Self-Organization in Living Cells

Benno Hess and Alexander Mikhailov

A living cell is an open system with a flow of energy passing through it. As shown by Schroedinger (*1*), the energy flow creates the conditions for strong deviations from thermodynamic equilibrium. This results in the phenomena of self-organization, the parameters of which are set by genetic as well as epigenetic constraints and opens up the possibility of autonomous pattern formation as revealed in the fundamental contributions by Turing (*2*) and Prigogine (*3*).

Recently, Lechleiter *et al.* (*4*) have observed propagating calcium waves inside single cells (frog eggs with a diameter of about 1 mm). Their properties were very similar to those of the spiral waves in the Belousov-Zhabotinsky reaction (*5*). Moreover, there are suggestions that stationary Turing patterns could also be found within biological cells (*6*).

However, it would be misleading to expect that the processes of self-organization in living cells represent simply a reduced copy of the pattern-formation phenomena in macroscopic reaction-diffusion systems. The laws of physics, when applied at a different scale typical for intracellular processes, can influence the mechanisms involved and produce a wealth of new properties, as demonstrated, for instance, by Purcell (*7*) for the case of the cell's motion.

The diffusion time for macromolecules in a cell or a cellular compartment with the linear size of 1 μm is about 10 ms. Because the turnover rate for many intracellular enzymatic reactions is a few hundreds per second, it means that within the duration of a single round of the catalysis the molecules can cross the entire reaction volume. This makes spatial pattern formation based on such reactions and diffusion at small length scales practically impossible. The finest spatial details of waves in the experiment (*4*) were still on the order of 10 μm. The estimates are less restrictive for fast enzymatic reactions with the turnover times below 0.1 ms. The possibility that under high enzyme concentrations these reactions may develop local spatial patterning should be further investigated (*6*).

The random Brownian motion of macromolecules inside cells is extremely strong. Our estimates of a characteristic traffic time show that within a compartment of a micrometer size, any two molecules meet each other within a time of about 1 s. Thus, simple diffusion fills the cellular compartment space with components always ready to react. At such smaller scales, the chemical system of a living cell is far from resembling a macroscopic unstirred chemical reactor. Rather, it should be viewed as a network formed by a population of active macromolecules that is characterized by a very high degree of "communication" between its members. This implies a different mode of self-organization similar to the collective behavior of insect societies or immune networks (*8*).

Another special aspect of self-organization in living cells is the abundance of energy contained in thermal fluctuations. For instance, random hydrodynamic flows induced by thermal fluctuations inside a cell have velocities on the order of 10 μm/s in the time range of 10 ms and the characteristic lengths of 1 μm. These strong thermal fluctuations can be employed by far-from-equilibrium subsystems inside the cells.

A pattern emerges. Self-similar organization of microtubules showing pseudohelical bands as part of a larger scale organization. Image is 1570 μm wide.

The laws of thermodynamics prevent the directed use of thermal fluctuations. However, as shown by Feynman (*9*) in his analysis of the thermal ratchet (a process allowing motion in one direction only), this does not generally apply to systems with some components that are far from thermal equilibrium (and thus, "Maxwell's demon" may operate if it receives and dissipates energy from external sources). The ratchet has already been proposed by Huxley (*10*) in his explanation of force generation by muscle fibers. Several further examples of rectification of thermal fluctuations by organelles have recently been considered [see, for example (*11*)]. It might be that the ratchet effects are involved to a much larger extent in the function of living cells and the organization of intracellular traffic.

The temporal self-organization of chemical processes, expressed in the generation of different periodicities and interactions between them, plays a fundamental role in living cells. Recent advances in the understanding of the mechanism of calcium oscillations not only revealed its widespread occurrence in cellular systems but showed its inherent response to frequency and coding to be a most important feature (*12*). This property might well be involved in the phenomenon of synchronization of synaptic boutons (*13*), in the shuttle streaming of *Physarum polycephalum* (*14*), in the propagation of calcium waves in *Xenopus laevis* oocytes (*4*), and mitotic cell cycles (*15*).

In this framework, the dynamic processes in larger cellular structures such as genomic components or the cellular actin and tubulin, the shape producing and controlling entities, should not be overlooked. Whereas the exploration of the first set of structures seems still to have a long way to go [although proper methodologies are coming up (*16, 17*)], investigations of the second class are already at hand.

The dynamic network of microtubules plays an essential role in the self-organization of cellular structures, turnover, and motility. The knowledge of its mechanisms and of the control of formation and decay (polymerization-depolymerization cycles) are prerequisites for understanding of the cellular interior. The complex organization of the microtubule turnover is revealed in the typical properties of arising dissipative structures. Recently, spatial pattern formation from oscillating microtubules has been observed (*18, 19*). Depending on the length and the frequency of oscillations, different spatial microtubulin patterns might readily be formed, leading to a variety of intracellular structures that fit well to the general size of the cellular reaction space as well as its time domains. Here, the biological design of suitable control mechanisms is a problem area open for future research. Studies reported by Tabony on p. 245 of this issue (*20*) clearly demonstrate the influence of reaction-diffusion instabilities and related bifurca-

B. Hess is at the Max Planck Institut für Medizinische Forschung, Jahnstrasse 29, D-69120 Heidelberg, Germany. A. Mikhailov is at the Fritz-Haber-Institut der Max-Planck-Gesellschaft, Faradayweg 4-6, D 14195 Berlin, Germany.

tions on the processes of pattern formation by cellular polymerization-depolymerization systems.

