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A functional equivalent of endoplasmic reticulum and Golgi in axons for secretion of locally synthesized proteins

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ABSTRACT

Subcellular localization of protein synthesis provides a means to regulate the protein composition in far reaches of a cell. This localized protein synthesis gives neuronal processes autonomy to rapidly respond to extracellular stimuli. Locally synthesized axonal proteins enable neurons to respond to guidance cues and can help to initiate regeneration after injury. Most studies of axonal mRNA translation have concentrated on cytoplasmic proteins. While ultrastructural studies suggest that axons do not have rough endoplasmic reticulum or Golgi apparatus, mRNAs for transmembrane and secreted proteins localize to axons. Here, we show that growing axons with protein synthetic activity contain ER and Golgi components needed for classical protein synthesis and secretion. Isolated axons have the capacity to traffic locally synthesized proteins into secretory pathways and inhibition of Golgi function attenuates translation-dependent axonal growth responses. Finally, the capacity for secreting locally synthesized proteins in axons appears to be increased by injury.

Introduction

Concentrating proteins and other macromolecules in subcellular regions generates the polarity and unique domains of neurons. Targeting membrane and secreted proteins to these regions establishes functional domains in dendrites and axons. Cotranslational secretion of newly synthesized proteins via the rough endoplasmic reticulum (RER) and Golgi apparatus is typically used to traffic membrane and secreted proteins to the correct subcellular locale. Membrane and secreted proteins synthesized in the neuronal cell body progress through RER and *cis*- and *trans*-Golgi, ultimately ending up in vesicles that are transported into the dendrites and axons. The endoplasmic reticulum (ER) and Golgi apparatus also provide sites for post-translational modifications, including disulfide bond formation and glycosylation. This soma-centric view for trafficking of nascent proteins into secretory pathways does not take the concept of localized protein synthesis into account (Gerst, 2008).

Neuronal proteins that are synthesized locally in dendrites likely make use of the ER- and Golgi-like structures that have been detected at the base of and within dendritic spines (Pierce et al., 2001). These structures are also the likely site of protein glycosylation that has been demonstrated in isolated dendritic processes (Torre and Steward, 1996). These 'outposts' of Golgi apparatus in dendrites appear to contribute to polarization of the dendritic compartment in developing neurons, but such has not been seen in the axonal compartment (Horton and Ehlers, 2003b). Axons have been argued to be devoid of such organelles.

In recent years, it has become clear that axonal processes of neurons can also synthesize proteins (Lin and Holt, 2008). Ultrastructurally, only smooth ER-like (SER) structures have been detected in axons and no Golgi or RER has been reported (Tsukita and Ishikawa, 1976, 1979). However, some ER proteins have been detected in axons by immunolocalization (Krijnse-Locker et al., 1995; Weclewicz et al., 1998) and we have shown that ER chaperone proteins can be locally synthesized in axons of cultured rat dorsal root ganglion (DRG) neurons (Willis et al., 2005). We have recently reported that mRNAs encoding membrane and secreted proteins are present in DRG axons in culture (Willis et al., 2007), and others have shown that kappa-opioid receptor mRNA is locally translated in rodent sensory axons (Bi et al., 2006; Tsai et al., 2006). Insertion of locally synthesized proteins into the axoplasmic membrane has been detected in both invertebrate and vertebrate neurons. Spencer et al. (2000) microinjected the mRNA

