Yin and Yang of Phosphorylation in Cytokine Signaling

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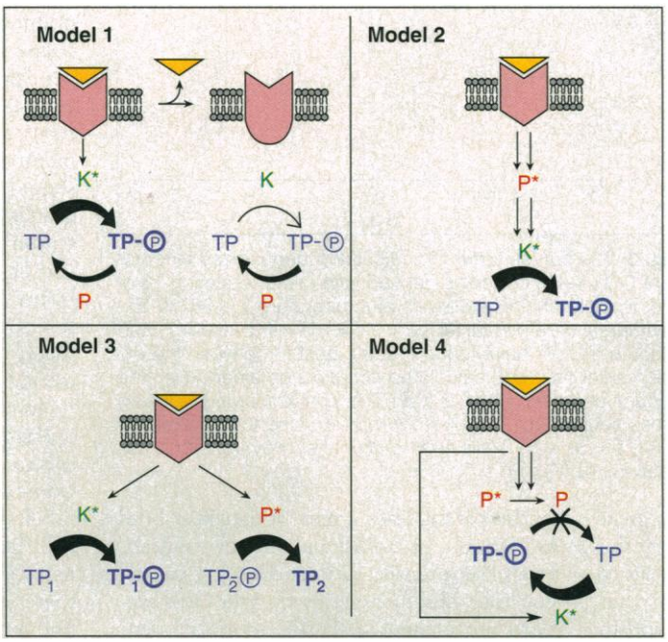
Signal transduction is the process by which extracellular molecules (such as cytokines) bind to receptors on the cell surface and generate intracellular regulatory signals that are transmitted to different parts of the cell. Initially, research on signal transduction sought to identify the soluble, intracellular second messengers. But now the emphasis is on the key enzyme participants in pathways that trigger gene transcription. Many second messengers [calcium and cyclic adenosine monophosphate (cAMP), for example] can stimulate the ubiquitous protein kinases, which are encoded by as much as 1% of the human genome. These enzymes fall into two categories: the serine-threonine protein kinases, which catalyze the addition of phosphate groups to the amino acids serine and threonine, and the tyrosine kinases, which add phosphate only to tyrosine residues in proteins. A third category of dual-specificity kinases can do both. Protein phosphatases, which remove phosphates from proteins, fall into similar categories on the basis of the amino acid residue from which the phosphate group is removed.



Kinases and phosphatases in cytokine signaling. (**Model 1**) The kinase (K) is activated (*) by ligand binding, while the phosphatase (P) restores target protein (TP) phosphorylation to equilibrium. (**Model 2**) The phosphatase is activated by ligand binding and activates a downstream kinase, which effects target protein phosphorylation. (**Model 3**) A kinase and a phosphatase are activated by ligand binding and act on different target proteins to effect signaling. (**Model 4**) Ligand binding results in phosphatase inactivation, possibly in conjunction with kinase activation to give a dual regulation effecting target protein phosphorylation. The form of target protein responsible for altered cell function in response to ligand binding is in bold.

Protein phosphatases and protein kinases have been considered the "yin" and "yang" of cytokine signal transduction: Kinases as active modulators (yang) and phosphatases as passive counter-modulators (yin) jointly maintain the phosphorylation of cellular proteins in homeostasis. Treatment of cells with cytokines changes this balance, rapidly sending signals from point to point in the cell. This dichotomy is like the yin and yang of traditional Chinese medicine in which yin, representing all that is "cold" or "passive," is in equilibrium with yang, which represents all that is "hot" or "active." Maintaining yin and yang in harmony is akin to attaining the homeostatic state.

