Uttar Pradesh Journal of Zoology

Volume 44, Issue 1, Page 22-36, 2023; Article no.UPJOZ.2418
ISSN: 0256-971X (P)

Establishing Transductional Relationships: Viral Vectors as an Experimental Tool in ALS (Amyotrophic Lateral Sclerosis)

Enrique Wulff [a*]

[a] *Instituto de Ciencias Marinas de Andalucía (CSIC), Spain.*

Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

DOI: 10.56557/UPJOZ/2023/v44i13388

Editor(s):
(1) Dr. P. Veera Muthumari, V. V. Vanniaperumal College for Women, India.
Reviewers:
(1) Lucky Jindal, Pt. BD Sharma University of Health Sciences, India.
(2) Nagwa Ali Sabri, Ain Shams University, Egypt.
(3) Mingxing Ding, China.

Review Article

Received: 07/11/2022
Accepted: 10/01/2023
Published: 11/01/2023

ABSTRACT

This contribution describes a number of historical aspects of discoveries made in the field of research called signal transduction by analyzing the meaning of the term and its relations to phenomena. Signal transduction refers to any process by which a cell converts one kind of signal or stimulus into another. For instance, that the epigenetic defect may result in additional genetic lesions has been associated with signal transduction pathways. These processes most often involve ordered sequences of biochemical reactions inside the cell, that are carried out by enzymes, and activated by second messengers. Thus suggesting the idea of reversibility at the leading edge of a transduction cascade as the most instrumental value in unveiling the ontology of its downstream elements. The purpose of this essay is to facilitate the historical and philosophical understanding of signaling pathways ultimately designed for the development of novel therapeutic

**Corresponding author: Email: enrique.wulff@icman.csic.es;*

strategies to treat neurological disorders. The repertoire of signal transduction pathways offered by nitric oxide (NO) and the strong ability of viral therapies to liaise effectively with the neurodegenerative sickness progression are researched. In particular, viral therapy research in ALS (amyotrophic lateral sclerosis) as a completely dominated by theory experimentation is taken as an example.

Keywords: ALS (amyotrophic lateral sclerosis); nitric oxide (NO); virotherapy for cancer; theory.

1. INTRODUCTION

From the mutational theory of cancer, and its emphasis on viral transformation, point mutations advanced the epistemic role of the expression of specific genes in the tumorigenic activity of the cell [1]. It may help us to understand it, to take into consideration the way a mechanistic explanation is allowed to deem its interplay vis-a-vis the environment [2]. This deregulation was thought to be the result of a viral infection, a vision consistent with the belief that cancer was mostly caused by a virus [3]. The history suggests that recombinant-work, with its origins in the study of transfection in bacteria, grew rapidly to the point in the early 1990s where virus engineering provided any scientific furtherance of virotherapy [4] (Fig. 1). As soon as the field of gene targeting became available in 1977 [5], a dynamic interaction between these strategies converge to the next big challenge of molecular biology. And 1989 saw the birth of several different knock-out mice.

Here we describe this change from genetic to genomic regulation through the use of the concept of transduction signalling. The essay includes an investigation of RNA interference mechanism as a part of rational gene silencing design. In the pursuit of research on a mechanism, researchers have formalized the organization graph-theoretically [6]. For example, the transmission of a signal from one neuron to another can be explained by a decomposition of the synapse into generalizations about the behavior of its component parts [7]. This descriptive knowledge intends to make explicit which individual parts or properties contribute to particular capacities, and exactly how they do so. Because it is encouraging to see that the genetic basis of different disorders that follow Mendelian inheritance has been elucidated and their cure can be a possibility, when gene therapy comes into play. Nevertheless, on the account of the biological support for the conception of the gene as a symbolic representation of the organism, a code script [8], it can be said that the code script contains only a description of the executive function, not the function itself. In this light, the treatment of the malignant properties in a neurodegenerative sickness through gene therapy is expected to afford with a good percentage of different genes associated with the disease which the function is still unknown [9].

To show that explaining a biological phenomenon like neurodegenerative disease implies a mechanistic level led to analyze which are the necessary and sufficient conditions that trigger the molecular cascade associated with the neuropathology. The type and spectrum of viral gene regulations and the broader interference mechanisms used, suggest distinct but not mutually exclusive pathways in the identification of the degenerative and regenerative processes in acquired neuropathies.

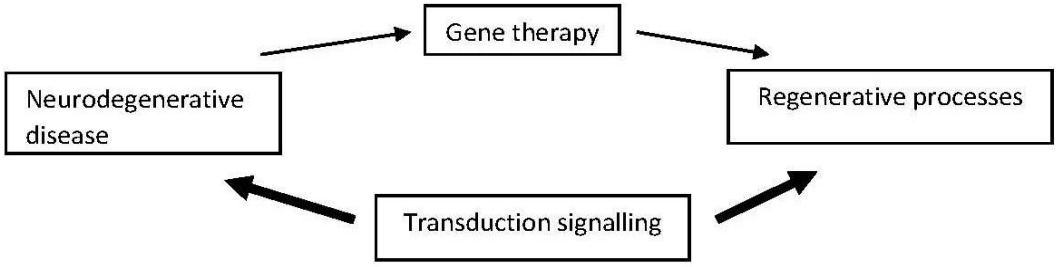

Fig. 1. At the mechanistic level neuropathies necessary and sufficient conditions trigger a cascade of transduction signals that favors viral gene therapies approaches

Our discussion first draws attention to implications for philosophical understanding of the role of transduction. To illuminate the conceptual transformations connected with this, the development of the improved models that arose out of mice and viral vectors is exposed. Through analyzing a case study, the acquired motor neuropathies, a number of historical aspects made in the field of NO research are discussed. We do provide detail to examine the concept of reversibility revealed by the rationale behind "loss of function" induction. These models of testing hypotheses investigative practices are finally connected with the history of RNA interference, a characterization that should prove helpful to philosophers of science who seek for more exploratory-driven modes of research.

2. TRANSDUCTION AND WHAT IT MEANS: DIFFERENT POINTS OF VIEW

In the early days of the techniques for determining amino acid sequences of proteins, 1974, the analysis of genes was limited to genetics. Indeed, the only way to assert that there was a gene in an organism was by finding a mutant allele for it. And when from 1985, the first discussions began about sequencing the human genome, as the genome had provided the inventory of gene loci, the work was to get on to the discovery of their products and the ways they integrate the physiology of the cell. Since the capacity to learn is a non-contingent capacity, the need to turn data into knowledge and the need for a framework to do it, resulted in the need to solve a instantiation problem. Multiple instantiations distinguished by different promoters make that many gene loci will specify the cell type. What means that to construct a framework for understanding functions the correct level of abstraction is the cell. The need to define non-contingent states of gene expression in an organism conducted to discuss a gene-centric view that had induced modern biology to forget that the real units of function and structure in an organism were cells and not genes.

