Bacteria Also Vote

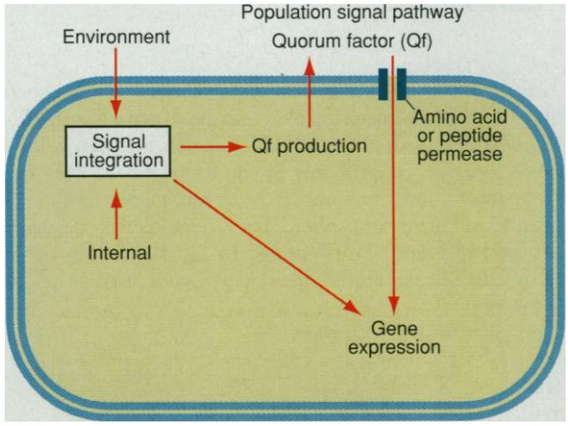
Dale Kaiser

Bacteria often release small extracellular signaling molecules, which are then taken up again by the same cells. This seemingly futile action adjusts the gene expression in the responding cells to a level appropriate for the local density of signaling cells. The best-studied examples of such "quorum sensors" are acylated homoserine lactones, members of the family of autoinducers. A report by Moré *et al.* in this issue describes the in vitro biosynthesis of a specific autoinducer catalyzed by affinity-purified TraI protein from *Agrobacterium tumefaciens* (*1*). Autoinducer from *A. tumefaciens* increases the spread from one bacterial cell to another of a plasmid that is responsible for producing a tumorous growth known as crown gall on host plants.

Autoinduction is one of the simplest of cell-cell interactions and may be a prototype for the more complex cell interactions that are a prominent feature of organismal development. Autoinduction, discovered in the luminescent *Vibrios*, was deduced from the finding that light production in culture is not proportional to cell density. In dilute cultures, the bacterial cells are very dim. As their cell density increases, however, the amount of light produced by each cell increases by as much as 100 times (*2*). During this induction phase, both luciferase protein and enzymes that synthesize its aldehyde substrate increase. The specific luminescence of dilute cultures of *Vibrio fischeri* or *V. harveyi* can be increased by the addition of medium conditioned by higher density cultures or by purified autoinducer (*3*). Inside the cell, autoinducer interacts with a transcriptional regulator, a member of the LuxR family (*4*, *5*). LuxR activates synthesis of the luciferase and a set of linked genes. The acylated homoserine lactones differ in the nature of their acyl group; each is specific for the bacterium that produces it, and each has its own LuxR partner.

In nature, the luminescent *Vibrios* inhabit the light organs of a squid or a bony fish (*2*, *6*). Confined within the animal's light organ, the concentration of autoinducer is believed to increase, and luminescence is thereby induced (*7*). Autoinduction thus discriminates between a free-living (low cell density) state and host-associated (high density) state. Luminescence requires extra energy; therefore, suppression of luminescense in the free-living state, when it is no longer required, conserves cell energy.

Autoinducers are not limited to the control of luminescence genes, as the *Agrobacterium* case illustrates (*1*). Extracellular lactones also control antibiotic production in streptomycetes. *Streptomyces griseus*, for example, releases isocapryl *d*-butryl lactone, which induces sporulation, streptomycin synthesis, and acquisition of streptomycin resistance (*8*).



Quorum sensing sharpens perception of external and internal signals.

Amino acids and short peptides can also function as quorum indicators, particularly when the task is to sense and respond to nutrient limitation. These examples illustrate another aspect of quorum sensing—sharpening perception for an important judgment call. When *Myxococcus xanthus* is limited for nutrients, it can either continue growth, but at reduced rate, or initiate a program of fruiting body development with sporulation. Cells that opt for fruiting body development instead of slow growth signify their choice by releasing a set of six amino acids (A-factor) into the medium (*9*). Unlike the regulated release of *Vibrio* autoinducer, each *Myxococcus* cell releases a fixed amount, and so the total amount of A-factor is directly proportional to the number of decisive cells per unit volume. Apart from a few early genes, expression of developmentally regulated genes depends on extracellular A-factor (see figure) (*10*).

A minimum concentration of A-factor (about 50 μM) is required to express A-factor–dependent genes. Signaling concentrations define a window from the 50 μM threshold to 10 mM. Above 10 mM A-factor, cells do not switch on their A-dependent genes; instead, they exit the fruiting body program and return to growth supported by the amino acids of A-factor (*9*). The A-factor threshold also sets a minimum cell density that can signal itself. Below this critical density, wild-type cells behave like a mutant that is deficient in A-factor production.

