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For the greater good: Programmed cell death in bacterial communities

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**ABSTRACT**

For a long a time programmed cell death was thought to be a unique characteristic of higher eukaryotes, but evidence has accumulated showing that programmed cell death is a universal phenomenon in all life forms. Many different types of bacterial programmed cell death systems have been identified, rivalling the eukaryotic systems in diversity. Bacteria are singular, seemingly independently living organisms, however they are part of complex communities. Being part of a community seems indispensable for survival in different environments. This review is focussed on the mechanism of and reasons for bacterial programmed cell death in the context of bacterial communities.

1. An introduction to programmed cell death

In the past, three different types of cell death were identified: necrosis, autophagic cell death and apoptosis (Liu and Levine, 2015; Clarke, 1990). Necrosis was regarded as an unregulated and uncontrollable process. Autophagic cell death or autophagy was considered to cause accidental cell death by over-activity, but is now also thought to promote cellular survival in response to stressors at normal levels. Only apoptosis was considered to intentionally lead to cell death and was therefore also thought to be the sole form of programmed cell death (PCD), where PCD is defined as all genetically encoded (hence, programmed) processes that lead to PCD (Bayles, 2014). It has now become apparent that apoptosis does not tell the complete story of programmed cell death. Purely unregulated necrosis was questioned for more than 15 years, currently there is more and more evidence for controlled necrosis (Berghe et al., 2014) and also for controlled autophagy (Marsh et al., 2007). Multiple pathways leading to programmed cell death have now been identified, such as apoptosis (Green and Reed, 1998), necroptosis or regulated necrosis (Pasparakis and Vandenabeele, 2015), parthanatos, accumulation of specific proteins as a consequence of genomic stress (Fatokun et al., 2014), autosis or regulated autophagy (Liu and Levine, 2015) and ferroptosis, an iron-dependent form of cell death (Dixon et al., 2012).

PCD in multicellular life is essential for four processes: (1) sculpting, of which an example is the formation of the interdigital regions in mammals (Jacobson et al., 1997), (2) removal of unwanted structures, a process which is for example very important during metamorphosis of a tadpole to a frog (Jiang et al., 1997; Nishikawa and Hayashi, 1995), (3) controlling cell numbers, by killing cells after they have served their purpose as happens with T-cells after the infection has subsided (4). Eliminating non-functional or harmful cells, by killing cells infected with a virus or cells damaged beyond repair (Ju et al., 1995; Cohen, 1991). PCD is therefore an important tool in the toolbox of multicellular life.

Programmed cell death intuitively seems to be the domain of multicellular organisms, as a single cell cannot benefit from their own demise. PCD was therefore long thought to be a unique characteristic of multicellular life, however there is increasing evidence showing that PCD is universal for all known forms of life (Berghe et al., 2014). The question therefore remains: why does programmed cell death exist in bacteria? The evidence and reasons for programmed cell death in bacteria are summarized in this review.

2. Uni- and multicellular life of bacteria

One of the main reasons for which programmed cell death in bacteria seems counterintuitive is the unicellular nature of bacteria. However, although many bacteria live as singular units, many species also spend part of their lives as part of a complex community, others have embraced multicellular lifestyles and have abandoned unicellular growth as reviewed by Claessen et al. (Claessen et al., 2014). To organize this type of multicellularity, organisms first have to cluster to become a coherent whole. In bacteria, this can be achieved in multiple ways, for example by aggregation in the form of biofilms (Hall-Stoodley et al., 2004), by incomplete cell fission after cell division resulting in chains of cells, a feature of filamentous cyanobacteria (Flores and

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forms of life, as can be demonstrated in the case of biological systems. When the three rules of autopoiesis apply to a physical object, it can be considered either a collective that is autopoietic or multicellular life and thus alive is not universally accepted (Luisi, 2003). Regardless, Maturana & Varela originally conceived the idea of autopoiesis in the context of biological systems. In a further development, Thompson further defines autopoiesis as follows (Thompson, 2007; Varela et al., 1974): (I) A semi-permeable barrier that separates the inside from the outside. (II) A network of reactions, where the components of this network are produced by the reactions within this network. (III) The barrier and the network are interdependent, where the barrier is produced by the network, and the network is regulated by the conditions created by the barrier. Physical systems which are alive are thought to adhere to these rules for autopoiesis may be applied. Maturana & Varela originally conceived the idea of autopoiesis in the context of biological systems. In a further development, Thompson further defines autopoiesis as follows (Thompson, 2007; Varela et al., 1974): (A) there is a semi-permeable barrier that separates the inside from the outside. (B) There also has to be a network of reactions, where the components of this network are produced by the reactions within this network. (C) The barrier and the network have to be interdependent, where the barrier is produced by the network, and the network is regulated by the conditions created by the barrier. Physical systems which are alive are thought to adhere to these rules and are therefore automatically thought to be autopoietic (Fleischaker, 1988). The reciprocal idea that something is autopoietic and thus alive is not universally accepted (Luisi, 2003). Regardless, when the three rules of autopoiesis apply to a physical object, it can be seen as alive.

