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Question 7: Modelling Minimal 'Lipid-Peptide' Cells

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Abstract This contribution is aimed to give support to 'bottom-up' approaches to the minimal or early cell research project. Even if, from this perspective, the most simple living cell still seems very far away, the analysis of less complex, infrabiological cellular systems (some of which could be relatively soon implemented in the lab) probably holds the key, or one of the fundamental keys, to the problem of origins of life. On these lines, we propose a simulation model to study the transition from proto-metabolic 'lipid' cells to 'lipid-peptide' cells, as a critical step in which self-reproducing vesicles could develop into more *functionalized* supramolecular systems

Keywords Minimal lipid-peptide cells · stochastic kinetics (Gillespie method) · proto-metabolism · osmotic burst · vesicle reproduction

The question on early cells, from our point of view, is not so much about the minimal amount of genes that can hold together a living cell, but about the type of material organization on which this should be based and how that organization can emerge/be constructed from simpler prebiotic cells. From this 'bottom-up' approach, the actual number of genes required to establish the most simple forms of DNA-RNA-protein metabolisms (i.e., genetically instructed metabolisms) is not such a crucial point. It is more important to try to determine what are the material requirements and evolutionary implications of the transition to cellular organisms based on a (code-mediated) 'genotype-phenotype' decoupling. The size of the most primitive genome will depend on the complexity of the

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systems (or, rather, populations of systems) that are ready to perform that transition. Thus, the discussion should cover the question of whether hypothetical 'RNA-protein' cells, or minimal RNA cells (Szostak et al. 2001), or even previous, molecularly less complex cellular proto-metabolisms could be full-fledged candidates for living beings.

Most minimal cell models and related in vitro experiments constitute 'semi-synthetic' approaches, in the sense that they combine self-assembled compartments (vesicles or liposomes) with biopolymers (genes, enzymes or other macromolecular machinery) extracted from extant living cells – see reviews in (Pohorille and Deamer 2002; Luisi et al. 2006). A significantly smaller amount of researchers is tackling at present the problem from a 'bottom-up' approach, and even less looking into the basic *chemical* (rather than biochemical) logic of a minimal protocell (Morowitz et al. 1988).

Nevertheless, given the importance of the latter type of approach, the work we present here explores theoretically (by means of a stochastic simulation model) a scenario where relatively simple chemical components and processes bring about minimal self-(re-) producing cells (for further details on our simulation platform and cell model assumptions see Mavelli and Ruiz-Mirazo 2007; Ruiz-Mirazo and Mavelli 2007). Starting from a situation where the membrane compartment of the system is just made of endogenously produced lipidic/amphiphilic molecules (e.g., fatty acids), we introduce in the scheme hydrophobic aminoacid molecules (e.g., alanine) that can oligomerize to give short peptide chains, some of which may interact with the lipid bilayer, get inserted and form aggregates in it, and – if they are long enough – even span it (see Fig. 1). This transition from 'pure lipid' to 'lipid-peptide' protocells does not involve a significant increase in the complexity of the building blocks of the system (i.e., does not threaten the prebiotic plausibility of the model), but should have important consequences for its dynamic behaviour, particularly due to the changes it is bound to provoke in the properties of the membrane (elasticity,

