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Stem cells can commit to divergent differentiate cell and it can be droven by different signaling pathways. But, in nature, a leading factor can dictated the way how cells can assume their fate. This leading factor, called as attractors, resemble that ones which participates on every signaling pathway. Here we consider Ca2+ and its perturbation as oscillations, amplitude, and frequency as leading attractor to guide stem cells fate.

Studying complex system:

Calcium oscillations as attractor of cell differentiation

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ABSTRACT

Biological processes, such as induction of undifferentiated cells to neurogenesis, provide complex mechanisms for studying. For further insights, subsets ruled by metabolic pathways or key molecules called attractors need to be elucidated. In this review, we have focused on the role of calcium as a driven force of neuronal differentiation. Calcium activity refers to peaks and waves, whose amplitudes and frequencies in stem and progenitor cells involve activation of a great variety of signaling pathways composed by neurotransmitters and their receptors, intracellular signaling factors and transcription factors, forming a complex network. The study of different subsets: From receptor-mediated calcium flux to transcription factor activation, can then combined to understand the process of neuronal differentiation.

INTRODUCTION

Emergent properties in complex systems

Emergent properties can be defined according to one's interests: physicists would consider liquid, solid and gaseous phases of water as emergent properties of water molecules in different energy states. In the present review, we intend to focus in complex entities that emerge from interactions of heterogeneous elements. These interactions generate properties that characterize the system as a whole.

The studying of emergent properties in complex systems cannot be accomplished properly only through reductionist approaches. However, it is also true that these properties cannot be studied without any reductionism. In order to understand and manipulate an emergent system, it is necessary to separate it into different constituent parts to and from what the emergent properties are generated [1]. However, after that, it is necessary to reconstruct the entire system. The use of quantitative models allows reconnecting the interactions between the subunits of the system [1, 2]. This approach allows the understanding, how properties of interest may arise from a dynamic organization [2]. Only through combined use of reductionism with synthesis, it is possible to acquire knowledge about emergent properties. In biological systems, the first one must be found and causally reduced to material subunits, from organs and tissues to cells, subcellular structures to molecules, atoms and their interactions, followed by reassembly of these subunits into a dynamic model [3, 4]. A better understanding of

how this organization works allows planning of most adequate and effective ways to interfere and obtain desired effects.

Limits of reductionism

The reduction of complex phenomena to understand its causes, natural laws, internal structure, energy, information involved in the process is the essence of science. Reductionism is authoritative, but it is not a panacea. Reduction is adequate when the phenomena of interest can be reduced to the properties of a physical unit, such as a particle of matter or a biological entities, such as i.e. bacteria or proteins [3]. Reduction is the substitution of a superficial appearance for a causal reality; the phenomenon of interest is demonstrated to be inherent to properties of its constituent parts [4, 5].

Successful reduction allows the discovery of microscopic entities, whose existence is necessary for the observed microscopic phenomena. The process of reduction does not, however, constitute the entire scientific operation when the object of interest is not a discrete entity, but allows the understanding of interactions [4]. The properties of subunits may not account for the behavior of a complex system. For example, the inhibition of the product of a gene known to be involved in blocking cell differentiation [6-10], does not necessarily decrease differentiation. The process of differentiation may apparently learn to reorganize its behavior using different subsets of molecules. A gene might become essential for a process only if the system organizes itself by being dependent on this gene, which is not the case in most of biological processes. The system auto-organizes itself in many levels, and for this to occur, the keys essential molecular interactions must also show functional plasticity [11-14]. In summary, systems may depend not only on the properties of its subunits, but also on their interactions [15, 16].

Some researchers may feel that the study of emergent properties does not provide true science, and that science is in fact an entire reduction in nature. The most striking example of an emergent property is life itself. Life is not a property of joining subunits such as DNA, proteins or carbohydrates, once they are not alive [17-19]. The alive differ from dead cells in interaction levels among its subunits. Life emerges from inert material as a consequence of metabolism, which has a continuous transfer of energy and information systematically packaged in cells in a way that leads to auto

perpetuation [18-20]. The complexity of the dynamic behavior generating metabolism, growth and genetic heritage is what we call life.

Attractors

Microscopic interactions, at the molecular and atomic level, constitute macroscopic matters. Molecular interactions are the essence of matter. What appears to be "structure" at one level might arises from what appears as "energy" in another level. These levels establish the operational limits of interactions. Entities interact more naturally when they occupy similar levels.

A surprising property of many systems is that make them to have certain stable states in which they may remain during a long amount of time [21, 22]. The emerging of such stability can be observed in natural systems. Some systems may never reach a stable condition, been always oscillating among unpredictable configurations. But in a stable state with a long duration is an "attractor". An attractor will alter the dynamic of interactions among the subunits of a system in order to stabilize the configuration [15]. The living cell may be considered an attractor of all the genetic and metabolic interactions of its subunits. Attractors are islands of order in an ocean of entropy. Natural systems are not equations formulated *a priori*. Rather then, they may or not stabilize around an attractor, without any previous design making them to behave in this way. A natural system is blind to its final state. Its behavior is not attracted to stability, but creates its own stability [21, 23].

Thus, an attractor is a global state, which generates long lasting stability. Stability is an operational property; attractors are states of the system that are invulnerable to perturbations. The loss of an attractor will not necessarily lead to a disordered end of the system. The system may stabilize in another attractor, or in another form of order. However, a very energetic perturbation may produce unexpected result; the impact of a perturbation is not proportional to its energy, but rather to the system's sensitivity to the perturbing energy or information [21, 22]. In other words, systems can be susceptible to the last modifications by specific states in critical moments.

Groups of perturbations, which will allow the return of the system to its normal attractor, form a system of attractors. Note that natural systems that contain other natural attractors tend to be mutually connected in such a way that their attractors are functionally dependent. In contrast to mathematically defined attractors, the attractors of natural systems are open and composed of subsystems, and can thus be creatively complex. An inherent problem of natural systems is the overlapping of its limits, which are all interconnected. A control element can be seen as the discrete information or energy necessary to maintain a specific set of interactions, a valley of attractors [24]. Therefore, the changing in a control element can lead a system to new attractors. The valley of attractors is, thus, a topological metaphor for the conditions, which delineate the stability of the system. It is desirable that the system persists in its behavior, and for such thing, there are sensitive points in the attractor valley, such as critical step ends must be avoided. If, on the other hand, one desires to perturbate the system, the impact must be directed towards these sensitive points. Each system has its own course, attractor topology, weaknesses and strengths, as well as unique limits of order and disorder [25].

Neuronal differentiation as a model

The study of factors driving cell differentiation remains among the most enigmatic of fields within biology. First, the egg after successive divisions originates three layers of cells, each of it with specific characteristics able to originate different tissues with their own organization, such as cells with highly specialized functions and properties. During this process, simultaneous cell migration occurs in the tissue and among the layers as well (especially in case of the vascular system), cell differentiation and maturation simultaneously progress resulting in tridimensional complexes, such as i.e. the brain with different properties in each area, exquisite cells types, specialization and properties. Here, we provide explanations for the spontaneous generation of many multiple neuronal cell types from stem cells, to then infer that natural systems tend to become greatly heterogeneous, and then explain the role of intracellular calcium fluxes and signaling in this process.

Grobstein classified some of the most important characteristics of the cell differentiation process [26].

- the initiation of a modification due to a signal
- stabilization after the initial signal has vanished
- the efficacy of various exogenous and endogenous substances as inductive stimuli
- a limit of 5 or 6 cells for the number of cell types that can be generated from any cell type
- a progressive limitation over time in the available number of pathways
- restrictive periods during which a cell is able to respond to inductive stimuli
- presence of unique features in each of the differentiated cell types
- necessity of a minimum heterogeneous cell mass to initiate differentiation under different circumstances
- presence of continuous division in undifferentiated, but not in differentiated cells

