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11. Glyceraldehyde-3-phosphate dehydrogenase as a moonlighting protein in bacteria

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Abstract. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is considered a housekeeping protein that is present in virtually all organisms, where it performs metabolic functions essential for survival. GAPDH plays an essential role in the process of energy production, and is also involved in numerous biological processes. GAPDH belongs to a subset of proteins called moonlighting proteins, in which different functions are associated with a single polypeptide chain. The multifunctionality of GAPDH has been described in pathogenic and probiotic microorganisms, in mammals and in plants. In this review, we summarize the moonlighting role of GAPDH in bacteria.

1. Moonlighting proteins

There are different mechanisms by which a gene may encode multiple functions, but there are also mechanisms that determine the ability of a given

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protein to perform more than one function. One subset of multifunctional proteins are called moonlighting proteins, in which the different functions are associated with a single polypeptide chain. A general feature of moonlighting proteins is that their different functions are unrelated and independent. Consequently, the inactivation of one of the functions does not usually affect the other functions and vice-versa [1]. It has been hypothesized that many of these proteins initially had just one role, but acquired additional functions during evolution. Proteins in which the different functions result from gene fusion, homologous protein families, splice variants or enzymes with promiscuous activity are not considered moonlighting proteins, nor are proteins that exhibit the same function in different cell types or subcellular localizations [2,3,4].

The first examples of moonlighting proteins were described in the early 1980s, when it was discovered that certain structural proteins in the lens of vertebrates were well-known metabolic enzymes such as argininosuccinate lyase [5], lactate dehydrogenase [6] or the glycolytic enzyme enolase [7]. At present, the concept of moonlighting protein is not restricted to enzymes, but also applies to other proteins such as receptors, membrane channels, chaperones and ribosomal proteins.

Different mechanisms have been proposed to explain how a moonlighting protein can exert different functions. Thus, the function of the protein may vary according to its subcellular localization, cell type, oligomeric state, interaction with other proteins or macromolecules, cell concentration of a ligand, substrate, cofactor or product. In these proteins, the binding of a metabolite, interaction with another protein or association into a multiprotein complex may induce a change in function. In most cases, these strategies are not mutually exclusive and a combination of them may be involved. In general, moonlighting proteins use different exposition surfaces for each function. In this context, the resolution of the three dimensional structure of multifunctional proteins is of great importance since, in many cases, it provides key information about the molecular mechanism associated with each of the functions [4].

An example of a moonlighting protein with a known switching mechanism between functions is PutA of *Escherichia coli or Salmonella typhimurium*. In the absence of proline, this protein remains in the cytoplasm where it acts as a transcriptional repressor through its interaction with a specific DNA sequence in the promoter of the *put* operon. However, binding of proline induces a conformational change that leads PutA protein to associate with the cell membrane where, by means of its proline dehydrogenase and pyrroline–5-carboxylate dehydrogenase activities, it catalyses the degradation of proline [8].

Until now, many of the functions of proteins could be inferred from known functions of homologous proteins. However, the existence of moonlighting proteins complicates this interpretation. Similarly, multifunctionality can hinder the analysis of results obtained from proteomic studies, since the same protein can have different expression patterns or multiple non-related interaction partners. Nevertheless, large-scale proteomic approaches can be very useful in identifying moonlighting proteins [4]. Thus, identification of a protein in a non-expected cell type or as a part of a new multiprotein complex may suggest a new function for it. For example, using a proteomic microarray-based approach, new DNA-binding activity was identified for the yeast mitochondrial enzyme Arg5,6(N-acetyl-gamma-glutamyl phosphate reductase/ acetylglutamate kinase) involved in the synthesis of ornithine [9]. In the immediate future, proteomic studies of this type, which use massive screening methods, are seen as tools of great potential for identifying new moonlighting proteins.

The existence of multifunctional proteins benefits the organism. It reduces the number of proteins to synthesize, and therefore the amount of DNA to replicate. In many cases, the combination of functions provides a mechanism to coordinate diverse cellular processes, such as metabolic and signalling pathways, which allows the cell to respond to changes in the environment.

