Available online at www.sciencedirect.com

SCIENCE DIRECT

Biochimica et Biophysica Acta 1744 (2005) 68–75

BIOCHIMICA ET BIOPHYSICA ACTA BBA

http://www.elsevier.com/locate/bba

Outer membrane protein 25-a mitochondrial anchor and inhibitor of stress-activated protein kinase-3

Naomi W. Court[a,1], Evan Ingley[b], S. Peter Klinken[b], Marie A. Bogoyevitch[a,*]

[a]*Cell Signalling Laboratory, Biochemistry and Molecular Biology (M310), University of Western Australia, Western Australia 6009, Australia*
[b]*Laboratory for Cancer Medicine, Western Australian Institute for Medical Research and Centre for Medical Research, University of Western Australia, Western Australia 6000, Australia*

Received 29 August 2004; received in revised form 29 November 2004; accepted 29 November 2004
Available online 31 December 2004

Abstract

Stress-activated protein kinase-3 (SAPK3) is unique amongst the mitogen-activated protein kinase (MAPK) family with its C-terminal 5 amino acids directing interaction with the PDZ domain-containing substrates α1-Syntrophin and SAP90/PSD95. Here, we identify three additional PDZ domain-containing binding partners, Lin-7C, Scribble, and outer membrane protein 25 (OMP25). This latter protein is localised together with SAPK3 at the mitochondria but it is not a SAPK3 substrate. Instead, OMP25 inhibits SAPK3 activity towards PDZ domain-containing substrates such as α1-Syntrophin and substrates without PDZ domains such as the mitochondrial protein Sab. This is a new mechanism for the regulation of SAPK3 and suggests that its intracellular activity should not be solely assessed by its phosphorylation status.

Keywords: Stress-activated protein kinase-3; OMP25; Mitochondria; PDZ domain

1. Introduction

A conserved group of signalling proteins known as mitogen-activated protein kinases (MAPKs) has been divided into several subgroups according to their sequence similarity and activating stimuli [1]. Within the p38 MAPK subfamily, the four members p38α, p38β, p38γ, and p38δ are activated in response to stress stimuli and are also referred to as stress-activated protein kinase (SAPK) 2a, 2b, 3, and 4 [1]. Of these, SAPK3 is distinguished by its resistance to the inhibitor SB203580 [1], its restricted tissue distribution [2–5], its subcellular localisation [5–8], and its substrate preferences [6–9].

The C-terminal tail of SAPK3 allows its interaction with the PDZ domains of its substrate proteins α1-Syntrophin and SAP90/PSD95 [7,8], although interaction with docking domains such as the Kinase Interaction Motif is possible [6]. The interactions of SAPK3 with PDZ domain-containing proteins provide a mechanism for the localisation of SAPK3 into distinct subcellular compartments. Thus, whilst SAPK3 interaction with α1-Syntrophin allows localisation at the neuromuscular junction of skeletal muscle [7], and interaction with SAP90/PSD95 allows localisation at the neuronal synapse [8], it is not clear whether PDZ domain interactions direct the mitochondrial localisation of SAPK3 in cardiac muscle cells or HEK293 cells [6].

Only one screen for SAPK3-interacting partners has been reported [7]. This, together with a recent study [8], suggests a preference of SAPK3 for PDZ domain-containing partners. Here, we report our findings from a yeast two-hybrid screen using full length wild type SAPK3 as bait. This revealed two new PDZ domain-containing partners. However, neither protein is reported to be mitochondrial in localisation and neither shows a tissue

Abbreviations: MAPK, mitogen-activated protein kinase; SAPK, stress-activated protein kinase; OMP25, outer membrane protein 25; PDZ, PSD-95/Discs-large/ZO-I; PSD-95, post-synaptic density-95; SAP90, synapsin-associated protein 90

* Corresponding author. Tel.: +61 8 6488 1348; fax: +61 8 6488 1148.
E-mail address: marieb@cyllene.uwa.edu.au (M.A. Bogoyevitch).
[1] Current address: Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Victoria 3800, Australia.

0167-4889/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2004.11.008

expression pattern like that of SAPK3. We therefore evaluated the binding of SAPK3 to a PDZ domain-containing outer mitochondrial membrane protein, outer membrane protein 25 (OMP25) [10]. OMP25 interacted with the SAPK3 C-terminus, but it was not a substrate like the other PDZ domain-containing binding partners reported to date. Instead, we show that this PDZ domain-containing protein inhibits SAPK3 activity. Importantly, this interaction prevents the SAPK3 phosphorylation of PDZ domain-containing proteins such as α1-Syntrophin and substrates that lack PDZ domains such as Sab. This provides a unique mechanism for the control of the activity of this p38 MAPK member.

2. Materials and methods

2.1. Yeast two-hybrid screening

Yeast 2-hybrid screening used the *Saccharomyces cerevisiae* L40 strain (*MATα*, *his3Δ200*, *trp1-901*, *leu2-3*, *112*, *ade2*, *LYS2::(lexAop)$_4$-HIS3*, *URA3::(lexAop)$_8$-lacZ*, *GAL4*) [11]. Full-length rat SAPK3 was subcloned into pBTM116 [11] as an in-frame fusion to the LexA DNA binding protein. pBTM116-SAPK3 was co-transformed into yeast with a cDNA library derived from the lymphohemopoietic progenitor cell line, EML clone.1 [12]. This library in the vector pVP16 produces fusions to the VP16 transcriptional activation domain. The transformed yeast colonies were subjected to *HIS3* and β-gal assays, and the library plasmids from these yeast colonies that showed SAPK3-dependent trans-activation of the *HIS3* and *lacZ* reporter plasmids were subsequently rescued into *Escherichia coli*. For secondary confirmation of interaction, the positive library plasmids were co-transformed with pBTM116-SAPK3, or with the controls pBTM116-Lamin A or pBTM116-ARL6, into the yeast L40 strain before being subjected to *HIS3* and β-gal assays. Colonies that showed SAPK3-dependent trans-activation of the *HIS3* and *lacZ* reporter genes, but no activation in the presence of ARL6 or Lamin A, were sequenced. To determine whether SAPK3-specific interactions were dependent on the C-terminal tail of SAPK3, a SAPK3 mutant where the 5 C-terminal amino acids were removed (SAPK3ΔC) was also subjected to *HIS3* and β-gal assays.

