Programmed Cell Death and Lysis in Bacteria and the Benefits for Survival.

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Introduction

Bacterial cell death is an interesting phenomenon because it plays a crucial role in many important processes. Bacterial cell death does not just occur in the late stationary phase when nutrient limitation can lead to starvation. Bacteria are capable of initiating autolysis during certain conditions, such as biofilm formation, competence, sporulation and stress response. One form of lysis is autolysis, bacterial suicide. Autolysis in bacteria constitutes programmed cell death (PCD) because death of the cell is the result of an internal mechanism that can be activated by internal or external signals. Another cause of death of a subpopulation of cells is allolysis; allolysis is the killing of sibling cells by bacteria. There are many different mechanisms that can cause lysis in bacteria. Which mechanisms are used depends on the species of bacteria and the circumstances. The possible benefits to killing sibling cells are obvious; killing other cells releases their nutrients into the environment. But what are the benefits to killing yourself? The answer to this lies in the complex nature of bacterial communities. Bacteria are for instance, capable of forming biofilms in which there exists a spatial and temporal difference in gene expression of genetically identical bacteria. The difference in gene expression patterns plays an important role in deciding which cells undergo autolysis. Bacteria also undergo complex developmental processes such as sporulation and competence in which autolysis and allolysis play a crucial role. The abovementioned aspects of bacterial cell death and lysis will be discussed in greater detail.

Cell Death in biofilms

There are many mechanisms involved in the lysis of cells in biofilms. Among these mechanism are, lysis through the action of prophages, autolytic proteins or holins.

Biofilm formation in *Pseudomonas aeruginosa* is influenced by cell-cell signalling and the alternative sigma factor of RNA polymerase, RpoN. Cell lysis in *P. aeruginosa* biofilm occurs in a spatial and temporal organization within microcolonies. Lysis in *P. aeruginosa* has been shown to be dependent on RpoN. In an *rpoN* mutant strain cell death in biofilm did not occur, cell death could be restored by in trans-expression of *rpoN* [29]. An important feature of the *rpoN* mutant is the lack of type IV pilli and flagella. Single *pilA* and *fliM* mutants did show cell death in microcolonies, but a *fliM pilA* double knockout did not [29]. Type IV pilli and flagella are common bacteriophage receptors and the effluent of flow cell biofilms of *P. aeuruginosa* contained a Pf1 like bacteriophage [29]. Double *pilA* and *fliM* mutants and *rpoN* mutants could produce the phage, but were not susceptible to infection [29].The genome of *P. aeruginosa* contains a Pf1 like prophage and these prophage genes are upregulated during biofilm production. The presence of a prophage in the genome of *P. aeruginosa* and the presence of a Pf1 like phage in the effluent indicate that the Pf1 phage plays a role in cell death in *P. aeruginosa* biofilm [29]. *P.aeruginosa* cells that contain the Pf1 prophage are normally resistant to super-infection by the phage, but in biofilm a specific subpopulation is killed by the phage [29]. The developmental stage of the cells might play a role in regulation of phage receptor expression. During biofilm formation of *P. aeruginosa* T4P expression is down regulated. In *Myxococcus xanthus* RpoN controls development and morphogenesis. Infection by Pf1 also requires the outer membrane protein TolA. TolA is differentially expressed in biofilm [29]. It seems likely that differential expression of phage receptors as a result of developmental stage restricts cell killing by phages to a subpopulation in the biofilm. Only cells that express the necessary receptors are killed by the phage.

Lysis through the activation of prophage genes is not the only way that bacteria can commit autolysis. Many bacteria possess autolysins that can induce lysis under certain circumstances. These autolysins degrade the cell wall which results in lysis. The autolysin AtlE of *Staphylococcus epidermidis* plays an important role in the release of DNA into the extra-cellular matrix of *S. epidermis*. A *ΔatlE* mutant showed a reduction of more than 90% in extra-cellular DNA and almost no dead cells were detected in the biofilm of *ΔatlE*. [24]It is likely that the release of DNA into the extra-cellular matrix is in a large part the result of cell lysis by AtlE activity [24]. The presence of extra-

cellular DNA appears to be important in biofilm formation of *S. epidermidis*. When DNase was added to the medium of *S. epidermidis* biofilm formation was severely reduced [24]. Cells that lacked the *atlE* gene were also compromised in their ability to form biofilm.

Another organism that commits autolysis through the action of autolytic proteins is *Pseudoalteromonas tunicata*. The autolytic protein AlpP of *P.tunicata* is responsible for the death of a subpopulation of *P. tunicata* cells in biofilm. Deficiency in AlpP does not lead to changes in the structure of the biofilm [3]. However cell death mediated by AlpP does play an important role in dispersal, metabolic activity of dispersed cell and in phenotypic variation of dispersed cells [21]. Wild type cells show a major detachment event that corresponds with a high level of cell death mediated by AlpP [21]. A *ΔalpP* mutant did not show such a major event; however when AlpP was added to *ΔalpP* a detachment event was induced [21]. AlpP deficient cells also showed less metabolic activity and lower phenotypic variation in dispersed cells [21]. Deficiency in AlpP does not lead to changes in the structure of the biofilm [21].

In *Enterococcus feacalis* biofilm formation is regulated by the *fsr* quorum sensing system. Two proteins are important for lysis in biofilm formation of *Enterococcus feacalis*, the secreted metalloprotease gellatinase GelE and the secreted serine protease SprE [4]. The *gelE* and *sprE* genes are co-transcribed, and their transcription is depended on the *fsr* regulatory pathway. In mutants that were deficient in GelE production a reduction in extracellular DNA was detected, which is consistent with a decreased rate of lysis in GelE deficient mutants [4]. Initiation of DNA release followed expression of GelE which indicates that GelE initiates lysis [4]. Mutants deficient in SprE showed an increase in extra-cellular DNA, indicating that SprE negatively regulates autolysis [4]. Thomas et al [4] propose two possible mechanisms for the effect of the proteases on biofilm production in *E. feacalis*. One mechanism is an autolytic process in which GelE localises to the cell wall of the producing cell where it initiates autolysis. If there isn't enough SprE present to regulate the activity of GelE the cell will undergo lysis. Another mechanism involves fratricide. GelE diffuses to the cell wall of neighbouring cells. If these cells have a delay in responding to quorum sensing signal GelE can initiate autolysis in these cells. In this case SprE is likely also present in the extra-cellular environment, but differences in diffusion and affinity for the cell wall would result in regions where there is not enough SprE and GelE can initiate lysis [4].

