COMMENTARY

Bacterial spore coat protein kinases: A new twist to an old story

John D. Scott[a,1] and Alexandra C. Newton[b]

"Plus ça change, plus c'est la même chose" (the more things change, the more they stay the same). Nowhere is this more apparent than in biology, where nature cleverly recycles and adapts the same chemical reactions to control complex physiology from bacteria to archaea, plants, and metazoans (1, 2). However, those of us who work on protein phosphorylation have been lured into thinking bacteria do it one way and the rest of nature another. It has been over 60 years since Fischer and Krebs (3) worked out the basic mechanisms of protein phosphorylation, 35 years since Tony Hunter connected tyrosine phosphorylation to oncogenesis (4), and 25 years since Susan Taylor gave us the iconic protein kinase fold (5). Structural genomic analyses have defined approaching 550 members of the protein kinase superfamily in humans and a burgeoning cohort of pseudokinases (1). This latter group look like their active counterparts but function as allosteric modulators or as enzyme scaffolds (6). So, it seems as if we know it all. However, an article published in PNAS (7) revealing the importance of bacterial Ser and Thr protein kinases in the phosphorylation of spore coat proteins may have begun a new chapter in the protein phosphorylation saga.

Regardless of their function, all archaea, plant, and metazoan kinases have evolved to enable the transfer a molecule of phosphate from ATP onto the hydroxyl of Ser, Thr, and Tyr residues. Conversely, bacterial protein kinases are traditionally considered to modify the imidazole of histidine residues on proteins as part of a two-component system. Nevertheless, a few bacterial Ser and Thr kinases were identified, but these enzymes were considered to be esoteric and were often relegated to annals of intellectual curiosity. However, Vincent Tagliabracci and Jack Dixon, who are among the authors of the study published in PNAS (7), set the stage to challenge this dogma when they discovered a family of divergent eukaryotic protein kinases called the Fam20 subgroup (8, 9). Members of this atypical protein kinase subgroup phosphorylate proteins within the lumen of the secretory pathway or modify proteoglycan substrates. Fam20C, the most celebrated member of this unconventional kinase cohort, turns out to be the physiological casein kinase. This enzyme plays a key regulatory role in the maturation of the milk protein casein, and human mutations in Fam20C are linked to Raine syndrome, a deadly osteosclerotic bone dysplasia (10). However, the link between Fam20C-like kinases and bacterial spore coat proteins had yet to be made.

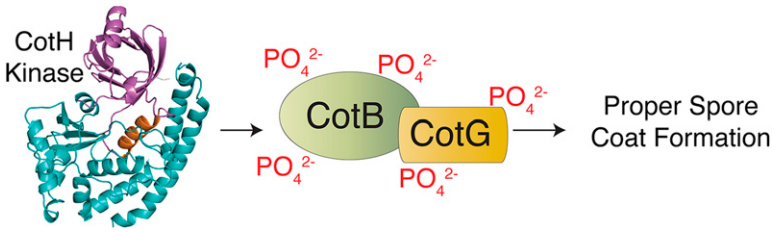

Fig. 1. Phosphorylation of spore coat proteins by the CotH family of kinases. CotH orthologs are found in many spore-forming prokaryotes and eukaryotes. In the model spore-forming organism *B. subtilis*, CotH is a component of the spore coat and phosphorylates two other coat proteins, CotB and CotG. Hyperphosphorylation of these proteins is important for the proper assembly of coat proteins and subsequent germination of the spore when environmental conditions become favorable for growth.

This conceptual link was sparked by a conversation between Tagliabracci and bioinfomatician Krzysztof Pawlowski at a protein kinase meeting in Warsaw, Poland (11). Pawlowski subsequently noted sequence similarities between members of the Fam20 family and the bacterial persistence kinase, HipA. Now, using HipA as a template, Pawlowski and his colleagues uncovered limited, but significant, hallmarks of eukaryotic protein kinases in CotH, one of ~70 proteins that form the bacterial endospore or spore coat. This proteinacious shield forms in response to nutrient deprivation and acts like a molecular sieve to exclude large toxic molecules that can damage the enzymes that catalyze bacterial germination. This informatics odyssey culminated with the demonstration by Kim Nguyen, a research technician at University of California at San Diego, and Anju Sreelatha, a postdoctoral fellow in the Tagliabracci group, that the *Bacillus subtilis* and *Bacillus cereus* CotH orthologs possess protein kinase activity. So, now we have Ser/Thr protein kinase activity in the bacterial spore coat—nature reminding us once again that the more things change, the more they stay the same.

[a]Howard Hughes Medical Institute, Department of Pharmacology, University of Washington, Seattle, WA 98195; and [b]Department of Pharmacology, University of California, San Diego, La Jolla, CA 92093

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[1]To whom correspondence should be addressed. Email: scottjdw@uw.edu.

