

Evolutionary Potential and Requirements for Minimal Protocells

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List of Abbreviations

AL	artificial life
EC	Enzyme Commission
GARD	graded autocatalysis replication domain
PNA	peptide nucleic acid
SCM	stochastic corrector model

1 Introduction

1.1 Units of Evolution and Units of Life

Evolution by natural selection is perhaps the most important process acting in populations of living systems. This is one of the reasons why it is so tempting to equate units of evolution (i.e. an abstract generalization that makes no reference whatsoever to any particular level of biological organization) to units of life.

Another reason is that units of evolution can be much more readily defined. There are a few known alternative formulations of the concept of units of evolution; here we stick to the version outlined by Maynard Smith [1, 2]: such units must multiply, show heredity across generations (like begets like), and heredity should not be exact. If some of the hereditary traits affect the chance of reproduction and/or survival of the units, evolution by natural selection can take place in a population of such units. The combination of survival and reproduction (translating into the expected number of descendants) is called fitness.

The above characterisation of Darwinian dynamics is deliberately general: note that it is not restricted to cover living systems only. (As a matter of fact some living systems do not – sometimes cannot – multiply: mules and neurons normally do not reproduce; we shall come back to this point.) Hence it is potentially applicable to molecules and cultural traits *as far as the criteria really apply*.

A general point about definitions is that they cannot be falsified. They have to be internally consistent, of course, but there can be an arbitrary number of such definitions for life, for example. It is the use of the alternative definitions that makes

the difference: some definitions are found helpful because they categorize natural phenomena in a way that is conducive to further insights. There is always an ingredient of arbitrariness in definitions: we have to live with this fact.

Coming back to the problems of units of life, it is obvious that if we think that they are equivalent to units of evolution, then replicating RNA molecules or some computer viruses would be alive. The first option would be endorsed by some protagonists of the RNA world, whereas the second alternative would be maintained by some AL researchers. We do not find this view useful: in short, we do not think that all replicators *sensu* Dawkins [3] are living systems. We appreciate the desire on behalf of researchers to move the goalpost so that a “living molecule” would provide us with the solution to the problem of the origin of life, but this would not explain much about the origin of cells (or “cellular life”, as some use to distinguish it from merely “molecular” life). Today every autonomous living system is cellular (prokaryotic or eukaryotic, uni- or multicellular) in nature, as advocated by the Schwann-Schleiden cell theory in the mid-nineteenth century. Viruses are not (autonomously) alive. Gánti’s [4, 5] analogy is very useful: a virus is to a cell what a replicating programme is to a computer. The cell is happily alive without the virus; the virus cannot do anything on its own (apart from slow disintegration). Thus if we search for the principles of life *sensu* Gánti we have to search for principles describing cellular life. The sensible relation, by our definition, between units of evolution and units of life is thus that of two partially overlapping sets [6].

As Gánti emphasizes, modelling of a living system in entire generality is not very rewarding, since there are (at least) two levels of life: multicellular organisms

consist of units that are living systems even if the multicellular organism is killed. Conversely, the death of many of its cells does not necessarily kill the organism. If we are interested in the origin of life, our ultimate target must be the explanation of the origin of the prokaryotic (bacterial) cell.

But a prokaryotic cell is far too complex for spontaneous self-assembly or self-organization. Even the simplest known living creatures (such as mycoplasmas) contain several hundred genes: they must be products of past evolution. And we have to assume that sometime, more than 3 billion years ago, there were no genes at all. The increase in complexity must have come about by duplication and divergence, and merging (recombination) [7]. We conclude that a lot of evolution must have preceded the origin of bacterial cells. Some of this evolution may have taken place even in a pre-cellular phase of evolution (such as the naked RNA world [8]). And the simplest cells must have been very remote from contemporary instantiations; hence a lot of evolution must have happened between the protocellular and bacterial phases of evolutionary history.

1.2 Criteria for Minimal Life

With the caveats above, we feel ready to head towards a working definition of minimal life. We base this working definition on the chemoton concept [4, 5]: a minimal living system is a chemical supersystem comprising three systems: a metabolic network, template replication and a boundary system. It is instructive to look at the abstract minimal version of the chemoton (Fig. 1). It is important that all

three systems are autocatalytic, and via the shown stoichiometric coupling, the chemoton as a whole is also autocatalytic. As we shall discuss later, such a system is not only chemically autocatalytic but also biologically reproducing by cell division. This biological minimal system will be our intellectual starting point. It is true that nobody has ever claimed that the system in Fig. 1 can be realized by a handful of reactions. This merely reflects that the chemoton is the result of abstraction and idealization. A pertinent question for the practical chemist is this: what is the requisite chemical complexity of a realistic chemoton? We do not know, but this question defines a research programme.

As mentioned above, this system is by definition not a general model of living systems: it applies (discounting a lot of evolutionary detail) to the bacterial level of organisation. But we should mention that it satisfies the so-called criteria for life, set up by Gánti [5]. Life criteria are phenomenological and verbal, but rigorous descriptions of those traits that apply to all kinds of living organisms. It is important for our discussion that Gánti distinguishes absolute from potential life criteria: the former are necessary for all units of life, the latter are necessary only if *populations* of living systems are to survive and evolve. A mule is a unit of life, but not a unit of evolution.

It follows from our foregoing discussion that such a system must be a culmination of a protracted period of prior evolution. This comprises chemical evolution (the complexification of chemical systems) and evolution by natural selection of chemical replicators of various kinds. It is likely that mineral surfaces have played an important role in precellular evolution (e.g. [9, 10, 11, 12]). Surfaces have favourable thermodynamic, kinetic and selective effects on

chemical and replicator evolution. Reviews of molecular selection dynamics on surfaces can be found elsewhere [13]. We mention this link because effects that surfaces can confer can be conferred even more efficiently by compartments: obviously, a reproducing protocell is the strongest form of population structure, conducive to group selection [14, 15] of the replicators included within.

Although we take the chemoton as the working definition of minimal life, it is important that its three subsystems can be combined to yield three different doublet systems (Fig. 2). It is historically interesting to note that the original formulation of a chemoton [4] consisted of the metabolic cycle and the replicating template only. By definition such a system is not actively compartmentalized, because it is lacking a self-generated boundary system, although it could have played a major role in precellular evolution (e.g. [13]). The combination of a metabolic cycle and a membrane was conceived also by Gánti [16], and called a self-reproducing microsphere. In contrast, Szostak et al. [17] conceived a protocell-like entity with a boundary and template replication but no metabolic subsystem.

Similar criteria for a protocell were suggested by Pohorille and Deamer [18]. It is instructive to look at their list:

- i. An information-carrying polymer must be synthesized by template polymerisation within a compartment delineated by a membrane.
- ii. Monomers of template synthesis and other raw materials must be provided from the outside, and must be able to pass through the membrane.
- iii. An external source of chemical energy must be present.

- iv. Hereditary variation of template replicators must affect the efficiency of catalysed reaction in the protocell.
- v. The membrane must be able to grow, either through direct incorporation of membranogenic molecules from the environment or through conversion of appropriate precursors.
- vi. Reproduction in space of the protocells must happen.
- vii. Catalysis, replication and growth must be synchronized well.

The chemoton model almost satisfies these criteria: it is less and more at the same time as the minimal cell envisaged by Pohorille and Deamer. Criterion (iv) is not satisfied by basic chemotons since templates are assumed to affect the system by means other than sequentially encoded enzymatic catalysis. In contrast, the chemoton has a *bona fide* metabolic subsystem, so it goes beyond criterion (ii) in this regard. It is remarkable that the protocell model suggested by the authors includes a pump in the membrane that actively maintains a certain concentration gradient. The issue is analysed in Ref. [149]. While a pump may not be strictly necessary, passage of charged molecules through protocell membranes warrants special considerations (see 2.5).

In a similar vein, Szostak et al. [17] propose that a protocell composed of a growing membrane, a general replicase ribozyme (able to replicate also another copy of itself), and another ribozyme involved at some stage in membrane formation would be truly alive. Once again, it is clear that this system is an “ultimate heterotroph” [19], completely devoid of a metabolic subsystem. We shall come back to this problem when discussing protocellular metabolism.

The aforementioned chemical supersystems we suggest to call *infrabiological* systems: they are not biological, since they always lack one essential component of a minimal living system, but they show a crucial subset of biological phenomena. Among the infrabiological systems those comprising a boundary belong to compartmentalized systems. In this chapter we are going to deal essentially with compartmentalized systems, including systems with chemoton-like organization. Section 2 concentrates on modes and feasibility of compartmentation, followed by detailed analysis of the nature of plausible genomes of reproducing compartments (Section 3). Section 4 deals with the difficult problem of running a metabolism in protocells. We would also like to draw attention to a complementary analysis of related issues by Ruiz-Mirazo and Moreno [149].

2 Compartmentation: Membranes, Reproducing Microspheres and the Lipid World

Without membranes there are no protocells. When discussing their role in early evolution, one should tackle the following issues: formation of membranogenic molecules, membrane growth and inheritance, microsphere division, and membrane permeability.

It is not our task here to comment on chemical evolution leading to membrane constituents. We rather focus on the remaining issues in turn.

2.1 Autocatalytic Membrane Formation

It is important to point out that membrane growth is an autocatalytic process [20]: membrane constituents are at a high energy state in the aqueous phase; hence they spontaneously insert themselves into pre-existing membrane surfaces. The larger the surface, the faster is the reaction. In this sense pieces of membrane can grow, and if there is some fragmentation, they can multiply. A good question is whether they can have some form of heredity. We mention in passing that in contemporary biological systems there exists the phenomenon of genetic membranes [21]: many biological membranes do not form *de novo*. For example, the membranes of plastids and mitochondria grow by the activity of specific import machineries composed of proteins. These import machineries recognize proteins destined to become parts of the specific cell organelles. The autocatalytic element comes in when we realize that parts of the import machineries are recognized in this way by the import machineries themselves. We are faced here with not only autocatalysis but membrane heredity, as the different kinds of membrane propagate their own kind.

Contemporary membrane heredity rests on the action of proteins, and it achieves only what has been called limited heredity [7, 22]: the number of possible types is limited. Hence evolution is also limited in such systems –this is not to say that they are unimportant. Contemporary heredity is limited because it is based on some subset of shape space, rather than on the whole of sequence space. Any molecule having the right conformation for recognition by the import machinery is dragged in, irrespective of its sequence. (One should not be confused in this discussion by the fact that the proteins involved are genetically encoded. One can

imagine the swapping of the import machineries of plastids and mitochondria. This manipulation would immediately redefine membrane identity and propagation without any change in the corresponding genes.) Two relevant questions emerge: (i) is it possible to have membrane inheritance without proteins? (ii) What are the limits of heredity in such systems?

2.2 Membrane Growth and Inheritance

An interesting line of research has been initiated by Doron Lancet with his group, conveniently referred to as the “lipid world” scenario [23]. The basic idea is as follows. We know that lipids (more generally: amphiphilic compounds, with a hydrophobic tail and a hydrophilic head) tend to form supramolecular structures, such as bilayers, micelles and vesicles. They can grow autocatalytically. Now imagine that we have a mixture of molecules in any one vesicle. Some of them may act as catalysts of certain reactions. It is theoretically possible that some will catalyze their own incorporation (direct autocatalysis), or that there will be a gang of molecules, each exerting some catalytic function, so that as a net result the incorporation of all members of the gang is ensured by the gang (reflexive autocatalysis). If this idea holds water, membrane heredity in the lipid world, and natural selection of vesicles without a genetic subsystem, would be feasible. The different, reflexively autocatalytic gangs would constitute “compositional genomes” [24]. Note that the model does not deal with the formation of the lipid constituents: they are assumed to be there in the surrounding soup.

Now, there is nothing mysterious about compositional genomes in the first place. Although relying on direct autocatalysis at the molecular level, the genome of the SCM (see section on genetics) is also a compositional genome: the genes there are unlinked, and the genome is characterized by gene composition. Formally, each protocell can be characterized by a genome vector with entries n_j , denoting the number of copies of the j th gene in that vesicle. Change in this number is a stochastic process, which can be characterized by mean and variance.

A similar approach is possible when considering questions in the lipid world; the issue is however complicated by the fact that we need to tackle the problem of reflexive autocatalysis. This has also precedence in the literature: the reflexively autocatalytic protein networks (e.g. [25]) is perhaps the best known example. We hasten to point out that nobody has seen a real reflexively autocatalytic protein set, apart from very small ones where replication is in fact modular and analogous to the complementary replication of oligonucleotides [26]. Let us see whether one can be more hopeful regarding autocatalytic lipid sets.

The process imagined is shown in Fig. 3. It displays a reflexively autocatalytic micelle with many components. The incorporation of amphiphile L_i may be catalyzed by amphiphile L_j at rate enhancement β_{ij} (the ratio of catalysed and uncatalysed reaction rates). The crucial question is this: where can one obtain the values of β_{ij} , considering the fact that no such system has been realized so far (the experimental cases, discussed below, are all directly autocatalytic and show no heredity)? Lancet and his colleagues suggest going around this problem as

follows. Fendler and Fendler [27] present a compendium of catalytic reactions documented in micellar systems, from which an empirical distribution of β values can be obtained. Is there a theoretical background to this distribution? The authors suggest translating the model developed for molecular recognition between receptors and ligands [28]. If catalysis depends on recognition of substrate by catalyst, the reasoning is sound: it shows that catalysis is a graded phenomenon. From this empirically constrained theoretical distribution, the authors obtain the β_{ij} values in their GARD model (Fig. 4).

It is instructive to contrast direct autocatalysis with reflexive (mutual) autocatalysis in the GARD model [23]. In direct autocatalysis, the diagonal elements in the catalytic enhancement matrix dominate. Simulations show that, as expected, one such direct replicator will dominate the population. If the diagonal elements are not dominating (as is most likely when using the inferred distribution), then we have the reflexively autocatalytic set.

It is imagined that every micelle (or vesicle) is a *sample* with replacement of a set of possible lipid molecule. Some samples will contain mutually autocatalytic gangs, others will not. The latter ones will not be able to grow. The former will grow and then fragment by some spontaneous process. Micelles containing more efficient gangs (characterized by higher β_{ij} values) will take over. Such sets do have heredity, and they are attractor-based [29]: the gangs maintain and propagate their identity by virtue of their mutual catalytic activity [24].

There are some acknowledged concerns with this model [23]. Reflexively autocatalytic network models (including protein nets [25]) are plagued by the side

reaction problem [22]. First, it seems natural to assume that many entries β_{ij} will be effectively zero (no catalytic effect). Worse still, several entries may be negative, which means that in the corresponding reaction an amphiphile catalyses a reaction *draining* the system. The real snag is that in chemical space the vast majority of reactions will be unwanted side reactions. They will presumably be catalyzed by values taken from the same distribution. Will this problem demolish the lipid world scenario?

