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How the living is in the world: An inquiry into the informational choreographies of life

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ABSTRACT

Understanding the nature of life has always been a fundamental objective of human knowledge. It is no wonder that biology, as the science of life, together with physics, has traditionally been the discipline that has generated the deepest philosophical and social repercussions. In our time, the major achievements in bioinformatics, systems biology, and *"omic"* fields (genomics, proteomics, metabolomics, etc.) have not only spurred a new biotechnological and biomedical 'postindustrial revolution', but they have also disclosed an intriguing molecular panorama of biological organization that invites us to reinterpret central themes of philosophy in the light of the new knowledge. Essential tenets of phenomenology may take an intriguing new turn when contemplated from these new biological perspectives: Does the living cell instantiate a unique biomolecular way of being in the world? How is life self-produced in continuous communication with the surrounding world? How can the incessant flows of mass, energy and information inherent of *embodiment* be coherently harnessed across billions of cellular individuals?

In this paper, based on the latest developments in cellular signaling, we will discuss the dynamic intertwining between self-production and communication that characterizes life at the prokaryotic, eukaryotic, organismic, and social levels of organization. An in-depth analysis of the particular transcriptional responses of a bacterium (*Escherichia coli K-12* strain), taking as a model system, will follow. It is the creation, transmission and reception of signals which, in all instances, provides guidance and orientation to the inner self-production activities of the living agent and connects it with the world. Transitions to new levels of organization are marked by the emergence of new forms of communication, embedded in the correspondingly augmented life-cycles of the more complex entities. As will be argued here, the ascending complexity of life is always information-based and recapitulates level after level, a successful "informational formula" for being in the world. The phenomenological basis for the naturalization of cognition has moved from the biological to a new scientific arena: informational. The philosophical notion of being-in-the-world (*Dasein*; Heidegger) is shown to be completely compatible with the latest advances in biology and information science.

1. Introduction: information and life

Throughout history, the phenomenon of life has fascinated philosophers, scientists, and thinkers of all kinds. In order to provide adequate responses to questions about the mysteriousness of life, the source of heredity, and the nature of human consciousness on the one hand, and the optimization of agricultural, botanical, and husbandry practices, as well as the demands of social health on the other, an enormous portion of mental energy has always been devoted to increasing the theoretical and practical knowledge of life (Gillispie, 1960). In fact, all philosophical doctrines and scientific worldviews have reserved an essential role for life and human reason, except in the dominant Newtonian framework, rather ironically. We have had to wait until the last two centuries to find expostulations on life couched in truly scientific terms. Solving the problem of "the origins of species", consolidating the evolutionary view, and approaching the "gene particles" of heredity were absolute prerequisites for the advancement of a genuinely scientific discourse in biology (Reid, 1985). Afterwards, the relationship of

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this 'fortified' biology with philosophy and the rest of disciplines and with the general fabric of social thought became deeper, more intense, and more controversial. During the crucial period of the "second scientific revolution", at the end of 19th century and first decades of 20th century, the new biology was caught in all sorts of philosophical and political debates, the object of heterogeneous catchwords and doctrines: *progress, competition, selectionism, racism, eugenics, hygienism, naturalism* … However there was little productive discussion of a possible interrelationship between biology and the nascent phenomenology.

Following Reid's narrative (1985) one could distinguish, though rather arbitrarily, the following main currents of biological thought at the time: (neo) Darwinism, physico-chemical positivism, vitalism, and holism. These currents were expressed more or less strongly in the different branches of biology: genetics and population biology, physiology, embryology, psychology–neuroscience, and ecology. From our perspective, the most interesting ideas came from a group of 'holistic' authors, disciplinarily not easily classifiable, who focus the integration of processes within the organism: Joseph Needham ("Order and Life", 1936), Jan Smuts ("Holism and Evolution", 1927), D'Arcy Thompson ("On Growth and Form", 1917), Samuel Alexander ("Moral Order and Progress", 1899), as well as Lloyd Morgan, Claude Bernard, Walter Cannon, Thomas Huxley, etc. However, neither the content of their work nor the personal influence of these authors provided firm ground for accompanying the new philosophical elaborations. Mainstream phenomenologists and semioticians remained blocked within their mostly anthropocentric positions—with important exceptions, e.g. Merleau-Ponty (1945). But they nevertheless contributed to inspire a new generation of integrative physiologists, ethologists, and systemic thinkers.

One philosopher well attuned to the phenomenological and biological reflections of that time was the Spanish thinker José Ortega y Gasset. He was widely read mostly through his internationally acclaimed book "The Rebellion of the Masses" (1930). Curiously, given his criticisms to Husserl's program based on language and logic (Husserl, 1911, 1970 trans.), phenomenologists considered him a stubborn existentialist, but existentialists disregarded his claims on both rationalism and vitalism and considered him a phenomenologist. An excellent analysis of the complex interaction between Ortega's *perspectivism* and Husserl's phenomenology is provided by Rodríguez Huescar (1994). Ortega, with neuroscientist Santiago Ramón y Cajal ("Textura del Sistema Nervioso del Hombre y los Vertebrados", 1899-1904), was a towering figure in Spanish intellectual life. A generation of philosophers, scientists, artists, and intellectuals of the Spanish culture (in those decades experimenting what has been known as a *'silver age'*) avidly followed Ortega's work, in particular, the painter Pablo Picasso and the poet Antonio Machado. This peculiar 'existentialist-phenomenological' track inspired wonderful paintings and poems from which an intense sentiment of life transpires, unfolding a passionate intellectual reflection. In painting, never had the deconstruction of symbolic elements conflated with unstructured human expressions under global generative processes of choral symmetries and symmetry-breakings had achieved such categorical representation of human tragedy—"The Guernica" (Leyton, 1992). In poetry, a few astonishing lines from one of Machado's poems will provide us a vivid metaphor about the meaningfulness of life … for a bacterium. Unfortunately, an in-depth analysis of that 'silver age' is outside the scope of the present essay. During brief periods, far less than a generation, some cultures get in touch with universal values of life, and these flashes of insight may be useful for us to give a human dimension to the abstracts findings of science.

The history of science, like that of humanity itself, is full of the improbable, of the unexpected, of the revolutionary. It happened in the science of the post-World War II generation: the sweeping revolution of *molecular biology* pushed the old, traditional physicochemical reductionism to a fascinating new direction, although it had to be re-elaborated under a completely new discourse. During the 1950s and 1960s, there emerged a collective commitment to represent genetic function as an information-storing system, and relentless energies were devoted to rewrite biology as an information science (Kay, 1993). Information technologies and their attendant computational discourse were permeating the wider scientific and cultural circles, loudly resonating in the work of the soon-to-be intellectual leaders of molecular biology: James Watson, Francis Crick, George Gamow, Henry Quastler, Jacques Monod, François Jacob, and Sydney Brenner. As the late historian Kay (2000) put it, this epoch implies the first historical triumph of the reductionist approach to life. It represented the weakening of holism and caused the fatal blow and total disappearance to vitalism. The information metaphor as enshrined by the founding fathers of molecular biology – expression, transcription, code, translation, messengers, transference, signaling, and so on – was giving way to the projection of the biological stuff within the ascending technology of the time, that of the disembodied binary bit.

At the same time, this first wave of revolutionary molecular-biological discoveries was planting the seeds for a series of influential non-reductionist approaches. The turmoil of discovery also put into action alternative ways of thinking that crafted new conceptual constructions: self-replication, self-organization, self-reference, autopoiesis, self-transcendence, autogenesis, autocatakinesis, etc., recapitulating the discoveries of that time from quite different angles. Factually, some fields of theoretical biology, physiology, thermodynamics, natural computation, and ecology were incorporating a plethora of alternative discourses during the last decades of the 20th century (well known authors such as Robert Rosen, Howard Pattee, Michael Conrad, Stuart Kauffman, Erick Jantsch, Humberto Maturana, Luis Varela, James Kay, Scott Kelso, Robert Ulanowicz, etc., to name but a few).

