Isolation of *CaSLN1* and *CaNIK1*, the genes for osmosensing histidine kinase homologues, from the pathogenic fungus *Candida albicans*

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Recent studies have revealed that fungi possess a mechanism similar to bacterial two-component systems to respond to extracellular changes in osmolarity. In *Saccharomyces cerevisiae*, Sln1p contains both histidine kinase and receiver (response regulator) domains and acts as an osmosensor protein that regulates the downstream HOG1 MAP kinase cascade. *SLN1* of *Candida albicans* was functionally cloned using an *S. cerevisiae* strain in which *SLN1* expression was conditionally suppressed. Deletion analysis of the cloned gene demonstrated that the receiver domain of *C. albicans* Sln1p was not necessary to rescue *SLN1*-deficient *S. cerevisiae* strains. Unlike *S. cerevisiae*, a null mutation of *C. albicans* *SLN1* was viable under regular and high osmotic conditions, but it caused a slight growth retardation at high osmolarity. Southern blotting with *C. albicans* *SLN1* revealed the presence of related genes, one of which is highly homologous to the *NIK1* gene of *Neurospora crassa*. Thus, *C. albicans* harbours both *SLN1*- and *NIK1*-type histidine kinases.

Keywords: *Candida albicans*, cloning, osmosensor, two-component system, histidine kinase

INTRODUCTION

Two-component systems which involve a phospho-relay from the histidine of the sensor kinase to the aspartic acid of the response regulator are widespread in bacteria (Bourret *et al.*, 1991; Stock *et al.*, 1991; Parkinson & Kofoed, 1992). Regulatory proteins similar to bacterial two-component systems are also found in some eukaryotes (Brown *et al.*, 1993; Ota & Varshavsky, 1993; Alex *et al.*, 1996; Chang *et al.*, 1993; Hua *et al.*, 1995; Wilkinson *et al.*, 1995). In *Saccharomyces cerevisiae*, Sln1p consists of an extra-cellular sensor, a kinase and a receiver domain (Ota & Varshavsky, 1993; Maeda *et al.*, 1994) and acts as an osmosensor protein (Maeda *et al.*, 1994). Under low osmolarity conditions, a specific histidine in the kinase domain is autophosphorylated. The phosphate moiety of this histidine is first transferred to a certain aspartic

acid within the receiver domain and then via a phospho-relay to the downstream proteins Ypd1p and Ssk1p, leading to the shut off of the HOG1 MAP kinase cascade (Brewster *et al.*, 1993; Maeda *et al.*, 1994; Posas *et al.*, 1996). Histidine kinase activity and phosphorylation of Sln1p are essential for growth at low osmolarity because a mutation of either the autophosphorylating histidine or the receiver aspartic acid of Sln1p is lethal under these conditions (Maeda *et al.*, 1994). Increased osmolarity hampers the histidine kinase activity of Sln1p, which in turn activates downstream HOG1 MAP kinase (Brewster *et al.*, 1993; Maeda *et al.*, 1994; Posas *et al.*, 1996).

Involvement of a histidine kinase in an osmosensing pathway has also been reported in *Neurospora crassa* (Alex *et al.*, 1996). The predicted product of the *N. crassa* *NIK1* gene possesses two domains that are related to sensor histidine kinases and response regulators of bacterial two-component proteins (Alex *et al.*, 1996). Nik1p is an apparent cytoplasmic protein with six repeats of about 90 aa that may form a coiled-coil structure. Deletion of the *NIK1* gene caused aberrant

Abbreviation: 5-FOA, 5-fluoroorotic acid.

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hyphal morphology, a phenotype more prominent under high osmotic conditions (Alex *et al.*, 1996).

To address whether a histidine kinase osmosensing mechanism is conserved in other yeasts, we attempted the functional cloning of *Candida albicans* *SLN1*. In addition, probing a *C. albicans* genomic library with the *C. albicans* *SLN1* gene identified a gene that is highly related to *NIK1* of *N. crassa*. Thus, it seems that *C. albicans* has both *Sln1p*- and *Nik1p*-type histidine kinases which may allow adaptation to different osmotic conditions.

