Pathogénie moléculaire et cellulaire des infections bactériennes

Leçon 5

Régulation de l'expression des gènes de virulence par «quorum-sensing» : science ou science-fiction ?

Professeur Jacques Mainil, Département des Maladies infectieuses et parasitaires, Bactériologie, Faculté de Médecine vétérinaire, Université de Liège

Correspondance. JG.Mainil@ulg.ac.be

Molecular and cellular pathogenesis of bacterial infections

Lecture 5

Regulation of virulence gene expression by « quorum-sensing » : science or science-fiction ?

Texte intégral en anglais (avec résumé en français) de la conférence donnée par le professeur Jacques Mainil le 24 mars 2005 dans l'auditoire Maximus de la *Diergeneeskunde Faculteit* (Université de Gand) à Merelbeke, dans le cadre de la Chaire Francqui au titre belge 2004-2005.

RESUME : L'expression des gènes bactériens est régulée par des mécanismes très stricts de contrôle sous l'influence des conditions du milieu extérieur. Les exemples classiques sont les régulations positives et négatives exercées par le lactose et le tryptophane respectivement sur les expressions des opérons *lac* et *trp*. Ce qui est beaucoup moins connu, est que l'expression de nombreux gènes dans une cellule bactérienne est sous la dépendance de la présence et de la concentration de molécules sécrétées par des cellules appartenant à la même espèce. Ces molécules ont été appelées «auto-inducteurs» (AI). Comme ce mécanisme de régulation dépend de la concentration de l'AI dans le milieu, donc du nombre de cellules bactériennes présentes dans l'environnement immédiat, il a aussi reçu la dénomination de «quorum-sensing» (QS).

Le phénomène de QS fut observé pour la première fois dans les années 1950 avec deux espèces bactériennes marines émettrices de lumière, *Vibrio fischeri* et *Vibrio harveyi*. De nombreuses expériences réalisées dans les années 1970 et 1980 ont permis de comprendre progressivement le fonctionnement général ainsi que moléculaire et la génétique du QS dans *Vibrio fischeri*. L'AI fut ainsi identifié à un dérivé d'homosérine lactone (HSL), sa concentration seuil mesurée et les gènes qu'il régule et qui sont responsables de l'émission de lumière, clonés et séquencés (gènes *lux*).

Par la suite, d'autres systèmes apparentés (systèmes Lux-like) de QS utilisant des dérivés d'HSL ont été décrits dans diverses bactéries Gram négatives. Des systèmes de QS de complexité variable existent aussi chez les bactéries Gram positives, mais les plus répandus utilisent des oligopeptides comme AI. De plus, un système de QS qui pourrait représenter une langue commune à de nombreuses espèces bactériennes a aussi été décrit et dénommé «Esperanto».

La plupart des systèmes de QS sont eux-mêmes soumis à différents circuits de régulation existant dans la cellule bactérienne et ces différents circuits sont eux-mêmes soumis aux influences de stimuli présents dans le milieu extérieur. Parfois, ces stimuli proviennent d'un hôte eucaryote avec lequel les bactéries développent une relation symbiotique ou pathogène. Les gènes régulés par QS codent pour diverses fonctions importantes pour la survie bactérienne, au niveau individuel ou de la population, dans l'environnement extérieur ou chez l'hôte, dans des circonstances précises, en règle générale lors de concentrations importantes en cellules bactériennes (microcolonies ou flore multi-bactérienne, par exemple) : production d'antibiotiques ou de bactériocines, conjugaison plasmidique, sporulation, formation de biofilms, mobilité, diverses propriétés de virulence…

Il est intéressant de comparer les systèmes bactériens de QS utilisant des AI avec les systèmes de communication entre individus basés sur des phérormones chez les plantes et les animaux ou entre organes d'un individu basés sur des hormones peptidiques chez les animaux.

Comme les systèmes de QS régulent l'expression de gènes en fonction de la concentration de l'AI (= science), est-il possible d'imaginer manipuler dans le futur ces systèmes en jouant sur la concentration de l'AI ou en apportant des antagonistes des AI (= science-fiction) ? Par exemple, réprimer l'expression de gènes impliqués dans la virulence de bactéries pathogènes en manipulant les systèmes de QS à la base de leur expression pourrait représenter un moyen de remplacer partiellement ou totalement les antibiotiques dans la lutte antibactérienne en médecine humaine et vétérinaire.

REGULATION OF GENE EXPRESSION

The genetic basis and some « classical » regulation mechanisms of the expression of different virulence-associated properties of pathogen bacteria were presented during the fourth lecture. During this fifth lecture a quite recently described regulation mechanism of bacterial gene expression, so-called « quorum-sensing », will be described. This basically represents the possibility of discussions between bacterial cells belonging not only to the same species, but also to other species. The quorum-sensing regulation mechanism was first associated with the expression of genes involved in the several ecological properties of bacteria, but is today also associated with the expression of genes coding for virulence properties.

