CHAPTER 14

RNA AS CODE MAKERS: A BIOSEMIOTIC VIEW OF RNAi AND CELL IMMUNITY

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Abstract: The development of the adaptive immune system as it is known in vertebrates relies on the highly coordinated program of cell differentiation achieved by such multicell organisms during their embryonic development, as well as during their functional physiology. This paper discusses the acquisition of an immune response by means of cell function specialization (recognizers, presenters, killers) in the light of biosemiosis. In particular, it will be argued that self/nonself differentiation rises in multicell organisms by a switch of organic codes and operating logic. In fact, double-stranded RNA molecules that induce a highly specific and selective mRNA degradation in non-vertebrates bring about an ubiquitary silencing of transcription and translation in differentiated vertebrate cells. This last response requires elements which are common to cell immunity, the so called interferon response machinery which is responsible by preserving cell genomes from mobile DNA fragments often generated during viral infection. This particular phenomenon will be extensively discussed to show the general point of how a major evolutionary change - invertebrates to vertebrates, in this particular case – requires the development and fixation of new organic codes. The pattern of embryonic and functional cell differentiation achieved by vertebrates' immune system will only be possible whenever, in evolution, cells are able to discriminate, recognize and integrate signs. We propose that the performance of these increasingly complex skills by cells is the hallmark of different levels of stabilization for living systems, the levels of CELL/SELF/SENSE. The way double-stranded RNA is dealt with by each of the levels proposed will be analyzed as a case study of a broader phenomenon: the contextual meaning of molecular signs as fixed by the combination of natural convention and natural selection as component mechanisms of the evolutionary process

Keywords: Cell immunity, RNAi, Organic codes, Natural conventions, tinkering

M. Barbieri (ed.), Introduction to Biosemiotics, 347–364.

La simplification, n'est pas dans le but dans l'art. On y arrive malgré soi en voulant faire des choses réelles qui ne soient pas la carcasse que nous voyons, mais ce qu'elle nous cache.

Constantin Brancusi

INTRODUCTION

The attempt to provide biological knowledge with a more explanatory conceptual framework is possibly Biosemiotics main motivation nowadays. Even though such enterprise, as mentioned in the editorial of this book, is not a homogeneous one. In such context, a dangerous trap for anyone trying to develop a research program in Biosemiotics becomes the difficulty of defining not only "how" this particular structural science (Artmann, 2005) can help Biology, but also, "which" particular brunch of Biosemiotics we are using as a structural science. Therefore, we should take the time to address the "which" question briefly, as a matter of methodological choice, and focus on the analytical development of the "How" question, where we do hope to make some concrete contributions.

There are many concepts in biology, which the widespread use seems to legitimate and vulgarize, but are still very problematic, lacking a precise definition. In the present work, we shall mention three of them: meaning, complexity and contingency. The discussion will be summary, only to establish formal links between different interpretations of these concepts and the various schools of structural sciences they refer to. By doing so, we should clarify to which sources in Biosemiotics and sciences of complexity we are related in the search for more precise and operational definitions of meaning, complexity and contingency in biological systems.

The difficulty dealing with the notion of meaning in biology is as remote as it has been overlooked. Here, we shall adopt the theory of organic codes (Barbieri, 2003) to address the question. According to this view, living systems are semiotic unities in the sense that they have the triadic structure of "sign, code, and meaning". All biological systems share conventional rules of correspondence between two different worlds (codes) that build up dimensional information (meaning) from linear information (signs). The cell and its triadic organization (genotype, ribotype, phenotype) should, in this scope, be understood as the simplest semiotic unity, maybe the first to be originated in the evolutionary process, but not the only one. From that perspective, the systematic search for collective rules that are not determined by individual features in their structures (organic codes) and the identification of functional unities of increasing complexity which convert signs into meanings by codification becomes a feasible research agenda. As pointed out in a previous chapter of this book (Artmaan, Computing codes versus Interpreting life), the key feature of this school of biosemiotics is its model-theoretical perspective on languages that are axiomatically described as computing codes. The emphasis, therefore, when investigating biological meaning is in the identification of organic codes, formally and systematically, and not – as opposed to other views – in the

quest for hermeneutic formulas that would allow us to interpret life itself in a rather transcendent way.

Complexity, as defined from a strictly informational standpoint, is the ability of some opened systems to use energy in order to increase its own order, creating a chain of information transfer (Shannon, 1948). If this is the concept of complexity one shall accept, it becomes almost natural for a biologist to understand the central dogma of molecular biology as a chain of causality leading from information in DNA segments to structure and function in protein polypeptide chains. In such perspective it would be acceptable to try the reduction of complexity to underlying causes, in fact the power of reductionist practices in providing scientific basis to our knowledge of natural phenomena is undeniable. Nevertheless, in agreement with Cohen's analysis of the subject (Cohen, 2004), we can assume that there are limits to the use of reductionism in the investigation of complexity in biological systems. These limits can be formulated in various terms and were indeed discussed by many authors (Brent and Bruck, 2006; Salthe, 2004; Westernhoff and Palson, 2004; Aderem and Hod, 2001; etc.). For our purposes, we should only stress the fact that an informational account of complexity does not take into consideration the role-played by codification in the building and maintenance of multiscale and self-organized biological systems. Coding is crucial for our understanding of meaning in biology and that is the reason we shall try to integrate it to any concept of complexity adopted.

