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COMMENTARY

An insight into future antibacterial therapy

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There is an urgent need for new strategies to combat the spread of drug-resistant bacteria worldwide. This commentary concerns, namely bacterial protein phosphorylation as a promising target for novel antibacterials.

The constant increase of bacterial resistance to conventional antibiotics has indeed become a dramatic public health problem, critically requiring the discovery of innovative antibacterial drugs with new modes of action. In the past decades, this situation has been worsened by the considerably reduced investment of large pharmaceutical companies in the research and development of antibiotics.[1]

Antibiotic resistance is acquired by chromosome mutation and/or integration of plasmids/transposons that carry resistance determinants by means of horizontal gene transfer.[2] The main mechanisms[3] through which resistance can develop are: (i) qualitative or quantitative modification of the target; (ii) enzymatic inactivation of antibiotics by hydrolysis or structural alteration; (iii) prevention of drug accumulation due to the impermeability of bacterial cell or increased efflux; and (iv) mutations in drug-activating enzymes.[4–6] Since different types of antibiotics have been frequently used simultaneously, several bacterial species have evolved toward multiresistance.

In the search for new antibacterials, different strategies have been explored. One of them has consisted in bringing incremental improvements to existing antibiotics by chemical modification, with, however, the risk for the corresponding derivatives being rapidly ineffective against the prevailing resistance mechanisms.[1,7] This strategy has been reinforced by extensive efforts made to better understand the mode of action at the molecular level of already known antibiotics, namely by using efficient techniques of structure determination such as nuclear magnetic resonance (NMR) and X-ray crystallography. In addition, new chemical classes have appeared, either natural or synthetic, and novel molecules have been assayed for therapeutic potential.[8] The use of combination therapies involving treatment of infections by sets of drugs rather than individual drugs has also been considered.[9] Still, in most cases, those different antibiotics have turned out to inhibit in fact the same four classical targets: nucleic acid biosynthesis, protein biosynthesis, cell wall formation and folic acid biosynthesis.[8]

From the mid-1990′s, the availability of a large number of complete bacterial genome sequences has provided an impressive tool to identify a variety of new putative targets.[10] The genome-based technologies and high-throughput screenings have generated a renewed interest in the search of novel antibiotics, especially in small biotechnology companies and academic centers. This concerns gram-negative, as well as gram-positive species, given the fact that there are less effective agents for treating gram-negative infections.[11] To cite a few, the new targets include fatty acid biosynthesis, lipoprotein biogenesis, efflux pumps, protein secretion, riboswitches and some specific antimicrobial peptides.[12] However, the results obtained to date indicate that minimal success has been met in converting theses targets into drugs, since none of them has reached advanced clinical development yet.[1,7] Revisiting the choice of targets and screen designs, and the compound libraries chosen for screens should help in improving the efficiency of this approach.[13]

Another target of special interest concerns bacterial protein phosphorylation by endogenous specific enzymes, which represents a promising way toward the discovery of non-conventional antibacterial drugs. This post-translational modification was long claimed to be restricted to eukaryotes until its occurrence was first demonstrated, simultaneously and independently, in *Escherichia coli*[14] and *Salmonella typhimurium*.[15] Since then, its existence has been described in a multitude of bacterial species and it is now considered an ubiquitous process in non-eukaryotic organisms.

Whereas eukaryotes utilize basically only one type of phosphorylating machinery that operates through the modification of proteins at serine/threonine or tyrosine residues at the expense of adenosine triphosphate (ATP), bacteria possess a diversity of phosphorylating systems. One system is similar, but not identical, to that of eukaryotes[16,17] (Figure 1A). Recent phosphoproteomics analyses have shown that bacterial serine/threonine and tyrosine kinases play vital roles in the molecular mechanisms of cell signaling and general regulation of cellular functions, such as central metabolism, cell growth, cell division and differentiation.[18] Therefore, these kinases and the cognate phosphoprotein phosphatases represent attractive antibacterial targets that deserve further investigation. Research in this field will obviously necessitate characterization of specific structural and functional features that differentiate the bacterial enzymes from their eukaryotic counterparts. On the other hand, bacteria are able to detect and transport different sugar substrates through their membrane, which are essential to their growth, by using the phosphoenolpyruvate: carbohydrate phosphotransferase system, which is a process strictly specific to prokaryotes[19] (Figure 1B). In this case, a phosphoryl group provided by phosphoenolpyruvate is passed down a cascade of five proteins or protein domains, and finally transferred to a sugar. Moreover, bacteria harbor a wide range of two-component systems (TCS) and their expanded variants known as phosphorelays

