NKFI-112788: The origin of mitochondria (A mitokondrium eredete) – Final report

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Short summary

The origin of mitochondria is a challenging and intensely debated issue. Mitochondria are ancestrally present in eukaryotes and their endosymbiotic inclusion was an extremely important step during the transition from prokaryotes to eukaryotes. However, because of the unknown order of eukaryotic inventions (cytoskeleton, phagocytosis, endomembranes, etc.) it is unknown whether they led to or followed the acquisition of mitochondria. I have published a review paper to critically evaluate origin of mitochondria hypotheses and point out the importance of early ecology of the host-symbiont partnership, with the unanswered questions in case of all current hypothesis. I have tested the farming hypothesis of mitochondrial origin in an ecological-evolutionary model. According to the farming hypothesis, the mitochondrial ancestor was captured by a phagocytic host, but the advantage was not direct metabolic help provided by the symbiont, rather, it was provisioning captured prey to farmers in poor times, like humans farm pigs. I have developed analytical and computational models to show that farming could lead to stable endosymbiosis without any further benefit assumed between partners. The latest results are accepted with revision at PNAS.

Összefoglaló

A mitokondrium eredete mind a mai napig erősen kutatott és vitatott kérdés. A sejtszervecske megléte ősi bélyeg az eukariótákban, és endoszimbiotikus integrálódásuk rendkívüli fontosságú volt a prokariótáktól az eukariótákhoz vezető nagy evolúciós lépés során. Az eukarióta találmányok (citoszkeleton, fagocitózis, endomembránok, stb.) kialakulási sorrendje nem ismert, így az sem világos, hogy a mitokondriumok megjelenése az ok vagy az okozat volt e nagy lépésben. A kutatásom során publikáltam egy áttekintő tanulmányt, amelyben a mitokondrium-eredet-hipotézisek kritikai értékelését végeztem el. Rámutattam a gazda-szimbionta partnerviszony korai ökológiájának fontosságára és a jelenlegi elméletek hiányosságaira. Ökológiai-evolúciós modellben teszteltem a mitokondrium-eredet egyik magyarázó elméletét, a farmoló hipotézist. A farmoló hipotézis szerint a mitokondriális őst egy fagocitotikus gazda kebelezte be. A partnerkapcsolat kezdeti előnye nem a szimbionta által nyújtott közvetlen metabolikus segítség volt. A gazda a bekebelezett baktériumokat úgy tartotta, ahogy az ember sertést: jó időben felszaporította, rossz időben elfogyasztotta őket. Analitikus és egyed-alapú modellek segítségével igazoltam, hogy a farmolás stabil endoszimbiózisra vezet, anélkül, hogy a partnerek között extra metabolikus kölcsönhatást (pl. fotoszintetikus termékek, ATP, metabolitok, stb. kicserélése) feltételeznénk. A legújabb eredményekből készült cikket revízióval elfogadta a PNAS folyóirat.

Summary

The project was aimed to understand the initial problems of mitochondrial integration at the emergence of eukaryotes, and model these problems by simulating plausible scenarios. In the last 50 years, since Margulis has cemented the endosymbiotic origin of mitochondria into evolutionary biology, a multitude of theories were raised to explain the origin of eukaryotes in relation with mitochondria. Unfortunately, the role of mitochondria during eukaryogenesis is more debated than ever: its capture was either the trigger for cellular complexification and nucleogenesis or an already complex proto-eukaryote captured the symbiont late in the game. Since the literature is theory-heavy, with minimal modelling, it was of extreme importance to design models that could 1) test relevant theories; and 2) polarize eukaryogenesis.

In my work, I have extensively reviewed the literature and critically compared mainstream theories to select the most important aspects that any theory must explain: initial ecological relationship and early benefit for the parties. According to my investigation, there is no present theory that could explain all raised questions and could also account for the early ecological relationship. Moreover, many of the theories are rendered implausible in light of new results from metagenomic analysis (cf. Asgard Archaea).