We propose that cell differentiation is governed by the behavior of random genetic networks (RGN) constructions. First studies presented this concept, in which signaling pathways follow in a linear fashion. But this is not a wrong concept. It is a point of view which consider just a part of the system, not at all. Many aspects of importance from biological effects have been obtained and are still being acquired by the linear information transfer. For increasing knowledge and obtaining different and wider perspectives, signaling pathway interactions can be modeled with the concept that they interact with other signaling pathways in order to exert their biological effects. These interactions result in complex networks and have a non-intuitive quality. The properties of these networks could be understandable through a systematic analysis of the signaling pathways interactions. Usually it is thought that cell differ due to their differential expression of genes, including short regulatory RNAs, and not due to structural changes [27, 28]. The study of differential activity of genes leads to two issues, which are often not carefully distinguished: the ability of the genome to behave in more than one model, and the mechanisms that guarantee the execution of the appropriate tasks for each cell type [29-31]. Networks of receptors, intracellular signals such as transcription factors, intracellular calcium levels and calcium-dependent proteins, and enzymes that synthesize these signals can be associated in a random way, behaving in multiple modes in the circuitry. Different periods of modes, when incorporated into a network, are isolated from each other so that none can be present in two periods simultaneously. Therefore, multiple mode periods, each with a different

profile of calcium induced gene expression, are expected to occur in random constructed genetic networks. That is why it is reasonable to identify a cell type within a period of a mode. Accuracy of this binary model, even though in its expression of a gene is potentially reversible at every moment, is based on common occurrence of multiple modes of behavior in a genetic system. If a cell type is identified with a particular period of a mode, the typical number of periods in a genetic network must be in the same order of magnitude of the number of cell types in organisms with the same number of genes, considering that oscillatory patterns of intracellular calcium levels regulate gene expression [29, 30, 32]. An example for a regulatory gene network regulating human embryonic stem cells growth and differentiation was previously published [33]. The existence of signaling pathways of different modules, such as WNT, LIF, NODAL and FGF, indicates that human embryonic stem cells (hES) are prepared to respond to a wide range of signals during their undifferentiated state. hES can undergo proliferation or differentiation in their niche or local where they allocate and by adjusting the balance of inhibitors and effectors (see a review about different types of Stem Cells [34]).

Relations between the local network properties and its global behavior are particularly interesting. Many parameters that specify these local properties can be modulated, which in turn makes the network operate in different regimes. In an orderly regime, the system behaves in a simple manner, and many of its components are static. In this regime, transfer of information between components is obstructed by contingents of its components. In the chaotic regime, the system behaves in the opposite way, so the perturbation of one component is propagated to other components, analogously to a signal cascade, where few components are static. Thus, networks in chaotic regimes are very sensitive to the initial conditions and perturbations [35, 36].

The frontier between order and chaos is defined as a complex regime or a critical phase, and networks are in a phase transition [23]. It is in this regime that networks are most evolved. Kauffman, a pioneer in debates on random Boolean networks, [22] argues that life must exist in the frontier of chaos, while networks representing real genetic regulatory interactions operate near the critical phase. As Kauffman wrote: “a living system must first find an internal equilibrium between stability and malleability

[37]. In order to survive in a changing environment, it is necessary to be stable, but not so stable as to become permanently static".

Mechanistic basis of calcium activity

Intracellular free calcium concentration $[Ca^{2+}]_i$ variations can control diverse cell physiology processes, such as cell proliferation, migration, gene transcription, determination of embryo segmentation, muscle cell contraction, glandule secretion, cell death and so on. $[Ca^{2+}]_i$ can vary in its levels for microseconds or during hours, and transients can propagate through cells and tissues [38-42]. As shown by the many examples of non-excitable cells, $[Ca^{2+}]_i$ oscillations can also regulate excitable cells in neuronal properties, such as excitability, excitotoxicity, synapse, gene expression and cell death [43]. Although diverse growth and neurotrophic factors may regulate neuronal cell differentiation, a wide variety of studies have demonstrated that neurotransmitter activation of cell membrane receptors produce $[Ca^{2+}]_i$ oscillations as one of the major cell differentiation regulatory mechanisms [44, 45]. $[Ca^{2+}]_i$ oscillations can result from spontaneous events or from cell regulated cascade, determining specific courses of cell physiology in diverse situations, including mesenchymal stem cell (MSC) differentiation into neuronal or osteoblast cell fates [46, 47].

Stem cell differentiation can be regulated through $[Ca^{2+}]_i$ transients, and we intend to demonstrate that these calcium oscillations determine cell fate. Beyond and contrary to the calcium oscillations for axial specification, quickly calcium variations to an increased concentration can initiate differentiation cell program, such as in neuronal and muscle cells [48]. In a model of development, using *Xenopus*, spontaneous calcium oscillations initiated by Ryanodine receptor (RYRs) activation determine cell fate of myocytes in somites [49]. Neuronal development can also be directed by these $[Ca^{2+}]_i$ oscillations, controlling cell physiology such as channel and neurotransmitter expression and activity [50, 51], deciding on growth cones growth [52] and specifying interneuron network communications [53].

$[Ca^{2+}]_i$ oscillations are constituted by elementary spontaneous calcium events that, overall, constitute intracellular Ca^{2+} signalling including puffs [54, 55], Ca^{2+} blips or quarks [56, 57], Intracellular waves [58], constituting the beginning process of cell networks signalling and communications [38, 59]. During brain development, calcium

signalling specifies cell fates. For instance, in neural cells from *Xenopus* embryos both, quickly increases of global calcium concentrations, similar to action potentials, and punctual and transient calcium increase, in the way of a wave, initiated at growth cone, have been demonstrated both *in vitro* and *in vivo*

Potassium channels and neurotransmitters gene expression are regulated by global increases in $[Ca^{2+}]_i$, and local calcium events control neurite extension [50, 52]. Calcium events lead to a cascade, which regulate protein phosphorylation [60]. Some different effects, in example, neurotransmitters specifying cell fate must be necessary to modify gene transcription [61].

Calcium concentration oscillations can be produced by both entry of extracellular calcium through receptor channels or others channels in plasma membrane or through calcium release from cell internal stores (e.g endoplasmic reticulum (ER) or sarcoplasmic reticulum (SR) in muscle cells. Calcium release from intracellular stores through ER/SR can be induced by metabotropic receptors. These receptors activate secondary messengers, such inositol-1,4,5-trisphosphate (IP_3) which activates ER/SR IP_3 receptors releasing calcium from these intracellular stores [62, 63]. Calcium entry through cell membrane surface receptors leads to a locally increase in $[Ca^{2+}]_{i,}$ called micro-domain increase, which dissipates when the channel receptor is closed [63, 64].

These rapid punctual events of calcium increase by channel opening and closing are denominated as calcium *sparks* or *puffs*, depending on the localization and nature of the activated channels. Intracellular calcium signaling is induced to promote both rapidly calcium increase in micro-domains for processes, which require elevated localized calcium concentrations, or recruiting diverse channels on the cell membrane, thus promoting a global rise in $[Ca^{2+}]_i$. However, sustained peaks in $[Ca^{2+}]_i$ for a long period induces cell death; however, this can be avoided when cells are using calcium signaling with low amplitude, or transient calcium signals. Thus, calcium signaling can be decoded through amplitude (AM) or frequency modulation (FM). Calcium-down stream signaling it is initiated by AM. Detection of $[Ca^{2+}]_i$ increases above noise levels may be difficult; however, this does not exclude this mode of calcium signaling action as controlling cell response [65]. In the other signaling modulation, as FM, information can be propagated through calcium oscillations. As an example, FM can induce

prolonged gene expression in activated T cells through nuclear transcription factors of activated T-cells (NF-AT) of the Oct/octamer-associated protein (OAP) and of nuclear factor κB (NF-κB) stimulation [66, 67].

Oscillatory patterns of calcium activity

Many published data from other groups and from our described that differentiation commitment can be controlled by calcium signaling which is mediated through local calcium increase that precede global calcium [7, 68-71]. Calcium signaling through local or punctual events may initiate intracellularly, in a specific trigger zone, nearby the plasma membrane. Calcium signaling studies analyzed by its AM and FM through calcium imaging techniques revealed characteristic patterns during neuronal differentiation **(Figure 1).** Cell signaling pathways involve coordinate activity of an interactive network. These signaling pathways interactions results in a complex network with divergent outputs depending on the combination of inductions. Natarajan and Berk [72] presented that a wide variety of individual or grouped receptors can mobilize Ca^{2+}, induce cyclic adenosine monophosphate (cAMP) production and cytokine synthesis, which then lead to protein phosphorylation regulating final cell signaling. This indicates that distinct extracellular stimuli induce similar intracellular signaling cues, which initially converge in some few interactions. Later on, these convergent signaling pathways can turn into a divergent process, ensuring cell physiology **(Figure 2).**

Intracellular calcium oscillations can be generated through external ligands as neurotransmitters, hormones, and others including external drug administration. Calcium oscillations can be modulated by frequency, which depends on how many and the type of excitations by those external ligands [73-76]. Spontaneous calcium oscillations and calcium transients present patterns, which can regulate specific gene expression [8, 66, 76, 77], stimulating cAMP oscillations promptly or through BDNF [77] also control gene transcription [78, 79]. Distinct calcium spikes pattern can lead to an increase of a cAMP [80] for decoding gene transcription. Calcium spikes present their high frequency modulation to control gene expression through Ca^{2+}-dependent kinases instead of calcium transients with low frequency modulation. This is the case for gene expression regulation in *Aplysia* neurons, where serotonin induces cAMP oscillations resulting in PKA translocation to the nucleus for long-term facilitation [79,

81]. As cAMP synthesis is regulated by a negative retro-feedback by calcium oscillations, a theoretical model [80] was constructed predicting that this combination is regulated through low-frequency cAMP events rather than by high-frequency ones [82, 83]. These data provide a novel concept for studying relations between both, calcium oscillations and other second messengers, as layers of intracellular signaling considering second messengers with distinct transient patterns produce unique cell responses.