Once molecular biologists make predetermined changes in a chosen gene, in a genotype, they look for inferences in the causal relation between genotype and phenotype [10]. But to reverse the progression in several neurodegenerative diseases, an increased information processing is in need. The focus is on reversibility. As dismantling the "irreversibility line" could be a possible breakdown of an intrinsic chronic effect, profoundly associated with cell membrane excitability parameters. In fact, the small, free radical molecule (NO) has been identified as a major signal transduction in vertebrates (animals). It is an essential element for normal cell function [11]. But, in light of the difficulties derived from considering radical superoxide implication in neurons ageing, it has been suggested that molecular biologists have employed a prototype concept of free radical, which they considered a cellular metabolic intermediate. And that when a nerve cell of any kind degenerates, at the time of its agony, an overproduction of free radicals is produced, also contributing to eliminate cellular debris. But this happens as much to a hepatocyte, as to a neuron. Thus the role of radical chemistry in certain cases of neurodegenerative illnesses is possible, but problematic. It is never an explosive phenomenon.

Indeed, the history of "protein machines" like the sodium-potassium pump, employ coupling reactions to power a motion that pushes ions across cell membranes against their concentration gradients. Thus, drawing an analogy with a machine that a competent engineer might construct from a static description, the research objective would be to identify downstream elements of the signalling pathway because it is this that will permit to decode which will be the plan. For it, in doing the experiments, it will be very important to identify where these signalling components reside within the cell. So, the processes by which is caused the synthesis of particular proteins that in turn take part in other complex interactions giving rise to neurological disorders suggest the idea of transduction cascade as the best to accommodate the scientific practice.

Consider the cascade, as a cellular network which can be decomposed into functional modules-each one functionally separable from the other. Signal transduction conceptual approaches in the nervous system provide a perspective on a robust ontology of processes that hangs our account on there being an objective way of individuating processes. The meaning of the thought lies in the reversibility of its formulations, and cannot exist apart from this exchange [12]. Consider one of the brain diseases, ALS (amyotrophic lateral sclerosis). While we have that the only proven cause of the disease occurs in just 2-3% of individuals, mutations to Cu-Zn superoxide dismutase

(SOD); the hypothesis has been formulated that Zn-deficient wild-type SOD is just as toxic as Zn-deficient ALS mutant SOD, suggesting that the loss of Zn from wild-type SOD could be involved in the other 98% of cases of ALS [13]. What this example seems to show is that an enormous number of outcomes could be reduced to a causal history problem, by taking it into account as a process that might be individuated by the evolutionary mechanisms driving its growth or by the distinct physiological mechanism that underwrites them. A historical overview of mammalian nitric oxide synthases, like in the case of signal transduction proteins, will permit to rapidly follow the reason of being of NO, whatever its content or quantity, as an innate agent of neuropathy in the face of genetic mutations [14].

3. THE CASE OF ACQUIRED MOTOR NEUROPATHIES

The enzymes responsible for the synthesis of NO in mammalian tissues are known as NO synthases (NOS) [15]. They were first described in 1989 [16], first purified in 1990 [17], and first cloned in 1991 [18]. As rapid progress in the field was possible because of the hypothesis that the formation of NO from L-arginine in the brain is a widespread transduction mechanism, molecular biologists and biochemists began to consider the implication of NO in neurodegeneration as an intriguing case. Because they researched in a situation quite similar to the one of cGMP (guanylyl cyclase, a second messenger that was discovered in urine in 1963), they tried to discover a function for a molecule [19]. The point was that inhibitors of NOS indicated that NO had the properties of a neurotransmitter, an unconventional one that was not stored in synaptic vesicles. And that its action in activation of guanylyl cyclase (cGMP is the enzyme that modulates phototransduction in rods and cones in the retina) depends on its free-radical character [19]. Thus, by the early 1990s, the mystery of soluble guanylyl cyclase and nitric oxide implied that NO in neurodegeneration provides an intriguing case whereby a molecule involved in numerous beneficial physiological processes in the CNS can also be transformed to promote tissue damage and disease progression [13].

Once this 'nitrergic' neurotransmission was demonstrated, the explosion of research into the investigation of NO pathway in every possible biological system (mainly the cardiovascular system, the nervous system and inflammation/immunology) acquired some of its most significant actions in the neuronal system. Relevant key roles in ALS pathology remain poorly understood, although an increase production of nitric oxide has been put in association with it [20]. As by now, taken together basic and clinical data support the notion of elevated levels of NO and its related species playing an important function in the pathology of ALS [21]. Because the understanding of NO signalling pathway provided evidence for mechanism of action of known drugs and identified novel targets for drug development. Indeed, the three main fields of research were based on the activity of the three isoforms of the NO synthase, originally called endothelial (eNOS) and neuronal (nNOS) after the tissues in which they were first identified, and inducible (iNOS) for the isoform which is expressed in macrophages following immunological stimulation [22].

Nevertheless, by 1993 it was known that lack of nNOS expression in the mutant mice (knockout) did not resulted in phenotypic changes [23]. The mutant NOS mice were viable, fertile, and with no abnormalities in the central nervous system. Despite this gene deletion no gross behavioral abnormalities were detected in the knockout mice as compared with the wild type mice. So a very specific question addressed within a tightly bound sphere of inquiry, if nitric oxide (NO) could act as a neurotransmitter in NOS-containing neurons, was not appropriately answered. May be experimenters did not observe a predicted phenotypic change because of the inherent risk involved in genetic engineering of knock-out animals, compensation. The compensatory mechanisms for the disrupted enzyme that results of atypical interactions with other genes or gene products, masks the effect of NO in neurological disorders. A local criterion of causal relevance that had driven the search for a mechanism was put in question, thus raising the relation between experiments and high theory [24]. When entering the field of the specific functions of NO in the nervous system, a theoretical test that seemed unclear made attractive the exploration of phenomena and processes far from the knockout animal models. The relative vulnerability of these mice to explain the mechanism of nNOS action opened the boundaries of this case study to a perspective for viral therapies. Accordingly, because of the relevance of some putative contextual factors, an alternative method went beyond the heuristic to which the scientific research was relegated [25].

In spite of concerns, like those Crusio reported on a November 1998 meeting on knock-outs and mutants, aimed at urging increased test standardization to avoid errors, and arguing that current strategies could identify general mechanisms related to "monomorphic genes identical in all individuals" [26], but not addressing individual variations in task-performance well. What transgenic mice did not tell Huang, two years later was established by Nelson [27]. They identified behavioral abnormalities in male mice lacking nNOS. These genetic knockout mice offered a sexuo-pathological behavior (violent and hypersexual), suggesting that fluctuation in their levels of NO, as neurotransmitters found in high densities in emotion-regulating brain regions, regulated their levels of self-esteem and place within the social hierarchy. Also they made a number of reconsiderations of fundamental problems in previous researches that overcame the use of mice with gene deletions. The behavioral interpretation has been obfuscated because NOS inhibitors that must be administered acutely, influence nNOS but can affect also other biological systems that use arginine.

