Erschienen in: Journal of Biosciences ; 46 (2021). - 9
https://dx.doi.org/10.1007/s12038-020-00129-z

Evolutionary Cell Biology (ECB): Lessons, challenges, and opportunities for the integrative study of cell evolution

PARSIFAL FIDELIO ISLAS-MORALES[1], LUIS F JIMÉNEZ-GARCÍA[1], MARIA MOSQUEIRA-SANTILLÁN[2] and CHRISTIAN R VOOLSTRA[3]*

[1]*Departamento de Biología Celular, Facultad de Ciencias, Universidad Nacional Autónoma de México, 04510 Mexico City, CDMX, Mexico*

[2]*Red Sea Research Center, Biological and Environmental Science and Engineering Division (BESE), King Abdullah University of Science and Technology (KAUST), Thuwal 23955-9600, Saudi Arabia*

[3]*Department of Biology, University of Konstanz, Konstanz, Germany*

**Corresponding author (Email, christian.voolstra@uni-konstanz.de)*

Evolutionary Cell Biology (ECB) has gained increasing attention in the last decades. Here we explore whether ECB is truly inter-disciplinary through the combination of cellular and evolutionary biology to offer evidence-based insights regarding the major questions of cell evolution. Since 2012, ECB asserts to utilize the increasing potential of high-throughput omics data (*in silico*) with morpho-functional (*in situ*) information, although challenges remain for a complete integration. For instance, the limited number of model organisms and cultivation techniques available excludes the majority of the extant diversity of cells from the scope of experimental inquiry. At the conceptual level, the simplification of evolutionary processes influenced by cultural views of evolution, such as adaptationism or Scala Naturae, challenges effective interdisciplinary work. Without a profound understanding of evolutionary theory and an integrative view of cell biology, the formulation of questions and experiments properly addressing evolution and diversification of cell complexities can become misleading. In 2009, we advanced the discovery of a nucleolus in the flagellated unicellular eukaryote *Giardia lamblia*, and studied nucleolus diversity in other lineages via electron microscopy. Since then, studying evolutionary questions at the cellular level became central to our research. We think that new methodological advances are re-shaping and strengthening the ECB research program and opening its door to experimental scientists. For example, the discovery of new archaea and protozoa and subsequent investigations that coupled *in situ* approaches with *in silico* approaches has proven that comprehensive morpho-functional information can be obtained that can only be understood through the merging of the cell biological and evolutionary discipline. Motivated by this, we here explore the history, the challenges, and the opportunities of ECB to motivate researchers to join this emergent field of research. We outline elements that contrast the current ECB discipline from previous integrative attempts. We conclude by elucidating the current disciplinary constraints of ECB and propose considerations towards successfully employing ECB to answer questions pertaining to the evolution of cellular complexity.

Keywords. Archaea; bacteria; eukaryotes; evolutionary cell biology; nuclear architecture; nucleolus

1. Introduction

> With the theory of evolution, the cell theory is the most important generalization in biology. There is, however, a missing link between these theories that prevents an even more general and unifying concept of life
>
> –Mazzarello 1999

What is evolutionary cell biology (ECB)? A general view is that ECB is an attempt to integrate evolutionary and cell sciences into a major discipline that can address the origins and diversity of cellular complexity, which spans the last centuries. A more recent view posits the name ECB entails a defined and consensual research program of 10 questions, based on the interdisciplinarity between omics, functional experiments, and phylogenetics (Lynch *et al.* 2012; Ford Doolittle 1980). In fact, ECB defines itself as: 'the study of patterns of variation in cellular features within and between species and of the mechanisms (molecular building blocks and population-genetic underpinnings) responsible for their establishment and maintenance' (Lynch *et al.* 2012). As with previous attempts, the 2012 research program of ECB cannot escape the fact that integration between two historical and contrasting epistemic entities is sought. Cell theory and evolutionary theory are probably the most important corollaries in biology, but they depart from different conceptual frameworks: empiricism and historicism, respectively (Mazzarello 1999). Thus, any ECB definition seeks implicitly the integration of two major biological disciplines and is, therefore, a motivation to develop a shared methodology taking into account authors, ideas, theories, techniques, and philosophical views in biology. Its purpose: the ability to study cells, their form, function, and changes.

Parallel to the consolidation of the ECB research program, our research group started working on the evolution of the nucleolus employing comparative Electron Microscopy among protozoa. In 2008, we could confirm that *Giardia lamblia* has a nucleolus (Jiménez-García *et al.* 2008) with the evolutionary implications to be studied using comparative functional and evolutionary approaches in line with the ECB proposed methodology. Evolutionary patterns in cellular diversity are addressed with hypotheses and experiments that transversally derive and use state-of-the-art techniques: 'big data' omics, experimental cell biology, and evolutionary phylogenetics (Lynch *et al.* 2014; Brodsky *et al.* 2012; Lynch *et al.* 2012). Here, omics is understood as the high-throughput acquisition and inventory of biomolecular metainformation of cells, organisms, or environments. These metadata should relate to a cellular context to become meaningful. Cellular context is often represented by morphological information and experiments on cells (*in situ*), but recently also by single-cell genomics. The availability of 'big data' omics technologies, with high-throughput evolutionary bioinformatics and functional *in situ* techniques (e.g., CRISPR-Cas, single-cell genomics) is the first argument that supports the technical capacity of ECB (Lynch *et al.* 2014). Lynch and collaborators have mentioned important technical and conceptual challenges for ECB: first, the lack of model organisms and cultivation techniques from most unicellular lineages; second, the over-simplification of evolution in experimental biosciences; and third, the difficulty to build research programs raising a mixed community of evolutionary and cell biologists (Lynch *et al.* 2014).

Thus, 8 years after the inception of ECB, we think it is time for an examination: how close are we to addressing evolution at the level of cellular organization? The question is intriguing and motivating for biologists that are addressing evolution at the cellular level.

