

Dendrites as separate compartment – local protein synthesis

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The article summarizes the most meaningful studies which have provided evidence that protein synthesis in neurons can occur not only in cell perikarya but also locally in dendrites. The presence of the complete machinery required to synthesize cytoplasmic and integral membrane proteins in dendrites, identification of binding proteins known to mediate mRNA trafficking in dendrites and the ability to trigger “on-site” translation make it possible for the synthesis of particular proteins to be regulated by synaptic signals. Until now over 100 different mRNAs coding the proteins involved in neurotransmission and modulation of synaptic activity have been identified in dendrites. Local protein synthesis is postulated to provide the basic mechanism of fast changes in the strength of neuronal connections and to be an important factor in the molecular background of synaptic plasticity, giving rise to enduring changes in synaptic function, which in turn play a role in local homeostatic responses. Local protein synthesis points to some autonomy of dendrites which makes them “the brains of the neurons” (Jim Eberwine; from the interview with J. Eberwine – Barinaga 2000).

Key words: local protein synthesis, synapse-associated polyribosomal complexes, mRNA translation repressors, selective photobleaching

HOW DID THE STORY BEGIN?

Neurons communicate through the synapses. Each neuron contains thousands of them. The dendritic tree of a typical projection neuron in the adult mammalian brain contains approximately 10 000 dendritic spines, small membranous protrusions from the central stalk of a dendrite. Onto each of them a single excitatory synapse with a single axon is formed. Synapses connect neurons into complicated networks which wire brain and spinal cord structures, enabling them to receive, processes and transmit informations. A synapse undergoes continuous modifications, changing efficacy of neurotransmission, depending on the preceding events. This particular feature is called synaptic plasticity and is considered to be, among others, the cellular basis for learning and memory (Bliss and Collingridge 1993). The ability to modify and reconstruct synaptic connections, strengthening ones and blunting the others, serves also to improve perception and to acquire and

refine motor abilities. Synaptic plasticity becomes apparent following injury to the nervous system, if reorganization of neuronal networks occurs; it is considered to be one of the conditions of morphological and functional recovery, although it may also accompany ectopic innervation and cause pathology (see Macias 2008, this issue).

Pondering over cellular mechanisms which drive plasticity of synapses led to obvious considerations that local content of proteins which are indispensable for altering synaptic activity and structure, must change. It was a conceptual challenge to assume that protein synthesis might occur and be regulated locally in dendrites and axonal terminals, in light of the classical view, that all the proteins encoded in cell nucleus undergo translation and processing in neuronal cell body, and only then are transported to specific dendritic regions. The drive for that alternative concept was that a classical view did not provide an explanation of two important features of fast and local synaptic changes: (1) a time scale of synaptic events and (2) their spatial specificity.

How to explain fast protein changes which occur in activated synapses within minutes, if local signals are

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to be communicated to the “headquarters” – a nucleus and perikaryon – and only then new proteins are to be transported and directed to the compartments of “higher demands”? What about tagging and distributing them, keeping them away from degradation? There may be many dead ends on their long way. Protein transport rate was calculated to be too slow to make it. Fast, local changes become feasible if we assume that local protein synthesis in subsynaptic region of dendrites is possible.

A discovery that high frequency, tetanic stimulation causes a long-term change in the efficacy of stimulated synapses led to an extensive research on the molecular basis of that phenomenon. Long term potentiation (LTP) and long-term depression (LTD) are two forms of persistent plastic changes in the brain which represent, respectively, an increase and a decrease, of excitatory neurotransmission (Bliss and Lomo 1973). Both forms develop within minutes and may be maintained for hours and days (see also Sala et al. 2008, this issue). Thus there must be a molecular recording of this information. There are two phases of plastic changes, each representing different molecular requirements and characteristics: short- and long-lasting ones. Short-term plasticity occurs owing to modifications (mostly by phosphorylations and dephosphorylations) of existing synaptic proteins (Goelet et al. 1986) whereas long-term plasticity and late phase of LTP require an increased protein synthesis; of those which are already active in a synapse, and those which are to be recruited to the process of making a synaptic change. Gathered data have shown that both LTP and other forms of neuronal activation stimulate protein synthesis in dendrites (see Pfeiffer and Huber 2006). It is now firmly established that local protein synthesis serves as a cellular mechanism by which neurons regulate physiological events in both dendrites and axons. The breadth and complexity of this capability has only been realized within the last few years. This review shows the beginning and highlights some of the exciting progress made within the last few years of local protein synthesis investigation.

WHAT CAN BE A RATIONALE FOR LOCAL PROTEIN SYNTHESIS IN DENDRITES?