Understanding how biomedical research led these defectors from structural biology to epigenetics, in large measure has been identified with impasses in knockout mice experimental approaches. For example, a role for peripheral and indirect influences such as dilated stomachs was the only phenotypic change detected by Huang et al. in the already indicated nNOS knockout animals. In furtherance of this research, to refine the inherent mutant mice validity, an already formulated hypothesis on the involvement of NO in brain damage connected with vascular stroke was tested. As has been reported [28], this was a misapplication of the materiality of specific standardized mice with the aim of enhancing the power of inference from previous experimental results. But the experiments could not rationalize such a practice, as long as neuronal networks were depicted like complex assemblies of interconnected nerve cells where certain synapses constitute central nodes in the network while others occupy more peripheral positions [29]. Another example where NO was implicated involves learning and memory in nNOS knockout mice. When pharmacological studies tried to test the idea that persistent stimulation of the increase in synaptic strength (connected with learning and memory) in the hippocampus of these mice resulted from the fact that long term potentiation (LTP) acted as a messenger in the process of neurotransmission. Far from meeting this expected phenotypic change successfully, all that this failed experiment was able to advance was that eNOS was not only present in endothelial cells but also in the neurons of the brain [30].

These researches on nitric oxide synthase were attempts to provide a connectionist solution to the instantiation problem. Where brain matter is considered simultaneously the actor and the result (through learning) of its own activity, as opposed to what is crucial in cognitivism (context independent) genetically driven micro-circuitry. The historical background in the instantiation argument fits the promises made in Benzer's, Brenner's and Streisinger's programmatic visions of explanations of physiological phenomena in terms of molecular regulation gained by mutational analysis [31]. It relies on the capacity to restrict gene expression to particular cells by working with molecular tools. And comes from the concept that spontaneous errors in DNA replication may be fundamental in transformation, as was put forward in the 1970s in an attempt to explain the genomic instability of cancer cells [32,33], that connects mutants and descriptive tools like the events that come downstream between genes and partial phenotypes. The metaphors in their scripts were those of dissection and surgery on the methodological side, and those of pathway, circuit and mechanism behind the analogical reasoning [34]. In other words, the use of mutations for the partial degradation of behavior in organisms to ascertain the molecular mechanisms that are behind them is not the only benefit, because it also helps to dissect processes on a higher physiological level, by providing support to the goals of classical anatomy and physiology.

Most of the information available in the case of the subtle forces that induce formation of the final architecture of the adult mammal central nervous system through the nitric oxide pathways of neurodegeneration, in amyotrophic lateral sclerosis (ALS), concerns the knockout mice. The stress is on experimental approaches to morphology and behavior in physical or functional disconnection of motor neurons from muscle. It starts from the mutational theory of cancer, and progresses to the accomplishments of the neuro-physiological advances on alteration of contractile characteristics of muscle by nerve cross-union. The representational features of

anatomy are used to model the protein machines related with this deprivation of neural support to the muscle. Both questions the genetic specification of nervous system and the way nervous systems work to produce behavior [35], are in line with the principle that despite the focus is on genes, cells are at the centre of explanations. A needed reconciliation between both advances make viruses with tiny genomes which grow on bacteria, the ideal 'model organisms' for this phase of research in molecular biology.

4. TRANSCRIPTIONAL MACHINERY TO REGENERATE TRAUMATIC INSULT IN MOTOR NEURONS

While historical patterns have made the characteristics of knock-out mice more convenient for a large range of problems than other organisms, the notion of instantiation suggests for the case of amyotrophic lateral sclerosis (ALS) the adoption of a viral strategy within a molecular level. Regulatory mechanisms of the transcriptional machinery explain the promoter activity of their target genes associated to regeneration in this neuronal injury. And different motor neuron behaviors, with the superoxidase dismutase (SOD) enzyme altered, explain physiological phenotypes. As Gzil has argued, the dichotomy between medical and biological models concerns the testing out of current hypothesis, but also pharmacological screening [36]. In the first case, mice are equated with sick men, and in providing a viral vector the mice support current knowledge on ALS. The medical researchers are working with a substitute reality for biochemical tinkering [37], that includes an anatomical dimension derived from the eventual implantation of human neurons into the mouse brain. Their aim is to suppress the neurodegeneration and that is why they study cellular processes. But in the biological sense, designers do not seek to produce an equivalent of ALS patients enabling the immersion in clinical results. They manage knock-out mice as a model of representation which allows to extrapolate to the human the knowledge gained from the animal. In general, we have here the two sides of meaning and denotation. An experimental work has an epistemic value, searching to obtain new knowledge, but first biologists develop new ways of measuring performances and deficits.

Like the approach to studying the interests in structures or functions that might correspond to the use of genetics for the first and chemistry for the latter, working with viral vectors could be considered a case of "bottom-up" causation. Because when a virus (molecular level) kill a person (level of macroscopic organism), such mode of reasoning has often been highly informative. Pathogenic involved in these biohazards with unwanted effects on the physiology and viability of transduced cells, due to viral vectors, comes from their capacity to activate the immune system, thus constraining their applicability for the treatment of autoimmune disorders. Usually these alleged risks are minimized by modifications that involve the elimination of part of the viral machinery necessary for their replication, so that the virus lacks the necessary proteins for the production of new viruses. Thus, an infection process has effectively been converted into a transduction one (i.e. non-replicative entrance of a virus into the cell), followed by expression of the desired genetic information from the recombinant vector into the host cell [38]. But the construction of these vectors is time consuming, associated with safety concerns [39]. And microinjection is often toxic to neurons commonly resulting in less than 10% cells surviving the transfection procedure. Thus, viral vectors currently applied in clinical research do not meet the demands for a safe gene transfer. Although gene therapy is still in its infancy, and it has been used in vivo with some success [40].

For the significance of this reported problem in ALS, the experimental research with viral vectors is organized adopting a mechanistic account of functional explanation. Following the classical physiological way entities and activities can be tested into lower levels. But the lower-level mechanisms may be responsible for some of the activities that feature in higher-level mechanisms. Like in the cases of development of leukaemia-like disorders in children triggered by the viral vector or, of death because of massive immune response. But the probative force of this hierarchical structure is not exclusive, and finds its limitation realizing that mechanisms may be horizontally linked; when the capacities turn out to be distributed, when any single type of component is crucial. Biochemical pathways are a model of this [41]. In our case, in ALS, in the regenerative processes that take place after nerve injury, Schwann cells mediated by a protein involved in cell shape plasticity and motility, GAP-43, change to a state where they are the cellular substrate to guide growing axons. Such is also the case with cell adhesion molecules, cytokines, neurotrophins, and growth

factors that promote axonal regeneration. As an experimental mechanism they share the salient properties of the physiology, although they need not coincide. The results go for a better mechanistic understanding, but they seem to lose physiological relevance (Fig. 2).

