Review

Regulation of Phenotypic Switching and Heterogeneity in *Photorhabdus luminescens* Cell Populations

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Abstract

Phenotypic heterogeneity in bacterial cell populations allows genetically identical organisms to different behavior under similar environmental conditions. The Gram-negative bacterium *Photorhabdus luminescens* is an excellent organism to study phenotypic heterogeneity since their life cycle involves a symbiotic interaction with soil nematodes as well as a pathogenic association with insect larvae. Phenotypic heterogeneity is highly distinct in *P. luminescens.* The bacteria exist in two phenotypic forms that differ in various morphologic and phenotypic traits and are therefore distinguished as primary (1°) and secondary (2°) cells. The 1 cells are bioluminescent, pigmented, produce several secondary metabolites and exo-enzymes, and support nematode growth and development. The 2° cells lack all these 1°-specific phenotypes. The entomopathogenic nematodes carry 1° cells in their upper gut and release them into an insect's body after slipping inside. During insect infection, up to the half number of 1° cells undergo phenotypic switching and convert to 2° cells. Since the 2° cells are not able to live in nematode symbiosis any more, they cannot re-associate with their symbiosis partners after the infection and remain in the soil. Phenotypic switching in *P. luminescens* has to be tightly regulated since a high switching frequency would lead to a complete break-down of the nematode-bacteria life cycle. Here, we present the main regulatory mechanisms known to-date that are important for phenotypic switching in *P. luminescens* cell populations and discuss the biological reason as well as the fate of the 2° cells in the soil.

Introduction

The genus *Photorhabdus*

Photorhabdus spp. are Gram-negative enteric bacteria, which are close symbionts of heterorhabditid soil nematodes and are in turn highly pathogenic toward insects. Based on molecular biological analyses, the genus has been divided into three bacterial species: *Photorhabdus luminescens*, *Photorhabdus temperata* and *Photorhabdus asymbiotica* [1]. Recently, genome sequencing of already described as well as 11 new isolates identified 14 new *Photorhabdus* subspecies, which led to the proposal of re-organizing the existing taxonomy by raising several subspecies to species level [2]. However, all *Photorhabdus* species live in a close symbiotic interaction with nematodes of the family *Heterorhabditidae*. While *P. luminescens* and *P. temperata* are only highly pathogenic toward insects, *P. asymbiotica* is additionally able to infect humans [3]. *Photorhabdus* bacteria are the only terrestrial bacteria known to be bioluminescent due to bacterial luciferase production [4].

The life cycle of *P. luminescens*

P. luminescens has a complex dualistic life cycle as on the one hand it maintains a mutualistic symbiosis with soil nematodes, and on the other hand, it is highly pathogenic toward a variety of insect species (Fig. 1). The bacteria colonize the upper gut of the infective juvenile stage (IJs) of the nematode *Heterorhabditis bacteriophora*. The IJs infect insect larvae by invading