The relationship between calcium and cAMP can be described as follows: some neurotransmitters or hormones activating adenylyl cyclase (AC), lead to an increase in cAMP concentration, activating PKA by separating their regulatory and catalytic subunits. Here, we need to have in mind that ACs are not the sole determinants of intracellular cAMP concentration; the family of phosphodiesterases (PDEs) degrading cAMP is also crucial for the dynamics of this second messenger [84]. Thus PKA can phosphorylate and activate VOOC promoting Ca^{2+} release from cytoplasmic stores. Furthermore, voltage-regulated and ATP-dependent potassium channels become inactivated. All of these events can generate an increase in $[Ca^{2+}]_i$ that further activates calcium-dependent ACs [83]. The cAMP transient triggers the first increase in calcium concentration, followed by synchronization of subsequent second messenger transients. Reinforcing the concept of bi-directionality in calcium and cAMP interaction, Tengholm and colleagues found that calcium removal abolished hormone-induced cAMP oscillations [70]. Stimulation of G-protein coupled receptors activates phospholipase C-β (PLC), elevating inositol 1,4,5-trisphosphate (IP_3) to concentrations that promote calcium release from internal stores. Following Ca^{2+} flux, Ca^{2+}/calmodulin (CaM) activates both, CaMKII and calcineurin (CaN), increasing cytosolic cAMP by CaM-dependent activation of adenylyl cyclases 1 and 8 (AC1-AC8) [83, 85]. CaMKII, the cAMP pathway, together with CaN present a persistent activation of CaMKII even after the calcium signal is withdrawn [86]. Bhalla and Iyengar developed a model of cAMP oscillations. They tested by simulation that an initial stimulus producing cAMP, which leads to an initial increase in $[Ca^{2+}]_i$ [86] caused activation of CaMKII, AC1, and CaN through CaM binding and of PKA through an increase in cAMP produced by activation of AC1-AC8. The simulations predict that activation of AC1-AC8 overcomes competing degradation of cAMP because of activation of CaM-dependent and PKA-activated PDEs. PKA activation leads to

phosphorylation of the Inhibitor-1 protein and hence to the inhibition of PP1 [87]. However, when $[Ca^{2+}]_i$ returns to normal levels, it leads, both PKA and CaN, to be inactivated. The balance between activation/deactivation of both PKA and CaN at basal level allow PP1 full activity to be restored 20 min after the end of the stimulus. As PP1 becomes active, it dephosphorylates and inactivates CaMKII [88, 89]. This, in turn, moves the system to its basal state again, within 20 min following the calcium flux. The cAMP-operated gate potentiates the amplitude of the CaMKII-response, prolonging its activity. Nevertheless, CaMKII does not undergo persistent activation. However, when CaMKII presents a balance between its state of autophosphorylation/dephosphorylation causing relative activities of PP1 and CaN, CaMKII present a persistent activation. This allows CaMKII to be activated as long as 20 min even when the stimulus has been interrupted, being sufficient to initiate a physiological response. However, when information needs to be withhold for longer periods, cells use repetitive signals known as calcium oscillations. Both transient events and global signals may oscillate, but they have different periods. The origins of spontaneous calcium oscillations in inducing neural differentiation are still debated. In some neurons, oscillations can be originated through calcium entry through VOCC, and by calcium release from intracellular stores contributing to its amplitude [90-93].

Modulating the frequency of spontaneous events of calcium activity provides a way to increase the susceptibility of local spontaneous calcium fluctuations. This effect has been observed in the proportion of active cells, in the frequency of its local increases and in the amplitude of these events. These results indicate that many effects of the local and global calcium signals in stem cells commit these to diverse cell fate determination including neurogenesis or differentiation into bone, myocyte and other cell types [8, 46, 47, 94-97]. Cell differentiation can be accelerated, or even co-ordinated, by increasing the frequency and modulating the amplitude of calcium oscillations.

Brief increases in calcium transients for cellular activation can be produced by the coordinated and direct opening of ryanodine and IP3 receptors. Diffusion of calcium through the cytosol exciting neighboring receptors lead to the production of a calcium wave, which may propagate long distances through continuous, but regenerating calcium releases from intracellular stores [98-100]. These propagation sites of calcium waves

were identified as being rich in ER proteins, such as SERCA, NAADP, calreticulin and IP3Rs, and as sites containing mitochondria [98, 99, 101-104]. A global calcium event is created through the coordinated release from all receptors, using calcium as a messenger.

Calcium can propagate from one cell to another one through gap junctions producing intercellular calcium waves. For instance, radial glial cells as a brain progenitor cell promote calcium waves passing through neighboring cells. Gap junctions are most abundant during mid-neurogenesis and declining during late-neurogenesis [105]. However, The decline in cell coupling by gap junctions is accompanied by a gain hemichannel junctions that also mediate calcium waves. It was demonstrated that these hemichannels communication between cells mediate calcium waves through developing ventricular zone mediated by the binding of the neurotransmitter ATP to its P2Y1 receptor, expressed in radial glial cells [98]. P2Y1 receptor-mediated PLC activations results in an increase in inositol IP3 levels and calcium release from ER store [106, 107]. A curious fact is that calcium wave properties, as frequency and amplitude, increase during late neurogenesis [107]. This occurrence can be due to the similar levels and types of connexins that are expressed during mid- and late neurogenesis [108-110] suggesting that expression of these proteins is regulated for modulating the clusters of gap junctions of the differentiating cells during mid-neurogenesis. Otherwise, during late neurogenesis, the spreading of calcium waves are mediated by hemichannels. In summary, three types of calcium signaling events exist: 1) local events, such as puffs and sparks; 2) global events (regenerative waves), which require temporal coordination of a sufficiently large number of local events; and 3) intercellular calcium waves, which are generated by global events from one cell and spread to their neighboring cells. A single calcium event can greatly increase calcium levels (10-100 μM) in a small region, that may represent less than 1% of the cell's volume, and that may increase global calcium levels by less than 2 nM [99-102]. Local calcium events have the potential to modulate calcium dependent processes that are not responsive to global increases in calcium, such as activation of kinases and phosphatases that are irregularly distributes in the cytoplasm. Activation of these proteins could lead to different transcriptional pathways.

Calcium signaling

We can divide calcium signaling networks in four functional stages. Firstly, by signal binding, physical-chemical, electrical, or mechanical activity can mobilize

calcium from cytosolic and extracellular stores acting in many mechanisms for increasing intracellular calcium levels. Secondly, increased calcium levels stimulate calcium sensitive processes that, thirdly, initiate many signaling pathways. Fourthly, the response is finalized by termination mechanisms such as pumps and transporters, which remove Ca^{2+} from the cytoplasm, decreasing calcium concentration to basal levels [103]. This signaling network is composed of many components, known as the *Ca^{2+} signaling toolkit* **(Figure 3)**. Since many of these molecular components of this toolkit are expressed in different isoforms, each cell type may explore this vast repertoire, constructing its own specific signaling mechanism [104, 105]. Differentiation produces distinct cell types specialized for specific functions. A key element in the differentiation process is the installment of components of the distinct calcium signaling toolkit for the need of the cell. Ca^{2+}, the simplest of all intracellular messengers, nevertheless succeeds in providing a specific link between a huge array of extracellular stimuli and an equally diverse collection of cellular responses. This is possible due to close interactions between calcium channels and calcium-regulated targets, leading to unique cell response. PLC, its substrate, and both products of the phosphoinositide pathway are important contributors in coordinating and regulating a wide variety of capacitative and non-capacitative calcium entry channels. This signaling pathway has a huge versatility that is necessary to allow calcium entry and efficiently couple specific extracellular signals to adequate cell responses.

