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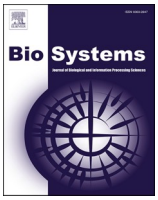

The major evolutionary transitions and codes of life

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ABSTRACT

Major evolutionary transitions as well as the evolution of codes of life are key elements in macroevolution which are characterized by increase in complexity Major evolutionary transitions ensues by a transition in individuality and by the evolution of a novel mode of using, transmitting or storing information. Here is where codes of life enter the picture: they are arbitrary mappings between different (mostly) molecular species. This flexibility allows information to be employed in a variety of ways, which can fuel evolutionary innovation. The collation of the list of major evolutionary transitions and the list of codes of life show a clear pattern: codes evolved prior to a major evolutionary transition and then played roles in the transition and/or in the transformation of the new individual. The evolution of a new code of life is in itself not a major evolutionary transition but allow major evolutionary transitions to happen. This could help us to identify new organic codes.

1. Introduction

The two most widely discussed codes of life (Barbieri, 2008a, 2008b, 2014, 2018) are the genetic code and human language. Their statuses as codes are undisputed. These two pivotal events in the history of life on Earth are also listed as major evolutionary transitions (METs) (Maynard Smith and Szathmáry, 1995; Szathmáry, 2015; Szathmáry and Maynard Smith, 1995). Major evolutionary transitions are characterized by (1) transition in individuality, and (2) by the emergence of a novel inheritance system. Transition in individuality means that a new evolutionary unit emerges either from evolutionary units that thereby lose their status as evolutionary units, or a new evolutionary unit emerges from within an existing evolutionary unit. A code of life is "a set of rules that establish *a correspondence between two independent world.*" (Barbieri, 2008b) (emphasis is in the original) or in more detail "a mapping between the objects of two independent worlds that is implemented by the objects of a third world called adaptors" (Barbieri, 2018). While the definition of codes of life does not imply change in individuality, it has an element of change in information transmission. Both codes of life and METs are strongly connected to information. With regard to METs, how information is used, stored or transmitted (see below) changes, and codes of life are concerned by the meaning of information (i.e. how information from one realm translates to another).

The original examples of major evolutionary transitions by Maynard Smith and Szathmáry (Maynard Smith and Szathmáry, 1995; Szathmáry and Maynard Smith, 1995) included the formation of the cell by the compartmentalization of independently replicating molecules; the linking of these independent replicators into chromosomes; the genetic code; the evolution of eukaryotes from prokaryotes; sexual reproduction; multicellularity; animal societies; and human language. This, apart from the genetic code and human language, is a different list, than the ones mentioned as codes of life. For example, Barbieri (2019) lists sequence code, signal transduction codes, splicing codes, compartment codes, tubulin code, nuclear signalling code, ubiquitin code, molecular codes, lamin code, Hox code, adhesion code, histone code, transcriptional codes, apoptosis code, bioelectric code and neural codes (the list is not exhaustive). The two lists are not the same, which imply that codes of life and major evolutionary transitions are two different and important elements in macroevolution (Barbieri, 2008b, 2019).

However, there are also striking similarities. Barbieri (2008b) lists five characteristics of codes of life that are important for the history of life. (1) *Discontinuity:* Codes of life represent something abruptly novel, not just gradual improvement of something that already exists. (2) *Invariance*: Codes of life do not change in the sense that there is a strong

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selection for their conservation. (3) *Additivity*: More than one types of code can be included in the same lineage, and one code does not erase the other. (4) *Stability:* Each code remains a viable form, and organism harbouring them still exist, thus the appearance of a new code does not invalidate former codes of life. And (5) *Complexity:* The evolution of a new code increases complexity. If we contrast this list with characteristics of the major evolutionary transitions (Maynard Smith and Szathmáry, 1995), then we nearly find the same list. They are fundamental events in the history of life (cf. discontinuity) which always increase complexity. METs are also mostly irreversible (cf. invariance). METs happen in succession too and an organism can be the product of multiple METs. Which also means that a previous MET is not erased by a successive MET (on the contrary, one can be a prerequisite to another). Thus, we can also identify the features additivity and stability. These are not the core of the definitions of codes of life and major evolutionary transitions, but at the core of what is an important event in macroevolution.

