Semiotic modelling of biological processes: Semiotic processes in the immune system

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General abstract: Here we introduce biosemiotics as a field of research that develops models of life processes focusing on their informational aspects. Peirce's general concept of semiosis can be used to analyze such processes, and provide a powerful basis for understanding the emergence of meaning in living systems, by contributing to the construction of a theory of biological information. Peirce's theory of sign action is introduced, and the relation between 'information processing' and sign processes is discussed, and, in fact, a semiotic definition of information is proposed. A biosemiotic model of genetic information processing in protein synthesis is developed.

'Signaling' has become in the last two decades a central concept in biological thought. This seems quite natural when we think of biology as an informational science, as 'systems biologists' now propose (Ideker et al., 2001). Biology has been increasingly conceptualized as a communication and information science, even though it is not clear at all what is meant by 'information' in biology (Griffiths, 2001; Jablonka, 2002). It is now quite clear that biological information operates at multiple hierarchical levels, in which complex networks of interactions between components are the rule. Consequently, the understanding of the structure and dynamics of entities and processes in living systems demands that they are located in complex informational networks and pathways (Ideker et al., 2001). Moreover, living systems should continuously communicate with each other, and, also, respond to cues from the environment in regular ways. We believe that biosemiotics can play an important role in this new wave of biological research, by offering invaluable conceptual and methodological tools for building models of informational processes in living systems.

In this lecture, we discuss functional and semiotic models of signaling pathways, focusing particularly on signal transduction in B-cell activation as a case study.

6.1. A semiotic model of signal transduction in B-cell activation

The B cell antigen receptor (BCR) is a multiprotein complex consisting of a membrane-bound immunoglobulin molecule (mIg), the ligand-binding part,

and an Ig-α/Ig-β heterodimer associated with mIg, which acts as a signaling subunit and couples the receptor to intracellular signal transducer elements (Reth & Wienands 1997). BCR has two functions in B-cell activation (Pierce 2002): it initiates signaling pathways that result in a series of intracellular actions in B-cells, including changes in gene expression patterns, which lead, in turn, to the activated B-cell phenotype; and it plays a role in the uptake and processing of antigens to be presented to T-helper cells, which will assist B-cells in achieving full activation (Figure 6.1).

