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Review article

Regulation and function of immediate-early genes in the brain: Beyond neuronal activity markers

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ABSTRACT

Long lasting forms of synaptic plasticity and long-term memory formation require new mRNA and protein synthesis. While activity-dependent expression of immediate-early genes has long been thought to account for such critical *de novo* macromolecular synthesis, experimental proof has been scarce until recently. During the past few decades, a growing number of genetic and molecular biological studies have started to elucidate essential roles of immediate-early genes in synaptic plasticity and cognitive functions. I here present an overview of the history and recent work on regulation and function of neuronal immediate-early genes, including *Arc*/*arg*3.1. This review provides a conceptual framework in which various immediate-early genes underlie several distinct processes required for long-term synaptic changes and memory formation.

Contents

1. Introduction

The brain stores information extracted from experiences and utilizes it to modify behaviors throughout the life span of an organism. This large mnemonic capacity is thought to depend on intrinsic neural networks whose synaptic connectivity and strength can be modulated by specific patterns of neuronal activity. Early behavioral studies using protein synthesis inhibitors indicated that newly synthesized protein is required for long-term memory but not for short-term memory (Davis and Squire, 1984). This conceptual framework has been expanded to synaptic plasticity; long-lasting forms of synaptic plasticity, such as long-term potentiation (LTP),

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require newly synthesized mRNA and proteins, while short-term plasticity does not (Bliss and Collingridge, 1993; Goelet et al., 1986; Kandel, 2001). This requirement has critical time windows for both memory formation and synaptic plasticity. Administration of protein synthesis inhibitors to animals just after learning effectively blocks long-term memory formation, while administration several hours later has little effect (Freeman et al., 1995; Nader et al., 2000; Rosenblum et al., 1993; Squire and Barondes, 1972; Suzuki et al., 2004). Similarly, LTP is prevented only when mRNA or protein synthesis is blocked immediately after LTP-inducing stimulation (Frey et al., 1988; Nguyen et al., 1994; Otani et al., 1989). Thus, gene expression occurring immediately after the events to be memorized appears to play critical roles for establishment and/or maintenance of long-lasting neuronal changes. Such inducible genes are mostly classified as a subset of genes called immediate-early genes (IEGs) (Lanahan and Worley, 1998; Morgan and Curran, 1991).

The term "immediate-early gene" originated from virology. When viruses infect a host cell, several viral genes are rapidly transcribed. This process requires only pre-existing transcription factors of the host cell and occurs in the absence of *de novo* protein synthesis (Watson and Clements, 1980). Through tremendous work on cellular differentiation and proliferation during the 1980s, it has become evident that various stimuli, such as growth/differentiation factors, hormones or cytokines, induce rapid and transient mRNA synthesis in fibroblasts and other cell lines even in the presence of protein synthesis inhibitors (Almendral et al., 1988; Curran et al., 1985; Greenberg and Ziff, 1984; Kelly et al., 1983; Kruijer et al., 1984; Lau and Nathans, 1985). By analogy to the viral IEGs, these cellular genes that are responsive to extracellular stimuli are called "cellular" IEGs. The cellular IEGs, simply referred to as IEGs, encode many functionally distinct proteins, including structural proteins, signaling molecules, and transcription factors.

In this review, I summarize recent expansion of our understanding of neuronal IEGs regarding their regulation and functions for neuronal plasticity and cognitive functions. In particular, I will focus on the neuron-specific IEG *Arc* (also known as *arg*3.1) (Link et al., 1995; Lyford et al., 1995) because recent studies on this gene have highlighted many characteristic and intriguing regulatory aspects of neuronal IEGs, although the biological function of these remains enigmatic.

2. Neuronal activity-dependent expression of IEGs

2.1. IEG expression in the brain

As in the case of intracellular responses to growth factors in mitotic cells, synaptic transmission and/or action potentials also initiate several intracellular signaling cascades, particularly those related to intracellular Ca^{2+} changes, in postmitotic neuronal cells (Morgan and Curran, 1991; Sheng and Greenberg, 1990). In the late 1980s, it was determined that the IEG encoded transcription factor c-Fos is rapidly induced in specific brain nuclei after pharmacological convulsive stimulation and physiological contexts (Morgan et al., 1987; Saffen et al., 1988; Sagar et al., 1988). As a consequence of these groundbreaking findings, two types of studies have been conducted on neuronal IEGs. One type is aimed at isolating and characterizing novel neuronal IEGs. Because many IEGs are implicated in neuronal plasticity and cognitive functions (discussed in Section 4), much effort has been invested to isolate novel IEGs, probably with the hope of finding "master genes" for learning and memory. The other type of study applies IEG expression as a tool to visualize neuronal activity in the brain. Because IEG expression in a neuron reflects the neuron's recent activity, detection of IEG mRNA or protein products in the brain provides information regarding where and when neurons were activated. A brief overview of both lines of work is described below.