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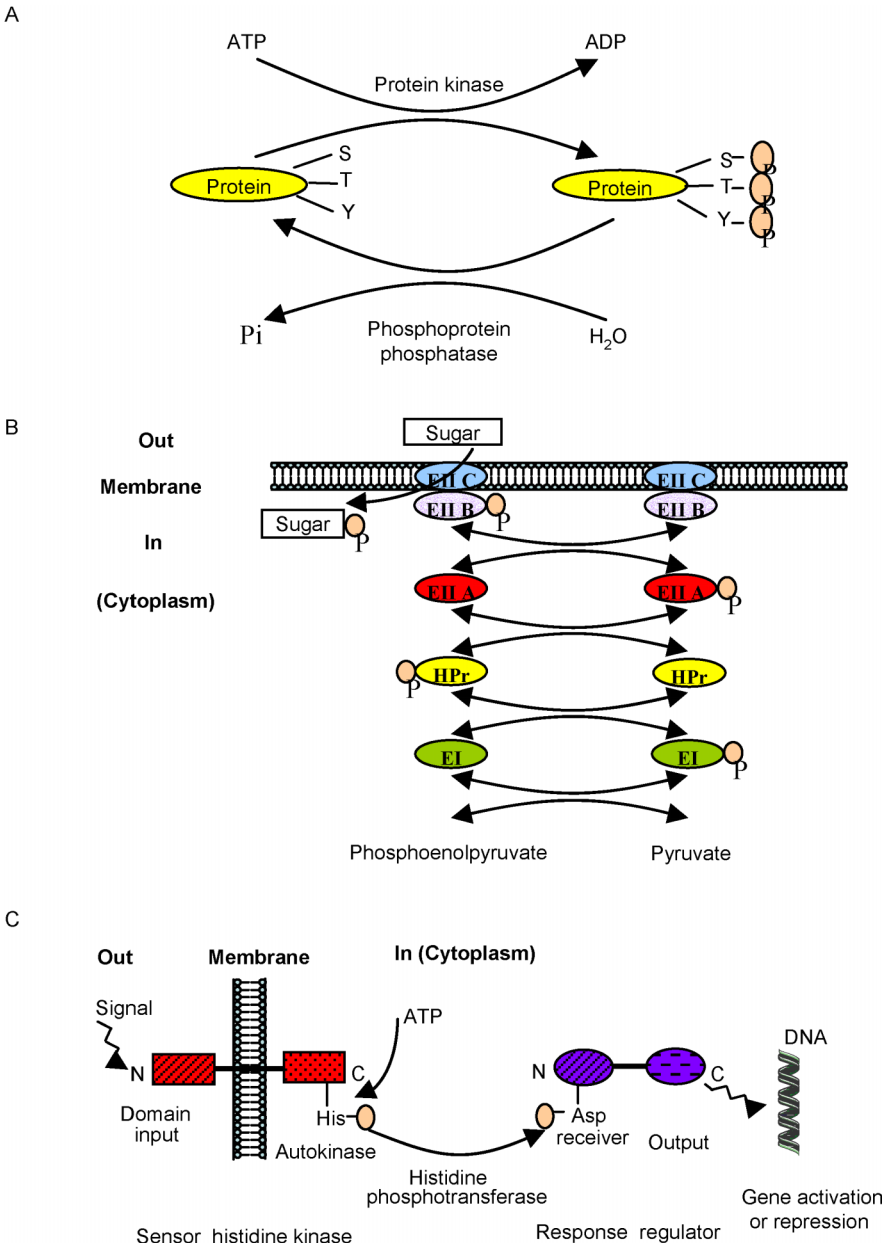