2.2. Cell culture and transfection

HEK293 cells were maintained in DMEM supplemented with 10% fetal calf serum and penicillin/streptomycin [5]. Transfections with plasmids allowing the expression of either myc-SAPK3 [5] or HA-OMP25 [10] were performed using Lipofectamine [5]. Cells were then cultured for an additional 40 h prior to immunoprecipitation experiments or immunocytochemical staining.

2.3. Immunoprecipitation experiments

Lysates from transfected HEK293 cells (100 μg) were incubated with anti-HA monoclonal antibody (5 μg; Roche) overnight at 4 °C, then HA-OMP25 was captured on protein G-sepharose. The immunoprecipitates were subjected to SDS-PAGE then Western blotting with an anti-SAPK3 antibody [5].

2.4. Immunocytochemical staining

HEK293 cells grown and transfected on glass coverslips were processed for staining as described [5]. The primary antibodies, the anti-HA mouse monoclonal (Roche) and the anti-SAPK3 rat monoclonal [5,6], were used at final concentrations of 12.5 μg/ml and 6.6 μg/ml, respectively. Staining was observed by confocal scanning microscopy [5]. No staining was observed in the control experiments, in which primary antibodies were omitted.

2.5. Recombinant proteins

SAPK3, α1-Syntrophin [7], and Sab(219–425) [13] were prepared as glutathione-*S*-transferase (GST)-fusion proteins. Full length OMP25 was subcloned into the pGEX-6P vector, introducing a stop codon to allow its preparation as GST-OMP25(1–177). This removed the OMP25 transmembrane domain and allowed its expression in reasonable quantities in *E. coli*. MKK6, a direct upstream activator of SAPK3, was expressed in its constitutively active form fused to the maltose binding protein, producing the protein MalE-MKK6(DD) [14].

2.6. SAPK3 activity assays

GST-SAPK3 was activated with MalE-MKK6(DD) as described [6]. Assays of SAPK3 activity were then performed essentially as described [6], with 5 μg of GST substrate for 1 μg of activated SAPK3 and, as required, pre-incubation of activated SAPK3 for 15 min at 30 °C with 1–10 μg of GST-OMP25 before assaying activity. The incorporation of the radiolabel into the substrate was quantitated by Cerenkov counting, and one-way analysis of variance was used to analyse the results using the Statview™ SE+ Graphics program. Values of $P<0.01$ were considered significant.

3. Results

3.1. Yeast two-hybrid analysis of SAPK3-interacting partners

In the only reported yeast two-hybrid screen for SAPK3-interacting partners, α1-Syntrophin was isolated from a skeletal muscle library and was shown to be a good in vitro substrate for SAPK3 [7]. SAPK3 has been previously shown

to be expressed in early hematopoietic progenitor cells [15], and we therefore undertook a two-hybrid screen using a library derived from the mouse lymphohematopoietic progenitor cell line EML clone.1 [12] that is well-validated in our laboratory [16,17]. The screening of 4×10^6 colonies resulted in the isolation of 148 positive SAPK3-specific interacting partners. Of these, 131 were partial clones of the PDZ protein Scribble (NP_598850), and 17 were partial or full length clones of the PDZ protein Lin-7C (NP_035829) (Fig. 1A and B). All isolated clones contained at least one Class I PDZ domain. We confirmed that these interactions were mediated by the C-terminal tail of SAPK3 when a SAPK3 truncation mutant (SAPK3Δ5C) did not interact with Scribble or Lin-7C (results not shown). These results are consistent with SAPK3 interacting with Class I PDZ domains [7].

Previous reports have shown Lin-7C to localise at cell–cell junctions and to be expressed highly in the brain, kidney, liver, spleen, and lung but not in the heart, testis, or

