Localization of mRNAs at Synaptic Sites on Dendrites

Oswald Steward[1] and Paul Worley[2]

1 Introduction

It is becoming increasingly clear that an important aspect of gene expression in neurons involves the delivery of mRNAs to particular subcellular domains. Specifically, although the majority of the mRNAs that are expressed by neurons are found only in the neuronal cell body, a select population of mRNAs are transported into dendrites, and certain neurons also transport mRNA into axons. The nature and significance of this RNA targeting is now under intense investigation, and it is clear that mRNA targeting plays a key role in several aspects of neuronal function.

The present chapter focuses on one aspect of RNA localization in neurons – the localization of protein synthetic machinery and particular mRNAs at synaptic sites on dendrites. We will briefly summarize what is known about the protein synthetic machinery that is localized at synapses, update the list of mRNAs that have been shown to be present in dendrites, and consider some of the key principles regarding dendritic mRNAs. We will focus especially on recent evidence regarding the mechanisms underlying mRNA sorting, transport, and selective localization at synapses as revealed by studies of the immediate early gene *Arc*. Finally, we will consider new information about the role that local translation plays in synaptic function.

2 The Machinery for Translation in Dendrites

The story regarding the targeting of mRNAs to dendrites had its roots in the discovery of synapse-associated polyribosome complexes (SPRCs). SPRCs are polyribosomes and associated membranous cisterns that are selectively localized beneath postsynaptic sites on the dendrites of CNS neurons (Steward and Levy 1982; Steward 1983; Steward and Fass 1983). Although it had long been known that polyribosomes were present in dendrites, these studies in the early 1980s were the first to note and document the highly selective localization

[1] Reeve-Irvine Research Center, Departments of Anatomy/Neurobiology and Neurobiology and Behavior, College of Medicine, University of California at Irvine, Irvine, CA 92697, USA
[2] Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

Results and Problems in Cell Differentiation, Vol. 34
D. Richter (Ed.): Cell Polarity and Subcellular RNA Localization

beneath synapses. These studies also laid out two key working hypotheses: (1) that the machinery might synthesize key molecular constituents of the synapse and (2) that translation might be regulated by activity at the individual postsynaptic site. Studies over the last 18 years have confirmed and extended these hypotheses, and a coherent story regarding local protein synthesis at synaptic sites is now beginning to emerge. In considering this story, it is useful to begin by summarizing some of the key features of SPRCs.

2.1 SPRCs Are Precisely Localized in the Postsynaptic Cytoplasm

A key feature of SPRCs is the selectivity of their localization. Quantitative electron microscope analyses have revealed that the vast majority of the polyribosomes that are present in dendrites are *precisely* positioned beneath postsynaptic sites, and are absent from other parts of the dendrite (Steward and Levy 1982). SPRCs are most often localized at the base of the spine in the small mound-like structures from which the neck of the spine emerges. Thus, SPRCS are located within or near the portal between the spine neck and the shaft of the dendrite – the route through which current must flow when spine synapses are activated. In this location, SPRCs are ideally situated to be influenced by electrical and/or chemical signals from the synapse as well as by events within the dendrite proper. An important implication of this selective localization is that there must be some mechanism that causes ribosomes, mRNA, and other components of the translational machinery to dock selectively in the postsynaptic cytoplasm. The mechanisms underlying this highly selective localization remain to be established.

Although most dendritic polyribosomes are localized beneath synapses, a few clusters of ribosomes are localized within the core of the dendritic shaft. It is not yet known whether these represent a different population than the synapse-associated polyribosomes. One possibility is that the polyribosomes in the dendritic core are associated with mRNAs that encode proteins that are not destined for synaptic sites, but which play some other role in dendritic function. This speculation is of particular interest given the functional diversity of the mRNAs that have been identified in dendrites (see below). Alternatively, the clusters of polyribosomes in the core of the dendrite may represent packets of mRNAs and ribosomes that are in transit from the cell body.