Another way bacteria can commit autolysis is through the action of bacteriophage holin antiholin homologues. The *lrgAB* operon and *cidABC* operon control cell lysis in *Staphylococcus aureus*. They do this by controlling the activity of murein hydrolase. Murein hydrolases are enzymes that cleave specific bonds in peptidoglycan. Some murein hydrolases are autolysins because their activity destroys the cell wall which results in cell lysis [24]. There is a high degree of secondary structural similarity between CdA and LrgA and bacteriophage holins and antiholins [3]. Bacteriophages control the timing of lysis through a mechanism containing a holin and an endolysin (murein hydrolase). The holin controls the activity of the endolysin, by one of two proposed mechanisms. In T4-like bacteriophages holins control the transport of the endolysins across the cytoplasmic membrane. The endolysins in this mechanism do not have a signal sequence for transport via bacterial transporters, and accumulate in the cytoplasm until holin transports them to the peptidoglycan [25]. The second mechanism is used by P1 bacteriophages. P1 endolysins possess a SAR domain which targets the endolysin to the Sec machinery and anchors the protein in an inactive form to the outer face of the membrane. The endolysin becomes active when it is activated by the holin [25]. The point at which holin triggers cell lysis is determined by the structure of the holin. A common feature of bacteriophage holins is the presence of a dual-start motif [25]. The presence of a dual-start motif results in the production of two proteins, a shorter and a longer version [25]. The shorter protein is the holin and the longer protein, which has only a few amino-acids more at the N-terminus functions as an antiholin [25]. The extra amino acids at the N-terminus of antiholin always contain the positively charged amino acid lysine or arginine [25]. The charge distribution of the energized

membrane orients the N-terminus of antiholin towards the cytoplasmic side of the cytoplasm membrane, while the N-terminus of the holin is oriented towards the periplasmic side of the membrane [25]. This orientation of the antiholin has an inhibitory effect on the holin's ability to activate endolysin [25]. Accumulation of the holin in the membrane leads to a gradual dissipation of the proton gradient. Eventually a point is reached where it becomes thermodynamically unfavourable for the N-terminus of the antiholin to reside at the cytoplasmic side of the membrane and the antiholin N-terminus flips to the periplasmic side of the membrane. When this happens the antiholin functions as a holin, and the membrane rapidly de-energizes and lyses [25]. As mentioned earlier for *S. aureus* there is a structural similarity between CidA and LrgA and holins and antiholins. CidA has a positive effect on murein hydrolase activity while LrgB has a negative effect. When the *cidA* gene of *S. aureus* is disrupted a decrease in extra-cellular murein hydrolase activity can be detected. Mutation of *lrgAB* increases murein hydrolase activity. Along with the structural similarity these findings indicate that the *cidA* encodes a holin and *lrgA* an antiholin [3]. Mutation of the *cidA* gene also leads to an increase in dead cells in biofilm and reduced cell lysis and reduction in extra-cellular DNA levels [25].The *lrgAB* operon is controlled by the two component system LytSR which responds to decreases in membrane potential. Expression of the *cidABC* operon is controlled by CidR in response to changes in carbohydrate metabolism [3].

Distribution of *lrgAB* and *cidAB* homologues

Genes	Phyletic distribution
lrgAB	Gram positive and Gram negative bacteria.
cidAB	Gram positive and Gram positive bacteria.

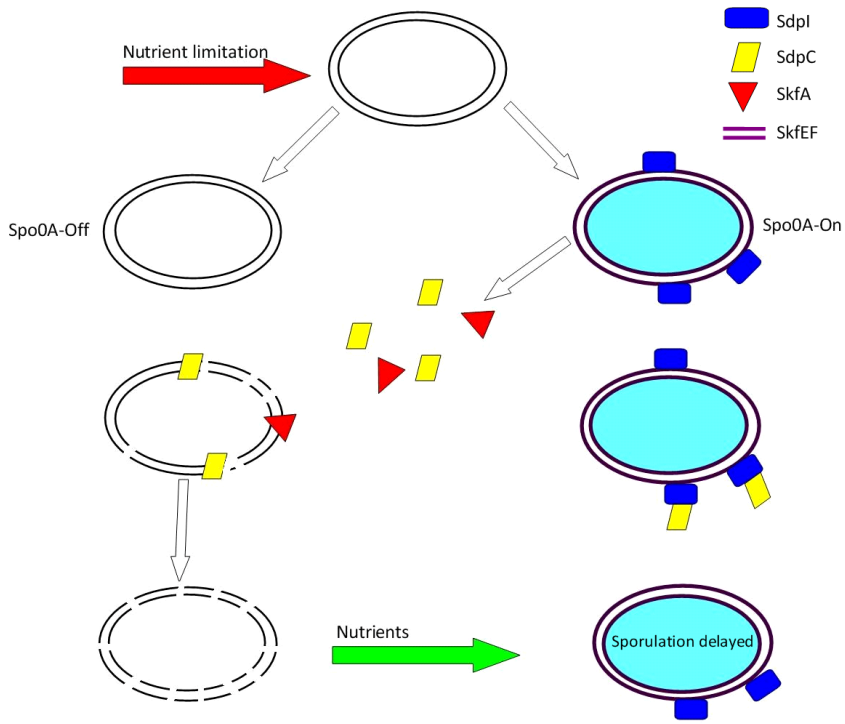
The previous examples show the diversity of mechanisms involved in lysis in biofilm and the role lysis plays in biofilm. Mechanisms can involve the expression of prophages that are expressed during certain developmental stages. Quorum sensing can also lead to lysis by initiating the expression of autolytic proteins that degrade the cell wall. Lysis by holin-antiholin systems also involves autolytic proteins, but is dependent on the holins and antiholins that alter the energy state of the membrane for the timing of autolysis. Lysis is important for the generation of extracellular DNA which can be used as a source of nucleotides during oligotrophic conditions and it may also enhance gene transfer [26]. Extracellular DNA is also involved in the attachment of biofilm to the surface and the structure of the biofilm. Other important functions of lysis are generation of nutrients and increasing phenotypic variation of dispersing cells, which are important for survival. Lysis also clears areas within the biofilm contributing to the structure.