In biology, it seems, boundary conditions are ever changing and are ever restricted to contingent resources inside their history (the narration of their uses). In biology, as opposed to Physics, time is not just a parameter but also the determinant one. In terms of evolution, time would be the ground for compromise between contingency and coherence, a compromise that has various ways such as replication, recombination, mutation, synchronization and hierarchization, yet "biological consistency" can only occur and is determined by the temporal scale of organic cycles, by this particular "cyclic-story-telling" temporal pace. **Figure 1** illustrates the ideas of "time" assumed in some of the Physics and Biology attempts to model the nature in movement. As for this article is concerned, we should just stick to the notion that biological time acts by diversification of agents, as a differentiation process generating specificity (or discriminatory competencies) and, in consequence, generating hierarchic levels. Arrows in a metabolic pathway, a philogenetic tree, or a signal transduction map do not establish equivalence between the points they connect, arrows in biology stand for realization of potentialities or, at the opposite direction, for the indetermination of potentials. In both cases a precise sense of time is at work, integrating it in a greater picture is an inevitable and inviting task for contemporary thinkers. It is our working hypothesis that the operational link between agents, states, structures and/or functions in biology accounts for the generation of meaningful information, based on the codification process that connects instances with no necessary-mechanic association (or material cause). In the study of complex systems the process we are referring to as codification is normally treated as emergence, a term very charged with philosophical enquires on

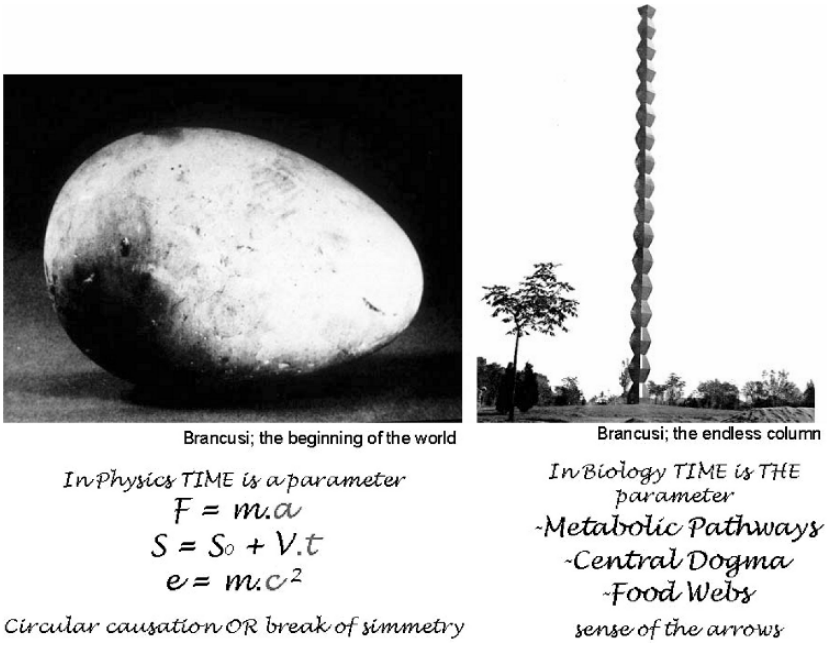

Figure 1. Time in Physics and Biology

how causality operates to build multiscale systems. It can be argued that codification is indeed a special instance of emergent phenomena but, since codification is much simpler to define and seems to be sufficient to the scope of biological complexity we are interested in, we shall adopt this concept. Nevertheless, there are some terms we can borrow from the sciences of complexity that are helpful for the understanding of scalar hierarchies in biology, the most useful is the notion of physical attractor: long-term stable states towards which complex systems tend (Huang et al., 2005 and Cohen, 2004). This definition should be addressed in more detail further.

Philosophical approaches to the question of stabilization levels in living systems also provide valuable contributions to the understanding of biological complexity. The theory of levels of reality and its various formulations has been reviewed in a recent paper (Polli, 2001). Initially developed by contemporary authors (Spencer, Morgan and Alexander) the categorization of reality into levels attempted to give the theory of evolution a metaphysical framework. Levels such as "Matter, Life and Mind" or ontological regions such as "Nature, Consciousness and Society" (Husserl) will follow this same rationale. These original levels were put forward by many thinkers, from Hartmann's "phylogenetic" layers where levels would be defined by their constitutive unites (atoms, molecules, cells, etc.) and corresponding structures, to Polli's "systemic" levels defined by groups of suitable categories and their underlying dynamics. To this last definition of levels and

the theoretical framework it seeds we shall from now on refer to as "Dynamic Ontology". Such view has an enormous heuristic power. The possibility of building up reality levels according to sensible classification of the dynamic categories, sets the scientist free from the fixed boundaries of material causes, but it also demands a new type of imagination, new ways of measuring, modeling and manipulating reality.

Edward Wilson, an eminent contemporary biologist, also recognized by his contributions into the fields of philosophy of science and methodology, has defined "complexity theory as the search of algorithms used in nature that display common features across many levels of organization" (Wilson, 1998). Assuming the terminology we have been using in the present work, this attempt would be equivalent to the search for organic codes in every scale in which living systems shall adopt long-term stability states (the previously mentioned notion of physical attractor).