'The Cell' is what we recognize as the structural and physiological unit of all living things since the 19th century (Gonzalez Recio 1990). With ECB, we want to explain the extant diversity of cells; how the forms and functions of their organelles evolved and contributed to organismal complexity, by taking advantage of state-of-the-art techniques in omics, microscopy, and cell biology. The challenges faced are not just technical, but also historical and discipline-inherent. While other reviews on ECB focus on recent perspectives or just mention ECB within a major debate between the integration of functional and evolutionary disciplines in biology, this review explores four centuries of ECB from a disciplinary and historical point of view to understand how the inquiry processes have changed. We are especially motivated to raise consciousness about the philosophical constraints, but also methodological and cultural challenges (figure 1).

2. Understanding of cell evolution through history

2.1 *Pre-disciplinary attempts*

The aspiration to integrate evolution – the notion of change in species — in the understanding of cells was

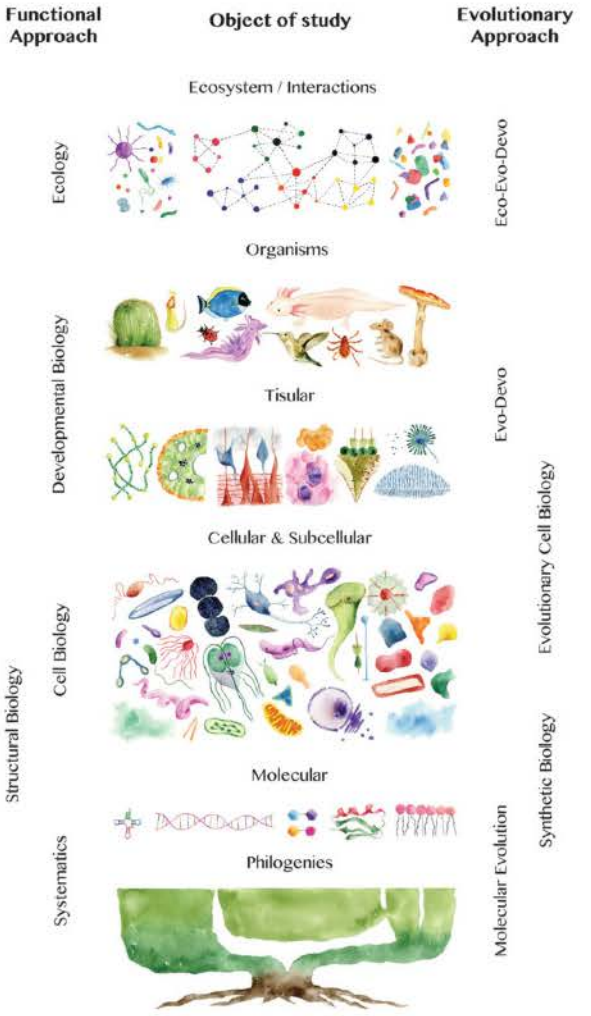

Figure 1. Theoretical position of Evolutionary Cell Biology (ECB). The figure shows an artistic representation of the living diversity emphasizing different levels of organization, which are study objects of biological disciplines. Considering the historical functional/evolutionary divide of biosciences, we show that novel disciplines such as ECB, Evo-devo, and Eco-evo-devo, have the potential to fulfill the gap of evolutionary approaches and research programs that are needed to address evolution at the cellular, tissular, developmental, and ecological level. We recognize the importance of already consolidated evolutionary disciplines (not shown) such as population genetics and comparative biology. Classical approaches are crucial as sources of transdisciplinary planning of new research strategies. Coincidences between disciplines are to be found in the first instance by sharing the same objectives and objects of study.

already present at the beginning of the 17th century. Robert Hooke's Micrographia (Hooke *et al.* 1667) and Anton van Leeuwenhoek's letters on 'Animalcules' opened a new world of (single) cells, followed by a philosophical revolution about life, its diversity, and its origin (Caspers 1964). These early works were driven by the empirical and realistic nature of Francis Bacon's research program, which aimed at the integration of empirical and theoretical knowledge. Therefore, Leeuwenhoek's proclivity to develop work based on experimental and observational evidence was revolutionary. Before Bacon, scholastics had been the principal philosophical program, based mostly on dissertation, hermetics, and logics, but without experimentation and truthful representations of what was seen. Scientific illustration became a technical advantage the new world unveiled by lenses (Alpers 1983). Microanatomical observations did not find a proper theory in the 17th century (Recio 2016). However, Leeuwenhoek's legacy provides interesting ideas that link the fact of microbial diversity to the idea of change and continuity in microscopic forms. He outlined important consiliences and discordances. For example, Leeuwenhoek proposed a divide between so-called gigantic monsters (protozoa) and little animalcules (bacteria) based on morphology, differential cultivation conditions, and their observed abundance. He pointed out similarities between the morphology and movement of bacteria and those of organelles in the protozoan *Colpidium colpoda*. The comparison may be considered the first, albeit unconscious, observations of mitochondria (Lane 2015). He also speculated on the ecology of microscopic organisms based on his observations, e.g. that rotifers disperse by means of transport on the feathers of aquatic birds from one pond to another, explaining the spread in geographical distribution and their relation to each other (Keilin 1959). Leuwenhoek's experiments on the anaerobic cultivation of *infusoria* (amoeba) led him to be convinced that the continuity of life in microbes is based on cell division. Based on this, he supported Reddi's ideas against spontaneous generation (Fildes 1951; Lane 2015) that dominated since Aristotle. Thus, even before disciplines such as cell biology and evolutionary biology were established, early microanatomists started an ontological survey detailing questions and ideas about single-cell organisms, their functions, diversity, and origins.

