Planta (2005) 221: 394–405
DOI 10.1007/s00425-004-1447-7

ORIGINAL ARTICLE

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The *Arabidopsis* SERK1 protein interacts with the AAA-ATPase *At*CDC48, the 14-3-3 protein GF14λ and the PP2C phosphatase KAPP

Received: 21 June 2004 / Accepted: 10 September 2004 / Published online: 9 December 2004

Abstract Leucine-rich repeat (LRR)-containing transmembrane receptor-like kinases (RLKs) are important components of plant signal transduction. The *Arabidopsis thaliana* somatic embryogenesis receptor-like kinase 1 (*At*SERK1) is an LRR-RLK proposed to participate in a signal transduction cascade involved in embryo development. By yeast two-hybrid screening we identified *At*CDC48, a homologue of the mammalian AAA-ATPase p97 and GF14λ, a member of the *Arabidopsis* family of 14-3-3 proteins as *At*SERK1 interactors. In vitro, the *At*SERK1 kinase domain is able to transphosphorylate and bind both *At*CDC48 and GF14λ. In yeast, *At*CDC48 interacts with GF14λ and with the PP2C phosphatase KAPP. In plant protoplasts *At*SERK1 interacts with GF14λ.

Keywords 14-3-3 Protein · *Arabidopsis* · Cell division cycle protein 48 · Kinase associated protein phosphatase (KAPP) · Signal transduction · Somatic embryogenesis receptor-like kinase 1

Abbreviations RLK: Receptor-like kinase · LRR: Leucine-rich repeat · MBP: Maltose binding protein · GST: Glutathione-S-transferase · FRET: Förster resonance energy transfer · FSPIM: Fluorescence spectral imaging microscopy · APB: Acceptor photobleaching · CFP: Cyan fluorescent protein · YFP: Yellow fluorescent protein

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Introduction

Receptor-like protein kinases (RLKs) are key elements in many signal transduction chains. RLKs consist of an extracellular domain, a transmembrane domain and an intracellular catalytic kinase domain. A large family amongst the plant RLKs consists of the leucine-rich repeat (LRR)-containing transmembrane receptors. We have previously identified the *Arabidopsis* somatic embryogenesis receptor-like kinase 1 (*At*SERK1), which is an RLK containing a leucine zipper, five LRRs, a single transmembrane domain and a functional serine-threonine kinase (Hecht et al. 2001; Shah et al. 2001b). In plants, *At*SERK1 is ubiquitously expressed, but shows highest expression in ovule primordia and the entire female gametophyte (Hecht et al. 2001). Ectopic expression of *At*SERK1 does not result in obvious phenotypes in *Arabidopsis* plants but increases somatic embryo formation in culture. Based on its expression pattern and its involvement in embryo formation, *At*-SERK1 is proposed to participate in an uncharacterised signal transduction cascade involved in ovule and embryo development (Hecht et al. 2001). Another member of the five-membered *At*SERK family was identified as the brassinosteroid insensitive 1 (BRI1) associated kinase 1 (BAK1) based on a suppressor screen as well as on a yeast interaction screen (Li et al. 2002; Nam and Li 2002). BAK1 is identical to *At*SERK3 and is proposed to function as the heterodimeric partner of the BRI1 receptor (Li et al. 2002).

The components of the *At*SERK1 signalling complex are mostly unknown. Previous work has shown that *At*SERK1 interacts with the PP2C kinase-associated protein phosphatase (KAPP), which was found to play a role in *At*SERK1 internalisation (Shah et al. 2002). To identify additional components of the *At*SERK1 signalling complex, we used the yeast two-hybrid system to find proteins interacting with the kinase domain of *At*-SERK1. Here we report that *At*SERK1 also interacts with the AAA-ATPase *At*CDC48 and the 14-3-3 protein

GF14λ. This shows analogy to the mammalian CDC48 homologue p97/VCP, which can be phosphorylated by the JAK-2 kinase and dephosphorylated by the phosphatase PTPH1 that associates with a 14-3-3 protein (Zhang et al. 1997, 1999).

Materials and methods

Yeast two-hybrid screening

The DuplexA yeast two-hybrid (Origene Technologies Inc.) system was used to screen for proteins interacting with *At*SERK1. An *Arabidopsis* (ecotype Landsberg erecta) cDNA library was prepared as a fusion to the activation domain in vector pJG4-5 (Origene Technologies Inc.) using mRNA from young silique tissue (Grebe et al. 2000). The primary library contained approximately 2×10^6 cDNA clones. The nucleotide sequence encoding the kinase domain of *At*SERK1 (GenBank accession no. A67827; a.a. 266–625) was cloned into the bait vector pEG202 (Origene Technologies Inc.) to produce a fusion protein with LexA (Shah et al. 2001a). Repression assays indicated that the LexA-*At*SERK1^{kinase} protein is transported correctly into the nucleus and autoactivation assays verified that the LexA-*At*SERK1^{kinase} protein does not autonomously activate the *lacZ* or the *LEU2* reporter genes. The cDNA library was transformed into yeast strain EGY48 (MATα, his3, trp1, ura3, $LexA_{op(x6)}$-*LEU2*) containing pEG202-*At*SERK1^{kinase} and the reporter plasmid pSH18-34. Colonies were judged positive when they grew in the absence of leucine at 30°C and showed visible β-galactosidase activity within 3 days. Screening of approximately 30×10^6 yeast transformants on selection plates containing 80 mg/l Xgal resulted in 200 clones that showed β-galactosidase activity and leucine auxotrophy. After excluding clones with *At*SERK1^{kinase}-independent activation or autoactivation of the reporter genes, 12 clones encoding putative *At*SERK1 interacting proteins remained.

Interacting proteins were identified as GF14λ (accession no. NP_568229), and *At*CDC48 (accession no. S60112). Interaction tests were performed with the *At*SERK1 kinase domain (a.a. 266–625), the entire mature *At*SERK1 protein without the N-terminal signal sequence (*At*SERK1$^{\Delta SS}$, a.a. 31–625), the *At*SERK1 extracellular domain (*At*SERK1^{LRR} ; a.a. 26–234) or the non-related human Lamin C protein in combination with GF14λ, *At*CDC48$^{747\text{-}809}$ or longer parts of *At*CDC48 encoded by EST cDNA clones 104O24 (accession no. T22005) and 180L9 (accession no. H36923) obtained from the *Arabidopsis* Biological Resource Centre. Interaction was also tested using *At*SERK1 cloned in pJG4-5 as prey, and the EST encoded peptides cloned in pEG202 as bait. The yeast two-hybrid system was also used to determine whether *At*CDC48 could interact with the *At*SERK1 interacting proteins GF14λ and KAPP. Therefore, pairwise interaction tests were performed using *At*SERK1^{kinase}, *At*CDC48$^{549\text{-}809}$, *At*CDC48$^{386\text{-}809}$, GF14λ, KAPP and KI-KAPP (Shah et al. 2002) cloned in pJG4-5 and pEG202. Interaction was determined after incubation at 30°C for 2 days.

