Order for Free: Molecular Diversity and Complexity Promote Self-Organisation

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In his book "At Home in the Universe", Stuart Kauffman describes a living organism as a system of chemicals that has the capacity to catalyse its own reproduction.[1] In his view, achieving autocatalytic closure among a collection of molecular species becomes the root property of life. Alone, each molecular species is "dead"; jointly, the collective system of molecules is "alive". The Kauffman thesis is very compelling for the chemical biologist since catalysis is no longer relegated to being a sustainer of life but instead becomes a key originator of life. However, catalysis alone cannot be enough to originate life. All living systems "eat"; they take in matter and energy in order to sustain and reproduce themselves. Therefore, living systems must be the equivalent of closed autocatalytic but open (nonequilibrium) thermodynamic systems. But even supposing that life can originate from autocatalytic closure among a collection of molecular species, how does this view of life account for the fearsome order that characterises living systems and seems so much at variance with the second law of thermodynamics? In Kauffman's view, molecular diversity and complexity in an open thermodynamic system themselves give order, "order for free" or self-organisation that arises naturally. In other words, order (self-organisation) is a direct consequence of molecular diversity and complexity.

Compartmentalisation is a key part of the self-organisation process, if for nothing else than to sustain spatial integrity and prevent dilution of the reacting molecules that comprise each open thermodynamic, closed autocatalytic living system. The ability of lipids to self-assemble into lipid bilayers and more complex mesophases is well established, and has long been accepted to form a key part of compartmentalisation in biology.[2] However, living cells also possess an intricate cytoskeleton involving proteins such as globular monomeric actin (G-actin) and the filamentous actin (F-actin). In this context, a recent publication by Wong et al. is interesting.[3] They described the spontaneous hierarchical self-assembly of F-actin filaments and cationic lipid membranes into tubules (approximately $0.25 \times 100\ \mu m$) consisting of stacks of composite membranes composed of three layers, a lipid bilayer sandwiched between two layers of actin; a structural organisation highly reminiscent of multilayered bacterial cell walls that exist far from closed equilibrium (Figures 1 and 2). Wong et al. suggested that these tubules with their composite membrane architecture could be considered as spontaneously assembled "artificial" bacteria. If we accept this conclusion then this publication should be regarded as important for providing evidence of molecular diversity and complexity leading to lifelike "order for free".

Amongst biologists, discussions about the origins of life have been focussed by arguments of natural selection. There has been an apparent tendency to ignore self-organisation. Most likely this is because there is a fundamental difficulty in recognising how living systems may be governed simultaneously by two sources of order, self-organisation born of molecular diversity and complexity, and the "forces" of natural selection. However if we are to understand properly the origin of life, a final theory of biology must allow for the commingling of self-organisation and selection processes as an expression of an even deeper order. Surely, as Kauffman argues, self-organisation precedes natural selection.[1] Stable, low-energy, self-organised states ("robust systems") such as lipid bilayers and mesophases,[2] the structures described by Wong et al.,[3] the DNA

Figure 1. *Long F-actin filaments spontaneously form two-dimensional crystal layers of F-actin that are able to sandwich cationic lipid bilayers forming three-layer membranes (inset) capable of self-assembly into ribbonlike tubule structures. Reproduced with permission from ref. [3]. Copyright (2000) American Association for the Advancement of Science.*

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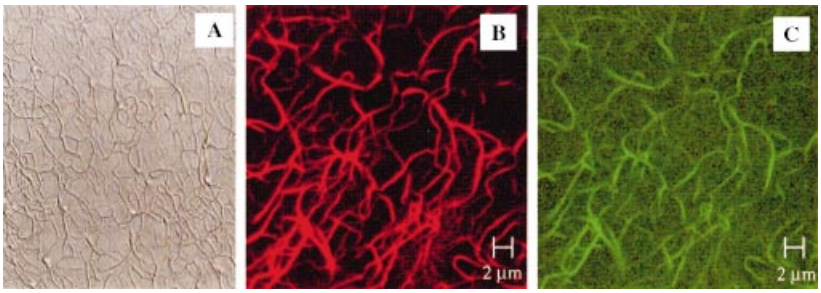