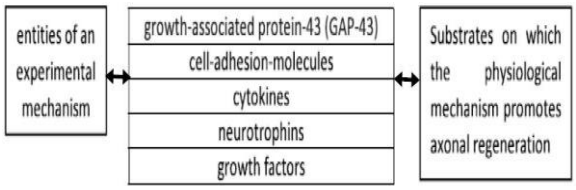

Fig. 2. Experimental mechanisms could lose physiological relevance. When ALS models' find mechanisms horizontally linked, the horizontal link is that they are entities of an experimental mechanism and substrates on which the physiological mechanism can act to promote axonal regeneration

At the highest level, a living brain has the ability to transduce the genes effectively delivered with viral vectors into the brain cells. The genes enter the neurons; they are even integrated into the host genome where the information enclosed in them is expressed to focus on long-term patterns. The conversion of these genes into patterns of neural activity in the brain of a mouse ALS model prolongs survival in these motor neuropathies. And another level down, dysregulated proteins, like eNOS or nNOS, emerge. Yet, even in the highly attractive approach that local eNOS inhibition with virus speeds up neuromuscular functional recovery, the actual role for nNOS is less clear, being its dysregulated behavior critically dependent on the behavior of others multiple altered proteins. It remains a challenging task. If transduction of mature neurons in a living brain is either resistant to these procedures or become damaged, the transfer of genes into brain in vivo, with viral delivery under the present scheme, is limited to address the problem on reliability of data in proteins to study the changes in functional properties of injured cells in pathological events. Using for that the patterns of counterfactual dependence involved in the definition of mechanism [42], the patterns of dependence involved in the contrast between the presence/absence of the genes products must be analyzed, as a comparison useful to shape the phenotypic aspects investigated. But the use of neuron-specific viral vectors to knockdown nNOS suggests the epistemic limits encouraged by the practice of only using increasingly simplified models. Bypassing these undesirable effects (this absence of new knowledge), it became ontologically relevant to consider that the engineering of virus vector has provided RNA-interference as a sophisticated measurement device with new instrumental value.

Recent developments have provided us with different kinds of vectors to deliver, remove or modify genes within individual cells and tissues, bypassing undesirable effects of broad knock-down or transgenic expression. One of the first successful infections with tumor inducing viruses was performed by Duran-Reynals in 1942 [3]. Additionally, those viruses, which kill the cells after replicating into them to propagate the infection, cannot be used as vectors. As the spontaneous evolution of a small lower dimensional level would lead to a confusion state once the dimensionality of the model is increased. In gene therapy, to stably introduce a gene into a cell, only a limited number of viruses can be used. They are modified from the retrovirus, adenovirus (AVV), and adeno-associated virus groups. The suggestion was made that ontological changes of entities at a low level differentially should affect other entities which occupy a higher level. But the interaction between the target gene and the viral vector anchor us at the molecular level we are observing. And thus such instruments are inter-level transducers, though they are strongly connected with other changes at other levels. Their production gives specific knowledge about how different means of access may break down, and under what conditions. What also helps us to distinguish modularity, which is a representation governed affair, from transduction, which is not. Their ontic (their different states and observable differences) puts into play the relation between mechanisms and representations.

5. VIRAL THERAPY RESEARCH IN ALS AS A COMPLETELY DOMINATED BY THEORY EXPERIMENTATION

In ALS, to identify Rho-kinase (ROCK, a NO-associated cellular motility regulator) pathway implication for axonal regeneration after CNS lesion, is to decipher a crucial module of the disease. One may argue that the relevant sense of function with which neuroscientists pursue the goal of scrutinizing ALS is based on ROCK's ability to induce cellular contractility reduction associated with the actomyosin protein (which regulates changes occurring along apoptosis).

But pathological synapse loss can also be induced by transduction of motor neurons with transgenic nNOS. As a mechanism of transfer, a signal transduction (the viral vectors transfer of genes) cascade can be seen like an information-processing module involving a succession of interactions, where the separation is accomplished by the specificity of the interactions, rather than that all components co-localize in time and space [43]. This idea is useful to make the equivalence between motor neurons transduced with transgenic nNOS, and a model of acquired motor neuropathy induced by the traumatic insult in the motor neurons, that follow the effect of crushing in motor neuron sensitivity. That means that a theory describing mechanisms, to identify modules by using visual as well as verbal representations, enables to fit phenomena into the discernible patterns of the delivery of genetic material by a virus.

To identify modules, criteria of functionality are employed. Retroviruses insert the genetic material in the form of a RNA molecule, which will produce a DNA copy by the action of the enzyme reverse transcriptase. Thus reverse transcriptase is a module in the mechanism by which retroviruses copy their RNA back into DNA. As retrovirus insertions activate adjacent genes, this module has been used for directed mutation in gene therapy, by using a work-notion where some consider a need to characterize the causal structure independently of the relative abundance of its counterexamples [44]. The engineering of virus vectors has allowed the development of gene vaccines for a wide range of infectious diseases and cancer. Actually, gene therapy is mainly being focused on the study of genetic disorders such as cancer, diabetes mellitus, cardiovascular diseases and nervous system pathologies. And regarding the applications in the nervous system (specifically at the level of peripheral nerve injury and central cardiovascular control), the potential of viral vectors is to be highlighted as tools for the investigation of the role of altered proteins in neuropathological processes. In spite of that, their capacity to activate the immune system restricts their applicability for the treatment of autoimmune disorders.

However, most retroviruses (like HIV, a Lentivirus (LVV)) can only infect dividing cells. Despite this limitation, lentiviral vectors can be extremely useful for gene-therapy experiments, because of their role of cell-mediated (epigenetic) gene silencing at the post-transcriptional level (for example, in the form of RNA interference (RNAi)). A number of aspects of the technology required for this combination, which selectively reduces the messenger RNA leading to its translational repression, are common with the use of adenovirus vectors (not derived from retroviruses but often referred to as dependovirus). Adenoviruses (AVV) insert their genome in the form of a double-stranded DNA molecule. But it does not replicate, and it remains as an extra-chromosomal molecule. Like the retroviruses, adeno-associated viruses, so called because they need the help of adenoviruses to replicate, integrate the host genome. As many differentiated cells in the brain and other tissues lack the capability to divide, LVVs, AVVs, and adeno-associated viruses are acquiring increasing popularity in gene therapy applications because they can transduce non-dividing cells. Their tentative role is good to address the study of cell lineage formation by non-mutational mechanisms. These observations suggesting mechanisms other than mutations responsible for non-dividing cells, can contribute to map NO’s split personality in the brain, to find the reversible mechanisms in ALS.