The major aim of my project was to test one particular hypothesis of mitochondrial origins: the farming hypothesis. According to it, the host, being already phagocytotic, acquired the symbiont not for its energetic boost or other (syntrophic) metabolic interaction but purely for food. The farming hypothesis posits that the early benefit was shelter for the symbiont and food stock for the host in resource-poor periods. Since newly discovered Archaea are equipped with many of the proteins related to the cytoskeleton, membrane folding and intracellular transport, it is not unlikely that the ancestral host was capable of phagocytosis. I have designed two models, to simulate dynamics of the early ecology of host and symbiont, according to the farming hypothesis. The first model provides a minimum model of continuous dynamics with implicit bacterial farm. The second model is individual-based, and gives a better understanding of the microscale dynamics of host and symbiont. The models successfully prove that in an environment that fluctuates between resource-poor and resource-rich periods, farming is an effective bet-hedging strategy that reduces the overall risk of extinction. Moreover, we have proven, that emergent farming it is a winning strategy against resident non-farmers when evolution is allowed in the models. The only assumption that the farming theory is based on is the early evolution of minimal phagocytotic capacity – and no energetic coupling is posited *ab initio*. This concludes the project as was promised, and also opens up future research where other theories and hypotheses can be tested within the same model.

Scientific publications

The following publications acknowledge the funding from NKFI:

Zachar I, Szathmáry E (2017) Breath-giving cooperation: critical review of origin of mitochondria hypotheses. Biology Direct 12(19). doi: 10.1186/s13062-017-0190-5.

Zachar I, Szilágyi A, Számadó S, Szathmáry E (2017) Farming by the host cell as the origin of mitochondrial endosymbiosis by natural selection. [accepted with revision at *PNAS*].

Further publications published during the reported period 2014-2017, but which lack funding acknowledgement due to not being directly related to the project:

Kun Á, Szilágyi A, Könnyű B, Boza G, Zachar I, et al. (2015) The dynamics of the RNA world: insights and challenges. *Annals of the New York Academy of Sciences*, 1341(1): 75–95. doi: 10.1111/nyas.12700.

Vasas V, Fernando C, Szilágyi A, Zachar I, Santos M, et al. (2015) Primordial evolvability: Impasses and challenges. *Journal of Theoretical Biology*, 381: 29–38. doi: 10.1016/j.jtbi.2015.06.047.

Szilágyi A, Zachar I, Fedor A, de Vladar HP, Szathmáry E (2016) Breeding novel solutions in the brain: A model of Darwinian neurodynamics. *F1000Research*, 5. doi: 10.12688/f1000research.9630.2.

De Vladar HP, Fedor A, Szilágyi A, Zachar I, Szathmáry E (2016) An attractor network-based model with Darwinian dynamics. In: *Proceedings of the 16th Annual Conference on Genetic and Evolutionary Computation*. New York, NY, USA: ACM, GECCO '16. doi: 10.1145/2908961.2931672.

Fedor A, Zachar I, Szilágyi A, Öllinger M, de Vladar HP, et al. (2017) Cognitive architecture with evolutionary dynamics solves insight problem. *Frontiers in Psychology*, 8: 427. doi: 10.3389/fpsyg.2017.00427.

Szilágyi A, Zachar I, Scheuring I, Kun d, Könnyű B, et al. (2017) Ecology and evolution in the RNA world: Dynamics and stability of prebiotic replicator systems. *Life*, 7(4). doi: 10.3390/life7040048.

Detailed report

Evaluating the various hypotheses of the origin of mitochondria

The integration of mitochondria was a major transition, and a hard one. It poses a puzzle so complicated that new theories are still generated 100 years since the endosymbiotic origin was first proposed by Konstantin Mereschkowsky [Mereschkowsky (1905)] and 50 years since Lynn Margulis cemented it into evolutionary biology [Sagan (1967)]. The challenge and singularity of eukaryotic origins lie in the fact that the resulting new unit is not just an amalgamation of organisms of different ancestry but also because lower-level units overtook energy metabolism [Blackstone (2013)]. New phylogenetic data are trickling in each year, shining light to new pieces of the puzzle, and old and buried theories are dusted again (e.g. [Davidov and Jurkevitch (2009)].

One would expect that by this time, there is a consensus about the transition, but far from that, even the most fundamental points are still debated. Major discrepancies are in the nature of the host and inclusion mechanism, but of course these aspects have far reaching dependencies. While there are strong arguments on all sides, the debate about engulfment or infection, and early or late phagocytosis is still ongoing (see [Ball et al.(2016)] and comment [Gould (2016), Pittis and Gabaldón (2016)] and responses [Degli Esposti (2016), Martin et al.(2016)], respectively).

In the last few decades, some have realized that the real question lies in the initial relationship that predated the nucleotide translocase insertion. Blackstone's scenario points out the fact that even if the metabolic coupling is feasible, one has to comply with ecological considerations and – to run a selectively superior joint – one has to deal with occasional subordinate partners (mutants).

