

Competition between model protocells driven by an encapsulated catalyst

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The advent of Darwinian evolution required the emergence of molecular mechanisms for the heritable variation of fitness. One model for such a system involves competing protocell populations, each consisting of a replicating genetic polymer within a replicating vesicle. In this model, each genetic polymer imparts a selective advantage to its protocell by, for example, coding for a catalyst that generates a useful metabolite. Here, we report a partial model of such nascent evolutionary traits in a system that consists of fatty-acid vesicles containing a dipeptide catalyst, which catalyses the formation of a second dipeptide. The newly formed dipeptide binds to vesicle membranes, which imparts enhanced affinity for fatty acids and thus promotes vesicle growth. The catalysed dipeptide synthesis proceeds with higher efficiency in vesicles than in free solution, which further enhances fitness. Our observations suggest that, in a replicating protocell with an RNA genome, ribozyme-catalysed peptide synthesis might have been sufficient to initiate Darwinian evolution.

Modern cells are thought to have evolved from much earlier protocells, simple replicating chemical systems composed of a cell membrane and an encapsulated genetic polymer, that were the first cellular systems capable of Darwinian evolution¹. Evolvability may have emerged in such systems via competition between protocells for a limiting resource^{2,3}. As protocells lacked the complex biochemical machinery of modern cells, such competition was necessarily based on simple chemical or physical processes⁴. To gain further insight into such competitive processes, various model protocell systems were developed. Fatty-acid vesicles were widely employed as models of early protocellular systems^{5–11} because fatty acids can be generated in plausible prebiotic scenarios, and because membranes based on fatty acids have physical properties that are well suited to primitive forms of life^{12,13}. The presence of small molecules can induce size changes and fusion of lipid vesicles^{14,15}, and it was shown previously that a membrane-based reaction can drive size changes of self-assembled lipid vesicles¹⁶.

The nature of the primordial genetic material remains uncertain; competing schools of thought support either RNA or some alternative nucleic acid as a progenitor of RNA. Irrespective of the nature of the original genetic material, an important question in considering the origins of cellular competition is how that genetic material could impart a selective advantage to a primitive protocell. An early model¹⁷ for such a scenario postulated an autocatalytic self-replicating genetic material, such as an RNA replicase, that would accumulate within vesicles at a rate corresponding to its catalytic efficiency. Mutations that led to greater replicase activity would result in a more rapid increase in internal RNA concentration and thus internal osmotic pressure, which would lead to faster vesicle swelling, which in turn would drive competitive vesicle growth. This model was supported by experimental observations that osmotically swollen vesicles could grow by absorbing fatty-acid molecules from the membranes of surrounding relaxed vesicles¹⁷. Although the simple physical link between mutations that lead to faster replication of the genetic material and the consequent osmotic swelling and vesicle growth is attractive, this model suffers from the lack of a plausible mechanism for the division of osmotically swollen vesicles.

More recently, we observed that low levels of phospholipids can drive the competitive growth of fatty-acid vesicles in a manner that circumvents this problem by causing growth into filamentous structures that divide readily in response to mild shear stresses¹⁸. This model implies that a catalyst for phospholipid synthesis, such as an acyltransferase ribozyme, imparts a large selective advantage to its host protocell because faster growth, coupled with division, results in a shorter cell cycle. A model system that illustrates the potential of an encapsulated catalyst to drive vesicle growth in a similar manner would, therefore, be a significant step towards realizing a complete model of the origin of Darwinian evolution.

Here we show that the simple dipeptide catalyst seryl-histidine (Ser-His) can drive vesicle growth through the catalytic synthesis of a hydrophobic dipeptide, *N*-acetyl-L-phenylalanine leucinamide (AcPheLeuNH₂), which localizes to the membrane of model protocells and drives competitive vesicle growth in a manner similar to that demonstrated previously for phospholipids. Ser-His catalyses the formation of peptide bonds between amino acids and between peptide nucleic acid monomers^{19,20}. Although Ser-His is a very inefficient and nonspecific catalyst, we found that it generates higher yields of peptide product in the presence of fatty-acid vesicles. As a result, vesicles that contain the catalyst generate sufficient reaction product to exhibit enhanced fitness, as measured by competitive growth, relative to those that lack the catalyst (Fig. 1). Therefore, we can observe how a simple catalyst causes changes in the composition of the membrane of protocell vesicles and enables the origin of selection and competition between protocells.

Results

Vesicles enhance Ser-His synthesis of AcPheLeuNH₂. The catalytic efficiency of the dipeptide Ser-His is extremely low, and quantitation is difficult because of precipitation of the hydrophobic product¹⁹. In addition, Ser-His appears to be a more-effective catalyst of the hydrolysis of the AcPheOEt ethyl ester than of peptide-bond formation, which further limits product yield. We decided to characterize the Ser-His-catalysed synthesis of AcPheLeuNH₂ in the presence of fatty-acid