So, unmasking the role of imbalanced proteins in anatomo-functional alterations of neurons in neurodegenerative disorders has deserved attention, by trying to view them through those "bits of understanding" that makes the primary function of models, to assemble pieces of the world as a thing more or less useful [45]. Not theoretical claims, but "adaptable tools", for building on more or less accurate predictions to know what is preprogrammed and which are the factors affecting the cell from outside. Because it continues to be fairly misunderstood a number of pitfalls related with the acquisition of the mutation (and not its inheritance), where the disease is originated and the current absence of its cause. What is missing in the way the scientific practice has been framed is what other dimensions within exploratory experimentation were taken into account. For instance, reverse transcriptase has not been found to play a role in producing directed mutation. By taking Steinle’s suggestion to consider discover-oriented work which is typically practiced in periods in which no conceptual framework is readily available, the search for NO inhibitors to reduce damage in mice and cultured nerve cells is from first to last an experiment completely dominated by that theory [24]. Unfortunately, germ cells can be altered by the insertion of functional genes that are inherited by later generations, thus ascribing

to the genetic lesion any phenotypic difference between mutation carriers and non-carriers. But the eventual loss of the somatic life introduces an ontological difference, at the price of a considerable loss of flexibility and openness to unexpected experimental outcomes (as far as they can be relevant to human neurodegenerative disorders). And numerous are the ethical, religious and technical reasons that restricts this for application in human beings. By way of consequence, although using viral vectors is an effective and efficient experimental methodology to manipulate the various functions of nitric oxide (NO) in the nervous system, when viral vectors are used for the gene delivery, they are introduced into somatic cells. It is somatic gene therapy, so that the effects will be restricted to the individual and will not be inherited by the offspring.

6. MICE AND VIRAL VECTORS

As research tool, the most traditional form of gene therapy involves mice transgenic biology to insert a functional gene at a specific location in the host genome in order to replace a mutated or missing gene. This is accomplished by isolating and amplifying the gene of interest, generating a construct containing the necessary elements for the right expression, and inserting this construct into the host organism. In this respect, knockout gene therapy works like viral vector systems. The analysis is concentrated on the way the mechanism is constructed, and its progress is conceived as a process of adjustment and revision of modules. They promote mice as models for identifying and studying human gene functions, as medical instruments. Moreover, the modules by which gene therapy in its mechanisms, first knockout-oriented and then in viral genes, recapitulate or resolve knowledge available consider transgenic mice as biological equivalents, in particular of neuropathology. So the requirements of the new and the old gene therapies have their common ground in the need to understand the direction of continuities and discontinuities in biomedical sciences. Although often the preclinical research in a knock-out animal, in which the resulting phenotype might give the keys regarding gene function, is the most popular approach to infer a causal relation between genotype and phenotype [10]. In it a specific gene is turned off through a targeted mutation, and the resulting phenotype of the knock-out mouse may provide valuable information regarding the function of the missing gene. Nevertheless, this way of producing predetermined changes in a chosen gene is costly and time-consuming. A link between space and time which makes the mechanisms of nitric oxide mediated neurotoxicity in the brain follow-up difficult. As in some cases the targeted genes are necessary for the embryonic development in which case the knock-out of that gene is not feasible. Alternatively, individual development may be directed towards compensating for that knock-down gene (another gene or genes has compensated for the loss-of-function), and the phenotype would not provide much relevant information. So in spite of scientists observe a predicted phenotypic change, always could happen that the targeted gene is not responsible. A limit to the inactivation of genes for checking predicted phenotype designs, that consequently puts on value viral other tools for the job.

These notions unsaid, this inability to understand, resulted in another research instrument, in the study of gene function by creating a transgenic organism, in which foreign, recombinant DNA (i.e., a transgene) is transferred directly into embryos to result in modified or novel genes. Transgenic organisms are broadly used for agriculture, production of pharmaceutical drugs and proteins such as insulin, biomedical research, and gene therapy in experimental medicine. However, transfer of genes into an intact brain in vivo remains a challenging task. Transduction of mature neurons in a living brain is either resistant to these procedures or become damaged (pathways inside the cell become broken down [46]). By contrast, viral vectors can effectively deliver genes into brain cells and even integrate them into the host genome for long-term expression making this a highly attractive approach. Especially when it comes to understand highly complicated central nervous system pathways, rendering them more affordable.

Vectors used in gene therapy for release of specific genes are classified in a dichotomous way; they can be viral or non-viral. Non-viral vectors include naked DNA, oligonucleotides and nano-engineered, organically modified silicates. The main advantage of non-viral methods is that the time-consuming process of constructing recombinant viruses is avoided. But, artificial DNA transfer methods have low efficiency (because they are rapidly cleared from the circulation by the tissues with a role in the removal of foreign matter) and are not useful in some type of cells. This gives a suggestion on

the topic of finding strategies for scope determination. Accordingly, that generated inheritable phenotypic diversity is not efficiently altered by non-viral vectors in its DNA sequence is an empirical issue, whereas natural evolution has led viruses to develop special molecular mechanism to transport their genomes into the cells that they infect. Thus, the scope of viral vectors as a mechanism by which theory testing can be settled is reinforced. The theory domination of the nerve regeneration experiment in ALS is reflected in a high specificity that is a standard term for the ideas used in viral vectors. As the virus can infect non dividing cells like neurons in which their genome is expressed for a long time. Although viral transduction is far more efficient than nucleic acid based approaches, functional tests are required in areas away from the injection site to minimize the contaminating effects of nNOS in transduced nerve cells. Because the functional component may intervene in space containing parts performing other operations or even entities that are not part of the mechanism responsible for the relative specificity of nNOS inhibitor.

7. DESIGN PHILOSOPHY

In this section we describe viral vectors from its design philosophy as a feasible tool for synaptic recovery after nerve injury, without systemic side effects. By design we understand the architecture of instantiations and molecules in the cell with which we can develop a predictive system. Ideally, a system for reproducing a plausible treatment discusses the extent to which it is possible to propose objective criteria to define the processes of functional recovery after nerve injury. As such it should be useful to justify the way genuine independent experiments tell how things ought to be to regain function. A viral vector, whose design permits NOS antagonism without side effects, inhibits wild-type NOS which interfere with its function. And like a recommendation intended to have instrumental value, the work to do can be viewed as conditional to ascertain the desired goal [41], i.e. that the system should take under account that there are several approaches to induce "loss of function" using viral transgenesis.

As far as the predominant source of NO in the brain, the targets of interest are nNOS and eNOS. Chronic systemic administration of NOS inhibitors is not advised because they cause motor deficits in mice and rats. Values in this dose-response model makes prediction a too heterogeneous category from a methodological perspective. Nonetheless, promising results have been obtained from the intraneural administration of a viral gene therapy. This renders viral transfection more amenable to use by biologists and enables its employ as an efficient and effective experimental approach to manipulate NOS expression both in vitro and in vivo. In this signalling pathway, where connections in the cascade are established independently of any physical interactions between enzymes [47], the viral approach takes practical steps to produce comparable data, as a technique in a series of manipulations aimed at setting raw data.