Protein expression and affinity purification

To express the proteins *At*CDC48$^{747\text{-}809}$, *At*CDC48$^{386\text{-}809}$, GF14λ, GF14$\lambda^{39\text{-}248}$ and A *t*SERK1^{kinase} translational fusions were made to glutathione-S-transferase (GST) and in the case of *At*CDC48$^{747\text{-}809}$, *At*CDC48$^{386\text{-}809}$ also to the maltose binding protein (MBP). Protein expression of GST-fusion proteins and GST was induced with 0.1 mM isopropyl β-D-thiogalactopyranoside. Proteins were solubilised by sonification and GST-fused proteins were bound to glutathione sepharose 4B beads following the manufacturer's instructions (Pharmacia Biotech Inc.). The GST-GF14λ and GST-GF14$\lambda^{39\text{-}248}$ fusion proteins were digested for 7 h with thrombin, releasing GF14λ and GF14$\lambda^{39\text{-}248}$ from glutathione sepharose 4B bound GST. GST and GST-*At*SERK1^{kinase} proteins were eluted from the beads with 15 mM reduced glutathione in 50 mM Tris pH 8.0 and 150 mM NaCl. Protein expression of MBP-fusion proteins and MBP was induced with 0.3 mM IPTG. The proteins were solubilised by sonification and bound to amylose resin (New England Biolabs). Soluble MBP-fusion proteins were purified from the supernatant by affinity chromatography using amylose resin chromatography according to the manufacturer's instructions. Proteins were eluted from the resin with 15 mM maltose in column buffer (20 mM Tris–HCl, 200 mM NaCl, 1 mM EDTA and 1 mM DTT). All fusion proteins were dialysed against 50 mM Tris pH 7.4, 1 mM DTT and 0.1 mM EDTA after purification. Protein concentrations were determined by Bradford micro-assay (Bio-Rad) using BSA as a standard.

Production of αGF14λ, αGST-*At*SERK1^{kinase} and α*At*SERK1^{kinase} antibodies

αGF14λ and αGST-*At*SERK1^{kinase} antibodies were generated by immunising rabbits with the purified full length GF14λ protein without the GST-tag or with the purified fusion protein GST-*At*SERK1^{kinase}. The rabbit serum was purified from antibodies directed against bacterial proteins by passing the serum over immobilised bacterial protein extract. For Western analysis, the αGF14λ antiserum and the αGST-*At*SERK1^{kinase} antiserum were used at a 1:15,000 dilution. Because the αGST-*At*SERK1^{kinase} antiserum was raised against the complete GST-*At*SERK1^{kinase} fusion protein, it recognises *At*SERK1^{kinase} as well as GST. The αGF14λ antiserum shows cross-reactivity with *At*SERK1^{kinase} and an unidentified 50 kDa protein, but not with GST.

Protein binding assays

Protein binding assays were performed with phosphorylated or non-phosphorylated GST-*At*SERK1^{kinase}. GST-*At*SERK1^{kinase} was phosphorylated by adding 100 μM ATP and incubated for 30 min at 30°C in a total volume of 30 μl protein binding buffer (PBB: 20 mM HEPES pH 7.5, 150 mM KCl, 1 mM DTT, 0.1 mM EDTA, 5 mM $MgCl_2$). GST and GST-*At*SERK1^{kinase} (3 μg) were bound to glutathione sepharose 4B and *At*CDC48$^{747-809}$ and *At*CDC48$^{386-809}$ (300 ng) were bound to amylose resin by incubation at room temperature for 30 min. After immobilising the proteins, the glutathione sepharose 4B beads were first blocked with 1% (w/v) skimmed milk, 0.1% (v/v) Triton X-100 and then with 2% (w/v) BSA, 0.1% (v/v) Triton X-100 both for 30 min at 4°C. For the binding assays, 1 μg of GF14λ or GF14λ$^{39-248}$ was added to immobilised GST or GST-*At*SERK1^{kinase} and 100 ng of GST or GST-*At*SERK1^{kinase} was added to immobilised *At*CDC48$^{747-809}$ and *At*CDC48$^{386-809}$. The proteins were then incubated for 2 h at room temperature in 300 μl PBB. The immobilised protein complexes were washed three times with 1 ml PBB. Bound proteins were removed from the beads by adding SDS-PAGE sample buffer to the beads and incubating at 100°C for 5 min. The proteins were then separated by 12% SDS-PAGE and detected by Western analysis.

Phosphorylation assays

Transphosphorylation of GF14λ, GF14λ$^{39-248}$, *At*CDC48$^{747-809}$ and *At*CDC48$^{386-809}$ was tested by incubating 500 ng protein in the presence of 500 ng GST-*At*SERK1^{kinase} for 45 min at 30°C in phosphorylation buffer (20 mM Tris, pH 7.5, 150 mM KCl, Triton, 10 mM $MgCl_2$) containing 50 μM unlabelled ATP and 3.7×10^5 Bq of [$\gamma-^{32}$P]ATP in a final volume of 30 μl. The reaction was stopped by adding SDS-PAGE sample buffer and incubated for 5 min at 100°C. The proteins were separated by 10% SDS-PAGE. Radioactivity was detected with a PhosphoImager using the Image Quant program (Molecular Dynamics). *At*SERK1^{kinase}-specific phosphorylation was tested by incubating the fusion proteins with ATP in the absence of GST-*At*SERK1^{kinase}.

Localisation and interaction of CFP/YFP fusion proteins in protoplasts

The cDNA sequence encoding complete GF14λ was amplified from the EST cDNA clone 190N19 in plasmid λZiplox with a gene-specific primer on the transcription start of GF14λ containing an *Eco*RI site (5′-GGG AAT TCG GCG GCG ACA TTA GGC AGA G-3′) and a λZiplox-specific primer containing a *Xho*I site (5′-CCG CTC GAG GGC CAG TGA ATT GAA TTT AGG-3′). The PCR fragment was digested with *Eco*RI and *Xho*I and cloned in the corresponding sites of vector pMON999 containing the cyan fluorescent protein (CFP) gene, resulting in the CFP-GF14λ construct. In addition, the PCR fragment was also digested with *Eco*RI and *Bam*HI and cloned in the corresponding sites of vector pEYFP*-C1 (Clontech). The resulting construct was then digested with *Nhe*I and *Bam*HI and the yellow fluorescent protein (YFP)-14-3-3 fragment was cloned in the *Xba*I and *Bam*HI sites of vector pMon999, resulting in the YFP-GF14λ construct. The CFP/YFP fusion proteins were constructed and transiently expressed in cowpea (*Vigna unguiculata* L.) mesophyll protoplasts as described (Shah et al. 2001a). The CFP and YFP fluorescence was analysed as described previously by Shah et al. (2002). For DAPI staining, protoplasts were fixated for 10 min in phosphate-buffered saline with 4% paraformaldehyde and washed three times with phosphate-buffered saline (pH 7.5). Protoplasts were stained with 1 μg/ml 4,6-dianidino-2-phenylindole (DAPI) in Vectashield antifade solution (Vector Laboratories Inc.) and analysed with a Zeiss epi-fluorescent microscope equipped with a CCD-camera. Pictures were taken with a DAPI filter and a FITC filter using Genus software (Applied Imaging).

Förster resonance energy transfer (FRET) between the fluorescently labelled *At*SERK1^{kinase} and 14-3-3 proteins was measured by fluorescence spectral imaging microscopy (FSPIM) as described by Shah et al. (2002). Spectral images were acquired using a 60× oil immersion objective with a 150 groove/mm grating on the entrance slit of the spectrograph, set at a central wavelength of 500 nm and a slit width of 100 μm corresponding to 5 μm in the object plane. Typical exposure and CCD integration time was 3 s. The resulting fluorescence spectra were corrected for background fluorescence and camera bias by background subtraction using an extracellular region from the same spectral image. Acceptor photobleaching (APB) (Wouters and Bastiaens 1999) was performed by bleaching the YFP fluorophore in a previously defined region for 2 s with 50–100% laser power at 514 nm. Images of the protoplast were taken with a 3.25 s time interval.

Results

The *At*SERK1 kinase domain interacts with *At*CDC48 and GF14λ in yeast

To identify proteins that physically interact with *At*SERK1 (Hecht et al. 2001), a yeast two-hybrid cDNA library from *Arabidopsis* young silique tissue (Grebe et al. 2000) was screened with the kinase domain of *At*SERK1 (a.a. 266–625, *At*SERK1^{kinase}). Two clones were found to contain the coding sequence for the C-terminal 62 and 65 residues of the *At*CDC48A protein (At3g09840). *At*CDC48A is a protein of 809 residues with a predicted molecular mass of 89 kDa and of the

three *At*CDC48 isoforms the most abundant one (Rancour et al. 2002). We used ESTs encoding the C-terminal 260 (*At*CDC48$^{549\text{-}809}$) and 423 (*At*CDC48$^{386\text{-}809}$) residues of *At*CDC48 to confirm the interaction with *At*SERK1. All *At*CDC48 peptides interacted with the kinase domain of *At*SERK1 and not with *At*SERK1^{LRR} or the human Lamin C protein used as negative controls (data not shown).

