetic acid stress [120]. Furthermore, a decrease of more than 50% of its expression levels was observed in *Cghaa1*Δ cells under acetic acid stress compared to the parental strain, suggesting that *CgHRK1* activity is also modulated by the transcription factor CgHaa1 (*CAGL0L09339g*) [120]. This transcription factor is an essential determinant of *C. glabrata* tolerance and response to acetic acid stress, and an orthologue of *S. cerevisiae* Haa1 transcription factor [120].

HRK1 and *HAL4* (see Section 2.5) were identified as genetic determinants of lipid accumulation in the oleaginous yeast *Rhodotorula toruloides* through fitness analysis of deletion mutants [121]. This yeast species can produce lipids and carotenoids from diverse carbon sources, including xylose, and displays relatively high tolerance to inhibitory compounds present in lignocellulosic hydrolysates, making it an attractive host for the production of biotechnological relevant compounds [122].

2.5. HAL5 (YJL165C) and HAL4 (YCR008W) stabilize several cation and nutrient plasma membrane transporters

The partially redundant kinases Hal5 and Hal4 (alias Sat4) were first identified through a genetic screen to confer tolerance to inhibitory concentrations of NaCl and LiCl upon overexpression, but not to osmotic stress in media with high concentrations of KCl or sorbitol [123]. Deletion of either *HAL5* or *HAL4* leads to salt sensitivity (Table 1), which is enhanced in the *hal4*Δ*hal5*Δ double mutant [82,123]. These kinases were therefore described as key determinants of ion homeostasis and salt tolerance. *S. cerevisiae* makes use of complex homeostatic pathways for the modulation of cellular ion homeostasis, which are essential to ensure the correct function of several cellular systems. In yeast, potassium (K^+) is the major intracellular cation, retained intracellularly at high concentrations. In contrast, the intracellular accumulation of other monovalent cations such as sodium (Na^+) or lithium (Li^+) must be kept low due to their toxicity [123]. Potassium is required for essential physiological functions, including the regulation of cell volume and maintenance of plasma membrane electrochemical potential and intracellular pH [124]. At the yeast plasma membrane, the alkali metal cation transport systems comprise the potassium uptake transporters Trk1 and Trk2, the potassium channel Tok1, the K^+-Na^+/H^+ antiporter Nha1, and the Ena Na^+-ATPases efflux systems [125]. The tolerance mechanism of Hal4 and Hal5 to salt stress results from the modulation of cation uptake through the Trk1 and Trk2 potassium transporters and independently from the Ena Na^+-ATPases activity [123]. The regulation exerted by Hal5 and Hal4 is a result of the stabilization of Trk1 at the plasma membrane: the double mutant strain *hal4*Δ*hal5*Δ displays a rapid degradation of the Trk1 transporter in limiting potassium conditions, and overexpression of *HAL5* leads to Trk1 accumulation at the plasma membrane [126,127]. Overexpression of *HAL5* was also described to suppress lithium-sensitive mutations of genes involved in sporulation and meiosis, in the biosynthesis of ergosterol, in the Rho1 signaling to the actin cytoskeleton, and in the Hal3/Ppz1/Calcineurin pathway [128]. The transcription regulation of *HAL5* gene expression, and consequently the control of Trk1 activity in the cell, was shown to be induced in response to salt stress and alkaline pH conditions in a calcineurin/Crz1-dependent manner [124,129,130]. Moreover, *HAL4* (*IPF11548*) expression was also found to be activated by calcium in a calcineurin/Crz1-dependent manner in the pathogenic yeast *C. albicans* [131]. Calcineurin is a Ca^{2+}/calmodulin-dependent phosphatase that modulates the activity of the transcription factor Crz1 and is activated under specific conditions, including exposure to high concentrations of Ca^{2+} or Na^+, high temperatures or prolonged incubation with α-factor [129]. Furthermore, both Hal4 and Hal5 are determinants of susceptibility to formic acid (Table 1), possibly acting through the stabilization of the Trk1 transporter [98].