Sporulation

Lysis not only plays an important role in the formation of biofilm, but is also very important in sporulation processes. When certain bacteria are exposed to environmentally stressful conditions a subpopulation starts to produce spores. During sporulation, instead of dividing, the mother cell generates a spore and undergoes lysis. In the case of Bacillus *subtilis* sporulating cells, the mother cell is actively lysed before release of the spore. There are three identified autolysins involved in lysis of the mother cell of *B. Subtilis*. These are CwlB, CwlC and CwlH [19]. CwlB is produced at the end of the exponential phase and CwlC is the major autolysin at the final stage of sporulation. Double mutants of *cwlB cwlC* and double mutants of *cwlC cwlH* are defective in mother cell lysis, but single mutants are not. The *cwlB* operon is transcribed by $E\sigma^A$ and $E\sigma^D$; these are the predominant transcription factors in the exponential phase [18]. CwlC and the minor autolysin CwlH are transcribed by $E\sigma^K$ a sporulation specific polymerase; they also require the coat protein transcriptional activator GerE for

expression. GerE likely interacts with the σ^K subunit of RNA polymerase [28]. The σ^K subunit is the final regulator in a pathway that is initiated by Spo0A. Spo0A is the master regulator for sporulation initiation. Spo0A integrates nutritional and cell cycle signals and activates a cascade of interdependent sigma factors in the fore spore and the mother cell [19]. While lysis of the mother cell during sporulation is necessary for the release of the spore, it also releases nutrients which can be used by non-sporulating cells. Mother cell lysis of *B. subtilis* constitutes PCD because it is the result of an internal mechanism resulting in autolysis.

Not all cells enter the sporulation pathway; some cells undergo lysis without producing a spore. Lysis of a subpopulation of cell is advantageous, because it would make nutrients available for the remaining cells. Sporulation is a very time and energy consuming process. If during the time that a cell undergoes sporulation the nutrient availability were to increase, sporulating cells would be at a disadvantage compared to non-sporulating cells that would start to divide rapidly. For this reason cells try to delay sporulation. Cells that have entered the sporulation pathway, but that are not yet irreversibly committed to sporulation are able to keep other cells from sporulating and kill them for their nutrients [25]. This killing of siblings by *B. subtilis* is called cannibalism. Cannibalism, like sporulation involves the activity of Spo0A. The promoters of the sporulation operons spoIIA, spoIIE and spoIIG require high levels of Spo0A for their induction, because they have a low affinity for Spo0A. Aside from triggering the sporulation pathway Spo0A also regulates the transcription of two operons called sporulating delay protein (*sdp*) and sporulating killing factor (*skf*). The promoter of the *skf* operon has a high affinity for Spo0A. The *sdp* operon is activated indirectly by repression of the gene *abrB*, AbrB is a repressor of the *sdp* operon [7]. Low levels of Spo0A repress transcription of *abrB* resulting in depletion of AbrB which allows transcription of *spd* [7].This mechanism ensures that the products of the *skf* and *sdp* operons are present at high levels early in the sporulation process. The eight gene *skf* operon (*skfABCDEFGH*) contains a killing factor that kills non-sporulating cells and resistance factors that protect the cells that produce the killing factor [7]. The product of *skfA* is a small 55 amino acid bacteriocidin like peptide. The second gene encodes a protein that is homologous to AlbA, a protein that is necessary for creating active subtilosin, an antimicrobial peptide produced by several *B. subtilis* strains. AlbA is probably involved in the post-translational modification of subtilosin, it is possible that SkfA is related to this bacteriocin [7]. The *spd* operon encodes a toxic signalling protein SdpC. SdpC controls the transcription of a two genes *yvbA* and *yvaZ*. These genes are located downstream of the *sdpABC* operon. Artificial induction of *yvbA* and *yvaZ* or *yvbA* alone causes a delay in sporulation [12]. The transcription factor YvbA turns on operons involved in ATP production and lipid oxidation. This leads to an increase in energy production which could delay sporulation [12]. SdpC also activates YvbA synthesis in Spo0A inactive cells. By stimulating energy production in non-sporulating cells it makes them more sensitive to the killing factor, metabolically active cells are more sensitive to antimicrobials. YvbA is also involved in inhibition of σ^W transcription. The regulatory protein σ^W activates transcription of genes involved in detoxification and antibiotic resistance [12]. By combined action of the killing factor and induction of YvbA synthesis by SdpC Spo0A inactive cells are lysed, making nutrients available for Spo0A active cells allowing them to delay sporulation. Immunity to SdpC is the result of an integral membrane protein SdPI. SdpI is part of the *yvaZA* operon (*sdpRI* operon). In Spo0A inactive cells the production of SdpI is blocked by the AbrB repressor, making them susceptible to SdpC. The *sdpR* gene of the *sdpRI* operon encodes an autoregulator that represses transcription of the *sdpRI* operon [7]. When SdpC binds SdpI repression of the operon is relieved, allowing increased production of SdpI and SdpR. In this way the cell only produces as much SdPI as it needs [7]. Expression of the *skfEF* genes also contributes to immunity. SkfEF resembles an ABC-type transporter. The regulatory mechanisms that control phosphorylation of Spo0A function as a bistable switch. This ensures that only a subpopulation of *B. subtilis* activates Spo0A, and starts the sporulation process [10].