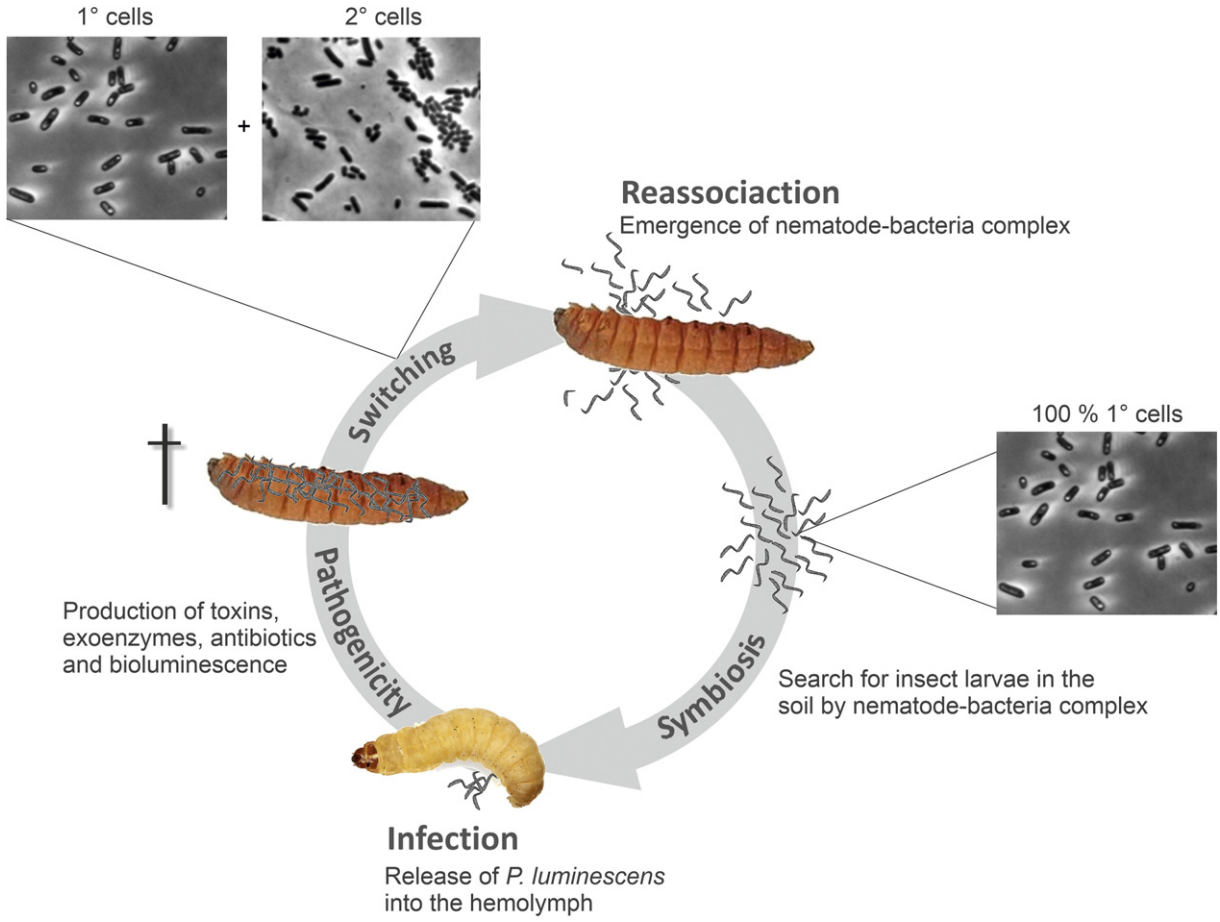


Fig. 1. Infection cycle and phenotypic heterogeneity of *P. luminescens*. After injected from the nematodes into the insect hemolymph, the bacteria replicate exponentially. After 48 h, the insect dies and the cells enter a prolonged stationary growth phase. In this growth phase, single cells undergo phenotypic switching. After 28 days, when the nutrients are depleted and novel generations of nematodes emerge from the cadaver, a significant subset of *P. luminescens* have converted to 2° cells. Among other phenotypic traits (e.g., pigmentation, bioluminescence, secondary metabolites, exoenzymes), 1° cells produce crystalline inclusion proteins that are visible in the light microscope, whereas 2° cells do not.

into the hemocoel of the insect. Once inside, the bacteria are directly released from the gut of the IJs into the insect's hemolymph by regurgitation. Then, the bacteria replicate rapidly, quickly establish a lethal septicemia in the host by production of a broad range of different toxins that effectively kill the insect victim within 48 h. Furthermore, *P. luminescens* produces several exoenzymes that bioconvert the insect's body into a rich food source, which is used for growth by the bacteria as well as by the nematodes. The bacteria support nematode development and reproduction, probably by providing essential nutrients that are required for efficient nematode proliferation [5,6]. In addition, the bacteria produce a huge range of secondary metabolites like several antibiotics to defend the insect cadaver from being affected by other bacteria and fungi [7]. Furthermore, the bacteria produce bacterial luciferase, which causes the cadaver to glow. When the nutrients are depleted, the IJs and bacteria re-associate and emerge from the carcass in search for a new insect host (Fig. 1; see Refs. [8,9] for reviews).

