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PERSPECTIVES

OPINION

Morphological plasticity as a bacterial survival strategy

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Abstract | Bacteria have evolved complex systems to maintain consistent cell morphologies. Nevertheless, in certain circumstances, bacteria alter this highly regulated process to transform into filamentous organisms. Accumulating evidence attributes important biological roles to filamentation in stressful environments, including, but not limited to, sites of interaction between pathogenic bacteria and their hosts. Filamentation could represent an intended response to specific environmental cues that promote survival amidst the threats of consumption and killing.

Microorganisms have large arsenals of tools to withstand a multitude of environmental insults. In most cases, exposure to harsh elements results in the production of specific enzymes that aid the alleviation of stresses and allow survival and/or growth[1,2]. Strikingly, a number of laboratories that study unrelated aspects of bacterial biology have converged on a similar observation: filamentous morphology provides survival advantages. Filamentation has been implicated in bacterial survival during exposure to environmental stresses, which include host effectors, protist predators and antimicrobial therapies (TABLE 1). It remains unclear whether the molecular mechanisms that lead to filamentation are shared by these scenarios; in fact, there is evidence to suggest that multiple mechanisms are involved in the inhibition of septation that leads to filamentation. For *Burkholderia pseudomallei*, the enhanced survival traits that are associated with filamentation can be conferred upon the daughter cells that are produced following the restoration of septation[3]. This survival allows typical bacterial-cell division, despite the continued presence of the cell-division inhibitor. This occurs even in the presence of other harsh environmental effectors that have not been previously encountered. These observations challenge our traditional views of bacterial survival and killing strategies and could drive the development of new and improved approaches to control bacteria in a number of environmental and clinical settings, particularly the treatment of human bacterial infections.

For many years, filamentous bacteria have been considered to be the over-stressed, sick and dying members of the population. On closer examination, the filamentous members of some communities have vital roles in the population's continued existence. In this Opinion, we discuss recent observations that describe filamentation as a survival mechanism that is a direct response to lethal environments. Observations that suggest a role for filaments in many pathogenic organisms will also be discussed. An understanding of the pathogenic advantages of morphological plasticity will illuminate new and improved anti-infective strategies.

Uniformity of cell shape and size

The typical size and shape of bacterial cells is dependent on numerous variables that are unique to the particular environments that are associated with the lifestyle of each species (reviewed in REF. 4). For each species, the dimensions of cell diameter and length are strictly controlled, as manifested by the minimal size variation that is detected among bacteria that are grown under similar growth conditions[5]. This has led to the inclusion of cell size and shape as 'classical' descriptors of bacterial species. In general, bacteria have developed complex genetic and biochemical programmes to maintain their classical shapes. However, despite these strict control mechanisms, bacteria have also retained genes that encode proteins that are dedicated to the purposeful alteration of the overall bacterial cell length under certain conditions (reviewed in REF. 6).

Filamentation occurs when cell growth continues in the absence of cell division, and results in the formation of elongated organisms that have multiple chromosomal copies. In most cases discussed here, filament lengths are typically 10–50 times longer than their bacillary counterparts. There are many conditions that lead to bacterial filamentation, including metabolic changes and DNA damage[7] (FIG. 1). Filamentation also results from the mutation and/or alteration of the stoichiometry of the cell-division components[6].

There are several examples of proteins (such as ZipA) that affect the frequency of cell division owing to modulation of the polymerization of the earliest known cell-division component, FtsZ[6]. Although the inhibition of cell division protects daughter cells from receiving damaged copies of the bacterial chromosome, the filamentous phenotype can also involve other programmes that are designed to promote bacterial survival. In the cases we discuss below, the filamentous phenotype confers protection against a different host immune mechanism — predation by eukaryotic cells.

Pathogenic bacterial filaments

***Subversion of innate immunity* in vivo.** Strains of uropathogenic *Escherichia coli* (UPEC) are the predominant cause of urinary-tract infections (UTIs)[8]. During acute infection of the mammalian bladder, UPEC invade the superficial epithelial cells of the bladder[9] and, following a complex developmental cycle[10] that is associated with morphological plasticity at each stage (FIG. 2), grow to form intracellular bacterial communities (IBCs). During IBC maturation, a subpopulation of the community progresses into a distinct developmental programme in

Table 1 | **Filamentous bacteria**

Species	Niche or disease	Filamentation observed	Other observations	Mechanism	Biological role
Flectobacillus spp.	Freshwater plankton	*Ochromonas* spp. grazing[28]	None	Unknown	Protection against consumption
Comamonas acidovorans	Freshwater plankton	*Ochromonas* spp. grazing[28]	None	Unknown	Protection against consumption
Betaprotobacteria	Freshwater and marine	*Bodo saltans* grazing[28]	None	Unknown	Protection against consumption
Caulobacter crescentus	Freshwater	Prolonged growth[34]	Resistance to heat, oxidative stress and pH[34]	Decreased FtsZ concentration	Survival
Uropathogenic *Escherichia coli*	Urinary-tract infections	Mouse bladder and human urine[11]	None	SulA and, possibly, the SOS response	Protection against phagocytosis
Haemophilus influenzae	Otitis media	Chinchilla inner-ear biofilm[12]	Polymorphonuclear leukocytes in biofilm[13]	Unknown	Unknown
Legionella pneumophila	Legionnaires' disease	*In vitro* biofilm[16]	None	Unknown	Unknown
		Intracellular (Vero cells)[17]	None	Unknown	Unknown
Mycobacterium tuberculosis	Tuberculosis	Intracellular (macrophages)[18]	None	FtsZ rings absent and, possibly, the SOS response	Unknown
Salmonella enterica serovar Typhimurium	Dysentery	Vacuoles of macrophages[20]	None	Histidine metabolism	Unknown
	Vesicles of macrophages	Vacuoles of macrophages[20]	Transcriptional profiling only[23]	SOS	Unknown
Shigella flexneri	Enteritis	Unknown	Transcriptional profiling only[22]	SOS	Unknown
Burkholderia pseudomallei	Melioidosis	Unknown	Intracellular macrophages[24]	RecA and, possibly, the SOS response	Intracellular survival
		Antibiotic exposure[31]	Intracellular macrophages[24]	SOS	Resistance to similar classes of antibiotics
Proteus mirabilis	Urinary-tract infections	Mouse bladder[45]	None	Quorum sensing	Swarming to evade host immune cells

which cell division is inhibited, which leads to the formation of filamentous bacteria that are up to 70 μm in length[10].