Local protein synthesis in dendrites may, theoretically, serve fast control of synaptic strength. If neuron did not have the capability of local protein synthesis,

neuronal plasticity would have to rely on two multistep mechanisms (Schuman 1999): (1) the one, directing each signal, which would render an activated synapse enriched in a given protein, from an activated synapse to a cell body; (2) the second one, driving newly synthesized proteins back from the cell body and directing them to active dendritic processes. To make a plastic change possible within a short time, the communication between cell periphery and cell perikaryon must be efficient and fast. Dendritic synthesis should be, *ex definitione*, more effective. In this case a neuron would produce *in situ* the proteins required for a synaptic response, without expenditure of energy related to long-distance transport, and a time lapse due to this process. The benefit would rely also on that if mRNA was transported in a translationally dormant state, sequestered from the translational apparatus in the cytoplasm, and derepressed only in dendrites, it would provide a mechanism of a tight *in situ* regulation of protein synthesis. The proteins, synthesized subsynaptically or in dendritic shafts would translocate on short distances only, towards molecularly labeled, active synapses (Frey and Morris 1997). If present, the process would indicate some autonomy of the dendritic compartment.

To occur, protein synthesis requires presence of several components of the translation system in place: messenger RNA (mRNA) – the template encoding the primary structure of the future protein – and a complex “molecular equipment” which makes translation possible: polyribosomes, transfer RNAs, and a set of enzymes that initiate and regulate translation and elongation of the peptide chains. In the next step newly synthesized proteins undergo processing, which may take place only if endoplasmic reticulum and Golgi apparatus cisterns are in place. What do we know on selective localization of protein synthetic machinery at postsynaptic sites?

THE MACHINERY FOR TRANSLATION IN DENDRITES

Local protein synthesis as a mechanism to modify specific adult synapses was first proposed in 1965 by David Bodian (1965). Bodian, known mostly from his work on neuropathology of poliomyelitis, was the first to find ribosomes in dendrites; this finding was soon followed by reports of ribosomes in axons (Zelena 1970). It remained ignored until Steward and Levy

(1982) reported a presence of synapse-associated polyribosomal complexes (SPRCs) in hippocampal pyramidal and granular neurons. SPRCs are polyribosomes connected with endoplasmic reticulum cisterns that are selectively and precisely localized beneath postsynaptic sites on dendrites of CNS neurons (Steward 1983, Steward and Fass 1983). In their article Steward and Levy reported: "polyribosomes appeared primarily in two locations within the dendrite: (1) beneath the base of identified spines just subjacent to the intersection of the spine neck with the main dendritic shaft and (2) beneath mounds in the dendritic membrane which had the appearance of the base of a spine which extended out of the plane of section" (Steward and Levy 1982). Such location make SPRCs ideally situated to be influenced by electrical and/or chemical signals from the synapse as well as by events within the dendrite proper (see Fig. 1). Since a small fraction of polyribosomes has been found also within the core of dendritic shaft, these may represent a population associated with mRNAs that encode proteins that play a different role in dendritic shaft than those destined to synaptic sites. Also, as shown recently by Ostroff and coauthors (2002), polyribosomes may translocate from dendritic shafts into spines in response to electrical stimulation. The reported change in dendritic content and distribution of polyribosomes after LTP induction, provides evidence that in dendrites they remain in the dynamic state related to the synaptic activity (Ostroff et al. 2002). Electron microscopy studies have shown that spines of different morphology differ in polyribosomal content.

The pioneering study by Steward and Levy (1982) as well as a vast majority of subsequent investigations concerned excitatory synapses on spines. But polyribosome distribution was also reported in non-spiny neurons, beneath asymmetric (presumed excitatory) and symmetric (presumed inhibitory) synapses. These observations became a premise to reactivate a hypothesis on the local dendritic mRNA translation and pointed to the possibility that not only excitatory synapses are plastic. They also laid out a working hypothesis that translation might be regulated by activity at the individual postsynaptic site.

What is the incidence of polyribosomes at spine synapses? Estimates vary, depending on the quantitative methods used and counting criteria, from 25% of spines on dendrites of the hippocampal dentate gyrus

granular cells (Steward and Levy 1982) to 75–82% of spines on pyramidal neurons in the cerebral cortex, (Spacek and Hartmann 1983, Spacek 1985). These numbers indicate that polyribosomes are ubiquitous component of the dendroplasm and that local protein synthesis may be a common process in multiple classes of brain neurons. Although there are no later studies which would verify those estimates, the possibility of dendritic protein synthesis has been supported further by the quantitative electron microscopic studies that revealed colocalization of mRNAs, polyribosomes and tubular cisterns identified as endoplasmic reticulum (ER) subsynaptically and at dendritic shafts (Palacios-Pru et al. 1981, Steward and Reeves 1988). Further studies confirmed that dendrites contain ER and provided evidence of subsynaptic occurrence of ribosomal RNA (rRNA) and proteins, translation initiation and elongation regulatory factors and cisternae of Golgi apparatus (Tiedge and Brosius 1996, Gardiol et al. 1999, Pierce et al. 2000, Wang et al. 2002). Albeit most data were obtained in brain studies, Gardiol group (Gardiol et al. 1999) carried out a solid morphological and immunocytochemical analysis of rat spinal cord neurons. It showed that at the cellular level, *in vivo*, protein synthesis macrocomplexes (ribosomes and eukaryotic elongation factor-2, eIF-2) as well as the system implicated in cotranslational and post-translational modifications (endoplasmic reticulum identified with specific marker BiP, and Golgi cisternae identified with rab1, CTR433, TGN38) penetrated some dendrites of ventromedial horn neurons. The works which showed that dendrites often contain the core elements of the secretory pathway, necessary for the synthesis and transport of integral membrane proteins, are of particular importance as they show the potential of dendrites to process newly synthesized protein (Gardiol et al. 1999, Pierce et al. 2000, Steward and Schuman 2001, Horton and Ehlers 2003). Isolated dendrites can incorporate sugar precursors indicative of Golgi function (Torre and Steward 1996). These results and direct imaging of ER-to-Golgi transport in dendrites (Horton and Ehlers 2003) suggest that integral membrane proteins, and not merely cytoplasmic proteins, can be locally synthesized in dendrites. Investigations of the protein composition of postsynaptic densities (PSD) revealed that 11% of all the proteins present in PSD are the proteins related to mRNA translation (Peng et al. 2004). The described findings indicate that synapses are equipped with the essential