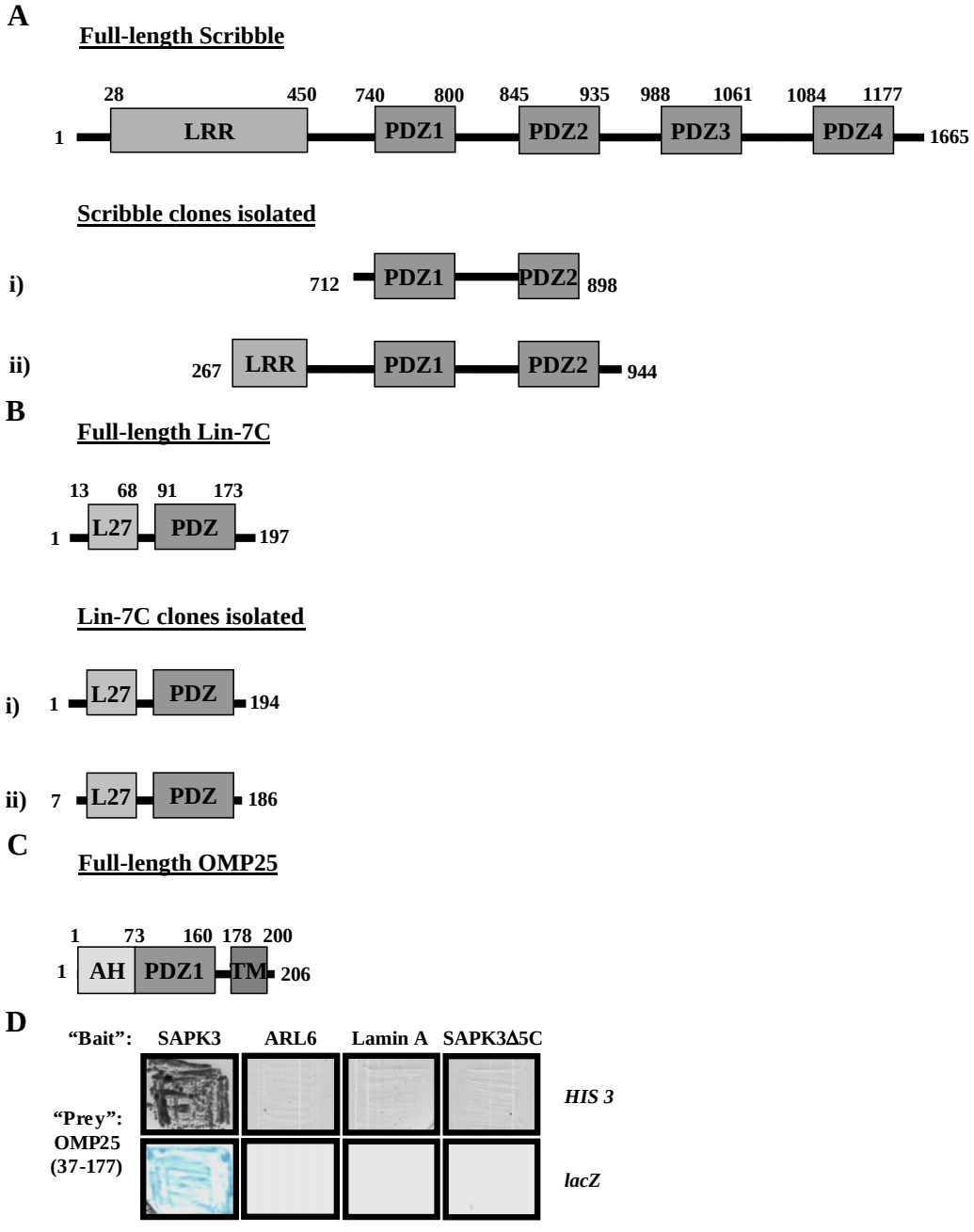

Fig. 1. SAPK3 interacts with PDZ domain-containing proteins by yeast 2-hybrid analysis. SAPK3 interacted with (A) Scribble (NP_598850) or (B) Lin-7C (NP_035829) by yeast 2-hybrid analysis. Schematic diagrams of the full-length proteins and constructs isolated from the screen (i, ii) are presented. LRR=leucine-rich region. (C) Schematic diagram of full-length OMP25 (AF107295). AH=amphipathic helix; TM=transmembrane domain. (D) pVP16-OMP25 DNA was transformed into yeast and tested for interaction with pBTM116-SAPK3, pBTM116-ARL6, pBTM116-lamin, and SAPK3Δ5C. Interaction was assessed by the activation of the *HIS3* or *lacZ* reporters.

skeletal muscle [18]. Using a specific anti-Scribble monoclonal antibody, we have detected its presence in the thymus, spleen, kidney, liver, and brain, but not in the heart, testis, or skeletal muscle (results not shown), and have confirmed its localisation at the basolateral cell membrane ([19,20] and results not shown). These results suggest that Lin-7C and Scribble are not likely to be mitochondrial binding partners for SAPK3.

We therefore evaluated the available literature for reports of mitochondrial PDZ domain-containing proteins. Outer membrane protein 25 (OMP25) is both mitochondrial in localisation and contains one Class I PDZ domain [10]. Furthermore, OMP25 expression is reported for a variety of tissues, with highest levels in skeletal muscle [10]. These features would place OMP25 as a good candidate as a SAPK3 binding partner of physiological relevance.

OMP25 (AF107295) is inserted into the outer mitochondrial membrane, with its PDZ domain facing the cytosol [10]. Its domain structure is shown schematically in Fig. 1C. Its structure is consistent with performing an anchoring role at the mitochondria, and this has been shown for its ability to localise the inositol phosphatase Synaptojanin 2A to the mitochondria [10]. We tested the interaction of a truncated OMP25 with SAPK3 in our yeast two-hybrid system. Specifically, we chose an OMP25(37–177) mutant, which does not include the mitochondrial targeting sequence that would likely confound the nuclear interactions that drive the transcriptional reporter systems in yeast two-hybrid analysis. OMP25(37–177) specifically interacted with SAPK3, and this was dependent on the C-terminal tail of SAPK3, since the SAPK3Δ5C mutant failed to interact with OMP25 (Fig. 1D).