In this paper, I will discuss the relation between codes of life and major evolutionary transitions from the perspective of METs. Barbieri (2008b) has offered a perspective from the codes of life point of view. Later he expresses that codes of life are necessarily leading to METs as part of their evolution: "When the ambiguity of a code is completely eliminated, the system starts producing components that have biological specificity and set in motion a major transition in evolution." (Barbieri, 2019) But while codes are important in allowing the transition, they do not lead to major evolutionary transitions. There are more codes of life, than major evolutionary transitions. Also, one type of code can be involved in multiple METs, and one MET might require multiple codes of life. Major evolutionary transitions and codes of life are intimately intertwined, and thus their relationship should be discussed. Here, we discuss them from the point of view of METs.

This paper does not strive to be a comprehensive review on the major evolutionary transitions nor on codes of life. Here, I would just want to make a connection between the two and identify areas worth further research.

2. The major evolutionary transitions

The major evolutionary transitions (METs) (Table 1) are key elements in the history of life on Earth which are characterized by (1) transition in individuality, and (2) a novel inheritance system. Out of these two requirements, the first is more concrete. A new evolutionary unit needs to emerge either from evolutionary units that thereby lose their status as evolutionary units, or within an evolutionary unit that does not lose its status as evolutionary unit. This new evolutionary unit is novel one and not just a copy or slight variation on its predecessor. New evolutionary units form from other evolutionary units either via fraternal or egalitarian transitions (Queller, 1997). In fraternal METs, evolutionary units from related individuals come (or stay) together to produce the new evolutionary unit (e.g. multicellularity, eusociality), whereas in egalitarian METs, the constituent evolutionary units are unrelated (e.g. mitochondria and plastids and their eukaryote host). The key characteristic of these transitions is the loss of the ability to reproduce (get into the next generation) for at least some of the constituent units, and thus reproduction happens at a higher level. The third form of METs is called a filial transition, and language (Szathmáry, 2015) and the adaptive immune system (Müller et al., 2018) are examples of it. Here, the evolutionary unit in which the new evolutionary unit evolves remains an evolutionary unit (like chordates in case of the adaptive immune systems or humans in case of language), and the new evolutionary units form within the existing one.

The second defining characteristic of METs is the evolution of a new inheritance system, which is a novel way of using, storing and transmitting information. This is the less concrete definition as information is so fundamental to living beings, their usage, storage and transmission changes a lot. The evolutionary transitions proposed by Maynard Smith and Szathmáry (Maynard Smith and Szathmáry, 1995) contain the transition from an RNA-world to a DNA-protein world. This – as far as I can tell now – is not a transition in individuality, but an informational transition. Instead of RNA, genetic information is stored in DNA afterwards. Instead of relying solely on ribozyme enzymes as in the RNA world proper, metabolism now also includes peptide enzymes. While previously information was only copied from the genetic storage to produce the enzyme (ribozyme), in the DNA-peptide system (in an RNA-peptide system too!) translation connects these molecular realms. If we accept that the evolution of the genetic code, which allows mapping between nucleotide triplets and amino acids sequences, is a major evolutionary transition then we need to accept that there are METs that are purely informational, and there is no transition in individuality. Consequently, we can distinguish two types of major evolutionary transitions: *transitions in individuality*, which also involve changes in information usage, storage or transmission; and *major informational evolutionary transitions* which does not require new individual, but nevertheless result in a fundamentally new entity.

Table 1
Identified major evolutionary transitions.

Major transition	Type of transition	Change in information usage/storage/ transmission	How many times?
Genetic information	Informational	genetic inheritance	1
Membranes	Informational	structural inheritance	1
Metabolism	Informational	metabolic feedback loops	1
Informational – metabolic infrabiological system	Egalitarian or filial	information is used to produce ribozymes	1
Origin of the cell	Egalitarian	–	1
Chromosome	Egalitarian	genes linked into a few molecules; the first and second error thresholds are solved	1
Genetic code and translation	Informational	genetic code, protein enzymes	1
Mitochondrion as a cell organelle	Egalitarian	genetic information in the cell nucleus and in the mitochondrion	1
Obligate sexual populations	Fraternal	genetic information is stored in the population	many times
Plastid as cell organelle	Egalitarian	genetic information also in the plastid	8–10
Multicellularity	Fraternal	epigenetics	20–22
Neural replicator	Filial	information transmission via electric sign	1
Adaptive immune system	Filial	specialized cells can have a different genome	1
Eusociality	Fraternal		17–18
Animal culture	Filial or Informational	behavioural inheritance	4
Human language	Filial	symbolic inheritance	1