The role of protein kinases in cytokine signaling is well recognized. For example, binding of mitogen to cells activates a receptor tyrosine kinase that activates Ras, Raf, MAP kinase kinase, MAP kinase, Rsk, and several transcription factors in a phosphorylation cascade (*1*). Less is known about how protein phosphatases participate in signal transduction. The expectation has been that phosphatases passively return cytokine-activated pathways to equilibrium (see figure, Model 1). However, our view of phosphatases is changing rapidly; it seems that protein phosphatases are not merely passive partners after all. Through the use of inhibitors of serine-threonine phosphatases (*2–4*) and an increased understanding of protein tyrosine phosphatases (PTPs) (*5*), it is becoming clear that direct regulation of phosphatases can be a primary signaling mechanism.

Type 1 interferon (IFN) increases intracellular diacylglycerol and activates protein kinase C (PKC) isoforms (*6*, *7*), and changes in early protein phosphorylation can be induced in cells by IFN-γ (*8*). A year ago, IFN was also shown to activate a protein tyrosine kinase (PTK) (*9*). The activated PTK phosphorylates an IFN-specific transcription factor (ISGF-3) on tyrosine. This phosphorylation is inhibited by staurosporine (a PKC inhibitor) and genistein (a PTK inhibitor), suggesting the involvement of both serine-threonine and tyrosine kinases in IFN signaling. Moreover, the phosphorylation and activation of ISGF-3 and γ-activated factor (a transcription factor activated by IFN-γ) are also unexpectedly inhibited by vanadate, phenylarsine oxide, and zinc chloride (PTP inhibitors); thus, a PTP may be involved in IFN and IFN-γ signaling (*10*). Interferon signaling could therefore occur by the putative tyrosine phosphatase activating the tyrosine kinase that targets ISGF-3 (see figure, Model 2). In this case, the traditional ying-like protein phosphatase of cell signaling would act more yang-like in stimulating a tyrosine kinase, perhaps by direct dephosphorylation of the PTK. A precedent for this mechanism was recently established for a receptor-like PTP, PTPα, in the activation of the proto-oncogene product c-Src (*11*). When overexpressed in primary rat fibroblasts, this phosphatase dephosphorylates c-Src at Tyr^{527}, activating the tyrosine kinase activity of c-Src. In the process, the transfected rat cells become virulently tumorigenic (*11*).

The emerging importance of protein phosphatases is also illustrated by recent work on T cells. Stimulated T cells lacking the tyrosine phosphatase CD45 are unable to generate the phosphatidylinositol-derived second messengers inositol trisphosphate and Ca^{2+}, probably due to the cell's inability to activate tyrosine kinases that couple the T cell receptor to the phosphatidylinositol pathway (*12*). Lymphokines that regulate the development and differentiation of the hematopoietic system also activate a tyrosine phosphatase (*13*). Addition of interleukin-4 (IL-4) (a lymphokine that triggers the proliferation and differentiation of human B cells) to leukemic cell lines causes the rapid and dose-dependent dephosphorylation of an 80-kD protein on tyrosine. The ability of vanadate to block IL-4–induced dephosphorylation suggests that IL-4 signaling also includes a PTP, perhaps acting in concert with IL-4–activated kinase (see figure, Model 3).

The importance of tyrosine phosphatases in cytokine signal transduction in

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the whole animal is further emphasized by the the mouse mutant *Motheaten*. Mice carrying this mutation show marked abnormalities in hematopoiesis and dysfunction of the immune system (*14*). The mutation was recently traced to the *Hcph* gene, which encodes the hematopoietic cell protein (HCP) tyrosine phosphatase (*15*). Defective HCP function may abrogate colony stimulating factor (CSF)–1 signaling, because wild-type HCP is rapidly phosphorylated in macrophages after the binding of CSF1-1 to its receptor (*16*).