Yet another transforming way of discoveries was arriving. At the turn of the millennium, amazing achievements in bioinformatics, systems biology, the "omic" fields (genomics, proteomics, metabolomics, etc.) and signaling science were spurring a new biotechnological and biomedical scientific/industrial revolution, refocusing biological thought on highly specialized and even more technologically-entrenched grounds.

In the wake of the Human Genome Initiative, developments in automation, the explosive growth of data, and the introduction of information science tools to master these very data have changed the biological playing field forever (Lenoir, 1998). In the futuristic agenda of the revamped discipline there appear a variety of new fields: synthetic biology, high yield sequencing, artificial synthesis of complete microorganisms and chromosomes, personalized medicine, nanobiosensing, artificial cells and artificial organs, ecosystem remediation, and geoengineering. Even the teleportation of organisms is envisioned by the most imaginative leaders (Venter, 2013). As the bioinformatic champions proclaim: "In terms of discipline biology has become an information science; institutionally, it is becoming "Big Science" (Lenoir, 1998). And more sharply: "*biology is an information science*" (Leroy Hood, cited in Smaglik (2000)), or *"the living is digital"* (Hood and Galas, 2003). For Eric Lander: "Biology is undergoing one of the most fundamental revolutions that any science has seen … the whole 20th century can be read, in some sense, as the prelude to this information biology" (Nature advertisement, ©AB Applied Biosystems, 2003).

Needless to say that, as in the previous biomolecular turmoil, alternative ways of thinking are needed to re-examine more attentively the achievements of this new wave of scientific

knowledge. That is the essential goal of the present paper: to re-examine critically and constructively the new panorama on cellular communication –a smallish part of it, actually– looking for a philosophically rich interpretation, addressed from an informational perspective rather than a computational one. Just echoing the scientific leaders of the bioinformatic transformation, *information* continues to be the key word. But this time it is not as a metaphor: it is life itself, even more abstract, thoroughly digitalized and deprived of any kind of purpose or teleonomic shadow, and without any philosophical nuance on meaning. As a noted biophysicist had put it, not without a tinge of irony: *"on question of meaning, the tools of science are still a bit coarse for such delicate matters"* (Morowitz, 1968). A "bit" coarse, indeed.

Our interpretive attempt is not far from those carried by phenomenologists and biosemiotician thinkers in Peircean terms, our focus will be about the communication that the living cell establishes with the world. There are plenty of data about that—mountains of data. In fact, most ongoing research has been about complete mapping of the total flows of cellular combinatorial components, and also about ascertaining the effects of the massive communication exchanges within and between biological systems. Signaling science, the scrutiny of cellular communication, has clearly become a booming field. Just as seminal work on bioenergetics during the 1940's and 1950's recapitulated a series of basic discoveries in biochemistry and biophysics on "energy flow" in the living cell and the whole biosphere (for instance, the work of F. Albert Lipmann and Hans Kreb, Nobel Prizes in 1953), the suggestion herein is that we should attempt the same for "information flow", both in prokaryotic and eukaryotic systems, basically revolving around the findings of signaling science and systems biology. In the extent to which this work may succeed, an interesting dialog with the philosophical positions attempting the naturalization of cognition could be maintained.

2. The information flow in prokaryotic cells

The 'informational' perspective advanced here requires a number of prior clarifications. Considering the energetic/metabolic aspects of biological self-production, they were far better understood once the *energy flow* became properly recognized, characterized, and charted (Morowitz, 1968). Taking this as a model, the *information flow* of communication would demand a series of in-depth conceptual changes.

2.1. The centrality of molecular recognition

Molecularly, how can life be so astonishingly complex? Counting the number of different molecular species teeming up at the interior of a prokaryotic cell (the multiple classes of peptides, enzymes, receptors, phospholipids, RNAs, DNA, metals, nutrients, ions, etc.) the figure is staggering: in the order of 20,000 species, unthinkable for any regular physical system that magnitude—a mere cubic micron. A number of singular conditions are involved: the special properties of water, the combinatory polymerization strategies, the organization in processing 'architectures' (the structural, the sequential, and the diluted), the coded correspondence between RNA triplets and amino acids, the folding process, the catalytic properties of enzymes, the semi-permeable membrane, the detection and capture of signals and nutrients from the environment … At the very bottom, however, *molecular recognition* appears as the essential phenomenon from which the whole fabric of biological complexity derives (Marijuán, 2003). Myriads of specific recognition encounters take place in the water matrix of the cell in a highly self-organized and systematic way: no 'insulating wires' are needed to organize complex functions among multiple molecular partners. In comparison to artificial systems, this informational 'wirelessness' is one of the most remarkable processing resources of the living cell.

In biomolecular recognition instances, an astonishing variety and combinatorial classes of chemical interactions are involved: hydrogen bonds, hydrophobic/hydrophilic forces, dipole forces, van der Waals forces, ionic Coulomb forces, etc. Determining molecular recognition and establishing its crucial variables can only be realized biologically on a case-by-case basis. Nevertheless, symmetry considerations (Lin, 2001; Marijuán, 2003) allow a rough classification of biomolecular recognition instances by means of three ordering categories: *identity*, *complementarity*, and *supplementarity* (or facultative complementarity). They respectively mean recognition by sharing identical molecular properties (e.g., self-organization of phospholipids in membranes, and of microtubules and microfilaments in cytoskeletons), recognition by means of complementary properties of the molecular partners (e.g., nucleotides specific coupling in the double helix), and recognition through a combinatory-based capability to wrap or envelop any molecular shape by means of scaffolds of predominantly weak bonds (e.g., enzymatic active sites, protein complexes). In the latter case of 'supplementarity' or facultative complementarity (not contemplated by Lin's approach), the partial surfaces involved are inherently sloppy in their specificity and display a variable affinity with respect to the very clean and holistic matching of strict complementarity (an important element of stochasticity is introduced); but at the same time they become highly tunable by mutational events. And that's the basic evolutionary wisdom of enzymes: out from clever combinations of 20 amino acids, everything molecular can be recognized and acted upon—with more or less probability. Everything else biological derives from that facultative capability.

2.2. 'Productive' information flow

As pictured by the "central dogma" (the term was coined by Crick (1956)), information is flowing from DNA to RNA, and from RNA to protein. But that flow is not so relevant here. It points out to where the *action* is not. Informationally we can concur with enzymologist Kornberg (1989): "DNA and genes captured the spotlight from enzymes; but in my theater enzymes kept the leading role. DNA and RNA provide the script, but the enzymes do the acting…" (Maybe the problem with enzymes has been how to pick the characteristics of that 'acting'; how to pick the enormous variety of their collective 'dynamic' information.) Notwithstanding the present mystique about the bit, life is about production of stuff, about self-production of living matter. In fact, the central dogma speaks about the information that has to feed into the *universal constructor*, the ribosome. It is in the ribosome where the ephemeral information of mRNA becomes *flesh*, in the form of robust enzymes and proteins acting as workhorses of life. Disregarded by theoreticians, but eagerly sought by endless legions of biomolecular 'predators' (viruses and bacteria vying for the conquest of that privileged site), the ribosome is what makes life *real,* and capable of transforming the surrounding world. Like modern 3D printers, ribosomes transform the received bit serials unto tangible stuff. This transformation becomes an utmost essential aspect of life: the massive, delocalized, parallel, capability to produce *in situ* the appropriate active agents, needing only some script easily portable that encodes the required composition.

The delocalized self-production, however, has to be accompanied by another essential aspect: the cellular recycling of the stuff. From the ribosome 'cradle', enzymes and proteins after quite many working cycles finally disappear into the *proteasome* 'grave'. It is

another molecular machine utterly disregarded by theoreticians, but of utmost necessity for adapting to the environment: for rapidly changing the existing populations of active elements and for recycling the amino acids of the wasted stuff. These two production machines, ribosome and proteasome, symbolize the real life-cycle of the cell's acting components, which in their own are continuously involved within a dense molecular mesh of substrates, products, and effectors—all of them agitated by Brownian motion.