2. Bacterial secreted moonlighting proteins and virulence

An increasing number of reports show that certain pathogenic microorganisms (bacteria, fungi, and parasites) display typically considered cytoplasmic proteins on the cell surface, where they exert functions related with virulence. These are highly conserved housekeeping proteins with basic metabolic functions essential for survival that fit into the category of moonlighting proteins. Considering bacterial moonlighting proteins in the context of bacteria-host interactions, besides their basic metabolic function, when secreted these proteins play a role in host adhesion and/or virulence. Once in the extracellular medium, these proteins have been shown to interact with host components, or interact directly with the host cells to elicit signal transduction events, and in this way enable pathogens to colonize and modulate the host immune response. Examples of these moonlighting proteins are glycolytic enzymes such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), enolase, fructose-bisphosphate aldolase or pyruvate kinase, other metabolic enzymes such as malic enzyme or succinyl-CoA synthase subunits, chaperones like DnaK or GroEL, and the elongation factor EF-Tu [10,11].

These cytoplasmic moonlighting proteins lack any typical secretion signal and the mechanism responsible for their secretion remains in many cases unknown. Although several studies point to cell lysis as the mechanism involved in the release of these non-classically secreted proteins into the extracellular medium [12], specific secretion processes have been reported [13]. For instance, in *Mycobacterium tuberculosis* and *Listeria monocytogenes* the SecA2-dependent system has been shown to be involved in the secretion of some of these cytoplasmic proteins [14,15]. In *staphylococcus aureus*, the export of such proteins depends on the major autolysin Atl [16].

3. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a moonlighting protein

GAPDH (EC 1.2.1.12) is a constitutively expressed enzyme that plays an important role in the glycolytic pathway, due to its catalytic activity in the synthesis of 1,3-bisphosphoglycerate. This enzyme is considered a housekeeping protein that is present in practically all organisms, where it performs metabolic functions essential for survival. The enzyme is even expressed in organisms that lack the tricarboxylic acid cycle, which provides essential metabolic activity for anaerobic or microaerophilic growth. Although GAPDH plays an essential role in the process of energy production, it cannot be considered only as a classic glycolytic protein. Several studies described GAPDH as a multifunctional protein involved in numerous biological processes in pathogens [10], probiotics [17,18], mammals [19,20] and plants [21]. Indeed, GAPDH can be considered a model of a moonlighting protein that displays a wide variety of cellular functions, due to their ability to form complex interactions. The multifunctionality of GAPDH has been extensively documented in human cells, where it was shown to be involved in numerous cellular processes, such as transcriptional and post-transcriptional gene regulation, chromatin structure, intracellular trafficking, DNA replication and DNA repair [19,20,22]. Each novel function requires GAPDH association into specific protein complexes and may involve different regions of the protein structure. The different moonlighting functions of GAPDH may also depend on specific post-translational modifications, which can define its oligomeric state, subcellular localization and/or different binding partners [19,20,23]. In fact, GAPDH was shown to be the target of several covalent modifications such as S-glutathionylation, S-nitrosylation, phosphorylation, acetylation and ADP-ribosylation among others. These changes give rise to various forms of the protein, which differ

in their isoelectric point, and therefore can be separated by two-dimensional gel electrophoresis. Most of these modifications are associated with oxidative stress responses and may have important physiological consequences [24].

4. Extracellular GAPDH in Gram-positive pathogens

As stated above, GAPDH is one of the proteins secreted and exposed on the bacterial surface, which enables pathogens to colonize and manipulate host cells [10].

