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Review

Intra-axonal protein synthesis in development and beyond

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ABSTRACT

Proteins can be locally produced in the periphery of a cell, allowing a rapid and spatially precise response to the changes in its environment. This process is especially relevant in highly polarized and morphologically complex cells such as neurons. The study of local translation in axons has evolved from being primarily focused on developing axons, to the notion that also mature axons can produce proteins. Axonal translation has been implied in several physiological and pathological conditions, and in all cases it shares common molecular actors and pathways as well as regulatory mechanisms. Here, we review the main findings in these fields, and attempt to highlight shared principles.

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1. Introduction

Asymmetric expression of proteins through the subcellular localization and translation of their mRNAs is an evolutionary conserved mechanism found in essentially all cells (Donnelly et al., 2010; Holt and Schuman, 2013; St Johnston, 2005). In the bacterium *Escherichia coli*, some transcripts are differentially localized between the cytoplasm and the inner membrane, according to the destination of their protein products (Nevo-Dinur et al., 2011). In Drosophila embryos, *in situ* hybridization analysis showed that 71% of the more than three thousand analysed transcripts were specifically localized, and their distribution was tightly correlated with

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the expression pattern of their protein products (Lécuyer et al., 2007). In rat hippocampal neurons an astonishing greater than 50% of the total mRNA content is estimated to be localized to neurites (Cajigas et al., 2012).

Neurons are highly specialized cells, able of conveying information over large distances. They typically possess several highly branched dendrites and a single long effector process, the axon. Due to this extremely polarized morphology, neurons are an ideal cell type to study the regulation and functional consequences of localized protein synthesis. In fact, their unique shape and the extreme distances covered by their neurites prompted already at the end of the 19th century the suggestion that axons might be able to synthesize at least parts of their materials in a manner independent of their cell bodies (Barker, 1899). Axons can span over distances thousands times the neuronal soma's diameter, and the fastest anterograde velocity reported in living neurons is a little less than 40 cm in a day (Kaether et al., 2000), with most cytoskeletal and cytosolic proteins moving only a few millimetres per day (Campenot and Eng, 2000). As a consequence, it can take days for somatically derived proteins to reach their targets in distal axons, raising the issue of protein stability during these long transport times. Slowly transported proteins are indeed degraded over time within mouse retinal ganglion axons (Nixon, 1980). Based on these considerations, the hypothesis was formulated that axonal protein synthesis was needed to overcome the slow transport rates of proteins from the neuronal cell bodies (Alvarez, 1992). Intensive research efforts aimed at understanding the functional requirements of individual axonally localized mRNAs, and more recently transcriptome analyses have changed this view. Instead of being merely an adaptation to slow axonal transport (Alvarez et al., 2000), local protein synthesis is now understood as a mechanism for the spatial-temporal regulation of gene expression, allowing neurons to react acutely to changes in their environment, to compartmentalize signalling events, and to communicate over long distances from the periphery to the cell body. Here we review the functional importance of local translation in developing axons and discuss the rapidly emerging evidence for the requirement of axonal translation beyond the developmental period.

2. Local translation in dendrites

Even though intuitively the longer length of axons means that they may benefit most from a local source of proteins, the existence of local translation in dendrites has been far less controversial, likely due to the ease which with it is detected. It has been known for more than 50 years that disrupting mRNA transcription or translation impedes the long-term storage of memory (Agranoff et al., 1967; Flexner et al., 1963). In the same decade, Bodian (1965) identified ribosome particles in proximal dendrites in monkey spinal cord motorneurons and speculated about a possible functional significance for local protein synthesis in synaptic function. Roughly twenty years later, using electron microscopy, polyribosomes were identified under the base of dendritic spines (Steward and Levy, 1982), and their number and localization showed changes following lesion and during synaptogenesis (Steward, 1983; Steward and Falk, 1986). Metabolic labelling studies in both synaptosomal fractions and compartmentalized cultures soon established that proteins could actually be produced outside of the neuronal soma (Rao and Steward, 1991; Torre and Steward, 1992; Weiler and Greenough, 1991). Initially, the number of proposed dendritically localized mRNAs was no more than a few dozens. New interest in the matter soon revealed an unexpectedly greater number of localized RNAs (Miyashiro et al., 1994), and large-scale microarray studies identified hundreds of transcripts (Poon et al., 2006; Zhong et al., 2006). More recently, RNA-sequencing analysis uncovered 2550 mRNAs localized to neurites of rat hippocampal neurons (Cajigas et al., 2012).

