Oxygen-utilizing reactions and symbiotic colonization of the squid light organ by *Vibrio fischeri*

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A major goal in microbiology is to understand the processes by which bacteria successfully colonize host tissue. Although a wealth of studies focusing on pathogenic microorganisms has revealed much about the rare interactions that result in disease, far less is known about the regulation of the ubiquitous, long-term, cooperative associations of bacteria with their animal hosts.

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One of the most prevalent and successful bacterial lifestyles is a long-term association with the surface tissues of animal and plant hosts. Contrary to popular belief, only a small minority of these associations have a negative impact on host fitness and, in fact, interaction with bacteria is increasingly being recognized as a requirement for normal host development and growth[1]. In spite of their importance, we know remarkably little about colonization by cooperative bacteria, especially those of the complex consortia found on the skin and digestive-tract epithelia of virtually all animals. Experimental model systems have emerged that utilize certain natural associations between single species of bacteria and specific host tissues[2–5]. These models are especially useful in defining the stages by which such associations develop, as well as defining the mechanisms underlying specificity and signaling. Interestingly, these studies provide evidence that similar biochemical activities and effectors are used by both cooperative and pathogenic microorganisms.

The colonization determinants of animal–bacterial associations, although diverse, can be divided into two broad classes: specific receptor–ligand, signal–response systems and certain physiological conditions in the environment of the bacteria–host interaction. Included in the latter are the presence of molecular oxygen and the reactive oxygen species (ROS), most notably superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and nitric oxide. Oxygen concentration has been described as a modulator of cooperative host–microorganism interactions in several distinct symbiotic associations, including those between root-nodule bacteria and leguminous plants[6], photosynthetic algae and marine invertebrates[7], chemoautotrophic bacteria and their hosts[8], and luminous bacteria and fish[9]. In each of these systems, via concerted control of oxygen concentration by the two partners, the association is believed to achieve a valuable symbiotic activity (e.g. nitrogen fixation, photosynthesis, chemosynthesis or bioluminescence). However, our knowledge of the mechanisms by which oxygen and its derivatives exert their effects remains rudimentary. For example, the possible role of oxygen in bacteria–fish bioluminescent associations is based primarily on experiments with luminous bacteria grown in laboratory culture. Such studies have shown that reduced oxygen availability increases the specific activity of bacterial light emission and depresses bacterial growth rate. These results have led to the hypothesis that the host might increase the efficiency of its metabolic investment in the association by controlling oxygen concentration[9].

In this review, we examine what has been discovered in the past five years about the dynamic and diverse nature of oxygen metabolism in the bioluminescent association between the sepiolid squid *Euprymna scolopes* and the luminous bacterium *Vibrio fischeri*. Experimental studies have revealed that not only oxygen but also its reactive derivatives play important roles in promoting both the efficiency and specificity of the association. We believe that the concepts presented here represent phenomena that might also apply to many other cooperative associations.

A brief background to the *E. scolopes–V. fischeri* association

Who are the players?

E. scolopes belongs to the Sepiolidae mollusc family, the members of which are commonly called bobtail squids[10]. Although there are over a dozen species in this widely distributed family, *E. scolopes* is restricted to the Hawaiian archipelago and is the only sepiolid species reported in Hawaiian waters[11]. This nocturnal predator hides during the day, buried in the sands surrounding nearshore coral reefs, but each night emerges to forage in the water column for its prey. The bioluminescence produced by the squid originates from the luminous bacterial symbionts that live within an internal light-emitting organ. The symbionts

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provide the squids with a camouflage strategy called counterillumination, which is used by the squid host for hunting in the transparent shallow-water environment above the reef. Counterilluminating animals emit light from their ventral surface, mimicking downwelling starlight and moonlight, and thereby cast no shadow when viewed from below[1]. *E. scolopes* achieves this by using its light organ, which is composed of a complex set of tissues, including both a central core of epithelium-lined crypts that harbor the extracellular bacterial symbionts and a series of accessory tissues that dynamically direct, modulate and diffuse the luminescence[1].