One can say that a similar problem arises in the RNA world: the side reaction problem applies to protocells harbouring ribozymes as well. Not quite, because ribozymes, by virtue of their direct modular replication, can undergo microevolution based on digital information. Genetic information in GARD models is not digital and *small* variations are not heritable (see [30] for a detailed analysis of inheritance in the GARD model).

On the experimental side it is worried [23] that Fendlers' compendium cites mostly hydrolytic reactions. It may well be the case that different types of reaction (such as biosynthetic ones) should be taken from a different, unknown distribution. This is an exciting problem that should be tackled, irrespective of the lipid world.

2.3 Vesicle Division

The division of protocells is an important issue. In general it should be the result of insertion of membranogenic molecules into the membrane. This can be achieved from outside [31], or from inside. Needless to say, growth from inside is the

favoured solution. Whether growth occurs from outside or inside, amphiphiles must be able to jump from one side of the bilayer to the other, otherwise the two layers of the membrane would grow out of proportion. The latter could happen transiently, but not notoriously. Amphiphiles are known to jump from one layer of the membrane to the other (flip-flop mechanism): this reaction must be sufficiently fast in a protocell to render growth and division possible.

In the chemoton model there is stoichiometric coupling between metabolism, template replication and membrane growth, ensuring strict synchrony among these autocatalytic systems. Under such an assumption Rashevsky's [32] early observation on doubling the surface area and volume of a sphere is relevant. Imagine a spherical protocell, kept in that shape by osmotic pressure (turgor) of the metabolites that cannot leave the protocell through the membrane. Due to chemoton coupling, both surface area and the mass of internal material are doubled in growth. But this is incompatible with the maintenance of a spherical shape: surface area scales with the square, volume scales with the cubic of the radius of the sphere. A sphere with double surface area has more than double volume. Since the quantity of osmotically active inner material is only doubled, it follows that the shape of the chemoton becomes continuously distorted. One resolution is that the cross-section becomes dumbbell shaped and ultimately the system divides into two spheres, which together have doubled surface and volume of those of the original [33].

This qualitative reasoning has received confirmation by calculations which assume that the growing microspheres go through a series of quasi-equilibria of different shapes. If the rate of ancient metabolism is not high, this is a fair

approximation. Verhás [34] as well as Tarumi and Schwegler [35] were able to calculate the equation of motion for the membrane surface, taking curvature into account. The latter is important since large curvature has higher energy: this is why in general symmetrical shapes are favoured over asymmetric ones.

Božič and Svetina [36] analyzed a different situation, where addition of membrane constituents happens from the external milieu, and there is no metabolism inside, but there is limited permeability. They supposed that the membrane assumes spontaneous membrane curvature. This is nonzero if the properties of the inside and outside solutions differ, or if the two layers of a bilayer membrane differ in composition, or if some membrane-embedded constituents are asymmetrically shaped. They were able to show that under these assumptions membrane division is possible provided $TLkC^4 \geq 1.85$, where T is the time taken to double the membrane area, L is the hydraulic permeability of the membrane, k is the bending modulus, and C is the spontaneous membrane curvature. In this model growing vesicles first retain spherical shape, then are distorted to a dumbbell, then to a pair of asymmetric vesicles coupled by a narrow neck, and finally to a pair of spherical vesicles linked by a narrow neck. Separation of the two daughter vesicles occurs as a result of mechanical agitation in the solution.

Heterogeneity of membrane constituents may also play an important role in the stabilisation of vesicles. Amphiphiles with cationic and anionic head groups can assemble into vesicles that are stable over a year [37]. This effect may be explained by assuming an asymmetric distribution of the two constituents between the two layers. Note that the two layers have curvatures of equal magnitude but

opposite sign. How such an asymmetric membrane structure would be maintained through generation of protocells is not obvious, however.

It must be said also that temperature can also change the equilibrium shape of membranes because the two layers react to temperature change differently [38], due perhaps to molecular impurities. The obtained shapes are very suggestive. Considering the fact that according to one scenario the origin of replication of longer templates was tied to temperature oscillation [39], it would be important to look at the concomitant changes in membrane shape. Replication as well as membrane division could have been helped by the same oscillation of temperature. Döbereiner et al. [40] demonstrated that budding of sphingomyelin vesicles can be triggered by increasing the area-to-volume ratio of the vesicles by heating. The explanation is that heating induces a gel to sol transition in the membrane. Another way is to induce budding and fission osmotically, which reduces the volume, hence increases the area-to-volume ratio again in the favoured direction. Note that there is no membrane growth in these experiments.

Experiments have confirmed the idea that micelles as well as vesicles could grow autocatalytically (see [41] for a good overview). In a landmark paper Bachmann et al. [42] observed the formation of autocatalytically replicating micelles from sodium caprylate. The micelles could be converted into more stable vesicles by pH change. Oleic acid/oleate vesicles can also multiply autocatalytically, and they show a remarkable “template” or “matrix” effect in the size distribution: somehow the newly formed vesicles inherit, with “good accuracy” the size distribution of the preexisting vesicles [43, 44]. Microscopic investigation

provided evidence for vesicle fission, but whether fission is binary or not is uncertain [45, 46].

2.4 Compartment/Template Infrabiological Systems

Growing membrane systems have been used to obtain artificial infrabiological systems. Walde et al. [47] have carried out the synthesis of polyadenylic acid in self-reproducing vesicles [48], in which the enzyme polynucleotide phosphorylase carried out the synthesis of poly-A, and membrane vesicle multiplication was due to the hydrolysis of externally provided oleic anhydride to oleic acid. The snag is that the enzyme component is not autocatalytic. Enzymatic RNA replication in vesicles [49] suffers from the same problem. It is also not known whether redistribution of the entrapped enzymes into newly formed vesicles occurs or not. An affirmative answer would be evidence for vesicle reproduction by fission.

The demonstration that the polymerase chain reaction can be carried out in liposomes [50] is important because it demonstrates that liposomes can resist the required temperature changes. In the light of the lipid world model it is useful to ask what catalytic functions Luisi's structures show behind direct autocatalysis. Binding of peptides to and polymerisation of amino acids in liposomes was demonstrated in various systems [51]. We are not aware of a similar effect on nucleic acid synthesis.

The direct autocatalytic multiplication of both caprylate and oleic acid vesicles received a simplified kinetic analysis [52]. It was shown, in agreement

with elementary reasoning that the catalytic effect is due to large growth of the reaction surface.

Another line of research demonstrated the catalysis of membrane formation from micelles by montmorillonite surfaces [53]. Such vesicles grow in size but spontaneous division (without externally enforced extrusion) was not demonstrated. RNA absorbed to clay can be taken up together with clay particles, but neither clay nor RNA is autocatalytic in this experimental system. Fatty acid vesicles containing RNA have a higher internal osmotic concentration. This is expected to strain the vesicle membrane and facilitate its growth. RNA-containing vesicles are shown to grow in size at the expense of vesicles that do not contain RNA [54]. The process is likely due to fatty acid molecules leaving the isotonic vesicles (which hence shrink in size) and joining the RNA-containing vesicles (which hence increase in size). The process continues until a new equilibrium is reached. Competition between protocells is thus an intriguing possibility not yet experimentally demonstrated.

In an attempt to design a protocell, a Los Alamos group proposed a system essentially composed of non-enzymatic template replication coupled to micelle growth [55, 56]. The micelle aggregate is assumed to incorporate from the medium precursors of lipids and template building blocks (monomers or oligomers). The authors assume that for this particular construct PNAs [57] would serve better because of their hydrophobic nature. It is assumed that single-stranded molecules face the hydrophilic exterior whereas double-stranded molecules immerse into the hydrophobic interior of the micelles. Alternation between these two states is assumed to facilitate replication.

A possible form of coupling between membrane growth and template replication is assumed to proceed as follows. PNA molecules are planned to carry photosensitizer molecules. PNA acts as a catalyst for the light-driven fragmentation of the lipid and template monomer precursors. It is also assumed that certain PNA sequences provide the efficient charge transfer system necessary to realize the dynamic coupling.

It remains to be seen whether this clever proposal can be chemically realized. If yes, it would serve as a proof-of-principle, but two main limitations remain. Due to the envisaged template replication, template length is assumed to be less than about 10 [56]. It seems that the growth dynamics, essentially obeying the “survival of everybody” rule for parabolic replicators [58], would result in a diversity of short oligomers, but the situation is more complicated. As mentioned above, only certain sequences would act as efficient charge transfer systems. Thus selection for efficient growth would result in very few winning, short sequences. Presumably, one would arrive at a system with limited heredity [7] and evolution would not be open-ended. It is doubtful whether any functionality of PNAs other than charge transfer could evolve in the system. It is not easy to see how the Los Alamos Bug could be modified to become a unit of evolution.

2.5 Membrane Permeability

Membrane permeability is one of the most difficult problems for protocells to solve. Electrolytes, amino acids and sugars permeate liposomes at a very small rate.

Today we find highly evolved transport mechanisms, resting on evolved proteins, which solve this problem for the cell. protocells must have resorted to more basic, but sufficiently functional tricks.

A number of suggestions have been made. A simple mechanism was proposed by Stillwell [59], resting on the following components: (i) increase the permeability of a molecule by a transient chemical modification, which renders the molecule more lipophilic; (ii) maintain a gradient by lowering the concentration of the material in question by metabolizing it (Fig. 5). In concrete terms, amino acids [59] and sugars [60] could react with aldehydes (such as formaldehyde and pyridoxal) to form a so-called Schiff base, which then could pass through more readily through the lipid bilayer. Experiments have confirmed the feasibility of this idea. Another possible route, requiring a H^+ gradient across the bilayer (acidic inside), was suggested by Chakrabarti and Deamer [61], pertaining to amino acids. The most common form of amino acids is zwitterionic, and the less common form is the neutral (uncharged) molecule (Fig. 6). The proposed transport mechanism consists of the following steps: (i) neutralize the $-COO^-$ group by creating amides or methyl ester derivatives; (ii) at basic pH the $-NH_3^+$ groups lose their H^+ ; (iii) let the neutral molecule pass through the bilayer; (iv) acidic pH inside restores $-NH_3^+$ and renders the amino acid again impermeable; (v) use up the amino acid in metabolic reactions. The increased transport rate of amino acids thus neutralized has also been experimentally demonstrated.

An interesting mechanism for the establishment of a pH gradient in growing fatty acid vesicles was recently shown by Chen and Szostak [62]. Fatty acid

vesicles are usually very permeable to cations, including H^+ . Maintenance of a gradient thus requires a non-permeant cation, such as arginine. Incorporation of protonated (neutralized) fatty acid molecules from the *external* medium results in acidification of the internal milieu, by a flip-flop mechanism of H^+ transfer, performed by the fatty acid molecules (Fig. 7). The energy released in membrane growth can thus be stored in the pH gradient thus built up. Such a gradient could be coupled to some other processes, as discussed above.

3 Primordial Genomes

The genome is the total genetic constitution of an organism. In present-day cells information encoded in DNA is transcribed into RNA and translated into proteins, the absolutely essential polymers for almost all cellular functions. But such flow of genetic information and relatively clear-cut boundary between replicating and catalytic systems was a later invention. Primitive cells (protocells) may have been entirely RNA-based, which circumvents the historical dilemma of which came first, proteins or DNA. There was little separation between molecules acting as functional units and those acting as genetic material. Nevertheless, even if difficulties related to prebiotic RNA synthesis and stability can be eventually solved, an important problem still remains: How could information have been preserved when templates were confined to isolated vesicles of finite size and replicated by a low copying fidelity RNA polymerase ribozyme?

3.1

Error Thresholds

The pioneering works of Eigen [63] called attention to the fact that the length of selectively maintained genetic information is limited by the copying fidelity. Without the aid of peptide enzymes the upper bound of copying fidelity per nucleotide per replication was likely around 96-99% [64, 65]. With such a high mutation rate the number of mutated offspring molecules far exceeded the number of non-mutated ones, resulting in the well-known quasispecies concept of Eigen [7, 63, 66, 67, 68] where a stable cloud of mutants formed around a master sequence as far as the maximum chain length (N) was below the critical error rate per site per replication (q^*) as determined by the following simplified expression:

$$N < \ln s / (1 - q^*),$$

where s is the superiority of the master. The occurrence of thresholds for error propagation was originally derived as a deterministic kinetic theory that is only valid in the limit case of an infinite number of molecules. Alves and Fontanari [69] have extended this to finite populations and found that q^* decreases linearly with the inverse of population size.

3.2 Vesicle Models

Proposals to circumvent the information crisis have focused on networks of non-encapsulated cooperative molecules (i.e. hypercycles, Fig. 8), or

compartmentalization of competitive unlinked templates (i.e. vesicle models, Fig. 9). As originally formulated [63, 66] the hypercycle is a catalytic feedback network in which each template helps in the replication of the next one, in a secondary cycle closing on itself (second-order autocatalysis). The enzymatic function (replicase activity) in the hypercycle was thought to be carried out by the encoded proteins (the “realistic” hypercycle [70]), but recently also the hypercycle was projected into the RNA world [8], with RNA molecules acting as templates as well as enzymes [71]. Problems arise, however, due to the dynamical instability of large hypercycles (e.g. they are vulnerable to extinction via fluctuations [72]), and the evolutionary conflicts among its members no matter how small hypercycles can be. Spatial implementations of hypercycles simulated by cellular automata are of little aid in this matter as it was shown [13] that the spatial patterns (spirals) that would supposedly increase the robustness of hypercycles against parasites [73] are unstable against a trivial form of “patchy environment”, namely differential death rates of replicators in the cells of the grid. As first recognized by Maynard Smith [74], to provide catalytic support in a molecular catalytic feedback network is an altruistic behaviour doomed to exploitation by parasitic molecules and eventual extinction. Apparently this criticism still holds.

The initial stimulus behind vesicle (compartment, protocell) models was to solve the conundrum of the evolutionarily dynamic coexistence of unlinked genes in view of the problems faced by hypercycles. Sooner or later hypercycles would have had to escape into compartments (as already accepted by Eigen et al. [75]), but in those circumstances alternative systems such as a group of competing species of molecules (genes) that happened to be enclosed in a vesicle and were

replicated by a non-specific replicase could have been a more efficient alternative for accumulating information and for forming a catalytic network that otherwise could be unstable in homogeneous solution [17, 19, 76, 77, 78, 79]. Elaborating on the package model proposed by Niesert et al. [80], Szathmáry and Demeter [81] described the SCM as a dynamic system where an optimal template composition ensures the fastest growth and division of protocells. The protocell grows due to replication of templates and by adding the necessary membrane building blocks due to the metabolism of the compartment. The fission size corresponds to a certain level of polyploidy of the genes (i.e., gene redundancy). As genes are distributed randomly to the daughter cells dead offspring can arise, and their average fraction increases with decreasing polyploidy of the slowest replicating template. For this reason the initial formulation of the SCM assumed that the number of different template types per protocell must have been small.