Phenotypic Switching in *P. luminescens* Cell Populations

P. luminescens exists in two phenotypic different cell forms called primary (1°) and secondary (2°) cells. In the nematode gut, the population exclusively consists of 1° cells. During the infection, a large portion of the of the cells switch from 1° to 2° cells. Both cell forms are genetically identical but differ in distinct phenotypic traits [10]. 1° cells exhibit several phenotypical characteristics that are absent in all 2° cells [11,12]. Among these, most apparent is the production of extracellular enzymes like proteases, pigments, secondary metabolites like antibiotics, bioluminescence, the crystalline inclusion proteins CipA and CipB, and cell clumping factor [11–13].

Moreover, both variants are morphologically distinct as 1° cells are long-shaped rods, whereas 2° cells are smaller short rods [14]. Interestingly, while both cell forms are equally virulent toward insects, only 1° cells are known to associate with the nematodes. Furthermore, 2° cells are unable to support nematode growth and development both in the insect cadaver and in culture (Fig. 2).

Moreover, heterogeneity of *P. luminescens* colonies has been described and the different colony forms were designated as P- and M-forms, which form larger and smaller colonies, respectively [15]. The pathogenic P-form switches to the mutualistic M-form to initiate mutualism in host nematode intestines. However, since a stochastic promoter inversion of the *mad* locus causes the switch between the two distinct forms, this phenomenon is not due to true phenotypic heterogeneity. The respective P_{mad} promoter direction is assumed to be similar, so that both directions might be found in 1° and 2° cells.

Phenotypic switching of *P. luminescens* has previously also been referred to as phase variation [12]. However, this phenomenon is different from classical bacterial phase variations as both cell forms are genetically homogeneous [16]. Furthermore, classical phase variation involves reversible genetic events, occurs at significant frequency and is almost reversible. Both 1° and 2° cells are genetically identical. DNA rearrangements or modifications, genetic instability, or the loss of plasmids is not involved in *P. luminescens* 2° cell formation [10,16–18]. However, genome sequencing of several switched 2° colonies and comparison of their genome(s) to the respective 1° cells should be performed to finally prove that phenotypic switching of *P. luminescens* is due to true phenotypic heterogeneity of 1° and 2° cells.

The 1°-specific phenotypes are most distinct in the early post-exponential growth phase, which correlates with the establishment of *P. luminescens* as a saturating monoculture in the insect host and with the initiation of nematode feeding and development within the insect cadaver. After a couple of days in the insect host, 2° cells that are unable to support nematode development occur spontaneously. Therefore, it is argued that the 1°-specific characteristics are required for the symbiotic interaction with the nematode rather than for the pathogenic interaction with the insect. Since 2° cells develop not only inside the insect larvae but also after prolonged cultivation in the laboratory, it was suggested that phenotypic switching is a response to environmental stress [19,20]. It has been observed that low osmolarity of the culture medium seems to trigger phenotypic switching [16,21]. However, co-cultivation assays of labeled 1° and 2° cells revealed that 1° cells overgrew the 2° cells in the exponential phase, while 2° cells outcompeted the 1° cells in the stationary growth phase, regardless of the initial composition (A. Langer, R.H., LMU, unpublished). This is in accordance with the observation that after periods of starvation, 2° cells were able to restart growth 10 to 12 h earlier than 1° cells [22]. In *Escherichia coli*, the universal stress protein UspA is known to play an important role in the recovery upon periods of nutrient starvation [23]. Conformingly, in *P. luminescens* 2° cells, up-regulation of UspA was observed [24]. UspA also plays an important role in the protection of the cell against superoxide-generating agents. Comparison of the 1° and the 2° proteome

Phenotype	Primary cells (1°)	Secondary cells (2°)
Bioluminescence	+++	+
Clumping	+	-
Pigmentation	+++	-
Crystal proteins	+	-
Pathogenicity	+++	+++
Symbiosis	+++	-

Fig. 2. Phenotypic differences of the *P. luminescens* 1° and 2° cells. In contrast to 1° cells, only reduced bioluminescence is visible in 2° cells. Cell clumping, protease activity, crystal protein production and symbiosis are absent from 2° cells. 1° and 2° cells are both pathogenic toward insects, whereas only insect cadavers that were infected with 1° cells are pigmented due to bacterial anthraqinone production. The figure was modified after Ref. [8]. "+++": high; "+": low; "-": no.

identified up to 450 potential factors that are characteristic for either the one or the other cell form in the stationary growth phase [24]. In 2° cells, specific proteins involved in oxidative stress response, alternative energy metabolism and different translation factors are produced that are absent from 1° cells. Furthermore, the protein amounts of iron transporters and iron binding proteins as well as those responsible for consumption of several sugars and amino acids were affected in 2° cells. Moreover, compared to 1° cells, the number of molecular chaperones was strongly reduced in 2° cells [24].