Positive regulation of the *lac* operon

The most classical example of the regulation of gene expression is the positive regulation of the *lac* operon by lactose. In the absence of lactose in the growth medium, the specific repressor molecule LacI binds to the promoter region of the *lac* operon and prevents the transcription of the *lac* genes. In the presence of lactose in the environment some molecules passively diffuse into the bacterial cytoplasm and bind to the LacI repressor, initiating a conformational change. The complex repressor/lactose cannot bind anymore to the promoter region and the transcription of the *lac* genes can be initiated by RNA polymerase, with synthesis of enzymes involved in the catabolism of lactose.

Negative regulation of the *trp* operon

In contrast, the expression of the *trp* operon is negatively regulated by the presence of tryptophane in the growth medium. In the absence of tryptophane, the repressor molecule cannot bind to the promoter region and the *trp* operon can be therefore transcribed, leading to synthesis of enzymes that are involved in the anabolism of tryptophane. When present in the environment, tryptophane diffuses inside the bacterial cytoplasm and binds to the repressor. After conformational change, the repressor/tryptophane complex can bind to the promoter region of the *trp* operon, whose transcription is now prevented, saving energy for other syntheses.

In these two classical examples lactose and tryptophane are present in the external environment, but their precise origin remains undetermined. Until the late 1980s it was very rare to propose that the expression of genes in a bacterium could be regulated by effectors originating from other bacterial cells belonging to the same species with a dose/effect relationship, i.e. depending on the cell population density. However, this phenomenon had already been observed and described in the 1950s with two marine bacteria, *Vibrio fischeri* and *Vibrio harveyi*. The effectors at the basis of this regulation process were named « auto-inducers » and the phenomenon itself « auto-induction » until the term « quorum-sensing » was proposed in 1994.

Definitions

Before presenting the history of quorum-sensing, the identity of the auto-inducers, and a few examples of regulation systems, let us first linguistically define the terms « auto-induction » and « quorum-sensing ». In cellular biology and in bacteriology, « induction » refers to the triggering of a physiological reaction inside the cell under the influence of a physical or chemical stimulus, and « auto » means « oneself ». Auto-induction thus refers to the triggering of a physiological reaction inside the cell under the influence of a stimulus produced by the cell itself or by a population of identical cells. The stimulus is called the « inducer ». In « quorum-sensing », « sensing » derives from « to sense » and also means « to perceive, to feel » and « quorum », originating from Latin, means « a minimal number or quantity ». « Quorum-sensing » thus refers to the perception of a stimulus from a threshold.

Combining all definitions, Schauder and collaborators wrote in 2001 that « quorum-sensing or the regulation of gene expression in response to cell population density is a process that bacteria use to co-ordinate the gene expression of the community. Presumably, the ability to control behaviour on a collective scale enables bacteria to behave like multicellular organisms. Quorum-sensing involves the production of extra-cellular signalling molecules called auto-inducers ».

Vibrio fischeri AND *Vibrio harveyi*

The first research studies on quorum-sensing were on the regulation mechanism of the production of light by two marine bacterial species, *Vibrio fischeri* and *Vibrio harveyi*.

Early studies

During the early 1950s, Farghaly and collaborators observed that the curve of light emission by these two bacterial species dissociates from the curve of bacterial growth. The emission of light actually begins with a lag phase, compared to the bacterial growth curve, and at a population density of 10^7 bacterial cells per ml. The conclusion at the time was that light emission was inducible. Other experiments showed that the composition of the growth medium, the pH and the temperature influenced the production of light. However, the inducer molecule

was not identified before the 1970s and the concept of « auto-induction » was of course not even mentioned.

Identification of the auto-inducer

Starting from the early 1970s, Nealson and his collaborators conducted several experiments with *Vibrio fischeri* to finally conclude that the inducer is a molecule produced by *Vibrio fischeri* itself. In an interesting series of experiments, they first demonstrated that the supernatant of a culture of *Vibrio fischeri* collected when bacterial population density is below 10^7 cells per ml and when there is thus no light emitted (time A), cannot induce the emission of light by cells of a fresh culture of *Vibrio fischeri*. In contrast, the supernatant collected when bacterial cell density is high and when light is already emitted (time B), induces easily the production of light by a fresh culture, even when the population density is below 10^7 cells per ml. Their conclusion was that the inducer is present in the culture supernatant and is produced by the cells themselves of the species *Vibrio fischeri*. The idea of auto-induction was on its way, but not yet the concept of a threshold concentration.

