Structural studies on the regulation of paralogous signal transduction pathways

Author: *Gábor Glatz*

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Glatz Gábor

ELTE, PhD School of Biology, PhD Program of Structural Biochemistry

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Introduction

Mitogen activated protein kinases (MAPKs) are activated through phosphorylation by MAPK kinases (MKK). MKK5 is the specific activator of extracellualr regulated kinase 5 (ERK5), which fulfills non-redundant physiological roles compared to its ERK1/2 MAPK paralogs. Mammals possess seven MKKs, where MKK1/2, MKK3/6, MKK7 activate ERK, p38 and JNK, respectively, MKK4 phosphorylates both p38 and JNK, and MKK5 is the specific activator of the ERK5 MAPK. While ERK1/2 is generally involved in mediating mitogenic signals, ERK5 controls distinct and somewhat more specific physiological responses in neuronal survival and differentiation, or in cardiovascular development where it plays a major role in the maintenance of blood vessel integrity for example . Both MAPKs are expressed ubiquitously and their activation patterns in different tissues often determine physiological outcomes in a synergistic or combinatorial manner.

ERK5 contains an N-terminal MAPK domain homologous to ERK1/2 and it also contains a long non-catalytic C-terminal tail that is involved in the regulation of its activity and cellular localization. Present-day MAPKs have emerged through whole genome or by individual gene duplication events during evolution. Interestingly, the phylogenetic tree of MAPKs and their MKK activators concurs well. The MAPK family – a branch of the CMGC protein kinase group - arose in the dawn of eukaryotic evolution. Separation of ERK-like and p38-like groups predates the divergence of animals and fungi and the four animal-specific MAPK subgroups (ERK1/2, ERK5, JNKs, p38s [α-β and γ-δ]) came into existence early in Metazoan evolution as recent genetic research into choanoflagellates, sponges and cnidarians show. The ERK5 pathway is intact in all Deuterostomes, but protostomes secondarily lost all components of this signaling pathway. With the emergence of vertebrates, full genome duplications created many similar MAPK paralogs also found in the human genome. MKKs belong to a separate branch of the kinome compared to MAPKs (STE7 protein kinase group) and MKK5 appears to be the closest homolog of ERK1/2 activating MKKs. Therefore, MKK5 likely appeared first in Metazoans following the separation of MAPKs involved in ERK or p38/JNK MAPK activation (MKK1/2 versus MKK7/4/3/6, respectively).

I investigated the molecular mechanisms determining the signaling specificity of ERK5 versus ERK1/2 MAPK modules. These two systems are evolutionarily clearly related, however they play distinct physiological roles. We concentrated only on the differences of ERK5 and ERK2 kinase domains

because these play the major role in the specific activation of MAPKs by MKKs. Although ERK5, in contrast to ERK2, has a long C-terminal non-catalytic tail, this unique ERK5 region have been suggested to play a role in the downstream signaling events that follow ERK5 phosphorylation by MKK5. In this study we examined the contribution of the MKK5 PB1 domain and the MKK5 linear docking motif towards ERK5-specific binding and activation in particular. We solved the crystal structure of the ERK5 kinase domain in complex with an MKK5 construct comprised of the MKK5 PB1 domain and the putative MAPK binding linear motif. This structure revealed that an ERK5 unique C-terminal kinase domain extension contacts the MKK5 PB1 domain surface by beta-strand addition and a linear motif with a long intervening region between consensus motif positions binds in the ERK5 docking groove. The ERK5 and ERK2 docking grooves are topographically different and we showed that this contributes to the specificity of MKK-MAPK and MAPK-substrate interaction and activation. We also reconstituted the three-tiered ERK5 MAPK module (MEKK3-MKK5-ERK5) in vitro and showed how that the MKK5 PB1 domain can work as a bivalent adaptor between the upstream activator kinase and a downstream substrate. These mechanisms described here for the human ERK5 MAPK module may have collectively contributed to the emergence of a distinct ERK-based signaling pathway in most organisms during evolution - at least in relation to the better studied and more canonical MKK1/2-ERK1/2 system.