Bacterial emergence from epithelial cells and exposure on the luminal surface of the bladder coincides with an influx of neutrophils and the phagocytosis of bacillary forms of UPEC, which results in enrichment of the filamentous UPEC on the surface[10]. Time-lapse-video microscopic evaluation of infected mouse bladders has revealed that filamentous UPEC survive killing by innate immune effector cells, and specifically by evading neutrophil phagocytosis[10]. Moreover, the bacterial cell-division inhibitor SulA (FIG. 1) is essential for UPEC pathogenesis in immunocompetent hosts[11] and mediates bacterial filamentation[10]. Bacterial lipopolysaccharide (LPS) is recognized by the innate immune system through Toll-like receptor 4 (TLR4). Mouse hosts that are defective in signal-transduction responses to LPS through TLR4 do not trigger UPEC filamentation during acute UTIs[10], which suggests that bacterial filamentation is a response to the effectors that are produced as part of host innate immunity. Conversely, SulA-mediated filamentation is dispensable for the establishment of UTIs in these TLR4-deficient mice[11], which indicates that filamentation is unnecessary in the absence of immune-mediated host pressure. Thus, the fundamental physical increase in cell length (which is probably accompanied by other alterations in the molecular properties of the bacterium) might contribute to the survival advantage that is provided during the transient exit and entry of epithelial cells, and increases the probability of a sustained infection.

The concept that filamentous organisms are resistant to phagocytosis is not new — filamentous forms of fungi are known to be resistant to killing by preventing phagocytosis (BOX 1). Additionally, UPEC are not the only organisms to display filamentous forms in pathogenesis. Although a biological role has not yet been confirmed, filamentous forms of non-typable *Haemophilus influenzae* were recently observed in biofilms that were isolated from the inner ear in a chinchilla model of otitis media[12]. As neutrophils are known to be in direct contact with *H. influenzae* biofilms *in vivo*[13], it is reasonable to speculate that the filaments may also promote protection from phagocytosis during otitis media.

Proteus mirabilis is an opportunistic urinary pathogen that forms elongated, filamentous cells that demonstrate swarming motility in response to flagellar contact with solid surfaces, such as urinary catheter materials[14]. The swarming filamentous phenotype is associated with increased virulence, including the upregulation of flagellar expression and production of pathogenic enzymes, such as a metalloprotease that degrades mammalian antibacterial peptides[15].

Pathogen filamentation in* in vitro *models. An increasing number of pathogenic organisms seem to undergo filamentation under *in vitro* conditions that resemble those found

within a host (TABLE 1). In these models of infection, it is unknown whether the observed filamentous morphology reflects bacterial responses to intracellular stress, nutrient limitation or inhibited metabolic pathways. It is also unknown whether the morphological change that is demonstrated by these pathogens is a virulence attribute. In light of the studies discussed above that describe filamentation as a survival tactic, these observations are noteworthy and merit further investigation in *in vivo* model systems. In contrast to the scenario described above, in which filamentation precedes, and confers protection against, phagocytosis, some observations reviewed in this section take place after phagocytosis.

The aetiological agent of Legionnaires' disease, *Legionella pneumophila*, resides within the lung and is able to survive phagocytosis by alveolar macrophages. *L. pneumophila* was recently shown to form mycelial-like biofilms at 37°C that consist of filamentous bacteria, whereas biofilms that were grown at 25°C were composed of rod-shaped organisms[16]. Moreover, multiple species of *Legionella* invade intracellular compartments of Vero cells *in vitro,* fail to divide and, subsequently, filament[17]. The propensity for filamentation at body temperature and within human cells offers the opportunity to consider a role for filamentation in Legionnaires' disease.

As for *L. pneumophila*, *Mycobacterium tuberculosis*, the aetiological agent of tuberculosis, is highly persistent in the host and survives within macrophages. *M. tuberculosis* transforms into non-septate filamentous bacteria that are devoid of FtsZ rings after engulfment by human-derived macrophages *in vitro*[18]. As described for UPEC, the lack of FtsZ rings is consistent with, but does not provide proof of, the induction of SulA as a mechanism for filamentation. Because the inhibition of cell division also leads to resistance to certain antimicrobial therapies (discussed below), it is intriguing to speculate that the clinical need for prolonged antituberculosis therapy might result, in part, from the resistance of intracellular filaments to these agents.

Salmonella enterica serovar Typhimurium (*S. typhimurium*) is a pathogen that also has the capacity to thrive within epithelial cells and survive phagocytosis by macrophages. Much attention has been given to the type III secretion system that is encoded within *S. typhimurium* pathogenicity island 2 and that mediates survival by impeding phagosome–lysosome fusion[19]. The survival of *S. typhimurium* within intracellular compartments of macrophages *in vitro* is associated with a filamentous phenotype. Cell-division inhibition occurs in response to macrophage production of nitric oxide radicals, which suggests that filamentation is mediated by the DNA-damage response system (the SOS response)[20]. Indeed, the expression of standard components of the SOS response (*sulA*, *recA* and *lexA*) was upregulated during intracellular growth within the *Salmonella*-containing vesicles of macrophages *in vitro*[21]. The upregulation of SOS components further indicates that the oxidative radicals that are produced by phagocytic cells can lead to DNA damage, an observation that is mirrored in the UPEC system described above. In another system, the *Salmonella* spp. filamentation that was observed within Lamp-1-containing vesicles of melanoma cells *in vitro* was SulA independent and was related to histidine metabolism[22].