Three identical clones were found to contain the complete coding sequence of GF14λ, a member of the *Arabidopsis* family of 14-3-3 proteins, while a fourth clone lacks the N-terminal 38 residues and was designated GF14$\lambda^{39\text{-}248}$. Control experiments in yeast showed that GF14λ interacts with *At*SERK1^{kinase} but not with *At*SERK1^{LRR} nor with the Lamin C protein (data not shown). We could not perform the reciprocal interaction experiment, because GF14λ auto-activates reporter gene expression when fused to LexA. This was also observed for other 14-3-3 proteins and is probably due to the acidic nature of 14-3-3 proteins (Wu et al. 1997; Wang et al. 1999). Because GF14λ activates the reporter genes in an *At*SERK1^{kinase}-dependent way when fused to the DNA-binding domain we conclude that *At*SERK1 does interact with GF14λ in yeast.

The *At*SERK1 kinase domain interacts with *At*CDC48 and GF14λ in vitro

To confirm the existence of a direct interaction between *At*SERK1 and the proteins *At*CDC48 and GF14λ, we expressed the *At*SERK1 kinase domain (*At*SERK1^{kinase}) as C-terminal fusion protein with GST in *Escherichia coli* followed by affinity purification on glutathione resin. Because GST is known to form homodimers by itself, we expressed and affinity-purified the C-terminal MBP fusion proteins MBP-*At*CDC48$^{747\text{-}809}$ and MBP-*At*CDC48$^{386\text{-}809}$. GF14λ and GF14$\lambda^{38\text{-}249}$ were expressed as GST-fusion proteins but had their GST-tag removed after purification.

GST-*At*SERK1^{kinase} was added to amylose resin with immobilised *At*CDC48 and tested for its ability to bind to *At*CDC48. Binding assays were performed using phosphorylated or non-phosphorylated *At*SERK1^{kinase}. Bound proteins were eluted and subjected to SDS-PAGE and Western blot analysis using the αGST-*At*SERK1^{kinase} antiserum (Fig. 1). GST-*At*SERK1^{kinase} was phosphorylated by incubating the protein with ATP at phosphorylating conditions. Autophosphorylation activity of the *At*SERK1 kinase domain was verified by incubating the GST-*At*SERK1^{kinase} protein in the presence of [γ–^{32}P]ATP. Figure 1 shows that MBP-*At*CDC48$^{747\text{-}809}$ does not interact with non-phosphorylated GST-*At*SERK1^{kinase} (Fig. 1, lane 2) or with GST (Fig. 1, lane 1). Upon phosphorylation of *At*SERK1, MBP-*At*CDC48$^{747\text{-}809}$ and GST-*At*SERK1^{kinase} do interact (Fig. 1, lane 3). MBP-*At*CDC48$^{386\text{-}809}$ interacts both with non-phosphorylated (Fig. 1, lane 6) and phosphorylated GST-*At*SERK1^{kinase} (Fig. 1, lane 7) and

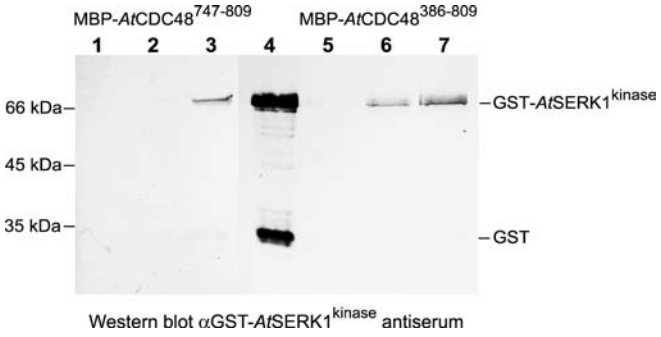

Fig. 1 Protein binding assays for binding of GST-*At*SERK1^{kinase} to immobilised MBP-*At*CDC48$^{747\text{-}809}$ (*lane 1–3*) or MBP-*At*CDC48$^{386\text{-}809}$ (*lane 5–7*). Proteins bound to the sepharose beads were pelleted, washed, eluted, subjected to SDS-PAGE and detected by Western blotting using αGST-*At*SERK1^{kinase} antiserum. The αGST-*At*SERK1^{kinase} antiserum cross-reacts with GST. *Lane 1* GST, *lane 2* non-phosphorylated GST-*At*SERK1^{kinase}, *lane 3* phosphorylated GST-*At*SERK1^{kinase}, *lane 4* GST and GST-*At*SERK1^{kinase} (100 ng), *lane 5* GST, *lane 6* non-phosphorylated GST-*At*SERK1^{kinase}, *lane 7* phosphorylated GST-*At*SERK1^{kinase}

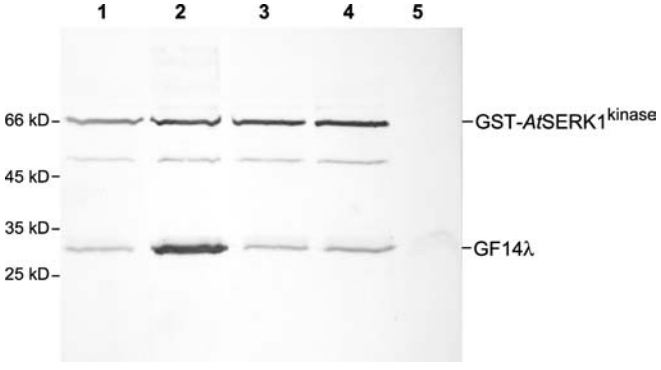

Fig. 2 Protein binding assays for binding of GF14λ and GF14$\lambda^{39\text{-}248}$ to immobilised GST-*At*SERK1^{kinase}. Proteins bound to the sepharose beads were pelleted, washed, eluted, subjected to SDS-PAGE and detected by Western blotting using αGF14λ antiserum. The αGF14λ antiserum cross-reacts with the GST-*At*SERK1^{kinase} and an unidentified 50 kDa protein. *Lane 1* GF14λ and non-phosphorylated GST-*At*SERK1^{kinase}, *lane 2* GF14λ and phosphorylated GST-*At*SERK1^{kinase}, *lane 3* GF14$\lambda^{39\text{-}248}$ and non-phosphorylated GST-*At*SERK1^{kinase}, *lane 4* GF14$\lambda^{39\text{-}248}$ and phosphorylated GST-*At*SERK1^{kinase}, *lane 5* GF14λ and GST

not with GST (Fig. 1, lane 5). Upon phosphorylation of *At*SERK1^{kinase} there was an increase in interaction with *At*CDC48$^{386\text{-}809}$. Lane 4 contains GST-*At*SERK1^{kinase} and free GST. From these results, we conclude that MBP-*At*CDC48$^{386\text{-}809}$ and *At*CDC48$^{747\text{-}809}$ bind to the kinase domain of *At*SERK1. Although phosphorylation of *At*SERK1 enhances the interaction, it is not clear whether the interaction of *At*SERK1 with the C-terminal amino acids of *At*CDC48 is phosphorylation dependent.

The results of the protein binding assay employing immobilised GST-*At*SERK1^{kinase} and GF14λ are shown in Fig. 2. GF14λ interacts with GST-*At*SERK1^{kinase} (Fig. 2, lane 1), but not with GST alone (Fig. 2, lane 5). Phosphorylated GST-*At*SERK1^{kinase} protein binds more

GF14λ (Fig. 2, lane 2) than non-phosphorylated GST-*At*SERK1^{kinase} (Fig. 2, lane 1). Through comparison of the band intensities on the Western blot, we estimated that interaction of GF14λ with phosphorylated *At*-SERK1 is about fivefold higher compared to non-phosphorylated *At*SERK1.