The first description of the location of GAPDH on the surface of a pathogen was in *Streptococcus pyogenes*, a Gram-positive bacterium that causes pharyngitis and skin infections [25-27]. The protein located on the bacterial surface is enzymatically active and able to bind several human proteins such as fibronectin, lysozyme, laminin, cytoskeletal proteins (myosin and actin) [26] and proteins from the human fibrinolytic system (fibrinogen) [26,28,29]. In addition, in pharyngeal cells the membrane protein uPAR/CD87 (urokinase plasminogen activator receptor) was identified as a receptor for *S. pyogenes* extracellular GAPDH [30]. It has been shown that interaction of group A *Streptococci* with human plasminogen through surface exposed GAPDH improves adhesion of these pathogens to the pharyngeal cells [29]. Plasminogen is the zymogen form of plasmin, an enzyme with serine-protease activity that is abundant in human plasma and extracellular fluids, and plays a fundamental role in the dissolution of fibrin clots, the extracellular matrix and other key proteins involved in immunity and tissue repair [31]. In this context, extracellular GAPDH either in the surface of the pathogen or secreted near the host cell promotes activation of plasminogen to plasmin, which can degrade extracellular matrix proteins and facilitate bacterial migration. GAPDH has also been found on the surface of other *Streptococci*, such as the swine pathogen *Streptococcus suis* serotype 2 and *Streptococcus pneumoniae* [32-35]. In these pathogens, GAPDH also acts as a plasminogen receptor. The involvement of GAPDH in the infection mechanism by means of its ability to interact with plasminogen is not specific for *Streptococci* isolates, but has also been reported for other Gram-positive pathogens such as *Bacillus anthracis.* In this pathogen, GAPDH has been detected both in the secretome and at the spore surface [36]. In *Mycoplasma pneumoniae*, cell surface exposed GAPDH mediates interactions with the extracellular matrix proteins of the human host and contributes to the colonization of the respiratory tract [37].

Other studies report the ability of extracellular *Streptococci* GAPDH to interact with the C5a component of the complement system, which promotes its degradation, in coordination with the bacterial surface protease (SCPA).

This strategy allows the pathogen to escape from detection by the host immune system [38]. In Group B *Streptococci*, comprised of human commensal bacteria with the capacity to cause life-threatening meningitis and septicaemia in newborns, GAPDH is exported outside the cell [39]. In this extracellular location, GAPDH can induce activation of polyclonal B cells and secretion of interleukin IL-10. The ability of microbial components to induce the activation of polyclonal B cells of the infected host is an immune evasion mechanism employed by pathogens to suppress specific immune responses. IL-10 has anti-inflammatory effects, and therefore its stimulation by GAPDH may decrease the immune response and facilitate colonization by the pathogen [40].

Soluble GAPDH was identified as the main protein secreted into the extracellular medium by *Streptococcus gordonii* FSS2, which produces endocarditis. In this pathogen, secretion of GAPDH is regulated by pH. This protein remains associated with the cell surface at pH 6.5, whereas a shift to pH 7.5 causes its secretion to the medium [41]. New functions of extracellular GAPDH have been reported in other Gram-positive pathogens. In *Listeria monocytogenes*, cell wall-associated GAPDH was shown to catalyse the ADP-ribosylation of Rab5a [42], a protein that localizes to early endosomes. ADP-ribosylation of Rab5a blocked the Rab5a-exchange factor (Vps9) and impaired maturation to late endosomes [43]. In *Streptococcus oralis*, extracellular GAPDH can bind the major fimbriae of the Gram-negative pathogen *Porphyromonas gingivalis*. This interaction is important for colonization of the oral cavity by this pathogen [44].

Moreover, the ability of antibodies against bacterial GAPDH to mediate opsonophagocytosis and to provide protection against bacterial infection by Gram-positive pathogens such as *Streptococcus pyogenes* [30,45], *Streptococcus agalactiae* [40] or *Bacillus anthracis* [36] points to GAPDH as a potential vaccine target. The proposed functions in host interaction of extracellular GAPDH of Gram-positive pathogens are summarized in Table 1.

5. Extracellular GAPDH in Gram-negative pathogens

As stated above, the first description of GAPDH on the surface of Gram-positive pathogens was published in 1992 [25,26]. However, reports describing the extracellular localization of GAPDH in Gram-negative pathogens and the involvement of this protein in the interaction with the host appeared more than 10 years later. Our group has contributed to this field. We showed that GAPDH is localized on the surface of enteropathogenic (EPEC) or enterohemorrhagic (EHEC) *Escherichia coli* strains and that this protein is also secreted into the medium in a soluble and active form [46].