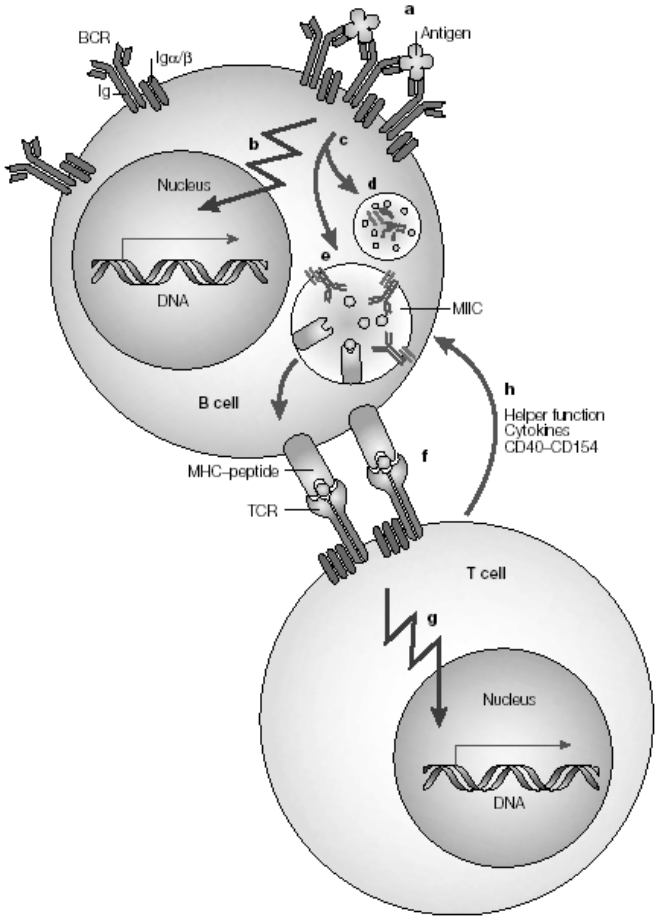

Figure 6.1.: The function of BCR in B-cell activation. Following antigen binding (a), the B-cell receptor (BCR) triggers a signal-transduction cascade (b), which regulates the transcription of genes associated with B-cell activation. BCR is internalized (c) and either degraded (d) or trafficked to an intracellular compartment (MIIC) (e), where complexes containing the antigen bound to BCR are formed. These complexes are transported to the cell surface, where they are recognized by the T-cell receptor (TCR) of T-helper cells (f), leading to T-cell activation (g), by triggering another signal-transduction cascade. The activated T cell provides 'help' to the B cell, leading to full B-cell activation (h). Ig, immunoglobulin. (From Pierce, 2002).

Reth and Wienands (1997) proposed a model of molecular interactions in signaling pathways based on functional definitions, intended to express the roles played by several elements in such pathways, acknowledging (as it is proper of functional definitions) that different elements can fulfill those roles, or, to put it differently, be the occupants of the functional roles

described in the model in different signaling processes. Such a functional model has the important characteristic of being general, in comparison to molecular, mechanistic models of particular signaling pathways. Reth and Wienands characterize eight functional categories of signaling elements (Figure 6.2).

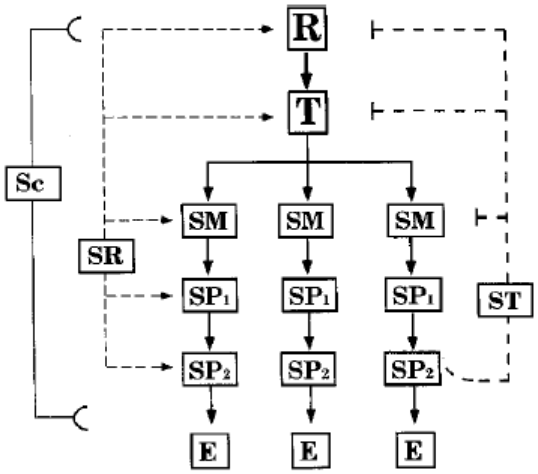

Figure 6.2. Reth and Wienands' (1997) functional model of signaling pathways. Arrows represent different types of functional connections between signaling elements. Dashed arrows represent regulatory relationships. R, receptor; T, transducer; SM, signal manager; SP, signal processor; SR, signal regulator; ST, signal terminator; Sc, scaffold protein; E, effector.

Through signal transduction, living systems are capable of internalizing a cue to a certain aspect of the environment, by producing intracellular signs in response to an extracellular sign. *Receptors* play a central role in the processes through which a cell shows the capacity of answering to its surroundings. A receptor is in most cases a transmembrane protein that undergoes, when bound by an extracellular ligand, a conformational or topological (e.g., receptor aggregation) change, which is, according to Reth and Wienands (1997, p. 456) "transmitted into the cell". But how is the molecular change suffered by the receptor communicated to the intracellular milieu? Here, *transducers* enter into action. But notice that the issue of how the reference to the same cue or signal is maintained in the several changes in the material basis of the message remains open, and is indeed the matter to be dealt with in semiotic models. Receptors usually do not have an intracellular catalytic domain and, thus, are dependent on transducer elements to carry out their signaling function. In most cases, transducers are enzymes physically associated with the intracellular part of the receptor. In its resting state, the receptor often represses signaling activity of the associated transducer, but, when it is activated by ligand binding, it suffers a conformational or topological change that leads to the activation of the transducer.

Each signaling pathway is switched on by the activity of the transducer and controlled by a *signal manager*, the third category in Reth and Wienands' model, located at the start of a particular signaling route. There can be

several signaling pathways arising from the same receptor. There are cases in which a signal manager interacts directly with an effector, which instantiates an action under the regulation of the signaling pathway. When this is not the case, the signal manager activates a signal cascade consisting of one or several *signal processors*. *Signal regulators*, in turn, modify the efficiency and duration of signals traveling down a signaling pathway, by amplifying or decreasing the signal. Such changes in the intensity of a signal can have major biological effects. As we can see in Figure 6.2., signal regulators can act at the level of receptors, transducers, signal managers or signal processors, *i.e.*, at all functional levels of the signaling system.

