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Point of VIEW

Defining MAPK Interactomes

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ABSTRACT The mitogen-activated protein kinase (MAPK) signaling network is integrated into most, if not all, defined homeostatic and regulatory responses of eukaryotic cells. A systematic experimental and computational approach has been used to identify more than 2,000 protein interactions related to functions and regulation of the MAPK signaling network. This discovery process provides a rich data and reagent resource for defining complexities of protein networks involving MAPKs and control of cellular physiology.

Mitogen-activated protein kinases (MAPKs) are expressed in all eukaryotic cells. Despite their ubiquitous expression, they regulate very specific biological responses that differ between cellular contexts. The classical view of MAPK pathways is a three-kinase module consisting of a MAP3K → MAP2K → MAPK. In fact, MAPKs, MAP2Ks, and MAP3Ks are part of a large, integrated signaling network and represent a rich target set for small molecule inhibitors to treat many human diseases (*1*). The recent work of Bandyopadhyay *et al.* (*2*) published in *Nature Methods* provides a large new data and reagent resource for investigators to discover the complex protein interactome involving the MAPK signaling network. The unique aspect of this work is the combinatorial methods used to define the MAPK interactome. The approach involved developing a physical protein interaction map using 86 MAPK network bait proteins in yeast two-hybrid screens against the proteome. This was followed by a second screen with 21 secondary baits identified as preys in the primary two-hybrid screen. Using this experimental data set a core MAPK network of 641 high-confidence interactions was defined. The investigators then used a siRNA screen for expression knockdown of 14 proteins where there was no previous evidence based on literature search for being part of the MAPK interactome. A robust reporter gene assay measuring AP-1 or NFκβ activity, representing two activities commonly regulated by the MAPK network, showed that a third of the RNAi target genes indeed regulated activity of the MAPK signaling network. RNAi targeting of 45 random genes gave only a 4% hit rate in the AP-1 or NFκβ activity screen. In addition, the interactome analysis predicted specific proteins to function as scaffolds that assemble MAPK signaling units. Several of these proteins were biochemically validated to have scaffolding function to regulate MAPK signaling. Overall, more than 2,000 protein interactions within a MAPK interactome were defined. The cDNA clone libraries characterizing the protein interactions are available upon request for further study and characterization.

Understanding the MAPK interactome is a formidable task and one that will vary in different cellular contexts. Thus, the MAPK interactome is more accurately represented as a dynamic, large interactome that varies with stimulus and cell type. There are 11 MAPKs, 7 MAP2Ks, and at least 20 MAP3Ks in human cells (Figure 1). This represents approximately 8% of the human kinome. MAPKs regulate many physiological processes in response to diverse stimuli including cytokines, growth factors, antigens, toxins, drugs, cell shape, adherence to extracellular matrix, and cell−cell interactions. The activation of MAPKs in response to these diverse stimuli contributes to the control of transcription, proliferation, development, cell death, motility, and many other important regulatory responses in cells. To control such diverse biological responses, MAPKs are activated and inactivated with spatial and temporal accuracy within the cell. Loss of this spatiotemporal control of MAPK signaling by mutation or altered expression of proteins regulating MAPKs can

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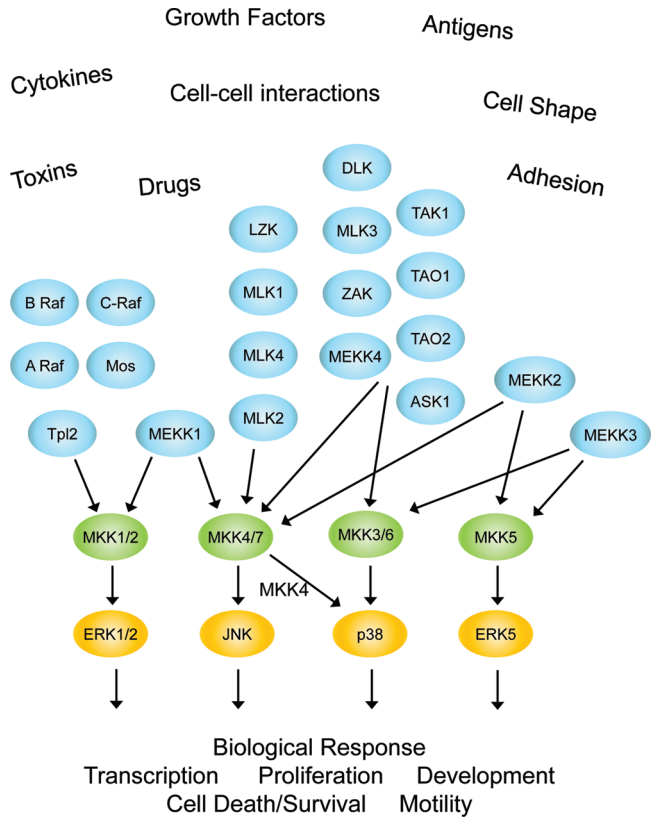