Calcium channels present a wide range of versatility due to their differential regulation and expression, their distinct permeability to different cations and linked to distinct cell physiology processes. Considering the cellular level, calcium can enter from different channels leading to distinct cell responses. This is due to random distribution of calcium channels on the cell surface, and intracellular calcium buffering produces spatial gradients of calcium. There are many examples of such targeted Ca^{2+} signals, not at least the selective coupling of capacitative Ca^{2+} entry (see below) to regulation of adenylyl cyclase and nitric oxide synthase [106]. Calcium channels located in the plasma membrane make calcium, the simplest and widely used of all intracellular messengers, to select specific physiological stimuli and couple it to adequate cellular responses.

However, calcium can be a hazardous messenger having in mind the high expression levels of calcium channels on cell membrane; therefore, calcium entering must be tightly regulated. Diverse calcium channels can be inhibited by high cytosolic ,calcium concentration, and this negative feedback loop can control and regulate excess calcium entry. In example, t L type calcium channels are blocked when interacting with tethered calmodulin. This feedback loop is operating with high calcium concentrations near the channel opening leading to its inhibition. Channel activity is confined by this way, but it does not control the various calcium entry mechanisms through different pathways, having this coordination mediated by complex interactions **(Figure 4).**

Calcium binding sites can be found in the primary sequence of ryanodine receptors (RYR) and inositol-1,4,5-trisphosphate (IP_3). Release of calcium from Ca^{2+}-induced Ca^{2+}-release channels (CICRs) occurs through direct binding of calcium to the channel. In addition to direct binding, calcium exerts some effects through accessory proteins, such as calmodulin. Ryanodine and IP_3 receptors exist as macromolecular complexes with kinases and phosphatases, which regulate the sensitivity of these complexes to calcium **(Figure 5)**. Examples of CICRs in non-excitable cells include calcium oscillations and waves induced by IP_3, which occur after fertilization in mammals, and the spread of calcium waves in pancreatic cells, which lead to exocytosis of zymogen granules and the secretion of fluid.,

Ryanodine receptors can also be activated by cyclic ADP-ribose (cADPR), which stimulates the release of calcium by ER stores. Differently from cADPR, NAADP acts on acidic lysosomal-like stores instead of stimulating ER stores [107]. NAADP acts on its own receptor, and this binding cooperates with the IP_3 and the ryanodine receptors in order to generate nucleoplasmic Ca^{2+} oscillations. There are evidences that the nuclear envelope is a Ca^{2+} store in neurons, sensitive to NAADP. The NAADP-specific receptor is still unknown; however, there are suggested targets such as the lysosomal Trp-like channel mucolipin I [108, 109].

Many antagonists bind to G-protein coupledkinase receptors, which in turn activate PLC, leading to release of 1,2-diacylglycerol (DAG) and soluble IP_3 generated from phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2). IP_3 diffuses into the cytosol and binds to the IP_3 receptor, IP_3R, which stimulates calcium release from intracellular stores. Depletion of calcium from intracellular stores is sensed by store-operated Ca^{2+} channels (SOC), in the plasma membrane. Other plasma membrane calcium channels

are regulated by stretch, membrane potential voltage-operated Ca^{2+} channels (VOCC) and receptor-operated channels (ROC).

It has been shown that increases in extracellular calcium levels or exposure to agonists that promote calcium influx through calcium receptors (CaR) trigger intracellular calcium signals through interactions between CaR and PLC, which is mediated by $G\alpha_q$ or $G\alpha_{11}$ subunits of heterotrimeric G-proteins [110, 111] These interactions result in IP_3 and DAG production. CaR interacts directly with $G\alpha_I$ too, which results in the inhibition of adenylate cyclase. CaR is also associated with many signaling kinases, such as MAPK (Mitogen-Activated Protein Kinase), ERK ½ (Extracellular signal-Regulated Kinase) [112-115] and kinase Jun-amino terminal (JNK) [116], responsible for many effects of CaR agonists in cell proliferation [113, 117]. However, the mechanism of how a single receptor activation (e.g. CaR) can inflect a wide range of cell physiology response, including secretion, proliferation, apoptosis, and differentiation depends on the cell type, which expresses the receptor. A delineation of the specific mechanisms of signal transduction that allow CaR to influence diverse cellular processes is a promising area for future investigation.

Ca^{2+} Homeostasis and Signaling

Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is maintained around 100 nM at resting state through homeostatic mechanisms. This concentration permits changes in $[Ca^{2+}]_i$ rapidly, reaching a concentration of several hundreds of nanomolar to a few micromolar [118, 119].

$[Ca^{2+}]_i$ levels are maintained by Ca^{2+}-ATPase with the balance of internal stores from mitochondria, endoplasmic reticulum and nucleoplasma and extrusion out of the cell [120]. In addition, Na^+/Ca^{2+} exchanger [121] switches internal Ca^{2+} with extracellular Na^+. In the plasma membrane of stem cells, some Ca^{2+} channels are voltage-dependent Ca^{2+} channels (VDCCs) and neurotransmitter-gated channels, many of which are involved in regulating cell proliferation and differentiation.

Some members of transient receptor potential (TRP) family of proteins also form channels that are permeant to Ca^{2+} [122]. At the resting potential, these TRP channels may open depending on extracellular signals, such as the neurotrophin brain-derived neurotrophic factor (BDNF) [123], or components of internal stores. Indeed, depletion of intracellular Ca^{2+} stores can induce influx of extracellular Ca^{2+} through plasma membrane channels, a mechanism known as store-operated Ca^{2+} entry (SOC) [124].

Different cell types express different SOC channels that may also be differentially regulated, thus the signal that links empty stores to their opening have not been unambiguously identified [125]. RNA interference screening detected two proteins, which are important for SOC activation: one present in the endoplasmic reticulum (ER), STIM, [126, 127] and the other one present in the plasma membrane (PM), Orai [127-129]. The first one is comprised by membrane proteins often present in the ER, which is capable to sense luminal Ca^{2+} changes promoting their translocation into junctional areas of the ER, juxtaposed with the PM [130]. In the other way, Orai proteins are channels located in the P, and translocated to the ER junctions, where STIM is present. Both, Orai and STIM, interact becoming active [130].

Some reviews addressed both the evidence that oligomeric assemblies of TRP proteins form SOC channels [130-132] and alternative proposals for the links between internal stores and the Ca^{2+} entry [132]. The coupling seems to be mediated either by IP_3 receptors physically interacting with the SOC channel (conformational coupling) or by release of a diffusible messenger. Interestingly, both models are supported by persuasive evidence, but neither is consistent with the results from different cell types.

Increase of $[Ca^{2+}]_i$ is first achieved by Ca^{2+} influx through the plasma membrane triggered by several extracellular stimuli. This $[Ca^{2+}]_i$ elevation is often amplified by Ca^{2+} released by internal stores, which seems to depend on the activation of ryanodine and IP_3 intracellular receptors [38]. Owing to the abundant immobile cytoplasmic Ca^{2+}-binding proteins [133, 134], Ca^{2+} diffuses very slowly in the cytoplasm (diffusion coefficient at ~10 $\mu m^2\ s^{-1}$; see [135, 136]. Therefore, Ca^{2+} signals are typically localized due to limited diffusion, but may become global when substantial internal release is involved **(Figures 1 and 5)**. Activation of Ca^{2+} channels in the plasma membrane or the ER can result in high $[Ca^{2+}]_i$ near the channels, creating a microdomain of $[Ca^{2+}]_i$ elevation, or Ca^{2+} "sparks." The spatiotemporal properties of cytoplasmic Ca^{2+} signals depend on the activation of Ca^{2+} channels in both the plasma membrane and the membrane of internal stores. During stem cell differentiation, diverse mechanisms for regulating $[Ca^{2+}]_i$ enable Ca^{2+} signals of different spatiotemporal patterns to serve for unique cell fate determination.

Among other factors, bradykinin and nerve growth factor (NGF) open vanilloid receptors (TRPV1) by stimulating hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP_2) that normally inhibits the channel [137]. Conversely, because PIP_2 stimulates the TRPM7 channel, this channel is inactivated by receptors that activate PLC [138]. In

fact, several TRP channels are directly activated by DAG, [139] and in smooth muscle cells, one of these channels (TRPC6) contributes to the Ca^{2+} entry evoked by α_1-adrenoceptors [140]. In other cells, DAG stimulates non-capacitative Ca^{2+} entry via PKC [141]. Arachidonic acid-stimulated Ca^{2+} entry pathway is the major route for the Ca^{2+} entry evoked by physiological stimuli in both HEK cells and A7r5 vascular smooth muscle cells [142].

