Osmoregulated Periplasmic Glucans

Sébastien Bontemps-Gallo, Jean-Pierre Bohin, Jean-Marie Lacroix

Osmoregulated Periplasmic Glucans

Sébastien Bontemps-Gallo[1,2], Jean-Pierre Bohin[1] and Jean-Marie Lacroix[1,*]

[1]Univ. Lille, CNRS, UMR 8576 – UGSF - Unité de Glycobiologie Structurale et Fonctionnelle, F 59000 Lille, France

[2]Laboratory of Zoonotic Pathogens, National Institute of Allergy and Infectious Diseases, National Institute of Health, Hamilton, MT, United States of America.

*Corresponding author.
E-mail: jean-marie.lacroix@univ-lille1.fr

Abstract

Among all the systems developed by Enterobacteria to face osmotic stress, only osmoregulated periplasmic glucans (OPGs) were found to be modulated during osmotic fluxes. First detected in 1973 by E.P. Kennedy's group in a study of phospholipid turnover in *Escherichia coli*, OPGs have been shown across alpha, beta and gamma subdivision of the Proteobacteria. Discovery of OPG-like compounds in the epsilon subdivision strongly suggested that the presence of periplasmic glucans is essential for almost all Proteobacteria. This chapter offers an overview of the different classes of OPGs. Then, the biosynthesis of OPGs and their regulation in *E. coli* and other species are discussed. Finally, the biological role of OPGs is developed. Beyond structural function, OPGs are involved in pathogenicity, in particular by playing a role in signal transduction pathways. Recently, OPG synthesis proteins have been suggested to control cell division and growth rate.

Introduction

Escherichia coli and *Salmonella enterica* are found in many environments with a wide range of osmolarity: intestinal tract of mammalian host (> 300 mosM), urinary tract (500/800 mosM for an adult with a normal diet, but may vary from 40 to 1,400 mosM)(1-3), in sea (1,000 mosM)(4) or freshwater (10 mosM)(5). To adapt to these osmotic fluxes, Enterobacteria have developed many tools to maintain the osmotic pressure inside of the cell (see EcoSal chapter Osmotic Stress (6)). Among these systems/molecules involved in osmoadaptation, the only periplasmic components whose synthesis was found to be

modulated by osmotic pressure in various bacterial species are osmoregulated periplasmic glucans (OPGs).

OPG structures found in different bacterial species share several common characteristics: i) D-glucose is the only constituent sugar; ii) glucose units are linked, at least partially, by β-glycosidic bonds; iii) there are a limited number of glucose units (5 to 24); iv) in most cases, but with a few exceptions, OPG concentration in the periplasm increases in response to a decrease in environmental osmolarity. In many but not all, species, the OPG backbones can be decorated to various extents with a variety of substituents. These substituents appear to belong to two classes: i) residues originating from membrane phospholipids like phosphoglycerol, phosphoethanolamine, or phosphocholine; ii) residues originating from intermediary metabolism like acetyl, succinyl and methylmalonyl. For the latter, acyl-coenzymes A are the likely donor molecules but this has not been demonstrated (7).

OPGs were detected during the study of phospholipid turn-over in *E. coli* by E. P. Kennedy's group (8). In this bacterium, rapid phosphatidylglycerol turn-over is associated with the transfer of *sn*-1-phosphoglycerol to a new class of oligosaccharides, accordingly named "membrane-derived oligosaccharides" (MDOs, reviewed in (9)). The homology between the linear β-glucans found in the periplasm of *E. coli* and the cyclic β-glucans found in the periplasm of *A. tumefaciens* was established in 1986 with the demonstration that the syntheses of both kinds of molecules are osmoregulated (10). We now know that these compounds are found in many Proteobacteria and most do not contain any substituent derived from membrane lipids. The term ‘MDO’ is confusing because, despite being part of the envelope, these compounds may not be derived from the membrane. For

this reason, the term OPGs was preferred and the gene and protein nomenclatures were changed accordingly (11).

OPG structures

OPG structures were described in several species of the alpha, beta and gamma subdivisions of the Proteobacteria. Recently, Szymanski's group demonstrated the presence of OPG-like compounds, called free oligosaccharide (fOS), in the epsilon subdivisions of the Proteobacteria (12, 13). No information is available for other Gram-negative bacteria. Beyond their common features, OPGs show an unexpected structural diversity. Four families (I to IV) have been defined on the basis of backbone organization (Fig. 1) (7).

Family I

OPGs of family I are found in almost all γ-Proteobacteria such as *E. coli* ((9, 14); and references therein), , *Salmonella enterica* serovar Typhimurium (unpublished data) *Dickeya dadantii* (15), *Pseudomonas aeruginosa* (16), *Pseudomonas syringae* (17). The structures found in these bacteria are very similar. In *E. coli*, OPGs are heterogeneous in size and appear to range from 5 to 12 glucose residues, with the principal species containing 8 or 9 glucose residues. The sugar backbone consisting of β-1,2-linked glucose units is highly branched by the irregular addition of one glucose units attached by β-1,6 linkages (Fig. 1). In *E. coli* and probably in *S. enterica*, a heterogeneous substitution by phosphoglycerol, succinyl and phosphoethanolamine residues is superimposed to this heterogeneity of backbone structures (9, 11, 18, 19). A specific

pattern of substitution was found depending on bacterial species (20). As example, in *D. dadantii*, OPGs are substituted by succinyl and acetyl residues (15, 21).

Family II

OPGs of family II are found in various members of the family Rhizobiaceae ((22) and references therein). *Agrobacterium*, *Rhizobium*, *Sinorhizobium*, *Brucella* and *Mesorhizobium* species synthesize periplasmic glucans with similar structures (23, 24). In these genera, OPGs are composed of a cyclic β-1,2-glucan backbone containing 17 to 25 glucose residues (Fig. 1). Much larger molecules (up to 40 glucose units) were detected within cultures of a particular strain of *Sinorhizobium meliloti* (22). The predominant substituent of OPGs of *S. meliloti* and *A. tumefaciens* strains is phosphoglycerol, but substitutions by succinyl and methylmalonyl residues were also found in other related species or strains. The degree of substitution may vary greatly among different strains and the stage of growth also influences the degree of substitution.

Family III

OPGs of family III were first described in *Bradyrhizobium japonicum* as β-1,6- and β-1,3-cyclic glucans containing 10 to 13 glucose units per ring (22, 25). Highly similar OPGs were found in *Azorhizobium caulinodans* (26) and in *Azospirillum brasilense* as judged from NMR analysis (27). For the latter, high-performance anion-exchange chromatography allowed for the separation of three distinct structures: glucan I, comprised of 12 glucose units linked by 3 β-1,3, 8 β-1,6 and one β-1,4 linkages; glucan II derived from glucan I by the addition of a glucose linked by an α-1,3 linkage; glucan III derived from glucan II by the addition of a 2-O-methyl group onto the α linked glucose unit (Fig. 1). Thus, the OPGs of family III differ from those of the family II not only by

the nature of the glycosidic linkage but also by a strict control of the ring size. While OPGs of family III are predominantly uncharged, OPGs of *B. japonicum* also demonstrate substitutions with phosphocholine (25).

Family IV

OPGs of family IV have very similar structural features. They were found in *Ralstonia solanacearum* (28), *Xanthomonas campestris* (28, 29) and *Rhodobacter sphaeroides* (30). These OPGs are cyclic and have a unique degree of polymerization (DP = 13, 16, and 18, respectively). One linkage is α-1,6 whereas all the other glucose residues are linked by β-1,2 linkages (Fig. 1). The presence of this α-1,6 linkage induces structural constraints in these kind of molecules, which contrast with the very flexible structures of the cyclic all β-1,2 OPGs of family II (31). While OPGs found in *X. campestris* and *R. solanacearum* are not substituted, those found in *R. sphaeroides* can be substituted by one to seven succinyl esters residues, and by one or two acetyl residues.

Thus, OPG diversity is very high not only among different bacterial species, due to different genomic capacity (see below), but also in a particular bacterial strain, because control of the backbone structure is often not stringent and because backbone substitution (if any) is heterogeneous. Consequently, OPGs may be neutral or moderately to highly anionic molecules.

Mechanisms of OPG biosynthesis in *E. coli*

OPG backbone enzymes

In *E. coli*, two specific proteins are necessary for glucan backbone biosynthesis (Fig. 2). They are encoded by the *opgGH* operon (32). *In vitro*, the presence of OpgH in inner membrane vesicles is necessary to obtain the production of linear β-1,2 polyglucose chains from the precursor UDP-glucose. For unknown reasons, the acyl carrier protein, which normally functions in fatty acid synthesis, is also necessary for this activity (33). OpgH (97 kDa) is composed of three large cytoplasmic regions linked by eight transmembrane segments (34). The central cytoplasmic region shows structural features of a glucosyltransferase of family 2 where several aspartic acid residues are necessary for OPG synthesis (35). It is postulated that the eight transmembrane segments in OpgH could form a channel for OPG translocation to the periplasm during synthesis (34). OpgG (56 kDa) is a periplasmic protein whose function has not been established. In the *in vitro* test of OpgH activity, OpgG was absent and the product showed a higher degree of polymerization and no branching (9, 33). However, OpgG defective mutants form neither mature OPG molecules nor any precursor forms (32). The 2.5Å crystal structure of OpgG has been reported (36). The protein is composed of two distinct β-sandwich domains. The N-terminal domain shares some similarities with carbohydrate-active enzymes and the C-terminal domain could interact with other molecules. Modeling analyses suggest interaction between both proteins (37). These data support the hypothesis that OpgG interacts with OpgH for the translocation of nascent molecules and catalyzing the addition of branches.