The bacterial species exclusively found in these crypts is *V. fischeri*[12], a marine heterotroph belonging to the Vibrionaceae, a large family of γ-proteobacteria that comprises many species characterized by both cooperative and pathogenic interactions with animal tissue[13]. *V. fischeri* has a worldwide distribution, principally in temperate and subtropical waters, occupying a variety of niches[14]. In addition to being a light-organ symbiont of several species of squids and fishes, *V. fischeri* also occurs enterically in many marine animals, as a pathogen of certain invertebrates and as a 'free-living' saprophyte growing on dissolved and particulate organic matter. In locations where they form light-organ symbioses with animals, free-living *V. fischeri* in seawater are the inoculum for the juvenile animal host[15].

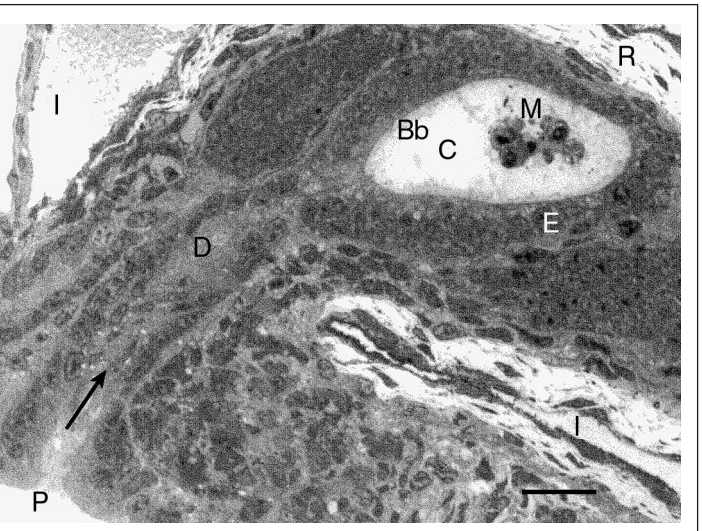

Fig. 1. Histological section through one crypt of the nascent light organ of a newly hatched juvenile *Euprymna scolopes*. The light organ lies within the mantle, or visceral, cavity of the animal, embedded in the ink sack (I). I diverticula and a thick reflector (R) control bioluminescence produced by the symbionts[1]. An external pore (P) leads via a cilia-lined duct (D and arrow) to each of six internal crypts (C), in which the symbiotic population of *Vibrio fischeri* cells is maintained. In the crypts, *V. fischeri* interact primarily with two host cell types: epithelial cells (E) lining the crypts, whose apical surface bears a dense brush-border (Bb) and granulocyte macrophages (M) that are free within the crypt spaces. This type of mollusc hemocyte can phagocytose bacterial cells and produce an oxidative burst, and might be important in assuring the specificity of the association. Scale bar = 50 μm.

How do they interact?

The association between *E. scolopes* and *V. fischeri* begins within hours of the juvenile squid hatching from its egg. For this reason, it is easy experimentally to initiate the colonization of laboratory-reared juvenile squids. When added to vials of seawater containing the 2-mm-long newly hatched animals, *V. fischeri* enter pores on the exterior of the nascent light organ and use their polar flagella (and perhaps chemotaxis) to swim through ducts that lead to the interior crypts (Fig. 1). The presence of a ciliated surface epithelium surrounding the pores apparently potentiates inoculation by bringing the bacteria closer to the crypt entrances. The *V. fischeri* that initiate colonization adhere to the crypt epithelium, grow by means of host-supplied nutrients and, within 12 h, fill the crypt spaces with a population of between 10^5–10^6 bacteria[16]. Thereafter, every day at dawn the squid expels most of the crypt contents, including 90%–95% of the bacterial population, through the pores and into the external environment[17]. By the following evening, the residual *V. fischeri* have proliferated, repopulating the crypts and restoring the full potential for bioluminescence. This continuous cycle of purging and regrowth maintains a metabolically robust population of symbionts and selects bacterial strains that not only are capable of continued growth within the crypts, but also are competitively dominant under those particular conditions[18].

