How did these different binding affinities evolve in the apparent absence of a duplicate gene copy that would mask the loss of function caused by a change in DNA binding selectivity? Sayou *et al*. show that a LFY protein from hornworts, a group thought to have branched off from the rest of the land plants early in their evolution (*7*, *8*), is promiscuous in its binding activity: It binds preferentially to type III DNA sequences but can also bind to both type II and type I sequences.

Based on these observations, the authors propose a model by which a single-copy transcription factor can evolve novel binding sequences without losing function. In this model, the DNA binding site selectivity did not change abruptly during evolution of the LFY family. Rather, an intermediate that can bind multiple sites arose. This intermediate could still regulate its original target genes but could also regulate new target genes. This process would avoid potential deleterious effects arising from loss of LFY-regulated gene activity. In the lineage leading to the mosses, the type II binding specificity became fixed, whereas in the lineage leading to liverworts, seedless vascular plants, gymnosperms, and flowering plants, type I binding specificity became fixed.

Left unanswered is how the DNA binding sites bound by the LFY protein evolved and how this reflects the function of LFY in various organisms. In the moss *Physcomitrella*, *LFY* controls cell division (*9*). In contrast, LFY's main role in flowering plants is regulation of reproduction. The functions of *LFY* genes in other plants and streptophyte algae are unknown. It is also unclear whether core LFY target genes are shared between organisms with different LFY DNA binding specificities. The changes in DNA binding specificity may have led to a shift in gene targets and thus in LFY function; alternatively, binding sites may have evolved along with changes in the DNA binding domain.

It remains to be shown whether other transcription factors also evolve through promiscuous intermediates. Another open question is what drives *LFY* to be a single-copy gene in most plant genomes; most transcription factors are preferentially retained after duplication, whereas genes with an essential housekeeping function are more often reduced to a single copy (*10*). The potential for disruption of multiprotein complexes by the presence of mutated duplicate proteins might lie behind this reduction. This process has been shown to influence the evolution of other transcription factors (*11*). In addition, the contribution to DNA site selection and dimer stabilization of another conserved region found in LFY proteins should be explored. This domain mediates dimerization independently of DNA binding and contributes to stable DNA binding in AtLFY (*12*), but its function in other plants is unknown. It may be important for the stabilization of LFY dimers on type III sites. Clearly, much remains to be learned about the evolution of this important transcription factor.

References

1. S. H. Shiu, M. C. Shih, W. H. Li, *Plant Physiol.* **139**, 18 (2005).
2. C. Sayou *et al*., *Science* **343**, 645 (2014); 10.1126/science.1248229.
3. E. A. Schultz, G. W. Haughn, *Plant Cell* **3**, 771 (1991).
4. C. Hamès *et al*., *EMBO J.* **27**, 2628 (2008).
5. A. Maizel *et al*., *Science* **308**, 260 (2005).
6. I. R. Gonçalves *et al*., *Genetics* **196**, 149 (2014).
7. D. L. Nickrent, C. L. Parkinson, J. D. Palmer, R. J. Duff, *Mol. Biol. Evol.* **17**, 1885 (2000).
8. O. Malek, K. Lättig, R. Hiesel, A. Brennicke, V. Knoop, *EMBO J.* **15**, 1403 (1996).
9. T. Tanahashi, N. Sumikawa, M. Kato, M. Hasebe, *Development* **132**, 1727 (2005).
10. R. De Smet *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **110**, 2898 (2013).
11. C. R. Baker, V. Hanson-Smith, A. D. Johnson, *Science* **342**, 104 (2013).
12. N. S. Siriwardana, R. S. Lamb, *Plant J.* **71**, 736 (2012).

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CELL BIOLOGY

Cells Listen to Their Inner Voice

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A signaling circuit that controls the release and detection of the same signaling molecule can trigger diverse behaviors in a cell population.

Picture yourself on top of a mountain shouting out aloud—alone. You will hear only your own voice and its echoes. Now, picture yourself in a crowded stadium cheering for your team. You will hear the collective voice of all those cheering around you. Scaled down a million times, this phenomenon can occur in a population of cells. On page 628 of this issue, Youk and Lim demonstrate (*1*) two distinct modes of communication in yeast cells: self-communication, in which a cell primarily senses the signal produced by itself, and neighbor communication, in which a cell senses the signal collectively produced by all cells nearby. These properties have important implications for understanding cell dynamics in different biological contexts and across species, and for exploring design principles of biological networks.