When the three rules are applied to bacterial communities they can be considered either a collective that is autopoietic or multicellular life forms of life, as can be demonstrated in the case of a biofilm. Biofilms have a semi-permeable barrier in the form of the extracellular matrix. (Rule A) and contain a network of reactions maintaining the extracellular matrix, where the reactions are performed by the bacteria contained within the biofilm (Rule B). The biofilm barrier and this network are also interdependent, where the extracellular matrix is produced by the bacteria and the bacteria are regulated by the conditions created by the extracellular matrix (Rule C). Bacterial communities, such as can be found in biofilms, can therefore also be considered as multicellular, as these also conform to the rules of an autopoietic system (Fig. 1a). A biofilm is composed of bacteria as first order autopoietic subunits, and the biofilm itself can then be considered to be a second order autopoietic system. Biofilms as multicellular and biofilms as autopoietic units is not a new concept when viewed in the context of autopoiesis (Luisi, 2003).

Bacterial “multicellularity” is different from eukaryotic multicellularity in two striking ways: it can be transient and patchy (Claessen et al., 2014). It can be transient, as many bacteria exist in a multicellular state such as in filamentous cyanobacteria (Claessen et al., 2014). It can be transient, as many bacteria exist in a multicellular state such as in filamentous cyanobacteria (Claessen et al., 2014). Multicellularity in bacteria can also be patchy, because not all members of a bacterial autopoietic system have to be of the same species, which is the case in many biofilms (Stewart and Franklin, 2008). The patchy nature of biofilms is promoted by the electrical signalling of bacteria within the biofilm that non-specifically attracts bacteria from outside the biofilm, but also by the micro-environments created by the consumption of nutrients and oxygen (Humphries et al., 2017; Stewart and Franklin, 2008).

3. Mechanisms of bacterial programmed cell death

Many different types of bacterial programmed cell death have been identified, rivalling mammalian eukaryotes in diversity. Similar to eukaryotes, these systems range from very well studied to obscure.

3.1. MazEF toxin-antitoxin system mediates cell death

Almost all organisms contain genes that have been identified to inhibit cell growth when expressed. The most well studied type of these are toxin-antitoxin (TA) systems, which are widely found in both bacteria and archaea (Sevin and Barloy-Hubler, 2007). Multiple types of
toxin-antitoxin systems have been identified and are classified by their mode of action, from type I to V. For an excellent review of bacterial TA systems see the review by Wen et al. (2014). A TA system consists of a stable toxin and a degradation prime antitoxin encoded in an operon with tight co-transcription of both the toxin and anti-toxin. They were first discovered as a plasmid addiction mechanism (Gerdes et al., 1986; Masuda et al., 1993). When a bacterium loses such a plasmid, the cell dies due to the action of the stable toxin after the degradation of the unstable antitoxin.

TA systems contribute to the regulation of bacterial survival through control of growth, biofilm formation, general stress response and persistence (Anantharaman and Aravind, 2003; Wen et al., 2014). Persistence has been described as the ability of a few cells in a homogeneous population to survive different types of stressors and manifests in the form of metabolic stasis, in which state the cell is metabolically inactive making it insensitive to stressors such as antibiotics. Populations of Escherichia coli TA system mutants have a greatly reduced numbers of persisters, indicating that these systems play an important role in the formation of persisters (Gelen et al., 2013).

Apart from a role in persistence and biofilm formation, some of the TA systems have been shown to be directly involved in PCD. The type II toxin-antitoxin system MazEF is widely found in the bacterial kingdom (Engelberg-Kulka et al., 2005), for example in species such as Lactobacillus plantarum, Staphylococcus aureus, Mycobacterium tuberculosis and Escherichia coli. The MazE antitoxin is a labile protein that neutralizes the MazF toxin, and under certain conditions is degraded by the ClpAP protease. In E. coli, the MazEF mediated cell death pathway is triggered when the cell is challenged with stressors, such as the alamarone ppGpp, severe amino acid starvation (Marimonovsky et al., 2001), DNA damage and quorum-sensing signalling factor (extracellular death factor or EDF), a linear pentapeptide (NNWNN) produced by ClpXP (Hazon et al., 2004; Kolodkin-Gal et al., 2007). These stressors either trigger mazEF directly, or trigger mazEF indirectly by a reduction in global transcription and translation which allows the labile antitoxin to be degraded, releasing the toxin (Fig. 2, Table 1).