Regulation of Phenotypic Switching

Phenotypic switching of *P. luminescens* has to be tightly regulated. In principle, all cells are exposed to similar environmental conditions, stress or signal(s) in the insect cadaver. If 100% of the 1° cells would convert to 2° cells in the insect cadaver during nematode development, this would have fatal consequences on the viability of the bacteria-nematode symbiosis. Consequently, the complete life and infection cycle of the symbiosis partners would break down. Therefore, heterogenous regulation mechanisms have to ensure that phenotypic switching is only induced in individual cells of a population to induce phenotypic switching during the life cycle of *P. luminescens* ensuring that a sufficient portion of the bacteria stays 1°.

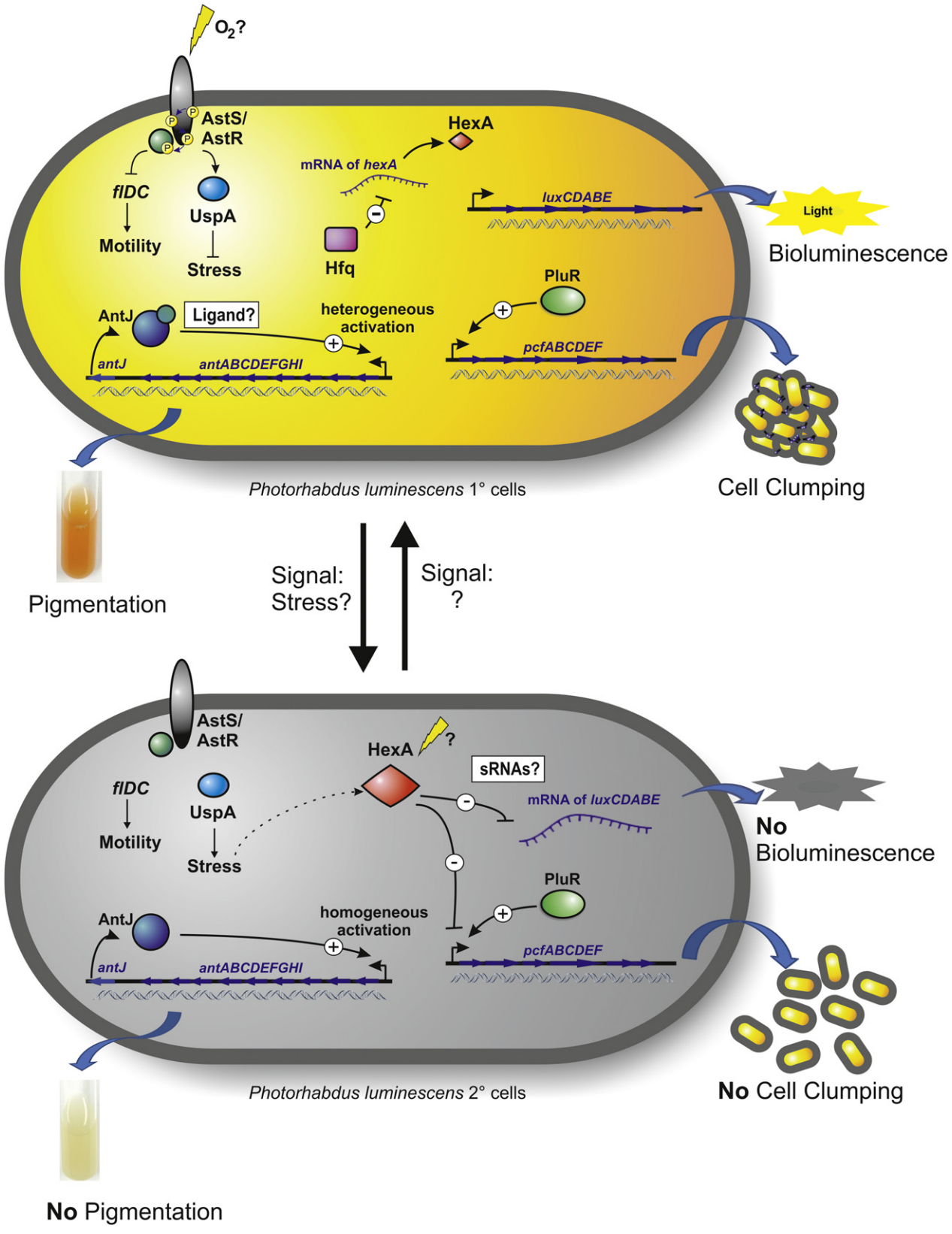
Global regulation by the LysR-type receptor HexA