Chemical-mediated communication is critical for controlling the physiological function of diverse organisms, from bacteria to mammalian cells. Particularly, this notion has transformed the way we perceive microbes. Rather than being truly "single-celled," microbes often engage in extensive communication to carry out functions essential for their survival (*2*), including biofilm development, production of virulence factors, and antibiotic resistance. Likewise, mammalian cells also communicate to regulate functions (*3*) that include homeostasis, growth, and cell-fate decisions. These diverse functions in bacteria and mammalian cells, although seemingly unassociated, share a common core module comprising the production, secretion, and detection of diffusible signals (*4*), or a "secrete-and-sense" module according to Youk and Lim.

The crucial role of chemical-mediated communication in social behaviors is reflected in terms like "quorum sensing" and "sociomicrobiology" (*5*, *6*). In synthetic biology, this mechanism has been extensively used to program dynamics involving one or multiple cell populations (*7*–*9*). When discussing such communication, it is often implied that each cell in a population senses the signal collectively produced by all cells in the vicinity. However, this implicit assumption is not always valid (*10*). For example, an isolated bacterium can also trigger a quorum-sensing response due to physical confinement—such as that of a mammalian cell or a small droplet of a cell culture (*4*, *11*). In this case, the bacterium is "listening" only to itself.

Youk and Lim demonstrate that even in the presence of other signal-producing cells, a cell may still realize self-sensing. The fundamental principle underlying this self-sensing is

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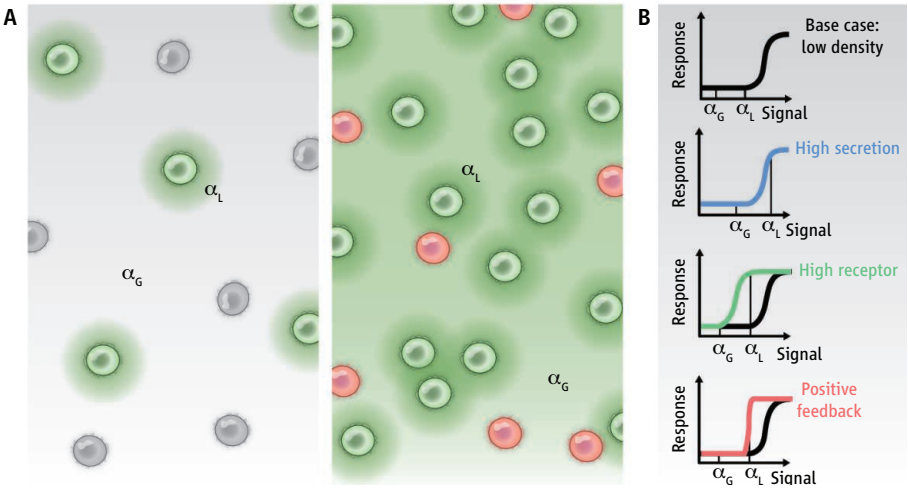

Tunable communication. (**A**) At a low cell density (left), each cell forms a cloud of local signal concentration (α_L). The global signal concentration (α_G) is lower than α_L because α_G increases at a slower rate. "Secrete-and-sense" cells (green) are self-activated; their activation by neighboring cells (gray) is limited. At a high cell density (right), more cells contribute to α_G, leading to activation in "sense-only" cells (red). (**B**) In the base case, α_G is lower than α_L and both are lower than the activation threshold to trigger a cellular response. Faster signal secretion increases both α_G and α_L such that α_L can trigger a response, leading to self-communication. Higher receptor abundance can reduce the activation threshold, leading to differential responses triggered by α_L and α_G. However, further increase in secretion and receptor abundance could diminish this difference and reduce self-communication. Positive feedback can make the response steeper and the activation threshold lower, facilitating self-activation. This can result in bimodal switching behavior at a low cell density.

the incomplete mixing of the culture medium around individual cells. At any moment, there is always a "cloud" of liquid surrounding a cell that is not well mixed, defining a local environment. The dimension of this cloud is characterized by the Kolmogorov mixing length scale (*12*) (see the figure). At a low cell density, cells are much farther apart than the mixing length scale, and the local signal concentration (α_L) is much greater than the global signal concentration (α_G). As a result, each cell primarily senses the signal produced by itself. At a high cell density, however, more cells contribute to the global pool of the signal, and α_G becomes comparable to α_L in magnitude, enabling communication between neighboring cells.

