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This Euroconference focuses on tyrosine kinase inhibitors, but this presentation will rather focus on protein-tyrosine phosphorylation in bacteria and I won't talk really about tyrosine kinases inhibitors. The reason is very simple: While this modification is widely observed in eukaryotic organisms, bacteria were thought instead to be devoid of tyrosine kinases until only ten years ago. Now, tyrosine phosphorylation in bacteria has been clearly evidenced, but enzymes that catalyze tyrosine phosphorylation are special. We are just beginning to understand their functioning and we are far from talking about their precise mode of action thus allowing design of inhibitors. Therefore, my purpose today is to present different data that have been obtained in bacteria with regard to tyrosine phosphorylation and to bring the message that in the future there will be a challenge to design inhibitors of bacterial tyrosine kinases and to control the development of bacteria.

In fact, from the beginning, it's only after a long period of controversy that, in the late 70s, the occurrence of phosphorylation has been observed in bacteria and, since then, despite some persuasive evidence for the presence of serine and threonine kinases in certain bacteria, most studies have essentially focused on the characterization of proteins phosphorylated on histidine and aspartic acid, known today as the two-component systems, a hallmark of bacterial signaling. A clear-cut picture was provided in those early years: eukaryotes use Ser/Thr/Tyr phosphorylation for signal transduction, and bacteria use the Asp/His two-component systems. The first serious crack in that picture came out in the early 1990s, with the characterization of the first serine/threonine eukaryotic-like protein-kinase (Pkn1) in the cyanobacterium *Myxococcus xanthus*. Today, due largely to genomics, the widespread presence of eukaryotic-like Ser/Thre kinase- and phosphatase-genes in bacteria is indisputable.

Concerning the existence of bacterial tyrosine phosphorylation, data have not accumulated as quickly, and nearly none of the eukaryotic-like kinases identified so far had been shown to modify a protein substrate at tyrosine. Concerning the existence of proteins phosphorylated at tyrosine, the first indication was only reported in 1983 in the case of *Escherichia coli*. Then, phosphotyrosine was detected in proteins from a large and still increasing number of bacterial species. However, most of proteins phosphorylated at tyrosine

or most of the corresponding protein-tyrosine kinase activities have not been identified. Nevertheless, the presence of phosphotyrosine in bacteria has suggested that bacteria were encoding enzymes that catalyzed this modification. Concerning phosphotyrosine-phosphatases, a number of them have been detected or identified in bacteria. They are divided essentially in three main families. The first two families share strong similarities with their eukaryotic counterparts. However, in bacteria, it has been recently described a new class of PTP: the Polymerase and Histidinol Phosphatase family (PHP). These enzymes harbor different activities and, it was only 3 years ago that the first bacterial PHP harboring a tyrosine phosphatase activity has been identified. Concerning the characterization of tyrosine-kinases, the same did not hold true. To date, only three eukaryotic like tyrosine kinases have been identified and characterized in bacteria.

From these observations concerning the detection of phosphotyrosine, phosphotyrosine phosphatases and only 3 tyrosine-kinase activities, a question arises: Which enzymes catalyze tyrosine phosphorylation in bacteria. This question has begun to be answered and bacteria seem to do it on their own by using a new type of enzyme to catalyze tyrosine phosphorylation.

This was the second crack in the picture that came out 10 years ago in 1996 with the characterization of a bacterial protein-tyrosine kinase in *Acinetobacter johnsonii*. Interestingly, database searches showed that this PTK was sharing strong sequence similarity with a number of bacterial proteins involved in the production and transport of polysaccharides both in Gram-negative and Gram-positive bacteria. The analysis of the sequences of these proteins revealed the presence of Walker A and Walker B motifs, which are generally found in ATP or GTP binding proteins. So, we were facing a situation where a series of bacterial kinases exhibited a catalytic mechanism different from that of their eukaryotic counterparts. Since then, an important work was performed concerning the organization of these particular kinases. The membrane topology has been determined for some of them. The kinases consist of two main structural domains, one located in the membrane and the other in the cytoplasm. It appears that, in Gram-negative bacteria, kinases are expressed each as a single protein, whereas, in Gram-positive bacteria, they are found in the form of two separate polypeptides, which are encoded by two distinct genes. When comparing with Gram-negative kinases, interestingly, one can observe that the peptide homologous to the N-terminal part of Gram-negative bacteria is located in the membrane and, that the peptide homologous to the C-terminal domain of Gram-negative is in the cytoplasm. This suggests that in Gram-positive bacteria both peptides need to interact with each other to mimic the Gram-negative situation.

