suspect the way forward lies in examining the ecological context in which the lebinthine communication system evolved. For example, it would be exciting to empirically test whether and how males mitigate bat predation costs incurred by their high song frequency plus exposure during searching, as the song frequency shift seems likely to have been a key step in establishing the conditions necessary for sensory exploitation and sexual selection to shape multimodal duetting in this system. More information about acoustic competitors, the relative risk of bat predation, mechanical limitations of vibrational transmission, and close-range pairing dynamics will undoubtedly inform the selective forces at play. Sexually antagonistic selection to reduce female predation risk by echolocating bats may have been an important factor in the evolution of multi-modal duetting in lebinthine crickets, as was suggested in *Onomarchus uninotatus* [8]. Experimentally demonstrating sex reversal in fitness costs of mate searching, and relating this to the reversal in search roles seen in lebinthines, would strengthen the argument for sensory exploitation.

Evolutionary switches involved in establishing multi-modal duetting in lebinthine crickets seem to have warped the cost and benefit structure of each sex's signal and response. The mystery of this is how the system progressed through seemingly non-adaptive evolutionary intermediates — valleys between adaptive peaks — and ter Hofstede *et al.* [7] have illuminated an important role of sensory exploitation in that process. Future insights from the all-singing, all-dancing lebinthine crickets can inform how sexual communication systems using different modalities and exquisitely time-sensitive duetting behaviour evolve, and ultimately how such signal complexity contributes to broader patterns of divergence and speciation.

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Microbiology: Social Suicide for a Good Cause

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Extracellular DNA is an important component of the biofilm matrix. Now, *Pseudomonas aeruginosa* is shown to control autolysis through the production of HQNO, a quorum-sensing-regulated respiratory poison. Thus, HQNO-driven autolysis links programmed cell death with quorum sensing and biofilm formation.

If one starts a liquid culture of *Pseudomonas aeruginosa*, it becomes turbid overnight as the bacteria grow through log phase and into stationary phase. Leave the culture to grow over the weekend, however, and the culture will clear as the bacteria spontaneously lyse. The phenomenon of bacterial autolysis, a form of programmed cell death, evokes many questions. What is the relationship between autolysis and bacterial density? Is there a population benefit to autolysis regulated by quorum sensing? Does bacterial autolysis parallel early events in eukaryote programmed cell death? A new report by Hazan *et al.* [1] published in this issue of *Current Biology* addresses these questions by elucidating the molecular mechanism of bacterial autolysis, and in doing so provides an adaptive rationale for what seems like an evolutionarily futile occurrence. The report also clarifies a role for the quorum-sensing molecule HQNO in *P. aeruginosa* physiology, and provides a

tantalizing clue that bacterial programmed cell death may represent a predecessor to the mitochondrial depolarization seen in eukaryotic apoptosis.

Autolysis and Cell Density

Bacteria sense the presence of their neighbors through quorum sensing. In quorum sensing, each individual secretes specific small molecules that accumulate (Figure 1A, B), and when these molecules reach a threshold concentration, large transcriptional changes are triggered (reviewed in [2]). The small molecule 2-n-heptyl-4-hydroxyquionoline-N-oxide (HQNO) is an anthranilic-acid derivative that is produced by *P. aeruginosa* in concert with the quorum-sensing molecules 4-hydroxy-2-heptylquinoline (HHQ) and 3,4-dihydroxy-2-heptylquinoline (PQS) (reviewed in [3]). Unlike PQS and HHQ, HQNO does not have an associated transcriptional regulator, and thus does not have a known autocrine function. HQNO inhibits cytochrome bc_1 (respiratory chain complex III) and has been used since the 1970s as a tool to study mitochondrial respiration [4]. As a respiratory inhibitor, HQNO has been previously described as a virulence factor that inhibits the growth of other bacteria (such as *Staphylococcus aureus*), thus providing *P. aeruginosa* with a competitive advantage by poisoning organisms more sensitive to HQNO [5]. However, how (or rather, if) *P. aeruginosa* escapes growth inhibition by HQNO has remained unclear.

Hazan *et al.* [1] now demonstrate that HQNO is essential for autolysis in *P. aeruginosa,* which is indeed sensitive to respiratory inhibition of cytochrome bc_1 by endogenously produced HQNO. Respiratory inhibition by HQNO leads to a burst of reactive oxygen species and, ultimately, the loss of membrane potential and bacterial cell death (Figure 1C). *P. aeruginosa* has a highly branched respiratory chain. HQNO binds specifically to the Qi site of cytochrome bc_1, which blocks respiration through the principal cytochromes used during aerobic and microaerobic growth. Despite the presence of alternative cytochromes bo_3 and *bd*, which bypass cytochrome bc_1, HQNO inhibition at bc_1 is sufficient to cause cell death, highlighting the role of reactive oxygen species in autolysis in *P. aeruginosa* [6].