Accordingly, I have extensively reviewed the literature and compiled the relevant data, and as a result, in a review paper (Zachar & Szathmáry 2017 *Biology Direct* 12(19)), we have objectively investigated eight theories of mitochondrial origins, some mainstream some less famous, through the same twelve questions (see Table 1). These questions refer to processes that once happened thus have to have a purely mechanistic explanation, that complies with bioenergetics but also with ecology. All questions stem from the evolutionary drive behind endosymbiosis, accordingly all of them had to be accounted for by a plausible mechanistic model (existing or future) of mitochondrial origin. We realized, that there is no single theory that can adequately answer all questions (see Table 2). Furthermore, and more importantly, some answers have turned out to be untenable in light of new results (Asgard archaea).

Table 1. Possible combinations of components and scenarios discussed in this paper. The + and - signs in the second column indicate ecologically beneficial (+) or costly (-) interactions for the host/guest. From [Zachar and Szathmáry (2017)].

		host (cytoplasm and possible nucleus)		
		primitive eukaryote	**archaeon**	**bacterium**
ecological relationship (inclusion mechanism)	**syntrophy (+ / +) (engulfment)**	• **ox-tox model**	• **hydrogen hypothesis** • **sulfur-cycling hypothesis**	• **syntrophy hypothesis** (+archaeon as nucleus)
	predation (+ / -) (phagocytosis)	• **pre-endosymbiont hypothesis**	• **phagocytosing archaeon theory**	• **photosynthetic symbiont theory**
	parasitism (- / +) (invasion)	•	• **origin-by-infection hypothesis**	•

Table 2. Summary of hypotheses and how they account for the unavoidable questions of mitochondrial origins. A checkmark indicates that the hypothesis reasonably accounts for the observed facts and complies with empirical data (even if debatable). A blank cell indicates that it is unclear how the theory deals (if at all) with the given question. From [Zachar and Szathmáry (2017)].

	Hydrogen hypothesis	Photosynthetic symbiont theory	Syntrophy hypothesis	Phagocytosing archaeon theory	Pre-endosymbiont hypothesis	Sulfur-cycling hypothesis	Origin-by-infection hypothesis	Oxygen detoxification hypothesis
Eukaryotic singularity	✓			✓				✓
Lack of intermediates	✓						✓	
Chimaeric nature (membrane	✓ (✓)	✓ (✓)	✓ (✓)	✓ (?)	✓ (?)	✓ (?)	✓ (?)	✓ (?)
Lack of membrane bioenergetics in host	✓	✓	✓		✓			
Non-photosynthetic mitochondria	✓	✓	✓					
Variety of mitochondria	✓	✓	✓					✓
Metabolism of host	✓	✓	✓	✓	✓	✓	✓	✓ (?)
Metabolism of symbiont	✓	✓	✓	?	✓	✓	✓	✓
Initial relationship	✓	✓	✓	✓ (?)	?	✓	✓	✓

Early selective advantage	✓	✓	✓		✓			✓ (untenable)
Mechanism of inclusion	✓	✓	✓	✓	✓	✓		
Vertical transmission							✓	

The theory that provides the most answers is the hydrogen hypothesis [Martin and Müller (1998)], but it does not mean that it is the single valid hypothesis. It still has some holes and debatable claims (lack of a host-derived membrane wrapping mitochondria; vertical transmission of intermediate syntrophic stages; membrane replacement; primarily derived mitochondria-related organelles). No syntrophic case is known where the strong metabolic coupling actually lead to endosymbiosis. From a mechanistic point of view, phagocytosis is more likely than syntrophic inclusion [Jékely (2007)]. Furthermore, the bioenergetic argument put forward by Nick Lane, supportive of early mitochondria, is debated [Lynch and Marinov (2016), Szathmáry (2015)]. We have also included an argument against the bioenergetic argument in the supplementary material of the paper; for a brief description, see Figure 1. The important point is that gaining energy cannot be explained with the mitochondrion, as initially it did not provide much. Consequently, any reasoning about the energy requirements of early eukaryogenesis must rely on a *gradual increase of energy*. In this light, the source of extra energy might as well came from the simplest possible source: increasing energetic membrane surfaces by internalizing respiration. Endomembranes evolved by e.g. photosynthetic cyanobacteria are able to power multicellularity (though still far from eukaryotic levels). There exist phagocytotic eukaryotes lacking active mitochondria proving that phagocytosis can be sufficiently powered without the powerhouse.