The morphology of the symbiotic organ changes dramatically over the first several days following inoculation with *V. fischeri*[19]. The entire ciliated surface epithelium regresses, the epithelial cells lining the crypt interior swell[20] and the density of microvilli along their apical surfaces increases fourfold, enhancing the intimacy between the host and bacterial cells[21]. Experimental manipulation has shown that each of these early developmental events is induced only when the crypts are colonized by *V. fischeri*. Both the swelling (J.A. Doino, PhD thesis, University of Southern California, 1998) and the increase in microvilli density[21] quickly revert to the non-symbiotic state if the bacterial population is removed by antibiotic treatment. However, the loss of the ciliated surface epithelium is irreversible[22].

V. fischeri also undergo a differentiation process during the initial hours of their association with the host. Within 12 h of the start of colonization, they exhibit a loss of flagellation, reduction in cell size, decrease in growth rate and enhancement of cell-specific luminescence[23]. The onset of these events is apparently controlled by the crypt environment, which the bacterium reacts to by changes in gene expression[24]. Recent ultrastructural studies of both juvenile and adult light organs[25] have revealed that *V. fischeri* interact not only with the crypt epithelium but also with a population of amoeboid granulocytes (phagocytes) that move freely within the crypt spaces (Fig. 1). This interaction might play a role in removing other bacterial species, thereby contributing to the characteristic specificity of the association.

As it grows and matures during the months following hatching, the squid nourishes and maintains a

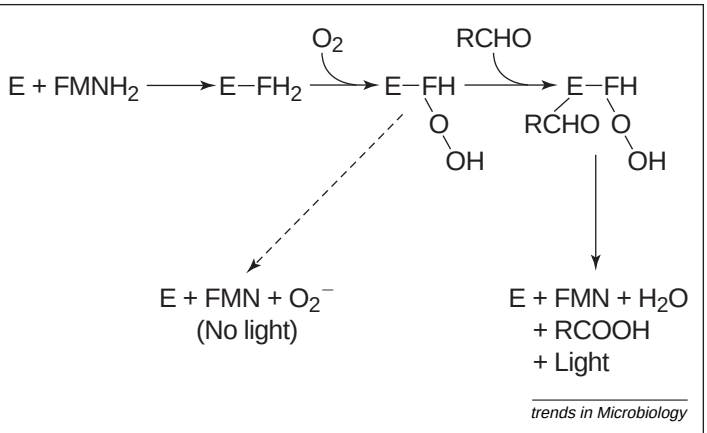

Fig. 2. Reactions catalysed by bacterial luciferase. Luciferase (E) is a mixed-function oxidase that uses molecular oxygen (O_2) to simultaneously oxidize reduced flavin mononucleotide ($FMNH_2$) and a long-chain aliphatic aldehyde (RCHO), producing water, oxidized flavin (FMN) and the corresponding acid (RCOOH). The typical reaction begins when luciferase binds first $FMNH_2$ and then O_2, forming an unstable flavin peroxide. If RCHO is present, it is bound next and the oxidase reaction proceeds, with the emission of light energy. If no RCHO is present, the flavin peroxide decomposes in an alternative pathway (dashed arrow) that regenerates FMN and produces hydrogen peroxide (H_2O_2) rather than water; in this 'dark reaction' no light is emitted. (Adapted from Ref. 29.)