In 1977, a mutant strain of *Vibrio fischeri* was isolated, strain B61, which could not produce any light (or at least 100 times less), but which could still be induced, as demonstrated in another series of experiments based on the same protocol. If the supernatant from a culture of a wild type *Vibrio fischeri* strain collected at time A has no effect on the production of light by the mutant strain B61, the supernatant collected at time B can induce light production by the mutant strain B61 at normal intensity, again even when the population density is below 10^7 cells per ml. The conclusion was that the inducer is not only produced by the bacterial cells but is also active *in trans*, since it can induce light emission in a mutant strain. Moreover, another series of experiments confirmed that the stimulus is directly synthesised by the bacteria and is not a side product of metabolism.

The identification of this auto-inducer was now just a question of time. The molecule is a derivative of homo-serine-lactone (HSL) and is called 3-oxo-N-(tetrahydro-2-oxo-3-furanyl)hexanamide or 3-oxo-C6-HSL. Additional experiments with synthetic HSL showed that 3-oxo-C6-HSL induces light emission by *Vibrio fischeri* within minutes and from the threshold concentration of 3.10^{-10} Molar. With such results, the microbiologists had at their disposal everything they needed to begin to talk of « auto-induction by quorum-sensing ».

Molecular mechanism of regulation by quorum-sensing

The cloning in *Escherichia coli* of the operon encoding the production of light by *Vibrio fischeri* not only confirmed all previous observations and experimental results, but also helped the understanding of regulation at the molecular level. DNA sequencing identified a total of eight *lux* genes forming two transcription units. The *luxC*, *luxD* and *luxE* genes code for the subunits of a reductase of fatty acids to produce the aldehydes that are the substrate of the luciferase enzyme, whose two subunit-encoding genes are *luxA* and *luxB*. The function of the *luxG* gene is still unknown. The seventh gene of the *lux* operon, *luxI*, codes for the synthase enzyme responsible for the synthesis of the 3-oxo-C6-HSL auto-inducer. Finally the eighth gene, *luxR*, is transcribed independently and codes for an activator of the transcription of the *luxI-G* operon acting by binding to the promoter region. But the LuxR effector is only active in the presence of and after binding to the auto-inducer, according to the following model.

The *luxI* gene is expressed and the auto-inducer is synthesised at a basic level in any bacterial cell. 3-oxo-C6-HSL diffuses freely from the cytoplasm of the bacterium to the external environment where it accumulates. However, when the bacterial population density is low, the concentration of the auto-inducer remains well under 3.10^{-10} Molar. But when the population cell density rises, the concentration of the auto-inducer reaches threshold concentration. Since the auto-inducer also diffuses freely back inside the bacteria, its intra-cytoplasmic concentration is now high enough to bind to LuxR and the complex LuxR-LuxI binds in turn to the promoter of the *luxI-G* operon to activate its transcription at a high level. The luciferase enzyme is synthesised and, at the end of the metabolic pathway, light is produced. Since this first simple model, different external parameters from the environment and from the host influencing the action of the auto-inducer have been identified and their mechanism of action partly uncovered. Additional networks of internal regulation of expression of the *lux* gene cluster have also been identified.

In vivo role of quorum-sensing

What is the ecological role of light emission by bacteria and why do bacteria regulate this emission of light by the quorum-sensing mechanism and auto-induction ? *Vibrio fischeri* and *Vibrio harveyi* live in marine environments freely or in symbiosis with different marine hosts, calamares and fish, at the level of specialised external organs. In these organs, they are safe from deleterious external conditions, have access to large amounts of nutriments, reach very high population cell density and produce light. The hosts utilise the light to repulse predators or to attract preys or sexual partners.

In the external environment, bacteria living freely never reach a high population density. The concentration of the auto-inducer never reaches the threshold, the transcription of the *lux* operon is not activated and light is not produced. Light emission by bacteria would actually be useless, since the host is not present, and would represent a waste of energy that is urgently needed for more vital functions in this nutriment-poor environment. On the other hand, in the host specialised organs, bacteria reach a very high population density, up to 10^{11} cells per ml. At such a population cell density, the auto-inducer is present in concentrations higher than the threshold, the transcription of the *lux* operon is initiated and light is emitted and utilised by the host. If light emission is more useful to the host than to the bacteria, let us not forget that the former provides « Bed and breakfast » for the latter.

LUX-LIKE SYSTEMS

In parallel with these studies on *Vibrio fischeri*, and thanks to the development of molecular biology, different research teams have described quorum-sensing regulation mechanisms using *lux*-like systems in other different Gram negative bacterial species (table 1).

