Sab (SH3BP5), a novel mitochondria-localized JNK-interacting protein

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Abstract

The JNK (c-Jun N-terminal kinase) pathway is activated by diverse stresses and can have an effect on a number of different cellular processes. Protein–protein interactions are critical for efficient signalling from JNK to multiple targets; through a screen for interacting proteins, we identified a novel JNK-interacting protein, Sab (SH3BP5). Sab has previously been found to interact with the Src homology 3 domain of Bruton’s tyrosine kinase; however, the interaction with JNK occurs through a mitogen-activated protein KIM (kinase interaction motif) in a region distinct from the Bruton’s tyrosine kinase-binding domain. As with c-Jun, the presence of this KIM is essential for Sab to act as a JNK substrate. Interestingly, Sab is associated with the mitochondria and co-localizes with a portion of active JNK after stress treatment. The present study and previously reported work may suggest a possible role for Sab in targeting JNK to this subcellular compartment and/or mediating crosstalk between different signal-transduction pathways.

JNK interacts physically with a number of different proteins

The best-known and the most well-studied JNK substrate is c-Jun, a transcription factor that is activated by phosphorylation on two serine residues located in the N-terminal transactivation domain [2]. Efficient modification of c-Jun depends on JNK binding to a docking domain (the delta region) located just N-terminal to these phospho-acceptor sites [3]. JNK also phosphorylates a number of other transcription factors, including Elk-1, ATF-2 (activating transcription factor-2) and NFAT (nuclear factor of activated T-cells). All of these proteins contain docking or KIMs (kinase interaction motifs) similar to those in the delta region of c-Jun, through which JNK can bind [4]. Although some of the factors determining specificity are not well understood, protein–protein interactions are known to be important for JNK substrate selection.

In addition to substrates, JNK can physically interact with a number of upstream activators, phosphatases and scaffolds. Scaffolds such as JIP1 (JNK-interacting protein 1) and JIP2 can bind not only JNK but also upstream and downstream regulators such as MAP kinase kinase 7, mixed-lineage kinases and MAP kinase phosphatase 7 [5]. As such, scaffolds may increase the efficiency and specificity of JNK signalling by forming modules that are not subject to interference by crosstalk from other pathways. Scaffolds may also play a role in the targeting of particular JNK modules to specific subcellular localizations or to a restricted set of substrates [1,6].

Sab (SH3BP5) is a novel JNK-interacting protein, identified through a protein–protein interaction screen, which was originally isolated through its ability to bind to the SH3 (Src homology 3) domain of Btk (Bruton’s tyrosine kinase) [7,8]. Only the C-terminal portion of Sab was captured in the JNK interaction screen and, through sequence analysis, two possible KIMs and four putative phosphorylation sites could be identified (Figure 1). Sab can interact with JNK in vitro through the most N-terminal of these two docking motifs, and it has been shown that this KIM is located N-terminal to the preferred phospho-acceptor site [8,9]. As with c-Jun, the ability of Sab to act as a JNK substrate is dependent on the presence of the KIM [8]. It has been reported recently that this is also the case for Sab to act as a substrate for one of the less well-known members of the p38 family, p38γ [9]. Interestingly, this interaction is mediated through

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Abbreviations used: Btk, Bruton’s tyrosine kinase; JNK, c-Jun N-terminal kinase; JIP1, JNK-interacting protein 1; KIM, kinase interaction motif; MAP, mitogen-activated protein; ROS, reactive oxygen species; SH3 domain, Src homology 3 domain.

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Figure 1 | Structural features of the Sab protein
The N-terminal portion is predicted to form an extended coiled coil (light shading) and contains the segment shown previously to bind to the SH3 domain of Btk (dark shading). The potential KIMs (black bars) and phospho-acceptor sites are located within the C-terminus. The preferred KIM (shaded arrow) and phospho-acceptor site (open arrow) are indicated.

Figure 2 | Co-localization of Sab and phospho-JNK to the mitochondria in stressed cells
Untreated (A) or anisomycin-treated (B) chicken-embryo fibroblasts were immunostained for Sab (red) and phospho-JNK (green). Merged images show the extent of co-localization between Sab and phospho-JNK.

the same docking motif for both kinases. However, further scrutiny of the Sab protein sequence gives little clue as to the function of these interactions. The N-terminus is predicted to form an extended coiled coil, includes the SH3-binding domain required for Btk interaction and shares some sequence similarity with the rod domain of the myosin heavy chain. However, except for the KIMs and putative phospho-acceptor sites, the C-terminus does not have any recognizable features, nor does it share any extended similarity to other known proteins (Figure 1) [7,8]. Coiled-coil domains are frequently implicated in dimerization or other protein-protein interactions; therefore, it is possible that Sab has multiple binding partners that have not been identified so far.

JNK can co-localize with Sab at mitochondria under stress conditions
A potentially informative property with respect to assigning a function to the Sab protein is its subcellular localization. In both stressed and unstressed cells, Sab is localized to mitochondria [8]. In addition, when cells are treated with anisomycin to activate JNK, a significant proportion can be seen to localize with Sab (Figure 2). (p38γ is also localized to the mitochondria, although stress-mediated activation is not required for this to occur [9].)

Stress-induced association of JNK with mitochondria has been reported previously, and it is associated with the modulation of apoptosis [10,11]. Both antiapoptotic (Bcl-2 and Bcl-X<sub>L</sub>) and proapoptotic (Bim and Bmf) Bcl-2 family members have been identified to be JNK substrates, although only Bcl-2 and Bcl-X<sub>L</sub> are localized at mitochondria when they interact with JNK [10–12]. However, mitochondrial events are integral to stress-mediated apoptosis, with JNK mediating apoptosis in mouse-embryo fibroblasts by promoting mitochondrial cytochrome c release [13].

In addition to their function during apoptosis, mitochondria constitute the major site of ROS (reactive oxygen species) generation in the cell, both during normal respiration and stress responses [14]. It has been suggested that ROS form a self-amplification loop under certain conditions, and this can result in sustained activation of JNK [15]. ROS have also been implicated in activating the mitochondrial apoptotic pathway [14].

Evidently, JNK is targeted to mitochondria, in some way, to fulfill these roles. However, no mitochondrial scaffolds are known for the JNK pathway, although JIP1 and a number of JNK pathway components have been found in the mitochondria-containing fraction of activated cells [16]. One possibility is that Sab acts as an anchor molecule to recruit JNK to the mitochondria and to facilitate phosphorylation of mitochondrial targets. Alternatively, JNK may be held at the mitochondria to allow activation by ROS to transmit a
signal between organelles. In addition, the fact that the three identified binding partners of Sab are all kinases could be highly significant, and may suggest a role for Sab in crosstalk between signalling pathways. It is hoped that construction of a DT40 chicken B-cell line deficient in Sab expression will allow some of these possibilities to be investigated.

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