HexA has been identified as a major regulator that is supposed to act as master regulator of phenotypic heterogeneity in *P. luminescens* [19]. HexA belongs to the LysR-type transcriptional regulator family and is present in high amounts in 2° cells. Positive autoregulation of HexA is supposed to keep the high level and therefore maintaining the phenotype of the 2° cell form [19]. Accordingly, HexA is assumed to act as a repressor of 1°-specific genes. Deletion of the *hexA* gene in 2° cells resulted in the de-repression of the 1°-specific factors and restored the ability to support nematode growth and development [19]. Moreover, the overproduction of HexA in 1° cells was sufficient to induce the 2° phenotype, which supports the idea that high levels of HexA are mandatory for undergoing phenotypic switching [20]. Deletion of *hexA* in 2° cells caused attenuation in virulence against insect larvae, suggesting that HexA is also required for regulation of pathogenicity [19]. In contrast, in the plant pathogen *Erwinia carotovora* deletion of *hexA* increased virulence [25]. Moreover, the production of stilbene-derived small molecules that are important for symbiosis is up-regulated in the Δ*hexA* mutant, further indicating that HexA is involved in regulation of both symbiosis and pathogenicity in *P. luminescens* [26].

HexA is a homolog of the LysR-type regulator LrhA of *E. coli*, which is described to control stress response and motility via regulation of translation of the alternative sigma factor RpoS, small RNAs and the chaperone Hfq [27]. As HexA includes a predicted helix–turn–helix DNA-binding domain, interaction with the DNA was very likely. Similar to LrhA also for HexA, it is assumed that it has a highly complex regulation mechanism including small RNAs (Fig. 3) [19,27]. It has recently been described that HexA controls phenotypic heterogeneity in a versatile way, directly and indirectly [13]. It has been demonstrated that HexA does not directly affect bioluminescence. The respective *luxCDABE* operon is repressed at the post-transcriptional level, and transcriptional levels of the RNA chaperone gene *hfq* are also enhanced in 2° cells. This underlined the idea that small regulatory RNAs are presumably involved in regulation of phenotypic switching, which are under control of HexA [13]. That small regulatory RNAs are mainly involved in the regulation of the switching process is also supported by the fact that upon deletion of *hfq*, the bacteria are no longer able to maintain a healthy symbiosis with nematodes due to the abolishment of the production of all known secondary metabolites [28]. The *hexA* gene was highly up-regulated in the 1°Δ*hfq* strain, so that Hfq mediates regulation of secondary metabolism in *P. luminescens* via HexA. A further deletion of *hexA* besides *hfq* fully restored secondary metabolism [28].

Another phenotypic trait that is specific for 1° cells is cell clumping (see also below). The corresponding *pcfABCDEF* operon could be identified as the first direct target of HexA, since the regulator binds to the *pcfA* promoter region and thereby blocks expression of the target operon. However, the binding kinetics of

Fig. 3. Model for the regulation of phenotypic switching in *P. luminescens.* Hfq mediates stability of *hexA* mRNA in 2° and not in 1° cells, which causes high levels of HexA in 2° cells repressing 1°-specific genes. Low HexA levels in 1° cells control cell clumping by directly controlling expression of the *pcfABCDEF* operon and indirect by controlling translation of the *luxCDABE* mRNA, which leads to light production. The heterogeneous strong activation of the *antABCDEFGHI* operon in 1° cells might be mediated via the ligand-bound AntJ. Enhanced HexA levels in 2° cells prevent the formation of cell clumping via repression of the P_{pcfA} activity and diminish bioluminescence via impaired translation of the *luxCDABE* mRNA presumably via small RNAs. A basal homogeneous P_{antA} activity might result from a missing ligand for AntJ and causes non-pigmentation. The AstS/AstR two-component system controls timing of phenotypic switching via a probable stress or oxygen-derived signal (see text for details).