elements required for the synthesis and insertion of a well folded and glycosylated transmembrane proteins.

EXPORT OF mRNAs TO NEURONAL PROCESSES

RNA-containing granules

If mRNA is to be transported to dendrites, there must be an efficient system to carry it on and a precise mechanism regulating it. To disclose the mechanism of mRNA transport to dendrites, pioneering studies of Knowles and coworkers (1996), Kohrmann and colleagues (1999) and others (Kiebler and DesGroseillers 2000, Huang et al. 2003, Tiruchinapalli et al. 2003) used nucleic acid stains and green fluorescent protein fused to RNA-binding proteins to visualize mRNA translocation in live neurons. These studies showed that localized mRNA are transported in the form of large granules which contain mRNAs, RNA helicases, RNA-binding proteins, translational factors, ribosomes, motor proteins and small non-coding RNA (Kim et al. 2005, reviewed by Bramham and Wells 2007). At least three types of RNA-containing granules have been characterized: ribonucleoprotein particles (RNPs), stress granules (SGs) and processing bodies (PBs). A model for RNA transport is only emerging (for review see Bramham and Wells 2007) but it is widely accepted that most mRNAs are transported into dendrites as part of large RNPs. Over 40 proteins that constitute the large RNA-containing granules have been identified until now (Kanai et al. 2004). That number reflects the complexity of possible interactions and variability of their targets.

SGs and PB are thought to be functionally distinct; SGs form under stress conditions, to help reprogram mRNA metabolism by storage of mRNAs when cell recovers from oxidative or metabolic stress, whereas PBs are thought to function in mRNA degradation (Bramham and Wells 2007).

RNA-containing granules translocate in a rapid (0.1 $\mu\text{m/s}$), bidirectional and microtubule-dependent manner (Knowles et al. 1996). Recent studies by Hirokawa group revealed that mRNA is transported to dendrites by means of molecular motors of the kinesin family KIF5, which bind RNA-binding proteins by a recognition motif (Kanai et al. 2004, see also Hirokawa 2006 for review).

Dendritic targeting

In order for specific mRNAs to be dendritically targeted, they must be first sequestered from translational machinery in the cytoplasm and organized into RNPs. Logically, mRNAs destined to dendrite translation, must be identified shortly after transcription. Then, during transport to dendrites, mRNAs must be in a dormant state and require specific activation mechanisms afterwards to make translation possible. It is now clear that both processes take place and involve mRNA binding proteins. Most of the sequences recognized by RNA-binding proteins have been identified within 3' untranslated region (3' UTR) of responsive transcripts. Experimentally, a proposed scenario has been proven for mRNA-binding protein zip-code binding protein 1 (ZBP1). This RNA-binding protein binds in the nucleus to a 54 nucleotide "zipcode" in the 3' UTR of beta-actin mRNA, translocates to the cytoplasm and acts to repress translation, possibly by preventing the interaction of the 40S and 60S ribosomal subunits. Although the above mechanism has been described for neuroblastoma cell line, partial colocalization of ZBP1 and beta-actin mRNAs in dendrites (Table I) suggests that such a mechanism may also take place in neurons (Tiruchinapalli et al 2003, see Wells 2006).

Although the detailed description of the state of the art on regulation of transcription and translation steps related to dendritic protein synthesis is beyond the scope of this review (it is the subject of excellent and extensive reviews recently published, e.g. Wells 2006, Bramham and Wells 2007) two important sets of data have to be mentioned. Firstly, there are already several members of the heterologous nuclear ribonucleoprotein family of proteins (represented here by ZIP1) identified, including hnRNP A2 and Fragile X mental retardation protein (FMRP), which have been shown to shuttle between nucleus and cytoplasm in neurons and to be transported into dendrites. Secondly, there is the other protein family of RNA-binding proteins, represented by Staufen 1 and 2 proteins. Disruption of ZBP 1 and 2, and Staufen, impairs localization of targeted RNA, suggesting a critical role of these classes of proteins as molecular adapters between cis-acting mRNA localization sequences and microtubule-based transport machinery (Zhang et al. 2001). A striking example of consequences of disturbances in regulation of the cellular machinery involved in mRNA targeting

Table I

mRNAs that have been shown to be localized within dendrites of neurons *in vivo* by *in situ* hybridization. Not shown are mRNAs that are localized only in the most proximal segments of dendrites.

