



PERSPECTIVES: OSCILLATING SYSTEMS

On Emerging Coherence

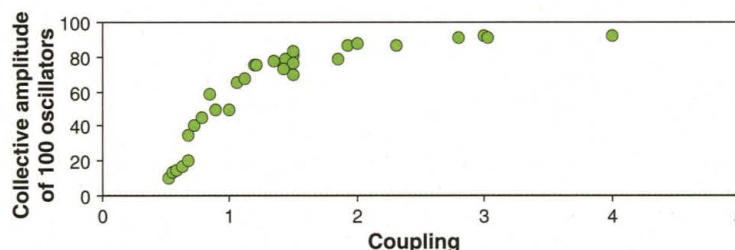
Arthur T. Winfree

Many diverse processes cycle regularly. If coupled in pairs, such oscillating systems tend to “mutually entrain”—that is, get in step with each other or alternate. The effect is not limited to pairs: Large populations of similar oscillators can interact in this way. Rhythmic clapping in a large crowd is one example of emerging coherence of this kind. An inorganic example has been described by Vanag and Epstein (1), who showed that when nanodroplets of an oscillating aqueous Belousov-Zhabotinsky reaction solution are suspended in oil, they appear to mutually entrain each other. In a recent report (2), Kiss *et al.* have described another inorganic system with sufficient accuracy to test the prevailing theory.

The first tractable mathematical model of emerging coherence was developed in 1966 (3, 4). In this severely simplified model, the unit oscillator progresses along a fixed cycle of successive states that eventually lead back to a prior state. The oscillator is always somewhere on the unique continuous cycle, the shape and amplitude of which cannot change. A pendulum, for example, is not like that; although it swings with fixed period through a cycle of angular positions and velocities, both can have arbitrary amplitude, determined by the initial impetus that started the oscillation. Oscillators in general need not be “on the cycle”: They can be at rest or engaged in alternative dynamics, depending merely on initial conditions.

Is there anything in the world that is like this idealized oscillator? The answer is yes. Chemical clocks go through a fixed sequence of successive concentration states, leading back to a prior state after a fixed time. In Josephson junctions driven by a constant current, the phase shift of a quantum wave function changes progressively, eventually completing a cycle. Biochemical oscillations in living cells or organisms, such as the cell mitotic cycle or the menstrual cycle, may progress through a fixed cycle of states, although the rate at which this happens may vary.

In idealized models of such processes, the oscillators can be coupled together by weak interactions—for example, by diffusive exchange of chemical concentrations or of electrochemical potential. In the 1960s models, it was found that such oscillators not only can attract one another in phase but can also mutually repel, thus avoiding synchronization even when all are driven by an external rhythm. They only mutually entrain when the intensity of mutual coupling is sufficient compared with the range of their individual native frequencies. Below a threshold, anarchy prevails; above it, there is a collective rhythm as some oscillators lock together. If the coupling is intensified, more



Coherence of coupled oscillators. The long-term collective amplitude for a population of 100 oscillators that are identical except for their native periods is shown as a function of their mutual coupling. The coupling is normalized to the range of native frequencies, such that the threshold for mutual synchronization is 0.5. Numerical simulation data replotted from figure 8 of (4).

oscillators join in until the whole population marches as one (see the figure) (3, 4).

According to an analytical model of such dynamics (4), the collective amplitude in a population of 100 oscillators of unit amplitude should rise from 0 to 100 as a sigmoidal function of the ratio of coupling intensity to the dispersion of native periods. A mathematically more tractable special case emerged in 1975 (5), prompting an avalanche of theoretical literature exploring its ramifications (6). But no quantifiable illustrations emerged from natural science experiments.

Arrays of electronic oscillators bore out qualitative expectations (7, 8). It was shown that aggregates of beating heart cells couple electrically to beat as one (9) and that in multinucleate “slime molds,” all nuclei divide at once as a result of chemical diffusion among them (10). Suspensions of yeast cells synchronously metabolize glucose in half-minute cycles (6, 11), and suspensions of cellular slime mold cells synchronously emit pulses of cyclic AMP (adenosine 3',5'-monophos-

phate) (12), if there are enough cells per milliliter to communicate adequately. Fireflies signal one another to flash together, and crickets and frogs chorus (2, 12). But for diverse technical reasons, none of these systems lent itself to the quantitative observations needed to test the theory.