The same experiments were repeated for GF14λ$^{39\text{-}248}$. Interaction between non-phosphorylated GST-*At*-SERK1^{kinase} and either GF14λ or GF14λ$^{39\text{-}248}$ is observed with equal intensities (Fig. 2, lanes 1 and 3). When the binding assay is performed using phosphorylated GST-*At*SERK1^{kinase} (Fig. 2, lane 4) no difference was observed in the ability to bind GF14λ$^{39\text{-}248}$ compared to non-phosphorylated GST-*At*SERK1^{kinase} (Fig. 2, lane 3). Due to the gel system we used, we did not see the expected difference of 4 kDa in molecular size between GF14λ and GF14λ$^{39\text{-}248}$. From these results, we conclude that there is a phosphorylation-independent interaction between GF14λ and the *At*SERK1 kinase domain as well as a phosphorylation-dependent interaction. This phosphorylation-dependent interaction requires the first 38 amino acids of the GF14λ protein.

*At*SERK1^{kinase} is able to transphosphorylate *At*CDC48 and GF14λ

*At*SERK1^{kinase} is able to transphosphorylate proteins in vitro (Shah et al. 2001b). Therefore, phosphorylation assays were performed to further substantiate the interaction between *At*SERK1 and *At*CDC48. *At*CDC48$^{747\text{-}809}$ was expressed as fusion protein with MBP but due to the size of MBP (42.5 kDa) also as fusion proteins with GST (25 kDa). To allow *At*SERK1 to transphosphorylate *At*CDC48, both the MBP- and GST-derived fusion proteins were incubated with GST-*At*SERK1^{kinase} in the presence of [$\gamma-^{32}$P]ATP. The reaction was stopped by adding protein sample buffer and the samples were subjected to SDS-PAGE and subsequent autoradiography. The phospho-images in Fig. 3 show that *At*SERK1 is able to transphosphorylate MBP- and GST-*At*CDC48$^{747\text{-}809}$ (Fig. 3b, lanes 1 and 3). *At*SERK1^{kinase} does not transphosphorylate GST nor MBP (not shown) and *At*CDC48 is not phosphorylated in the absence of *At*SERK1^{kinase} (Fig. 3b, lane 2). *At*SERK1 autophosphorylation is stronger than transphosphorylation on *At*CDC48. One explanation for this is the presence of multiple phosphorylation sites in the *At*SERK1^{kinase} protein (Shah et al. 2001b) in comparison to only one (or two) phosphorylation site(s) in *At*CDC48$^{747\text{-}809}$ (next section).

In yeast, phosphorylation of a tyrosine residue in the C-terminal DDDLYS sequence of *Sc*Cdc48 was shown to regulate cell-cycle-dependent shuttling of the protein between nucleus and cytoplasm due to exposure of the N-terminal nuclear localisation signals (Madeo et al. 1998). Therefore, we determined which residue(s) in *At*CDC48 is phosphorylated by *At*SERK1 using α(p)Thr, α(p)Ser and α(p)Tyr antibodies. α(p)Thr antibodies recognised the GST-*At*CDC48$^{747\text{-}809}$ peptide after phosphorylation by GST-*At*SERK1^{kinase} (Fig. 3c) while α(p)Ser (Fig. 3d) and α(p)Tyr (Fig. 3e) antibodies did not. According to the prediction of phosphorylation sites by NetPhos 2.0 (http://www.cbs.dtu.dk/services/NetPhos) there are two putative threonine phosphorylation sites within the C-terminal 62 amino acids of *At*CDC48. One of these sites is conserved amongst the *Arabidopsis* *At*CDC48 family (Thr^{796} of *At*CDC48A) but not in yeast. Although *At*SERK1 is able to phosphorylate on tyrosine residues of the common substrate myelin basic protein (Shah et al. 2001b), it clearly does not phosphorylate the tyrosine residue in the C-terminal DDDDLYN sequence of *At*CDC48 (Fig. 3e). Therefore, we conclude that *At*SERK1 phosphorylates *At*CDC48 on at least one threonine residue within the C-terminal 62 amino acids, which may have a comparable function as phosphorylation of the tyrosine residue at the C-terminus resulting in cellular translocation of the *At*CDC48 protein.

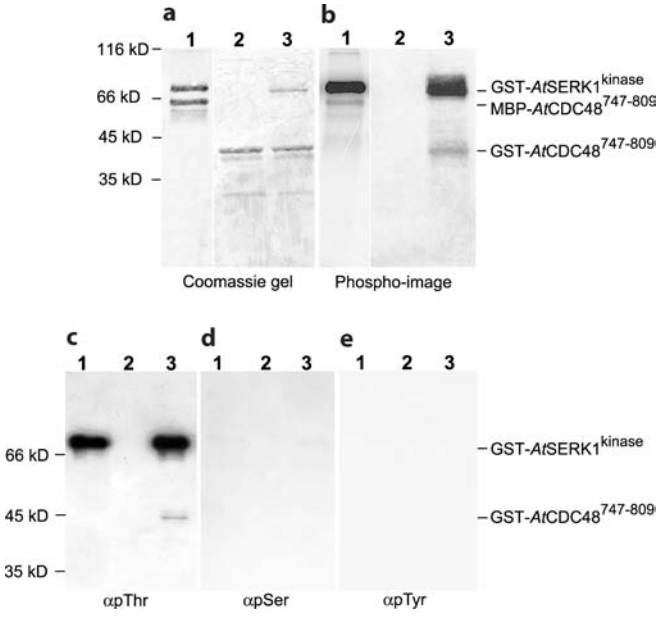

Fig. 3 Transphosphorylation of *At*CDC48 by GST-*At*SERK1^{kinase}. Gels were stained with Coomassie brilliant blue (**a**) and radiolabelling detected with a PhosphoImager (**b**). **a**, **b** *Lane 1* GST-*At*SERK1^{kinase} and MBP-*At*CDC48$^{747\text{-}809}$, *lane 2* GST-*At*CDC48$^{747\text{-}809}$, *lane 3* GST-*At*SERK1^{kinase} and GST-*At*CDC48$^{747\text{-}809}$. Transphosphorylation of *At*CDC48$^{747\text{-}809}$ was detected by Western analysis using α(p)Thr antibodies (**c**), α(p)Ser antibodies (**d**) or α(p)Tyr antibodies (**e**). *Lane 1* GST-*At*SERK1^{kinase}, *lane 2* GST-*At*CDC48$^{747\text{-}809}$, *lane 3* GST-*At*SERK1^{kinase} + GST-*At*CDC48$^{747\text{-}809}$

It has been reported that some protein kinases can use a 14-3-3 protein as substrate (Lu et al. 1994; van der Hoeven et al. 2000). Therefore, in order to further substantiate the interaction between *At*SERK1 and GF14λ, we determined whether *At*SERK1 is also able to transphosphorylate GF14λ. Figure 4 shows that besides autophosphorylation, GST-*At*SERK1^{kinase} can transphosphorylate both GF14λ and GF14λ$^{39\text{-}248}$ with comparable intensity; thus phosphorylation is not restricted