2.2 SPRCs Are Present at Spine Synapses on Different Neuron Types

Quantitative analyses of polyribosomes have been carried out on dentate granule cells, hippocampal pyramidal cells, cortical neurons, and cerebellar Purkinje cells. These analyses reveal that SPRCs are present in a roughly similar configuration in all of the spine-bearing neurons that have been evaluated.

Estimates of the incidence of polyribosomes at spine synapses vary depending on the quantitative methods used. In evaluations of single sections, about 11–15% of the identified spines have underlying polyribosomes (Steward and

Levy 1982). However, this is clearly an underestimate because not all of the area under a spine is contained within a single section. For example, serial section reconstructions of dendrites in the dentate gyrus reveal that the actual incidence of polyribosomes in spines on mid proximo-distal dendrites is about 25% (Steward and Levy 1982).

The estimates of incidence also depend on the counting criteria. Studies that have used serial section reconstruction techniques to evaluate the distribution of individual ribosomes (not polyribosomes) yield higher estimates of incidence (Spacek and Hartmann 1983). For example, in pyramidal neurons in the cerebral cortex, 82% of the reconstructed spines had ribosomes in the head, 42% had ribosomes in the neck, and 62% had ribosomes at the base. In cerebellar Purkinje cells, 13% of the spines had ribosomes in the head and 22% had ribosomes at the base. It is likely that an important reason for the higher incidence values in this study is that singlet ribosomes were counted along with polyribosomes. In any case, it is clear that polyribosomes are a ubiquitous component of the postsynaptic cytoplasm in a variety of neuron types.

2.3 SPRCs Are Often Associated with Membranous Organelles in an RER-Like Configuration

Serial section reconstructions of dendrites of dentate granule cells and hippocampal pyramidal cells have revealed that about 50% of the polyribosomes are associated with tubular cisterns (Steward and Reeves 1988). A common configuration is one in which the ribosomes surround a blind end of a cistern. These configurations suggested that the SPRC/cisternal complex may be a form of RER that could allow the synthesis of integral membrane proteins or soluble proteins destined for release. In support of this hypothesis, recent studies have provided evidence for RER-associated proteins and also proteins of the Golgi apparatus in dendrites, some of which appear to be localized in these subsynaptic cisterns (see below).

Interestingly, the cisterns with which SPRCs are associated are sometimes connected with a spine apparatus (Steward and Reeves 1988). The significance of these connections is not known. One interesting hypothesis is that the spine apparatus may involved in some aspect of post-translational processing of proteins that are synthesized at SPRCs. So far, however, there is no definitive evidence in support of this hypothesis.

2.4 SPRCs Are Also Present at Non-Spine Synapses

There have been no detailed quantitative evaluations of polyribosome distribution in the dendrites of non-spiny neurons. Nevertheless, it is clear that the same basic relationships exist as in spiny dendrites. For example, polyribosomes are often present beneath shaft synapses in association with submembranous cisterns, and are found beneath both asymmetric (presumed excitatory) and symmetric (presumed inhibitory) synapses.

Polyribosomes are also localized beneath synapses on axon initial segments (Steward and Ribak 1986). This localization is noteworthy for two reasons. First, it extends the generality of SPRCs to yet another type of postsynaptic location. Second, most (perhaps all) synapses on axon initial segments use GABA as their neurotransmitter, and are thus inhibitory.

It is noteworthy that axon initial segments also contain organelles that appear identical to spine apparatuses. When in the axon, such organelles are termed cisternal orgenelles. Because of the similarity in appearance between spine apparatuses and cisternal organelles beneath synapses on axon initial segments, we suggest a new term that would apply to both – *subsynaptic cisternal organelles*. Based on the localization of subsynaptic cisternal organelles beneath both excitatory and inhibitory synapses on axon initial segments, it may be worthwhile to reconsider the possible functions of these enigmatic organelles. This is especially true because previous hypotheses have focused on functions that would be especially important at excitatory synapses and perhaps of minimal importance at inhibitory synapses (for example Ca^{2+} sequestration).