mRNA	Cell type	Localization in dendrites	Class of protein	Protein function	References
MAP-2	Cerebral cortex, Hippocampus proper, Dentate gyrus	proximal one third to one half	cytoskeletal	microtubule-associated	Garner et al. 1988 Blichenberg et al. 1999
CaMKII alpha subunit	Cerebral cortex, Hippocampus proper, Dentate gyrus	throughout	soluble	multifunctional kinase (e.g. catalysis of AMPA, NMDA and CPEP phosphorylation), Ca ²⁺ signaling	Burgin et al. 1990 Steward 1977 Ouyang et al. 1997, 1999 Bagni et al. 2000
Arc/Arg 3.1, inducible	Cerebral cortex, Hippocampus proper, Dentate gyrus (depending on stimulus)	throughout (when induced)	cytoskeleton associated	actin binding, synaptic junctional protein (regulates maintenance/consolidation of LTP, BDNF activated)	Lyford et al. 1995 Steward et al. 1998, 2001
Dendrin	Cerebral cortex, Hippocampus proper, Dentate gyrus	throughout	putative membrane protein	non identified	Herb et al. 1997
G-protein gamma subunit	Cerebral cortex, Hippocampus proper, Dentate gyrus, striatum	throughout	membrane-associated	metabotropic receptor signaling (LTD?)	Watson et al. 1994
calmodulin	Cerebral cortex, Hippocampus proper, cerebellum (Purkinje cells)	proximal-middle (during synaptogenesis)	cytoplasmic and membrane associated	Ca ²⁺ signaling in conjunction with CamKII	Berry and Brown 1996
NR1 subunit of NMDA receptor	Dentate gyrus	proximal-middle	integral membrane	ionotropic receptor signaling, associated with Ca ²⁺ channel	Benson 1997, Gazzaley et al. 1997
Alpha subunit of Gly receptor	Spinal cord motoneurons	proximal	integral membrane	inhibitory, glycinergic signaling	Racca et al. 1997
Vasopressin	Hypothalamo-hypophyseal axis	proximal-middle	soluble neuropeptide	regulates blood pressure	Prakash et al. 1997
Neurofilament protein 6	Vestibular neurons	proximal-middle	cytoskeletal	neurofilament	Paradies and Steward 1977

L7	Cerebellum, Purkinje cells	throughout	cytoplasmic?	homology to cis-PDGF signaling	Bian et al. 1996
Receptor IP3	Cerebellum, Purkinje cells	throughout (concentrated proximally)	integral membrane (endoplasmic reticulum)	Ca ²⁺ signaling	Bannai et al. 2004

is a mutation in *fmrl* gene, encoding FMRP, which leads to loss of FMRP mRNA binding ability; it may result in mental retardation known as fragile X syndrome (Jin and Warren 2000).

INITIATION AND CONTROL OF TRANSLATION IN DENDRITES

If mRNAs translation is to be initiated in their target place, derepression of dormant mRNAs has to take place. There are at least two mechanisms identified, by which translation of specific mRNAs can be regulated in neurons. The first is through stabilization of the message. The best described example is that of ELAV-like protein HuD, which binds to a 26-nucleotide AU-rich element in the 3-UTR of the mRNA encoding growth-associated protein (GAP-43) and stabilizes the mRNA. The biological consequences of HuD-induced stability of GAP-43 have been described recently, showing its role in spatial memory formation in hippocampus (see Wells et al. 2006). The second mechanism, especially important for the process of dendritic translation, involves a derepression of mRNAs that are maintained in a translationally dormant state; well characterized protein of this type to be described in neurons is the cytoplasmic polyadenylation element binding protein CPEP (Huang et al. 2003, for further review see Wells 2006). Studies on this protein provided data indicating that RNA-binding proteins may play a dual role: facilitation of mRNA targeting to dendrites, and triggering “on-site” polyadenylation and translation in response to external signals (Atkins et al. 2004, Shin et al. 2004). CPEB undergoes regulation by phosphorylation. In *Xenopus* oocytes and in neurons, CPEB can be regulated by the Aurora kinase, whereas Atkins showed that in hippocampal neurons CPEB is phosphorylated by alpha calcium/calmodulin-dependent protein kinase II (α CaMKII) *in vitro* and in postsynaptic density fractions, providing evidence that α CaMKII can constitute a regulator of dendritic protein synthesis (Atkins et al. 2004).

Activity-dependent translation, as postulated for localized protein synthesis from localized mRNAs, likely occurs *via* activation of the general protein synthetic machinery in dendrites and release from repression of specific mRNAs. mRNA translation is generally divided into three steps: initiation, elongation, and termination. There is evidence that suggests local translation in neurons is regulated at the initiation step.

In eukaryotic cells, synthesis of most proteins is driven by cap-dependent mRNA translation, which requires the interaction of eukaryotic initiation factor (eIF) 4 F complex with the 5'-m⁷G-cap (see Fig. 1). eIF4F recruits the preinitiation complex, including 40S ribosomal subunit, to the 5'-UTR for the AUG start codon. Here, part of the initiation complex is dissolved and the 60S ribosomal subunit joins the 40S subunit to form a 80S ribosome which is translationally competent. Such a translational control, occurring via MAPK signaling, that enhances phosphorylation of the eIF4F and related 4E-BP protein, has been described by Kelleher and coauthors (2004) and Banko and others (2005) in the hippocampus following synaptic stimulation.

