© 2022. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2022) 225, jeb243214. doi:10.1242/jeb.243214

COMMENTARY

Evolution of plasticity: metabolic compensation for fluctuating energy demands at the origin of life

Frank Seebacher[1,*] and Julian Beaman[2]

ABSTRACT

Phenotypic plasticity of physiological functions enables rapid responses to changing environments and may thereby increase the resilience of organisms to environmental change. Here, we argue that the principal hallmarks of life itself, self-replication and maintenance, are contingent on the plasticity of metabolic processes ('metabolic plasticity'). It is likely that the Last Universal Common Ancestor (LUCA), 4 billion years ago, already possessed energy-sensing molecules that could adjust energy (ATP) production to meet demand. The earliest manifestation of metabolic plasticity, switching cells from growth and storage (anabolism) to breakdown and ATP production (catabolism), coincides with the advent of Darwinian evolution. Darwinian evolution depends on reliable translation of information from information-carrying molecules, and on cell genealogy where information is accurately passed between cell generations. Both of these processes create fluctuating energy demands that necessitate metabolic plasticity to facilitate replication of genetic material and (proto)cell division. We propose that LUCA possessed rudimentary forms of these capabilities. Since LUCA, metabolic networks have increased in complexity. Generalist founder enzymes formed the basis of many derived networks, and complexity arose partly by recruiting novel pathways from the untapped pool of reactions that are present in cells but do not have current physiological functions (the so-called 'underground metabolism'). Complexity may thereby be specific to environmental contexts and phylogenetic lineages. We suggest that a Boolean network analysis could be useful to model the transition of metabolic networks over evolutionary time. Network analyses can be effective in modelling phenotypic plasticity in metabolic functions for different phylogenetic groups because they incorporate actual biochemical regulators that can be updated as new empirical insights are gained.

KEY WORDS: Metabolism, Acclimation, Environmental change, Network, Cell division

Introduction

The phenotype of an individual organism reflects interactions between its genotype and the environment. However, phenotypes can be modified independently from their DNA nucleotide sequence by epigenetic mechanisms that act both within and between generations (Burton and Metcalfe, 2014). Such 'phenotypic plasticity' (see Glossary) is a regulated response that can be beneficial because it adjusts the rates of physiological functions to compensate for the immediate impacts of changes in the environment (Havird et al., 2020; Loughland et al., 2021; Schulte, 2014). Physiological rates often shift with acute changes in temperature, salinity, pH and other environmental parameters. Plastic responses can modulate the sensitivity of physiological rates to this environmental variability, and can do so at different time scales (Schulte et al., 2011). Within the lifetime of an organism, the environment experienced early in development can influence the adult phenotype ('developmental plasticity') (Burggren, 2018). In post-embryonic organisms, relatively long-term (weeks) environmental changes can induce shifts of physiological rates ('acclimation') (Guderley, 2004). Developmental plasticity and acclimation can act together or individually to reduce variance in physiological rates across environmental gradients, and thereby potentially increase organismal resilience to environmental change (Beaman et al., 2016). Additionally, environments experienced by previous generations can have lasting and heritable effects on offspring phenotypes ('transgenerational plasticity') (LeRoy et al., 2017).

In this Commentary, we present a framework for the evolution of phenotypic plasticity in metabolic functions (which we will refer to as 'metabolic plasticity') in terms of fundamental cellular mechanisms. Specifically, we focus on compensatory plasticity in energy (adenosine triphosphate, ATP) production to maintain cellular function (Guderley, 1990). We argue that metabolic plasticity is constitutive to cells, and that it is an ancient pre-requisite for life. However, metabolic regulation became more complex over evolutionary time, and we suggest that phylogenetic lineages can have differing capacities for plasticity because their regulatory networks (see Glossary) differ.

All biochemical reactions that lower the entropy of a reaction system require input of energy. Energy metabolism (see Glossary) is therefore fundamental for life, and energy transduction systems that produce ATP even precede the advent of life (Milshteyn et al., 2019). An evolutionary perspective on metabolic plasticity must therefore start at the origin of life itself and we anchor the origin of metabolic plasticity and of Darwinian evolution to around the Last Universal Common Ancestor (LUCA; see Glossary). We review chemical energy (e.g. ATP) production in pre-biotic structures and discuss how metabolic pathways became more complex in living organisms to form networks that now underlie whole-organism performance such as locomotion and metabolic rates. We suggest that a Boolean network analysis (see Glossary) can be useful in modelling plasticity in networks with different topologies, and we provide worked examples to demonstrate this approach.

Metabolism at the origin of life

The use of free energy to build complex molecules from smaller building blocks is essential for all life (Kaufmann, 2009). This process is not unique to life and also occurs in non-living systems, where molecules are synthesised from simpler precursors. For example, reduced gas mixtures produced amino acids and

[1]School of Life and Environmental Sciences, A08, University of Sydney, Sydney, NSW 2006, Australia. [2]College of Science and Engineering, Flinders University, Bedford Park, SA 5042, Australia.

*Author for correspondence (frank.seebacher@sydney.edu.au)

F.S., 0000-0002-2281-9311

Journal of Experimental Biology (2022) 225, jeb243214. doi:10.1242/jeb.243214

Glossary

Amphiphilic
An amphiphilic compound has both hydrophilic (water attracting) and lipophilic (lipid attracting) properties. Cell membranes are amphiphilic, and amphiphilic compounds form liposomes which may resemble early protocells.

Attractor
Favoured state or series of states of a network.

Boolean network
Analytical network approach that links different network nodes with logical statements.

Anabolism and catabolism
During anabolism, chemical building blocks and energy-rich substrates are stored, while catabolism denotes the breakdown of stored compounds to synthesise ATP.

Chemiosmotic energy
Chemical energy produced by electron transport coupled with a proton gradient across a membrane-like structure. Mitochondria produce ATP by chemiosmosis.

Darwinian threshold
A theoretical stage when vertical gene transfer (i.e. across cell generations) increased in importance, leading to an improved genealogy of cells and ultimately to Darwinian evolution.

Founder enzymes
Generalist enzymes that could react with a broad range of metabolites. Founder enzymes are thought to give rise to more complex networks, and are well connected in extant metabolic networks.

Last Universal Common Ancestor (LUCA)
Hypothetical cellular structure at the transition between abiotic chemistry and biology.

Metabolism
We use this term here to refer to energy metabolism; that is, the production of ATP or cellular storage of high-energy molecules.

Phenotypic plasticity
Here, we use a relatively narrow definition of plasticity as the expression of different phenotypes by the same genotypes in response to environmental signals. Plasticity may be induced during early embryonic stages (developmental plasticity), or via the gametes of previous generations (transgenerational plasticity). Phenotypes may also change within juvenile and adult organisms in response to environmental variation (acclimation).

Primordial soup
Mixture of organic chemicals from which life may have originated.

Promiscuous reactions
Reactions of a single enzyme with multiple metabolites.

Regulatory networks
Network of interacting molecules that regulate fluxes of metabolites and gene expression. ‘Metabolic networks’ refer specifically to networks that regulate energy metabolism.

Replicator
Molecules that could make identical copies of themselves thought to comprise an information carrier such as a nucleotide plus a catalytic peptide.

Underground metabolism
Pool of promiscuous reactions by founder enzymes that do not have current physiological importance, but which provide a source for potentially beneficial reactions in response to environmental change.

nucleobases in the Miller–Urey experiment (Miller and Urey, 1959). Similar compounds were present in a 4.5 billion year old meteorite, indicating that the chemical composition of reagents and products in the Miller–Urey experiment resembles chemical conditions before the origin of life (Kauffman et al., 2020; Sephton, 2002). Interestingly, under certain physico-chemical conditions, the abiotically produced ‘primordial soup’ (see Glossary) from the Miller–Urey experiment contained sufficient nutrients to support life (Xie et al., 2015). An alternative to the heterotrophic primordial soup scenario is that complex chemical structures arose autotrophically in hydrothermal vents or on dehydrated metal surfaces, and obtained their energy from H_2 oxidation on Fe(Ni)S surfaces, for example (Camprubi et al., 2017).