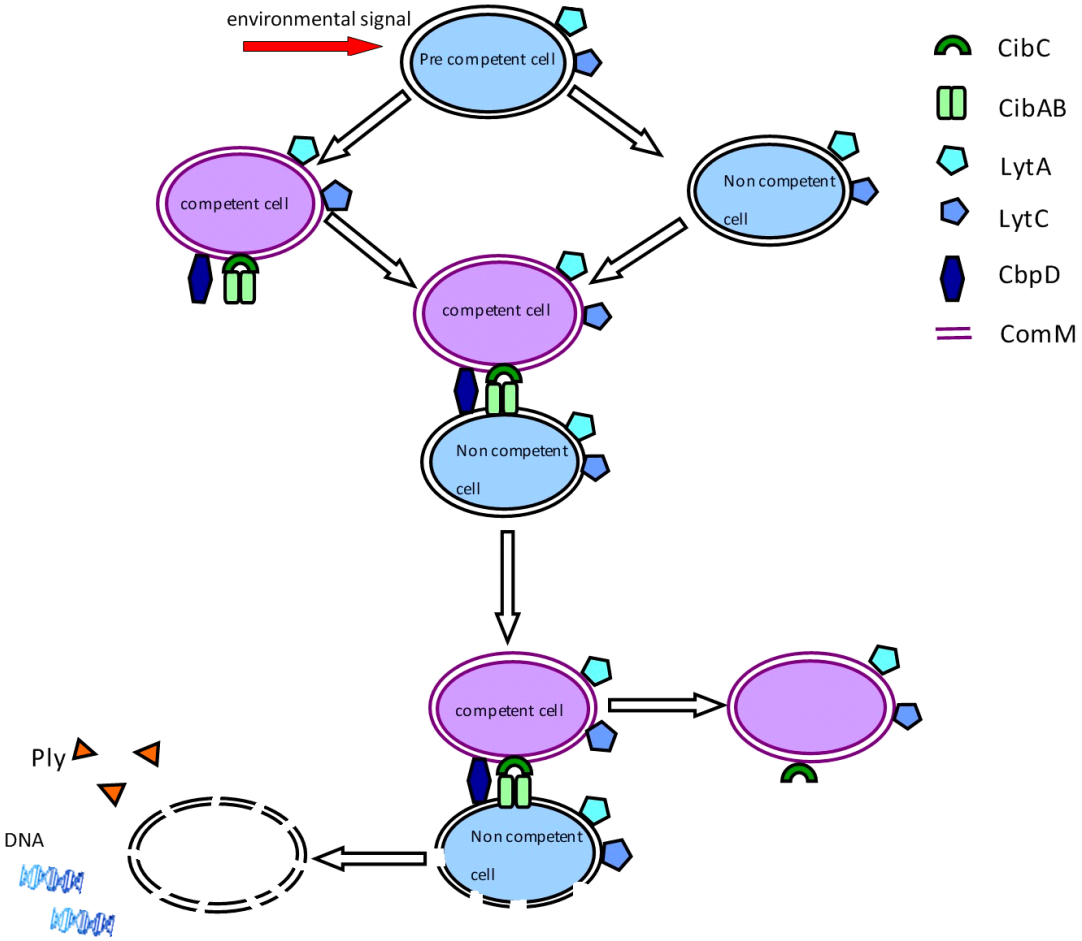
In reaction to nutrient limitation a subgroup of cells activates Spo0A. The Spo0A on cells protect themselves from the killing factors SkfS and SdpC with SdpI and SkfEF. The spo0A off cells do not have these immunity factors and are susceptible to the lethal action of SdpC and SkfA.

Because sporulation is such an energy-intensive process there is a clear advantage in lysing sibling cells. Lysis provides nutrients to continue the sporulation process, but it can also be used to delay it. Delaying the sporulation process gives the cell an advantage should nutrients suddenly become more abundant. The delay allows the cells to rapidly adapt to the new situation, while the cells that are past the irreversible point in the sporulation process cannot take advantage of the new situation.

Competence

Another process that involves lysis is competence. In *Streptococcus pneumoniae* killing of siblings is called fratricide. Fratricide occurs when *S. pneumoniae* becomes naturally competent for genetic transformation. Competence occurs in a subpopulation *of S. pneumoniae* during logarithmic growth. It is regulated by a quorum sensing dependent pathway. There is also evidence that competence in *S. pneumoniae* is induced in response to environmental stress such a low pH and the presence of antibiotics [7].Before *S. pneumoniae* becomes competent it secretes a competence stimulating peptide (CSP). CSP is detected by the two-component signalling system ComDE [11]. ComD is a membrane bound histidine kinase receptor which binds CSP, and ComE is a cytoplasmic cognate response regulator. When the extracellular CSP reaches a threshold concentration ComD autophosphorylates and activates ComE by transfer of the phosphoryl group to ComE [25]. Phosphorylated ComE activates the early competence genes. Among the early competence genes is comX, which encodes the alternative sigma factor σ^X. σ^X controls the transcription of the late com genes[25].Post transcriptional regulation of *comX* is mediated by the early com gene product ComW. ComW is involved in the stabilization and activation of sigmaX. Sigma X recognizes a conserved sequence in the promoter region of the late com genes [6]. The products of six genes that respond to CSP are implicated to be directly involved in fratricide. These genes are: *cpbD, cidA, cidB, cidC, comM, lytA* and the σ^{70} dependent gene lytC. LytA and LytC are cell wall associated murein hydrolases[25]. Fratricide was tested with three different assays, release of pneumolysin on agar plates and release of chromosomal DNA or cytoplasmic β-glactosidase in liquid medium [25]. Clumping, which occurs when competent cells are resuspended in a mild acid was also studied [25]. Clumping relies on the presence of extracellular chromosomal DNA [25]. These assays showed the effects of inactivation