General presentation

All Lux-like systems described so far are based upon the production of HSL derivatives with an even number of C

Table 1. Examples of Lux-like systems in Gram negative bacteria

Bacterial species	Auto-inducer Homo-Serine-Lactone (HSL)	LuxI/ LuxR systems	Regulated function(s)
Aeromonas hydrophila	C4-HSL	AhyI/AhyR	synthesis of serine proteases and of extracellular metalloproteases
Agrobacterium tumefaciens	3-oxo-C8-HSL	TraI/TraR	conjugation of Ti plasmid
Erwinia carotovora	3-oxo-C6-HSL	ExpI/ExpR	synthesis of exoenzymes
		CarI/CarR	synthesis of antibiotic (carbapenem)
Escherichi coli	?	?/SdiA	replication of chromosome and cell division
Pseudomonas aeruginosa	3-oxo-C12-HSL	LasI/LasR	synthesis of exoproteases and of virulence factors, formation of biofilms
	C4-HSL	RhlI/RhlR	synthesis of exoenzymes, cyanides, lectins and pigments
Rolstania solanacearum	C6-HSL, C8-HSL	SolI/SolR	?
Salmonella Typhimurium	?	?/SdiA	resistance of the bactericidal activity of the complement
Xenorhabdus nematophilus	3-hydroxy-C4-HSL	?/ ?	synthesis of a bacterial lipase and of other virulence factors
Yersinia pseudotuberculosis	3-oxo-C6-HSL	YpsI/YpsR	?
	C8-HSL	YtbI/YtbR	aggregation and motility

atoms between C4 and C14, sometimes with modifications or substitutions along the side chain. The HSL derivatives are synthesised by *luxI*-like genes and activate by binding to LuxR-like molecules the transcription of many different genes involved in various bacterial properties : production of exoenzymes, of antibiotics, of bacteriocins and of pigments, formation of aggregates and biofilms, motility, plasmid conjugation, chromosome replication and cell division and several virulence properties, including production of adhesins and of toxins. In *Pseudomonas aeruginosa*, the 3-oxo-C12-HSL and the C4-HSL, respectively, act as auto-inducers of the Las and Rhl quorum-sensing systems (table 1). Not only do these independently regulate expression of two different sets of genes, but they also interact, since the Las system modulates the expression of the Rhl system.

Pseudomonas aeruginosa

The Las system of *Pseudomonas aeruginosa* is almost identical to the Lux system of *Vibrio fischeri*. The auto-inducer LasI freely diffuses into the external environment and back into the bacterial cytoplasm in population density-dependent concentrations. When reaching the concentration threshold, LasI binds to LasR, the gene transcription activator. In contrast with the Lux system of *Vibrio fischeri*, the genes activated by Las R are multiple and are not located adjacently to the *lasR/lasI* genes. The activated genes code for an elastase, a protease, the exotoxin A (described during the third lecture), an alkaline phosphatase and other factors implicated in the virulence of *Pseudomonas aeruginosa* and in the formation of biofilms.

The complex LasR/LasI also positively regulates the expression of the second quorum-sensing system, or Rhl, by activating the transcription of the *rhlR* gene, which codes for the transcription activator. The RhlR activator binds the RhlI auto-inducer, which can also freely diffuse outside and back inside the bacterial cells. The RhlR/RhlI complex in turn acts as a transcriptional activator of several different independent genes coding for a haemolysin, a lectin adhesin, pigments and bacteriocins, i.e. several properties implicated in the defence against other microbes and in the virulence of *Pseudomonas aeruginosa*. But the interactions between the Las and Rhl systems go further, since LasI negatively influences the formation and the stability of the RhlR/RhlI complex.

To understand the ecological meaning of quorum-sensing in *Pseudomonas aeruginosa*, let us remember that this bacterial species is ubiquitous in nature and an opportunistic pathogen in man and animals, responsible for many different clinical conditions, especially localised infections. At the height of the infection sites, the population density reaches cell concentrations above $10^9/10^{10}$ bacteria per ml, with enough auto-inducers to activate the transcription of the regulated genes, in particular those coding for virulence factors active on the host tissue and for biofilm formation, initially via the Las system. To reach such a cell population density in the external environment is a rarer event but, when this happens, it is also important to activate some of those genes to form biofilms, for example. In either case, it also becomes important to activate the Rhl system, which will activate genes coding for some additional virulence factors but, more importantly, for antimicrobial defences, since other microorganisms are often present either on the host tissues or in the external environment. This explanation is of course an oversimplification of the quorum-sensing regulation systems of *Pseudomonas aeruginosa*, which represent a complexification in comparison with *Vibrio fischeri*. Taking into account the great variety of ecosystems in which *Pseudomonas aeruginosa* can live, it is easy to understand that additional networks of auto-regulation, inter-regulation and regulation after signals from the external environment, including the host tissues, exert a fine control of the expression of each set of genes. This occurs upstream or downstream of the general regulation by the Las and Rhl quorum-sensing systems. For example, the transcription of the *toxA* gene coding for exotoxin A is influenced by the iron concentration and is regulated by a Fur-like system (described during the fourth lecture).

OTHER QUORUM-SENSING SYSTEMS

In parallel with the studies on the Lux-like systems, other quorum-sensing

regulation mechanisms have been progressively uncovered amongst different Gram negative and Gram positive bacterial species.