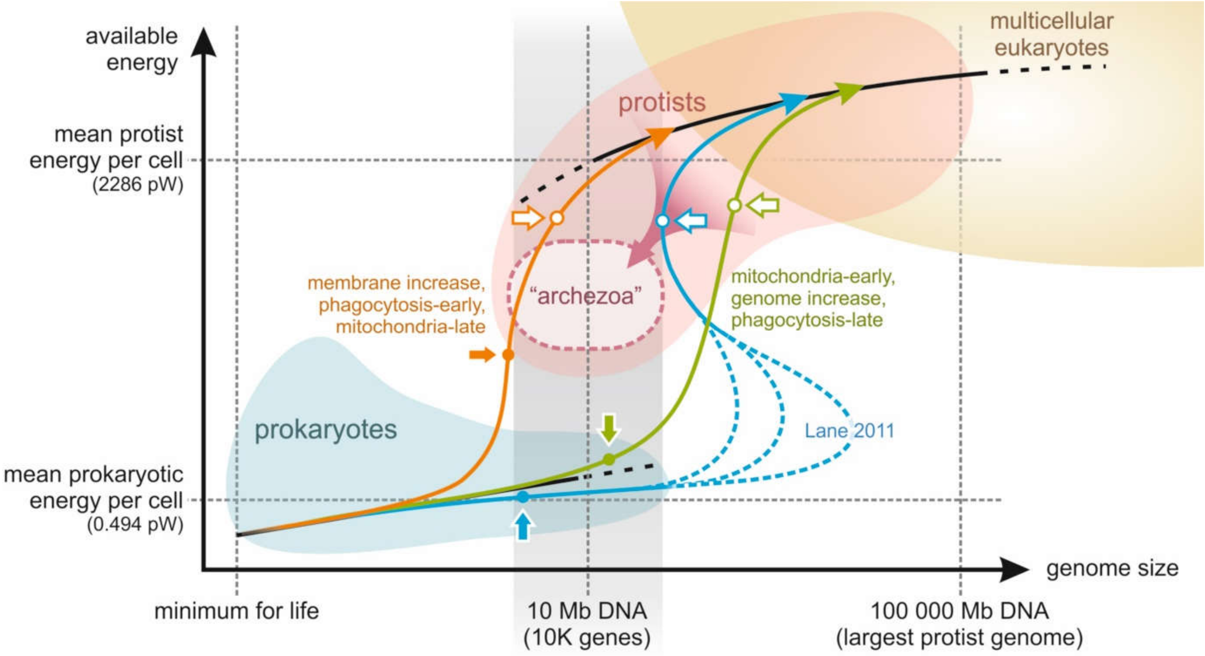

Figure 1. Various energetic scenarios of eukaryotes based on mitochondrial acquisition. Filled arrows indicate the first eukaryotic common ancestor (FECA) and the acquisition of mitochondria, empty arrows stand for the last eukaryotic common ancestor (LECA). Black lines roughly indicate averages in prokaryotes and eukaryotes. Prokaryotes cannot have genomes much larger than ~10Mb (or ~10K genes); smallest unicellular eukaryotes overlap with prokaryotes at this complexity. According to Lane and Martin [Lane and Martin (2010), Lane (2011)], there is an energetic barrier that prevents prokaryotes to maintain larger genomes (energy per cell values are from [Lane and Martin (2010)]. They claim that the early acquisition of mitochondria permit the transition of this barrier by temporarily increasing the gene count (blue curve;

though the multiplier factor is only guessed by Lane, hence the dashed curves) to be able to experiment with new gene families. They maintain that amitochondriate eukaryotes cannot evolve directly from prokaryotes, only by losing the endosymbiont. Another possible scenario is to increase the area of internal respiratory membranes which provides extra energy with no additional genes (orange curve). This might just have been enough to power primitive phagocytosis. Mitochondria had to be acquired at a point where respiratory membranes could not be further exploited. Early mitochondria might induce gradual genome increase that progressively made inventions possible (green curve), though if this happened at low energetic levels, the archezoan niche (dashed oval) again could only be reached reductively. Theoretically, any trajectory between the orange and green curves is possible, either with early or late mitochondria. Ultimately, all scenarios lead to the same LECA, though starting from different FECAs. Present amitochondriate eukaryotes are secondarily derived (purple arrow), but some scenarios allow (orange and dark green) the existence of primarily amitochondriate "archezoan" eukaryotes. Image from [Zachar and Szathmáry (2017)].