The MazF toxin is an mRNA interference, which inhibits protein synthesis by preferentially cleaving mRNAs at specific ACA or ACU sequences (Engelberg-Kulka et al., 2005). Due to the degradation of mRNA, the cells are thought to first enter a state of metabolic stasis, until a point of no return is reached and the effects go from bacteriostatic to bactericidal. The metabolic stasis also explains why these systems form persisters as discussed previously. Interestingly, the synthesis of a subset of proteins (10%) are not affected by induction of the MazEF module (Vesper et al., 2011). These are grouped into two classes coined “death proteins” that are required for the death of the majority of the population and “survival proteins” that are required for the survival of a small subset of the population (Amaiti et al., 2009).

The survival proteins are mostly responsible for the protection against reactive oxygen species (ROS). The death proteins cleave the MazE antitoxin, are involved in RNA processing and some death proteins protect other ROS-sensitive proteins from ROS. The selective translation of survival and death mRNAs upon MazF activation relies on the cleavage of 16S ribosomal RNA by MazF on an ACA site. This cleavage results in the loss of the rRNA anti-Shine and Dalgarno sequence that is required for initiation of canonical translation. This 3’-truncation leads to the formation of stress ribosomes that selectively translate leaderless mRNAs (Temmel et al., 2016). Upon stress relief the modified ribosomes no longer serve a purpose. The RNA ligase RtcB has been shown to regenerate these ribosomes by ligating the cleaved RNA to the modified ribosome, restoring standard translational proficiency of the ribosomes (Temmel et al., 2016). The balance between MazF and RtcB is therefore thought to determine the ratios of truncated and unmodified ribosomes (Temmel et al., 2016).

MazEF induced PCD therefore happens in a few steps: (1) expression of MazEF is induced by an increase in the alamarone ppGpp upon severe amino acid starvation and DNA damage. (2) MazF cleaves RNA at specific sites, reducing transcription and translation of essential proteins, producing leaderless mRNA and causes the formation of stress ribosomes that selectively translate leaderless mRNAs. (3) The resulting proteins are required for survival in a small subset of the population or required for death in the majority of the population. Death is eventually caused by ROS and DNA damage.

The formation of specialized ribosomes is not unique. Ribosomes can be modified in function by posttranslational modification (acetylation, phosphorylation, etc.), through ribosome associated factors and the expression of different versions of rRNA genes during different phases of life (Xue and Barna, 2012). The MazEF system is unique in that it modifies the specificity of the ribosomes to prefer leaderless transcripts. A system like MazEF does not exist in eukaryas, as no toxin-antitoxin systems exist in eukaryotic life. There are however parallels between bacteria and eukaryas in the initiation and effector functions of the PCD pathways. MazEF mediated PCD is induced by alamarone ppGpp, which is analogous to the “death ligand” tumor necrosis factor (TNF) in apoptosis and necroptosis or the beclin-1-autophagic pathway in autosis in mammalian eukarya (Qu et al., 2003; Vandenabeele et al., 2010; Pasparakis and Vandenabeele, 2015; Green and Reed, 1998). In addition, DNA damage can lead to PCD in both bacteria via the MazEF-pathway in and in eukarya via the intrinsic pathway of apoptosis (Green and Reed, 1998). Initiation of PCD in eukaryotes and bacteria is therefore similar in that both can be induced by a dedicated ligand or by DNA damage. MazEF mediated cell death seems almost accidental, and occurs only after a point of no return. It that sense, this type of PCD is similar to autophagy, which is hypothesised to be a self-preserving process that is taken too far (Liu and Levine, 2015; Marsh et al., 2007).

