

What history tells us VI. The transfer of behaviours by macromolecules

MICHEL MORANGE

Centre Cavailles, Ecole normale supérieure, 29 rue d'Ulm, 75230 Paris Cedex 05, France

(Fax, 33-1-44 323941; Email, morange@biologie.ens.fr)

1. Introduction

From the end of the 1950s to the mid-1970s, a huge amount of work and various controversies focused on the isolation and characterization of the “molecules of memory”. Did such molecules exist, and if so, what was their nature? Was it possible to transfer a specific acquired behaviour from a trained organism to a naive one by means of pure macromolecules, RNAs or proteins (peptides)? Was there a molecular code of memory, and if so, what was the code? These experiments have been largely forgotten, although they were published in the best journals and in a plethora of books, and were the subject of active debates in many symposia and meetings. In retrospect, this period in the history of neurobiology appears as an aberration.

We now have at our disposal good descriptions of these controversies. What we have yet to do though is to position the entire episode in its scientific, historical and epistemological context, to consider what it can teach us about the value of concepts such as ‘information’ which still have a very important place in contemporary biology. Finally, it forms an interesting case-study of how science reacts to extraordinary scientific claims (Stern 1999).

2. A brief historical account

Three different kinds of observations supported the existence of a molecular code of memory. The first were initiated in Sweden by Holger Hyden at the end of the 1950s, and consisted of a very careful study of the macromolecular syntheses – of RNAs and proteins – which occur when rats learn behaviours (Hyden and Egyhazi 1962). Hyden was able to show that the newly synthesized RNAs had a specific nucleotide composition, and were not the simple result of

brain activation. The observation that specific changes took place in RNA synthesis during learning, was not totally unexpected. But the interpretation given by Hyden was more provocative: he hypothesized that different RNA molecules were synthesized during different learning tasks, and that their structure was the result of a process of encoding linked with the retention of the learned behaviours. The credit given to the elegant experiments of Hyden indirectly favoured the conceptual leap that he made.

Hyden's experiments were supported by the observations made by the Flexners on the effect on memory fixation of drugs altering RNA and protein synthesis (Flexner *et al* 1963, 1967). These studies were crucial in distinguishing different phases in the process of memory consolidation. Similar results were obtained by other laboratories (Agranoff *et al* 1965). The opposite positive effects of drugs enhancing RNA metabolism confirmed the previous observations and held out the promise of future therapeutic approaches. As in the case of the results of Hyden, the newer observations demonstrated only the importance of macromolecular syntheses in learning, not the occurrence of molecular encoding of memories and behaviours. The hypothesis, that the latter existed at all, was the simplest interpretation of the results of a third line of research. Most strikingly, that third line appeared to demonstrate that memories and behaviours could be transferred directly, in the form of partially or fully purified macromolecules, from a donor animal to a recipient. In these experiments three successive phases can be distinguished by the nature of the animals used and the molecules under scrutiny.

The first series of experiments was carried out by James McConnell on the flatworm *Planaria* (McConnell *et al* 1959). Flatworms have two unusual properties which were exploited in these experiments: the capacity to regenerate, and a propensity for cannibalism. During regeneration from

the tails of *Planaria*, learning of specific behaviours was lost if the animals were preincubated with RNase (Corning and John 1961). *Planaria* were able passively to acquire specific behaviours by ingestion of fragments of animals trained in these behaviours (McConnell 1962).

Many doubts were voiced concerning the specificity of the behaviours and even the capacity of these animals to learn (Travis 1981). The provocative attitude of McConnell in the mid-1960s raised serious doubts within the scientific community regarding the significance of the observations which had been made until then.