The idea of contingency underlies both of the concepts that are essential in Darwinian theory of evolution: Natural selection and adaptation. It is also present in the new – Darwinian notion of exaptation (Gould) and, although not explicitly defined in any case, contingency intuitively accounts for the role played by chance during evolution. Once again the problem of such definition in the framework of our analysis is that it does not take into consideration coding, or natural convention, as one of the mechanisms of evolution. Therefore, we will try to define biological contingency as related to the previous definitions adopted for biological meaning and complexity in the framework of the organic codes theory.

François Jacob has proposed the notion of "evolution as tinkering" in the mid 70's (Jacob, 1976). He claimed that the way living things are shaped by evolution is not a balance of teleonomic coherence, replicative invariance and chance variation (as stated in Monod's chance and necessity and broadly accepted), but rather by "the constant reuse of the old to make new" (Jacob, 1986). Tinkering, as opposed to engineering, has to deal with the contingency of resources and their history; therefore, it does not and cannot aim a predetermined output. Stefan Artmann and other structuralist semioticians tried to develop the tinkering concept from a semiotic perspective (Artmann, 2004). There, the materials to be recycled by tinkering become signs and their syntax, semantics and pragmatics. The theory of tinkering assumes the concept of process consistency as the relation between contingency and coherence, as the formal determinant of evolution. This would be equivalent, using Barbieri's terminology of the organic codes theory, to admit that evolution proceeds by natural convention and by natural selection. The pragmatics of any evolution (of living beings, living institutions, living theories etc.) can be analyzed by means of its consistency. Biopragmatics, as a research agenda, should be the search for the set of coherent transformation of contingent boundaries given limited resources. Such investigation done by semiotic means becomes the search for context-dependent transformation of all processes that diversify potentials in the precise sense of originating organic codes.

Our understanding of biological meaning, complexity and contingency is intimately linked to the theory of organic codes in Biosemiotics by one hand, and to some accounts of multiscale emergence formulated by sciences of complexity and philosophy of sciences by the other hand. These notions will frame our discussion of the acquisition of cell immunity and the role played by repetitive RNA sequences in different levels of organization displayed by living systems. By doing so, we will come up with a new attempt to classify functional unities of life into categories of increasing complexity. Kinetic constants, structural limits, and ontological drifts no longer define the frontiers amongst levels. The frontiers become rather a matter of dynamics as the ground for the origin and evolution of semiotic systems as stable states.

The notion of categories that bear a formal correspondence with the stabilization levels adopted by biological systems is seminal in our reasoning and will be developed in detail on the next sections. Briefly, we propose that there are three major levels of stabilization for the living:

1) the CELL, whereas by the discriminatory competences of a semiotic unity a functional autonomy towards the environment is first achieved, The CELL level is able to provide environmental change with biological meaning;
2) the SELF, whereas recognition tasks are added to the previously acquired discriminatory competencies and more complex semiotic unities rise, being able to couple environmental change and Cell fate either by the triggering of life cycles or differentiation programs;
3) The SENSE, whereas cognition skills are added to previously acquired discrimination and recognition ones, giving rise to more complex semiotic unities that display metabolic, developmental and somatic autonomy towards the environment, being able to make dynamic use of information to remodel their own function and structure.

Typically, in each level of organization there is no unique solution for the dynamics of a system compatible with the production of long-term stability states. In articulating our analysis, a dichotomized repertoire of solutions will be discussed for the three categories proposed (Cell/Self/Sense): a Fancy one and a Frugal one. The fancy/frugal distinction refers to alternative pathways taken by living unities under the pressure of natural selection and the synthetic power of natural convention. In both cases, the dynamics of constitutive elements bring about structures and behaviors at higher levels. Nevertheless, the way higher level dynamics constrain lower level structures and behaviors seem to be different in each case. Downward determination is streamlining in frugal solutions, conversely, when fancy pathways are adopted higher levels tend to be more permissive in the determination they perform, pragmatically that will allow to more flexibility concerning structures and behaviors to be naturally selected and conventionned. Therefore, we shall propose this instrumental dichotomy between "Frugal/Fancy" to proceed through the analysis of different levels of life organization. **Figure 2** produces a formal representation of alternative configurations adopted by the living at the levels of: Cell, Self and Sense as categories in an analytical framework (Körner, 1999).

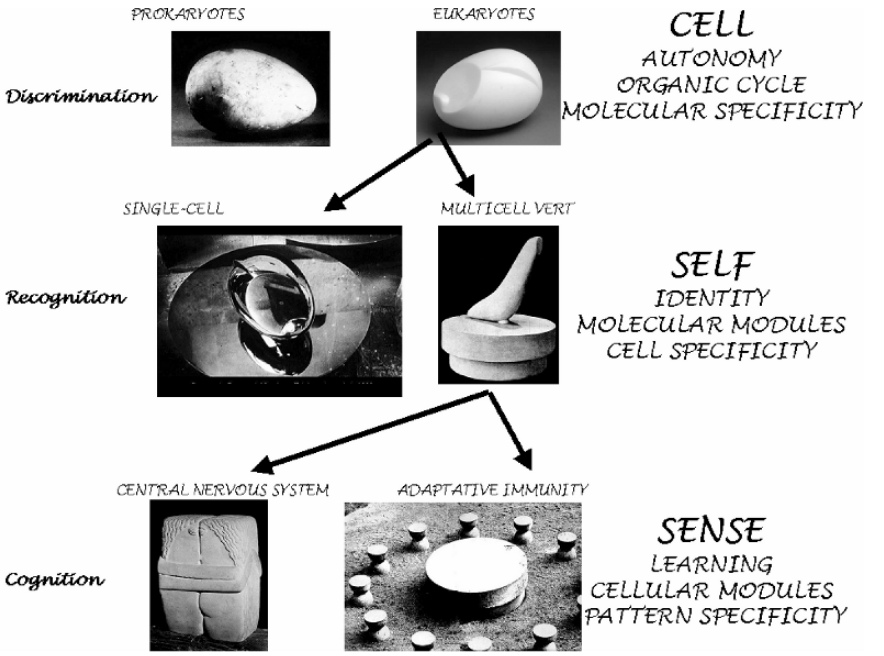

Figure 2. The Cell/Self/Sense categories, levels of stabilization adopted by living systems