3.2. The PezAT toxin-antitoxin mediates cell death

The PezAT toxin-antitoxin operon is found on pneumococcal pathogenicity islands together with antibiotic resistance and virulence factors (Harvey et al., 2011). The PezA antitoxin is a labile protein that neutralizes the toxin PezT until certain conditions result in the degradation of the antitoxin. The PezT mechanism of action was a mystery, until Mutschler et al., showed that PezT directly targets cell wall synthesis by expressing PezT in E. coli (Mutschler et al., 2011). They showed that PezT phosphorylates the metabolite UDP-N-acetylmuramylpentaglycaminosine (UNAG), which is an essential precursor in muropeptide synthesis. The modification creates a stable product that can no longer be used for muropeptide synthesis and then accumulates intracellularly. In addition phosphorylated UNAG inhibits cell wall synthesis enzyme MurA, required for the first committed step in peptidoglycan synthesis. PezT therefore causes the cell wall to lose integrity and if PezT persists the cell eventually lyses.

The bacterium S. pneumoniae expresses a cytosolic pneumolysin that can only be released through lysis. Based on this finding Mutschler et al., hypothesise the PezAT operon may be used as an additional option in S. pneumoniae in order to lyse the cell and release the pneumolysin into the environment. If this is true, then the ultimate end result of the PezAT operon is improved fitness of the rest of the population through lysis of human host cells by release of the pneumolysin (Mutschler et al., 2011). Later Chan et al., found that using PezAT in the natural host and under physiological conditions causes a decrease in competence and a decreased resistance against β-lactam antibiotics. They therefore hypothesized that S. pneumoniae has traded off survivability for an increased virulence (Chan and Espinosa, 2016).

Just like the MazEF system when the stress is temporary the cells can recover and even under longer term stress persisters may survive and can then be selected for (Mutschler et al., 2011; Engelberg-Kulka et al., 2005). The comparisons between mammalian and PezAT PCD are similar enough to the comparison between MazEF and mammalian PCD to not warrant much elaboration, with the exception of the absence of a specific inducing signal like the alamarone ppGpp. Any comparisons between that are dependent on an extracellular inducing signal
3.3. Apoptosis-like cell death is recA mediated

Different bactericidal antibiotics induce apoptotic hallmarks such as DNA fragmentation, chromosomal condensation, exposure of phosphatidylserine to the outer leaflet of the plasma membrane, dissipation of membrane potential, rRNA degradation by an endoribonuclease, upregulation of extensive-damage-induced (Edin) genes, a decrease in the activities of complexes I and II of the electron transport chain, and the formation of high levels of superoxides, which in turn damage iron-sulfur clusters freeing up ferrous iron for the Fenton-reaction (Erental et al., 2014), producing OH*.

The OH* contributes to additional double strand (DSB) DNA breaks, further activating RecA and possible resulting in cell death (Erental et al., 2014). PezAT mediated cell death is induced by general inhibition of transcription which allows the labile antitoxin PezA to be degraded while the toxin PezT persists (Chan and Espinosa, 2016; Mutschler et al., 2011). The PezT toxin phosphorylates UNAC, a muropeptide precursor resulting in the buildup of intracellular UNAC-P. In addition to substrate depletion, cell wall synthesis is also inhibited because UNAC-P directly inhibits MurA, the enzyme responsible for the first committed step in peptidoglycan synthesis (Mutschler et al., 2011).

Therefore do not apply.
RecA mRNA degradation through MazF activation. The crosstalk between the ClpXP protease is thought to indirectly inhibit ALD by stimulating the ALD pathway. Cells that survive MazEF-mediated cell death are likely resistant against ROS and DNA damage. Cells that survive ALD are likely resistant against a much larger variety of stressors, as this pathway is proposed mechanisms: 1) Controlling the release and activation of murein hydrolases, or mediating the translocation murein hydrolases across the cytoplasmic membrane both via cidA insertion into the membrane (Ranjit et al., 2011; Rice et al., 2003; Zhang, 2016). High population densities lead to high levels of CSP (sensed by comDE) and to an increased expression of CipB, which is autolytic (Dufour and Lévesque, 2013; Perry et al., 2009). The exact mechanism is unknown, but involves the activation of amidases and results in cell cycle arrest after chromosome segregation but before constriction (Dewachter et al., 2017).