Besides Trk1, Hal4 and Hal5 are also presumably responsible for the stabilization of different nutrient transporters at the plasma membrane (some of them regulated by the ART-Rsp5 pathway), such as amino acid permeases (Can1, Fur4, Mup1, and Gap1), and glucose permeases (Hxt1), some of them dependent on the intracellular potassium levels [13,126], but the underlying molecular mechanism remains unclear. In fact, the *hal4*Δ*hal5*Δ mutant displays a constitutive activation of the GCN pathway and decreased uptake of amino acids, and glucose [127]. This double mutant also has an altered metabolic state toward respiration [127]. Interestingly, a small fraction of the Hal4 protein was described to localize to the mitochondria, while Hal4 protein is mainly cytosolic [132–134]. Although deletion of *HAL4* does not significantly affect mitochondrial functions or mitochondrial proteome, its overexpression does lead to impaired growth on non-fermentable carbon sources and significant changes in the mitochondrial proteome; its regulatory role was proposed to involve the regulation of late steps of the maturation of mitochondrial iron-sulfur cluster proteins [132]. Hal5 was found to be a nutrient-responsive kinase that localizes to the plasma membrane depending on the availability of specific nutrients such as amino acids [13]. Excess concentration of certain amino acids in the media reduces Hal5 localization to the plasma membrane in a TORC1-independent manner (increasing the Hal5 cytosolic pool), while exposure to stress-inducing salt concentrations has the opposite effect. Furthermore, the N-terminal region (upstream of the kinase domain) was shown to be essential for the recruitment of Hal5 to the plasma membrane and regulation of endocytosis [13]. *HAL5* overexpression suppresses the lithium sensitivity phenotype displayed by *S. cerevisiae* cells deleted for genes encoding proteins involved in the vacuolar targeting of

nutrient-permeases [128,135]. This is consistent with the role attributed to Hal5 in sorting and stabilization of nutrient transporters at the plasma membrane.

2.6. RTK1 (YDL025C) and KKQ8 (YKL168C) remain largely uncharacterized

Information concerning the protein kinase Rtk1 is very limited. Rtk1 may play a role in the peroxisomal biogenesis process since deletion of *RTK1* leads to fewer and enlarged peroxisomes; however, the derived morphological defects did not affect peroxisome functionality [136]. Deleting of either *RTK1* or *PTK2* (see Section 2.3) results in high-impact consequences in the lipidome of *S. cerevisiae*, suggesting a role in lipid homeostasis regulation. Lipid homeostasis modulation is highly dynamic and represents an essential mechanism for yeast cell adaptation to environmental challenges [137].

The protein abundance and phosphorylation levels of Rtk1 were found to be increased in yeast cells exposed to acetic acid stress [138]. In addition, overexpression of *RTK1* led to enhanced acetic acid tolerance, ethanol productivity, and better fermentation performance when yeast cells were grown in a medium containing a corn stover hydrolysate-simulated inhibitory mixture [138]. Curiously, deletion of *RTK1* does not cause a phenotype upon acetic acid stress [97], which likely indicates its activity is compensated by another kinase (Hog1, Hrk1, and Ptk2 are possible candidates). Indeed, a yeast two–hybrid assay revealed that Hog1 – known to play a role in acetic acid stress tolerance [97,139–141] – interacts with Rtk1 in vivo, suggesting that these kinases might belong to the same signaling pathway in response to acetic acid stress [138]. *RTK1* overexpressing strain response to other stresses was also tested: exposure to salt stress (NaCl) did not significantly affect its growth, whereas growth upon exposure to hydrogen peroxide (H_2O_2) was significantly improved compared to the wild-type counterpart [138]. These results are in agreement with the phenotypes displayed by strains deleted for *RTK1* exposed to oxidative stress or toxic cations (Table 1). Deletion of *RTK1* renders the cell resistant to toxic cations such as Hygromycin B, spermine, tetramethylammonium, lithium chloride, and sodium chloride and sensitive to oxidative stress-inducing compounds such as H_2O_2, potassium dichromate, cadmium chloride, sodium fluoride, and sodium arsenate. [15,60,82,142]. Moreover, *RTK1* expression is significantly increased in cells exposed to a combination of citrinin and ochratoxin A and in response to selenide stress, which mainly trigger a response to oxidative stress [143,144]. Yap1, a transcription factor essential for oxidative stress response and tolerance, was also described to bind the *RTK1* gene promoter *in vivo* [144]. Altogether, this data suggests involvement of Rtk1 in the yeast cell response to oxidative stress.

The Kkq8 kinase is the most uncharacterized kinase from the NPR/Hal family. Phylogenetically, it is the closest kinase to Hal5; however, it does not seem to function in a similar manner. Contrarily to *HAL5*, overexpression of *KKQ8* does not confer salt tolerance [123]. Deletion of *KKQ8* was described to render *S. cerevisiae* cells sensitive to anti-fungals such as clotrimazole and nystatin (Table 1) [60]. Additionally, the absence of *KKQ8* suppresses the plasma membrane localization of the drug efflux transporters Pdr5 and Yor1, which are under the control of the transcription factors Pdr1/3, in cells treated with the anti-fungal and PDR substrate atorvastatin [145].