METHODS

Plasmid construction and yeast strain. A 700 bp *HindIII*–*HindIII* fragment that harbours the *tetO*–*HOP1* chimaeric promoter, UAS and URS, was excised from p97t (Nagahashi *et al.*, 1997), ligated with the DNA fragment containing the *hisG*–*URA3*–*hisG* module isolated from pMPY-ZAP (Schneider *et al.*, 1996) and cloned into Bluescript SKII+ to generate p97tZAP. Replacement of the cognate *SLN1* promoter with a tetracycline-controllable promoter (Nagahashi *et al.*, 1997) was achieved by the one-step gene replacement method (Baudin *et al.*, 1993; Schneider *et al.*, 1996) with slight modification. DNA fragments harbouring target sequences of the *SLN1* and tetracycline-regulated promoters and the *hisG*–*URA3*–*hisG* module were amplified by PCR using p97tZAP as a template and a pair of primers, 5' CATCGAAAACAGCACGAACAAAAGCCAACTCAC-TACATTTTAGAACAGCTATGACCATG 3' and 5' TCC-AATTTTGATGGCAGGCCAAATCGCATTTGTATT-GGAATCTTTTCTGAGATAAAG 3'. The resulting DNA fragment was transfected into an *S. cerevisiae* strain, YPH499 (*MATa ade2 his3 leu2 lys2 ura3*) that had been transfected with pINFAGAL4 and which constitutively expressed the *tetR*–*GAL4* fusion activator (Nagahashi *et al.*, 1997). After confirming the correct integration of the *tetO*–*HOP1* chimaeric promoter by PCR and Southern blotting, transfectants were selected by 5-fluoroorotic acid (5-FOA) and used for experiments. For convenience, we designated the above strain as Tet-*SLN1*. To determine the region of *CaSLN1* essential for complementing *ScSLN1*, deletion mutants of *CaSLN1* were cloned into the unique *Bam*HI site of YEp24T (Yamada-Okabe *et al.*, 1996) and transfected into another *S. cerevisiae* strain, A451 (*MATa can1 leu2 trp1 ura3 aro7*) in which the original *SLN1* locus was disrupted by *LEU2* but where episomal copies of *SLN1* in pYES2 (Invitrogen) were maintained under the control of the *GAL1* promoter. This strain, termed pYES-*SLN1*, was unable to grow in the presence of either 5-FOA or glucose as sole carbon source. Primers used to amplify *ScSLN1* were 5' CCCGGGGAATTCATGCGAT-TTGGCCTGCCA 3' and 5' CCCGGGGAATTCATCTCATT-TGTTATTTTCTT 3'. For *ScSLN1* disruption, the 2.3 kb *PstI*–*PstI* region of *ScSLN1* was replaced by *LEU2*.

Screening the *C. albicans* *SLN1* and *NIK1* genes. Tet-*SLN1* cells were transfected with a *C. albicans* genomic DNA library and spread on plates containing 50 µg tetracycline ml⁻¹. After incubation at 30 °C for 3 d, colonies appeared on the plates, cells were collected and plasmid DNA was recovered from them. After a second screening, the essential region of the insert DNA that conferred tetracycline-resistant growth of Tet-*SLN1* was determined by cloning each DNA fragment in YEp24T (Yamada-Okabe *et al.*, 1996) and in pRS416 (Stratagene).

For cloning the *NIK1* gene of *C. albicans*, a *C. albicans* genomic DNA library was screened with the 2.1 kb *KpnI*–*Sall* fragment of the *C. albicans* *SLN1* gene as probe. Hybridization was carried out under low stringency conditions in a buffer containing 0.25 M sodium phosphate (pH 7.2), 2 × SSC (1 × SSC contains 150 mM NaCl and 15 mM sodium citrate), 1% (w/v) bovine serum albumin, 1 mM EDTA, 0.1% (w/v) SDS and 25% (w/v) formamide at 37 °C for 12 h. Radio-labelling of DNA with [α -³²P]dCTP and DNA sequencing were carried out as described by Sambrook *et al.* (1989). Construction of *C. albicans* genomic DNA library was reported previously (Yamada-Okabe *et al.*, 1996).

Disruption of *CaSLN1*. The 2.3 kb *KpnI*–*KpnI* fragment of *CaSLN1* was cloned in pUC19 and the resulting plasmid digested with *BalI* and *SnaBI* followed by ligation with a 3.8 kb *Bam*HI–*XbaI* fragment carrying the *hisG*–*URA3*–*hisG* module, generating pCASLN1U. Thus, the 0.6 kb *SnaBI*–*BalI* region of *CaSLN1* was replaced by the *hisG*–*URA3*–*hisG* module in pCASLN1U. After pCASLN1U was linearized by digestion with *PvuII*, 10 µg DNA was transformed into *C. albicans* CA14 (*ura3Δ::imm434/ura3Δ::imm434*) cells by the lithium acetate method (Sanglard *et al.*, 1997). Before and after a second round of transformation, the *URA3* gene was excised by 5-FOA as described previously (Mio *et al.*, 1996). Unless otherwise specified, *C. albicans* cells were cultured in YPD (1%, w/v, yeast extract; 2%, w/v, peptone; 2%, w/v, glucose) in the presence or absence of 1.5 M NaCl.