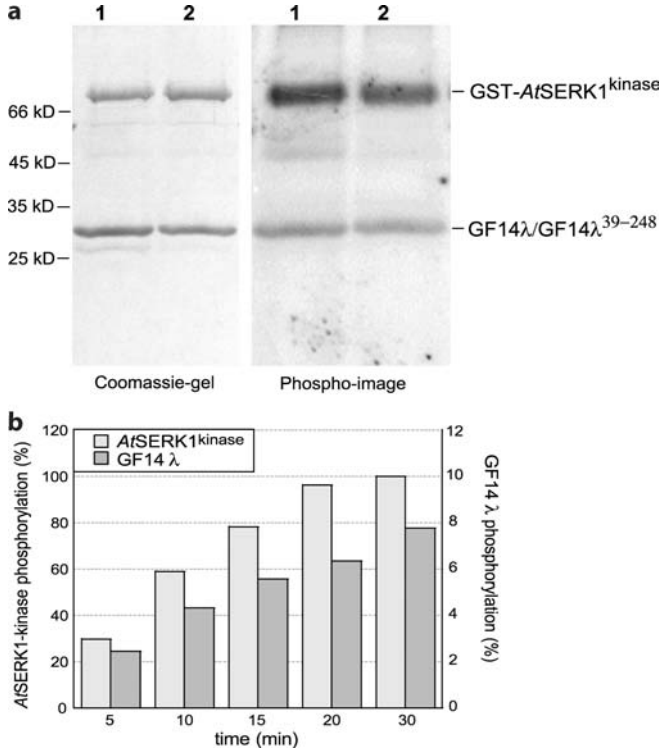

Fig. 4 Transphosphorylation of GF14λ and GF14$\lambda^{39\text{-}248}$ by GST-*At*SERK1. Gels were stained with Coomassie brilliant blue (**a**) and radiolabelling detected with a PhosphoImager (**b**). *Lane 1* GST-*At*SERK1^{kinase} with GF14λ, *lane 2* GST-*At*SERK1^{kinase} with GF14$\lambda^{39\text{-}248}$. **c** Quantification of GST-*At*SERK1 autophosphorylation and GF14λ transphosphorylation

to the N-terminal 38 residues of GF14λ. The observed GF14λ phosphorylation requires the presence of *At*SERK1 as no phosphorylation was observed after incubating GF14λ or GF14$\lambda^{39\text{-}248}$ with [$\gamma-^{32}$P]ATP in the absence of *At*SERK1^{kinase} (data not shown). This suggests that GF14λ can be a natural substrate of *At*SERK1.

Although the biological significance of all interactions with 14-3-3 proteins that have been detected is not known, it is generally agreed that 14-3-3 proteins have a regulatory function through protein–protein interactions. Therefore, we determined whether binding of GF14λ to the *At*SERK1 kinase domain has an effect on phosphorylation. We compared the kinetics of GF14λ phosphorylation by *At*SERK1^{kinase} to *At*SERK1^{kinase} autophosphorylation (Fig. 4b). In a standard assay with 10 mM $MgCl_2$ and 500 ng *At*SERK1^{kinase} autophosphorylation is complete within 30 min (Shah et al. 2001b). Thus, we set the pixel intensity of the *At*SERK1^{kinase} autophosphorylation band at 30 min at 100% and compared the intensity of the other bands on the autoradiogram with this band. In the time-interval we used, GF14λ is being transphosphorylated as soon as *At*SERK1 is autophosphorylated and *At*SERK1 autophosphorylation is not impaired by the presence of phosphorylated GF14λ. We cannot state with certainty whether non-phosphorylated GF14λ does not interfere with *At*SERK1 autophosphorylation. However, we consider this to be unlikely due to the comparable autophosphorylation kinetics of *At*SERK1 in the presence and absence of partially phosphorylated GF14λ such as present in the reaction mixture after 10 min (data not shown). Like observed for *At*CDC48, GF14λ transphosphorylation is lower than *At*SERK1^{kinase} autophosphorylation. This could also be due to a lower number of target phosphorylation sites on GF14λ in comparison to *At*SERK1. From these results, we conclude that the binding of GF14λ to the kinase domain of *At*SERK1 does not affect its phosphorylation activities in vitro.

Table 1 Pairwise interaction tests between *At*SERK1^{kinase}, *At*CDC48$^{549\text{-}809}$, GF14λ, KAPP and KI-KAPP cloned in pJG4-5 (BD) and pEG202 (AD) using the yeast two-hybrid system

	AD-*At*SERK1^{kinase}	AD-*At*CDC48$^{549\text{-}809}$
BD-*At*CDC48$^{549\text{-}809}$	+	+ +
BD-GF14λ	+	+ +
BD-KAPP	+ +	+
BD-KI-KAPP	+ +	–

Interaction tests were done in two independent experiments using a minimum of five colonies per interaction + + Strong interaction, + interaction, – no interaction

The *At*SERK1 interacting proteins also interact with each other

In addition to *At*CDC48 and GF14λ, the kinase domain of *At*SERK1 can also bind the KAPP (Shah et al. 2002). The phosphatase KAPP was determined to physically and functionally interact with the *At*SERK1 kinase domain, as it is able to dephosphorylate phosphorylated *At*SERK1 and actively prevents autophosphorylation of the kinase (Shah et al. 2002). In addition, yeast two-hybrid interaction tests showed that KAPP binds to *At*SERK1 with its kinase interaction domain (KI-KAPP). To determine whether one or more of the *At*SERK1 interacting proteins can also interact with each other, we performed a pairwise yeast two-hybrid interaction test using *At*SERK1^{kinase}, *At*CDC48$^{549\text{-}809}$, GF14λ, KAPP and KI-KAPP cloned in pJG4-5 and pEG202. Unfortunately, not all interactions could be tested due to autoactivation of the reporter genes by KI-KAPP, KAPP and GF14λ when fused to the LexA DNA-binding protein of pEG202. As summarised in Table 1, *At*CDC48$^{549\text{-}809}$ appears to interact not only with *At*SERK1 but also with itself, with GF14λ and with KAPP. While *At*SERK1 interacts with the kinase interaction domain of KAPP, *At*CDC48$^{549\text{-}809}$ only interacts with the complete KAPP protein. Yeast CDC48p (Fröhlich et al. 1995) as well as *Arabidopsis* CDC48 (Rancour et al. 2002) homohexadimerise to form a functional complex in vivo. Based on our experiments, we cannot state whether the observed