For an evolutionary adaptation to go to fixation it must be preceded by an ecological equilibrium where partners coexist for a prolonged time. The solution to mitochondrial origins and eukaryogenesis lies in this early relationship and, in turn, due to a probably unstable proto-nuclear host lineage, it is a question of ecology rather than evolution. If, however, early ecology was costly for the host, as the symbiont was rather a parasite at the time, the host had to receive some indirect benefit from the relationship to achieve the unprecedented success eukaryotes exhibit today.

The review paper entitled **Breath-giving cooperation: critical review of origin of mitochondria hypotheses** was submitted to Biology Direct in 14 March 2017 and was published 14 August. It has received two positive and one rather harsh critique from the reviewers (reviews are published with the paper as Biology Direct endorses an open review process). The response from the community was overly positive, the article was accessed 2327 times on its homepage (with an Altmetric Attention Score of 34) and has already received 2 independent citations. The paper also made it to the top three highly accessed articles in Biology Direct in November.

Testing the farming hypothesis

At the earliest stage, the bacterium was either a parasite (as was suggested by many, e.g. Lynn Margulis [Sagan (1967)], Siv Andersson [Andersson et al.(1998)] and Dennis Searcy [Searcy (2003)] or it was a photosynthetic slave [Cavalier-Smith (1983), Cavalier-Smith (2007)]. A third option is that it was neither: the host simply was feeding on the bacterium and had the ability to store it for later times. The second pillar of the project was to develop models to test this particular hypothesis, the farming origin, suggested by Maynard Smith and Szathmáry [Maynard Smith and Szathmáry (1995)]. According to the hypothesis, before any advantage could be reaped directly from extra mitochondrial energetics, the symbionts must have coexisted with their hosts. The interactions between species is depicted in Figure 2.

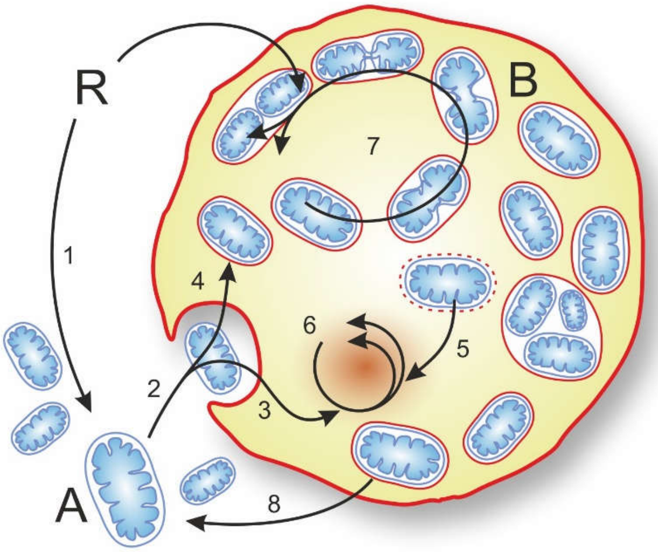

Figure 2. ***Predator-prey interactions in the farming archaeon*** *and interactions in the individual based model. Free living prey bacteria (blue cells) have density A that is explicitly defined by the abiotic resource density R (1). Predatory archaeon capable of farming (red cell) captures prey (2). The predator either consumes the captured prey to fuel its growth (3) or store prey in the farm (farming, 4) that has density B. Stored living cells can also be digested by the host to grow (culling, 5) which eventually leads to the reproduction of the host (6). Stored bacteria can also reproduce within the host depending on resource R (7). Bacteria multiply in separate phagosomes (red wrappers). Farmed cells could escape the host (8) to reseed environments where prey species has been extinct (omitted in the model). Predators unable to maintain a farm (non-farmers) lack processes 4, 5, 7, 8; predators unable to cull their farm lack 5. Any explicit benefit the farm provides for the host (metabolites, energy) is not displayed; if there is no explicit benefit, hosted bacteria are parasites. Image from [Zachar et al.(2017)].*

The traditional answer for the benefit of eukaryotes is predation by phagocytosis that would have considerably increased the efficiency relative to any bacterial predation (with external digestion) [Cavalier-Smith (2002), Martijn and Ettema (2013)]. The benefit in this case is the energy saved for processes other than the synthesis of many organic compounds. Although the phagocytotic machinery and its functioning demand energy, (secondarily) amitochondriate eukaryotes, once termed Archezoa [Cavalier-Smith (1987)], are phagocytosing without mitochondria. Note, however, that lack of evidence is not the evidence of lack; the recently discovered Asgard archaea [Zaremba-Niedzwiedzka et al.(2017)] shows signs of a cytoskeleton. Mitochondria, in this scenario, were acquired phagocytotically, and provided photosynthates for the host to help survive hard times.