RESULTS

Functional cloning of the *C. albicans* *SLN1* gene

To isolate a *C. albicans* *SLN1* gene by functional cloning, we generated an *S. cerevisiae* strain in which *SLN1* expression was conditionally repressed. This strain, designated Tet-*SLN1*, grew normally in the absence of tetracycline, while its growth was severely impaired by the addition of 50 µg tetracycline ml⁻¹. Transfection of Tet-*SLN1* with intact *S. cerevisiae* *SLN1* restored normal growth even in the presence of 50 µg tetracycline ml⁻¹, demonstrating that the growth defect of Tet-*SLN1* caused by tetracycline was largely due to the repression of *SLN1* expression.

Tet-*SLN1* cells were transfected with a *C. albicans* genomic DNA library that was constructed with a vector harbouring the *S. cerevisiae* *TRP1* gene and a 2 µ replication origin (Yamada-Okabe *et al.*, 1996) and transfectants were selected using 50 µg tetracycline ml⁻¹. From 10⁴ independent colonies, three clones grew in the presence of 50 µg tetracycline ml⁻¹. The plasmid DNA was recovered from these clones and a restriction map of each insert DNA determined. Although the restriction maps of these three clones differed from each other, the map of clone 1 coincided with the pattern of a *C. albicans* genomic Southern blot that was obtained using the *S. cerevisiae* *SLN1* gene as a probe and this clone was analysed further.

Deletion analysis with this clone demonstrated that a 5.6 kb *EcoRV*–*XhoI* region was sufficient to rescue Tet-*SLN1* cells in the presence of tetracycline (Fig. 1a). Sequencing of this region revealed that it contained a long ORF of 3.6 kb, with a coding sequence possibly

(a) *CaSLN1* (b) *CaNIK1*

+

+

+

-

-

-

Fig. 1. Restriction maps of *C. albicans* genomic DNA fragments that contain *CaSLN1* (a) and *CaNIK1* (b) are illustrated together with the expected structures of their products. The ability of deletion mutants to rescue pYES-SLN1 cells (*sln1Δ*) in the presence of 5-FOA is indicated as '+' and '-', respectively. Ba, *Bam*HI; Bg, *Bgl*II; Ec, *Eco*RI; Ev, *Eco*RV; P, *Pst*I; S, *Sal*I; X, *Xho*I; TM, transmembrane domain; HK, histidine kinase domain; REC, receiver domain; REP, repeats of an approximately 90 aa motif.

extending further downstream. To obtain a clone containing the missing 3' end of the ORF, we screened the same *C. albicans* genomic DNA library using a 0.5 kb *SalI*-*XhoI* fragment as a probe. Sequencing of a clone containing the complete ORF revealed that the predicted product of the gene is a 150 kDa protein highly similar to *S. cerevisiae* Sln1p (37% identity) (Fig. 2a) and the gene was designated *C. albicans* *SLN1* (*CaSLN1*). To avoid confusion, the *S. cerevisiae* *SLN1* was termed *ScSLN1* in this study. Like *ScSln1p*, *CaSln1p* has no N-terminal signal sequence, but possesses two potential transmembrane helices in its N-terminal half (Fig. 3a). Sequence identity between *CaSln1p* and *ScSln1p* was remarkably high within the histidine kinase and receiver domains located in the middle of the protein and near the C-terminal end, respectively, and both the essential phosphorylating histidine at position 576 and receiver aspartic acid at position 1144 of *ScSln1p* are conserved in *CaSln1p* (Fig. 2a).

As described above, the initially isolated *CaSLN1* clone lacked the C-terminal receiver domain, suggesting that the receiver domain of *CaSln1p* is not crucial to complement a *ScSLN1* deletion. We confirmed this by making a series of deletion mutants and transfecting them into another *S. cerevisiae* strain, pYES-SLN1. Consistent with the results of the functional cloning of *CaSLN1*, the 5.6 kb *EcoRV*-*XhoI* fragment of *CaSLN1* that eliminates the receiver domain from *CaSln1p* still rescued pYES-SLN1 in the presence of 5-FOA or glucose, while further deletion from C terminus, destroying half of the probable ATP-binding site within the histidine kinase domain lead to loss of the ability to complement *ScSLN1* (Figs 1a and 4).

Disruption of *CaSLN1*

To study the function of *CaSLN1*, we generated the homozygous *casln1Δ* null mutant strain by means of the ura-blaster protocol (Fonzi & Irwin, 1993). Using this

strategy, the first one-third of the histidine kinase domain, including the probable autophosphorylating histidine at position 519 of *CaSln1p*, was replaced by the *hisG* sequence of *Salmonella typhimurium* (Fig. 5a). The correct integration of the *hisG* sequence into the expected loci was confirmed by Southern blotting (Fig. 5b). Unlike *S. cerevisiae*, the homozygous *casln1Δ* null mutant of *C. albicans* grew under both normal and high osmotic conditions and sustained an ability to form hyphae. However, the homozygous null mutation, but not the hemizygous mutation of *CaSLN1* caused weak growth retardation in the presence of 1.5 M NaCl (Fig. 6a). Moreover, the homozygous *casln1Δ* null mutant cells elongated in the presence of 1.5 M NaCl (Fig. 6b). Similar morphological changes of the homozygous *casln1Δ* null mutants were also observed in the presence of 1 M sorbitol or 1 M KCl (data not shown).