Most of the other quorum-sensing systems have been described in only one or a few bacterial species. They are involved in the regulation of different ecological and life cycle properties. The main exception is represented by the quorum-sensing systems of several Gram positive bacteria, which are based upon the production of modified oligopeptides as auto-inducers (table 2) and which, like the Lux-like systems, regulate ecological as much as pathological properties.

Bacillus subtilis

Two interacting quorum-sensing systems, ComX and CSF, have been described in *Bacillus subtilis* (table 2). The first auto-inducer of *Bacillus subtilis* is a decapeptide, ComX, which derives by hydrolysis by the ComQ enzyme from a 55 amino-acid precursor peptide. ComX does not freely diffuse between the bacterial cytoplasm and the external environment, but is actively secreted by an ABC-like transporter. Moreover, after reaching threshold concentration in the external environment, ComX interacts with a membrane protein receptor, ComP, which is a sensor kinase. Just as in the BvgAS regulon of *Bordetella* species (described during the fourth lecture), ComP auto-phosphorylates on a histidine residue and transfers the phosphate residue onto an aspartate residue of the so-called response regulator protein, ComA. The phosphorylated form of ComA activates the synthesis of ComS that inhibits the proteolysis of ComK. ComK is the activator of the expression of genes involved in the transformation competence, i.e. the uptake of nude DNA fragments after passage through the different membranes of the bacteria.

The second auto-inducer of *Bacilus subtilis*, CSF derives from a 40 amino-acid precursor peptide (table 2), which appears to be secreted by the general Sec secretion system into the external environment and hydrolysed during the secretion process to give birth to the pentapeptide auto-inducer. In contrast with ComX, CSF re-enters the bacterial cytoplasm after reaching the threshold concentration by an ABC-like active transporter. Inside the cytoplasm, CSF acts in different ways, depending on its concentration. At low concentration, CSF inhibits the action of RapC phosphatase, whose role is to dephosphorylate ComA, thereby inhibiting its activity. At low concentration, CSF thus reinforces the action of the Com system. On the other hand, at higher concentrations, CSF inhibits directly the role of ComS and therefore negatively interacts with the Com system. At similar high concentrations, CSF also inhibits another phosphatase, RapB, whose target is Spo0A. The phosphorylated form of Spo0A normally activates the transcription of genes initiating bacterial sporulation. Thanks to the action of CSF on RapB, *Bacillus subtilis* is able to sporulate.

Bacillus subtilis is a spore-forming bacterium living in soil and forming microcolonies. For any spore-forming bacterium, acquisition of transformation competence and initiation of sporulation are two mutually exclusive states of life. At the beginning of growth, *Bacillus subtilis* possesses neither transformation competence, nor sporulation capacity. Acquisition of transformation competence begins to be interesting when the population cell density increases, i.e. when acquisition of DNA becomes possible following the death of other cells within the population. The ComX auto-inducer reaches high concentrations while the CSF auto-inducer concentration is still low, reinforcing the action of the Com system. Later, the population ages and the CSF auto-inducer in turn reaches in turn high concentration, probably following activation of the transcription of its encoding-gene under the influence of external parameters, such as nutriment shortage. The high concentration of CSF activates the transcription of the genes initiating sporulation and inhibits the action of the Com system, which is good, since a bacterium does not need to express genes involved in transformation competence when it begins to sporulate. Expressions of both Com and CSF systems are of course submitted to additional networks of auto-regulation, inter-regulation and regulation after signals from the external environment. To ensure the best chance of population survival and evolution, the Com and CSF systems of *Bacillus subtilis* must in fact act sequentially and hierarchically on the transcription of different genes coding for opposite functions.

DO BACTERIA SPEAK ESPERANTO ?

In all examples described so far, each bacterial species uses its own vocabulary to be understood only from its species mates. In order to dialogue with other species, bacteria must use a more common language, like Esperanto, which is supposed to be the common language of Europe. Such a possible bacterial Esperanto language was identified by Bassler and her research team while working on the Lux-like system of *Vibrio harveyi*.

In contrast with other Gram-negative bacteria in general and with *Vibrio fischeri* in particular, the auto-inducers produced by *Vibrio harveyi*

Table 2. Examples of "quorum-sensing" systems of Gram positive bacteria using oligopeptides as auto-inducers

Bacterial species	Auto-inducer peptides	Regulated function(s)	Secretion pathway	Induction system
Bacillus subtilis	5 AA (CSF)	sporulation	General Sec system	ABC-like transporter
	10 AA (ComX)	competence (transformation)	ABC-like transporter	Receptor/Regulator
Enterococcus faecalis	?	plasmid conjugation virulence properties	ABC-like transporter	ABC-like transporter
Lactobacillus plantarum	26 AA	production of bacteriocins	ABC-like transporter	Receptor/Regulator
Staphylococcus aureus	8 AA	virulence properties	ABC-like transporter	Receptor/Regulator
Streptococcus pneumoniae	17 AA	competence (transformation)	ABC-like transporter	Receptor/Regulator