V. fischeri population, which, in the adult host, can contain as many as 10^9 cells. This non-pathogenic infection is characterized by two remarkable traits: firstly, although in contact with the surrounding seawater, the symbiont population remains monospecific, resisting any detectable contamination from other bacteria in the external environment and, secondly, there is no opportunistic invasion of adjacent host tissues by *V. fischeri*[23]. These findings indicate the presence of a specific, tightly regulated recognition process between the bacteria and the host tissues. Further proof of this specificity comes from population genetics studies showing that, although strains of *V. fischeri* isolated from different species of *Euprymna* are capable of initiating and sustaining a symbiosis with *E. scolopes*, when presented in mixed inoculations these strains are outcompeted by the native symbiont strains[10]. Thus, squid–*Vibrio* associations appear to be complex and ancient, apparently resulting from coordinated evolution between pairs of host species and their symbionts[10,26–28].

Oxygen metabolism of bioluminescent bacteria

Symbiotic strains of *V. fischeri* typically grow aerobically in laboratory culture but they are, in fact, facultative anaerobes capable of multiplying in natural environments in which oxygen might not be available[14]. *V. fischeri* has been the focus of studies of bioluminescence, another major oxygen-utilizing reaction of this bacterium[29]. Bacterial luciferase catalyses a unique mixed-function oxidation reaction that normally leads to the complete reduction of oxygen to H_2O, with the concomitant emission of a blue-green light (Fig. 2). An intriguing enzymatic characteristic of bacterial luciferases is their unusually high affinity for oxygen, with Michelis–Menton constants (K_ms) in the nanomolar range[30]. As a result, luminescing bacteria have a relatively high specific activity of oxygen consumption that supports both aerobic respiration and light emission. Under conditions in which the supply of oxygen is limiting, bioluminescence activity might outcompete respiration by maintaining the ambient concentration of oxygen below that required to support the activity of other oxidases[31,32]. The high oxygen affinity of luciferase, which might constitute up to 5% of the total soluble protein of *V. fischeri*[14], suggests that luminous bacteria have the potential to play an important role in lowering the oxygen concentration in their ambient environment.

Other enzymatic studies of luciferase have shown that, under certain conditions, it can also catalyse a reaction that produces ROS (Fig. 2). If the final substrate to bind the enzyme complex – aldehyde – is not present, luciferase will catalyse a non-luminescent reaction in which the oxygen molecule is only partially reduced, resulting in the release of O_2^-. This 'dark pathway' has been demonstrated with purified luciferase, with mutant strains of luminous bacteria that are incapable of aldehyde biosynthesis, as well as with *Escherichia coli* cells carrying the luciferase genes but not the genes required for aldehyde synthesis[29,33]. However, the relevance of the pathway to the normal metabolism of luminous bacteria has remained essentially unexamined.

We hypothesize that the dark pathway might provide clues to the process by which bacterial luciferase evolved. Perhaps its ancestor was an enzyme that did not catalyse a light-producing reaction but performed the simpler, O_2^--producing dark reaction (Fig. 2). Interestingly, several pathogenic *Vibrio* species have been shown to carry the genes for luciferase, yet produce no light[34]. In the case of the fish pathogen *Vibrio salmonicida*, high levels of luciferase are synthesized but luminescence is emitted only when aldehyde is added experimentally[35]. It is possible that the principal function of the luciferases of these pathogens is not the production of light but the generation of O_2^- that damages host tissue and thereby encourages nutrient release and colonization. Such a strategy is employed by pathogenic mycoplasma, which use a different class of O_2^--generating enzymes[36].

Manipulation of ROS by mammalian pathogenic bacteria

The determinants of bacterial virulence include several proteins that allow bacterial pathogens to withstand oxidative stress, a common host defense strategy. The mechanisms by which oxidative stress is imposed are varied but commonly involve a 'respiratory (or oxidative) burst' produced by antimicrobial macrophage-like cells[37]. In this reaction, oxygen is partially reduced by an activated membrane-bound NADPH–oxidase complex, producing O_2^- that is exported either into an intracellular lysosome or out of the host cell. O_2^- can either act directly on its target or can give rise to other toxic ROS, such as H_2O_2. Similarly, H_2O_2 can be a substrate for a secreted halide peroxidase such as mammalian myeloperoxidase (MPO),