Kiss *et al.* have produced such a system. It is electrochemical: a polished nickel electrode in sulfuric acid with an electrical potential that varies sinusoidally in less than 1 s. Neighboring electrodes interact electrically in a common bath, and all interact equally with all others through a single resistor. Their individual native frequencies can be tuned by individual resistors. When tuned to a sufficiently narrow distribution, the array is susceptible to mutual entrainment at or above a critical level of global coupling.

This is the first laboratory system in which collective amplitude has been reported quantitatively as a function of coupling intensity. The outcome resembles that shown in the figure: There is a sigmoidal relation-

ship that rises sharply near the threshold for mutual entrainment. Wiesenfeld *et al.* (13) predicted the same sort of threshold effect and sigmoidal rise of collective amplitude for Josephson junctions, but a lab experiment has not yet been reported.

Can such experiments be done *in vivo*? Much is now known about the molecular mechanisms of circadian clocks that was completely hidden a decade ago. Circadian clocks in individual cyanobacteria, just like cells of the mammalian suprachiasmatic nucleus, synchronize with one another when closely enough coupled by proximity. Can a threshold level of coupling be established? And can the agent of communication be isolated?

Thirty years ago, both these questions were explored by biochemists interested in the regulation of glycolysis in yeast cells. Under anaerobic conditions, glycolysis oscillates with half-minute periodicity; cells in dense suspension oscillate synchronously but not in more dilute suspension. Ghosh *et al.* (11) found the expected threshold of dilution and enthusiastically imposed the foregoing diagrams (see the figure) on dubious biochemists. But it turned out recently (6) that the threshold was measured incorrectly by two orders of magnitude. Dano *et al.* (14) have refined the experimental methods and identified the coupling agent as acetaldehyde (which had seemed excluded by the first measurements), so that delicately quantitative experiments are now feasible.

The author, formerly of the Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721, USA, has recently passed away.

The remarkable precision achieved by Kiss *et al.* (2) is thus within reach for glycolysis and may eventually come to the world of circadian clocks with the use of suspensions of cyanobacteria or suprachiasmatic nucleus neurons in tissue culture.

References and Notes

1. V. K. Vanag, I. R. Epstein, *Science* **294**, 835 (2001).
2. I. Z. Kiss, Y. Zhai, J. L. Hudson, *Science* **296**, 1676 (2002).
3. A. T. Winfree, *A Progress Report* (Department of Biophysics, Johns Hopkins University, Baltimore, MD 1966).
4. ———, *J. Theor. Biol.* **16**, 15 (1967).
5. Y. Kuramoto, *Chemical Oscillations, Waves, and Turbulence* (Springer, Berlin, 1984).
6. A. T. Winfree, *The Geometry of Biological Time* (Springer, New York, ed. 2, 2001), chapter 12, pp. 119–134.
7. E. Basar, *EEG Brain Dynamics* (Elsevier/North-Holland, Amsterdam, 1980), pp. 176–200.
8. A. T. Winfree, *The Geometry of Biological Time*, (Springer, New York, ed. 2, 2001), chapter 11.
9. L. Glass, M. C. Mackey, *From Clocks to Chaos: The Rhythms of Life* (Princeton Univ. Press, Princeton, NJ, 1988).
10. J. Tyson, W. Sachsenmaier, *J. Theor. Biol.* **73**, 723 (1978).
11. A. K. Ghosh, B. Chance, E. K. Pye, *Arch. Biochem. Biophys.* **145**, 319 (1971).
12. A. T. Winfree, *The Geometry of Biological Time* (Springer, New York, ed. 2, 2001), chapter 1.
13. K. Wiesenfeld, P. Colet, S. H. Strogatz, *Phys. Rev. Lett.* **76**, 404 (1996).
14. S. Dano, P. G. Sorensen, F. Hynne, *Nature* **402**, 320 (2001).
15. Steven H. Strogatz (Cornell University) assisted in the final preparation of the manuscript.

