Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Research Report

Tau mRNA is present in axonal RNA granules and is associated with elongation factor 1A

Tony Malmqvist, Karen Anthony[1], *Jean-Marc Gallo*[*]

Department of Clinical Neuroscience, Centre for Neurodegeneration Research, King's College London, Institute of Psychiatry, De Crespigny Park, London, SE5 8AF, United Kingdom

ARTICLE INFO

Article history:
Accepted 24 December 2013
Available online 2 January 2014

Keywords:
Tau
mRNA
Axon
eEF1A

ABSTRACT

The microtubule-associated protein tau is predominantly localized in the axonal compartment over the entire length of the axon in neurons. The mechanisms responsible for the localization of tau in axons at long distance from the cell body are not properly understood. Using fluorescence *in situ* hybridization, we show that tau mRNA is present in the central and distal parts of the axons of cultured rat cortical neurons. Axonal tau mRNA is associated with granules which are distributed throughout the entire length of the axon, including the growth cone. We also show that tau mRNA-containing axonal particles are associated with elongation factor 1A, a component of the protein translation machinery. The presence of tau mRNA in axons might be at least part of the process by which tau is localized to distal axons.

This article is part of a Special Issue entitled RNA Metabolism 2013.

1. Introduction

The microtubule-associated protein tau is predominantly localized in the axonal compartment over the entire length of the axon. The mechanisms responsible for the axonal localization of tau are not properly understood. Tau could be synthesized in the cell body only and transported down the axon. Tau can diffuse from the cell body into the axon, however diffusion is only effective over short distances and can only explain tau presence in the proximal part of the axon (Konzack et al., 2007). Over longer distances tau is, at least in part, transported bound to microtubule fragments moving along the axon through a dynein/actin dependent mechanism (Konzack et al., 2007). Another proposed mechanism to explain the localization of tau in axons at long distance from the cell body, not mutually exclusive with co-transport on microtubule fragments, is that tau is synthesized in the axon following transport of its mRNA. A body of evidence has emerged showing that mRNAs are present and locally translated in axons, in particular during elongation and in response to injury (Donnelly et al., 2010; Sotelo-Silveira et al., 2006; Taylor et al., 2009; Willis et al., 2005). Tau mRNA has been detected in the axon of cultured rat primary neurons, but its presence was restricted to the proximal part of axons (Litman et al., 1993). The only data supporting the presence of tau mRNA in further distal parts of axons were obtained in transfected P19 teratocarcinoma cells overexpressing tau. Overexpressed tau mRNA in these cells is present in granules along processes (Aronov et al., 2001, 2002). Clearly, a definite answer regarding the localization of tau mRNA in

Abbreviations: eEF1A, eukaryotic elongation factor 1A; DIG, digoxigenin

[*]*Corresponding author*. Fax: +44 207 708 0017.
E-mail addresses: jean-marc.gallo@kcl.ac.uk, j.gallo@iop.kcl.ac.uk (J.-M. Gallo).
[1]Present address: Dubowitz Neuromuscular Centre, UCL Institute of Child Health, London, WC1N 1EH, United Kingdom.

0006-8993/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2013.12.033

axons requires analysis of endogenous tau mRNA in primary neurons.

In this study, we explored the localization of tau mRNA in axons using fluorescence *in situ* hybridization (FISH) in primary rat cortical neurons. We show that tau mRNA is not only present in the cell body and proximal axon, but also in granules throughout the axon, including the growth cone. Importantly, we show that tau mRNA-containing axonal granules are associated with the eukaryotic elongation factor 1A, a component of the protein translation machinery.

2. Results and discussion

2.1. *RNA granules are present in axons*

To establish the presence of RNA in the axon, primary rat cortical neurons were stained with the nucleic acid dye, SYTO 14. Neurons were seeded at very low density; hence individual neurons were easily distinguished. Axonal processes were defined by morphological assessment as the longest process in each neuron. Furthermore, to distinguish axons from dendrites, neurons were stained with an antibody against the neurofilament heavy subunit (NF-H). Neurons were brightly labeled by SYTO 14 with the cell body staining intensely (Fig.1A). In addition, SYTO 14 labeled granular material along the length of axons (Fig.1A). To confirm the specificity of the staining, neurons were treated with RNases after labeling with SYTO 14 (Fig. 1B). Treatment with RNases reduced SYTO 14 staining in the cell body and completely abolished granular staining in processes thus confirming that the SYTO 14-positive granules contain RNA.

