

Gene expression in learning processes

Leszek Kaczmarek

Department for Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Poland,
Email: leszek@nencki.gov.pl

Abstract. It has repeatedly been shown that long-term memory formation involves neuronal gene expression. In this article several different roles for neuronal gene function in a context of learning are considered: maintenance of neural functioning, replenishment of cellular elements that are exhausted in response to massive neuronal stimulation accompanying behavioral training, maintenance of the plastically reorganized neuronal connections, and finally integration of information at the level of transcription factor-promoter interaction. It is strongly advocated that only careful scrutiny of learning-related gene expression phenomena may aid in understanding of the complex learning process.

Key words: transcription factors, *c-fos*, AP-1, *zif268*, behavior, information processing, long-term memory

INTRODUCTION

Recent developments in the molecular neurobiology of learning and memory have profoundly changed our understanding of these phenomena. Although the connection between gene expression and learning was already observed almost forty years ago (Flexner et al. 1963, see also: Davis and Squire 1984, Matthies 1989), only further experiments, as well as studies implicating specific genes and their protein products in neuronal plasticity, have provided unequivocal evidence for a role for gene expression in learning and memory (see, e.g.: Dash et al. 1990, Rose 1991, Kaczmarek 1993, Nguyen et al. 1994, Lamprecht and Dudai 1996, Lamprecht et al. 1997). Moreover, the notion of a pivotal link between gene function and neural plasticity has been widely accepted. Presently, the question of whether such a link exists has been replaced by the question - "what is this link?". Two facets of this problem could, in theory, be considered: (1) effects of alterations in gene structure and expression on learning and memory; (2) effects of learning and memory on gene structure and function.

Whereas the first of the aforementioned two issues has attracted considerable attention recently, studies on the second aspect may be at least as important as the previous one. At the moment, there is no reliable proof for learning-related changes in gene structure. On the other hand, there are multiple lines of evidence that learning-evoked changes (and neuronal plasticity in general) are accompanied by alterations in gene expression (see: Rose 1991, Robertson 1992, Kaczmarek 1993, Kaczmarek and Chaudhuri 1997 for review). It has repeatedly been shown that identifying genes that are activated under certain physiological and pathological conditions may reveal molecular mechanisms driving those conditions. Thus, it is believed that studies on learning-evoked gene expression may provide clues to understand the learning process itself, by identifying proteins that are involved in its molecular and cellular mechanisms.

At the same time, it is also clear that learning is a complex phenomenon that often correlates with many other events, not directly related to memory formation. Hence, it is important to dissect the behavioral training in order to isolate processes such as sensory information, arousal, motivation, emotion, reward, etc., that have to be integrated in memory formation (Gibs and Ng 1977, Gold and Zornetzer 1983, McGaugh 1989). The integration of various inputs reaching a neural network can be taken as the essence of the learning process. Similarly, it is also of

great importance to examine gene expression events that correlate with learning, especially its various components. This article is an attempt to analyze this issue theoretically, and to focus on experimental data on transcription factors that control gene expression. The transcription factors are taken as an example that is of particular interest, as they are believed to be the most revealing in disclosing changes in molecular programs involving many genes to be regulated in a concerted manner.

GENE EXPRESSION AND NEURONAL MAINTENANCE

The simplest explanation for a link between gene expression and learning is a role of the former in maintenance of neural functioning. Every living cell requires gene expression to make up for the proteins that are lost during physiological metabolic turnover, and thus there is a continuous need for proteins that are responsible for the homeostatic maintenance of nerve cells. The genes that are involved in such processes should be ubiquitously expressed, however their levels may be modulated according to neuronal activity (Fig. 1). Expression pattern of *zif268* in neocortex can be used to exemplify such a possibility. This gene has been extensively studied in the central nervous system, and it encodes a transcription factor (ZIF268) whose recognition element was found in genes coding for ubiquitously expressed neuronal proteins such as synapsins, and glutamate dehydrogenase (Das et al. 1993, Thiel et al. 1994,