Therefore, quite different forms of information appear and 'flow' in many ways within the cellular system: as sequences, as structures, as mixed concentration gradients … In general, this is the whole information world enclosed in the self-production of the cell, the flow of ions and small molecules being the most dynamic part. And also, as observers from the outside, we may consider that the latter flow, involving the direct interaction with enzymes and proteins, is the inner *'productive' information flow*. Given that additionally the cell needs *ad hoc* energy and specific matter inputs from the environment to keep running, we may refer to these other inputs from the environment just as *energy flow* or material flow.

2.3. 'Communicative' information flow

Although apparently similar, the information flow of communication is utterly different from, but tightly related to, the energy and matter flows that are necessary for self-production. This is another evident fact that has not received due attention yet. In general, communication signals are treated in a very different way than energetic metabolites. Rather exceptionally, the singular 'hands-off' processing that characterizes signaling pathways was highlighted by Gerhardt (1999), p. 228):

> *"As information transfer pathways, these signaling pathways are basically different from metabolic pathways, even though both are called 'pathways'. In a biosynthetic metabolic pathway, a carbon compound passes through a series of enzymatic steps, with appropriate energy inputs, undergoing modifications until it emerges as an end product ready for incorporation into a macromolecule or complex lipid. But in a signal transduction pathway, carbon atoms and energy are not passed along. Only an impulse is relayed by way of successive reversible changes of state of switch-like intermediates. At the end of the pathway, the transduced signal activates or inhibits some target protein […] the most frequent target of signaling is transcription, and some pathways affect only transcription."*

Although the communication flow and the energy flow may partially overlap in their constitutive components, the living cell systematically processes them by following separated strategies. Metaphorically, 'reading' the environment becomes utterly different from, and prior to, 'eating' it. Or more conventionally, the high-energy, highly valuable flows related with apportioning the materials needed for self-production will be anticipated, detected, and captured by means of the faster and cheaper communication flows tended with the surrounding environment. See Fig. 1 (del Moral et al., 2014a).

The mutual congruence in functional terms between the two flows is crucial for the viability of the cell and, in general, for the viability of informational entities whatsoever. In the more complex entities, a frequent commonality of flowing forms occurs as well, manifested in supporting structures that often display fractal forms derived from the necessity to cover a region of space and to transport the affordances of both the material and the communicational to a center (Bejan and Peder, 2012).

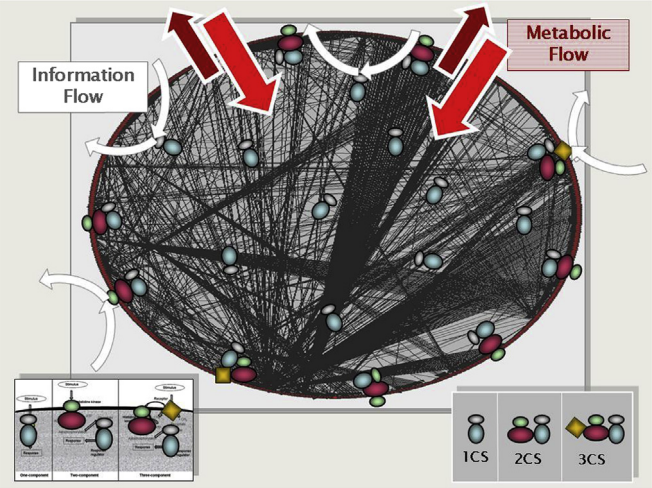

Fig. 1. Metabolic flows and information flows are shown respectively as thick (red) arrows and as thinner (white) ones. The representation highlights the difference in kind between the two flows, mediated by receptor binding, and bulk metabolic flows, which are actively introduced by permeases, transporters, and pumps. The figure also shows the three characteristic signaling pathways developed by prokaryotes: One Component Systems (1CS), Two Component Systems (2CS), and Three Component Systems (3CS). These three different options imply very different information processing capabilities and metabolic costs. (Modified from del Moral et al., 2014b.)

2.4. The concept of information

Our previous use of the term information, rather unspecific, requires some comment. Although we concur with Marcin Schroeder (this issue), in the sense that information is a *relative* term, we need specify some sufficiently general notion addressed to the biological and capable of making sense on how communication and self-production dovetail. Somewhere else (Marijuán, 2004; del Moral et al., 2014b), we have proposed understanding information as *"distinction on the adjacent"*. In this compact notion, the *distinction* term refers to the processing capabilities of the biological subject engaged in the 'receiving the information' game, what internalizing a portion of the information flow involves for the living system. Contrary to metabolic items, as wisely pointed out by Gerhardt (1999), signaling objects are not internalized by the living: only some special relationships extracted in the reception process are circulated inwards. Via specialized receptors, sensory surfaces, or dedicated 'agents' situated at its real or symbolic surface, the biological-informational entity interacts with the impinging signaling flows and simultaneously creates preliminary streams of relationships, distinctions, derived from the structure of the incoming signals. These distinctional relationships are successively transduced or translated onto the operational order of the bigger entity as they circulate throughout the interior to participate in further processes.

The *adjacent* term of the proposed notion of information refers to the physical contact to be achieved, and the need of counting with sensory elements or excitatory surfaces to be impinged upon by the incoming communication signals. Given that the signaling objects are not internalized, and only their distinctional consequences are circulated, increasing the potential reach of adjacency becomes one of the most strategic and cheapest assets for the informational entity. Extending the portion of the environment potentially controlled through communication flows is a formidable drive of biological evolution: cellular pili, flagella, cilia, arborization of axons and dendrites, multiple neuronal sensors and receptors, multiple brain mappings, etc. By transcending the limits of their immediate space-time adjacency, biological subjects

propitiate a myriad of further distinctions and cognitive operations.

Presumably, after these preliminary considerations on information itself, on the information (communication) flows, and on the energy and self-production flows, all of them based on specific molecular-recognition instances, we are a little bit better equipped to attempt the toughest challenge: analyzing how a real prokaryotic cell is in the world.

3. How *Escherichia coli* is in the world

In today's mainstream biology, the central goal of the sequencing efforts is the full annotation of genomes. It means that the one-dimensional list of components (comprising gene functions, positions of genes, promoters, terminators, and transcription factor binding sites) must add descriptions of cellular networks, the spatial orientations of the network components, and the genome changes occurred during the evolution of the organism (Reed et al., 2006; Karp et al., 2007). Besides, the annotation must incorporate experimental versus computational information and clearly distinguish the fraction of the annotations that has been validated experimentally. One of most accurate, complete and multidimensional genome annotations corresponds to KEGG and EcoCyc databases (www.kegg.jp; www.ecocyc.org). The latter is centered on the *E. coli K-12* genome (Karp et al., 2007). It will be our main source together with RegulonDB v8.0 (Salgado et al., 2013) that covers the regulatory aspects of the bacterium. Both databases have addressed the challenges of incorporating regulatory data generated through the high-throughput technology, achieving a much better home for integrating knowledge on gene-regulation from the sources of information currently available.

What do EcoCyc and RegulonDB tell us about the life of *E. coli K-12*? We learn that the bacterium has 4460 genes, of which 2941 have an attributed function experimentally determined (66%) and up to 3384 (76%) if we include those functions determined through other procedures. These percentages are the highest for any living organism. Close to one thousand genes (991) are devoted to metabolic tasks, with a similar number of distinct metabolic enzymes (918, of which 482 are monomers and 436 are multimers) which are formed out from the expression of those genes. The metabolism is organized around 194 well-defined pathways, with 1008 individual reactions of small-molecule metabolism. A distinct structure of linked metabolic reactions emerges in the form of a giant component with star-like topology; it is the "high flux backbone" of the metabolism that includes most of the metabolites produced under the given growth condition (Almaas et al., 2004). Apart, a number of transport activities are assigned to 214 different transporters (encoded in 355 genes) that are in charge of handling more than 180 different, mostly metabolic substrates —the particular constellation of substrates basically defines the growth condition, and includes a very long list of carbohydrates, lipids, metals, amino acids, peptides, phosphates, nucleotides, etc.