Youk and Lim tested this idea by engineering yeast cells to secrete-and-sense the α-factor, the mating pheromone of yeast. These cells were designed to produce a green fluorescent protein in response to the pheromone signal. To distinguish self- and neighbor communication, the authors engineered a "sense-only" strain for comparison that does not produce the signal but can respond to it by expressing a red fluorescent protein. By quantifying the responses of the two strains, Youk and Lim demonstrate varying degrees of self- and neighbor communication. The balance between the two modes can be readily tuned by controlling the amount of signal receptors expressed by cells, modulating the signal degradation rate, and introducing feedback control. In particular, at a low cell density, higher receptor abundance, faster signal secretion, or positive feedback control can promote self-communication. The common consequence of these strategies is an increased difference between the response to a local signal concentration and that to a global signal concentration. Conversely, faster degradation of the signal leads to diminished self-communication.

An appealing property of chemical-mediated communication is its potential to coordinate gene expression among a population of cells. At a low cell density, however, accumulation of signaling molecules around individual cells reduces the effective strength of neighbor communication, thus limiting the ability of the latter to reduce cell-cell variability. This variability becomes amplified by positive feedback. Indeed, Youk and Lim observed that their positive-feedback circuit led to distinctive bimodal switching behavior at a low cell density. By contrast, a high cell density enhanced neighbor communication, which in turn led to coherent activation by the same positive-feedback circuit.

Why do these modes of communication matter? The tunability of cell communication enables programming of tremendously versatile functions, as demonstrated by Youk and Lim. Similar dynamics may occur in the natural setting, resulting from signaling networks built around the same core secrete-and-sense regulatory motif. In this regard, the study of Youk and Lim represents an example of building synthetic gene circuits to enable quantitative exploration of design principles of biological networks (*13*, *14*). In a natural system, the function of a secrete-and-sense motif can be masked by complex interactions with other cellular components, making it difficult to analyze the design features of the motif. To this end, the circuits engineered by Youk and Lim provide a unified framework to examine the quantitative and qualitative properties of chemical-mediated communication.

The study by Youk and Lim highlights the underappreciated role of the transport property of molecules in defining the nature of biological interactions. Past studies have shown that the length scale of diffusible signals can constrain the outcomes of inter- or intrapopulation communication (*11*, *15*). Youk and Lim show that a fundamental constraint arising from liquid mixing enables cells to switch between social and asocial modes of interactions, using the same signaling molecule. This raises a cautionary note against equating the presence of a secrete-and-sense module to social interaction (*10*, *16*). Whether the module controls asocial or social interactions depends on kinetic rate constants associated with the module and the cell density. This property has profound implications for understanding the evolutionary dynamics of bacterial cooperation, where apparently cooperative traits are often regulated by such secrete-and-sense modules.

References

1. I. H. Youk, W. A. Lim, *Science* **343**, 1242782 (2014); 10.1126/science.1242782.
2. J. A. Shapiro, *Annu. Rev. Microbiol.* **52**, 81 (1998).
3. W. G. Junger, *Nat. Rev. Immunol.* **11**, 201 (2011).
4. A. Pai, L. You, *Mol. Syst. Biol.* **5**, 286 (2009).
5. C. M. Waters, B. L. Bassler, *Annu. Rev. Cell Dev. Biol.* **21**, 319 (2005).
6. M. R. Parsek, E. P. Greenberg, *Trends Microbiol.* **13**, 27 (2005).
7. F. K. Balagaddé *et al.*, *Mol. Syst. Biol.* **4**, 187 (2008).
8. T. Danino, O. Mondragón-Palomino, L. Tsimring, J. Hasty, *Nature* **463**, 326 (2010).
9. W. Weber, M. Daoud-El Baba, M. Fussenegger, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 10435 (2007).
10. R. J. Redfield, *Trends Microbiol.* **10**, 365 (2002).
11. E. C. Carnes *et al.*, *Nat. Chem. Biol.* **6**, 41 (2010).
12. L. D. Landau, E. M. Lifshitz, *Course of Theoretical Physics*, vol. 6, *Fluid Mechanics* (Butterworth-Heinemann, Oxford, UK, 1987).
13. S. Mukherji, A. van Oudenaarden, *Nat. Rev. Genet.* **10**, 859 (2009).
14. J. B. Xavier, *Mol. Syst. Biol.* **7**, 483 (2011).
15. H. Song, S. Payne, M. Gray, L. You, *Nat. Chem. Biol.* **5**, 929 (2009).
16. S. A. West, K. Winzer, A. Gardner, S. P. Diggle, *Trends Microbiol.* **20**, 586 (2012).

10.1126/science.1250244