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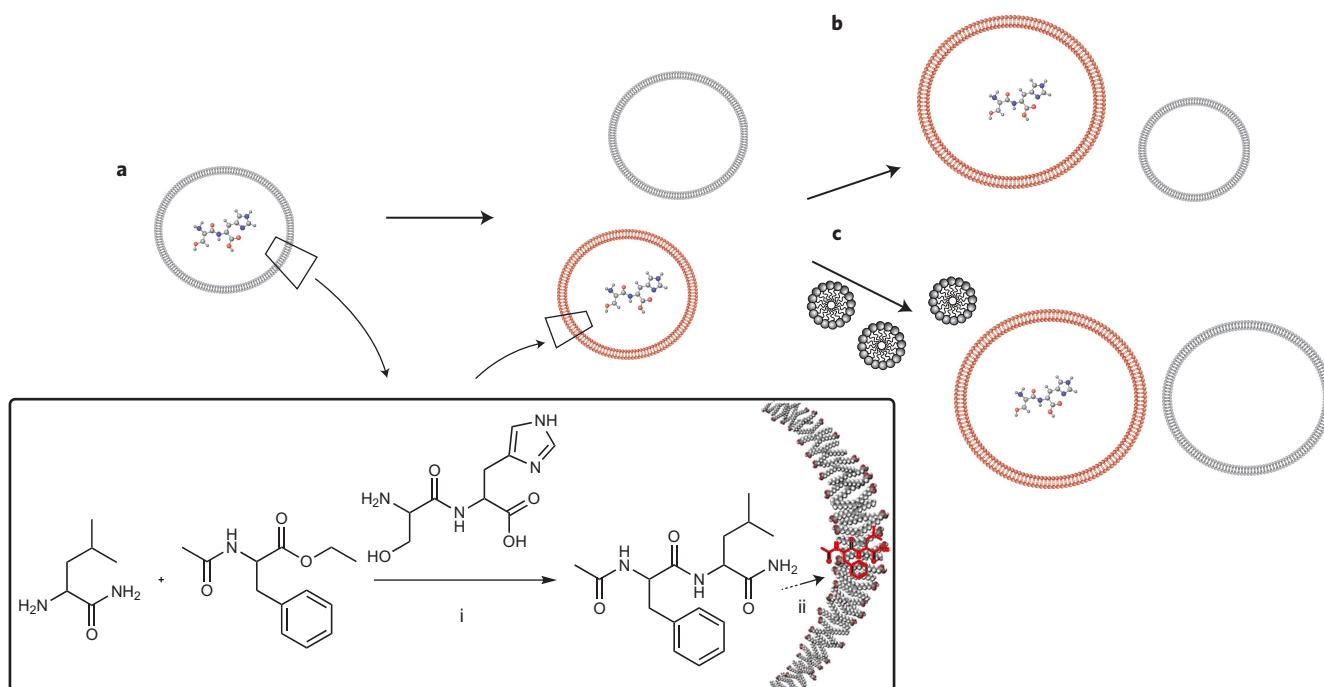


Figure 1 | Schematic representation of adaptive changes and competition between protocell vesicles. **a**, Synthesis of AcPheLeuNH₂ by catalyst encapsulated in fatty-acid vesicles. The dipeptide Ser-His catalyses the reaction between substrates LeuNH₂ and AcPheOEt (i), which generates the product of the reaction, AcPheLeuNH₂. The product dipeptide AcPheLeuNH₂ localizes to the bilayer membrane (ii). **b**, Vesicles with AcPheLeuNH₂ in the membrane (red) grow when mixed with vesicles without dipeptide (grey), which shrink. **c**, After micelle addition vesicles with AcPheLeuNH₂ in the membrane grow more than vesicles without the dipeptide.

