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Cell signaling regulation by protein phosphorylation: a multivariate, heterogeneous, and context-dependent process

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Proper spatiotemporal regulation of protein phosphorylation in cells and tissues is required for normal development and homeostasis, but aberrant protein phosphorylation regulation leads to various diseases. The study of signaling regulation by protein phosphorylation is complicated in part by the sheer scope of the kinome and phosphoproteome, dependence of signaling protein functionality on cellular localization, and the complex multivariate relationships that exist between protein phosphorylation dynamics and the cellular phenotypes they control. Additional complexities arise from the ability of microenvironmental factors to influence phosphorylation-dependent signaling and from the tendency for some signaling processes to occur heterogeneously among cells. These considerations should be taken into account when measuring cell signaling regulation by protein phosphorylation.

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Cell signaling regulation by protein phosphorylation

Cell signaling is the biochemical process by which cells are cued to respond to perturbations in their environment. In one example, ligand binding to the extracellular domain of a receptor initiates a network of intracellular biochemical reactions that affect cell phenotypes by modifying gene expression, metabolism, or cytoskeletal arrangements. Signaling regulates most normal cell processes, but improper signaling results in numerous diseases.

Many biomolecules participate in signaling, including proteins, amino acids, lipids, and second messengers (e.g., cyclic AMP, inositol triphosphate). The focus here is signaling regulation by protein phosphorylation, which plays several mechanistic roles. Phosphorylation generally receives greater attention than other post-translational modifications, in part because kinases are often overexpressed or mutated in disease, especially cancer. As a result, many inhibitors and antibodies have been developed to antagonize the activity of kinases.

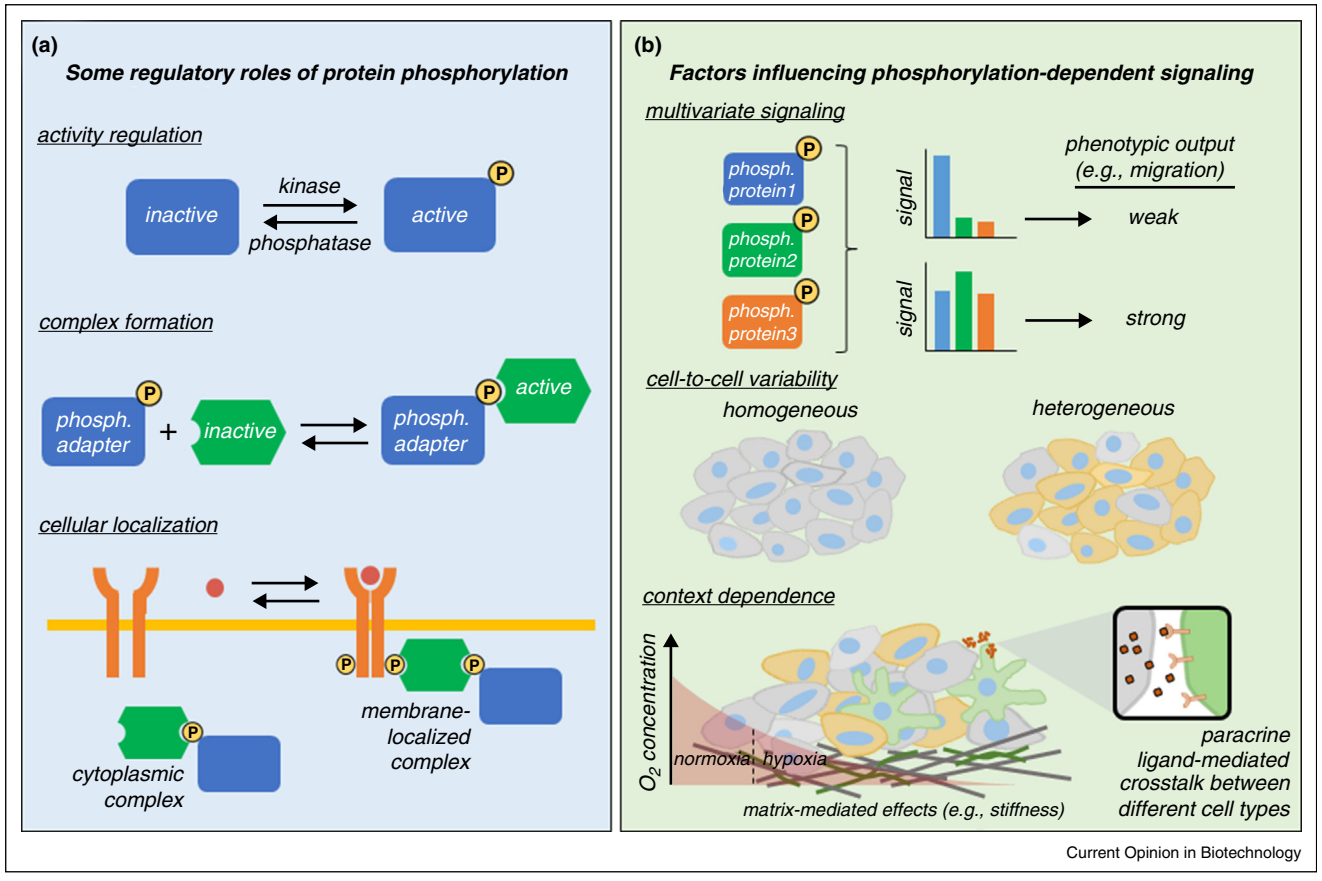
Protein phosphorylation by kinases: mechanistic aspects and signaling regulatory roles