A common criticism faced by the SCM is that it is a very sloppy system of information integration because the major difficulty for conserving a complete set of genes is the growth rate difference between replicators [80, 82, 83]. The snag with such criticism is that it overlooks evolution. Thus, if we reasonably assume an evolving population of protocells where there is initially genetic variation for replication rates, only those lineages with a reduced variance in growth rates among unlinked genes would eventually survive [84]. Those lineages could likely host many more genes than the alleged upper limit of 3 (e.g. [80, 82]). Actually, in the limiting case of an infinite number of vesicles, each one carrying a finite number of templates that replicate at the same rate, Fontanari et al. [85] have recently found that there is no fundamental impediment to the coexistence of an

arbitrary number of template types inside a vesicle (except of course for the vesicle capacity). This is in sharp contrast with the inherent dynamical instability of large un-packed hypercycles (see above). Since the total information content is the product of the number of different templates and the maximum information coded per template, the information gain due to the coexistence of different templates in stable hypercycles relative to the theoretical upper limit in vesicle models is therefore negligible.

Compartmentalization thus offers the most natural and efficient way of information integration, but it does not still solve the original problem raised by Eigen [63]: the information crisis in primitive genomes. The first attempt to compare q^* between “conceptually analogous” versions of compartmentalized hypercycles and the SCM was carried out by Zintzaras et al. [71]. They found that a population of SCM protocells can tolerate a higher input of deleterious mutation rates, and reaches an equilibrium mutational load (i.e. the decrease in average fitness of a population exposed to deleterious mutations relative to an error-free population) lower than that in a population of protocells hosting hypercycles. Hence, given our current understanding a working-model for the protocell scenario in the origin of life would enclose a persistent cloud of mutants around master sequences of genes competing for within-group common resources. Selection on stochastically produced offspring variants would favour those lineages with non-competitive molecular assemblies, thus stabilizing the population against random loss of essential genes after compartment fission. But a critical question still remains: Could such a primitive protocell sustain the “minimum” informational

length required for the basic features of life with a putative low copying fidelity RNA polymerase ribozyme?

3.3 Recombination (Sex) and Gene Redundancy

Within the classical framework of vesicle models, the previous question was approached by several authors [71, 86] and the answer was clear: compartmentalization *per se* was not sufficient to overcome the information bottleneck imposed by the error threshold. However, Lehman [87] raised the issue that recombination – a frequently ignored player in models of early evolution – could have been crucial to build up primeval genomes of sizeable length. In the article that coined the phrase “the RNA world” Gilbert [8] already speculated that «the RNA molecules evolve in self-replicating patterns, using recombination and mutation to explore new functions and to adapt to new niches.» In this context the discovery of spontaneous rearrangements and recombinations of sequence-non-specific RNAs in solution is important [88]. According to the experimental evidence, RNA chains of diverse sequences can recombine at a rate of 10^{-9} h^{-1} per site and the reaction is not due to cryptic ribozyme structures that might be formed by some RNAs, but is an intrinsic chemical property of polyribonucleotides. More recently, Riley and Lehman [89] have shown that *Tetrahymena* and *Azoarcus* ribozymes can promote RNA recombination.

This capability of RNA to potentially minimize the burden imposed by the error threshold – along with [87] – has been recently analyzed by Santos et al.

[90]. They assumed that recombination in protocells took place via copy-choice means; i.e. that the replicase switched between RNA-like templates as occurs frequently in RNA viruses and is crucial for retroviral replication during reverse transcription (e.g. [91, 92, 93, 94, 95]). They ignored, however, the possibility of gene chimerisation resulting from illegitimate recombination – a putative source of major evolutionary innovations as discussed by Cavalier-Smith [96] – because it would introduce many technical and theoretical problems in a protocell scenario. The numerical results showed that there is a quite intricate interplay between mutation, recombination and gene redundancy, but the conclusion from the fitness function they used was that the informational content could have increased by ~25% at most by keeping the same mutational load than that for a population without recombination. Even so, the upper bound of ~75 nucleotides reached in that work is still far from the minimal life provisions.

The consequences of imperfect replication in vesicle models are somewhat puzzling [85, 90]. For small mutation rates increased level of polyploidy favours the persistence of protocell lineages since the random loss of essential genes after fission is attenuated. However, for large mutation rates the situation is reversed, resulting in that those lineages with low levels of polyploidy are better able to cope with higher mutation rates, particularly when recombination is allowed. This means that gene redundancy was indeed costly. Therefore, selective forces favouring the linkage of genes to make the first chromosomes would eventually outweigh the advantage of faster replicating single genes because linked genes are less likely to be lost by random assortment when protocells divide [97].

The role of the number of gene copies in a primitive cell was investigated by Koch [98], who pointed out the existence of two conflicting forces: (i) higher copy numbers act as a safeguard against random loss of all copies of a gene; (ii) but such copy numbers slow down adaptive evolution because a newly arisen favourable mutant is diluted out and cannot be “seen” efficiently by natural selection acting on cells. He further observed that a moderately high (<100) copy number per gene is not only optimal, but is confers some additional evolvability by the “duplication and divergence” scenario, as first emphasized by Ohno [99].

3.4 Lessons from “Bags of Genes” in Contemporary Genetic Systems

It is important to point out that unlinked, independently replicating and reassorting genetic elements (replicons), exist even today. For example, the macronucleus of ciliates (unicellular protists) is essentially a bag of genes in high copy number [100]. Bacteria can harbour a number of chromosomes and plasmids. It is important that high- and low-copy-number plasmids follow different assortment strategies: the former rely on stochastic assortment, whereas the latter apply cell wall-mediated accurate segregation mechanism [101], in line with conclusions on analogous protobiological systems. A further remarkable example is that of the unigenic mini-circles of the dinoflagellate plastid [102, 103]. It is important to stress that all these systems are only partially analogous, rather than homologous to the assumed primordial “bag-of-genes” genomes.

It is potentially rewarding to have a closer look at the ciliate case. Ciliates usually have one or a few micronuclei and one or many macronuclei (Fig. 10). It is only the former that participate in sexual recombination; we are not aware of a case of macronuclear fusion. Typically, a round of sex is followed by hundreds of clonal cell divisions (called the vegetative phase). During vegetative reproduction the micronucleus is (almost) completely inactive and the macronucleus serves the transcriptional needs of cells. DNA in the macronucleus is highly amplified, presumably to meet the transcriptional demand of the large ciliated cells (they can be larger than 100 micron in length). DNA in the macronucleus originates from one of the micronuclei after fragmentation, elimination and specific amplification [100]. *Paramecium* and *Tetrahymena* species show these phenomena to a moderate extent: In the latter, the macronucleus contains about 57 copies of the unique sequences found in the micronucleus. Out of the 5 chromosomes up to 200 different subchromosomal DNA molecules are generated. Thus each such fragment still contains thousands of genes.

Macronuclear reorganisation is much more radical in the so-called hypotrich ciliates, in which fragmentation occurs down to the level of the gene. Finally, each unigenic minichromosome is endowed with the sequences necessary for replication. For example, in *Oxytricha*, the macronucleus harbours about 24,000 different unlinked genes, each with an average copy number of 950.

The odd fact is that upon division bulk DNA material, as well as each copy of genes, is *randomly assorted* into offspring nuclei. Thus there can be 10-15% difference in the total DNA content of sister macronuclei. This calls for some correcting mechanism at the genomic as well as the genic levels. For the former,

chromatin extrusion, extra full or partial rounds of DNA replication, and/or skipped rounds of replication all seem to contribute to varying extent, although the molecular basis for DNA content regulation is unknown.

At the genic level we find that different genes can be maintained at various copy numbers [104]. This set number can change abruptly, presumably because of mutation in the regulatory sequences [100]. It seems that the added telomeres at the ends of the minichromosomes are sufficient for the initiation of replication [105]: chromosome-internal sequences do not seem to influence the regulation. This of course renders the puzzle of copy number control *specific* for different (groups of) genes even greater.

We wish to draw some important conclusions from the ciliate case. Apparently, the strategy of the “bag-of-genes” is a viable one, and is a satisfactory base of cellular inheritance. (Note that the objection that macronuclei are doomed to death and rejuvenation from sexually recombining micronuclei is mandatory is flawed —there are known cases of amiconucleate clones that can be maintained apparently indefinitely [100]). This does not resort to maintenance of linkage groups or accurate segregation, but does require some mechanism for suppression of replication and copy number control. It seems that the basic adaptation to ensure that most molecules are replicated exactly once in hypotrichs is the existence of so-called replication bands, in which individual DNA molecules cannot migrate and there is a directional wave of DNA replication [100]. Nevertheless, inaccurate segregation requires an active correction mechanism for copy number, as discussed above.

The hypotrich ciliate is very suggestive for early evolution studies. First, it should not escape our attention that one suggested mechanism for initiating replication of RNA molecules is by telomers; noting that telomerases have a ribozyme component and that some RNA viruses adopt a similar strategy [106]. Ciliates testify that a very large number of genes can be maintained when copy number control sufficiently reduces the assortment load. It would be very important to find out the molecular details of the ciliate mechanism, since some analogous mechanism could readily ameliorate the earliest genomic conflicts. The apparent independence from chromosome-internal sequences is particularly encouraging.

Multicopy plasmids offer a limited, but still instructive comparison. Plasmids are replicating clusters of genes in bacteria. Under certain conditions they confer beneficial traits (such as resistance against antibiotics) on the bacterial host. They can also be passed on by conjugation between cells. A well elucidated mechanism of copy number control of abundant plasmids relies on a *trans*-acting inhibitor and *cis*-acting activator. For example, the ColE 1 plasmid in *Escherichia coli* blocks the action of the RNA primer (activator) by a constitutively produced, unstable inhibitor RNA. The concentration of the latter is a good indicator of plasmid copy number in the bacterium [101]. It is true that several unrelated plasmids can coexist in the same bacterium, but closely related plasmids cannot be stably maintained in the same population, unless reintroduced by horizontal transfer. This reminds one of the facts that ciliate macronuclei usually become homoallelic because of chance segregation of the alleles of any given gene; but there is a crucial difference. The latter process happens even if the alleles in the same cell do not compete. In contrast, closely related plasmids vary in the strength of activation and inhibition

elements, and there is therefore within-cell competition [107]. Between-cell competition is influenced by at least three factors: (i) whether the plasmid is essential for survival in the given medium; (ii) very high copy number entails a metabolic burden; (iii) horizontal spread of plasmids is possible.

A final remark here is that in vesicle models the classical source of intragenomic conflict (i.e. conflicts among different elements of the genome) is thought to arise because natural selection acts at two levels: within- (i.e. selfish replicators can reap the benefits of a common metabolism to enhance their own survival) and between-protocells. Sex (broadly defined as the exchange of genetic material between genomes or between two sources [87, 108]) poses yet another riddle because genetic systems that involve fusion between organisms (protocells) offer higher prospects for parasitic genes. The menace of horizontal transfer of parasites between protocells was studied by Santos et al. [84]. They concluded that a population of protocells is able to resist invasion of parasites and, in some situations (i.e. when an over-exploiting parasite invades the population), extensive cellular fusion would have been beneficial and the argument by Hamilton et al. [109] for sex as an adaptation to parasites applies (Fig. 11). An important point is that the scenario they explored numerically is fully consistent with the idea that life may have begun as a series of ever-changing, swapping committees of proto-organisms that exchanged much genetic information [110, 111]. What remains to be explored is the flip side of the coin: the potentially important role of co-opting genetic material from other protocells to significantly speed up evolution.

3.5

Minimal Set of Genes for Cellular Life: the Top-Down Approach

Comparative genomics shows that most bacterial proteins are highly conserved in evolution. From this standpoint an increasingly appealing issue is the identification of «the smallest possible group of genes that would be sufficient to sustain a functioning cellular life form under the most favourable conditions imaginable, that is, in the presence of a full complement of essential nutrients and in the absence of environmental stress» [112]; in other words, to define “the minimal gene set”. The latest suggestion by Gil et al. [113] is a minimal gene set composed of 206 genes. It is highly illustrative to list some of the molecular features apparently needed for the hypothetical simplest bacterial cell:

- i. A virtually complete DNA replication machinery.
- ii. A rudimentary system for RNA repair.
- iii. A complete transcriptional machinery.
- iv. A nearly complete translational system.
- v. Protein-processing, -folding, secretion and degradation functions.
- vi. Machinery for cell division.
- vii. A basic substrate transport machinery.
- viii. etc...

From this catalogue it is painfully obvious that a great deal of molecular and protocellular evolution preceded the hypothetical “minimal cell” in the context of comparative genomics. Our point here is that the top-down approach to design a minimum cell in terms of molecular biology is a worthwhile exercise, but logically

and evolutionary comes later, and not instead of, the chemoton. In addition, the comparative genomic approach might be flawed for the following reason. Suppose that genomes consist of four genes: A, B, C and D in bacterium B1, and A', B', E and F in bacterium B2. Genes C, D, E and F show no homology whatsoever. This does not mean that (A, B) and (A', B') organisms would be viable. They may have a problem, which must be solved somehow, and it happens to be solved by genes D and C in B1 and genes E and F in B2.

Metabolism

4.1

All Living Systems Today are Metabolic

All living systems contain metabolism consisting of at least one autocatalytic cycle.

An autocatalytic cycle is a set of consecutive reaction steps that has A_i

constituents ($i > 1$), takes as material inputs a set of reagents, X, and produces a set of products Y. A cycle has an autocatalytic stoichiometry if, after a finite

number of turns, each constituent multiplies in quantity [5]. In the simplified

description of the formose reaction (Fig. 12), in one turn of the cycle, two

molecules of glycolaldehyde are formed for each glycolaldehyde molecule present

at the beginning. Therefore it has autocatalytic stoichiometry. The products, Y, are

used in constructing the organism (e.g. membrane, templates), or expelled as

waste materials from the chemoton. Chemical cycles are homogeneous catalysts.

Catalysts accelerate chemical reactions without being used up or changed in

nature. They act at low concentrations by reducing the activation energy of the reaction. All living systems are catalysts, e.g. yeast catalyses the production of CO_2 from sugars, but in addition they use some of the chemical matter of the reagents to construct their constituents hence they are autocatalytic.

Why should metabolism be an absolute characteristic of life? An organism without metabolism would be one that did not synthesize any of its materials from precursors, but obtained them all pre-formed from the environment and from its parent(s). Such an entity could only exist if all its materials were present in the environment in sufficient concentrations. Heterotrophic theories popularized by Oparin [114] and Haldane [115] assume such concentrations could have been found in a rich prebiotic soup. Lancet's GARD model [23, 24] and other models of reflexive autocatalytic sets such as Eigen's hypercycle [66], Farmer's et al. [116] autocatalytic binary strings, Fox's microspheres [117], and more recently Szostak's protocell [17] all make the same assumption. How much time would it take the biosphere to deplete this free gift of complex molecules? This would depend on the "gross primary production" of the primitive biota [118]. Although the hypothesised entities do not metabolise X in order to synthesise their components, any complex organization requires some energy for self-construction, i.e. to reduce internal entropy. Unless all this energy could be obtained from sources external to X (e.g. light or redox potentials), X would have to be used as a chemical energy source, and so degraded to waste products. Also X decays to simpler molecules at a finite rate. This happens independently of any reactions required for the maintenance of the organism. Therefore, long-term persistence of non-metabolising entities assumes the existence of mechanisms in the

environment able to replenish X. No known mechanisms, other than living systems with metabolism, are capable of synthesizing complex organic molecules continuously. Since there is no continued influx of complex organics from outer space, non-metabolising organisms can therefore only exist as transients in a system initialized with abundant complex organic molecules, because eventually these will run out. Dyson suggested that life may indeed have started in this way [119]. Alternatively, non-metabolizers can be “parasitic” upon complex organics produced by entities capable of synthesising complex organic molecules from a subset of X, as are viruses for example. In the long-term the concentration of these metabolizing entities will be the rate-limiting factor for the non-metabolizing entities. So metabolism should be considered a characteristic of living systems because understanding metabolism is crucial for explaining how living systems work as dissipative structures [120].