Important elements in the regulation of translation is rapamycin-dependent signaling (Klann and Dever 2004, see also Swiech et al 2008, and Urbanska et al. 2008, this issue) and small non-messenger RNAs (snmRNAs) the role of which is only emerging. This heterogeneous group of non-coding RNAs has a variety of regulatory functions including regulation of protein expression and guidance in RNA modifications. Brain-specific snmRNAs have been identified in mammals and they seem to contribute to brain functions subserving learning and memory. Among them are micro RNAs (miRNA) abundant in neurons (125 different miRNAs were identified in brain) candidates for repression over specific mRNA (Ashraf and Kunes 2006, Ashraf et al. 2006).

Although many of the molecular players involved in compartmentalized protein synthesis are being elucidated, how these mRNA-binding proteins interact and cooperate is far from full understanding.

THE SEARCH FOR DENDRITIC mRNAs

With improvement of sensitivity of older methods, and the advent of new mRNA amplification techniques, an increasing number of mRNAs have been identified in neuronal dendrites (Table I). Initially, *in situ* hybridization was used to localize mRNAs to dendrites. These experiments were performed by incorporating radioactive nucleotides into cDNA or cRNA probes followed by their hybridization to tissue sections or harvested cells and subsequent emulsion dipping. To visualize the signal exposure, times of weeks and even months were often required. Because of the limitations of this methodology, only the top of an iceberg could be bared and only a handful of mRNAs, like MAP2 (Garner et al. 1988) and α CamKII (Burgin et al. 1990), have been identified. By combining new mRNA amplification methodologies with single dendrite analysis, the group of Eberwine (Miyashiro et al. 1994, Crino and Eberwine 1996) have been able to identify over 100 mRNAs in dendrites of cultured neurons, although far fewer such mRNAs have been experimentally confirmed in adult non-cultured brain neurons (Eberwine et al. 1992, Miyashiro et al. 1994, Crino and Eberwine 1996, Ju et al. 2004). Out of these studies the picture emerges of multiple functional classes of dendritic mRNAs, including various cytoskeletal proteins like β -actin, MAP2 and Arc (Link et al. 1995, Lyford et al. 1995), those encoding cytoplasmic regulatory proteins such as α CamKII alpha (Ca-calmodulin dependent protein kinase, present predominantly in dendrites) and integral membrane signaling proteins: glutamate NMDA receptors (Miyashiro et al. 1994), AMPA GluR1 and GluR2 receptor subunits (Ju et al. 2004, Grooms et al. 2006), GABA receptors and Ca^{v2} channels (Crino and Eberwine 1996) and their scaffolds such as GRIP1 and PSD-95 (Schratt et al. 2004, Lee et al. 2005). More unexpectedly, dendrites also contain mRNAs of secretory proteases of extracellular matrix: tissue plasminogen activator (tPA) and metalloproteinase-9 (MMP-9) (Nagy et al. 2006, Konopacki et al. 2007) (see Table II). It becomes less surprising if we take into account recent data showing that an important function of extracellular tPA is the proteolytic conversion of proBDNF to mature BDNF a neurotrophic factor, which is considered crucial for LTP consolidation (Pang et al. 2004), whereas MMP-9 modulates synaptic efficacy in the hippocampus through the activation of integrin receptors, which were indirectly implicated in

actin polymerization that underlies LTP consolidation (Kramar et al. 2006).

NEWLY SYNTHESIZED PROTEINS LOCALIZE TO DENDRITES

Although the described investigations indicated that local protein synthesis can occur, until the year 2001 there was no unequivocal evidence for it. Pioneering studies in this field were carried out with the use of radioactive leucine, labeled with tritium [³H] (Kiss 1977). The ³H-leucine was injected to brain ventricles of anesthetized animals and localization of proteins that incorporated radioactive leucine was determined shortly afterwards with autoradiography. The results did show presence of newly synthesized proteins in dendritic compartment, but the experiment did not elucidate the origin of the labeled protein and thus a possibility that proteins were transported from the cell body compartment could not be excluded (Kiss 1977).

A vast majority of further studies, aimed to provide evidence of local protein synthesis and to document its role in long-term synaptic plasticity, was carried out *in vitro* (Rao and Steward 1991, Weiler and Greenough 1993, Weiler et al. 1994). They were based on the analysis of incorporation of radiolabeled amino acids to newly synthesized proteins in synaptoneurosomal preparations or in isolated dendrites (Rao and Steward 1991, Torre and Steward 1992, 1996, Weiler and Greenough 1993, Weiler et al. 1994, Crino and Eberwine 1996). When dendritic processes were first separated from cell bodies and then the isolated dendrites were incubated with radiolabeled leucine or transfected with mRNA, there was a strong labeling of proteins, indicating that local protein synthesis takes place (Torre and Steward 1992, 1996, Crino and Eberwine 1996). That latter technique, designed and worked out by Oswald Steward (Torre and Steward 1992), was not devoid of drawbacks: there was a risk of contamination with non-dendritic fractions, the dendrites were taken out of the physiological context, and biochemical analyses excluded the possibility to follow the changes in function of time. A common limitation of these approaches was also a possibility to contaminate dendrites with glial cell fibers (Henn et al. 1976). Therefore these studies faced the criticisms, that the observed events are artifacts. More convincing results were provided later on by the laboratories of Cattaneo (Tongiorgi et al. 1997) and Schuman

