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Subcellular localization of the histidine kinase receptors Sln1p, Nik1p and Chk1p in the yeast CTG clade species *Candida guilliermondii*

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ABSTRACT

Fungal histidine kinase receptors (HKR) sense and transduce many intra- and extracellular signals that regulate a wide range of physiological processes. *Candida* CTG clade species commonly possess three types of HKR namely Sln1p (type VI), Nik1p (type III) and Chk1p (type X). Although some recent work has demonstrated the potential involvement of HKR in osmoregulation, morphogenesis, sexual development, adaptation to osmotic stresses and drug resistance in distinct *Candida* species, little data is available in relation to their subcellular distribution within yeast cells. We describe in this work the comparative subcellular localization of class III, VI, and X HKRs in *Candida guilliermondii*, a yeast CTG clade species of clinical and biotechnological interest. Using a fluorescent protein fusion approach, we showed that *C. guilliermondii* Sln1p fused to the yellow fluorescent protein (Sln1p-YFP) appeared to be anchored in the plasma membrane. By contrast, both Chk1p-YFP and YFP-Chk1p were localized in the nucleocytosol of *C. guilliermondii* transformed cells. Furthermore, while Nik1p-YFP fusion protein always displayed a nucleocytosolic localization, we noted that most of the cells expressing YFP-Nik1p fusion protein displayed an aggregated pattern of fluorescence in the cytosol but not in the nucleus. Interestingly, Sln1p-YFP and Nik1p-YFP fusion protein localization changed in response to hyperosmotic stress by rapidly clustering into punctuated structures that could be associated to osmotic stress signaling. To date, this work provides the first insight into the subcellular localization of the three classes of HKR encoded by CTG clade yeast genomes and constitutes original new data concerning this family of receptors. This represents also an essential prerequisite to open a window into the understanding of the global architecture of HKR-mediated signaling pathways in CTG clade species.

1. Introduction

Since the end of the 1990s it has been accepted that the majority of yeast species belonging to the *Candida* genus have adopted a particular codon usage (Kawaguchi et al., 1989). More recent phylogenic studies based on whole genome analysis indicate that *Saccharomycotina* could be subdivided into two major groups: (i) the Saccharomycetaceae clade including *C. glabrata* and other yeasts from the *Saccharomyces* genus and (ii) species that translate CTG as serine instead of leucine (referred to as the CTG clade). A number of CTG clade species such as *C. albicans*, *C. dubliniensis*, *C. tropicalis*, *C. parapsilosis*, *C. maltosa*, *C. famata*, *C. guilliermondii*, *C. lusitaniae*, *C. oleophila*, *C. rugosa*, and *Scheffersomyces stipitis* are currently studied in depth due to their clinical incidence, their biotechnological importance, and their biological control potential (Fitzpatrick et al., 2006; Butler et al., 2009; Papon et al., 2013a, 2014).

Fungal histidine kinase receptors (HKR) sense and transduce many intra- and extracellular signals that regulate a wide palette of physiological processes including osmoregulation, morphogenesis, sexual development, adaptation to osmotic stresses and drug resistance (Santos and Shiozaki, 2001; Chauhan et al., 2006). In fungal cells, these receptors commonly constitute initial sensing proteins of a three-component multistep phosphorelay signaling pathway composed of HKR, histidine phosphotransfer (Hpt) shuttle

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proteins and response regulators (RR). In most cases, these transduction pathways are branched through RRs to one or more mitogen-activated protein kinase (MAPK) cascades (for a review, see Li et al., 2010).