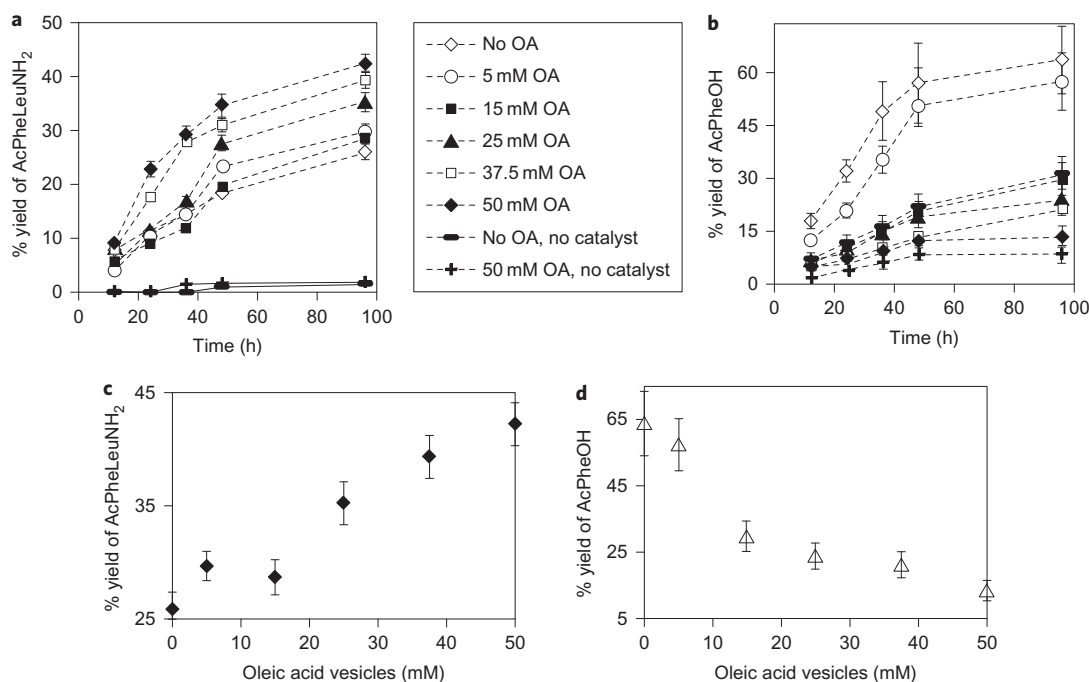


Figure 2 | Ser-His catalysis in the presence of fatty-acid vesicles results in increased synthesis of the hydrophobic dipeptide product and decreased substrate hydrolysis. **a**, Ser-His-catalysed synthesis of AcPheLeuNH₂ is faster and results in a higher yield in the presence of increasing concentrations of oleate vesicles (OA). **b**, Ser-His-catalysed hydrolysis of the substrate AcPheOEt is progressively slower in the presence of increasing concentrations of oleate vesicles. **c**, Yield of dipeptide AcPheLeuNH₂ versus concentration of oleate vesicles. **d**, Yield of hydrolysed substrate AcPheOH versus concentration of oleate vesicles in the same reactions. Data points are the arithmetic average of two independent experiments and error bars show extreme values. For all experiments, 10 mM of each substrate, 5 mM Ser-His catalyst, 0.2 M Na⁺-bicine, pH 8.5, 37 °C. In these experiments, the Ser-His catalyst and the AcPheOEt and LeuNH₂ substrates were present both inside and outside the oleate vesicles.

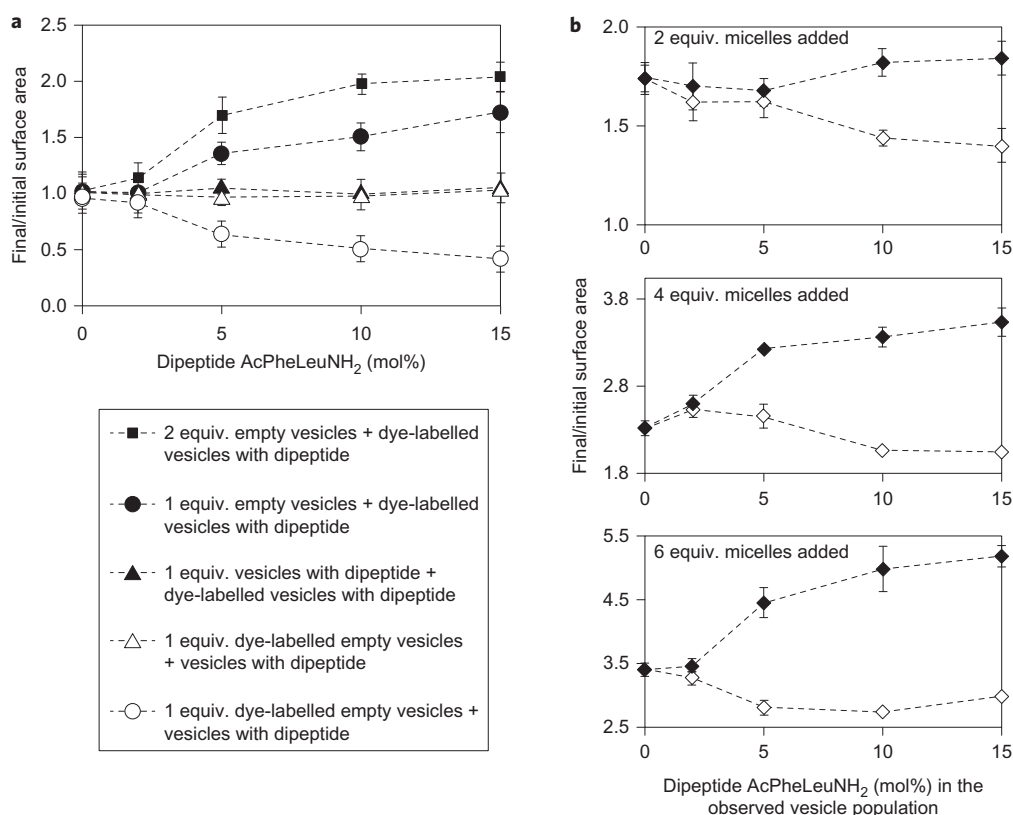


Figure 3 | Competition between vesicles with and without the hydrophobic dipeptide AcPheLeuNH₂. **a**, Oleate vesicles prepared without added buffer or salt and containing the indicated amount of AcPheLeuNH₂ were mixed with 1 or 2 equiv. empty vesicles. After 15 minutes the membrane surface area was measured using a FRET-based assay. FRET dyes on vesicles with AcPheLeuNH₂ show growth (filled symbols) and FRET dyes on vesicles without AcPheLeuNH₂ show shrinkage (open symbols). **b**, Competition for added oleate micelles in a pH-buffered high-salt environment (0.2 M Na⁺-bicine, pH 8.5). Equal amounts of vesicles with and without AcPheLeuNH₂ were mixed, then oleate micelles were added and the surface area measured after 15 minutes. FRET dyes on vesicles with AcPheLeuNH₂ show growth. FRET dyes on vesicles without AcPheLeuNH₂ (open symbols) show less growth than that for the corresponding peptide-containing sample (filled symbols). Error bars indicate the standard deviation. This experiment could only be carried out in the presence of added buffer, as without buffer the alkaline oleate micelles (1 equiv. NaOH relative to fatty acid versus 0.5 equiv. per fatty acid in a vesicle) quickly and excessively changed the pH of the mixture, which destabilized the preformed vesicles.