Myxococcus xanthus initiates fruiting body development when it lacks any one of the charged amino acyl-tRNAs and synthesizes the polyphosphorylated guanine nucleotide, (p)ppGpp (signal integration in the figure) (*11*, *12*). Releasing A-factor is the way that a cell votes its particular assessment of nutritional conditions. When each cell contributes its vote to the extracellular pool of A-factor, a more reliable choice of response can be made because responsive genes receive dual input (gene expression in the figure).

Bacillus subtilis integrates its response to nutrient limitation through a network of protein kinases and phosphatases (signal integration in the figure). Both sporulation and the acquisition of competence for DNA transformation depend on cell density. Oligopeptides that accumulate in the medium during growth must reach a critical concentration before the cells enter stationary phase in order for them to differentiate starvation-induced spores (*13*). Among these peptides are competence stimulating factor and ComX pheromone (*14*, *15*), both of which increase competence by stimulating synthesis of a transcription factor (Com A~P). Another transcription factor, SpoOA~P, is required for sporulation gene expression (*16*). Accumulation of SpoOA~P to its critical concentration is prevented by one or more cellular phosphatases. When oligopeptides derived from the products of genes such as *phrA* reach an extracellular level sufficient to provide a quorum signal, the effect of the phosphatases is overcome and sporulation ensues (*17*). Specific, hydrophobic oligopeptides at concentrations less than 10^{-11} M act as conjugation pheromones for *Enterococcus faecalis*, indicating the potential for high sensitivity to such signals (*18*).

Quorum-signaling extracellular molecules and the regulatory proteins they control are formal analogs of hormone–hormone receptor systems in eukaryotic cells.

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Perhaps hormone–hormone receptor pairs evolved from acylated lactones (or hydrophobic amino acids or peptides) and their target transcriptional regulators. Such origins are attractive because acylated lactones, owing to their hydrophobicity, can diffuse into a cell without a specialized transmembrane receptor, as well as diffuse out without a specialized secretion apparatus (*19*). Similarly, hydrophobic amino acids and peptides are transported by permeases with a rather low degree of specificity. *Rhizobium* nodulating a leguminous plant plays on an autoinducer-related theme in which flavonoids from the plant enter and control NodD transcriptional activators in the bacterium (*20*). NodD defines another family of activator proteins structurally unrelated to LuxR. Nevertheless, the number and variety of LuxR-like proteins are already large, as is the number of lactones with which they interact (*21*). Each element in a quorum-sensing system has the freedom to evolve greater complexity or specificity without compromising the overall system function, provided that it retains a capacity to interact effectively with its partners.

References

1. M. I. Moré *et al.*, *Science* **272**, 1655 (1996).
2. K. H. Nealson and J. W. Hastings, *Microbiol. Rev.* **43**, 496 (1979).
3. A. Eberhard *et al.*, *Biochemistry* **20**, 2444 (1981).
4. H. B. Kaplan and E. P. Greenberg, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 6639 (1987).
5. G. S. Shadel and T. O. Baldwin, *J. Bacteriol.* **173**, 568 (1991).
6. M. J. McFall-Ngai and E. G. Ruby, *Science* **254**, 1491 (1991).
7. E. G. Ruby and M. J. McFall-Ngai, *J. Bacteriol.* **174**, 4865 (1992).
8. S. Horinouchi and T. Beppu, *Gene* **115**, 167 (1992).
9. A. Kuspa, L. Plamann, D. Kaiser, *J. Bacteriol.* **174**, 3319 (1992).
10. ———, *ibid.*, p. 7360.
11. C. Manoil and D. Kaiser, *ibid.* **141**, 305 (1980).
12. M. Singer and D. Kaiser, *Genes Dev.* **9**, 1633 (1995).
13. A. D. Grossman and R. Losick, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4369 (1988).
14. R. Magnuson, J. M. Solomon, A. D. Grossman, *Cell* **77**, 207 (1994).
15. J. M. Solomon, R. Magnuson, A. Srivastava, A. D. Grossman, *Genes Dev.* **9**, 547 (1995).
16. D. Burbulys, K. A. Trach, J. A. Hoch, *Cell* **64**, 545 (1991).
17. M. Perego and J. A. Hoch, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 1549 (1996).
18. G. M. Dunny, B. A. B. Leonard, P. J. Hedberg, *J. Bacteriol.* **177**, 871 (1995).
19. H. B. Kaplan and E. P. Greenberg, *ibid.* **163**, 1210 (1985).
20. S. R. Long and B. J. Staskawicz, *Cell* **73**, 921 (1993).
21. W. C. Fuqua, S. C. Winans, E. P. Greenberg, *J. Bacteriol.* **176**, 269 (1994).