The first MET on the list of examples by Maynard Smith and Szathmáry is the origin of cell by the encapsulation of independently replicating genes. According to the RNA world hypothesis (Joyce, 2002; Kun et al., 2015; Yarus, 2011), RNA replicators acting as ribozymes were encapsulated by a lipid membrane to form the first cell. Replicators are those capable of autocatalytic growth, and they are used here only as such, a more nuanced discussion of the can be found in (Zachar et al., 2013; Zachar and Szathmáry, 2010). This transition led from the realm of supramolecular chemistry to that of biology. This is an egalitarian transition, as different, independently replicating genes form the new evolutionary unit, the cell.

The formation of the cell is the moment from which living beings grace the surface (or depth) of Earth. However, there had to be major evolutionary transitions before it. The appearance of the genetic inheritance system (Jablonka and Lamb, 2005), which is the most well-known inheritance system, being a major informational

evolutionary transitions. But potentially there are more METs before the first cell. According to Tibor Gánti (Gánti, 1971, 2003) a living cell should at least consist of an informational, a metabolic and a compartment subsystem. In contemporary organism, the informational subsystem is the DNA, metabolism is mostly run by peptide enzymes, and the cell is surrounded by a lipid membrane. The compartment subsystem, the cell membrane, has limited heredity (Cavalier-Smith, 2000; Jablonka and Lamb, 2005), but as it is novel heredity system we can consider it a MET. Similarly, the metabolic subsystem is also an autocatalytic system exhibiting heritable variance (Kun et al., 2008). Thus, the formation of all subsystems can be considered an informational MET in their own right.

As the first cell harboured all three subsystems, and all three coming together at the same time to firm the first cell is unlikely, there was a stage when an infrabiological system (*sensu* (Szathmáry, 2005; 2006; Szathmáry et al., 2005)) existed. An infrabiological system consists of two of the three subsystems. The most probable sequence of event was a formation of an informational – metabolic infrabiological system before encapsulation. Replicating ribozymes are such infrabiological system, as they both have information storage in RNA and a metabolism run by ribozymes. The formation of this infrabiological system is also a MET (either a filial or an egalitarian, depending on how it has originated, details of which are not discussed here).

Chromosomes, the stringing of independently replicating genes together is the second MET mentioned in the original list. Since the seminal work by Manfred Eigen (1971), it is known that mutation rate limits the amount of information that can be maintained in one molecule. The critical mutation rate above which the information required by the entity cannot be maintained is called the (first) error threshold. The error threshold might not be as severe as first suggested by Eigen, it can allow nearly a thousand nucleotides instead of a mere 100 to be replicated (Kun, 2021; Kun et al., 2005; Takeuchi et al., 2005). This is still a far cry from the potential minimal metabolism needed by the first ribo-cell (Kun, 2021). If information is stored not in one long strand but in several shorter one, i.e. independently replicating genes, then the information content of the cell could be higher. But at the same time the independent replication and transmission of the genes introduces another error threshold. The second error threshold (Hubai and Kun, 2016; Kun et al., 2015) sets a limit on the number of independently replicating genes, as during random assortment into daughter cells, replicators can be lost. The chromosome solves this problem but cannot evolve before the first error threshold (the one stemming from high copying inaccuracy) is solved. As different genes form a chromosome, this is an egalitarian transition.

The next MET is the evolution of eukaryotes by the endosymbiosis of the mitochondrion (an alpha proteobacterium) with the host cell. But the evolution of the other eukaryotic features such as phagocytosis, endomembrane system, nucleus, etc. is less understood. The origin of eukaryotes proves to be a difficult question (Zachar and Boza, 2020; Zachar and Szathmáry, 2017). Basically, there is no consensus about the nature of the host. Irrespective of these problems, it is an egalitarian transition.

The other METs involving endosymbiosis are the evolution of plastids. In this case, both the host and the symbiont are known. Primary endosymbiosis of a eukaryote and a cyanobacteria happened twice in the history of life on Earth. The ancestors of *Archaeplastida* some 1.5 billion years ago (Parfrey et al., 2011; Yoon et al., 2004) acquired a new cell organelle which is now employed in most photosynthetic eukaryotes. The sole known exception is *Paulinella chromatophora* (*Rhizaria*) which has a plastid of cyanobacterial ancestry independent of the other plastids (Marin et al., 2005). This MET happened a mere 60 million years ago (Nowack et al., 2008). Outside of *Archaeplastida*, other eukaryotes acquired the ability of photosynthesis by endosymbiosis with a photosynthetic eukaryote of primary plastid (for example *Euglenophyta*, photosynthetic *Stramenopiles* or *Chlorarachniophyta*) or endosymbiosis with eukaryotes having secondary plastids (in *Dinophyta*) (Keeling, 2010, 2013). These are also egalitarian transition.