cpbD, cidA, cidB, cidC, comM, *lytA* and the *lytC* genes on fratricide. Fratricide leads to the release of intracellular components such as β-galactosidase, DNA and pneumolysin (Ply) [25]. Pneumoslysin is produced by many Gram-positive bacteria, it is a member of the cholesterol dependent pore forming toxin family [11]. Simultaneous inactivation of LytA and LytC results in a strong reduction in fratricide. These results lead to the conclusion that fratricide involves lysis [6]. Inactivation of LytA and LytC in competent cells does not lead to a reduction in fratricide, as long as LytA and LytC are still active in non-competent cells. This indicates the presence of a mechanism for the trans-activation of LytA and LytC [7]. Lysis of one cell by another was termed allolysis. CbpD another murein hydrolase and the CibA and CibB (cibAB) a possible two-peptide bacteriocin are involved in triggering allolysis. CibAB is necessary for the release of pneumolysin from killed cells, but it can not do this in the absence of the lytic enzymes CbpD, LytA and LytC [7]. CibAB possibly triggers allolysis by inserting itself into the membrane of non-competent cells [7]. If *cibAB* encodes a bacteriocin then the competent cell would require an immunity factor for this bacteriocin. A possible immunity factor is the product of a third gene in the c*ibAB* operon, *cibC*. Only competent cells have the full length transcript. Inactivation of the *cibC* gene makes the competent cell susceptible to allolysis in a mixed culture of competent and non-competent cells [25]. Another immunity factor is ComM. Inactivation of *comM* makes competent cells sensitive to self lysis [25]. The mechanism that makes some cells *S. pneumoniae* cells competent but not others is not yet known. But it is possibly similar to the mechanism of competence in *B. subtilis*, which is regulated by a bistable switch that controls the expression of *comK* [25]. In nature *S.pneumoniae* does not normally exist in a single species environment. *S. pneumoniae* shares its natural environment, the mucosal tissue of the human upper respiratory tract, with other Streptococcus species such as *Streptococcus mitis* and *Streptococcus oralis*. Different strains of these Streptococcus species produce slightly different CSPs, they belong to different pherogroups [14]. When competence is triggered in one of these pherogroups members of another pherogroup who are insensitive to the CSP will remain non-competent [14]. Insensitivity of a pherogroup to another strain's CSP is a result of the specificity of the ComD receptor [14]. It has been shown that Streptococci belonging to one pherogroup can induce lysis in another pherogroup insensitive to the CSP of he competent pherogroup [14]. The DNA of the susceptible pherogroup is released into the environment, and the competent bacteria can absorb this DNA. Killing members of another pherogroup during competence allows *S. pneumoniae* to acquire antibiotic resistance genes and capsular genes from other strains. By absorbing capsular genes from other strains it can undergo serotype conversion [14].



An environmental signal causes subpopulations of competent and non competent cells to arise. The bacteriocin CibAB initiates the lytic action of CbpD, LytA and LytC in the non-competent cell, while CibC and ComM provide the competent cell with immunity to the lytic factors. Lysis of the non competent cell releases DNA and Ply.

Susceptibility or resistance to lysis is not the result of difference in the genetic code; rather it is caused by a difference in gene expression patterns. The difference in gene expression is initiated by cell-cell communication. Differences in gene expression allow bacteria to perform tasks they can not do on their own. Induction of competence under stress condition can be very beneficial to *S. pneumoniae*. By killing members of another pherogroup during competence *S.pneumoniae* can acquire genes that can give it a selective advantage, such as antibiotic resistance or serotype conversion, which can make capsule based vaccines ineffective. Another possible reason for *S. pneumoniae* fratricide is population control: too high a population density might activate the immune system of the host.

Toxin-antitoxin systems

There are two types of bacterial toxin-antitoxin (TA) systems. The toxin of a TA system is always a protein, but the antitoxin can be either a protein or a small RNA that is complementary to the toxin mRNA. The antitoxin of type I inhibits the translation of the toxin. The antitoxin is a small RNA and the toxin a small hydrophobic protein that damages the cell membrane [27]. The antitoxin of type II TA systems is a small unstable protein that inhibits the toxin by binding it. The TA systems are located in operons in which the antitoxin is usually located upstream of the toxin [23]. Some bacteria like *E.coli* have multiple TA systems on their chromosome. The distribution of the TA systems in bacteria is very diverse. Almost all free living bacteria have TA systems in there genome, sometimes in very high numbers. *Nitrosomonas europaea* has 43 intact TA systems and 2 solitary toxin genes [23]. Although most free living bacteria have TA systems there are exceptions; *Lactococcus lactis* does not

have any TA loci [23]. A lack of TA systems is common in obligate host associated bacteria. These bacteria appear to have lost their TA systems over the course of their evolution [23].