The above results clearly demonstrate that *CaSLN1* is not an essential gene in *C. albicans* and may imply that *C. albicans* harbours other genes, possibly histidine kinases, to adapt to high osmolarity.

Cloning the *C. albicans* *NIK1* gene

In an attempt to search for other histidine kinase genes in *C. albicans*, we performed genomic Southern blotting with a part of *CaSLN1* DNA corresponding to the histidine kinase domain of *CaSln1p* and detected several discrete bands that were not derived from *CaSLN1* alleles. A genomic DNA library was again screened with the 2.1 kb *KpnI*-*EcoRI* fragment of *CaSLN1* as a probe and a clone distinct from *CaSLN1* was obtained. This clone contained an ORF that could encode a 119 kDa protein highly similar to *N. crassa* *Nik1p* (50% identity) (Fig. 2b) and the gene was termed *C. albicans* *NIK1* (*CaNIK1*). Like *N. crassa* *Nik1p*, *CaNik1p* contains five repeats of about 90 aa within the N-terminal half (Figs 1b and 2b) and a hydropathy plot of *CaNik1p* lacks any apparent transmembrane domain (Fig. 3b). However,

(a)

ScSlnlp	1	:	MRFGLPKLE	LTTPPRIGIR	<u>QTLTALVSV IV</u>	<u>ALGSLIILAV</u>	<u>TTGVVYFNSY</u>	KNLRSDRLYI	AAQLKSSQID	QTLNLYYYQA	YYLASRDALQ	SSLTSYVAGN
			**	****	** *	* * * * *	* * * *	****	**	*** *	* * * *	* * * *
CaSlnlp	1	:	MR-----	---RLKIGIR	<u>PQLIIIVCFA</u>	<u>SLFSLIILGI</u>	<u>VTGIYFSANL</u>	KNLRRLRLLV	ISQLKRTQVQ	QAIQYIAYQV	MTVSEVDSL	VPLSNVRAGN
ScSlnlp	101	:	KSADNWVDSL	SVIQKFLSSS	NLFYVAKVYD	SSPNAVLNAT	NNGTGDLIPE	DVLDLSLFLPS	TDTPLPSSLE	TIGIL--TDP	VLNSTD----	-YLMMSLPI
			*	*	* * *	*	* * *	*	*	* * *	* *	*
CaSlnlp	90	:	NSKAVFSEAQ	NYLQQYVLTT	DSFTAARLYD	LDLQVASSF	DNMT--LISE	SAQDVVYPLQ	PNRSMPPVLG	TPSGLYFTGP	IANNSDNFNS	RYPMGITVPV
ScSlnlp	194	:	FANPSIILTD	SRVYGYITII	MSABGLKSVF	NDTIALEHST	IAISAVY--	-----NSQ	GKASGYHFV-	-FPYPGSRSD	LPQKVFSIKN	DTFISSAFRN
			*****	** **	* * *	* * *	* * *		* * *		* *	**
CaSlnlp	188	:	LNSSSIILSQ	PSISGYLTIV	AAAESIRSAL	NSTSEDDYQA	MA-VQPVYGD	PQKGIDNLSQ	NAYNGDNEVI	GFKLVFPVEN	SLLEAGTIYN	INSSSSMKTA
ScSlnlp	283	:	GKGSSLKQTN	ILS--TRNTA	LGYSPCS--F	NLVNWWAIVS	QPESVFLSPA	<u>TKLAKIITGT</u>	<u>VIAIGVFVIL</u>	<u>LTLPLAHWAV</u>	QPIVRLQKAT	ELITEGRGLR
			*	*	*	* * *	****	* * * *	* * * *	* * * *	* * *	*
CaSlnlp	287	:	LASNSGTATG	VKSFP GKVA	IGFSRISVQD	NL-NWSIVIV	QSNSVFNGPA	<u>NKLKRITIGV</u>	<u>VIGIGAFMCF</u>	<u>VTFPLAVWFI</u>	RPITKLKEAT	EAIT-----
ScSlnlp	379	:	PSTPRTISRA	SSFGRKFSSG	FAVPSSLLQF	NTAEAGSTTS	VSGHG-GSGH	GSGAASFANS	SMKSAINLGN	EKMSPPEEEN	KIPNNHTDAK	ISMDSGLNHD
				*	*	*	* * * *	***	***			* * * *
CaSlnlp	380	:	-----	---KYKKEKL	NSVNS----	-----NSPTS	GSGSGSGSGS	GSGSGSRANS	-----	-----	-----D	SSADQSLSLD
												+
