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In the Beginning, There Was Protein Phosphorylation

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The importance of reversible protein phosphorylation to cellular regulation cannot be overstated. In eukaryotic cells, protein kinase/phosphatase signaling pathways regulate a staggering number of cellular processes, including cell proliferation, cell death (apoptosis, necroptosis, necrosis), metabolism (at both the cellular and organismal levels), behavior and neurological function, development, and pathogen resistance. Although protein phosphorylation as a mode of eukaryotic cell regulation is familiar to most biochemists, many are less familiar with protein kinase/phosphatase signaling networks that function in prokaryotes. In this thematic minireview series, we present four minireviews that cover the important field of prokaryotic protein phosphorylation.

The phenomenon of reversible protein phosphorylation was discovered >50 years ago by Edwin Krebs and Edmond Fischer and described in a series of classic papers published in this journal. These papers described a protein kinase activity that converted phosphorylase *b* to phosphorylase *a* and laid the groundwork for dissecting the molecular signaling pathway by which epinephrine could inactivate muscle glycogen synthase (1, 2).

The notion of a multistep protein kinase pathway triggered by the production of second messengers and inactivated by protein phosphatases also set a paradigm for understanding signal transduction by protein phosphorylation cascades. Numerous protein kinase cascades have been described in the intervening years, including the MAPKs, the Akt and mTOR pathways, the NF-κB pathway, the JAK/STAT pathway, and others. Each of these pathways is recruited by extracellular stimuli acting through receptors that transduce these signals through the generation of second messengers (cyclic nucleotides, inositol phosphates, etc.), receptor Tyr kinase autophosphorylation (a form of second messenger in which such phosphorylation, at the receptor intracellular extensions, prompts the binding and membrane recruitment of downstream adaptors), or the more recently discovered stimulus-induced formation of second messengers consisting of free Lys^{63}-linked polyubiquitin chains (3–9).

Eukaryotic protein kinases fall into three broad classes: Ser/Thr-specific protein kinases that phosphorylate Ser or Thr residues exclusively, Tyr kinases that phosphorylate Tyr exclusively, or dual-specificity kinases (exemplified by MEKs) that can phosphorylate Tyr and Ser/Thr concomitantly (10, 11).

All eukaryotic protein kinases contain a conserved phosphotransferase catalytic domain consisting of 12 conserved subdomains designated with Roman numerals (I–XII). These fold into a bilobed structure consisting of a smaller N-terminal lobe comprised of subdomains I–IV, which is involved primarily in anchoring and orienting nucleotide triphosphate (in most cases, ATP) (10, 11).

Within this lobe is a classic glycine-rich subdomain I motif ($G^{50}XGXXGXV^{57}$ in PKA) that forms part of the nucleotide-binding pocket. A subdomain II invariant Lys residue (Lys^{72} in PKA) coordinates the γ-phosphate of ATP and is critical for the phosphotransfer reaction (10, 11).

A larger C-terminal lobe consisting of subdomains VI–XII binds substrate protein and initiates phosphotransfer. Subdomain V serves as a linker region between the two lobes (10, 11).

A portion of subdomain VI contains a conserved motif (H^{164}RDLK*XX*N^{171} in PKA) that includes an Asp residue that likely functions as a catalytic base. A conserved subdomain VII triplet ($D^{184}FG^{186}$ in PKA) chelates the Mg^{2+} ions that bridge the β- and γ-phosphates of ATP. Subdomain VIII contains an activation loop that includes a conserved triplet (APE, amino acids 206–208 in PKA) and distinct residues that are conserved among different protein kinase families. This region often contains phosphoacceptor sites targeted by upstream regulatory protein kinases that control the activation state of the target protein kinase. This domain is also key to the recognition of peptide substrates (10, 11).

There is conservation between the catalytic domains of Ser/Thr, Tyr, and dual-specificity kinases; however, Tyr kinase catalytic domains also have distinct structural features that mediate their specificity for tyrosine residues. For example, the classic G*X*G*XX*G motif in subdomain I is more narrowly defined in Tyr kinases (G*X*G*X*PG), whereas the HRDLK*XX*N motif in Ser/Thr kinases is HRDL*XXX*N in Tyr kinases (10–12).

Nevertheless, the conserved eukaryotic protein kinase domain retains its bilobed structure throughout the family. This structure and its components have been reviewed extensively (10–12).

Protein kinases in prokaryotic cells are a more recent discovery, originating from work beginning in the 1970s. Emerging evidence indicates that, as with eukaryotes, prokaryotic cell protein phosphorylation networks regulate a wide variety of cell functions.

Unlike mammalian protein kinase signaling, which has seen remarkable progress in the last 30 years, progress in dissecting and understanding prokaryotic protein kinase and phosphatase signaling has been slower. This is a shame because understanding these pathways could be of considerable clinical (many such pathways are key to the survival and physiology of pathological microorganisms) or industrial importance. Moreover, understanding the structural similarities (and notable differences) between eukaryotic and prokaryotic protein phosphorylation

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polypeptides and between their respective physiological functions provides important insight into the evolution of protein phosphorylation as a mechanism of cellular regulation. This is key inasmuch as the existence of whole protein kinase groups is not evenly distributed among eukaryotes; for example, among eukaryotes, receptor Tyr kinases are found only in metazoans.

In this thematic minireview series, we present four exciting minireviews on prokaryotic protein kinases. In the first minireview, Yossef Av-Gay and colleagues discuss prokaryotic protein-Tyr kinases. Of particular note, these fall into two broad categories: one that resembles structurally eukaryotic Tyr kinases and the other that includes the so-called BY-kinases (bacterial tyrosine kinases) and the "odd" kinases. The BY-kinases are unrelated to any known eukaryotic kinases, suggesting an early evolutionary divergence in protein kinase structure (13).