2.2 *Early interdisciplinary attempts: cell theory encounters Darwinian methodology*

Two centuries after Leeuwenhoek, cell theory was converting progressively into the major axiom

experimental life sciences, hosted under the term physiology. The great advantage was defining cells as the anatomical, functional, and ontogenetic unit of plants and animals. Paraphrasing Nurse, with the cell theory *'The living found its atom'*, cells became a model to explain or grasp the basic units of organisms (Nurse 2003a). In parallel, the Darwinian Theory of Evolution revolutionized biology. In fact, evolution as changes in biological species through time had been the subject of study for centuries, similar to the cells described by Hooke, but it was not until these two theories were accepted that biology became a scientific discipline. The search for a major generalization of both theories became immediately a challenge. It meant the chance to unify biology, a single theory linking form, function, and change, and solving the issue of the origin of cells in the light of evolution (Albarracín 1983). However, interactions between evolutionists and cell physiologists were unilateral rather than interdisciplinary. Cell physiology and cytochemistry built an understanding that the basic unit of reproduction and life continuity was a cellular process, such as mitosis, leaving questions on variation at the cellular level still pending and relying on ideas such as *gemmules* and protoplasm. This remained until 1930 when the detailed mechanisms of inheritance by chromatin and chromosomes were discovered and contextualized as the cellular explanation of Darwinian variation.

Another challenge was that cell evolution and microbial diversity were hard to assess from a Darwinian perspective at first. Ernst Haeckel attempted a comparative approach to link elements of the cell theory with those of Darwin's theory systematizing uniformities, sequentialities, consiliences, and discordances in his observations of microbial diversity (Dayrat 2003). His classification of protozoa and monera at the base of the tree of life represented a major change to the prevalent Linnaean classification, where microbes were still allocated according to Aristotle under the names of Vermes (Worms) and Chaos (formless) (Lane 2015). With Haeckel, single cells became the origin of life and the cells' uniform character relating all life forms. It became evident that cells are diverse and that subcellular characters relate to the classification of unicellular organisms, but also functions of different cell types and tissues in animals, plants, and fungi. To address this evolution of cellular types and functions, Haeckel tried to extrapolate cell generation and evolution with embryonic development.

Haeckel´s biogenetic law is an interpretation of Virchow´s corollary '*Omni cellula e cellula*' suggesting that animal development is the result of cell proliferation. Accordingly, the simplest state of development is a single cell, in this case, the fertilized egg (Gonzalez Recio 1990). This was unfortunately misleading because cell proliferation and differentiation were just starting to be understood, and certainly not all single cells are the same. However, Haeckel thought of the fertilized egg as the homolog of the ancestral unicellular state, and therefore it should resemble the most ancient state of the phylogeny. Logical at first, he was unable to find evidence for his biogenetic law among protozoa due to their diverse and complex cellular organization, which made it impossible to associate their cell morphology with any of the developmental steps of animals. Boveri´s work on the organization of chromatin contributed to the understanding of the complexity in the nuclear structure during development. But even at the level of the cell nucleus, common to all eukaryotes, Haeckel noticed that the wide diversity of morphologies in the nuclei of protozoa was incompatible with his biogenetic law and could not be related to any ontogeny steps in animals.

To the discredit of the biogenetic law, its notion of less and more evolved organisms by means of complexity (from bacteria to protozoa to invertebrates to vertebrates to humans as the most evolved organisms), became one of the most common misunderstandings in biology (Maehle 2011). This representation, also called *Scala Naturae*, is strongly misleading because it depicts evolution as stepwise progress mirroring animal development, rather than the sum of changes over time following a directionless diversifying process.

2.3 *Modern attempts: the concept of bacteria cell in comparative biochemistry*

For long, cell theory and evolutionary theory concentrated mostly on eukaryotes, despite bacteria being the most abundant and diverse group of unicellular organisms, and leaving out their economic and medical relevance. In the early 20th century, bacterial cells started to be understood and classified by comparative biochemistry. Alfred Kluyver's work on fermentations advanced the knowledge on metabolic diversity and prompted the use of biochemical characters in a new bacterial taxonomy (Lane 2015). In 1962, van Niels and Stanier published '*The concept of bacterium*' (Stanier and Van Niel 1962) incorporating the advances of biochemistry and electron microscopy to begin to provide a definition of bacteria. The relevance of biochemical characteristics in phylogenies was stated by Kluyver as follows: 'What is true for the butyric acid

bacteria is true for elephants'. In reference to this, years later Jacques Monod stated: 'What is true for *E.coli* is true for elephants'. Bacterial model organisms became the experimental platform for cellular metabolism (Lane 2015). Evolutionary implications are present in these statements. Namely, that metabolism is conserved across lineages as distant as bacteria, elephants, and plants. Metabolic characters might be useful for a functional taxonomy of bacteria, but are not necessarily evidence of natural groups, since they are present in different lineages. In contrast, the fine *organization of the cellular machineries is more diverse* across the tree of life and evidences specific characteristics of some groups of organisms. Thus, Steiner and Niel concluded that, despite sharing basic principles of metabolism with other organisms, bacteria are a monophyletic group, because they have substantial differences in cell architecture compared to eukaryotes, and these differences are uniform among bacteria, and thus, make them a natural group (Stanier and Van Niel 1962; Lane 2015). Bacteria were no longer called *monera* and thought of as an artificial group.

Recognizing the value of characters as homologies and homoplasies at the cellular level is important in not misunderstanding evolution. While many molecular functions are conserved (homolog), cell structures can evolve independently linked to different functions (homoplastic). Thus, a certain metabolic pathway can be found in different cell compartments across species.

A common misunderstanding in early biochemistry was simplifying the evolution of a certain pathway to the 'adaptation towards thermodynamic perfection'. For example, Hans Krebs suggested that the origin of his homonymous pathway in the prebiotic world took place because multiple steps of degradation and oxidation are energetically 'perfectly adapted' in contrast to direct acetate oxidation (Baldwin and Krebs 1981). Similarly, Jacques Monod was convinced that allosteric regulation was the result of linear adaptation towards perfect enzyme and substrate interaction (Morange 2010). (This would mean that a metabolic pathway that involves several steps and degrees of molecular and cellular complexity can only evolve in one direction and should be the same in all livings, rather than being part of a larger process of metabolic diversification. Following this notion, how would one explain the exceptions and alternative pathways to the Krebs cycle that are found in the extant microbial diversity? Furthermore, the above posits remain to explain how 'imperfect' parts of the Krebs cycle remained over evolutionary time or were recruited into the pathway in the first place.