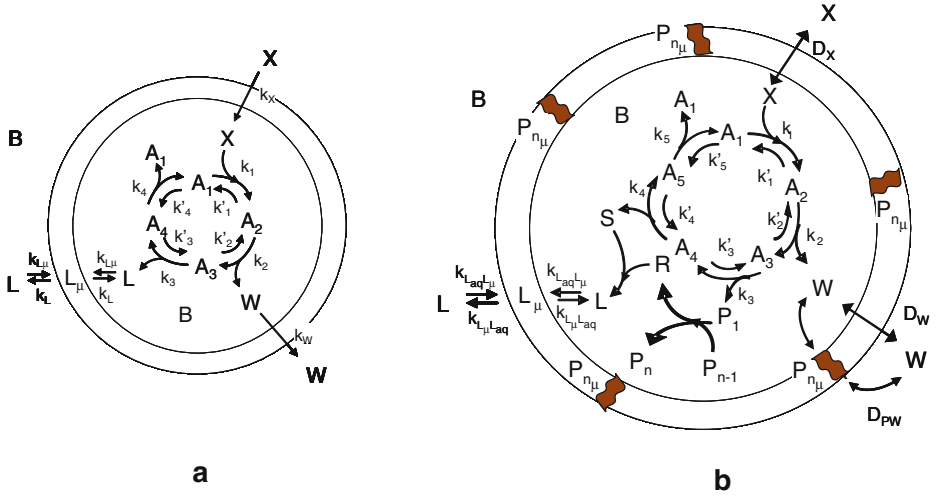

Fig. 1 Scheme drawings: **a** Scheme for the 'minimal lipid cell' scenario (analysed in Mavelli and Ruiz-Mirazo 2007, inspired partly by Ganti 2002). **b** Scheme for the 'minimal lipid-peptide cell' scenario. *L* stands for lipid (amphiphilic molecule, in general); *B* for buffer; *X* and *S* for different lipid precursors; *W* for waste product; *R* for a byproduct of oligomerization processes, coupled to internal lipid synthesis; P_1 is the aminoacid (monomer); P_n an oligomer; P_{n_μ} stands for a trans-membrane oligomer channel; the A_i stand for the minimal set of metabolites required in the internal autocatalytic cycle

permeability,...). For instance, polyalanine and polyleucine embedded in lipid bilayers have already been shown to induce proton-conducting pathways (Oliver and Deamer 1994).

Other current bottom-up attempts to the construction of minimal lipid-peptide systems (Rasmussen et al. 2004) consider an even simpler protocell scenario: they just model the compartment as a different phase (a hydrophobic domain), where certain reactions (in particular, the polymerization of PNA) may be favoured. Instead, we opt for a somewhat more elaborate minimal cell simulation model, in which oligo-peptides are produced within lipid vesicles (i.e., in their aqueous interior or in the internal lipid–water interphase), because that cell topology is the key to provide a plausible and continuous account of the origin of biological cells. In those conditions, while oligomerization processes are