It cannot be stressed too strongly that without exception, all known cellular life possesses an autocatalytic metabolism, even if the cells are heterotrophic. Thus for the autocatalytic nature of the whole metabolic network it is not necessary to be able to identify a smaller autocatalytic core as the reductive citric acid cycle or the Calvin cycle. Imagine the following thought experiment. Take away all metabolites from a cell but leave all the water and the informational macromolecules in place. Can the network be recreated from the food materials only, or not? Let us be generous and provide enough ATP also for the supposed kick-start. The fact is that no contemporary cell could resume its activity in this experiment. Consequently, all cells today possess a *distributive* autocatalytic

network then *cannot be seeded from outside*, because some of its seed components cannot be taken up from medium.

It is easy to see that such a system has certain advantages under bad times, when food is depleted. Because some key components of the network cannot permeate the membrane, they cannot leak out either, thus a package of metabolites will be preserved in the cell interior (until it is degraded by side reactions). In contrast, a cell whose metabolic network could be re-created entirely from outside could also lose all its metabolites under harsh conditions since they would simply be lost by reactions running in the reverse. We suggest the term *endogenous autocatalysis* to describe contemporary metabolic networks.

Living systems exist in a biosphere that to a first approximation is a closed thermodynamic system (i.e., conserves matter but not energy, like a greenhouse). To explain life's persistence, we must explain how a finite set of chemicals can be recycled effectively and indefinitely by a biosphere. As a prerequisite for the persistence of life, we require entities that are capable of obtaining energy from outside the system in order to re-cycle the chemical system (autotrophs or non-living 'autotrophic' metabolic systems). Entities capable of recycling a subset (X1) of chemicals using only energy from another subset (X2) of chemicals will not be able to do so for very long, since X2 will run out. Heterotrophs are an example of such entities. If they preceded autotrophs historically, then we can conclude that they could only persist if either autotrophs or non-living 'autotrophic' metabolic systems could evolve before X2 was used up, killing all the heterotrophs. Let us consider the requirements for the evolution of non-living metabolic entities of both "heterotrophic" and "autotrophic" types.

4.2 Evolution of Autocatalytic Cycles

Imagine an experiment that simulates early earth conditions [121, 122]. Construct multiple “micro-environments” each with different characteristics. These could use different abiotic energy sources, for example. Some might use UV light (oscillating as night and day) or redox potentials (e.g. FeS_2 / FeS surfaces). They could also have different temperatures, salinity, pH, local chemical concentrations or other attributes. Together, these disparities will make the equilibrium positions and chemical reaction rates vary between these micro-environments. The chemical network is massively reconfigurable and non-linear. Spatial factors can establish chemical gradients, so some degree of specificity over and above that provided by the chemical network can be obtained. In this way, spatial properties of the chemical network enable “vectorial metabolism” [123]. In essence, we can keep the system very far from equilibrium, in many different ways, physically and chemically. Initialize the system with a subset of atoms and small molecules e.g. C, H, N, O, P, S, and leave it for some time. *Under what circumstances will the system settle down into a boring point attractor, e.g. tar, and under what circumstances will it produce life? Would the “tar” be the same boring tar, or different and still boring tar, if the “tape were re-run”?* Under what circumstances would an autocatalytic cycle arise? And for what subset of parameters would an autocatalytic cycle evolve into life? How would the “platonic space” of all possible chemical reactions be explored [122]?

King [124] modelled a recycling chemical network (i.e., where every molecule type is produced in at least one reaction, and consumed in at least one reaction) of bimolecular reactions (i.e., where two reagent molecules react to produce two product molecules) and showed that the number of “platonic” autocatalytic cycles C is given by

$$C = \sum_{i=0}^r J_i - r ,$$

where J_i is the number of reactions that the i th reactant takes part in, and r is the number of reactant types. One can demonstrate this by induction from cases with few reactants. But for the cycle to exist materially, the constituents’ rates of decay must equal their rate of creation from reagents. The rates of decay are increased by “OR-reactions” that tap the cycle [125], i.e. reactions where a constituent may undergo side-reactions. The factor limiting this creation rate is very likely to be one “limiting reagent”. Exponential growth of the autocatalytic cycle will only occur when the limiting reagent (whichever one it is at the time) is present in excess, so for the cycle to persist, this limiting reagent must be generated in sufficient quantity. Reagents may be the products of other autocatalytic processes occurring under different equilibrium conditions in other micro-environments or may be due to non-autocatalytic recycling of some components of the system by solar radiation [126].

How specific must the reactions of an autocatalytic cycle be for it to grow? Imagine a cycle with n constituents, and m other active substances in the medium (which also include the reagents). Considering all possible reactions between the

constituents and the other substances, King found that the cycle grows exponentially only if

$$\prod_{i=1}^n [1 + 1/S_i] \leq 2$$

for all n constituents, where S_i is the specificity of the i^{th} constituent, given by

$$S_i = \frac{\alpha_{ij}}{\sum_{j=1, j \neq i}^m \alpha_{ij}},$$

where $\alpha_{ij} = \beta_{ij} R_j$, β_{ij} being the rate coefficient of the reaction between the i^{th} constituent and the reactant j , and R_j being the concentration of the reactant j . In particular, the steady state of the limiting reagent R_L is given by

$$R_L = \sum_{j=1, j \neq L}^m \alpha_L / \beta_L,$$

where β_L is the rate coefficient for uptake of the limiting reagent, and α_L is the rate of decay of the autocatalytic constituents.

How probable is it that a randomly generated autocatalytic cycle of size n will persist? Assuming randomly assigned rate coefficients and concentrations, King defines a “kinetic complexity” to a cycle ($Y = n(m-1)$), where n is the number of constituents and m is the number of the active substances in the medium including reagents, and calculates the probability that a cycle of size n will persist under these conditions. King assumes an exponential distribution of specificities of reaction, with most reactions having low specificity. For an

autocatalytic system with 4 uptake reactions and in a medium containing *only* the 4 appropriate reagents the chance of the cycle persisting is only 9×10^{-10} . See the table below for other values of Y .

Y	2	4	6	12	20	30
Probability	0.26	0.019	6×10^{-4}	9×10^{-10}	2×10^{-19}	2×10^{-33}

In conclusion, *selection on rate coefficients and concentrations of reagents are needed to make an autocatalytic cycle that persists with more than a very small number of constituents*. Random search will not do for anything but the smallest cycles.

Does an autocatalytic cycle conform to Maynard Smith's [1, 2] definition of a unit of evolution? Szathmáry classified autocatalytic cycles as replicators of the "holistic" type, and predicted that their heredity would be limited to a small number of alternative forms (basins of attraction in the chemical space of constituents), which showed only infrequent macromutations [22]. To what extent can autocatalytic cycles evolve as "holistic replicators" in chemical space? Obviously as a prerequisite, the cycle intermediates must not be lost, and therefore the limiting reagent must remain above its threshold at all times. King suggests selection would be largely confined to the specificity of the reaction for uptake of the limiting reagent. This could be achieved by loss of those materials that disrupted the recycling of the limiting reagent, or by exclusion of the m other species from the medium for which physical separation would be helpful. All else being equal, simpler autocatalytic cycles are easier to maintain. Separate

autocatalytic cycles can compete for the same reactant, with competition in the growth phase being dependent on rate of limiting reactant usage, and competition in the decay phase being dependent on the comparative decay rates [127]. Since growth is exponential, there is “survival of the fittest” during the growth phase, and co-existence is not possible, assuming a well-mixed reactor. During the decay phase there is exponential decay, with selection for autocatalytic particles with low decay rates. In a well-mixed reactor, co-existence can only occur if autocatalytic cycles are not competing for the same limiting reagent [127]. Co-operative interactions between autocatalytic cycles occur when their reactions are consecutive (i.e., the product of one is the reactant of the other) or where the constituent of one autocatalyst is the reagent for another autocatalyst. Such a coupling has been hypothesised by Kalapos, in which the formose cycle could have been anaplerotic of pyruvate (it supplied the limiting reagent) to the reductive citric acid cycle, therefore explaining the ubiquitous presence of the (methyl) glyoxalase pathway in living systems [128]. King has claimed that evolution from the first autocatalytic cycles to prokaryotes was due to a relatively small number of symbioses between cooperative autocatalytic cycles. Some sort of physical coupling between cycle constituents to form a combined “particle” would have been necessary in order for symbiosis to occur. King demonstrates that symbiosis would have been selectively advantageous when the limiting reagents of the original cycles were running low [126]. The chemoton is just such an example of three coupled autocatalytic cycles.

One crucial feature of the evolution of autocatalytic cycles is the bioenergetic constraint on the existence of continued recycling [122]. Evolution of

metabolism seems to be rate-determined by its discovery of new “prime movers”, just as cultural evolution seems to be rate-determined by the discovery of fire, water wheels, coal and nuclear power. An explanation of self-sustaining ecosystems of autocatalytic cycles must explain how novel energy sources were utilized for “complexification” of the metabolic network, using for example, electrical discharges, redox potentials, UV light, concentration by drying in intertidal zones, mineral surface films, or gradients across vesicles. Returning to the giant pre-biotic synthesis experiment, early attempts may have failed (i.e., have reached a point attractor of tar, or experienced the curse of combinatorial explosion [129]) because they used an ecosystem that was unable to exploit these novel energy sources: fatty acids aggregated and underwent the browning reaction, the mixture became disordered, pyrophosphates degraded, redox potentials were not utilizable, light energy could not be utilized by the constituents of the medium. So effectively *the ecosystem was not recycling*, it was not *collectively autotrophic* [130]. How can a sufficiently complex chemical network be recycling? Work has to be done on the network so that a constant flux of limiting reagents is available for autocatalytic cycles.

4.3

The Formose Cycle and the Reductive Citric Acid Cycle

Two candidates for the first pre-biological autocatalytic cycle are the formose cycle and the reductive citric acid cycle. The formose cycle was discovered by Butlerow [131] and has been investigated by others since [132,133, 134, 135, 136]. A simplified version of the cycle is shown in Fig. 12. It converts formaldehyde to a

mixture of complex molecules in alkaline (and only very slowly in neutral) solution containing a divalent metallic ion catalyst e.g. Ca^{2+} or Pb^{2+} . Two formaldehyde molecules form glycolaldehyde to enter the cycle but formaldehyde can also react by the Cannizzaro reaction in alkaline solution producing methanol and formic acid, so reducing the specificity of the critical reagent reaction. The reaction does not proceed exponentially until a limiting concentration of formaldehyde is exceeded, as expected from the models of King [127]. Glycolaldehyde is converted to glyceraldehyde which is then converted to tetrose, pentose and hexose sugars. The sugars decompose to hydroxy-acids and related compounds that are lost from the cycle, and may also interfere with the cycle. Many “mutant” related cycles exist that recycle various versions of the sugars. Each sugar can exist in D- or L- form, each enantiomer inhabiting in its own version of the cycle. Important tapping side-reactions include the Cannizzaro reduction of sugars. The existence of these side-reactions increases the limiting concentration of formaldehyde required to run the cycle. If the kinetic conditions described previously are satisfied, two molecules of glycolaldehyde are produced for every one entering the cycle. If the concentration of formaldehyde is not maintained by continuous supply, the cycle runs down, but as long as it is maintained the formose cycle can be run in a flow reactor [132]. Leslie Orgel [136] describes the side-reactions of the formose reaction as “notorious”. It is thought that formaldehyde occurred through atmospheric oxidation of methane [132]. How could this have occurred at high rates? How is it possible to reduce the side-reactions of the formose cycle, such that its constituents can persist at low formaldehyde concentration?

Wächtershäuser [11] argued that the reductive citric acid cycle (reductive tricarboxylic acid cycle, rTCA) was the first autocatalytic cycle that evolved using the reducing power of pyrite surfaces. Smith and Morowitz [130] also argued that the rTCA cycle was a likely pre-biotic autocatalyst that could have worked without enzymes in the reducing atmosphere of the early earth. They propose it could have been a part of the “relaxation pathway” for the free energy “bottleneck” of redox couples created from volcanic magma. There are multiple possible synthetic pathways for sugars, lipids and amino acids, starting from the rTCA cycle, so possibly increasing their probability of evolution. Constituent reactions are first order so increasing turning rate, and acetate could be converted to lipids allowing vesicles to form [130]. These properties also apply to the formose cycle. They relate the energetic features of the rTCA cycle to its evolvability, «harmful side reactions that cannot be eliminated cost additional metabolic energy to handle. Thus, a metabolic core with high intrinsic efficiency and statistically favored reactions would in general leave more free-energy for the synthesis of higher-level regulatory structures than less intrinsically efficient alternatives.»

4.4 Encapsulation of Autocatalytic Cycles

«Physical structure is an essential aspect of even the simplest autocatalysts in solution, ensuring their stability against side-reactions and enhancing their turnover rate. The kinetic characteristics of autocatalysis are such that, under some conditions, there may be feedback that alters the physical structure, building complexity [127].» For example, fire is autocatalytic and it spreads as a “front”.

Chemical waves are fronts of progressive energy dissipation. Bacterial growth is also autocatalytic, and spreads as a front. Spatial factors can have complex and non-linear effects on selection. These effects have been explored in models of co-evolution between metabolism and template replicators *in silico* [13] and for contemporary self-encoding macromolecular systems *in vitro* [51], but not for autocatalytic cycles, nor for autocatalytic cycles coupled with protocell growth under realistic conditions, e.g. side-reactions, and reagent re-cycling.

Encapsulation of metabolism in protocells could allow high metabolite concentrations to be maintained, especially those concentrations of large and charged molecules. It could allow increased specificity of reactions (by excluding poisons that can pass through the membrane), and selection of the autocatalytic cycle as a spatially discrete unit as well as a unit in chemical space. Limited membrane heredity could have operated with peptides and amphiphiles. On the road to vesicle encapsulation, intermediate steps might have been: encapsulation in less well-organized structures such as coacervates or diffusion limited on mineral surfaces. Ingeniously, Wächtershäuser's "bubble wrap" (abstriction) scenario involves the production of membrane caps by surface lipid metabolism of rTCA chemo-autotrophs, allowing the subsequent evolution of cytosol metabolism desorbed from the surface [11]. He proposes these could eventually produce vesicles. Wächtershäuser has argued that a heterotrophic origin in a prebiotic broth is implausible because cleavage reactions are favoured by increasing entropy; whereas on surfaces the increase in entropy caused by cleavage is small, so surface metabolism would be inherently synthetic.