Catlett et al. (2003) provided a classification of several HKR genes identified in Ascomycota and revealed that fungal HKRs fall into eleven classes. More recent analyses suggest that fungal species belonging to Saccharomycetaceae clade usually encode a unique class VI HKR (namely Sln1p) while in contrast, species belonging to CTG clade commonly encode three HKRs from class III (Nik1p), VI (Sln1p), and X (Chk1p), respectively (Chapeland-Leclerc et al., 2007). Class VI HKRs are currently known as main osmosensing receptors communicating with the high-osmolarity glycerol (HOG) MAPK pathway. The class III HKR likely corresponds to secondary osmosensing receptors regulating optimal phenotypic switching during growth in high salt through the HOG MAPK cascade. Due to its unconventional structure, the class X HKR was extensively studied, particularly Chk1p in *C. albicans*, for which a crucial role in virulence, morphogenesis (particularly yeast to hyphae switch), peroxide adaptation, cell wall composition, quorum sensing, biofilm formation, and triazole resistance has been established. During fungal infection, *C. albicans* Chk1p is required for survival in human neutrophils and adherence to *ex vivo* human esophageal cells (for a review see Li et al., 2010).

To date, although the potential involvement of HKRs in various physiological processes in distinct *Candida* species has been demonstrated (Li et al., 2010), very little data is available concerning their distribution within yeast cells. It has currently been suggested that awareness of the subcellular distribution of these proteins could provide precious insight into their specific role or function. While the anchoring of Sln1p to the plasmalemma has been clearly demonstrated in *Saccharomyces cerevisiae* (Reiser et al., 2003), no confirmation of this localization has been reported in *Candida* species. Furthermore, no subcellular localization has been described for Chk1p. The cytosolic localization of Nik1p from *C. famata* (*Debaryomyces hansenii*) is the only report concerning the subcellular compartmentalization of HKRs from *Candida* species (Meena et al., 2010). By contrast, localization of the HKR downstream partners has been more clearly documented and it has been established in *C. guilliermondii* that Hpt (Ypd1p) is located in the nucleocytosol while RR (Skn7p) is confined to the nucleus suggesting that the phosphotransfer between these two proteins occurs preferentially in this subcellular compartment (Courdavault et al., 2011).

Due to lack of consistent localization data of HKRs precluding the fine elucidation of their function, we describe in this work and for the first time, the comparative subcellular localization of class III, VI and X HKRs in a yeast CTG clade species. Since a complete toolbox in *C. guilliermondii* is now available (Foureau et al., 2012a, 2012b, 2013a, 2013b, 2013c; Papon et al., 2012), notably molecular tools dedicated to dual fluorescent protein labeling for protein localization monitoring (Courdavault et al., 2011), we report on the subcellular localization of Sln1p, Nik1p and Chk1p orthologues in this species of clinical and biotechnological interest (Butler et al., 2009; Papon et al., 2013b).

2. Materials and methods

2.1. Strains and standard growth conditions

C. guilliermondii reference strain ATCC 6260 (American Type Culture Collection, ATCC Manassas, USA) was used as a source of genomic DNA for PCR amplifications. Yeast strains (Table 1) were routinely cultivated in liquid YPS medium (1% yeast extract, 2% peptone, 2% sucrose) at 30 °C under agitation (150 rpm). Minimal medium (0.67% Yeast Nitrogen Base with ammonium sulfate and without amino acids) was supplemented with 200 μg/ml uridine or 200 μg/ml L-leucine. Solid media were prepared with 2% agar.

Table 1
Candida guilliermondii strains.