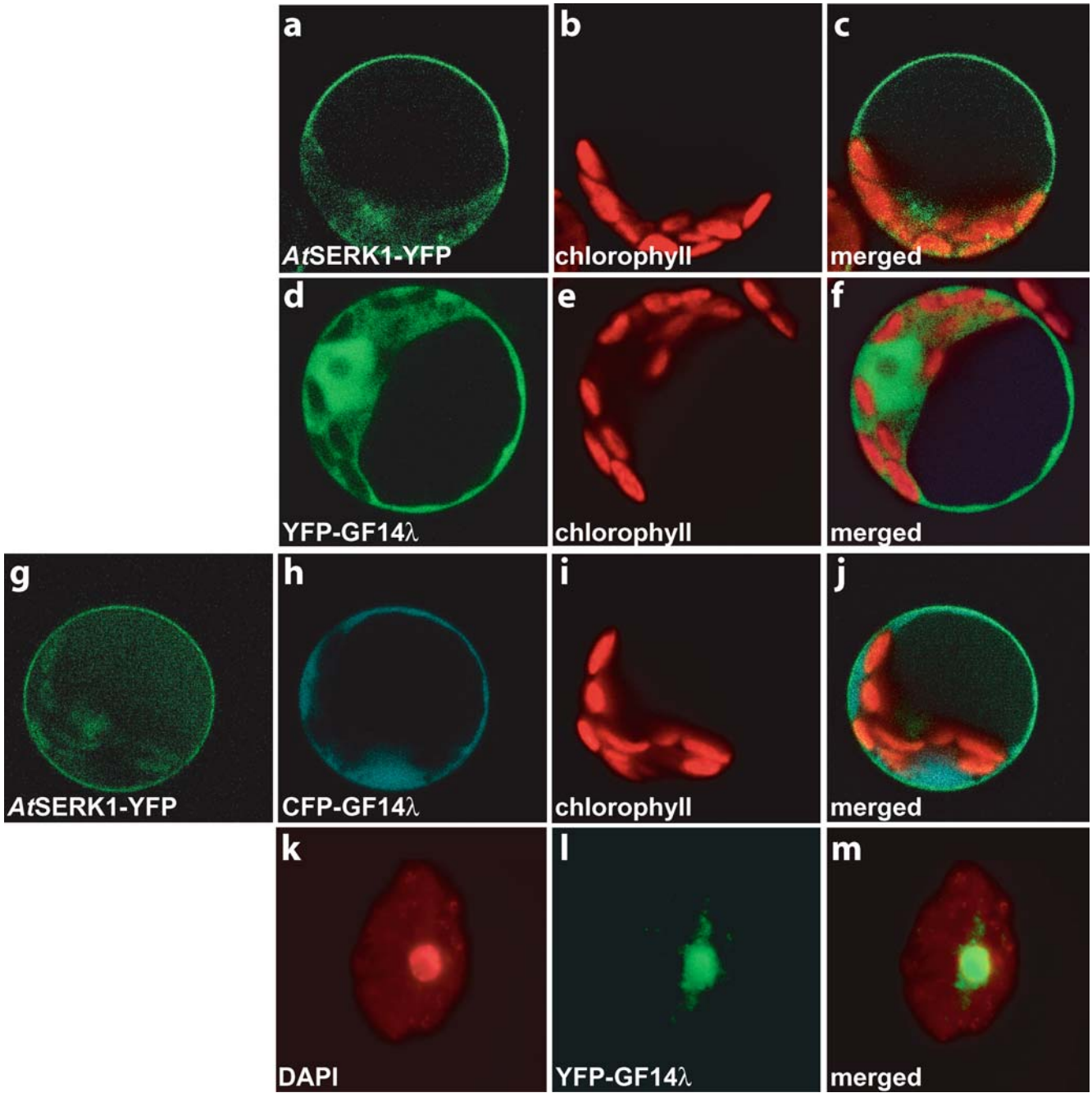

Fig. 5 Cellular localisation of fluorescently labelled *At*SERK1 and GF14λ in cowpea mesophyll protoplasts. All images were obtained by CLSM. Chlorophyll auto-fluorescence is shown in *red*, YFP fluorescence is shown in *green* and CFP fluorescence is shown in *cyan*. **a**–**c** Cellular localisation of *At*SERK1-YFP. The superimposed images of (**a**) and (**b**) result in (**c**). **d**–**f** Cellular localisation of YFP-GF14λ. The superimposed images of (**d**) and (**e**) result in (**f**). **g**–**j** Co-localisation of *At*SERK1-YFP and CFP-GF14λ. The superimposed images of (**g**), (**h**) and (**i**) result in (**j**). **k**–**m** DAPI staining in a fixated protoplast (**k**) and localisation of YFP-GF14λ fluorescence in the nucleus of the same protoplast (**l**). The superimposed images of (**k**) and (**l** result in (**m**)

interaction between CDC48 peptides and the *At*SERK1 receptor kinase also occurs with the entire hexameric complex.

Co-localisation and interaction of *At*SERK1 and GF14λ in plant protoplasts

The GF14λ protein was found to be constitutively expressed in *Arabidopsis*, although expression can be enhanced with cold treatment (Jarillo et al. 1994; Zhang et al. 1995). Therefore, we determined the occurrence of co-localisation and a molecular interaction of GF14λ and *At*SERK1 at the cellular level in cowpea mesophyll protoplasts. For this we fused the entire coding region of GF14λ to the C-terminus of CFP and YPF (CFP-GF14λ and YFP-GF14λ). The entire coding region of *At*SERK1 was fused to the N-terminus of YFP (*At*SERK1-YFP). The fusion proteins were then

transiently expressed in cowpea protoplasts. Confocal laser scanning microscopy (CLSM) was used to image cells expressing the fusion proteins. Figure 5 shows the localisation of fluorescent signals for *At*SERK1-YFP (Fig. 5a–c), YFP-GF14λ (Fig. 5d–f) and CFP-GF14λ together with *At*SERK1-YFP (Fig. 5g–j). For *At*SERK1-YFP, it has previously been shown that a fluorescent signal can be detected at the plasma membrane and to a lesser extent at endomembranes (Shah et al. 2001a). Figure 5a, c confirm the previously noted location of the *At*SERK1 protein. For YFP-GF14λ, fluorescence can be detected in the nucleus and the cytosol. Nuclear localisation of YFP-GF14λ was confirmed by co-localisation of YFP-GF14λ fluorescence and DAPI staining (Fig. 5k–m). The 14-3-3 proteins do not contain any obvious cellular localisation signal, although many differences in cellular organisation exist among the 14-3-3 proteins. For example the *Arabidopsis* GF14κ isoform is found in the nucleus and the plasma membrane region, and GF14υ is found in the cytoplasm and in the chloroplast stroma (Sehnke et al. 2002). To examine the subcellular localisation of *At*SERK1 in the presence of GF14λ, we co-expressed CFP-GF14λ with *At*SERK1-YFP in protoplasts. The superimposed image (Fig. 5j) shows co-localisation of the proteins at the plasma membrane. No obvious difference in localisation of either protein is observed upon co-expression.

The occurrence of a molecular interaction between *At*SERK1-YFP and CFP-GF14λ was studied by measuring the FRET between the CFP (donor) and YFP (acceptor) fluorophores. The FRET occurs when the fluorophores interact based on dipole–dipole interaction, which is only possible at close proximity (5 nm for CFP and YFP). In the case of FRET, the CFP fluorescence will be quenched and the YFP fluorescence will be increased, which indicates a molecular interaction of the proteins that are fused to the fluorophores. FRET was measured using FSPIM as a detection method. Spectral images were taken from small regions of protoplasts co-expressing *At*SERK1-YFP and CFP-GF14λ at approximately equal expression levels and fluorescence emission spectra were generated. As a control spectral images were taken from protoplasts expressing only CFP-GF14λ. Figure 6 shows the emission spectra of CFP and YFP fluorescence in protoplasts. The peak at 480 nm corresponds to CFP emission and the peak at 520 nm to YFP fluorescence. The ratio of 480 to 520 nm emission intensity acts as an indicator of the extent of FRET. In order to compare the different measurements, the CFP emission peak at 480 nm is normalised to 1. The emission intensity ratios deduced from measurements at several sites in the cell, but not at the plasma membrane, of 35 different protoplasts co-expressing *At*SERK1-YFP and CFP-GF14λ is close to 1.2 (Fig. 6a). This is comparable with measurements obtained from ten protoplasts only expressing CFP-GF14λ. The emission intensity ratios obtained from measurements at the plasma membrane of the 35 different protoplasts co-expressing *At*SERK1-YFP and CFP-GF14λ showed that there was an increase in YFP fluorescence in 46% of the measurements performed with a maximum of 1.6 (Fig. 6b). The FRET energy transfer efficiency was calculated from the emission intensity ratios between Fig. 6a, b and is roughly 23%. These results show that at the plasma membrane *At*SERK1 and GF14λ can interact.

