Motion to Form a Quorum

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Bacterial gene expression is frequently regulated by small molecules secreted into the surrounding medium. These autoinducers build up with increasing cell number until a critical population density or "quorum" is achieved, at which point the cells produce a response such as virulence or biofilm formation that requires the coordinated activity of large numbers of individuals. It has generally been assumed that quorum formation derives primarily from conditions favorable for growth to high cell density (*1*).

Numerous studies have shown that motility promotes biofilm formation, but this dependence has been attributed to random transport of cells from the bulk medium to a surface (*2*). Such experiments have been conducted with smooth surfaces that have no preferred surface sites or flow chambers where gradients of attractant chemicals will be dispersed. Here we show that, given appropriate surface topologies, bacteria can use chemotaxis to associate and form a quorum. Thus, chemotaxis provides an important mechanism for establishing the high local cell densities required for quorum-dependent interactions.

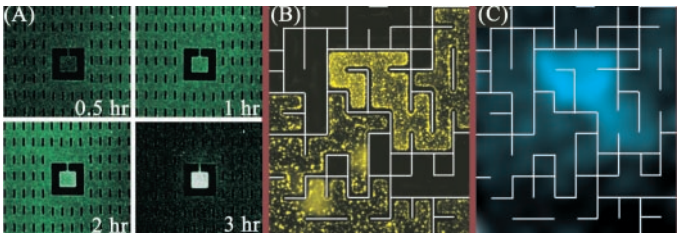

Fig. 1. *E. coli* and *V. harveyi* accumulation and quorum sensing. **(A)** Epifluorescence images of green fluorescent protein (GFP)–labeled *E. coli* in M9 minimal media as they accumulate into a central 250 μm by 250 μm enclosure via a 40-μm-wide channel through 100-μm-wide walls. After 3 hours the density of cells is more than seven times greater inside than outside. The rectangles are silicone pillars that support the roof of the chamber. **(B)** Dark-field image of *V. harveyi* after 8 hours in the maze. The narrowest passages are 100 μm wide. Lines corresponding to the walls of the maze are overlaid for clarity. **(C)** Photon-counting image of the intrinsic luminescence, indicating active quorum sensing in areas where the cells have accumulated at high density (*9*).

A culture of *Escherichia coli* grown to moderate density (approximately 2×10^8 cells/ml) in either rich media or in minimal media was used to uniformly fill a microfluidic chamber (7 mm by 3 mm by 30 μm) with a small central enclosure (250 μm by 250 μm) constructed from silicone elastomer. Over the course of 1 to 3 hours (depending on the media), the cells migrate from the chamber into the central enclosure through a narrow (40 μm) channel (Fig. 1A). This behavior is not observed with a mutant strain that is motile but deficient in chemotaxis.

Cells accumulate in the enclosure because they are attracted to each other due to their secretion of amino acids, such as glycine, that are chemoattractants. We detected this secretion by analysis of the free amino acid content of the growth media over time. The serine receptor, Tsr, is the most abundant chemotaxis receptor in *E. coli*, whereas the aspartate receptor, Tar, is also present at relatively high levels (*3*). The redox-sensing aerotaxis receptor, Aer, is present at low levels but still effectively modulates chemotaxis. Tsr binds L-serine with the highest affinity, but it also binds L-alanine, L-cysteine, and glycine (*4*). Whereas strains with *tsr* deleted were unable to accumulate in the enclosure, *tar* or *aer* deletion had little or no effect. Moreover, addition of saturating levels of L-serine (0.5 mM), which effectively competes with glycine, completely blocked accumulation of wild-type cells. Saturating concentrations of L-aspartate had no effect. The particular amino acid that is most important in mediating self attraction seems to depend on the conditions of growth before nutrient depletion. It has previously been shown that *E. coli* grown in succinate secrete aspartate, which acts through Tar to cause cells to associate into dense colonies in soft agar (*5*). Chemotaxis has generally been considered as a mechanism for cell dispersal. In nutrient-depleted environments, however, the cells themselves become sources of attractant molecules. Movement toward the amino acids secreted by the cells is enhanced by the ability of the chemotaxis system to adjust its sensitivity so that it can respond to very low concentrations of attractant chemicals (*6*). Accumulation of a high local density of cells may offer advantages such as enhanced genetic exchange or communal degradation of antibiotics, as well as the enabling of quorum-dependent behaviors.

The sites at which cells accumulate depend on the geometry of their surroundings. Results with cells in percolated lattices formed from silicone elastomer indicate that *E. coli* tend to accumulate in any areas of these mazes where the geometry provides a sufficiently enclosed space, such as dead ends and cul-de-sacs. Self-attractive behavior in chemotaxis has been modeled using the Keller-Segel equations (*7*). Our analysis of these equations indicates that for a small volume connected by a small opening to a large volume, such as our enclosure within a relatively large microfluidic chamber (Fig. 1A) or the dead ends in a maze (Fig. 1B), random fluctuations in cell number that cause an increase in the density of bacteria inside the small volume can increase irreversibly to produce a dense accumulation of cells.

Our results suggest that self attraction could readily produce local cell densities that exceed the threshold necessary for quorum-dependent processes. This notion was supported by the observation that, after coming together within the chamber, wild-type *E. coli* tended to exhibit a further association to form dense granular aggregates. A proposed *E. coli* quorum sensing signal, AI-2, is produced by the LuxS enzyme (*8*). A strain with *luxS* deleted accumulated into the enclosure just like wild type, but was never observed to form dense aggregates. *Vibrio harveyi*, a highly motile marine bacterium, exhibits a similar tendency to accumulate in confined spaces. These cells produce light in regions of high population density (Fig. 1, B and C). This bioluminescence, which is one of the most well-studied of quorum-dependent responses (*8*), confirms that chemotaxis-mediated associations facilitated by closed geometries can lead to activation of quorum sensing–dependent genes and their associated behaviors.

References and Notes

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Materials and Methods
References

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