Multicellularity is the most well-studied MET. The origin and diversification of animals and higher plants (*Embryophyta*) (well-known examples of complex multicellular organisms) is a focus of considerable research. Multicellularity evolved several times, mostly by cells not fully separating after cell division. Such colonial organism can then further evolve to have a distinction between gametes and soma, which concludes the transition. *Volvox* is a prime example of the most primitive form of multicellularity: it has two cell types, gametes and flagellate somatic cells (Kirk, 2005). The flagellate somatic cells are important for the individual, also there could be a lot of them, but they cannot produce the next generation of *Volvox*. Contrary to common belief, multicellularity is not confined to Eukarya. Multicellular bacteria exist among cyanobacteria (Schirrmeister et al., 2011), myxobacteria (Reichenbach, 2005), actionomycetes and *Bacillus* (van Gestel et al., 2015). These are fraternal transitions.

Sex as a major evolutionary transition appeared in the original formulation of the theory (Maynard Smith and Szathmáry, 1995; Szathmáry and Maynard Smith, 1995), but its status as MET was questioned in the update (Szathmáry, 2015). I agree with Michod that obligatory sex is an evolutionary transition in individuality (Michod, 2011). Individuals can no longer reproduce by themselves, only a pair can produce offspring. Thus, the requirement of transition in individuality is fulfilled. These are fraternal transitions.

Jablonka and Lamb (2006) proposed the evolution of the nervous system as a MET. Animal nervous system evolved in *Bilateria* (Hirth, 2010). Associative learning which could have been the novelty permitting the Cambrian explosion (Ginsburg and Jablonka, 2010) evolved in *Nephrozoa*. Associative learning helps the individual to learn about its environment, but the novel knowledge cannot by itself be transmitted to new generations. Thus, associative learning while important for behavioural inheritance is not a new hereditary mechanism, and thus it is not a MET. Furthermore, it might not be confined to animals with a central nervous system, as plants can demonstratedly learn by association (Gagliano et al., 2016) and molecular circuits could also exhibit associative learning (Fernando et al., 2009). Still, there could be novel replicators in a central nervous system, that can fulfil our criteria of a MET. Fernando and Szathmáry (Fernando et al., 2012) proposed a mechanism by which neuronal patterns can be copied and selected for in the brain. This can lead to a novel replicator, the neuronal replicator, which can be a new evolutionary unit. Consequently, the nervous system, at least some forms of it, can be considered a MET. In this case, the transition is a filial one.

The adaptive immune system is a well-known evolutionary unit working inside *Vertebrata*. *Agnatha* (lampreys and hagfish) and *Gnathostomata* (the rest of *Vertebrata,* including us) have a different mechanism of generating variability for their antibodies (Cooper and Alder, 2006), but they stem from the same root. Müller and colleagues have recently proposed the adaptive immune system to be a major evolutionary transition of the filial variety (Müller et al., 2018).

Eusociality is defined as an animal society having a sterile caste which is morphologically different from the reproductive caste. The well-known examples of honeybees (*Apinea*), ants (*Formicidae*) and termites (*Isoptera*) fully conform to this definition. Lesser known examples of sterile castes can be observed among wasps (*Vespinae*), *Allopodini*, *Encyrtidae*, aphids (*Aphididae*) and bark beetles (*Scolytinae*). In these insect clades, individuality transitioned to the hive/colony. While the queen reproduces, she cannot do it without the workers. There is a new evolutionary unit. The picture is less clear in the considerably more clades in which females do not lose their ability to reproduce but forfeit reproduction, and a queen produces the offspring.

Behavioural heredity (Jablonka and Lamb, 2005) is a novel hereditary system and it allows animal culture to evolve. Animal culture then can be an informational MET. Gregarious animals exhibiting culture are not eusocial, thus no new individual has emerged, the group is not an evolutionary unit (unlike in eusocial animals). However, culture and its

artefacts can be considered as new evolutionary replicators (Zachar and Szathmáry, 2010). If so then it is a filial transition in individuality. This possibility is not yet fully explored.