At any given moment, only 30% or 40% of genes can be active. It means that due to the limited solvation capacity of internal water, to the translation limits of the existing ribosomes, and to the energy budget of the whole cell, only 1/3 of the genes in that big genome are expressible simultaneously. A careful logistics has to be imposed upon transcription so that the cell may adaptively respond to changes in the environment and to changes in its own networked processes. Interestingly, the way *E. coli* organizes its gene transcription processes depends, first, on its generalized internal conditions, in the form of some overall 'mood' affecting the entire cell. What we mean is that there are a few general states that strongly orientate the whole activities of transcription: adequate growth conditions, thermal stress, osmotic stress, starvation, lack of iron, etc. These states are captured by a few "sigma" factors, each one in charge of activating the transcription of numerous genes devoted to take care of the state particular conditions (they are identified by their molecular weight in kDalton, so in *E. coli* the existing 7 different "sigmas" are respectively known as: 70, 54, 38, 32, 28, 24, and 19). These factors link RNA polymerases to gene promoters. In particular, Sigma 70 is constitutively expressed and promotes generic translation of an astonishing number of genes: 1803, almost half of the genome. The other sigma factors translate around one hundred genes or less. All these factors are carefully kept in check: there exist anti-sigma proteins and anti-anti-sigma proteins that together determine, according to specific molecular presences and absences, what should be the reigning mood for transcription and what cohorts of genes should be targeted for expression (Karp et al., 2007; Salgado et al., 2013).

The general OK to gene transcription given by those few sigma factors has to be 'revised' by far more numerous Transcription Factors (TFs) with higher specificity and combinatory capability that also recognize the specific DNA sequences of the promoters. In general, these transcription factors play inhibitory roles. They repress with higher specificity the expression of some precise genes that sigma factors have previously activated, and only let them be transcribed when some further molecular signals that activate the TFs themselves are detected. There are also a number of enhancers, or DNA-binding proteins that in spite of being positioned far away from the promoter can influence positively (in general) the transcription of specific genes. Sigma factors, transcription factors, and enhancers act together, forming genuine 'molecular machines' tightly associated to DNA sequences and to RNA Polymerases and capable of integrating a number of molecular signals (Davidson, 2006, 2010).

As a result of all this interplay of specific recognition of DNA sequences and environmental signals, an essential aspect of the cell's being in the world, the active agents that will constitute the cell itself, is elucidated. The way external and internal signals are processed and integrated depends mainly on a number of transcription factors, the presence of which depends on the trajectory followed by the life cycle. These transcription factors are indeed 'two head' molecules. One head comprises the DNA-binding site domain and the other head contains an allosteric site to which a metabolite or environmental signal binds non-covalently (Salgado et al., 2013). Thus, in spite that TFs are situated inside the cell, there is direct environmental sensing through them—actually, scores of different molecules of the environment are detected. In the signaling literature (Navarro and Marijuán, 2010), however, this direct, single molecule sensing or "one component system" was disregarded in favor of the well-known bacterial "two component systems", where the receiver domain constitutes an independent protein situated at the membrane and the response element containing the DNA-binding domain becomes activated by the former component via phosphorylation. Most cellular proteins that sense the environment belong to the one-component system category (Galperin, 2005). In a review by the present authors (Marijuán et al., 2010), the different sensing modalities have been compared, including a very brief label about the *"1-2-3 Component Systems"* classes. These three systems have different signaling advantages and disadvantages: 1CSs imply simplicity and robustness, 2CSs provide faster responses and easier evolvability of receptors, and 3CSs imply a better handling of mixed signals and superior integration of complex responses.

Concerning the transcriptional repercussions of their DNA-binding activity, not all TFs are 'created equal'. In EcoCyc we find description of 171 factors that control 718 Transcription Units (each TU may contain either single genes or groups of them, i.e., operons), in average each TF controls 1.7 genes. Vice versa, a given TU may be regulated by several TFs conjointly, and a total of 156 TUs appear as

served by three or more TFs (Karp et al., 2007). Power laws emerge in the mutual relationships between TUs and TFs. A select minority of TFs has a disproportionate regulatory impact; they are known as "global regulators" (ArcA, Lrp, Hns, IHF, FIS, FNR, and CRP), as most of them are constitutively expressed and regulate their own transcription; they also co-regulate the transcription of sigma factors (Ishihama et al., 2014). In the hierarchy of gene transcription they occupy the top level, transcribing numerous genes (in the hundreds) and not being transcribed by any other gene. They are also at the top the level of translation, as their presence outnumbers all other TFs and most proteins (around 50,000–100,000 units per cell, far above the average of a few hundred or even a few dozen for other TFs). In the second level of the hierarchy, around 140 authenticated TFs are transcribed by the previous global TFs, and in their own turn they transcribe scores of other genes as well (Balderas-Martínez et al., 2013). Finally, at the bottom level, all the rest of genes are just transcribed and do not contribute to any further transcription.

Numerous feed-back and feed-forward loops co-regulating the target genes are established among TFs in different levels. Fig. 2 represents in a simplified way the whole interactions among the three hierarchic levels (Yan et al., 2010). It is interesting that the figure also compares the hierarchy of transcription with another hierarchical system: the controlling interaction within a computer's operating system, the Linus OS actually. The two pyramids are inverted, and this is a consequence of the different processing strategies: based on specific molecular recognition and 'wirelessness' within a water matrix agitated by unending Brownian motion in one case, versus the sequential chaining of logical gates in insulated electronic circuits in the other.

A more detailed view of the whole transcription process and TFs' roles appears in Fig. 3; it also represents in different colors the distinct sensing realms that impinge upon gene transcription: the external sensed, the external transported, the hybrid external–internal, the purely internal, and the DNA sensing. See Fig. 3 from RegulonDB (Salgado et al., 2013).

The transcriptional scheme of *E. coli K-12* constitutes, as we have reiterated, the essence of the way this bacterium *is* in the world: how it 'feels', stays, reproduces, associates, and transforms the world around. This cell is far more complex than the average prokaryote as it must survive occasionally in inanimate harsh environments but it mainly proliferates inside the gut of animals, being involved in dense exchanges with other microbial communities (of the *microbiome*, and also the *virome* and *mycome*) and with the immune defenses of the host as well. The numerous two components systems (29 actually) present in the bacterium reveal an active, vigilant sensing of its environment, including the social exchange with congeners by means of quorum sensing (QseC-QseB). However, the far more numerous one-component TFs devoted to inner sensing –around 140, from an estimated total of 300 TFs (Ishihama et al., 2014) – reveal a rich metabolic network and a vast transportation system of external fluxes to be carefully controlled. All this complexity has been acquired adaptively, along the evolutionary co-construction of microbial niches with the other interrelated species. The way metals are sensed by *E. coli*, for instance *iron*, (Fig. 4) discloses eons of evolutionary coupling with the medium (Anbar, 2008). This strategic metal –the lack of it– is object of defensive strategies amidst microbial communities and host–prey interactions. No wonder that the sensory–genomic complex dealing with iron involves scores of transporters, siderophores, storage elements, heme cofactors, numerous transcription factors, and two sigma factors as well. Similar stories could be told about the sensing of phosphorus and phosphates, of nitrogen and nitrates, and about the sensory control of the motility system—the latter, a conspicuous three component system, 3CS, subject to countless modeling exercises in the recent biocomputational literature.

All this wealth of biomolecular information may be connected with the general ideas sketched in Section 2. Perhaps the most general statement about this bacterial way of life consists in the continual adjustment that the ongoing life-cycle performs with respect to the medium, the continuous 'reading' on the external and the internal environments it performs. Depending on its sigma factors, on the external signals impinging on its external and internal receptors, on the transported flows of metabolites and nutrients, and on the inner DNA-sensing modalities *E. coli* will express cohorts of genes that will be translated into the ribosomes. In Fig. 3 we can clearly appreciate these different realms of transcriptional sensibility. In line with what we have stated in Section 2, the *communication flow* (1CS, 2 CS, 3CS), the *energy flow* through transporters, and the *productive information flow* of multiple activators and inhibitors operating on enzyme networks, including protein degradation by proteasomes, harmoniously contribute to obtain a life cycle that culminates in reproduction. Perturbing in whatever manner this scheme of flows directly affects the multitude of sensors related to protein synthesis and protein degradation, informing in different ways the ongoing self-production process.

