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Crosstalk between casein kinase II and Ste20-related kinase Nak1

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Introduction

Ste20 kinase in S. cerevisiae functions as a MAP4K in the mating pheromone-response MAP kinase pathway.1 There are 28 kinases in this family in mammals, with some members functioning similarly to Ste20 in MAP kinase signaling cascades.2,3 The Ste20 family of protein kinases is further subdivided into p21-activated kinases (PAKs) and germinal center kinases (GCKs) based on their structural differences. Ste20 in budding yeast, Pak1/Shk kinases in fission yeast and corresponding PAK family homologs in mammals are regulated by the Rho/Cdc42 family of small GTPase.4,5 GCKs in mammals include MST1/2 and MST3/4, which act upstream of two NDR kinase signaling pathways (Hippo/LATS1/2 and NDR1/2), which have distinct roles in control of cell growth and apoptosis.6,7 In S. pombe, the GCK family members (Nak1, Sid1 and Ppk11) also function in distinct NDR kinase signaling pathways. Nak1 and Ppk11 kinases function in the MOR (morphogenesis Orb6 network) signaling pathway that regulates interphase polarized growth and cell separation at the end of cytokinesis through activation of the NDR1/2-related kinase Orb6.8-13 In contrast, Sid1 activates the LATS1/2-related kinase Sid2 during late mitosis to trigger cytokinesis through constriction of the actomyosin ring. Work from S. pombe showed that crosstalk between these signaling pathways is important for proper

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Although the sterile 20 (Ste20) serine/threonine protein kinase was originally identified as a component of the S. cerevisiae mating pathway, it has homologs in higher eukaryotes and is part of a larger family of Ste20-like kinases. Ste20-like kinases are involved in multiple cellular processes, such as cell growth, morphogenesis, apoptosis and immune response. Carrying out such a diverse array of biological functions requires numerous regulatory inputs and outputs in the form of protein-protein interactions and post-translational modifications. Hence, a thorough knowledge of Ste20-like kinase binding partners and phosphorylation sites will be essential for understanding the various roles of these kinases. Our recent study revealed that Schizosaccharomyces pombe Nak1 (a conserved member of the GC-kinase sub-family of Ste20-like kinases) is in a complex with the leucine-rich repeat-containing protein Sog2. Here, we show a novel and unexpected interaction between the Nak1-Sog2 kinase complex and Casein kinase 2 (Cka1, Ckb1 and Ckb2) using tandem-affinity purification followed by mass spectrometric analysis. In addition, we identify unique phosphosites on Nak1, Sog2 and the catalytic subunit of casein kinase 2, Cka1. Given the conserved nature of these kinases, we expect this work will shed light on the functions of these proteins both in yeast and higher eukaryotes.
Specifically, there appears to be mutual antagonism between the SIN and MOR pathways, whereby the SIN inhibits MOR signaling through phosphorylation of the Nak1 kinase. Nak1 is in a complex with the leucine-rich repeat-containing protein, Sog2, which might also be a target of SIN inhibition. Therefore, studying Nak1 phosphorylation and interacting proteins has provided insights into crosstalk between conserved NDR-kinase signaling networks.

Interestingly, the genetic screen for cell polarity mutants, which identified Nak1 and other MOR pathway components, also identified mutants in casein kinase 2. However, how casein kinase 2 fits in to the MOR signaling pathway has remained mysterious.

Results and Discussion

In order to identify proteins that physically interact with the Nak1 kinase, we constructed functional tandem affinity purification (TAP)-tagged Nak1 (Nak1-TAP) according to our protocol described at http://mendel.imp.ac.at/Pombe_tagging/. We used a TAP protocol to purify Nak1-TAP, and co-purifying proteins were identified by mass spectrometry. Among proteins with at least two unique peptides, we identified leucine-rich repeat protein Sog2, an uncharacterized protein SPBC2G5.02c, as well as catalytic (Cka1) and regulatory subunits (Ckb1) of casein kinase 2 (Fig. 1A). To confirm the specificity of these interactions, we also performed reciprocal purifications using functional TAP-tagged Sog2 (Sog2-TAP) and Cka1 (Cka1-TAP). Indeed, we found that Nak1, Cka1, Ckb1 and SPBC2G5.02c co-purified with Sog2-TAP and Nak1, Sog2, Ckb1 and SPBC2G5.02c co-purified with Cka1-TAP (Fig. 1A). Our finding that Nak1 co-purifies with Sog2 is consistent with our recent study and the observation from budding yeast that the Sog2 ortholog is known to interact with the Nak1 ortholog Kic1. More intriguing is our observation that Nak1 co-purifies with casein kinase 2 (CK2). CK2 is an evolutionarily conserved serine/threonine protein kinase that regulates many cellular processes, including cell signaling and proliferation, DNA repair, apoptosis and senescence. Extensive evidence of its involvement in various cancers as well as neurodegenerative diseases had led to its emergence as a promising...
are indicative of a loss of bipolar growth.30 However, crosstalk between protein kinases from the Ste20 family and CK2 kinases is poorly understood.