Although secretion of GAPDH is regulated by environmental pH in other bacteria [41,47], this factor does not affect GAPDH secretion in EHEC and EPEC strains. In these pathogens, secretion depends on the external medium and temperature. The protein is secreted at 37ºC by cells grown in LB or in eukaryotic culture media such as DMEM or Ham's F12, but not in glucose minimal medium [46]. Western blot and ELISA assays have revealed the capacity of this protein to interact with the human proteins fibrinogen and plasminogen, as well as its association with Caco-2 cells upon infection [46]. Pathogens need to attach to host components as the first step in the establishment of infection. As mentioned above, these interactions with human proteins confer GAPDH a function in the degradation of extracellular matrix proteins that, in the case of enteropathogens, would facilitate their migration through the intestinal mucosa. Another aspect of GAPDH function in the interaction with the intestinal mucosa is linked to post-translational modifications, such as ADP-ribosylation. This is a reversible, covalent modification in which the ADP-ribose moiety of NAD^+ is enzymatically transferred to a specific amino acid of the target protein. We have shown that *E. coli* GADPH catalyses its own modification and that this modification affects Cys149 at the active site. ADP-ribosylation assays have shown that the *E. coli* enzyme is modified by NAD^+ both in the cytosol and in the extracellular medium, and that nitric oxide stimulates the NAD^+-dependent post-translational modification of GAPDH [48]. ADP-ribosylation of extracellular GAPDH may play an important role in bacteria-host interaction, as proposed for other pathogens. The secreted protein may protect bacteria against the oxidative host response during infection and/or it may be involved in signal transduction events in the host [26,48].

Studies performed in our group have provided genetic and biochemical evidence that at least two different secretion pathways mediate GAPDH secretion in EPEC, depending on the growth conditions. In cells grown in DMEM, GAPDH secretion depends on T3SS, whereas in cells grown in LB, conditions that do not induce the expression of T3SS proteins, GAPDH is secreted by a T3SS-independent system that has not yet been identified [49]. EPEC and EHEC are members of a related family that intimately attach to the intestinal epithelial cells and induce characteristic attaching and effacing (A/E) lesions on the host cells, causing diarrhoea. In EPEC, the ability to induce A/E effects is encoded in a 41-gene pathogenicity island, called the locus of enterocyte effacement (LEE). The T3SS components are encoded in this locus together with transcriptional regulators, chaperones and effector proteins. EPEC can secrete and translocate multiple effector proteins to the infected cells through the T3SS. The secretion process is dependent frequently on a bacterial chaperone that is not secreted. One chaperone that

displayed broad substrate specificity and plays a central role in recruiting multiple type III effectors to the T3SS is CesT. This soluble, small protein interacts with type III ATPase EscN and promotes the recruitment of cognate effectors to the T3SS apparatus for efficient secretion [50]. Our studies showed that secretion of GAPDH in DMEM is abolished in a mutant defective in the type III ATPase EscN, but restored upon complementation with the *escN* gene. It is well-known that *escN* mutations abolish secretion of both translocator and effector proteins through the T3SS apparatus. In cells that are defective in SepD protein, secretion of GAPDH is increased, which is in accordance with previous results for other T3SS effectors. Direct binding of CesT with GAPDH was shown by means of different methodologies such as pull-down experiments, overlay immunoblotting and bilayer interferometry. We evidenced that this interaction is strong and slow dissociating. Modelling of the N-terminal sequence of *E. coli* GAPDH revealed that this protein displays in this region the structural determinants of the CesT targets. We proposed that during the synthesis of GAPDH in ribosomes, interaction of its nascent N-terminal sequence with CesT may stabilize a population of GAPDH molecules in a secretion competent-state and target them to the T3SS secretion apparatus.