A crucial question is how these early chemical structures transitioned to living organisms. The capacity for self-replication and self-maintenance is an essential requirement for life and Darwinian evolution (Koonin et al., 2020). At a prebiotic level, molecules called ‘replicators’ (see Glossary) had the capacity to make identical copies of themselves. The exact characteristics of prebiotic replicators are not known, but self-replicating RNA molecules show that this is chemically possible (Wochner et al., 2011; Wolk et al., 2020). It is likely that replicators immediately preceding the origin of life were nucleopeptides, where peptides provided the catalytic activity necessary to replicate the molecule based on the chemical information contained in an information carrier such as RNA (Piette and Heddle, 2020).

For effective replication, it is critical to keep the information carrier and catalyst in close proximity. Some form of compartmentalisation was therefore essential (Schreiber et al., 2019). The first prebiotic protocells were thought to be surrounded by amphiphilic lipid membranes (see Glossary) (Deamer, 2017). However, it is also possible that protocells were formed by amphiphilic protein building blocks that self-assembled from prebiotic amino acids (e.g. Miller–Urey-type amino acids), which are robust to environmental variation (Schreiber et al., 2019). Compartmentalisation by the formation of ‘protocells’ in prebiotic chemistry permitted accumulation of small molecules, including ATP analogues (Deamer, 1997; 2017; Sugiyama et al., 2020). Consequently, molecules travelled between the internal and external environments. However, maintaining the interior milieu of the protocell at a different chemical composition from the exterior environment would require the regulation of transport of substances through the envelope (membrane) of protocells. Liposomes, which are spherical vesicles bounded by an amphiphilic lipid bi-layer, are likely to closely resemble prebiotic protocells and are relatively impermeable to most substances (Gibard et al., 2018). In living cells, transport is facilitated by proteins that exchange ions or actively transport target molecules using energy (e.g. ATPases). However, even in prebiotic cells, substances could traverse the liposomal membrane in the absence of transport proteins, facilitated by biophysical factors alone (Sugiyama et al., 2020).

The compartmentalisation of protocells and the transport of molecules into and out of the protocell compartment required structural complexity (i.e. low entropy) that relied on energy input. At a more complex level than simple storage, anabolism may be defined as the use of free energy to assemble pre-existing building blocks into larger structures that reduce the entropy of the system (Clarke, 2019; Kaufmann, 2009). Increasing complexity in (prebiotic) chemical structures would require sustained sources of energy to facilitate assembly of more complex structures, so that the system had to be coupled to a source of free energy (Ducluzeau et al., 2014). Potential environmental energy sources include mechanical energy (Hansma, 2010), electromagnetic radiation, chemical energy, heat and ionic potentials (Deamer, 1997).

Submarine hydrothermal vents with their unique chemistry and heat input from volcanic activity are a possible site for the transition from prebiotic to biotic systems (Brunk and Marshall, 2021; Martin et al., 2008). The amphiphilic lipid bilayer of protocell liposomes or similar structures also enabled a chemiosmotic energy source (see Glossary), established by proton gradients coupled to electron transport (Ducluzeau et al., 2014; Milshteyn et al., 2019; Simakov

et al., 2021). This chemiosmotic energy source is reminiscent of electrochemical gradients seen in mitochondria of extant eukaryotic organisms (Milshteyn et al., 2019). The universal prokaryotic ancestor possessed a rotor–stator ATP synthase that could couple proton flow to ATP production (Sousa et al., 2016), so that ATP synthesis driven by electron transport and proton gradients was already present very close to the origin of life. Similarly, the Krebs cycle, which is central to energy metabolism in modern organisms, originated under prebiotic conditions (Ritson, 2021). Hence, there was considerable 'metabolic' complexity in the prebiotic world, which was eventually co-opted into energy metabolism of living cells.

At a more complex structural level, transport processes would require considerable energy to import necessary building blocks and keep ions inside the protocell at different concentrations from the outside environment (Melkikh and Sutormina, 2019). Ion transport in protocells may have involved using the energy from hydrolysation of ATP to ADP+P to pump protons across the cell membrane. The resulting proton gradient could then facilitate transport of Na^+, K^+ and Cl^- (Melkikh and Sutormina, 2019). It is likely that LUCA possessed transport mechanisms such as ATPases in addition to transport mediated by proton gradients (Melkikh and Sutormina, 2019). Similarly, building complex structures from simple precursors (e.g. polymerase activity) requires energy, which in simple replicators would have been supplied by external environmental sources as outlined above (Kaufmann, 2009; Toner and Catling, 2020). However, as complexity grew from replicators to protocells and cellular organisms, a reliable supply of ATP became essential, particularly because the compartment (protocell, cell) itself also needed to replicate (Schreiber et al., 2019). Some configuration of chemiosmotic pathways (electron transport associated with proton pumping) is likely to have supplied ATP in LUCA (Ducluzeau et al., 2014).

Darwinian evolution is contingent on metabolic plasticity

Darwinian evolution occurred sometime after LUCA. LUCA is a theoretical construct that links abiotic chemistry to the first microbial life (Weiss et al., 2018). The consensus is that LUCA was a compartmentalised entity that possessed a version of the nucleotide–protein genetic code as well as the metabolic capacity for self-replication and for structural maintenance (Weiss et al., 2016). However, early protocells leading up to LUCA possessed only imprecise information processing (e.g. translation) and had weak genealogy across cell divisions, generating imprecise proteins referred to as 'statistical' proteins with variable amino acid sequences (de Farias et al., 2015). Instead of a single line of ancestors, the predecessors of LUCA are best thought of as communities of interacting prebiotic protocells (Woese, 1998). The weak genealogy diminished the efficacy, or even precluded the possibility of natural selection. Nonetheless, there was biological innovation, which is thought to have arisen from horizontal transfer of materials and information (genes) among communities of early protocells (Levin and West, 2017; Woese, 2002). As cell design became more complex chemically, cellular components became more integrated with each other, forming interacting modules. Horizontal transfer of cellular components became more difficult, because of the greater functional interdependence of individual components within the modules (Sambamoorthy et al., 2019). At this critical point, referred to as the 'Darwinian threshold' (see Glossary), greater integration of individual components also increased the effectiveness of information processing systems (e.g. translation), and vertical gene transfer (i.e. across cell generations) increased in importance, leading to an improved genealogy of cell lines and ultimately to Darwinian evolution (Woese, 1998; 2002).

The transition from chemistry to biology, and the adaptation of biological systems to novel environments are tightly linked to energy metabolism (Dibrova et al., 2012; Woronoff et al., 2020). The increasing connectedness of the components within cells increased the demand for more precise metabolic regulation to maintain different components and to sustain the energy levels necessary for cell division (Zhao et al., 2019). Replication of cells and genetic material to produce the next generation would have caused considerable variation in energy status. The cell would have to switch from anabolism (see Glossary), when chemical building blocks and energy-rich substrates are stored, to catabolism (see Glossary) that release ATP for biosynthesis and transport during cell division. Switching between anabolic and catabolic states ensured that energy levels remained relatively constant despite fluctuating requirements, and it represents the first manifestation of metabolic plasticity (Fig. 1). The principal components of metabolic plasticity, energy sensing and regulated energy production by chemiosmosis, for example, were present in pre-biotic systems. This capacity for metabolic plasticity enabled replication and transmission of genetic material to subsequent generations, or at least greatly facilitated it. Without compensatory adjustments of energy balance, replication would have had to rely on the haphazard availability of energy in excess of that necessary for maintenance.