Current data also suggest that components of the SOS response are induced in other intracellular pathogens, although filamentous organisms have not been investigated. *Shigella flexneri* is a pathogen that causes enteritis. Evaluation of the intracellular transcriptional profile of *Shigella* spp. growing within macrophages revealed an increase in the expression of *sulA* and *recA*[23]. *B. pseudomallei* infects a number of epithelial cell lines and, reportedly, survives phagocytosis by alveolar macrophages[24]. Recently, *recA* was identified as an essential gene for the pathogenesis of *B. pseudomallei* in a mouse model of melioidosis[25]. Although RecA participates in multiple cellular programmes, its involvement in the SOS response is the only known essential role for this protein. Although this observation is open to interpretation, it is interesting to speculate that the SOS response may be essential for melioidosis. A formal consideration of the cell morphology of these pathogens should be undertaken in conjunction with an investigation of their resistance to harsh environments within the host. In general, there are many examples that highlight the relationship between the induction of constituents of the SOS response and the virulence of filamentous bacterial forms.

Resistance to predator protists

Model for pathogenesis. Many opportunistic bacteria have environmental niches that require survival against predation by free-living protists. For example, *L. pneumophila* increases in number while it resides in a number of protist species[26]. It is notable that the pathways that are important for pathogenesis in mammals are also important for survival of protist consumption (reviewed in REF. 27). This has led a number of investigators to use protist grazing on bacteria as a model to further characterize infections by human pathogens, such as species of *Legionella, Mycobacterium, Pseudomonas* and *Vibrio*. Although filamentation has not been directly investigated in these protist models, it has been clearly shown that

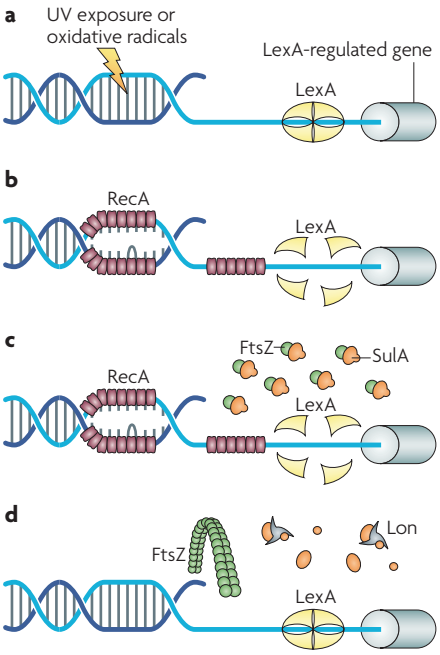

Figure 1 | **The SOS response. a** | Exposure to ultraviolet (UV) radiation or oxidative radicals[38] can damage chromosomal DNA, thereby leading to the induction of the DNA-damage response system (the SOS response). The transcription of over 30 unlinked genes[39] is coordinately activated, which facilitates the repair of damaged DNA without transmission to daughter cells. **b** | On a molecular level, mismatched base pairs produce regions of single-stranded DNA that serve as a polymerization platform for RecA, the major bacterial recombinase. Polymerized RecA is activated in the presence of free nucleotide triphosphates and stimulates the autoproteolysis of the SOS transcriptional repressor LexA. Most of the genes that are transcriptionally activated are DNA-repair enzymes. **c** | The LexA regulon also includes a cell-division inhibitor, SulA, to prevent the transmission of mutant DNA to new daughter cells. SulA specifically inhibits polymerization of the division protein FtsZ[40,41] by binding to the FtsZ monomer[42,43]. This blocks FtsZ ring formation at mid-cell, which results in the formation of non-septate bacterial filaments. **d** | Once DNA repair is complete, LexA repression of the SOS genes is restored. In addition, the general cytoplasmic protease Lon degrades SulA, thereby restoring cell-division capacity.

