Supplement

Supplement Methods

Tissue culture, immunoprecipitation and mass spectrometry of Mst3 protein complexes

Tissue culture, cell lysis and immunoprecipitations were performed as previously described (Figeys *et al.*, 2001). Briefly, human embryonic kidney cells, HEK293, were transfected with pMST3-FLAG or with the pCMV-TAG4A vector, and the cells were lysed 48 hours after transfection. FLAG-tagged proteins were immunoprecipitated from clarified lysates using anti-FLAG M2-agarose (SIGMA). Proteins were eluted with free FLAG peptide, resolved by SDS-PAGE and stained with Coomassie blue (GelCode Blue, Pierce). Stained bands were excised from the gel and the proteins were digested with trypsin. Peptides were analysed by mass spectrometry using either an LCQ Deca XP ion trap (Finnigan) or a QSTAR PULSAR hybrid Qq-TOF (PE Sciex) mass spectrometer.

Supplement Results and Discussion

Evidence for a Kic1p-Mob2p-like network in mammalian cells. Analyses of genomic and EST databases indicate that there are Cbk1p-, Mob2p-, Tao3p-, Hym1p- and Kic1p-related proteins in mammalian cells. As an initial test for conservation of the RAM signaling module, we immunoprecipitated epitope-tagged MST3 (GI: 2582413/23274191**)** (Schinkmann and Blenis, 1997), a human Kic1-like protein, from human embryonic kidney cells (HEK293) and identified the co-precipitating proteins by mass spectrometry (see methods). We found that MST3 co-precipitates with human phocein (GI: 11691896/14730060), protein phosphatase 2a (PP2a) catalytic subunit (GI:

4506017) and regulatory subunit A (GI: 7657475) and the striatin family proteins, striatin (GI:4507283), SG2NA (GI: 11128017) and zinedin (GI: 7019573) (Fig. S1). None of these proteins were present in immunoprecipitated material from similarly treated untagged HEK293 cells (Fig. S1) or from cells expressing epitope-tagged pICln, Skb1, SmD3 and protein 4.1 (data not shown).

Phocein is a class II Mob protein that is distantly related to Mob1p and Mob2p and was originally identified in early mouse embryonic cDNA libraries (Temeles *et al.*, 1994; Luca and Winey, 1998). It was shown to bind striatin family proteins and PP2a subunits (Baillat *et al.*, 2001; Moreno *et al.*, 2001; Baillat *et al.*, 2002). Members of the striatin family are thought to function as scaffolds for signal transduction pathways and may be involved in membrane trafficking and vesicle transport (Castets *et al.*, 2000; Gaillard *et al.*, 2001; Baillat *et al.*, 2002).

Striatin family proteins are highly related proteins that contain caveolin and calmodulin-binding domains, a coiled-coil domain and WD40 repeats (Castets *et al.*, 2000; Gaillard *et al.*, 2001). These proteins bind calmodulin in the presence of calcium and may act both as scaffolds and as calcium-dependent signaling proteins.

It is not yet known which cellular processes the Mst3 and phocein-containing complex regulates. It has been suggested that phocein, striatin family proteins and phosphatase 2a participate in regulating Golgi function and vesicle transport (Baillat *et al.*, 2001; Baillat *et al.*, 2002). In contrast, other data suggest that some Mst3 kinase is activated in Jurkat cells by caspase cleavage, which allow it to translocate into the nucleus and promote apoptosis (Huang *et al.*, 2002). Whether phocein, striatin or other Mst3-binding proteins participate in apoptosis remains to be determined.

Our data indicate that two human proteins that share sequence similarity with yeast RAM proteins interact in human cells and may suggest that a conserved role for RAM-like signaling networks is to regulate vesicle transport in yeast and vertebrate cells. Based on conservation to RAM and MEN proteins, it seems probable that future experiments will lead to the discovery of a Cbk1p/Dbf2p related protein kinase that associates with phocein. Along these lines, perhaps a Sog2p- or a Hym1p-related protein will be found to interact with Mst3, as for the RAM protein Kic1p.