The problem with both the formose cycle and the rTCA cycle is that we do not know how either could evolve to produce their own membrane constituents. Gánti had to hypothesise a separate reaction sequence from the formose cycle for synthesising the amphiphiles. Vesicles may be produced under primordial conditions, for example montmorillonite clay catalyses the formation of micelles which form into vesicles and trap some of the clay inside them [137] However, demonstration of self-replicating vesicles capable of exponential growth has been limited to caprylic acid vesicles that catalyse the hydrolysis of ethyl caprylate into caprylic acid which then spontaneously incorporates into the vesicle causing growth [42]. Caprylic acid has no plausible pre-biological synthesis. It has not yet been demonstrated that an autocatalytic cycle can continuously produce amphiphiles that self-organise to enclose the metabolites at stoichiometric ratios that coordinate the exponential growth of the vesicle with that of the metabolism. What is the minimum metabolism, and the minimum synthetic procedure required to allow metabolism to enclose itself in a protocell that replicates exponentially and can evolve?

We can safely assume the existence of whole 'families' of vesicles without autocatalytic metabolisms that were not exponentially growing. Some of these vesicles may even have had H^+ gradients providing a source of energy for metabolism [17]. Some of these vesicles may have learnt themselves to colonization by autocatalytic metabolisms which had evolved to a significant degree already independently of the vesicles, e.g. on surfaces. However in this case there would be the problem of impermeability. How would the highly evolved autocatalytic metabolism get into the vesicle? Although transient chemical transfer

groups could solve this problem it seems unlikely these could occur all at once, and if they occurred incrementally, the autocatalytic cycle would presumably be compromised. Alternatively, it may be the case that evolution of autocatalytic metabolism may have been so difficult outside vesicles that even the earliest most inefficient autocatalytic metabolism required vesicles to evolve in; either vesicles produced by their own amphiphiles, or more likely, vesicles produced by other chemical systems. This may have been particularly beneficial if these vesicles possessed a method of utilizing energy. Presumably a large amount of permeability variation would exist for selection to act upon. Heterogeneous membranes are pre-adapted for stability. At an early stage, perhaps walls of amphiphiles could separate compartments of autocatalytic cycles on a 2D surface, or vesicles could clump together in the presence of ions.

One experimental approach would be to generate heterogeneous vesicles in broths containing the constituents of the formose reaction, initially limiting the concentration of formaldehyde. After leaving the system for a while, formaldehyde would be added to the solution. Those vesicles that grew and divided fastest would be selected for. There may be rare conditions under which the constituents of the formose reaction are permeable to the membrane. They enter the vesicle and become trapped as soon as those conditions change. Formaldehyde could then enter the vesicles and allow the autocatalytic cycle to run.

Deamer [121] and others have advocated peptide evolution in early protocells capable of energy-transduction. He suggests the first membranes may have been made of monocarboxylic acids and alcohol. However, peptide evolution in protocells lacks any plausible mechanism for heredity of sequence. New

sequences (e.g. coding for ligases or proteases) would have to be rediscovered in each lifetime.

4.5 Post-Enzymatic Evolution of Metabolism

Let us assume for now that an exponentially growing protocell with an enclosed autocatalytic metabolism could form and eventually evolve RNA enzymes. RNA enzymes would have co-evolved with the original metabolic pathways. After the evolution of protein enzymes, further takeover and transformation of pathways would have occurred. Pohorille and New [138] observed «since there is no relationship between the RNA catalytic power of a given RNA and the protein for which that RNA can code, there is no clear path from the RNA world to the protein world.» Therefore, protein cladistics can only make conclusions about metabolism after protein enzymes have evolved [139]. To infer the state of living systems before protein enzymes, further theoretical assumptions are required.

The chemoton model suggests that a general motif of metabolism evolution was the stoichiometric coupling of reaction cycles and chains, shaped first by the underlying chemical bioenergetics, but shaped later by enzymes (leading to so-called catalytic supercycles [125]). The advantage of translated protein enzymes would have been the decoupling of the difficult process of their own self-replication from cross-catalysis. Vast catalytic spaces would become easily searchable by protein enzymes, after the underlying nucleic acid replication problem had been solved for all possible sequences. Unlimited heredity and microevolution of metabolism would be possible. Enzymes would only have been able to catalyse

reactions for which at least one molecule of reactant existed. Only then could the enzyme have an effect. Therefore, *evolution of metabolism can be seen as a genetic assimilation of underlying metabolic pathways*. It is possible that non-functional enzymes (i.e. enzymes in cells without any suitable reactants) could exist, and ‘cultivate’ the platonic space yet unexplored by the cells’ metabolism.

Wächtershäuser [11] has produced a classification of elementary variational motifs describing evolution at the level of biochemical phenotypes (Scheme 1). Presumably because Wächtershäuser was interested in autotrophs, he did not consider the fundamental pathway operation of retro-evolution, discovered by Horowitz [140], which we include here as an additional fundamental operation **i)**. Horowitz assumed that D, a complex organic molecule was present in the soup that heterotrophs first used, but that it ran out. Heterotrophs then evolved to use D’s precursor C to synthesise D, and so on for B and A. Horowitz’ mechanism is important if the end product D is indeed available at an early stage, and if C, B and A are available in excess in the environment also. This is only likely where autotrophs produce these components, in which case retro-evolution may be an important mechanism where a heterotroph co-evolves closely with an autotroph.

i) Retro- Extension: **B → C ⇒ A → B → C**

It is assumed that loss of pathway components is also a fundamental operation **j)** that may occur due to stochastic loss during replication for example.

j) Loss of Pathways: **A → B → C → D ⇒ A → B--||**

Using various combinations of these primitives, hypotheses can be formed regarding the evolutionary trajectory that led to extant metabolic systems. Because these primitives allow us to trace multiple pathways to extant metabolism, other assumptions must be made to limit the space of possible evolutionary trajectories. Melendez-Hevia et al. [141] suggest some additional assumptions of evolutionary “opportunism”.

- i. Any intermediate evolutionary metabolic pathway should be possible, albeit running slowly, without enzymes.
- ii. Intermediates should be stable to rapid decomposition, otherwise the pathway could not exist before enzymes or when only the earliest enzymes were present.
- iii. The existence of material inputs to the new pathway must be due to another metabolic process that had previously been selected for.
- iv. The new pathway cannot involve a reaction that is thermodynamically or kinetically incompatible with any other pathway in the same space simultaneously.

Using these constraints and the principle c) of pathway recruitment (Scheme 1), they argued that the Krebs cycle evolved opportunistically, originally as a biosynthetic pathway allowing synthesis of amino acids from pyruvate, and that this required the evolution of only one enzyme to catalyse the conversion of succinyl-CoA to succinate. This solution rests on the assumption that amino acid

biosynthesis preceded the Krebs cycle, for which there is evidence [139]. They also suggested several alternative mechanisms for catabolizing acetate groups (the current role of the Krebs cycle) and showed that these would have been more difficult to evolve or less efficient, given the principles of “opportunism”.

The question whether there is a historical trace of the retroevolution of pathways [140] or another alternative, the so-called patchwork mechanism [142] has been asked repeatedly. The first evolution scenario predicts that enzymes catalysing steps of the same metabolic pathway should be phylogenetically related (homologous). The latter scenario states that enzymes evolve by increasing their specificity: first enzyme E catalyses reactions of chemically similar substrates S1 and S2, then its genes duplicates and the two enzymes diverge, E1 and E2 evolving specificity towards S1 and S2, respectively. Thus homologous enzymes should be functionally similar, but they may or may not catalyze neighbouring reactions in metabolic pathways. Skipping the whole history of more and more refined analyses, we just mention the latest result. Light and Kraulis [143] found, analysing the complete available dataset from *E. coli*, that homologous enzyme pairs abound at the minimal path length of one (i.e. the product of one is the substrate of the other, or vice versa). This may corroborate the retroevolution scenario. Not so, for two reasons. First, there is a small degree of homology between enzyme pairs with a mean path length of 2, and negligible homology between pair of mean path length of 3 or greater. Second, the majority of homologous pairs with mean path length of 1 have similar EC numbers, hence they are functionally related. Therefore the most recent analysis seems to support the patchwork model of enzyme evolution.

Note that analysis of contemporary protein enzymes do not necessarily shed light on the primordial build-up of metabolic networks, if modern metabolism is a palimpsest of the RNA world [144]. If there was a metabolically rich RNA world, then a large part of the network must have been built up before the advent of encoded proteins. By the time of the origin of translation primary heterotrophy (if there was one), feeding on the prebiotic environment, must have been over. Hence the fact that we find no strong evidence for retroevolution in contemporary enzymatic metabolism may say nothing about its original significance in the RNA world.

Let us point on a perhaps even more severe objection. Historical retroevolution is fully compatible with the patchwork type of enzyme recruitment, provided at least two pathways are present and evolving in parallel. The higher the number of pathways, the more “patchworky” the recruitment can be, even though each individual pathway is retroevolving.

It is also important to realize that the Horowitz scenario makes sense only if, although intermediates of contemporary pathways have been present without enzymatic aid in the milieu of protocells, they were synthesized elsewhere. In contrast, if they could be chemically synthesized in the same milieu, then autotrophy would have been easy simply by letting the reactions run *inside* protocells. It is no miracle that in Wächtersäuser’s [10, 11] scenario, where everything is formed *in situ* on the pyrite surface, autotrophy is given for free.

Following the considerable recent interest in scale-free networks, Jeong et al. [145] have shown that the metabolic networks of extant living systems are “scale-free networks” sharing the same metabolite “hubs” over evolutionary time. Wagner

and Fell [146] suggest that there are three reasons why metabolic networks may be scale free.

- i. Metabolism may be scale-free because of chemical constraints of the underlying chemical network. They dismiss this possibility, claiming as evidence the fact that in different organisms the metabolic network takes many different forms. However this does not rule out the existence of more general chemical constraints that may produce the scale-free network. For example, it is generally the case that small molecules have more possible synthesis routes than large molecules and so we expect connectivity to scale as a function of size.
- ii. Metabolism may be scale free because during evolution, metabolites with more connections are more likely to make further connections, implying that older metabolites are those with the largest number of connections. This is based on Barabási's algorithm for producing scale-free networks [147]. Morowitz uses the assumption that older metabolites should be more highly connected to support the idea of an early amino acid metabolism. The metabolites with highest connectivity are glutamate, pyruvate, and coenzyme A. However, this simple algorithm for constructing scale-free networks is one of an infinite number of methods, many of which involve pruning as well as addition of connections or metabolites, e.g. it is likely that pathway recruitment c) (Scheme 1) would tend to produce networks with "scale-free" properties. This would mean that it is not possible to conclusively infer phylogenetic age from connectivity.

iii. Metabolism may be scale-free as a result of selection for functional properties, i.e. the rapid propagation of perturbations resulting in developmental robustness and evolvability. Jeong et al. [145] support this adaptive interpretation. They showed that the diameter of the network, i.e. the average path distance from one metabolite to another, did not increase with the number of metabolites in a given organism. They claimed that scale-free metabolic networks should be more evolvable than random metabolic networks because deleting nodes randomly (i.e., randomly removing metabolites) would not have a great effect on network diameter, and that networks with larger diameters would reduce the organisms' ability to respond effectively to external changes or internal errors because «offsetting these changes would involve a longer alternative biochemical pathway and consequently the synthesis of more new enzymes than within a metabolic network with a smaller diameter.» However, there is no evidence relating network diameter and rate of reaction to perturbations, to evolvability. Bioinformatic evidence is against this explanation. Metabolic network analysis of yeast has provided fascinating results [148]. First, the majority of genes that looked dispensable turn out to be such only under laboratory conditions. Second, gene duplicates catalysing the same reaction are not more common for indispensable reactions, suggesting that the reason for their retention is not to provide compensation; instead, their presence is better explained by selection for high enzymatic flux. Third, only 4-17% of *in silico* deleted, dispensable gene products are buffered by metabolic network flux reorganization. In fact a different, more chemically minded adaptive

reasoning may turn out to be more fruitful. The main hubs in metabolism are molecules like water and the coenzymes. Coenzymes carry important functional groups, thus connecting different pathways in the network. It seems rational to argue that this is a better adaptive design than running a network without them. Coenzymes (like ATP) play the same role in metabolism as money does in economy.

More work is required to understand the adaptive significance of the small-world character in experiments in which the above explanations can be independently controlled in an evolutionary model of dynamic autocatalytic metabolism.

5 Outlook

We have witnessed spectacular development revolving around the minimal life/protocell idea in the last few years, although the roots of this vision go back to more than thirty years ago. A fairly unusual interplay between theory and experiment seems to unfold: theoretical models investigate possible dynamics, and experiments either confirm them or come up with surprising, unexpected novelties, but increasingly often reflecting back on theoretical considerations.

Most experimental studies attempt at the synthesis of infrabiological systems, i.e. all three subsystems (metabolism, template polycondensation, and membrane) of the chemoton are not yet figuring in those trials: practically everybody is now concentrating on membrane/template systems. Metabolism seems to be an especially hard problem, since we do not know where a sufficient

amount of channelling could have come from without enzymes. We strongly urge the initiation of another experimental line, aiming at a metabolism/membrane infrabiological system, preferably using the formose reaction for metabolism.

One cannot deny the fact that the origin of unlimited heredity is an unsolved problem. Perhaps compartmentation will help solve this problem as well, so that long templates could self-replicate within vesicles without enzymatic aid.

We emphasize that top-down approaches to a “minimal genome” do not solve our problem: the spontaneous generation of cells with several hundred genes can be safely ruled out. We must adopt a bottom-up strategy instead: this is exactly the attempted synthesis of various infrabiological systems. The ultimate goal is, of course, to arrive at a chemical supersystem which at the same time is a *bona fide* biological system, conforming to Gánti’s chemoton model.

Another top-down approach, namely phylogenetic analysis of contemporary protein enzymes is also of limited help. Very early primordial systems must have been enzyme-free, and later early systems could have been catalysed by ribozymes. Consequently, a substantial part of basic metabolism could have originated in an era about which there simply cannot be memories in protein coding genes.

Synthesis of a living chemical system may not shed too much light on the historical process of the origination of life, but we are optimistic that work in progress will contribute to the solution of one of the outstanding unsolved problems of science.

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Scheme 1. Classification of elementary variational motifs describing evolution at the level of biochemical phenotypes. ' \rightarrow ' indicates enzyme catalysed chemical reactions, and ' \Rightarrow ' indicates evolutionary progression. The fundamental operations are shown in bold.