Table 1. Halide peroxidase activity in host tissues of other symbioses[a]

Species	Tissue[b]	Symbionts present[c]	Mean specific activity[d] (range)
Fish			
Cleidopus gloriamaris (pinecone fish)	Light organ (2)	Yes	5.6 (5.1–6.2)
	Intestine (2)	Yes	250 (240–260)
	Muscle (2)	No	3.4 (3.1–3.6)
Leiognathus equulus (ponyfish)	Light organ (14)	Yes	47 (18–91)
	Intestine (13)	Yes	360 (110–1400)
	Muscle (14)	No	0.5 (<0.1–3.3)
Molluscs			
Crassostrea virginica (American oyster)	Gill (9)	Poss.	8.3 (<0.1–27)
	Muscle (9)	No	0.1 (<0.1–1.1)
Mercenaria mercenaria (quahog)	Gill (10)	Poss.	680 (129–1600)
	Foot (10)	No	2.1 (<0.1–8.9)
Mytilus edulis (edible mussel)	Gill (11)	Poss.	7.4 (0.8–13)
	Foot (10)	No	0.7 (<0.1–5.0)
Solemya reidi (chemosynthetic clam)	Gill (14)	Yes	61 (11–200)
	Foot (14)	No	0.9 (<0.1–5.5)
Insect			
Leucophaea maderae (African cockroach)	Fat body (2)	Yes	0.6 (0.4–0.8)
	Midgut (5)	Yes	140 (75–230)
	Muscle (2)	No	0.2 (0.1–0.3)
Vestimentiferan			
Riftia pachyptila (vent tube worm)	Trophosome (6)	Yes	160 (94–270)
	Vestimentum muscle (6)	No	20 (<0.1–97)
Cniderian			
Aiptasia pulchella (coral polyp)	Tentacle tips (14)	Yes	13 (<0.1–85)
	Pedal base muscle (14)	No	1.1 (<0.1–4.1)

[a]Dianisidine-type peroxidase activity measured as previously described[42].
[b]Number of samples assayed is given in parentheses.
[c]Yes, always present as cooperative and/or commensal; No, not present; Poss., possibly present because gills serve to clear pathogens.
[d]One unit of activity = 1 mM *O*-dianisidine dihydrochloride oxidized/min/mg protein.

which binds to the surface of bacterial cells and generates highly toxic HOCl (Ref. 37). Together, these various ROS present a formidable defense against unwanted bacterial colonization.

Many pathogenic bacteria either remove ROS or inhibit the host enzymes responsible for their generation[38]; for example, catalase rapidly converts H_2O_2 to oxygen and water. In several pathogens, catalase is either periplasmic or is secreted extracellularly, where it can detoxify any host-generated H_2O_2 before it has the opportunity to enter the bacterium. By contrast, some pathogens inhibit the synthesis of ROS by directly inhibiting NADPH-oxidase activity. For example, one of the effects of cholera toxin (Ctx), the principal virulence factor of *Vibrio cholerae*, is believed to be interference with the G-protein signaling cascade that activates NADPH oxidase[39].

Discovery of the squid haloperoxidase

Early in the study of the squid–*Vibrio* association, it was discovered that the light organ contains abundant mRNA encoding a squid halide peroxidase (HPO) with sequence[40] and enzymatic[41] similarities to mammalian MPO. Interestingly, this same protein is found associated with the gills of *E. scolopes*, which is the site at which unwanted bacteria are removed from squid tissues[42]. An examination of a number of other animal species, whose tissues support cooperative bacterial associations, has revealed that halide peroxidase activity might be common to these symbioses (Table 1). The discovery that a protein usually associated with antimicrobial activity is present in cooperative bacterial associations at first seems paradoxical. However, the abundance of HPO in the light organ could indicate that this enzyme is used not simply as a means to keep tissues axenic but is a part of a strategy that selects against undesired bacteria, while specifically permitting colonization by the appropriate symbiont.

