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Methods for analyzing MAPK cascades

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Mitogen-activated protein kinases (MAPKs) and their activating kinases transduce an enormous variety of extracellular and intracellular signals into cellular responses in all eukaryotic organisms. They play critical roles in proliferation, development, response to stress, and cell survival, often serving as the key relayers of information from the cell surface to transcriptional events in the nucleus. MAP kinases and their activators are being studied as drug targets for therapeutic intervention in a variety of diseases.

The goal of this issue of *Methods* is to provide readers both tried and true and state-of-the-art methods to analyze MAPK cascades *in vitro* and *in vivo*. The analysis of MAPK cascades involves assessment of potential target proteins and potential regulatory proteins on MAPK activity. Charles Heise and Melanie Cobb provide a generally applicable step-by-step method for expressing and activating the best studied MAPK, ERK2, in bacteria by a constitutively active mutant of MEK1, using thiophosphorylation for a more durable active version resistant to phosphoprotein phosphatases for analysis of downstream targets and activation capacity. Lee Bardwell and Kandarp Shah describe *in vitro* translation for monitoring and delineating MAPK interactions with docking partners that can be followed by the uninitiated. MAPKs were among the first protein kinases to be crystallized, and crystallization is used widely to analyze their function. Seung-Jae Lee, Tianjun Zhou, and Elizabeth Goldsmith provide an in depth compilation of methodologies used to crystallize a wide variety of MAPKs in inactive and active forms, with or without inhibitors or peptide docking partners that will serve as an excellent starting point for any investigator. Vera Cherkasova describes the use of tried and true immunoprecipitation to identify or confirm binding partners of MAPKs and follow their activity *in vivo*. Blagoy Blagoev and Matthias Mann provide a step-by-step protocol of state-of-the art quantitative MS-based proteomics they have developed using stable isotope labeling by amino acids in cell culture (SILAC), a versatile methodology that can be used to study numerous aspects of MAPK pathway signaling *in vivo*.

Significant advancement in our knowledge of MAPK pathways has been gained through genetic analysis in model organisms such as yeast, *Drosophila*, and *C. elegans*. Hiten Madhani outlines the basics of genetic analysis and key issues to consider in analyzing MAPK function *in vivo* by knock out and knock down techniques. Functional analysis of MAPKs can be difficult in instances of functional redundancy among related MAPK isozymes activated by common stimuli, such as is found with ERK, JNK, and SAPK families of MAPKs. David Engleberg and Oded Livnah describe a strategy of complementation of yeast MAPK mutants by MAPKs from other organisms for identifying intrinsically active MAPKs that can be used to define function. With the development of genome-wide RNAi and ShRNA libraries it is now possible to screen for new components of mitogen-activated protein kinase pathways in cell culture. Adam Friedman and Norbert Perrimon provide an elegant description of generally applicable high-throughput approaches to assaying MAPK signaling in the *Drosophila* receptor tyrosine kinase (RTK)/extracellular signal-regulated kinase (ERK) pathway with

examples of secondary validation screens and methods for managing large datasets for future *in vivo* functional characterization. A major role of MAPKs is now appreciated to be transcriptional control on chromosomes. Amparo Pascual-Ahuir, Kevin Struhl, and Markus Proft describe generally applicable chromatin immunoprecipitation in combination with microarrays (ChIP-Chip) first used to identify the transcriptional targets of yeast Hog1 on a genomic scale.

Finally, it is of great interest to analyze MAPK activation and localization in single cells as a function of various stimuli, tissue type, and interacting molecules. Stephen Michnick, Marnie MacDonald, and John Westwick describe the clever approach of protein-fragment complementation assay (PCA) in simple and high-throughput quantitative microscopic methods to locate and quantify MAPK pathway protein complexes. Qiang Ni, Denis V. Titov, and Jin Zhang provide an informative description of emerging FRET reporters that could be applied to studying MAPK dynamics.

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