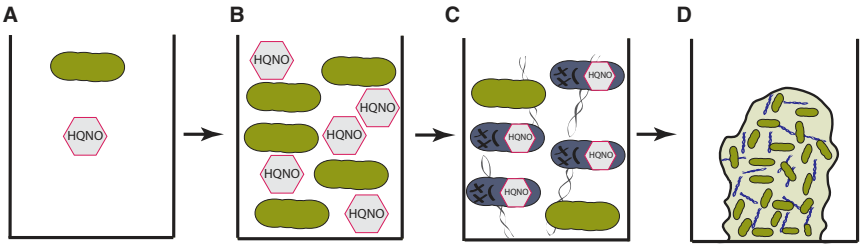

Figure 1. Self-poisoning leads to biofilm formation.
(A, B) *Pseudomonas aeruginosa* (green ovals) secretes the quorum-sensing molecule HQNO upon reaching high densities. (C) After HQNO accumulates, it inhibits bacterial respiration, causing bacterial death (dark gray ovals) and autolysis, releasing bacterial DNA. (D) The released DNA (shown as helical lines between bacteria) contributes to the biofilm matrix.

Population Benefit of Autolysis

Biofilms are communities of microbes encased in a matrix of extracellular DNA (eDNA), exopolysaccharides and proteins (reviewed in [7]). In experimental systems, loss of quorum sensing in *P. aeruginosa* impairs biofilm formation, yet Hazen *et al.* [1] showed that the defect in biofilm formation, due to loss of quorum sensing, can be partially rescued by addition of HQNO. Increases in local eDNA concentrations promote bacterial biofilm formation, and lysed bacteria are a key source of eDNA [8–10]. Hazan *et al.* [1] demonstrate that HQNO-mediated bacterial autolysis releases eDNA, rescuing biofilm formation in bacteria deficient in quorum sensing (Figure 1D). Intrinsic in this model is that some individuals must escape poisoning by HQNO to form and grow in the biofilm, where they may be protected from HQNO's effects. The respiratory adaptations by which some individuals escape death to grow in the biofilm remain to be determined. However, one could envision a model whereby protection from HQNO-mediated killing may be a result of the decreasing oxygen gradient seen toward the center of the biofilm [11]. Bacteria growing in biofilms exhibit extreme antibiotic resistance, and thus biofilm growth spurred on by autolysis may protect a population from annihilation by antibiotics [12].

A second possible benefit of the autolysis regulated by quorum sensing is in the policing of cheaters. Quorum sensing regulates about 300 genes, many of which are secreted enzymes that can liberate nutrients from the environment for the benefit of the population as public goods. Because the production of proteins regulated by quorum sensing is an energetically costly endeavor, cheaters arise in a bacterial population to take advantage of the public goods without paying the price of producing costly enzymes. *P. aeruginosa* also produces cyanide, which is subject to quorum-sensing control. Cyanide significantly inhibits *P. aeruginosa* respiration at different sites, but to a similar extent as HQNO. A recent study by Wang *et al.* demonstrated that cyanide serves a crucial role within *P. aeruginosa* populations, by poisoning cheaters that use public goods without paying the price of producing energetically expensive enzymes [13]. These studies raise the question of whether bacteria deficient for quorum sensing are more sensitive to HQNO, and if HQNO serves a similar role in policing cheating on a population level.

Autolysis versus Apoptosis

Beyond the role of HQNO in autolysis, Hazen *et al.* [1] discovered that cyclosporine A blocks autolysis, and thus the authors draw parallels between bacterial autolysis and the mitochondrial permeability transition (MPT; reviewed in [14]). Mitochondrial depolarization is an early, initiating event in eukaryotic programmed cell death (apoptosis). During the MPT, the inner mitochondrial membrane (analogous to the inner bacterial membrane in Gram-negative organisms) depolarizes. The molecular identity of the mitochondrial transition pore has remained elusive despite detailed electrophysiological characterization of the pore. Cyclosporine A blocks the MTP by binding to ubiquitous cyclophilin D proteins, which modulate

MPT activity without being structural components of the MPT pore. Cyclophilins are broadly present in bacteria [15], although they have not been specifically studied in *P. aeruginosa*. The observation that autolysis is inhibited by cyclosporine A suggests that *P. aeruginosa* has a cyclophilin that fulfills a similar physiological function to that in eukaryotes. It also suggests that the fundamental regulation of membrane depolarization in autolysis is similar to MPT in mitochondria. These exciting findings are potentially far-reaching and open the door for future studies examining the relationship between bacterial autolysis and eukaryotic programmed-cell-death processes.

Despite being a biologically fundamental occurrence, bacterial programmed-cell-death processes are remarkably poorly understood. In *P. aeruginosa*, autolysis is a highly regulated and complex process. The study by Hazan *et al.* [1] offers key insight into the physiological role of HQNO in *P. aeruginosa*, both by regulating and directly causing autolysis through endogenous respiratory inhibition. The direct relationship between quorum sensing and programmed cell death adds another facet to *P. aeruginosa*'s wide repertoire of intercellular community behaviors. Finally, the authors' conclusion that bacterial autolysis shares similarities with eukaryotic programmed cell death is provocative and provides exciting new directions for future work.

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