3.2. Confirmation of the interaction of SAPK3 with OMP25

We evaluated the interaction of SAPK3 with OMP25 in three ways. First, HEK293 cells were transfected with HA-OMP25 [10] and myc-SAPK3 [5]. The expression of OMP25 and SAPK3 has been detected in the kidney

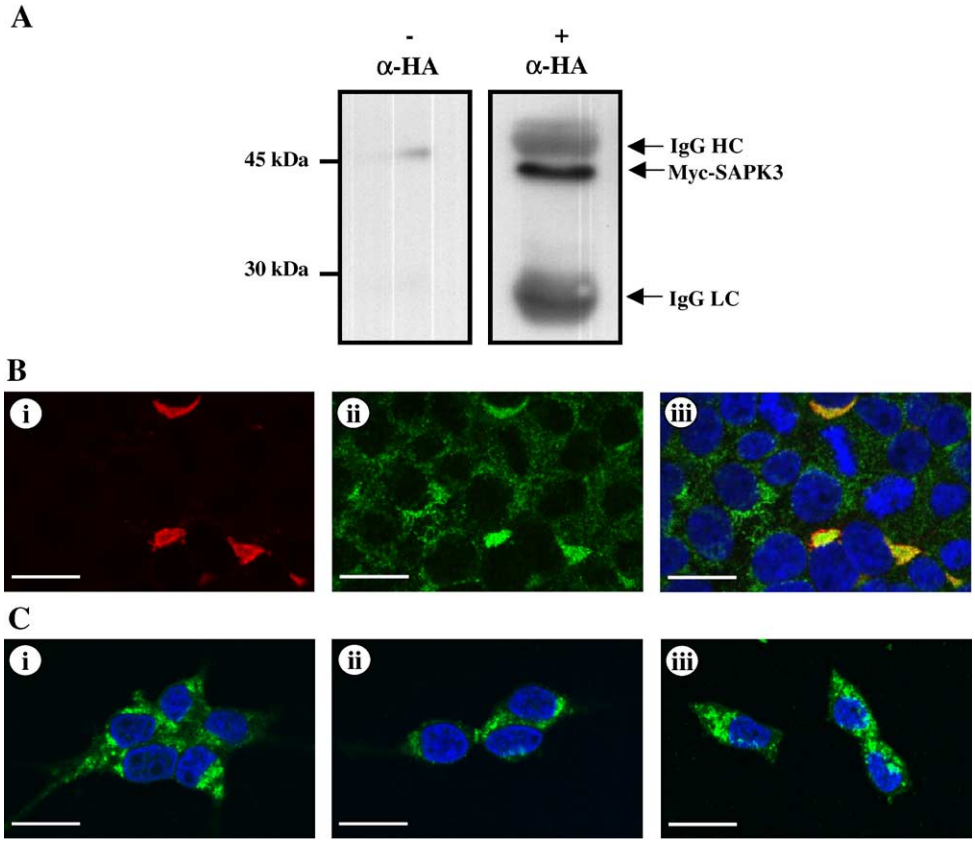

Fig. 2. SAPK3 and OMP25 interact by co-immunoprecipitation and co-localise in cells. (A) Lysates from HEK293 cells, transfected with myc-tagged SAPK3 and HA-tagged OMP25, were subjected to immunoprecipitation with HA antibody (+αHA) or in the absence of the HA antibody (−αHA) as a negative control. The resulting immunoprecipitates collected on Protein G-sepharose were subjected to Western blotting with the SAPK3 antibody. Results shown are representative of at least 4 independent experiments. IgG HC=immunoglobulin heavy chain; IgG LC=immunoglobulin light chain. (B) HEK293 cells, transfected with HA-OMP25, were fixed, permeabilised, and stained with HA (i) and SAPK3 (ii) antibodies. Panel (iii) shows an overlay of Panels (i) and (ii), with yellow staining indicating where SAPK3 (green) and HA-OMP25 (red) co-localise. Hoescht 33258 staining is also included to visualise the cell nuclei. Results shown are representative of 2 identical experiments. Scale bar=18 μm. (C) HEK293 cells were treated with 0.5 M sorbitol for 5 (i), 15 (ii), or 30 min (iii), then fixed, permeabilised, and stained with SAPK3 antibody. Hoescht 33258 staining is also included to visualise the cell nuclei. Results shown are representative of 2 identical experiments. Scale bar=18 μm.

previously [5,10], therefore, HEK293 cells were thought to be a suitable model for studying the interaction between these two proteins. Immunoprecipitation following the addition of an anti-HA antibody, then immunoblotting for SAPK3, showed the association of the two proteins (Fig. 2A). No SAPK3 could be detected when the anti-HA antibody was omitted from the procedure (Fig. 2A). It was not possible to assess this interaction by immunoprecipitation with the myc-antibody or SAPK3-antibody, followed by blotting with the HA antibody, due to the antibody light chain interfering with OMP25 detection (results not shown). Furthermore, when we immunoprecipitated HA-OMP from HEK293 cells, the low transfection efficiency (~20%), the less than 50% immunoprecipitation efficiency, and the lower levels of endogenous SAPK3 in these cells compromised the evaluation of the interaction of HA-OMP25 with endogenous SAPK3. No commercially available OMP25 antibodies are yet available to confirm interactions of the endogenous proteins in these protocols.

Next, we confirmed the co-localisation of OMP25 and SAPK3 within intact cells. HEK293 cells were again transfected with HA-OMP25, then HA-OMP25 and SAPK3 localisation was detected by confocal scanning microscopy (Fig. 2B). In transfected cells, SAPK3 and HA-staining co-localised in the aggregated mitochondria that are the result of the overexpression of HA-OMP25 as previously described [10].

Lastly, the localisation of OMP25 would not be expected to change when cells are exposed to various stresses due to its transmembrane domain that acts as its anchor to the mitochondria. However, it was of interest to evaluate the localisation of SAPK3 under conditions that result in its activation. We have previously shown that osmotic stress activates SAPK3 in HEK293 cells [5]. When we evaluated the localisation of SAPK3 following exposure of HEK293 cells to 0.5 M sorbitol for 5, 15, or 30 min, no change in SAPK3 from its mitochondrial localisation could be observed (Fig. 2C).