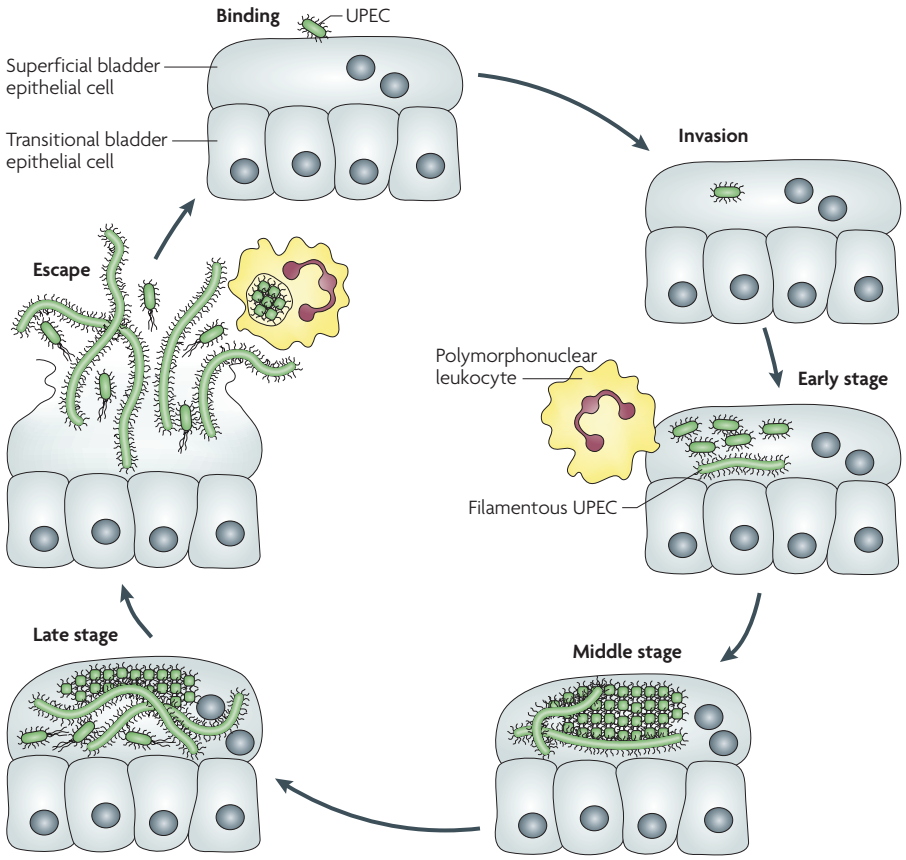

Figure 2 | **Model of the UTI pathogenic cascade.** The sequence of events that take place during the progression of the establishment of a urinary-tract infection (UTI). The infection is cyclical in nature and the events that occur in one cycle of the acute infection are depicted. Uropathogenic *Escherichia coli* (UPEC) bind to, and invade, superficial bladder epithelial cells. At 'early' stages, the average bacterial length is 3 μm and the community doubles in size every 30 minutes[10]. Transition to the 'middle' intracellular bacterial-community stage is hallmarked by a decrease in the average bacterial length to 1 μm and a doubling time of 45 minutes. In 'late' stages, the bacteria return to the typical rod shape (3 μm)[10]. Filamentous and late-stage rod-shaped bacteria detach from the community, 'escape' from epithelial cells and either attach to naive epithelial cells or exit the host during micturition. Filamentous UPEC are induced intracellularly in the urothelial cells that are capable of recognizing bacterial lipopolysaccharide[11]. The acute infection can progress through additional cycles (indicated by arrows). This figure is modified, with permission, from REF. 10 © (2004) National Academy of Sciences.

filamentous prey are resistant to protist predation in a number of marine environments (reviewed in REF. 28).

Grazing-resistant filaments. In marine ecosystems, bacteria represent a major component of the food chain. The quality of the bacterial food source in an ecosystem has a substantial impact on the diversity of higher marine organisms[28]. Numerous groups who evaluated predator–prey interactions in natural aquatic ecosystems[28] or laboratory-controlled ecosystems within chemostats[29] have observed that the presence of eukaryotic protists alters the distribution of bacterial morphologies. The protists feed or 'graze' on the numerous bacterial species that are present in the water. There is a size-class preference for grazing that varies for each species of protist[29]. In general, filamentous bacteria that are larger than 7 μm in length are inedible by marine protists[29], which has led to the use of the term grazing-resistant filaments to describe this morphological class. According to one hypothesis, the size preference is related to the kinetics of phagocytosis, whereas another suggests that size restriction is due to the size of the phagocytic vesicle (reviewed in REF. 28). Although grazing-resistant filaments have been recognized for the past 30 years, controversy persists regarding whether the changes in bacterial morphology are due to the unavailability of nutrient supplies or to effectors that are produced by protists during grazing.

Protist by-products induce filamentation. The morphological plasticity of prey bacteria is a direct consequence of the by-products that are produced by grazing protists[30]. The morphological phenotypes of *Flectobacillus* spp. were evaluated in the presence and absence of the flagellate predator *Ochromonas* spp., in a laboratory-controlled environment within a chemostat (FIG. 3). When grown alone, *Flectobacillus* spp. remain a typical 6.2 μm in length. By contrast, *Ochromonas* spp. predation alters the overall cell length of the *Flectobacillus* spp. to an average of 18.6 μm, and they are resistant to grazing. Interestingly, an increase in the length of *Flectobacillus* spp. to an average of 11.4 μm is observed if the bacteria are exposed to the soluble by-products that are produced by grazing *Ochromonas* spp. and pass through a dialysis membrane. Although the molecular details of this interaction have yet to be elucidated, the filamentous phenotype is a direct consequence of predator-produced effectors.

UPEC and* Flectobacillus *spp. UPEC and *Flectobacillus* spp. share striking similarities during their interactions with eukaryotic cells in a predator–prey relationship (FIG. 4). In both cases, filamentation provides a survival advantage against phagocytosis. Additionally, filamentation occurs as a direct response to effectors that are produced by the predator or host. For *Flectobacillus* spp., it is the predation on neighbouring bacteria that produces molecules that induce filamentation. For UPEC, the host produces effectors on recognition of the invading pathogen that directly lead to filamentation. In both cases, phagocytosis leads to the killing and degradation of the bacteria. Although the primary functions of degradation are different (food source or protection of the host), it is reasonable to predict that protists also use oxidative radicals in the killing of ingested bacteria in a similar manner to that used by mammalian immune cells. This would lead to DNA damage and theinduction of the SOS response, thereby resulting in filamentation. Hence, DNA damage may serve as a signal that the bacteria are present in specific lethal environments and require transcriptional and morphological changes to survive. The observation in three diverse systems that filamentation leads to the prevention of phagocytosis and killing suggests that this could be a global survival strategy for microorganisms such as UPEC, *Flectobacillus* spp. and *Candida albicans* (BOX 1).

Resistance to antibiotics

In addition to resistance to biological stresses that are native to the host, filamentation may also contribute to a pathogen's resistance to antimicrobial agents, which has obvious implications for therapeutics. Filamentous organisms are commonly observed in clinical specimens taken from patients who are undergoing antibiotic treatment. The molecular correlate of this was described by Cohen and colleagues[31], who reported that exposure to β-lactam antibiotics induces the SOS response in *E. coli*. In this case, the inhibition of cell division does not satisfy the need to permit DNA-damage repair, but instead obviates the need for cell-wall synthesis during the period of exposure to these cell-wall synthetic inhibitors.

