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Structure-Function Analysis of Transformation Events

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1. Introduction

At the beginning of the twenty-first century, critical reforms were outlined in biology. This was due to the fact that the increase of information garnered from the sequences of informational molecules (DNA, RNA and proteins) and their perception by the scientific community were incompatible with the narrow horizons of the dogmas and paradigms that shaped the ideology of biological knowledge at the end of the last century. The clear linear representations that were invoked during the initial period of the development of molecular biology were at first encountered with numerous exceptions to these "clear" rules and then lost in the avalanche of new representations that were incompatible with the linear schemes. As a result, new directions of study and even meta-directions (e.g., epigenetics, epigenomics and biosemiotics - for reviews, see Allis et al., 2007; Ferguson-Smith et al., 2009; Hoffmeyer, 2008) have arisen, and functionally oriented divisions of biological science have formed that are named as various "–omics" (including the extravagant "biblioma"- Abi-Haidar et al., 2007), the RNA machine (Amaral et al., 2008), and the molecular mechanisms of cell cycle regulation and individual development. In turn, these disciplines have demanded new theoretical implementations involving novel approaches from the theory of networks and systems (Barabasi et al., 2000; West & Brown, 2005; Zaretzky & Letelier, 2002).

The last two decades have been particularly rich in discoveries concerning the mechanisms of the expression of biological information, such as new ways of alternative splicing (Rodríguez-Trelles et al., 2006), a variety of functions for non-coding transcripts and the role of short RNAs as forward and reverse regulators (Mattick et al., 2009). Presently, we have a situation in biology in which it would be nearly impossible to publish a book with the title "DNA Makes RNA Makes Protein" (Pentris et al., 1983). However, another book, with a title equally as concise and perfectly reflecting the new biological paradigm, has not yet been written. In other words, a revision of the old concepts has not been completed with a new and clear way of structuring the data from the quickly growing "body" of biological science. Theoretical biology is also at a critical state, which is characterized by attempts to formulate or reformulate the basic concepts and axioms of the discipline. Signs of such attempts may be the revival of interest in the definition of life, the revision of "sets" and even "types", signs that distinguish the living from the non-living and increased attention to the genotype-phenotype relationship.

This specific creative climate is generated in the interaction zone of theoretical biology and directions relating to the theory of artificial intelligence[1], and it is packed with the new fundamental knowledge that artificial intelligence has absorbed from the different fields of mathematics, physics, and logic (see reviews in Bersini, 2009; Cárdenas et al., 2010; Longo, 2010). It is in this climate that a systems approach to the analysis of biological phenomena is the most productive, yet difficult, for biologists. In journals, the contents of which always gravitate toward theoretical biology, publications on autopoesis, autonomy and incompleteness, and the determinism and unpredictability of biological systems have become commonplace. Notable among these reports is a group of publications that explore the phenomenon of causal closure in living systems as one of the key events on the way to biological complexity (Cárdenas et al., 2010; Kauffmann et al., 2008; Longo, 2010).

Practical achievements of new biological technologies are also impressive. Transgenic plants with useful properties have spread across the globe, displacing more than half of the varieties generated by conventional breeding (Godfray et al., 2010). Information about "cloned" mammals and "replacement components" of mammalian organisms has become standard (e.g., see Ficz et al., 2009; Morgan, et al., 2005). Nevertheless, the theoretical basis for even the most impressive achievements in biotechnology remains underdeveloped, and many promises remain unfulfilled or have been replaced with surrogate solutions.

There is a similar situation with regard to experiments on the genetic transformation of biological objects, where many publications use the terminology and views of the last century. Although many of these terms and concepts are still relevant, it seems appropriate to review the most common events of transformation from a position of a new system of knowledge. We believe that such an analysis will be able to correctly classify several important areas of transformation, to discuss the reasons for failures in some cases and to outline ways to achieve the assigned goals. Therefore, we do not advocate a quick alteration of the terminology and concepts or the creation of a new theoretical foundation of biological science. Personally, we cannot proceed without the concepts of gene, species and many other supposedly "outdated" terms. The task of this publication is to gain a deeper understanding of examples of genetic engineering, those acquired in biotechnology and often having analogs in the wild, with help of a structure-function analysis and to apply new knowledge for the planning of experiments and the prediction of their results. Likely, even this limited goal can scarcely be solved in one study. However, we hope that even the smallest success in this direction will soon be in demand.

We associate certain expectations with the operational presentation of a biological object, where, in the most general mode, the program elements (see below for content on the term "program element" and other elements of the triad) are mapped into the observables. Application of an operational definition of a biological object can help to distinguish (rather conditionally) the mappings associated with the transformation of structures (actual objects) from those associated with the transformation of functions (relations). Such a distinction plays an important role in the interpretation of the transformation events because the result of transformation can be involved primarily in the structural flow or, conversely, in the functional flow of the individual development of the transformed object.

[1] Henceforth, terms and metaphors will be used, which may create the impression that the authors present an organism similar to a computer. In fact, it is not, and we do not know exactly to what extent the notion about the organism can be reduced to the notion about the machine.

In this work, we logically elaborate on the basic ideas of the entity-set representation of biological objects, which has been performed earlier in the framework of plant morphogenesis (Zhuravlev & Omelko, 2008), and show how they can be exploited to describe the individual development of transformed organisms. The chapter is organized as follows. In the first two sections, we determine which transformation events will be included in our analysis and what the relationship is with the individual development of the object. The next two sections present a description of the operational triad as a means of creating a biological object and as a target for transformation. The fifth and largest section provides an analysis of the changes of individual development, which are induced by transformation in the transgenic organisms. Some examples from experiments with producers of secondary metabolites and from experiments on the re-programming of the somatic cells of plants and animals are scrutinized. In the sixth section, the results of the performed analyses are summarized to allow some predictions and suggestions. Last, the conclusion section draws attention to the complex and convoluted character of the mappings between programmed and phenotypic characters that allows for deterministic and probabilistic manifestations.

2. What is a transformation event?

The problem specified in the title of this section is more complex than it seems. This is well illustrated by the example of attempts to define life itself (Luisi, 1998; Ruiz-Mirazo et al., 2004; Zhuravlev & Avetisov, 2006), and defining transformation is equally as difficult. Of course, we can agree to interpret transformation as a particular biotechnological method, consisting of the construction of recombinant DNA and the subsequent introduction of the resulting structure into a living system, but this interpretation does not coincide with all of the similar phenomena in the wild. This idea relates to questions of whether we consider transformation as an exclusively human invention (i.e., as one of the techniques in the arsenal of our human exploration of reality) or an invention of nature, achieved long before the human mind and related to the arsenal of 'becoming' in the living world? The depth of our understanding of transformation events will depend greatly on what point of view is preferred.