3.3. OMP25(1–177) is not a SAPK3 substrate but rather inhibits SAPK3 activity in vitro

The PDZ domain-containing SAPK3 binding partners α1-Syntrophin and SAP90/PSD95 are good in vitro substrates for SAPK3 [7,8]. An examination of the OMP25 sequence does not reveal any consensus MAPK phosphorylation sites (Ser/Thr-Pro) [21], in contrast to the 9 and 6 sites in either α1-Syntrophin or SAP90/PSD95, respectively [7,8]. We expressed OMP25(1–177) as a recombinant GST-fusion protein because the expression of the full-length protein in *E. coli* produced poor protein yields (unpublished observations). When we tested GST-OMP25(1–177) as a substrate in in vitro kinase assays using SAPK3 as the kinase, this OMP25 construct did not appear to be phosphorylated (Fig. 3). Under the same conditions, recombinant α1-Syntrophin was an excellent substrate (Fig. 3). Although it remains possible that full-length OMP25 can be phosphorylated by SAPK3 in the

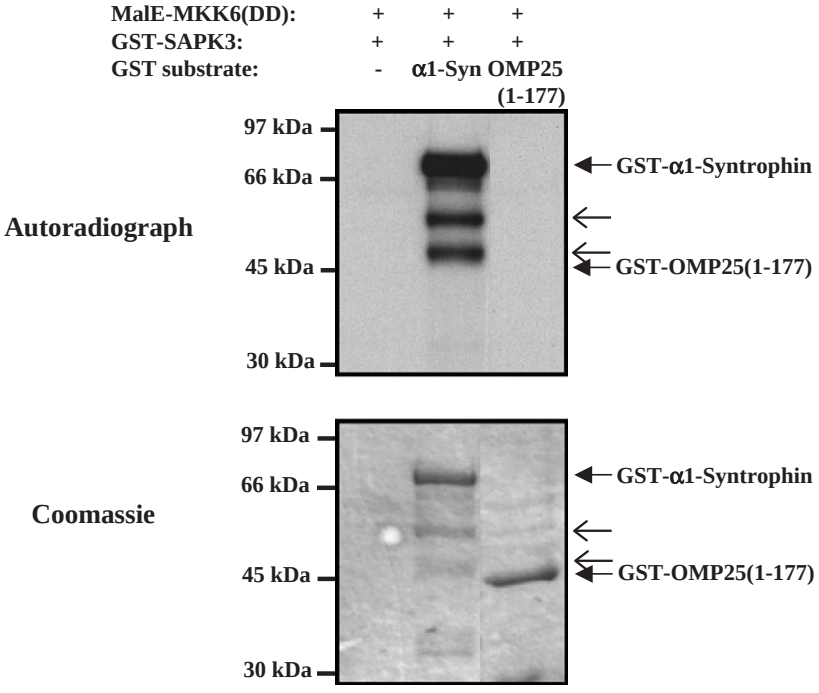

Fig. 3. OMP25 is not a substrate of SAPK3 in vitro. GST-SAPK3 was activated by MalE-MKK6(DD) and subjected to kinase assays with GST-α1-Syntrophin or GST-OMP25(1–177). The lower panel shows the Coomassie-stained gel to illustrate the even loading of GST substrates. The open arrows indicate bands which likely represent partially transcribed GST-α1-Syntrophin or GST-α1-Syntrophin degradation products. Results shown are representative of 2 identical experiments.

intact cell, for example with other proteins acting to promote binding and/or phosphorylation, our results in the in vitro kinase assay raised the question of whether the interaction of SAPK3 with OMP25 could affect the activity of SAPK3 in other ways.

Active SAPK3 was therefore incubated with GST-OMP25(1–177), and its activity was assayed towards two different substrate proteins. As shown in Fig. 4, SAPK3 activity towards α1-Syntrophin was inhibited by the inclusion of as little as 1 μg of OMP25(1–177) in the incubation mixture. The inclusion of 10 μg of OMP25(1–177) inhibited SAPK3 activity by up to 85% (Fig. 4). We then determined the effects of the inclusion of OMP25(1–177) on the activity of SAPK3 towards the substrate protein Sab. The phosphorylation of Sab requires a Kinase Interaction Motif rather than a PDZ domain [6]. However, as shown in Fig. 4, the activity of SAPK3 towards Sab was inhibited to an extent comparable to the inhibition observed using α1-Syntrophin as substrate. Therefore, the ability of OMP25(1–177) to inhibit SAPK3 was not dependent on the presence of a PDZ domain in its substrate.

4. Discussion

The archetypical MAPK pathway consists of a cascade of protein kinases, namely, MAPK kinase kinases, MAPK kinases, and the MAPKs themselves [22]. Thus, the activation of each kinase in the pathway relies on its phosphorylation by an upstream kinase and the deactivation would presumably require their dephosphorylation by phosphatases. For the MAPKs, serine/threonine phosphatases, as well as dual-specificity phosphatases, have been implicated in these dephosphorylation events [23,24]. However, as the MAPK family is more extensively studied, other mechanisms for control are being revealed. For example, the protein kinases ERK3 and ERK7 have recently been shown to be acutely regulated by proteolytic degradation rather than

