HHS Public Access

Author manuscript

Bioessays. Author manuscript; available in PMC 2017 April 01.

Published in final edited form as:

Bioessays. 2016 April ; 38(4): 306–315. doi:10.1002/bies.201500132.

Tissue repair in myxobacteria: a cooperative strategy to heal cellular damage

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Summary

Damage repair is a fundamental requirement of all life as organisms find themselves in challenging and fluctuating environments. In particular, damage to the barrier between an organism and its environment (e.g., skin, plasma membrane, bacterial cell envelope) is frequent because these organs/organelles directly interact with the external world. Here we discuss the general strategies that bacteria use to cope with damage to their cell envelope and their repair limits. We then describe a novel damage-coping mechanism used by multicellular myxobacteria. We propose that cell-cell transfer of membrane material within a population serves as a wound-healing strategy and provide evidence for its utility. We suggest that – similar to how tissues in eukaryotes have evolved cooperative methods of damage repair – so too have some bacteria that live a multicellular lifestyle.

Keywords

damage dilution; damage repair; lipopolysaccharide; myxobacteria; outer membrane exchange; rejuvenation

Introduction

Multicellularity serves as the cornerstone for the specialization of cells and tissues that function synchronously for the benefit of the organism. Multicellularity also necessitates resource sharing between cells. Each cell contributes to the organism's success by the use of communication and cooperation networks with other cells. This includes a concerted response to cellular or tissue damage during which multiple cells, through signaling and response pathways, organize a wound repair program. Cooperation provides an advantage over independent cells responding to their own survival, as resources can be allocated from healthy to damaged tissue. Although the multicellular contribution to wound repair in higher eukaryotes is widely recognized [1,2], its contribution to damage repair in cooperative bacteria is largely unexplored.

Like all organisms, bacteria must maintain a highly controlled compartment that is separated from their environment and must sustain homeostasis in the face of external stresses.

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Successful organisms have evolved the ability to adapt to common stressors and to actively repair molecular- and macro-scale damage. Radiation, desiccation, osmolality, pH, temperature, metabolic errors, predators, reactive molecules and physical insults all threaten the integrity of the individual. Molecular and structural damage caused by such stressors must be either avoided or repaired to prevent fitness loss. For example, damage to DNA is an immediate threat to the fitness of the organism and is also heritable. For this reason, cells devote many resources to the repair of DNA, which has been well studied [3] and will not be discussed further here. However, damage can occur to every component of the cell, and maintaining health in response to this damage is an essential feature of the cell.

Although a number of self-repair mechanisms that address non-DNA damage have been described in bacteria, their ability to carry out repairs has limits. Moreover, it has been postulated that it would be impossible for a cell to address each type of damage with a dedicated repair system, because there are too many forms of damage [4]. As a result, damage slowly accumulates during an organism's lifetime. This notion helps to explain the observed phenomenon of cellular aging—the gradual loss of cell function and the inability to recover from damage inflicted by stress that ultimately leads to senescence or cell death. In recent years, aging has been described in single-celled organisms that reproduce asymmetrically or symmetrically by binary fission [5,6]. This finding implies that the known bacterial stress response systems that shield or repair cell damage are incomplete and thus damage accumulates over time. For single-celled organisms this necessitates other strategies such as damage segregation or shedding [7–9], although these strategies are not ideal (see below). Alternatively, multicellular bacteria can potentially share resources to aid damaged kin and to distribute a damage burden, making it plausible that multicellular repair mechanisms have evolved among certain bacteria. Until recently this possibility was unexplored.

We found that myxobacteria can use a cooperative strategy to cope with damage to their outer membrane (OM) [10]. Myxobacteria live in groups that move and function as a single unit with synchronized behaviors, including the formation of macroscopic multicellular fruiting body structures [11]. Because their main habitat is soil, they are exposed to many insults that can damage their cell envelope. We have reported the curious finding that myxobacteria fuse their OMs and exchange membrane proteins, lipids and lipopolysaccharide (LPS) [12]. Importantly, this fusion and exchange of contents can support the life of otherwise lethally damaged cells in a population [10]. Aside from the ability to exchange protein and LPS material to complement certain mutant phenotypes [13], we have also directly shown that fluorescently labeled lipids and proteins can be transferred between cells [14,15]. From these results we propose the following as a central thesis of this essay: OM exchange (OME) is used as a cooperative multicellular behavior to replenish cells with critical components and to dilute damaged factors among the population to allow cell damage to be buffered. In turn this might increase the efficiency of how damage components are repaired, recycled and/or removed. In other words, the nature of multicellularity provides a platform for ailing cells to be supplied with healthy components from other cells, and this facilitates repair. Indeed, direct cell-to-cell content sharing has been shown to play a role in eukaryotic stress and damage coping mechanisms [1,2]. By analogy, we propose that OME in myxobacteria acts as a multicellular wound-healing

strategy by infusing healthy membrane and diluting damaged material at the wound site, and we discuss the implications in terms of multicellular bacterial repair.