2.5 SPRCs Are Especially Prominent at Developing Synapses

If protein synthetic machinery is localized at synapses in order to synthesize some of the components of the synaptic junction, one would expect SPRCs to be especially prominent at synapses during periods of synapse growth. This is the case. Polyribosomes are very abundant in the dendrites of developing neurons, and again appear to be preferentially localized beneath postsynaptic sites, although the degree of selectivity has not been evaluated quantitatively (Steward and Falk 1986).

3 Types of Proteins That Are Synthesized at SPRCs

The discovery of SPRCs raised the question of what proteins were synthesized in the postsynaptic cytoplasm. The approaches that have been used to address this question include:

1. Biochemical studies of proteins synthesized by subcellular fractions enriched in pinched-off dendrites;
2. In situ hybridization analyses of the subcellular distribution of mRNAs in neurons; and
3. Molecular biological analyses of the complement of mRNAs in isolated dendrites from immature neurons grown in culture.

3.1 Biochemical Studies of Proteins Synthesized by Subcellular Fractions Enriched in Pinched-Off Dendrites

Biochemical approaches take advantage of subcellular fractionation techniques that allow the isolation of synaptosomes with attached fragments of

dendrites that retain their cytoplasmic constituents, including polyribosomes and associated mRNAs. These fractions were initially called heavy synaptosomes (Verity et al. 1980), but we prefer the term synaptodendrosomes to emphasize the fact that the fractions contain pinched-off dendrites (Rao and Steward 1991a). Others have used similar fractions prepared by filtration, which are termed synaptoneurosomes (Weiler and Greenough 1991, 1993; Weiler et al. 1997).

The major limitation in using synaptodendrosome or synaptoneurosome fractions to study dendritic protein synthesis is that the fractions are contaminated with fragments of neuronal and glial cell bodies. For example, high levels of the mRNA encoding glial fibrillary acidic protein are present (Chicurel et al. 1990; Rao and Steward 1991b), and it is likely that there are also fragments of neuronal cell bodies that contain mRNAs that are normally not present in dendrites.

One way to circumvent the problem of contamination is to focus on proteins that are synthesized in these fractions and then assembled into synaptic structures. For example, when synaptosomes are incubated with [^{35}S]-methionine, a number of protein species become labeled. Subcellular fractionation and detergent extraction techniques can then be used to prepare synaptic plasma membranes and fractions enriched in synaptic junctional complexes (the postsynaptic membrane specialization and associated membrane). Polyacrylamide gel electrophoresis combined with fluorography can then be used to reveal the proteins that were synthesized within the synaptodendrosomes and that had become associated with synaptic plasma membrane and synaptic junctional complex. This strategy has revealed characteristics of the labeled bands (Rao and Steward 1991a; Leski and Steward 1996), but so far, the approach has not provided definitive identification of the proteins. This combined strategy also has the limitation that it is only useful for proteins that are assembled into the synaptic membrane or synaptic junctional complex. Thus, proteins that are not assembled into the synapse are not detected.

Studies using synaptoneurosome fractions without the secondary purification step of subcellular fractionation have provided evidence for the dendritic synthesis of one novel protein that had not previously been identified – fragile X mental retardation protein (FMRP; Weiler et al. 1997). FMRP is encoded by a gene called *fmr1*, which is affected in human fragile X syndrome. Treatment of synaptoneurosomes with agonists for metabotropic glutamate receptors cause a rapid increase in the amount of FMRP in the synaptoneurosome fractions by Western blot analysis. These data suggested that FMRP was being synthesized within the fractions, and that the synthesis was enhanced by mGluR activation. This evidence has led to the interesting idea that the neuronal dysfunction that is part of fragile X syndrome may result from a disruption of local synthesis of protein at synapses (Comery et al. 1997; Weiler et al. 1997).

There are some inconsistencies in the story regarding FMRP, however. In the first place, FMRP mRNA does not appear to be present in dendrites at detectable levels (Hinds et al. 1993; Valentine et al. 2000). One can conceive of