The last MET in the sense of being the closest to present in time, is the emergence of human language and the symbolic inheritance system.

We do not know how many times did the first few METs happened. As, for example, all current genetic codes have the same ancestry, it has evolved once for all extant organisms. There could have been independently evolved genetic codes, but there is no trace of them. Major evolutionary transitions in the middle of list (Table 1) has multiple occurrences. While some think it is because these are easy transitions (e.g. (Grosberg and Strathmann, 2007)), I think these are the transitions that had enough time to appear multiple times, but not too long ago so that all other instances are gone because of drift, catastrophes or other reasons. For example, human language evolved a few tens of thousands of years ago, and we cannot expect other lineages discovering it in the same time span. Multicellularity, obligatory sex, plastid endosymbiosis and eusociality are the METs we know the most about, and most of the codes of life discussed below are related to them.

3. Codes of life

A code of life is "a mapping between the objects of two independent worlds that is implemented by the objects of a third world called adaptors" (Barbieri, 2018). I prefer to use the lengthier "code of life" phrase instead of simply "code", as the latter has everyday meanings, including computer codes. Barbieri distinguishes three major groups of codes of life: organic codes, neural codes and cultural codes (Barbieri, 2019). Organic codes are "relationship between two worlds of organic molecules and are necessarily implemented by other molecules, called adaptors, that build a bridge between them." (Barbieri, 2003) Most known codes of life are organic codes. Many of these affect the transcriptional state of a cell. Consequently, it is not a surprise that most organic codes are somehow involved in multicellularity as cell differentiation requires long term change in transcriptional status. Here I will go through the codes of life according to what kind of major evolutionary transitions are they involved in. While above, I have introduced the METs in roughly the same order as they have evolved, here I will start the discussion with multicellularity, and then go on with the other METs.

3.1. Multicellularity

Multicellularity requires cell adhesion (Gumbiner, 1996), extracellular matrix (Özbek et al., 2010; Seifert and Blaukopf, 2010), cell-cell communication (Rokas, 2008), programmed cell death (Fuchs and Steller, 2011; Pennell and Lamb, 1997) and cell differentiation. The most obvious feature of a multicellular organism is the multiplicity of cell types. At its origin, the cells of a multicellular organism had the same genome, yet cell had to differentiate. This require change in transcription, cytoskeletal structure, extra cellular matrix composition, etc. There are signals from other cells or the environment, which taken together determines the fate of the cell. There are organic codes operating in this process. The signals are often chemicals in nature, processed by some protein (the adaptor) which in turn emits another signal (another chemical) or changes its structure which is a signal. Thus, the components of a code are in place. Quite many were already found and described.

A signal transduction code maps signals from the extracellular environment to signals in the internal environment (producing secondary messengers) (Barbieri, 2003, 2008a). The secondary messengers (Newton et al., 2016) in turn can activate tertiary messengers, and so on, producing signal transduction cascades. They are essential to multicellular organisms, but it needs not to be restricted to them. Prokaryotes have their complex signalling pathways (Marijuán et al., 2018), already demonstrating that signal transduction have roles much broader than making multicellularity possible. The usage of cAMP, a prominent secondary messenger, across the tree of life is telling. It was the first secondary messenger to be discovered (Sutherland, 1972), and it is involved in how hormones affect glycogen metabolism. It is also employed as a signal in *Dictyostelium* (*Amoebozoa*) to aggregate and form a multicellular body. Furthermore, unicellular organism belonging to the clade *Excavata*, which is thought to be nearest to the root of Eukaryotes (Cavalier-Smith, 2009, 2013; Cavalier-Smith et al., 2014; He et al., 2014; Moreira et al., 2000), also have cAMP signalling (Ross et al., 1991; Seebeck et al., 2001) along with other unicellular eukaryotes (Shemarova, 2009). This suggest that this form of signal transduction was present when eukaryotes emerged. This claim is further corroborated by the fact that cAMP signalling is also found in bacteria (Gomelsky, 2011).