Bacterial cell envelopes are susceptible to damage

Molecular damage can occur to all structures of a cell; however, in this essay we focus primarily on damage inflicted to the cell envelope of gram-negative bacteria. The cell envelope is a key organelle because it serves as a protective barrier to the cytoplasm, and its location puts the envelope in harm's way as it is directly exposed to insults from the external world [16]. The envelope not only functions as a barrier but also senses and relays extracellular information to the cytoplasm, provides structural integrity to the cell and is the gateway for nutrient acquisition. The canonical envelope consists of the inner membrane, the cell wall and the OM [16]. The OM is an asymmetric bilayer made up of LPS in the outer leaflet and phospholipid in the inner leaflet. This membrane houses lipid-anchored lipoproteins and integral membrane proteins that typically assume a β-barrel fold. In addition, large protein complexes anchored in the cell envelope extend through the OM to function outside the cell (e.g. flagella, pili).

The components that make up the cell, which exist as complex assemblies of countless molecules, inevitably have imperfections and the cell envelope is no exception. Envelope damage, caused by environmental factors and internal errors in metabolism, can be manifest at a molecular or organizational (e.g. disruption of OM asymmetry) level. In particular, proteins are susceptible to damage by different means (reviewed in [17]). Physical stresses, such as heat, lead to misfolding and aggregation of envelope proteins. Oxidative reactions can result in protein and lipid oxidation [18,19]. Enzymatic, oxidative or hydrolytic reactions can damage the LPS sugars or acyl chains. Still other insults result in the release of OM proteins (OMPs) and LPS [20]. For instance, antibiotics or chelating agents can destabilize lateral LPS bridges, resulting in the release of proteins and LPS [21]. As a cell inevitably takes on and accumulates these and other forms of damage there are three possible outcomes: (i) cell senescence or death; (ii) cellular repair; or (iii) dilution, segregation or shedding of damage. Both restoration of function (direct repair) and loss of damage (indirect repair) are used by bacteria as single-cell recovery strategies (Fig. 1A).

Bacteria have evolved repair systems to directly address cell envelope damage

Perturbations to the cell envelope are sensed and responded to by signaling pathways in the individual cell. Of these pathways, the best studied are in the model organism *Escherichia coli* and related species. In *E. coli*, damage cues such as protein misfolding [22], abnormal LPS [23] and antimicrobial peptides [24] trigger responses including the Cpx [25], σ^E [26], Rcs [27] and Bae [28] regulons, which direct gene expression to enhance the repair programs [29]. These regulons include proteins that function as envelope chaperones or proteases or are involved in OMP and LPS transport, assembly and maintenance [29]. For instance, the periplasmic chaperone-protease DegP monitors the periplasm for unfolded proteins and can either refold or degrade them [30,31]. Oxidative damage in the cell envelope can be repaired by a number of reducing systems [32]. For example, the OM

MsrA/B proteins of *Neisseria* provide protection against reactive oxygen species by reducing methionine sulfoxide residues in oxidatively damaged proteins [33]. Furthermore, proteases that reside in the OM, such as OmpT, can cleave foreign (antimicrobial) peptides that bind and inhibit LPS function [34]. Although these types of systems allow bacteria to respond and adapt to envelope stressors, they can nevertheless be overloaded, leading to permanent envelope damage and death. Indeed, cells that have lost their reproductive ability because of the accumulation of oxidative damage have upregulated stress responses, indicating that these mechanisms are not always sufficient for cell rejuvenation [35].

Certain stressors (temperature, antibiotics, EDTA) cause organizational damage to the OM such as the loss of protein and LPS molecules [21]. Damage to LPS or β-barrel proteins can cause phospholipids to mislocalize from the inner leaflet to the outer leaflet. The resulting OM loses its asymmetry and consequently reduces its permeability and protective properties [36]. In turn the cell responds to this damage. For instance, when phospholipids are mislocalized in the outer leaflet, some bacteria adapt by destroying them with PldA phospholipase [37,38]. In another example, the conserved OMP PagP of *Salmonella* can cleave stray outer leaflet phospholipids and in turn adds the resulting palmitoyl chain to lipid A of LPS, giving the molecule a hepta-acylated lipid anchor [39–41]. This palmitoylation also provides an adaptive response to enhance survival [42]. The Mla system provides yet another means for maintaining bilayer asymmetry, apparently by retrograde transport of excess phospholipids from the outer to the inner membrane [38]. These are examples of mechanisms that maintain an asymmetric and hence functional OM bilayer, but there are fundamental limits to their healing ability. Either the systems can become overwhelmed, or the cell lacks a repair pathway for the damage that has been acquired. In fact, cells often require more drastic and costly strategies—indirect repair mechanisms—that shed or segregate damaged material in the bilayer.