In contrast to the consensus eukaryotic Tyr kinase domain, the catalytic domains of BY-kinases consist of Walker A loops (for nucleotide binding) and Walker B loops (for nucleotide hydrolysis) (13). As one might expect, the non-conserved BY-kinases regulate functions that are seemingly unique to bacteria such as biofilm formation and capsule formation, whereas there is an interesting (if admittedly simplistic) similarity between the conserved prokaryotic Tyr kinase functions and the functions of eukaryotic Tyr kinases (*i.e.* regulation of cell growth, metabolism, and development) (13).

Av-Gay and colleagues provide special focus on the odd non-conserved *Mycobacterium tuberculosis* Tyr kinase PtkA. This kinase contains neither the conserved G*X*G*X*(*X*/P)G motif of eukaryotic Tyr kinases nor the Walker A motifs of BY-kinases. Of note, a key substrate of PtkA is a prokaryotic Tyr phosphatase (PtpA) required for virulence (13).

In the second minireview, Virginie Molle and her colleague, Marc J. Canova, discuss prokaryotic Ser/Thr kinases. Ser/Thr kinases are present in a broad range of microbial pathogens, including *Streptococcus*, *Mycobacteria, Yersinia*, and *Listeria*, where they participate in host-pathogen interactions (14). By and large, the catalytic domains of prokaryotic Ser/Thr kinases are structurally within the consensus defined by eukaryotic Ser/Thr kinases (14).

Many of these kinases are secreted, and a signature function of many Ser/Thr kinases in microbial pathogens is the phosphorylation of host cell proteins, a process that disrupts host cell function and contributes to pathogenicity. Thus, *Yersinia pestis* YpkA consists of an N-terminal kinase domain and a C-terminal guanine nucleotide exchange inhibitor domain. The former domain can phosphorylate and inactivate the host cell $G\alpha_q$ protein, whereas the guanine nucleotide exchange inhibitor motif can inactivate the mammalian Ras superfamily protein RhoA. These events can lead to a distortion of cell shape and motility. The *Legionella* kinase LegK1 phosphorylates the host cell IκB protein, thereby triggering activation of the NF-κB pathway and producing an inappropriate and potentially lethal hyperinflammatory response (14). Others of this group of kinases, including the four Pkn kinases of *M. tuberculosis*, are more poorly understood, although they appear to be antagonistic to pathogenicity (14). The obvious clinical significance of these kinases makes them ideal candidates for drug development.

In the third minireview, Peter J. Kennelly discusses the protein-Ser/Thr and protein-Tyr kinases of the most ancient of species, the archaea. Interestingly, archaea, which share more similar properties with eukaryotic cells than do eubacteria, contain both protein kinases and phosphatases that structurally resemble their eukaryotic cell distant cousins. The protein kinases of archaea are all structurally related and resemble both eukaryotic Ser/Thr and Tyr kinases. Indeed, the phosphoproteomes of archaea that have been examined contain phospho-Ser, phospho-Thr, and phospho-Tyr. By contrast, the phosphatases in these organisms diverge structurally and fall into either the protein-Ser/Thr phosphatase or protein-Tyr phosphatase superfamily (15).

Still, it is important to note that the structural resemblance between eukaryotic and archaeal protein kinases and phosphatases suggests, as Kennelly notes, that the first eukaryotic protein kinases likely appeared after the divergence of the combined archaeal/eukaryotic line but prior to the divergence of the archaea from eukaryotes (15).

Much less is known of the functions of archaeal protein phosphorylations. Much of what is known arises from studies of the Sulfolobales order. *Sulfolobus solfataricus* PK3, a kinase from this group, appears to be Ser/Thr-specific. Studies of the phosphoproteomes of Sulfolobales indicate a variety of potential targets, including aminoacyl-tRNA synthases, DNA helicases, gyrases, and other proteins, and an abundance of Tyr phosphorylation. The stoichiometry of each phosphorylation event and its physiological significance are somewhat unclear; however, the sheer number of phosphoproteins in these archaea indicates relevance. Indeed, substantial alterations in the *S. solfataricus* phosphoproteome change with alterations in the nutrient milieu, and *in vitro* studies indicate that kinases in these organisms can regulate metabolic enzymes in a phosphorylation-dependent manner (15).

Finally, Nicole A. LaRonde presents a minireview on microbial RIO (right open reading frame) kinases. The RIO kinases may be the most ancient (they may have emerged before the divergence of archaea and eubacteria) and widely expressed kinases, with examples in all prokaryotes and in eukaryotes, including humans (16).

Structurally, the catalytic cores of RIO kinases resemble the canonical eukaryotic structure; however, in the RIO family, the consensus domain is considerably truncated, missing the key subdomain VIII activation loop sequences. However, all of the nucleotide-binding and phosphoryl transfer loops seen in eukaryotic kinases are present. Many RIO kinases also contain extracatalytic domains that are required for enzymatic function (16).

Although a function for eukaryotic RIO kinases in ribosome biogenesis has been identified, little else is known about RIO kinase substrates or functions, especially in prokaryotes. Some archaeal RIO kinases may modulate ribosomal activity, serving as ribosome-processing factors, whereas others may play a role in modulating the proteasome (16).

This thematic minireview series provides a survey of prokaryotic protein kinases and sheds light on the wide conservation of protein phosphorylation as a mode of cellular regulation. With this series, we hope to provoke greater interest in this

emerging and exciting field. The elucidation of functions for these enzymes will prove important in clarifying the molecular evolution of protein kinases and could prove critical to the development of novel clinical approaches to deal with microbial pathology. One lesson is already clear. It is important that we broaden our thinking about protein phosphorylation to consider non-eukaryotic cell mechanisms.

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