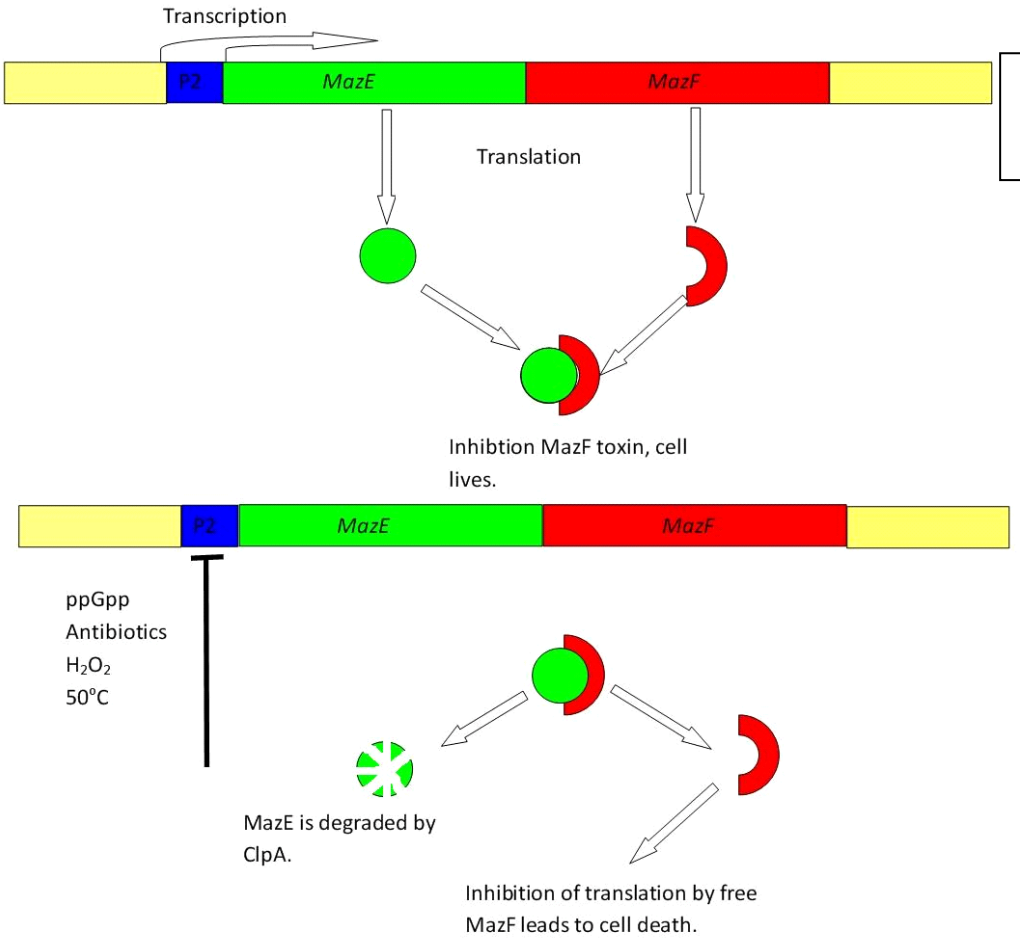
Distribution of TA systems

TA system	Toxin	Antitoxin	Mechanism toxin	Phyletic distribution
mazEF	MazF	MazE	Inhibition of translation by specific cleavage of mRNA.	Gram negative and Gram positive bacteria.
relBE	RelE	RelB	Inhibition of translation by specific cleavage of mRNA.	Gram negative, Gram positive bacteria and archea.
HipBA	HipA	HipB	Inhibition of macromolecular synthesis.	Gram negative bacteria.

The MazEF toxin-antitoxin system

MazEF is an example of a bacterial type II toxin-antitoxin system (TA). A toxin-antitoxin system is a system of two genes were one of the genes encodes an unstable antitoxin that is degraded faster than the more stable toxin encoded by the other gene. The antitoxin is able to inhibit the lethal activity of the toxin. Toxin-antitoxin systems were first discovered in *Escherichia coli* on low copy number plasmids. When the bacterium loses the plasmid the cell dies, because the unstable antitoxin is degraded faster than the more stable toxin. Because the cell can not survive without the plasmid it is also called an addiction module, the cell is addicted to the continual transcription of the antitoxin [9].The *mazEF* operon is not only found on plasmids. In *E.coli* the *mazE* and mazF genes are located downstream of the *relA* gene on its chromosome. The *relA* gene is responsible for the production of 3',5'-bipyrophosphate (ppGpp) during the stringent response. MazF is the stable toxin and MazE is the unstable antitoxin. MazE inhibits the lethal properties of MazF through direct interaction. MazE is degraded by the serine protease ClpA [9]. The promoter P2 of *mazE* is active during exponential growth and under normal circumstances MazEF dependant cell death does not occur during the stationary phase. In the stationary phase transcription is controlled by the stationary phase transcription factor σ^S. σ^S activates the transcription of reactive oxygen species (ROS) detoxifying enzymes. These enzymes antagonize the MazEF death system by removing ROS [15]. During amino-acid starvation the P2 promoter is inhibited by high levels of ppGpp. If mazEF is deleted the cells show an increased survival rate after induction of ppGpp[1]. MazF has an endoribonucleolytic effect; it preferentially cuts single-stranded mRNA at ACA sequences. It also cuts tmRNA, the tRNA-mRNA hybrid that binds to the A side of the ribosome [9]. By cutting mRNA and tmRNA MazF inhibits translation. The bacteriocidic effect might be the result of inhibition of translation of proteins essential for cell survival. It is also possible that the preferential cutting of MazF leads to the specific translation of mRNAs that lack the ACA sequences or in which the ACA sequences are protected from MazF. These mRNAs might encode proteins that are involved in a cell death mechanism [9]. After

MazF is overproduced, there is a time-frame in which MazE can reverse the effects of MazF. How long this time-frame is depends on the growth conditions, in nutrient rich LB medium MazE can almost completely reverse the effects of MazF, but in minimal medium this time-frame is shorter [2]. MazE is capable of reversing the initial step of MazF mediated cell death; however it can not stop the downstream cascade that MazF initiated [2]. This means that if the activity of is not stopped in time, the process can not be reversed and the cell dies. MazEF induced cell death is not only induced during amino-acid starvation. Other stressful conditions such as exposure to high temperatures can also cause MazEF dependant cell death. At high temperatures the normal σ^{70} transcription factor becomes inactive and is replaced by the periplasmic σ^{E} transcription factor, which should not be able to initiate transcription of *mazEF* [2]. Inhibitors of transcription/translation such as rifampicin, chloramphenicol and spectinomycin also inhibit mazEF transcription and cause cell death. DNA damage and oxidative stress also cause mazEF dependent cell death [2]. On the ability of the *mazEF* system to induce cell death under various stress conditions the programmed cell death model is based. Activation of MazF leads to the death of at least 95% of the bacterial population. The death of these cells is proposed to provide nutrients for the remaining cells [27].



Schematic overview of the MazEF mechanism.

MazF dependent cell death also occurs when P1 prophages in the bacterial genome become active and go from the lysogenic state to the lytic state. In a wild type population, cells in which the lytic cycle of the prophage is initiated undergo MazF enabled cell death before the cells can be lysed by the phage. By committing suicide the cell protects the population from infection [9].