- a) **Terminal Extension:** $\rightarrow \mathbf{A} \rightarrow \mathbf{B} \Rightarrow \rightarrow \mathbf{A} \rightarrow \mathbf{B} \rightarrow \mathbf{C}$
- b) **Lateral Branching:** $\rightarrow \mathbf{A} \rightarrow \mathbf{B} \rightarrow \mathbf{C} \Rightarrow \rightarrow \mathbf{A} \rightarrow \mathbf{B} \rightarrow \mathbf{C}; \mathbf{B} \rightarrow \mathbf{D}$
- c) Pathway Recruitment: $\rightarrow \mathbf{A} \rightarrow \mathbf{B} \rightarrow \mathbf{C} \rightarrow \mathbf{D} \Rightarrow \mathbf{A} \rightarrow \mathbf{B} \rightarrow \mathbf{C} \rightarrow \mathbf{D}; \mathbf{A} \rightarrow \mathbf{X} \Rightarrow \mathbf{A} \rightarrow \mathbf{B} \rightarrow \mathbf{C} \rightarrow \mathbf{D}; \mathbf{A} \rightarrow \mathbf{X}; \mathbf{X} \rightarrow \mathbf{B}' \rightarrow \mathbf{C}' \rightarrow \mathbf{D}'$
- d) Pathway Abandonment: $\mathbf{A} \rightarrow \mathbf{B} \rightarrow \mathbf{C} \Rightarrow \mathbf{A} \rightarrow \mathbf{B} \rightarrow \mathbf{C}; \mathbf{A}' \rightarrow \mathbf{B}' \rightarrow \mathbf{C}' \Rightarrow \mathbf{A}' \rightarrow \mathbf{B}' \rightarrow \mathbf{C}'$
- e) Pathway Takeover: $\mathbf{A} \rightarrow \mathbf{B} \Rightarrow \mathbf{A} \rightarrow \mathbf{B}; \mathbf{C} \rightarrow \mathbf{B} \Rightarrow \mathbf{C} \rightarrow \mathbf{B}$
- f) Pathway Insertion: $\mathbf{A} \rightarrow \mathbf{B} \rightarrow \mathbf{C} \Rightarrow \mathbf{A} \rightarrow \mathbf{B} \rightarrow \mathbf{C}; \mathbf{D} \rightarrow \mathbf{E} \rightarrow \mathbf{F} \rightarrow \mathbf{C}; \Rightarrow \mathbf{A} \rightarrow \mathbf{B} \rightarrow \mathbf{C}; \mathbf{A} \rightarrow \mathbf{D} \rightarrow \mathbf{E} \rightarrow \mathbf{F} \rightarrow \mathbf{G};$
 $\Rightarrow \mathbf{A} \rightarrow \mathbf{D} \rightarrow \mathbf{E} \rightarrow \mathbf{F} \rightarrow \mathbf{C}$
- g) Retrograde Mimicry: $\mathbf{A} \rightarrow \mathbf{B} \rightarrow \mathbf{C} \Rightarrow \mathbf{A} \rightarrow \mathbf{B} \rightarrow \mathbf{C}; \mathbf{E} \rightarrow \mathbf{D} \rightarrow \mathbf{C}; \Rightarrow \mathbf{E} \rightarrow \mathbf{D} \rightarrow \mathbf{C} \Rightarrow \mathbf{E} \rightarrow \mathbf{D} \rightarrow \mathbf{C}; \mathbf{F} \rightarrow \mathbf{D} \Rightarrow \mathbf{F} \rightarrow \mathbf{D} \rightarrow \mathbf{C}$
- h) Pathway Reversal: $\mathbf{A} \rightarrow \mathbf{B} \rightarrow \mathbf{C} \Rightarrow \mathbf{C} \rightarrow \mathbf{B} \rightarrow \mathbf{A} .$

FIGURE LEGENDS

Fig. 1. Gánti's abstract chemoton model for minimal life. A_i are intermediates of the metabolic cycle, pV_n is a template molecule consisting of n pieces of monomer V , V' is the activated monomer, T' is the precursor to the membranogenic molecule T . T_m denotes a membrane consisting of m pieces of T . The system works at the expense of the difference between X and Y . Note that all three subsystems are autocatalytic.

Fig. 2. Elementary combinatorics of infrabiological systems. The chemoton is a biological minimal system comprising three qualitatively different subsystems (metabolism, membrane, and template).

Fig. 3. The GARD model. Catalyzed micelle growth and fission. L_i and L_j molecules are different amphiphilic compounds, k_i and k_{-i} are rate constants for spontaneous insertion and emigration of amphiphile L_i , and β_{ij} is the rate enhancement of getting in and out of this molecule from the micelle, catalyzed by L_j . Note that the model does not deal with the primary origin of L_i molecules per se.

Fig. 4. Visualises example of a β_{ij} matrix generated from a distribution based on receptor/ligand interaction. Darker blocks indicate higher numerical values. Note that typically off-diagonal elements are not dominant in this model. (From [23].)

Fig. 5. Enhancement of amino acid transport into vesicles by reversible chemical transformations. Amino acid reacts with aldehyde to form a more permeant molecule.

Inside the vesicle the reaction runs in reverse and the amino acid can be incorporated into other molecules, such as peptides.

Fig. 6. Another mechanism of amino acid transport rests on the idea of the reversible formation of the neutral form, which passes through the membrane much faster.

Fig. 7. Membrane growth (A) can create a pH gradient. At the pK value of the fatty acid head half of the incoming fatty acid molecules will be protonated (B), hence neutralized. Neutralized molecules flip more readily (B). Approximately half of the flipped molecule will be deprotonated (C). A pH gradient (D) builds up provided the membrane is rather impenetrable to other cations. (From [62].)

Fig. 8. The hypercycle (a) and its parasite (b). Each member I_i is autocatalytic for its own growth and heterocatalytic for the replication of the next member. The parasite P shown accepts the catalytic help from I_2 but does not give anything back. If the arrow leading to P is stronger than that leading to I_3 , the system is doomed to extinction in a spatially homogeneous dynamical system.

Fig. 9. One of the vesicle models (as depicted by the SCM). Different templates (labelled by open and closed circles) contribute to the well being of the compartments (protocells) in that they catalyse steps of metabolism, for example. During protocell growth ($\cdots >$) templates replicate at differential expected rates, but stochastically. Upon division (\rightarrow) there is chance assortment of templates into offspring compartments. Stochastic

replication and reassortment generate variation among protocells, on which natural selection at the compartment level can act and oppose to (correct) internal deterioration due to within-cell competition.

Fig. 10. Micrograph of the ciliate *Paramecium*. Macronuclei of ciliates are more or less fragmented genomes (bags of chromosomes or genes) (Source of the image: <http://www.bio.umass.edu/biology/conn.river/parameci.html>.)

Fig. 11. Sex between protocells. The model assumes that protocells host essential replicators (genes) with metabolic function that are replicated by non-specific replicases (R). According to the replication rate constants the replicase can be “selfish” (R1; i.e. reaps the benefits of a common metabolism and swiftly outgrows the metabolic genes), “cooperative” (R2; i.e. all genes inside a vesicle grow at nearly similar rates) or “altruistic” (R3; i.e. helps the metabolic genes but at the expense of deviating the whole compartment from the optimal gene composition). The figure plots some sample simulations showing the average number of different replicases per protocell according to the proportion of cells that undergo random cellular fusion (sex). R3 is quickly lost, but the frequencies of both R1 (grey lines) and R2 (black lines) oscillate according to the “amount of sex” (proportion of cell fusions/generation). The important point here is that a protocell population could resist invasion of rapid exploitation by a potentially lethal (at the compartment level) parasite (R1 in this case). (After [84].)

Fig. 12. The formose reaction contains an autocatalytic core, in which the amount of cycle intermediates (such as glycolaldehyde) grows autocatalytically.

Fig.1. Szathmáry et al.

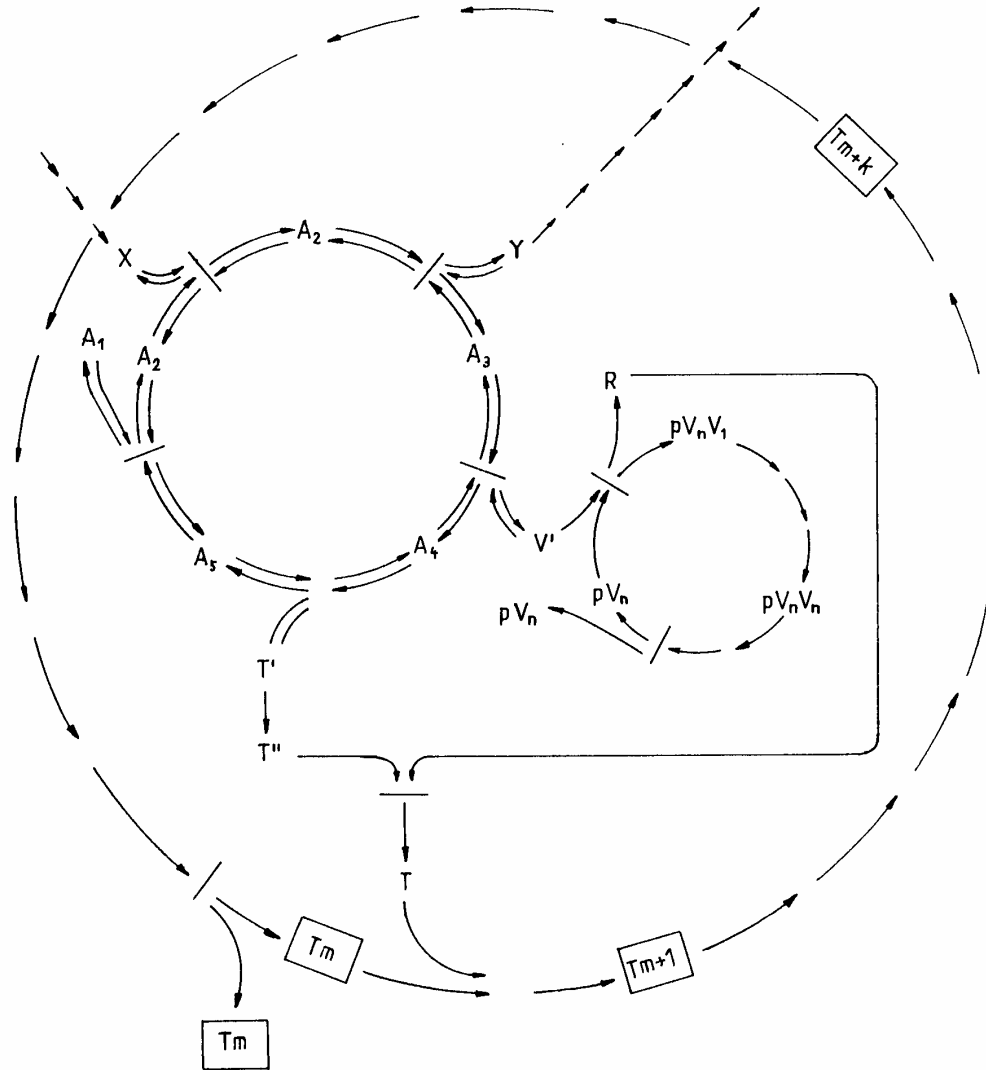


Fig. 2. Szathmáry et al.

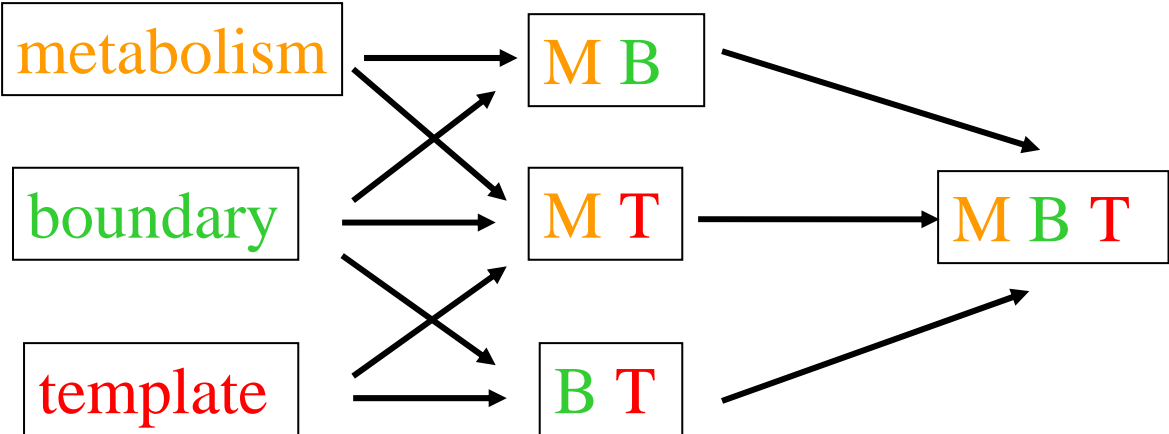


Fig.3. Szathmáry et al.

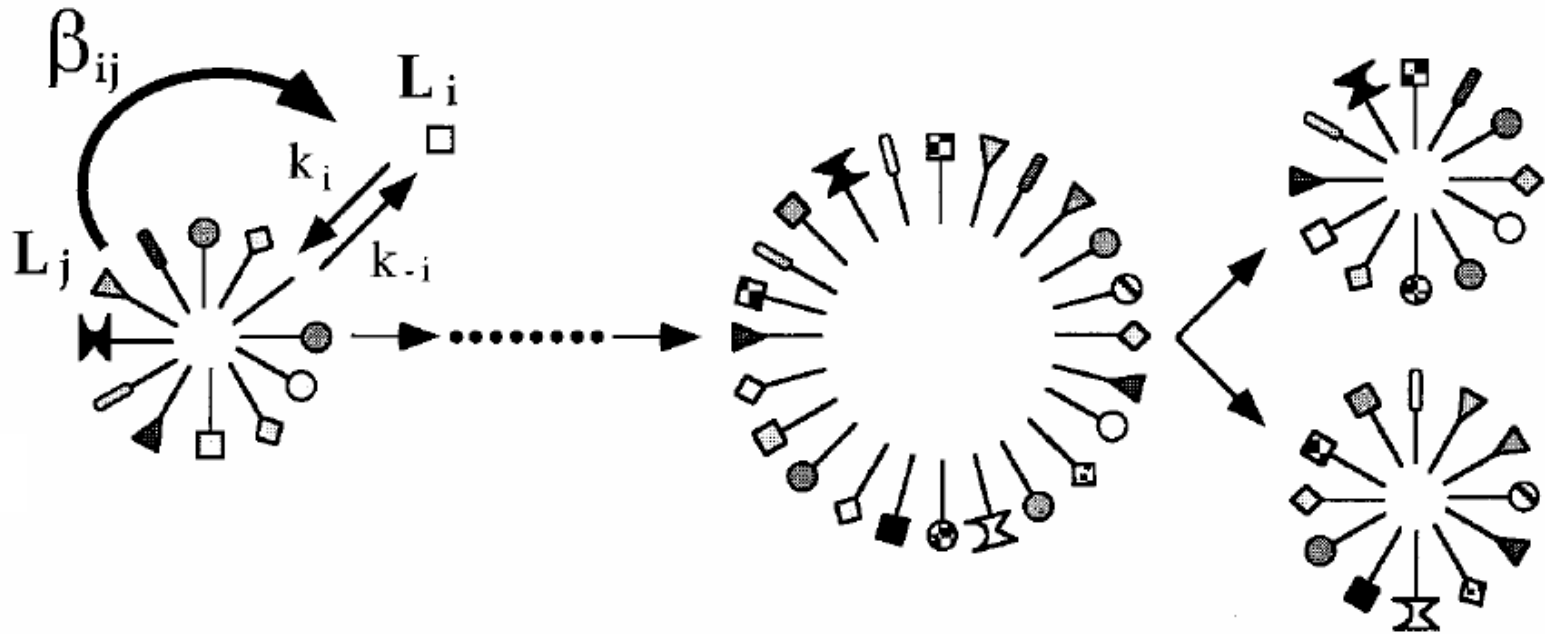


Fig.4. Szathmáry et al.