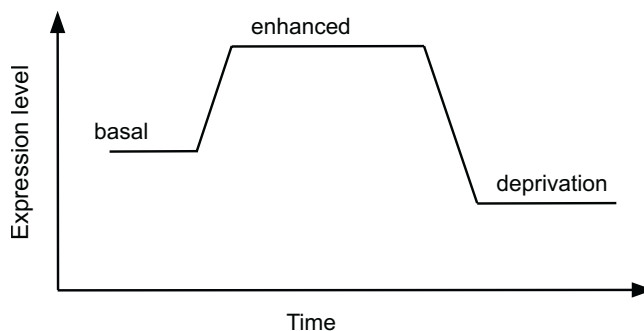


Fig. 1. Graphic representation of the gene expression exemplary to the concept of maintenance. Expression of a "maintenance" gene is always present, but can be modulated according to neuronal activity, e.g. enhanced in such situation as sensory stimulation in a novel environment, and decreased after sensory deprivation.

Petersohn et al. 1995). The expression of the Zif268 protein in cortical neurons is maintained at relatively high levels with ongoing synaptic stimulation. However, it can be rapidly down-regulated by, e.g., sensory deprivation (for review see: Kaczmarek and Chaudhuri 1997), or by treating neuronal cell cultures with tetrodotoxin (Murphy et al. 1991).

THE MAINTENANCE HYPOTHESIS DOES NOT EXPLAIN ALL THE FINDINGS

Despite the fact that the maintenance as an explanation for gene expression requirement in learning may clarify a number of experimental data, it does not elucidate such findings as training-related time-windows, in which the inhibitors of either protein or RNA synthesis operate (Davis and Squire 1984, Matthies 1989). Multiple studies documented that inhibition of protein and/or RNA synthesis affects acquisition of long-term memory. Long-term memory is defined operationally as memory which lasts longer than a few hours and that is protein synthesis dependent. Treating animals with drugs blocking *de novo* protein synthesis does not affect shorter lasting memory traces. To be effective, the inhibition of protein synthesis has to occur around the time of training or a few hours afterwards. Inhibitors given either a couple of hours or 10 or more hours after training do not affect memory formation (Squire and Davis 1984, Matthies 1989, Rose 1995). Therefore, during and immediately after training as well as several hours later, there are two waves of *de novo* synthesis of proteins in-

involved in memory formation/consolidation. On the other hand, the aforementioned results indicate that there is a period, within a few hours after the training, when inhibition of gene expression does not affect the memory trace. Thus, the scenario in which there is a constant requirement for gene expression/mRNA accumulation/protein synthesis that if blocked results in inhibition of memory formation, can not hold. In conclusion, the experimentally proven phenomenon of learning-evoked gene expression is not compatible with the maintenance of neural functioning hypothesis defined as above.

REPLENISHMENT AS AN EXPLANATION FOR LEARNING-RELATED GENE EXPRESSION

The term replenishment may capture the events surrounding learning evoked gene expression. Although this may be considered to be an aspect of homeostasis - included in the previously described maintenance hypothesis - it should be viewed separately as it reflects the sudden needs of a neuron entering a period of intense activity. Most learning conditions involve abrupt bursts of spiking activity - following relative quiescence periods - related to information processing. This may result in (1) rapid depletion of neuronal components (e.g., synaptic release machinery, metabolic enzymes, etc.) and in (2) the need to replace these exhausted elements (see: Rainbow 1979). Obviously, one would expect that a replenishment-related wave of gene expression should follow stimulation conditions such as behavioral training (Fig. 2). It is conspicuous that tasks based on aversive, rather than appetitive conditioning - the former resulting probably in more robust neuronal response than the latter - were shown to be the most effective in triggering elevations in the expression of certain genes (for review see: Kaczmarek 1993). It is noteworthy that there are transcription factors whose expression pattern appears to be compatible with the concept of replenishment. AP-1 may serve as an example in this regard (Fig. 3).