O_2?
AstS/
AstR
flDC
Motility
UspA
Stress
HexA
mRNA of hexA
Hfq
luxCDABE
Light
Bioluminescence
PluR
AntJ
Ligand?
heterogeneous
activation
antJ
antABCDEFGHI
pcfABCDEF
Cell Clumping
Photorhabdus luminescens 1° cells
Pigmentation
Signal:
Stress?
Signal:
?
AstS/
AstR
HexA
?
sRNAs?
flDC
Motility
UspA
Stress
mRNA of luxCDABE
No
Bioluminescence
PluR
AntJ
homogeneous
activation
antJ
antABCDEFGHI
pcfABCDEF
No Cell Clumping
Photorhabdus luminescens 2° cells
No Pigmentation

HexA to the DNA revealed that a ligand is missing, which modulates DNA binding activity of HexA, because HexA alone was observed to have a high dissociation rate from the P_{pcfA} promoter [13]. This is underlined by the fact that HexA has a C-terminal putative ligand binding site, which is believed to somehow affect HexA activity. Primary metabolites that indicate nutrient limitation have been speculated as HexA ligands, but overall, the chemical nature of this ligand is not yet known. In summary, HexA fulfills the task as repressor of 1°-specific features in 2° cells in a versatile way, directly and indirectly [13].

Regulation of timing of phenotypic switching

It has been shown that *lrhA* expression, a homolog of *hexA* in *E. coli*, is under control of the Rcs phosphorelay system. Interestingly, AstS/AstR, a homologous system to Rcs, has been identified to control timing of phenotypic switching in *P. luminescens* [29]. *P. luminescens* cells lacking the response regulator AstR start to undergo phenotypic switching in culture 7 days earlier than the respective wild-type strain. However, in contrast to the situation in *E. coli*, expression of *hexA* is not under control of AstS/AstR. Proteome analysis of the Δ*astR* strain revealed that the AstS/AstR system positively regulates the expression of the gene encoding the universal stress protein UspA [29]. Such proteins occur in high concentrations during periods of stress, like oxidative and osmotic stress, as well as under stasis [30,31]. Therefore, the AstS/AstR pathway is believed to protect the cell from stress and thus prevent or delay phenotypic switching [20]. This supports the suggestion that global stress has a major impact on the signal inducing the switching process. Furthermore, the Δ*astR* mutant is hypermotile as the functional AstS/AstR system represses flagella formation [29]. However, motility is not under the control of HexA [19], revealing a different regulation mechanism for HexA than LrhA, which directly regulates motility in *E. coli* [32]. As this is only true under anaerobic conditions [33], it was suggested that there are at least two pathways controlling phenotypic switching, a HexA-dependent pathway and an O_2-dependent pathway via AstS/AstR. Although both pathways are somehow activated under global stress, no direct connection between the HexA and AstS/AstR regulation pathways is known to date [20] (Fig. 3).