An important consideration in traumatic neuropathy recovery to choice viral control is its capacity to restrict gene expression to particular cells by working with two molecular tools. First and foremost, as we have tried to show, the central problem of regeneration in these models has been the proposal of a neuroprotective tool in a key attempt to discover the causes of neurodegenerative diseases (Fig. 3). This is a case of negative control. The inducer, the NO inhibitor, interferes with an instance of “causation by disconnection” [48], as the intrinsic property that promotes motor function recovery after nerve injury is the dependence between the production of NO and the molecular mechanism involved in the motor pathology. The hypothesized “causal mechanism” [44] could be the right one to justify the tie. First, and unlike positive controls, because it ensures the expression of a marked dominant negative effect on the NOS enzyme activity, itself a key determinant of NO production; the experiments proving that a truncated expression of endothelial NOS (eNOS) inhibited wild type eNOS [49]. The viral format of this construction was first used in the hippocampus. Truncated mutants of eNOS were injected in the nervous system as a conceivable therapeutic tool. Moreover, these earlier experiments with a recombinant adenoviral vector revealed the isoform of NO involved in learning and memory [50]. Because memory deficits in ALS are relevant, it is important to know that pharmacological studies supported the idea that in the hippocampus, long term potentiation (LTP), a persistent increase in synaptic strength, results from eNOS activity. This is good thing because eNOS supports motor neuron survival and it was unexpected because neuronal NO synthase (nNOS) was discarded. Furthermore, in an attempt to look for some empirical feature that underlies the mechanism that may induce synaptic detachment from motor

neurons, another fundamental activity of NO, that it acts as a messenger for stimulating further neurotransmitter release [10], reveals the two faces of NO in motor neuron biology as evidenced through support for survival as well as induction of apoptosis.

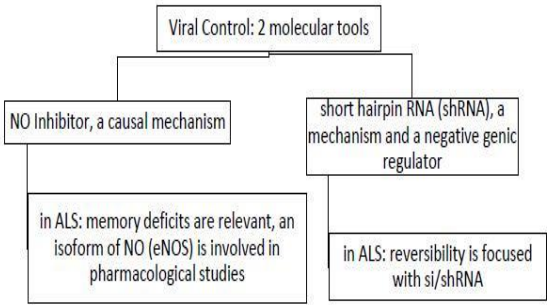

Fig. 3. The involvement of viral therapies in ALS, involves its capacity to restrict gene expressions to certain cells with two molecular tools: NO (nitric oxide) inhibitor and short hairpin RNA (shRNA). Both searched to provide a neuroprotective agent facing the central problem of regeneration

Secondly, another molecular instrument that can be studied as a viral agent is short hairpin RNA (shRNA, a small interfering RNA (siRNA)), which is as much a tool and a mechanism as a negative genic regulator. RNA molecules were originally regarded as simple messengers responsible for handing down the genetic information contained inside the cell nucleus to the ribosomes, to make the protein synthesis. Nowadays, the discovery of microRNAs, and that of an associated multiprotein complex (RISC (RNA induced silencing complex)), has led to the identification of translation repression in human cells by microRNAs new roles. A single of these small RNA molecules (miRNA) may contribute to regulate the simultaneous expression of several genes, thus controlling the expression of complete transduction cascades. The miRNA case provides a source of insight for philosophers of science who are seeking to develop more pluralistic and pragmatic models of scientific practice than were achieved in earlier hypothesis-focused accounts [51]. In the case of ALS, the mechanism of signal transduction details the effects of the administration of a neuron-specific LVV to knock-down nNOS, an aim to achieve therapeutic gain. Contaminated effects of nNOS transduction alter axonal properties so reducing those of the synapsis. This reduction is prevented by targeting the gene responsible with si/shRNA. This level of abstraction is associated with reversibility, and is useful in preventing the more probable pattern of peripheral neuropathies and neurodegenerative disorders.

8. A SILENT REVOLUTION

Silence lends credence to the assertion that through a loss of function can be recognized the function of a gene. This is the opportunity offered by RNA interference (RNAi), the capacity to silence many genes in a quick and simultaneous way, a promising strategy in molecular biology oriented to the identification of the unknown function of thousands of genes. In a cell or organism, the introduction of a double-stranded RNA (dsRNA) produced by a RNA virus or artificial is the onset of a complex cascade of events, and concludes with the suppression of that messenger RNA (mRNA) homologous to the originally introduced dsRNA [52]. In the field of neural regeneration, this emphasis on the complete control of transduction cascades revisits the viral approach and, as it possesses counterfactual force, the new instrument can be considered in converging to the “true” answer. That's why the ALS phenomena would be within an "intelligible" area, a mechanism where bottom out activities in molecular neurobiology fall into identified categories [42]. At this post-transcriptional level, a cytoplasmic ribonuclease (DICER) acts on the dsRNA. A different mode of research practice is introduced where dsRNA is fragmented in small interfering RNA (siRNA) portions. siRNA induces the formation of a RNA-Induced Silencing Complex (RISC), and RISC includes a helicase that cleaves the two strands of siRNA. The direct strand is degraded through a RISC component, Argonaute. Then RISC identifies and silences the messenger RNA complementary to the antisense strand. A new protein will not be involved in this mRNA degradation; the particular protein has been depleted. Thus a search for universal interrupters that serves as guides to inhibit protein synthesis, to control the complex space-temporal coordination that assures central nervous system, digestive tract, and lower and upper extremities development, resulted in the identification of the involved gene, associated to the discovery of the first microRNA. And scientists were surprised to discover that this gene did not codify for a protein. The product of this gene was a small size RNA which formed an imperfect loop-shaped structure similar to a hook to the hair (shRNA, small hairpin). Exploring the generalizability of this phenomenon was not so much a matter of testing a specific hypothesis but rather of investigating a general question:

how many other short RNAs might play an important regulatory role in other multicellular organisms? [51].

This potential relevance of somatic gene therapy for the understanding of neuropathy development argues in favor of the prevalence of viral vectors in such a medically related biological research as ALS investigation. Indeed, shRNA and siRNA are both able to guide nuclear events, resulting in transcriptional silencing. But they are not functionally interchangeable. The recognizing of a target mRNA by interfering RNAs (siRNAs) after introduction in the silencing machinery, leads to its degradation. But this type of silencing effect, this dark side, can only be transient and is therefore not adequate for the treatment of a long and progressive neurodegenerative disease. In contrast, sustained gene suppression in neurons can be successful with lentiviral vectors (LVV) encoding short hairpin RNAs (shRNAs), which will be processed into siRNAs in the infected cells. In addition, this kind of viral delivery favors easy internalization by cells, and avoids extracellular degradation, and immune reaction.

So it can be explained, by using the causal model where a necessary and also sufficient condition to trigger the molecular cascade leading to synapse withdrawal is up-regulated nNOS in injured axons, that after injecting a neuron-specific LVV, referred to as LVV-miR-shRNA/nNOS, the RNA-induced pathway is silenced and nNOS is knockdown. RNA shows the route that leads to protect neurons against the changes induced by the expression of nNOS. But much is to say on these efforts to see a story of mutants explained in terms of the epigenetic machinery evidence-conditions that have to be met by modules inside mechanisms [44]. Anyway, the discovery of miRNA and its system liaison with shRNA is not trivial to stop nNOS expression in neurons. And the viral therapy associated, bypassing undesirable effects that may be the result of atypical interactions with other genes or gene products, will be highlighted as a tool for the investigation of the role of altered proteins in neuropathological processes.