PERSPECTIVES: STRUCTURAL BIOLOGY

LDL Receptor's β -Propeller Displaces LDL

Thomas L. Innerarity

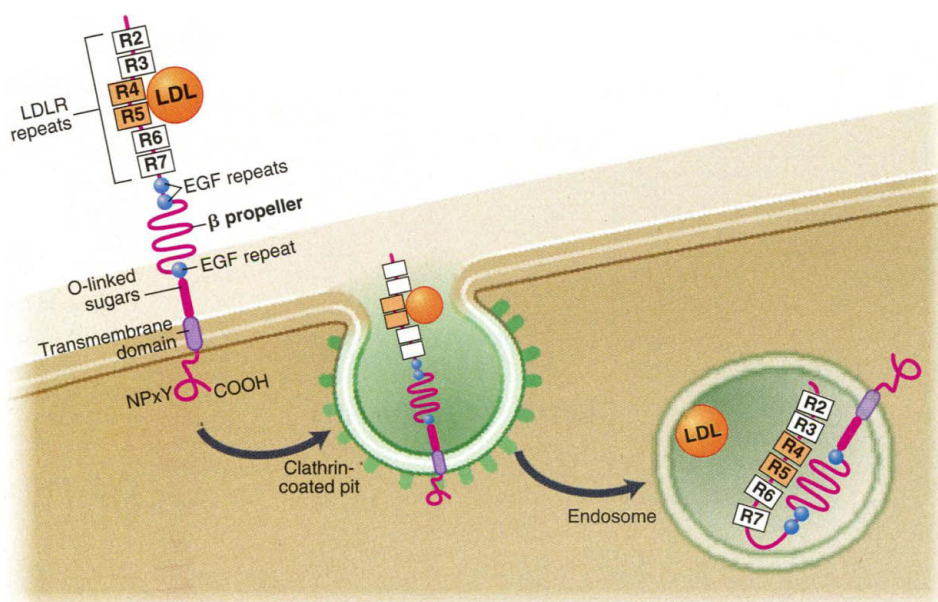
Much of what is known about receptor-mediated endocytosis comes from studies of the low density lipoprotein receptor (LDLR) pathway (1). LDLR binds cholesterol-carrying LDL, associates with clathrin-coated pits, and is internalized into acidic endosomes where it separates from its ligand. The ligand is degraded in lysosomes, while the receptor returns to the cell surface. Mutations in the LDLR gene can lead to elevated plasma cholesterol levels, resulting in coronary heart disease and atherosclerosis (1). Seminal observations by Rudenko *et al.* on page 2353 in this issue (2) shed light on a mystery of LDLR recycling—how the LDLR releases its lipoprotein ligand in the endosome.

The LDLR has several domains (see the figure). The ligand-binding domain contains seven imperfect repeats, each with three disulfide bonds and a coordinated Ca^{2+} ion. Extracellularly, it binds two ligands: apolipoprotein (apo) B100 (the only protein in LDL) and apoE (a protein in other lipoproteins). The second domain (411 amino acids in length) is analogous to the membrane-bound precursor of the epidermal growth factor (EGF). It consists of two EGF repeats, followed by a β -propeller region that contains the consensus sequence Tyr-Trp-Thr-Asp, and another EGF repeat (see the figure). LDLR with the EGF precursor domain deleted still binds apoE, but not LDL. However, apoE is not released in the endosome, and the ligand-receptor complex is degraded in the lysosome. Thus, the EGF precursor domain is critical for ligand release and recycling of the receptor, but until now the mechanism remained a mystery (3).

The solution came from the structure of the extracellular domain of the human LDLR crystallized at pH 5.3. In this structure, the β -propeller region of the EGF precursor domain interacts with the main ligand-binding repeats of the LDLR (R4 and R5) (see the figure). Rudenko *et al.* (2) propose that in the endosomes, the β -propeller region displaces the bound lipoprotein ligand by acting as an alternate substrate for the ligand-binding domain. This compelling model is supported by other key evidence: mutations in the ligand-binding and EGF precursor regions that abolish function, phylogenetic evidence of conserved amino acids, and biochemical evidence that the ligand-binding repeats associate with the EGF

precursor at pH 6 but not at pH 8. Clusters of histidines in the β -propeller region likely act as pH-sensitive switches for the domain interactions. The histidines carry no net charge at pH 7.3, but are partially charged at pH 5.3 and participate in the formation of salt bridges in the crystal structure.

The interaction of the β -propeller region with repeats 4 and 5 appears to have much in common with the interactions between lipoprotein ligands and the ligand-binding repeats and clarifies a controversy about lipoprotein-receptor interactions. The interaction of the two domains of the LDLR, as shown by the crystal structure, is based on six hydrophobic bonds and seven salt bridges between R4/R5 and the β -propeller region. Previous studies indicated that ionic or salt bridges are also critical for LDLR-ligand interactions (4, 5), with conserved acidic amino acids in the ligand-binding repeats forming ionic interactions with positively charged amino acids in the receptor binding site of the lipoprotein ligands. However, this ionic interaction model has



Catch and release. A model for how LDLR releases LDL. A crystal structure of the extracellular domain of LDLR at pH 5.3 (2) shows that ligand-binding repeats R4 and R5 interact with the β -propeller region of the EGF precursor domain. This interaction may displace LDL from the receptor in acidic endosomes.