3. Concluding remarks

This review article compiles the currently available information on the NPR/Hal kinases, including their integration into signaling pathways responsive to environmental changes. It also makes use of data obtained by high-throughput analyses, whose main goal was not to examine specifically those kinases. Although a significant amount of information was put together, much remains to be uncovered and explored. The study of protein kinases is not straightforward. Most of the experimental evidence regarding their regulation targets is provident from genome-wide analyses, such as phosphoproteomic analysis, *in vitro* protein chip analysis, and quantitative genetic interaction mapping. Despite uncovering possible phosphorylation targets, phosphoproteomic analyses do not offer information regarding the functional and biological relevance of the uncovered phosphorylation sites. Furthermore, studies focused on specific kinases often overlook relevant connections and crosstalk beyond the different kinases and the involved signaling pathways.

The fungal-specific kinases from the NPR/Hal family have key roles in regulating nutrient transport and ion homeostasis. These kinases display several overlapping and complementary functions. The most prominent examples of function overlap are the kinase paralogue pairs Npr1/Prr2 and Ptk2/Ptk1. Npr1 and Prr2 both have roles in the pheromone-response and filamentous growth pathways (see Sections 2.1 and 2.2), whereas Ptk1 and Ptk2 regulate polyamine uptake (see Section 2.3). Additionally, the kinase pair Hal4 and Hal5, although not paralogues, are partially redundant in regulating plasma membrane transporters' stabilization and potassium homeostasis (see Section 2.5). Hrk1, despite having a higher similarity to Rtk1, Npr1 and Prr2, appears to function more similarly to the Ptk2 kinase (see Section 2.4). The remaining kinases, Rtk1 and Kkq8, remain functionally uncharacterized and unexplored (see Section 2.6). The majority of NPR/Hal kinases appear to function in a coordinated manner in regulating plasma membrane nutrient transporters and ion homeostasis in *S. cerevisiae*. As an example, alterations in potassium availability or regulation of its uptake have influence on the modulation mechanisms of phosphate uptake and metabolism [146,147]. Perturbations in the potassium uptake lead to the hyperactivation of Pma1 and affect the phosphate metabolism by triggering a response similar to phosphate starvation [146]. The activation of *PTK2* transcription by the transcription factor Pho4 (active in phosphate-limiting and alkaline pH conditions) in these conditions might have a role in the increased activity levels displayed by Pma1. Furthermore, the TORC1-Sit4-Npr1 pathway (described in Section 2.1) activity is also linked with intracellular potassium levels. The observation was based on the hypersensibility displayed by the *hal4*Δ*hal5*Δ and *trk1*Δ*trk2*Δ mutants to rapamycin and the Trk1/2-independent decreased potassium accumulation resultant from TORC1 inhibition [56,148]. A model displaying currently known complex regulation mechanisms of the NPR/Hal kinases is shown in Fig. 3.