Signal transduction occurs in an organized microenvironment, in which different elements of a signaling pathway are connected both functionally and spatially. This architecture of signaling elements can be established before or after the activation of a receptor. In the former case, *scaffold* or *adaptor proteins* play an important role in organizing the spatial and functional architecture of signaling elements, by bringing them together in a preformed protein complex.

Even if the stimulus is persistent, signal transduction through many receptors is terminated after some time, due to the activity of *signal terminators*, which can be phosphatases as well as kinases or GTPases. They establish a negative feedback loop that changes the activity of the receptor, transducer, and/or a particular signal manager.

At the endpoint of a signaling pathway, one finds one or several *effectors*, which can be enzymes, transcription factors, or cytoskeletal elements. They are the elements whose behavior is modulated by the signaling pathway.

Signal transduction is a process through which living systems can answer in a regular and (usually but not always) adaptive manner to the environment, by producing intracellular signs in response to an extracellular sign. The mechanistic interactions involved in this process are aptly modeled by Reth and Wienands in functional (and, thus, properly general) terms, but, if the series of mechanistic interactions that take place in a signaling pathway amounts to a process of *signal transduction*, a description in terms of molecular interactions or even functional definitions will not be enough. It is not that some additional element, besides the molecules themselves, should be added to the mechanistic and material aspect of the signaling pathway; rather, what should be added to the picture is a model of the semiotic relation by means of which a molecule such as an antigen can be a sign that stands for something else, say, a virus-infected cell, and, in turn, lead to the production, within the living system, of other (signaling) molecules which stand in the same relation to that object in which the antigen itself stood. Only in this manner we will be able to explain not only the molecular interactions and functional roles in a pathway, but also the maintenance of the reference to the same object, namely the virus-infected cell, while several different signaling molecules are engaged in the pathway. This is clearly a fundamental property to account for, if we want to explain why this is a signal transduction process.

In more details, to model in Peircean terms the maintenance of the reference to an extracellular sign throughout the several changes in intracellular signs that characterize a signaling pathway, one should consider how the processes described by Reth and Wienands instantiate a triadic relation in which a sign (the extracellular signal, an antigen), which refers to an object in the world (a dynamical object, say, a virus-infected cell) through a feature semiotically available in its representation (the molecular form of the antigen, as an immediate object that indicates the infected cell, as a dynamical object), is recognized by a receptor, which acts as an interpreting system. Receptors act as interpreting systems by activating transducers in response to ligands (signs). That is, the receptor communicates the sign process to the interior by coupling to transducers, catalytic molecules that triggers the production of another sign inside the cell in response to the extracellular sign. This subsequent sign is the interpretant of a first triadic relation, and it takes the role of a sign for a subsequent triadic relation, allowing signaling to proceed. This happens through a series of intracellular signals that can diverge, if several signaling pathways are triggered from the transduction of a single extracellular sign, and are amplified by signal regulators along the pathways. Each pathway ends in an effector, which produces the final interpretant in the process, an action through which sign interpretation has an effect on the cell phenotype.