MazF-mx: The toxin-antitoxin system of *Myxocoxxus xanthus*

The MazF gene is not always accompanied by the *mazE* gene. *Myxococcus xanthus* is a bacterium that under starvation conditions aggregates to form a fruiting body. During fruiting body development approximately 80% of the cells undergo lysis; the remaining cells can form spores [22]. In *M. xanthus* the key developmental regulator of early developmental genes MrpC, functions as the antitoxin and transcription activator of *mazF-mx*. MrpC is highly expressed during early and middle development [22]. In this stage it inhibits the endoribonucleolytic activity of *MazF-mx*, but at the same time it continues to upregulate mazF-mx transcription [22]. Before *M. Xanthus* sporulation is initiated, MrpC is likely degraded by LonD and/or other unidentified proteases [22]. *MazF-mx* is now no longer inhibited, and can cleave mRNA. The *mazF-mx* gene is essential for the formation of a fruiting body because a *ΔmazF-mx* mutant show a dramatic reduction in the formation of myxospores [27]. The role of *mazf-mx in* the development of *M. xanthus* gave rise to the development model. In this model the lethal action of MazF on some cells makes nutrients available for the remaining cells that enter the sporulation process [27].

The RelBE toxin-antitoxin system

Another toxin-antitoxin is encoded by the *relBEF* operon. The *relBEF* operon in *E.coli* contains three genes, *relB, relE* and *relF* [25]. The *relE* gene encodes a global inhibitor of translation, and the *relB* gene the antitoxin. The *relF* encodes a homologue of the R1 plasmid *hok* gene, the *hok* gene of R1 is involved in the post-segregational killing of cells that lose the R1 plasmid [25]. RelF causes a cessation of cell growth, collapse of the cell membrane potential and inhibition of respiration [13]. RelE dependent inhibition of translation can be induced by glucose starvation and the translation inhibitor chloramphenicol. Amino-acid starvation also leads to inhibition of translation by *relE* but it is not dependent on ppGpp [25]. RelB autoregulates the *relBEF* operon at the transcription level [13]. Induction of RelBE is dependent on the Lon protease; Lon protease degrades RelB [5]. The *relBEF* operon has a structural similarity to proteolytic plasmid systems and was able to stabilise an unstable R1 plasmid [13]. The *relBEF* operon did not stabilise a plasmid in a wild type *E.coli* with a chromosomal *relBEF* operon, because the continued expression of *relB* prevents activation of RelE [13]. Inhibition of translation by RelE induces a reversible bacteriostatic condition, and not cell death [25]. The bacteriostatic effect of *relBE* supports the growth-modulation model. In this model amino-acid starvation leads to an inhibition of cell growth until more favourable conditions occur [27].

HipBA toxin-antitoxin and its role in persistence

The *hipBA* TA consists of the toxin *hipA* and the antitoxin *hipB.* The antitoxin *hipB* not only inhibits the toxic properties of HipA, but also regulates the promoter of *hipBA*. The *hipBA* operon appears to be involved in the generation of persister cells. Persister cells are a small fraction (~10^{-6}) that are resistant to a variety of stress conditions, including antibiotics [27]. Cells containing a mutant of the *hipA gene*, *hipA7* display increased persister cell formation [25]. The *hipA7* allele encodes a non-toxic version of the HipA protein and its ability to confer high level persistence is independent of HipB [25]. When the *relA* gene in a hipA7 mutant is inactivated the high level persistence is lost. This suggests that the *hipA7* gene helps to establish the persister state by inducing the production of ppGpp synthesis [16]. The change in basal levels of ppGpp synthesis leads to altered gene expression of the genes involved in entering the persistent state [16]. Expression of *hipA* in excess of hipB leads to inhibition of protein, DNA and RNA synthesis in vivo. The expression of *hipA* induces a transient dormant state in a large portion of the cells, the remaining cells enter a prolonged dormant state that can be reversed by expression of *hipB* under the right conditions [17].The expression of hipA7 under these conditions does not lead to a notable inhibition of these processes, but still causes a significant potion of cells to enter the transient dormant state [17]. This suggests that the ability of

hipA to induce persistence might be independent of its ability to inhibit macromolecular synthesis [17]. Persistence is growth-state dependent, during stationary phase bacteria generate the most persisters [20]. The dormant state induced by *hipA* provides the cells with an increased ability to survive stress conditions, metabolically active cells and cells that undergo division are for instance more susceptible to antibiotics. The dormant state also requires fewer nutrients allowing the cell to survive longer under minimal nutrient conditions, so that it can resume growth when nutrients become available. The ability of *hipBA* to induce persistence is consistent with the persistence mode [27]. No *hip* mutants have been found in natural *E. coli* isolates which suggests that increased resistance is detrimental to survival. Mutations in *hip* also lead a lower death rate due to DNA damage. The ability to eliminate defective cells might be more advantageous to a clonal population than surviving severe DNA damage [19]

All the above mentioned TA systems have in common that the toxin inhibits translation, however the mechanism by which the transcription of the operon is inhibited and the type of antitoxin differs. In the case of MazEF the *mazEF* operon is inhibited by ppGpp. Initiation of the bacteriostatic effect of the RelE involves relieve of the autoregulation of the RelBEF operon by degradation of the RelB protein by Lon protease. MrpC in the MazF-mx system is a transcription activator of *mazF-mex*, in late development stages of mixococcus development mrpC transcription is reduced which also reduces the regulation of the *mazf-mz* gene. MrpC is degraded and can no longer neutralise the MazF resulting in lysis. The different mechanisms by which the TA systems are controlled make it possible that they are initiated by different circumstances. That bacterial TA systems are evolutionary successful is confirmed by there presence in a large number of bacterial species. Programmed cell death through the action of TA systems is obviously not beneficial to the individual cell undergoing cell death. However there are a variety of possible benefits bacterial TA systems can have on survival of bacterial populations. The possible benefits of TA systems on bacterial population survival appears to be dependent on the type of TA system and the bacterial species and if it is located on the genome or on a plasmid. It is also possible that the effects of a TA system vary under different developmental and environmental conditions. Some TA systems are able to inhibit growth which gives an advantage during times of nutritional stress, because growth requires a lot of energy. By inhibiting growth and cell division, the cell increases its chances to survive until more nutrients become available. TA induced lysis during nutritional stress provides the remaining cell with nutrients. Having multiple TA systems activated under different circumstances also gives bacteria an advantage.