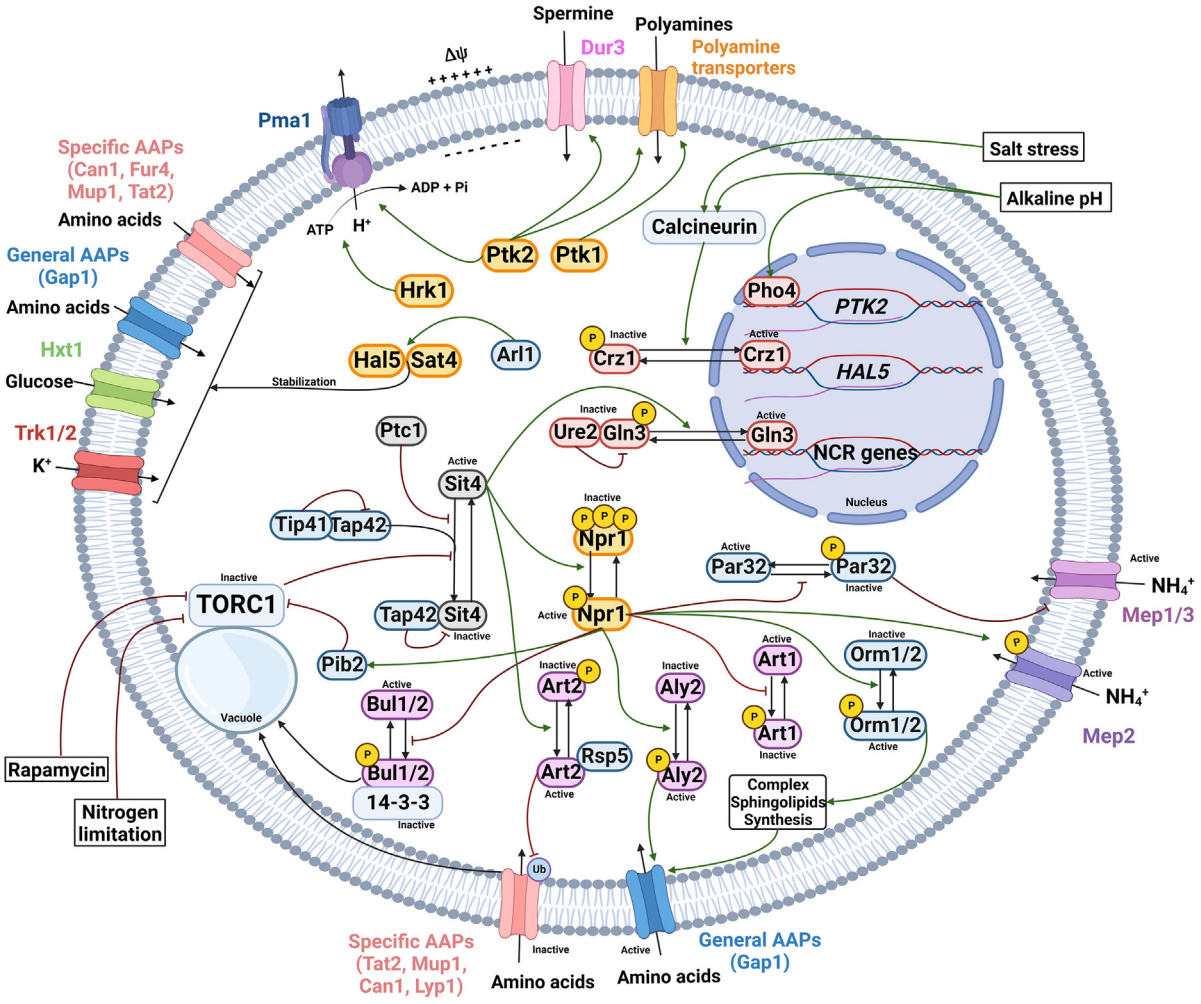

Fig. 3. Model of NPR/Hal kinases mechanisms of regulation. Schematic model displaying a simplified version of the known molecular mechanisms and signaling pathways underlying NPR/Hal kinases regulation of cellular processes in *S. cerevisiae*. Kinases are represented in orange, phosphatases in gray, α-arrestins in pink, and transcription factors in red. Regulations are indicated by activating (green) or inhibitory (red), and when relevant proteins are marked as "Active" or "Inactive". P designates phosphorylation and Ub ubiquitylation. AAP stands for amino acid permease. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Knowledge of the mechanisms of regulation of these kinases, their phosphorylation targets, and involvement and organization in signaling pathways in yeast is valuable in understanding the processes underlying ion homeostasis maintenance and regulation of intracellular pH and plasma membrane nutrient transporters. Some applications include the identification of potential targets for genome manipulation to generate more robust yeast species for producing high-value metabolites with high tolerance capacity to stresses occurring during industrial bioprocesses. The characteristics of the NPR/Hal kinases in pathogenic yeast species also make them attractive candidates as therapeutic targets. Protein kinases play essential roles in the regulation of the pathogenicity of *Candida* species. A recent *in silico* study identified the protein kinases Npr1 and Ptk2 as potential drug targets and tools to discover new lead compounds to fight fungal infections, such as candidiasis [149]. Npr1 and Ptk2 were selected due to their key roles in the mechanisms regulating Candida spp. pathogenicity, their fungal specificity, and lack of human homologues [62,63,149–151]. More in-depth molecular and cellular studies are fundamental to better understand the overlooked role of the NPR/Hal kinases in the regulation of cellular processes in yeasts with impact in biological knowledge and in biotechnological and clinical applications.

CRediT authorship contribution statement

Miguel Antunes: Writing – original draft, Writing – review & editing, Visualization. **Isabel Sá-Correia:** Conceptualization, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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