In a narrow sense, signal transduction is about the transformation of a signal (the first messenger) into an internal signal, the secondary messengers. But there could be a more holistic view that encompasses all organic codes that ultimately effect transcriptional status (Faria, 2008). Accordingly, histone modification is also a code of life (Kühn and Hofmeyr, 2014; Prakash and Fournier, 2018) affecting transcriptional status. By modifying histones, an epigenetic change, the eukaryotic cell can modify the transcriptional level of genes. This, along with a few other mechanisms, is the most inward in the sense of affecting the DNA itself. Going from the DNA toward the cell membrane, we first find the nuclear membrane in eukaryotes. Lamins – proteins associated with the nuclear membrane – are important in animals and they have a role in differentiation (Maraldi, 2018). They are also candidates for being organic codes. It was thought that they are unique to animals (*Metazoa*), but similar elements were found in *Amobeozoa* (Krüger et al., 2012) and later in diverse other Eukaryotes as well (Kollmar, 2015). These findings hint that the primordial elements were present in stem eukaryotes, and later employed in animals for developmental control. The outside of the cell also hosts molecules for recognition and thus can be organic codes. Cell adhesion molecules (Faria, 2018) has been proposed to be part of a code. Components of the cell wall were also proposed to be codes (the glycomic code (Buckeridge, 2018; Buckeridge and de Souza, 2014)), albeit I'm not convinced of them being codes of life as defined here.

As yet another example of a code of life employed in multicellularity, we can look at the splicing code. Eukaryotic genes could have introns, which needs to be excised during mRNA maturation. This process is called splicing. Most splicing is done by the spliceosome, and it was suggested to be an organic code (Baralle and Baralle, 2018). It has its own ambiguity mostly in the possibility of alternative splicing, i.e. one pre-mRNA could yield many different final mRNA. The Dscam gene of *Drosophila melanogaster* can be sliced in 38016 different ways (Schmucker et al., 2000). Alternative splicing is not random, and can be tissue specific and employed in tissue differentiation (Baralle and Giudice, 2017). Thus, the splicing machinery reduces the ambiguity present. The splicing code is quite complex, and a simple sequence feature in itself is unable to predict where splicing will occur (Baralle and Baralle, 2018). However, this is mostly true only for spliceosomal splicing. There is a probably more ancient form of splicing that is catalysed by self-splicing RNAs. These are just catalysed reaction, as self-splicing hinges on RNA based catalysis alone without the involvement of other macromolecule (i.e. there is no adaptor). Moreover, the reaction is very specific, it has to be tailored to each sequence or sequence-structure moiety. Thus, while evolution can produce new self-splicing introns, it is slower than with spliceosomal splicing. The key difference is arbitrariness (see below).

All these examples have a theme in common: they have evolved in unicellulars and were co-opted in multicellularity.

3.2. Major transitions in the origin of life

Major transitions in the origin of life happened mostly in the RNA world. The very first, the appearance of the genetic inheritance system,

is not a code of life (Barbieri, 2019). In later ones, codes could potentially be involved, but as most processes were catalysed or facilitated by RNA molecules, there was less need to link worlds. But signal transduction can give us a hint: while most of the primary signals are proteins (Heldin et al., 2016), so they could not have existed prior to the evolution of protein synthesis, the secondary messengers (nucleotides (mostly in cyclic form), ions, lipids (Newton et al., 2016)) could have existed in the RNA world. The adaptors, the receptors, are now proteins in nature, but sensing can also be achieved by RNAs (Frommer et al., 2015; Winkler and Breaker, 2003). Riboswitches (Vitreschak et al., 2004) are case in points. They have different conformations based on the environment, i.e. the presence of an effector. Thus, there could have been true codes prior to the evolution of protein synthesis, but it is not a necessity. As discussed above, splicing can be achieved by ribozymes acting on very specific substrates. That system is less flexible, and consequently less evolvable, but it still does what needs to be done.

RNA enzymes (ribozymes) fold according to thermodynamical and kinetic rules (Lorenz et al., 2011; Mathews et al., 1999, 2004; Schuster et al., 1994). Thus, the mapping between RNA sequence and ribozyme structure is a direct physicochemical one. The mapping between sequence and structure is complex enough (Kun and Szathmáry, 2015; Schuster, 1997, 2002; Schuster and Fontana, 1998) so that ribozymes are highly evolvable (Kun et al., 2015). Still, there are probably only so much constraints that can be placed on a sequence and its structure. But an organism can adapt its DNA to different environments by changing its GC content and independently adapt their proteins (Radványi and Kun, 2021).