In particular, if we want to ascertain the effect that a given signal produces, we must count the new molecular presences and absences derived from the gene expression consequences of the signaling event. The *meaning* of a particular signal is thus established through "molecular mining". But there is no fixed reference there: the life cycle itself, in all its enormous multiplicity of possible 'moods' and trajectories, can only be established in retrospect, by 'freezing' it; at every instant we might look behind, the reference

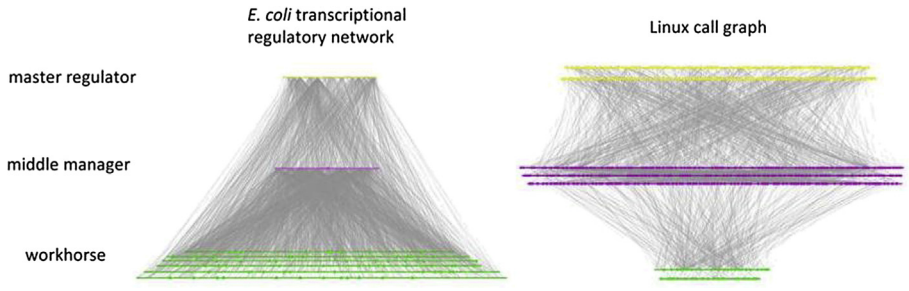

Fig. 2. Representation of the hierarchical layout of *E. coli* transcriptional regulatory network (*Left*) and the Linux call graph (*Right*). Functional nodes (genes/instructions) are classified into three categories on the basis of their location in the hierarchy: master regulators (nodes with zero in-degree, *Yellow*), workhorses (nodes with zero out-degree, *Green*), and middle managers (nodes with nonzero in- and out-degree, *Purple*). Interestingly the two hierarchical constructions show an opposed configuration. (From Yan et al.(2010), with permission.)

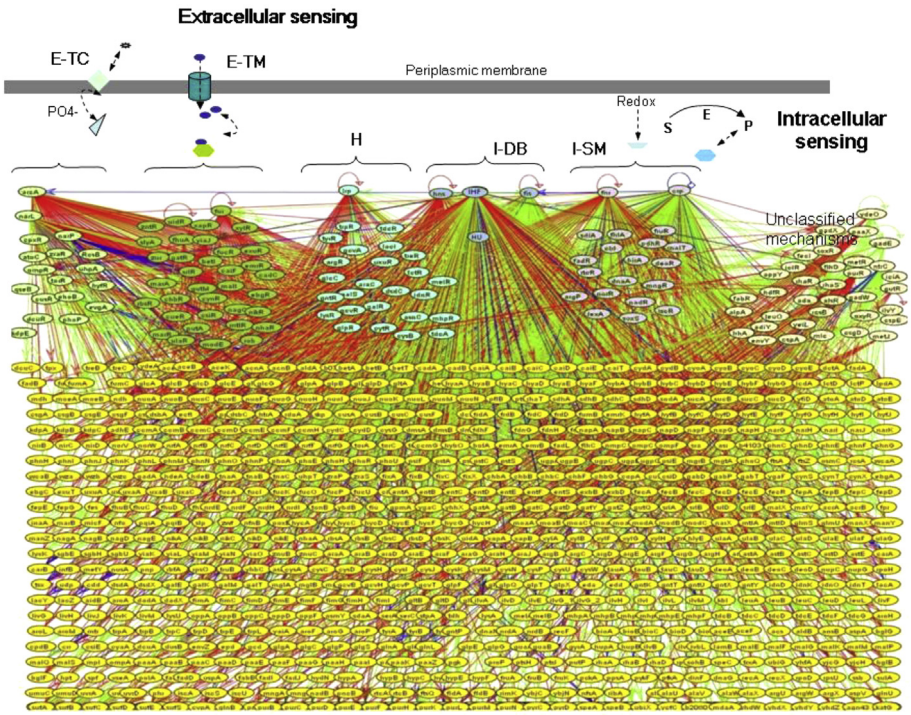

Fig. 3. The *Escherichia coli* transcriptional regulatory network for sensing the extracellular and intracellular environment. In the upper part and from left to right, in green, are those TFs corresponding to the *extracellular class of sensing*; in light green, are those TFs from *two-component systems* (E-TC) and in dark green, are those TFs using exogenous metabolites transported into the cells by *transport systems* (E-TM). In light blue are those TFs corresponding to *hybrid system of sensing* (H); i.e those TFs using metabolites synthesized inside the cell and incorporated from the milieu. In dark blue are those TFs for DNA-bending or chromatin architectural TFs, they do not sense metabolites directly. In pink are those TFs for sensing intracellular conditions or sensing the internal cellular redox-state. Finally, in light orange are those TFs without metabolites or unknown mechanisms to modulate their activities. Global TFs (ArcA, Lrp, Hns, IHF, FIS, FNR and CRP) are at the top level. The connections: green lines represent *activation*; red, *repression*; blue, *dual* (activation and repression). In yellow (low) are those genes which do not code for TF products. Abbreviations: S, substrate; E, enzyme and P, product. (From RegulonDB, with permission.)

that provides, generates, and fabricates the meaning has changed … The whole life cycle is but a temporal sequence of instantaneous meanings continuously churned out from the entire self-production processes and apparatuses of life. Looking from the angle of semiotic conventions, *signals* appear as compositional structures of the *objects* themselves, quite indistinguishable and inseparable from them and from the outer world as well; only with the advent of quorum signals and inter-species communication, we may partially distinguish between signals that denote the presence of a very important 'animate' object. As for the *subjects*, they appear themselves as life cycles in progress, and only that which pertains to the advancement of the life cycle has been evolutionarily incorporated as being part of the subjects' own communication and energy flows. These flows, we should emphasize, are always maintained and renewed by Brownian motion, acting as the crucial engine for whatever action at the bacterial dimension.

Therefore, at the cellular scale, the semiotic triangle looks rather 'flat' – just the cell and the world directly contacted through Brownian motion – and the occurring communication flow is much focused and singularized to the functional needs of self-production; everything else is blatantly ignored or blindly suffered. In the informational parlance we are trying to establish, the way a bacterium is in the world becomes a 'proto-phenomenon', the primary instantiation of the informational way of existence that constitutes life.

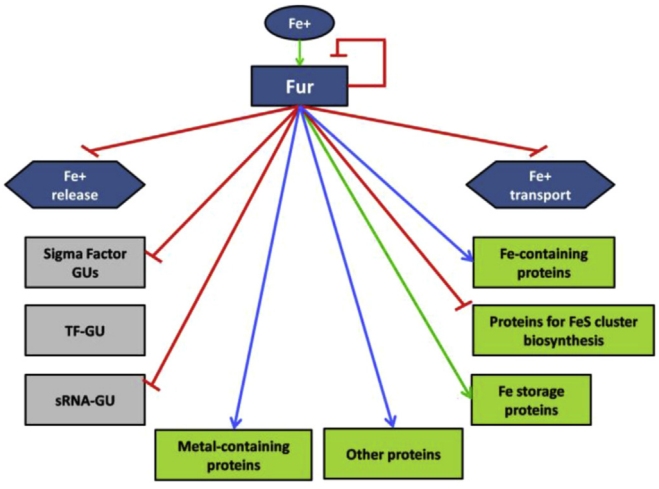

Fig. 4. In the presence of Fe^{+}, Fur represses genes involved in transport and release of Fe^{+} from siderophores and genes for biosynthesis and assembly of FeS clusters; in addition, it activates genes involved in Fe^{+} storage and activates/represses genes that encode proteins that contain Fe^{+} or a group heme as a cofactor. Fur also regulates transcription of 9 TFs, as well as the 19 and 38 sigma factors and others metal binding proteins. (From RegulonDB, with permission.)