Regulation of pigmentation

Pigmentation caused by anthraquinones (AQ) is a distinct 1°-specific feature and absent from 2° cells. A novel type of transcriptional regulator named AntJ was found that activates expression of the *antABCDEFGHI* operon [34], which encodes the enzymes for AQ synthesis [34,35]. The development of a novel genetic tool for stable reporter gene integration into the *P. luminescens* genome made is possible to analyze reporter gene activity at the single cell level [36]. Using P_{antA} reporter strains generated with this tool, bimodality of AQ production could be identified [34]. AntJ heterogeneously activates the AQ production in single *P. luminescens* 1° cells and blocks AQ production in 2° cells. AntJ is one of the rare examples of regulators that mediates heterogeneous gene expression by altering activity rather than copy number in single cells. AntJ contains a so called WYL-domain, which has yet only been found in bacteria [37]. The WYL-domain is predicted to be a putative ligand-binding domain, although any ligand(s) are unknown to date. The AntJ levels in 1° as well as in 2° cells are constant, which showed that transcriptional activation of the *antA-I* operon in single 1° cells is not mediated by a simple increase of AntJ. Therefore, a specific activation of AntJ was proposed to be mandatory for binding of AntJ to the P_{antA} promoter and to promote heterogeneous AQ production. For that reason, AQ production in single 1° cells was suggested to be mediated by ligand-dependent activation of AntJ by a specific metabolite or protein, which is not present in 2° cells. A simple overexpression of *antJ* leads to a homogeneous activation of AQ production in 2° cells, which is though ligand-independently and only caused by the enhanced AntJ copy number in the cells. However, one additional chromosomal copy of *antJ* under control of its native promoter did not lead to AQ production in 2° cells but was sufficient to decrease heterogeneity of P_{antA} activity in 1° cells. Only upon strong overexpression of *antJ*, AQ production was detectable in 2° cells, assuming that the influence of a putative inhibiting ligand might be out-competed by high AntJ copy numbers [34]. The presence or absence of a putative ligand must therefore drive activation of AntJ in single cells and mediate heterogeneity of P_{antA} activation as a noise generator. Since no differences in heterogeneity of P_{antA} activity in 1°Δ*hexA* cells were observed, heterogeneity of AQ production is presumably independent of the master regulator HexA [34] (Fig. 3).

Quorum sensing and regulation of cell clumping

Cell-clumping caused by production of the *Photorhabdus* clumping factor (PCF) is another feature that is only detectable in 1° and not in 2° cells [13]. PCF is produced by enzymes that are encoded in the *pcfABCDEF* operon and is under control of bacterial quorum sensing (QS) [38]. QS via LuxI/LuxR-type systems with acyl-homoserine lactones as signals is well studied in many Gram-negative bacteria [39]. However, *P. luminescens* communicates via α-pyrones named photopyrones (PPYs) instead of acyl-homoserine lactones. The PPYs are produced by the pyrone synthase PpyS and sensed by the LuxR-type receptor PluR. At high cell density the PpyS/PluR system positively regulates the expression of the

pcfABCDEF operon in 1° cells, which then leads to the production of PCF [38]. In *P. asymbiotica*, the *pcf* operon is under the control of the DarABC/PauR QS system that uses dialkylresorcinols instead of PPYs for communication [40]. As described above, it was shown that the expression of the *pcfABCDEF* operon was directly blocked upon HexA binding to the cognate P_{pcfA} promoter in *P. luminescens*, so that HexA acts as a direct repressor for the *pcf* operon. The binding site of HexA is located upstream of the PluR binding site in the *pcfA* promoter region [13]. Furthermore, PPYs production is also reduced in 2° cells, so that cell–cell communication via the PpyS/PluR system is assumed to be predominantly present in 1° and not in 2° cells. Since *P. luminescens* harbors 40 LuxR solo receptors that have been supposed to be involved in cell–cell communication as well as inter-kingdom signaling [41], it is possible that 2° cells use another chemical language than 1° cells for QS.

Biological Function of Phenotypic Switching

Phenotypic switching of *P. luminescens* might be a classical bet-hedging strategy that ensures survival of the community in any case. However, little is known about the role and the fate of the 2° cells. Since 2° cells are not able to re-associate with the nematodes, it has been suggested that they are better adapted to a life independent from their symbiosis partners. Therefore, phenotypic variation might be an adaptation for survival of the bacterial population that remains in soil after the nutrients of the insect are depleted and the nematodes together with the 1° cells have emerged from the cadaver [22]. However, 2° cells of *P. luminescens* have never been isolated from soil. For that reason, it is conceivable that 2° cells change into a kind of persister state to outlast periods of nutrient depletion until they somehow can re-enter the life cycle, but this has never been shown yet. However, it can be observed that nearly the complete *P. luminescens* population (1° and 2° cells) undergoes a switch into a non-culturable state upon pro-longed cultivation, meaning that the cells are viable but non-culturable (VBNC) any more (S.E. and R.H., unpublished). Those VBNC cells have also been described for other bacteria like *Salmonella* Typhimurium, *Vibrio cholerae*, *E. coli* or *Pseudomonas aeruginosa* [42–45], whereas only a small portion of the cells form VBNCs, which is in clear contrast to *P. luminescens*. The formation of VBNCs is often mediated by toxin/antitoxin systems in bacteria [46]. Since in *P. luminescens* more than 90 copies of those systems have been identified [47], it is likely that VBNC formation plays a major role to outlive long periods of starvation for *P. luminescens*. However, a correlation between VBNC formation and the high number of toxin/antitoxin systems in *P. luminescens* has not been found yet.