In *E. coli*, a third protein, OpgD, was described (11), which is encoded by a paralog of *opgG*. When *opgD* was inactivated, normal amounts of OPGs were recovered

but backbone structure was altered, showing a higher degree of polymerization. Thus, OpgD seems to control (or interfere with) the OPG biosynthetic machinery (Fig. 2).

The functions of genes similar to the *E. coli opgG and opgH* genes have been demonstrated in *P. syringae* (38) and *D. dadantii* (39), which produce linear OPGs of the family I, and also in *R. sphaeroides* (40) and (very likely) in *X. campestris* (41), which produce cyclic OPGs of the family IV. The *opgD* gene can be absent from certain genomes, while in some cases, *opgG* is absent but *opgD* is present (see above). Therefore, one can envisage that interactions between OpgH, on the one hand, and OpgG/OpgD, on the other hand have evolved differently in different species.

Highly similar OPG genes were found in *A. tumefaciens* (*chvB* and *chvA*), *S. meliloti* (*ndvB* and *ndvA*) and *B. abortus* (*cgs* and *cgt*) (22, 42-45). These genes are strict homologues; genes from one species complementing OPG biosynthetic defects in other species. While the two genes are adjacent to each other in *A. tumefaciens* and *S. meliloti*, they are separated by 857 bp in *B. abortus*. The biosynthetic protein of *B. abortus* (Cgs) is a large cytoplasmic membrane protein (316 to 319 kDa). It is composed of six transmembrane segments, which determine four large cytoplasmic domains and three very small periplasmic regions (46). The biosynthetic enzymatic activity can be assayed efficiently *in vitro*. Unpurified membrane preparations are able to catalyze the formation of cyclic β-1,2 glucans from UDP-glucose and a high molecular-weight membrane protein is labeled when radioactive UDP-glucose is present (22, 47). The second protein (Cgt) is a 66- or 67-kDa inner membrane protein which shares amino-acid sequence similarity with several ATP-binding cassette transporters. When the protein is absent, cyclic glucans are formed but they are not substituted. Since substitution occurs in the

periplasm (see below), it can be concluded that the function of Cgt is the translocation of the cyclic molecules towards the periplasm.

Using the hypothesis that structurally different OPGs could functionally compensate for OPG defect in a *S. meliloti ndvB* mutant, Bhagwat *et al.* identified a gene they named *ndvB* from *B. japonicum* (48). Later, two others genes were characterized, *ndvC* (49) and *ndvD* (50), which form a locus of three monocistronic genes with *ndvB*. NdvB (102 kDa), NdvC (62 kDa) and NdvD (26 kDa) are predicted to be membrane-bound with NdvB and NdvC showing several transmembrane segments and NdvD only one. When *ndvB* was inactivated, no OPGs were synthesized *in vivo* and mutant membrane preparations failed to produce glucans *in vitro*. In contrast, when *ndvC* was inactivated normal amounts of OPGs were produced, but their structures contained almost only β,1-3 linkages. Inactivation of *ndvD* abolished OPG synthesis *in vivo* but did not affect the glucan synthesis by membrane preparations *in vitro*. Thus, NdvB and NdvC are most likely two membrane-bound biosynthetic enzymes, while the function of NdvD remains elusive.

OPG substitution enzymes

In *E. coli*, the OPGs produced by the biosynthetic enzymes are very heterogeneous, and backbone variety can be further modified by 1, 2, or 3 residues of phosphoglycerol, and/or succinyl, and/or phosphoethanolamine. Thus, a majority of OPG molecules have a high anionic character (up to 5 negative charges), while a minority are neutral (Fig. 2). To date, only three genes have been recognized to participate in OPG substitution: *opgB* (51), *opgC* (19), *opgE* (18). Phosphoglycerol transfer was shown to occur in the periplasmic compartment (52). Two phosphoglycerol transferase (PGT)

activities have been measured *in vitro*: the membrane bound PGT I and the periplasmic PGT II. Soluble OPGs behave as phosphoglycerol acceptors for the latter but not for the former enzyme. Jackson and Kennedy have proposed a two-step model to account for phosphoglycerol substitution: first, PGT I transfers residues from the membrane phosphatidylglycerol to nascent OPG molecules; second, PGT II swaps residues from one OPG molecule to another (53). OpgB (85 kDa) consists of a large periplasmic sulfatase domain anchored in the inner membrane by three putative transmembrane segments and corresponds to PGT I. This protein was found to be highly sensitive to proteolytic cleavage and PGT II (OpgB') is actually a soluble form of OpgB (54).

OpgE (60 kDa), like OpgB, consists of a periplasmic sulfatase domain anchored in the inner membrane by four putative transmembrane domains. OpgE was proposed to transfer phosphoethanolamine residues from phosphatidylethanolamine to OPG molecules (18).

If one considers the probable viscosity of the periplasm, OpgB and OpgE should greatly facilitate the transfer of phosphoglycerol or phosphoethanolamine residues from the surface of the inner membrane toward OPGs molecules located in the external part of the periplasm (Fig. 2).

OpgC (44 kDa) is a polytopic protein with ten putative transmembrane segments. OpgC was proposed to catalyze the transfer of succinyl residues from the cytoplasmic side of the membrane to the nascent glucan backbones on the periplasmic side of the membrane (19).

In *R. sphaeroides*, $OpgC_{Rsp}$ (44 kDa) exhibits stretches of hydrophobic amino acids over its entire length and eleven transmembrane segments are predicted. In *D.*

dadantii, $OpgC_{Dda}$ (48 kDa) is also an inner membrane protein with 10 putative transmembrane domains (55) Thus, $OpgC_{Rsp}$, $OpgC_{Dda}$ and $OpgC_{Eco}$ share several common characteristics; however they do not show any significant sequence similarities (55).

In conclusion, despite the fact that they carry out very similar functions, OPG substitution enzymes described to date are not phylogenetically related but appear to result from convergent evolution.

Genomic overview of OPG biosynthesis

Genetic analyses of OPG synthesis were done in a limited number of bacterial species and OPG genes were defined with unrelated names: *mdo* in *E. coli* (56) and *chv* in *A. tumefaciens* (for chromosomal virulence) (44). Later, mutants were also obtained in other genera: *R. sphaeroides* (40) and *X. campestris* (41) where the genes were named *opg*. Complementation experiments were used to identify homologous genes in related organisms of the Rhizobiales group, essentially S. *meliloti* (42) and *B. japonicum* (48) where the genes were named *ndv*, for nodule development. This strategy also allowed the isolation of a locus similar to *chvB*, *cviB*, in *Azospirillum brasilense* while the *chv* and *ndv* nucleotide sequences of *A. tumefaciens* and *S. meliloti* were highly similar, they did not share any significant similarity with the *opg* genes. Moreover, the *ndv* genes found in *B. japonicum* share some similarities with some *opg* genes but no similarity with the *chv* genes. Thus, three distinct sets of genes were described and similar genes can be recognized in various completely sequenced genomes of Proteobacteria (Fig. 3). The first set of genes includes *opgG*, *opgH*, *opgD*, *opgI*, which synthesize linear OPGs. *opgG*,

opgH and *opgD* were described initially in *E. coli and opgI* in *R. sphaeroides*. Phylogenetic analyses were previously published for OpgH (40, 55), OpgG (11, 55) and OpgD (11). The second set of genes includes *ndvB*, *ndvC* and *ndvD*, initially described in *B. japonicum*, and the third includes *chvA* and *chvB*, initially described in *A. tumefaciens*. Both the second and third gene sets are involved in cyclic OPG synthesis.

Several conclusions can be drawn from the data shown in Fig. 3. Several bacterial species do not possess any of the OPG genes already described, as exemplified by *Yersinia pestis*. *Yersinia* species do not synthesize OPGs regardless of the presence and the expression of the *opgGH* operon (58). The *opgH* gene which encodes a protein belonging to the glycosyltransferase family 2 (59-61) is present in many bacteria of the alpha, beta, and gamma subdivisions. The *opgG* and *opgD* genes are diversely distributed with many possible combinations; *opgG alone*, *opgG* and *opgD*, or only *opgD*. In some cases, two copies of *opgH*, *opgG*, or *opgD* are detected. The *opgI* gene encodes another accessory protein only present in *Rhodobacter*. The combination of all three *ndvB*, *ndvC* and *ndvD* genes is found only in related alpha Proteobacteria in addition to *opgH* and *opgG* while *ndvB* and/or *ndvC* encoding proteins belonging to the glycosyl hydrolases family 17 (61-66) can also be found in *Pseudomonas*. The *chvB* gene encodes a protein belonging to the glycoside hydrolase of family 94 (61-66) and the *chvA* gene encodes an ABC transporter. These genes are found together among Rhizobiaceae and *Burkholderia*. The *chvA* gene is found in association with the *ndvB*, *ndvC* and *ndvD* genes for the transport of cyclic glucans. The *chvB* gene is found in various other bacteria. However, until the functions of such genes are demonstrated, their assignations to OPG synthesis should be approached with caution since they can encode biosynthetic as well as

degradative enzymes. For example, an *ndvB* defective mutant of *R. sphaeroides* synthesizes normal OPGs (40).