A first functional role for dendritic protein synthesis was revealed when it was found to be involved in a neurotrophin-dependent form of long-lasting synaptic enhancement in the hippocampus: neuropil isolated from its cell bodies could undergo brain-derived neurotrophic factor (BDNF)-induced long term plasticity, and, more importantly, this process was sensitive to inhibitors of protein synthesis (Kang and Schuman, 1996). Dendritic protein synthesis is now considered a requisite process in synaptic plasticity (Sutton and Schuman, 2005), especially for the late phase of long-term potentiation (LTP). LTP reflects a persistent strengthening of synaptic connections and is regarded as the main molecular mechanism underlying memory consolidation, or the conversion of short-term and working memory into long-term memory (Kandel, 2001; McGaugh, 2000). Accordingly, the dysregulation of dendritic protein synthesis has been implied in several neurological disorders (Swanger and Bassell, 2013).

3. Local translation in developing axons

3.1. Early evidence and controversy

Unlike dendritic translation, the existence of a localized system of protein synthesis in axons and nerve terminals was not easily accepted. The first experiments that argued against the exclusive somatic origin of axonal proteins were conducted on vertebrate neurons. If the soma was the only source of an axonal protein such as acetylcholinesterase (AchE), after an irreversible inactivation of AChE, its reappearance in axons should follow a proximal to distal gradient. However, in cat cholinergic neurons both proximal and distal axonal segments displayed an homogeneous recovery rate (Koenig and Koelle, 1960). Also, metabolic labelling studies showed incorporation of [^{3}H]leucine into proteins of desomatized rabbit axons (Koenig, 1967). But after initial enthusiasm and several studies conducted on synaptosomal fractions, the idea lost widespread support mainly because, differently from dendrites, only in very few studies were ribosomes identified in axons by electron microscopy (Peters et al., 1970; Zelená, 1972). Thus based mainly on the perceived absence of clearly identified ribosomes in electron micrographs of mature axons the source of newly synthesized proteins found in synaptosomal fractions was assumed to be exclusively glial, post-synaptic, free mitochondria and membranous contaminants (reviewed in Alvarez et al., 2000). On the contrary, ribosomes were easily identified in growth cones of developing dorsal root ganglion neurons (Tennyson, 1970; Yamada et al., 1971) and in the neurite endings of sympathetic neurons (Bunge, 1973). Brain synaptosomes prepared from young rats had a higher proportion of polyadenylated RNAs and seemed to show higher protein synthetic ability (DeLarco et al., 1975). Further evidence showed isolated axonal growth cones could incorporate radiolabelled amino acids (Davis et al., 1992). Polyribosomes were later identified in the growth cones of developing hippocampal axons by ultrastructural analysis (Deitch and Banker, 1993), even though at a much lower density. Indeed, this paucity of polyribosomes was pointed as one of the earliest and most reliable feature that distinguishing a developing axon from other minor processes (Deitch and Banker, 1993).

Evidence for axonal protein synthesis also started to accumulate from invertebrate models. The squid giant axon was found to possess ribosomal RNAs (Giuditta et al., 1980), mRNAs (Giuditta et al., 1986), tRNAs (Ingoglia et al., 1983), and actively translating polysomes (Giuditta et al., 1991). More compelling still, squid axons separated from their soma could incorporate radioactive amino acids into newly synthesized proteins (Giuditta et al., 1968). Similar

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