Table II

Other mRNAs identified in dendrites of mammalian neurons without use of mRNA amplification techniques. Respective RNA binding proteins are also shown.

mRNA	Cell type	Class of protein	Protein function	RNA binding protein	References
Beta-actin	hippocampus	cytoskeletal	cytoskeletal	Zip-code-binding protein 1 (ZBP1)	Tiruchinapalli et al. 2003
FMR1	hippocampus			Fragile-X mental retardation protein FMRP	Weiler et al. 1997, Feng et al. 1997
BDNF	Cerebral cortex, hippocampus	soluble	neurotrophic		Dugich-Djordjevic et al. 1992, Wetmore et al. 2004, Crino and Eberwine, 1996, Tongiorgi et al. 1997
TrkB receptor	hippocampus	integral membrane	conveys neurotrophin (BDNF/NT-4) signaling		Tongiorgi et al. 1997, 2004
Rho subunits of GABA C receptor	spinal cord motoneurons	integral membrane	conveys GABAergic signaling		Rozzo et al. 2002
Glu R1/Glu R2 AMPA receptor subunits	hippocampus	integral membrane	conveys glutamatergic signaling	CPEB 3	Ju et al. 2004
Tissue plasminogen (tPA)	hippocampus	soluble	proteolysis	CPEP 1	Shin et al. 2004
Matrix metalloproteinase MMP-9	hippocampus	soluble	proteolysis		Nagy et al. 2006, Konopacki et al. 2007

(Ouyang et al. 1999). The Cattaneo group showed that stimulation of hippocampal neurons with high KCl concentrations results in a significant increase of dendritic level of mRNAs for two proteins: BDNF neurotrophin and BDNF high affinity receptor, TrkB, both involved in modulation of excitatory neurotransmission, and related to synaptic plasticity (see Macias 2008, this issue). They also showed that accumulation

of dendritic BDNF mRNA and TrkB mRNA, found predominantly in distal segments of dendritic shafts, was accompanied by an increase of BDNF and TrkB proteins (Tongiorgi et al. 2004). Importantly, the experiments on the effect of electrical stimulation of hippocampal neurons on compartmental concentration and distribution of α CaMKII (Steward 1997, Ouyang et al. 1999), revealed a strong increase of

α CaMKII protein in CA1 dendritic fields as early as 5 min after tetanic stimulation of Schaffer collaterals (fibres projecting from hippocampal CA3 to CA1 dendritic fields). That increase occurred at a distance of 100–200 μ m away from a cell body (Ouyang et al. 1999). Since the transport of α CaMKII from cell body to dendrites is too slow to explain fast increase of protein level in distal parts of dendrites, this result could be interpreted, with high probability, as a demonstration of dendritic protein synthesis. But a real breakthrough in the field were next experiments carried out by Erin Schuman.

THE KEY EVIDENCE: EXPERIMENTS FROM SCHUMAN LABORATORY

BDNF stimulates synthesis of GFP reporter protein

In 2001, the group of Erin Schuman published results on the fascinating experiment carried out on the isolated hippocampal neurons and dendrites (Aakalu et al. 2001). To study local protein synthesis in hippocampal dendrites, a construct of protein synthesis reporter in which destabilized coding sequence of green fluorescent protein (GFP) is flanked by the 5' and 3' untranslated regions from CAMKII- α subunit, conferring both dendritic mRNA localization and translational regulation (Mayford et al. 1996, Mori et al. 2000) was developed. GFP was used as a reliable, fluorescent reporter of protein synthesis. Transfection of cells with this construct allowed to follow changes in localization and intensity of GFP fluorescence with two-photon microscopy, to assess GFP protein synthesis following stimulation.

Neurons were isolated from the brains of 3 days-old pups, and kept in culture for 14–21 days, until they revealed a mature phenotype. Six hours after transfection of mature neurons with GFP-UTRCaMKII α construct, GFP protein synthesis was estimated and the compartments of GFP localization were identified. First, the synthesis of the reporter protein was evaluated after pharmacological stimulation of neurons with BDNF. As already mentioned, BDNF is a neurotrophic protein, produced by numerous classes of neurons; hippocampal neurons produce BDNF and respond to it. The experiment showed that neurons exposed to BDNF revealed an increase in GFP fluorescence 60 min after exposure; an increase in fluorescence intensity and GFP

distribution along dendrites amounted to 60%, when estimated for the whole volume of dendritic processes, and occurred simultaneously in cell perikarya and in distal dendrites. That result strongly suggested protein synthesis in both compartments, but did not exclude dendritic translocation of the newly synthesized proteins to the dendrites. What was intriguing, the image analysis revealed the occurrence of “hot spots”, which were distributed along dendritic shafts and demonstrated much stronger fluorescence signal, up to 8 times higher from the average. This pointed to the possibility that dendrites possess regions of extensive, local protein synthesis! Going the step further by using inhibitors of protein synthesis which blocked those effects, the authors proved that the changes observed were indeed the consequence of protein synthesis.