ScSlnlp	478	:	LLGPHSLRHN	DTRSSNRSH	ILTTSANLTE	ARLPDYRRLF	SDELSDLTET	FNIMTDALDQ	HYALLEERV	ARTKQLEAAK	IEAEAAANEAK	TVFIANISHE
			.	** .	* *	* * *	* * *	* * *	* * *	* * *	* * *	*****
CaSlnlp	428	:	TGKRNS----	--INSSSFSS	SYSTGIRL-P	ARIPRSKKIF	KDELTELSEA	<u>FNIMTEELDK</u>	<u>QYTHLEDVRK</u>	<u>LRTKELEASK</u>	<u>IQAEAAANEAK</u>	TVFIANISHE
ScSlnlp	578	:	LRTPLNGILG	MTAISMEETD	VNKIRNSLKL	IFRSGELLHL	ILTELLTFSK	NVLQRTKLEK	RDFCITDVAL	QIKSIFGKVA	KDQVRVLSIS	LFPNLIRIMV
			*****	** * *	* * *	* * *	*****	* * *	*	* * * *	****	* * *
CaSlnlp	521	:	LRTPLNGILG	MTSIAMEEED	PNVIHDSLKL	IHRSGELLHL	ILTELLTYSK	NTLNRSKLEK	SNFQILEIVY	QVRSIFNKLA	HDQRVNFKIL	VKPNIFRKLI
ScSlnlp	678	:	LWGDNSRIIQ	IVMNLVSNAL	KFTPVGDGTD	VRMKLLGEYD	KELSEKKQYK	EVYI-----	-----	-----	-----	-----
			*****	*****	* * *	* * *	* * *	*				
CaSlnlp	621	:	IYGDNSRIIQ	IVMNLVSNSL	KFTPVGDSVS	VSFKLLGEYD	HERSKKLDYK	KVCILNDSSS	STVAVPPPTP	PSDTKPNPKP	KSTPTPKPDP	TRSHLVDHNN
ScSlnlp	732	:	-----	--KKGTEVTE	NLETT-----	-----	-----	-DKYD-LPTL	SNHRKS----	-----	-----V	DLESSATS-L
				* * *	*			*	* * *			* * *
CaSlnlp	721	:	RSANITTSPLT	PVRKPTNQTK	NKSITNNVTK	QNMKIRKXXX	TNKNLHNNNN	NNKQNSDFL	MNRRLSGSHK	FNNINDEELS	PTAIEKNIDK	YLTSSADSDN
ScSlnlp	769	:	GSNRDTSTIQ	EEIT-----	-----	-----	KRNTVANE--	-SIYKVNDR	EKASN-----	-----DDVS-	-----S	IVSTT---TS
			*	* * *	*		* * *	* * *	*	*		* * *
CaSlnlp	821	:	ISVTTLSTVQ	YETTIFESQF	KSKPLPALPV	DAKPQVSGKI	DENDVNDEDP	SGGSIKDDDS	EDTINEKQGI	SSSPSSSSSS	NEKQENSPRS	NDSTTVTVTR
ScSlnlp	817	:	SYDN-----	A IFNS--QFNK	AP-----GSD	DEEGGN----	LGRPIENPKT	WVISIEVEDT	GPGIDPSLQE	SVFHPFVQGD	QTLRSRQYGGT	GLGLSICRQL
			*	* * *	* *	* *	* *	* * * *	* * * *	* * * *	* * * *	*****
CaSlnlp	921	:	PRHNMPSAQ	DFKSYPYTFDK	KPEYDSNMNS	NEIVQNNRVY	RIRNMYQPKV	WVIQIEVITD	GPGIEPALQE	KVFEPFVQGD	QTLRSRSGGT	GLGLSICRQL
ScSlnlp	901	:	ANMMHGTMKL	ESKVGVSQKF	TFTLPLNQTK	EI--SPADME	FPFEDEFNPE	SRKNRRVKF-	-----	-----SVAKS	IKSRQSTSSV	ATPATNRSSL
			. *****	* * * *	* * * *	* * *	*****	* * *		*	* *	* *
CaSlnlp	1021	:	ATMMHGTLTL	KSTIGKSGTF	TLTLPLPQTG	EDMVPPEDMA	EFCEDEFNPA	AKINRKVAFE	DGDIIDTESQQ	QENPSSEEDT	QGDRNVQSST	SSSPPN-SSS
ScSlnlp	983	:	TNDVLEPVRS	-----KGKGE	TKDVGNPNMG	REEKNDNGGL	EQLQEKNIK-	PSICLTGAEV	NBQNSLSSK			

Fig. 2. For legend see facing page.

there are some structural differences between the two proteins. As mentioned above, *N. crassa* Nik1p contains six repeats of 90 aa that always start with tryptophan, but the first repeat in CaNik1p begins with glutamic acid

and the fourth repeat observed in the *N. crassa* Nik1p is absent from CaNik1p (Fig. 2b). The regions from amino acid residues 490 to 641 and from 886 to 990 of CaNik1p share a high degree of sequence identity with the sensor