9. CONCLUSIONS

The intricate pathways of signal transduction, whose alteration ultimately confers its malignant properties to the neuromuscular function in neurodegenerative sicknesses, requires a dichotomous approach to delivery of genetic material, that should be interpreted at the light of their validity in animal models. And partial simultaneous instantiation of ground dichotomies is supported by viral manipulation and knock-out mice which are at the same time techniques that support 'models for' and 'models of' nervous system pathologies. As none of the current knock-out models are equated with the desired phenotype, due to compensation mechanisms and embryonic conflicts associated with the fact that the capacity to learn is a non-contingent property of neurons, results out of the experimental work with virus leads action. The standard view in amyotrophic lateral sclerosis (ALS) accords with a particular experimental design that attempts to provide a solution through the nitric oxide pathways of neurodegeneration. Such a connectionist solution uses the representational features of anatomy to model the protein machines related with the deprivation of neural support to the muscle.

Thus a model which allows extrapolating to the human the strategies gained from the animal searched as a daily practice in medicine is a specific type of equivalent that recapitulate and materialize current knowledge. And viral therapies are as much pharmacological screening instruments as they test out current hypothesis derived from interference RNA. What helps to distinguish modularity, a representational governed affair from transduction. Otherwise within the large context of clinical therapeutics, viral instruments derived from neuropathology have both an epistemic value (gaining new knowledge on neuropathies), and an instrumental value coming from biologists discussions on measures of cognitive deficits.

ACKNOWLEDGEMENT

Research included in this review was partially completed at the University of Cádiz, Spain, under the supervision of Dr. Bernardo Moreno-López (hdl.handle.net/ 10261/ 256003).

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Knudson AG. Mutation and cancer: Statistical study of retinoblastoma. Proceedings of the National Academy of

Sciences of the United States of America. 1971;68:820-823.
Available:https://doi.org/10.1073/pnas.68.4.820

2. Roe SM, Baumgaertner B. Extended mechanistic explanations: Expanding the current mechanistic conception to include more complex biological systems. Journal for General Philosophy of Science. 2017;48:517–534.
Available:https://doi.org/10.1007/s10838-016-9356-6
3. Duran-Reynals F. The reciprocal infection of ducks and chickens with tumor inducing viruses. Cancer Research. 1942;2:343-369.
4. Kelly E, Russell SJ. History of oncolytic viruses: Genesis to genetic engineering. Molecular Therapy. 2007;15: 651-659.
Available:https://doi.org/10.1038/sj.mt.6300108
5. Wigler M, Silverstein S, Lee LS, Pellicer A, Cheng Y, Axel R. Transfer of purified herpes virus thymidine kinase gene to cultured mouse cells. Cell. 1977;11:223-232.
Available:https://doi.org/ 10.1016/0092-8674(77)90333-6
6. Bechtel W. Generalizing mechanistic explanations using graph-theoretic representations. In: Explanation in Biology. History, Philosophy and Theory of the Life Sciences, Vol. 11. Springer, Dordrecht. 2015;199-225.
Available:https://doi.org/10.1007/978-94-017-9822-8_9
7. Barendregt M, van Hezewijk R. Adaptive and genomic explanations of human behaviour: might evolutionary psychology contribute to behavioural genomics? Biology and Philosophy. 2005;20:57-78.
Available:https://doi.org/10.1007/s10539-005-0388-2
8. Brenner S. Turing centenary: Life's code script. Nature. 2012;482:461.
Available:https://doi.org/10.1038/482461a
9. Bertolaso M, Olsson J, Picardi A, Rakela J. Gene therapy and enhancement for diabetes (and other diseases): The multiplicity of considerations. Diabetes/Metabolism Research and Reviews. 2010;26:520-524.
Available:
https://doi.org/10.1002/dmrr.1116
10. Culp S. Establishing genotype/phenotype relationships: Gene targeting as an experimental approach. Philosophy of Science. 1997;64:S268-S278.
Available: https://doi.org/10.1086/392606
11. Culotta E, Koshland DE Jr. NO news is good news. Science. 1992;258:1862-1865.
Available:
https://doi.org/10.1126/science.1361684
12. Dillon MC. Merleau-Ponty and the reversibility thesis. Man and World. 1983;16:365-388.
Available:https://doi.org/10.1007/BF01252651
13. Drechsel DA, Estévez AG, Barbeito L, Beckman JS. Nitric oxide-mediated oxidative damage and the progressive demise of motor neurons in ALS. Neurotoxicity Research. 2012;22:251-264.
Available:https://doi.org/10.1007/s12640-012-9322-y
14. Mallon R, Weinberg JM. Innateness as closed process invariance. Philosophy of Science. 2006; 73(3):323-44.
Available:https://doi.org/10.1086/515414.pdf
15. Portillo F, Moreno-López B. Nitric oxide controls excitatory/inhibitory balance in the hypoglossal nucleus during early postnatal development. Brain Structure and Function. 2020;225(9):2871-2884.
Available:https://doi.org/10.1007/s00429-020-02165-9
16. Knowles RG, Palacios M, Palmer RM, Moncada S. Formation of nitric oxide from L-arginine in the central nervous system: a transduction mechanism for stimulation of the soluble guanylate cyclase. Proceedings of the National Academy of Sciences of the United States of America. 1989;86:5159-5162.
Available:https://doi.org/10.1073/pnas.86.13.5159
17. Bredt DS, Snyder SH. Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. Proceedings of the National Academy of Sciences of the United States of America. 1990;87:682-685.
Available:https://doi.org/10.1073/pnas.87.2.682
18. Bredt DS, Hwang PM, Glatt CE, Lowenstein C, Reed RR, Snyder SH. Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. Nature. 1991;351:714-718.
Available:https://doi.org/10.1038/351714a0
19. Kots AY, Martin E, Sharina IG, Murad F. A short history of cGMP, guanylyl cyclases,

and cGMP-dependent protein kinase. Handbook of Experimental Pharmacology. 2009;191:1-14.
Available:https://doi.org/10.1007/978-3-540-68964-5_1