More recent data for *B. pseudomallei* also suggest that the developmental changes that result from antibiotic exposure provide unanticipated protection against various insults[3]. Exposure of *B. pseudomallei* to levels of ceftazidime (a β-lactam antibiotic) at, or below, the minimal inhibitory concentration resulted in the appearance of filamentous bacteria that remained viable within the vacuoles of immortalized human macrophages. By contrast, the bacillary form was susceptible to killing under the same experimental conditions. Moreover, after removal of the antibiotic and restoration of cell division, the filament-derived, bacillary daughter cells maintained cell-division capacity and viability despite re-exposure to ceftazidime and antibiotics of other classes[3] (FIG. 4). Thus, the developmental changes that accompany filamentation may provide additional survival advantages. Furthermore, the induction of the SOS response can augment the horizontal transfer of DNA, particularly antibiotic-resistance determinants, among pathogenic bacteria[32]. These findings underscore the importance of informed antibiotic prescribing, and patient adherence to dosing schedules, to the successful treatment of infectious diseases. Strategies to identify and develop small-molecule inhibitors of filamentation might lead to new therapeutics and will be valuable for chemical genetic studies that examine aspects of filamentation in model systems.

Other environmental stresses

Caulobacter crescentus is a dimorphic aquatic bacterium that has been investigated owing to the developmental changes that occur during its growth. The 'stalk' cell is the reproductive form that produces daughter 'swarmer' cells that are incapable of cell division until they differentiate into a stalk cell[33]. Prolonged growth of *C. crescentus* results in a decrease in the FtsZ concentration, which leads to the formation of long, helical, filamentous organisms that have acquired resistance to heat, oxidative stress and changes in alkalinity[34]. Although the mechanism of enhanced survival is unknown, there is evidence to indicate that this process is an additional developmental pathway that does not involve permanent mutation, which further implicates filamentation as a component of bacterial survival strategies.

Perspective

It is our hypothesis that the morphological plasticity of pathogenic bacteria is a direct and adaptive response to the sensing of environmental changes, which would yield fitter organisms that can survive multiple forms of external stress. It is evident from the published data summarized here that multiple systems are involved in the regulation of cell-division inhibition and filamentation. Although the molecular mechanisms that led to filamentation in each of these examples have not been

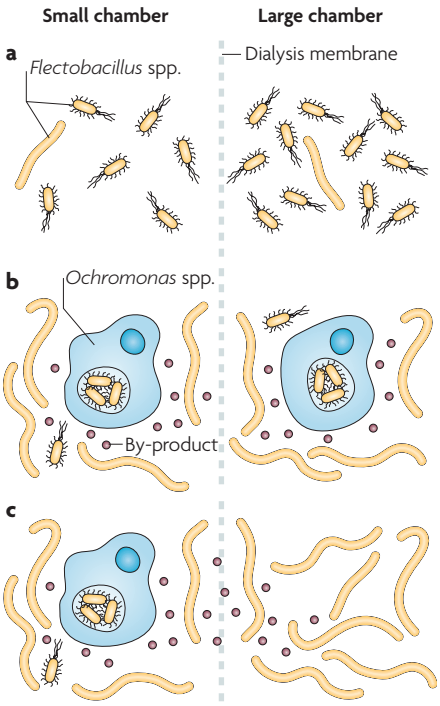

Figure 3 | **Protist by-products induce filamentation.** A schematic representation of a chemostat, showing the two chambers (a small chamber and a large chamber) that are divided by a dialysis membrane[30]. **a** | Growth of *Flectobacillus* spp. occurs in both chambers, typically to the normal bacterial-cell length (6.2 μm). **b** | The growth of *Flectobacillus* spp. and the protist *Ochromonas* spp. within both chambers results in an increase in the average bacterial-cell length (18.6 μm), which leads to the prevention of phagocytosis of that form. **c** | Bacterial-cell length of the filamentous form of *Flectobacillus* spp. increases (11.4 μm) in the large chamber, owing to the presence of *Ochromonas* spp. by-products that are derived from protist feeding on the bacteria in the small chamber. The by-products that are produced by the grazing protists subsequently pass through the dialysis membrane, which has a molecular-weight cut-off of 15,000 daltons. The nature of the by-product is unknown. This figure is modified, with permission, from REF. 30 © (2006) American Society for Microbiology.

Box 1 | **Morphological plasticity and fungal survival**

Fungi are, perhaps, the most recognized organisms that use morphological plasticity in different environments. This phenomenon is so common that the term dimorphic is used to describe fungal species. Although only a small proportion of fungi are pathogenic, most of the pathogenic species are dimorphic. There is no set rule for the dimorphic form that is pathogenic (reviewed in REF. 35). In most cases, the yeast form is pathogenic (for example, in *Histoplasma capsulatum* and *Blastomyces dermatitidis*). However, there are examples in which the hyphal or filamentous form is pathogenic (for example, *Candida albicans*). In fact, *C. albicans* is associated with the highest incidence of invasive fungal infection[35]. It is also a common cause of nosocomial infection and is becoming an increasing threat to immunocompromised patients, particularly as strains are emerging that are resistant to standard antifungal agents. Filamentation is crucial for *C. albicans* biofilm formation and virulence in the host, and gene-specific control mechanisms are being dissected to drive the development of anti-filamentation strategies that may be effective in combination antifungal therapies[36,37].