(b)

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NcNik1p 1 : MTDGPTLAAI AALVKS LAVD PATTQTSGLR PSTHVRLPGP YTREKGDLEL ELSALVVRIE QLETAALAAAS PPAMPDTPNA PTDALFNGT LSPSSETPDA
          *  **                *  **                *  *                *  *                *  *
CaNik1p 1 : M--NPT----- -KKPRLSPMQ PS----- -VFEI----- -LS DP----- -ELYSQHC HS-----

NcNik1p 101 : RYPAPLPRNG FIDEALEGLR EHVDQSKLL DSQRQELAGV NAQLIEQKQL QEKALAIIEQ ERVATLEREL WKHQKANEAF QKALREIGEI VTAVARGDLS
              *  *          *  *          *  *          *  *          *  *          *  *          *  *          *  *
CaNik1p 33 : -----LR E-----TLL D-----HF NHQ----- -ATLI----- -DTYEHEL EKSKNANKAF QQALSEIGTV VISVAMGDLS

NcNik1p 201 : KKVRMNSVEM DPEITTFKRT INIMMDQLQV FSSEVSRRAR EVGTGEGILGG QAQIEGVDGT WKELTDNVN MAQNLTQDVR EIASVTTAVA HGDLTCKIER
          ***  **  ***  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
CaNik1p 86 : KKVEIHTVEN DPEILKVKIT INIMMDQLQT FANEVTKVAT EV-ANGELGG QAKNDGSGVI WRSLTDNVNI MALNLTNQVR EIADVTRAVA KGDLSRKINV

NcNik1p 301 : PAKGEILQLQ QTINIMVDQL RTFASEVTRV ARDVGTGEGIL GGQADVEGVQ GMWNELTVNV NAMANNLTQ VTDIIRVTTA VAKGDLTQKV QAECRGEIFE
          *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
CaNik1p 185 : HAQGEILQLQ RTINIMVDQL RTFAFEVSKV ARDVGVGLGIL GGQALIEVNE GIWEELTDNV NAMALNLTQ VRNIANVTTA VAKGDLSSKV TADCKGEILD

NcNik1p 401 : LKKTINSMVD QLQQFAREV KAREVGTGEG RLGGQATVHD VQGTWRDLTE NVNGMAMNLT TQVREIAKVT TAVAKGDLTK KIGVEVQGEI LDLKNTINTM
          **  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
CaNik1p 285 : LKLTINQMD RLQNFALAVT TLSREVGLG ILGGQANQVD VEGAWKQVTE NVNLMATNLT NQVRSIATVT TAVAHGDLQK KIDVHAQGEI LQLKNTINKM

NcNik1p 501 : VDRLGTFAFE VSKVAREVGT DGTGGAQV DNVGKWKDL TENVNIMASN LTSQVRGIST VTQAIANGDM SRKIEVEAKG EILILKETIN NMVDRLSIFC
          *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
CaNik1p 385 : VDSLQLFASE VSKVAQDVGI NGKLGIAQV -----

NcNik1p 601 : NEVQRVAKDV GVDGIMGGQA DVAGLKGRW EITTDVNIMA NNLTAVRAF GDITNAATDG DFTKLVEVEA SGEMDELKKK INQMVYNLRD SIQRNTQARE
          *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
CaNik1p 415 : -----S DVDGL--WK EITSNVNIMA SNLTSQVRAF AQITAAATDG DFTRFITVEA SGEMDALKTK INQMVFNLRD SLQRNTAARE

NcNik1p 701 : AAELANKTKS EFLANMSHEI RTPMNGIIGM TQLTLDLDTL QYQREMLNIV NSLANSLLTI IDDILDLSKI EARRMVIEEI PYTLRGTVFN ALKTLAVKET
          *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
CaNik1p 493 : AAELANSKAS EFLANMSHEI RTPLNGIIGM TQLSLDTELT QYQREMLNIV HNLANSLLTI IDDILDLSKI EARRMTVEEI DFSLRGTVFN ALKTLAVKAI

NcNik1p 801 : EKFLDLTYRV DHSVPDHVVG DSFRLRQIIL NLVGNAIKFT EHGEVSLTIQ KASSVQCSTE EYAEFVUSD TGIGIPADKL DLIFDTFQQA DGSMTKRFGG
          *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
CaNik1p 593 : EKNLDLTYQC DSSFPDNLIG DSFRLRQVIL NLAGNAIKFT KEGKVS-VSV KSKDKMVLDS KILLEVCVSD TGIGIEKDKL GLIFDTFCQA DGSMTKRFGG

