Scaffold Proteins in Mammalian JNK Signaling

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This article provides a synopsis of JNK signaling followed by a brief discussion of the scaffold proteins, JIP-1/2, IB1, JSAP-1 and β-arrestin 2, which coordinate the formation of the JNK signaling networks.

Introduction

The kinases involved in cell signaling networks are often tethered in close proximity to one another by binding to scaffold proteins. This physical localization creates a multi-enzyme complex that enhances the specificity of the kinases by minimizing extraneous interactions. The known scaffold proteins for JNK signaling are JIP-1/2 (JNK Interacting Protein-1/2), IB1 (Islet-Brain 1 or JIP-1b), JSAP-1 (JNK/ Stress-Activated Protein kinase associated protein-1), β-arrestin 2 and perhaps MEKK1 (MAPK/ERK Kinase Kinase-1). This article provides a short introduction to JNK signaling followed by a brief discussion of these scaffold proteins.

The c-Jun N-terminal Kinase (JNK) is, along with p38 kinase, one of the stress-activated kinases that is sensitive to tumor necrosis factor alpha (TNF-α), UV light and osmotic shock (1–4). JNK is found in the cytosol, where it phosphorylates serine residues 63 and 73 of the transcription factor c-Jun and thereby activates it. JNK has three isoforms, JNK-1,-2 and -3, with several splice-variants of each for a total of ten different kinases ranging in molecular mass from 46 to 57kDa (5). Activators of JNK include JNK Kinases (JNKK1 and 2), also known as MKK4 and MKK7, that can in turn be activated by Mixed Lineage protein Kinase (MLK) and MEKK1, among others (6).

JIP-1

Let us first consider JIP-1. Immunoprecipitation studies have shown that JIP-1 forms complexes with hemopoietic progenitor kinase (HPK), MLK and MKK7. The association of JNK with the 72kDa JIP-1 and these other kinases is illustrated in Figure 1. JIP-1 and JIP-2 can form homo- and hetero-oligomers in vitro as well as in vivo, and both proteins are expressed in many tissues including brain. Studies of neuronal tissue have shown that JIP-1 and JIP-2 interact with the apolipoprotein receptor 2 (7). Co-expression experiments in COS-7 cells indicated that full-length JIP-1 and JIP-2 greatly enhance the activation of JNK (8). In contrast, transfection of PC12 cells with the JNK Binding Domain (residues 127–281) of JIP-1 decreased the pro-apoptotic activity of JNK (9). Thus, the JIP scaffold proteins appear to have both functional and structural roles in JNK signaling.

IB1

A second scaffold protein for JNK, cloned from a pancreatic beta cell cDNA library and named IB1 for Islet-Brain 1, is a JIP-1 homologue with a 47 amino acid, C-terminal insert. IB1 is a DNA-binding protein that can transactivate a glucose transporter gene, GLUT2, by binding to its *cis*-regulatory element, GTII (10). Preliminary evidence indicates that a mutation near the JNK Binding Domain of IB1 is associated with adult-onset, type II diabetes (11). In the brain, IB1 is localized to synapses, and its expression declines after day 15 postpartum. These findings suggest that IB1 may play a role in developing neurons (12). Recently, another isoform of IB1 has been identified and designated IB2. IB2 resembles IB1 in sequence, tissue expression and function as a scaffold protein (11).

JSAP-1

A third scaffold protein for JNK is JSAP-1, which is distinct from JIP-1 in that JSAP-1 has a leucine zipper motif but not an SH3 domain. In addition, the 144kDa protein JSAP-1 forms a complex with MEKK1, MKK4 and JNK, while JIP-1 organizes MLK, MKK7 and JNK (13). Cotransfection studies in COS-7 cells first demonstrated the binding of JSAP-1 to MEKK1, MKK4 and JNK, and these results were confirmed for MEKK1 and JNK by in vitro binding studies. MEKK1 itself may be a scaffold protein, because it binds to NCK interacting kinase (NIK), MKK4 and JNK. However, the significance and function of this MEKK1 binding has not been established (6).