AP-1 is a protein dimer made of Fos and Jun components (Morgan and Curran 1991, Hughes and Dragunow 1995). Some of these proteins, especially c-Fos, have been extensively characterized with respect to their expression pattern in nerve cells (Hughes and Dragunow 1995, Kaczmarek and Chaudhuri 1997). In the brains of naive animals, the expression of c-Fos remains very low. However, stimulation of various kinds results in a rapid,

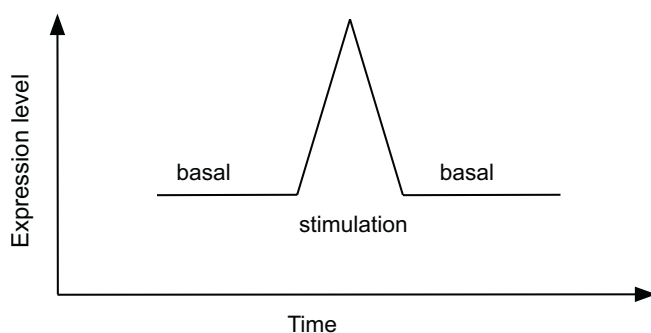


Fig. 2. Graphic representation of the gene expression exemplary to the concept of replenishment. Expression for "replenishment" gene is at low levels under basal conditions, and is transiently upregulated as a result of stimulation, such as, e.g., behavioral training, sudden sensory exposure, etc.

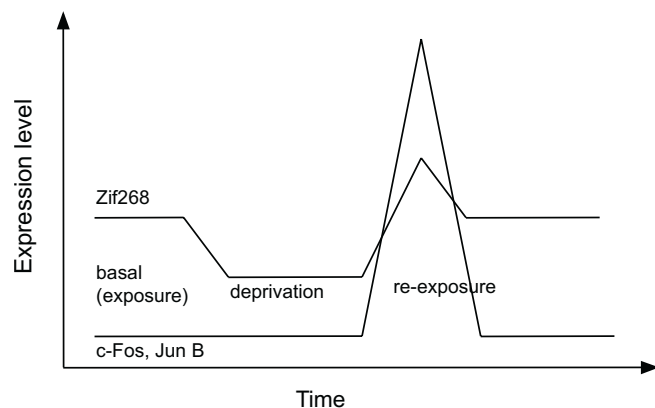


Fig. 3. Graphic representation of expression patterns of Zif268 and AP-1 transcription factor proteins in a context of various sensory conditions.

and usually very transient, activation of gene expression and protein accumulation. Given that c-Fos is a component of the AP-1 transcription factor and that it can initiate gene expression, even the transient nature of c-Fos expression may have a prolonged impact on neuronal physiology.

Upregulated expression of c-Fos (and AP-1 containing c-Fos) has been observed in the context of neural plasticity (Morgan and Curran 1991, Hughes and Dragunow 1995, Kaczmarek and Chaudhuri 1997). This transcription factor is elevated at the time of intense plastic changes in the neocortex (critical periods), and can be induced upon activation of glutamate receptors, especially the NMDA receptor. It is also upregulated by electrical stimuli leading to long lasting LTP as well as sensory stimuli, and behavioral training. It is noteworthy that during the aforementioned massive neuronal activation of various kinds, there is also a massive release of synaptic vesicles. Therefore, it can be suggested that replenishment of synaptic vesicles is one of the reasons for the subsequent gene expression.

MAINTENANCE OF PLASTIC CHANGES

The concept of replenishment implies that learning- (or rather neuronal activity-) evoked gene expression is involved in the metabolic recovery triggered by depletion of key cellular components during learning and thus serves to reinstate the same situation as before the training. On the other hand, learning is thought to be based on the reorganization of specific synaptic connections that

cause a lasting change in neural functioning. Hence, another possible way to explain learning-evoked gene expression is that it serves to produce proteins, whose function is to maintain the plastically reorganized neuronal connections. Most obviously, these proteins should be targeted to specific synapses to support their newly gained functions (these may include formation/loss as well as their strengthening/weakening of synapses).