Protein phosphorylation is a reversible process wherein phosphate from ATP (or other nucleoside phosphates) is esterified to amino acids by protein kinases. Serine and threonine phosphorylation are the most common phosphorylation events, with tyrosine accounting for <1% of the total esterified phosphate. Other amino acids, including histidine and lysine, can be phosphorylated, but roles of these events have not been deeply investigated.

In humans, more than 500 kinases control protein phosphorylation. Of the 90 tyrosine kinases, 56 are transmembrane receptors (receptor tyrosine kinases, RTK). Most kinases are serine/threonine kinases. Some of these are receptors, and some have dual specificity (tyrosine and serine/threonine). Protein kinases have conserved structural motifs including an activation loop, catalytic domain, and ATP binding domain. Many kinase structures have been solved, which has aided inhibitor design.

Protein phosphorylation regulates signaling in multiple ways (Figure 1a). Phosphorylation of residues in kinase activation loops promotes an active kinase conformation (e.g., Thr^{202}/Tyr^{204} in the extracellular regulated kinase, ERK). Conversely, some phosphorylation events negatively regulate kinase activity (e.g., SRC Tyr^{527} phosphorylation, which promotes inhibitory intramolecular tethering). Thus, some phosphorylation events are probed as proxies for host protein kinase activity. Protein phosphorylation also creates binding sites for proteins containing cognate motifs. For phosphotyrosines, these include SRC homology 2 (SH2) and phosphotyrosine binding (PTB) domains. Domains for phosphorylated serine or threonine also exist. Phosphorylation-dependent protein complex formation has regulatory roles including protein localization, activation of constituent proteins, and protein trafficking.

Figure 1



(a) Protein phosphorylation (primarily on serine, threonine, or tyrosine) can influence cell signaling in multiple ways including: regulation of biochemical activity of host proteins; reversible formation of protein complexes (e.g., through SH2 domain–phosphotyrosine binding interactions); and regulation of protein localization. **(b)** Complete analysis and understanding of phosphorylation-dependent signaling processes may need to account for: the abilities of multiple signaling pathways to coordinate to determine complex cellular phenotypes; the tendency for cell signaling processes to occur heterogeneously among cells in populations; and the ability of the local cell context (microenvironment) to influence signaling processes through numerous effects including matrix mechanical effects on adhesion proteins, hypoxia-initiated transcriptional regulation, and ligand-mediated intercellular crosstalk.

Protein phosphatases

Phosphorylation-dependent signaling is tightly regulated by protein phosphatases, which hydrolyze phosphate esters (Figure 1a, top panel). 147 protein phosphatases are encoded in the human genome. There are approximately 100 protein tyrosine phosphatases (PTPs). 37 are phosphotyrosine-specific, including 21 receptor-like PTPs. 65 PTPs have dual specificity for phosphorylated serine or threonine and tyrosine. While phosphatase biochemical functions have been well studied, substrates of specific phosphatases remain largely unknown. Phosphatases are often referred to as non-specific, but some recent findings may argue against this notion [1].

Phosphatases are critical for normal cell signaling, and alterations to phosphatase expression or localization and phosphatase mutation arise in numerous diseases and can alter response to therapy [2,3]. Interestingly, elevated phosphatase activity can sometimes promote, rather than antagonize, pathogenic signaling. SH2-domain containing phosphatase 2 (SHP2), which is required for complete ERK activation downstream of most RTKs, provides a well-known example [4,5]. Although phosphatase catalytic domains have been difficult to drug with specificity, inhibition through allosteric mechanisms may be a viable approach [6].

An underappreciated aspect of phosphatase regulation is that dephosphorylation rates can be fast compared to other signal transduction rates, including those for receptor trafficking and signaling persistence. This issue has been studied for epidermal growth factor (EGF) receptor (EGFR) signaling using experimental and computational modeling [7,8]. These studies have revealed that EGFR