Researh Goal

As present-day MAPKs evolved by gene duplication, it is an interesting question how new MAPK paralogs acquire novel signaling functions.
MKK1/2-ERK1/2 and MKK5-ERK5 systems serve as a paradigm how the latter MAP2K→MAPK transition became distinct by evolving novel protein-protein interactions and how signaling cascades using common components and/or similar paralogues enzymes can achieve functionally distinct, specific outcomes.

Results

1. All interacting regions (PB1 domain, docking motif, kinase domain) of MKK5 contribute to ERK5-specific binding.

2. Pull-down and fluorescence polarization (FP) assays showed minimally two MKK5 interacting regions are required to bind ERK5 and PB1 domain and a linear motif (PB1-D) cooperate to mediate high affinity binding to ERK5.

3. PB1-D discriminates between ERK5 and ERK2.

4. Crystal structure of ERK5 - MKK5 PB1-D complex (PDB code: 4IC7) a classical MAP2K→MAPK docking interaction, where positively charged and hydrofobic amino acides fit into the docking groove of ERK5 and a unique β-sheet-medieted interaction between the MKK5 PB1-domain and the C-terminus of ERK5 kinase domain.

5. Interaction assays showed that PB1 domain of MKK5 organizes MEKK2/3, MKK5 and ERK5 into one, signaling competent complex Model of the ERK5/PB1-D/MEKK3(PB1) ternary complex.

6. XIAP-mediated polyubiquitination of MEKK2/3 interferes with ERK5 phosphorylation, but not with MKK5 activation. Hence ERK5 pathway has an ubiquitin-dependent regulation compared to JNK pathway which is not inhibited by MEKK2/3 ubiquitination.

Abstract

Mitogen-activated protein kinases (MAPKs) are well-conserved elements of human signal transduction. They play critical roles in many biological processes, e.g. proliferation, differentiation, stress-induced signaling and apoptosis. MAPKs are activated through phosphorylation by MAPK kinases (MKKs). MKK5 is a specific activator of ERK5, which fulfills non-redundant physiological roles compared to its ERK1/2 paralogs. MKK1/2-ERK1/2 and MKK5-ERK5 pathways are clearly evolutionarily related, and they serve as a paradigm on how functionally distinct pathways can be built using paralogous signaling enzymes.

Pull-down and fluorescence polarization (FP) based assays showed that minimally two MKK5 interacting regions are required to bind ERK5. A Phox and Bem1 (PB1) domain and a linear motif (D-motif) from MKK5 cooperate to mediate high affinity binding to ERK5. MAPK activation depends on a linear binding motif found in all MAPK kinases (MKK). I present the crystal structure of ERK5 in complex with an MKK5 construct comprised of the PB1 domain and the linear binding motif. The structure reveals that ERK5 has distinct protein-protein interaction surfaces compared to ERK2, which is the closest ERK5 paralog. The two MAPKs have characteristically different physiological functions and their distinct protein-protein interaction surface topography enables them to bind different sets of activators and substrates. Structural and biochemical characterization revealed that the MKK5 PB1 domain cooperates with the MAPK binding linear motif to achieve substrate specific binding. In addition this domain also enables co-recruitment of the upstream activating enzyme and the down-stream substrate into one signaling competent complex. The upstream activator kinase for MKK5 is MEKK2/3, which also activates MKK7 that in turn activates c-jun N-terminal kinase (JNK) signaling. ERK5 and JNK signaling is functionally distinct and I demonstrate that ERK5 pathway activity is diminished upon MEKK2/3 ubiquitination by XIAP (X-linked Inhibitor of Apoptosis Protein). Interestingly, JNK activation is not inhibited by ubiquitination of this shared upstream activator kinase.