membranes, which we suspected would dissolve the product and prevent precipitation. We also suspected that colocalizing the hydrophobic substrates for the synthesis of the AcPheLeuNH₂ within or on fatty-acid membranes might improve the yield and possibly minimize substrate hydrolysis. We found that the Ser-His catalyst produced progressively more AcPheLeuNH₂ in the presence of increasing concentrations of oleate vesicles, with the conversion of substrate into product increasing from 25% in free solution to 44% in the presence of 50 mM oleate vesicles (Fig. 2a). The presence of vesicles also diminished substrate hydrolysis from 65% in free solution to 10% in the presence of 50 mM oleate vesicles (Fig. 2b). One possible explanation for these results is that the hydrophobic substrates partition to the membrane, which allows the reaction to occur at the solvent-lipid bilayer interface, or even within the bilayer, and thereby minimize ester hydrolysis and enhance product formation.

Also, we studied the catalysis of AcPheLeuNH₂ synthesis by the tripeptide Ser-His-Gly, a less-effective catalyst than Ser-His, which produced about half as much product dipeptide in the presence of oleate vesicles (with 50 mM vesicles, the yield of AcPheLeuNH₂ synthesis from 10 mM of each substrate and with 5 mM Ser-His-Gly was 27% after 96 hours in 0.2 M Na⁺-bicine, pH 8.5 at 37 °C, compared to 44% for Ser-His).

Slow exchange of Ser-His and AcPheLeuNH₂ peptides between vesicles. Competition between protocells is expected to result in

the selection of adaptations that are beneficial to the protocells in which those adaptations originated. Thus, the adaptive property must not be shared with other protocells in a population. It was therefore important to establish, in our simplified model system, that the Ser-His catalyst and the dipeptide product AcPheLeuNH₂ remained localized within the vesicles that originally contained the catalyst.

We first asked whether the hydrophobic peptide AcPheLeuNH₂ remained localized within an initial set of vesicles or was exchanged between vesicles. Our results indicate that dipeptide AcPheLeuNH₂ exchanges only slowly between vesicles when the vesicles are prepared from oleic acid plus 0.5 equiv. NaOH, with >80% remaining in the original vesicles over a period of eight hours (Supplementary Fig. S1a). In contrast, the presence of additional buffer (0.2 M Na⁺-bicine, pH 8.5) led to an accelerated exchange of peptide between vesicles. Therefore, all subsequent experiments were carried out over timescales that were short relative to peptide exchange. We performed similar experiments to show that encapsulated Ser-His and Ser-His-Gly were also retained within vesicles and did not exchange between vesicles (Supplementary Fig. S1b,c).

Interaction of AcPheLeuNH₂ with membranes. As a means of examining the interaction of AcPheLeuNH₂ with the vesicle membrane, we measured the fluorescence anisotropy of the molecular probe 1,6-diphenyl-1,3,5-hexatriene as a probe of membrane order. We found that the fluidity of oleate membranes

a Competitive growth and corresponding shrinking

Reaction scheme	Peptide in vesicle A	Peptide in vesicle B	Final/initial surface area
	S-H	None	1.24 (0.009)
	S-H	S-H	1.00 (0.013)
	S-H-G	None	1.14 (0.009)
	S-H-G	S-H-G	1.00 (0.014)
	S-H	None	0.74 (0.007)
	S-H	S-H	1.01 (0.011)
	S-H-G	None	0.85 (0.008)
	S-H-G	S-H-G	1.00 (0.006)

b Competitive micelle uptake

	S-H	None	4.18 (0.025)
	S-H	S-H	3.41 (0.024)
	S-H-G	None	4.01 (0.036)
	S-H-G	S-H-G	3.43 (0.021)
	S-H	None	2.78 (0.022)
	S-H	S-H	3.44 (0.017)
	S-H-G	None	2.96 (0.027)
	S-H-G	S-H-G	3.41 (0.026)

c Competition between vesicles with different catalysts

Reaction scheme	Final/initial surface area
	1.09 (0.01)
	0.92 (0.005)
	1.16 (0.011)
	1.09 (0.01)
	0.69 (0.006)

Figure 4 | Competition between populations of protocell vesicles. **a**, Vesicle size changes following 1:1 mixing of the indicated vesicle populations. **b**, Vesicle size changes following competitive oleate uptake after the addition of 6 equiv. oleate micelles. Populations of vesicles contained either Ser-His (S-H), Ser-His-Gly (S-H-G) or no catalyst, as indicated. All vesicle populations were incubated separately with amino-acid substrates for 48 hours to allow for synthesis of the hydrophobic dipeptide product prior to mixing. **c**, Each sample contained two or three populations of vesicles, as indicated, in a 1:1 or 1:1:1 ratio. In each case, the FRET dye pair was placed in one of the populations to measure the size change after the reaction. Vesicles that contained Ser-His outcompeted vesicles that contained Ser-His-Gly. The italic values are standard deviations (σ), $N = 8$.

decreased in the presence of the dipeptide AcPheLeuNH₂ (Supplementary Fig. S2), similar to the previously observed effects of phospholipids on oleate membrane fluidity¹⁸. The presence of the hydrophobic dipeptide also decreased the off-rate of fatty acids from fatty-acid membranes (Supplementary Fig. S9). We found that the desorption rate of oleate from fatty-acid vesicles decreased with increasing concentrations of dipeptide AcPheLeuNH₂ in the membrane, consistent with previously reported observations of the effects of phospholipid on fatty-acid vesicles¹⁸. The similar effects of AcPheLeuNH₂ and phospholipid on fatty-acid desorption suggests that the dipeptide might, as for phospholipid, also drive vesicle growth at the expense of surrounding pure fatty-acid vesicles.