Preliminary data suggest that colonization by *V. fischeri* dramatically changes the overall oxidative signature of the light organ, a response that includes a significant reduction in levels of HPO mRNA[42]. Presumably, by reducing transcription of this enzyme, the potential for the generation of microbicidal HOCl is reduced.

A model for ROS modulation during colonization of the squid light organ

The evidence suggests that, by working together to modulate the levels of host-generated ROS, the

symbiotic partners accomplish two mutually beneficial goals: selection against colonization by inappropriate bacteria (i.e. non-*V. fischeri* bacteria or *V. fischeri* strains that are not light emitting) and the provision of sufficient oxygen to support bioluminescence. The presence of bioluminescent *V. fischeri* in the crypts appears to initiate four independent but overlapping mechanisms that reduce the rate of HOCl synthesis: (1) reducing the availability of molecular oxygen to phagocytes in the crypts (Fig. 1), (2) inhibiting the enzyme activity responsible for the H_2O_2-generating respiratory burst, (3) removing any H_2O_2 that is produced and (4) depressing the expression of HPO activity (Fig. 3). By reducing the amount of H_2O_2 available for the HPO reaction, and/or reducing the concentration of the enzyme itself, *V. fischeri* apparently limit the production of this potent antibacterial molecule for which there is no known strategy for direct detoxification.

Oxygen is provided by the host to the symbiotic bacterial population via the vascular system of the light organ. *V. fischeri* cells in the crypts use this oxygen both for respiratory activity and for bioluminescence (Fig. 2). The demand for oxygen by these two cellular activities apparently exceeds its rate of supply[17] and, because the oxygen affinity of bacterial luciferase is relatively high[30], the oxygen concentration is likely to be maintained below the μM level. At such a level, the activity of the host NADPH oxidase is significantly lowered[31], thereby reducing the opportunity for O_2^- production. This strategy has also been documented in pathogenic gonococci[43].

V. fischeri also secrete two proteins, called halovibrin A and B, that have ADP-ribosyltransferase activity[44]. Because Ctx, another ADP-ribosylating enzyme, inhibits oxidative-burst activity, we hypothesize that these excreted halovibrins might have a similar role. Such a role could be particularly important when the concentration of bacterial symbionts in the light organ has been lowered by 95% following the daily expulsion process. At this time, the diminished abundance of bacterial cells might be expected to limit their ability to control the oxygen concentration in the crypts. Thus, the activities of these ADP-ribosyltransferases might promote recolonization of the light organ by *V. fischeri*. Interestingly, colonization experiments with *Vibrio logei*, a closely related but symbiotically deficient relative of *V. fischeri*[45], have shown that the addition of Ctx significantly enhances the ability of this species to colonize the squid light organ[46].

Either spontaneously, or via the activity of superoxide dismutase, any O_2^- produced by respiratory-burst activity is converted into H_2O_2. H_2O_2 could be a substrate for the squid HPO, which is exported into the light-organ crypt space and associates with the surface of *V. fischeri* cells[42]. The resulting production of HOCl might then kill the bacteria. However, the presence of a highly active catalase in the periplasm of *V. fischeri* cells would rapidly reduce the effective concentration of H_2O_2 near the bacteria[18], serving as a final safeguard against any residual HPO activity present in the *V. fischeri*-colonized light organ[42].