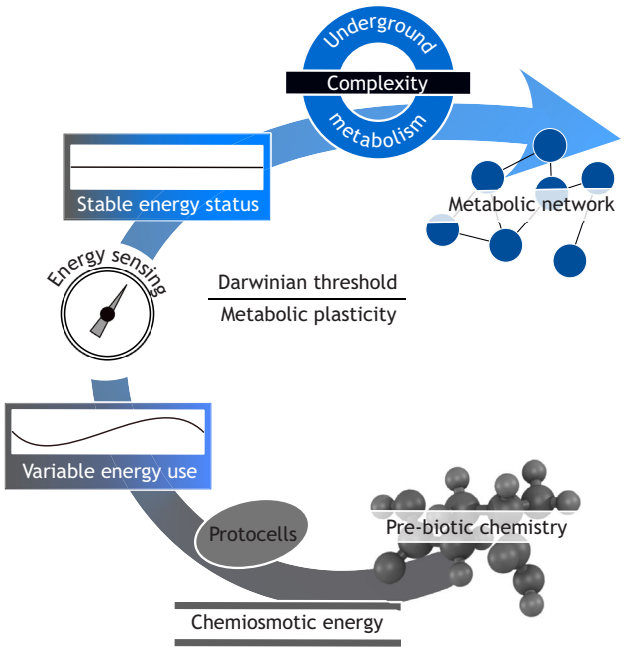

Fig. 1. Conceptual summary. In prebiotic chemistry, the potential for chemiosmotic ATP production, similar to that in modern mitochondria, already existed and facilitated more complex structures such as protocells. Division of protocells and replication of genetic material in protocells caused variable energy demands, including cycling and stochastic variation. Sensing of cellular energy status and adjusting energy production to fluctuating demand so that energy status remained relatively constant represents the first manifestation of metabolic plasticity. Metabolic plasticity supported vertical (i.e. across cell divisions) information transfer and cell genealogy on which Darwinian evolution depended (the Darwinian threshold). The underground metabolism in living cells provides a vast pool of reactions that can be harnessed for adaptation to novel environments and thereby promotes complexity. Increased complexity manifests in metabolic networks.

Journal of Experimental Biology (2022) 225, jeb243214. doi:10.1242/jeb.243214

Hence, metabolic plasticity would have changed the nature of vertical gene transfer across generations from a random process driven by environmental fluctuations to a constituent characteristic of the cell, thereby heralding Darwinian evolution.

Increasing complexity of metabolic systems

Energy metabolism requires the concomitant evolution of multiple enzymes and enzyme–metabolite interactions. Ancestral 'founder' enzymes gave rise to more complex metabolic interactions and networks. Founder enzymes (see Glossary) are likely to have started as generalist enzymes that are now well connected in complex networks. Generalist enzymes can react with multiple metabolites. These so-called 'promiscuous' reactions (see Glossary) by generalist enzymes comprise the 'underground' metabolism (Guzmán et al., 2019) consisting of a myriad of reactions and metabolites. Most promiscuous reactions do not have any current physiological benefits. However, the 'underground metabolism' (see Glossary) is important because it provides a vast source of potentially beneficial reactions, which may be harnessed when organisms are exposed to different environments (Copley, 2020; Guzmán et al., 2019). Promiscuous reactions therefore promote new enzyme activities, and support adaptation to new environments.

Novel pathways mediated by promiscuous reactions could theoretically occur at any stage of evolution, and all organisms carry reagents (e.g. proteins, nucleotides) that do not have known functions. Yeast cells and *Escherichia coli* display novel reactions in response to environmental changes via non-specific enzyme reactions (Grassi and Tramontano, 2011; Guzmán et al., 2019), and hidden reactions in the central carbon metabolism of *E. coli* are recruited in response to accumulation of intermediate metabolites (Nakahigashi et al., 2009). The extent to which promiscuous reactions contribute to novel physiological functions across taxa remains unresolved, although the potential is huge considering the vast number of molecules with unknown functions in cells (Noda-Garcia et al., 2018; da Silva et al., 2015). Diversification of pathways can be further enhanced by genome duplications and mutations (Copley, 2020). Consequently, metabolic network structures and complexity changed in response to the specific contexts within which different phylogenetic lineages evolved (Fig. 2). These molecular dynamics in response to novel environments may also promote metabolic plasticity.

A guiding principle for even the simplest metabolic reactions is that they have to be thermodynamically favourable. An energy source such as ATP in catabolic pathways is therefore essential to provide the energy necessary for reactions to proceed. Not surprisingly, ATP, ADP and ATP synthase are among the most connected compounds in the metabolic network of *E. coli* (Light and Kraulis, 2004). Self-replication and ultimately vertical gene transfer and cell division relied heavily on ATP availability that could sustain the complete replicatory cycle. The challenge of sufficient energy supply for replication would have increased as cellular structures became more complex, with their greater requirements for transport of building blocks and synthesis of macromolecules (Clarke, 2019). A mechanism that could match cellular ATP demand to ATP supply, and in some sense even predict it, would have greatly facilitated the transition from abiotic chemistry to cell genealogy and organismal evolution. Any such adjustments would represent compensatory phenotypic plasticity. The most ancestral known mechanisms by which this could be achieved are cystathionine β-synthase (CBS) domains, which could function as stand-alone energy sensors (Scott et al., 2004). CBS domains are present in Archaea, Bacteria and Eukaryota, indicating

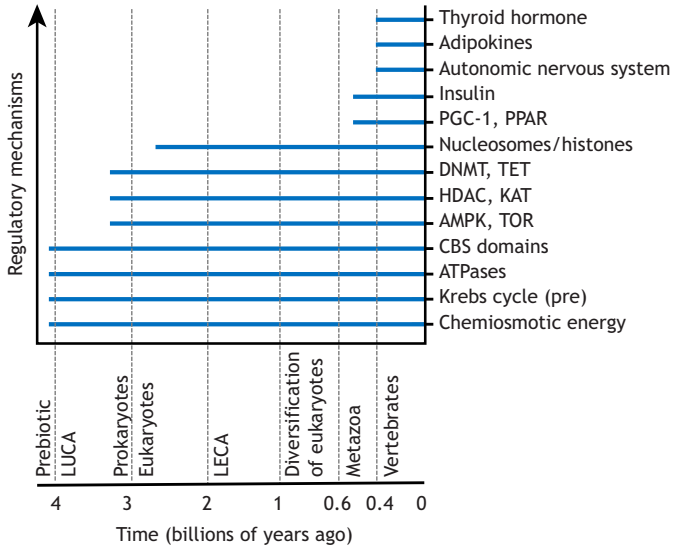

Fig. 2. Evolution of major cellular regulators of metabolism. The timeline on the *x*-axis summarises major evolutionary events from a prebiotic world and the Last Universal Common Ancestor (LUCA) to the appearance of eukaryotes and diversification of eukaryotes from the Last Eukaryotic Common Ancestor (LECA). The right *y*-axis lists some major regulators, and their evolutionary origin is indicated by the horizontal blue lines (see main text for more details). Figure based on an idea in Seebacher (2018); note that the *x*-axis is not to scale. PGC-1, peroxisome proliferator-activated receptor gamma coactivator 1; PPAR, peroxisome proliferator-activated receptor; DNMT, DNA methyltransferases; TET, ten-eleven translocation enzymes; HDAC, histone deacetylases; KAT, lysine acetyltransferases; AMPK, AMP-activated protein kinase; TOR, target of rapamycin; CBS, cystathionine β-synthase.

that they arose in a common ancestor close to LUCA (Gómez-García et al., 2010; Hardie, 2007). CBS domains bind adenosine derivatives (AMP, ADP), and their deletion causes metabolic disruption and disease in prokaryotes and eukaryotes (Alexandre et al., 2019; An et al., 2020; Anashkin et al., 2017). AMP-activated protein kinase (AMPK) is a more derived energy sensor in all eukaryotes (Fig. 2), and it switches the cell from an anabolic state to a catabolic state when the energy status decreases (González et al., 2020; Roustan et al., 2016). The regulatory AMPKγ subunit is composed of four CBS domains, and archaeal CBS domains are structurally similar to those on AMPKγ, and bind AMP in a similar manner to AMPKγ, indicating a conserved function (Gómez-García et al., 2010; King et al., 2008).