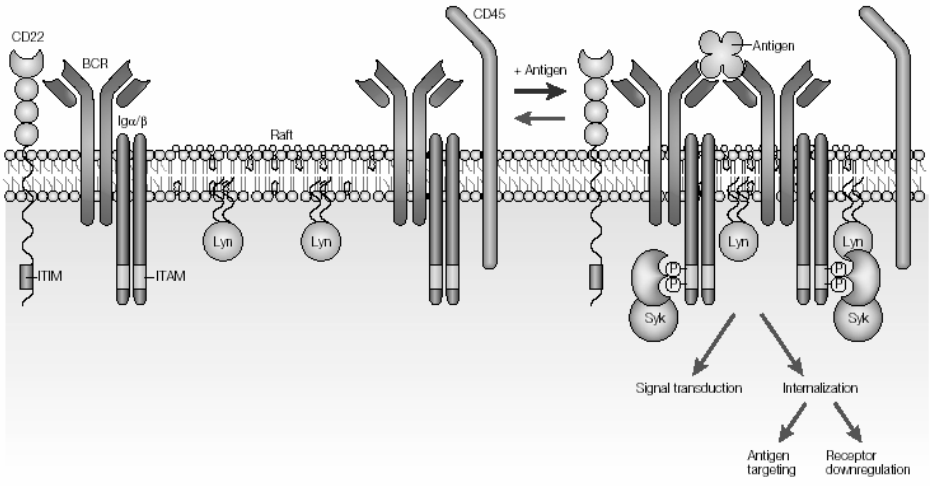

Figure 6.3.: Model of the initiating events in the signal-transduction pathways leading to B-cell activation. (From Pierce 2002).

Let us take now a closer look at initiation events at the BCR signaling system. Figure 6.3 presents a model of the main events at stake. In resting B cells, BCR is excluded from membrane domains (lipid rafts) that concentrate the transducer *Lyn*. In the absence of antigen, the BCR monomer has a weak affinity for lipid rafts, but antigen binding makes BCR molecules associate with each other, increasing affinity for the domains. Stable residency in the domains results in association with *Lyn*, which phosphorylates BCR, initiating several signaling pathways. In Figure 6.3., another kinase is shown, named

Syk, which initiates one of the signaling pathways resulting from BCR activation.

When interpreted from a Peircean perspective, an antigen is a sign that stands for something else, say, a virus-infected cell, and a receptor such as BCR acts as an interpreting system in the cell membrane, triggering processes by means of which new signs, i.e., interpretants, are produced inside the B-cell. The first interpretant in this case is the phosphorylated state of BCR, which is a sign that stands for the virus-infected cell as the antigen itself stood for it. This generates a new triad, linked to the previous one by the double role played by the phosphorylated state of BCR, which is both the interpretant of a first triad, and the sign of a second triad (Figure 6.4). We are dealing, thus, with a first transition accounting for the dynamical nature of semiosis, namely, the interpretant-sign (I-S) transition. By this "transition" we simply mean that the same element that plays the role of the interpretant in a triad will play in a subsequent triad the role of the sign. After all, from a Peircean perspective, to perform sign processing and interpretation is to produce further (or, as Peirce says, more developed) signs. The I-S transition is a basic process underlying the generation of chains of triads. When it takes place, there is also a change in the occupant of the functional role of the immediate object. In the case we are modeling here, the aspect of the virus-infected cell which was represented in the antigen (O_i) is now represented in the phosphorylated state of BCR (O_{i+1}). To put it differently, following the I-S transition, there is a change in the occupant of the functional role of O (Figure 6.4.). It is this latter change that makes it possible that the same entity or process is kept as a stable referent throughout the signaling process, despite the several changes in the material bases of signaling, i.e., in the signs involved. The maintenance of the reference to the virus-infected cell in a signaling pathway can be modeled as such changes of occupants because all the immediate objects in a chain of triads stand for the same dynamical object, the virus-infected cell. The fact that the reference to the same dynamical object is maintained can be explained on the basis that the latter is, in a Peircean framework, the primary constraining factor in semiosis, since its form – understood as a regularity or habit – is communicated through several semiotic, triadic relations. Such a communication of the form of the dynamical object, as semiotically available in a series of immediate objects, is *information* in a signaling pathway. After all, information is conceived, in the Peircean framework developed here, as a triadic-dependent process through which a form embodied in the object in a regular way is communicated to an interpretant through the mediation of a sign.

Biochemical and genetic evidence has shown that *Syk* has a key role in a well-defined pathway of B-cell activation, which results in the release of Ca^{2+} from the endoplasmic reticulum (Reth and Wienands 1997). In this case, the binding of *Syk* to the phosphorylated BCR makes a specific interpretative process proceed. When *Syk* is activated, it leads to the activation of another enzyme, phospholipase Cγ (*PLC*-γ), which is an effector, converting the membrane component phosphatidylinositol 4,5-biphosphate into the two second messengers diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3). This illustrates a case of divergence of intracellular signals, modeled in

semiotic terms by means of the production of more than one interpretant from a single sign, namely, the phosphorylated state of BCR.