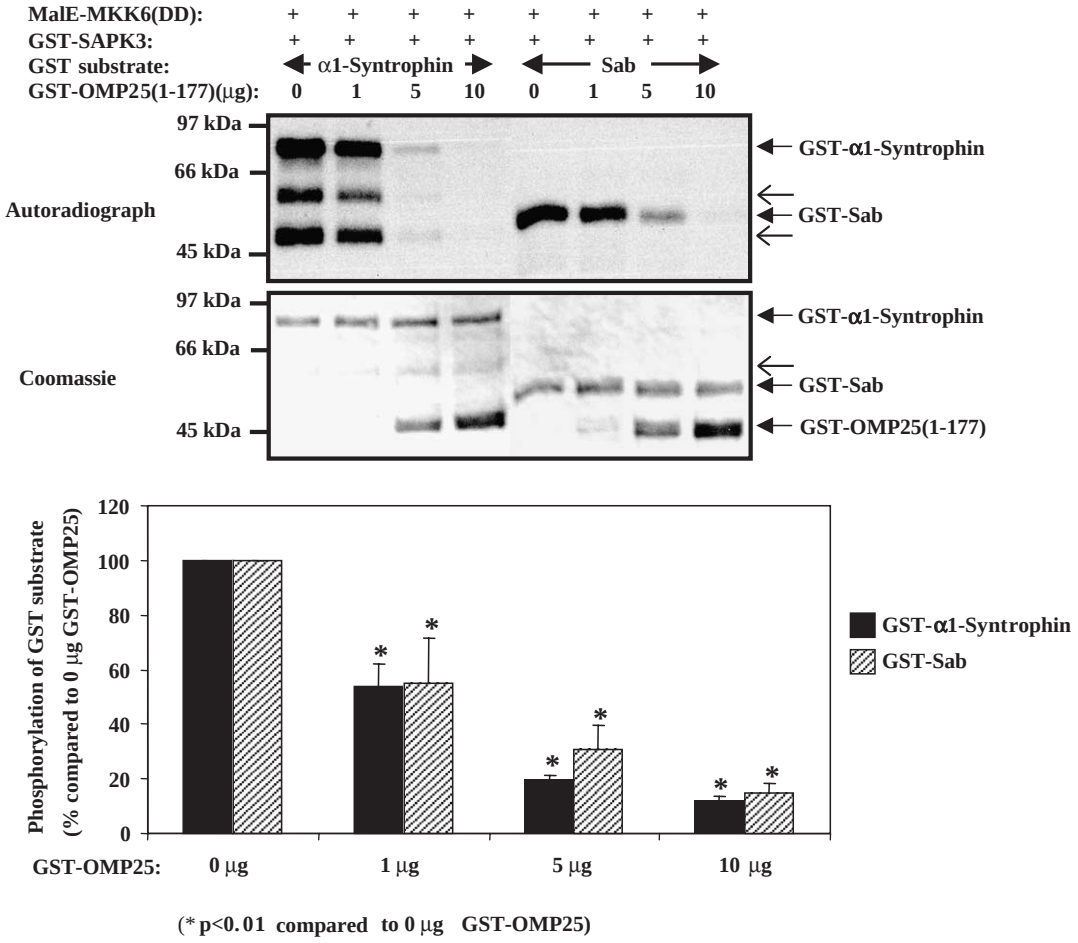

Fig. 4. GST-OMP25 inhibits the phosphorylation of GST-α1-Syntrophin and GST-Sab by SAPK3 in vitro. GST-SAPK3 was activated by MalE-MKK6(DD) and pre-incubated with GST-OMP25(1–177) (0–10 μg) for 15 min, prior to kinase assays with GST-α1-Syntrophin or GST-Sab. An autoradiograph of a representative gel is shown in the top panel, and the middle panel shows the Coomassie-stained gel to illustrate the even loading of GST-α1-Syntrophin and GST-Sab, and the increasing amount of GST-OMP25 in each assay. The bottom panel shows the quantitation of 3 assays as determined by Cerenkov counting of the GST-α1-Syntrophin or GST-Sab bands. The open arrows indicate bands which likely represent partially transcribed GST-α1-Syntrophin or GST-α1-Syntrophin degradation products. Results are shown as means±S.E.

dephosphorylation [25,26], whereas 14-3-3 proteins interact with a phosphorylated serine in the C-terminal tail of ERK5, stabilising it in an inactive form by blocking the interaction with the upstream activator MEK5 [27].

Additional levels of control in the MAPK pathway come with the involvement of proteins with anchoring or scaffolding roles [28,29]. These interactions can place kinases, their substrates, or their regulators in the correct intracellular location. A variety of protein interaction domains can be involved in these processes, including SH2 domains, SH3 domains, or PTB domains [30]. For the SAPK3 pathway, PDZ domain-mediated interactions are also possible due to the unique C-terminal tail of SAPK3 [7]. This interaction has been implicated in SAPK3 substrate recognition, as well as maintaining its unique intracellular localisation [7,8].

Here, we have identified 3 additional PDZ domain-containing proteins, Lin-7C, Scribble, and OMP25, as binding partners for SAPK3. Importantly, both Lin-7C and Scribble, but not OMP25, have possible sites for phosphorylation by SAPK3. Indeed, we have now shown that OMP25 prevents the ability of activated SAPK3 to phosphorylate substrates that contain PDZ domains such as α1-Syntrophin as well as those that lack PDZ domains such as Sab. This provides the first evidence for protein–protein interactions preventing the actions of active SAPK3. OMP25, which anchors the inositol phosphatase Synaptojanin 2A [10], can therefore perform more than an anchoring role at the mitochondria for the SAPK3 pathway.

There are at least three implications of this finding. The first relates to the assessment of SAPK3 activation. Whilst the use of phospho-specific antibodies has been widely adopted as a means to assess the activities of kinases within the MAPK cascades, caution is warranted when negative regulatory proteins, other than phosphatases, are present within the cell. In the case of SAPK3, changes in phosphorylation (4-fold increase) have not strictly corresponded with changes in the activity of SAPK3 (1.5-fold increase) in skeletal muscle samples [31]. Even if OMP25 is not the endogenous binding partner for SAPK3 in all tissues, other PDZ domain-containing partners could inhibit its activity. This, together with the observation that higher levels of MKK6 activity are required to activate SAPK3 [14], sets SAPK3 apart from the other p38 MAPKs.