It is also crucial to investigate the potential for distinct morphological forms of fungi to evade host responses during pathogenesis, such as for uropathogenic *Escherichia coli* infection. For *C. albicans*, the filamentous or hyphal form invades the tissue and therefore escapes phagocytosis. However, there is no universal rule for the form that is able to subvert host innate immune effectors. The filamentous form of *B. dermatitidis*, for example, is sensitive to killing by phagocytosis, whereas the yeast form is resistant. Thus, the molecular mechanisms for morphological switches, and the evasion of host responses and killing, differ among fungal species, just as they probably differ among bacterial species. Yet, the use of morphological plasticity remains a common theme for survival.

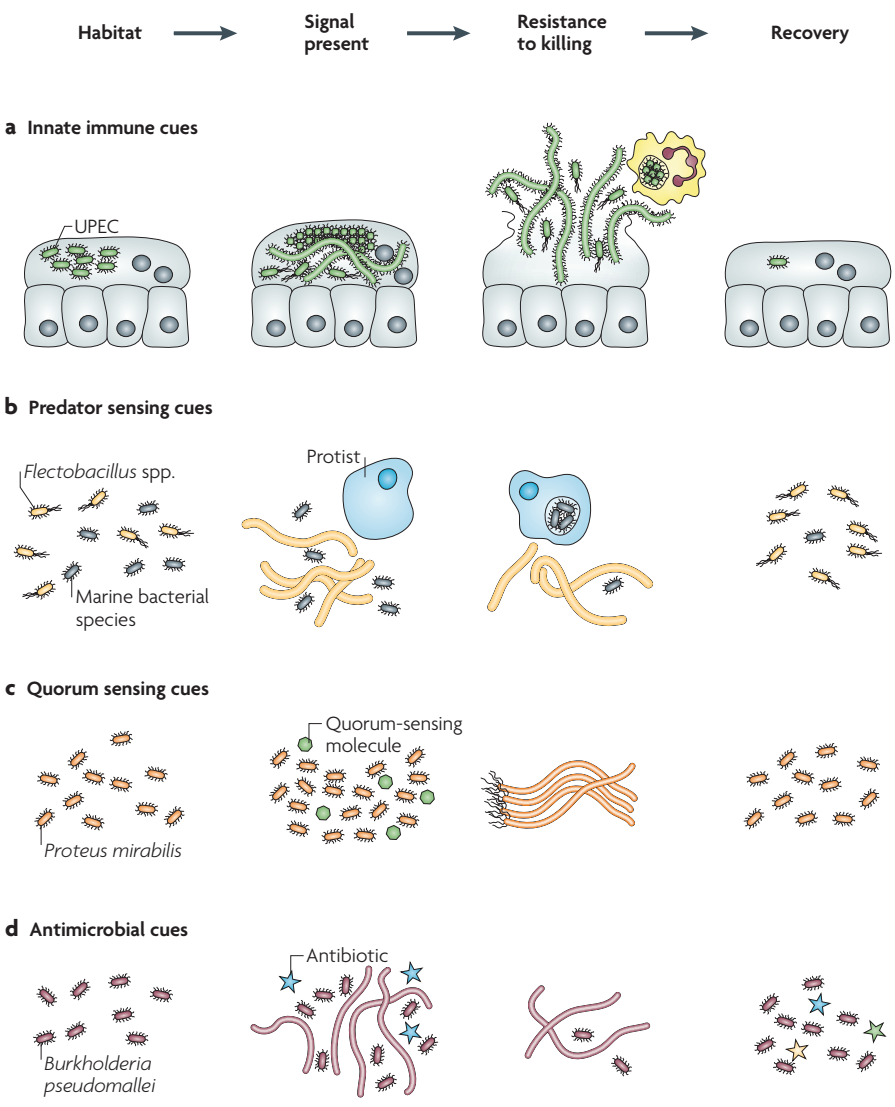

Figure 4 | **Filamentation of bacteria in response to environmental cues.** A schematic representation of the known triggers and biological effects of filamentation. **a** | Innate immune cues. Uropathogenic *Escherichia coli* (UPEC) reside intracellularly within bladder epithelial cells. A small number of intracellular bacteria respond to the activation of host immune effectors by filamentation. Epithelial-cell death accompanies bacterial growth, thereby resulting in the exposure of filamentous and bacillary organisms on the surface. The filamentous form is resistant to neutrophil phagocytosis[11]. The recovery from filamentation results in the invasion of naive epithelial cells to begin the process again. **b** | Predator sensing cues. Marine bacterial populations are made up of multiple species, including the prototypical *Flectobacillus* spp. Protist grazing on all species stimulates filamentation in *Flectobacillus* spp. Filamentous forms cannot be grazed by marine protists. Recovery from filamentation results in an alteration in the diversity of bacteria in the environment and the depletion of non-*Flectobacillus* species[28]. **c** | Quorum sensing cues. *Proteus mirabilis* grows as a bacillary form that can count the number of similar species in the vicinity by quorum sensing[44]. If a quorum of organisms is verified, the bacteria respond by initiating the differential gene expression that leads to filamentation. Although a mechanistic reason for filamentation in *Proteus* spp. is under debate, the evidence suggests that this morphology leads to enhanced invasion of the urothelium, thereby providing protection from the host immune response[44,45]. Dissemination into the tissue disperses the swarming filaments. Cell division is restored owing to low levels of quorum-sensing molecules in the tissue. **d** | Antimicrobial cues. Filamentation occurs if bacteria are exposed to certain β-lactam antibiotics *in vitro* and *in vivo*. Filamentation allows for survival until the antibiotic is diluted or becomes inactive. The restoration of cell division occurs once the antibiotic activity is lost. In some cases, for example, *Burkholderia pseudomallei*, cell-division capacity is maintained even in the presence of antibiotics of similar and dissimilar classes, which indicates that protection has been conferred to daughter cells. The mechanisms and consequences of this response have not yet been explored.

completely resolved, filamentation seems to be a survival tactic (FIG. 4). The observation that filamentation can result in bacterial cells that are simultaneously resistant to multiple unrelated insults (that is, phagocytosis and antibiotics) suggests that filamentation could be a multi-factorial survival strategy.