Figure 1 Schematic presentation of three major phosphorylating systems in bacteria. (**A**) ATP-dependent phosphorylation/dephosphorylation of proteins at serine, threonine or tyrosine residues. (**B**) Phosphoenolpyruvate: carbohydrate phosphotransferase system. (**C**) Two-component system. Phosphoryl groups are indicated by orange oval symbols.

to adapt to environmental conditions.[20] To do so, a sensor histidine protein kinase autophosphorylates in response to signal ligands and subsequently transfers its phosphate to an invariant aspartate residue of a response regulator that is generally a transcription factor (Figure 1C). Here again, since TCS are absent in higher eukaryotes, they can be selectively targeted by antibacterial molecules.

Of particular importance is the fact that the activity of these three major phosphorylating systems, which are not mutually exclusive, is closely connected with the virulence of pathogens.[21] Thus, the possibility to attenuate virulence by acting on this relationship represents a notably interesting approach to prevent the expression of pathogenicity without causing the arrest of growth or death of bacteria, as do conventional antibiotics. The ensuing advantage is the preservation of the host endogenous normal flora and the limitation of the selective pressure, which results in a decreased capacity of drug resistance.

To date, a number of antibacterial agents have already proven to impair virulence, although to a varying extent, by interfering with phosphorylation. For instance, the low-molecular-weight inhibitor AX20017 blocks selectively the activity of the serine/threonine protein kinase PknG, a virulence factor of *Mycobacterium tuberculosis*, by interacting with a unique set of amino-acid side chains in the kinase domain that are not found in any human kinase.[22] Also, the production and transport of bacterial surface polysaccharides that are potent virulence factors controlled by the activity of the tyrosine kinases called BY-kinases, are inhibited by peptide nucleic acid analogues able to bind

specifically to the ATP-binding pocket of these enzymes.[23] In both phosphotransferase system and TCS systems, a variety of molecules stemming from library screens and structure–activity relationship programs have been checked for potential inhibition. But, so far, most of them have been shown not to be selective enough or even to have sometimes unexpected side effects.[24] However, in TCS systems, a small molecule of the benzenesulfonamide family, LED 209, has recently been shown to inhibit the binding of signals to the histidine protein kinase QseC, which prevents its autophosphorylation and consequently the virulence gene expression mediated by this kinase in several pathogens.[25] In a similar way, the infection process by *Streptococcus pneumoniae* is impaired by a series of chemical compounds, namely, furan and thiophene derivatives, which inhibit the histidine protein kinase VicK.[26] In general, the blockade of histidine kinase activity is not lethal to the bacterial cell. Nevertheles, the deranged regulation that occurs consequently results in a bacteriostatic effect that can be sufficient to hinder infection. This applies as well to serine/threonine and tyrosine kinases. Thus, for example, in-frame deletions of the *stk1* gene that encodes serine/threonine kinase Stk1 in *Staphylococcus aureus* cause a strong reduction of bacterial growth in mouse kidneys compared to the parental strain.[27]

The concept that protein phosphorylation in its various facets could be a good drug target is not new *per se*. However, the data summarized here, as well as other similar reports, confirm that further investigation of this protein modification raises hope for the future discovery of novel antibacterials. Obviously, a number of technical questions will have to first be answered such as the availability of drugs with broad spectrum activity or the immediacy of their action in clinical use. However, the combination of bacterial genomics, biochemistry coupled with bioinformatics, and physiology can be expected to facilitate valuable progress in this field. In particular, the recent determination of the intimate three-dimensional structure of a few serine/threonine kinases (reviewed in Ref. 16) and tyrosine kinases,[28–30] and more to come, should offer significant opportunities for designing previously unexploited molecules that would efficiently combat bacterial diseases by acting on this protein modification.

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