We also examined the effects of the peptide on membrane permeability; neither AcPheLeuNH₂ nor reaction substrates of the Ser-His catalyst affected vesicle permeability to the small molecule calcein or to oligonucleotides (Supplementary Fig. S4).

Competitive growth of vesicles that contain AcPheLeuNH₂. Given that AcPheLeuNH₂ decreases membrane fluidity and fatty-acid dissociation in a manner similar to that observed previously for phospholipids, we investigated whether AcPheLeuNH₂ also affected membrane-growth dynamics. The dipeptide AcPheLeuNH₂ is practically insoluble in water, so the dilution of the insoluble peptide fraction present in fatty-acid membrane is favoured entropically. That, in addition to the decreased fatty-acid off-rate (monomer efflux from the membrane), could lead to the accumulation of fatty acids in the dipeptide-containing membrane, when fatty-acid vesicles without the dipeptide are present to provide the oleate monomer.

We monitored vesicle growth using an assay based on fluorescence resonance energy transfer (FRET) for the real-time measurement of membrane surface area²¹, in which the dilution of membrane localized fluorescent donor and acceptor dyes causes decreased FRET (see the Supplementary Information for details of the materials and methods). We found that vesicles containing AcPheLeuNH₂ grew at the expense of those that lacked this dipeptide when the two were incubated together (Fig. 3a and Fig. 4a). Similarly, when 'fed' with added fatty-acid micelles, vesicles that contained AcPheLeuNH₂ grew preferentially, taking up more micelles than vesicles without dipeptide (Fig. 3b and Fig. 4b). The time course of competitive vesicle growth, and the corresponding time course of the shrinking of vesicles without the dipeptide, match phospholipid-driven growth reported previously, which suggests a similar fatty-acid exchange mechanism (Supplementary Fig. S3).

Surprisingly, we only observed competitive growth between vesicles with and without peptide in self-buffered vesicles (vesicles with only the carboxyl groups of the fatty acids as buffering agents). After the addition of 0.5 molar equiv. NaCl to self-buffered vesicles (relative to oleate), less than half as much growth was observed, and when 1 equiv. NaCl was added to self-buffered vesicles, no significant competitive growth was observed (Fig. 5). The addition of 1 equiv. tetramethylammonium chloride (TMAC) affected the observed surface-area change to a lesser extent (~20% inhibition) (Fig. 5), which suggests that surface ionic interactions strongly affect fatty-acid exchange processes. In contrast to competitive vesicle-vesicle growth, we were only able to measure competitive micelle-induced growth in high-salt Na⁺-bicine-buffered vesicle samples (Fig. 3b).

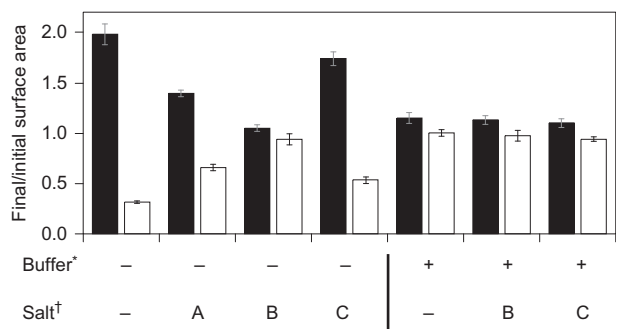


Figure 5 | Inhibitory effect of salt and buffer on competitive growth of vesicles. Equal amounts of oleate vesicles with and without 10 mol% AcPheLeuNH₂ were mixed and growth (or shrinkage) was monitored by a FRET-based surface-area assay. Vesicles that contained AcPheLeuNH₂ were labelled with FRET dyes and the growth monitored (black bars). Vesicles without AcPheLeuNH₂ were labelled with FRET dyes and shrinkage monitored (white bars). The presence of either salt or buffer strongly inhibited both growth and shrinkage after the vesicles were mixed. Error bars indicate s.e.m. ($N = 5$). *+, vesicles in a high-salt buffer (0.2M Na⁺-bicine, pH 8.5); -, self-buffered vesicles (50 mol% NaOH). †-, no salt added; A, 50 mol% NaCl (relative to oleate); B, 100 mol% NaCl; C, 100 mol% TMAC.

In a preliminary effort to correlate structure and activity, we examined the effect of several different small peptides on vesicle growth. Although other small hydrophobic dipeptides (for example, Phe-Phe) led to some competitive growth, none was as efficient as AcPheLeuNH₂ (Supplementary Fig. S5). *N*-terminal peptide acetylation was quite important, as Phe-LeuNH₂ was less effective than AcPheLeuNH₂. Log P_{calc} (calculated logarithm of octanol-water partition coefficient) values suggest that the more lipophilic peptides induce a greater competitive growth effect.

