Exploring the role of c-Fos protein in memory-related processes

Molecular Roots of Memory

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Our understanding of learning and memory has been greatly aided by molecular biology research on the complex processes which regulate gene expression

It was shown in the 1960s that inhibitors of de novo protein synthesis, when introduced into the brains of animals, prevent the formation of memories lasting longer than a few hours. This led to the conclusion that certain proteins that were produced during the process of learning were crucial for long-term memory. Soon thereafter it was demonstrated that a similar inhibitive effect on memory was also exerted by substances that block RNA synthesis (i.e. inhibiting gene expression).

Entirely independently, it was discovered in the late 1970s and early 1980s that oncogenes (the genes that give rise to cancer) have normal counterpart genes called proto-oncogenes which are involved in controlling the cell-division cycle in healthy cells. The present author's own post-doc research in the United States in the mid-1980s showed that the protein c-Myc, coded by what is called a nuclear proto-oncogene, is capable of initiating the cell division cycle. Because the nucleus, the part of the cell which contains genes, is also where the protein c-Myc is found, it was immediately hypothesized that this and similar proteins (such as c-Fos) have an impact on the regulation (e.g. stimulation) of genes, thus entailing that these proteins are very important for the function of the entire cell.

PHOTOTAKE/BEAW

Research on the appearance of new proteins in model learning situations in rats has proven extraordinarily significant for molecular neurobiology

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Using this specially designed cage, the protein c-Fos has been shown to appear in the central amygdaloid nucleus in mice brains as a consequence of their learning to recognize in which corner of the cage sweet water is available

It should be stressed that essentially all of our body's cells are equipped with the same set of genes - the vast diversity seen in the structure and function of various cells stemming mainly from differences in gene expression, which translate directly into the characteristic set of proteins for each cell. Gene expression is controlled firstly on the level of transcription (the initial stage, involving the "copying" of genetic information), by certain proteins called transcription factors.

Important proto-oncogenes

Upon returning from the US to Poland, the present author published a review paper on proto-oncogenes in the cell cycle, suggesting that proto-oncogenes might also play a great role in other biological processes thought to involve gene expression. Other researchers expressed similar views in the same year (1986). That encouraged our team and others to seek out which genes were activated in the brain under the influence of external stimuli to the nerve cells (neurons). Several publications appeared on the topic nearly immediately, including our own - in it we showed that when glutamic acid, an important neurotransmitter which stimulates neurons to fire, is introduced into the brain, it very quickly causes the activation of the gene coding the c-Fos protein.

Encouraged by that observation, we raised the question of whether behavioral training, here simply meaning a learning episode in rats, also has the same effects. Indeed that proved to be the case. Interestingly, the first experiments in this regard were carried out in Poland and East Germany, as Prof. H.J. Matthies from Magdeburg was then probably the world's best specialist on the appearance of new proteins in model learning situations in rats. The present author persuaded Prof. Matthies to collaborate in 1987. Similar experiments were also carried out in Moscow by Dr. K.V. Anokhin and his team, arriving at similar results (activation of the gene encoding c-Fos). And although our findings were not accepted by the leading journals - as I now presume, this was due to their novelty combined with "provincial" provenance - they were soon repeated and confirmed by others.

This laid the groundwork for the molecular neurobiological study of learning and memory, a domain of science that is now advancing very rapidly. Its development has been greatly assisted by molecular biology's vast methodological contribution to the brain research, with the ground-breaking applications of genetically modified mice, making it possible to trace the involvement of individual genes in the memory process. No less important were experiments carried out in parallel on such model animal species as the snail *Aplysia californica* and the fruit

fly *Drosophila melanogaster*. A fundamental consistency has been discovered among varied vertebrate and invertebrate species in terms of the most fundamental molecular mechanisms involved in learning. Other research, in turn, has shown that the protein we have studied so intensively, c-Fos, does indeed regulate the activity of various genes making up the transcription factor AP-1.