Ca^{2+} entry by this pathway mediates cleavage of PIP2 and release of IP_3, which in turn increase $[Ca^{2+}]_i$ through the IP_3-R1 receptor. Elevated Ca^{2+} levels then activate NFAT transcription via calcineurin **(Figure 3)**. In addition, neurotrophin signaling through NFAT appears to regulate expression of the IP_3-R1 receptor [143], and one of four NFAT consensus site identified within the IP_3-R1 promoter was able to bind and shift NFAT from hippocampal nuclear extract or recombinant NFATc4 [144]. Interestingly, in cultured spinal cells, NFAT was able to activate COX-2 expression via BDNF signaling, but did not increase expression of IP_3-R1 receptor transcripts [145]. It is possible that distinct NFAT transcriptional complexes are used to activate different sets of genes during the developing and the adult nervous system.

Indeed, initial Ca^{2+} release, which stimulates NFAT-dependent transcription, can also be triggered by L-type channels or NMDA receptor. Their activation leads to an upregulation of IP_3-R1 through calcineurin in cerebellar granule neurons and in cultured hippocampal cells [144, 146, 147].

Considering these evidences, a positive feedback loop might exist whereby an initial Ca^{2+} increase caused by neurotrophin signaling or L-type Ca^{2+} channels would activate NFAT transcriptional activity and feedback to modulate Ca^{2+} amplitude over time by the upregulation of IP_3-R1 expression (**Figure 3**)

Cellular differentiation: Regulatory networks in cell phenotype determination

The cellular phenotype is controlled by the interaction of signals and transcription factors that lead to the expression of specific groups of genes. This interaction may be summarized and represented graphically as a genetic regulatory network (GRN). A typical connection consists of the binding of a transcription factor to the regulatory elements of a target gene. If this gene encodes a transcription factor, it may also take part in similar interactions, leading to the creation of complex networks. Additional entries to the network include signaling molecules that activate target

transcription factors. Reducing the GRN to its target components allows the construction of networks from a limited quantity of information, even though GRNs have been commonly created from large amounts of data [148-150].

As the embryonic cell number increases, this is accompanied by a reduction in cell size, and so individual cells become surrounded by an increasing number of neighboring cells. We suggest that at this stage, when developmental events requiring coordinated activity are initiated, occurs an increase in the degree of intercellular communication. The generation of organs depends on successive programs of gene expression during development. Temporal regulation of transcription is critical to acquire a spatial pattern, as observed in vertebrate generation of somites [151]. Temporal regulation is also essential to integrate the progression of events that accompany specification and differentiation of a cell [152-154]. Regulatory networks that depend on a wide range of transcription factors, placing some questions of how transcriptional circuits can control and determining the temporal development, guide these processes. In some cases, the order of activation of transcription factors is structured hierarchically to establish sequential patterns of gene expression [155-161]. All regulators can only be in active state during a brief period of time in development promoting a regular response in cells that express them. Often, each regulatory module induces a unique spatio-temporal pattern of gene expression, but when many modules are involved they can result in complex patterns of gene expression [162]. Since each module is regulated by many transcription factors and each of these interacts with many other modules, it is possible to represent developmental patterns of gene expression as an integration network [149, 163-166].

In unicellular organisms, recurring patterns or motifs occur with a higher frequency than expected by chance **(Figure 4)**. Each motif has a limit of biological properties, such as establishing handles of retro feeding, multicomponent handles; maintenance of gene expression, auto regulation; or introduction of a delay between the activation of a gene and its next target, feed forward motifs [167]. Analysis of hematopoietic networks, however, has not revealed any isolated motif; they are highly interconnected and interregulated [168, 169]. It is common to consider a network as being a grouping of motifs, determining biological functions for each component, thus revealing how a network controls transitions between differentiation stages [170].

Multicellular organisms, in the other hand, have multiple networks active in every moment, generating different cell types. Davidson [149] suggested two models of networks for organisms: the genome model and the nucleus model. The first describes all possible interactions while the latter characterizes interactions that occur in individual nuclei in specific stages of development or differentiation. The genome model acts as a repository of all known interactions, while those that are active in a particular nucleus require experimental verification [168]. An interesting property of GRNs is that there are not any limits of scales [171], because there are few genes that are highly connected and many that are poorly connected. This arrangement leads to the concept of central networks and one can correlate this with the idea that important genes regulate subgroups within the network [171]. When a central gene is inactivated, it can lead to a significant break within the system, while the same would not happen to a poorly connected gene.

The importance of neurotransmitters, growth factors, miRNAs and specification signals of each cell line are recognized, but the mechanisms through which individual cells make the choice of their lineage remain unknown. This leads to the question of whether these components select specific lineages from a group of progenitors that stochastically express programs of specific cell lineages or if these components instruct individual cells to activate specific programs [172].

A mathematical theory for access data presented in time scale of stimuli and triggered spikes

Sensory information can be codified by temporal structure of neuronal activity varying over brief period, about 10 ms. Calcium spikes may be just determined by the time when stimulus were presented adding knowledge about sensory stimuli, which can be missed if timeline of spikes are not determined with sufficient temporal resolution [173]. Some data suggest that even spikes times can decode sensory information beyond that is presented in spikes it count properly and is registered throughout long period, this was described in distinct brain structures, including cortex and sensory areas [174-176].

The alignment assembly spikes and sensory events can study these acquired data from time-lapsed spike trains containing their structural and knowledge content through a computer clock that can registers neuron activity and induced stimuli with great

accuracy. However, to get the information presented in timing of stimuli during exactly when spikes is triggered requires that this timely lapse is maintained during responses analysis. For this, it is necessary to have at least two computer analyses for the decoder to realize. Firstly, to decode it is essentially to get precise information relating to the precise time of sensory events, which can be translated to the beginning of the stimulus or a specific time during the course of the stimulus). And second, data acquiring with accuracy from time intervals must be registered for the decoder [177].

Studying sensory decoding it is essential to use a quantitative analysis keeping all information carried by distinct coding schemes. An equation by Shannon, referred as information, introduce a way to calculate differently single-trial stimulus

$$I(S;\mathbf{R}) = \sum_{\mathbf{r},s} P(\mathbf{r},s)\log_2 \frac{P(\mathbf{r},s)}{P(\mathbf{r})P(s)}, \quad (1)$$

where the probability of join together P(r,s) to present a stimulus (s) and perceiving a response (r), and their specific probabilities P(r), P(s). These data acquire a quantitative information reducing uncertainty (i.e. knowledge acquiring) about the induced stimuli produced from a neural response to a single-trial observation (the averaged ratio for induced stimuli and acquired responses). Data acquisition enter in bits (one bit is related to the reduction of uncertainty by a factor of two) becoming an upper limit of information referring to the stimuli that can be obtained by any neuronal responses which is decoded by an algorithm operating. There is a situation in that equivalent data acquisition can quantify single-trial stimuli information, which in neuronal systems often differentiate or recognize stimuli on a single encounter.

Considering the data acquired from neuronal codes *r* related on distinct response feature (frequency and timing of spikes events) and determined relative to distinct reference frames, it is possible to calculate the capacity of distinct neuronal codes. We describe bellow the comparative between the data acquired from diverse responses *r* determined with experimental clock with responses *r*, which is outlined with an internal reference signals.

Data acquisition metrics could be analyzed with intermediary decoding phases in case of difficulties to compute stimuli-responses probabilities from few experimental data

[178]. In this case, computing for anyone obtained response *r* the most seemly stimulus *sP* that produce this response taking a decoding method to be executed (e.g. the corresponding template) and cross-validation [179]. Following this, data obtained from stimuli reconstruction procedure is possible to be quantified by the equation [179]:

$$I(S;S^P)=\sum_{s,s^P} Q(s^P,s)\log_2\frac{Q(s^P,s)}{Q(s^P)Q(s)}, \quad (2)$$

where is the ensemble probability that in a process could be decoded and describes the presence of stimulus been the true value the stimulus *s*. Data decoded by can be quantified (as it uses a computer, in bits) by average information obtained, for each trial, when stimuli is predicted for a given algorithm, taking in consideration both the part of corrected decoding and the decoding scatter.

FUTURE PERSPECTIVES AND CONCLUSIONS

Using linear signaling pathways to describe development and stem cell differentiation, control is determined by actual data processing in a specific point of view in development biology leading us to rapid reach its limits. However, this point of view is not incorrect, it can serve for reducing the system to a more easy study of complex system. This can achieved through experimental demonstration using soluble hormones and neurotransmitters, which exert functional effects on cell physiology by their binding to membrane cell surface receptors. Nowadays, many studies demonstrated that induced calcium oscillations can control cell process to differentiate in a specific cell fate [180]. Moreover, distinct cell fate can be controlled by cell shape, which can induce the same gene network and calcium signaling-dependent protein activation through specific signaling transmitters and their oscillating patterns. This vast available information can be joint with previous data, but not in a full view, been necessary to get cell signaling networks process their produced data and turn it understandable. For this it is essentially to build an integrative model of cell networks regulation.