Bacteria discard damaged components during indirect repair

The mentioned direct repair mechanisms are involved in the degradation or restoration of defective material, but these repair strategies are not sufficient for all forms of damage. For example, these response pathways do not address the repair of mature OMP and LPS molecules. Whereas phospholipid turnover has been described in some detail [43], the question of how damaged, undesired or excessive integral OMP and LPS components are dealt with still remains. In *E. coli* it was recently shown that older OMPs are displaced to the cell poles, and thus the mother cell partitions old and new OMPs upon cell division [44,45]. This implies that, at least in *E. coli*, there is no mechanism to turn over OMPs. In addition, as LPS transport is apparently unidirectional [46], there is no known mechanism for recycling this component. This suggests that OMP and LPS genesis and insertion are in a sense irreversible and that the default repair mechanism for the OM involves shedding (OM vesicle or tube secretion [47,48]) and asymmetric distribution of damage to repository (aged) cells [7,9]. It has been shown in *E. coli* that the amount of vesicle formation correlates with the amount of protein accumulation in the cell envelope and increases in the absence of active stress response pathways. Furthermore, vesiculation enhances survival under stressful conditions and preferentially packages damaged proteins [47]. OMV production has been observed as a general stress response in a variety of bacteria, and has

been shown to respond to misfolded proteins, accumulating peptidoglycan or LPS fragments and oxidative stress, all of which indicate cell damage or aging [49]. Aside from shedding, damaged molecules can be partitioned into one of two cell poles thereby creating a healthy daughter cell and a damage repository daughter cell after cell division. This was demonstrated by the observation of asymmetric division of protein aggregates [7], the reduced fitness of old-pole derived daughters [5,6], and that old OMPs migrate to only one pole [44]. These studies were mostly done in *E. coli* and more work is needed to determine if this is a general strategy to combat aging. We designate these healing strategies "indirect repair" to distinguish them from the restorative activity that takes place in direct repair (Fig. 1A). The existence of indirect repair strategies indicates that direct repair is not always adequate. During indirect repair, irreparable damage is intentionally discarded or segregated. In the case of segregation, the daughter cells that inherit the old pole accumulate damage following repeated cell divisions. Consequently these cells have decreased fitness and over time reach senescence [6,50].

All mechanisms of repair described so far have drawbacks if one considers a cell in a nutrient-limited habitat, as is typically found in nature [51]. Mechanisms that involve simple dilution or segregation of damage by cell division require growth to outpace damage accumulation (Fig. 1A). De novo synthesis of stress response pathways is similarly metabolically costly, and, although it may keep the cell alive for a period of time, it may hamper the ability of the cell to seek a more favorable habitat by exhausting local nutritional resources. Segregation of damage to a repository cell is particularly costly considering it demands the biosynthesis of a daughter cell. Similarly, OM shedding is sustainable only when the lost material is replaced with newly synthesized macromolecules.

Multicellular bacteria have the potential for unique solutions to these problems. For instance, damaged material that is shed could be re-metabolized by sibling cells akin to a bacterial autophagy-like process [52]. In addition, cannibalism or the sacrifice of some cells for the benefit of the population has been described [53,54]. If ‘old’ or senescent cells or vesicles were cannibalized, it would also provide a mechanism to remove damage and salvage community material. However, there are drawbacks to content recycling, considering competitors could also consume these external resources. To our knowledge, the recycling of released extracellular material specifically from damaged cells has not yet been demonstrated. Another multicellular damage coping mechanism could rely on direct content exchange between individuals to dilute a damage burden without the need for cell division or shedding. In direct content exchange a pool of healthy material would be available to be freely distributed to ailing cells to buffer and combat damage. This scenario works providing there is a mechanism to exchange content between cells and an incentive to redistribute resources.

Content exchange as a strategy for multicellular repair

Indeed, content exchange is observed as a strategy for eukaryotic damage repair both at the cell-to-cell and organelle-to-organelle levels. For example, transfer of mitochondria between cells is able to rescue respiration in recipient cells with defective mitochondria [55]. Transfer of organelles can occur through tunneling nanotubes (TNTs), which define a broad

collection of cell-to-cell tube-like connections found in eukaryotes. TNTs formed between ischemically damaged cardiomyoblasts and mesenchymal stem cells facilitate the transfer of mitochondria to rescue injured cells [56]. Cytoplasmic and organelle transfer via cell-cell contacts is also involved in the restoration of cell function of renal tube cells [57]. Furthermore, selective transfer of lysosomes between endothelial progenitor cells and damaged endothelial cells via TNTs rescued lysosome recipients [58]. Recently it was demonstrated that stressed cells preferentially form specialized TNTs to facilitate rescue from apoptosis by mitochondrial transfer from healthy cells [59]. Aside from mitochondria and lysosomes, TNTs also transfer Golgi and endoplasmic reticulum (ER) [60]. In addition to organelles, TNTs are involved in the transfer of membrane proteins [61,62], endosomes [63] and RNA [64,65]. Stress appears to play a major role in inducing TNT formation between cells [66,67]. Though the in vivo contribution of TNTs to cell repair is not fully described, clearly cell-to-cell transfer of contents via TNTs can contribute to health and homeostasis in multicellular organisms through content exchange.