The phenomenon of transformation was discovered in the late 1920s, when F. Griffith established that pneumococcal cells could convert from a harmless form to a disease-causing type. This transformation was heritable, and its "transforming principle" was identified as DNA. Since then, and until very recently, transformation events have been associated with DNA. The Encyclopedia Britannica describes transformation in biology as[2]: 'one of several processes by which genetic material in the form of 'naked' deoxyribonucleic acid (DNA) is transferred between microbial cells. Its discovery and elucidation constitutes one of the significant cornerstones of molecular genetics. The term also refers to the change in an animal cell invaded by a tumour-inducing virus.'

In microbiology, transduction events are accepted as distinguishable and considered separately as a process very similar to transformation where genetic recombination in bacteria results from the incorporation of a fragment of bacterial DNA into the genome of a bacteriophage. Then, during infection, this fragment (together with bacteriophage DNA) is

[2] http://www.britannica.com/EBchecked/topic/602613/transformation

carried into another host cell; this process will be covered in more detail in Section 5.1. The field of transformation is closely connected with genetic engineering, which assumes the development of approaches to manipulate the genetic material of a cell to produce new characteristics in an organism. Genes from plants, microbes, and animals can be recombined (recombinant DNA) and introduced into the living cells of any of these organisms. Organisms that have had genes from other species inserted into their genome (the full complement of an organism's genes) are called transgenic.[3]

The abovementioned examples are positioned inside the scope of transformation, but they illustrate a trend toward a greater differentiation in the scope of transformation. For example, there is a tendency in the understanding of transgenic organisms to exclusively those obtained by so-called gene cloning. However, this differentiation gives rise to restrictions. In the specific case of transgenes, primary meaning is associated with the mechanism of the creation of the transgene, and the definition sets aside the result of that action: whether or not a transformed phenotype was created. We emphasize here that the first transformations were found at the phenotypic level, and therefore, the restriction of the notion of transformation strongly constricts the number of examples of transformation and narrows our outlook on this issue. Based on such a narrow view, it is impossible to determine what constitutes a "transformation event". Assume that we have transformed cells using recombinant DNA and have shown the presence of the inserted fragment in the DNA of recipient cell. Can we consider the event of transformation accomplished? Of course, the appearance of the expected sign inclines us to a positive answer, but what can we say when the recombinant DNA is integrated successfully and the expected observable sign does not appear? Some specific cases of transgene silencing will be discussed below, but here, we note only that the transformation event can be viewed as the action itself: the transformation and its result, the manifestation of a new trait. This is a common situation where a function is identified with the result of its action, and this situation tells us that we are dealing with events that can be modeled as a function or as a map. This circumstance will be used in the next section.

It should be emphasized that to understand the mechanism of the expression of observable traits in the cell and to assess the results of an experimental intervention in the operation of this mechanism, we must take the broadest approach possible. To do this, we must replace the concept of the gene with the concept of a hereditary trait, thus referring to the events in transformation as whether the creation (or loss) of the trait is unusual (or usual) in the antecedent cycles of the development of the individual. A direct consequence of such a change will be that the terms of our consideration will include all of the operations that result in the transformation of an organism, whatever their origin may be. This situation will be complicated by the fact that all types of mutations, lateral transfer and hybridization may fall within such operations. We must, for example, classify in this way the phenomenon of the spread of unrelated traits in plants with pollen (in both experimental and natural conditions, equally). Strictly speaking, according to our viewpoint, all of these events *must* be considered in their relation to transformation in the wide sense. For example, viral diseases must be considered here because they induce heritable changes of phenotypic traits. This effect is especially interesting with regard to functional shifts in the host, and it is appropriate place to remember that RNA-directed DNA methylation was discovered in viroid-infected tobacco plants (Wassenegger et al., 1994).

[3] http://www.britannica.com/EBchecked/topic/463327/plant-disease

However, it is clear that such a broad analysis cannot be the subject of merely a single chapter. Therefore, for our analysis, we have chosen only a few examples of the range of events that relate to the production of transgenic organisms, corresponding to the set $\{P_r,P_d\}$, where P_r and P_d denote the sets of recipient and donor characters, respectively. We will include constant reminders that we have consciously limited the scope of our research and that the events analyzed, in fact, can be assessed using scales of different dimensions. Of course, this is a fairly broad idea, but it is this breadth that allows us to make the necessary generalizations.

3. Schematic representation of the development of multicellular organisms

In describing the process of transformation, schemes and diagrams are a common means of representation, but only the structure of the recombinant DNA is usually represented on the schemes. The second participant (i.e., the object of transformation) is usually absent, apparently due to the complexity of its presentation with the same level of detail as for the recombinant DNA. However, to analyze transformation events in this manner, refinement is not required. We need a level of generalization (modeling) of individual development, which will illustrate the relationships of the host and transforming principle.

All of the possible alternatives for the development of an individual can be reduced to a single module, which is the transition (of cells, tissues, organisms, systems) from the less differentiated state to a more differentiated one. The transition itself can occur as a division, multiplication or death. Consequently, this versatile module can be represented as an expression (1):

$$\{(G,Ph)_s \rightarrow (G,Ph)_f\}, \quad (1)$$

where *G* indicates genotype, *Ph* indicates phenotype, and s and f indicate the initial (start) and final states, respectively.

A schematic representation of the plant organism *in vitro* has been published previously (Zhuravlev & Omelko, 2008). Additionally, a rather similar schema, based on the conventional binate idea of genotype-phenotype interactions and also demonstrating EVO:DEVO relationships, has been recently published (Andrade, 2010). Here, we present a modified scheme, which provides the relationship between genotype and phenotype as relationships between sets of inherited and observed properties (Fig. 1).

The scheme in Fig. 1 illustrates the relationship between genotype and phenotype in the rather classical representation, namely from the viewpoint that the genotype, *G*, is a pre-image of the phenotype, *Ph*. However, the first attempt to differentially trace the relationships of these two moieties already meets with some difficulties. We cannot trace their transmutations as two parallel lines, where each previous state of *G* or *Ph* has been converted into the next state. Instead, every division induces changes in *G* between two states that correspond to the active and condensed state of chromatin. Only the active state of chromatin opens the possibility for *G* to be mapped into *Ph.* This type of mapping is dead-end in some sense.

An absence of direct connections between the states of phenotype is of importance in this schema because, for the external observer, the ontogeny of the object appears as a succession of observables. However, we cannot figure the continuous mapping, such as $Ph_1 \rightarrow Ph_2 \rightarrow Ph_3 \ldots$, as the appearance of every new state of *Ph* is a result of a rather long route of mappings, each starting from new state of *G*. The diagram (2) below is not commutative.