In a second paper we show by explicit modelling that the farming scenario is a viable route to protomitochondrial establishment: farming by regulated internal digestion could have led to stable endosymbiosis without any other benefit (such as ATP or other metabolic currency from the symbiont) in an environment alternating between rich and poor in prey bacteria. We present an analytical minimum model of farming and a more comprehensive, but consonant, individual-based model. Each model investigates how two types of phagocytotic predatory archaea, one conventional (*non-farmer*) and one capable of storing prey for delayed digestion (*farmer*), compete in an ecological-evolutionary setup. To my knowledge, this is the first ecologically explicit dynamical model of the establishment phase of the prokaryote-to-eukaryote major transition.

The ecological modelling turned out to be rather complicated. The extensive simulation unveiled that the issue at hand cannot be directly modelled with continuous differential equations: the mean field approximation, does not accurately model the internal state of the predators capable of farming. The explicit representation of different size of internal farm per host is necessary, as otherwise there could be no selective difference between individuals. Thus while an analytical minimum model was designed, I have also developed an individual-based model to back up results of the analytical model. The minimal model assumes continuous-time dynamics and uses standard differential equation-techniques, while the individual-based model is discrete in time and state and uses a closed Moran process to simulate population dynamics. Both models use the same coupled ecological-evolutionary principles. Individuals are governed by the same actions and events. In the individual-based model, they also have internal states (amount of internally farmed prey cells and growth state).

We have found in both the analytical and the computational models that no explicit benefit is required from the partners for a stable integration to evolve, provided parties receive *implicit benefit* (food for the host and shelter for the symbiont in poor times). Farming is a form of bet-hedging: the host applies different strategies in good and hard times to minimize its overall risk of extinction. In consequence, relative fitness becomes higher in poor environments and overall temporal variance of fitness is reduced in expense of reduced fitness in rich environments [Ripa et al.(2010)]. While no examples of bet-hedging are known in Archea, it is prevalent and well documented in Bacteria and Eukarya. As there is no need to assume any further metabolic interaction, our bet-hedging strategy can explain stable integration of endosymbiont with host without pre-existing metabolic coupling. Given that in both of our models, there is a wide range of parameters where farmers can spread and dominate the population, we claim that ours is a general result that could explain many cases of stable endosymbiosis (see Figure 3).