Our existing arsenal of antibiotics has substantially exhausted the known targets of microbial cell biology and biochemistry, thereby obliging us to turn in a new direction. Further anti-infective development will, therefore, be driven by the discovery of the pathogenic processes of infectious diseases at the cellular and molecular level. Along these lines, candidate drug targets include other essential, ubiquitous and highly conserved bacterial processes, such as cell division (which includes the FtsZ protein). Although such a new inhibitor might be effective in halting planktonic growth, the association of filamentation with virulence and antimicrobial resistance supports the notion that the inhibition of filament formation might be more effective at interrupting pathogenesis. In fact, agents that interfere with yeast filamentation are currently being considered as antifungal therapies (BOX 1). Anti-filamentation agents that are specifically directed against pathogenic organisms might be less effective against the patient's normal flora, thus decreasing the probability of antimicrobial resistance and increasing patient compliance to complete treatment.

Most studies on bacterial physiology and pathogenesis have been carried out using organisms that are in classically described morphological states. In addition, much of the data regarding bacterial systems that promote filamentation have been gathered *in vitro*. Given the evidence summarized here, a comprehensive understanding of bacterial-survival strategies will require a more global perspective and consideration of distinct morphological forms within the host and in other host-relevant environmental conditions.

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1. Duguay, A. R. & Silhavy, T. J. Quality control in the bacterial periplasm. *Biochim. Biophys. Acta* **1694**, 121–134 (2004).
2. Siu, L. K. Antibiotics: action and resistance in Gram-negative bacteria. *J. Microbiol. Immunol. Infect.* **35**, 1–11 (2002).
3. Chen, K., Sun, G. W., Chua, K. L. & Gan, Y. H. Modified virulence of antibiotic-induced *Burkholderia pseudomallei* filaments. *Antimicrob. Agents Chemother.* **49**, 1002–1009 (2005).
4. Young, K. D. The selective value of bacterial shape. *Microbiol. Mol. Biol. Rev.* **70**, 660–703 (2006).
5. Zaritsky, A. On dimensional determination of rod-shaped bacteria. *J. Theor. Biol.* **54**, 243–248 (1975).
6. Harry, E., Monahan, L. & Thompson, L. Bacterial cell division: the mechanism and its precison. *Int. Rev. Cytol.* **253**, 27–94 (2006).
7. Rothfield, L., Justice, S. & Garcia-Lara, J. Bacterial cell division. *Annu. Rev. Genet.* **33**, 423–448 (1999).
8. Foxman, B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Dis. Mon.* **49**, 53–70 (2003).
9. Mulvey, M. A. *et al.* Induction and evasion of host defenses by type 1-piliated uropathogenic *Escherichia coli*. *Science* **282**, 1494–1497 (1998).
10. Justice, S. S. *et al.* Differentiation and developmental pathways of uropathogenic *Escherichia coli* in urinary tract pathogenesis. *Proc. Natl Acad. Sci. USA* **101**, 1333–1338 (2004).
11. Justice, S. S., Hunstad, D. A., Seed, P. C. & Hultgren, S. J. Filamentation by *Escherichia coli* subverts innate defenses during urinary tract infection. *Proc. Natl Acad. Sci. USA* **103**, 19884–19889 (2006).
12. Leroy, M. *et al.* Multiple consecutive lavage samplings reveal greater burden of disease and provide direct access to the nontypeable *Haemophilus influenzae* biofilm in experimental otitis media. *Infect. Immun.* **75**, 4158–4172 (2007).
13. Jurcisek, J. A. & Bakaletz, L. O. Biofilms formed by nontypeable *Haemophilus influenzae in vivo* contain both double-stranded DNA and type IV pilin protein. *J. Bacteriol.* **189**, 3868–3875 (2007).
14. Belas, R. & Suvanasuthi, R. The ability of *Proteus mirabilis* to sense surfaces and regulate virulence gene expression involves FliL, a flagellar basal body protein. *J. Bacteriol.* **187**, 6789–6803 (2005).
15. Belas, R., Manos, J. & Suvanasuthi, R. *Proteus mirabilis* ZapA metalloprotease degrades a broad spectrum of substrates, including antimicrobial peptides. *Infect. Immun.* **72**, 5159–5167 (2004).
16. Piao, Z., Sze, C. C., Barysheva, O., Iida, K. & Yoshida, S. Temperature-regulated formation of mycelial mat-like biofilms by *Legionella pneumophila*. *Appl. Environ. Microbiol.* **72**, 1613–1622 (2006).
17. Ogawa, M., Takade, A., Miyamoto, H., Taniguchi, H. & Yoshida, S. Morphological variety of intracellular microcolonies of *Legionella* species in Vero cells. *Microbiol. Immunol.* **45**, 557–562 (2001).
18. Chauhan, A. *et al. Mycobacterium tuberculosis* cells growing in macrophages are filamentous and deficient in FtsZ rings. *J. Bacteriol.* **188**, 1856–1865 (2006).
19. Abrahams, G. L. & Hensel, M. Manipulating cellular transport and immune responses: dynamic interactions between intracellular *Salmonella enterica* and its host cells. *Cell. Microbiol.* **8**, 728–737 (2006).
20. Rosenberger, C. M. & Finlay, B. B. Macrophages inhibit *Salmonella typhimurium* replication through MEK/ERK kinase and phagocyte NADPH oxidase activities. *J. Biol. Chem.* **277**, 18753–18762 (2002).