In addition to *E. coli*, there are descriptions of GAPDH on the surface of other gram-negative pathogens, as in *Neisseria meningitidis* [51], *Brucella abortus* [52] and *Edwardsiella tarda* [53]. *Neisseria meningitidis* has two genes (*gapA1* and *gapA2*) encoding GAPDH. From them, *gapA1* has been reported to be up-regulated upon bacterial contact with human epithelial cells. Constitutive expression of *gapA1* has been seen among diverse isolates of *Neisseria* species, whereas the GapA1 protein can only be detected on the surface of capsule-deficient strains. GapA1 is not necessary for *Neisseria* growth *in vitro*, but deficiency in GapA1 significantly reduces adhesion of the pathogen to human epithelial and endothelial cells through a capsule-independent mechanism. From these results, the authors suggested a role of GapA1 in the pathogenesis of meningococcal infection [51]. In *Brucella abortus*, recombinant GAPDH protein was shown to have immunoreactive properties and induce a protective T-cell mediated immune response in mice and natural hosts as well as in acute brucellosis patients [54]. The *Brucella* recombinant GAPDH protein was recognized by IgG antibodies from naturally infected sheep and cattle, which supports its potential use as a vaccine. In fact, partial protection against *Brucella* infection was achieved in mice upon immunization with *gap* and IL-12 genes [52]. The use of recombinant GAPDH as a protective antigen in vaccination processes has also been explored in *Edwardsiella tarda*, a Gram-negative pathogen which causes systemic infection in turbot. The increasing frequency of edwardsiellosis in

fish farming has stressed the need to develop prevention and control strategies. A vaccine based on *E. tarda* recombinant GAPDH has been proven to significantly protect zebrafish not only against *E. tarda*, but also against *Aeromonas hydrophila*, *Vibrio anguillarum*, *Vibrio alginolyticus* and *Vibrio harvei*, thus showing its potential as a vaccine candidate against polymicrobial infections in the aquaculture industry [55]. The proposed functions in host interaction of extracellular GAPDH of Gram-negative pathogens are also summarized in Table 1.

6. Extracellular GAPDH in probiotics

Certain extracellular activities are advantageous in the colonization of the intestinal mucosa and may in fact be a mechanism of competition between commensal or probiotic bacteria versus pathogens. In this context, certain housekeeping proteins secreted by pathogens, initially related to their pathogenesis mechanisms, have been identified in recent years in the secretome of probiotic strains. Probiotics are a group of non-pathogenic intestinal microorganisms that, when administered to humans, produce positive effects on the microbiota balance and increase gastrointestinal homeostasis. They are utilized as dietary supplements or as pharmaceutical products in the treatment of intestinal alterations and diseases. Some probiotic strains reduce colonization by pathogens through direct competition for adhesion sites, the production of antimicrobial agents, or modulation of the host's acquired immune response. Furthermore, they have a beneficial influence upon the epithelial cells, thereby regulating their development and function. By virtue of their extracellular localization, proteins secreted by probiotic strains may be responsible for some of these probiotic traits [56,57]. In order to identify proteins related with probiotic effects or the adaptation of bacteria to changing environmental conditions, several studies have been carried out to characterize the secretome of Gram-positive probiotics [58-60]. Among the housekeeping proteins secreted by Gram-positive probiotics are EF-Tu, enolase, GroEL chaperone, or GAPDH.

Regarding GAPDH, several reports describe GAPDH secretion by some Gram-positive probiotic *Lactobacillus* species [56,60,61], and by the Gram-negative probiotic *Escherichia coli* Nissle 1917 [49]. In *Lactobacillus plantarum*, export of GAPDH to the bacterial cell surface was shown to be closely related to plasma membrane permeability [62]. In *E. coli* Nissle 1917, GAPDH was found in the proteome of outer-membrane vesicles isolated from LB cultures [63]. *L. plantarum* extracellular GAPDH can bind mucin [17]. The adhesion mechanism can be partly attributed to GAPDH binding to human ABO-type blood group antigens expressed on human colonic mucin

Table 1. Bacterial extracellular GAPDH and the proposed function in bacteria-host interaction.