Table 1

<table>
<thead>
<tr>
<th>Genes</th>
<th>Triggers</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>masEF</td>
<td>Alarome ppGpp (Aizenman et al., 1996), amino acid starvation (Ahmad et al., 1996), DNA damage (Hazen et al., 2004), extracellular death factor (Kolodkin-Gal et al., 2007), others (Hazen et al., 2004; Pedersen et al., 2003).</td>
<td>Stress inhibits transcription and translation, which allows the labile antibiotic MazE to be degraded and frees the toxin MazF. MazF cleaves RNA and leads to the formation of a subpopulation of ribosomes which cause cell death through the selective translation of death proteins (Engelberg-Kulka et al., 2005; Erental et al., 2012).</td>
</tr>
<tr>
<td>recA</td>
<td>DNA damage, reactive oxygen species, mutations in MazEF pathway components (Bell and Kowalczykowski, 1998; Erental et al., 2012; Erental et al., 2014).</td>
<td>Stress triggers RecA, which deactivates LexA while activating induction of din and Edin genes. Transcription of din genes leads to cell cycle arrest and DNA repair, while Edin genes cause cell death through the generation of reactive oxygen species (Bell and Kowalczykowski, 1998; Erental et al., 1998; Erental et al., 2014).</td>
</tr>
<tr>
<td>pezAT</td>
<td>General transcription/translation inhibition (Chan and Espinosa, 2016; Mutschler et al., 2011).</td>
<td>Stress causes a decrease in general transcription and translation, which allows the labile antibiotic PezA to be degraded, freeing PezT. PezT phosphorylates UNAC, a muropeptide precursor and inhibits the enzyme MzaA, required for the first committed step of peptidoglycan synthesis (Mutschler et al., 2011).</td>
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<tr>
<td>cidA/lrgA (holin/antiholin)</td>
<td>The operon is induced by acetic acid (Rice et al., 2005). Cell death is induced by membrane depolarization and changes in carbohydrate metabolism (Zhang, 2016).</td>
<td>Controlling the release and activation of murein hydrolases, or mediating the translocation murein hydrolases across the cytoplasmic membrane both via cidA insertion into the membrane (Ranjit et al., 2011; Rice et al., 2003; Zhang, 2016).</td>
</tr>
<tr>
<td>cipB/CSP-comDE</td>
<td>High population densities and high concentration of CSP (Perry et al., 2009; Dufour and Lévesque, 2013).</td>
<td>The exact mechanism is unknown, but involves the activation of amidases and results in cell cycle arrest after chromosome segregation but before constriction (Dewachter et al., 2017).</td>
</tr>
<tr>
<td>obgE*</td>
<td>Disruption of cell division (Dewachter et al., 2015; Dewachter et al., 2017).</td>
<td>The exact mechanism is unknown, but involves the activation of amidases and results in cell cycle arrest after chromosome segregation but before constriction (Dewachter et al., 2017).</td>
</tr>
</tbody>
</table>

the ClpXP protease is thought to indirectly inhibit ALD by stimulating the RecA mRNA degradation through MazF activation. The crosstalk between these two PCD pathways is very interesting. What situations would determine the use of one or the other? Erental et al. (Erental et al., 2012) hypothesised that the ALD pathway is meant to kill individuals that lose the MazEF pathway through mutation. They state that these so-called “cheaters” would be cheating themselves, as the loss of the MazEF system would also result in the loss of ALD inhibition. They also hypothesise that the MazEF pathway is preferred over the ALD pathway, which they base on the type of stressor that initiates each pathway. Cells that survive ALD are likely resistant against ROS and DNA damage. Cells that survive MazEF-mediated cell death are likely resistant against a much larger variety of stressors, as this pathway is triggered by a wider variety of stressors. It is therefore likely that the population benefits from using the MazEF pathway over the ALD pathway, as survival from MazEF implies resistance against a larger number of stressors than survival from the ALD pathway. Erental et al. therefore suggest that the ALD pathway works on the individual level, as opposed to the MazEF pathway that they suggest works on the population level.

3.4. cidA/lrgA holin-antiholin mediated cell death

Two homologous operons in S. aureus encoding putative holin-like proteins, designated cid and lrg, have shown to contain pro-apoptotic and anti-apoptotic effector functions (Fig. 3, Table 1), much like the BCL-2 protein family in mammalian eukaryotes (Rice et al., 2003; Groicher et al., 2000; Ranjit et al., 2011). The cidABC and lrgAB operons have been shown to regulate death and lysis through one of the two proposed mechanisms: 1) Controlling the release and activation of membrane-associated murein hydrolases or 2) Mediating the translocation of murein hydrolases across the cytoplasmic membrane (Rice et al., 2003). The CidA gene encodes for a holin, which has a stimulating effect on death and lysis, while the LrgA gene that encodes for an antiholin that blocks death and lysis (Ranjit et al., 2011). The cidA/lrgA operon is triggered by acetic acid, but it is not clear whether the cell death pathway dependent on the genes in this operon is also activated by acetic acid (Rice et al., 2005). The operon has been shown to play a role in biofilm integrity in S. aureus through PCD mediated DNA release (Mann et al., 2009). It is therefore likely that the operon is controlled by a quorum sensing mechanism, where PCD is induced as a function of population density in the biofilm.