UNITY OF LIFE – CELL MAKING

Let us take the cell as the first (in time and space) dynamic configuration that behaves as a living unity. The cell is the minimal thing conserved through evolution capable of:

– Multiplication, variation and heredity (life definition by Herman Muller 1966);
– Assembling functioning units in a structural hierarchy that has acquired trough evolution the ability to store and process the information necessary to its own reproduction (Lila Gatlin, 1972);
– Transforming an external energy and matter flow into an internal flux of self-maintenance and self-reproduction (Varela and Maturana, 1974);
– Assembling and perpetuating artificial structures from natural ones (Barbieri, 2002).

Regardless of the definition of life we shall adopt, just to illustrate a few possibilities, any cell (prokaryotic or eukaryotic, autonomous or living in an organism, differentiated or not) will fulfill the criteria. A cell is a unity of life, a whole which dynamic configuration displays stability relative to its elements.

The organic cycles based on ATP recycling were developed and fixed using cells as photographic paper. Energy gradients tend to dissipate by organized and/or periodic means in nature, so that relative to the energy flow a cell is also a plausible

level of synthesis, as much as it is so for molecular cycling (Salthe, 2005). Different levels of analysis and of synthesis, at the same time.

Assuming that, following Neuman terminology based on the work of Bateson, cells are recursive-hierarchical systems (Neuman, 2004) that enable organized/periodic use of molecules and electrons, we must admit causality to proceed bottom-up and top-down (Ellis, 2005a and b and El-Hani, 2005). The compromise between information coming from different levels in hierarchic systems exists everywhere (thanks to feedback loops, patterns are recursive in nature), but only living systems, can use this compromise to dynamically change their own behavior in various levels (thanks to "evolution" understood as the possible output of two operating mechanisms: "natural convention" and "natural selection").

The term "natural convention" presents a broad range of applications that goes beyond the central dogma of molecular biology, presumably the first organic code fixed by natural selection and natural convention. The intersection in an imaginary Venn diagram displaying these two evolutionary processes would be the actual (selected and conventioned) evolving unites of life. The synthetic integration of bottom-up causation and downward determination in hierarchical systems allows by its own nature – synchronic determination from the bottom and diachronic constraining from the top – multiple solutions in the higher levels. At the dynamic level of cells, which is under analysis here, solutions as diverse as non nucleated Eubacteria and Archaebacteria, or the nucleated cells arranged as single cell and as multicell organisms are equally compatible with supporting life.

Cells' autonomy relies on their creating compartments to make cyclic use of energy. The prokaryotic solution is frugal in that its streamlining nature constrains further changes in form, despite their remarkable adaptability to changing environmental conditions. The eukaryotic solution can be referred as fancy, in that its extra-compartmentalization opens up windows of opportunity for alternative controls. In eukaryotic cells form is not as constrained from within, the structure seems permissive to adaptation to the same extent as it is the case in prokaryotes, but it is also permissive to complexification into new logical typing (Bateson, 2002), into creation of new forms and patterns.

At the Cell stabilization level, double-stranded RNAs (dsRNAs) are constitutive structures in all RNA species of the intracellular environment: messenger RNA, transporter RNA, ribosomal RNA and small nuclear RNA. Base-pairing between complementary regions of different RNA molecules, or even intramolecular links, seem to be essential for many control-steps of RNA metabolism, namely: translation initiation and termination; messengers stability; messengers editing (only in nucleated cells); and transcription termination (Lewin, 2000). It is textbook common sense that local RNA-RNA interactions at the Cell level are RNA metabolism signs. In Prokaryotes, these controls are restricted to the steps of protein synthesis, in nucleated cells they also account for RNA processing events.

UNITY OF LIFE – SELF-MAKING

The following level of integration from the perspective of nucleated cells deals with the setting of increasingly abstract compartments. Autonomy towards the indiscriminate external world is necessary and sufficient for creatures to live, but eukaryotic cells could and did discriminate further: between cell types and between cell types in time. We shall refer to that kind of discriminatory property as "recognition" (which literally means "an awareness triggered by contact"), a property essential for what will be called "self-making". Two very different general strategies seemed to be selected to cope with the cell-to-cell discrimination/recognition problem. Unicellular eukaryotes, as yeast and parasites, have taken the frugal way and multicell organisms have taken the fancy one.

The making of self in single cells seems to require differentiation: alternative cell stages attuned in adaptative life cycles. Environmental conditions become integrated into signals that control growth, but also, functions of a diverse logical typing as differentiation, migration, latency, mating, invasion, which are not clonal. Such processes encompass the positioning of single cells in their own life cycle. By the comparison between alternative stages of the same cell and among different cells and their pattern of contact: the notion of identity unfolds in each and every cell.