Recent advancements in studies on targeting proteins and mRNAs to well defined subcellular domains - including dendrites and even synapses - suggest how neurons may target key proteins and mRNAs to specific synapses (Frey and Morris 1997, Martin et al. 1997, Steward et al. 1998, Casadio et al. 1999).

INFORMATION INTEGRATION

The maintenance of plastic changes hypothesis can be extended further to suggest that learning-evoked gene expression may be central to the learning process. Gene regulatory regions always contain sites for binding multiple transcription factors and interactions between these factors are believed to be mandatory to drive gene expression. Thus, the regulatory regions of genes encoding proteins directly subserving synaptic reorganization ("effector" proteins) may act as coincidence detectors allowing a convergence of information provided by various transcription factors, activated by different signaling pathways of behavioral relevance, such as sensory information, arousal, motivation, reward, etc. (Kaczmarek 1993, 1995). There is a vast literature suggesting that there are separate neurotransmitter/receptor systems for conveying these inputs (Gold and Zornetzer 1983, McGaugh 1989, Decker and McGaugh 1991). The molecular meaning of this transcriptional coincidence detection may be to trigger and elaborate long-term plastic changes.

In this context it is important to stress that the upregulation of AP-1 does not always correlate with just neuronal excitation (Kaczmarek and Chaudhuri 1997). On the other hand, c-Fos expression appears to strictly correlate with propensity to a long-term change in neuronal function. A similar role has been previously proposed for c-Fos in driving phenotypic changes in other cells in the cell cycle and cell differentiation (Goelet et al. 1986, Kaczmarek 1986, Marx 1987, Kaczmarek and Kaminska 1989, Angel and Karin 1991, Kaczmarek and Chaudhuri 1997). Moreover, there are

indications that upregulation of c-Fos is a prerequisite for long term changes both in neurons and other cells (Fagarasan et al. 1991, Colotta et al. 1992, Riabowol et al. 1992, Grimm et al. 1997).

Another transcription factor that has been functionally implicated in memory is CREB (Kaang et al. 1993, Bourtchaladze et al. 1994, Frank and Greenberg 1994, Yin et al. 1994, 1995, Bartsch et al. 1995, Carew 1996, Guzowski and McGaugh 1997, Lamprecht et al. 1997). There are multiple pieces of evidence that down-regulation of its levels adversely affect long-term memory formation. Moreover, upregulation of CREB results in enhanced transition from short-term to long-term memory in *Drosophila* (Bartsch et al. 1995, Yin et al. 1995).

CONCLUSION

This paper presents an attempt to dissect a complex phenomenon of learning-related gene expression. Major conclusions to be drawn from this analysis are following. First, the careful scrutiny of various aspects of a pattern of gene expression in a context of learning has to be applied as a necessary step before any learning-related roles could be ascribed to specific genes. Second, as a result of such a scrutiny, one may expect to select genes whose protein products indeed subserve the learning process. Finally, it might be envisioned that identification of such genes, and especially their regulatory regions, might reveal the molecular mechanisms of coincidence detection at the levels of transcription regulation.