4. The new realms of eukaryotic complexity

In some sense, the further complexity growth of multicellular organisms is a *déjà vu* of the prokaryotic phenomenology. In another sense, the uncanny complexity of signaling and transcriptional processes in all the eukaryotic kingdoms of life challenges the meaningfulness of whatever simplified scheme we may propose. Herein we will merely discuss a few evolutionary guidelines on the fundamentals of the 'new eukaryotic order': symbiosis,

signaling expansion, cell-cycle modularity, and ontogenetic multicellular development. We will basically follow a review on 'eukaryotic intelligence' recently made by the present authors (Marijuán et al., 2013).

4.1. Symbiosis

As Margulis (1970) forcefully argued in her theory of Endosymbiosis, the eukaryotic cell became itself a symbiotic contraption or 'composite' of other cellular systems. Because of this sort of inner multicellularity, the eukaryotic cell was forced to handle its own organization of processes in new *modular* ways that expanded controlling systems and later on allowed the emergence of external, true multicellularity. A far more effective problem solving was emerging, to be based on specialization of different cell types and massive communication flows through multiple pathways—networks. This was a genuine 'information revolution' which implied the development of far more complex *cellular signaling systems* in order to capture the external information flows and distribute them inside each cell type (Marijuán et al., 2013). It took place in cellular systems around 1200 Mys ago; it was made possible by the tremendous 'energy revolution' derived from the symbiotic capture of mitochondria. The outcomes of which were but staggering: an average protozoan has nearly 5000 times more metabolic power than a single bacterium, and can support a genome several thousand times larger with more than two orders of magnitude in the energy devoted to expression and translation of each gene (Lane and Martin, 2010).

Whereas prokaryotes had already made a start towards cellular complexity eukaryotic style, they could not exhibit more than one complex trait at a time, given the energy costs implied. Novel protein folds, far more protein interactions, and enlarged regulatory cascades were required for putting together the isolated complexity traits that bacteria had already explored but in too restricted a way: separate nucleus, dynamic cytoskeleton, endocytosis, linear chromosomes, introns and exons, massive intracellular and intercellular signaling, etc. The increase in protein repertoire by the eukaryote common ancestor was dramatic: It represented some 3000 novel gene families—the most intense phase of gene invention since the origin of life (Lane and Martin, 2010). We might argue that prokaryotes had already used some of those very capabilities, or at least their incipient evolutionary traits, but mainly towards the direct solution of molecular assimilation problems (in their encounter with environmental substances), while eukaryotes were to achieve a fascinating developmental complexity by evolving towards a quasi-universal solution of molecular organization problems of the organism, always under the guidance of cellular signaling systems which now were enormously expanded (Marijuan et al., 2013).

4.2. Signaling expansion

The amazing signaling novelties of eukaryotic cells, later on excelling in nervous systems, were due to processes of protein-domain recombination that allowed old prokaryotic resources and new eukaryotic tools to be put together within longer, mixed pathways that liberally cross-talked with each other. Osmotic tools (i.e., ion channels) were liberally cobbled together with detection of solutes by protein receptors and with hierarchic chains of protein kinases, as well as with the recycling of proteins in endosomes—finally connecting with ubiquitination and degradation systems. The synaptic processing of neurotransmitters appears as one of the best examples of such intercombination of heterogeneous signaling resources, often involving stages of information integration by means of transportation systems, force fields, and – above all – electric fields.

A good portion of the new signaling system was directly inherited from prokaryotes, but many other parts were invented through domain-recombination *bricolage* and were cobbled together among highly complex controlling apparatuses unrelated to prokaryotes (Marijuán et al., 2013). Overall, four main classes of functional resources were used in the expansion of eukaryotic signaling systems—four "roots" that supported the fast branching of all the new complexity:

- Prokaryotic signaling pathways, actually devoted to detection of solutes, which comprise: receptors, protein kinases, phosphatases, and regulated transcription factors.
- Prokaryotic osmotic apparatuses counteracting the Donnan (osmotic) effect, actually devoted to solvent sensing, which comprise: stretch ion-channels, voltage ion-channels, ligand-gated channels, water transporters, and pumps.
- The cell-cycle controlling system, with hierarchies of protein kinases, checkpoints, cyclins, and protein degradation systems.
- The cytoskeleton plus the endocytic matrix, with mechanical support, adhesion, and force-field detection on the one side; compartments, inner transportation, and vesicle formation on the other.

Additionally, strategic areas of prokaryotic metabolism were also providing key substances previously involved in detecting the energetic state of the cell (cAMP, cGMP) and in the synthesis and integrity of membrane systems (IP3, DAG, arachidonic acid, ceramide acid), plus the key enzyme ionic-effector (Ca^{2+}). All of them would be reused inside the eukaryotic signaling pathways as second messengers to amplify the information flow, conveying integrative messages by diffusion into localized regions of a far bigger cell, in connection with all the new membrane systems, compartments, and inner transportation mechanisms.

Functionally, at least 20 main classes of signaling pathways can be distinguished in multicellular eukaryotes. According with Gerhart's scheme (1999), in which we have added a few further classes (Marijuán et al., 2013), they would cover the following stages:

- Early development: Wnt, Hedgegog, Notch, TGF-β, Neurotrophins.
- Mid development and organogenesis: Integrins, Cadherins, Nuclear Hormones, Reelin.
- Tissue physiology: G-Protein Coupled Receptors, Guanylate Cyclase, Adenylate Cyclase, Electromolecular Transmission (4 main classes).
- Stress and criticality: NFkB, Cytokines, Autophagy, Apoptosis, Hippo, Complement Cascade.

None of those pathway classes acts in isolation. A series of balances and symmetry breakings between opposed pathways are systematically crossed along the processes of development, morphology, and physiology. Frequent symmetry players are Wnt and Hedgehog, Hippo and Wnt, Notch and Hedgehog, Hippo and TGF-β, etc. Such balances and interconnections between pathways, far from being linearly organized, are enmeshed in networks and circuits of fiendish complexity. In general, pathway coalitions are subtended by the most complex intracellular networks, where inner controlling apparatuses and outer signaling apparatuses turn out to be inextricably mixed with the machinery taking care of the cell cycle.

4.3. Cell-cycle and modularity

The heavy investment in signaling resources by eukaryotes was

necessary in order to produce a new kind of life cycle amenable to controlled dissociation or modularization amidst the far more complex internal and external happenstances. Most cellular functions had to change from a temporal context to a spatial one, tightly controlled by specific signals; while some functions were delayed or directly suppressed, others became augmented and specialized (Nedelcu and Michod, 2004). The decoupling of cell division from cell reproduction, organizing successive levels of "potency" along the developmental process, was one of the central achievements. The cell cycle became contingent on signals received from other cells, whereas on single cells these processes had no such dependence (Gerhart, 1999). Thus, the capability to keep cells in a quiescent state, facultatively and reversibly by way of signaling instances, is what made possible the advent of true multicellularity (Davidson, 2006, 2010).

Looking from the outside, the cell cycle appears as the main 'user' of the whole signaling information system, its genuine 'master' – and as such it has finally mixed and hybridized its own organization with the structure of the most strategic signaling pathways, endowing them with the most powerful downstream processing sets. It is as if the cell cycle machinery would have projected itself towards the surface in order to take the most relevant guidance cues from the external environment, subordinating thereafter most of the other signaling pathways to its own machinery.