The interaction was confirmed using APB (Wouters and Bastiaens 1999). APB was performed by bleaching the YFP fluorophore in a defined region. Images of the protoplast were taken before and after the bleaching event. When YFP is inactivated due to bleaching, the CFP fluorescence energy can no longer be transferred to YFP in the case of FRET between CFP-GF14λ and *At*SERK1-YFP. This will result in an increase of the 480 nm CFP emission signal and a decrease of the 520 nm YFP emission signal. When we bleached the YFP fluorescence signal at a small portion of the plasma membrane where CFP-GF14λ and *At*SERK1-YFP co-localise, an increase in CFP intensity was observed (Fig. 6c). As a control we bleached YFP at a region in the cell where the two proteins do not interact (Fig. 6d). This did not result in an increase of CFP intensity. To compare the changes in fluorescence intensity between Fig. 6c, d, the fluorescent counts were converted to fluorescent percentages. Energy transfer efficiencies can be calculated based on the release of quenching of donor fluorescence due to FRET, measured by comparing the intensity of donor fluorescence before and after complete photobleaching of the acceptor (Bastiaens and Jovin 1996). For *At*SERK1-YFP and CFP-GF14λ the FRET energy transfer efficiency is roughly 20%. However, the fluorescence of *At*SERK1-YFP was not completely bleached, therefore the actual energy transfer will be higher. The FRET efficiency using acceptor photobleaching or FSPIM as a detection method is comparable. Therefore, we conclude that the interaction between GF14λ and *At*SERK1 as observed in yeast can also occur at the plasma membrane of transfected plant cells.

Discussion

A yeast two-hybrid screen was performed to identify proteins interacting with the *At*SERK1. As a result of this screen, *At*CDC48 and GF14λ were found to interact with the kinase domain of *At*SERK1. The binding of *At*CDC48 and GF14λ to *At*SERK1 was confirmed with protein binding assays. The interactions are phosphorylation dependent, because *At*CDC48 and GF14λ preferentially bind to phosphorylated *At*SERK1. In addition, *At*SERK1 is able to transphosphorylate *At*CDC48 as well as GF14λ. Pairwise interaction tests in yeast between *At*SERK1 and its binding partners showed that besides binding to *At*SERK1^{kinase}, *At*CDC48 can also bind to GF14λ and the phosphatase KAPP. GF14λ also interacts with *At*SERK1 at the plasma membrane of transfected plant cells.

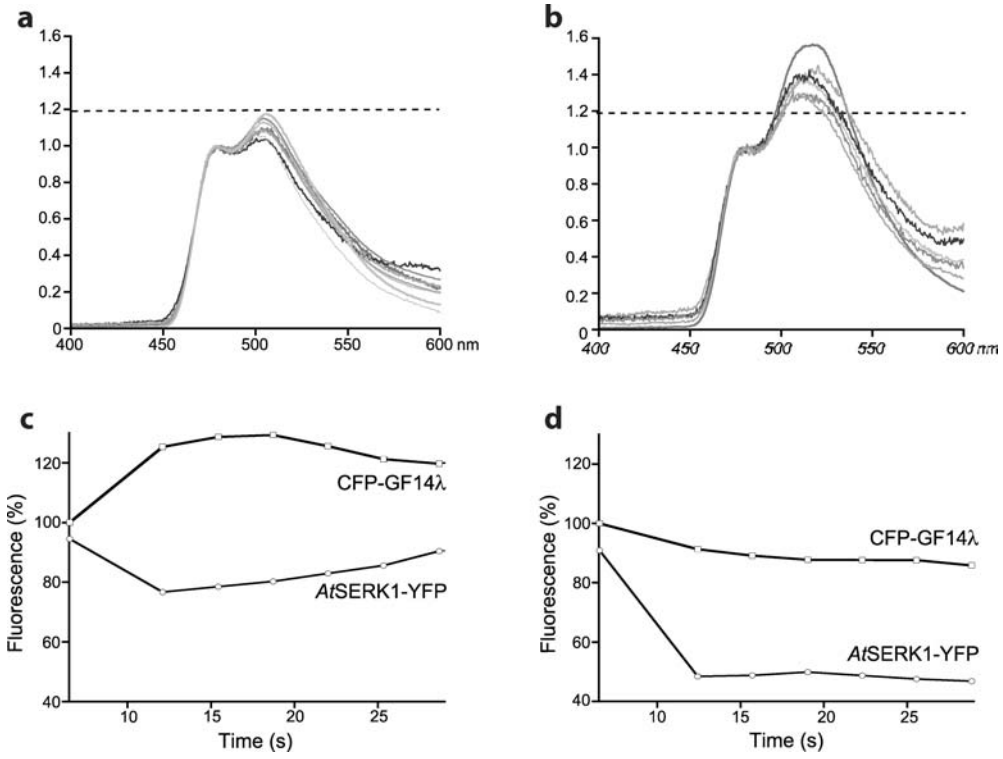

Fig. 6 FRET-SPIM and FRET-APB analysis of protoplasts expressing GF14λ and *At*SERK1 fused to CFP and YFP. For the FRET-SPIM analysis fluorescent intensities were normalised to 1.0 at 480 nm for comparison. Emission spectra from protoplasts co-expressing *At*SERK1-YFP and CFP-GF14λ obtained at several sites in the cell where the two proteins do not interact (**a**) and at the plasma membrane where they do interact (**b**). Typical FRET-APB analysis of protoplasts co-expressing *At*SERK1-YFP and CFP-GF14λ at the plasma membrane (**c**) and at an intracellular site where they do not interact (**d**). For comparison of the figures, the fluorescent counts were recalculated to the percentage of fluorescence. The original intensities of CFP-GF14λ and *At*SERK1-YFP fluorescence in (**c**) were at 2,025 and 1,840 counts respectively and in (**d**) at 840 and 790 counts respectively

GF14λ function

GF14λ belongs to the 14-3-3 protein-family, which consists of highly conserved 28–31 kDa proteins. The 14-3-3 proteins are broadly expressed in a wide range of eukaryotes and at least 13 different isoforms have been identified in *Arabidopsis* (Rosenquist et al. 2001). Interaction of plant 14-3-3 proteins with kinase domains was found for the calcium-dependent protein kinases CDK1 and CDPK2 (Camoni et al. 1998; Moorhead et al. 1999) and for the wheat protein kinase WPK4 (Ikeda et al. 2000). So far no interaction with a transmembrane-located receptor kinase has been reported in plants.

Under native conditions, the 14-3-3 proteins predominantly exist as dimers (Wu et al. 1997). The crystal structure of the 14-3-3 dimer was resolved for the human isoforms 14-3-3ζ and 14-3-3τ (Liu et al. 1995; Xiao et al. 1995). The 14-3-3 dimer forms a U-shaped structure. The N-terminal domains of the two 14-3-3 monomers form the dimerisation interface and the floor of the cleft; the C-terminal domains form the sides of the channel (Yaffe et al. 1997). Part of the N-terminal dimerisation domain containing the first two α-helices is deleted in GF14$\lambda^{39\text{-}248}$. We showed that for phosphorylation-dependent interaction with the kinase domain of *At*SERK1 the complete 14-3-3 protein is needed, suggesting that the 14-3-3 dimerisation domain (Yaffe 2002) or other residues in the first 38 amino acids are required for interaction with *At*SERK1. We also showed that *At*SERK1 is able to transphosphorylate GF14λ. Phosphorylation of the animal 14-3-3β and 14-3-3ζ isoforms by casein kinase 1α (CK1α) affects their ability to bind specific targets, such as Raf kinase (van der Hoeven et al. 2000). Phosphorylation of plant 14-3-3 proteins has been described as well but a role was not identified. The *Arabidopsis* GF14ω 14-3-3 isoform is phosphorylated by endogenous membrane kinase activity (Lu et al. 1994) and was proposed to have a function in the cell cycle based on its binding to fission yeast Cdc25 (Sorell et al. 2003).