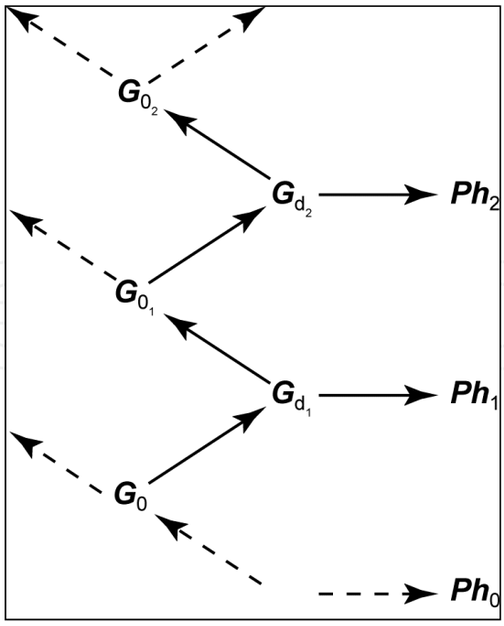

Fig. 1. Scheme of genotype-phenotype interactions during the succession of events in one particular cell line. G_0 corresponds to the compact chromatin state in the course of cell division, and G_d corresponds to the active chromatin state when the expression of the genes responsible for the phenotypic traits is permitted; Ph_i - the phenotypic character.

$$\begin{array}{l} G_1 \rightarrow Ph_1 \\ \searrow \quad \nearrow \\ G_2 \rightarrow Ph_2 \end{array} \tag{2}$$

As we will demonstrate, this idea reflects and explains some of the difficulties of dedifferentiation in experiments on nuclear transfer. We can suppose that this also was a reason why G. Longo and P.-E. Tendero asserted that the existence of empirical correlations between genotypic and phenotypic modifications does not demonstrate the existence of a direct causal relationship between them (Longo, 2009; Longo & Tendero, 2007).

If we use the elaborated diagram (2) and scheme of the development of an individual cell line (Fig. 1) to determine the position of a foreign gene in the transformed genome, we will see that the only place for transgene incorporation is in the set of the oscillating states of G. However, this set is inhomogeneous, and the dynamics of expressions in the transition from G_0 to G_d is not equal to that in the opposite direction because the composition of chromatin, its architecture and the ways of its decoding change in this way. Therefore, in some definite cases, the fate of a transgene will be dependent on its position and the direction of chromatin modeling.

4. Operational triad and individual development

For further analysis of transformation, we require a definition of a biological object. We hypothesized that the definition of a biological object is a task comparable with that for the definition of life itself. Insurmountable obstacles for the latter definition have been reviewed in many publications (Luisi, 1998; Ruiz-Mirazo et al., 2004; Zhuravlev & Avetisov, 2006).

Therefore, we will attempt to define a biological object operationally, in a rather narrow sense, which reflects the individual development of the object. We believe that it will be sufficient to restrict our analytical purpose to the scope of ontogeny. Moreover, we believe that the operational representation of a biological object can be reduced to the analysis of the relationships between the hereditary characteristics of an object and their manifestations; in other words, between the genotype and phenotype. However, the notions of genotype and phenotype have both been subjected to a recent radical revision (Costa, 2008; Fox Keller & Harel, 2007; Gerstein et al., 2007; Snyder & Gerstein, 2003).

With the new molecular knowledge that was partially reviewed in the Introduction section and is partially presented below, it seems problematic to associate the content of an inherited character with a single DNA fragment. As a reflection of the problem, the publications in which the conventional concept of the gene was proposed should be replaced by a "more functional" concept, such as the genon (Scherrer & Jost, 2007) and the deme-bene concept (Fox Keller & Harel, 2007). Within these ideas, the notion of the irreducibility of the content of heritable characters of an organism to a single molecule of DNA has been developed, causing researchers to suggest hereditary mechanisms "beyond the genes" (Amaral & Mattick, 2008; Fox Keller & Harel, 2007), whereas others have defined the genome as an RNA machine (Amaral et al., 2008).

In this context, it seems reasonable to interpret the informational content of DNA and other molecules possessing informational content as a databank or "polytypic library" that causes or begets (in an operational sense) the observable characteristics of the biological object. With this interpretation, hereditary characteristics are not directly associated with certain nucleotide sequences but with a set of characters with respect to which the observable characteristics, such as phenotypic traits, are understood as an operational image of this set. In other words, hereditary characteristics are understood as (remote or direct) operational pre-images of the observable characteristics. However, the cause-and-effect relationships between the pre-images and the observables, even in the case of "typical" transcription/translation, are not (unambiguously) determined. The observable characteristics of the same objects, such as observables of insects during metamorphosis, can vary greatly at different stages of development, while their DNA or other informational structures (pre-images) remains the same. To make the relationship between the pre-image and the image clearer, we must introduce the operational component, which can be attributed neither to pre-image characters nor to the observable characteristics. One can find one of the first rationales of the need for such a similarity of the operating system in Hoffmeyer's early publication, where he stated 'that the conversion of a one-dimensional sequence of symbols, e.g. "DNA inscription" … in a three-dimensional organism' has to be deciphered (Hoffmeyer, 1996, p. 20).

To meet these requirements, we introduce the notion of a function in a broad sense, *F*. Thus, the biological object can be operationally represented by a short universal list:

$$O=(P,F,Ph) \tag{3}$$

The symbol *G*, used above in schemes (2) and (3), is replaced with *P* in expression (3). This may lead to confusion, meaning that we "extract" DNA from the complex image of chromatin to obtain a pure program. Indeed, for our task, we must separate the DNA moiety from the other content of chromatin. However, we interpret the program character more broadly. The programs, *P*, are understood as characteristics of an informational nature

(e.g., instructions or directives) that present the informational content inherent in biological objects. In particular, the programs constitute the operational pre-images of the observables. The most famous, but not the only possible carriers of instructive information, are fragments of DNA that encode proteins. The functions, *F*, are the operations per se that implement interrelationships between the programs, as well as the relationships between the programs and the observables. From the latter, it becomes clear that the part of chromatin released after "DNA extraction" can be considered as a property of *F*.

The observables are the structural and/or functional characteristics of a biological object that can be established by measurements of the object in its interaction with the environment. Phenotypic characteristics of an organism, *Ph*, as observables, are well known, but are not the only possible examples of such characteristics. Therefore, any current state of a biological object is referred to as the particular combination of *P*-, *F*-, and *Ph*-characters (a triad), i.e., the object itself and its current states can be represented by the composition of the following general notations:

$$F: P \rightarrow Ph \tag{4}$$

Such organization allows the object to be represented as both a self-making entity and an element of a self-making entity of higher rank. Similarly, the cell in its operational representation can be considered as an individual entity and as an element of an entity of higher rank (e.g., a tissue or organ). In the biological world, before the appearance of mankind, a biological object was solely executor of any its representation. An object creates itself, displays itself in the surroundings as a successive representation of states of creation (becoming) and interacts with its surroundings in nature.

