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# Functional analysis of protein kinase networks in living cells: Beyond "knock-outs" and "knock-downs"

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**Abstract**

The identification of over 500 protein kinases encoded by the human genome sequence offers one measure of the importance of protein kinase networks in cell biology. High throughput technologies for inactivating genes are producing an awe-inspiring amount of data on the cellular and organismal effects of reducing the levels of individual protein kinases. Despite these technical advances, our understanding of kinase networks remains imprecise. Major challenges include correctly assigning kinases to particular networks, understanding how they are regulated, and identifying the relevant *in vivo* substrates. Genetic methods provide a way of addressing these questions, but their application requires understanding the nuances of how different types of mutations can affect protein kinases. The goal of this article is to provide a brief introductory primer into these issues using examples from yeast MAPK cascades and to motivate future systematic genetic analysis focusing on individual residues of protein kinases.


*Keywords:* Kinase; Signaling; Genetics; Yeast

## 1. Introduction

The generation of gene "knock-outs" and the application of RNAi technology have been the most widely used methodologies for assessing the functions of protein kinases in cells. Less generic but of major clinical importance are small-molecule inhibitors of protein kinases. Although highly useful, each method has its drawbacks. Knock-out mutations can fail to yield effects because of genetic redundancy. RNAi can be incomplete and display off-target effects. Small-molecule inhibitors can affect both the intended target and unintended targets. Beyond these technical issues, there are more fundamental limitations to these often-utilized approaches because of the inherent sophistication of protein kinases as regulatory enzymes.

## 2. Thinking about the genetics of protein kinases

Several properties of protein kinases are important for the interpretation of mutations in the corresponding genes. These include (but are not limited to) the following (Fig. 1):

(A) Protein kinases often have multiple states, such as an enzymatically active phosphorylated state and an enzymatically inactive unphosphorylated state.
(B) The enzymatically inactive state may have "kinase-independent" functions.
(C) Protein kinases often contain so-called "autoinhibitory" domains that inhibit the activation of the enzymatic kinase domain. Activation of kinases can result from inactivation of the autoinhibitory domain.
(D) Protein kinases frequently have multiple substrates. For some kinases, associated proteins control substrate specificity.

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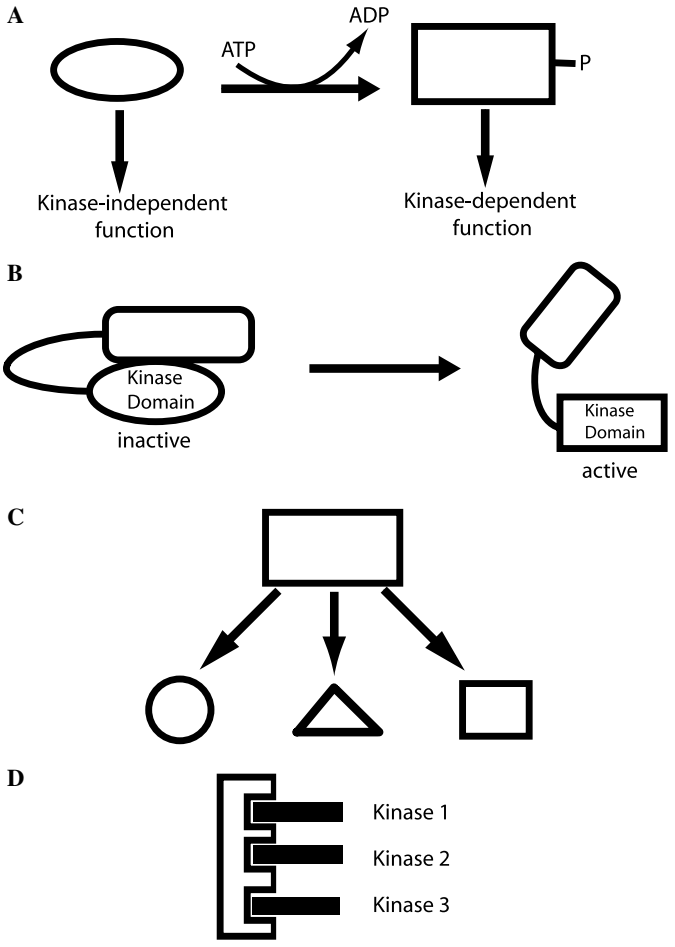


Fig. 1. Protein kinases are sophisticated enzymes. (A) Kinases can have kinase-independent functions. (B) Kinases can be regulated by autoinhibitory domains. (C) Kinase can have multiple substrates. (D) Kinases can be controlled by interactions with scaffolding proteins.

(E) Many protein kinases bind to scaffold proteins that can influence their activities.

The complexity of regulation and substrate specificity of protein kinases means that mutations that eliminate a protein kinase from a cell will have different effects from those that affect a particular state of a kinase, an inhibitory domain, or a residue in the kinase involved in substrate specificity. This means that considering only the effects of reducing the levels of a protein kinase will lead to an incomplete picture of regulation and function.

## 3. Classes of mutations: terms and meanings

Placing mutations into categories is helpful for interpreting the results of genetic experiments. The categories that follow were originally described by H.J. Muller based on his analysis of mutants in *Drosophila* in the early part of the 20th century (Table 1). Note that I use the term "mutation" to refer to a change in the DNA sequence of a gene, whereas "mutant" refers either to a cell containing a mutation or a protein encoded by the gene harboring that change.

Table 1
Classes of mutations

| | |
|---|---|
| *Loss-of-function* (*recessive*) | |
| Null | No gene product synthesized |
| Hypomorph | Reduced levels of normal gene product and/or reduced activity of gene product |
| *Gain-of-function* (*dominant*) | |
| Hypermorph | Increased amount of normal gene product and/or increased activity of gene product |
| Neomorph | Abnormal activity of gene product and/or expression in wrong time or place |
| Antimorph | "Dominant-negative" inhibits activity of normal gene product |

### 3.1. Loss-of-function mutations

A mutation in a gene that precludes synthesis of the encoded polypeptide is termed a "null allele" or "deletion allele." In recent years, the boxing term "knock-out" has been used synonymously. A mutation that reduces (but does not eliminate) the levels of a protein or its activity is termed a "reduced-function" or "hypomorphic" allele. RNAi techniques induce degradation of mRNAs, reducing protein levels. The reduced-function effect of RNAi is sometimes called a "knock-down." These classes of mutations are generally recessive to wild-type; that is, in the presence of the normal or wild-type allele of a gene, the mutation has no effect. This is interpreted to mean that the mutation reduces or eliminates the function of protein, hence the name "loss-of-function" mutation.

### 3.2. Gain-of-function mutations

These mutations are "dominant" to the wild-type allele meaning that they produce a phenotype even when the wild-type allele is present. They can be classified as follows. Those that increase the levels of the wild-type protein or the activity of the wild-type protein are called "increased wild-type function" or "hypermorphs." A second class comprises mutations that produce a protein with an abnormal activity (a protein that does something that the normal protein does not do or does it at the wrong time or place)—these mutations are termed "neomorphs." A third class comprises mutations that produce a phenotype similar to a null or hypomorphic allele, yet are dominant to the wild-type allele: these are called "antimorphs" or, more commonly, "dominant-negative" mutations.

This vocabulary falls short when a protein has more than one function, a common occurrence in biology. In these cases, the effect of a mutation is generally considered with respect to a particular function.

## 4. Interpreting mutants of protein kinases: three examples from yeast MAPK cascades

The considerations outlined above are not merely theoretical. Below I describe examples that illustrate how the analysis of specific alleles of protein kinases (as opposed to