Competitive growth can facilitate division of protocell vesicles. It was shown previously that the growth of multilamellar oleate vesicles after micelle addition²² results in the development of fragile, thread-like structures that can fragment easily to produce daughter vesicles. Similar filamentous growth of phospholipid-containing vesicles was observed after mixing with excess pure fatty-acid vesicles¹⁷. We therefore asked whether competitive growth caused by the presence of the hydrophobic AcPheLeuNH₂

can also result in the formation of filamentous vesicles and subsequent division. We found that initially spherical vesicles with 10 mol% AcPheLeuNH₂ in their membranes developed into thread-like filamentous vesicles after mixing with 100 equiv. empty oleate vesicles (Fig. 6a,b). Gentle agitation of the filamentous vesicles resulted in vesicle division into multiple smaller daughter vesicles (Fig. 6c). We showed previously that the division of filamentous vesicles occurs without significant loss of encapsulated content, and therefore is a plausible mechanism for spontaneous protocell division^{17,23}.

Competitive vesicle growth induced by Ser-His-catalysed synthesis of AcPheLeuNH₂. The results described above show that Ser-His can catalyse the formation of AcPheLeuNH₂ in vesicles and that AcPheLeuNH₂ can cause or enhance vesicle growth. We sought to combine these phenomena to effect Ser-His-driven vesicle growth. We prepared oleate vesicles that contained encapsulated Ser-His dipeptide and FRET dyes in their membranes. Unencapsulated catalyst was removed either by dialysis against a vesicle solution of the same amphiphile concentration that lacked the catalyst or, alternatively, by purification on a Sepharose 4B size-exclusion column. Purified vesicles that contained 5 mM Ser-His were then mixed with 10 mM of the amino-acid substrates AcPheOEt and LeuNH₂, and incubated at 37 °C for 24–48 hours to allow for synthesis of the dipeptide AcPheLeuNH₂. High-performance liquid chromatography analysis of vesicles after 48 hours of incubation showed that in vesicles with Ser-His the dipeptide product AcPheLeuNH₂ was synthesized with 28% yield; parallel experiments with vesicles that contained Ser-His-Gly showed that the product was synthesized in 16% yield (Supplementary Fig. S8). After incubation, samples with catalyst were mixed with 1 equiv. oleate vesicles that had been incubated with amino-acid substrates, but without the Ser-His catalyst. After the mixing, vesicle-size changes were measured using a FRET assay for the surface area, as described above. We found that vesicles containing the peptide catalyst Ser-His already incubated with substrates (to allow for the accumulation of AcPheLeuNH₂) increased in surface area by 24% when mixed with 1 equiv. empty oleate vesicles. When the added empty oleate vesicles were labelled with FRET dyes, we saw a decrease in their surface area, as expected. In parallel experiments, vesicles that lacked Ser-His or were incubated without substrates did not grow after the addition of oleate vesicles (Fig. 4a). We also examined vesicles that

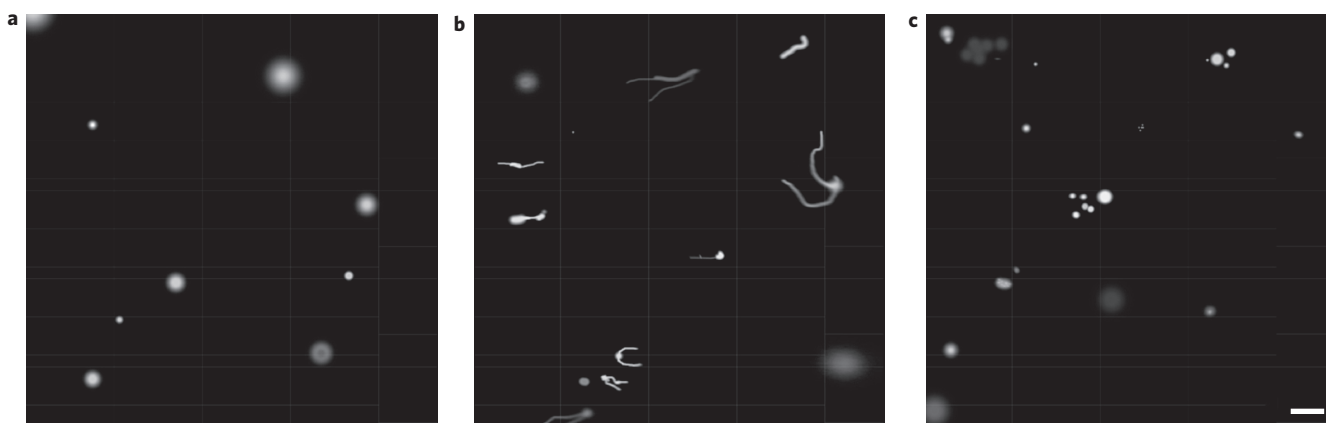


Figure 6 | Vesicle growth and division. **a**, Large multilamellar vesicles with 0.2 mol% Rh-DHPE (rhodamine B 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine) dye and 10 mol% dipeptide AcPheLeuNH₂ in the membrane were initially spherical. **b**, Ten minutes after mixing with 100 equiv. unlabelled empty oleic acid vesicles without the dipeptide, thread-like filamentous structures developed. The development of filamentous vesicles from initially spherical vesicles is caused by the more rapid increase of surface area relative to the volume increase, which is osmotically controlled by solute permeability. To recreate this effect in the absence of additional buffer, we used sucrose, a non-ionic osmolyte, to provide an osmotic constraint on vesicle volume. **c**, After gentle agitation, the filamentous vesicles fragment into small daughter vesicles. Scale bar, 10 μm.

contained the less-active catalyst Ser-His-Gly; these grew, but to a lesser extent than the vesicles that contained Ser-His (Fig. 4a).