The triad is understood by us as an operational unit that helps us to represent the individual development as a succession of directed mappings of operational units working in sequential and parallel modes, with cis- and trans-interactions between the units, their compositions, and nests. This can be implemented in the example of a promoter DNA fragment. This fragment can be considered as an argument for several different functions. Particularly, in the course of DNA replication, it is a part of the DNA strand in a chromosome; it is a small part of the bigger argument. However, the promoter can be considered as an entirely independent argument when it is modified by a methyltransferase or other nuclear enzyme. Finally, when specific regulators of transcription, such as protein repressor complexes (Bantignies & Cavalli, 2006), bind to the chromatin, the promoter fragment of DNA can again be considered as part of the larger argument (of collective body of all repressed promoters).

Such manifold representation is characteristic of many informational molecules and their fragments in the cell. A more detailed analysis in this vein requires the assistance of relevant mathematical languages, as was validated in Mossio et al. (2009). To obtain a general image of the diversity of representations characteristic to elements of the triad, we confine ourselves to the following schematic representation (Fig. 2).

The scheme in Fig. 2 is based on the distributed representation of the existence of two functionally different types of cells (germ and somatic) in a multi-cellular organism. Divisions of cells in the germ line are divisions where non-differentiated cells are obtained as a result of the division of pre-existing (meristem or stem) non-differentiated cells. Landscapes of methylation and other labels distributions are very similar in generations of both germ cell lines and meristematic cells. The totipotency of these kinds of cells has been demonstrated in experiments with both plants and animals (Batygina, 2009; Nagy et al.,

1993; Takebe, 1968). Actually, every division in germ cells can be regarded as the identity map or the identity function.

Conversely, the mappings that lead to the creation of observables form a different class of maps, which is performed by different functions. To distinguish between these functions, we introduce the symbols φ and f (see Fig. 2). Both classes of functions can be divided into more-detailed subclasses in accordance with their role in the development of the individual. Thus, the function of the substitution of histones with polyamines in developing sperm can be considered as a subclass of the function φ-class. In turn, functions f_1, f_2, f_3... can be considered as subclasses of functions in different tissues and organs.

It must be emphasized that functional activities corresponding to symbols φ and f are often inconsistent with each other. It can be a consequence of the fact that chromatin expression activity is inconsistent with some stages of the cell cycle (Jacobs, 1995). In contrast to scheme 1 and diagram 2, the relationship between genotype and phenotype is not visible in Fig. 2. This relationship is symbolized by the differential activity of genes in cells in different states (note the different color of the dots in the nuclei). The attempt to represent the relationship in more detail leads to a catastrophic growth in the complexity of the representation, as can be seen in an article representing the genetic landscape of a cell (Costanzo et al., 2010).

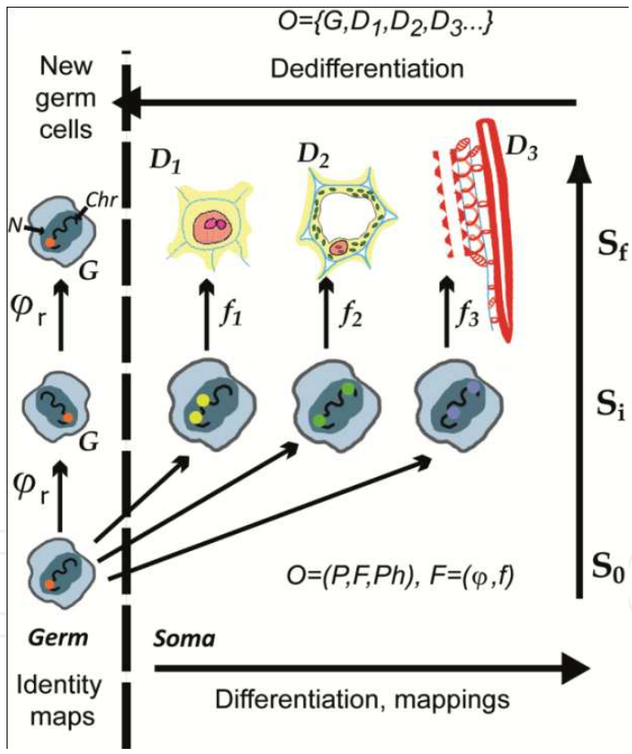

Fig. 2. Scheme illustrating the interrelationships between the elements of the operational triad in two lines of cells in a developing organism. The set of P elements is roughly the same for both germ and somatic cells. However, two different types of functions are associated with these two main directions of cell division in the embryo. Thus, the symbols φ and f are assigned the divisions of the germ and somatic cells, respectively. S_0, S_i and S_f symbolize the initial, intermodal and final states of the object, respectively. The dots of different colors in the nuclei of the cells denote the differential activity of the chromatin.

However, the relationships represented in Fig. 2 are sufficient to argue that these relationships cannot be interpreted as a one-to-one mapping in the majority of cases. Moreover, most of the maps that precede the final (producing the observable) map cannot be expressed in terms of states. The latter entails a specific architecture of the biological object where the set of programs and data are "wrapped" in several shells of specifically arranged mappings. Only the outer shell of observables participates in the contact with surroundings, though many inner layers include the measurable structures that can be identified as observable.

4.1 How many units in dividing cell are portable?

In the beginning of this section, we will emphasize the complex design of chromatin. This complexity is specific to biology because this feature is not an invariant measure, but it develops in the course of the individual development of the object. Moreover, this complex body builds itself of its own accord. During this building, the different structures of chromatin may be interpreted as functions and, thereafter, can be applied to other structures of chromatin as to the arguments. Thus, the repressive complex PRC1 acts as a function for methylated promoter though both, the complex and promoter, are constituents of chromatin. Due to this activity, an inseparable complex of DNA and other chromatin structures carrying general function, *F*, are created.

The idea of the inseparability of DNA and other chromatin structures is not yet generally acknowledged. Researchers and philosophers, seeking to understand the role of DNA in a living organism, have considered the portability feature of DNA as an indication that it belongs to the world of software. For example, G. Longo (2009) writes '…Ending with portability of software: even on different, but suitable environment, a fortiori over identical environments, programs may be repeated at will. And it works'. We contend that this argument is not universal. Any enzyme will work in a suitable environment. The idea of inseparability consists of the affirmation that no single, inseparable part of an object can be extracted from the object (or system) without the complete destruction of the object. In other words, the physical extraction of DNA from the object will destroy this object as an individual.