The fancy path leads to bigger wholes; many cells are assembled in organisms. Here the making of self also requires differentiation, but in organisms there seem to be synchronic life cycles for different cell types. Populations of cells as they dynamically associate in tissues, organs, systems, follow rather diverse programs of differentiation, latency, senescence, programmed cell death. The notion of identity unfolds in a cell-to-cell basis but emerges for the whole organism as well. Once again, the fitness of frugal and fancy strategies is equivalent, but the fancy of multicellularity broadens the spectra for future change. The Cambrian explosion, for example, illustrates the diversity of forms triggered in multicell organisms whenever the fancy path was the substrate for further change.

The role of RNAs as code-makers has been previously stated by Barbieri (Barbieri, 2003) in the scope of the central dogma, bridging the gap between DNA and protein, essential in the making of the Cell level, as mentioned in the previous section, local dsRNA structures, in particular, act as RNA metabolism signals at the Cell level. We shall analyze some mechanisms that cells have developed to deal with double-stranded RNA in different context to elucidate the role of RNAs as code-makers also in the making of Self. RNA interference (RNAi) is a physiological phenomenon widely conserved through evolution by which double-stranded RNA (dsRNA) triggers the silencing of cognate genes (reviewed in Faria et al., 2004). The process was first observed in *Caenorhabditis elegans* after the realization that the injection of dsRNA into this worm brought about the specific degradation of homologous endogenous mRNAs. The evidence of other dsRNA induced homology-dependent gene-silencing mechanisms as chromatin remodeling, chromosome rearrangements, genome *de novo* methylation and translation

inhibition emerged later, making it compulsory to enlarge the scope of the investigation (for extensive review see Agami, 2002). According to the currently accepted model, dsRNA can trigger RNAi following their conversion into small, 21–25 nucleotide (nt), interfering RNAs (siRNAs) by members of two families of enzymes: the *rde-1* (for RNAi defective)/*ago-1* (for Argonaute) family and the Dicer multidomain RNAse-III family. The siRNAs will then guide another enzyme complex, the RNA-induced silencing protein complex (RISC) to homologous mRNAs and induce their cleavage and degradation. It is worth mentioning that dsRNAs are physiological intermediates of processes as diverse as viral infection, the expression of transgenes, and the transcription of repetitive sequence gene arrays (endogenous or exogenous, single or multi copy). We will develop the idea that the way different cells deal with such a "polisemic" signal will ultimately reflect their tolerance against genome instability. In the case of differentiated vertebrate cells, dsRNAs induce the interferon response, which activates protein kinase R (PKR) and 2′5′(A)$_n$-synthetase and triggers, as final consequences, the ubiquous inhibition of translation and the induction of mRNA degradation, respectively (Leaman et al., 1998 and Clemens et al., 1997). The toxic effects of dsRNAs in somatic vertebrate cells can be overcome by the use of siRNAs (the shorter versions of dsRNAs) as the input signal to trigger specific-gene silencing. Interestingly, bypassing the interferon response shows that RNAi, thought not visibly triggered by long dsRNAs sequences (the interferon response is just prevalent), is still perfectly functional after cell differentiation (Elbashir et al., 2001).

Two enzymes seem critical for the logical shift that takes place during vertebrates somatic cell differentiation. In single cell eukaryotes and invertebrates PKR homologues do not exist. As for 2′5′ (A)$_n$-synthetase, the enzyme is highly conserved amongst vertebrate, but only poorly homologous putative sequences are found restricted to two species of sponges, among the invertebrates. In embryonic and stem cells, the response to dsRNA is restricted to the silencing of homologous endogenous genes because these two classes of enzymes are inactive or not expressed. Let us dissect the functional structure of PKR, which is, at present, better characterized than 2′5′(A)$_n$-synthetase. PKR is a kinase dependent on dsRNA binding for its activation, the catalytic kinase activity lies in a C-terminal domain and the dsRNA binding is mediated by a N-terminal domain (Lemaire et al., 2005). Upon dsRNA binding PKR undergoes auto-phosphorylation and dimerization, once activated it phosphorylates the eukaryotic initiation factor eIF2α and inhibits translation initiation, in addition PKR induces proinflammatory genes (such as type I interferon) by activating the NF-κB pathway (this issue will discussed in more detail in the "sense making" section of the article). Interestingly, the catalytic domains of other kinases that phosphorylate eIF2α such as HRI, GCN2 and PEK, are highly conserved, but their regulatory domains are different (Rothenburg et al., 2005). It seems that the association of a dsRNA binding activity with a catalytic kinase domain in the same enzyme enabled differentiated vertebrate cells to connect the presence ds-RNA necessarily to translation inhibition, PKR links two otherwise separated sets of information, this happens by natural convention. Many proteins

are composed of modular functional units which combined through evolution achieve the conformational flexibility required for regulation without sacrificing the specificity essential for catalysis. In that sense, neither dsRNA recognition, nor translation initiation factors phosphorylation are major evolutionary novelties, but their assembly into the same protein that is alternatively expressed depending on the identity of the cell is new, it is exclusive to vertebrates and it stands for a new organic code.