2.2. Isolation of neuronal IEGs

Early following studies revealed that several IEGs that were initially identified in fibroblasts and cell lines are in fact also expressed and activity-regulated in neurons in the brain (Dragunow et al., 1992; Herdegen et al., 1991; Morgan et al., 1987; Saffen et al., 1988; Worley et al., 1991). Thus, it is reasonable to expect that there might be more dynamically regulated and more neuron-specific IEGs that could be relevant to synaptic plasticity and memory formation. In the early 1990s, several laboratories extensively explored new IEGs that could be induced by neuronal activity (Table 1). A group led by Paul Worley at the Johns Hopkins University isolated IEGs from a subtraction cDNA library made from control and electroconvulsive shock-treated hippocampi. Through this strategy, they isolated more than 10 novel IEGs; the clones encode transcription factors (*egr*-3) (Yamagata et al., 1994a), signaling molecules (*rheb*, *rsg*2, *cox*-2) (Ingi et al., 1998; Yamagata et al., 1993, 1994b), and several functionally unknown proteins at that time (*Arc*, *homer*1a, *narp*, etc.) (Brakeman et al., 1997; Lyford et al., 1995; Tsui et al., 1996). Dietmar Kuhl and colleagues at Columbia University and later in Germany isolated several IEGs using a similar differential screening strategy. Their identified clones include *tPA* (Qian et al., 1993), *SNK* (Kauselmann et al., 1999) and *arg*3.1 (Link et al., 1995). Inokuchi's group in Japan independently started to search for activity-induced IEGs through a PCR-based differential cloning strategy and isolated several novel neuronal IEGs, including *vesl*-1s (Kato et al., 1997) and activin-β (Inokuchi et al., 1996). Elly Nedivi and colleagues isolated multiple candidate-plasticity genes (*CPG*s), some of which were shown to be IEGs (Fujino et al., 2003; Nedivi et al., 1993, 1996). These studies used the protein synthesis inhibitor cycloheximide to stabilize or enrich activity-induced mRNAs, which also ensured the definition of IEGs, i.e., *de novo* protein-synthesis independent expression of transcripts. Some of these genes have turned out to be identical. Table 1 presents a list of representative neuronal IEGs with a brief descriptions of structures and function of their products; neuronal IEG products can be classified into several categories including transcription factors, postsynaptic proteins, signaling molecules, secretory factors, and membrane proteins. It is noteworthy that most of the IEGs that were reported by earlier studies encoded transcription factors, while many of those reported more recently encoded non-transcription factor proteins whose function might be directly associated with synaptic properties. The roles and functions of these IEGs *in vitro* and *in vivo* remain central topics in the field (see Section 4).

2.3. Mapping IEG expression in the brain

IEG expression mapping is a powerful method to visualize activated neuronal populations in the brain of animals. Importantly, this technique has been applied to the identification of brain loci related to learning and memory. Historically, c-Fos immunohistochemistry (IHC) and c-*fos* mRNA in situ hybridization (ISH) have been used (Brennan et al., 1992; Rosen et al., 1992, 1998; Vann et al., 2000; Wisden et al., 1990; Zhu et al., 1995). However, because the induction threshold of c-*fos* appears to be rather high compared to those of other IEGs (Waltereit et al., 2001; Wisden et al., 1990; Worley et al., 1993), c-*fos* mapping tends to be applied to behavioral paradigms with a relatively strong cognitive or emotional burden. Expression of *zif*268 is more responsive to synaptic activities at physiological levels (Cole et al., 1990; Worley et al., 1993). Both contextual and cued fear conditioning evoke *zif*268 induction in the amygdala, the center of emotional memory, as well as in the CA1 region of the hippocampus in rodents (Hall