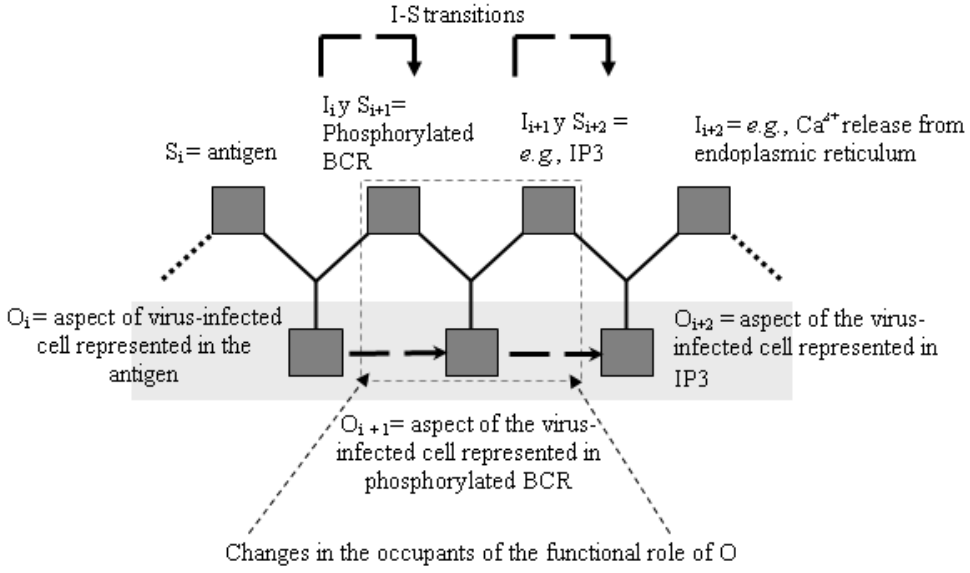

Figure 6.4.: A model of one of the signaling pathways triggered by activated BCR as a chain of triads. Notice the I-S transition and the changes in the occupants of the functional role of O. The maintenance of the reference to the virus-infected cell in a signaling pathway is modeled in terms of these changes of occupants, since all the immediate objects stand for the same dynamical object, the virus-infected cell, throughout semiotic, triadic relations that communicates the form of the object and are conceived, in accordance to the theoretical framework developed here, as *information* in a signaling pathway.

DAG remains attached to the inner side of the plasma membrane and recruits and activates the cytosolic protein kinase C (PKC). IP3 binds to receptors on the endoplasmic reticulum, causing the release of Ca^{2+} ions. The release of Ca^{2+} ions is a new interpretant in the signaling pathway managed by *Syk*. The number of different PKC substrates (for example, CD20, c-Raf, IκB) and the multifunctional role of Ca^{2+} ions in cell metabolism and, also, in signaling, make it clear how an original sign-response can be broadly diversified by the signaling systems of a cell. As we can see in Figure 6.5, the pathway managed by *Syk* in which IP3 is involved does not end in Ca^{2+} ions, but continue through further I-S transitions, which we will not model here for reasons of space. The final interpretant of this (and other) signaling process amounts to the regulation of gene expression, leading to B-cell activation.