AA = amino-acids

do not diffuse freely from the external environment back into the bacterial cell to activate the *lux* operon. Moreover, two interacting two-component receptor/regulator quorum-sensing systems (table 3) are present in *Vibrio harveyi* to regulate bioluminescence via an elaborate phosphorylation cascade, similar to those already described in Gram positive bacteria. The first system is a Lux-like system with 3-hydroxy-C4-HSL or LuxLM, as auto-inducer, and the LuxN protein, as membrane sensor receptor. The second auto-inducer, AI2 or LuxS, which is a unique furanosyl borate diester, is detected by the membrane sensor receptor LuxQ, after binding to another bacterial protein, LuxP. Both LuxN and LuxQ transfer phosphate residues onto the LuxU regulator. A further step of the activation cascade is the transmission of phosphate residue onto LuxO. LuxO would inactivate the transcription of a gene coding for a still unidentified putative inhibitor of the transcription of the *lux* operon. The actual transcription activator, LuxR, can now initiate the transcription of the *lux* operon and the production of light.

But the most interesting finding of this series of experiments was the possibility for other *Vibrio* species to induce the production of light by *Vibrio harveyi*, via the synthesis of AI2. After cloning of the *luxS* gene, which codes for a synthase catalysing the synthesis of AI2, genetic studies also identified highly conserved *luxS* genes in several different Gram negative and Gram positive bacterial species. While the HSL of the Lux-like systems are used for intraspecies communication, AI2 could thus be a unique alphabet of a universal language for bacterial interspecies communication, just as the Esperanto language was supposed to become for the different peoples of Europe. The functions controlled by AI2 in other species are : production of virulence factors, iron acquisition, antibiotic production, motility and the formation of mixed-species biofilms. However, the functions that are controlled by AI2 have not been identified in many other bacteria and there exist today questions about the actual function of this AI2 system in those species. Some scientists, however, have expressed general doubts about the actual role of AI2 in quorum-sensing mechanisms and see AI2 more as a bi-product of bacterial metabolism than as a true auto-inducer.

MANIPULATION OF QUORUM-SENSING

The role played by different quorum-sensing systems in the regulation of expression of numerous genes, whose products are involved in different ecological and pathological properties, has raised hope regarding new tactics : (i) in the anti-bacterial warfare in human and veterinary medicine, especially since the development of multi-resistant bacterial strains and (ii) in the industrial production of bacterial metabolites. We will now look at three different aspects of the manipulation of quorum-sensing regulation mechanisms.

In theory

In theory, influencing quorum-sensing systems positively or negatively is possible at different levels, since they are themselves quite strictly regulated by loops of retro-acting controls, by other interacting quorum-sensing systems (as illustrated in *Pseudomonas aeruginosa* and *Bacillus subtilis*), by chemicals and physical parameters of the external environment, by chemical signals produced by the cells of the eukaryotic hosts, and also by physical parameters of the hosts.

In nature

A first example of natural interaction between two quorum-sensing systems of different bacterial species is the inhibition of the auto-inducer C4-HSL of *Aeromonas hydrophila* (involved in the quorum-sensing regulated expression of genes coding for different exoproteases that represent virulence factors of this bacterial species) by the auto-inducer 3-oxo-C10-HSL of *Vibrio anguillarum*. These two species are marine bacteria, which are pathogenic for fish and which share the same ecosystems. A second example is the production by different species of the Gram positive genus *Bacillus* of an enzyme called lactonase, which hydrolyses the HSL auto-inducers produced by Gram negative bacteria, an interesting function in ecosystems with mixed bacterial populations competing for nutriment sources.

In 2004, Tron and collaborators also reported that strains of *Pseudomonas aeruginosa* isolated from cases of *otitis externa* in dogs are deficient in the production of elastase. The origin of the deficiency is to be found in mutations in one of the quorum-sensing systems of this bacterial species. Although the relationship between the deficiency and the virulence of the strains is not yet understood, the observation may be very important. It could mean that strains of one opportunistic bacterial species associated with a specific localised or systemic disease would express differently genes coding for different virulence properties, although all would actually harbour those genes, which would simply be permanently silenced. If this kind of finding were confirmed in the future, it would also have important implications for diagnostic methodology in bacteriology, since using only PCR assays targeting the genes coding for

Table 3. "Quorum-sensing" systems in *Vibrio harveyi*

Bacterial species	Auto-inducers	Two component systems	Phosphorylation cascade	Regulated function
Vibrio harveyi	3-hydroxy-C4-HSL (= LuxLM)	LuxN/LuxU	LuxO/ ? /LuxR	bioluminescence
	furanosyl borate diester (= AI2 ou LuxS)	LuxQP/LuxU		

HSL = homo-serine-lactone

the virulence properties would not be appropriate.