AMPK is now integrated within complex networks of metabolic regulation (Roustan et al., 2016). AMPK in modern taxa restores energy balance in response to cell division, exercise, cold exposure or a change in diet, for example (Craig et al., 2018; Zhao et al., 2019). It does so partly by blocking its counterpart, target of rapamycin (TOR), which promotes protein synthesis and growth (González et al., 2020). In modern taxa, the long-term effects of AMPK activation include enhanced capacities of mitochondria, and a shift to slow, oxidative muscle fibre types in vertebrates (Hardie, 2007; Ljubicic et al., 2011). AMPK thereby induces compensatory plasticity that buffers metabolic and locomotor performance from environmental variability (Box 1). Even though AMPK and other regulatory proteins are evolutionarily ancient, there are considerable differences in metabolism and metabolic plasticity between phylogenetic groups (Box 1; Fig. 2). This metabolic diversity may be captured in a network analysis, which could be a useful modelling tool, particularly if it incorporated interactions between actual proteins and pathways.

Journal of Experimental Biology (2022) 225, jeb243214. doi:10.1242/jeb.243214

Box 1. Interactions between regulators

Different metabolic regulators appeared over evolutionary time (Fig. 2) and their interactions now form metabolic networks that influence a wide range of physiological systems beyond energy production. These networks are far too complex to do justice to here, but some examples may be useful to illustrate the point. An evolutionarily ancient interaction occurs between AMPK and histone deacetylases (HDAC), which are a protein family of prokaryotic origin. HDAC together with their counterpart, lysine acetyltransferases (KAT), remove or add acetyl groups to proteins, respectively, thereby altering protein function (Vancura et al., 2018). In the case of histones, addition and removal of acetyl groups by KAT and HDAC relaxes or condenses chromatin structure to facilitate or block access of transcriptional regulators to DNA, respectively (Sheikh and Akhtar, 2018). Consequently, gene expression programmes change, and HDAC can modulate plastic responses to temperature change (Seebacher and Simmonds, 2019). AMPK and HDAC also interact to modulate muscle phenotypes. Activated AMPK triggers the transport of HDAC out of the nucleus and thereby lifts repression of myocyte enhancer factor 2 (MEF2) (Gaur et al., 2016). MEF2 promotes a shift from fast to slow muscle fibre types, which enhances aerobic metabolism and endurance (Dial et al., 2018).

In vertebrates, there are several interacting regulators, including the autonomic nervous system, adipokines, insulin and thyroid hormone, which have broad regulatory functions across numerous physiological systems (Fig. 2). For example, thyroid hormone is a regulator of metabolism and metabolic plasticity that is interlinked with AMPK (Little, 2021). Thyroid hormone induces AMPK activity in skeletal muscle (Mullur et al., 2014), and both AMPK and thyroid hormone induce the expression of the 'metabolic master controller' PGC-1α (peroxisome proliferator-activated receptor gamma coactivator 1 alpha) (Branvold et al., 2008). PGC-1α co-activates transcription factors such as PPARγ to induce metabolic plasticity by increasing metabolic activity in response to cold and exercise (LeMoine et al., 2010).

DNA methyltransferases (DNMT) and demethylases (e.g. ten-eleven translocation enzymes; TET) are other groups of proteins of ancient origin (Fig. 2) that alter access to DNA by, respectively, adding methyl groups to or removing them from the DNA molecule (Aliaga et al., 2019; Catania et al., 2020). DNA methylation patterns can be modified during early development by environmental stimuli such as temperature and diet, and these patterns can be passed on across generations (Mendizabal et al., 2014). DNA methylation is therefore a key mechanism mediating both developmental and transgenerational plasticity. AMPK links different types of plasticity by also regulating DNA methylation via phosphorylation of DNMT and TET (Fiedler and Shaw, 2018; Marin et al., 2017). The mechanisms briefly discussed here represent only a small number of nodes and vertices in metabolic networks, but demonstrate how diversification leads to increasingly integrated regulatory networks.

Boolean network analysis of metabolic plasticity

Phenotypes emerge from underlying biochemical networks. Networks consist of nodes connected by edges. Nodes comprise molecules that interact with one another as defined by the connecting edges. Regulatory molecules such as transcriptional regulators (e.g. transcription factors, chromatin modifiers) influence a large number of downstream nodes, and thereby play an important role in determining metabolic phenotypes (Ortmayr et al., 2019). The function of the network is largely dependent on its stability, and decreasing stability also decreases the predictability of the regulatory outcomes (Shmulevich et al., 2002). Modelling interactions between known regulators within networks is an effective way to explore their function, and modelling networks of different phylogenetic groups can provide insights into the evolution of plasticity. The challenge of this approach lies in uncovering the mechanisms regulating metabolism in different taxa, but its strength is that newly discovered regulators can be incorporated easily into network models.

Boolean networks are a simple way to model interactions between known regulators qualitatively (Dutta et al., 2019). The evolutionary histories of some regulators such as AMPK and TOR are well known (Roustan et al., 2016) and can therefore serve as an example of how interactions can be incorporated into Boolean network models. Conceptually, metabolic plasticity is induced by an environmental input that changes the energy status of a cell (e.g. cell division, temperature or exercise). A decrease in energy status (i.e. an increase in AMP) activates AMPK and switches the cell between anabolic and catabolic states in a compensatory response to increase ATP production and restore energy balance (Fig. 3). This process can be modelled in a Boolean network as a simple switch between anabolism and catabolism induced by increasing AMP levels (Fig. 3). From this basic on–off switch, the network may be enhanced by including other known metabolic moderators, thereby building more complex interactions between nodes that more closely resemble what is known from experimental data. For example, TOR may be included as a mediator of anabolism, and chemiosmosis as the principal catabolic ATP-production mechanisms. Network complexity increased over evolutionary time: mitochondria became the dominant site for chemiosmosis and, in animals, regulators such as thyroid hormone and peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1) appeared, and muscle fibre type (e.g. different myosin heavy chains) became linked to metabolic phenotypes (Fig. 2; Box 1). These different molecules are known interact with each other, and these interactions can be added as nodes and edges to the network. In addition to positive interactions, such as the induction of PGC-1 by thyroid hormone, there are also trade-offs. Upregulation of ATP production introduces inefficiencies in energy transduction (Salin et al., 2018) and induces potential costs such as the production of reactive oxygen species (ROS) (Loughland and Seebacher, 2020), both of which would influence ATP production negatively. These different interactions are known from the literature, and adding them to the network builds an increasingly realistic representation of metabolic plasticity. The networks produced here (Fig. 3) (Müssel et al., 2010) are factually correct, but are oversimplified. However, they serve the purpose of demonstrating the utility of network analyses.

Biochemical networks are perturbed by environmental impacts, and the capacity of the network to resume its core functions (e.g. compensatory ATP production) is important for the overall success of the cell. The robustness or stability of Boolean networks can be analysed by estimating the Hamming distance, which represents the number of transitions or 'flips' needed to move from the disturbed state back to the most favoured states or cycle of states ('attractors'; see Glossary) of the network (Wang et al., 2012). Robustness increases, i.e. Hamming distance decreases (Fig. 3), with increasing complexity of the network, because complexity increases functional redundancy. Functional redundancy means that the same outcome may be achieved via different pathways, which makes the system less vulnerable to perturbation of any one pathway (Ross et al., 2021; Sambamoorthy et al., 2019). Hence, robustness depends on the number of nodes in a network as well as on their interactions (Wilmers, 2007). Compared with random networks of the same complexity (comparisons with 100 simulated random networks), only the more complex biological networks are statistically more robust than random (i.e. the probability that the robustness in a biological network equals that of a random network is <0.05). This makes sense in terms of network design principles (Sambamoorthy