Since OpgB and OpgE are only found in *E. coli*, it's currently not possible to analyze the origin of these genes. OpgC was found and characterized in three different bacteria (19, 40, 55). In a phylogenetic analysis, two separate origins were demonstrated between *E. coli* and *D. dadantii*, while $OpgC_{Rsp}$ was outside of these two groups. Both groups also had a specific synteny. Interestingly, a functional complementation occurred between $opgC_{Dda}$ and $opgC_{Eco}$ (55).

There is no simple correlation between the natures of the OPG genes present and the structures and substitution of OPG produced by a particular bacterial species and sequence-based predictions are not possible. Likely, the need for substitutions on OPGs may reflect the lifestyle of bacteria.

Regulation of OPG biosynthesis

OPG synthesis was shown to be osmotically regulated in a wide range of Proteobacteria (7, 14) except for *B. abortus* (23) and strains of *R. leguminosarum*. The observations in *R. leguminosarum* were complicated because these strains secrete large amounts of OPG when grown in media of high osmolarity, suggesting outer membrane modifications (see below) (22).

OPG amount increases as the osmolarity of the medium decreases to an extent depending on the species. In *E. coli*, periplasmic glucans represent up to 5% of the dry weight of the cell in a medium of low osmolarity (50 to 100 mosM), while it decreases to 0.5% in a medium of high osmolarity (600 to 700 mosM).

Osmotic regulation takes place at an early stage of OPG backbone biosynthesis at the transcriptional level and/or at the post translational level depending on the species considered. *In vitro*, the membrane bound activities found in extracts isolated from bacteria of *S. meliloti*, *R. leguminosarum*, *A. tumefaciens* and *E. coli* grown in media of low or high osmolarity were very similar and were very sensitive to elevation of ionic strength. In *E. coli*, the rate of OPG synthesis measured in a strain (*zwf*, *pgi* – Fig. 4) unable to synthesize UDP-glucose in the absence of glucose, falls abruptly when osmolarity increases suddenly (67). Increase of medium osmolarity correlates with an increase in the cytoplasm ionic strength. OPGs are necessary for hypoosmotic adaptation of most Rhizobiaceae, since mutants devoid of OPGs are impaired for growth in media of low osmolarity. This is consistent with an OPG synthesis regulated solely at the enzymatic level. Nevertheless, in *E. coli*, growth of mutants devoid of OPG remains unaffected in media of low osmolarity and when the osmolarity of the medium decreases suddenly, almost one generation time is necessary to adjust the level of OPG. This observation is inconsistent with a regulation limited to an enzymatic modulation. Thus, transcription of the *opgGH* operon of *E. coli* was found to be osmoregulated (32), and membrane bound activity of *A. brasilense* was reduced in extracts of cells grown in a medium of high osmolarity (47).

Independently of this osmotic regulation, a feedback regulation exerted by the end product was observed whatever the osmolarity is. After addition of glucose to the *E. coli zwf, pgi* strain, OPGs are accumulated rapidly until they reach the concentration observed in the wild type strain. *In vitro*, glucosyltransferase membrane bound activity of *R. leguminosarum* was severely reduced when 15 mM OPG was added. Thus, in the

secretory strains of this species, secreted OPGs escape from feedback control, explaining the large accumulation of OPG in the medium (68).

However, factors other than osmolarity affect the regulation of OPG biosynthesis in several bacterial species. OPG synthesis was found to be upregulated by a diffusible signal factor in *X. campestris* pv. campestris (69). The *opgH* gene was downregulated in *Xanthomonas axonopodis* pv. citri grown *in planta* (70), but *opgH* was found to be induced in *Xylella fastidiosa* during biofilm formation (71), and *opgG* is one of the 153 genes induced during growth of *Ralstonia solanacearum* in tomatoes (72). In *D. dadantii*, OPG biosynthesis is only regulated at the enzymatic level by an as yet unknown signal *in planta* (21) and osmolarity *in vitro* (15, 39). A previous study demonstrated that the amount of OPGs increased abruptly *in planta*, while the amount of both OpgG and OpgH protein remained unchanged (21, 73). Finally, the *opgGH* operon of *E. coli* was found to be activated by σ^E, the sigma factor which responds to misfolding of proteins in the envelope (74).

The *opgD* gene of *E. coli* is regulated by growth phase because OpgD was preferentially observed in stationary growth phase (75). This gene contains a cytosine base in position -13 of its promoter (one important characteristic of σ^S dependence), and is partially transcribe by the σ^S RNA polymerase (35). Factor σ^S is activated when growth slows down, particularly in stationary growth phase (76). However, *opgD* was not observed amongst the forty-one genes whose expression was reduced in a σ^S defective mutant (77). The *opgD* homolog of *X. axonopodis* pv. *citri* was shown to be overexpressed *in planta* (77).

In *E. coli*, none of the substitution genes were demonstrated to be osmoregulated (19, 52, 55). In *D. dadantii*, only succinylation was demonstrated to be osmoregulated, while acetylation of OPGs wasn't affected by the osmolarity or *in planta* (15, 55). *opgC*$_{Dda}$ gene expression occurred at high osmolarity and required activation of both EnvZ-OmpR and RcsCDB phosphorelays (55). In *S. meliloti*, the *cgmB* gene, encoding the phosphoglyceroltransferase, was shown to be osmoregulated (78).

Regulation of OPG synthesis is complex not only because osmoregulation does not follow common features among species but it is complicated by the existence of additional regulatory factors.

Connection with trehalose synthesis

Trehalose is a non-reducing disaccharide of glucose. It is the most widespread disaccharide in nature, occurring in bacteria, fungi, insects, and plants (79). It serves as a carbon source and/or can be accumulated as a protectant against high osmolarity, heat, desiccation (80). In *E. coli*, externally supplied trehalose is only used as a carbon source and not as an osmoprotectant because of the synthesis of a high activity periplasmic trehalase (the product of *treA*) upon exposure to high osmolarity media and a PTS system synthesized at low osmolarity and repressed at high osmolarity (81). Thus, osmoprotective trehalose must be endogenously synthesized for accumulation in media of high osmolarity. Trehalose is synthesized from UDP-glucose (like OPGs) and glucose-6-Phosphate and accumulated up to 400 mM (Fig. 4) (80). Because large accumulation of OPGs and trehalose occurs in media of low and high osmolarity respectively, no competition for UDP-glucose availability exists for the synthesis of both kinds of

molecules. Since OPGs are never degraded within cells, they never serve as a glucose source for trehalose synthesis. On the contrary, one could imagine that trehalose serves as a glucose donor for OPG biosynthesis after hydrolysis by the cytoplasmic trehalase, the *treF* product, in media of low osmolarity despite its low affinity for trehalose (Km: 1.9 mM) (82). In *E. coli*, little is known about what becomes of trehalose once cells are in media of low osmolarity. Release of osmoprotectants such as betaine and trehalose is known to occur through mechanosensitive channels in response to osmotic downshock (83), thus it is possible to hypothesize the hydrolysis of trehalose by TreF. In *B. japonicum*, *in vivo* NMR studies on cells in stationary growth phase clearly reveal that no connection exists between OPGs and trehalose (84). After a hypoosmotic shock, OPGs are synthesized from glycogen in stationary growth phase and from glucose in exponential growth phase. When no glucose was present in the medium, synthesis of OPGs does not occur. Nevertheless, trehalose was synthesized in media of high osmolarity but was released into the medium during the hypoosmotic shock. Thus, the disaccharide cannot serve as a precursor for OPG biosynthesis. Because osmoprotectants are often released into the medium after an osmotic downshock and because OPGs cannot be degraded, one can consider that in a majority of bacterial species, trehalose and OPGs cannot serve as a precursor to each other.

Pleiotropic phenotypes of OPG defective mutants

OPG defective mutants characterized in various bacterial species have in common a highly pleiotropic phenotype, which is indicative of a global alteration of their envelope properties. In *E. coli* and *S. enterica* but also in most species tested, Opg^- colonies

generally exhibit a mucous phenotype and a defect in motility and chemotaxis. This defect was shown to be the consequence of a reduced number of flagella in *E. coli* (85, 86). A proteomic analysis of Opg$^-$ mutants of *D. dadantii* confirmed several years later by the same approach in *S. enterica* indicated a constitutive induction of a general stress response and an altered envelope structure (87, 88). In a similar way, Opg$^-$ mutants showed increased sensitivity to hydrophobic antibiotics in *S. meliloti* (89), biliary salts in *D. dadantii* (39), sodium dodecyl sulfate in *E. coli* (90), or increased resistance to endogenously produced lysis protein of phage MS2 in *E. coli* (91). Depending upon medium composition, other phenotypic changes were found in envelope proteins in *E. coli* (85) and A. *tumefaciens* (92). Moreover, Opg$^-$ mutants of *D. dadantii* produce and secrete lower amounts of plant cell wall-degrading enzymes (proteases, cellulases and pectate lyases) (39).