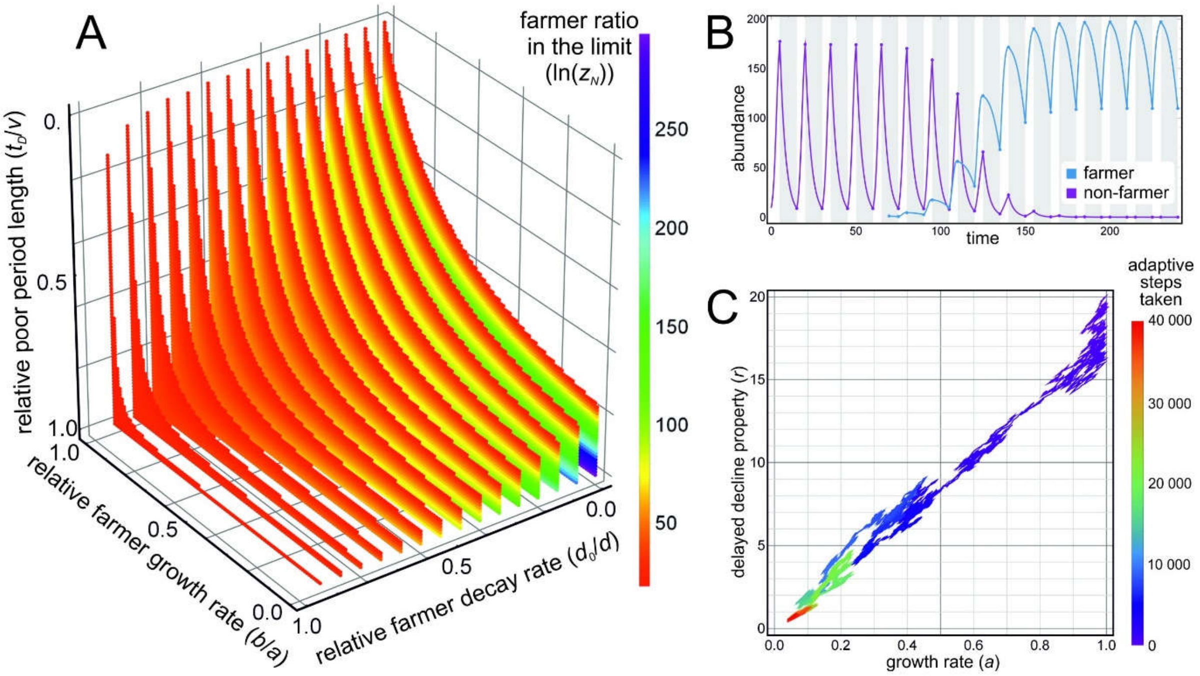

Figure 3. ***A**: **Equilibrium distribution of invading farmers**. Colored points indicate cases where farming cells dominate over non-farmers after 100 cycles of rich-poor periods. Color indicates the logarithm of the ratio of farmers in the population. Points are not shown where farmer is practically extinct.* ***B**: **Time evolution of farmers invading non-farmer population.** Mutant (farmer) is introduced at $t = 70$.* ***C**: **Adaptive dynamics of invading farmers.** Successive farmer mutants can invade a non-farmer population only if parameters a (intrinsic growth rate) and r (delayed decline by farming) are in trade off: if r decreases (better delay of death in poor periods), a must also decrease (worse growth in good periods). In this case, there exist viable evolutionary trajectories toward establishing farming. Image from [Zachar et al.(2017)].*

Recall, that no intermediate of any stage toward eukaryotes is known (neither mitochondriate prokaryotes, nor primarily amitochondriate eukaryotes are known to exist). It is obvious however, that some must have existed. An early appearance of phagocytosis in Archaea is increasingly, albeit as yet inconclusively, supported by finding the necessary components [Ettema et al.(2011), Godde (2012), Lindås and Bernander (2013), Spang et al.(2015), Yutin and Koonin (2012), Zaremba-Niedzwiedzka et al.(2017)]. Assumption of phagocytosis implies that the farming strategy can be applied to the establishment of mitochondria. What makes our models specific to mitochondrial origins, are 1) the complete lack of any pre-existing metabolic interaction or preadaptation (that could provide any explicit benefit), that are certainly there in any modern eukaryote harbouring endosymbionts; 2) the lack of synchronised cell cycles for host and symbiont; 3) phagocytosis; and 4) farming and delayed digestion of the farm.

A serious problem of farming, which we have identified, is divisional dilution: even in good times (when the farm is not supposed to be culled), the actual farm size will reduce in successive divisions, unless something counters it. (This is even more pronounced in case of nonsynchronous host and symbiont cell cycles.) Storing more prey does not help, as it reduces the relative reproduction rate of farmers compared to non-farmers, since farmers can eat less in unit time (see above). The only factor that can counter divisional dilution at the start of the partnership is autonomous growth of the farm. Accordingly, the farm's ability to grow inside the host must have been paramount in countering occasional culling *and* halving at every division (in the minimal model, this is implicitly assumed). Furthermore, farmed bacteria directly depend on the external resource (i.e. the environment), so in poor periods they can only grow very slowly (or not at all). Therefore, the farm will not last indefinitely in poor times as the host will ultimately consume faster than it can grow. If the poor period is any longer than the provisions, it means a death sentence for farmers (even if non-farmers have already been starved to death). Thus, farmers must balance between building up a farm, paying costs and competing with non-farmers in good periods, and rationing their farms in poor periods such that in the long run, they outcompete non-farmers.

To model a worst-case scenario, we deliberately implied costs on *everything* the farmer does to prevent any trivial advantage over non-farmers, so as not to beg the question. Farming has an explicit cost, dependent on farm size, that the host must pay in the form of reduced growth. Furthermore, we also added an implicit cost of farming: farmers cannot store *and* cull in the same timestep (modelling increased handling time). Since any food stored is not consumed right away, it means that farming equals giving up eating. This ensures that farming is not a zero-sum process and has a disadvantage in good periods: farmers grow less in unit time compared to non-farmers (even if farming has zero explicit cost). Consequently, farmers must have superior reproductive rates compared to non-farmers in poor environments, otherwise they will go extinct or cannot invade. This is achieved by delaying death in poor periods (or even being able to reproduce in the individual-based model) when non-farmers simply starve. Thus, according to our models, hosts do not receive free lunch – nevertheless, proto-mitochondria are able to stably integrate.