Of course, naked DNA transferred to another suitable environment (hardware) can realize itself through the interaction with this new environment, but the probability that this realization will result in the creation of an initial object is negligible. Indeed, such is only the case with rather complex entities. We can take numerous examples of the transmission of infectious agents by the naked DNA of viruses and bacteriophages. However, these objects are devoid of individuality hereupon one cannot decide whether the initial object was reproduced or not.

In all cases, when the matter is the division or multiplication of cells, where we have to use the term chromatin, the individuality of the object is connected with the DNA-protein complex. At the current level of knowledge, we cannot exclude the fact that this complex is even more complicated and involves an RNA component (Amaral & Mattick, 2008; Yao, 2008). Of course, these two components (protein and RNA) possess a portable nature.

For example, what is observed in experiments on vegetative plant hybridization? We know that grafting to cold-resistant stock increases the cold resistance of the graft. The graft of a tall plant becomes stunted even if there is a small insertion of the dwarf plant between the root and stem. It is unlikely that we would have reason to believe that these changes in phenotype are associated with the transportability of DNA. The transfer of these properties

can be assumed to involve two other components of chromatin. There is experimental evidence that certain transcription factors (Kim et al., 2002) and short RNAs may be regarded as mobile signaling molecules (Dinger et al., 2008).
The examples of vegetative hybridization discussed here indicate that very distinct and specific phenotypic traits can be created by means of these signaling molecules. However, if this is so, we can no longer assert that all of the specific information is concentrated in the DNA. Thus, we would need to accept the idea that the transportability of the other components of chromatin is relevant for the phenotypic manifestations of living organisms.
We hope that due to this short analysis of chromatin dynamics, we succeeded in the elaboration of the chromatin image as a supramolecular and complex (in chemical sense) body. This body, however, is an indispensible part of the cell and has multifold connections with the rest part of the cell (and organism). Nevertheless, chromatin, as a specific formation of living cell, possesses its own intrinsic topology and dynamics. DNA and some proteins are the regular constituents of chromatin whereas many others signal molecules can incorporate into the system, reactivate and remodel corresponding sites and leave the system. The aggregation of signal molecules with the meaning-bearing fragments of constituent parts can radically convert the functional meaning of these fragments as transmutation functions in arguments. As a result, composition and orientation of operational units in the chromatin body will change too. The individual development of a biological object is, to a large extent, offered by the dynamics of the functional part of chromatin body.

5. Operational triads as targets for transformation

From the operational definition of a biological object established in the previous section, it follows that the individual development can be understood as successions of continuous mappings that build the structure of the object as a complex web, with branchings and the interactions between the operational units. However, these branchings and interactions entangle the web. Even the task of finding one particular fragment of DNA that encodes a necessary protein, among 35,000 such fragments, which together make up scarcely more than 1.5% of the total DNA, cannot be solved by a simple search. This creates a problem that is characteristic of computing science, called data typing, which has some analogs in biology (e.g., blood typing and DNA fingerprinting). In large-scale typing in cell, transposons apparently take part (von Sternberg & Shapiro, 2005). However, in this specific case, DNA typing is a function-oriented process where the typing is performed from the viewpoint (and on behalf) of becoming an operating system. The typed data, P, can be properly treated with the corresponding functions, F, and the results of this processing are handled by other functions. Only after numerous iterations, recursions and destruction of intermediate states are a set of observables, Ph, corresponding to the current state of the object, created.
Only this moment of the creation of a set of observables is critical mapping, in the sense of the creation of Hoffmeyer's three-dimensional organism. However, this critical mapping is preceded by a large and complex task for the proper organization of data and the creation of the necessary operating system. This work remains directly uncommitted in the final mapping, but its role in producing the final observable factor is determinative.
Due to this, any intervention in the structure and organization of the database or in the composition of functions can modify the result of transformation.

5.1 Recombinant DNA carrying one to several genes of known function

The reports described below originated with a study of a phenomenon that Joshua Lederberg and his student Norton D. Zinder termed transduction. In 1952, they revealed that certain bacteriophages were capable of carrying a bacterial gene from one strain of Salmonella to another. Although the capacity of the bacteriophage genome is low and because most bacteriophages can only infect restricted variants of bacterial species, a number of microorganisms that produce important proteins were nonetheless obtained. Worthy of mention are those proteins, hormones, interleukins and other enzymes whose genes are not complex structures and that presented no issues with folding or solubility in the microbe or in the laboratory. These transformations, though producing metabolites unusual for the microbes, ought to be classified as the simplest transformation events because the route from recombinant DNA fragment to observable product is relatively short and because the microbial set of DNA-regulating mechanisms is simple due to their unicellular nature.

Similar results have been obtained in the transformation of plant cells growing in a bioreactor as separated cells or small cell aggregates. However, these cells, their DNA, nucleus and the cell as a whole are equipped with more complex gene expression control mechanisms than bacteria. Furthermore, some intercellular effects can even be observed in thick cell suspensions.

Very often, plant cell transformation is directed at increasing the yield of secondary metabolites that are a characteristic of the plant species. In this case, some evolutionarily developed mechanisms may help the cell to avoid the over-expression of individual genes, including the transgene. A useful way to evade this controlling mechanism is to use viral promoters, which often are unresponsive to host control. This approach protects the transgene from the *cis*-control of the host and from individual *trans*-control, whereas the expression of the transgene can be blocked during the more general form of chromatin remodeling. However, the remodeling of chromatin by such a general transforming principle (e.g., a plant oncogene of a Ti-plasmid) can result in the over-expression of certain sets of host genes (Bulgakov et al., 2009; Zhuravlev et al., 1990). The slow *cis*-control of host DNA (DNA reparation) can destroy the transgene because mutations seem to occur more often in the transgene that in the host DNA (Kiselev et al., 2009).

Several products can be obtained from plant cells through transformation (Godfray et al., 2010). Thus, a certain resonance has been caused by the cultivation of transgenic crops that produce proteins of the human immune system, modified fragments of infectious agents, and other important products of a proteinaceous nature. Important industrial progress has been made in the field of plant protection against pests by the transformation of agricultural crops with the bacillotoxin gene, initially detected in *Bacillus thuringensis.*

The progress of this research was largely determined by the ability of plants to regenerate an entire plant from transformed cells, i.e., it depended on the implementation of the totipotency of plant somatic cells. In cases where the production of somaclones from transformed cells is a complex task, embryogenic culture, embryo or meristem cells are bombarded with microparticles loaded with the corresponding recombinant DNA. Less commonly, the transformation is accomplished by introducing the vector constructs in the course of the "normal" sexual process. This last method is one of few that are suitable for the transformation of multicellular animal species when examples of their somatic embryogenesis are unknown or rare.