The doubling times for growth in hypoosmotic media were twice as higher for Opg$^-$ mutants of *A. tumefaciens* (92) and *S. meliloti* (89) compared to the wild type, and increasing the medium osmolarity compensated for that defect. In contrast, growth of Opg$^-$ mutants in *E. coli* (52), *D. dadantii* (39) or *B. Japonicum* (50) is only slightly affected.

Several groups have isolated suppressor mutations capable of restoring a wild-type phenotype for Opg$^-$ mutants. Some *E. coli* chemotactic pseudorevertants mainly involved in osmoregulation of the outer membrane porins (85) were mapped by transduction in the *envZ-omp*R locus. However, this locus is not involved in regulation of OPG synthesis. Similarly, pseudorevertants of *S. meliloti ndv* mutants were selected for restoration of osmotolerance, motility, or symbiosis. Pseudorevertants for vegetative

properties regained only minor symbiotic ability while symbiotic pseudorevertants were unrestored for vegetative properties and remained highly impaired in the first steps of the symbiotic interaction (89).

Thus, in some cases, OPGs may serve in osmoadaptation but they also appear to play other roles unrelated to osmoadaptation. This is not surprising since OPG synthesis is regulated by additional factors in several bacteria.

OPGs and pathogenicity

Mutants defective in OPG synthesis were primarily obtained during the screening or the selection of attenuated or avirulent mutants of plant or animal pathogens. Thus, mutants of *A. tumefaciens* (*chvA* and *chvB*, (44)), *P. syringae* pv. syringae (*hrpM*, an OpgH homolog (93)) and *X. campestris* pv. vesicatoria (*opgH*, (41)) are completely or severely impaired in their virulence toward their host plants. The *P. syringae* mutant is severely impaired in its ability to grow in the plant host and failed to elicit a nonhost hypersensitive response when inoculated on tobacco. Similarly, mutants of *S. meliloti* (42) form defective nodules where tissue differentiation occurs in the absence of bacterial invasion. As mentioned earlier, OPGs can be recovered in the external medium under certain conditions. However, addition of purified OPGs to the inoculum cannot restore attachment and virulence or symbiotic ability of Rhizobiaceae Opg^{-} mutants.

Random mutations affecting OPG biosynthesis were also recovered during investigation of animal pathogens. *opgH* was found among chromosomal loci expressed by *Y. enterocolitica* at an early stage of the infection process (94). However, inactivation of the *opgGH* operon in *Y. pseudotuberculosis* didn't affect the virulence (58).

Furthermore, *Y. pestis* expressing *opgGH* grew normally and was able to complete its infectious cycle (flea-host) (58). Knowing that OPGs cannot be detected in other *Yersinia* species, even if the *opgGH* operon is expressed, , suggests that the role of OPGs was lost in an ancestral *Yersinia* strain.

One of several *P. aeruginosa* PA14 mutants severely impaired in virulence toward *Caenorhabditis elegans* possesses transposon insertion in *opgH* (95). This mutant also has a dramatic effect in a mouse model, causing no mortality, and is severely affected in its ability to grow in *Arabidopsis* leaves.

A mutant of *P. aeruginosa* was identified that, while still capable of forming biofilms with normal architecture, does not develop high-level biofilm-specific resistance to antibiotics (96). The mutation was identified in the *ndvB* gene (see Fig. 3) and the mutant is impaired in the synthesis of cyclic glucan. Is this glucan corresponding to OPGs? While the authors accept this hypothesis, and discuss the possibility that its cyclic nature allows sequestration of antibiotics, we can raise objections. OPGs of the closely related species, *P. syringae,* belong to family I and both species have highly similar *opgGH* genes. Moreover, in *R. sphaeroides*, genes similar to *opgGH* and to *ndvB* coexist (see Fig. 3), and only the former is involved in the synthesis of a cyclic OPG. Thus, further structural investigations are necessary. Nonetheless, one can imagine that *P. aeruginosa* can produce two kinds of OPGs, either simultaneously or independently in response to different environmental signals.

The implication of OPGs in pathogenicity was further confirmed by directed inactivation of *opg* genes in various species. Thus, nodule development was severely impaired in *ndvB* mutants of *B. japonicum* which are devoid of OPGs, and to a lesser

degree in *ndvC* mutants which produce OPGs with altered structures (97). Arellano-Reynoso *et al.* have reported that Opg^{-} mutants of *B. abortus*, an intracellular pathogen, are unable to evade phagosome-lysosome fusion (98). In pathogenic strains of *E. coli* devoid of OPG, virulence is affected (unpublished data) and in *S. enterica*, virulence of Opg^{-} mutants is severely reduced (86). Similarly, Opg^{-} mutants of *D. dadantii* are non-virulent on potato tubers, chicory leaves, and carrots, and this lack of virulence was correlated with their inability to grow and to survive in the plant host (39, 99).

In conclusion, every time their role in host-bacteria interactions was tested, OPGs appeared to be essential component of the bacterial envelope necessary for virulence or symbiosis.

OPGs and stress signaling

OPGs appear to be important intrinsic components of the Gram-negative bacterial envelope, which can be essential in extreme conditions found in nature, especially when bacteria must interact with a eukaryotic host. But, what is the fundamental function of these compounds?

A Periplasmic osmoprotectant?

Stock *et al.*, in their seminal study of the enterobacterial periplasm, have shown this compartment to comprise 20 to 40% of the total cell volume (100) and that a Gibbs-Donnan equilibrium exists between the periplasm and the extracellular medium, allowing isoosmolality between periplasm and cytoplasm (101). Thus, E. P. Kennedy proposed that OPGs function as periplasmic osmoprotectants on the basis of their anionic character, where OPGs would participate in the Gibbs-Donnan equilibrium through the

outer membrane, and he found, accordingly to this hypothesis, that OPG synthesis is osmoregulated in *E. coli* (102).

However, conflicting data and interpretations have been published concerning the volume and concentration of periplasm (84, 103-106). It is necessary to make a distinction between transient states following rapid upshift or downshift of external osmolarity, and steady-state adaptation to a particular medium where the bacterial cell can grow. Only the first situations can be considered as stress conditions essentially because fluxes of water are extremely rapid, as are those of small molecules in the case of abrupt downshift in osmolarity. Cayley *et al.* have calculated the OPG concentrations in steady-state cells of *E. coli* grown at different external osmolarities (103). The obtained values, from 50mM at 70 mosM to 3 mM at 800 mosM, are in the same range as the estimation of 15 mM reported for cells of the family Rhizobiaceae (22). The amount of OPGs is sufficient to quantitatively explain the observed Gibbs-Donnan potential maintained across the outer membrane (107). However, even in the case of *E. coli*, the anionic character of OPGs is only effective when ionic strength is high (104), and OPGs produced by several other bacteria are not charged molecules. Moreover, *E. coli* mutants defective in OPG substitution (*opgB, opgC, opgE*), as well as mutants defective in OPG backbone synthesis (*opgH* or *opgD*), do not show a particular osmosensitive phenotype (56) (unpublished data). More intriguing is the fact that during osmotic stress OPG concentration is only slowly adjusted to that found in steady-state: after abrupt downshift it takes at least half a generation time for *E. coli* cells to accumulate OPGs up to the ten-fold-higher steady-state level (67), and following upshift, OPG content decreases to a ten-fold-lower level by dilution due to successive cell divisions, because degradation of

OPGs was never observed (14, 108). Thus, osmoregulation of OPG synthesis, when it exists, appears more likely to be a way to detect diluted media rather than a true osmoadaptation.

A structural role?

OPGs may have a structural role in the envelope organization similar to carbohydrate molecules interacting with other structural components like phospholipids and/or peptidoglycan (109). The periplasm is generally described as a highly viscous gel-like compartment where peptidoglycan, protein junctions between inner and outer membranes, periseptal and polar annuli would drastically limit molecular movements. However, evidence of free movement of the jellyfish protein within the periplasm have been reported (110). Similarly, the use of high-resolution NMR spectroscopy under magic angle spinning revealed that the major fraction of OPGs detected *in vivo* in *R. solanacearum* undergoes significant rotational diffusion (111); they are not associated with macromolecules.

OPGs could be seen as molecular agents necessary to maintain a minimal periplasmic space at low medium osmolarity. However, *E. coli* Opg$^-$ mutant cells exhibited larger periplasmic space than wild type cells when subjected to gentle plasmolysis (91). Thus, they seem necessary to maintain interactions between the outer and inner membranes. OPGs induce the porin channels to close (112), while changing the Gibbs-Donnan potential has no effect on the permeability of porin channels (107).

A role in protein folding, association and function?

OPGs by their presence may participate in a macromolecular crowding determinant for protein folding, association and function (113). From this point of view,

it is noteworthy that *opgGH* transcription is controlled, at least in part, by the sigma E factor whose synthesis is induced following accumulation of unfolded proteins in the periplasm. An *opgH* mutant exhibits partially increased expression of *osmY* (114). Similarly, in a *dsbA* mutant that does not form disulfide in the envelope proteins, a stress is perceived that results in a reduced amount of OpgG and a higher amount of OsmY , two proteins which do not contain disulfide bonds (115). The *dsbA* cells behave as if they perceived a high external osmolarity.