Journal of Experimental Biology (2022) 225, jeb243214. doi:10.1242/jeb.243214

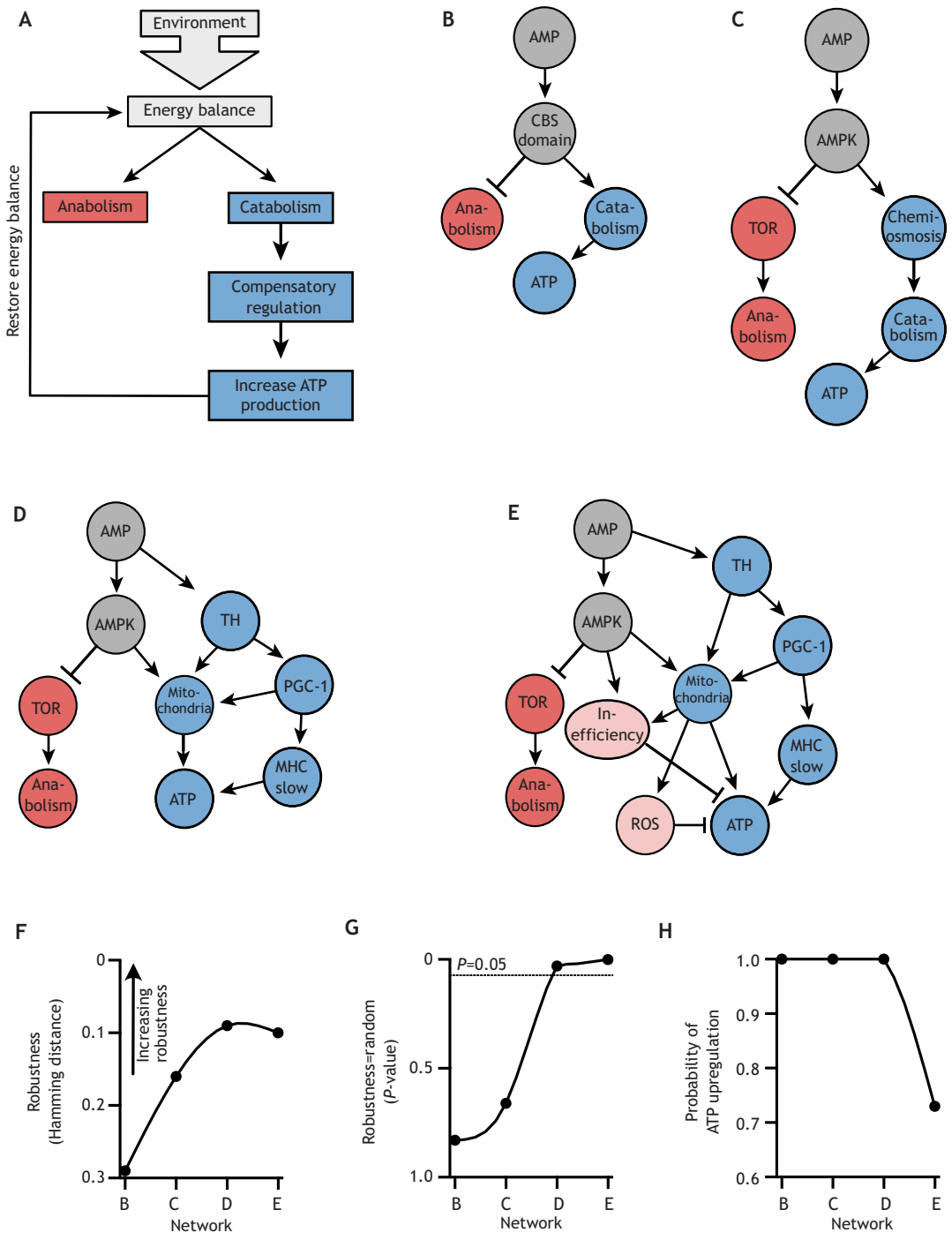

Fig. 3. See next page for legend.

et al., 2019), and maybe because the most basic manifestation of metabolic plasticity arose spontaneously under the right environmental conditions in pre-biotic chemistry, while the more complex networks were subject to selection.

The probability of achieving particular attractors in any particular network can be modelled (Shmulevich et al., 2002). In the simple networks with a single switch from the anabolic to the catabolic pathway, the outcome is always an upregulation of ATP (P=1). However, the situation changes in more complex networks that also incorporate inefficiencies and costs. For example, ROS production and mitochondrial inefficiencies reduce the probability of increased ATP production (Fig. 3). This result indicates that when ROS production is high and energy transduction efficiency is low, there is a possibility that upregulating catabolic pathways may not lead to net increases in ATP. An extreme example of this is brown adipose tissue, where increased catabolic flux does not result in ATP production (but heat production instead) because of the extreme inefficiency of mitochondria (Jastroch et al., 2018).

In summary, the strengths of Boolean network analyses are: (i) networks are constructed based on empirical relationships; (ii) they can be modelled explicitly for different evolutionary stages and phylogenies; and (iii) they can be updated as new empirical information becomes available. Where the data are available, the

Fig. 3. Metabolic plasticity as a Boolean network. (A) Phenotypic plasticity of energy metabolism (metabolic plasticity) can be represented conceptually (A) by an environmental stimulus (e.g. decrease in temperature, cell division or exercise) that decreases the energy status in the cell. In a compensatory response, the cell increases catabolic reactions (blue) and slows anabolism (red), thereby increasing ATP production to restore energy balance. (B) In a hypothetical protocell, an environmental impact that decreased energy status would have increased AMP concentrations, which would have been detected by an energy-sensing molecule, the cystathionine β-synthase (CBS) domain. The CBS domain would have switched the cell towards catabolism and ATP production. (C) From this ancestral state, metabolic networks became more complex to incorporate anabolic regulators such as target of rapamycin (TOR), and the simple CBS domain evolved into the AMP-activated protein kinase (AMPK) to regulate ATP production by chemiosmosis. (D) In animals, increasingly complex metabolic networks incorporated other regulators such as thyroid hormone (TH) and peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1). Chemiosmosis is performed by mitochondria, and muscle function and fibre type (e.g. myosin heavy chains, MHCs) became closely linked to metabolic phenotype and ATP production. However, metabolic networks are not perfectly efficient and there may be potential costs of metabolic regulation such as production of reactive oxygen species (ROS), both of which (E, magenta nodes) would decrease ATP production. (F) The robustness of a network to perturbations increases with increasing complexity (decreasing Hamming distances indicate increasing robustness), and only the more complex biological networks could be more robust than a randomly assembled network with the same components (G; $P<0.05$ is based on simulations of 100 networks and indicates the probability that robustness of biological networks equals that of random networks). (H) Markov chain simulations of the networks show that the probability of achieving compensatory upregulation of ATP decreases when inefficiencies and ROS production are present in the network. Note that the networks shown here (B–E) are realistic empirically but not comprehensive, and serve to exemplify the utility of applying network analyses to study plasticity. Networks were produced in the BoolNet package in R (Müssel et al., 2010).

networks can be expanded in many ways. For example, they can be used to test hypotheses about how metabolic plasticity (compensatory ATP production) is affected by upstream regulators of the nodes shown here (e.g. thyroid stimulating hormone, thyroid releasing hormone), and how feedback from the output (i.e. ATP production) may modify attractor states. In a sense, the Boolean network represents a synthesis of the literature that can be modelled under different contexts. Ideally, a more quantitative approach such as representing relationships between nodes with differential equations would provide more quantitative insights into metabolic plasticity than the logical Boolean approach, which cannot model continuous quantitative responses. However, at this point, empirical data quantifying these relationships are not available, so the logical approach is a good first approximation.

Conclusions

We argue that a fundamental form of metabolic plasticity was a pre-condition for the emergence of cell genealogy and Darwinian evolution at the origin of life. Phylogenetic differences and diversity in the manifestation of metabolic plasticity then arose via the elaboration of underlying mechanisms. Within our framework, the evolutionary origin of metabolic plasticity was not a response to particular environmental conditions, such as variable or stable environments, but is essential in all environments. It is interesting and relevant, however, to understand which environmental contexts promoted the diversity in metabolic plasticity that is evident among modern taxa. For example, genetic drift may have produced diversity (Nielsen, 2009) and changed the pool and expression of underground reactions. Alternatively, selection may have favoured particular reaction systems or networks in particular environmental contexts.