3.3. Cell organelles

Membrane bound organelles exist mostly in eukaryotes. Their proper function requires that molecules are delivered to the organelle they are destined for (Sakhrani and Padh, 2013). Generally, proteins have a sequence feature or group attached to them as targeting signal. To be specific, such proteins are synthesized as pre-proteins with a short terminal signal sequence, the signal peptide, that is recognised by the target location and then cleaved from the protein by a signal peptidase. For example, peptides destined to the mitochondrion have, for example, an N-terminal extensions of some 15–40 amino acids in case of most of the proteins destined to the matrix of the organelle (Stojanovski et al., 2003). As a further example, mannose-6 phosphate is a signal that allow enzymes to be transported to the lysosome (Ni et al., 2006). This targeting is proposed to be a code of life (Barbieri, 2008b).

Examples of adaptors like sortilin (Nielsen et al., 2001), TIM/TOM complexes (Wiedemann and Pfanner, 2017) and TIC/TOC complexes (Andrès et al., 2010; Kovács-Bogdán et al., 2010) have evolved in Eukaryotes. However, there are some known membrane bounded prokaryotic cell organelles such as the magnetosome (Greene and Komeili, 2012), the anammoxosome (Jetten et al., 2009), the acidocalcisome (Docampo et al., 2005), the chlorosome (Oostergetel et al., 2010; Orf and Blankenship, 2013), thylakoids (Vothknecht and Westhoff, 2001) and the internal membranes of *Planctomycetes* (Fuerst, 2005; Sagulenko et al., 2014). This implies that they have to have some targeting as well.

Compartment targeting is then a prerequisite for the functioning of cell compartments, including those that formed via endosymbiosis. Some have probably evolved prior to the evolution of Eukaryotes, again suggesting that codes of life predate the major evolutionary transition they are involved in.

3.4. Obligate sexuality

The origin of sexual reproduction can be traced back to the origin of eukaryotes, but I think the major transition came when some groups become obligately sexual (or when without sexual reproduction population would dwindle (Lajos Rózsa, personal communication)). Sexual reproduction happens by the fusion of cells, consequently those cells need to recognise each other as partners. *Chlamydomonas reinhardtii* cells meet randomly, but only ones having different mating types begin the process of sexual reproduction (Ende, 1985). Here, the flagellar surface acts as a recognition mechanism. These adhesion molecules are codes of life, like the adhesion molecules acting in multicellular organisms (see above in section 3.1). Other unicellular organisms employ active attraction, which is done by chemical signals (e.g. yeast (Merlini et al., 2013); diatoms (Moeys et al., 2016); heterogamous green algae (Starr et al., 1995)). Preparation for the exchange of genetic material (either by full cellular fusion or by a cellular bridge between the partners) is a cell differentiation process. Organic codes similar to the ones acting in multicellularity are making this major transition possible.

Animals can both employ various chemical signals (pheromones) and use their evolved nervous system to find and attract mates. In animals with nervous system, environmental stimuli translate to an electrical signal and processed in this form. Accordingly, neural receptors act as adaptors that map environmental stimuli to the realm of the neural system. We can distinguish neural codes for smell (Grabe and Sachse, 2018), sounds (Farina, 2018; Farina and Pieretti, 2014) and all the other senses as well. Chemicals can act as sexual pheromones. For example, *Bonellia viridis* (an annelid worm) has mostly environmental sex-determination (Jaccarini et al., 1983). Without cues a larva would attach itself onto the bottom and develop as a female. In the presence of a female's pheromones it develops into a male. Insects employ a battery of volatile compounds to attract mates (Roelofs, 1995). Our own species also make use of pheromones (Grammer et al., 2005; Wysocki and Preti, 2004). Furthermore, sounds are employed to attract mates, especially among birds (Eriksson and Wallin, 1986; Kroodsma and Byers, 1991). Other examples can come from whales (Smith et al., 2008), New World monkeys (Snowdon, 1989) and frogs (Kelley, 2004).

As obligate sexuality can be found at vastly different branches of the tree of life, the codes involved are also very diverse. As discussed above, some are organic codes, while animals employ neural codes.

3.5. Eusociality

Workers and soldiers of eusocial animals are sterile or at least they have underdeveloped reproductive organs, and they do not reproduce. This strict reproductive division of labour has to be maintained in primitively eusocial species, that still have potentially reproduction capable workers. Here, I gave just two examples of neuronal (sensory) codes taking part in the maintenance of a eusocial organisation.