The problem is to dismiss variation and diversification (there is only perfection in a given context), which are found in cellular diversity that evidences cases of co-evolution and adaptive radiation. Thinking of evolution just as a process of selective pressure shaping a perfect adaptation is a common mistake in biochemistry (Morange 2009, 2010). Metabolism evolves, but it is not disentangled from a changing cellular context. To address these issues and to be consistent with evolutionary theory, one has to understand the importance of randomness, imperfection, which becomes readily visible if one moves away from model organisms and focuses on diversity across organisms. In fact, evolution has no direction and not every feature is the product of adaptation to the environment. A conclusion of these early attempts integrating evolution and biochemistry is that biologists, despite recognizing evolutionary theory, still struggle to apply it to their understanding of the molecular biology of the cell, which is a complex and diverse collection of features, not just an adaptive trait. Effective ECB should therefore emphasize that teleological and panadaptationist views are to be avoided.

2.4 *Direct antecedents of ECB*

Systematic and evolutionary biology were revolutionized when biochemical and morphological taxonomies were extended through the use of molecular characteristics. Shortly after the discovery of the DNA double helix structure in 1953, Francis Crick wrote: 'Biologists should realize that before long we shall have a subject which might be called 'protein taxonomy'—the study of amino acid sequences of proteins of an organism and the comparison of them between species (Crick 1958; Cobb 2017). In 1962, the Zuckerkandl and Pauling paper on Molecules as documents of evolutionary history outlined how changes in amino acid and nucleotide sequences can be used to build phylogenies and construct evolutionary relationships between species (Zuckerkandl and Pauling 1965).

In the next decade, advances in DNA and protein sequencing were developed and gave rise to the discipline of molecular evolution (Stent 1968). The informative value of molecular characters relies on the uniformity of the central dogma (DNA-RNA-Protein) across species, which also explains the source of variation at the molecular level. Was the cell substituted by DNA as the basic unit of life? In molecular evolution, characters are condensed into 4 letters (A, C, G,

T) and phylogenies are inferred using statistical approaches (Grahame and Avise 1995).

However, linking molecular phylogenies with complex cellular features became not meaningful until after Woese's 1990 paper on the tree of life (Woese, Kandler, and Wheelis 1990). With a vision of microbial diversity, Woese suggested a revolution to Steiner & Niel's concept of bacteria (Stanier and Van Niel 1962). Life is divided in three domains Eukarya, Bacteria, and Archaea based on the supremacy of one molecular character –the ribosomal gene - over morphology and metabolism. But what happens with the history of all complex cell features: Do they rely only on gene sequences? Luckily, the ribosomal gene phylogeny of Woese was consistent with later investigations on archaeal cell structure; structural differences sustained the vision of a three domains tree of life (Zillig 1991). However, the tree remained un-rooted. A one-gene phylogeny is reductionist and unable to explain gradual changes in cell complexity. One has to ask the impossible question of how archaea derived from bacteria. If the tree was rooted, the question would be how archaea had originated within bacteria, and the same applies to how the eukaryotes originated within the archaea. When Lynn Margullis proposed endosymbiosis, she took the distance of Woese's molecular reductionism and developed her theory based on comparative morphology (Sagan 1967). Later her theory got validated by molecular data, evidencing the chimeric nature of genomes and organelles. She concluded that genes are not enough to explain evolution, it is necessary to incorporate morphology and cell biology to phylogenomics in order to address the evolution of different degrees of cell complexity (Margulis and Fester 1991; Dyall and Johnson 2000). In 1980, Doolittle already anticipated the need for an integrative view of cell evolution: 'Developments in micropaleontology, RNA and protein sequencing, and the analysis of genome organization suggest a view of early cellular evolution radically different..' (Ford Doolittle 1980). Thirty years later, the tree of life is an always-changing conception due to the integration of more data, in particular big data coming from *omics* approaches. However, our view of early cell evolution is still debating between Woese´s un-rooted tree domain and the never-ending question: how did eukaryotic features arise? (Nick 2009). Gene evolution alone does not explain complex features, and comparative cell biology may not be simplified enough to the level at which gene evolution operates.

The use of metagenomics and high throughput phylogenomics has contributed to the solving of this problem. Only in the last ten years, five new phyla of archaea have been discovered due to metagenomics and were classified into a new superphylum embracing Thaumarchaeota, Aigarchaeota, Crenarchaeota, Korarchaeota, and recently Lokiarchaeota (TACK) (Nunoura *et al.* 2011; Guy and Ettema 2011; Spang *et al.* 2015). This new diversity, analyzed with novel supermatrix approaches that include molecular and morphological characters, led to propose the hypothesis of a two-domain tree of life. This means that eukaryotes are rooted within Archaea because TACK is its sister lineage. (Williams *et al.* 2013; Gribaldo *et al.* 2010; Guy and Ettema 2011). This rooting enables us to explore the cellular nature of LECA, which probably evolved gradually within the archaeal diversification. Such types of approaches are direct antecedents of modern ECB because they evidence that new tools in evolutionary biology enable the integration of molecular, functional, and morphological data that can become biologically meaningful to address questions from a cell biology perspective advancing the understanding of cell evolution.