Some interesting questions for future research include: (i) what were the regulatory mechanisms in early biotic cells, and how did they emerge from pre-biotic chemistry?; (ii) how did those regulatory systems evolve to produce the metabolic diversity in extant taxa and (iii) what was the relative importance of genetic and epigenetic mechanisms (Ashe et al., 2021) in shaping regulatory networks within individuals and across phylogenies?; (iv) what is ultimately the best mathematical approach to model these regulatory networks?; and (v) do phylogenetic differences in regulatory networks constrain plasticity and affect the vulnerability of different lineages to environmental change? We think that a mechanistic approach like the one we present here can make an important contribution to answering these questions, and to understanding plasticity and its evolution.

Acknowledgements

We thank R. B. Huey for input on earlier versions of the manuscript.

Competing interests

The authors declare no competing or financial interests.

Funding

F.S. was supported by Australian Research Council Discovery Grant DP190101168.

References

Alexandre, T., Lupan, A., Helynck, O., Vichier-Guerre, S., Dugué, L., Gelin, M., Haouz, A., Labesse, G. and Munier-Lehmann, H. (2019). First-in-class allosteric inhibitors of bacterial IMPDHs. *Eur. J. Med. Chem.* **167**, 124-132. doi:10.1016/j.ejmech.2019.01.064

Aliaga, B., Bulla, I., Mouahid, G., Duval, D. and Grunau, C. (2019). Universality of the DNA methylation codes in Eucaryotes. *Sci. Rep.* **9**, 173. doi:10.1038/s41598-018-37407-8

An, H., Wang, Y., Qin, C., Li, M., Maheshwari, A. and He, L. (2020). The importance of the AMPK gamma 1 subunit in metformin suppression of liver glucose production. *Sci. Rep.* **10**, 10482. doi:10.1038/s41598-020-67030-5

Anashkin, V. A., Baykov, A. A. and Lahti, R. (2017). Enzymes regulated via cystathionine β-synthase domains. *Biochem. (Mosc.)* **82**, 1079-1087. doi:10.1134/S0006297917100017

Ashe, A., Colot, V. and Oldroyd, B. P. (2021). How does epigenetics influence the course of evolution? *Phil. Trans. R. Soc. B* **376**, 20200111. doi:10.1098/rstb.2020.0111

Beaman, J. E., White, C. R. and Seebacher, F. (2016). Evolution of plasticity: Mechanistic link between development and reversible acclimation. *Trends Ecol. Evol.* **31**, 237-249. doi:10.1016/j.tree.2016.01.004

Branvold, D. J., Allred, D. R., Beckstead, D. J., Kim, H. J., Fillmore, N., Condon, B. M., Brown, J. D., Sudweeks, S. N., Thomson, D. M. and Winder, W. W. (2008). Thyroid hormone effects on LKB1, MO25, phospho-AMPK, phospho-CREB, and PGC-1α in rat muscle. *J. Appl. Physiol.* **105**, 1218-1227. doi:10.1152/japplphysiol.00997.2007

Brunk, C. F. and Marshall, C. R. (2021). 'Whole Organism', systems biology, and top-down criteria for evaluating scenarios for the origin of life. *Life* **11**, 690. doi:10.3390/life11070690

Burggren, W. (2018). Developmental phenotypic plasticity helps bridge stochastic weather events associated with climate change. *J. Exp. Biol.* **221**, jeb161984. doi:10.1242/jeb.161984

Burton, T. and Metcalfe, N. B. (2014). Can environmental conditions experienced in early life influence future generations? *Proc. R. Soc. B* **281**, 20140311. doi:10.1098/rspb.2014.0311

Camprubi, E., Jordan, S. F., Vasiliadou, R. and Lane, N. (2017). Iron catalysis at the origin of life. *IUBMB Life* **69**, 373-381. doi:10.1002/iub.1632

Catania, S., Dumesic, P. A., Pimentel, H., Nasif, A., Stoddard, C. I., Burke, J. E., Diedrich, J. K., Cooke, S., Shea, T., Gienger, E. et al. (2020). Evolutionary persistence of DNA methylation for millions of years after ancient loss of a de novo methyltransferase. *Cell* **180**, 263-277. doi:10.1016/j.cell.2019.12.012

Clarke, A. (2019). Energy flow in growth and production. *Trends Ecol. Evol.* **34**, 502-509. doi:10.1016/j.tree.2019.02.003

Copley, S. D. (2020). Evolution of new enzymes by gene duplication and divergence. *FEBS J.* **287**, 1262-1283. doi:10.1111/febs.15299

Craig, P. M., Moyes, C. D., LeMoine, C. M. R. (2018). Sensing and responding to energetic stress: Evolution of the AMPK network. *Comp. Biochem. Physiol.* **224**, 156-169. doi:10.1016/j.cbpb.2017.11.001

da Silva, R. R., Dorrestein, P. C. and Quinn, R. A. (2015). Illuminating the dark matter in metabolomics. *Proc. Natl Acad. Sci. USA* **112**, 12549-12550. doi:10.1073/pnas.1516878112