Concerning their mechanism of autophosphorylation, although the Walker A and B motifs have been shown to constitute the catalytic site for all of them, still the functioning of these kinases appears to differ from one bacterial species to the other. When looking at Gram-positive bacteria, here again, some differences are emerging. In Gram-positive bacteria, two distinct proteins are required together for phosphorylation.

Therefore, taken together, the data obtained on the mechanism of phosphorylation of bacterial tyrosine kinases show that there is a common root for functioning, but some differences seem to exist. These differences could be explained by differences in the structural organization of the proteins, or by the presence of additional factors, which remain to be characterized, or eventually by the different sensitivities of the methods used to examine this phosphorylation.

Concerning the role of these kinases in the bacterial cell, a large amount of work has been performed. It is noteworthy that immediately upstream or downstream of the kinase genes, some other genes encoding low-molecular-weight or PHP phosphatases have been identified. The presence of such two opposing activities closed to each other has suggested the occurrence of a regulation process based on protein phosphorylation/dephosphorylation.

In Gram-negative bacteria, the phosphorylation of tyrosine-kinase *Wzc* from *E. coli* K30 seems to be required for the formation of the capsule whereas in *E. coli* K12 and *A. lwoffii*, the phosphorylation of *Wzc* would act as a negative regulator. In addition, other data observed in *S. meliloti* indicate that the phosphorylation of the ExoP kinase is connected with an increase in the amount of high-molecular-mass polysaccharides, thus suggesting that phosphorylation regulate the size of the sugar polymer. Concerning Gram-positive bacteria, results have been obtained essentially with *S. pneumoniae*, and discrepant observations have been made depending on the type of strain analyzed. These differences could be explained by the involvement of certain cellular factors, not yet identified, which would be present in some strains but not in others.

Besides these differences, tyrosine kinase phosphorylation / dephosphorylation is thought to be part of a dynamic process which initiates and stops alternatively the polymerization and transport of polysaccharide polymers. Other observations, some of them quite recent, have been made which render even more complex the role of tyrosine phosphorylation catalyzed by these kinases.

In some bacteria, the presence on the chromosome of a second copy of the kinase and phosphatase genes has been identified, but located outside the polysaccharide operon. Thus, in *E. coli*, it has been shown that the *Etk* kinase is expressed by EPEC, ETEC and EHEC strains suggesting a possible role in virulence. In addition, a recent study has revealed that *Etk* and

Etp could be involved in the regulation of the heat shock response by phosphorylating/dephosphorylating regulators of transcription of its two heat shock regulons. These data suggest the occurrence of a new mechanism of heat shock regulation involving tyrosine phosphorylation.

Second, in *E. coli* and *B. subtilis*, an endogenous substrate has been characterized for PTKs. This substrate is an UDP-glucose dehydrogenase whose activity is enhanced upon phosphorylation. Since this protein is involved in the sugar unit composition, one can envisage that tyrosine kinase would regulate the amount of polysaccharide produced. It is noteworthy that this substrate is also involved in other biological pathways such as the production of compounds which render bacteria resistant to some particular antibiotics, namely polymixin. Thus, tyrosine phosphorylation could be involved in such a resistance pathway.

Another interesting example of the role of tyrosine phosphorylation comes from protein BipA. This protein is a ribosome-binding elongation factor G, which harbors the Walker A and B motifs. It was found phosphorylated on tyrosine in clinical isolates of *E. coli*, but not in laboratory strains.

Finally, it has been recently demonstrated that in *B. subtilis*, protein SSB was phosphorylated on tyrosine, thus increasing SSB affinity for DNA. Knowing the role of this protein in DNA metabolism, this could be of particular importance for bacteria physiology.

In conclusion, it appears that research carried out during the last ten years has not only confirmed that tyrosine phosphorylation is actually an ubiquitous biological modification important for the life of bacteria, but also that its role would be as complex as that observed in eukaryotic systems. One can imagine that in the near future, a new class of kinases, sharing more homology with the ATP and GTP binding proteins than with the classical eukaryotic kinases will be identified. The identification of these new kinases, the characterization of their catalytic mechanism and of their targets, the characterization of the factors that would trigger their activity and the characterization of their role in the bacterial cell should lead to better understanding of certain cellular events in the physiology of bacteria. At this moment, the use of specific inhibitors to modulate tyrosine phosphorylation in bacteria would then constitute an interesting approach to control the development of bacterial cells.