The main problem in research of this type is not only the transformation itself but also the regeneration of the transgenic organism from a transformed cell. Until recently, such regeneration from somatic cells was known only in plant systems. Although numerous transformed plants have been obtained by means of somatic embryogenesis, many details of the induction mechanism remain unclear.

We are more interested in the fate of the gene in the context of its relationship with the process of the expression of genetic information of the host cell. A transgene integrated in DNA, as would be assimilated by the database, is indistinguishable from "its own data" in most cases, which (as we shall see below) we cannot say about the transfer of a nucleus. However, in mixed populations, the transformed cells and organisms may differ from untransformed ones by such factors as growth rate and sensitivity to abiotic factors (i.e., being subject to selection). The best results can be obtained when the selection scheme includes the production of cellular clones, such as in the following scheme:

a cell suspension enriched with super-producers → single-cell suspension → the seeding on solid media of a highly diluted suspension → the selection of colonies originating from a single cell → receipt and comparison of clonal lines.

This means that the cell population must originate from a single cell. However, this choice is fraught with the unlikelihood that a uniform population will be obtained with the same resistance characteristics for all of the cells that may result in the decline of the population stability.

5.2 Transfer of the nucleus (nuclear transplantation)

As it is understood in biotechnology and classical genetics (*i.e.* offspring production of one and the same cell), cloning does not belong to the events of transformation. The term cloning, which is often used in the procedure for the reprogramming of a nucleus of the somatic cells of large animals, is used inappropriately, whereas the terms regeneration and hybridization are more adequate in most cases. However, some attempts toward the regeneration of adult animals from somatic cells, including a procedure of transformation with recombinant molecules or nucleus transfer, in the broadest sense, can also be viewed as a transformation. The first phase of experiments with somatic cells is represented by attempts to reprogram somatic cells as a result of a merger with an undifferentiated cell (often an oocyte), enucleated or with an inactivated nucleus (Jaenisch & Gurdon, 2007).

In plant biotechnology, such manipulations are called somatic or parasexual hybridization. Well before the end of the twentieth century, the problem of dedifferentiation (reprogramming) was shown to be less acute in experiments with the cells of higher plants, so the studies were primarily conducted as examples of inter-specific hybridization (Gleba & Sytnik, 1984). However, these experiments have only had a limited theoretical yield. As far as we know, the hybrid Brassica napus was constructed in this way.

In the biotechnology of vertebrates, The Encyclopedia Britannica (2011) cites experiments performed by the British molecular biologist J.B. Gurdon as one of the landmark studies in this direction. J.B. Gurdon transplanted a mature nucleus from an intestinal cell of a tadpole into an enucleated egg of a frog, which subsequently developed into a normal, adult frog. ’Gurdon thus demonstrated that a highly differentiated intestinal cell nucleus, with only intestinal cell genes functioning, could undifferentiate in the environment of the enucleated egg cell and could reactivate those genes necessary to create an entire frog. The frog that was produced was a "clone" in the sense that the entire genome of the donor tadpole was

present in all the cells of the newly formed frog'[4]. For a more detailed history and the recent state of the field, see Jaenisch & Gurdon (2007).

Approximately 20 years later, reports began appearing about the successful development of transplanted mammalian nuclei. In these experiments, the acceptor cell usually originated from germ line cells; more often, it was an oocyte. After fusion, successfully induced embryos were transferred into a surrogate female to promote the full-term development of the new animal. Just as with frogs, the obtained organisms are incorrectly called clones (see, for example, Eggan et al. 2001, 2004).

From the view of knowledge obtained in experiments on plant cells, all of the experiments to obtain "clones" of large animals should be classified as a special case of intra-specific somatic hybridization, where hybridization with undifferentiated enucleated cells was used for the induction of totipotency of a differentiated donor nucleus. All of the examples where the nucleus was taken from one animal and an undifferentiated enucleated cell from another animal are essentially examples of the receipt of a chimeric genotype because cytoplasmic hereditary factors belong to the acceptor cell. For this reason, it is logical to expect that the hybrid offspring would not be an exact copy of the donor; just this has been observed in practice.

The most significant differences of the phenotypic "somatic" descendant from the nucleus-donor phenotype can be assumed to be associated with cytoplasmic hereditary factors, chief of which are the mitochondria. In endothermal animals, mitochondria are inherited through the maternal line. Therefore, when the donor of a somatic nucleus is a male, genome mixing is inevitable because the oocyte can only be obtained through the female line.

However, this is only a *design* part of the problem, whereas a *functional* part also exists. It is important to remember the following details in the technique of cell hybridization (Egli et al., 2007): for the successful induction of embryogenic development of the hybrid cell, the phase of the cell-cycle in which the enucleation of acceptor cell was performed is important. Notably, the state of the nuclear membrane and the level of compactness of chromatin are decisive. The authors believe that the 'removal of the pronuclei during enucleation would deplete these factors and prevent development. In contrast, removal of the condensed chromosomes from a metaphase II, meiotic egg would not do so' (Egli et al., 2007, p.683).

It suggests that some components of the cell-acceptor nucleus, which passed into the cytoplasm in this stage (see subsection 4.1) and, therefore, had the opportunity to interact with the transplanted nucleus, were needed for reprogramming the donor chromatin. Among such important factors, methyltransferase Dnmt1 is suspected. This enzyme is present in the nucleus in the course of one cell cycle only and then is removed from it (Surani & Reik, 2007). This, or some other, transferase can be crucial for the establishment of the specific architecture of donor chromatin as making it available for further control during embryogenesis. In one viewpoint, the post-nuclear transfer development of the hybrid cell can be understood as an interaction between the transplanted nucleus with a soluble part of the acceptor chromatin (i.e., *as an interaction of the nucleus with the nucleus*, which, of course, contradicts the intention of the experiment). It is also important to remember that many successful experiments with nuclear transfer in frogs were performed by the UV inactivation of the recipient nucleus, and such inactivation does not exclude the possibility that some low molecular weight chromatin structure of the recipient remained intact and played some role in reprogramming the donor nucleus.