CDC48 function

In yeast, Cdc48 is essential for cell-cycle progression and is presumably involved in spindle pole body duplication or separation (Fröhlich et al. 1991). In addition, *Sc*Cdc48 participates in homotypic fusion of ER membranes (Latterich et al. 1995). *Sc*Cdc48 shuttles between cytoplasm and nucleus in a cell-cycle-dependent manner. This is regulated by phosphorylation of the tyrosine residue in the C-terminal DDDLYS sequence, resulting in the exposure of a N-terminal nuclear localisation

signal (Madeo et al. 1998). The mammalian CDC48 homologue p97/VCP is tyrosine phosphorylated in response to T-cell antigen receptor activation (Egerton et al. 1992) and p97 phosphorylation coincides with its relocalisation from the ER to the centrosome (Lavoie et al. 2000). Like in *Sc*Cdc48, the N-terminal region of *At*CDC48 contains two putative nuclear localisation signals ($KKX_{(8)}RKK$ and KGKRKD) and the C-terminus contains the conserved negatively charged sequence DDDDLYN (Feiler et al. 1995). *At*SERK1 phosphorylates a threonine residue of *At*CDC48 within the C-terminal 62 amino acid residues. Although *At*SERK1 does not phosphorylate the tyrosine residue in the sequence DDDDLYN of *At*CDC48, phosphorylation of a threonine residue at the C-terminus might have a comparable function as phosphorylation of tyrosine residue in the yeast DDDLYS sequence in changing the three-dimensional structure of the CDC48 protein, possibly resulting in cellular relocalisation.

*At*CDC48 was originally described by Feiler et al. (1995) as an *Arabidopsis* cell division cycle protein based on functional complementation studies in yeast Cdc48 mutants. *Saccharomyces cerevisiae Cdc48-1* mutants arrest as large budded cells with an undivided nucleus and microtubules spreading aberrantly throughout the cytoplasm from a single spindle plaque (Fröhlich et al. 1991). The protein is also annotated as a putative endoplasmic reticulum ATPase (accession no. AC015985) due to its homology with the mammalian CDC48 isoform p97 or VCP, which was shown to regulate membrane fusion and assembly of endoplasmic reticulum in vitro together with the adaptor protein p47 (Pècheur et al. 2002; Rabinovich et al. 2002). Like yeast Cdc48, p97/VCP is also involved in targeted proteolysis of ubiquitinated retro-translocated proteins from the ER to which it is targeted by a protein complex, Ufd1/Npl4 (Jarosch et al. 2002), that in turn competes with p47 for binding p97/VCP (Meyer et al. 2000). Cdc48/p97/VCP was proposed to affect different cellular processes via specific adaptor proteins. The commonality between these processes seems to be the ATP-dependent protein folding/unfolding activity of Cdc48/p97/VCP (Golbik et al. 1999).

In *Arabidopsis* *At*CDC48 and *At*SERK1 expression patterns coincide

In *Arabidopsis*, in situ hybridisation experiments showed that CDC48 is highly expressed in proliferating cells of the vegetative shoot, root, floral inflorescence and flowers and in rapidly growing cells (Feiler et al. 1995). *AtCDC48* mRNA expression is also high in developing ovules and microspores and appears down-regulated in most differentiated cell types. At the subcellular level, the *At*CDC48 protein is localised at the division plane in dividing *Arabidopsis* cells and in punctuate, cytoplasmically distributed structures in both dividing and interphase cells (Rancour et al. 2002). During interphase *At*CDC48 was also found to be associated with the nuclear envelope. *At*CDC48 was detected in both soluble and membrane fractions and was found to cofractionate with the SNAREs KNOLLE and SYP31 in a sucrose density gradient (Rancour et al. 2002). KNOLLE can be found at the cell plate and in cytoplasmic punctuate structures (Lauber et al. 1997). SYP31 localises to the division plane during cytokinesis (Rancour et al. 2002). Disruption of the *KNOLLE* gene results in an embryonic defect with cells containing incomplete cross walls and enlarged cells with polyploid nuclei (Lukowitz et al. 1996). At the division plane *At*CDC48 co-localises and interacts with the SYP31 (Rancour et al. 2002). *At*CDC48 also co-localises but does not interact with the KNOLLE protein at the division plane nor in the cytoplasmic punctuate structures. In these cytoplasmic punctuate structures *At*CDC48 also co-localises with the endosomal-specific marker protein SNARE SYP21 (Rancour et al. 2002).

The *At*SERK1 gene is ubiquitously expressed in seedlings, flowers and shoot meristems, but shows highest expression in ovule primordia and the female gametophyte (Hecht et al. 2001). In many cells, *At*SERK1 expression therefore coincides with *At*CDC48 expression. However, *At*SERK1 is a transmembrane receptor protein that localises to the plasma membrane (Shah et al. 2001a). *At*CDC48 can be found in the cytoplasm as well as attached to membranes (Rancour et al. 2002). Fluorescently labelled *At*SERK1 and KAPP proteins both localise to the plasma membrane in protoplasts when they are separately expressed. When the proteins are co-expressed, *At*SERK1 and KAPP were found to become sequestered into intracellular vesicles similar to early endosomes (Shah et al. 2002), where the proteins were found to physically interact. Thus, an interaction between *At*SERK1 and *At*CDC48 is possible after *At*SERK1 internalisation at the endosomes.

*At*SERK1 may function in a complex containing *At*CDC48, GF14λ and KAPP

Many of the interactions with a 14-3-3 protein are mediated through binding of the 14-3-3 protein to a sequence-specific 14-3-3 binding domain. These binding sequences are based on the consensus motif $[RK]X_{(2,3)}pSXP$, where X stands for any amino acid and p denotes a phosphorylated amino acid (Muslin et al. 1996; Yaffe et al. 1997; Sehnke et al. 2002). Phosphorylation of the serine residue in the 14-3-3 binding domain has been shown to be important for the interaction with a 14-3-3 protein (Muslin et al. 1996; Yaffe et al. 1997). In the kinase domain of *At*SERK1, we identified the sequence $RPPS^{394}QPP$ as putative 14-3-3 binding motif. KAPP contains a putative 14-3-3 binding sequence ($RLPSS^{105}SP$) in the cytoplasmic domain of the KAPP protein located upstream of the FHA domain (Shah et al. 2002). A putative 14-3-3 binding domain, $KARQS^{572}AP$, corresponding to the consensus 14-3-3

binding motif can also be found in the *At*CDC48 protein. The sequence is located in the second AAA-module (Feiler et al. 1995) and is conserved in all three *Arabidopsis* CDC48 family members but not in yeast Cdc48.

The presence of putative 14-3-3 binding sites in KAPP and *At*CDC48 and the proposed interactions between *At*CDC48, *At*SERK1, KAPP and GF14λ suggest that there is analogy to a mammalian complex that consists of the CDC48 homologue p97/VCP, that can be phosphorylated by the JAK-2 kinase (Lavoie et al. 2000) and dephosphorylated by the PTPH1 phosphatase, which in turn associates with a 14-3-3 protein (Zhang et al. 1997; Zhang et al. 1999). We propose that *Arabidopsis* CDC48 can function together with GF14λ, KAPP and *At*SERK1 in a similar way. In dividing *Arabidopsis* cells, *At*CDC48 is found at the phragmoplast (Rancour et al. 2002). Phosphorylation of *At*CDC48 by *At*SERK1 may mediate cytokinesis by relocalisation of the *At*CDC48 protein to the cell plate (Lavoie et al. 2000; Rancour et al. 2002). In that scenario dephosphorylation of *At*CDC48 by KAPP, possibly regulated by 14-3-3 binding, would then prevent cytokinesis. Based on this, the reported role of the *At*SERK1 protein in promoting embryogenic competence could be interlinked with an aspect of cell cycle regulation.

Acknowledgements We are grateful to the following colleagues from Wageningen University, Laboratories of Biochemistry and Molecular Biology: Casper Vroemen and Vered Raz for the many useful discussions and critical comments on the manuscript, Ingrid Vleghels for her help with the yeast two-hybrid system, Jeroen Pouwels for his help with isolation and transformation of the cowpea protoplasts, Boudewijn van Veen, Jacques Vervoort and Olga Kulikova for their help with the figures in the manuscript and Gerard van der Krogt for providing us with the YFP/CFP cloning vectors. This work was supported by grant 805.18.302 from the Netherlands Organisation for Scientific Research NWO (I. M. R), grant QLG2-2000-00603 from the EU Quality of Life and Management of Living Resources program (E. R).

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