Strains	Genotype
ATCC6260 [a]	Wild type
leu2 ^{REP}[b]	*ura5*, *leu2::REP*
SLN1-YFP	*ura5*, *leu2::REP*, pLEU2-SLN1-YFP
CHK1-YFP	*ura5*, *leu2::REP*, pLEU2-CHK1-YFP
NIK1-YFP	*ura5*, *leu2::REP*, pLEU2-NIK1-YFP
YFP-CHK1	*ura5*, *leu2::REP*, pLEU2-YFP-CHK1
YFP-NIK1	*ura5*, *leu2::REP*, pLEU2-YFP-NIK1
SLN1-YFP/CFP	*ura5*, *leu2::REP*, pLEU2-SLN1-YFP, pURA5-CFP
SLN1-YFP/CFP-SKN7	*ura5*, *leu2::REP*, pLEU2-SLN1-YFP, pURA5-CFP-SKN7
CHK1-YFP/CFP	*ura5*, *leu2::REP*, pLEU2-CHK1-YFP, pURA5-CFP
CHK1-YFP/CFP-SKN7	*ura5*, *leu2::REP*, pLEU2-CHK1-YFP, pURA5-CFP-SKN7
NIK1-YFP/CFP	*ura5*, *leu2::REP*, pLEU2-NIK1-YFP, pURA5-CFP
NIK1-YFP/CFP-SKN7	*ura5*, *leu2::REP*, pLEU2-NIK1-YFP, pURA5-CFP-SKN7
YFP-CHK1/CFP	*ura5*, *leu2::REP*, pLEU2-YFP-CHK1, pURA5-CFP
YFP-CHK1/CFP-SKN7	*ura5*, *leu2::REP*, pLEU2-YFP-CHK1, pURA5-CFP-SKN7
YFP-NIK1/CFP	*ura5*, *leu2::REP*, pLEU2-YFP-NIK1, pURA5-CFP
YFP-NIK1/CFP-SKN7	*ura5*, *leu2::REP*, pLEU2-YFP-NIK1, pURA5-CFP-SKN7

[a] Reference strain from American Type Culture Collection, ATCC Manassas, USA.
[b] From Courdavault et al. (2011).

2.2. Nucleic acid isolation and PCR amplifications

Genomic DNA was extracted following the protocol described by Scherer and Stevens (1987). All PCRs were performed with Phusion polymerase (Fermentas). PCR conditions for amplification were those indicated by the supplier. Primers were synthesized by Eurofins MWG Operon and are listed in Table 2. The PCR and endonuclease-digested products were purified using the Nucleospin Extract II kit (Macherey–Nagel) according to the manufacturer's instructions.

2.3. Plasmid construction

The *SLN1* coding sequence was amplified using primers SLN1S and SLN1R (Supplemental Fig. 1A, Table 2), digested at its extremities with *Bam*HI and *Spe*I and cloned into the *Bam*HI and *Nhe*I sites of pLEU2-YFP (Courdavault et al., 2011) yielding plasmid pLEU2-SLN1-YFP (Supplemental Fig. 1B).

The *NIK1* coding sequence was amplified using primers NIK11 and NIK12 (Supplemental Fig. 1A, Table 2), digested at its

Table 2
Primers.

Primers	Sequence
CHK15	GCGGCGCCGGATCCATGAATTTGTCCCAAAGTTGCGCC
CHK16	GCGGCGCCCCTAGGCCCCTTTTGCTGGTATATCTTTTCACC
CHK17	CTGAGACCTGCAGGATATGAATTTGTCCCAAAGTTGCGCC
CHK18	CTGAGACCTGCAGGCTACCCCTTTTGCTGGTATATCTTTTCACC
CHK1R	AGTACCCACGACCAGTACCAGGATAAGG
CHK1S	TGGTTTCCTGATTCAGTTCGCTATACCC
CHK1S	TGGTTTCCTGATTCAGTTCGCTATACCC
FRNPS2	CTGAGAGGCGCCCTGCAGGTCGACTTTGTACAATTCATCCATACCA
FSBNX1	CTGAGAGGATCCGCTAGCCTCGAGATGTCTAAAGGTGAAGAATTAT
NIK11	GCGGCGCCGCTAGCATGAGTGCAACGGAGTTGATAGGC
NIK12	GCGGCGCCCTCGAGATCTAGATGTTCAAAACGAGATTC
NIK14	CTGAGACCTGCAGGATATGAGTGCAACGGAGTTGATAGGC
NIK15	CTGAGACCTGCAGGCTAATCTAGATGTTCAAAACGAGATTC
NIK16	TGAGTATACAGTGCCAGGATCAGC
SLN11	ATCTCAGCCGATTGTTCGCATTGC
SLN1R	CTGGTCGACACTAGTCTGCAGGTAGCCCAAGCGCTTGTCCTCACTTGG
SLN1S	CTGGTCGACGGATCCATGAGACGGCTCAAAATCGGAATCC