Until recently little was known of serine-threonine phosphatases in cytokine signaling, although tumor necrosis factor (TNF) and IL-1 activate multiple protein kinases by means of hsp27 kinase, MAP kinase, β-casein kinase, and other unidentified tyrosine kinases in primary human fibroblasts (*3*, *17*). Treatment of primary human cells with TNF and IL-1 initiates the rapid enhancement of phosphorylation of more than 100 phosphoproteins, including several transcription factors (*3*). Among the cytokines tested (TNF, IL-1, epidermal growth factor, and IFN-α, -β, and -γ), the pattern of changes in early protein phosphorylation is ligand-specific, with TNF and IL-1 (both have similar biological activities) producing almost identical changes in cellular protein phosphorylation. Treatment of cells with inhibitors of protein phosphatase–1 and –2A (okadaic acid, calyculin A, and cantharidin) but not inhibitors of protein phosphatase–2B also produces changes in early protein phosphorylation and gene transcription, which are remarkably similar to those observed with TNF and IL-1 treatment (*3*). This striking symmetry in the effects of cytokines and inhibitors of protein phosphatases (*3*, *4*) implies that the phosphorylation of cellular substrates by TNF- and IL-1–activated protein kinases is tightly regulated by these inhibitor-sensitive phosphatases. The inactivation of the phosphatases by the inhibitor alters the balance between the opposing phosphorylation and dephosphorylation reaction in favor of net phosphorylation. Similarly, TNF and IL-1 treatment could lead to the inactivation of inhibitor-sensitive protein phosphatases (see figure, Model 4). This inhibition of dephosphorylation would increase the phosphorylation of cellular protein substrates by the opposing protein kinase. Thus, the inactivation of a protein phosphatase could serve as a signaling mechanism (*3*). Indeed, the protein phosphatase that is rapidly inactivated in primary human fibroblasts after TNF treatment is a redox-sensitive class of protein phosphatase–2A or related phosphatases regulated by the cellular redox potential (*3*).

In these several examples, phosphatases do not behave in a way consistent with their previous yin-like status as simple counter-regulators for kinases and are taking a more active yang-like role in cytokine signaling. It will not be surprising if they turn out to be as ubiquitous and crucial as the protein kinases.

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Plant Biotechnology in China

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China is the largest agricultural country in the world. The nation's farmers, who account for about two-thirds of China's 1.2 billion inhabitants, face an enormous challenge: They must produce enough food for 20% of the world's population in a country with only 7% of the world's cultivable land. Plant biotechnology, which draws on the molecular biology methods developed during the 1960s and 1970s, is likely to play an important role in meeting this challenge.

From the beginning, scientists in China have made many important contributions to the development of plant biotechnology, particularly in the area of tissue, cell, and protoplast culture (see figure). This research has received strong support from the Chinese government. In 1986, the government launched a National High Technology Plan in which plant biotechnology was listed as a top priority. Currently, more than 100 laboratories are supported by grants from this plan as well as other government funds. Several foreign governments and international organizations such as the World Bank and the Rockefeller Foundation have also provided financial assistance to plant scientists in China. With this support, China has become a leader in plant biotechnology among Asian countries (*1*, *2*). In this perspective, we provide a brief overview of the agricultural problems to which this technology is being applied.

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Developing pathogen-resistant plants

Plant viruses have caused significant reductions in crop yield in China, and consequently there is great interest in developing new antiviral strategies. After the observation by Beachy's laboratory in 1986 that tobacco plants transformed with the coat protein gene of tobacco mosaic virus (TMV) acquire resistance to the virus (*3*), scientists in China began testing coat protein genes from the Chinese strains of pathogenic viruses for similar antiviral activity. This is now the most active area of plant biotechnology research in the country (*1*).

The coat protein genes of TMV, cucumber mosaic virus (CMV), potato viruses X and Y, soybean mosaic virus, rice dwarf virus, and other viruses have been cloned and characterized (*4*). Some of these genes have been engineered into plants and are being tested for antiviral activity in the field. Tobacco transformed with the TMV coat protein gene has been planted in 500 hectares of land in 12 Chinese provinces (*5*), and transgenic tomatoes carrying the CMV coat protein are being similarly tested (*6*). Early results from researchers at the Institute of Microbiology, Chinese Academy of