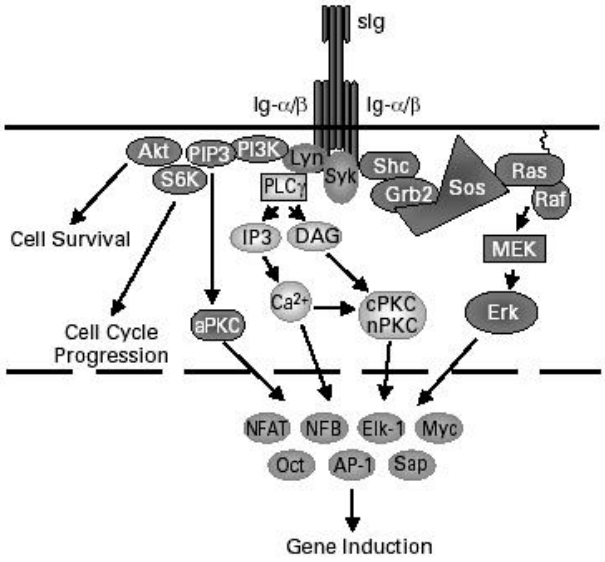

Figure 6.5. Several intracellular signaling pathways are initiated by cross-linking of B-cell receptors by antigen (From Goodridge and Harnett, 2005). In the center of the figure, one can see the signaling pathways modeled above, involving *Syk*, *PLC*γ, IP3, and Ca^{2+} release. Notice the integration between this signaling pathway and the one involving DAG, which leads to the activation of *cPKC* and *nPKC*. Notice, also, that the pathway involving IP3 and Ca^{2+} regulates patterns of gene expression in B-cells.

DAG and IP3 stand for the virus-infected cell in the same way as the antigen and the phosphorylated state of BCR stood, maintaining the reference of the signaling process through changes of occupants of the functional role of the immediate object. IP3, for instance, acts as a sign to a subsequent triad, triggering the production of Ca^{2+}, which, in turn, will occupy the role of sign in a further triad, up to the final interpretant of this particular semiotic process.

From a global perspective, the overall result of the semiotic process modeled above can be grasped in terms of a triad containing the antigen as a sign, the virus-infected cell as represented, say, in the three-dimensional form of the antigen as an immediate object, and changes in the pattern of gene expression in B-cells, as an interpretant (Figure 6.6).

To stress the necessity of semiotic modeling of signaling processes, we can ask why molecules such as DAG and IP3 can be called 'second messengers'? What is the 'message' and how is it preserved in them? The message refers to the presence of a non-self entity, for instance, a virus-infected cell, within the organism. But how is the reference to such an entity preserved in the messengers? In order to successfully model the maintenance of reference throughout the process we should go beyond the pairwise or dyadic interactions between molecules and their substrates, and build a semiotic model capable of showing how the reference to a non-self entity external to the cell can be maintained during the processing of signs within the cell. A semiotic analysis allows us to go beyond a metaphorical usage of the expression 'second messenger': DAG and IP3 are second messengers precisely because they are interpretants produced as a result of the processing of an

extracellular sign (a 'first messenger'), in this case, an antigen. In turn, the changes in the occupants of the functional role of O in chains of triads corresponding to the signaling pathways managed by *Syk* show how the reference to the virus-infected cell is maintained while the material bases of the message, namely the signs, keep changing throughout the process.

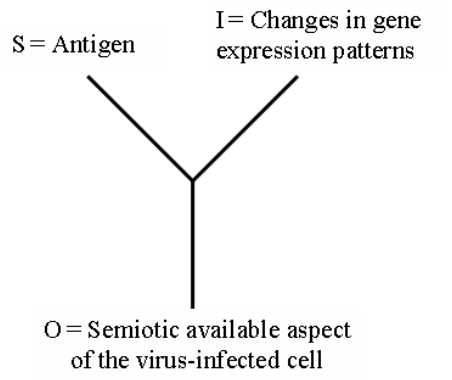

Figure 6.6. A global semiotic analysis of a semiotic process triggered by antigen-binding to BCR.

Conclusion

To understand signaling processes, we need at least three properly connected, but different models: (i) molecular, mechanistic models of particular signaling pathways, in which the molecular interactions that take place in them are properly represented and explained; (ii) general, functional models, such as the one proposed by Reth and Wienands (1997), which represent and explain in general terms how different occupants can play the several functional roles in a signaling pathway; and (iii) semiotic models, such as the one proposed by El-Hani, Arnellos and Queiroz (2007), and reviewed and extended here, which represent and explain in semiotic terms how different occupants can play the semiotic roles in a signaling pathway.

Finally, consider the role of signaling processes, as a higher-level semiotic network, in the actualization of genes as potential signs, by affecting the likelihood of their transcription, or the patterns of splicing of pre-mRNA, or post-translational changes of functional products. Accordingly, the next step in our research will be to employ the basic framework developed above to model signal transduction in connection with gene actualization, combining in a single model the accounts we developed in separate papers.

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Next lecture (Non-human primate communication): In the next lecture we approach 'the meaning of alarm calls in vervet monkeys' according to our model of biosemiotic processes.

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