This first wave was immediately followed by a second in which it was demonstrated that different types of behaviours could also be transmitted in rats by RNAs extracted from the brains of trained animals (Babich *et al* 1965; Jacobson *et al* 1965). In the case of rats, there were no doubts about their capacity to acquire specific behaviours or about the possibility of distinguishing such behaviours from non-specific behaviours. Although the initial observations were confirmed by other laboratories, many other groups were unable to reproduce them. Serious doubts arose from the observation that RNAs injected into the peritoneal cavity – the procedure used in most of these experiments – did not reach the brain (Luttges *et al* 1966). The next year, twenty-three authors from eight laboratories jointly sent the journal *Science* a letter stating that they had been unable to reproduce the experiments of transfer of behaviours by RNAs (Byrne *et al* 1966). Although the signatories pinpointed the major difficulties of these experiments, they did not appeal for a moratorium on them, nor for the hypothesis of molecules of memories to be abandoned.

This second wave was immediately followed by the development of a third approach by Georges Ungar, an eminent pharmacologist at the Baylor College of Medicine. He confirmed the occurrence of a transfer, but explained the discrepancies between the different experiments by the demonstration that the active molecules were not RNAs, but peptides which contaminated the preparations of RNAs (Ungar and Ocegüera-Navarro 1965). He linked the results of the experiments of transfer of behaviours by molecules with what was known of the neurophysiological bases of memory processes – something that most of the authors of the previous studies had not attempted. He saw the role of the active peptides as connectors, signposts in the circuits of neurons involved in the storage of memories and behaviours. These signposts were related to the peptides already known to control many functions in the nervous system. But they were specific to certain neural circuits and to certain behaviours. They encoded information in a universal language, a property which explained the possibility of inter-species memory transfer. The most remarkable feature of Georges Ungar's work was his isolation and characterization of these peptides. After years of effort and

the use of four thousand brains (Ungar *et al* 1972a), he was able to sequence a peptide, christened 'scotophobin', that was said to be involved in the fear of darkness. He obtained a synthetic peptide which when injected into recipient animals mimicked the activity of the peptide extracted from trained animals. This peptide was sent to different laboratories in order for them to reproduce the experiments in different animal models.

Despite these achievements, the results were heavily criticized. Although some laboratories were able to reproduce the results of Ungar, others were not. The isolation and characterization of four new peptides involved in other behaviours, and even the accomplishment of the first step in 'cracking' the code according to which these molecules were synthesized and assembled (Tate *et al* 1976), did not convince the scientific community (Misslin *et al* 1978). Ungar's ambitions appeared excessive. The experiments describing the purification of scotophobin were published in *Nature* 18 months after their submission, and were followed by the negative comments of a referee (Stewart 1972), and the reply of Ungar (Ungar *et al* 1972b).

The hypothesis that memories and behaviours could be encoded in macromolecules, and could be passively transferred from one organism to another, was abandoned in the following years. The early death of Ungar in 1978 was certainly responsible in part for the cessation of this work. In the mid-1970s, peptides with essential roles in the central nervous system were discovered. Among them were endorphins and enkephalins, the natural analogs of morphine. These discoveries convinced most biologists that the observations of previous years were of the same nature as those recently described: the error had been to label as specific, variations in behaviours which were non-specific.

3. A very particular scientific context

The episodes related above have been extensively described by the participants and by historians (Travis 1981; Morange 1985; Setlow 1997). However, they have not yet been fully placed in their right context. The characterization of molecules of memories has been linked with the general process of molecularization of biology which occurred during these years, but it has not been noticed by most observers (except J P Gaudillière 1988) that a similar process of identifying complex intercellular interactions with informational macromolecules – RNAs – occurred in at least two other fields of biology. In embryology, the inductive effects were reinterpreted as being due to the transfer of RNA informational molecules from the inducing cells to the induced ones (Niu 1958). In immunology, the complex intercellular interactions which participate in the production of antibodies, in particular the interaction between macrophages and B lymphocytes, were interpreted

as the production of informational RNA molecules by macrophages and their transfer to lymph node cells (Fishman *et al* 1963). In both fields, the preliminary observations were received very favourably and were published in prestigious journals, some years before similar observations were made on the transfer of memories. In both cases, the observations were criticized and progressively disappeared from the research landscape, before being (it seems) completely forgotten.