3. The core of evolutionary cell biology

Modern ECB comes with the challenge of relating high throughput molecular approaches with a spatial context of cell structures and functions. This is essential to give a biological meaning to omics data at the cellular level of evolution. Here, the concept of the cell as the functional and structural unit becomes useful as its methodological framework expands and profits from new interdisciplines. Summarizing the biological meaning of cell evolution, according to Michael Lynch '*All aspects of biological diversification ultimately trace to evolutionary modifications at the cellular level'* (Lynch *et al.* 2014). This is a central corollary at the core of contemporary ECB. Cellular level means not just diversity of organelles and functions, but considering interactions and *in situ* phenomena that happen at the levels of the molecular and the organismic scale. Certainly, the exploration of cell dynamics *in vivo* and *in situ* with regards to diversity and evolution is an almost pristine field of study previously limited by the availability of tools that we have today such as time-lapse microscopy, *in situ* omics, and integrative phylogenomics. Thus, the modern ECB as proposed by Lynch and collaborators in 2012 claims that transdisciplinary interactions between cell biology and evolutionary biology are now possible, and it is useful to study intriguing jumps in cell evolution, such

as in eukaryogenesis and the origin of multicellularity (Lynch *et al.* 2014). Both are also considered major transitions in evolution (Smith and Szathmary 1997). As in previous attempts, these new interdisciplinary challenges need to encompass epistemic differences between functional and evolutionary biology.

Within Lynch's corollary, there is an implied assumption: we can use the mechanistic nature of the cell and molecular biology to explain the phenotypic diversity of cells. This is consistent with the fact that cell biology is a reductionist advancing explanation through empirical evidence and analysis of parts and finally synthesis. In contrast, evolutionary biology builds upon historical evidence and narrative as a methodology to confirm its theory. Can this evolutionary approach be integrated into the process of investigation of cell biology? First, one should recognize that evolutionary biology has become more reductionist due to the realm and acceptance of statistically resolved phylogenies. For example, analytical approaches can be applied to gene or protein sequences to predict an ancestral protein function, concluding that some protein domains can be considered evolutionary building blocks for certain biological functions. Furthermore, the results of these types of analysis can be proven using tools from cell biology, e.g. synthesizing ancient proteins in the laboratory and confirming functional properties. This is in fact a reductionist approach that addresses a question in evolution using functional biology. This interdiscipline has been called 'the functional synthesis' (Dean and Thornton 2007; Morange 2011a, b). At this level, the evolutionary approach (e.g., phylogenomics) does not conflict with the reductionist nature of functional disciplines such as cell biology or structural biology, because both have developed tools that are reductionist for the study of evolution and mechanisms of proteins.

It becomes more complex, functionally and evolutionary, when we focus on the gap, still unexplored, between single molecules and cellular landscapes. Cells provide a rich universe of study objects in evolution at different complexity levels (Nurse 2003b). This is linked but cannot be reduced to the study of biomolecules in the spirit of the functional synthesis. To this end to complex cellular features are converted into supramolecular and morphological characters using evolutionary bioinformatics and then hypotheses can be tested with experiments using cell biology approaches

Technical and disciplinary challenges are to be faced in modern ECB. The technical challenge is that the diversity of model organisms should increase in order to fairly represent the known cellular diversity. The disciplinary challenge is that for cell biology to be complementary to evolutionary inquiry, it has to look after information in diversity at the cellular level. A comparative approach, the same way as Darwin, is necessary for both disciplines to converge methodologically into the same object of study: evolution at the cellular level of organization.

The core of the Lynch *et al.* proposal takes this into account and asks four carefully formulated questions: (1) Why are cells the way they are and why are they not perfect? (2) How do cellular innovations arise? (3) Where do cellular innovations map onto the tree of life? (4) How can ECB be effectively implemented? (Lynch *et al.* 2014).

4. Inquiry process in evolutionary cell biology

A common example of ECB in action is the reconstruction of ancestral states of an organelle or the architecture of a cellular pathway (Lynch *et al.* 2014; Richardson *et al.* 2015). According to the authors, the inquiry process in ECB departs from metagenomics and metaproteomics analysis. One should compare the functional cellular constraints known in model organisms with the clues from meta-analysis of the unknown diversity. As in functional synthesis, protein domain analysis can be used as a powerful tool to address the homology of functional entities in the different cell types or lineages of microbes. Once, proteins, protein domains, and related cell features are mapped as characters on comprehensive phylogenies, one can formulate new questions based on bioinformatics analysis, for example, about the biology of a common ancestor. Here is where functional studies from cell biology (*in situ* experiments, microscopy, etc.) can be conducted to answer specific questions in non-model organisms. Comparative morphology using modern high throughput imaging tools plays two major roles in this process: first, it helps to revise and correct morphological characters with natural meaning in the new phylogenies, and second, it helps to resolve experimental studies regarding structure, topology, and localization of cell functions at the nano- and microscale.

As a result, evolutionary narratives will be able to rely on more statistical and functional evidence. Using cell features as hierarchical characters in addition to molecular characters, evolutionary bioinformatics can encompass complex cellular features into its analysis and formulate questions that are addressable using

recent advances in cell imaging and environmental microbiology, especially *in situ* approaches, given the lack of model organisms. Little by little more detailed cellular processes can be incorporated into this methodology and unveil consiliences and discordances at different levels of cellular complexity, advancing the understanding of cells.

5. Perspective for ECB in coming years

> The uniqueness of biology relies on the epistemology of evolutionary theory, an aspect every functional biologist should understand to grasp the nature of life
>
> –Mayr 2007

Based on this review, we think that technical challenges in microscopy, in situ omics, and bioinformatics will experience continuous improvements. However, the most prominent challenges in the near future of ECB are connected to educational and cultural constraints among biologists. In particular, our concern is that evolutionary theory can effectively pervade scientists from entering ECB.

The scholar Michel Morange has made a philosophical analysis of how evolutionary biology has pervaded functional biology in the last decades. He claims that previous attempts in the 19th and 20th to unify bio-disciplines with evolutionary biology failed, because of an improper understanding of evolution by experimental biologists. Morange explains that: 'The first obstacle was clearly an ignorance of the complexity of evolutionary theory, and of the transformations it underwent throughout the twentieth century (…). The vision of Darwinian evolution held by most functional biologists was closer to that of Herbert Spencer—in which progress is the motor of evolution than to the vision of Darwin himself' (Morange 2010). This has been reflected in historical examples provided in this review. Often in the history of cell biology, evolutionary processes have been over-simplified in the cultural understanding of biological adaptationism.