[4] http://www.britannica.com/EBchecked/topic/262934/heredity

The number of transcription factors required for the induction of embryonic development in mammals has recently been reduced to three (Ficz et al., 2009). However, we have no reason to believe that these transcription factors (and only these transcription factors) were released into the acceptor cytoplasm. Taking into account the possibility that their first "products" can be enzymes of DNA demethylation (Bhutani et al., 2010; Popp et al., 2010), we cannot exclude the possibility that these enzymes are themselves passed into the cytoplasm.
For the hybridization of animal somatic cells, it has been assumed that the donor DNA, as the database, remains intact. Therefore, the main problem is to create a new operating unit, which would start operating from scratch. However, operating from scratch is impossible because the chromatin of the transplanted nucleus is a complex combination of different structures (see above sections), and even the DNA in transplanted chromatin is quite different from that in a zygote. Using the language of artificial intelligence, the problem includes the following: i) a database state that is inappropriate to the initial one, and ii) the remnants of the previous operational structure that support the state available at the moment of nuclear transfer. There is reason to believe that the operating structure is presented mainly by the short-lived regulatory elements. In addition, continuous division, being the basis of development and differentiation, may lead to the rapid dilution of relatively stable regulatory elements. In this respect, experiments on secondary embryogenesis in some plants, rarely giving somaclonal variants, are very informative. For instance, for ginseng (*Panax ginseng* C.A. Meyer), it is relatively easy to obtain vegetative shoots *in vitro*, but they root poorly, presumably because the required level of dedifferentiation in somatic cells was not reached. We can suppose that these effects were associated with DNA demethylation, as demonstrated in other experiments (Bhutani et al., 2010; Laurent et al., 2010; Popp et al., 2010). However, if the ginseng embryoids deficient in root formation were transplanted to a new medium, then after a short period of callus growth, the secondary embryos would develop into normal plants with roots. The molecular mechanism of root initiation was recently revealed to be connected with a movable agent (Schlereth et al., 2010). Thus, additional divisions can result in a decrease in the level of methylation of DNA or in the dilution of some hypothetic factor(s) blocking root initiation. In *Arabidopsis* embryogenesis, an extra-embryonic cell (of the suspensor) is specified to become the founder cell of the primary root meristem, the hypophysis, in response to signals from adjacent embryonic cells (Schlereth et al., 2010). In the course of somatic embryogenesis, no suspensor structure can usually be specified in the callus cells around the embryo (Batygina, 2009). Consequently, two competent cells have to occur in contact to give rise to the somatic embryo and the extra-embryonic root meristem. Of course, this contact is easier when a movable signal exists. Similar events may have taken place in the experiments with vertebrates, where the improvement of embryo formation was obtained due to a procedure known as ”serial cloning”.

5.3 Transformation instead of transplantation

It is known and was reviewed above that the induction of embryo development is a critical phase in experiments on nuclear transfer (Bhutani et al., 2010; Ficz et al., 2009). In this regard, the induction of embryogenesis in plants with phytohormones, antitubulin factors and abiotic factors seems to be more understandable (Zhuravlev & Omelko, 2008). Nevertheless, the problem of induction of embryogenesis remains a major issue for biotechnology in both animal and plant cells. It is particularly urgent in cases that require the generation of a transformed organism in which all of the cells are modified. Currently,

this can only be achieved through the induction of embryogenesis in a separate transformed cell. If the transformant is not a zygote, the difficulties of its induction to embryogenic development may become an insurmountable obstacle to obtaining a transgenic organism. Even in the biotechnology of plant cells, we are faced with fundamental difficulties. For example, the possibility of somatic embryogenesis in such important cultivated plants as soybeans (for a review, see Dos Santos et al., 2006) is severely limited. However, many obstacles impede the application of somatic embryogenesis in the biotechnology of economically important animals. Above, we discussed the problems associated with this so-called cloning. Here, we focus on receiving the reprogrammed fibroblasts through their transformation. As we show below, this process can also be interpreted as applying an induction function, but it is a significantly more economical and accurate attempt than in nuclear transplantation.

The direction of this research came in 2006, after K. Takahashi and S. Yamanaka demonstrated that transformation with vectors carrying genes encoding the Oct4, Sox2, c-Myc and Klf4 transcription factors induced the transformation of mouse fibroblasts into pluripotent stem cells (induced pluripotent stem [iPS] cells). These factors play an important role in the early stages of embryogenesis. Thus, the Oct3/4 and Sox2 genes encode regulatory proteins required for the maintenance of the properties of stem cells (induced in zygote and embryonic tissues). The c-Myc and Klf4 factors maintain the self-renewal of stem cells, and Klf4 also increases the level of Oct4. After the experiments by Takahashi and Yamanaka (2006), similar experiments were performed (Okita et al., 2007; Wering et al., 2007), which successfully demonstrated live-born chimeras using an injection of mixed iPS cells into blastocysts. It became clear that there was a new prospect for cloning, namely the replacement of the nuclear transfer procedure for the induction of somatic embryogenesis from minimally differentiated cells of the connective tissue of animals (i.e., fibroblasts). It should be noted that the wound tissue of plants, callus, is even less differentiated, and the use of this tissue in plant biotechnology has yielded progress in the attainment of somaclonal and gametoclonal variants. Therefore, we expect that this model (induced fibroblasts) will lead to comparable success, though the calli of plants and the connective tissue of animals should not be equated.

Nevertheless, the few achievements obtained with this model confirmed its promise. There is, however, one major difficulty. Transformation, causing an induction, can be considered as the creation of a certain intermediate operating unit, whose fate is not indifferent to the further development of the transformed cells. If it continues to function, it can become an obstacle to further development. We are faced with a similar problem when using the transformation of callus cells with the *rol*C gene to induce embryogenesis in ginseng. The transformed cells are successfully induced and form an embryo-like structure. However, without reaching the torpedo stage, the structures revert to embryogenesis, now secondary, and the cycle is repeated over many passages (Gorpenchenko et al., 2006). We understand the results of this experiment as follows. Under the influence of *rol*C, cells are exposed to dedifferentiation and take the form and properties of embryonic cells. The inductive effect is, however, permanent. With the development of the embryo, and in the course of differentiation, these cells are again faced with factors produced by the recombinant DNA causing the induction. As a result, they revert to an undifferentiated state and, thus, give rise to a new cycle of embryogenesis. In terms of programming, one can say that the operational structure causing the induction is extremely stable, and its function is performed whenever the cells reached a certain degree of differentiation.

Although the molecular mechanism of the induction of embryogenesis with the transformation by *rol* genes or transcription factors genes is still not clear, the fundamental aspect of the matter is plain enough. In addition, the significance of these results for planning experiments on morphogenesis is also obvious: when transformation is used for induction, the transformation must be temporary. How can we halt or dilute the inducing signal?

The number of induction (parental) factors in a "normal" zygote can be assumed to be limited and reduced with each division (perhaps by half). A rapid dilution eliminates the possibility of secondary induction. However, what about a situation where (at this stage of knowledge) transformation seems the only possibility for the implementation of an induction? We see the solution in the creation of short-lived vectors unable to integrate into the host DNA (Zhuravlev & Omelko, 2008). The creation of such structures in plants (e.g., the use of attenuated tobacco mosaic virus, containing the mRNA from transcription factors genes) can be used as a method in the induction of embryogenesis in crop plants, whose somaclonal variants are difficult to obtain.