β-arrestin 2

Recently, immunoprecipitation and yeast two-hybrid studies have shown that β-arrestin 2 is a scaffold protein for apoptosis signal-regulating kinase (ASK1), MKK4, and JNK3 (14). Thus, β-arrestin 2 has two opposing functions. The first is to prevent further signaling once G-protein coupled receptor kinases have phosphorylated β-adrenergic receptors. The second function is to localize three kinases (ASK1, MKK4 and JNK3) together in the cytosol to facilitate activation of c-Jun in brain tissue.

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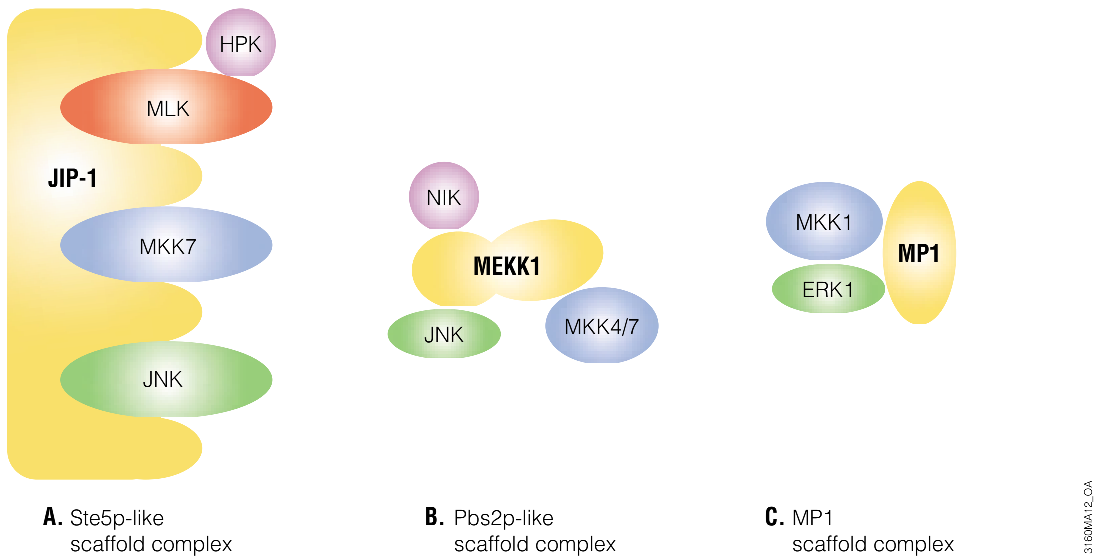

▲ Figure 1. Mammalian MAP-kinase-scaffold complexes. Depicted are three classes of mammalian MAP-kinase-scaffold proteins. (A) The JNK-interacting protein (JIP-1) appears to coordinate components of the JNK MAP-kinase module. The JIP-1 scaffold complex includes the Ste20p-like protein kinase HPK-1, the MLK group of MAP-kinase-kinase kinases (MKKK), the MAP-kinase kinase (MKK) isoform MKK7, and the MAP-kinase JNK. The mammalian complex coordinated by JIP-1 interacts with multiple components of the MAP-kinase module, facilitates MAP-kinase activation in response to specific signals and insulates the bound MAP-kinase module against activation by irrelevant stimuli. (B) The second class of mammalian MAP-kinase scaffold complex is represented by the MKKK isoform MEKK1, which interacts with the Ste20p-like protein kinase NIK, the MKK protein kinases MKK4 and the MAP kinase JNK. Whether this complex has any physiological significance remains to be established. (C) The protein MP1 represents a third class of mammalian scaffold protein. MP1 binds to MAP kinase ERK1 and to the MKK isoform MKK1. MP1 appears to function by facilitating the activation of ERK1 by MKK1. Image reprinted with the kind permission of Dr. Roger Davis and Elsevier Science from Whitmarsh, A.J. and Davis, R. (1998) *TIBS*. **23**, 481–485.

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