Olfactory cues maintain the "rule" of the queen over a colony of paper wasps (*Ropalidia marginata*). In this primitively eusocial species, the queen and the workers are morphologically similar, the difference is the presence or absence of functional ovaries. As long as the queen is present, others won't develop their ovaries (Kardile and Gadagkar, 2003; Shukla et al., 2014), thus maintaining the reproductive division of labour. The queen produces a volatile compound which is present on the surfaces of the colony (Sumana et al., 2008), and signals her presence.

Naked mole rats (*Heterocephalus glaber*) are eusocial mammals (Jarvis, 1981). Only the queen give birth to pups, the other members of the colony help and defend it. As resources are scarce, they are very protective of their underground home (O'Riain and Jarvis, 1997). Member recognition depends on odour (O'Riain and Jarvis, 1997) as well as sound (Barker et al., 2021).

The involved codes are prerequisites in the sense, that communicating the presence of the queen, and colony membership cues need to be present. These mechanisms evolved much earlier and are employed here for a specific purpose.

3.6. Animal society

Groups of gregarious animals are not new units of evolution, but as discussed above, animal culture can be considered a new evolutionary replicator, and behavioural heredity (Jablonka and Lamb, 2005) a new

informational major evolutionary transition. The evolution of the nervous system is a prerequisite for such social system. With the advent of the nervous system, a new code has emerged: the neural code maps the incoming signal from a sensory neuron to that of an effector neuron via intermediate neurons (Barbieri, 2019). This complex mapping (the neuronal network) plays a role in a variety of ecological situation from predation through predator avoidance, foraging, searching for mate and coping with conspecifics. Group living animals use their nervous system to navigate the intricacies of gregarious life. I think, as far as coding is concerned, no new coding has arisen as they are in their conservation phase (*sensu* (Barbieri, 2019)). Barbieri identify phases of a code's evolution: beginning, evolution, optimization, major transition, conservation. I think major transition is not a necessity, rather a possibility in the evolution of a code. The neural codes rarely lead to a major evolutionary transition, albeit they have entered their evolution's conservation phase. There might also be a next step: repurposing or rewiring of a code of life. Codes' strength, from an evolution perspective, is their arbitrariness. Arbitrariness is evident in the mapping, but also in the meaning (purpose) of the code. I have highlighted the role of olfactory and acoustic codes in obligate sexuality. I could as well list examples of olfactory and acoustic communication in animal societies (Seyfarth and Cheney, 2017) and also in eusociality (see above).

4. Evolutionary potential

The pattern inferred from the previous overview is clear: codes of life evolved prior to a major evolutionary transition and then played roles in the transition and/or in transformation of the new individual (*sensu* (Bourke, 2011)) afterwards. This means that the evolution of codes of life is not in itself a major evolutionary transition, but they allow major evolutionary transitions to happen. They do not lead to a major evolutionary transition in themselves, and thus the evolution of an organic or neural code is not the same as a major evolutionary transition. But we find organic codes as prerequisites for METs (Barbieri, 2008b), except in the case of the genetic code and language in which case the evolution of a code of life is a MET. These two transitions, however, are unique: the genetic code is universal, and if there were another genetic code, it left no trace; and there are only one species with full language capability: humans.

Codes of life can facilitate major evolutionary transitions because of their arbitrariness. Marcello Barbieri, the founding father of code biology, emphasises that a code is not just a mapping between two worlds, but an arbitrary mapping. This means that the mapping can also evolve (change). Evolution can proceed without arbitrary coding. As we have seen, splicing by self-splicing RNAs is not arbitrary, and thus it is not a code of life. But spliceosome catalysed splicing is a code of life. Alternative splicing, which in theory can be executed by both systems, has a role in multicellularity. But all the alternatively spliced genes are spliced by spliceosomes and not by individual ribozymes. The flexibility afforded by an arbitrary code increase evolvability. If we just look at how chemical signals can help in multicellularity, searching for a mate or control workers in a colony, then we can understand how versatile codes can be.

I think we should expand on the evolutionary sequence of a code, and add repurposing/rewiring to the end. Codes emerge (beginning), their ambiguity is reduced (evolution) and the mapping is optimized. Here I think the next step is conservation for a given purpose. That purpose might not change, and could be conserved in the lineage, but the code could later be used for other purposes. This phase might be called reuse or repurposing. There might not be many codes of life, or at least they could be fit into a limited number of board categories, but their flexibility help evolution to tinker with them, and use them again and again to increase complexity.

Declarations of competing interest

None.

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