The role of Cid/Lrg as holin/antiholin puts bacteriophages in a very interesting position. The classification of Cid/Lrg as holin and antiholin suggests that these genes are of viral origin, which would mean that the bacteriophage has provided bacteria with a means to control the phage infection through controlled cell suicide by expressing these genes (Ranjit et al., 2011).

Strikingly, there are functional parallels between eukaryotic BCL-2 proteins and the Lrg/Cid operons (Ranjit et al., 2011). Both the holins and the BCL2 family of proteins have been shown to oligomerise upon insertion in the membrane (Smith et al., 2016; Zagotta and Wilson, 1990). Both the holins and the BCL2 family are regulated by homologous proteins (Pang et al., 2011). Insertion of these proteins into a membrane leads to membrane depolarisation and activation of proteins causing cellular disassembly (control of murein hydrolases in bacteria and caspases in eukaryocytes) (Elmore, 2007; Ranjit et al., 2011; Pang et al., 2011). The similarities are more fundamental than functional similarities, as eukaryotic apoptotic effectors BAX and BAK were shown to oligomerize in the E. coli membrane and were able to induce death and lysis (Pang et al., 2011). Finally, the native holin in a lambda bacteriophage was replaced by the gene that encodes BAK, resulting in fully functional infectious bacteriophage particles (Pang et al., 2011). Combining these findings, the authors hypothesise that BCL2 may function as a holin (Ranjit et al., 2011).

3.5. cipB-mediated cell death

The bacteriocin CipB is required for altruistic lysis in Streptococcus mutans biofilms (Perry et al., 2009). Lysis occurs after the intracellular accumulation of bacteriocin CipB, which is hypothesised to function similarly to other bactericides by depleting the membrane potential by creating pores or channels and therefore causing cell death by cellular energy depletion (Hossain and Biswas, 2011). CipB is inhibited by CipI sequestration (Dufour et al., 2011). This CipI/B system is under control of the ComDE and LiaSR regulatory mechanisms and is summarized in Fig. 3. At low population densities CipI is up-regulated through the...
action of the quorum sensing LiaSR two-component signal transduction system, protecting the cell against CipB via sequestration of CipB (Dufour et al., 2011; Dufour and Lévesque, 2013). Due to high population densities in biofilms the competence-stimulating peptide (CSP) is present in a high concentration. The quorum sensing ComDE two-component signal transduction system senses CSP and up-regulates CipB in response, leading to CipI saturation. CipB can then act by forming pores or channels, depleting the membrane potential and causing cell death or metabolic stasis by cellular energy depletion (Perry et al., 2009; Dufour and Lévesque, 2013). CidA mediated cell death is thought to be initiated by high population density, which would then activate CidA to translocate to the cytoplasmic membrane allowing murein hydrolases to pass into the periplasm, degrade peptidoglycan, and lead to cell lysis by weakening the cell wall (Rice et al., 2003). (ObgE*) The ObgE GTPase is thought to link and coordinate many cell division processes (Morimoto et al., 2002). Expression of mutant ObgE leads to a highly coordinated process leading to cell death with chromosome condensation, DNA fragmentation, and membrane depolarization, exposure of phosphatidylserine on the cell surface and the formation of membrane blebs (Dewachter et al., 2017). The exact mechanism of ObgE induced cell death is unknown, but it has been suggested that disruption of cell division may lead to ObgE to display the ObgE* phenotype (Dewachter et al., 2017).

Just like in cidA/lrgA-mediated cell death, this type of altruistic behaviour may provide surviving cells with nutrients or provide stability to the biofilm through structural DNA in the biofilm. Different from the holins is that a state of metabolic stasis precedes cell death. In this state, the cells are extremely resistant against antibiotic effects (Maisonneuve and Gerdes, 2014). This pathway may therefore also
serve as a mechanism for persistence. Even more interesting is that this pathway has been implicated in allogyis in *Streptococcus pneumoniae*, where lysis is induced by the non-competent bacterial population by a group of competent and epigenetically different cells of the same species. This is thought to provide the DNA required for natural transformation (Claverys et al., 2007).