By man

There are three potential applications of these principles and observations by man. The first potential application is the blockade of quorum-sensing systems regulating the expression of genes coding for virulence factors, by using chemical analogues of the auto-inducer molecules or enzymes destroying auto-inducers, just like in the two natural examples presented. Secondly, production of mutant strains in one quorum-sensing system might also be of value in the production of either a vaccine component or a probiotic-like bacterial strain. In contrast, the third possibility is the over-expression of quorum-sensing regulated genes coding for the production of antibiotics or other metabolites by addition to the growth medium of appropriate auto-inducers before the population cell density reaches the threshold value. These applications are of course for the future, but they might be not so far away.

DISCUSSION

Bacterial quorum-sensing systems and auto-inducers have analogues in the world of multicellular eukaryotic organisms : they are named pheromones and hormones. The definitions of auto-inducers, pheromones and hormones indeed differ very little. **Auto-inducers** are chemicals secreted by a bacterium into the external environment, inducing from a threshold concentration specific modifications of gene expression in its species mates. **Pheromones** are chemicals secreted at very low doses by a multicellular organism into the external environment, inducing from a threshold concentration specific behaviours in its species mates. **Hormones** are chemicals secreted at very low doses by a cell of a multicellular eukaryotic organism into the extracellular environment, inducing specific biochemical reactions in other cells of the same organism.

Moreover, the definition of bacterial oligopeptide toxins does not differ so much from the previous ones. Bacterial **oligopeptide toxins** are in fact chemicals secreted by a bacterium into the extracellular environment, inducing specific metabolic perturbations in cells of multicellular organisms after binding to a transmembrane receptor. But where do they come from ? Today's hypothesis is that bacterial genes coding for oligopeptide toxins derive from eukaryotic genes coding for oligopeptide hormones. During the third lecture, the similarity between the STa enterotoxin of *Escherichia coli* and the guanylin hormone has already been mentioned. And where do the eukaryotic genes coding for oligopeptide hormones themselves come from ? Would they derive from genes coding for oligopeptide auto-inducers of Gram positive bacteria ? This is an interesting proposition, but purely speculative so far.

This discussion finishes the presentation of quorum-sensing regulation mechanisms. Even though they have opened up new frontiers in the genetics of the microbial prokaryotic world, quorum-sensing and auto-induction are not science-fiction, but science, particularly if we consider the ever growing numbers of publications referred to in PubMed since 1994 (figure 1).

WHY DOES A BACTERIUM CAUSE DISEASE ?

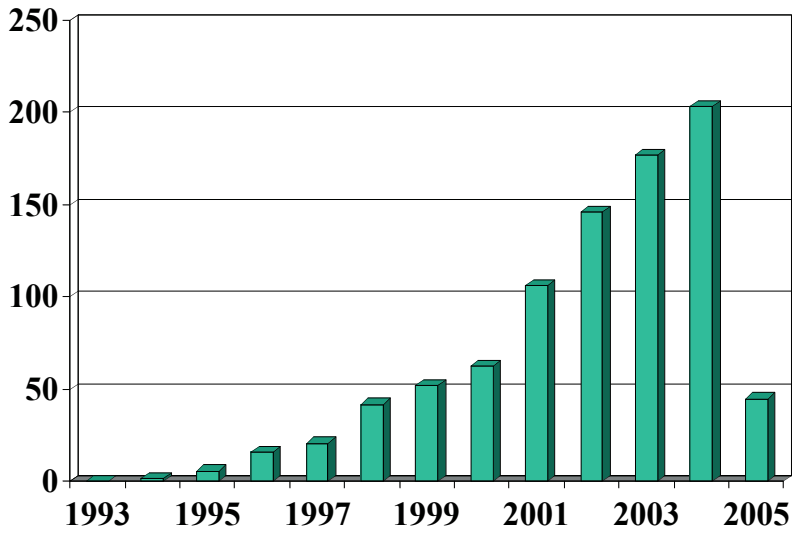
No straight answer exists to this question, but the following one has been proposed. In order to get lunch and to survive, bacteria have adapted and evolved like all living organisms following the principle of Jacques Monod : « Le hasard et la nécessité ».

Lunch and survival

Over the course of time, different prokaryotes developed very close relationship with multicellular eukaryotes and ended up living on the surface of their skin and mucosae. During this process, these commensal bacteria acquired new genes from other prokaryotes, but also from eukaryotes, including their hosts, thanks to transformation, transduction and conjugation. One day, at random, one bacterial species acquired genes allowing the following : a more efficient colonisation of the host surfaces, the crossing of the host epithelial layers, survival against the host defences and/or the production of toxins. This last acquisition is very intriguing, since these toxins can cause very severe damages to the host, even killing it. So why did bacteria keep toxin-encoding genes in the course of evolution ?