Yeast, single cell parasites, invertebrates and non-differentiated vertebrate cells are Eukaryotic cells, but based on "single cell" logic. Their response to double-stranded RNA is selective to its sequence; RNAi operates by inhibiting the expression of cognate messengers without killing the triggered cell. The notion of self unfolds allowing to some plasticity of the genome in the behalf of keeping cell stability. At the multicell level of mature vertebrates, the selective response to ds-RNA is no longer enough; these systems would rather spare the affected cell than risking genome stability. The notion of self unfolds privileging the stability of the bigger whole, the organism.

The similarities between invertebrates and vertebrates are very striking for many dimensions of self development that were not mentioned in the present work and should be discussed in depth in the future, namely: body plans, embryology and the pattern specificity of most organs and systems. The dichotomizing exception is the development of more or less complex cell-mediated adaptative immunity and of central nervous systems, exclusive to vertebrates. The differentiated response of vertebrates to dsRNAs segregates along with their acquisition of adaptative cell systems able to produce somatic change, memory and learning. Some of the consequences of such achievements we shall examine in the following section.

UNITY OF LIFE – SENSE MAKING

The following level of integration from the perspective of multicell organisms deals with the setting of compartments increasingly abstract. Identity provided by discrimination between cell types and synchronic differentiation programs are essential features in self-making, but organisms could and did discriminate further, by building classes of differences and dealing with hierarchic levels of classes by integrating simplified versions of those (coded information, memory and decision-making). We shall refer to discriminatory properties of that kind as "cognition", they are essential to what we will call "sense making".

Two very different general strategies seemed to be selected to cope with the hierarchic multicell integration/cognition problem in vertebrates. The complexification of a central nervous system is the frugal solution. Adaptative cell immunity is the fancy way.

Nervous and Immune system development, both require the differentiation of very specialized cells to mediate somatic adaptation to integrated signals

and learning. The two systems care for protecting the whole organism against foreigners and for its body maintenance, only they use very different topological strategies. In Cohen's formulation "The nervous system houses spatially fixed non-renewable neurons, with a hard-wired network geometry. The immune system is composed by constantly renewing, physically flowing population of cells" (Cohen, 2004).

In the nervous system the prototypical cells are neurons, despite differences due to the nature of the specific sensorial structures they connect, these cells share minimal features concerning their structure and function. In every case, dendritic region, cell body, axons and synapses will be respectively responsible for the reception, integration, conduction and propagation of the nervous impulse. These cells of ectodermic origin undergo three main irreversible transitions during embryogenesis until becoming functional neurons. First, there is the determination to a neuronal pathway, then the migration and, eventually the synaptogenesis. The three events are controlled by cell context sensed as neurotrophic factors and cell adhesion molecules relative presence, at each step there is a decrease in the potential destinies the cells can follow. The frugality of vertebrate's nervous system has nothing to do with their complex functionality. They respond as a robust network of information processing and integration which plasticity is only comparable with that of their own immune system. The economical nature of nervous system has to do with the relatively constrained form of their cellular unities, and their being unable to regenerate after differentiation.

As the immune system ontogeny evolves, the adaptative system accumulates a population of mesodermic origin lymphocytes equipped with unique surface receptors able to recognize nonself epitopes in cognate interactions. Recognition will trigger proliferation and further differentiation (clonal selection) and after stimulation by cytokines or other by-products of innate immunity the lymphocytes progeny acquire effector's functions. The antigen-specific receptors, Immunoglobulins and T-cell receptors are generated in a somatic process of gene rearrangement that constructs the variable part of the molecule bearing specificity towards the epitope. It is worth mentioning that a complex selection check, by means of the major histocompatibility complex molecules presented, avoids self-recognition. Therefore, when in action, adaptive immunity accounts for specificity and memory. The fancy of the system is not much in these properties, shared by the nervous system, but in its functional organization. The unfolding of responses to antigens in vertebrates is a clear example of somatic evolution at the scale of ontogeny and at the scale of physiology; cells are under the pressure of the same laws of mutation and selection as individuals in a species (Du Pasquier and Flajnik, 1999). The immune system of vertebrates also exploits innate immunity and the nature of some of the mediators that are common to innate and adaptative pathways. This cross talk will be of particular interest to our understanding of RNAs role in the building of sense. Innate response to double-stranded RNA (integrated by the system as a sign of viral infection) includes inhibition of viral replication (by PKR and $2'5'(A)_n$-synthetase activation) and a canonical inflammatory response,

shared by invertebrates. The novelty is that Interferon and interleukins secreted as part of the innate response by infected cells will trigger signal transduction pathways guiding alternative differentiation of lymphocytes B and T, NK and TAP, and ultimately recruiting the adaptative cell immunity into the scene. In this sense, dsRNA is also dealt with by triggering the rearranging machinery to generate antibodies against the cognate viral antigens. A link that has become necessary by natural convention/selection based on the modular association of receptors in immune cells and the modular association of cell types into discrete functions.