Bacteria	Host target	Functions	References
Gram-positive			
Streptococcus pyogenes	Fibronectin, lysozyme, laminin, myosin, actin, fibrinogen, uPAR/CD87	Adhesion and degradation of extracellular matrix proteins. Bacterial migration	[26,28,29,30]
	C5a component of the complement system	C5a degradation. Evasion of the host immune system	[38]
Streptococcus suis serotype 2	Plasminogen	Degradation of extracellular matrix proteins	[32,34]
Streptococcus pneumoniae	Plasminogen	Degradation of extracellular matrix proteins	[33,35]
Streptococci group B		Induction of polyclonal B cells and secretion of IL-10. Decrease of immune response. Contribution to the colonization process	[39,40]
Bacillus antracis	Plasminogen	Degradation of extracellular matrix proteins	[36]
Mycoplasma pneumoniae	Fibrinogen.	Interaction with extracellular matrix. Colonization of the respiratory track	[37]
Lysteria monocytogenes	Rybosylation of Rab5a	Impaired maturation to late endosomes	[43]
Gram-negative			
Escherichia coli (EHEC and EPEC)	Fibrinogen, plasminogen	Adhesion and degradation of extracellular matrix	[46,48]
Neisseria meningitidis		Adhesion to epithelial and endothelial cells	[51]
Brucella abortus		Induction of protective T-cell mediated immune response	[52,54]
Probiotics			
Lactobacillus plantarum	Mucin	Adhesion to the intestinal mucosa	[17,18]

[18]. It has been suggested that GAPDH interaction with mucin helps the probiotic to colonize the human intestinal mucosa, and provides a mechanism for competition with pathogens that also secrete this protein (Table 1). Studies performed with 30 lactobacilli isolates from human intestinal samples revealed a good correlation between extracellular GAPDH activity and the adhesion capacity to human colonic mucin. This fact has led authors to propose a new screening method based on extracellular GAPDH enzymatic activity to predict highly adhesive lactobacilli without the need to perform adhesion tests, which are complex and time-consuming [64]. In a similar study, adhesion to mucin was investigated in 43 human lactobacilli (32 vaginal and 11 intestinal isolates). GAPDH was found among the bacterial proteins recovered from mucin binding experiments [65].

7. Moonlighting functions of intracellular GAPDH in bacteria

Besides the role of secreted GAPDH in virulence or host colonization processes, few studies have dealt with new GAPDH intracellular functions that are unrelated to glycolysis in bacteria.

As stated above, GAPDH export to the bacterial surface is an essential step in the pathogenesis of *Streptococcus pyogenes*. Studies performed with a strain expressing a modified GAPDH form that prevents its export to the cell surface showed down-regulation of *emm1*, a gene encoding the cell wall protein M, which is one of the main virulence factors of this pathogen [29]. A transcriptomic analysis of this mutant, which displays higher GAPDH intracellular levels than the wild type strain, revealed down-regulation of genes encoding virulence factors as well as genes involved in carbohydrate and amino acid metabolism, whereas genes involved in lipid metabolism were up-regulated. These results prompted the authors to suggest a new role for intracellular GAPDH in the transcriptional control of virulence genes [45] (Fig. 1). In *E. coli*, some studies also suggest that GAPDH could interact with DNA and act as a regulator of gene expression. In a study performed by Green *et al*. [66] using the methodology of Dynabeads, GAPDH was identified among the proteins that could interact with the promoter of *ndh* gene, which encodes NADH oxidase-2. However, no functional characterization of this interaction has been performed so far.