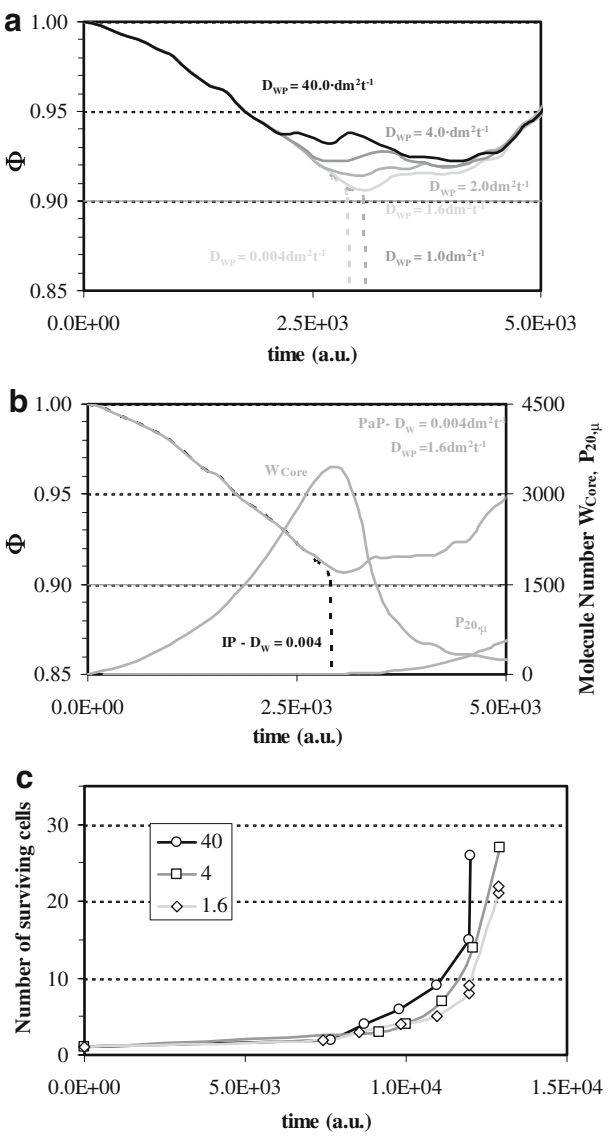

Fig. 2 Main results. Time course of the stability factor Φ for different waste diffusion constants (D_{PW}): **a** The *upper plot* shows a threshold below 1.6 dm^2 t^{-1}. **b** In the *lower plot* a comparison between the 'inert peptide' or IP cell (D_W= 0.004 dm^2 t^{-1}, *black line*) and the 'permeability-altering-peptide' or PaP cell (D_W=0.004 dm^2 t^{-1}, D_{PW}=1.6 dm^2 t^{-1}, *gray line*) is reported on the *right axis*; the time evolution of the number of molecules of W and P_{20} in the core volume and in the membrane are reported on the *left axis* (*gray lines*). In the *last plot* (**c**), the number of surviving cells for different waste diffusion constants (D_{PW}) above the threshold is shown: D_{PW}=40.0 dm^2 t^{-1} (*circles*), D_{PW}=4.0 dm^2 t^{-1} (*squares*) and D_{PW}=1.6 dm^2 t^{-1} (*diamonds*)

facilitated, the vesicles may also benefit from the situation to become more elaborate and functional cell compartments, including, for instance, pores or elementary trans-membrane channels. The formation of these pores/channels has proved instrumental to overcome the energetic-nutrient limitations of artificial cell bioreactors (Noireaux and Libchaber 2004). It remains an open question whether this is also the case at earlier stages, when the molecular complexity of the system is strongly reduced. But that is precisely the hypothesis we would like to explore, convinced that the capturing and channelling of matter and energy resources into the system is pivotal for its autonomous construction and robust maintenance, and that the membrane must play central role in this task from very early stages (Ruiz-Mirazo and Moreno 2004).

So we simulated the dynamics of minimal (hypothetically very primitive) lipid-peptide cells and, in particular, as part of a more complete analysis (Ruiz-Mirazo and Mavelli 2007), how the presence of polypeptides in the lipid bilayer could change its permeability to a certain compound that is critical for the osmotic stability of the system. Given our previous results in a scenario without peptides (Mavelli and Ruiz-Mirazo 2007), according to which the rate of disposal of the metabolic waste product (W) was crucial in that sense, we considered the possibility that peptide chains assist in the passive transport of that compound. Thus, polypeptides of a certain critical length (20 residues) will be assumed to span the bilayer and form selective channels for waste molecules (or just new transport pathways for them – since channels would surely require the self-assembly of different α-helices, made of different aminoacids), while the permeability of the lipidic membrane to W is set very low (D_W=0.004 $dm^2\ t^{-1}$, to observe more clearly the peptide effect).

Our results confirm, as shown in the upper plot of Fig. 2, that there is a threshold in the values of the aided or mediated diffusion coefficient ($D_W/dm^2\ t^{-1} \geq 1.6$), below which the cell is instable and bursts (due to spontaneous water inflow for overall osmotic balance). The comparison with the case in which similar peptides are produced and inserted in the membrane but do not contribute to change its permeability to W is reported in Fig. 2b. Up to the incorporation of peptides into membrane, the behaviour of the stability coefficient (Φ) is equal for both. But as soon as alternative W transport pathways are formed in the membrane, the amount of waste in the cell aqueous core rapidly decreases. Accordingly, the core volume growth rate is decreased. Therefore, if a cell is able to produce peptide chains fast enough, so that their insertion in the membrane induces a relatively quick change in the diffusion constant of the waste product, an eventual osmotic crisis can be avoided.

Within a population of diverse lipid and lipid-peptide cells, this could confer an obvious evolutionary advantage to those cellular systems that manage to produce channels rapid or effectively enough (see results in Fig. 2c). Quite interestingly, in these conditions it is not the cell that grows and multiplies more rapidly that has the advantage: stability or robustness as a cellular entity is crucial (and implies precisely control on growth and division rates). So our approach also allows to investigate pre-Darwinian evolutionary dynamics in which the capacity for robust 'self-maintenance' or 'self-production' of the systems involved (i.e., their organizational structure) is more important than their bare reproductive success. We consider that further work on these lines (instead of following the traditional approaches of 'molecular replicator' models) should be carried out to provide a more complete understanding of the origins of biological evolution mechanisms.

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