Although this model remains unproven, it makes several predictions that can be directly tested using a variety of approaches, including bacterial genetics and further experimental manipulation of the host. For example, mutant *V. fischeri* strains defective in luciferase or catalase synthesis have been constructed by gene replacement. Although the luciferase mutant initially colonizes the light organ to the same extent as the parent strain, after 48 h it has dropped to only 20% of the normal number of cells[47]. This result suggests that the absence of functional luciferase leads to a symbiotic defect. Similarly, colonization assays using mixtures of a catalase-defective *V. fischeri* mutant and its parent result in competitive dominance by the parent strain within 48 hours[18], indicating that the absence of catalase also decreases the fitness of the bacterium in the light-organ crypts. Currently, we are creating another *V. fischeri* strain that is mutated in both of the genes encoding the halovibrin proteins to determine its ability to colonize and its effect on the concentration of ROS in the light-organ crypts[42]. Using such approaches, it should be possible to examine the robustness of our current hypothesis, and extend its predictions about the role of oxidative stress in this cooperative bacteria–host association.

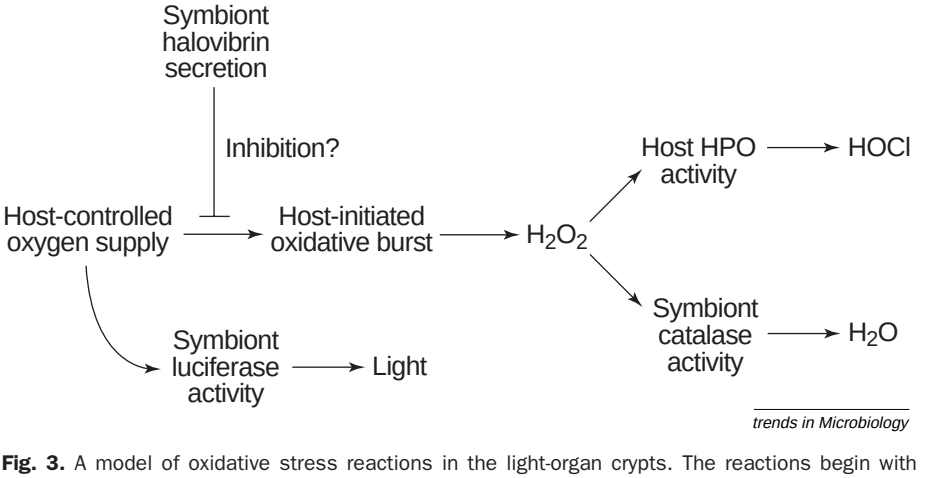

Fig. 3. A model of oxidative stress reactions in the light-organ crypts. The reactions begin with host-supplied oxygen, which is the substrate for both bacterial luciferase and an oxidative burst catalysed by a host cell (perhaps phagocytic) NADPH–oxidase complex. The ADP-ribosyl-transferase enzymes halovibrin A and B, secreted by *Vibrio fischeri*, might have an inhibitory effect on the potential for an oxidative burst. Hydrogen peroxide (H_2O_2), an eventual product of the host oxidative burst, can be used by a halide peroxidase (HPO), unless removed by catalase activity.

Outlook

Colonization of light-organ crypts by the luminous bacterium *V. fischeri* appears to be specified and controlled by oxygen and its metabolism. Our present hypothesis

suggests that the mechanisms for this, at least in part, include host production of an antibacterial HPO, the modulation of HPO expression by *V. fischeri*, the control of oxygen concentration by bacterial luciferase, and the activities of bacterial catalase and ADP-ribosylating proteins that might provide defense against host ROS. Such factors, and their spatial and temporal patterns of expression in the light-organ crypts, apparently play a role in the stability of a successful benign tissue infection.

This strategy of regulating ROS synthesis and detoxification, which appears to stabilize a cooperative bacterial association, reveals a number of parallels with the responses of host tissues to disease-causing microorganisms. These parallels further suggest that one must take care not to infer that evidence of a host oxidative response is necessarily an indication of the presence of a pathogenic infection. Furthermore, the possibility that ADP-ribosylating activities might have evolved in *V. fischeri* as symbiotic modulators of invertebrate host responses could indicate that similar bacterial products, such as Ctx, did not evolve as toxins; instead, they might have appeared in this role only in recent history and function most importantly to promote benign relationships with as-yet-undescribed aquatic hosts.