NcNik1p 901 : TGLGLSISKR LVNLMGGDVM VKSEYKGSK FFPTCVVRLA NDDISLIAQ LNPYKSHQVL FIDKGRGTGHG PEIAKML--- -HGLGLVPIV VDSERNPALE
          *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
CaNik1p 692 : TGLGLSISKQ LIHLMGGEIW VTSEYSGGSN FYFTVCVSPS NIRYTRQTEQ LLPFSSHYVL F ---VSTEHT QEELDVLRDG IIEGLGLPII V---RN--IE

NcNik1p 997 : KARAAGQAPY DVIIVDSIED ARRLRSVDDF KYLPVL-LA PVVHSLKSC LDLGITSYMT TPCQLIDLGN GMVPALENRA TPSLADNTKS FEILLAEDNT
          *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
CaNik1p 784 : DATLTEPVKY DIIMIDSIEI AKKLRLLESEV KYIPLVLVHH SIPQLNMRVC IDLGISSYAN TPCSIIDLAS AIIPALESRS ISQNSDESVR YKILLAEDNL

NcNik1p 1096 : VNQLRAVKIL EKYHHVTVTV NGEEAVEAV KRKKFDVILM DVQPMIMGGF EATAKIREYE -----RSLG SQRTPIIALT AHAMMGDREK CIQAQMDEYL
          *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
CaNik1p 884 : VNQKLAVRIL EKQGHSEVVV ENGLEAYEAI KRKNYDVILM DVQPMVMGGF EATEKIRQWE KKSNPIDSL- TFRTPPIALT AHAMMGDREK SLAKGMDYV

NcNik1p 1190 : SKPLQONHLI QTILKCATLG GQLEKNRER ELTRAADAVT GGRRDNGMYS ASQAAQHAAL RPPLATRGTL AADSLVSGLE SPSIVTADKE DPLSRARASL
          *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
CaNik1p 983 : SKPLKPKLIM QXINKCIHNI NQKELSRNS RGSDFAKKMT --RNTPG-- -STTRQGSDE GSVEDMIGDT PRQGSVEGGG TSS---RPV QRRSATEGSI

NcNik1p 1290 : SEPNIHKAS

CaNik1p 1073 : TTISEQIDR

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Fig. 2. (a) The amino acid sequence of *S. cerevisiae* Sln1p (ScSln1p) is compared with that of *C. albicans* Sln1p (CaSln1p). Identical amino acids between ScSln1p and CaSln1p are marked by asterisks. Probable transmembrane regions of ScSln1p and CaSln1p are double-underlined. (b) The amino acid sequence of *N. crassa* Nik1p (NcNik1p) is compared with that of *C. albicans* Nik1p (CaNik1p). Identical amino acids between CaNik1p and NcNik1p are marked by asterisks. The first two amino acids of each repeat of about 90 aa in NcNik1p and CaNik1p are double-underlined. Amino acid sequences were aligned using the BLAST and FASTA programs. Predicted histidine kinase and receiver domains are indicated by bold underlining and dashed bold underlining, respectively. '+' represents the positions of the histidine and aspartic acid residues that correspond to the autophosphorylated histidine and phosphorylated aspartic acid residues of ScSln1p. According to the report of Santos & Tuite (1995), the CTG codon is decoded as serine instead of leucine.

Fig. 3. Hydropathy plots of *C. albicans* Sln1p (a) and Nik1p (b) were calculated as described by Kyte & Doolittle (1982) using a window of 12 aa. It should be noted that two short domains with hydrophobicity indices of above 3 in CaSln1p (a) are considered as transmembrane helices.

kinase and response regulator domains of two-component systems, respectively. Sequence comparisons with *S. cerevisiae* Sln1p and the BarA protein of *Escherichia coli* implicate the histidine at position 510 and the aspartic acid at position 924 as sites of phosphorylation (Fig. 2b).

DISCUSSION

We have isolated and sequenced a *C. albicans* homologue of *ScSLN1*. Like *ScSln1p*, *CaSln1p* possesses extracellular sensor, histidine kinase and receiver domains. When expressed from a multicopy plasmid, *CaSLN1* overcame the growth defect of *S. cerevisiae* cells caused by the repression of *ScSLN1*. Since *CaSln1p* also shares significant sequence identity with the probable ATP-binding site within the kinase domain of *ScSln1p*, it should be able to autophosphorylate a histidine residue of the protein.