This is only a misunderstanding, because we are analysing the disease from our side. Let us switch position and analyse the situation from the bacterial side, and in particular, not from the individual's point of view, but from that of the whole population. There is only one rule in nature: SURVIVAL of the population. That is exactly what pathogenic bacteria are doing in their hosts - SURVIVING. After being confined to a few niches for generations,

Figure 1. Number of publications referenced on PubMed since 1994 using « quorum-sensing » as key word (Year 2005: only January and February)



they can now multiply, thanks to favourable conditions, and have access to many nutriment sources, thanks to different virulence factors, in particular enzymes and toxins. But they are so numerous that some must migrate to other places and hosts. In the course of random evolution, some bacterial strains and clones have been favoured in their dissemination in the external environment by the production of different clinical conditions : diarrhoea, coughing, hypersecretion of mucus… Once in the external environment, they can contaminate new hosts. Even the death of the host presents advantages : it now represents a huge mass of nutriments, enough for thousands and thousands of bacterial generations, and no more danger exists, since it has stopped trying to defend itself ! And if other animals come and eat the carrion, they represent either new hosts or a means of disseminating even further in the environment. So, from the bacterial point of view, disease and death of the host is no more a problem than killing a herbivore is for a carnivore : this just means lunch and survival of the population.

« Le hasard et la nécessité »

This evolution occurred as usual by « hasard et nécessité ». If a newly acquired property does not help survival, it will be rejected, or the clone population will die at the end of the day. The problem with this kind of discussion is that, if nobody has any difficulty in understanding the adaptation and the evolution of bacteria facing negative selective pressure (as in the presence of antibiotics), not many people can understand that the same bacteria can adapt and evolve in a similar way in their pathogenic power. Still, in both cases, adaptation and evolution proceed from the same purpose : « Survival of the population ».

FURTHER READINGS

BASSLER B.L. Small talk: cell-to-cell communication in bacteria. *Cell*, 2002, **109**, 421-424.

DUNNY G.M., WINANS S.C. Cell-cell signalling in bacteria. ASM Press : Washington, 1999, 367 p.

EBERHARD A., BUURLINGAME A.L., EBERHARD C., KENYON G.L., NEALSON K.H., OPPENHEIMER J.J. Structural identification of autoinducer of *Photobacterium fischeri* luciferase. *Biochemistry*, 1981, **120**, 2444-2449.

FARGHALY A.H. Factors influencing the growth and light production by luminous bacteria. *J. Cell. Comp. Physiol.*, 1950, **36**, 165-184.

McFALL-NGAI M.J. Negotiations between animals and bacteria : the « diplomacy » of the squid-vibrio symbiosis. *Comp. Biochem. Physiol. A*, 2000, **126**, 471-480.

MILLER M.B., BASSLER B.L. Quorum sensing in bacteria. *Annu. Rev. Microbiol.*, 2001, **55**, 165-199.

MILLER R.V., DAY M.J. Microbial evolution : gene establishment, survival, and exchange. ASM Press : Washington, 2004, 374 p.

NEALSON K.H. Autoinduction of bacterial luciferase : occurrence, mechanism and significance. *Arch. Microbiol.*, 1977, **112**, 73-79.

NEALSON K.H., HASTINGS J.W. Bacterial bioluminescence : its control and ecological significance. *Microbiol. Rev.*, 1979, **43**, 496-518.

NEALSON K.H., MARKOVITCH A. Mutant analysis and enzyme subunit complementation in bacterial luminescence in *Photobacterium fischeri*. *J. Bacteriol.*, 1970, **104**, 300-312.

PESCI E.C., IGLEWSKI B.H. Quorum sensing. In : Burns D.L., Barbieri J.T., Iglewski B.H., Rappuoli R. (Eds), Bacterial protein toxins. ASM Press : Washington, 2003, 55-66.

SCHAUDER S., BASSLER B.L. The language of bacteria. *Genes & Development*, 2001, **15**, 1468-1480.

SCHAUDER S., SHOKAT K., SURETTE M.G., BASSLER B.L. The LuxS family of bacterial autoinducers : biosynthesis of a novel quorum sensing signal molecule. *Mol. Microbiol.*, 2001, **41**, 463-476.

STOCK J.B., STOCK A.M., MOTTONEN J.M. Signal transduction in bacteria. *Nature*, 1990, **344**, 395-400.

TAGA M.E., BASSLER B.L. Chemical communication among bacteria. *Proc. Natl. Acad. Sci. USA*, 2003, **100S2**, 14549-14554.

TRON E.A., WILKE H.L., PETERMANN S.R., RUST L. *Pseudomonas aeruginosa* from canine otitis externa exhibits a quorum sensing deficiency. *Vet. Microbiol.*, 2004, **99**, 121-130.

ZHANG L.H., DONG Y.H. Quorum sensing and signal interference : diverse implications. *Mol. Microbiol.*, 2004, **53**, 1563-1571.