de Farias, S. T., Rêgo, T. G. and José, M. V. (2015). A proposal of the proteome before the last universal common ancestor (LUCA). *Int. J. Astrobiol.* **15**, 27-31. doi:10.1017/S1473550415000464
Deamer, D. W. (1997). The first living systems: A bioenergetic perspectives. *Microbiol. Mol. Biol. Rev.* **61**, 239-261. doi:10.1128/mmbr.61.2.239-261.1997
Deamer, D. (2017). The role of lipid membranes in life's origin. *Life* **7**, 5-17. doi:10.3390/life7010005
Dial, A. G., Ng, S. Y., Manta, A. and Ljubicic, V. (2018). The role of AMPK in neuromuscular biology and disease. *Trends Endocrinol. Metabol.* **29**, 300-312. doi:10.1016/j.tem.2018.02.010
Dibrova, D. V., Chudetsky, M. Y., Galperin, M. Y., Koonin, E. V. and Mulkidjanian, A. Y. (2012). The role of energy in the emergence of biology from chemistry. *Orig. Life Evol. Biosph.* **42**, 459-468. doi:10.1007/s11084-012-9308-z
Ducluzeau, A.-L., Schoepp-Cothenet, B., Baymann, F., Russell, M. J. and Nitschke, W. (2014). Free energy conversion in the LUCA: Quo vadis? *Biochim. Biophys. Acta Bioenerg.* **1837**, 982-988. doi:10.1016/j.bbabio.2013.12.005
Dutta, P., Ma, L., Ali, Y., Sloot, P. M. A. and Zheng, J. (2019). Boolean network modeling of β-cell apoptosis and insulin resistance in type 2 diabetes mellitus. *BMC Syst. Biol.* **13**, 36. doi:10.1186/s12918-019-0692-0
Fiedler, E. C. and Shaw, R. J. (2018). AMPK regulates the epigenome through phosphorylation of TET2. *Cell Metabol.* **28**, 534-536. doi:10.1016/j.cmet.2018.09.015
Gaur, V., Connor, T., Sanigorski, A., Martin, S. D., Bruce, C. R., Henstridge, D. C., Bond, S. T., McEwen, K. A., Kerr-Bayles, L., Ashton, T. D. et al. (2016). Disruption of the class IIa HDAC corepressor complex increases energy expenditure and lipid oxidation. *Cell Rep.* **16**, 2802-2810. doi:10.1016/j.celrep.2016.08.005
Gibard, C., Bhowmik, S., Karki, M., Kim, E.-K. and Krishnamurthy, R. (2018). Phosphorylation, oligomerization and self-assembly in water under potential prebiotic conditions. *Nat. Chem.* **10**, 212-217. doi:10.1038/nchem.2878
Gómez-García, I., Oyenarte, I. and Martínez-Cruz, L. A. (2010). The crystal structure of protein MJ1225 from *Methanocaldococcus jannaschii* shows strong conservation of key structural features seen in the eukaryal γ-AMPK. *J. Mol. Biol.* **399**, 53-70. doi:10.1016/j.jmb.2010.03.045
González, A., Hall, M. N., Lin, S.-C. and Hardie, D. G. (2020). AMPK and TOR: The yin and yang of cellular nutrient sensing and growth control. *Cell Metab.* **31**, 472-492. doi:10.1016/j.cmet.2020.01.015
Grassi, L. and Tramontano, A. (2011). Horizontal and vertical growth of *S. cerevisiae* metabolic network. *BMC Evol. Biol.* **11**, 301. doi:10.1186/1471-2148-11-301
Guderley, H. (1990). Functional significance of metabolic responses to thermal acclimation in fish muscle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **259**, R245-R252. doi:10.1152/ajpregu.1990.259.2.R245
Guderley, H. (2004). Metabolic responses to low temperature in fish muscle. *Biol. Rev.* **79**, 409-427. doi:10.1017/S1464793103006328
Guzmán, G. I., Sandberg, T. E., LaCroix, R. A., Nyerges, Á., Papp, H., de Raad, M., King, Z. A., Hefner, Y., Northen, T. R., et al. (2019). Enzyme promiscuity shapes adaptation to novel growth substrates. *Mol. Syst. Biol.* **15**, e8462. doi:10.15252/msb.20188462
Hansma, H. G. (2010). Possible origin of life between mica sheets. *J. Theor. Biol.* **266**, 175-188. doi:10.1016/j.jtbi.2010.06.016
Hardie, D. G. (2007). AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nat. Rev. Mol. Cell Biol.* **8**, 774-785. doi:10.1038/nrm2249
Havird, J. C., Fox, C., Neuwald, J. L., Shah, A. A., Mauro, A., Marshall, C. A. and Ghalambor, C. K. (2020). Distinguishing between active plasticity due to thermal acclimation and passive plasticity due to Q_{10} effects: Why methodology matters. *Funct. Ecol.* **34**, 1015-1028. doi:10.1111/1365-2435.13534
Jastroch, M., Oelkrug, R. and Keipert, S. (2018). Insights into brown adipose tissue evolution and function from non-model organisms. *J. Exp. Biol.* **221**, jeb169425. doi:10.1242/jeb.169425
Kaufmann, M. (2009). On the free energy that drove primordial anabolism. *Int. J. Mol. Sci.* **10**, 1853-1871. doi:10.3390/ijms10041853
Kauffman, S. A., Jelenfi, D. P. and Vattay, G. (2020). Theory of chemical evolution of molecule compositions in the universe, in the Miller–Urey experiment and the mass distribution of interstellar and intergalactic molecules. *J. Theor. Biol.* **486**, 110097. doi:10.1016/j.jtbi.2019.110097
King, N. P., Lee, T. M., Sawaya, M. R., Cascio, D. and Yeates, T. O. (2008). Structures and functional implications of an AMP-binding cystathionine β-synthase domain protein from a hyperthermophilic archaeon. *J. Mol. Biol.* **380**, 181-192. doi:10.1016/j.jmb.2008.04.073
Koonin, E. V., Krupovic, M., Ishino, S. and Ishino, Y. (2020). The replication machinery of LUCA: common origin of DNA replication and transcription. *BMC Biol.* **18**, 61. doi:10.1186/s12915-020-00800-9
LeMoine, C. M. R., Lougheed, S. C. and Moyes, C. D. (2010). Modular evolution of PGC-1α in Vertebrates. *J. Mol. Evol.* **70**, 492-505. doi:10.1007/s00239-010-9347-x
LeRoy, A., Loughland, I. and Seebacher, F. (2017). Differential effects of developmental thermal plasticity across three generations of guppies (*Poecilia reticulata*): Canalization and anticipatory matching. *Sci. Rep.* **7**, 4313. doi:10.1038/s41598-017-03300-z
Levin, S. R. and West, S. A. (2017). The evolution of cooperation in simple molecular replicators. *Proc. R. Soc. B* **284**, 20171967. doi:10.1098/rspb.2017.1967
Light, S. and Kraulis, P. (2004). Network analysis of metabolic enzyme evolution in Escherichia coli. *BMC Bioinf.* **5**, 15. doi:10.1186/1471-2105-5-15
Little, A. G. (2021). Thyroid hormone regulation of thermal acclimation in ectotherms: Physiological mechanisms and eco-evolutionary implications. *Mol. Cell. Endocrinol.* **530**, 111285. doi:10.1016/j.mce.2021.111285
Ljubicic, V., Miura, P., Burt, M., Boudreault, L., Khogali, S., Lunde, J. A., Renaud, J.-M. and Jasmin, B. J. (2011). Chronic AMPK activation evokes the slow, oxidative myogenic program and triggers beneficial adaptations in mdx mouse skeletal muscle. *Hum. Mol. Gen.* **20**, 3478-3493. doi:10.1093/hmg/ddr265
Loughland, I. and Seebacher, F. (2020). Differences in oxidative status explain variation in thermal acclimation capacity between individual mosquitofish (*Gambusia holbrooki*). *Funct. Ecol.* **34**, 1380-1390. doi:10.1111/1365-2435.13563
Loughland, I., Little, A. G. and Seebacher, F. (2021). DNA methyltransferase 3a mediates developmental thermal plasticity. *BMC Biol.* **19**, 11. doi:10.1186/s12915-020-00942-w
Marin, T. L., Gongol, B., Zhang, F., Martin, M., Johnson, D. A., Xiao, H., Wang, Y., Subramaniam, S., Chien, S. and Shyy, J. Y.-J. (2017). AMPK promotes mitochondrial biogenesis and function by phosphorylating the epigenetic factors DNMT1, RBBP7, and HAT1. *Sci. Sig.* **10**, eaaf7478. doi:10.1126/scisignal.aaf7478
Martin, W., Baross, J., Kelley, D. and Russell, M. J. (2008). Hydrothermal vents and the origin of life. *Nat. Rev. Microbiol.* **6**, 805-814. doi:10.1038/nrmicro1991
Melkikh, A. V. and Sutormina, M. (2019). Protocells and LUCA: Transport of substances from first physicochemical principles. *Prog. Biophys. Mol. Biol.* **145**, 85-104. doi:10.1016/j.pbiomolbio.2018.12.011
Mendizabal, I., Keller, T. E., Zeng, J. and Yi, S. V. (2014). Epigenetics and evolution. *Integr. Comp. Biol.* **54**, 31-42. doi:10.1093/icb/icu040
Miller, S. L. and Urey, H. C. (1959). Organic compound synthesis on the primitive earth. *Science* **130**, 245-251. doi:10.1126/science.130.3370.245
Milshteyn, D., Cooper, G. and Deamer, D. (2019). Chemiosmotic energy for primitive cellular life: Proton gradients are generated across lipid membranes by redox reactions coupled to meteoritic quinones. *Sci. Rep.* **9**, 12447. doi:10.1038/s41598-019-48328-5
Mullur, R., Liu, Y.-Y. and Brent, G. A. (2014). Thyroid hormone regulation of metabolism. *Physiol. Rev.* **94**, 355-382. doi:10.1152/physrev.00030.2013
Müssel, C., Hopfensitz, M. and Kestler, H. A. (2010). BoolNet—an R package for generation, reconstruction and analysis of Boolean networks. *Bioinf.* **26**, 1378-1380. doi:10.1093/bioinformatics/btq124
Nakahigashi, K., Toya, Y., Ishii, N., Soga, T., Hasegawa, M., Watanabe, H., Takai, Y., Honma, M., Mori, H. and Tomita, M. (2009). Systematic phenome analysis of *Escherichia coli* multiple-knockout mutants reveals hidden reactions in central carbon metabolism. *Mol. Syst. Biol.* **5**, 306. doi:10.1038/msb.2009.65
Nielsen, R. (2009). Adaptionism - 30 years after Gould and Lewontin. *Evolution* **63**, 2487-2490. doi:10.1111/j.1558-5646.2009.00799.x
Noda-Garcia, L., Liebermeister, W. and Tawfik, D. S. (2018). Metabolite–enzyme coevolution: From single enzymes to metabolic pathways and networks. *Annu. Rev. Biochem.* **87**, 187-216. doi:10.1146/annurev-biochem-062917-012023
Ortmayr, K., Dubuis, S. and Zampieri, M. (2019). Metabolic profiling of cancer cells reveals genome-wide crosstalk between transcriptional regulators and metabolism. *Nature Comm.* **10**, 1841. doi:10.1038/s41467-019-09695-9
Piette, B. M. A. G. and Heddle, J. G. (2020). A peptide–nucleic acid replicator origin for life. *Trends Ecol. Evol.* **35**, 397-406. doi:10.1016/j.tree.2020.01.001
Ritson, D. J. (2021). A cyanosulfidic origin of the Krebs cycle. *Sci. Adv.* **7**, eabh3981. doi:10.1126/sciadv.abh3981
Ross, S. R. P.-J., Arnoldi, J.-F., Loreau, M., White, C. D., Stout, J. C., Jackson, A. L. and Donohue, I. (2021). Universal scaling of robustness of ecosystem services to species loss. *Nat. Comm.* **12**, 5167. doi:10.1038/s41467-021-25507-5
Roustan, V., Jain, A., Teige, M., Ebersberger, I. and Weckwerth, W. (2016). An evolutionary perspective of AMPK–TOR signaling in the three domains of life. *J. Exp. Bot.* **67**, 3897-3907. doi:10.1093/jxb/erw211
Salin, K., Villasevil, E. M., Anderson, G. J., Selman, C., Chinopoulos, C. and Metcalfe, N. B. (2018). The RCR and ATP/O indices can give contradictory messages about mitochondrial efficiency. *Integr. Comp. Biol.* **58**, 486-494. doi:10.1093/icb/icy085
Sambamoorthy, G., Sinha, H. and Raman, K. (2019). Evolutionary design principles in metabolism. *Proc. R. Soc. B* **286**, 20190098. doi:10.1098/rspb.2019.0098
Schreiber, A., Huber, M. C. and Schiller, S. M. (2019). Prebiotic protocell model based on dynamic protein membranes accommodating anabolic reactions. *Langmuir* **35**, 9593-9610. doi:10.1021/acs.langmuir.9b00445
Schulte, P. M. (2014). What is environmental stress? Insights from fish living in a variable environment. *J. Exp. Biol.* **217**, 23-34. doi:10.1242/jeb.089722