The second implication of this study is that SAPK3 must be released from interactions with PDZ domain-containing proteins such as OMP25 if it is to phosphorylate its substrates. This provides an additional control mechanism in the SAPK3 pathway and parallels the release of inward rectifier K^+ channel Kir 2.3 from the PDZ domain of SAP90/PSD95 [32]. In this case, it is the phosphorylation of a single amino acid within the C-terminal tail by cAMP-dependent protein kinase that allows this disruption of binding. The C-terminal tail of SAPK3 is Lys-Glu-Thr-Ala-Leu [3]. The phosphorylation of the Threonine within this sequence which conforms to the consensus site of phosphorylation by cGMP-dependent protein kinase [33] could provide a release mechanism.

The final implication of this study is that the interaction of the C-terminal tail of SAPK3 with PDZ domain-containing proteins provides a unique means for control of SAPK3 activity. Other SAPKs lack the C-terminal sequence recognised by Class I PDZ domains. For example, the C-terminal tail sequence of SAPK4 is Ser-Gly-Met-Lys-Leu. Thus, SAPK4 or SAPKs other than SAPK3 would not be expected to interact with the PDZ domain containing proteins, nor be inhibited by them.

Taken together, our results reinforce the emerging idea of additional layers of complexity in the regulation of the MAPKs. Not only will phosphatases be negative regulators of these pathways [34], but interactions with 14-3-3 proteins [27], degradation by the proteasome [25,26], and interaction with other non-substrate proteins such as seen here with the interaction of SAPK3 and OMP25 will provide alternative mechanisms for control.

Acknowledgements

We thank P. De Camilli for the OMP25 construct, C. Wiltshire for the Sab construct, A. Cuenda for the α1-Syntrophin construct, and A. Nebreda for the MKK6(DD) construct. We thank Paul Rigby for assistance with confocal microscopy. NWC gratefully acknowledges the support of a National Heart Foundation of Australia Postgraduate Research Scholarship and a Jean Gilmore Grant from the Australian Federation of University Women. EI gratefully acknowledges the support of National Health and Medical Research Council project grants (#139008 and #303101).

References

[1] P. Cohen, The search for physiological substrates of MAP and SAP kinases in mammalian cells, Trends Cell Biol. 7 (1997) 353–361.
[2] C. Lechner, M.A. Zahalka, J.F. Giot, N.P. Moller, A. Ullrich, ERK6, a mitogen-activated protein kinase involved in C2C12 myoblast differentiation, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 4355–4359.
[3] S. Mertens, M. Craxton, M. Goedert, SAP kinase-3, a new member of the family of mammalian stress-activated protein kinases, FEBS Lett. 383 (1996) 273–276.
[4] Z. Li, Y. Jiang, R.J. Ulevitch, J. Han, The primary structure of p38 gamma: a new member of p38 group of MAP kinases, Biochem. Biophys. Res. Commun. 228 (1996) 334–340.
[5] N.W. Court, C.G. dos Remedios, J. Cordell, M.A. Bogoyevitch, Cardiac expression and subcellular localization of the p38 mitogen-activated protein kinase member, stress-activated protein kinase-3 (SAPK3), J. Mol. Cell. Cardiol. 34 (2002) 413–426.
[6] N.W. Court, I. Kuo, O. Quigley, M.A. Bogoyevitch, Phosphorylation of the mitochondrial protein Sab by stress-activated protein kinase 3, Biochem. Biophys. Res. Commun. 319 (2004) 130–137.
[7] M. Hasegawa, A. Cuenda, M.G. Spillantini, G.M. Thomas, V. Buee-Scherrer, P. Cohen, M. Goedert, Stress-activated protein kinase-3 interacts with the PDZ domain of alpha1-syntrophin. A mechanism for specific substrate recognition, J. Biol. Chem. 274 (1999) 12626–12631.