The most powerful set of protein kinases in the entire signaling system is, thus, directly associated with mitotic control: the MAPK cascade (MAPKKK, MAPKK, MAPK). Depending on the cellular context, this cascade will be divided into three branches: MAPK/ERK, SAPK/JNK, p38/MAPK. Whatever receptors and transducers happen to be associated with the successive kinase hierarchies of these cascades, they come to occupy a highly privileged position in the control of the cell cycle and the life and death decisions. See Fig. 5 (Marijuán et al., 2013). And this may happen regarding an ample variety of inputs. Precisely, one of the functions attributed to

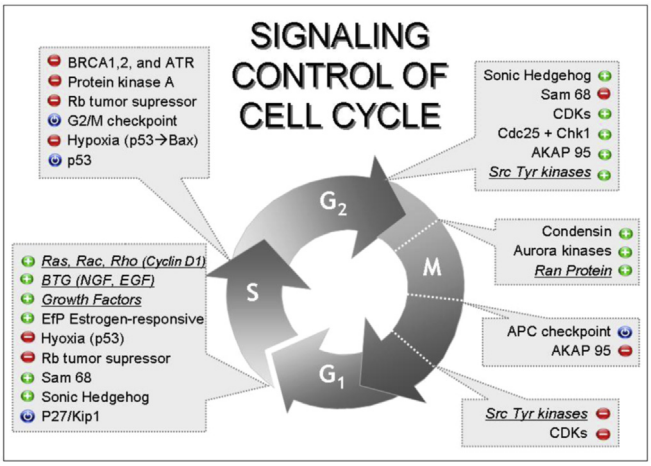

Fig. 5. Cell-cycle control. A tight signaling control is established on the different phases of the cell cycle (G1, gap; S, synthesis; G2, interphase gap; M, mitosis) and on their respective transitions. The modular organization of the multicellular organism allows the space-time separation between cell-cycle phases, mediated by a number of controlling signaling pathways. The signaling control is ultimately based on a cloud of internal and external signals, usually of opposed signs (activators vs. inhibitors), that carefully regulate the reproductive and specialization trajectories of cells and tissues. In the figure, activating signaling pathways promoting progress of the cell-cycle bear the ⊕ sign, while the inhibiting ones bear the ⊖ sign. In the case of cellular checkpoints the sign is ⊙, as they can result in progress or in arrest, depending on the incoming factors. The signaling pathways associated to MAP kinases appear in italics. (From Marijuan et al. (2013), with permission.)

MAPK cascades is to coherently insert a large variety of cross-talk signaling inputs from other pathways, but endowed with the appropriate relevance and hierarchical order throughout the different signal amplification values of the successive kinase hierarchical levels.

A populational control of the different phases of the cell cycle (G1, S, G2, M) and their respective transitions takes place along the modular organization of multicellular organisms. Such populational control is ultimately based on a cloud of internal and external signals, usually of opposed signs, that carefully regulate the reproductive and specialization trajectories of cells and tissues. The balance between growth factors and apoptotic factors becomes essential for the developmental and physiological pruning up of the organism. It is this balance what propels cellular growth, eliminates transformed, senescent or redundant cells, and keeps organs and tissues within their functional bounds. As a sort of reminder of the symbiotic origins of eukaryotes, the control center regarding the irreversibility of death decisions along the cell cycle (once the balance or symmetry between growth and apoptotic signals is broken) locates in mitochondria – the Bcl-2 protein linked with the integrity of the respiratory chain. It makes a lot of evolutionary sense thus, the involvement of respiratory proteins as a form of permanent selection for cells and organisms endowed with the formidable power of mitochondrial symbiosis (Blackstone and Green, 1999; Lane, 2011). The metabolic centrality of mitochondria makes them an important target of a number of signaling pathways, a cross-roads where metabolic state, cell cycle state, and external signals are gauged together, converging in fundamental "checkpoints" where the fate of the whole cell is decided.

4.4. Multicellular development

In multicellular – vertebrate – development, signaling is everywhere: from the earliest steps of axis specification, to the diverse kinds of morphogenesis, organogenesis, and growth in the embryo; from sexual maturation and regular tissue renovation to the ongoing physiology in the adult (Gerhart, 1999). Actually, each phylum is characterized by a body plan, *bauplan*, which is a unique topological configuration of secreted signals, active signaling pathways, and expressed genes, all of them dynamically self-organized along the development and life cycle of the individual. A *signaling master plan* could be envisioned too; but it would hardly take any formal expression. Some tools for symmetry and asymmetry compositions in group theory, however, could provide some help in the theoretical-biological approach to developmental symmetry-breaking processes (Leyton, 2001).

In development, the epithelial–mesenchymal transition (EMT) is critical for the formation of many tissues and organs as well as for physiological processes such as wound healing and initiation of metastasis in cancer. As a result of this transformation, well-stacked epithelial cells lose their polarity and adhesion-junctions and gain invasive and migratory capabilities becoming mesenchymal cells. Processes such as gastrulation, neural tube formation, heart formation, as well as different types of cancer occur in dependence of those signaling pathways, basically a series of inputs and outputs around the Par complex signaling, involving Wnt, Notch, TGF-β, Tyrosine kinases, and the cytoskeleton (McCaffrey and Macara, 2011). In other cases, mesenchymal cells experiment the reverse process (MET) in order to participate in the formation of many epithelial mesodermal organs. The flexible conjugation of both EMT and MET events is an essential feature of the developmental process.

Within the functioning organism, perhaps the best way to characterize signaling complexity is to go to those tissues where most signaling pathways could be caught into action. Neurons, for

instance, which contain a superabundance of signaling elements above any other eukaryotic tissue or cellular specialization, are the best specialists in the use of extra/intracellular computing. All the main classes of signaling pathways previously mentioned are potentially involved in one or another aspect of nervous system development, structure or function. Having to deal with the most subtle biological stuff, information, and having to organize a macroscopic system for memory storage, has probably represented the highest evolutionary challenge and has involved the most complex, sophisticate signaling solutions. The molecular machinery subtending memory, mostly in the postsynaptic site of excitatory neurons, is perhaps one of the best instances of such evolutionary signaling complexity. In every postsynaptic site of excitatory synapses, a re-enaction of the very basic evolutionary mechanisms of life takes place after repeated volleys of electrical discharges have caused membrane depolarization: it includes a number of receptor classes, channels, and pathways, but also the frantic translational activity of ribosomes, for local protein synthesis occurs *in situ*, with a number of proteasomes in action too, and microtubules and microfilament mesh involvement, and finally the mitochondrial presence empowering the whole game.

Let us note that, outside the sophisticate information-processing use of electric fields in nervous systems, all possible *channels* for conveying functional information flows —beyond Brownian motion, but always using it at the final end— have been used by animal organisms. From fixed signposts (extracellular matrix), to local paracrine diffusion, distant endocrine transport (via blood and lymph), distant transport via other functional fluids (urine, sweat, bile, mucus, milk, tears, etc.), gaseous exchanges (pulmonary respiration, endothelial control, synaptic gap), electric field conduits (nervous system, cardiac tissue), and force field conduits (skeletal and muscular systems). The coupling between the individual cellular agents and these communication channels always involve the presence of signaling pathways for reception but also the existence of dedicated pathways and export mechanisms for emission of the responses. The way signals are emitted and ascend toward integration centers have received far less attention than the opposed descending order —often coming from nervous system commands— which results more attuned to reductionist analyses. The crisscrossing of ascending, descending, and lateral communication flows among hundreds of cell types in the organism defies any reasonable representation.

After the basic eukaryotic complexity guidelines presented in this Section, an interesting question to formulate is: How a meaningful interpretation of the whole eukaryotic organization might be crafted theoretically? Beyond the customarily rigid arguments about evolutionary theory, almost useless in this context, a new bioinformational or biocomputational interpretation of eukaryotic organization seems ineludible. We have argued (Marijuán et al., 2013) that the tight eukaryotic coupling among transcription, alternative splicing, domain recombination, modularity, and cell differentiation, all of them under signaling system's guidance, integrate an abstract problem-solving system, the power of which transcends the biomolecular realm. Rather than following approaches based on Turing machines and computational analogies (Danchin, 2009; Yan et al., 2010), we should discuss the eukaryotic self-construction and communication capabilities in a completely new way. As we have seen here, the mixing assimilation of the different information flows takes place inside a unitary 'wetware' all along the *scala naturae* of life, conversely to the 'hardness' of artificial systems for information processing and their strict separation between hardware and software. The whole living structures become permeable to the information introjected from the environment, and physically adapt to it in a continuous way. Thus, the information flows of life are not just 'processed': they acquire a changing embodiment as they circulate through the processing structures and are entered into the life cycles of the entities. In the biological information processing, the processors own material structures are changed, and often 'sculpted', by the circulating information flow. Signals are traded by new structures; and structures themselves resolve in the creation of new signals. It is quite different from, and far more powerful than, the information processing paradigms of artificial systems.