To explore new GAPDH functions in *E. coli*, we carried out protein interaction studies. In the area of systems biology, the identification of protein-protein interactions within cells is of prime importance to understand biological processes at the molecular level. Indeed, most human GAPDH functions were identified through the detection of GAPDH as a component of protein complexes involved in the cellular processes under study [20]. We

investigated new intracellular functions of *E. coli* GAPDH following a proteomic approach, to identify the proteins that interact with GAPDH using *in vivo* formaldehyde cross-linking followed by mass spectrometry-based protein identification. This methodology covalently fixes interaction partners in living cells, thus allowing identification of even transient interactions or weakly binding proteins [67]. Experiments were performed with *E. coli* cells that express GAPDH fused to the V5 epitope to facilitate immunoaffinity purification of the protein complexes with anti-V5 beads. Experiments in the absence of cross-linking were also performed. From these experiments, several proteins were identified to putatively interact with GAPDH. The identified proteins include the metabolic enzymes phosphoglycolate phosphatase, enolase, tryptophanase, the ATP synthase β- and α-subunits, aldehyde dehydrogenase, pyruvate kinase, transketolase, aconitase, anthranilate synthase component II, dihydrolipoyl dehydrogenase, D-tagatose-1-6-bisphosphatase, the ATP binding proteins AraG and RbsA, the chaperones DnaK and trigger factor, the periplasm D-ribose binding protein (RbsB), the outer membrane protein OmpC, as well as factors involved in protein synthesis such as EF-Tu [68]. Four proteins (trigger factor, DnaK, phosphoglycolate phosphatase and aldehyde dehydrogenase) also appeared as candidates to interact with GAPDH in other large-scale studies performed in *E. coli* following different approaches [69,70] or in databases such as DIP (database of interacting proteins) or IntAct. Since proteomic studies aimed at identifying protein interactions are intended to assign new functions to a given protein, we further characterized GAPDH interaction with phosphoglycolate phosphatase (Gph). This enzyme is involved in the

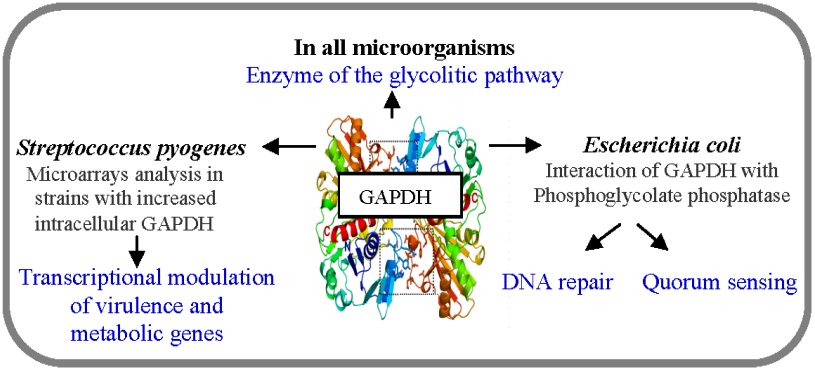

Figure 1. Intracellular functions of GAPDH in bacteria. The experimental evidences for function assignment are indicated in gray and the proposed functions in blue.

metabolism of 2-phosphoglycolate formed in the DNA repair of 3- phosphoglycolate ends generated by bleomycin damage [71]. We showed that interaction between Gph and GAPDH increases in cells treated with bleomycin, which suggests that GAPDH may be involved in cellular processes linked to DNA repair mechanisms. Moreover, since 2-phosphoglycolate is also generated in the degradation of the autoinducer-2 precursor 4,5-dihydroxy-2,3-pentanedione (DPD) [72,73], interaction between GAPDH and Gph may suggest that GAPDH participates in quorum sensing signalling processes [68] (Fig. 1).

8. Conclusion

GAPDH, a key glycolysis enzyme, is a moonlighting protein performing additional functions unrelated with its original metabolic role. In bacteria, moonlighting GAPDH functions have been mainly associated with its extracellular location. This protein is secreted and exposed on the bacterial surface enabling pathogens and probiotics to colonize and/or modulate the host immune response. Multifunctionality of intracellular GAPDH has been widely studied in humans; however such kind of studies is scarce in bacteria. Recent reports on *S. pyogenes* and *E. coli* provide evidence that bacterial GAPDH may also be involved in intracellular cell processes like transcriptional regulation, DNA repair and quorum sensing signaling.

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