A successful implementation has been achieved by K. Kaji et al. (2009) in their attempt to obtain the virus-free induction of pluripotency in mouse and human fibroblasts and a subsequent excision of reprogramming factors. A drug-inducible lentiviral reprogramming strategy has also been designed to achieve the tight control of transgene expression in iPS cells (Boland et al., 2009). In this work, the four original reprogramming factors (Oct4, Sox2, Klf4 and c-Myc) were placed under the control of the tetO promoter, which is activated by the reverse tetracycline transactivator (rtTA) protein in the presence of the tetracycline analogue doxycycline (dox). This construct makes possible the control of the level of expression of reprogramming factors.

However, the task of managing the development of an individual is not always reduced to the necessity of the rapid dilution or direct removal of initiation factors; occasionally, the opposite is necessary. The low content of such factors in the zygote has apparently caused the failure of attempts to split the embryos of cattle. If this is predominantly due to the lack of transcription factors it is possible that their direct injection into blastomeres will be decisive in the embryo splitting technique for rapid breeding of cattle.

6. The problems of transformation and some routes to success

This section concerns the question of the unpredictability of the results of transformation and, in more general terms, the uncertainty and unpredictability of the individual development of any organism. This question in the aspect of embryogenesis in plants *in vitro* has been partially discussed in a previous publication (Zhuravlev & Omelko, 2008).

Those working in biotechnology are often faced with a problem when a transformation does not produce the desired result and the target product accumulates only in small amounts. These phenomena are associated with the uncertainty of the location of transgene insertion, variations in the number of integrated copies and the active defense of the cell against the expression of foreign DNA fragments. The first two facts are usually clear and established by experiments. With regard to the protective measures, there are less data, and these data are more difficult to interpret. Indeed, the number of mutations in the DNA inserts from plasmids is significantly above the average mutation rate in the non-transformed DNA of ginseng (Kiselev et al., 2009).

Perhaps these considerations may have a more general nature, and the very unpredictable results of transformation may be associated with the uncertainty of the developmental paths

of the transformed organism. Another basis for the unpredictability of the results of morphogenesis *in vitro* can often be the indeterminate number of induced cells. The point here is that when the induction covers not just one but a number of neighboring cells, each characterized by different states of chromatin activity, the overall result is difficult to predict. Simultaneous operation of many sources of signal molecules complicates the model and deprives its predictive power.

Another important condition that leads to unpredictability in plant morphogenesis is the low level of specificity of the signal that induces the start of the morphogenetic transformations. Such signals in plants may be various biotic and abiotic factors, such as stress, phytohormones, a shift of the ionic environment, or an electrical impulse. To induce morphogenesis in animal cells *in vitro*, most of these factors have proven to be ineffective, indicating the greater specificity of the primary signal in animal cells. The structural features of plant cells, especially the cytoskeleton, predispose the cell to the perception of external signals of an abiotic nature (see review in Rowat et al., 2008). This structural feature, among others, likely makes the induction of morphogenesis in plants easier but less selective in comparison with the systems of an animal nature. The induction of somatic embryogenesis in animals, with the many known limitations, requires more specific endogenous inducers. In addition to transcription factors, which have already been mentioned, transcribed and non-transcribed RNAs of the mother organism may be such highly specific inducers, which are deposited in the egg and direct the early stages of development. Maternal RNA degrades during embryogenesis and is replaced by zygotic RNA as the transcription of zygotic genes is triggered. Before this, the zygotic genome in animals is transcriptionally inactive (Amaral & Mattick, 2008; Schier, 2007; Yao, 2008).

Taking these facts into consideration, it is logical to expect that the unpredictability of results of transformation is associated with the indirect mode of mapping the genotype on the phenotype, which was apparently a historical necessity because the organisms were placed in front of an intractable task to increase and diversify the phenotype without significantly increasing the genotype. In artificial intellect programs, this problem occurs very rapidly. One possible solution may be to use an indirect genetic encoding that takes the form of a developmental process (Nowacki et al., 2008; Roggen et al., 2007).

In this chapter, we developed a dual idea representing the organism as the following: i) an integral, indivisible entity, and ii) a complex construction in which three functionally entangled bodies can be abstracted, namely, the *P*, *F* and *Ph* sets. The task to describe the relationships of these sets resembles the Poincare's three-body problem, which was recently analyzed by G. Longo (2009) in its relation to biological objects.

7. Conclusion

The current shift of paradigm in the science of complex systems has affected our view of biological object, its development and its transformation. Depending on the construct used, transformation can relate to different intracellular processes, which are characterized by non-linearity and self-referencing, self-construction and self-modification. The problem now is how long distance is between the introduced DNA fragment and the desirable phenotypic character and how many side effects will be induced on this route.

This route is short only in the simplest unicellular organisms. In more complex organisms and especially in those organisms that manifest individuality, the route includes numerous iterations and alternatives, which are incompatible with the idea of predictability of results

of transformation. In situation where the transformation aims for induction (of embryogenesis) only, the problem of eliminating the transforming agent after the execution of its signal function necessarily arises. However, recent publications have given us confidence that these problems can be solved in the biotechnology of both mammals and seed plants.

Transformation, with the aim to produce a specific product, has been more successful when the site of incorporation of the transforming agent is closer to the final map in observables. In this case, however, there are some additional uncertainties. First, the introduction of one or two genes is not always sufficient to produce the final product. Second, the over-accumulation of the product may be harmful to the host (the conflict of phenotypes). Third, the conditions of the host may be inconsistent with the demands for correct labeling and folding of target product (usually of protein nature) and, therefore, inactivate it.

In addition to these features, there is another uncertainty of a more common nature, which is peculiar to the individual development of biological systems in general. It lies in the fact that there is not a strict determinism in the ways in which and in what order the information of a DNA fragment to its phenotypic embodiment will be realized. Nevertheless, there are apparently still some obscure channels that allow a biological object to successfully realize its individual development by the creation of the phenotype with properties similar to those expected.

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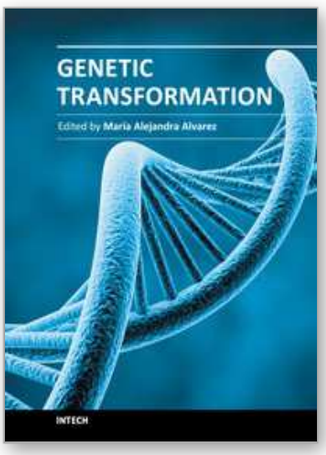

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Genetic transformation of plants has revolutionized both basic and applied plant research. Plant molecular biology and physiology benefit from this power fool, as well as biotechnology. This book is a review of some of the most significant achievements that plant transformation has brought to the fields of Agrobacterium biology, crop improvement and, flower, fruit and tree amelioration. Also, it examines their impact on molecular farming, phytoremediation and RNAi tools.

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