Genetic theory of memory

In the late 1980s, I proposed that the complexity of gene expression regulation processes (each gene being controlled by many transcription factors, and not only by them) might form the basis for the information integration process that comprises the essence of learning and memory. Memory, after all, is chiefly the ability to link various elements (sensory, emotional, motivational, attention) of the incoming message to the brain with one another, and then to store and recover that information. Attempting to verify this "genetic" theory of memory has therefore led to a search for which genes become activated during learning, followed by subsequent study of their function and regulation.

Over the past 20 years, our work has predominantly focused on the expression of the c-Fos gene and protein in the brains of laboratory animals. Based on the findings obtained both by the present author's team and by many others in the world, it may be concluded that the protein c-Fos appears in nerve cells only when a given cell undergoes transformations that may lead to what is called "plastic change." This means that the cell's interaction with other neurons is altered, which as a consequence alters the entire neural network. We can imagine, therefore, that the path followed by a signal in the brain becomes changed, and this change can be viewed as the memory trace. c-Fos is therefore an indicator of nerve cells that have been sent down the path of plastic change. Importantly, the protein c-Fos does not occur in active yet unaltered neurons, since its gene is then not expressed.

Following this line of reasoning in recent years, we have evidenced the specific involvement of certain regions of the cortex (the anterior cingulate) in very long-term memory lasting at least several weeks (research performed with A.J. Silva's team in Los Angeles), as well as the specific involvement of the central portion of the amygdala in learning pleasant situations (in conjunction with T. Werka's team at the Nencki Institute). These findings enabled us to advance last year a new theory of amygdala function.

Synaptic plasticity

Because the protein c-Fos is a gene regulator, the next big challenge was to identify which genes are controlled by c-Fos - currently the most important objective of our research. For example, we have shown that c-Fos activates the gene encoding TIMP-1 (tissue inhibitor of matrix metalloproteinases), a protein controlling the extracellular enzyme MMP-9 (matrix metalloptoteinase-9). In recent years we have demonstrated both the proteins TIMP-1 and MMP-9 to be important in long-term memory, which in a certain sense bears out the hypothesis the present author put forward more than 20 years ago. Moreover, we found the protein MMP-9 to be active at the synapses, i.e. the points of neuron-neuron contact, and to be secreted there when neurons are stimulated with glutamic acid. Those results fit in well with the currently prevalent theories of learning, which posit that modifications in effective information transfer between neurons via the synapses (synaptic plasticity) are crucial for learning and memory.

The research findings described above seemed to be of a purely basic nature, helping us to grasp the true essence of memory, a fascinating aspect of our own minds. Yet they have also turned out to have another medical aspect, of potential application. Our recent work indicates that when studying the expression of c-Fos and MMP-9/TIMP-1, we are touching upon the biological foundations of serious pathologies: epilepsy, mental illness, and ethyl alcohol and cocaine addiction. All of these disorders involve disturbances in neural plasticity, including synaptic plasticity, and the study of such phenomena is now slowly beginning to dominate our team's efforts. ■

Further reading:

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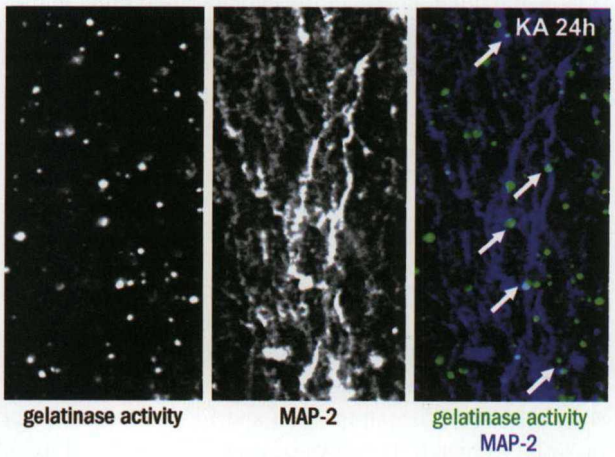

Enzymatic activity of the protein MMP-9 (green) on the synapses in a rat brain. Nerve cell projections (blue) marked with MAP-2