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Minireview

Towards understanding of the role of transcription factors in learning processes

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Recent advances in application of molecular biology to studies on learning and memory formation suggest that understanding of these seemingly elusive phenomena may be within our reach. This mini-review summarizes the present knowledge on activation and possible functions of transcription factors in learning processes with a focus on studies performed in the author's laboratory.

Gene expression, protein biosynthesis and learning-related phenomena

The neural mechanisms of learning and memory are under intensive multidisciplinary study involving an integrative approach based on molecular biology, cell biology, pharmacology, electrophysiology and behavior. The prevalent working hypothesis is that memory formation requires modulation of the connectivity of specific synapses in particular brain regions [1, 2]. Despite major scientific efforts made over the last three decades, the molecular bases underlying learning phenomena still remain largely elusive, although it has been proposed that the cascade of molecular processes critical for memory formation involves:

- i. activation of receptors for neurotransmitters, neurotrophins and cell adhesion molecules,
- ii. formation of intracellular and intercellular messengers,
- iii. gene expression,
- iv. synthesis of membrane glycoproteins which, when inserted into synapses, may influence their efficacy.

It is becoming increasingly clear that long term memory formation involves specific gene expression [3–9]. Central to explanation of this phenomenon is the role of transcription factors (TF), controlling gene expression.

The role of gene expression in learning processes or, more generally, neuronal plasticity, has been postulated repeatedly, and well documented since seminal studies published in early sixties. At that time it was shown that inhibitors of protein biosynthesis (puromycin, cycloheximide, anisomycin), and then also inhibitors of RNA synthesis (actinomycin D) blocked long term memory formation, if injected into the brain (see [3, 10]). These studies were not, however, without their critics, rightly pointing out that these drugs influenced the well being of the animals, clearly impairing

Abbreviations used: AP-1, activator protein 1, a transcription factor; CREB, cyclic AMP responsive element binding protein(s), a transcription factor; LTP, long term potentiation of synaptic efficacy; NMDA, *N*-methyl-D-aspartate; TF, transcription factor.

their behavioral performance. More recent development of *in vitro* models enabling studies of neuronal plasticity confirmed, however, those initial observations under conditions where well being of the subject could not be considered as a factor [3, 5].

Precise investigations onto effects of the inhibitors on neuronal plasticity showed that there are two clear time-windows during which treatment with the inhibitors is effective, i.e. at the time of training and then several hours later. Inhibition of protein biosynthesis, e.g., a couple of hours after the training session does not interfere with memory formation [7, 8].

c-Fos protein in long term cellular responses

Since middle eighties, several investigators, including ourselves [11–16] have suggested that long term phenotypic changes, based on reprogramming of gene expression, involve induction and activity of certain regulatory genes, such as nuclear protooncogenes (see e.g., [17, 19]). Although all of these genes seemed to be quite ubiquitously expressed in a wide range of biological phenomena, the question arose whether their protein products (later shown to be *bona fide* transcription factors) might serve a regulatory role controlling the expression of genes specific to those phenomena [5, 11, 13–16]. For instance, proteins of the Jun family (c-Jun, Jun B, Jun D), together with proteins of the Fos family (c-Fos, Fos B, Fra-1, Fra-2) form a transcription factor AP-1, known to influence the expression of a number of genes.

The physiological regulatory role of *c-fos* and AP-1 has been shown to be critical in certain experimentally amenable cellular responses like cell cycle [19] and β-endorphin release from pituitary neurons in culture [20].

Activation of *c-fos* in nerve cells

Possible involvement of *c-fos* in long term physiological responses of nerve cells prompted numerous researchers to study the role of various neurotransmitters in *c-fos* activation. These studies have repeatedly shown that glutamate (a major excitatory neurotransmitter in the mammalian brain) acting through ionotropic receptors, the NMDA (*N*-methyl-D-aspartate) receptor in particular, comprises a major, though not exclusive, pathway for *c-fos* activation (for review see [21]).