Molybdenum Bolsters the Bioinorganic Brigade

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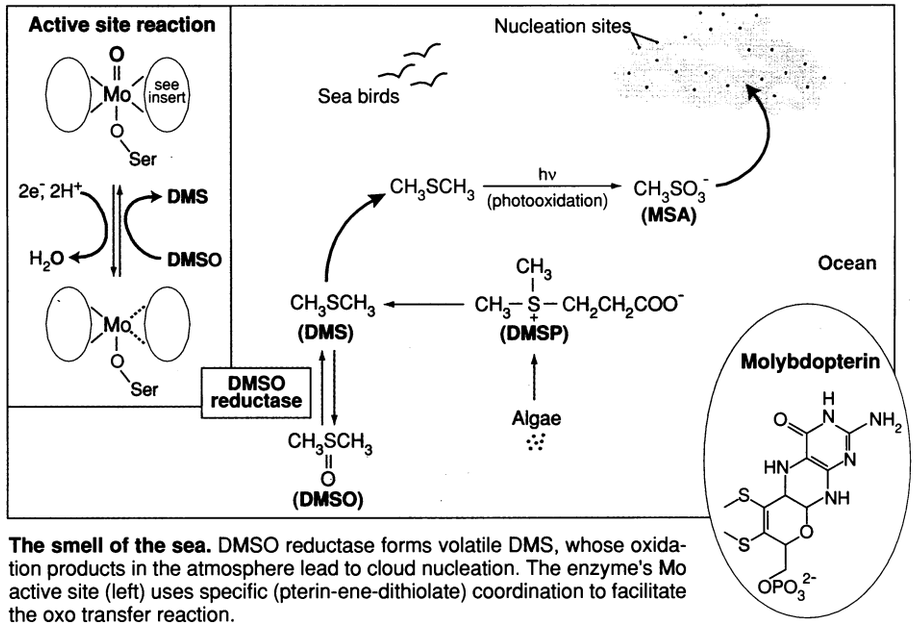
Plants, animals, and microorganisms need molybdenum (Mo) (*1*). Among the elements in the second transition row of the periodic table, only Mo has known biological functions, which involve more than 30 distinct enzymes (*1–3*). Although rare on Earth, Mo is abundant and soluble (as molybdate) in natural waters, exceeding in concentration such essential trace elements as manganese, iron, cobalt, copper, and zinc (*4*). This availability and a remarkable chemical versatility make Mo a crucial component of catalysts for both industrial (*2*) and enzymatic systems (*1–3*). In this issue, Schindelin *et al.* (*5*) report the crystal structure of the simplest known Mo enzyme, the dimethyl sulfoxide (DMSO) reductase (*6*) of *Rhodobacter spheroides*, which catalyzes the conversion of DMSO to dimethyl sulfide. The structure of this environmentally important enzyme should help elucidate how the tandem pair of protein and cofactor interacts to effect catalysis.

The Mo enzymes fall into two broad classes (*1–3*). Nitrogenase, responsible for biological nitrogen fixation, contains the special multimetal cluster called the iron-molybdenum cofactor and is the sole member of one class. The other class, embodying all other Mo enzymes, uses variants of the Mo cofactor (Moco), which contains a mononuclear Mo site. All Mo cofactors share a common nonprotein organic component, called molybdopterin, that acts as a ligand to Mo and was first shown to be a pterin with an ene-dithiolate (dithiolene) side chain (see figure) in the pioneering work of Rajagopalan and coworkers (*7*). Molybdopterin is also the ligand for tungsten (W) in enzymes from thermophilic microorganisms, which use tungsten, apparently in place of Mo (*8*). The full cofactor unit, metal plus specialized ligand, is designated Moco (or the tungsten cofactor).

DMSO reductase is one of several Moco enzymes—including sulfite oxidase, tetrathionate reductase, and polysulfide reductase—that are important in the sulfur cycle (*1–3*). Other Mo enzymes, such as nitrogenase, nitrate reductase, and a variety of heterocyclic *N*-oxidases, are critical for the global nitrogen cycle, highlighting the environmental and agronomic importance of Mo. The mammalian Mo enzymes xanthine oxidase (implicated in reperfusion injury and selectively inhibited in the treatment of gout), sulfite oxidase (necessary for sulfite detoxification and whose absence leads to



The smell of the sea. DMSO reductase forms volatile DMS, whose oxidation products in the atmosphere lead to cloud nucleation. The enzyme's Mo active site (left) uses specific (pterin-ene-dithiolate) coordination to facilitate the oxo transfer reaction.

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