The feedback regulation of OPG synthesis observed in *E. coli* cannot be explained by a simple hypothesis. A similar control is exerted at low and high external osmolarity at different OPG periplasmic concentrations. Since the membrane-bound glucosyl transferase OpgH is the ultimate target of this control, one can envisage that OpgH directly or indirectly senses a particular subfraction of the OPGs with similar properties, whatever the external conditions. Alternatively, OpgH, as other membrane-bound proteins, could sense mechanical deformation or alterations in the physicochemistry of the membrane bilayer (116) due to OPG accumulation.

A role in cell signaling?

OPGs may also have a function as informational molecules sensed by specific proteins present in the periplasmic compartment or bound to one or the other membrane. As discussed previously, when bacteria are shifted from a diluted medium to a concentrated environment, OPGs are slowly diluted. During the first steps of infection, having been exposed previously to media of low osmolarities, bacteria would possess, in their periplasmic space, OPG concentrations higher than those characteristic of their new environment. If OPG concentration could be monitored by one or several sensor proteins,

it could be used as a kind of internal counter of the number of cell division, and, indirectly, of bacteria at low population densities. This could control the expression of a set of genes in repsonse to an increase in cell numbers and it would be, in some way, the counterpart of the quorum sensing systems involved in the control of gene expression at high cell density.

OPGs were proposed as a possible signal for two *E. coli* inner membrane sensor proteins: EnvZ (85) and RcsC (117). Several phenotypic changes observed in OPG defective cells (e.g., increased synthesis of exopolysaccharides and decreased number of flagella) can result from activation of the RcsCDB sensor/regulator system (118). Genes belonging to the RcsCDB regulon appear essential to control the timing of infection processes and formation of biofilms. According to the proposed model, RcsC (a histidine kinase) and RcsD sense a signal and cooperate in a phosphorelay to phosphorylate the response regulator RcsB. In an *opgH* mutant this system is constitutively activated, and RcsC or/and RcsD could sense directly the OPGs present in the periplasm (117).

Recently, the concentration of OPGs was demonstrated to directly modulate the activation level of the RcsCDB system in *D. dadantii* (21, 119, 120). In this bacterium, RcsCDB activation is deleterious for virulence. Thus, OPG concentration dramatically increases at the early step of the infectious process to strongly repress the histidine kinase activity of RcsC (21). Moreover, RcsCDB and EnvZ-OmpR (37) controlled the succinylation of OPGs in *D. dadantii* (55). This data strengthen the interconnection between OPGs and the RcsCDB system in Enterobacteria. A relationship between OPGs and the RcsCDB phosphorelay was not demonstrated in *E. coli* or *S. enterica* but Opg$^-$

phenotypes observed for these bacteria strongly suggest a similar control of the RcsCDB phosphorelay by OPGs in these species.

Interestingly, *Y. pestis*, closely related to *D. dadantii*, lost the *opgGH* operon during the evolution from *Y. pseudotuberculosis* but the RcsCDB system of *Y. pestis* is also altered by a mutated *rcsD* gene (121). Taken together, both events - *i.e.* inactivation of the two sides of the same system - could be linked to ensure new virulence attributes of *Y. pestis.*

OPGs and cell division

Finally, OPGs may also be involved in cell size control. Recently, Hill *et al.* demonstrated that OpgH interacts with the FtsZ protein, one of the main components of the cell division ring (122). OpgH sequestered FtsZ to modulate the division and size of the cell depending on the nutrient availability, as sensed by OpgH through UDP-glucose concentration. An identical role is suggested in *Y. pseudotuberculosis*. An *opgH* mutant of *Y. pseudotuberculosis* was smaller than the wild-type (58). Interestingly, Hill *et al.* compared OpgH and UgtP from *Bacillus subtilis*. UgtP is an enzyme required for synthesis of the diglucosyl-diacylglycerol anchor for lipoteichoic acid (LTA), one of the major components of the Gram-positive cell wall (123, 124). UgtP was shown to inhibit FtsZ assembly and control cell size and growth rate (125). Even if both OpgH and UgtP don’t share homology or similar enzymatic properties, they are functional homologs (122). It was speculated that OPGs and LTA could play similar roles in Gram negative and Gram positive bacteria, respectively (122). All these data suggest a more complex role for OPGs and proteins involved in their biosynthesis than expected 45 years ago…

Acknowledgments

Jean-Marie Lacroix' group is funded by the University of Lille, the French National Center for Scientific Research, the French National Research Agency. S. Bontemps-Gallo is supported by the Intramural Research Program of the National Institute for Allergy and Infectious Diseases, National Institutes of Health. We thank Ryan Kissinger for help with graphics. We thank Dr. Ciaran Finn for English corrections.

Figure 1. Backbone structure of the four families of OPGs from Bohin, 2000 **(7)**

Figure 2. Working model of the OPG biosynthetic complex of *E. coli.* The variety of backbone structures and patterns of substitution are schematically represented (see text for details). Ptd-Gro: phosphatidylglycerol, Ptd-Etn: phosphatidylethanolamine, DG: diacylglycerol, Suc-CoA: succinyl-coenzyme A, CoA: coenzyme A, and UDP: uridine-diphosphate**.**

Figure 3. Occurrences of OPG genes in various representative genomes. Protein-protein BLAST program was used to search in the non-redundant databases sequences highly similar to OpgG, OpgH and OpgD from *E. coli*, OpgI from *R. sphaeroides*, NdvB, NdvC and NdvD from *B. japonicum*, and ChvB and ChvA from *A. tumefaciens*, OpgB and OpgE from *E. coli*, OpgC from *E. coli* and *D. dadantii*, and CgmB from *S. meliloti*.

Figure 4. Schematic of UDP-Glucose production, utilization and connection with trehalose and OPG biosynthesis in *E. coli*. The Kyoto Encyclopedia of Genes and Genomes (KEGG) was used to establish the metabolic pathway (126, 127). Glucose is transported into the cell via the phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS). During uptake, glucose is activated and transformed into Glucose-6-P. Glucose-6-P is used by Pgm and GalU enzymes to produce Uridine-diphosphate (UDP)-Glucose (in red). The UDP-Glucose can be used either to produce OPGs via the OpgH/OpgG complex or Trehalose-6-P via OtsA. Trehalose-6-P is transformed into Trehalose by OtsB enzyme. Then, it can either be excreted via the stretch-activated proteins (SAP) into the periplasm or maintained inside the cell. In both

cases, the trehalose will be degraded in 2 glucoses by TreF in the cytoplasm or TreA in the periplasm. P: Phosphate, OPG: Osmoregulated Periplasmic Glucan.

References

1. Altman PL. 1961. Physical properties and chemical composition of urine: mammals., p 363-369. *In* Dittmer DS (ed), Blood and other bodily fluids. Federation of American Societies for Experimental Biology, Washington, D.C.
2. Kunin CM. 1987. Detection, prevention and management of urinary tract infections, p 447. *In* Febiger L (ed), 4th ed, Philadelphia, PA.
3. Ross DL, Neely AE. 1983. Textbook of urinalysis and bodily fluids. Appleton Century Crofts, Norwalk, Va.
4. Russell PJ, Hertz PE, McMillan B. 2017. Regulating the Internal Environment., p 1041, Biology: The Dynamic Science, 4th Edition, 4th ed. Brooks Cole, Salt Lake City, UT.
5. Purves WK, Sadava D, Orians GH, Heller C. 2003. Salt and Water Balance and Nitrogen Excretion, Life: The Science of Biology, 7th ed. Sinauer Associates and W. H. Freeman, Sunderland, MA.
6. Altendorf K, Booth IR, Gralla J, Greie JC, Rosenthal AZ, Wood JM. 2009. Osmotic Stress. EcoSal Plus 3.
7. Bohin JP. 2000. Osmoregulated periplasmic glucans in Proteobacteria. FEMS Microbiol Lett 186:11-9.
8. van Golde LM, Schulman G, H., Kennedy EP. 1973. Metabolism of membrane phospholipids and its relation to a novel class of oligosaccharides in Escherichia coli. Proc Natl Acad Sci U S A 70:1368-72.
9. Kennedy EP. 1996. Membrane derived oligosaccharides (periplasmic beta-D-glucans) of Escherichia coli, p 1064-1074. *In* Neidhardt FC, Curtiss III R, Ingraham JL, Lin ECC, Low KB, Maganasik B, Reznikoff WS, Riley M, Schaechter M, Umbarger HE (ed), Escherichia coli and Salmonella Cellular and Molecular Biology, 2nd ed. ASM Press, Washington, D.C.