We think that evolutionary theory should not just superficially touch, but critically improve and pervade, the mechanistic explanations of biological phenomena in experimental disciplines and *vice versa*. This is critical for ECB to succeed. In this regard, increased attention to philosophy and the history of science in biological education should be improved to avoid oversimplification of ideas in evolution. Moreover, with a stronger philosophical and historical foundation, scientists realize that new disciplines such as ECB are objects of philosophical discussion and that research programs should benefit from a community prompting epistemological and historical culture in order to identify and critically examine common values between disciplines and achieve real transdisciplinarity.

We are convinced that ECB represents a promising opportunity for methodological transdisciplinarity and theoretical consilience between functional and evolutionary biology. ECB may not provide a revolutionary paradigm shift in biology, but the discipline shapes the convergence of two major theoretical frameworks towards robust theories around the origin and evolution of the cells, one of the most cryptic and unifying dimensions of life.

6. Conclusions

1. Since 2012 many research projects and programs are becoming consolidated around the conceptual framework of ECB. In order to remain successful, ECB should improve a profound understanding of evolutionary theory and its epistemic and methodological differences to functional cell biology. Among its scientific community, evolutionary theory must become a non-dogmatic and dynamic theoretical background. Thus, experimental hypothesis can organically incorporate evolution into functional approaches and *vice versa*.
2. ECB can also contribute to addressing the role of cellular modifications in the evolution of higher levels of biological organization such as tissues, or even the development of organisms.
3. Experimental advances in omics, microscopy, and cell biology are becoming essential for the process of inquiry in evolutionary cell biology because it facilitates the detailed study of cells from different lineages, enabling comparative approaches, and advancing evolutionary hypotheses with regards to the extant microbial diversity.
4. ECBs main challenge is educational and cultural. For ECB to be successful, coming generations of researchers and scientists need to acquire a broad and robust background in evolutionary theory and methodology, regardless of their specialization. However, misleading views and simplifications of evolutionary theory (e.g., adaptationism) are still common among the scientific community worldwide. Thus, education in biosciences should improve the teaching of evolution with the same

effort it has been improving technical skills in cellular, molecular, and computational biology. This is critical for biologists from different disciplines to understand each other methodologically and in order to establish integrative approaches built on common epistemic values.

Acknowledgements

We want to thank our colleagues: Alejandro Fábregas Tejeda from Instituto de Biología at UNAM for his critical reading and comments on History of Science; Silvia Juárez Chavero and Teresa Jiménez Segura for her technical assistance; Prof. Edmundo Calva from Instituto de Biotecnología and Prof. David Romero from Centro de Ciencias Genómicas at UNAM for their critical reading. We also want to thank the two anonymous reviewers who greatly contributed to the final form of the manuscript with their critical reading and input. Parsifal Fidelio Islas- Morales is doctoral student from the "Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México (UNAM) and has received CONACyT fellowship 495217

Appendix

Examples of ECB

Multicellularity and development: How organisms became multicellular is a question within the scope of inquiry of ECB that can contribute to the transdisciplinary research field of evolutionary developmental biology (informally, evo-devo). Evo-devo compares developmental processes across organisms to infer how developmental processes evolved. In the work of Nicole King on the origin of the multicellular condition, the cellular basis of development is split into characteristics and mapped onto the most recent eukaryotic phylogenies. Her work highlights that multicellularity has appeared many times in different lineages (e.g., across plants, fungi, and animals) and that many of the features of multicellularity were already present in unicellular ancestors. The different ways of how these ancestors evolved cell complexity sheds light upon the changes at the cellular level that prompted the evolution of developmental systems across the tree of life (King 2004).

Gradualism in eukaryogenesis – A focus for ECB: ECB has the potential of redefining fundamental questions in eukaryogenesis.

For example, gradualist scenarios are a more plausible way to explain the idiosyncratic cell complexity of eukaryotes, if we observe a rooted tree of life. Otherwise, the FECA-LECA transition is by itself a non-gradualist conception based on the assumption that ancient proto-eukaryotic lineages disappeared. In fact, if cell complexity was acquired gradually, we have limited understanding with regard to how simple or even how eukaryotic a hypothetical FECA should have been. In a gradualist scenario, the nature of the sister group of eukaryotes is critical to explore in order to define the attributes exclusively associated with eukaryotes and which attributes were already present in common ancestors. Based on this, we can ask: how are *in silico* evidenced homologs entities organized and functionally involved in the cells of the sister group of eukaryotes? Can we find them in any kind of ancestral compartment, or do they provide different functions related to a completely different cellular context?

In a two-domain tree of life, TACK-Archaea are the monophyletic sister group of eukaryotes. TACK-Archaea then represent a source of evolutionary characters and ancestral states through comparative approaches. As pointed out by Simonetta Gribaldo: in a two-domain tree of life late or early mitochondrial endosymbiosis does not affect a gradualist cellular diversification. FECA is no longer the un-rooted starting point of eukaryogenesis. And eukaryogenesis converts into a result of cell evolution within the TACK Archaea (Williams *et al.* 2013; Gribaldo *et al.* 2010).

The question for ECB would be: which eukaryotic cell features do TACK-Archaea have? Based on the extant and still unveiled diversity of TACK Archaea, how can we approach a real gradualist reconstruction for each common ancestor?

We should be aware of the possibility that many TACK-archaeal lineages, which represent intermediate states, might be already extinct or are highly modified. What is the proof of concept to establish homology between archaeal and eukaryotic compartments or structures? TACK archaeal genes and protein domains are heterogeneously conserved and derived among different eukaryotic families. The notion of a Dispersive Archaeal Eukaryome (Koonin and Yutin 2014) suggests that a comparative approach to prokaryotic and protist cell architecture is needed in order to relate molecular homologs to their cellular context, their putative function, and their evolutionary meaning. For instance, homology between structures can be established if the products of molecular homologs are localized in the same cellular feature(s) in two related lineages. Even if the compartment or structure is highly

derived, cell compartments of distant lineages can be homologous if molecular characters support a monophyletic relation and can be structurally related to a similar cellular context. Then the structure can be attributed to a common ancestor or be seen as a derived character.