RNA-Seq analysis of 1° and 2° cells revealed a significantly decreased transcription of genes encoding 1°-specific features in 2° cells. Others that are homologous to factors involved in bacterial plant-interaction were induced in 2° cells (S.E. and R.H., unpublished). Furthermore, the up-regulation of several stress-related genes/proteins was identified in 2° cells [24]. This reveals a complete alternative life style for 2° cells in the soil, where the bacteria have to cope with starvation, temperature and osmotic stress, and to compete with other bacteria (Fig. 4). After remaining in the soil, the cells have to adapt and sustain in the rhizosphere, suggesting also an interaction with, for example, plant roots. Increased chemotactic motility of 2° cells toward plant root exudates would be the next step to further support a *Photorhabdus*–plant interaction in the future. Furthermore, VBNC formation has also been found to be important for the resistance of several phytopathogenic bacteria [48], so that a VBNC lifestyle of 2° cells on plants also seems possible.

It still remains unclear if *P. luminescens* 2° cells can reverse the switch and become 1° again. From the current state of knowledge, the phenotypic switching of *P. luminescens* is unidirectional, occurring only from 1° to 2° cells. However, a switch back from 2 ° to 1° cells has been observed for the closely related genus *Xenorhabdus nematophila* [49], implying that there must be a need or at least the possibility to reverse the switching process also for *P. luminescens* in nature. Therefore, it is likely that *P. luminescens* can also undergo a back-switch from 2° to 1° under specific environmental conditions or in presence of a specific, yet unknown, signal. However, such a signal might be found in the rhizosphere. It is possible that the signal(s) is derived from plants, nematodes or insects, which could force a decision of 2° cells to become 1° again, so that the cells can somehow re-enter the entomopathogenic life cycle.

Conclusions and Future Work

The current knowledge of molecular mechanisms regulating phenotypic switching in *P. luminescens* cell populations has identified not only master regulators like HexA that are involved in the switching process but also those like AntJ that control further downstream regulation processes of phenotypic heterogeneity. Furthermore, regulation at the post-transcriptional level via small RNAs and the molecular chaperone Hfq seem to play a global role in the switching process. However, we are far away to fully understand this complex regulation network. It will be important in the near future to identify specific signals and ligands that control the activity of the specific regulators. This will help to understand how fine stochastic differences, concentrations or ligand affinities to specific regulators could make up the decision to induce the switching cascade in one cell, and to

Fig. 4. Model for phenotypic switching of *P. luminescens* in nature and putative fate of the 2° cells. During insect infection, a specific subset of the 1° cells undergo phenotypic switching and convert to 2° cells. Since 2° cells are not able to support symbiosis and cannot re-associate with the nematodes, they remain in the soil when the infection cycle is finished. In the new environment, they have to adapt to several stress conditions like osmotic stress, variation in temperature and starvation and to compete with other bacteria. To survive in the rhizosphere, it is possible that the 2° cells are adapted to plant derived nutrients.

block it in another cell in a homogenous environment. Furthermore, it will be important to understand the fate of 2° cells and therefore the biological reason for phenotypic heterogeneity in *P. luminescens*. A major focus will be laid on the molecular mechanism of VBNC formation, the interaction of *P. luminescens* with plants as well as the signal(s) and the back-switch of 2° to 1° cells. Since nematodes colonized by *P. luminescens* are commercially used as bio-insecticides in agriculture, the control of phenotypic switching and the signal(s) for the switching process are also major issues for the biotechnological use of *P. luminescens* as bio-insecticide.

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AntJ;
PluR

Abbreviations used:
IJs, infective juveniles; AQ, anthraquinones; PCF, *Photorhabdus* clumping factor; QS, quorum sensing; PPYs, photopyrones; VBNC, viable but non-culturable.

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