10. Miller KJ, Kennedy EP, Reinhold VN. 1986. Osmotic adaptation by gram-negative bacteria: possible role for periplasmic oligosaccharides. Science 231:48-51.
11. Lequette Y, Odberg-Ferragut C, Bohin JP, Lacroix JM. 2004. Identification of mdoD, an mdoG paralog which encodes a twin-arginine-dependent periplasmic protein that controls osmoregulated periplasmic glucan backbone structures. J Bacteriol 186:3695-702.
12. Nothaft H, Liu X, Li J, Szymanski CM. 2010. Campylobacter jejuni free oligosaccharides: function and fate. Virulence 1:546-50.
13. Nothaft H, Liu X, McNally DJ, Li J, Szymanski CM. 2009. Study of free oligosaccharides derived from the bacterial N-glycosylation pathway. Proc Natl Acad Sci U S A 106:15019-24.
14. Bohin JP, Lacroix JM. 2006. Osmoregulation in the periplasm, p pp. 325–341. *In* Ehrmann M (ed), The Periplasm. American Society for Microbiology, Washington, DC, USA.
15. Cogez V, Talaga P, Lemoine J, Bohin JP. 2001. Osmoregulated periplasmic glucans of Erwinia chrysanthemi. J Bacteriol 183:3127-33.
16. Lequette Y, Rollet E, Delangle A, Greenberg EP, Bohin JP. 2007. Linear osmoregulated periplasmic glucans are encoded by the opgGH locus of Pseudomonas aeruginosa. Microbiology 153:3255-63.
17. Talaga P, Fournet B, Bohin JP. 1994. Periplasmic glucans of Pseudomonas syringae pv. syringae. J Bacteriol 176:6538-44.
18. Bontemps-Gallo S, Cogez V, Robbe-Masselot C, Quintard K, Dondeyne J, Madec E, Lacroix JM. 2013. Biosynthesis of osmoregulated periplasmic glucans in Escherichia coli: the phosphoethanolamine transferase is encoded by opgE. Biomed Res Int 2013:371429.
19. Lacroix JM, Lanfroy E, Cogez V, Lequette Y, Bohin A, Bohin JP. 1999. The mdoC gene of Escherichia coli encodes a membrane protein that is required for succinylation of osmoregulated periplasmic glucans. J Bacteriol 181:3626-31.
20. Lee S, Cho E, Jung S. 2009. Periplasmic glucans isolated from Proteobacteria. BMB Rep 42:769-75.

21. Bontemps-Gallo S, Madec E, Dondeyne J, Delrue B, Robbe-Masselot C, Vidal O, Prouvost AF, Boussemart G, Bohin JP, Lacroix JM. 2013. Concentration of osmoregulated periplasmic glucans (OPGs) modulates the activation level of the RcsCD RcsB phosphorelay in the phytopathogen bacteria Dickeya dadantii. Environ Microbiol 15:881-94.
22. Breedveld MW, Miller KJ. 1994. Cyclic beta-glucans of members of the family Rhizobiaceae. Microbiol Rev 58:145-61.
23. Briones G, Inon de Iannino N, Steinberg M, Ugalde RA. 1997. Periplasmic cyclic 1,2-beta-glucan in Brucella spp. is not osmoregulated. Microbiology 143 (Pt 4):1115-24.
24. Choma A, Komaniecka I. 2003. Characterisation of Mesorhizobium huakuii cyclic beta-glucan. Acta Biochim Pol 50:1273-81.
25. Rolin DB, Pfeffer PE, Osman SF, Szwergold BS, Kappler F, Benesi AJ. 1992. Structural studies of a phosphocholine substituted beta-(1,3);(1,6) macrocyclic glucan from Bradyrhizobium japonicum USDA 110. Biochim Biophys Acta 1116:215-25.
26. Komaniecka I, Choma A. 2003. Isolation and characterization of periplasmic cyclic beta-glucans of Azorhizobium caulinodans. FEMS Microbiol Lett 227:263-9.
27. Altabe SG, Talaga P, Wieruszeski J-M, Lippens G, Ugalde RA, Bohin JP. 1998. Periplasmic glucans of Azospirillum brasilense, p 390. *In* Elmerich C, Kondorosi A, Newton WE (ed), Biological nitrogen fixation for the 21st century. Kluwer Academic Publishers,, The Netherlands.
28. Talaga P, Stahl B, Wieruszeski JM, Hillenkamp F, Tsuyumu S, Lippens G, Bohin JP. 1996. Cell-associated glucans of Burkholderia solanacearum and Xanthomonas campestris pv. citri: a new family of periplasmic glucans. J Bacteriol 178:2263-71.
29. York WS. 1995. A conformational model for cyclic beta-(1-->2)-linked glucans based on NMR analysis of the beta-glucans produced by Xanthomonas campestris. Carbohydr Res 278:205-25.

30. Talaga P, Cogez V, Wieruszeski JM, Stahl B, Lemoine J, Lippens G, Bohin JP. 2002. Osmoregulated periplasmic glucans of the free-living photosynthetic bacterium Rhodobacter sphaeroides. Eur J Biochem 269:2464-72.
31. Lippens G, Wieruszeski JM, Horvath D, Talaga P, Bohin JP. 1998. Slow Dynamics of the Cyclic Osmoregulated Periplasmic Glucan of Ralstonia solanacearum As Revealed by Heteronuclear Relaxation Studies. Journal of the American Chemical Society 120:170-177.
32. Lacroix JM, Loubens I, Tempete M, Menichi B, Bohin JP. 1991. The mdoA locus of Escherichia coli consists of an operon under osmotic control. Mol Microbiol 5:1745-53.
33. Therisod H, Weissborn AC, Kennedy EP. 1986. An essential function for acyl carrier protein in the biosynthesis of membrane-derived oligosaccharides of Escherichia coli. Proc Natl Acad Sci U S A 83:7236-40.
34. Debarbieux L, Bohin A, Bohin JP. 1997. Topological analysis of the membrane-bound glucosyltransferase, MdoH, required for osmoregulated periplasmic glucan synthesis in Escherichia coli. J Bacteriol 179:6692-8.
35. Lequette Y. 2002. Biosynthèse des glucanes périplasmiques osmorégulés chez Escherichia coli : analyse fonctionnelle des protéines MdoG et MdoH et caractérisation de deux nouvelles activités. PhD thesis. Université des Sciences et Technologie de Lille, France.
36. Hanoulle X, Rollet E, Clantin B, Landrieu I, Odberg-Ferragut C, Lippens G, Bohin JP, Villeret V. 2004. Structural analysis of Escherichia coli OpgG, a protein required for the biosynthesis of osmoregulated periplasmic glucans. J Mol Biol 342:195-205.
37. Bontemps Gallo S. 2013. Dickeya dadantii: Towards the understanding of the biological role of the osmoregulated periplasmic glucans (in French). PhD thesis. Université de Lille, France.
38. Loubens I, Debarbieux L, Bohin A, Lacroix JM, Bohin JP. 1993. Homology between a genetic locus (mdoA) involved in the osmoregulated biosynthesis of periplasmic glucans in Escherichia coli and a genetic locus (hrpM) controlling pathogenicity of Pseudomonas syringae. Mol Microbiol 10:329-40.

39. Page F, Altabe S, Hugouvieux-Cotte-Pattat N, Lacroix JM, Robert-Baudouy J, Bohin JP. 2001. Osmoregulated periplasmic glucan synthesis is required for Erwinia chrysanthemi pathogenicity. J Bacteriol 183:3134-41.
40. Cogez V, Gak E, Puskas A, Kaplan S, Bohin JP. 2002. The opgGIH and opgC genes of Rhodobacter sphaeroides form an operon that controls backbone synthesis and succinylation of osmoregulated periplasmic glucans. Eur J Biochem 269:2473-84.
41. Minsavage GV, Mudgett MB, Stall RE, Jones JB. 2004. Importance of opgHXcv of Xanthomonas campestris pv. vesicatoria in host-parasite interactions. Mol Plant Microbe Interact 17:152-61.
42. Dylan T, Ielpi L, Stanfield S, Kashyap L, Douglas C, Yanofsky M, Nester E, Helinski DR, Ditta G. 1986. Rhizobium meliloti genes required for nodule development are related to chromosomal virulence genes in Agrobacterium tumefaciens. Proc Natl Acad Sci U S A 83:4403-7.
43. Inon de Iannino N, Briones G, Tolmasky M, Ugalde RA. 1998. Molecular cloning and characterization of cgs, the Brucella abortus cyclic beta(1-2) glucan synthetase gene: genetic complementation of Rhizobium meliloti ndvB and Agrobacterium tumefaciens chvB mutants. J Bacteriol 180:4392-400.
44. Puvanesarajah V, Schell FM, Stacey G, Douglas CJ, Nester EW. 1985. Role for 2-linked-beta-D-glucan in the virulence of Agrobacterium tumefaciens. J Bacteriol 164:102-6.
45. Roset MS, Ciocchini AE, Ugalde RA, Inon de Iannino N. 2004. Molecular cloning and characterization of cgt, the Brucella abortus cyclic beta-1,2-glucan transporter gene, and its role in virulence. Infect Immun 72:2263-71.
46. Ciocchini AE, Roset MS, Inon de Iannino N, Ugalde RA. 2004. Membrane topology analysis of cyclic glucan synthase, a virulence determinant of Brucella abortus. J Bacteriol 186:7205-13.
47. Altabe SG, Inon de Iannino N, de Mendoza D, Ugalde RA. 1994. New osmoregulated beta(1-3),beta(1-6) glucosyltransferase(s) in Azospirillum brasilense. J Bacteriol 176:4890-8.