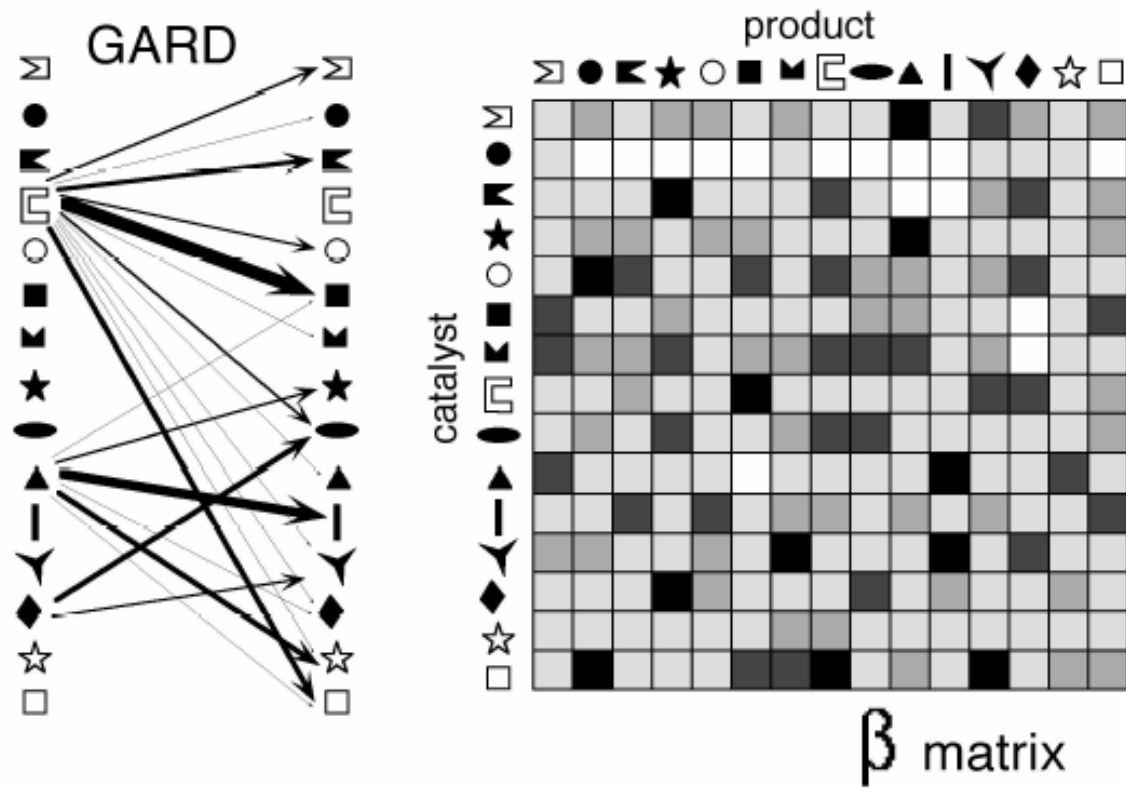


Fig.5. Szathmáry et al.

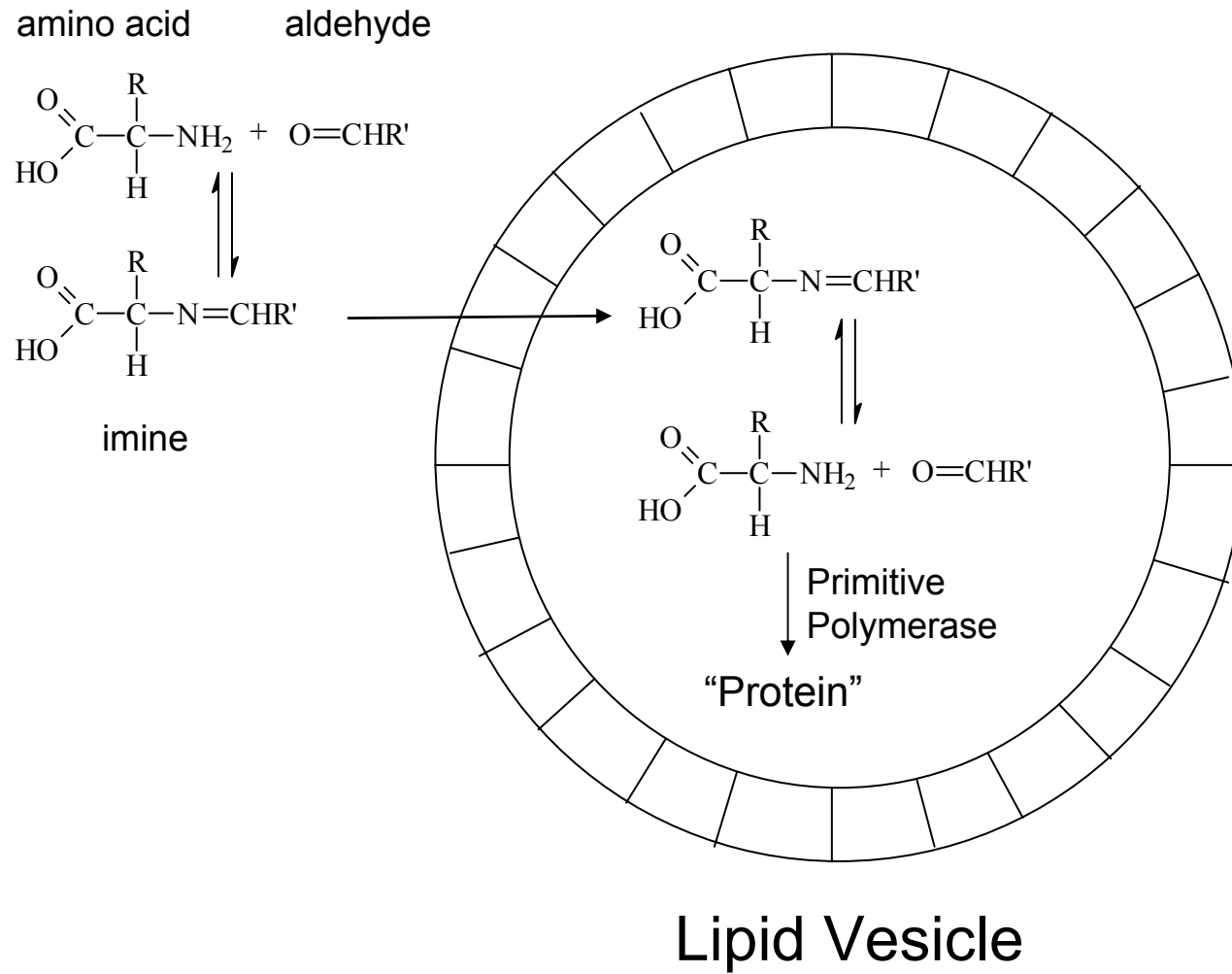


Fig.6. szathmáry et al.

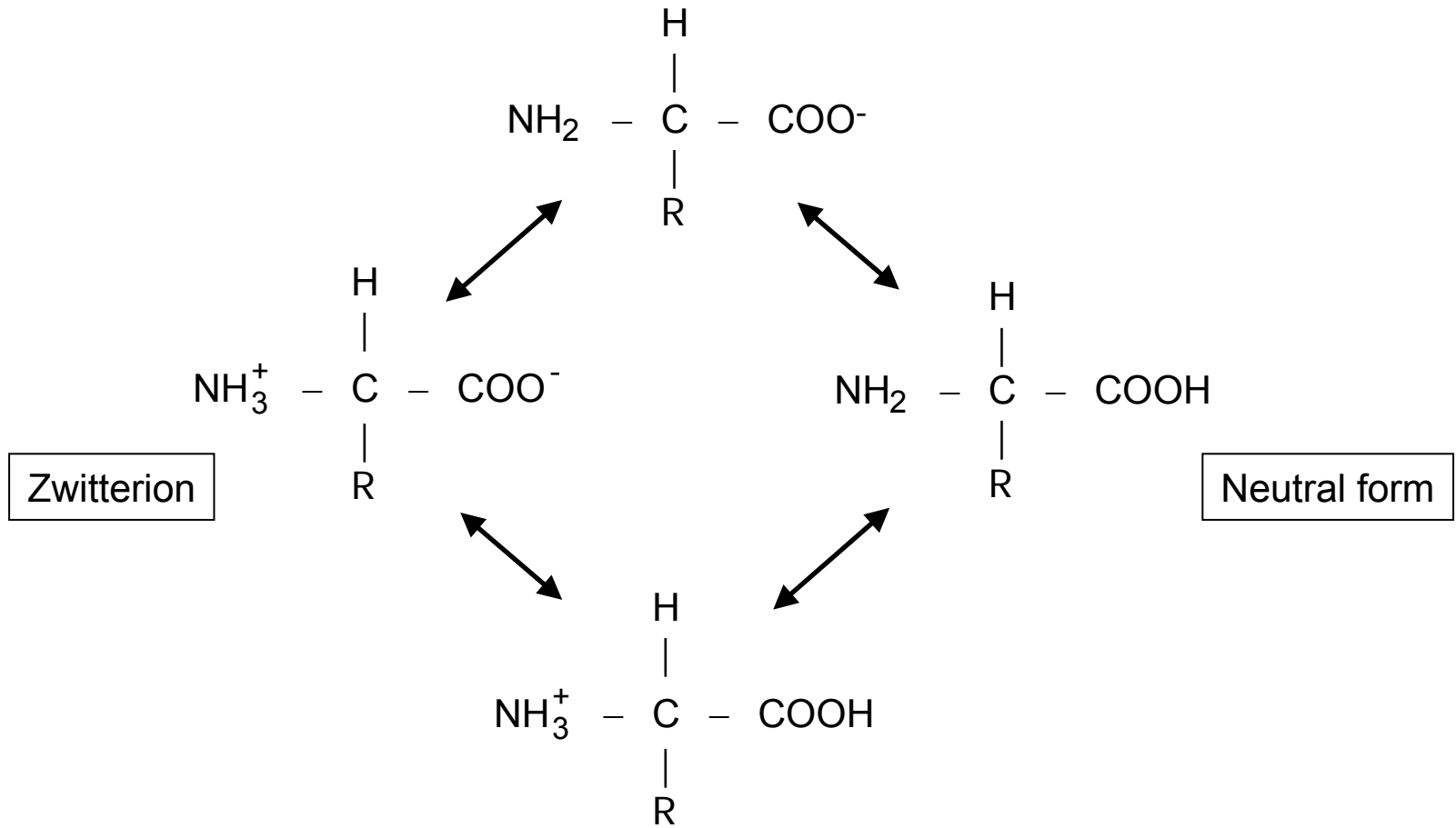


Fig.7. szathmáry et al.

