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Protein phosphorylation remains as a black box in signal transduction: developing new methods to search for substrates of protein kinases and the related database

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Protein phosphorylation is a major post-translational modification in eukaryotic cells that plays a critical role in various cellular processes. More than 500 protein kinases are encoded in the human genome and are classified into 7 major groups based on their catalytic domain sequences, with each kinase playing distinct roles through its specific substrates. Although several kinases have been well-characterized and demonstrated to be physiologically important, the functions of a number of kinases remain to be elucidated. For example, more than 200,000 human phosphorylation sites are registered in PhosphoSitePlus, a large database of post-translational modifications, but only 8,000 sites are linked to their respective kinases.

To identify the specific substrates for the specific kinases, we have recently developed an *in vitro* approach termed the kinase-interacting substrate screening (KISS) method (Amano et al. J Cell Biol 2015) and an *in vivo* approach termed kinase-oriented substrate screening (KIOSS) method (Nagai et al. Neuron 2016; Nagai et al. Trend Pharmacol Sci 2016). We determined a lot of specific substrates for kinases including PKA, PKC, MAPK, CaMKs, CDKs, PAK, LYN, FYN and Rho-kinases (ROCK). By using these methods, we identified the PKA substrates downstream of dopamine from mouse striatum, and found more than one hundred candidate substrates of PKA, including Rap1 GEF (Rasgrp2). We also found that PKA phosphorylation of Rasgrp2 activated its guanine nucleotide exchange activity on Rap1. Cocaine exposure activated Rap1 in nucleus accumbense in mice through dopamine. The expression of constitutively active PKA or Rap1 in accumbal D1R-expressing medium spiny neurons (D1R-MSNs) enhanced neuronal firing rates and behavioral responses to cocaine exposure through MAPK. Knockout of Rap1 in the accumbal D1R-MSNs was sufficient to decrease these phenotypes. These findings demonstrate a novel DA-PKA-Rap1-MAPK intracellular signaling mechanism in D1R-MSNs that increases neuronal excitability to enhance reward-related behaviors.

We also constructed an on-line database system, named “KANPHOS (Kinase-Associated Neural PHOspho-Signaling), that provides the phosphorylation signals identified by our methods as well as those previously reported in the literature. All data are controlled for quality via review and curated by our professional staffs. In the portal site, we can search for the data of interest in three ways: 1) Search for substrates phosphorylated by a specific kinase; 2) Search for kinases phosphorylating a specific protein; and 3) Search for kinases and their target substrates by a specific signaling pathway. Each substrate is associated with basic information provided by the UniProt Knowledgebase (UniProtKB) such as protein and gene name synonyms and description about physiological functions. Also, to allow the users for cross-searching with minimal efforts, each entry has hyperlink to external databases (e.g. KEGG PATHWAY Database, GeneCards, and Allen Brain Atlas).