A first interpretation is that what occurred was an extreme form of reductionism, favoured by the recent successes of molecular biology. The complex functions of organisms were directly reduced to the characteristics of macromolecules, as the complex characteristics of the genes had been reduced to the properties of the DNA molecule. However, with the rise of cell biology, this extreme form of reductionism was no longer tenable by the 1970s (Morange 1998). The complex functions of cells and organisms could no longer be directly reduced to the characteristics of one or a limited number of macromolecules, even if the explanation of these complex functions required the precise description of the structure of the macromolecules involved in their realization.

A second way to appreciate the transient importance of these transfer experiments is to situate them in a long tradition of experimentation. The protocol used to demonstrate the action of the memory molecules or of the inductive factors was the same as a traditional one used by pharmacologists, which had allowed, among other discoveries, the demonstration by Otto Loewy in the 1920s of the inhibitory action of acetylcholine. Subsequently, evidence for the genetic role of DNA was obtained by the transfer of the transforming principle between *Pneumococcus* bacterial cells (Avery *et al* 1944). The reference to Avery's work was frequent. It was not without impact at a time when Avery's contribution was rediscovered after twenty years of neglect in favour of the experiments of Alfred Hershey and Martha Chase on bacteriophages. At the end of the 1970s, similar transfer experiments in Robert Weinberg's hands played a major role in the first identification of oncogenes (Shih *et al* 1979; Morange 1993). Nevertheless, there was something specific to the transfer of memory molecules. In the field of neurobiology, there was a tradition of taking informational concepts seriously – and not in a metaphoric way as in most areas of molecular biology. The isolation and characterization of molecules bearing information therefore found its place in this field of research more naturally than in immunology or embryology.

4. What does this episode tell us about the role of information in biology?

Historians and philosophers of science are currently involved in active discussions of the significance of the

use of informational concepts in molecular biology. The first question is whether the use of informational concepts is purely metaphorical, as some historians believe (Keller 1995; Kay 2000), or whether it has more meaning. In the latter case, informational concepts can be used to express a certain form of causality. Or, they can have a semantic value and refer to the meaning the word 'information' has in linguistics and computer science (Sterelny and Griffiths 1999). Other debates are more oriented toward the potential consequences of the use of informational concepts. Does not this use contribute to a deterministic vision of gene action? Supporters of developmental systems theory argue that when used in the course of explaining the development and evolution of organisms, it might participate in an artificial disequilibrium between the genes and the environment (Oyama 2000). The abuse of informational terms is also seen by some philosophers as part of a process of disembodiment of organisms, in the abandonment of a materialistic vision of organisms and of their functions.

The arguments exchanged in these discussions are often difficult to grasp, at least by biologists, because they are not directly related to scientific practice. My personal conviction is that they could be simplified and clarified by greater attention to historical episodes as, precisely, the characterization of memory molecules. It is obvious that the identification and characterization of what were considered to be informational molecules supported a strong deterministic vision, whence the fears expressed by Georges Ungar regarding the consequences of his discoveries. But the evidence for informational molecules was not correlated with any tendency to disembodiment or discarnation. The molecules which were transferred were fully informational and fully chemical. There was absolutely no sign of disembodiment in these experiments. Nevertheless, the use of informational concepts was obviously more than metaphoric. The transferred molecules carried with them information which was causal: it induced a specific behaviour in the recipient animals. But it was more than that: it was also semantic, in the sense that, at least for Hyden and Ungar, these molecules encoded a certain information.

Retrospectively, the failure of these studies was a decisive moment in the history of molecular biology: it initiated the abandonment by most molecular biologists of a semantic view of information. Informational terms are still widely used in biology, but only in a metaphoric or weakly causal way. No longer is the semantic view considered seriously, in developmental biology, for instance, the only exception being the genetic code, the correspondence between a sequence of nucleotides and a sequence of amino acids. The years of lively controversy and 'dirty' experiments involving informational molecules was followed by the rapid abandonment, without any serious scientific justification, of the previous observations and hypotheses. This course

of events was too traumatic for the community of biologists to allow for the conservation of a semantic concept of information.

