Chapter 16
Complexity and Modularity of MAPK Signaling Networks

George V. Popescu
University Politehnica Bucharest, Romania

Sorina C. Popescu
Boyce Thompson Institute for Plant Research, USA

ABSTRACT

Signaling through mitogen-activated protein kinase (MAPK) cascades is a conserved and fundamental process in all eukaryotes. This chapter reviews recent progress made in the identification of components of MAPK signaling networks using novel large scale experimental methods. It also presents recent landmarks in the computational modeling and simulation of the dynamics of MAPK signaling modules. The in vitro MAPK signaling network reconstructed from predicted phosphorylation events is dense, supporting the hypothesis of a combinatorial control of transcription through selective phosphorylation of sets of transcription factors. Despite the fact that additional co-factors and scaffold proteins may regulate the dynamics of signal transduction in vivo, the complexity of MAPK signaling networks supports a new model that departs significantly from that of the classical definition of a MAPK cascade.

INTRODUCTION

Mitogen-activated protein kinase (MAPK) cascades are components of intracellular signaling activated in response to a wide array of external and internal signals, contributing towards development of diverse cellular responses such as growth, differentiation, response to pathogens, and cell death. A MAPK cascade contains several key components: a MAPK kinase kinase (MAP3K), a MAPK kinase (MAP2K) and a MAPK. MAP3Ks, activated by upstream kinases or receptor-associated molecules, activate in turn the MAP2Ks. Activated MAP2Ks phosphorylate the MAPK components which, in turn, phosphorylate diverse substrates such as transcription and translation factors, protein kinases and phosphatases, thus regulating many cellular processes in response

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to the initial stimulus (Chen & Thorner, 2007; Zhang et al., 2006).

In plants, the diversity of processes regulated through MAPK signaling cascades combined with the large number of predicted members within MAPK families implies a robust and well synchronized control of kinase signaling. In support of this hypothesis, recent large-scale screens to identify signaling proteins in eukaryotic organisms challenged the classical view of signaling cascades as simple, linear conduits composed of a handful of elements. Current models reflect the apparent complexity of signaling pathways, the cross-talk between parallel pathways, and the dynamic nature of the protein interactions (Friedman & Perrimon, 2006, 2007; Mackay, 2004; Popescu et al., 2007). A recent study (Popescu, Popescu, & Bachan et al., 2009) re-constituted a MAPK phosphorylation network based on experimental data generated using high-density protein microarrays. The study found that MAPKs are able to phosphorylate *in vitro* a large diversity of transcription factors with known or predicted roles in disease resistance, flower development, cellular differentiation and auxin signaling. The analysis of the re-constituted signaling network supported the hypothesis of a combinatorial control of cellular processes through MAP2Ks and MAPKs cascades.

In this chapter, we present a systems view of the molecular interactions of the MAPK proteins and review current results on the architecture and dynamics of MAPK signaling networks.

BACKGROUND

Signaling Components Represent a Major Part of Higher Eukaryotic Genomes

Identification of signaling networks is a central research topic in the systematic analysis of cellular organization. The employed methods range from direct, low throughput screening of kinase phosphorylation targets to indirect, large scale predictions of protein-protein interactions from protein microarrays data, protein similarity search, analysis of protein domains and gene co-expression. Signaling through MAPK cascades is a fundamental and conserved process in eukaryotes. The MAPK signaling network has a hierarchical structure (Figure 1) composed of at least three levels of nodes: (1) the MAP3K proteins which, when activated directly or indirectly by receptor molecules, phosphorylate MAP2K proteins; (2) the activated MAP2Ks which activate and phosphorylate MAPK proteins; (3) a multitude of cytoplasmic and nuclear substrates acted upon by activated MAPK; in addition, a MAP4K may activate the MAP3K component.

A prototypical multicellular model organism useful for the study of the architecture of MAPK signaling networks is *Arabidopsis thaliana*. Direct functional assays and analysis of sequence conservation with other eukaryotic organisms identified 128 *Arabidopsis* genes, putative members of four MAPKs groups (MAP4Ks, MAP3Ks, MAP2Ks, and MAPKs) (Champion et al., 2004; Ichimura et al., 2002). The large size of the *Arabidopsis* MAPK gene complement is comparable only with the mammalian one (Uhlik et al., 2004). Out of this large group of genes, only a handful have been verified functionally and proven to have roles in signal transduction within MAPK cascades or as adaptors (scaffolds) that bring together members of a MAPK module. The 10 *Arabidopsis* predicted MAP4K genes were classified in several groups such as PAK-like (p21 Ras-activated protein kinase), MST-like (mammalian sterile 20-like) and SOC-like (STE20 oxidant stress kinase), based on similarity with the yeast and mammalian counterparts (Champion et al., 2004). It is possible that some MAP4Ks function as adaptor proteins rather than enzymatic components of the MAPK signaling modules. The MAP3Ks constitute a heterogeneous family including 20 plant MEKKs ("true" MAP3Ks) and 58 plant MEKK-related genes (48