48. Bhagwat AA, Tully RE, Keister DL. 1993. Identification and cloning of a cyclic beta-(1-->3), beta-(1-->6)-D-glucan synthesis locus from Bradyrhizobium japonicum. FEMS Microbiol Lett 114:139-44.
49. Bhagwat AA, Gross KC, Tully RE, Keister DL. 1996. Beta-glucan synthesis in Bradyrhizobium japonicum: characterization of a new locus (ndvC) influencing beta-(1-->6) linkages. J Bacteriol 178:4635-42.
50. Chen R, Bhagwat AA, Yaklich R, Keister DL. 2002. Characterization of ndvD, the third gene involved in the synthesis of cyclic beta-(1 --> 3),(1 --> 6)-D-glucans in Bradyrhizobium japonicum. Can J Microbiol 48:1008-16.
51. Jackson BJ, Bohin JP, Kennedy EP. 1984. Biosynthesis of membrane-derived oligosaccharides: characterization of mdoB mutants defective in phosphoglycerol transferase I activity. J Bacteriol 160:976-81.
52. Bohin JP, Kennedy EP. 1984. Regulation of the synthesis of membrane-derived oligosaccharides in Escherichia coli. Assay of phosphoglycerol transferase I in vivo. J Biol Chem 259:8388-93.
53. Jackson BJ, Kennedy EP. 1983. The biosynthesis of membrane-derived oligosaccharides. A membrane-bound phosphoglycerol transferase. J Biol Chem 258:2394-8.
54. Lequette Y, Lanfroy E, Cogez V, Bohin JP, Lacroix JM. 2008. Biosynthesis of osmoregulated periplasmic glucans in Escherichia coli: the membrane-bound and the soluble periplasmic phosphoglycerol transferases are encoded by the same gene. Microbiology 154:476-83.
55. Bontemps-Gallo S, Madec E, Robbe-Masselot C, Souche E, Dondeyne J, Lacroix JM. 2016. The opgC gene is required for OPGs succinylation and is osmoregulated through RcsCDB and EnvZ/OmpR in the phytopathogen Dickeya dadantii. Sci Rep 6:19619.
56. Bohin JP, Kennedy EP. 1984. Mapping of a locus (mdoA) that affects the biosynthesis of membrane-derived oligosaccharides in Escherichia coli. J Bacteriol 157:956-7.
57. Raina S, Raina R, Venkatesh TV, Das HK. 1995. Isolation and characterization of a locus from Azospirillum brasilense Sp7 that complements the tumorigenic

defect of Agrobacterium tumefaciens chvB mutant. Mol Plant Microbe Interact 8:322-6.

58. Quintard K, Dewitte A, Reboul A, Madec E, Bontemps-Gallo S, Dondeyne J, Marceau M, Simonet M, Lacroix JM, Sebbane F. 2015. Evaluation of the Role of the opgGH Operon in Yersinia pseudotuberculosis and Its Deletion during the Emergence of Yersinia pestis. Infect Immun 83:3638-47.
59. Campbell JA, Davies GJ, Bulone V, Henrissat B. 1997. A classification of nucleotide-diphospho-sugar glycosyltransferases based on amino acid sequence similarities. Biochem J 326 (Pt 3):929-39.
60. Coutinho PM, Deleury E, Davies GJ, Henrissat B. 2003. An evolving hierarchical family classification for glycosyltransferases. J Mol Biol 328:307-17.
61. Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B. 2014. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res 42:D490-5.
62. Davies G, Henrissat B. 1995. Structures and mechanisms of glycosyl hydrolases. Structure 3:853-9.
63. Henrissat B. 1991. A classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem J 280 (Pt 2):309-16.
64. Henrissat B, Bairoch A. 1993. New families in the classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem J 293 (Pt 3):781-8.
65. Henrissat B, Bairoch A. 1996. Updating the sequence-based classification of glycosyl hydrolases. Biochem J 316 (Pt 2):695-6.
66. Henrissat B, Davies G. 1997. Structural and sequence-based classification of glycoside hydrolases. Curr Opin Struct Biol 7:637-44.
67. Lacroix J-M. 1989. Etude génétique et physiologique de la régulation osmotique de la biosynthèse du MDO chez Escherichia coli. PhD thesis. Université de Paris-Sud, Centre d'Orsay, France.
68. Breedveld MW, Zevenhuizen LP, Zehnder AJ. 1992. Synthesis of cyclic beta-(1,2)-glucans by Rhizobium leguminosarum biovar trifolii TA-1: factors influencing excretion. J Bacteriol 174:6336-42.

69. Vojnov AA, Slater H, Newman MA, Daniels MJ, Dow JM. 2001. Regulation of the synthesis of cyclic glucan in Xanthomonas campestris by a diffusible signal molecule. Arch Microbiol 176:415-20.
70. Astua-Monge G, Freitas-Astua J, Bacocina G, Roncoletta J, Carvalho SA, Machado MA. 2005. Expression profiling of virulence and pathogenicity genes of Xanthomonas axonopodis pv. citri. J Bacteriol 187:1201-5.
71. de Souza AA, Takita MA, Coletta-Filho HD, Caldana C, Yanai GM, Muto NH, de Oliveira RC, Nunes LR, Machado MA. 2004. Gene expression profile of the plant pathogen Xylella fastidiosa during biofilm formation in vitro. FEMS Microbiol Lett 237:341-53.
72. Brown DG, Allen C. 2004. Ralstonia solanacearum genes induced during growth in tomato: an inside view of bacterial wilt. Mol Microbiol 53:1641-60.
73. Rougemont B, Bontemps Gallo S, Ayciriex S, Carriere R, Hondermarck H, Lacroix JM, Le Blanc JC, Lemoine J. 2016. Scout-MRM: multiplexed targeted mass spectrometry-based assay without retention time scheduling exemplified by Dickeya dadantii proteomic analysis during plant infection. Anal Chem doi:10.1021/acs.analchem.6b03201.
74. Dartigalongue C, Missiakas D, Raina S. 2001. Characterization of the Escherichia coli sigma E regulon. J Biol Chem 276:20866-75.
75. Link AJ, Robison K, Church GM. 1997. Comparing the predicted and observed properties of proteins encoded in the genome of Escherichia coli K-12. Electrophoresis 18:1259-313.
76. Hengge-Aronis R. 2002. Stationary phase gene regulation: what makes an Escherichia coli promoter sigmaS-selective? Curr Opin Microbiol 5:591-5.
77. Lacour S, Landini P. 2004. SigmaS-dependent gene expression at the onset of stationary phase in Escherichia coli: function of sigmaS-dependent genes and identification of their promoter sequences. J Bacteriol 186:7186-95.
78. Wang P, Ingram-Smith C, Hadley JA, Miller KJ. 1999. Cloning, sequencing, and characterization of the cgmB gene of Sinorhizobium meliloti involved in cyclic beta-glucan biosynthesis. J Bacteriol 181:4576-83.

79. Kolbe A, Tiessen A, Schluepmann H, Paul M, Ulrich S, Geigenberger P. 2005. Trehalose 6-phosphate regulates starch synthesis via posttranslational redox activation of ADP-glucose pyrophosphorylase. Proc Natl Acad Sci U S A 102:11118-23.
80. Strom AR, Kaasen I. 1993. Trehalose metabolism in Escherichia coli: stress protection and stress regulation of gene expression. Mol Microbiol 8:205-10.
81. Boos W, Ehmann U, Bremer E, Middendorf A, Postma P. 1987. Trehalase of Escherichia coli. Mapping and cloning of its structural gene and identification of the enzyme as a periplasmic protein induced under high osmolarity growth conditions. J Biol Chem 262:13212-8.
82. Horlacher R, Uhland K, Klein W, Ehrmann M, Boos W. 1996. Characterization of a cytoplasmic trehalase of Escherichia coli. J Bacteriol 178:6250-7.
83. Levina N, Totemeyer S, Stokes NR, Louis P, Jones MA, Booth IR. 1999. Protection of Escherichia coli cells against extreme turgor by activation of MscS and MscL mechanosensitive channels: identification of genes required for MscS activity. EMBO J 18:1730-7.
84. Pfeffer PE, Becard G, Rolin DB, Uknalis J, Cooke P, Tu S. 1994. In vivo nuclear magnetic resonance study of the osmoregulation of phosphocholine-substituted beta-1,3;1,6 cyclic glucan and its associated carbon metabolism in Bradyrhizobium japonicum USDA 110. Appl Environ Microbiol 60:2137-46.
85. Fiedler W, Rotering H. 1988. Properties of Escherichia coli mutants lacking membrane-derived oligosaccharides. J Biol Chem 263:14684-9.
86. Bhagwat AA, Jun W, Liu L, Kannan P, Dharne M, Pheh B, Tall BD, Kothary MH, Gross KC, Angle S, Meng J, Smith A. 2009. Osmoregulated periplasmic glucans of Salmonella enterica serovar Typhimurium are required for optimal virulence in mice. Microbiology 155:229-37.
87. Bouchart F, Delangle A, Lemoine J, Bohin JP, Lacroix JM. 2007. Proteomic analysis of a non-virulent mutant of the phytopathogenic bacterium Erwinia chrysanthemi deficient in osmoregulated periplasmic glucans: change in protein expression is not restricted to the envelope, but affects general metabolism. Microbiology 153:760-7.