Even in invertebrates, RNAi seems to play a role in sense making, as it starts emerging in evolution. In *C. elegans* a remarkable aspect of the RNAi process is its ability to spread throughout the target gene beyond the sequence homology region harbored by the dsRNA trigger molecule, a phenomenon called transitive RNAi (Sijen et al., 2001). Besides, in the worm, RNAi also spreads throughout the organism, suggesting a mechanism to forward the signal from cell-to-cell. The sid-1 gene product is a Trans membrane protein that could act as a channel for such systemic silencing (Winston et al., 2002). Following this same rationale of amplification by spreading of the RNAi silencing to homologous sequences in the genome, is their targeted methylation or the methylation of associated histones. Two recent studies have shown that in the fission yeast the integrity of RNAi machinery is required for epigenetic silencing at centromers, and for initiation of heterochromatin formation at the mating locus, being also important for proper regulation of chromosome dynamics during cell division by meiosis and mitosis (Volpe et al., 2002 and Hall et al., 2002). We shall propose that by promoting intercellular communication, all attempts to spread the RNAi phenomenon could be regarded as incipient sense-making strategies. In "single-cell oriented" organisms the operating strategy for dealing with dsRNA is only the specific silencing of homologous sequences. "Sense making" appears by as the spreading of this strategy by multiple mechanisms to as many cells as possible. By the other hand, the possibility of displaying alternative reactions to dsRNA will be a privilege of "multicell oriented organisms" only to be fully realized along with the development of vertebrates immune and nervous adaptative systems. The Venn diagram in **Figure 3** summarizes the molecular partners of dsRNA associated with the different cell responses that can be triggered depending on cell context, the comparison stands for the differences between differentiated vertebrate cells and non-differentiated vertebrate cells or invertebrate cells.

As a concluding remark on the contextual nature of dsRNA signs in sense-making, we must discuss some evidence on the mechanisms that control dendritic protein synthesis in neurons. In 2002 it has been proposed that translational control could be achieved by means of ribosomal/mRNA interactions (Mauro and Edelman, 2002). In what the authors called "The ribosome filter hypothesis", the sub cellular localization of particular mRNAs would be a result of the complementarity between their non-coding regions and sequences on rRNAs associated with 40S ribosomal subunit, resulting in local dsRNA structures. More recently, the presence of cytoplasmic

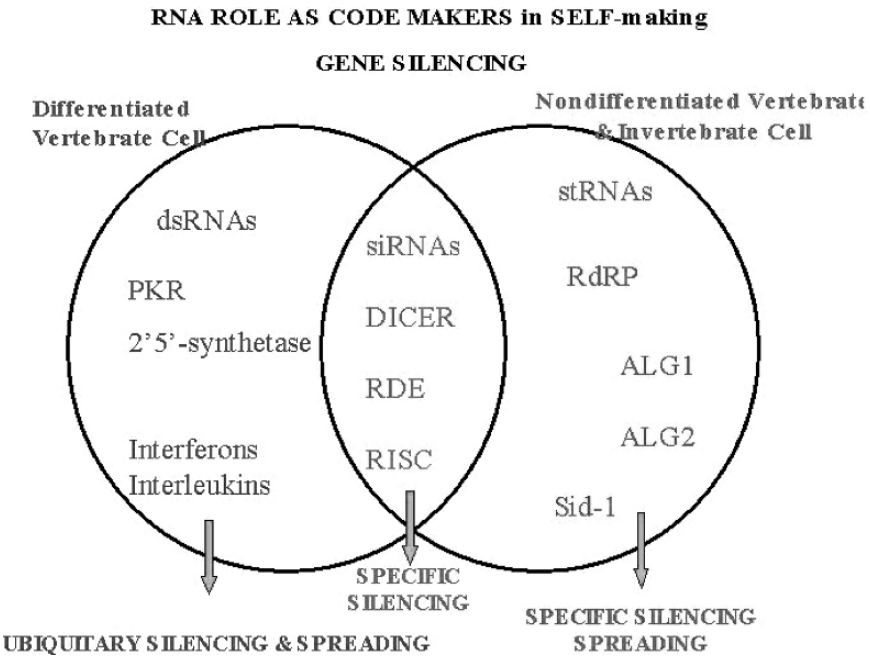

Figure 3. Molecular machineries associated to cell response to dsRNA, there are common and exclusive codes amongst different classes of cells

RNA granules has been associated with mRNA/rRNA interactions (Anderson and Kedersha, 2006) and their putative role in translational control is reinforced by the fact that such structures are restricted to certain cell types and cell regions where the selective translation of recruited messengers is carried out. Of particular interest is the fact that, in neurons, structures of that kind (neuronal granules) have emerged as important players in the targeting of specific protein synthesis to dendritic regions. The local translation performed in neurons seems to be dependent on microtubules integrity, mRNA/rRNA local double-stranded formation and RISC pathway integrity (Cristofanilli et al., 2006; Ashraf et al., 2006 and Pinkstaff et al., 2001). Moreover, such pattern of gene expression control is associated with long-lasting forms of memory, at least in Drosophila (Ashraf et al., 2006). The data is far from being conclusive, but the evidence suggests that dsRNA might have a precise role in nervous system sense-making, by targeting protein synthesis to synaptic regions and by favoring specific paths of cell cognition. In **Figure 4** we can see a Venn diagram illustrating that panic response is a common feature of differentiated vertebrate cells, and that neurons and immune cells have developed different pathways for dealing with dsRNA. In Neurons, these molecules can trigger dendritic protein synthesis, while in immune system they will trigger somatic cell differentiation.