5. The way science functions and... dysfunctions

The trauma that I refer to was partly due to the widely shared feeling that, during these years, too many experiments, poorly designed and with insufficient controls, were published in the so-called best journals. I have already described the scientific context which facilitated a favourable reception of observations that were too preliminary. There was a kind of fashion which pushed many biologists to try to reproduce and extend the first results. The phenomenon of fashion is frequently criticized as contrary to the rational behaviour which ought to be adopted by scientists. Fashion generates “badly cooked” experiments. But fashion also has a positive face: this concentration of means and efforts allows rapid discrimination between reproducible and irreproducible phenomena. The quick succession of three waves in the studies of memory molecules was the result, in the end positive, of the fashion generated by the description of the first experimental results.

Despite the fact that waves of enthusiasm were quickly followed by others of disappointment, Georges Ungar was, surprisingly, able to ensure the persistence of discussions for nearly ten more years. This is the consequence of his personality, and of the strategy he adopted. He had a high reputation as a pharmacologist, having participated with the Nobel Prize winner Daniel Bovet in the discovery of anti-histaminic drugs. Unlike James McConnell, he was, at least during the first part of his career, modest and reserved. His scientific work looked sound and well controlled. In addition, the explanation he provided for the previous controversial experiments – namely, that the active molecules were peptides – could account for the failure to replicate the experiments in the conditions previously held. Also, his explanation linked the observations with preexisting models of memory and behaviour-storage in the central nervous system. Without restricting the ambitions of those who preceded him – to discover a “code for memory” – he provided an explanation more likely to be accepted by all neurobiologists.

Nevertheless, the last episodes of the story, with the joint publication of the article of Ungar’s group on scotophobin and the criticisms of the referee, reveal dysfunctions in the normal process of peer review of scientific contributions. I propose two partial explanations for these dysfunctions. The first is the difficulty – still present today – of publishing negative results. As we saw, there were some publications of negative results, but they did not acquire the same status as the initial, retrospectively incorrect, publications. They were, in most cases, not published in the same prestigious

journals nor accorded the same attention. The refutation of the experiments of memory transfer in rats by eight laboratories and twenty-three researchers was half-a page long (Byrne *et al* 1966). The second explanation of the dysfunction was too strong an attachment to the facts, to the empiricist tradition of science. What was at stake in these studies was the nature of the mechanisms involved in information storage in the central nervous system, and the strength of an ‘old’ analogy between the memory of recent facts and the memory of evolution that organisms carry and transmit to future generations by genetic mechanisms. Was it reasonable to imagine the occurrence of similar mechanisms for these two forms of memory? Was it reasonable to imagine that a complex behaviour could be encoded in the structure of a molecule, and triggered by the simple addition of this molecule to the brain of a recipient animal? It would have been important to raise these questions in addition to those bearing on the experimental systems and protocols. They were only raised and discussed peripherally. “Free space” for this sort of discussion was clearly lacking in those years. It is interesting that scientific journals have since created new headings under which this kind of free discussion can now take place.