DNA damage can also cause the cell to commit suicide. Two genes involved in DNA damage induced cell-death are *sulA* and *marA*. The *sulA* gene is transcribed as part of the SOS-response; it is normally repressed by LexA. During the SOS-response LexA inhibition is relieved and *sulA* is transcribed. SulA binds to FtsZ the protein that forms the division ring [19]. By binding to FtsZ SulA inhibits cell division. Once DNA is repaired SulA is degraded by Lon protease, allowing cell division to resume [19]. The inhibition of cell division by SulA suggests that without SulA DNA will not be repaired properly before replication which would result in nonviable cells [19]. However a *sulA* mutant has a 1000 fold higher survival rate when exposed to mutagenic quinolones. SulA might thus be involved in initiating cell death in cells with severe DNA damage [19]. The global regulator MarA controls the expression of genes involved in multi-drug resistance (MDR). It inhibits synthesis of OmpF, a large porin, and it activates expression of the AcrAB-TolC pump. This enhances the efflux of toxins over the membrane [19]. Cells that express *marA* during exposure to quinolones have a 100 fold higher chance of survival, than cells that do not express *marA* [19]. It is possible that survival of DNA damage depends on a balance between SulA and MarA activities [19].

Too high a mutation rate could also lead to the generation of egoists that could take advantage of other altruistic cells [19]. Egoists would eventually be able to take over the colony. Reducing the mutation rate would protect the colony from takeover by egoists. A colony of egoists will lose in competition with a colony of altruists exhibiting cooperative behaviour. Bacterial colonies can

protect themselves from merging with other colonies. There is usually a clear zone between colonies; they also protect themselves with a layer of polysaccharide [19]. Formation of biofilms offers protection because they are difficult to penetrate [19].By limiting infiltration by cells from other colonies altruistic bacteria can protect themselves from takeover by egoistic bacteria from another colony. During stationary phase the population of *E. coli* is reduced to about 1% of its original size [19]. In this population mutants arise that can proliferate faster under stationary state conditions. The first mutation that leads to an advantage during stationary state is an allele of *rpoS*. This mutation causes reduced production of catalase, an enzyme that neutralises hydrogen peroxide [19]. Low catalase concentrations during stationary state leads to an increase in mutation rate due to the higher concentration of ROS. These mutants eventually take over the population. While these mutants have an advantage during the stationary state, they are at a disadvantage during conditions in which carbohydrate concentrations are high but amino-acid concentrations low [19]. Fast growing takeover mutants are more susceptible to lethal factors such as antibiotics produced by neighbouring species, while the wild type bacteria with a strongly reduced growth rate acquire a tolerance to lethal factors [19].

Discussion

There are many mechanisms in bacteria that are involved in cell lysis, some cause autolysis others allolysis. Autolysis can be the result of autolytic enzymes that degrade the cell-wall, expression of prophages or of toxin-antitoxin systems. Autolysin dependent programmed cell death can be initiated via several mechanisms such as quorum sensing, decrease in membrane potential and carbohydrate stress. Lysis through prophage expression appears to be dependent on developmental stage of cells in the biofilm. TA systems are activated by nutritional stress and other forms of stress such as antibiotics that inhibit translation. Autolysis, while not beneficial to the individual cell undergoing it, does provide an advantage to the population. Autolysis of a subgroup of cells in a biofilm reduces the strain on the available nutrients. It also provides sibling cells with nutrients that are released when the cell lyses. Autolysis during biofilm development is also important for the generation of extracellular DNA; extracellular DNA plays an important role in the structural integrity of the biofilm. Phenotypic diversity of cells dispersing from the biofilm is also positively influenced by lysis of a subpopulation of cells. Phenotypic diversity is very important for the survival of bacteria, because it allows greater adaptation to different environmental situations. Autolysis is also an important process during spore formation. Lysis of the mother cell during sporulation is necessary to release the spore; it also provides non-sporulating cells with nutrients. In some species such as *M. xanthus* a large subpopulation of cells undergoes programmed cell death which assists in the sporulation process of the remaining cells by providing nutrients. Allolytic events such as cannibalism and fratricide benefit the cells that commit it. By killing sibling cells during cannibalism the cells are able to delay sporulation because of the nutrients released by the lysed cells. Diversity such as sporulating and non-sporulating cells within a population of genetically identical cells has a strong advantage for survival; it ensures that there is always a subgroup of cells that survives. Fratricide of close relatives of a different pherotypes during competence allows cells to absorb DNA that might contain genes that useful for their survival. The presence of many different mechanisms involved in bacterial programmed cell death and lysis indicates that PCD and lysis are evolutionary successful. Bacteria exhibit multi-cellular behaviour in their ability to form biofilms and by undergoing autolysis which benefits the community instead of the individual. Programmed cell death as a result of DNA damage is not beneficial to an individual cell but it is advantageous to a clonal population on a long term. Accumulation of mutations would include detrimental mutations which would result in lower fitness, decreased adaptability and chances of survival. The great number of different mechanisms for programmed cell death and lysis are evidence that these processes play an essential role in bacterial development and survival. A bacterium undergoing programmed cell death for the benefit of the community is not that surprising because bacteria reproduce through binary fission, which results in a colony of clones. The subpopulation of bacteria undergoing autolysis sacrifices itself for a

population of genetically identical cells, and in this way they ensure the propagation of their genes. Programmed cell death greatly increases the adaptability and survival chances of bacteria.

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