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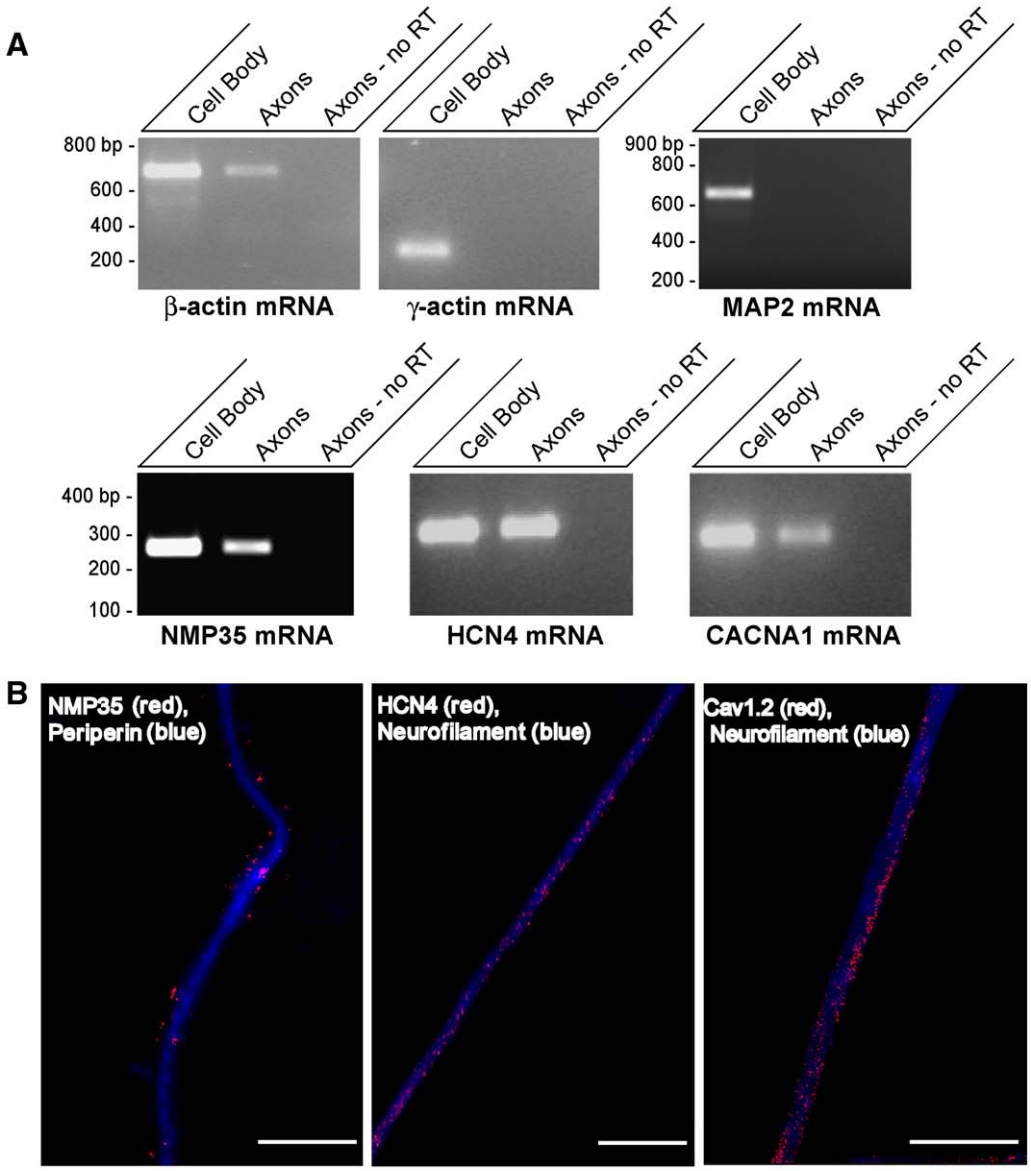

Fig. 1. DRG axons contain mRNAs encoding membrane and channel proteins. A, RNA isolated from DRG axonal and cell-body fractions was used for detection of β-actin, γ-actin and MAP2 mRNA by RT-PCR to assess purity of the axonal preparations (Willis et al., 2005). β-Actin was amplified from both cell body and axonal RNA templates, but γ-actin and MAP2 mRNAs could only be detected in the cell body RNA template. DRG axonal RNA processed for RT-PCR without the addition of reverse transcriptase shows that the β-actin PCR product detected in the axons is specific for amplification of mRNA. NMP35, HCN4, and CACNA1 primers were used for amplification from reverse transcribed cell body and axonal RNA templates. Each of the mRNAs encoding these transmembrane proteins was detected in the DRG axons. B, Immunolocalization was used to determine if proteins encoded by NMP35, HCN4, and CACNA1c ($Ca_v1.2\alpha$) are present in the sensory axons. Axonal signals for NMP35 (red) are coarsely granular and concentrated along the periphery of the peripherin (blue) in a single optical XY plane through a segment of distal axon. A reconstructed three-dimensional projection of optical planes of DRG cultures stained in HCN4 (red) is coarsely granular in the axon and is concentrated along the peripheral extents of the neurofilament (blue) signal (18 optical XY planes taken at ~0.21 μm intervals). A single optical XY plane through distal axon stained in CACNA1c (red) is also concentrated along the periphery of the neurofilament (blue) immunoreactivity. [Scale bar = 10 μm].

for a mammalian G-protein coupled receptor directly into anucleated *Lymnea* axons and showed physiologically functional receptor (Spencer et al., 2000). Brittis et al. (2002) transfected isolated axons from developing vertebrate neurons to show that an exogenous alkaline phosphatase mRNA can be locally translated and exported to the cell surface (Brittis et al., 2002). Thus, axonal processes undoubtedly have a secretory mechanism for locally synthesized proteins. However, without any ultrastructurally obvious RER or Golgi in axons the mechanism for secretion is not clear.

Axons could use non-classical mechanisms to target locally synthesized proteins for the axoplasmic membrane and secretion. Alternatively, functional equivalents of RER and Golgi apparatus are present but not recognized ultrastructurally because they lack classical morphological characteristics. To address these two possibilities, we have asked whether axonal processes have the capacity to target locally synthesized proteins to the cell surface, using cultures of DRG and retinal ganglion cells (RGC), two neuronal populations that have been shown to autonomously synthesize proteins in their axons. By immunolocalization studies, distal axons were found to contain components of the co-translational targeting machinery including: the signal recognition particle (SRP); ER proteins needed for protein translocation, folding, and post-translational modifications; and, Golgi apparatus proteins. Dyes that show enriched staining in ER and Golgi membranes also indicate that ER- and Golgi-like structures exist in the DRG and RGC axons. Using anucleated rat sensory axons for metabolic labeling (Zheng et al., 2001), axonally synthesized proteins of varying molecular weights are seen in membrane preparations from the DRG axons. The rat DRG neurons show a transition in growth capacity with