3.6. obgE*-mediated cell death

There are many genes involved in bacterial cell division, one of which is the GTPase encoding *obgE* (Verstraeten et al., 2011). *ObgE* is a cell cycle checkpoint protein that links together ribosome assembly, the stringent response, DNA replication and chromosome segregation during cell division (Persky et al., 2009). Expression of a *ObgE* mutant (K268I, from now on ObgE*) in *E. coli* led to a highly coordinated cell death process with chromosome condensation, DNA fragmentation, membrane depolarization, exposure of phosphatidylserine on the cell surface and the formation of membrane blebs (Dewachter et al., 2015), summarized in Fig. 3. The phenotype is therefore strikingly similar to that of apoptosis and ALD (Elmore, 2007; Erental et al., 2014). The exact pathway leading to this phenotype is unknown, although it was confirmed to be MazE and RecA independent (Dewachter et al., 2015).

At first glance, this appears to be a mutant that causes cell death. Not very special, as there are many mutants that have the same effect. What makes this mutant interesting are the similarities in cell death phenotype with apoptosis and the gene involved: *obgE* is a conserved gene that is also present in higher eukaryotes. The human gene with the highest homology is *obgH1*, which localizes to the mitochondria and is essential in the intrinsic pathway of apoptosis (Hirano et al., 2006). Based on this De Wachter et al. hypothesised that there may be an evolutionary link between *ObgE*-mediated PCD and apoptosis (Dewachter et al., 2015). Further strengthening this hypothetical link is the function of *ObgE* as a cell cycle checkpoint protein. Eukaryotic cell cycle regulators can also induce PCD when cell division is disturbed, similar to the situation with *ObgE* (Dewachter et al., 2017). This therefore indicates that the cell cycle regulator *ObgE* in *E. coli* may play a similar role to cell cycle regulators in eukarya.

4. Why bacterial programmed cell death exists

Now that the knowledge on the existence of PCD in bacteria has been established, the question remains: what advantage does it have to maintain genes involved in suicide in populations consisting of unicellular organisms? In short: What are the advantages of bacterial programmed cell death?

4.1. The advantages of death

In the first section we argue that most bacteria are, at least temporarily, part of a multicellular system. Considering that the individual cannot benefit from PCD the advantage is gained by the larger multicellular structure in which the individual take part. The absence of PCD during the unicellular phase of bacteria may then also be explained, as the bacterium is not part of a multicellular structure there is no benefit from PCD. This absence could also be explained by a research bias, as micro-organism are almost invariably studied when part of a group.

Multicellularity and PCD are in fact intrinsically linked according to some models. These models show that under viral pressure, the acquisition of PCD and multicellularity must coincide, as one without the other is not viable (Iranzo et al., 2014). In the absence of pathogens the faster unicellular reproduction is favoured. By contrast, in the presence of pathogens clusters of cells benefit from PCD as a defence mechanism additional to immunity.

Apart from defence against a viral attack, there are also other advantages of PCD. The death of a cell can provide nutrients for others an example of this is *Myxococcus xanthus*, which has swarming cells that move as a coordinated assembly on the hunt for prey or other substrates (Berleman et al., 2006). When nutrients are depleted the formation of aggregate centres is initiated, ending in the formation of fruiting bodies. Some of the surrounding cells undergo PCD and are thought to provide nutrients for the remaining nutrient deprived cells. Biofilms are also thought to be a scene for PCD as a means for nutrient generation through both decreasing the population density (increasing the amount of nutrients per cell) and by releasing nutrients via lysis that can provide nutrition for the remaining population (Monds and O’Toole, 2009; Bayles, 2014).

Instead of providing immunity or nutrients, PCD can also be used as a tool for construction. For example, in *Staphylococcus aureus* biofilms PCD plays an important structural role and genomic DNA from this bacterium is present in an ordered pattern in *S. aureus* biofilms. The DNA is thought to be a product of cell lysis that is mediated by PCD as a function of the cells spatial location in the biofilm (Perry et al., 2009). This DNA is a structural component of the biofilm matrix, as treatment with DNAse weakens the biofilm. A *S. aureus* *cidA* mutant is lysis defective and has a weaker biofilm that is not affected by DNAase treatment, indicating that cell lysis is required for DNA release. Less than one percent of cells undergo lysis during biofilm development, indicating that this small fraction provides a quantity of DNA that is enough for biofilm stability (Perry et al., 2009; Mann et al., 2009; Rice et al., 2007). PCD therefore plays an integral role in the development and stability of a biofilm.