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Supplement figure legend

Figure S1. **The Kic1p-like kinase, Mst3, co-immunoprecipitates with the phocein/striatin family/PP2a complex.** HEK293 cells were transfected with empty vector (vector) or pMST3-FLAG (MST3 bait). FLAG-tagged Mst3 and associated proteins were co-precipitated from cellular lysates and resolved by electrophoresis on a 4-15% polyacrylamide gel. Co-precipitating proteins were visualized with Coomassie blue staining and identified by mass spectrometry. The identified proteins are indicated. The asterisks indicate background bands, and Ig indicates immunoglobulin from the anti-FLAG antibody. Anti-FLAG Ig and phocein co-migrate on this gel, as do striatin, SG2NA, and zinedin.

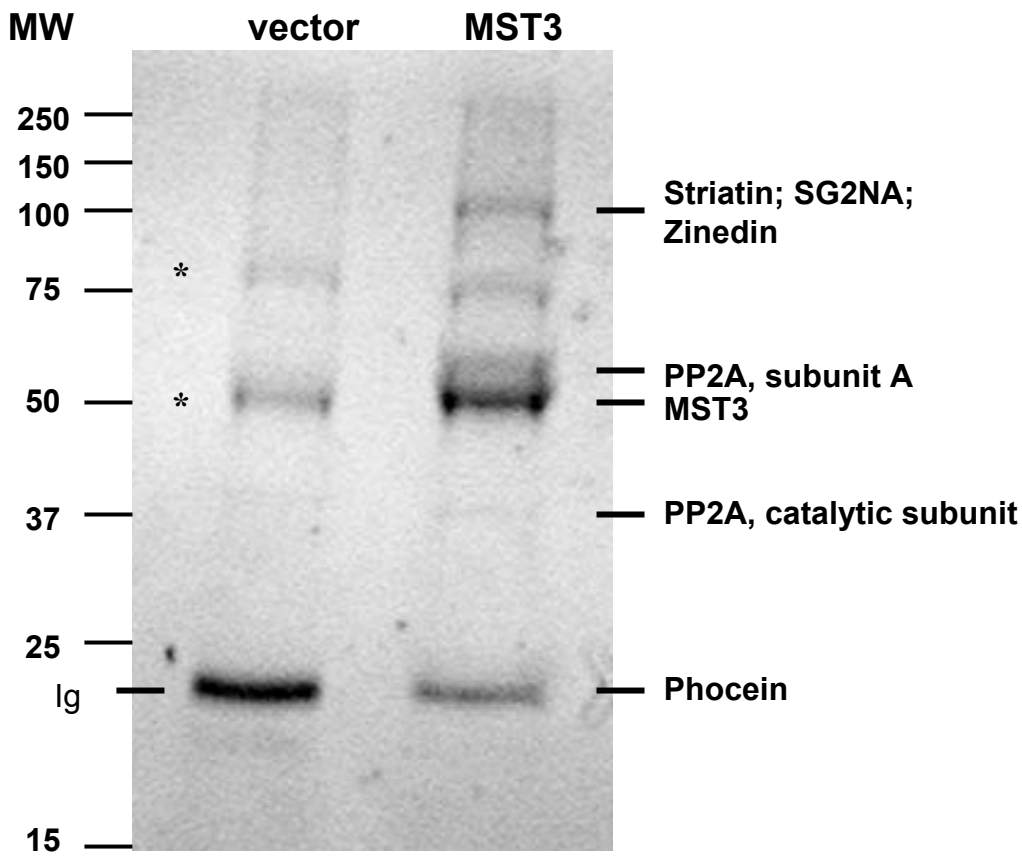
MW
vector
MST3
250
150
100
75
50
37
25
Ig
15
*
*
Striatin; SG2NA;
Zinedin
PP2A, subunit A
MST3
PP2A, catalytic subunit
Phocein

Fig. S1