Figure 2. A) *Video-enhanced differential interference contrast (DIC) optical microscopy image of F-actin membrane complexes formed with DOTAP/DOPC (1:1) cationic liposomes. The field of view is 50 μm by 65 μm. Laser-scanning confocal images of the same membrane complexes with observation of fluorescence emission from Texas red labelled lipid (B) and coumarin green labelled F-actin (C), respectively. DOTAP = 1,2-dioleoyloxy-3-(trimethylammonio)propane, DOPC = dioleoyl-L-α-phosphatidylcholine. Reproduced with permission from ref. [3]. Copyright (2000) American Association for the Advancement of Science.*

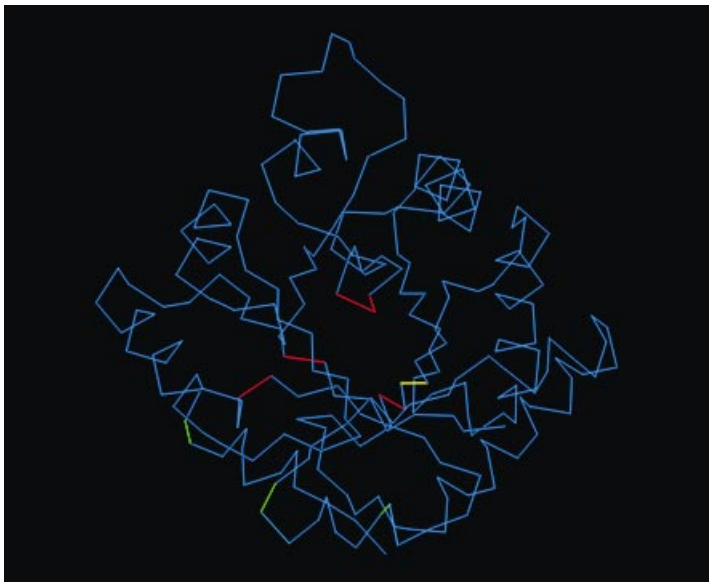

Figure 3. *X-ray crystal structure (Protein databank accession code: 1tim) of chicken triose phosphate isomerase displayed as an α-carbon backbone trace (blue). Shown in yellow is the key active site residue 165 (yellow) that was mutated [E165D] to impair the original catalytic function of the enzyme. The positions of six alternative single amino acid residue point mutations (including the second site-suppressor S96P) able to partially rescue this active-site catalytic lesion are shown (red), in or close to the active site. Finally the positions of three alternative single amino acid residue point mutations (the third site-suppressors G214V, A221V and P238S) able to improve further the catalytic activity of double mutant [E165D, S96P] are also indicated (green), at sites more remote from the active site on the protein surface. Amino acid abbreviations: A = Ala, D = Asp, E = Glu, G = Gly, P = Pro, S = Ser, V = Val.*

double helix and even folded proteins themselves intuitively provide a sound foundation upon which to exercise the forces of natural selection to create and mould organisms.

Directed evolution of enzymes provides clear experimental evidence for this moulding, not to mention the commingling of self-organisation and selection processes. For instance, work carried out on the triose phosphate isomerase (TIM) enzyme demonstrated that an active-site catalytic lesion [E165D] could be partially rescued by a cluster of six alternative single amino acid point mutations in or close to the active site (for example, S96P), selected from definably random libraries of mutations in response to a metabolic selection process (Figure 3).[4] A second cluster of three alternative single amino acid point mutations remote from the active site was identified in a subsequent selection process that was able to confer further improvements on the catalytic performance of the [E165D, S96P] double mutant (Figure 3).[5] In effect, external metabolic "natural" selection pressures were observed to have prompted the internal molecular evolution of a "robust" biological catalyst. In the first instance, such molecular level evolution by a succession of single-point mutations seems to be a satisfying way to account for the inner workings of natural selection pressures at the molecular level, consistent with the comments of John Maynard Smith.[6] However, if we combine this concept with the idea that natural selection pressures are preceded by self-organisation, then we have an integrated, holistic molecular hypothesis for the origin and development of life. Firstly, self-organisation that exists as a direct consequence of molecular complexity and diversity provides the means to compartmentalise closed autocatalytic "living" systems of reacting molecules. Secondly, self-organisation generates other stable, low-energy, robust systems such as folded proteins that can be moulded by a succession of single-point mutations under pressures of natural selection so as to promote catalysis or other functions necessary to originate and promulgate living organisms. This molecular hypothesis would not only be consistent with the Kauffman description of a living organism as a system of chemicals that has the capacity to catalyse its own reproduction, but must surely strike a chord with the chemical biologist who believes in the central role of chemistry in biology.

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