Omics-driven ECB: In 2016 Pittis and Gabaldón proposed that the acquisition of mitochondria was a late event in eukaryogenesis. Using an omics approach these authors identified monophyletic groups of genes to be present in LECA with their prokaryotic orthologs. So-called Eukaryotic Protein Families (EPF) are monophyletic and relate to specific cell compartments being able to explore its history between prokaryotes and LECA, namely in the FECA-LECA transition. For instance, alpha-proteobacterial proteins represent mitochondria, while fibrillarin orthologs in Archaea represent nucleoli. By reconstructing phylogenies of each EPF, and evaluating branch lengths separating eukaryotic and prokaryotic sequences with a molecular clock methodology, they conclude that EPFs of archaeal origin tended to be closer to FECA, whereas genes of mitochondrial were closer to extant eukaryotes. The authors suggested that with this measure, as mitochondria have the shortest phylogenetic distance, they would have been the last organelles to be incorporated in an evolving FECA-LECA transition. In contrast, membrane trafficking compartments and ribonucleoproteins had been acquired long before the mitochondrial acquisition (Pittis and Gabaldón 2016). These studies have been criticized by Martin and collaborators, pointing out that the difference in stem lengths of EPFs are not statistically significant, due to the use of a strict molecular clock, meaning evolutionary rates can be considered the same across the complete phylogeny (Martin *et al.* 2017). According to Roger and collaborators: 'this objection fails to acknowledge the possibility that even if no single gene evolved in a clock-like manner, increases and decreases in rates across lineages and proteins could cancel so that the average of stem lengths of a large protein set may be roughly clocklike' (Roger *et al.* 2017).

Despite this methodological discussion, Pittis and Gabaldón conclusions are interesting in the light of ECB. Based on a two-domain tree of life, if EPF homologs existing in TACK-archaeal phyla are mainly orthologs, the search for the ancestral states of eukaryotic compartments in the order suggested by these authors should rely on experimental studies of extant TACK-Archaea. An approximation to the nature of early cell compartments could be addressed with the following question: How are these orthologs distributed and organized cells of Archaea?

Nuclear architecture: Nuclear architecture involves the form and function of nuclear bodies (Misteli 2005). These concepts are inherently related to the question of the origin of the cell nucleus. A special example is the nucleolus. The nucleolus is a multiproteic complex of close to 300 different proteins that are involved with processes as important as programmed cell death, metabolic regulation, cell differentiation, stress, and aging (Boulon *et al.* 2010). All these processes converge to the core process occurring in the nucleolus, namely the transcription and maturation of ribosomal RNA. The nucleolus can be considered a domain of ribosomal gene expression.

The discovery that *Giardia lamblia* (a flagellated protozoan and intestinal parasite with pediatric relevance) harbors a nucleolus (Jiménez-García *et al.* 2008) was for us the motivation to address the evolution of nuclear architecture from an ECB perspective. Phylogenies placed *Giardia* as part of 'primitive' or 'early branching lineage', known as diplomonads. These notions were misleading because eukaryotic phylogenies lack a root. We don´t know if *Giardia* were among the first eukaryotes to branch, and certainly, since *Giardia* are parasites with a long history of secondary losses and modifications of their organelles, there is no compelling argument to assume that they are primitive in comparison to other protozoa. However, this ambiguity of the evolutionary meaning of Giardia revealed the real value of our contribution. Before our confirmation, *G. lamblia* was considered the only anucleolated eukaryote. The discovery of *Giardia* nucleoli implicated a major advance, namely that, regardless of un-rooted phylogenies, nucleoli represent a character shared among all eukaryotes (a synapomorphy). The Last Eukaryotic Common Ancestor (LECA) should also harbor a nucleolus.

References

Albarracín A 1983 *La teoría celular: historia de un paradigma*.

Alpers S 1983 With a Sincere Hand and a Faithful Eye: The Craft of Representation. In *Svetlana Alpers: The Art of Describing. Dutch Art in the Seventeenth Century.* University of Chicago Press: Chicago pp 67–69

Baldwin JE and Krebs H 1981 The evolution of metabolic cycles. *Nature 291* 381–382

Boulon S, Westman BJ, Hutten S, Boisvert F-M and Lamond AI 2010 The nucleolus under stress. *Mol. Cell* **40** 216–227

Brodsky FM, Thattai M and Mayor S 2012 Evolutionary cell biology: Lessons from diversity. *Nat. Cell Biol.* 14 651

Caspers H 1964 *Dobell, Clifford: Antony van Leeuwenhoek and his "Little Animals". New* York, N. Y.: Dover Publ. Inc. pp 503–503

Cobb M 2017 60 years ago, Francis Crick changed the logic of biology. *PLoS Biol.* **15** e2003243

Crick FH 1958 On protein synthesis. *Symp. Soc. Exp. Biol.* **12** 138–163

Dayrat B 2003 The roots of phylogeny: how did Haeckel build his trees? *Syst. Biol.* **52** 515–527

Dean AM and Thornton JW 2007 Mechanistic approaches to the study of evolution: the functional synthesis. *Nat. Rev. Genet.* 8 675–688

Dyall SD and Johnson PJ 2000 Origins of hydrogenosomes and mitochondria: evolution and organelle biogenesis. *Curr. Opin. Microbiol.* **3** 404–411

Fildes P 1951 Leeuwenhoek lecture; the evolution of microbiology. *Proc. R. Soc. Lond. B Biol. Sci.* **138** 65–74

Ford Doolittle W 1980 Revolutionary concepts in evolutionary cell biology. *Trends Biochem. Sci.* **5** 146–149

Gonzalez Recio JC 1990 Elementos dinámicos de la teoría celular. *Revista de filosofia (Madrid)* **3** 83–109

Grahame J and Avise JC 1995 Molecular markers, natural history and evolution. *J. Anim. Eco.* 64 538

Gribaldo S, Poole AM, Daubin V, Forterre P and Brochier-Armanet C 2010 *The origin of eukaryotes and their relationship with the Archaea: are we at a phylogenomic impasse? Nature Reviews Microbiology* **8** 743–752.