Like during OME, tunneling nanotubes form a continuous membrane between cells and can thus facilitate the transfer of lipids and lipid-anchored proteins [68]. Another process of plasma membrane exchange takes place during trogocytosis during which lymphocytes extract membrane patches and proteins from target cells. While trogocytosis certainly plays some role in immune modulation, it has been proposed that trogocytosis could have evolved from a primitive mechanism of specialized cells "feeding" off of other cells [69], or, put in other terms, kin cells donating lipid and protein components. Still other membrane-based content sharing platforms occur in plant cells in which a continuous ER transverses plasmodesmata [70] to share lipids and proteins via the ER membrane and lumen [71]. Intercellular protein transfer is not at all uncommon [72], but knowledge of its use as a platform for cell repair is limited.

Content sharing is used as a strategy to repair damage at the organelle level as well. For instance, metazoan cells repair lesions to their plasma membrane by the rapid fusion of cytoplasmic membrane-enclosed organelles to seal the hole at the site of damage, thereby sacrificing the organelle for the benefit of the cell as a whole [2,73]. In another example, ER-derived vesicles containing nuclear DNA-derived products are delivered to mitochondria to replenish mitochondrial membranes [74]. Also striking is the process of mitochondrial fusion and fission during which damaged material is diluted by content exchange [75,76]. Importantly, mitochondrial exchange dynamics allow the complementation of defective DNA, mRNA and proteins among individual organelles [77]. Disruption of mitochondria exchange and repair impedes homeostasis and leads to organelle dysfunction [78]. Mitochondria that display little motility and fusion such as those in cardiomyocyte cells can exchange contents via nanotubular extensions [79].

Maintaining health and homeostasis of the organism as a whole to preserve its fitness serves as the incentive for content exchange between individual cells and organelles in multicellular systems. The question then arises: can multicellular bacteria or bacteria that benefit from colonial growth use cell-to-cell content exchange to support the health of individual cells in their community? We hypothesize that this indeed can occur and that OME in myxobacteria

is a content sharing platform that allows cell rescue and rejuvenation and helps maintain OM homeostasis in a population.

Myxobacteria use content exchange to repair their damaged kin through OME

Our appreciation for cooperation among bacteria has rapidly increased [80]. With cooperation comes the potential for multicellular responses to damage. Eukaryotes have evolved to include multicellular damage responses such as wound repair and immune responses [2,81]. In microbes, relatedness in a population of cells provides an evolutionary basis for assisting kin by sharing resources [82]. For instance, the collaboratively built biofilm matrix of a related microbial community provides protection from external stressors [83]; in essence sacrificing the health of some cells for the benefit of others and for the good of the community. Although there are many examples of cooperative behaviors among bacteria, multicellular damage repair mechanisms in bacteria have been explored little.

Myxobacteria are an ideal system to study multicellular behaviors in prokaryotes. They are typically found in soil communities that rely on communication networks to perform synchronized behaviors, analogous to how tissues function in eukaryotes. One such interaction platform is the constitutive exchange of large amounts of OM material between cells by OME [84]. In a cell contact–dependent manner, myxobacteria transiently fuse their OMs [10], allowing rapid exchange by lateral diffusion of lipids, proteins and LPS from partnering cells. As a consequence, they can phenotypically complement OMP and LPS mutants by efficiently transferring OM components from a wild-type cell to a mutant [10,85–87]. Two proteins, TraA and TraB, which are localized on the cell surface, are the only factors known to be necessary for OME [14,88]. These proteins are required in both contacting cells, and presumed homotypic interactions between TraA receptors help to ensure that transfer takes place between kin cells [89]. Although the biological utility of OME is not fully understood, we hypothesize that two consequences of OME are the cooperative repair of damage and the maintenance of OM homeostasis in a population.