21. Eriksson, S., Lucchini, S., Thompson, A., Rhen, M. & Hinton, J. C. Unravelling the biology of macrophage infection by gene expression profiling of intracellular *Salmonella enterica*. *Mol. Microbiol.* **47**, 103–118 (2003).
22. Henry, T., Garcia-Del Portillo, F. & Gorvel, J. P. Identification of *Salmonella* functions critical for bacterial cell division within eukaryotic cells. *Mol. Microbiol.* **56**, 252–267 (2005).
23. Lucchini, S., Liu, H., Jin, Q., Hinton, J. C. & Yu, J. Transcriptional adaptation of *Shigella flexneri* during infection of macrophages and epithelial cells: insights into the strategies of a cytosolic bacterial pathogen. *Infect. Immun.* **73**, 88–102 (2005).
24. Jones, A. L., Beveridge, T. J. & Woods, D. E. Intracellular survival of *Burkholderia pseudomallei*. *Infect. Immun.* **64**, 782–790 (1996).
25. Cuccui, J. *et al.* Development of signature-tagged mutagenesis in *Burkholderia pseudomallei* to identify genes important in survival and pathogenesis. *Infect. Immun.* **75**, 1186–1195 (2007).
26. Fields, B. S., Shotts, E. B. Jr, Feeley, J. C., Gorman, G. W. & Martin, W. T. Proliferation of *Legionella pneumophila* as an intracellular parasite of the ciliated protozoan *Tetrahymena pyriformis*. *Appl. Environ. Microbiol.* **47**, 467–471 (1984).
27. Hilbi, H., Weber, S. S., Ragaz, C., Nyfeler, Y. & Urwyler, S. Environmental predators as models for bacterial pathogenesis. *Environ. Microbiol.* **9**, 563–575 (2007).
28. Pernthaler, J. Predation on prokaryotes in the water column and its ecological implications. *Nature Rev. Microbiol.* **3**, 537–546 (2005).
29. Jurgens, K. & Matz, C. Predation as a shaping force for the phenotypic and genotypic composition of planktonic bacteria. *Antonie Van Leeuwenhoek* **81**, 413–434 (2002).
30. Corno, G. & Jurgens, K. Direct and indirect effects of protist predation on population size structure of a bacterial strain with high phenotypic plasticity. *Appl. Environ. Microbiol.* **72**, 78–86 (2006).
31. Miller, C. *et al.* SOS response induction by β-lactams and bacterial defense against antibiotic lethality. *Science* **305**, 1629–1631 (2004).
32. Beaber, J. W., Hochhut, B. & Waldor, M. K. SOS response promotes horizontal dissemination of antibiotic resistance genes. *Nature* **427**, 72–74 (2004).
33. Wagner, J. K. & Brun, Y. V. Out on a limb: how the *Caulobacter* stalk can boost the study of bacterial cell shape. *Mol. Microbiol.* **64**, 28–33 (2007).
34. Wortinger, M. A., Quardokus, E. M. & Brun, Y. V. Morphological adaptation and inhibition of cell division during stationary phase in *Caulobacter crescentus*. *Mol. Microbiol.* **29**, 963–973 (1998).
35. Rooney, P. J. & Klein, B. S. Linking fungal morphogenesis with virulence. *Cell. Microbiol.* **4**, 127–137 (2002).
36. Saville, S. P. *et al.* Inhibition of filamentation can be used to treat disseminated candidiasis. *Antimicrob. Agents Chemother.* **50**, 3312–3316 (2006).
37. Nemecek, J. C., Wuthrich, M. & Klein, B. S. Global control of dimorphism and virulence in fungi. *Science* **312**, 583–588 (2006).
38. Janion, C. Some aspects of the SOS response system — a critical survey. *Acta Biochim. Pol.* **48**, 599–610 (2001).
39. Quillardet, P., Rouffaud, M. A. & Bouige, P. DNA array analysis of gene expression in response to UV irradiation in *Escherichia coli*. *Res. Microbiol.* **154**, 559–572 (2003).
40. Mukherjee, A., Cao, C. & Lutkenhaus, J. Inhibition of FtsZ polymerization by SulA, an inhibitor of septation in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **95**, 2885–2890 (1998).
41. Trusca, D., Scott, S., Thompson, C. & Bramhill, D. Bacterial SOS checkpoint protein SulA inhibits polymerization of purified FtsZ cell division protein. *J. Bacteriol.* **180**, 3946–3953 (1998).
42. Justice, S. S., Garcia-Lara, J. & Rothfield, L. I. Cell division inhibitors SulA and MinC/MinD block septum formation at different steps in the assembly of the *Escherichia coli* division machinery. *Mol. Microbiol.* **37**, 410–423 (2000).
43. Cordell, S. C., Robinson, E. J. & Lowe, J. Crystal structure of the SOS cell division inhibitor SulA and in complex with FtsZ. *Proc. Natl Acad. Sci. USA* **100**, 7889–7894 (2003).
44. Rather, P. N. Swarmer cell differentiation in *Proteus mirabilis*. *Environ. Microbiol.* **7**, 1065–1073 (2005).
45. Allison, C., Coleman, N., Jones, P. L. & Hughes, C. Ability of *Proteus mirabilis* to invade human urothelial cells is coupled to motility and swarming differentiation. *Infect. Immun.* **60**, 4740–4746 (1992).

DATABASES

Entrez Genome Project: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj

Burkholderia pseudomallei | *Candida albicans* | *Caulobacter crescentus* | *Escherichia coli* | *Haemophilus influenzae* | *Legionella pneumophila* | *Mycobacterium tuberculosis* | *Proteus mirabilis* | *Salmonella typhimurium* | *Shigella flexneri*

FURTHER INFORMATION

Sheryl S. Justice's homepage: http://www.nationwidechildrens.org/gd/applications/controller.cfm?page=3812&pname=bio&rID=682

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