88. Cooper B, Chen R, Garrett WM, Murphy C, Chang C, Tucker ML, Bhagwat AA. 2012. Proteomic pleiotropy of OpgGH, an operon necessary for efficient growth of Salmonella enterica serovar typhimurium under low-osmotic conditions. J Proteome Res 11:1720-7.
89. Dylan T, Nagpal P, Helinski DR, Ditta GS. 1990. Symbiotic pseudorevertants of Rhizobium meliloti ndv mutants. J Bacteriol 172:1409-17.
90. Rajagopal S, Eis N, Bhattacharya M, Nickerson KW. 2003. Membrane-derived oligosaccharides (MDOs) are essential for sodium dodecyl sulfate resistance in Escherichia coli. FEMS Microbiol Lett 223:25-31.
91. Holtje JV, Fiedler W, Rotering H, Walderich B, van Duin J. 1988. Lysis induction of Escherichia coli by the cloned lysis protein of the phage MS2 depends on the presence of osmoregulatory membrane-derived oligosaccharides. J Biol Chem 263:3539-41.
92. Cangelosi GA, Martinetti G, Nester EW. 1990. Osmosensitivity phenotypes of Agrobacterium tumefaciens mutants that lack periplasmic beta-1,2-glucan. J Bacteriol 172:2172-4.
93. Mills D, Mukhopadhyay P. 1990. Organization of the hrpM locus of Pseudomonas syringae pv. syringae and its potential function in pathogenesis, p 47-57. *In* Silver S, Chakrabarty AM, Iglewski B, Kaplan S (ed), Pseudomonas: biotransformation, pathogenesis, and evolving biotechnology. ASM Press,, Washington, D.C.
94. Young GM, Miller VL. 1997. Identification of novel chromosomal loci affecting Yersinia enterocolitica pathogenesis. Mol Microbiol 25:319-28.
95. Mahajan-Miklos S, Tan MW, Rahme LG, Ausubel FM. 1999. Molecular mechanisms of bacterial virulence elucidated using a Pseudomonas aeruginosa-Caenorhabditis elegans pathogenesis model. Cell 96:47-56.
96. Mah TF, Pitts B, Pellock B, Walker GC, Stewart PS, O'Toole GA. 2003. A genetic basis for Pseudomonas aeruginosa biofilm antibiotic resistance. Nature 426:306-10.

97. Dunlap J, Minami E, Bhagwat AA, Keister DL, Stacey G. 1996. Nodule development induced by mutants of Bradyrhizobium japonicum defective in cyclic B-glucan synthesis. Mol Plant Microbe Interact 9:546-55.
98. Arellano-Reynoso B, Lapaque N, Salcedo S, Briones G, Ciocchini AE, Ugalde R, Moreno E, Moriyon I, Gorvel JP. 2005. Cyclic beta-1,2-glucan is a Brucella virulence factor required for intracellular survival. Nat Immunol 6:618-25.
99. Bontemps-Gallo S, Lacroix JM. 2015. New insights into the biological role of the osmoregulated periplasmic glucans in pathogenic and symbiotic bacteria. Environ Microbiol Rep 7:690-7.
100. Stock JB, Rauch B, Roseman S. 1977. Periplasmic space in Salmonella typhimurium and Escherichia coli. J Biol Chem 252:7850-61.
101. Donnan FG. 1911. Theorie der Membrangleichgewichte und Membranpotentiale bei Vorhandensein von nicht dialysierenden Elektrolyten. Ein Beitrag zur physikalisch-chemischen Physiologie. Zeitschrift für Elektrochemie und angewandte physikalische Chemie 17:572-581.
102. Kennedy EP. 1982. Osmotic regulation and the biosynthesis of membrane-derived oligosaccharides in Escherichia coli. Proc Natl Acad Sci U S A 79:1092-5.
103. Cayley DS, Guttman HJ, Record MT, Jr. 2000. Biophysical characterization of changes in amounts and activity of Escherichia coli cell and compartment water and turgor pressure in response to osmotic stress. Biophys J 78:1748-64.
104. Koch AL. 1998. The biophysics of the gram-negative periplasmic space. Crit Rev Microbiol 24:23-59.
105. Oliver DB. 1996. Periplasm, p 88-103. *In* Neidhardt FC, Curtiss III R, Ingraham JL, Lin ECC, Low KB, Maganasik B, Reznikoff WS, Riley M, Schaechter M, Umbarger HE (ed), Escherichia coli and Salmonella Cellular and Molecular Biology, 2nd ed. ASM Press, Washington, D.C.
106. Wood JM. 1999. Osmosensing by bacteria: signals and membrane-based sensors. Microbiol Mol Biol Rev 63:230-62.
107. Sen K, Hellman J, Nikaido H. 1988. Porin channels in intact cells of Escherichia coli are not affected by Donnan potentials across the outer membrane. Journal of Biological Chemistry 263:1182-1187.

108. Ruby EG, McCabe JB. 1988. Metabolism of periplasmic membrane-derived oligosaccharides by the predatory bacterium Bdellovibrio bacteriovorus 109J. J Bacteriol 170:646-52.
109. Banta LM, Bohne J, Lovejoy SD, Dostal K. 1998. Stability of the Agrobacterium tumefaciens VirB10 protein is modulated by growth temperature and periplasmic osmoadaption. J Bacteriol 180:6597-606.
110. Santini CL, Bernadac A, Zhang M, Chanal A, Ize B, Blanco C, Wu LF. 2001. Translocation of jellyfish green fluorescent protein via the Tat system of Escherichia coli and change of its periplasmic localization in response to osmotic up-shock. J Biol Chem 276:8159-64.
111. Wieruszeski JM, Bohin A, Bohin JP, Lippens G. 2001. In vivo detection of the cyclic osmoregulated periplasmic glucan of Ralstonia solanacearum by high-resolution magic angle spinning NMR. J Magn Reson 151:118-23.
112. Delcour AH, Adler J, Kung C, Martinac B. 1992. Membrane-derived oligosaccharides (MDO's) promote closing of an E. coli porin channel. FEBS Lett 304:216-20.
113. Mogensen JE, Otzen DE. 2005. Interactions between folding factors and bacterial outer membrane proteins. Mol Microbiol 57:326-46.
114. Bohringer J, Fischer D, Mosler G, Hengge-Aronis R. 1995. UDP-glucose is a potential intracellular signal molecule in the control of expression of sigma S and sigma S-dependent genes in Escherichia coli. J Bacteriol 177:413-22.
115. Hiniker A, Bardwell JC. 2004. In vivo substrate specificity of periplasmic disulfide oxidoreductases. J Biol Chem 279:12967-73.
116. Poolman B, Blount P, Folgering JH, Friesen RH, Moe PC, van der Heide T. 2002. How do membrane proteins sense water stress? Mol Microbiol 44:889-902.
117. Ebel W, Vaughn GJ, Peters HK, 3rd, Trempy JE. 1997. Inactivation of mdoH leads to increased expression of colanic acid capsular polysaccharide in Escherichia coli. J Bacteriol 179:6858-61.
118. Majdalani N, Gottesman S. 2005. The Rcs phosphorelay: a complex signal transduction system. Annu Rev Microbiol 59:379-405.

119. Bouchart F, Boussemart G, Prouvost AF, Cogez V, Madec E, Vidal O, Delrue B, Bohin JP, Lacroix JM. 2010. The virulence of a Dickeya dadantii 3937 mutant devoid of osmoregulated periplasmic glucans is restored by inactivation of the RcsCD-RcsB phosphorelay. J Bacteriol 192:3484-90.
120. Madec E, Bontemps-Gallo S, Lacroix JM. 2014. Increased phosphorylation of the RcsB regulator of the RcsCDB phosphorelay in strains of Dickeya dadantii devoid of osmoregulated periplasmic glucans revealed by Phos-tag gel analysis. Microbiology 160:2763-70.
121. Sun YC, Hinnebusch BJ, Darby C. 2008. Experimental evidence for negative selection in the evolution of a Yersinia pestis pseudogene. Proc Natl Acad Sci U S A 105:8097-101.
122. Hill NS, Buske PJ, Shi Y, Levin PA. 2013. A moonlighting enzyme links Escherichia coli cell size with central metabolism. PLoS Genet 9:e1003663.
123. Chien AC, Zareh SK, Wang YM, Levin PA. 2012. Changes in the oligomerization potential of the division inhibitor UgtP co-ordinate Bacillus subtilis cell size with nutrient availability. Mol Microbiol 86:594-610.
124. Weart RB, Lee AH, Chien AC, Haeusser DP, Hill NS, Levin PA. 2007. A metabolic sensor governing cell size in bacteria. Cell 130:335-47.
125. Lazarevic V, Soldo B, Medico N, Pooley H, Bron S, Karamata D. 2005. Bacillus subtilis alpha-phosphoglucomutase is required for normal cell morphology and biofilm formation. Appl Environ Microbiol 71:39-45.
126. Kanehisa M, Goto S. 2000. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28:27-30.
127. Tanabe M, Kanehisa M. 2012. Using the KEGG database resource. Curr Protoc Bioinformatics Chapter 1:Unit1 12.