LPS is the outermost structure of the cell and directly interacts with other cells and with the environment. As such, LPS functions in a number of behaviors involving cell-cell and cell-substrate interactions. In *Myxococcus xanthus*, truncated LPS molecules caused by mutations in the biosynthesis of distal LPS sugars impair both type IV pili–mediated (social) motility and adventurous motility [90,91]. The impairment might involve defective contacts at the interface of the LPS and the gliding surface. As motility is critical for the synchronized behaviors of myxobacteria, these mutants also have severe defects in development [10,90]. Like other OMP defects, mutants with truncated LPS can be complemented by OME from a wild-type cell, thereby restoring motility and development to the mutant (Fig. 2A) [10].

To further investigate the ability of OME to repair damage, we created a genetic model of OM damage in *M. xanthus*. In gram-negative bacteria the acylated LPS component known as lipid A forms the essential hydrophobic module that anchors the distal polysaccharide moiety to the outer leaflet bilayer. To mimic OM damage, we constructed a strain that

replaced the promoter of *lpxC*, a critical lipid A biosynthesis enzyme [92], with a heterologous inducible promoter. This strain grows like its parent in the presence of the inducer. However, in the absence of inducer, LpxC and hence lipid A are no longer produced, and LPS levels are reduced in the OM as the cells grow. Similar to what occurs when external stresses or metabolic errors cause a loss of OM function, the *lpxC* strain likely contains mislocalized phospholipids in the outer leaflet to compensate for the absence of LPS. This destroys the permeability barrier and the structural integrity that LPS provides and the cells lyse [10]. With this *lpxC* conditional strain, we then asked if OME with cells that make wild-type LPS could replenish the missing molecules and restore viability to the damaged cells. In the absence of inducer, the *lpxC* mutant lysed when placed in a one-to-one mixture with OME-deficient cells (*traA* mutant) with similar kinetics as an *lpxC* monoculture. However, in a one-to-one mixed culture with isogenic OME-competent cells, the *lpxC* strain survives and grows [10]. As the LpxC enzyme itself is not transferred, we interpret this result to mean that the rapid flux of OM material between mutant and healthy cells relieves the damage burden of the mutant cells by replenishing wild-type LPS, while distributing the damaged membrane among healthy cells (Fig. 1B). Because the membrane damage, presumably including mislocalized phospholipids, is distributed equally between strains, newly synthesized LPS from the wild-type strain in concert with the OM homeostasis machinery of both strains can then supply the population with a functional OM. In essence, we hypothesize that an indirect form of repair (damage dilution) combined with direct repair allows rejuvenation of damaged cells in a cooperative system. We propose that these findings can be extended to other forms of OM damage, as lipids, OMPs and LPS are all transferred efficiently by OME [10,14,84,86].

Implications of multicellular repair in myxobacteria

Damage repair by OME requires a sub-population of healthy cells in spatial proximity to damaged kin. Often biofilms exposed to external stress have an unequal distribution of damage, as cells at the periphery are more exposed to stressors than those in the center [93,94]. Similarly, myxobacteria form tightly aligned and aggregated populations, which may spatially establish a damage gradient that is greater toward the periphery of the population or microcolony. Since myxobacteria move by gliding motility [95,96], the population members are constantly repositioning themselves in the colony and hence exchanging OM material with different individuals. In addition, when microcolonies of myxobacteria encounter one another during migration [97], the populations may bear different damage loads as they are exposed to different environmental conditions along their migratory path. In these scenarios, OME offers a means to homogenize the differentially damaged populations. However, for this to occur there must be an incentive (positive selection) for the healthy cells to share material. In this regard, by directing repair function toward TraA-compatible cells (kin) [89], the fitness benefit of increasing the size and density of a functional population may exceed the fitness cost to healthy cells of acquiring damage [10,12]. In this scheme, damaged or aged cells acquire undamaged OM materials that are necessary for viability and to remain a productive member of their microbial community. In turn these cells remain competent for multicellular interactions, leading to improved outcomes for the whole population. For example, although OM damage impairs

development (Fig. 2), we hypothesized that rejuvenated cells can participate effectively in development after OME. Indeed this idea was supported when a sub-threshold density of a healthy cell population, which cannot develop optimally, was mixed with a damaged population; the combined population effectively developed into spores, whereas a monoculture or OME-defective mixed population did not (Fig. 2B) [10]. Thus OME facilitated the formation of a developmentally competent population (size and density) by restoring function to damaged cells. This result explains how healthy cells can benefit by aiding ailing kin—ultimately the distribution of a damage load can increase the fitness of the entire population. Thus, like wound healing in multicellular eukaryotes, a part of the organism is healed to maintain the fitness of the whole organism, requiring resource sacrifice from kin cells. Although this sacrifice may establish a selective pressure to evolve cheating genotypes that do not participate in OME, the selective pressure to maintain *traAB* functionality, and hence receive aid and 'private goods' from kin cells, may be greater. In addition, there may be other benefits of *traAB* and OME that require myxobacteria to remain OME competent. For instance, OME may provide a competitive advantage since it has been demonstrated to be a platform to deliver toxins to competing myxobacteria [98]. Furthermore, TraA is an adhesin and may contribute to population viscosity, which limits migration away from the colony [99]. Therefore, the practical benefit to cheater genotypes may be outweighed by the selective pressure to maintain *traAB*.