Guy L and Ettema TJG 2011 The archaeal 'TACK'superphylum and the origin of eukaryotes. *Trends Microbiol.* **19** 580–587

Hooke R, Allestry J, Allestry J and Royal Society (Great Britain) 1667 *Micrographia, or, Some physiological descriptions of minute bodies made by magnifying glasses : with observations and inquiries thereupon.*

Jiménez-García LF, Zavala G, Chávez-Munguía B, Ramos-Godínez MP, López-Velázquez G, Segura-Valdez ML, Montañez C, Hehl AB, Argüello-García R and Ortega-Pierres G 2008 Identification of nucleoli in the early branching protist Giardia duodenalis. *Int. J. Parasitol.* **38** 1297–1304

Keilin D 1959 The Leeuwenhoek Lecture - The problem of anabiosis or latent life: history and current concept. *Proc. R. So. London. Se. B Biol. Sci.* **150** 149–191

King N 2004 The unicellular ancestry of animal development. *Dev. Cell* **7** 313–325

Koonin EV and Yutin N 2014 The dispersed archaeal eukaryome and the complex archaeal ancestor of eukaryotes. *Cold Spring Harb. Perspect. Biol.* **6** a016188

Lane N 2015 *The unseen world: reflections on Leeuwenhoek (1677) 'Concerning little animals.' Philosophical Transactions of the Royal Society B: Biological Sciences* **370** 20140344.

Lynch M, Field MC, Goodson HV, Malik HS, Pereira-Leal JB, Roos DS, Turkewitz AP and Sazer S 2014 Evolutionary cell biology: two origins, one objective. *Proc. Natl. Acad. Sci. U. S. A.* **111** 16990–16994

Lynch M, Goodson HV and Turkewitz A 2012 *Evolutionary Cell Biology: A report of the findings of the NSF-sponsored Workshop on Evolutionary Cell Biology held May 29-June 1.*

Maehle A-H 2011 Ambiguous cells: the emergence of the stem cell concept in the nineteenth and twentieth centuries. *Notes Rec. R. Soc. Lond.* **65** 359–378

Margulis L and Fester R 1991 *Symbiosis as a Source of Evolutionary Innovation: Speciation and Morphogenesis*. MIT Press.

Martin WF, Roettger M, Ku C, Garg SG, Nelson-Sathi S and Landan G 2017 *Late Mitochondrial Origin Is an Artifact. Genome Biology and Evolution* 9p.: 373–379.

Mayr E 2007 *What Makes Biology Unique?: Considerations on the Autonomy of a Scientific Discipline*. Cambridge University Press.

Mazzarello P 1999 A unifying concept: the history of cell theory. *Nat. Cell Biol.* 1 E13-5

Misteli T 2005 Concepts in nuclear architecture. *Bioessays* **27** 477–487

Morange M 2009 What history tells us XVIII. When functional biologists propose mechanisms of evolution. *J. Biosci.* **34** 373–376

Morange M 2011a *How Evolutionary Biology Presently Pervades Cell and Molecular Biology.How Evolutionary Biology Presently Pervades Cell and Molecular Biology*, in *Darwinism, Philosophy, and Experimental Biology.*

Morange M 2011b What will result from the interaction between functional and evolutionary biology? *Stud. Hist. Philos. Biol. Biomed. Sci.* **42** 69–74

Nick P 2009 Evolutionary cell biology. *Protoplasma* **235** 1

Nunoura T, Takaki Y, Kakuta J, Nishi S, Sugahara J, Kazama H, Chee G-J, Hattori M, Kanai A, Atomi H, Takai K and Takami H 2011 Insights into the evolution of Archaea and eukaryotic protein modifier systems revealed by the genome of a novel archaeal group. *Nucleic Acids Res.* **39** 3204–3223

Nurse P 2003 a *Systems biology: Understanding cells. Nature* **424** 883–883.

Nurse P 2003 b The great ideas of biology. *Clin. Med.* **3** 560–568

Pittis AA and Gabaldón T 2016 Late acquisition of mitochondria by a host with chimaeric prokaryotic ancestry. *Nature* **531** 101–104

Recio JLG 2016 La constitución de la citología y los modelos de la microanatomía celular. *Ludus Vitalis* **20** 25–32

Richardson E, Zerr K, Tsaousis A, Dorrell RG and Dacks JB 2015 Evolutionary cell biology: functional insight from "endless forms most beautiful." *Mol. Biol. Cell* **26** 4532–4538

Roger AJ, Muñoz-Gómez SA and Kamikawa R 2017 The Origin and Diversification of Mitochondria. *Curr. Biol.* **27** R1177–R1192

Sagan L 1967 On the origin of mitosing cells. *J. Theor. Biol.* **14** 255–274

Smith JM and Szathmary E 1997 *The Major Transitions in Evolution*.

Spang A, Saw JH, Jørgensen SL, Zaremba-Niedzwiedzka K, Martijn J, Lind AE, van Eijk R, Schleper C, Guy L and Ettema TJG 2015 Complex archaea that bridge the gap between prokaryotes and eukaryotes. *Nature* **521** 173–179

Stanier RY and Van Niel CB 1962 The concept of a bacterium. *Archiv für Mikrobiologie* **42** 17–35

Stent GS 1968 That was the molecular biology that was. *Science* **160** 390–395

Williams TA, Foster PG, Cox CJ and Embley TM 2013 An archaeal origin of eukaryotes supports only two primary domains of life. *Nature* **504** 231–236

Woese CR, Kandler O and Wheelis ML 1990 Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proc. Natl. Acad. Sci. U. S. A.* **87** 4576–4579

Zillig W 1991 Comparative biochemistry of Archaea and Bacteria. *Curr. Opin. Genet. Dev.* **1** 544–551

Zuckerkandl E and Pauling L 1965 Molecules as documents of evolutionary history. *J. Theor. Biol.* **8** 357–366