In this article, we mentioned the use of Boolean networks as simple idealized models for processing cellular information, mainly when it means in switching between different cell fates. It was shown by computer simulations that the diverse exhibited cell phenotypes, (e.g. cell growth, migration, differentiation, apoptosis, etc.,**)** may represent

attractors which can be self-organized within the dynamic network of molecular interactions that includes the living cell. The existence of attractors for a cellular state greatly increases the chances for evolution to connect the intricate biochemistry of cell fate regulation with the physical world that needs of a specific molecule-encoded information. This might have facilitated the evolution of larger organisms whose development and function must satisfy the laws of physics at all scales of size, however, regulated by genes.

More importantly, the concepts of Boolean networks and attractors systems capture many properties of the dynamics of cellular regulation that otherwise would have required to understand metaphorical descriptions, like the recognition of a "balance of survival and apoptosis signals", "cellular decision-making", "conflicting signals", and "default programs" [181-184]. Furthermore, the use of this type of complex dynamic networks by cells provides a formal basis for the analysis of tolerance and sensitivity of cellular systems to diverse types of conditions (e.g., pharmacological or infectious treatment) [185, 186].

The implications of the use of dynamic regulatory networks by cells go much further than control of cell fate determination by calcium oscillations. Very simply, existing forms of gene cluster and proteomic analysis [187], at best, transform data into information. To convert information into knowledge, novel approaches are needed that address the fundamental principle of cellular regulatory systems as a whole. An integrative network can be described, but with limit to basic principles, using Boolean networks and attractor systems.

Widening our paradigm beyond what really happens inside cell molecular interactions accepting physical control parameters in cell regulation, such as calcium oscillations patterns and growth factors interacting with cell membrane, also should help to bridge the gap between test tube biochemistry and the biology that we observe within intact living cells and organisms. Continued efforts of this type attempting to look over unique molecular signaling pathway placing molecular networks control in context of cellular and whole tissue structure it is essential to ensure that we got the path forward to this new paradigm in postgenomic era.

Calcium oscillations are considered as attractor that can trigger stem cell differentiation to a huge variety of distinct and specialized neural cells to compose a complex cyto-architecture better known as brain. A vast intrinsic signaling process is activated by neurotransmitter receptors, CaR, ROC, RYR, SOC, VOCC, NAADP or second messengers such as - DAG, IP3, cAMP, PtdIns(4,5)P_2 or even by modulation of kinase proteins, ERK or MAPK and enzyme, PLC. All of these signaling molecules are regulated through calcium oscillations, waves or spikes, restricted to micro-domains or the whole cell. Furthermore, calcium oscillations can vary in their amplitude and frequencies. These studies of signaling networks are considered a complex system as it is involved in distinct levels or organization and integrated with others networks, structured around different attractors, which compose a central regulatory genetic network, helping to solve, at least in part, the whole process.

We suggest that new advanced technics in photodynamic, image and "oma" areas will keep more information to our knowledge. The first area concerns calcium imaging and calcium reporters for each cell types during embryonic development and to subcellular compartments within these cells. There have been some encouraging advances in this area [188, 189]. The second area for advancement will be the generation of targeted reporters that can be used as probes for non-invasive imaging of gene expression patterns in living embryos. Dorsky et al. described a technique for long-term imaging of regulated gene expression in zebrafish [190]. Using these probes, it is possible to identify their transcription in embryos together with a calcium reporter, and to image their activity at the same time or almost. The third area is the development of calcium reporters that catch their sensitive targets recording their interactions between calcium signaling and specific development or differentiation signaling networks. Encouraging advances, such as the use of fluorescein (FL)- or green fluorescent protein (GFP)-linked calmodulin (CaM) probes [191, 192], and the development of fluorescence resonance energy transfer (FRET)-based biosensors to monitor agonist-induced phospholipase C activation, have been made in this area. The future, therefore, looks encouragingly bright for the continued exploration of developmental Ca^{2+} signaling.

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FIGURE LEGENDS

Figure 1: Calcium oscillation patterns depicted by frequency and amplitude on temporal scale. A) Cell-specific Ca^{2+} oscillations created by selecting components from the Ca^{2+}-signalling toolkit generate Ca^{2+} transients that are characteristic for each cell type and for differentiation states of stem cells. It is proposed that the Ca^{2+} transients have two functions. In addition to activating cellular responses, it also functions as part of a feedback mechanism to regulate the transcriptional events that are responsible for maintaining the oscillation patterns. This Ca^{2+}-dependent transcriptional regulation might have a central role in the compensatory mechanisms that enable cells to adapt to any modifications of their Ca^{2+}-signalling systems. It seems that Ca^{2+} can both positively and negatively adjust transcriptional activity. Furthermore, Ca^{2+} signalling can be modulated by frequency and amplitude of oscillations. Cells often respond to changes in stimulus intensity by varying the frequency of Ca^{2+} waves. To use

such a frequency-modulated signalling system, cells have evolved sophisticated 'molecular machines' for decoding frequency-encoded Ca^{2+} signals. Sparks and puffs contribute to intracellular Ca^{2+} signals, such as the Ca^{2+} waves that sweep through cells. For waves to occur, most of the $InsP_3Rs$ and the RYRs must be sufficiently sensitive to Ca^{2+} to respond to each other through the process of Ca^{2+}-induced Ca^{2+} release. B) Scheme depicting calcium oscillation patterns through neuronal differentiation of mesenchymal stem cells. See the text for a better description.

Figure 2: Ca^{2+} signalling pathways. Ca^{2+} signalling depends on the increase of intracellular free Ca^{2+} levels $[Ca^{2+}]_i$, derived from extracellular calcium $(Ca^{2+})_o$ sources or intracellular stores of the endoplasmic reticulum (ER Ca^{2+}). Ca^{2+} can enter through calcium channels operated by voltage (voltage-operated Ca^{2+} channels, VOCCs) in excitable cells such as neurons and muscular cells, or through calcium channels operated by receptors (receptor-operated Ca^{2+} channels, ROCs) in response to neurotransmitters. Ca^{2+} channels operated by storage (store-operated Ca^{2+} channels, SOCs), which open when internal Ca^{2+} stores are depleted. They are found mainly in non-excitable cells. The Ca^{2+} of the ER is released by two types of channels. Inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) is generated by the action of the enzyme PLC from phosphatidylinositol 4,5-bisphosphate ($PtdIns(4,5)P_2$) present in the plasma membrane in response to the action of growth factors, hormones or neurotransmitters on the receptors. The $Ins(1,4,5)P_3$ acts on receptors in the endoplasmic reticulum, promoting the release of Ca^{2+} from ER stores. In some cell types, such as embryonic stem cells, the production of $Ins(1,4,5)P_3$ is modulated by phosphatidylinositol 3-OH kinase (PI(3)K) signalling, which uses $PtdIns(4,5)P_2$ to produce $PtdIns(3,4,5)P_3$ that acts as a messenger to keep the activity of PLC. GPCR ligands act on cognate receptors (dark yellow ovals;

grouped by Ca^{2+} and cAMP stimulators) and activate appropriate Gα-subunits and subsequent signalling cascades. A receptor-dependent Ca^{2+} increase (symbolized as a logical AND gate) synergizes with the Gαs-stimulated production of cAMP. Feedback from cAMP inhibits the increase of levels of intracellular calcium by a PKA-dependent mechanism. An interaction agent summarizing and defining the conditional cross-talk between Ca^{2+} and cAMP is highlighted by a blue box.

Figure 3: Signal transduction networks mediating stem cell activity-dependent gene expression. Calcium influx through metabotropic receptors from neurotransmitters and neurotrophic factors, voltage-gated calcium channels (VOC), mainly L-type channel, store-operated channel (SOC), which open when the internal stores are emptied of Ca^{2+}, and other ligand-gated channels (LGC) are activated by neurotransmitters (nicotinic and purinergic receptor agonists and possible others). This leads to the activation of many calcium-regulated signaling enzymes, which set in motion several signal transduction cascades. These pathways converge with preexisting transcription factors in the nucleus and lead to their activation through direct posttranslational protein modifications. Several of the activity-regulated genes encode transcriptional regulators, which in turn promote the transcription of additional activity-regulated genes, in a wave model. Many other activity-regulated genes encode proteins functioning during differentiation process, augment their expression and thereby coordinate activity-dependent gene expression leading to a cell specific phenotype. Only a subset of the signaling pathways that mediate activity-dependent transcription is shown here.