An advantage of OME is that cells do not require de novo biosynthesis for damage dilution to take place; all that is needed is cell motility and the TraA/B proteins in partnering cells [14,84]. This makes the strategy of damage dilution by OME an efficient process in low-nutrient soil environments. In contrast, damage dilution by cell division or shedding mechanisms requires active metabolism and nutrients. We would like to add that although OME in myxobacteria does not directly exchange inner membrane or cytoplasm content, it is plausible that a secondary pathway(s) may facilitate the transport of specific cargo from the OM to proximal positions inside the cell.

Although from the literature it is unclear whether OMPs and LPS are ever turned over or recycled, in myxobacteria these components are distributed between cells [14,84]. In contrast, *E. coli* distributes old OMPs to the cell pole to be segregated upon cell division. As a result, cells have different aged poles, some of which are 'old poles', and with growth the population becomes heterogeneous [7]. Although there may be advantages and disadvantages to both strategies, the myxobacterial strategy supports a homogeneous population, which is likely important for their synchronous behaviors.

We have recently demonstrated that damaged or missing OMPs or LPS can be functionally restored to defective cells by OME from healthy donor cells. These findings are based on genetic approaches in which engineered mutants express defects in their OM. These genetic models serve as surrogates to mimic cellular damage inflicted by environmental insults. Future studies need to examine how OME can cope with specific damage to the OM imposed by physical or chemical insults. Such studies require an understanding of how particular insults damage the OM and how single-cell response pathways may lead to self-repair. Ideally these studies would ultimately determine whether OME simply buffers the population from damage or whether membrane sharing also augments autonomous cell

repair pathways. It is also of interest to know whether (i) old and new OM components are transferred equally by OME, (ii) whether young cells can revitalize old cells, and (iii) under what conditions OME provides a fitness advantage to cells propagated in natural soil habitats.

Is multicellular repair unique to the myxobacteria?

Myxobacteria are remarkable in that they are at an extreme example of multicellularity in the prokaryotic kingdoms. However, as noted before, our appreciation for multicellular behaviors between otherwise unicellular organisms is expanding. With the development of more advanced microscopy techniques, research into new bacterial ultrastructures has emerged giving us fresh insight of bacterial cell-to-cell exchange platforms. Intriguingly, there is accumulating evidence that some bacterial groups respond to stress by triggering the exchange of cellular material [100,101]. As these other groups exchange cytoplasmic material, they may also engage in multicellular repair. Notably, cell-to-cell connections have been observed in pathogens such as *Salmonella* [102] and *Francisella* [103]. In fact, membrane fusion events similar to myxobacterial OME has been observed in *Borellia*, though content transfer was not tested [104]. Cell-to-cell transfer of electrons has also been documented [105], which sometimes occurs through membrane tube-like structures [106]. In addition, observation of large structures appearing to connect cells can be observed in natural environments with cryo-electron microscopy [107]. Other bacteria, such as actinomycetes, cyanobacteria and magnetotactic prokaryotes, exist as multicellular organisms, where cellular components may be exchanged among tightly associated cells. For example, in *Anabaena* filaments the cells are enclosed by a continuous OM and the cells exchange molecules [108,109]. Whether *Anabaena* or other multicellular bacteria engage in multicellular repair remains an open question.

Though bacterial cell-to-cell transfer of components has been noted as far back as the discovery of bacterial conjugation, research into the role of content exchange in damage repair is lacking. However, advances in our understanding of inter-microbial interactions may yet lead us to the discovery of analogous strategies in other bacteria. A better understanding of how bacterial content sharing contributes to buffering or repairing cellular damage will provide insight to community dynamics. These processes include infection, microbiome dynamics, microbial fermentation and bioremediation.

Conclusions and perspective

We propose that OME expands the paradigm for how cell damage repair occurs in bacteria by adding the dimension of cooperation. Many multicellular organisms have evolved the ability to coordinate cell behavior to combat damage [2], thus setting a precedent for multicellular repair in bacteria. As diverse forms of bacterial cooperative behaviors come to light, microbiologists should now consider how multicellular interactions are used to combat stress. This notion is especially relevant for organisms in which cell number and density correlate with fitness, because there is a selective advantage for individuals to assist their kin. In addition, understanding how groups of cells cope with stress is fundamental in elucidating bacterial survival strategies, including how they respond to antibiotics and the

immune system of host organisms. We conclude that multicellular wound repair has evolved in prokaryotes, and this notion will hopefully stimulate and inform future studies on bacterial repair.