The receiver domain of *CaSln1p* was not necessary to rescue *sln1Δ S. cerevisiae* cells. However, the mechanism of the complementation of *ScSln1p* by C-terminally truncated *CaSln1p* is not clear. In *S. cerevisiae*, the phosphate moiety at the autophosphorylated histidine residue in the kinase domain of Sln1p is transferred to an acceptor aspartic acid residue in the receiver domain of the same protein, then to a histidine residue in the downstream Ypd1p and finally to an aspartic acid residue of the further downstream Ssk1p, with both the

Fig. 4. Functional complementation of *ScSLN1* by *CaSLN1*. *S. cerevisiae* pYES-SLN1 cells were transfected with YEp24T (vector) or YEp24T harbouring *CaSLN1*, which can encode full length *CaSln1p* [*CaSln1*(1–1377)], or C-terminally truncated *CaSln1p* [*CaSln1*(1–1200) and *CaSln1*(1–1016)]. The transformants were spread on plates with (+) or without (–) 5-FOA and were incubated for 3 d. The receiver domain is deleted in *CaSln1*(1–1200) and the receiver domain and half of ATP-binding site are destroyed in *CaSln1*(1–1016).

autophosphorylated histidine and receiver aspartic acid residues being essential for viability under low osmotic conditions (Posas *et al.*, 1996). One possibility is that *CaSln1p* can bypass the phospho-relay to the receiver domain and to Ypd1p, and can function by directly phosphorylating Ssk1p.

In addition to *CaSLN1*, another gene, *CaNIK1*, was isolated and sequenced. The product of *CaNIK1* is highly homologous to Nik1p of *N. crassa*, with regions that are highly related to the sensor kinase and response regulator domains of two-component systems. Although there is no apparent transmembrane helix in *CaNik1p*, we asked if *CaNik1p* has a similar function to *ScSln1p*. However, preliminary experiments did not support this hypothesis, because pYES-SLN1 cells harbouring *CaNIK1* in a multicopy plasmid were unable to grow in the presence of 5-FOA or glucose. This result implies that *CaNIK1* is functionally distinct from *S. cerevisiae* *SLN1* and that *CaSLN1* and *CaNIK1* may not act in the same pathway. In fact, sequence homology between *CaSln1p* and *CaNik1p* is restricted to short regions encompassing the phosphorylated histidine and receiver aspartic acid residues. However, we cannot yet rule out the trivial possibility that the *CaNIK1* gene failed to function in *S. cerevisiae*.

Unexpectedly, the homozygous *casln1Δ* null mutation

Fig. 5. Generation of the homozygous *casln1Δ* null mutant strain. (a) The strategy for disrupting *CaSLN1* is illustrated, with the *SnaI*/*Ball* region of *CaSLN1* replaced by the *hisG* sequences. (b) Southern blotting of *CaSLN1*. Twenty-five micrograms of genomic DNA from wild-type CAI4 (lane 1), the hemizygous *casln1Δ* mutant with *URA3* (lane 2), the hemizygous *casln1Δ* mutant without *URA3* (lane 3) and the homozygous *casln1Δ* null mutant (lane 4) was digested with *Bgl*/*I* and *Sal*/*I*, fractionated on an agarose gel and hybridized with the 0.9 kb *Ball*-*Sal* fragment of *CaSLN1*. Bands derived from the *CaSLN1* allele, the *casln1Δ::hisG-URA3-hisG* allele and the *casln1Δ::hisG* allele are indicated by a, b and c, respectively.

was not lethal in *C. albicans*. The homozygous *casln1Δ* null mutant cells grew even under high osmotic conditions, but growth in the presence of 1.5 M NaCl was somewhat impaired by disruption of *CaSLN1*. This phenotype resembles that of the *nik1Δ* null mutant of *N. crassa* (Alex *et al.*, 1996) and also that of the *hog1Δ* null mutant of *C. albicans* (Jose *et al.*, 1996) and is suggestive of a *CaSln1p* function under high osmotic conditions. In contrast, the histidine kinase activity of *ScSln1p* is necessary under low osmotic conditions and is repressed under high osmotic conditions, leading to activation of the HOG1 MAP kinase in *S. cerevisiae*. Thus, it seems to be puzzling why a disruption of *casln1Δ* caused a growth defect at high osmolarity. Although further experiments, including the disruption of *CaNIK1*, are under way to

Fig. 6. Effects of the disruption of *CaSLN1* on growth and morphology. (a) Effects of high osmolarity on the growth of wild-type CAI4 (*SLN1/SLN1*) (—), the hemizygous *casln1Δ* mutant (*SLN1/sln1Δ*) (....) and the homozygous *casln1Δ* null mutant (*sln1Δ/sln1Δ*) (----). Cells of the indicated strains were cultured in YPD medium in the absence (left) or presence (right) of 1.5 M NaCl and the growth of the cells was monitored using a Biophotorecorder (Advantec). (b) Morphological change caused by the disruption of *CaSLN1*. Cells of wild-type CAI4 (*SLN1/SLN1*) and of the homozygous *sln1Δ* null mutant (*sln1Δ/sln1Δ*) were cultured in YPD medium in the absence (upper panels) or presence (lower panels) of 1.5 M NaCl. Photographs of cells from overnight cultures are shown. Bar, 10 μ m.

address this question, the absence of a *NIK1* homologue in the *S. cerevisiae* genome suggests a divergence of osmosensing signal transduction mechanisms in fungi.

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