The calcineurin (CaN)/nuclear factor of activated T cells (NFAT) transcriptional cascade plays a direct role in a process of Ca^{2+}-induced transcription of components of the Ca^{2+} signalling toolkit.

Figure 4: Structural organization of transcriptional regulatory networks motives. A) The basic unit includes the transcription factors, their target genes with the site of DNA recognition and regulatory interactions between them. The units are often arranged in network motives, including inter-regulation specific patterns which are over-represented in the networks. Examples of motives consist of autoregulation, where a factor binds to and positively or negatively regulates its own expression, helping to direct the following networks. It acts as a check point when autoregulation is positive or stabilizes its expression if autoregulation is negative; feed-forward loop (FFL), an initial transcription factor regulates the next transcription factor, and both act together to regulate target gene(s). With eight possible subtypes; multicomponent loop, two or more transcription factors that regulate each other in a loop. If the handle is positive, it contributes to the next moment. A negative loop between two or more transcription factors represents the most basic competition form between two alternative routes; regulatory chain, an orderly chain of several transcription factors in series. A chain finishes if the gene does not have a target, or is autoregulated; simple input (SIM), a single transcription factor (which is usually autoregulatory) regulates a group of target genes and all the regulatory signals are equal. These motives are more common in unicellular organisms; multiple input (ME), some transcription factors regulate the same group of target genes; dense overlapping region (DOR), a group of transcription factors overlap to regulate a group of target genes and each of these targets are regulated by different combinations of transcription factors. This promotes individual intersection

points for integrating signals from various branching points, indicating different cell types.

B) Network motives can interconnect themselves to form semi-independent modules, many of which were identified in order to include regulatory integration data with gene expression data imposing evolutionary conservation.

Figure 5: Common calcium toolkit. Cells have an extensive signalling toolkit that can be mixed and matched to create Ca^{2+} signals of widely different properties. Ca^{2+}-mobilizing signals (brown arrows) are generated by stimuli acting through a variety of cell-surface receptors (R), including G-protein (G)-linked receptors and receptor tyrosine kinases (RTK). The signals generated include: inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$), generated by the hydrolysis of phosphatidylinositol-4,5-bisphosphate ($PtdIns(4,5)P_2$) by a family of phospholipase C enzymes (PLCγ); cyclic ADP ribose (cADPR) and nicotinic acid dinucleotide phosphate (NAADP), both generated from nicotinamide-adenine dinucleotide (NAD) and its phosphorylated derivative NADP by ADP ribosyl cyclase; and sphingosine 1-phosphate (S1P), generated from sphingosine by a sphingosine kinase. ON mechanisms (blue arrows) include plasma membrane Ca^{2+} channels, which respond to transmitters or to membrane depolarization, and the intracellular Ca^{2+} channels, such as the $Ins(1,4,5)P_3$ receptor ($InsP_3R$), ryanodine receptor (RYR), NAADP receptor and sphingolipid Ca^{2+} release-mediating protein of the ER (SCaMPER). The Ca^{2+} released into the cytoplasm by these ON mechanisms activates different Ca^{2+} sensors, which augment a wide range of Ca^{2+}-sensitive processes, depending on cell type and context. OFF mechanisms (red arrows) pump Ca^{2+} out of the cytoplasm: the Na^+/Ca^{2+} exchanger and the plasma membrane Ca^{2+}

ATPase (PMCA) pumps Ca^{2+} out of the cell and the sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA) pumps it back into the ER/SR. Ca^{2+} released from the NE enters the nucleoplasm either directly through Ins(1,4,5)P_3Rs or RyRs in the inner nuclear membrane (INM) or through the NPCs when released through Ins(1,4,5)P_3Rs or RyRs in the outer nuclear membrane (ONM). Three messengers can be produced locally inside the NE: Ins(1,4,5)P_3 generated by phospholipase C (PLC) or cADPR and NAADP generated by the CD38/ADP ribosyl cyclase (ARC). cADPR and NAADP bind different binding sites or receptors (orange arrows) and activate RyR Ca^{2+} channels. All three Ca^{2+} messengers can also be produced in the cytosol and then enter the nucleoplasm through the NPCs. Ca^{2+} is pumped into the NE and ER by the sarco-endoplasmic reticulum Ca^{2+}-activated ATPase (SERCA) on the ONM.

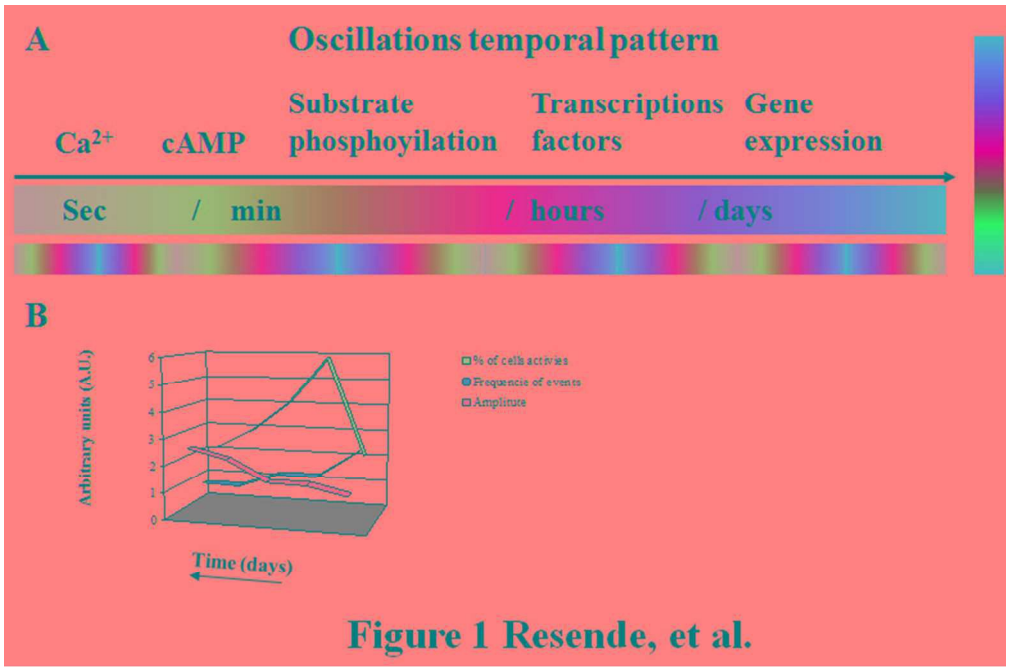

Figure 1 Resende, et al.

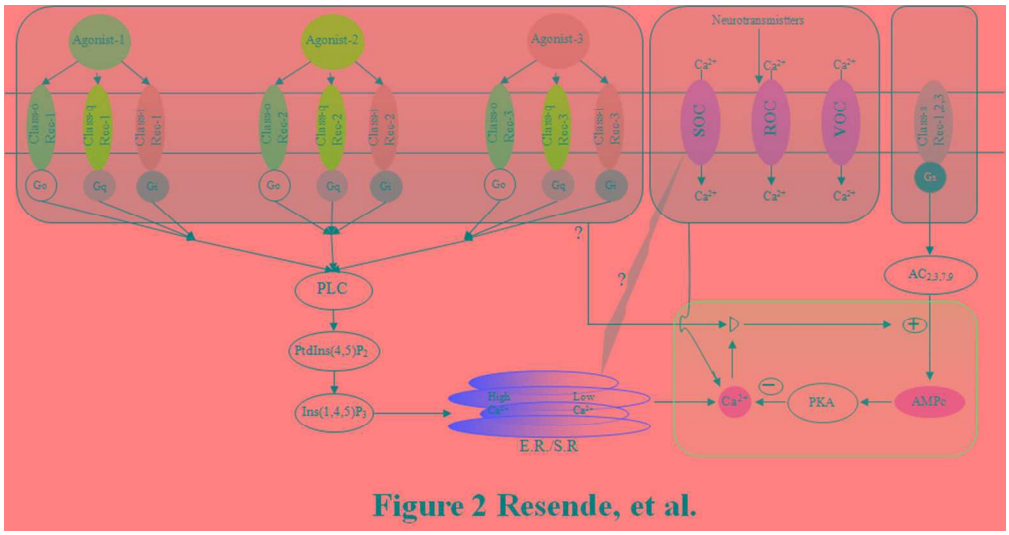

Figure 2 Resende, et al.