[8] G. Sabio, S. Reuver, C. Feijoo, M. Hasegawa, G.M. Thomas, F. Centeno, S. Kuhlendahl, S. Leal-Ortiz, M. Goedert, C. Garner, A. Cuenda, Stress- and mitogen-induced phosphorylation of the synapse-associated protein SAP90/PSD95 by activation of SAPK3/p38gamma and ERK1/ERK2, Biochem. J. 380 (2004) 19–30.
[9] M. Goedert, A. Cuenda, M. Craxton, R. Jakes, P. Cohen, Activation of the novel stress-activated protein kinase SAPK4 by cytokines and cellular stresses is mediated by SKK3 (MKK6); comparison of its substrate specificity with that of other SAP kinases, EMBO J. 16 (1997) 3563–3571.
[10] Y. Nemoto, P. De Camilli, Recruitment of an alternatively spliced form of synaptojanin 2 to mitochondria by the interaction with the PDZ domain of a mitochondrial outer membrane protein, EMBO J. 18 (1999) 2991–3006.
[11] A.B. Vojtek, S.M. Hollenberg, J.A. Cooper, Mammalian Ras interacts directly with the serine/threonine kinase Raf, Cell 74 (1993) 205–214.
[12] S. Tsai, S. Bartelmez, E. Sitnicka, S. Collins, Lymphohematopoietic progenitors immortalized by a retroviral vector harboring a dominant-negative retinoic acid receptor can recapitulate lymphoid, myeloid, and erythroid development, Genes Dev. 8 (1994) 2831–2841.
[13] C. Wiltshire, M. Matsushita, S. Tsukada, D.A. Gillespie, G.H. May, A new c-*Jun* N-terminal kinase (JNK)-interacting protein, Sab (SH3BP5), associates with mitochondria, Biochem. J. 367 (2002) 577–585.
[14] G. Alonso, C. Ambrosino, M. Jones, A.R. Nebreda, Differential activation of p38 mitogen-activated protein kinase isoforms depending on signal strength, J. Biol. Chem. 275 (2000) 40641–40648.
[15] S. Uddin, J. Ah-Kang, J. Ulaszek, D. Mahmud, A. Wickrema, Differentiation stage-specific activation of p38 mitogen-activated protein kinase isoforms in primary human erythroid cells, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 147–152.
[16] E. Ingley, D. Chappell, S.Y. Poon, M.K. Sarna, J.G. Beaumont, J.H. Williams, J.P. Stillitano, S. Tsai, P.J. Leedman, P.A. Tilbrook, S.P. Klinken, Thyroid hormone receptor-interacting protein 1 modulates cytokine and nuclear hormone signaling in erythroid cells, J. Biol. Chem. 276 (2001) 43428–43434.
[17] E. Ingley, M.K. Sarna, J.G. Beaumont, P.A. Tilbrook, S. Tsai, Y. Takemoto, J.H. Williams, S.P. Klinken, HS1 interacts with Lyn and is critical for erythropoietin-induced differentiation of erythroid cells, J. Biol. Chem. 275 (2000) 7887–7893.
[18] M. Irie, Y. Hata, M. Deguchi, N. Ide, K. Hirao, I. Yao, H. Nishioka, Y. Takai, Isolation and characterization of mammalian homologues of *Caenorhabditis elegans* lin-7: localization at cell–cell junctions, Oncogene 18 (1999) 2811–2817.
[19] D. Bilder, N. Perrimon, Localization of apical epithelial determinants by the basolateral PDZ protein Scribble, Nature 403 (2000) 676–680.
[20] L.E. Dow, A.M. Brumby, R. Muratore, M.L. Coombe, K.A. Sedelies, J.A. Trapani, S.M. Russell, H.E. Richardson, P.O. Humbert, hScrib is a functional homologue of the *Drosophila* tumour suppressor Scribble, Oncogene 22 (2003) 9225–9230.
[21] I. Clark-Lewis, J.S. Sanghera, S.L. Pelech, Definition of a consensus sequence for peptide substrate recognition by p44mpk, the meiosis-activated myelin basic protein kinase, J. Biol. Chem. 266 (1991) 15180–15184.
[22] M.J. Robinson, M.H. Cobb, Mitogen-activated protein kinase pathways, Curr. Opin. Cell Biol. 9 (1997) 180–186.
[23] J.D. Graves, J.S. Campbell, E.G. Krebs, Protein serine/threonine kinases of the MAPK cascade, Ann. N.Y. Acad. Sci. 766 (1995) 320–343.
[24] M. Camps, A. Nichols, S. Arkinstall, Dual specificity phosphatases: a gene family for control of MAP kinase function, FASEB J. 14 (2000) 6–16.
[25] P. Coulombe, G. Rodier, S. Pelletier, J. Pellerin, S. Meloche, Rapid turnover of extracellular signal-regulated kinase 3 by the ubiquitin–proteasome pathway defines a novel paradigm of mitogen-activated protein kinase regulation during cellular differentiation, Mol. Cell. Biol. 23 (2003) 4542–4558.
[26] W.L. Kuo, C.J. Duke, M.K. Abe, E.K. Kaplan, G. Suzanna, M.R. Rosner, ERK7 expression and kinase activity is regulated by the ubiquitin–proteasome pathway, J. Biol. Chem. 279 (2004) 23073–23081.
[27] Q. Zheng, G. Yin, C. Yan, M. Cavet, B.C. Berk, 14-3-3Beta binds to big mitogen-activated protein kinase 1 (BMK1/ERK5) and regulates BMK1 function, J. Biol. Chem. 279 (2004) 8787–8791.
[28] W.L. Wang, S.F. Yeh, Y.I. Chang, S.F. Hsiao, W.N. Lian, C.H. Lin, C.Y. Huang, W.J. Lin, PICK1, an anchoring protein that specifically targets protein kinase Calpha to mitochondria selectively upon serum stimulation in NIH 3T3 cells, J. Biol. Chem. 278 (2003) 37705–37712.
[29] D.K. Morrison, R.J. Davis, Regulation of MAP kinase signaling modules by scaffold proteins in mammals, Annu. Rev. Cell Dev. Biol. 19 (2003) 91–118.
[30] T. Pawson, P. Nash, Assembly of cell regulatory systems through protein interaction domains, Science 300 (2003) 445–452.
[31] M.D. Boppart, S. Asp, J.F. Wojtaszewski, R.A. Fielding, T. Mohr, L.J. Goodyear, Marathon running transiently increases c-*Jun* NH2-terminal kinase and p38 activities in human skeletal muscle, J. Physiol. 526 (2000) 663–669.
[32] N.A. Cohen, J.E. Brenman, S.H. Snyder, D.S. Bredt, Binding of the inward rectifier K+ channel Kir 2.3 to PSD-95 is regulated by protein kinase A phosphorylation, Neuron 17 (1996) 759–767.
[33] R.B. Pearson, B.E. Kemp, Protein kinase phosphorylation site sequences and consensus specificity motifs: tabulations, Methods Enzymol. 200 (1991) 62–81.
[34] M. Haneda, T. Sugimoto, R. Kikkawa, Mitogen-activated protein kinase phosphatase: a negative regulator of the mitogen-activated protein kinase cascade, Eur. J. Pharmacol. 365 (1999) 1–7.