REFERENCES

- Angel P., Karin M. (1991) The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochim. Biophys. Acta* 1072: 129-157.
- Bartsch D., Ghirardi M., Skehel P.A., Karl K.A., Herder S.P., Chen M., Bailey C.H., Kandel E.R. (1995) Aplysia CREB2 represses long-term facilitation: relief of repression converts transient facilitation into long-term functional and structural change. *Cell* 83: 979-992.
- Bourtchaladze R., Frenguelli B., Blendy J., Cioffi D., Schutz G., Silva A.J. (1994) Deficient long-term memory formation in mice with a targeted mutation of the cAMP-responsive element binding protein. *Cell* 79: 59-68.
- Carew T.J. (1996) Molecular enhancement of memory formation. *Neuron* 16: 5-8.
- Casadio A., Martin K.C., Giustetto M., Zhu H., Chen M., Bartsch D., Bailey C.H., Kandel E.R. (1999) A transient, neuron-wide form of CREB-mediated long-term facilitation can be stabilized at specific synapses by local protein synthesis. *Cell* 99: 221-237.
- Colotta F., Polentarutti N., Sironi M., Mantovani A. (1992) Expression and involvement of c-fos and c-jun protooncogenes in programmed cell death induced by growth factor deprivation in lymphoid cell lines. *J. Biol. Chem.* 267: 18278-18283.
- Das A.T., Arnberg A.C., Malingre H., Moerer P., Charles R., Moorman A.F.M., Lamers W.H. (1993) Isolation and characterization of the rat gene encoding glutamate dehydrogenase. *Eur. J. Biochem.* 211: 795-803.
- Dash P.K., Hochner B., Kandel E.R. (1990) Injection of cAMP-responsive element into the nucleus of Aplysia sensory neurons blocks long-term facilitation. *Nature* 345: 718-721.
- Davis H.R., Squire L.R. (1984) Protein synthesis and memory: A review. *Psychol. Bull.* 96: 518-559.
- Decker M.W., McGaugh J.L. (1991) The role of interactions between the cholinergic system and other neuromodulatory systems in learning and memory. *Synapse* 7: 151-168.
- Fagarasan M.O., Aiello F., Muegge K., Durum S., Axelrod J. (1990) Interleukin 1 induces B-endorphin secretion via fos and jun in AtT-20 pituitary cells. *Proc. Natl Acad. Sci. USA* 87: 7871-7874.
- Flexner J.B., Flexner L.B., Stellar E. (1963) Memory in mice as affected by intracerebral puromycin. *Science* 141: 57-59.
- Frank D.A., Greenberg M.E. (1994) CREB: a mediator of long-term memory from mollusks to mammals. *Cell* 79: 5-8.
- Frey U., Morris R.G. (1997) Synaptic tagging and long-term potentiation. *Nature* 385: 533-536.
- Gibbs M.E., Ng K.T. (1977) Psychobiology of memory: towards a model of memory formation. *Biobehav. Rev.* 1: 113-136.
- Goelet P., Castelluci V.F., Schacher S., Kandel E.R. (1986) The long and the short of long term memory - a molecular framework. *Nature* 322: 419-423.
- Gold P.E., Zornetzer S.F. (1983) The mnemon and its juices: neuromodulation of memory processes. *Behav. Neural Biol.* 38: 151-189.
- Hughes P., Dragunow M. (1995) Induction of immediate-early genes and the control of neurotransmitter-regulated gene expression within the nervous system. *Pharmacol. Rev.* 47: 133-178.
- Kaang B.K., Kandel E.R., Grant S.G. (1993) Activation of cAMP-responsive genes by stimuli that produce long-term facilitation in Aplysia sensory neurons. *Neuron* 10: 427-435.
- Kaczmarek L. (1986) Protooncogene expression during the cell cycle. *Lab. Invest.* 54: 365-377.
- Kaczmarek L. (1993) Molecular biology of vertebrate learning: is c-fos a new beginning? *J. Neurosci. Res.* 34: 377-381.