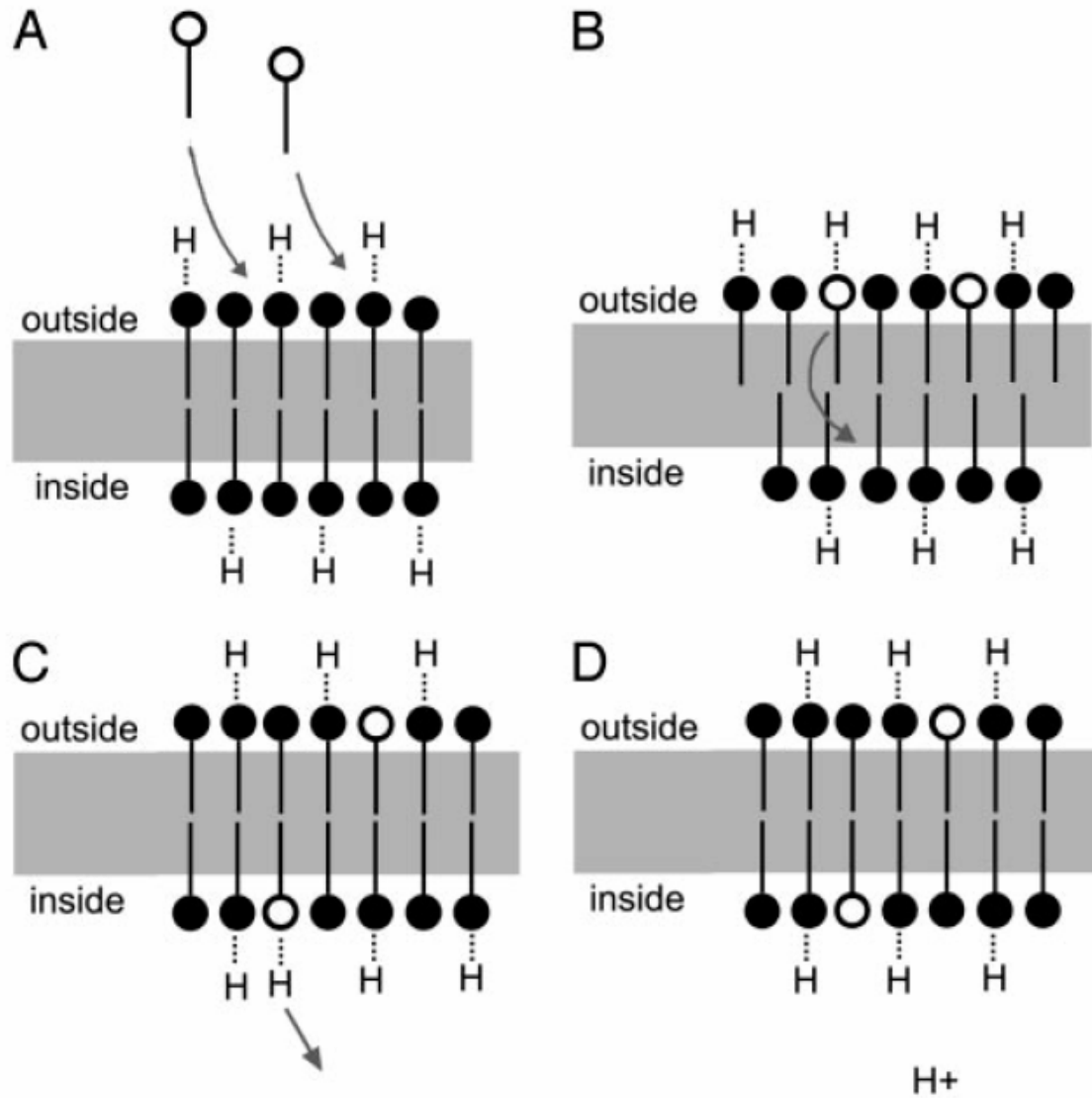
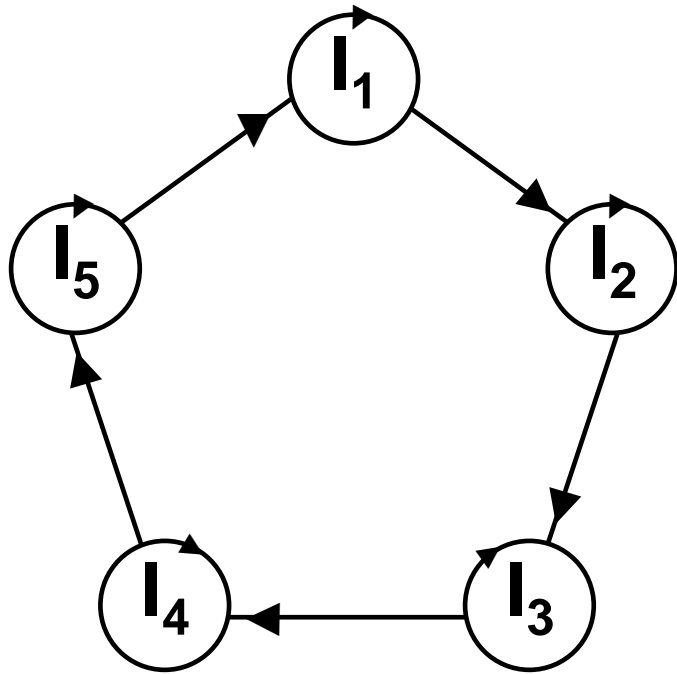
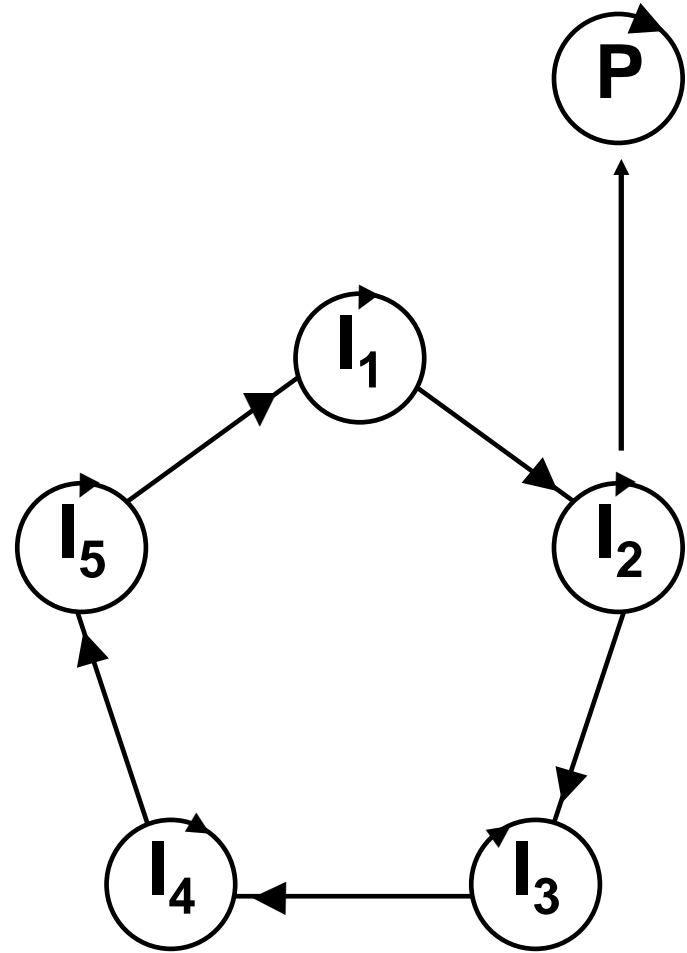


Fig.8. szathmáry et al.



a



b

Fig.9. szathmáry et al.

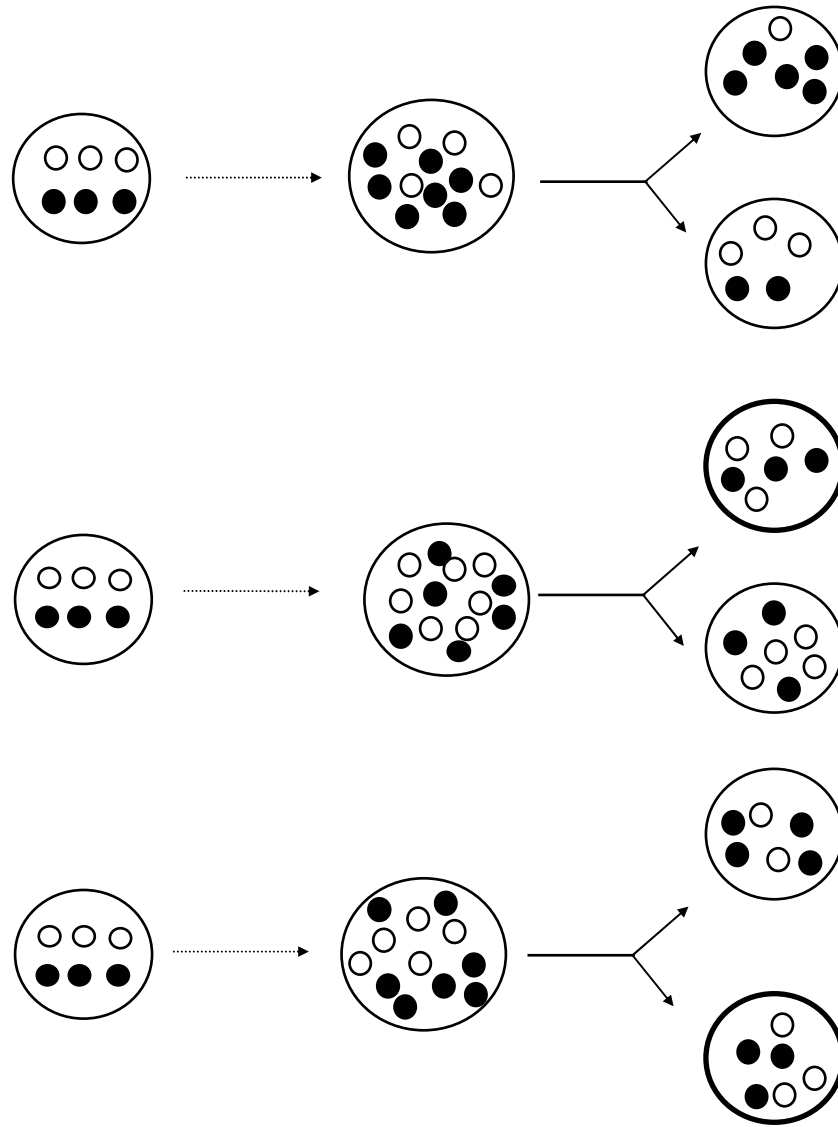
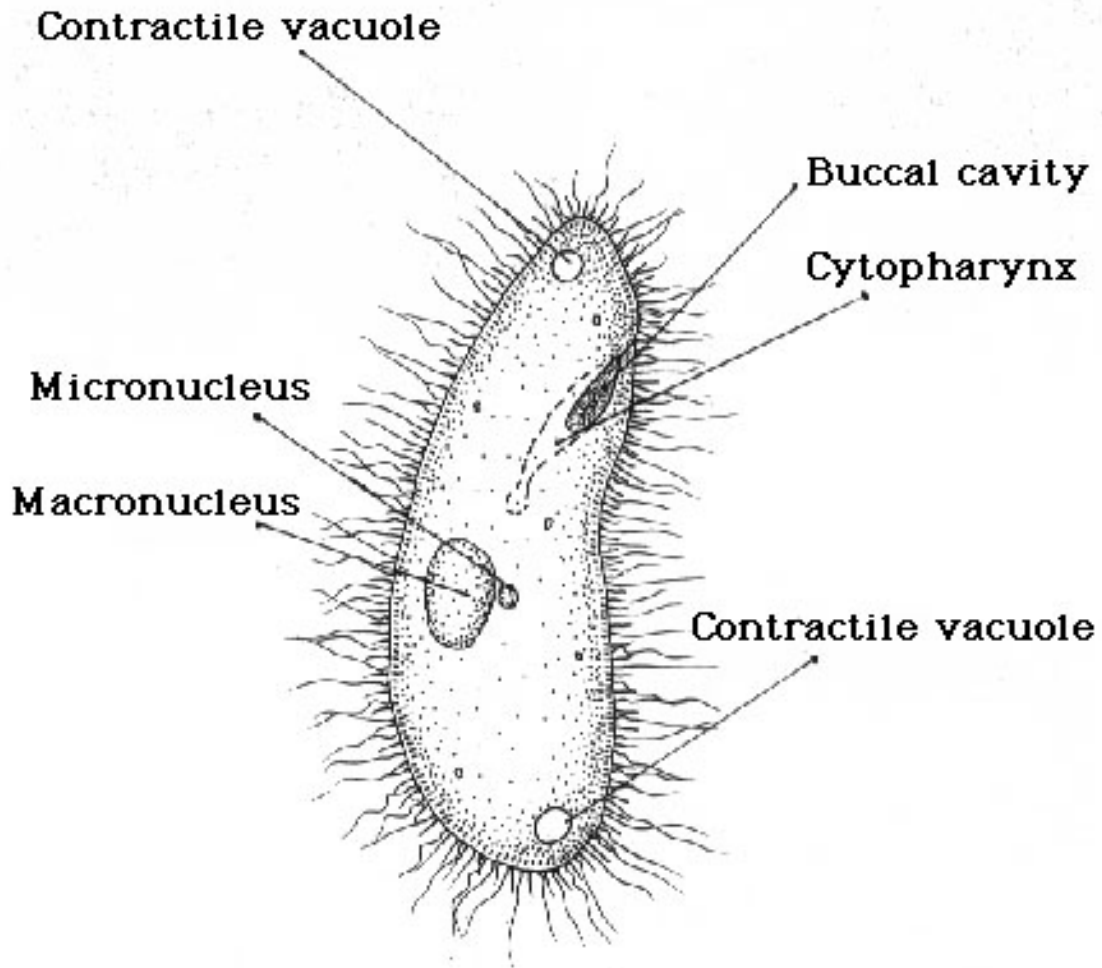
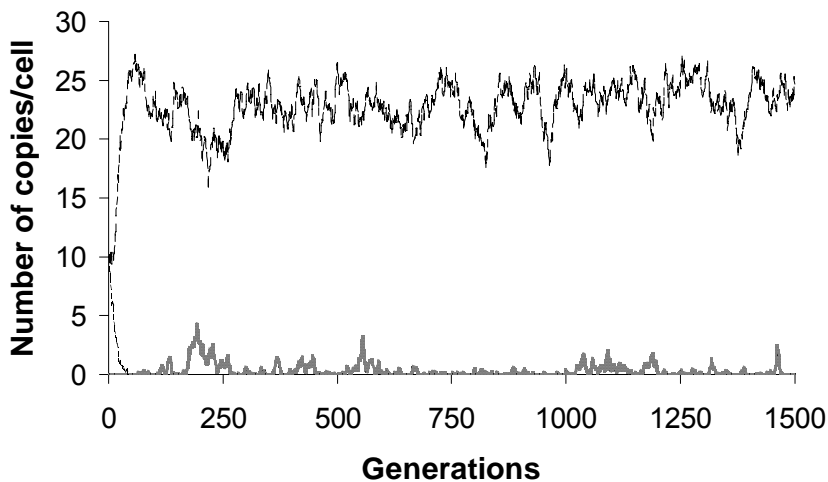


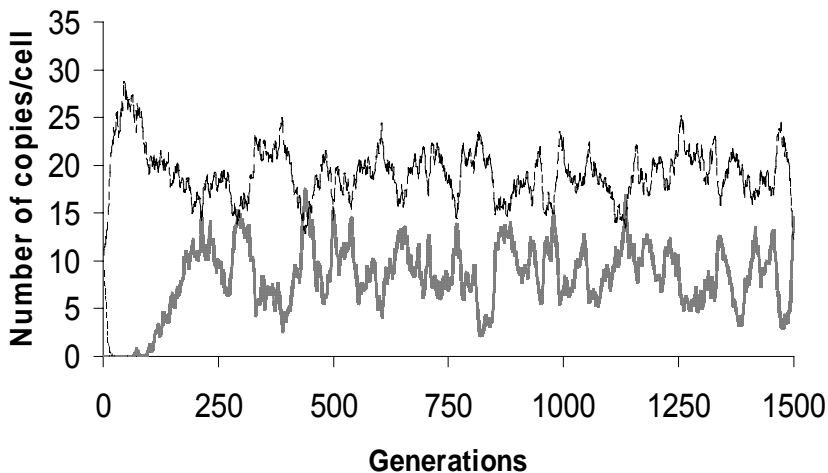
Fig.10. Szathmáry et al.



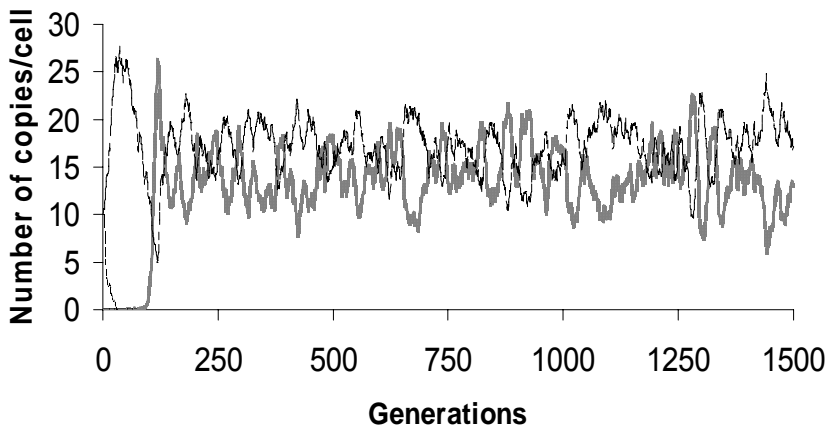
10% cell fusions/generation



20% cell fusions/generation



40% cell fusions/generation



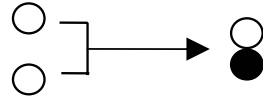
— R1(a) - - - R2 ···· R3

Fig.12. Szathmáry et al.

(a)

formaldehyde

glycolaldehyde



(b)