During our own studies on neuronal cultures [22–24] we have shown that L-glutamate activates the AP-1 DNA binding activity, as well as expression of *zif 268* (another gene encoding a transcription factor) through activation of both NMDA and non-NMDA ionotropic L-glutamate receptors, but apparently not through metabotropic receptors [23]. On the other hand, L-glutamate failed to activate DNA binding activities of other transcription factors tested, including CREB, NF-κB, AP-2, SP-1 [24].

We have confirmed these results *in vivo*, using epileptogenic stimulations (pentylenetetrazole or kainate), believed to involve L-glutamate receptors [25, 26]. The importance of these studies is underscored by the well established fact that Ca^{2+} influx subsequent to L-glutamate (in particular NMDA) receptor activation regulates *c-fos* expression [27, 28] as well as it is a prerequisite for long term memory formation for various tasks [29, 30], see also [31, 32].

***c-fos* in learning-related phenomena**

Guided by the aforementioned data and theoretical considerations, we have initiated studies on the expression of *c-fos* in learning phenomena. At the beginning, we found that multiple trains of the perforant path high frequency stimulation leading to long lasting long term potentiation (LTP) of the synaptic responses in the granule cells of the hippocampal dentate gyrus, regarded as an electrophysiological model for neuronal plasticity, provoked accumulation of *c-fos* mRNA. Low frequency stimulation (not leading to LTP) did not have this effect [33]. Several other research groups also reported increased TFs' gene expression following induction of long lasting LTP (for review and discussion of some discrepancies see [34]).

We have also found that there is a dramatic increase of mRNA levels of *c-fos*, as well as of *zif 268* in the parieto-occipital cortex and cerebellum of the rat brain, following acquisition of a 2-way active avoidance reaction, with a compound visual and auditory conditioned stimulus [35, 36]. Interestingly, performance of already learned behavior by itself was unable to elicit *c-fos* activation [35, 36]. We have also reported that acquisition of copulatory behavior in male rats correlates with elevated *c-fos* expression in the rat sensory cortex [37]. Similar findings of learning-related accumulation of

c-fos, c-jun, jun B and *zif 268* mRNAs and proteins have also been reported by others (for review see [6] and also [38–42].

Transcription factors in learning processes: expression and function

The fact that elevated expression of genes coding for transcription factors correlates with learning prompted us to ask whether activation of transcription factors themselves may follow. Using electrophoretic mobility shift assay (see e.g. [43]) we have recently obtained data that the AP-1 DNA binding activity, reflecting the functional form of transcription factor, and not just the level of mRNA or protein, does indeed increase significantly after a single session of two-way active avoidance training, and not much after long term training (Łukasiuk, K., Nikolaev, E. & Kaczmarek, L., submitted to publication).

Recent functional studies with transient or permanent blocking of AP-1 components further support a role of this transcription factor in learning-related phenomena. In particular, *c-fos* anti-sense oligonucleotides have been reported to block acquisition of long term memory for passive avoidance in chicks (Rose *et al.*, personal communication). Similarly anti-*c-jun* as oligonucleotides were found to disrupt acquisition of the brightness discrimination reaction in rats [41].

It has been also shown that genetically engineered mice [44], lacking *c-fos* gene, display learning disabilities [45]. Unfortunately, they were accompanied by other behavioral abnormalities thus is was impossible to clearly distinguish between the two sets of phenomena.

More precise results were obtained with CREB (cAMP responsive element binding protein, a transcription factor activated by protein kinase A). The role of CREB in long term neuronal plasticity has been strongly suggested by the data collected with invertebrate systems as well as LTP and regulation of circadian clock in mammals [46–52]. Using CREB-lacking mice Bourtchaladze *et al.* [52] were able to show that these animals may develop memory lasting up to 1.0–1.5 h, but not for a longer time. These results were interpreted as indicating that a specific transcription factor, like CREB, may control long- but not short-term memory formation.