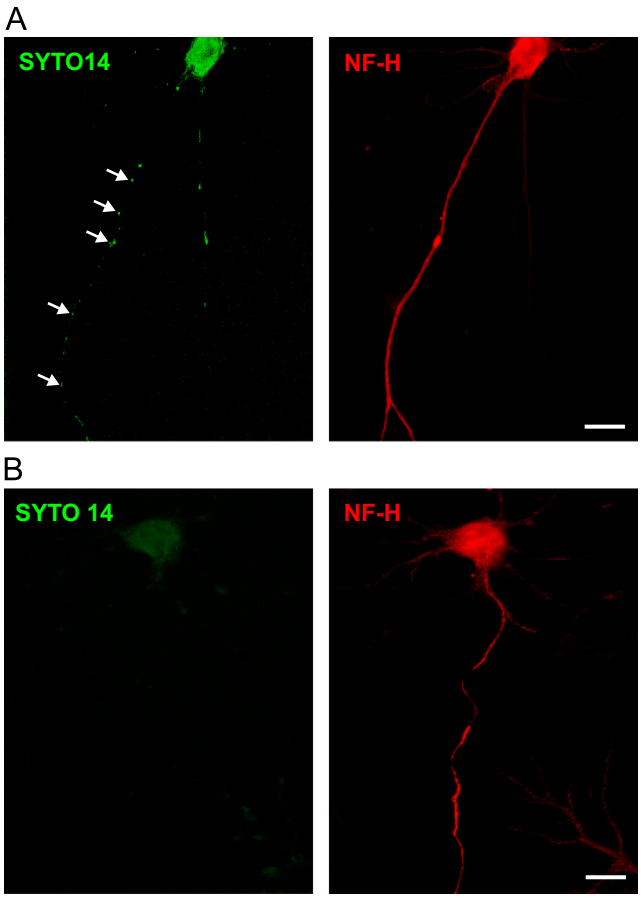

Fig. 1 – Presence of RNA granules in axons. Rat primary cortical neurons were stained for RNA using SYTO 14 before immunostaining. (A) Axons were visualized by immunostaining using an NF-H antibody. RNA is present in granules in neuronal processes along the length of the axon (arrows). (B) Treatment with RNase A and RNase T1 abolishes SYTO 14 staining. Scale bars, 20 μm.

2.2. *Tau mRNA is present in RNA granules in axons*

We next analyzed the distribution of tau mRNA in rat primary cortical neurons using FISH. A digoxigenin (DIG)-labeled RNA antisense probe corresponding to the last 400 bases of the coding region of tau mRNA was synthesized. This sequence encodes the C-terminus of tau, including the third and fourth microtubule-binding repeats, a domain not affected by alternative splicing and expressed in embryonic neurons. Using this probe, an intense staining for tau mRNA was obtained in cell bodies and proximal axons (Fig. 2A), a distribution in agreement with previous studies (Litman et al., 1993). Tau mRNA was also observed in dendrites, consistent with the reported dendritic presence of tau, particularly in dendritic spines, a localization more pronounced in pathological conditions (Pooler et al., 2014; Tashiro et al., 1997).

In addition, in all cells examined, tau mRNA was present in the distal part of the axon. High magnification revealed a granular staining pattern with tau mRNA granules distributed along the length of the axon (Fig. 2B). To validate the specificity of tau mRNA staining a number of control conditions were used (Fig. 2C–E). Only a weak signal was detected using a sense probe (Fig. 2C) or when cells were incubated with the anti-DIG antibody only, without any RNA probe (Fig. 2D). Finally, the tau mRNA signal detected with the antisense probe was reduced to a weak background when cells were treated with RNases before the FISH procedure (Fig. 2E). Importantly, no granular staining was visualized in axons in these controls. Thus, endogenous tau mRNA is associated with granules in axons of rat primary cortical neurons. Previous localization of tau mRNA in cultured neurons by *in situ* hybridization revealed the presence of tau mRNA in the proximal part of the axon only and not in the distal part (Litman et al., 1993). The lack of detection of tau mRNA in the distal part of the axon in the latter study may be due to the detection method used, alkaline phosphatase, that is not as sensitive as fluorescence. While our results are consistent with the observation of overexpressed tau mRNA in granules in processes of transfected P19 teratocarcinoma cells (Aronov et al., 2001, 2002), this is the first time that endogenous tau mRNA is shown to be present in granular material along axons in primary neurons. This represents a physiological situation as compared to overexpressed tau mRNA that could saturate mechanisms regulating mRNA compartmentalization. Although we found tau mRNA in the