In the extent to which these bio-informational generalizing ideas or similar ones could be adequately framed and formalized, they might help develop the new thinking needed and contribute to a more fruitful dialog with theoretical scientists and philosophers.

5. Concluding comments: life and the world

Now it is probably time to try to respond to the philosophical questions addressed at the beginning of our dialog: Does the living cell instantiate a unique biomolecular way of being in the world? How is life self-produced in continuous communication with the surrounding world? How could the incessant flows of mass, energy, and information inherent in *embodiment* be coherently harnessed across billions of cellular individuals?

Regarding the first question, the difference between living and inanimate matter has not always been considered clearly by phenomenologists, and is of utmost importance. Life *is* but, in some fundamentally different way, it appears continuously *in the making*. Life's permanence in time is like a thrilling theatrical play. Molecular characters cast in great numbers enter through the ribosome curtain, occupy the central stage for a while, and disappear into oblivion via proteasomes. Only the script remains more or less stable, but just in the role of a palimpsest, continuously recopied by troupes of the passing actors and slightly altered due to the many circumstances of the play (genetic-epigenetic-transfer-transposition). The play never ends. It generates a multitude of repetitions of a singular construct we call *cycle*, by means of which the living re-emerges renewed and achieves an amazing form of permanence, of *being in the world*.

In spite of its apparent fragility and cumbersome complexity, this particular way of continuous self-recreation in communication with the environment is comparable to the most persistent and resilient structures of inanimate matter itself—3.5 MM years of permanence by the phenomenon of life, at least in our planet, has cosmological significance, indeed. Are we getting at the roots of a phenomenological lived subjectivity? We think so. The cells' information-based structures, always in the making, support a new dynamics of matter where stochasticity, which is inseparable from molecular recognition, adds an intrinsic twist and a formidable source of variability to the conjunction of internal and external happenstances that are evolutionarily integrated within the life cycle. Never a life cycle is repeated twice—it always keeps changing. This fluid biomolecular stuff constitutes the only known substrate of life's subjectivity: its material cause.

A response to the second question of the surrounding world was actually given in Sections 2 and 3. The relationships of the bacterial subject with the compositional structures of the external world in its niche are unusually transparent. This is not the case for multicellular organisms where the mass of communication flows occurs inside their own structures, rendering them almost opaque to external happenstances. A response to the third question about how the organism's *embodiment*, the 'flesh', is organized has been described in Section 4 on eukaryotic complexity. When we contemplate in parallel the workings of eukaryotes and prokaryotes, we cannot help but consider that in spite of their organizational differences they have instantiated variants of a

successful *"informational formula" for being in the world*.

In essence, the living strategy for survival requires a functional congruence between energy flows and information flows. What is sensed should be used for guidance on what should be entered to feed in the own processes. This intertwining of differentiated self-production and communication processes may be taken as an approximation, for in a number of cases the communicative information flows are *physically mixed* with the productive information flows inside. That this occurs in prokaryotes is patent in different parts of Fig. 3—but it does not appear to be very consequential. In the case of multicellular organisms the consequence of that inner mixing, which is far more generalized, entails that different information flows, situated at different levels of organization, are put together systematically. The 'communicative' information flow collected from the external environment, after being properly transduced and internalized by specialized cells, circulates among different cell types and becomes irretrievably mixed with the 'productive' information flow that circulates inside these cells, interfering with their own private spheres of communication and self-production activities. Looking from the lower level, the consequence of receiving the new portion of transduced information implies a cascade of alterations in the ongoing internal self-production processes. Populations of these lower-level agents will be able to solve through their own adaptive mechanisms the corresponding portion of higher-level adaptive problems. But an upward direction is established too, as the autonomous changes of the low-level signaling flows are also capable of influencing and guiding the self-production and communication processes of the whole informational entity, changing its adaptive behavior and provoking the emission of specific high-level communication signals. Indeed the verb changes the flesh, but the flesh may change the verb as well: the strange informational formula of life trades virtual signals for pieces of structure, and *vice versa*.

The strategy for complexity growth by means of successive applications of that informational formula can now be generalized to supra-biological domains. More complex agents communicate about their collective activities by means of new communication channels that sustain the emergence of new aggregate entities; and these communications feedback to the self-production of the agents, which in their turn communicate downwards onto their own self-producing functional components. A whole series of countercurrent flows need to be established too. Informationally we may echo Richard Feynman's famous dictum: there is plenty of room at the bottom—but there is more new room at the top!

This coarse reflection on the dynamics of successive "informational entities" helps us make sense of fundamentals of social evolution. The transition to a new social order, more or less 'revolutionary', tends to be produced by new information channels and communication practices that support the emergence of new ways to organize the structures of social self-production. Thus, the development of social complexity appears as irreversibly linked to a chain of historical inventions for communication and knowledge generation: numbers, writing, alphabet, codices, universities, printing press, books, steam engines, means of communication, computers, Internet, etc. (Stonier, 1990; Hobart and Schiffman, 1998). This succession of fundamental inventions has dramatically altered the "infostructure" of modern societies, and subsequently the informational formula for *being in the world* has been applied with multiple variants along that complexity runaway: with plenty of room generated by the new information tools, not at the bottom but at the supra-individual top. We should not forget that the momentous Scientific Revolution was preceded by what has been called the silent "corporate revolution" (Huff, 2011), which opened the way for collective organizations legally autonomous in European cities during XIII and XIV centuries: universities, parliaments, counsels, municipalities, professional colleges, guilds, mercantile associations, charities, schools, etc. It was this Medieval awakening in the cities of Western Europe what made possible the later hyperinflation of autonomous collective organizations, —"information based"— growing exponentially and propelling all the further complexity of modern societies.

Let us emphasize that, like Brownian motion in living cells, the natural flow of human information, *conversation*, always remains present at the interfaces of all the new structures and forms of communication invented, and in the creation and dissemination of knowledge as well. Finally, it is the ephemeral life cycle of the individual, informationally grounded in human language, and amplified by all the amazing new tools, that has supported the emergence of a social world of unfathomable complexity, subtended by the most complex communication and energy flows and endowed with endless capabilities to master the surrounding world—as long as the planetary environment may keep affording the basic energy and material flows.

These reflections presented here, based in biology, are preliminary, and probably rather rigid and coarse to meaningfully contribute to philosophical discussion. They have not completed the 'extra mile' needed for mutual encounter in a fertile multidisciplinary dialog, so much needed in this time of fast and furious disciplinary change. The present approach has received ample inspiration from the idiosyncratic positions held by the Spanish philosopher and thinker José Ortega y Gasset. The "phenomenological" views of this author have not been subject of much discussion in the theoretical circles either, at least in recent decades. However, his views on *perspectivism,* about the doctrine of limitation (probably inspired by Nietsche), on *ratio-vitalism,* about the duality of the workings of our intelligence (anticipating contemporary neurophilosophical stances about the two systems: system 1, quick and dirty; system 2, quiet and reflective – Kahneman (2012)), and on the new *scientific-technological barbarianism* derived from narrower and narrower disciplinary specialization, as well as many other valuable insights about history, arts, esthetics, political life, etc., should make him intellectually present in our theoretical discussions and tentative interpretations of life (Ortega y Gasset, 2004-2010). For instance, how close the ideas defended herein are to his views on *primordial consciousness* as directly derived from the primary structures of life … A contemporary of his, the poet Antonio Machado, crafted one of the most impressive interpretations of the inherent phenomenological quality of life (Machado, 1999): "Caminante, son tus huellas/el camino y nada más; /Caminante, no hay camino,/se hace camino al andar./Al andar se hace el camino,/y al volver la vista atrás/se ve la senda que nunca/se ha de volver a pisar./Caminante no hay camino/sino estelas en la mar." An English translation would say: "Wanderer, your footsteps are the road, and nothing more; wanderer, there is no road, the road is made by walking. By walking one makes the road, and upon glancing behind one sees the path that never will be trod again. Wanderer, there is no road– Only waves upon the sea." Doesn't it forcefully describe the paradoxes of the bacterial life cycle and of our own?

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