A hypothesis: Neuron's nucleus as an information integration device in learning processes

On the basis of the above considerations as well as the repeatedly, demonstrated fact that learning in general, and consolidation of memory trace in particular, requires co-operation between several neurotransmitters and neuromodulators, acting through specific receptors [53–56], we have suggested that long-term memory formation could involve activation of transcription factors and, in consequence, expression of downstream "effector" genes in learning. These genes could code for proteins involved in strengthening of synaptic efficacy. According to this hypothesis regulation of expression of "effector genes" should be driven by simultaneous co-activation of a group of TFs, driven by different neurotransmitter/receptor systems [6, 57]. In this way, the regulatory promoter and enhancer regions of "effector" genes could act as molecular coincidence detectors in learning processes, providing a tool to integrate information.

Advocatus diaboli: gene expression in neuronal plasticity — maintenance, replenishment or regulation?

The reasoning presented above was focused on the data supporting the idea of gene expression playing *a regulatory* role in learning processes. However, it is also worthwhile to discuss major criticisms of this concept. There are at least two explanations, other than regulation, for the necessity of gene expression in learning, or more generally in neuronal plasticity. First, and an apparently most obvious one, can be termed *neuronal maintenance*. Every living cell requires gene expression to make up for the proteins lost during physiological metabolic turnover. Learning-disruptive effects of protein and/or RNA synthesis inhibitors could be explained by this notion. However, this still does not provide a good explanation for the specific training-related time-windows, in which the inhibitors operate, as well as for the learning- (or plasticity) evoked enhancement of gene expression.

The second possible explanation can be described by the word *replenishment*. In all of the situations when elevated gene expression correlated with phenomena of neuronal plasticity (studied with the aid of more or less

physiological learning models) was observed a massive neuronal activation. This seems to be also true for behavioral training. It is conspicuous that tasks based on aversive conditioning, rather than appetitive conditioning, were shown to provoke elevated gene expression. The opposite examples, like acquisition of sexual proficiency, are still a point of debate to what extent they are indeed learning phenomena. During the aforementioned massive neuronal activation, there is a massive release of the content of synaptic vesicles as well. It can be thus suggested that replenishment of this content is a reason for the subsequent gene expression. No wonder then that the genes coding for the synaptic release machinery and content were suggested to be stimulated as a result of enhanced activity of transcription factors [58].

Closer scrutiny of the functional studies suggesting involvement of transcription factors in learning can also reveal their weaknesses. In particular, the time course of the learning deficits observed in CREB mutants by Bourtchaladze *et al.* [52] (later than 0.5 h or 1.0 h, but sooner than 1.0–1.5 h) is difficult to reconcile with other data suggesting that gene expression is required for formation of memories lasting at least 3–4 hours [7, 8, 59]. Hence, results of these studies may be alternatively explained by down-regulation of pre-existing TF leading to inhibition of proper maintenance of neuronal functions, rather than by blocking of learning-evoked formation of the TF. Similarly, Tischmeyer *et al.* [41] have recently shown that down-regulation of c-Jun with specific antisense oligonucleotides disrupts the acquisition phase (as opposed to formation of a long lasting memory trace) of the brightness discrimination reaction in rats as well. Again, these results may imply that down-regulation of c-Jun interferes with basal functioning (maintenance) of brain cells, preventing them from modifying synaptic connections even for a short time — a phenomenon impossible to imagine to be gene expression-dependent.

In conclusion, in our opinion, it is impossible to refute the replenishment hypothesis, although there is no data to give up the idea of a regulatory role of gene expression, either. Obviously, possible co-existence of all three (maintenance, replenishment, regulation) molecular mechanisms of neuronal functioning in plasticity-related phenomena makes the experiments aiming to resolve this issue more difficult. Clearly, it is an exciting area of research in which recent developments lead to the belief that solving of the problem of molecular biology of learning processes is within our reach.

I would like to express my deep gratitude to my numerous colleagues for their enthusiastic involvement in the experiments we have carried out together, as well as to the present members of the group also for critical reading of the manuscript.

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