So what is so special about the CotH kinases? Perhaps not surprisingly the crystal structure of *B. cereus* CotH, bound to AMP, reveals an atypical protein kinase-like fold. Scrutiny of protein structure databases indicates that *B. cereus* CotH is most similar to HipA and the phosphatidylinositol 4-kinase family. Nguyen et al. also notes several unique features within the active site of CotH (7). For example, the adenine moiety of AMP is sandwiched by two aromatic residues. Additionally, CotH kinases have an Arg within the Gly-rich nucleotide binding loop that coordinates the alpha phosphate of AMP. This Arg is stabilized by a hydrogen bond with an Asn residue. This unique interaction functionally replaces the ion pair that is considered a hallmark of an activated protein kinase. Also, the authors note that *B. subtilis* CotH displays a preference for Mn2+ as the activating divalent cation. This is an interesting feature when one considers that *B. subtilis* is a soil-dwelling organism and may, depending upon the prevailing environmental conditions, have more access to Mn2+ than to Mg2+. Thus, CotH is a structurally distinct bacterial kinase whose optimization for a unique environment results in a slightly different mechanics of phosphotransfer than its metazoan counterparts. We refer readers to a more complete and scholarly analysis of the similarities and differences between CotH and metazoan protein kinases in the PNAS article (7).

A notable feature of the CotH structure is that it does not fully adopt the iconic bilobal fold of a canonical protein kinase. In fact, the N-lobe in *B. cereus* CotH is quite different. Most notably, the N-lobe of the kinase fold contains a seven-stranded β-barrel–like subdomain filled with hydrophobic residues (Fig. 1). These aforementioned findings are vaguely reminiscent of work on the *Shigella* effector kinase OspG, another distant relative of the protein kinase superfamily (12). This pathogenic kinase is assembled upon *Shigella* infection in the cytosol of eukaryotic cells to suppress the host inflammatory response. Bioinformatics analyses reveal that OspG encodes a minimal kinase domain with some of the essential elements required for catalysis. However, this is not enough for full activity. In fact, recruitment of ubiquitin to the catalytic core is necessary to stabilize an active conformation of the OspG kinase. This interesting variation on a theme was validated by Pruneda et al. (13) when they solved the cocrystal structure of the OspG/UbcH5c~Ub complex. This reveals a three-lobed kinase–ubiquitin assembly that constrains OspG in the active conformation. Although they are structurally diverse, parallels can be drawn between CotH and OspG. Thus, one can conclude that CotH and OspG are distant relatives of the metazoan protein kinase and have adapted to the prokaryotic world by taking advantage of unique features of their environment, whether it be accessibility to atypical divalent cations or the recruitment of other signaling proteins. This latter point raises an intriguing chicken-or-egg conundrum. Are CotH and OspG remnants of ancestral kinases that have evolved into their metazoan counterparts? Or, alternatively, do the atypical structural features of both enzymes provide clear evidence for convergent evolution toward utilization of the phosphotransfer reaction?

Another fascinating aspect of this work is the relationship of CotH to its substrates. Inspection of the *B. cerus* and *B. subtilis* genomes reveals that the gene for *cotH* is in the neighborhood of genes for two other spore coat proteins, CotB and CotG (Fig. 1). This led Nguyen et al. (7) to establish that CotB and CotG are substrates for the CotH kinase. In an elegant series of biochemical studies, the authors characterized the phosphorylation sites in CotG and noted that basic residues surround the phosphosites. A particularly innovative facet of the work was to take advantage of a phospho-PKC substrate antibody, which recognizes phosphorylated Ser in the context of basic residues, to demonstrate robust immunoreactivity in WT spores and virtually no phosphorylation in CotH null spores. The labeling of multiple bands suggests that CotG and CotB are only two of a number of other possible substrates for CotH (Fig. 1). Accordingly, protein phosphorylation of the spore coat must be a key regulatory event in its formation. Functional studies confirm this notion, showing that CotH kinase activity is required for the efficient germination of spores in *B. subtilis*. CotH orthologs are found in many spore-forming bacteria and eukaryotic species, including pathogenic species such as *Bacillus anthracis* and *Rhizopus oryzae*, the causative agents of anthrax and mucormycosis, respectively. Hence, the discovery of this new family of bacterial protein kinases not only reveals a previously unappreciated role for protein phosphorylation in spore biology, but it may ultimately have clinical implications.

So, where do we go from here? Understanding how phosphorylation of coat proteins by CotH contributes to the assembly of spores is likely to shed light on analogous processes in eukaryotic cells. For example, formation and maintenance of the extracellular matrix involves the concerted assembly of protein complexes and gels of polysaccharides. These extracellular substructures may prove to be ripe targets for the secreted Fam20C and Fam20B kinases, respectively. Finally, because several CotH-containing organisms are human pathogens, the new perspective on bacterial protein kinase activity provided by Nguyen et al. (7) may have clinical implications as we reassess how to combat human diseases such as anthrax and fungal infections including mucormycosis. This clear demonstration of bacteria using Ser/Thr phosphorylation, hitherto assumed to be a metazoan modification, for a prokaryote physiology provides a lot of food for thought. It underscores that although we still have much to learn about protein phosphorylation and the enzymes that catalyze this fundamental regulatory process some things do not change.

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