- Kaczmarek L. (1995) Towards understanding of the role of transcription factors in learning processes. *Acta Biochim. Pol.* 42: 221-226.
- Kaczmarek L., Chaudhuri A. (1997) Sensory regulation of immediate-early gene expression in mammalian visual cortex: Implications for functional mapping and neural plasticity. *Brain Res. Rev.* 23: 237-256.
- Kaczmarek L., Kaminska B. (1989) Molecular biology of a cell activation. *Exp. Cell Res.* 183: 24-35.
- Lamprecht R., Dudai Y. (1996) Transient expression of c-Fos in rat amygdala during training is required for encoding conditioned taste aversion memory. *Learn. Memory* 3: 31-41.
- Lamprecht R., Hazvi S., Dudai Y. (1997) cAMP response element-binding protein in the amygdala is required for long-but not short-term conditioned taste aversion memory. *J. Neurosci.* 17: 8443-8450.
- Martin K.C., Casadio A., Zhu H.E.Y., Rose J.C., Chen M., Bailey C.H., Kandel E.R. (1997) Synapse-specific, long-term facilitation of aplysia sensory to motor synapses: a function for local protein synthesis in memory storage. *Cell* 91: 927-938.
- Marx J.L. (1987) The fos gene as "master switch". *Science* 237: 854-856.
- Matthies, H. (1989) Neurobiological aspects of learning and memory. *Ann. Rev. Psychol.* 40: 381-404.
- McGaugh J.L. (1989) Involvement of hormonal and neuromodulatory systems in the regulation of memory storage. *Ann. Rev. Neurosci.* 12: 255-287.
- Morgan J.I., Curran T. (1991) Stimulus-transcription coupling in the nervous system: involvement of the inducible protooncogenes fos and jun. *Ann. Rev. Neurosci.* 14: 421-451.
- Murphy T.H., Worley P.F., Nakabeppu Y., Christy B., Gastel J., Baraban J.M. (1991) Synaptic regulation of immediate early gene expression in primary cultures of cortical neurons. *J. Neurochem.* 57: 1862-1872.
- Nguyen P.T., Abel T., Kandel E.R. (1994) Requirement of a critical period of transcription for induction of a late phase of LTP. *Science* 265: 1104-1107.
- Petersohn D., Schoch S., Brinkmann D.R., Thiel G. (1995) The human synapsin II gene promoter. Possible role for the transcription factor zif268/egr-1, polyoma enhancer activator 3, and AP2. *J. Biol. Chem.* 270: 24361-24369.
- Rainbow, T.C. (1979) Role of RNA and protein synthesis in memory formation. *Neurochem. Res.* 4: 297-312.
- Riabowol K., Schiff J., Gilman M.Z. (1992) Transcription factor AP-1 activity is required for initiation of DNA synthesis and is lost during cellular aging. *Proc. Natl Acad. Sci. USA* 89: 157-161.
- Robertson H.A. (1992) Immediate-early genes, neuronal plasticity, and memory. *Biochem. Cell. Biol.* 70: 729-737.
- Rose S.P.R. (1991) How chicks make memories: the cellular cascade from c-fos to dendritic remodelling. *Trends Neurosci.* 14: 390-397.
- Rose S.P. (1995) Cell-adhesion molecules, glucocorticoids and long-term-memory formation. *Trends Neurosci.* 18: 502-506.
- Steward O., Wallace C.S., Lyford G.L., Worley P.F. (1998) Synaptic activation causes the mRNA for the IEG Arc to localize selectively near activated postsynaptic sites on dendrites. *Neuron* 21: 741-751.
- Thiel G., Schoch S., Petersohn D. (1994) Regulation of synapsin I gene expression by the zinc finger transcription factor zif268/egr-1. *J. Biol. Chem.* 269: 15294-15301.
- Yin J.C., Del Vecchio M., Zhou H., Tully T. (1995) CREB as a memory modulator: induced expression of a dCREB2 activator isoform enhances long-term memory in *Drosophila*. *Cell* 81: 107-115.
- Yin J.C.P., Wallach J.S., Del Vecchio M., Wilder E.L., Zhuo H., Quinn W.G., Tully T. (1994) Induction of a dominant-negative CREB transgene specifically blocks long-term memory in *Drosophila*. *Cell* 79: 49-58.