The most important consequence of the phagosomal membrane is, however, that the symbiont could only reproduce clonally. Unless host was sexual (see later), symbiont is also exclusive to the host's lineage. As a result, the symbiont genome becomes closely linked with the host's genome, even before nuclear transfer of any genes. In the individual-based model, we assume asexual hosts. We also associate the farming ability with the host's genome (instead of the symbionts) as is the case with *Dictyostelium*, where carrying a farm is a clone-specific trait [Brock et al.(2011)]. Consequently, all evolutionary traits presently associated with the asexual host could equally be associated with its clonal symbiont, i.e., *farm allocation rate* of the host could in fact be *digestion-evasion rate* of the symbiont. The evolved trait of culling can thus be interpreted as the ability to slowly overcome this evasive strategy. If, however, bacteria can evade host's digestion, non-farmers lacking the culling ability can end up with internal bacteria that only imply costs and do not provide any benefit. This would be a parasitic scenario.

While the above argument holds, ancient archaea might have practiced sex and fused to share genes and farms. Eury- and Crenarchaeota are known to undergo fusion and fission [Naor and Gophna (2013), Rosenshine et al.(1989)]. We have also investigated a simple game theoretical model that nevertheless captures the essence of the situation. Assuming that fusion is triggered by starvation, we find that farmer-farmer interactions are less critical as non-farmers can practically "steal" part of the stock when fusing (and splitting) with farmers. We show that non-farmers can never build up stock larger than farmers, if diffusion is responsible for exchange. In other words, farmers in the poor period always have more stock than non-farmers, which maintains their selective advantage (in terms of survival) in poor times. There are other important considerations that favour farmers in the long-term. Repeated fusion-fission is costly (draining stocks), and leads to selective death of those running out of their reserves. Remorseless decline in population density entails an Allee effect that favours farmers: as densities drop (there is no reproduction in the poor period), mating probability (also of farmers and non-farmers) decreases hyperbolically. Ultimately, internal stock levels will decide who survives the poor period and in what density. That is, farmers must maintain a farm large enough to survive the poor period with an end period density that prohibits non-farmers to outgrow them in the following good period.

The farming hypothesis of Maynard Smith and Szathmáry [Maynard Smith and Szathmáry (1995), Szathmáry (2015)] is a plausible scenario for the origin of mitochondria. Our models provide strong support for the farming hypothesis, and – consequently – for the origin of mitochondria right after phagocytosis and before any metabolic coupling, especially before the invention of ANT. As a matter of fact, this is the first work that explicitly tests a mitochondrial origin hypothesis in a dynamical model. Our work is intended to bridge the gap between tell-tale evolutionary scenarios and ecological assumptions within the origin of eukaryotes. While our scenario does not explain all the issues of eukaryogenesis (neither of the hypotheses do, see the other, published paper), it provides a plausible explanation to the early relationship of partners and the emergence of relevant evolutionary innovations (farming, prudent predation).

The major highlights of the research project are:

- If early ecology was costly for the host, the symbiont had to provide indirect benefit compensating costs for the relationship to be stably maintained in evolutionary timescales.
- The original relationship need not have to be mutually beneficial (most symbionts started out as parasites), but it had to be ecologically stable for a prolonged time period for evolution to act on the partnership.
- These are the first models to directly simulate the emergence of mitochondria in an ecological-evolutionary context that complies with present knowledge of mitochondrial origins.

- Our models prove that farming can facilitate the evolution of symbiosis *without any kind of explicit benefit* provided by the enclosed partner.
- Our singular assumption about the partnership is that the host was a heterotrophic phagocyte that could delay or mitigate digestion.
- Our model provides support for the early appearance of mitochondria, right after phagocytotic capabilities were evolved, well before nucleogenesis.

The above results are part of the manuscript Zachar, Szilágyi, Számadó & Szathmáry: *Farming by the host cell as the origin of mitochondrial endosymbiosis by natural selection*. It was submitted to PNAS and has received positive reviews from two anonymous referees. The submitted manuscript, supplementary material and referee reports can be downloaded from the following links (please respect that this is still unpublished work and is therefore confidential):

- Manuscript: https://drive.google.com/open?id=1pIc-ojY-BfpDYGEBHkF7EY0zI4IAlSVC
- Supplementum: https://drive.google.com/open?id=1EajFaJezJ3kmYbiKDP0jCsYsNvl8MPuR
- Referees' report: https://drive.google.com/open?id=12vzN9ITu7_mDPmzuy1KI5x_0FgqYs22L

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