Acknowledgments

This work was supported by NIH grant GM101449 to D.W.

Abbreviations

OM	outer membrane
OME	outer membrane exchange
LPS	lipopolysaccharide
OMPs	outer membrane proteins
OMVs	outer membrane vesicles
TNTs	tunneling nanotubes
ER	endoplasmic reticulum

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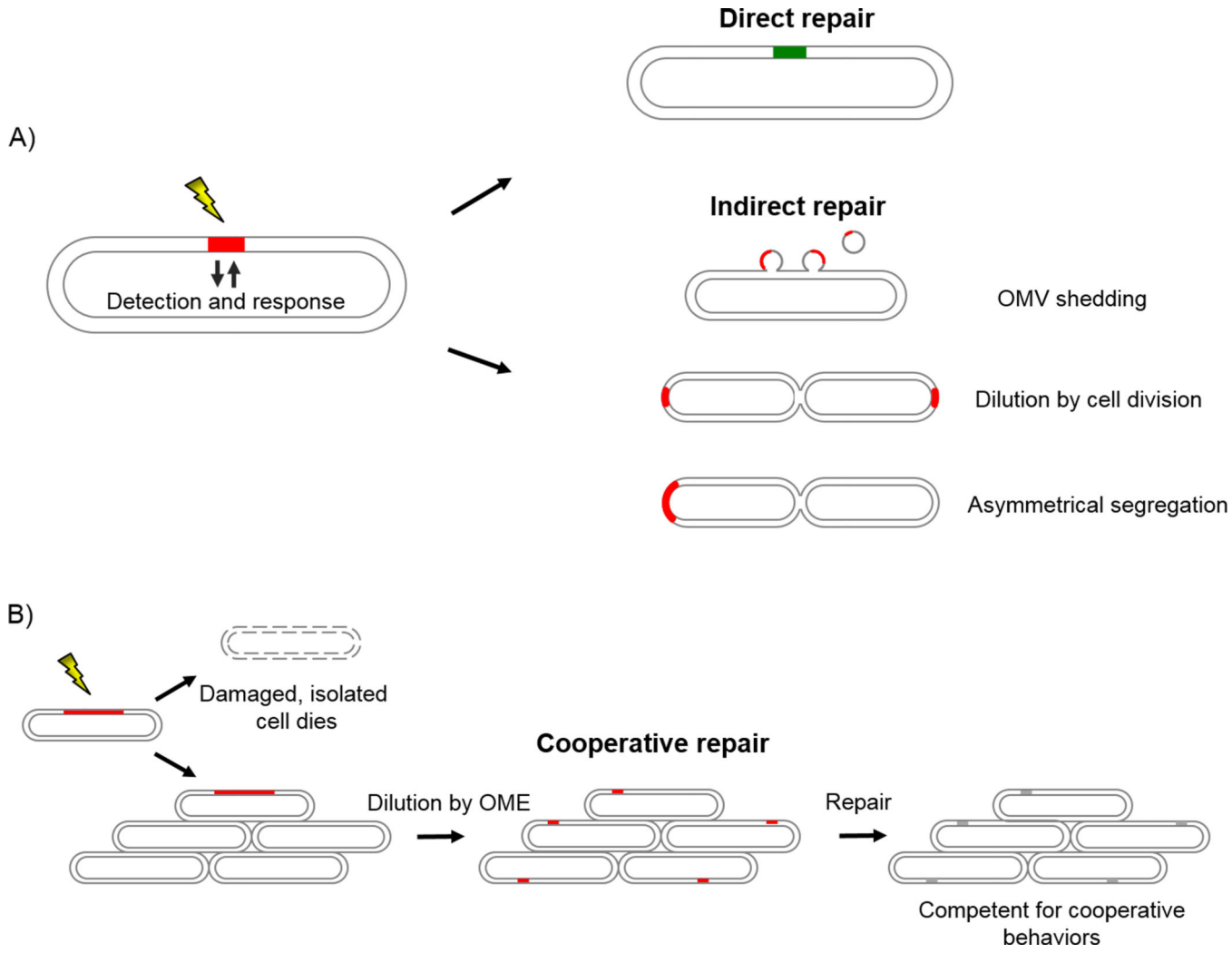

Fig. 1.

Envelope repair strategies. A: A stress inflicts damage (red) to the cell envelope. The direct repair pathways sense this damage and respond by the induction of cellular machinery which results in repair (green). In indirect repair, damaged material is shed by OM vesicles (OMVs), diluted by cell division or asymmetrically segregated. B: Multicellular myxobacteria dilute cell envelope damage by OME. In some cases the dilution of damage may be sufficient in and of itself to alleviate the stress. In other cases, dilution makes damage more manageable for direct autonomous repair and/or indirect repair (grey; indicating alternative possibilities). An isolated damaged cell cannot participate in OME and dies.