Questions for future research

- How can we use promoter–reporter constructs in *Vibrio fischeri* to define the changing oxidative conditions of the light organ during colonization and progression through the host's cycle of day-to-night interactions with its symbionts?
- What are the relative contributions of *V. fischeri*, host epithelial cells and host macrophages in the tripartite 'conversation' that sets the oxidative environment of the symbiosis?
- How do bacterial ADP-ribosyltransferases, such as the *V. fischeri* halovibrins, function in the dynamics of cooperative animal–bacterial association? Do the secreted halovibrins target specific host effector proteins for modification?
- What are the relative contributions of other reactive oxygen species, such as nitric oxide, to the development of the association?
- Is competitive dominance of native *V. fischeri* strains determined by their different abilities to withstand the oxidative stress imposed by their specific host species?

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Bacterial mechanosensitive channels: integrating physiology, structure and function

Paul Blount and Paul C. Moe

When confronted with hypo-osmotic stress, many bacterial species are able rapidly to adapt to the increase in cell turgor pressure by jettisoning cytoplasmic solutes into the medium through membrane-tension-gated channels. Physiological studies have confirmed the importance of these channels in osmoregulation. Mutagenesis of one of these channels, combined with structural information derived from X-ray crystallography, has given the first clues of how a mechanosensitive channel senses and responds to membrane tension.

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Imagine a bacterial cell being suddenly transported from the colon of an animal to a pool of water. This situation rapidly becomes life threatening because of the large and sudden downward shift in osmotic environment. Water rushes in, the cell turgor pressure increases and the membrane tension skyrockets. A decrease in the external solute concentration of 250 mM could increase the intracellular pressure by more than 6 atmospheres. Unless drastic steps are taken, these forces will cause the cell to rupture; however, the cell contains emergency release valves primed for such a crisis. The increased tension in the membrane opens large pores in the cell and solutes within the cytoplasm are rapidly and efficiently jettisoned into the pool of water. Although some metabolic solutes are lost, this is a small cost compared with the life of the cell.

Historically, several independent observations have been consistent with this scenario. Many bacterial species, including *Escherichia coli*, survive rapid decreases in their osmotic environment. A rapid efflux of cytoplasmic solutes has been measured in many species following such an osmotic insult, and large-conductance mechanosensitive (MS) channels are found in a diverse array of bacterial species. As the data accumulated, the links made between these observations allowed the integration of a physiological phenomenon – an MS channel activity – and a molecular entity. In addition, the identification and structure–function analysis of the MS channel MscL has allowed a first glimpse of a possible molecular mechanism of action of a physiologically important membrane-tension-gated channel.

Bacterial MS channels

Nearly 40 years ago, Britten and McClure[1] studied the bacterial amino acid pool and found that, when grown at high osmolarity, *E. coli* accumulates proline from the medium. This amino acid is jettisoned from the cytoplasm upon dilution of the medium with water (referred to as osmotic downshock). Other researchers have observed that, in addition to proline, several other cytoplasmic solutes, including potassium, glutamate, trehalose, ATP and even small proteins such as thioredoxin, are also rapidly and efficiently jettisoned upon osmotic downshock[2,3]. Further study demonstrated that this phenomenon occurs in a number of bacterial species, including Gram-negative and Gram-positive organisms[3].

With the advent of the patch-clamp technique, it was inevitable that methods would be developed to allow bacteria to be subjected to electrophysiological analysis. Using giant spheroplasts from *E. coli*, Martinac *et al.*[4] discovered that MS channels were the most prevalent channel activities in the *E. coli* membrane. Other studies demonstrated that these activities could also be observed in Gram-positive species[3,5,6]. Three channel activities have been found in *E. coli*: MscL (an MS channel of large conductance), MscS (smaller conductance) and MscM (mini conductance). Figure 1 shows a typical current trace for the two largest-conducting channels. The conductance of the *E. coli* channels is approximately 100-fold

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