Schulte, P. M., Healy, T. M. and Fangue, N. A. (2011). Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integr. Comp. Biol.* **51**, 691-702. doi:10.1093/icb/icr097

Scott, J. W., Hawley, S. A., Green, K. A., Anis, M., Stewart, G., Scullion, G. A., Norman, D. G. and Hardie, D. G. (2004). CBS domains form energy-sensing modules whose binding of adenosine ligands is disrupted by disease mutations. *J. Clin. Invest.* **113**, 274-284. doi:10.1172/JCI19874

Seebacher, F. (2018). The evolution of metabolic regulation in animals. *Comp. Biochem. Physiol. B* **224**, 195-203. doi:10.1016/j.cbpb.2017.11.002

Seebacher, F. and Simmonds, A. I. M. (2019). Histone deacetylase activity mediates thermal plasticity in zebrafish (*Danio rerio*). *Sci. Rep.* **9**, 8216. doi:10.1038/s41598-019-44726-x

Sephton, M. A. (2002). Organic compounds in carbonaceous meteorites. *Nat. Prod. Rep.* **19**, 292-311. doi:10.1039/b103775g

Sheikh, B. N. and Akhtar, A. (2018). The many lives of KATs - detectors, integrators and modulators of the cellular environment. *Nat. Rev. Gen.* **20**, 7-23. doi:10.1038/s41576-018-0072-4

Shmulevich, I., Dougherty, E. R., Kim, S. and Zhang, W. (2002). Probabilistic Boolean networks: a rule-based uncertainty model for gene regulatory networks. *Bioinf.* **18**, 261-274. doi:10.1093/bioinformatics/18.2.261

Simakov, S. K., Scribano, V., Mel'Nik, N. N. and Barone, G. (2021). Sicilian serpentinite xenoliths containing abiotic organics with nanodiamond clusters as key model for prebiotic processes. *Geosci. Front.* **12**, 255-263. doi:10.1016/j.gsf.2020.04.008

Sousa, F. L., Nelson-Sathi, S. and Martin, W. F. (2016). One step beyond a ribosome: The ancient anaerobic core. *Biochim. Biophys. Acta Bioenerg.* **1857**, 1027-1038. doi:10.1016/j.bbabio.2016.04.284

Sugiyama, H., Osaki, T., Takeuchi, S. and Toyota, T. (2020). Hydrodynamic accumulation of small molecules and ions into cell-sized liposomes against a concentration gradient. *Comm. Chem.* **3**, 32. doi:10.1038/s42004-020-0277-2

Toner, J. D. and Catling, D. C. (2020). A carbonate-rich lake solution to the phosphate problem of the origin of life. *Proc. Natl Acad. Sci. USA* **117**, 883-888. doi:10.1073/pnas.1916109117

Vancura, A., Nagar, S., Kaur, P., Bu, P., Bhagwat, M. and Vancurova, I. (2018). Reciprocal regulation of AMPK/SNF1 and protein acetylation. *Int. J. Mol. Sci.* **19**, 3314. doi:10.3390/ijms19113314

Wang, R.-S., Saadatpour, A. and Albert, R. (2012). Boolean modeling in systems biology: an overview of methodology and applications. *Phys. Biol.* **9**, 055001-055015. doi:10.1088/1478-3975/9/5/055001

Weiss, M. C., Sousa, F. L., Mrnjavac, N., Neukirchen, S., Roettger, M., Nelson-Sathi, S. and Martin, W. F. (2016). The physiology and habitat of the last universal common ancestor. *Nat. Microbiol.* **1**, 457-458. doi:10.1038/nmicrobiol.2016.116

Weiss, M. C., Achtman, M., Preiner, M., Xavier, J. C., Zimorski, V. and Martin, W. F. (2018). The last universal common ancestor between ancient Earth chemistry and the onset of genetics. *PLoS Gen.* **14**, e1007518-e19. doi:10.1371/journal.pgen.1007518

Wilmers, C. C. (2007). Understanding ecosystem robustness. *Trends Ecol. Evol.* **22**, 504-506. doi:10.1016/j.tree.2007.08.008

Wochner, A., Attwater, J., Coulson, A. and Holliger, P. (2011). Ribozyme-catalized transcription of an active ribozxyme. *Science* **332**, 209-212. doi:10.1126/science.1200752

Woese, C. (1998). The universal ancestor. *Proc. Natl Acad. Sci. USA* **95**, 6854-6859. doi:10.1073/pnas.95.12.6854

Woese, C. R. (2002). On the evolution of cells. *Proc. Natl Acad. Sci. USA* **99**, 8742-8747. doi:10.1073/pnas.132266999

Wolk, S. K., Mayfield, W. S., Gelinas, A. D., Astling, D., Guillot, J., Brody, E. N., Janjic, N. and Gold, L. (2020). Modified nucleotides may have enhanced early RNA catalysis. *Proc. Natl Acad. Sci. USA* **117**, 8236-8242. doi:10.1073/pnas.1809041117

Woronoff, G., Nghe, P., Baudry, J., Boitard, L., Braun, E., Griffiths, A. D. and Bibette, J. (2020). Metabolic cost of rapid adaptation of single yeast cells. *Proc. Natl Acad. Sci. USA* **117**, 10660-10666. doi:10.1073/pnas.1913767117

Xie, X., Backman, D., Lebedev, A. T., Artaev, V. B., Jiang, L., Ilag, L. L. and Zubarev, R. A. (2015). Primordial soup was edible: abiotically produced Miller-Urey mixture supports bacterial growth. *Sci. Rep.* **5**, 537-538. doi:10.1038/srep14338

Zhao, H., Li, T., Wang, K., Zhao, F., Chen, J., Xu, G., Zhao, J., Li, T., Chen, L., Li, L. et al. (2019). AMPK-mediated activation of MCU stimulates mitochondrial Ca^{2+} entry to promote mitotic progression. *Nat. Cell Biol.* **21**, 476-486. doi:10.1038/s41556-019-0296-3