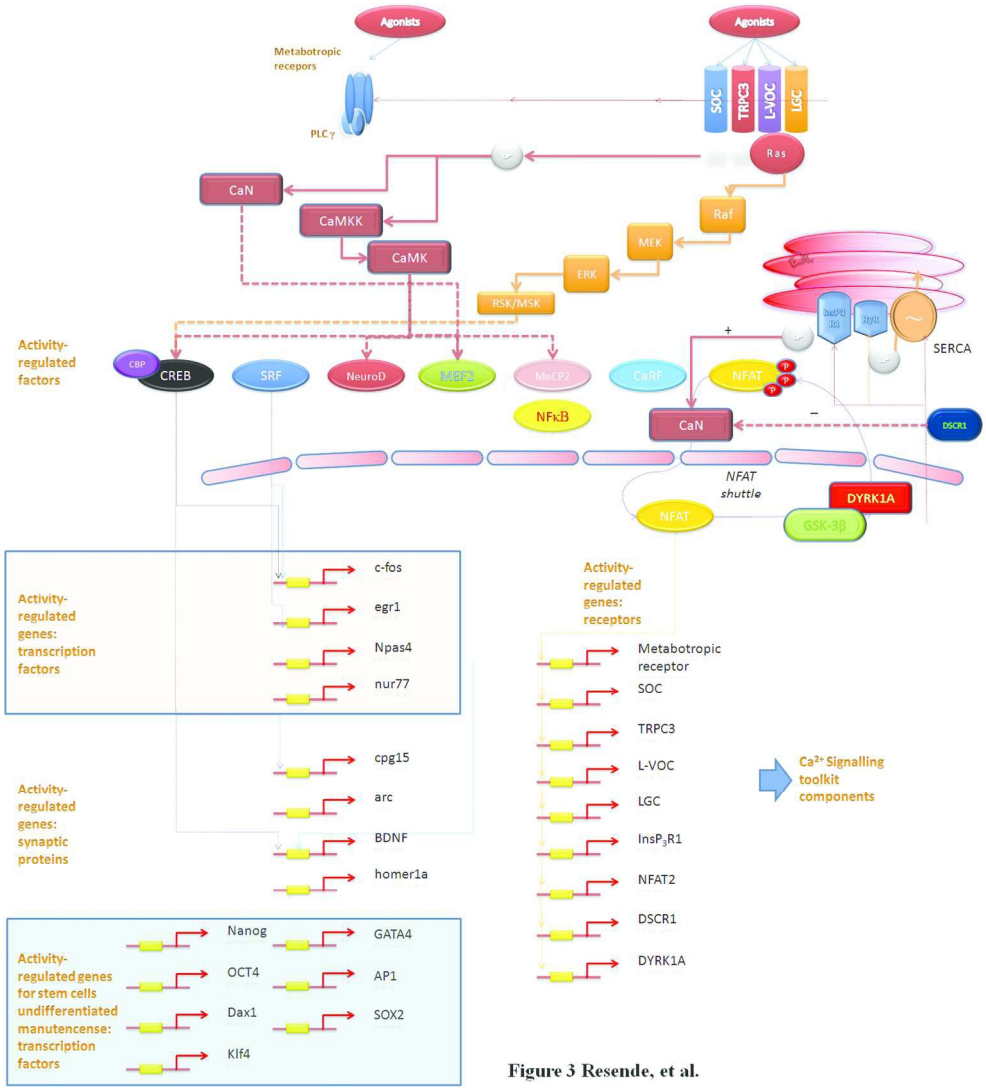

Figure 3 Resende, et al.

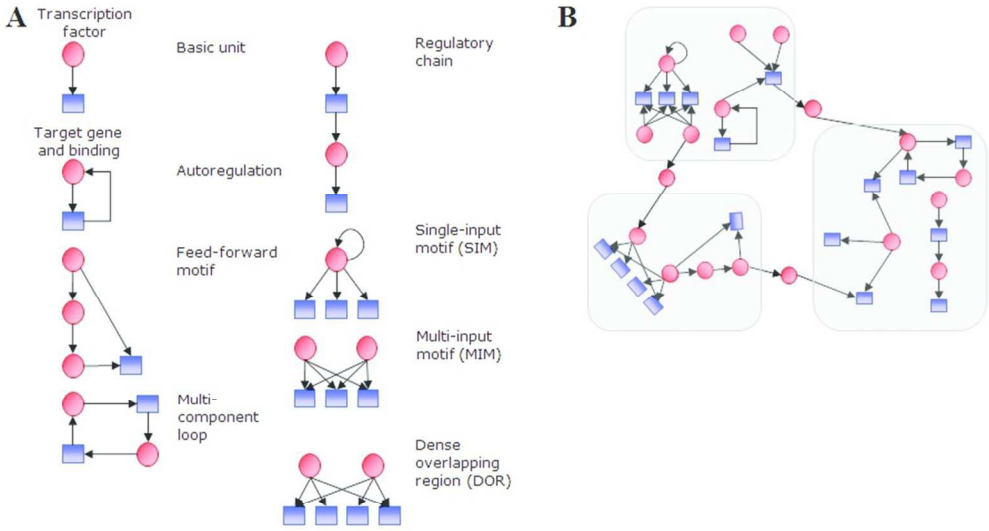

Figure 4 Resende, et al.

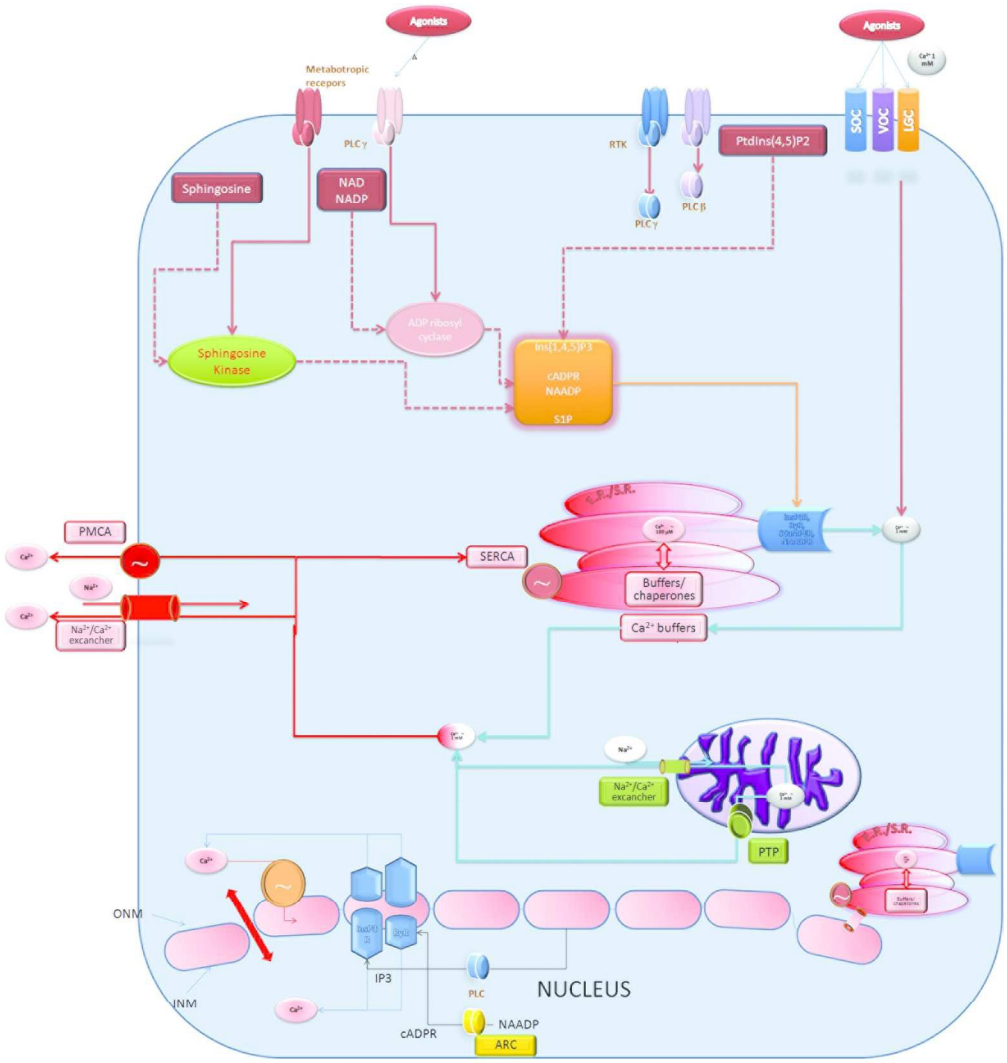

Figure 5 Resende, et al.