Next we examined the ability of Ser-His (and Ser-His-Gly) to enhance vesicle growth after AcPheLeuNH₂ synthesis and then oleate micelle addition. As expected from previous experiments in which vesicles were prepared with AcPheLeuNH₂ directly, vesicles in which AcPheLeuNH₂ was synthesized internally also showed enhanced oleate uptake from added micelles, and they therefore grew more than empty vesicles (Fig. 4b). Vesicles prepared with Ser-His-Gly showed a similar, but smaller, growth enhancement.

Vesicles containing AcPheLeuNH₂ exhibited enhanced chemical potential generation during competitive micelle uptake. As fatty-acid vesicles grow, protonated fatty-acid molecules flip across the membrane to maintain equilibrium between the inner and outer leaflets. Subsequent ionization of a fraction of the protonated fatty acids that flip to the inside of the vesicle acidifies the vesicle interior, which generates a pH gradient across the membrane. This pH gradient normally decays rapidly because of H⁺/Na⁺ exchange, but we showed previously that by employing a membrane-impermeable counterion, such as arginine, the pH gradient can be maintained for many hours ($t_{1/2} \approx 16$ hours)²¹. We therefore used arginine as a counterion (arg⁺) to study the effect of AcPheLeuNH₂ on the generation of a transmembrane electrochemical potential during micelle-mediated vesicle growth.

To monitor the effect of the peptide on pH-gradient formation induced by vesicle growth, we prepared two populations of vesicles in arg⁺-bicine buffer, one with and one without the hydrophobic dipeptide AcPheLeuNH₂ in the membrane. Consistent with the enhanced surface-area growth of AcPheLeuNH₂-containing

vesicles, after growth induced by the addition of oleate-arg⁺ micelles the vesicles that contained peptide developed a transmembrane pH gradient twice as large as that seen in the vesicles without peptide (Fig. 7).

Competition between vesicles that contain different catalysts. To examine competition between vesicles that harbour peptides of varying catalytic efficiencies, we prepared self-buffered Ser-His-containing vesicles and Ser-His-Gly-containing vesicles and incubated them separately in the presence of substrates to allow for the synthesis of the hydrophobic dipeptide AcPheLeuNH₂. These vesicles were mixed and, after 60 minutes of incubation, the change in the surface area of vesicles labelled with dye was determined from the change in the FRET signal. The vesicles that contained Ser-His increased in surface area, but those that contained Ser-His-Gly shrank. This directly demonstrates competition between protocells that contain two catalysts of varying efficiency (Fig. 4c).

In a separate experiment, we mixed vesicles with both catalysts and added 1 equiv. empty oleate vesicles to serve as a 'feedstock' for the vesicles with the dipeptide AcPheLeuNH₂. In this case, both vesicles with Ser-His and those with Ser-His-Gly increased in size, but vesicles with Ser-His grew almost twice as much as those with Ser-His-Gly. This demonstrates that where 'feedstock' is readily available (from empty vesicles), both populations of protocell grow, but the more-efficient catalyst allows for more growth (Fig. 4c).

Discussion

The ubiquitous role of proteins as the catalysts of metabolic reactions raises the question of the origins of protein enzymes. That all modern proteins are synthesized through the catalytic activity of the RNA component of the large ribosomal subunit²⁴ suggests that primitive enzymes might have been peptides synthesized by ribozymes. By extension, the earliest enzyme progenitors might have been simple peptides synthesized in a non-coding fashion by one or more ribozymes acting sequentially. If short, prebiotically available peptides played useful roles in the growth or division of early protocells, there would have been a strong selective advantage conferred by ribozymes able to synthesize more such useful peptides. We found that a short hydrophobic peptide, whether supplied exogenously or synthesized internally, can confer a growth advantage to fatty-acid vesicles. Thus, a ribozyme capable of synthesizing short hydrophobic peptides could accelerate protocell growth, and thereby confer a strong selective advantage. The short peptide Ser-His confers similar effects through its catalytic synthesis of the hydrophobic peptide product, which supports the idea that a ribozyme with similar catalytic activity would confer a selective advantage, but also raises the possibility that a ribozyme that makes a catalytic peptide product could amplify its own efficacy through the indirect synthesis of the functional end product. Similarly, a ribozyme that synthesizes a peptide with nascent phospholipid synthase activity would confer a strong selective advantage through phospholipid-driven growth¹⁸.