20. Trias E, Beilby PR, Kovacs M, Ibarburu S, Varela V, Barreto-Núñez R, Bradford SC, Beckman JS, Barbeito L. Emergence of microglia bearing senescence markers during paralysis progression in a rat model of inherited ALS. Frontiers in aging neuroscience.2019;11:42.
Available:https://doi.org/10.3389/fnagi.2019.00042
21. Steinert JR, Amal H. The contribution of an imbalanced redox signalling to neurological and neurodegenerative conditions. Free Radical Biology and Medicine. 2023; 194:71-83.
Available:https://doi.org/10.1016/j.freeradbiomed.2022.11.035
22. Moncada S. Adventures in vascular biology: A tale of two mediators. Philososophical transactions of the Royal Society of London. Series B, Biological Sciences.2006;361:735-759.
Available:https://doi.org/10.1098/rstb.2005.1775
23. Huang PL, Dawson TM, Bredt DS, Snyder SH, Fishman MC. Targeted disruption of the neuronal nitric oxide synthase gene. Cell.1993.75:1273-1286.
Available:https://doi.org/10.1016/0092-8674(93)90615-w
24. Steinle F. Entering new fields: Exploratory uses of experimentation. Philosophy of Science.1997;64:S65-S74.
Available:https://doi.org/10.1086/392587
25. Burian RM. The dilemma of case studies resolved: The virtues of using case studies in the history and philosophy of science. Perspectives on Science. 2001;9:383-403.
Available:https://doi.org/10.1162/106361401760375794
26. Crusio WE. Using spontaneous and induced mutations to dissect brain and behavior genetically. Trends in Neurosciences.1999;22:100-102.
Available:https://doi.org/10.1016/s0166-2236(98)01385-x
27. Nelson RJ, Demas GE, Huang PL, Fishman MC, Dawson VL, Dawson TM, Snyder SH. Behavioural abnormalities in male mice lacking neuronal nitric oxide synthase. Nature. 1995;378:383-386.
Available:https://doi.org/10.1038/378383a0
28. Huber L, Keuck LK. Mutant mice: Experimental organisms as materialised models in biomedicine. Studies in History and Philosophy of Biological and Biomedical Sciences. 2013;44:385-391.
Available:https://doi.org/10.1016/j.shpsc.2013.03.001
29. Papadopoulos D. The imaginary of plasticity: Neural embodiment, epigenetics and ecomorphs. Sociological Review. 2011;59:432-456.
Available:https://doi.org/10.1111/j.1467-954X.2011.02025.x
30. O'Dell TJ, Huang PL, Dawson TM, Dinerman JL, Snyder SH, Kandel ER, Fishman MC. Endothelial Nos and the blockade of LTP by Nos inhibitors in mice lacking neuronal Nos. Science. 1994;265: 542-546.
Available:https://doi.org/10.1126/science.7518615
31. Meunier R. Stages in the development of a model organism as a platform for mechanistic models in developmental biology: Zebrafish, 1970–2000. Studies in History and Philosophy of Biological and Biomedical Sciences. 2012;43:522-531.
Available:https://doi.org/10.1016/j.shpsc.2011.11.013
32. Loeb LA, Springgate CF, Battula N. Errors in DNA replication as a basis of malignant changes. Cancer Research. 1974;34:2311-2321.
33. Cairns J. Mutation selection and the natural history of cancer. Nature. 1975; 255:197-200.
Available:https://doi.org/10.1038/255197a0
34. de Chadarevian S. Mapping development or how molecular is molecular biology? History and Philosophy of the Life Sciences. 2000;22:381-396.
Available:https://www.jstor.org/stable/23332782
35. Bechtel W, Richardson RC. Discovering complexity: decomposition and localization as strategies in scientific research. The MIT Press, Cambridge, MA;2010.
Available:https://www.jstor.org/stable/j.ctt5hhhpx.14
36. Gzil F. Animal models of Alzheimer's disease and cognitive ageing'. In: First Conference of the European Philosophy of Science Association (Eds. San Pedro I, Solé A.) Madrid. 2007;14-17.
37. Bensaude-Vincent B, Loeve S, Nordmann A. Matters of interest: the objects of research in science and technoscience.

Journal for General Philosophy of Science. 2011;42:365-383.
Available:https://doi.org/ 10.1007/s10838-011-9172-y
38. Papale A, Cerovic M, Brambilla R. Viral vector approaches to modify gene expression in the brain. Journal of Neuroscience Methods. 2009;15:1-14.
Available:https://doi.org/10.1016/j.jneumeth.2009.08.013
39. Thomas CE, Ehrhardt A, Kay MA. Progress and problems with the use of viral vectors for gene therapy. Nature Reviews. Genetics. 2003;4:346-358.
Available:https://doi.org/ 10.1038/nrg1066
40. Wirth T, Parker N, Ylä-Herttuala S. History of gene therapy. Gene. 2013;525:162-169.
Available:https://doi.org/10.1016/j.gene.2013.03.137
41. Weber M. The crux of crucial experiments: Duhem's problems and inference to the best explanation. The British Journal for the Philosophy of Science. 2009;60:19-49.
Available:https://doi.org/10.1093/bjps/axn040
42. Woodward J. What is a mechanism? A counterfactual account. Philosophy of Science. 2002;69:S366–S377.
Available:https://doi.org/10.1086/341859
43. Pereira-Leal JB, Levy ED, Teichmann SA. The origins and evolution of functional modules: lessons from protein complexes. Philosophical Transactions of the Royal Society B: Biological Sciences. 2006;361 (1467):507-17.
Available:https://doi.org/10.1098/rstb.2005.1807
44. Psillos S. A glimpse of the secret connexion: Harmonizing mechanisms with counterfactuals. Perspectives on Science. 2004;12:288-319.
Available:https://doi.org/10.1162/1063614042795426
45. Keller EF. Models of and models for: theory and practice in contemporary biology. Philosophy of Science. 2000; 67:72-86.
Available:https://doi.org/10.1086/392810
46. Thagard P. Pathways to biomedical discovery. Philosophy of Science. 2003; 70:235-241.
Available:https://doi.org/10.1086/375465
47. Culp S, Kitcher P. Theory structure and theory change in contemporary molecular biology. The British Journal for the Philosophy of Science. 1989;40: 459-483.
Available:https://doi.org/10.1093/bjps/40.4.459
48. Woodward J. What is a mechanism? A counterfactual account. Philosophy of Science.2002;69:S366–S377.
Available:https://doi.org/10.1086/341859
49. Lee CM, Robinson LJ, Michel T. Oligomerization of endothelial nitric oxide synthase. Evidence for a dominant negative effect of truncation mutants. Journal of Biological Chemistry. 1995;270: 27403-27406.
Available:https://doi.org/10.1074/jbc.270.46.27403
50. Kantor DB, Lanzrein M, Stary DJ, Sandoval GM, Smith WB, Sullivan BM, Davidson N, Schuman EM. A role for endothelial NO synthase in LTP revealed by adenovirus-mediated inhibition and rescue. Science. 1996;274:1744-1748.
Available:https://doi.org/10.1126/science.274.5293.1744
51. O'Malley MA, Elliott KC, Burian RM. From genetic to genomic regulation: Iterativity in microRNA research. Studies in History and Philosophy of Biological and Biomedical Sciences. 2010; 41:407–417.
Available:https://doi.org/10.1016/j.shpsc.2010.10.011
52. Hannon GJ, Rossi JJ. Unlocking the potential of the human genome with RNA interference. Nature. 2004;431:371-378.
Available:https://doi.org/10.1038/nature02870