PCD can also be used as a way to gain an evolutionary edge. The human pathogen *Streptococcus pneumoniae* has been shown to practice heterolysis/allogyis (lysis of a bacterial cell by another cell), instead of autolysis (lysis caused by the cell itself) (Claverys et al., 2007). During competence development, the population of competent cells kill the population of non-competent *S. pneumoniae* cells, releasing chromosomal DNA that may be used for natural transformation (Steinmoen et al., 2009). This phenomenon of killing related cells has been coined “pneumococcal fratricide” and the pathway is mediated by the ComCDE regulatory system (Moscoso and Claverys, 2004), discussed in the section “Mechanisms of bacterial programmed cell death”. This bacterium therefore induces programmed cell death in siblings to gain an advantage on an individual and population level.

4.2. Programmed cell death in bacteria and mammalia

When linking these findings to the mammalian function of PCD, many similarities can be seen. As previously noted, PCD in multicellular life seems to be essential for tissue development and immunity and for host survival. The development of eukaryotic tissue can show similarities to the formation of a bacterial biofilm, as a group of cells together form a fairly fixed unit that collectively perform some function. The role of PCD in biofilm formation and stability therefore echoes the function PCD has in eukaryotic tissue development. If we assume that bacteria can be considered (transiently) multicellular, PCD plays a crucial role removing infected members of the multicellular system. PCD in eukarya and bacteria therefore plays a similar role in preventing the spread of a bacteriophage throughout the population by selective killing of the infected cells. Both options provide the bacterial population (but not the individual) with improved survival.

An important difference between multicellular eukarya and bacteria is PCD as a means of nutrient acquisition. In multicellular eukarya, the release of nutrients seems a beneficial secondary effect of PCD, but not the main goal. PCD for nutrients does seem to be performed in early stage autophagy, but only for the cell in question and not for the surrounding cells. In bacteria nutrient acquisition via PCD can be the main goal and not a secondary effect. This difference reflects a crucial divide between multicellular life and unicellular life. Multicellular life (and certainly mammals) require most of the tissues to be maintained, otherwise the organisms will succumb and die. Although bacteria can form multicellular structures, they are not dependent on certain tissues.
for survival. The death of all but one bacterial cell does therefore not spell doom for the bacterium, which is definitely not the case for most multicellular life, and may even be the starting point for a new population. Bacteria can therefore afford to use PCD for nutrients, as opposed to multicellular eukarya, who (mostly, disregarding sponges) cannot.

Another big difference between PCD in eukarya and bacteria is the use of and uses for DNA. When PCD occurs, the release of DNA is inevitable. In bacteria PCD is used to free DNA for biofilm formation and structural stability, and may also be freed by alloysis and used for natural transformation. The release of DNA can therefore be a goal in and of itself and PCD is a means to reach this goal. In mammalian eukarya the DNA released by PCD plays a role in immunity, but is not the raison d’etre for PCD. The difference between bacteria and eukarya is therefore that the release of DNA via PCD in bacteria is the goal, and in eukarya it is a side effect and not the main goal. Based on the information above, the question “what advantage does it have to maintain genes involved in the suicide of the free-living individual?” can be answered. In short: The advantages of maintaining suicide genes are mostly for population as a whole and not for the free-living individual. The genes are therefore maintained as evolution works on populations and not on individuals.

4.3. Using programmed cell death

The emergence of antibiotic resistant bugs and the presence of these suicide pathways in bacteria immediately lead to an important question: can these pathways be exploited for antimicrobial therapy? The study of suicide genes involved in these pathways may result in the identification of new and yet unexpected antibiotic targets not to kill by inhibiting a cellular process, but by stimulating a PCD pathway. Current literature shows that although antibiotics exist that cause cell death through the activation of PCD pathways, they do not target these pathways directly. Examples are the MazEF and RecA mediated cell death pathways. Activation of the MazEF pathway is induced by bêta-cycloluporins, aminoglycosides and rifampicin as a consequence of transcription and translation inhibition (Sat et al., 2001; Hazan et al., 2004). The RecA pathway is activated by bacterialicidal antibiotics such as the â-lactam antibiotics and fluoroquinolones (Dwyer et al., 2012). These antibiotics have been shown to lead to an increase in cytosolic DNA release by inhibiting gusA (corrected) and S-biprophosphate synthase in E. coli. A model for programmed bacterial cell death. Proc. Nat. Acad. Sci. 93, 6059–6063. Amitai, S., Koldokin-Gal, I., Hananya-Meltzabi, M., Sacher, A., Engelberg-Kulka, H., 2009. Escherichia coli MazF leads to the simultaneous selective synthesis of both bacterialicidal antibiotic proteins and survival proteins. PLoS Genet. 5, e1000390.


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