Mechanically isolated dendrites

To obtain the final proof of local dendritic synthesis, in the next step neuronal perikarya were separated from dendrites mechanically, with a use of glass microelectrodes. This procedure had to be carried out thoroughly not to cause functional impairment and neuronal death; otherwise the result could be an artifact. Therefore, before each experiment, the preparations were carefully checked, and propidium iodide was used to eliminate dead neurons. During a 2-year study, out of 300 neurons isolated and analysed there were only 10 which met the criteria (Aakalu et al. 2001). The assays carried out on these 10 neurons did prove, that dendrites cut off and separated from cell bodies are able to synthesize GFP protein under BDNF stimulation. At the same time Job and Eberwine (2001) provided evidence that dendrites, cut off and transfected subsequently with mRNA GFP, carry on protein synthesis under stimulation with glutamate.

“Optically” isolated dendrites

Since mechanical isolation of dendrites proved technically difficult and may cause functional impairment increasing in time postisolation, the approach is not optimal to investigate local protein synthesis. In the next step Schuman used an alternative approach. A version of the reporter which contained myristoylation consensus sequence that anchored the fluorescent protein to the cell membrane was created, and dendrites

were isolated optically from cell bodies by photobleaching perikarya for 1h with laser light of high energy. This way photobleaching destroys the component of protein signal derived from cell body, strongly reducing or eliminating contamination of dendritic signal, whereas newly synthesized GFP protein remains *in situ* owing to its myristoil anchor. In the designed experiment BDNF caused an immediate and strong (17×) increase of GFP translation in optically isolated dendrites.

Repeated imaging of dendrites led to a precise identification of regions where a strong increase of mRNA translation took place, and revealed that “hot spots” of protein synthesis waxed and waned consistently in the same place in dendrites for several hours. Immunocytochemical mapping of these spots revealed that they localize precisely at the base of dendritic spines and often colocalize with synaptic proteins. That intriguing observation pointed to an extensive protein synthesis addressed preferably to neighboring synapses (Aakalu et al. 2001).

As already described BDNF stimulates protein synthesis in dendrites and is synthesized in this compartment of stimulated neurons in culture (Aakalu et al. 2001). More recent data provided evidence that BDNF accumulates in dendrites also *in vivo*, following kindling or pharmacological treatment with pilocarpine or kainate, that cause a strong stimulation of NMDA receptors in hippocampal neurons and evoke seizures (Tongiorgi et al. 2004). Dendritic accumulation of BDNF has to be, in this case, explained by dendritic targeting of a protein synthesized in a cell body, as well as of its local synthesis.

DENDRITIC PROTEIN SYNTHESIS IS REQUIRED FOR THE PERSISTENCE OF SYNAPTIC CHANGE

Activity-dependent synaptic changes are a mechanism by which the brain encodes and stores information. As already mentioned at the introductory remarks, the most well established models for activity-dependent strengthening and weakening are LTP and LTD. Although early studies on molecular background of these processes demonstrated a role of protein transcription and translation in the maintenance of the late phase of LTP, it is now evident that intermediate stages of late LTP are maintained by new protein synthesis in dendrites which is independent of transcription. Moreover the findings that LTD also requires

protein synthesis indicate that synaptic change is maintained, regardless of the polarity, by new protein synthesis. It is intriguing, at what level the specificity for synthesizing “LTP” or “LTD” proteins is determined. Pfeiffer and Huber (2006) discuss two hypotheses, which are under experimental verification: either distinct patterns of synaptic activity regulate distinct signaling cascades and translation machinery as well as RNA-binding proteins, which target specific mRNAs for translation, or both LTP-specific and LTD-specific proteins are translated but the specific pattern of stimulation determines, which proteins are used or captured. Current evidence indicates a particular role for LTP and LTD of newly synthesized proteins in ionotropic glutamate receptor trafficking (Fig. 1).

DENDRITES ARE ALSO SITES OF LOCAL PROTEIN DEGRADATION

If local protein synthesis contributes to plasticity of synapses that involves their strengthening and weakening, there should be a local machinery that down regulates synthesis and mediates fast degradation of proteins. Indeed, Steward and Schuman (2003) and Bingol and Schuman (2006) provided evidence that there is a local protein degradation and that synaptic stimulation *via* NMDA (N-methyl-D-aspartate) receptor causes redistribution of proteasomes from dendritic shafts to synaptic spines. Thus these large multi-unit cellular machines that recognize, unfold and degrade ubiquitinated proteins become available subsynaptically. Again, using restricted photobleaching of individual spines and dendritic shafts, they showed that activity modestly enhances the entry rate of proteasomes into spines, while dramatically reducing their exit rate (Bingol and Schuman 2006). Proteasome sequestration is persistent, reflecting an association with the actin-based cytoskeleton. Together, data indicate that synaptic activity can promote the recruitment and sequestration of proteasomes to locally remodel the protein composition of synapses. A novel form of translational regulation in dendrites that may also contribute to synaptic remodeling is an already mentioned repression of the local synthesis of protein with small noncoding RNAs. A recent study from Greenberg laboratory (Schratt et al. 2006) has shown that miR-134, a brain-specific microRNA, is present in dendrites and represses the local synthesis of the protein kinase

Limk1, that controls spine development. Again, BDNF reveals its potential showing that exposure of neurons to BDNF relieves miR-134 inhibition of Limk1. These new data disclose dendritic potential to control protein content in the synapse.