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The Evolution of Genetic Intelligence

David S. Thaler

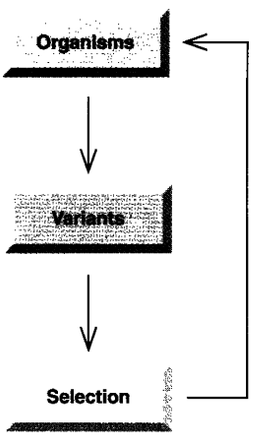
In 1988 Cairns and his colleagues (*1*) published a scientific paper that provoked heated discussion, even among philosophers (*2*). They suggested that mutations arise more frequently when the organism is under selective pressure for that mutation, in apparent contradiction to the conventional idea that mutations arise without regard for their utility. In this proposal, the environment not only selects among preexisting variants, it also interacts with the organism in a sophisticated way to generate the variation on which selection acts. This phenomenon of mutagenesis under selection has been variously called "Cairnsian," "selection-induced," "adaptive," "post-plating," "late-arising," or "selection-promoted" mutation and has been much polemicized. A paper by Harris and co-workers in this week's *Science* (*3*) helps to move beyond the polemic that has obscured our study of the molecular mechanisms that generate variation.

In their classic works, Luria and Delbruck (*4*) and Lederberg and Lederberg (*5*) studied mutation in the context of lethal selection—in which only preexisting mutants have a chance to survive. In contrast, the experiments of Cairns and of others inspired by him are designed to detect the frequencies and types of mutations arising under conditions of nonlethal selection, in which the organism has a chance to react to the selective conditions. These conditions do not kill counterselected cells but prevent an increase in their number (for example, in the paper by Harris and co-workers in this issue, cells mutant for *lacZ*, the gene for the lactose-utilizing enzyme, are plated onto medium with lactose as the only carbon source). The frequencies, timing, and types of mutations arising under selective conditions are then compared with those that occur during nonselective growth.

The most biting criticism directed at the Cairnsian school has been that, under conditions selective for the appearance of scorable mutants, there is enough "normal" growth to account for the mutants, assuming only that selection-induced mutations are created similarly to those of nonselective growth (*6*). This "growth-on-the-plate" argument, however, is not an adequate explanation of the results, because the types of mutations arising under selection seem to be distinct from those that occur during nonselective growth (*7*). For example, transposon excisions are seen only under stressed conditions, and the ratio of recombination to point reversion is different under selection. In addition, distinct sets of genes are required in the two conditions—strong evidence that different pathways of mutagenesis operate during mutation under selection. Cairns and Foster (*8*) found that the DNA recombinase gene *recA* is required for mutagenesis only during selection. Cairns has pointed out that this requirement for *recA* (*9*) indicates that there is a specialized pathway for mutagenesis under selective conditions.

The work by Harris and co-workers in this issue of *Science* (*3*) confirms the necessity for *recA* in selection-promoted mutagenesis and strengthens the conclusion by using a deletion allele of *recA*. They also find that the *recB* recombination gene is required for selection-induced appearance of mutants and that a *recD* null mutation increases mutagenesis only under the selective conditions. (RecD is an inhibitory subunit of the recombination complex.) The $recA^+$ and $recB^+$ alleles are required for the *recD* (null)–stimulated mutation. These requirements for mutation occurring under selection are the same as those for homologous recombination via the wild-type (RecBCD) recombination pathway of *Escherichia coli*. The absence of effect of *recJ* and *recQ* genes implies that an alternative *E. coli* recombination pathway called RecF does not participate in the formation of selection-induced mutants. Because *recA* participates in processes other than recombination, the reported involvement of other RecBCD pathway genes provides important new support for the idea that mechanisms that underlie homologous recombination also underlie mutation during selection.

Numerous models, all of which account for the necessity of the homologous recombination machinery, have been invoked to account for the apparently Lamarkian evolution seen under selective conditions. These proposals include reverse-transcribed mRNA interacting with the host genome (*1*), heteroallelic interactions (*7*) analogous to cassette switching (*10*), immunoglobulin diversity generation (*11*) or heterochromatic interactions (*12*), and gene amplification (*8*, *13*). The "toe-in-the-water" proposal invokes transcripts as replication primers when growth is the consequence of a revertant transcript (*13*). Homologous interactions may be



A conventional view of evolution. The environment functions only at the selection step.

The author is in the Laboratory of Molecular Genetics and Informatics, Rockefeller University, 1230 York Avenue, New York, NY 10021-6399, USA.