The polarity of nucleic acids means that ribozymes might have difficulty catalysing reactions between membrane-localized substrates; thus, the synthesis of intermediate catalytic peptides could be an effective strategy for the synthesis of membrane-modifying products. The hydrophobic environment found in the interior of vesicle bilayers could provide a favourable reaction milieu for the catalysis of many chemical reactions, similar to that afforded (in a much more sophisticated fashion) by the interior of folded proteins in contemporary biochemistry. This speculation is supported by our observation that the presence of vesicles increases the yield of AcPheLeuNH₂ from amino-acid substrates with Ser-His as a catalyst. This increased yield most probably results from the greatly decreased extent of AcPheOEt substrate hydrolysis when it is

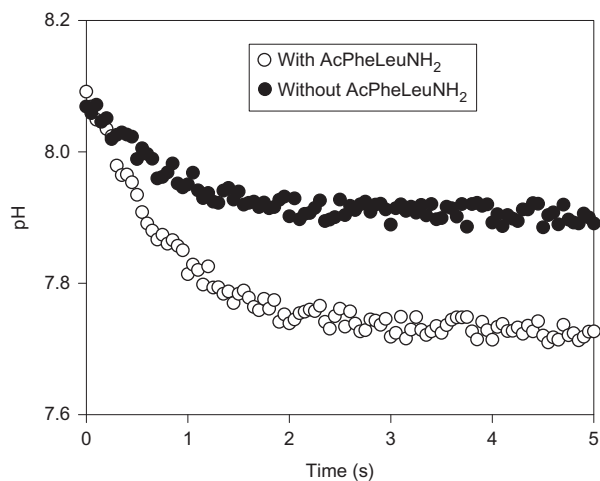


Figure 7 | Transmembrane pH gradient generated by growth of vesicles during competitive micelle uptake. Equal amounts of vesicles with and without 10 mol% AcPheLeuNH₂ were mixed in high-salt buffer (arg⁺-bicine, pH = 8.1), and 1 equiv. arginine-oleate micelles was added to the mixture to initiate growth. In both experiments, the peptide-containing vesicles that carried the pH-sensitive water-soluble dye HPTS (trisodium 8-hydroxypyrene-1,3,6-trisulfonate) encapsulated in the vesicle interior (open circles) and the peptide-free vesicles that contained the HPTS (filled circles) were mixed together and incubated for 30 minutes. No competitive growth occurred at this stage because vesicles were in a high-salt arg⁺-bicine buffer. We then added 1 equiv. oleate-arg⁺ micelles to the mixed vesicle sample, which triggered growth of both sets of vesicles. We measured the change in pH inside the vesicles versus time by monitoring the change in the fluorescence emission of the HPTS dye (see the Supplementary Information). Vesicles that contained AcPheLeuNH₂ developed a larger transmembrane pH gradient as a result of greater growth.

localized to the hydrophobic membrane interior and is thereby protected from attack by water (Fig. 2), much as labile intermediates are protected from water within the active sites of enzymes. Membrane localization of the leucyl-carboxamide substrate may also decrease the pK_a of the *N*-terminal amino group, and so enhance its reactivity by increasing the fraction of nucleophilic deprotonated amine. The phenomena of enhancing yields of chemical reactions by colocalizing substrates, altering pK_a values and limiting side reactions will probably be observed for many other membrane-associated substrates, which would allow protocells to start membrane-localized metabolism with assistance from very simple peptide catalysts.

We describe here a laboratory model system designed to illustrate the principles of competition between protocells. As the catalyst we used is not heritable, our system cannot yet evolve. Nevertheless, we have demonstrated adaptive changes that arise from encapsulated primitive catalysts acting at the protocell level, as well as competition between populations of protocells that contain two catalysts differing in primary structure (Ser-His and Ser-His-Gly). Direct vesicle-vesicle competitive growth is analogous to a predator-prey interaction, in which one population acquires nutrients from another population so that the 'predatory' population grows and the 'prey' population shrinks. The competitive micelle uptake is analogous to a 'competition for feedstock' among two populations. It is interesting that, in the model system we describe, these two mechanisms operate under different environmental conditions: direct competitive growth occurs only under low-salt conditions, but competitive micelle uptake occurs only under high-salt/buffer conditions. In each case, adaptive changes occur as a result of an encapsulated reaction, the rate of which is enhanced by an encapsulated catalyst. Thus, this system links intraprotocell chemistry (and catalysis) to the ability of a model protocell to adapt to selective pressure. As AcPheLeuNH₂ exchanges only slowly between vesicles, the presence of this hydrophobic dipeptide is truly adaptive, in that the product produced by the encapsulated catalyst remains with its original vesicle, at least for a few hours, which affords it an advantage (here, an enhanced affinity for membrane components, which allows growth). Competitive protocell vesicle growth can also result, under certain circumstances, in the development of a higher transmembrane pH gradient (Fig. 7), which potentially could be linked to the development of useful energy sources for protocell metabolism²¹. Furthermore, rapid competitive vesicle growth leads to the development of thread-like filamentous vesicles, which can subsequently divide into small daughter vesicles as a result of gentle agitation (Fig. 6). This process was observed previously for oleate vesicles grown by either micelle addition²² or by competitive lipid uptake driven by membrane composition⁴, and thus appears to be a general route to a cycle of growth and division. Here we show that growth can be induced by the activity of a catalyst encapsulated within the protocell, which thus brings the system one step closer to an internally controlled cell cycle. If such a system exhibited heredity, for example, via the activity of a self-replicating ribozyme that forms peptide bonds, it would amount to a fully functioning protocell capable of Darwinian evolution.

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Author contributions

Both authors contributed to the design of the experiments and to writing the paper. Experiments were conducted by K.A.

Additional information

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Competing financial interests

The authors declare no competing financial interests.