ROLE OF DENDRITIC PROTEIN SYNTHESIS IN HOMEOSTATIC PLASTICITY: SYNAPTIC SCALING?

Synaptic scaling is a form of homeostatic plasticity (Turrigiano and Nelson 2004), that scales synaptic strengths up or down to compensate for prolonged changes in activity. The idea is relatively new and local protein synthesis is a good candidate to be involved, but there are not many data available that could prove it. Wierenga and coworkers (2005) showed that after 1 or 2 d of activity blockade at excitatory synapses in visual cortical cultures, there was a significant increase in postsynaptic accumulation of synaptic AMPA receptors via proportional increases in glutamate receptor 1 (GluR1) and GluR2. Time-lapse imaging of enhanced GFP-tagged AMPA receptors revealed that receptor accumulation increased progressively over 2 d of activity blockade and affected the entire population of imaged synapses. The strength of synaptic connections between pyramidal neurons was more than doubled after activity blockade. Schuman group reported after a prolonged blockade of action potentials alone, that exposed dendrites show diminished protein synthesis (Sutton et al. 2004). When miniature excitatory synaptic events (minis), which do not lead to action potential, were blocked additionally to action potential blockade, local protein synthesis was enhanced. This suggests that minis inhibit dendritic translation and stabilize connections. Furthermore, when minis were acutely stimulated or blocked, an immediate decrease or increase, respectively, in dendritic translation was observed. Together these results reveal a role of miniature synaptic events in the acute regulation of dendritic protein synthesis.

Activation of hippocampal neurons with dopamine, which involves binding to D1–D5 receptors, also stimulates local protein synthesis. As a consequence an increase of AMPA GluR1 subunit synthesis and membrane insertion was reported. (Smith et al. 2005). This mechanism may lead to awaking silent synapses, exemplifying the use-dependent modification of synapse and an increase of synapse sensitivity.

DENDRITIC AUTONOMY

The discovery of differential subcellular RNA distribution patterns in neurons has widened the view of the determinants of neuronal polarity. Before, selective cytoplasmic organelle and protein targeting have long been thought to constitute the sole determinants of this feature. Dendritic spines in particular, are discrete structural, physiological and biochemical compartment; the presented studies demonstrated that dendritic spines afford a necessary degree of autonomy during information processing and storage. mRNA for a glutamate receptor subunit is found in subsynaptic regions of the fly neuromuscular junction providing an example that the local control of glutamate receptor synthesis is an evolutionary conserved mechanism (Sigrist et al. 2000). The question may be asked to which extent dendrites are autonomous in their functioning and what their autonomy relies on? One of the answers point to a limited number of the mRNAs operating there: while all of dendritically localized mRNAs are also present in the cytoplasm of neurons, the bulk of cytoplasmically localized mRNAs have not been detected in dendrites. Obviously, provided evidence of local protein synthesis does not exclude the possibility that a vast majority of proteins which are not involved in fast synaptic changes is synthesized within cell body and contribute to protein content of dendritic compartment.

Novel dendritic mRNAs do not change much with this respect: those described first and detected repeatedly in number of studies, are privileged to form most probably the core of messages indispensable in dendrite fast responses and plasticity.

The observations by Malenka group (Ju et al. 2004) that over 95% of physically isolated dendrites exhibited local synthesis of GluR1 and GluR2 and some surface expression of AMPAR occurred in synaptic regions of these preparations suggests that ER, Golgi and associated components of the secretory pathway are ubiquitously expressed in dendrites (at least in cultured neurons) and that retrograde transport from dendritic ER to somatic Golgi may not be required for the processing of integral membrane proteins that are synthesized in dendrites (Ju et al. 2004).

CONCLUSIONS

Local protein synthesis in dendrites is now a well documented phenomenon, and available data suggest

that it is involved in plasticity of fundamental nature. However many questions, important for our understanding of the modulation of dendritic events remain unanswered. A crucial question concerns the translation limiting factors. As there are not many ribosomes at an individual synapse, the number of polyribosomes seem to be such a limiting factor in protein translation. How is that limited machinery allocated to the different mRNA potentially available for translation and is it running a full capacity, or can be modulated, remain to be elucidated. A theme that only emerges is the possibility of reciprocal interactions between actin dynamics and dendritic protein synthesis in the control of synaptic plasticity. A major goal of current research into dendritic protein synthesis is to determine how dendritically synthesized proteins cooperate to regulate synaptic efficacy.

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