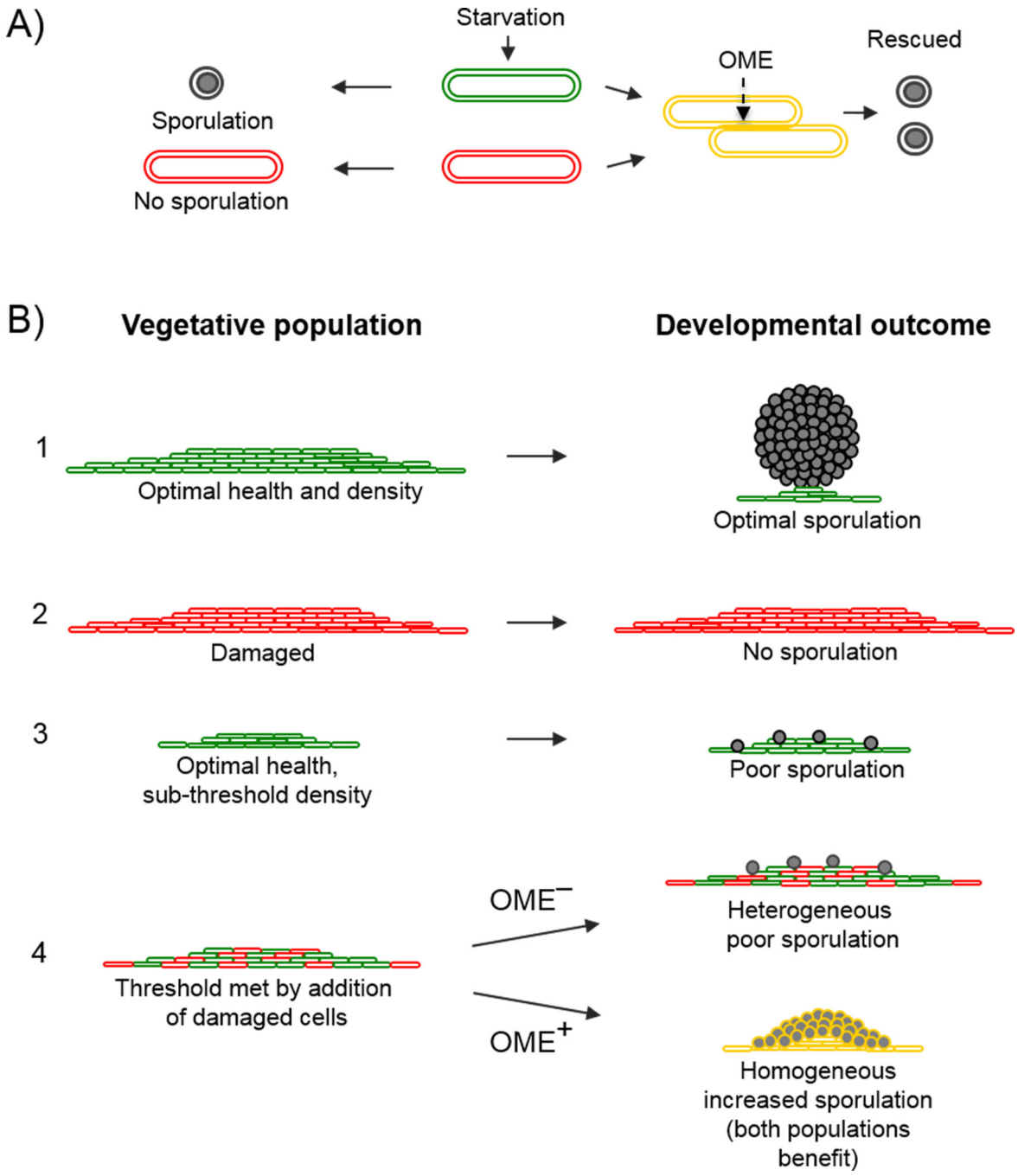

Fig. 2.

Multicellular damage repair by OME benefits the individual and the population. A: In response to starvation (center), a healthy cell (green) enters a developmental program and sporulates, whereas a cell with a damaged OM, e.g. truncated LPS (red), cannot (left). In contrast, when damage is exchanged, diluted and repaired by OME (yellow), the damaged cell is rescued (right). Note: development occurs in fruiting bodies that are not depicted. B: Schematic of vegetative myxobacteria populations. The corresponding developmental outcomes that depend on cell health and density are shown in rows one through three. Row

four depicts how surpassing the density threshold by the addition of damaged cells leads to increased sporulation outcomes only when damaged cells can exchange membrane material and be repaired (OME^+); otherwise sporulation does not improve (OME^-).