References

- Agranoff B W, Davis R E and Brink J J 1965 Memory fixation in the goldfish; *Proc. Natl. Acad. Sci. USA* **54** 788–793
- Avery O T, MacLeod C M and McCarty M 1944 Studies on the chemical nature of the substance inducing transformation of pneumococcal types; *J. Exp. Med.* **79** 137–158
- Babich F R, Jacobson A L, Bubash S and Jacobson A 1965 Transfer of a response to naive rats by injection of ribonucleic acid extracted from trained rats; *Science* **144** 656–657
- Byrne W L, Samuel D, Bennett E L *et al* 1966 Memory transfer; *Science* **153** 658–659
- Corning W C and John E R 1961 Effects of ribonuclease on retention of conditioned response in regenerated planarians; *Science* **34** 1363–1365
- Fishman M, Hammerstrom R A and Bond V P 1963 *In vitro* transfer of macrophage RNA to lymph node cells; *Nature* **198** 549–551
- Flexner J B, Flexner L B and Stellar E 1963 Memory in mice is affected by intracerebral puromycin; *Science* **141** 57–59
- Flexner L B, Flexner J B and Roberts R B 1967 Memory in mice analyzed with antibiotics; *Science* **155** 1377–1383
- Gaudillière J P 1988 Un code moléculaire pour la différenciation cellulaire: la controverse sur les transferts d’ARN informationnel (1955–1973) et les étapes de diffusion du paradigme de la biologie moléculaire; *Fundamenta Scientiae* **9** 429–467
- Hyden H and Egyhazi 1962 Nuclear RNA changes of nerve cells during a learning experiment in rats; *Proc. Natl. Acad. Sci. USA* **48** 1366–1372
- Jacobson A L, Babish F R, Bubash S and Jacobson A 1965 Differential approach tendencies produced by injection of ribonucleic acid from trained rats; *Science* **150** 636–637

- Kay L E 2000 *Who wrote the book of life? A history of the genetic code* (Palo Alto: Stanford University Press)
- Keller E F 1995 *Refiguring life: metaphors of twentieth-century biology* (New York: Columbia University Press)
- Luttges J, Johnson T, Buck C *et al* 1966 An examination of « transfer of learning » by nucleic acid; *Science* **151** 834–837
- McConnell J V 1962 Memory transfer through cannibalism in planarians; *J. Neuropsychiat.* **3** 42–48
- McConnell J V, Jacobson A L and Kimble D P 1959 Effects of regeneration upon retention of a conditioned response in the planarian; *J. comp. Physiol. Psychol.* **52** 1–5
- Misslin R, Ropartz P, Ungerer A and Mandel P 1978 Non-reproducibility of the behavioural effects induced by scotophobin; *Behav. Proc.* **3** 45–56
- Morange M 1985 La recherche d'un code moléculaire de la mémoire; *Fundamenta Scientiae* **6** 65–80
- Morange M 1993 The discovery of cellular oncogenes; *Hist. Phil. Life Sci.* **15** 45–59
- Morange M 1998 *A history of molecular biology* (Cambridge: Harvard University Press)
- Niu M C 1958 Thymus ribonucleic acid and embryonic differentiation; *Proc. Natl. Acad. Sci. USA* **44** 1264–1274
- Oyama S 2000 *The ontogeny of information: Developmental systems and evolution* (Durham: Duke University Press)
- Setlow B 1997 Georges Ungar and memory transfer; *J. Hist. Neurosci.* **6** 181–192
- Shih C, Shilo B Z, Goldfarb M P *et al* 1979 Passages of phenotypes of chemically transformed cells via transfection of DNA and chromatin; *Proc. Natl. Acad. Sci. USA* **76** 5714–5718
- Stern L 1999 *The reception of extraordinary scientific claims: Georges Unger, scotophobin and the search for a molecular code of memory*, ISHPSSB meeting, Oaxaca, Mexico
- Sterelny K and Griffiths P 1999 *Sex and death: an introduction to the philosophy of biology* (Chicago: University of Chicago Press)
- Stewart W W 1972 Comments on the chemistry of scotophobin; *Nature (London)* **238** 202–209
- Tate D F, Galvan L and Ungar G 1976 Isolation and identification of two learning-induced peptides; *Pharmacol. Biochem. Behav.* **5** 441–448
- Travis G D L 1981 Replicating replication? Aspects of the social construction of learning in planarian worms; *Soc. Stud. Sci.* **11** 11–32
- Ungar G and Ocegüera-Navarro C 1965 Transfer of habituation by material extracted from brain; *Nature (London)* **207** 301–302
- Ungar G, Desiderio D M and Parr W 1972a Isolation, identification and synthesis of a specific-behaviour-inducing brain peptide; *Nature (London)* **238** 198–202
- Ungar G, Desiderio D M and Parr W 1972b; *Nature (London)* **238** 209–210

ePublication: 8 August 2006