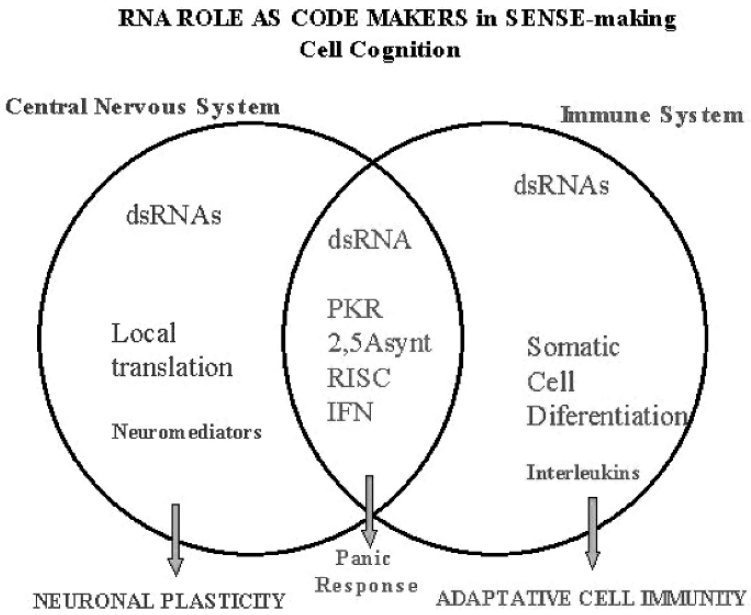

Figure 4. Molecular machineries associated to cell response to dsRNAs, there are common and exclusive codes amongst vertebrates Nervous and Immune Systems

CELL, SELF, SENSE – CONCLUSION AND RESEARCH PERSPECTIVES

Levels of reality based on denoting categories imply that the structuring of such levels "does not respect *a universal principle of linearity,* then one is forced to *restrict* the multidynamic frames *to their linear fragments"* (Polli, 2001, emphasis mine). Because of this assumption we have the fact that properties of higher hierarchical levels bear a causal dependency towards lower hierarchic ones, but are categorically independent from those. Another way to phrase the same statement, only adopting other terminology (El-Hani and Queiroz, 2005), is to say that properties from higher hierarchical levels in biological systems are not reducible to lower level ones from a synthetical standpoint, but are reducible from an analytical standpoint. The only research agenda that seems fruitful assuming these dynamics of multiple causalities in hierarchic systems comes from a balance of analytical and synthetical procedures, of descriptive and categorial classifications. Methodologically one shall proceed through analytical reductionism in order to identify lapses of the living system that can be linearly explored, but then, the integration of such "horizontal cut" into the greater picture to build up complexity, restore context and probe deductibility, will be no less than necessary.

The CELL/SELF/SENSE account of the unity of life is an attempt to pull forward the type of research agenda mentioned on the previous period.

The analysis of the molecular partners RNAs are able to recruit as:

a) mediators of genetic coding into proteins
b) mediators of sequence-specific gene silencing by RNAi;
c) mediators of global cell response to integrative signs;

is clearly an analytical reductionist approach. The integration of each RNA-molecular machinery-"partnership" into categorial frameworks (CELL/SELF/SENSE), the classification of properties and dynamics accordingly, in respect to the categories they are embodied in, is clearly a deductive categorial approach. The biological meaning of repetitive RNA sequences evolves by means of the physiological processes that are associated to their presence at different levels:

a) dsRNA are RNA processing signs at the CELL level, able to recruit either only translational machinery (in Prokaryotes) or translational machinery and splicing machinery (in Eukaryotes);
b) dsRNAs are selective gene silencing signs at the SELF level, able to recruit either only selective nucleases (in single or non-differentiated cells) or selective nucleases and ubiquitary transcriptional and translational machinery (in differentiated cells of multi-cell organisms);
c) ds RNAs are cell cognition signs at the SENSE level, able to trigger localized protein synthesis modulation (in multi-cell organisms neurons) or the recruitment of adaptative cell immunity for targeted cell destruction (in all other differentiated systems of multi-cell vertebrates)

Research in theoretical biology aims testing the explanatory, predictive and heuristic power of scientific theories. In this scope, the following steps in our research program would be testing the proposed categorical framework by means of:

– the analysis of other case-studies that could validate the "organic code/ level transition hypothesis";
– the formalization of the attributes that segregate into each category in a less natural language;
– the application of the Cell/Self/Sense categories to other disciplines in search for overcoding.

In fact, these three approaches are currently under investigation. Meanwhile, let us summarize some of the principles that are conclusive in the study of contextual meaning of dsRNAs and shall be seminal for future projects.

The assembly of dynamic configurations into stabilization levels applies to complex systems in general. This tendency to build tangled hierarchies as means to accommodate energy flows, could be the missing link (and the common material ground) between Physics and Biology. Therefore, it seems, if one wishes to attack the emergence of biological tinkering and of biological timing (as canonical indexes of biological contingency and complexity), it might be worth analyzing how natural hierarchization (the assembly of levels) rise. In particular, it would be helpful to investigate the specificities of coded hierarchies. Following Barbieri's formulation,

this track will lead us to the first organic codes, the first semiotic unity and its minimal conformation: a cell.

Copying first and coding later (replication, mutation, differentiation, and others being just instantiations of these two relational patterns) are new functions, restricted to the realm of living things. Once again, this two relational patterns are reducible in analysis to their physical grounds, though not strictly deducible from them. Copying and coding are new, emergent properties, coherent once contingent, and once coherent and contingent, necessarily consistent. Natural selection, Natural convention, adaptation, evolution and even life itself would be corollaries of those relational patterns originated some 4.5 billion years ago with the first triadic cells (composed by genotype, ribotype, phenotype).

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