Figure 1. Model depicting the MAPK signaling network. Shown are MAPKs, MAP2Ks, and MAP3Ks. The kinases interact with many different proteins to regulate the spatial and temporal control of the MAPK network. Shown are representative types of stimuli that differentially activate and responses controlled by the MAPK network. Activation and responses in the different cell types are regulated by the MAPK interactome characterized by Bandyopadhyay (*2*).

lead to diseases including cancer, neurodegeneration, inflammation, and developmental defects. It is the selective interactions of the 40 kinases within the MAPK network with themselves and, just as importantly, with other proteins that orchestrates the spatiotemporal control and integration of the signaling system. Hence, defining the dynamic nature of MAPK interactomes is extremely important for understanding homeostatic regulatory mechanisms and how deregulation of the MAPK interactome contributes to human disease.

An important aspect of the work by Bandyopadhyay *et al.* (*2*) was the choice of the initial 86 baits used for the yeast two-hybrid screen. The baits included 27 known MAPKs, 14 transcription factors defined to be downstream of MAPKs, and 4 proteins associated with membrane receptors that activate MAPKs. A number of additional kinases, phospholipases, GTPases, and scaffolds known to regulate the MAPK network were also used as baits. The primary screen yielded a large set of 1,496 protein–protein interactions that was expanded to 2,269 interactions with a second yeast two-hybrid screen using 21 secondary baits detected as preys in the first screen. Confirmation that the screens worked to identify protein interactions relevant for the MAPK interactome was evident from the observation of significant overlap with proteins identified in Drosophila for ERK1/2 activation defined by siRNA screens and proteins phosphorylated in HeLa cells in response to EGF, which gives a strong activation of ERK1/2 and JNK. This information was assimilated in an attempt to identify proteins with multiple protein interactions that might function as MAPK signaling scaffolds, with multiple kinase interactions as one criterion used to define a scaffold protein. Ten such proteins were identified, including the known MAPK scaffold, the filamin protein FLNA. Several of these proteins including NHE1 and RANBP9 were shown to have properties of protein scaffolds, illustrating the richness of the experimental and data mining resource.

What might the MAPK interactome resource be used for by other investigators? An *in silico* benefit is simply to scan the protein–protein interaction data set for proteins of interest for an investigator. Identifying binding partners can provide invaluable insight into function and novel regulatory mechanisms for a protein of interest. In analyzing such data, it is important to understand that protein–protein interaction domains control the formation of multiprotein complexes that cumulatively make up an interactome. There are at least 81 defined protein interaction domains providing a rich diversity of interactions within an interactome (*3, 4*). Even a given protein interaction domain has complexity, and sequence changes within a domain can alter affinity and specificity of protein interactions. In addition to the modular characteristics of interaction domains, protein interactions are often fine-tuned by variations of amino acid sequences in surface patches outside the interaction domain (*5*). Changes in surface patch sequences provide a means in addition to the architecture of interaction domains to alter affinities of protein–protein interactions. The more than 2,000 protein–protein interactions defined for the MAPK interactome need to be analyzed for known protein–protein interaction domains and the possible discovery of new protein–protein interaction motifs. Poten-

tial regulatory mechanisms should be examined, such as defining possible phosphorylation sites, by searching protein interactor sequences with phosphoproteomic databases. Other possible covalent modifications such as ubiquitination should also be analyzed.

Experimentally, the MAPK interactome can provide an interesting resource for investigators performing RNAi screens, particularly synthetic lethal screens or more complex cell response RNAi screens where the relationship between two proteins may be unknown but found to be interactors or part of a larger multiprotein complex defined within the MAPK interactome. The MAPK interactome can also be useful as a reference for defining proteins identified in antibody co-immunoprecipitation studies and other co-affinity purification type experiments. The experimental challenge is to understand the behavior and dynamics of the MAPK interactome in different cellular contexts. This will require experiments that parse out dominant nodes within the MAPK interactome important for specific biological responses that will probably require a number of different genetic, cellular, biochemical, and computational approaches. Accomplishing this task will identify new targets for small molecule development. Finally, as the domain/motif/surface patch sequences controlling protein−protein interactions within the MAPK interactome are defined, it should be possible to use this information for rationale design of synthetic signaling systems (*6*). Such synthetic systems will be engineered in the future to function as disease sentinels or coupled to metabolic sensors to regulate physiologic responses to treat disease.

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