So far, the only evidence linking these two kinase families in humans appears to be from Chaar et al., who observed that human CK2 can phosphorylate the Ste20-like kinase family member SLK in vitro.24 It has also been shown that CK2 subunits in S. cerevisiae interact with either Ste20 and/or with Bem1, a cell polarity establishment protein that acts as a scaffold for Ste20.35,36 Few other studies in yeast and humans show interaction of CK2 subunits with Ste20-associated proteins. For instance, budding yeast Cka1 subunit associates with Ste20-tar-

Figure 2. Cka1-GFP localizes similar to MOR pathway proteins like Nak1. (A) Fixed Cka1-GFP-expressing cells (YDM2969) were stained with DAPI. White arrows in the merge panel indicate localization of Cka1-GFP to dots that might correspond to the SPB. Red arrows indicate Cka1-GFP localization to cell septum and tips. (B) Cka1 co-localizes with the SPB marker Sad1. Fixed Cka1-GFP Sad1-RFP expressing cells (YDM3463) were stained with DAPI.

Protein kinase CK2 is a tetramer composed of two catalytically active (CK2 α isoforms) and two regulatory (CK2 β isoforms) subunits. In fission yeast, one catalytic subunit (Cka1) and one regulatory subunit (Ckb1) have been characterized.31 Our analysis showed that Cka1, in addition to Ckb1, co-purifies with an unchar-

Figure 1B, indicated in bold). Sites with an RXXS consensus motif are targeted by NDR kinases. We previously showed that the RXXS sites on Nak1 are phosphorylated by the SIN pathway NDR kinase, Sid2. All Sid2 phosphorylation sites identified by this study were mutated previously (ref. 15 and unpublished data; Fig. 1B, shown in red). Analysis of the mutants showed that Sid2 phosphorylation of Nak1 causes removal of Nak1 from the spindle pole bodies, which prevents premature activation of the SIN in early mitosis. Moreover, Sid2 phosphorylation of Nak1 blocks MOR signaling by preventing interaction of Nak1 with the scaffold protein Mor2.35 The presence of RXXS site phosphorylation on the Nak1 binding partner Sog2 suggests that it might also be phosphorylated by the SIN kinase Sid2. To completely understand the role of phosphorylation in the regulation of Nak1, Sog2 and Cka1, it will be important to identify relevant protein kinases and counteracting phosphatases, analyze possible periodicity during the cell cycle and study functional consequences of mutating these phosphorylation sites. We suggest that sites with the phospho-

motif pSP may be targets of the Cdk1 kinase.34 Furthermore, both Nak1 and Sog2 contain the consensus phosphoryla-
tion motif S/T-X-X-D/E, which is recognized by CK2.31,35 Some of these sites have been identified by our analysis (Fig. 1B, indicated by asterisks). This indicates a possibility that Nak1 and Sog2 may be potential CK2 substrates. However, fur-
ther mutational studies will be required to confirm this. Because most of the phospho-
ylation sites on Nak1 cluster in the C-terminal non-kinase half of the protein, we expect that this region is a key regulatory module targeted by multiple kinases.

Taken together, our results presented in this study provide a thorough biochemical
analysis of Nak1-interacting partners and their post-translational modifications. We confirmed that Nak1 co-purifies with Sog2. Significantly, Nak1 shows interaction with the CK2 complex. We therefore established a physical interaction between members of two major kinase families, namely Ste20-related kinases and the Casein kinase 2 family. Further exploration should reveal a better understanding of the functional relationship between them.

Materials and Methods

Strains. S. pombe strains expressing either Nak1-TAP (JG15615, h+ naka1-TAP::KanMX), Cka1-TAP (JG15429, h+ cka1-TAP::KanMX) or Sog2-TAP (JG16552, h+ sog2-TAP::KanMX) were grown in complete yeast extract medium (YE + 5S). TAP-tagging was confirmed by PCR and immunoblotting. The TAP epitope was detected using PAP antibodies (rabbit antiperoxidase antibody linked to peroxidase, Dako) at 1:50,000 dilution in PBS-T.

Protein purification. Six-liter cultures of strains expressing TAP-tagged proteins were grown to mid-log phase (OD ~0.8) and filtered at the XCorr values to an FDR of 1% on the peptide level.

Peptide identification was performed using the SEQUEST algorithm in the Proteome Discoverer 1.3 software package (Thermo Fisher Scientific). Spectra were searched against the Sanger S. pombe database (04/23/2012). Following search parameters were used: peptide tolerance was set to 10 ppm, MS/MS fragment tolerance to 50 munits, trypsin was selected as protease and two missed cleavages were allowed, carbamidomethylation of cysteines and oxidation of methionine and phosphorylation of serine, threonine and tyrosine as a variable modification. The results were filtered at the XCorr values to an FDR of 1% on the peptide level.

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