Genome wide identification of Staufen2-bound mRNAs in embryonic rat brains

Marjolaine Maher-Laporte & Luc DesGroseillers*
Département de Biochimie, Université de Montréal, PO Box 6128 Centre Ville, Montréal, Québec, Canada. H3C 3J7

INTRODUCTION

mRNA transport is critical in many situations to differentially control protein content in domains distant from the cell body (1). In particular, transport and subsequent local translation are important for different aspects of synaptic functions and plasticity (1-3). Several mechanisms of mRNA transport have been described in dendrites ranging from a constitutive transport pathway to an activity-induced transport mechanism of newly transcribed mRNAs (3). It is believed that different mechanisms of mRNA transport are linked to different forms of synaptic activity and/or plasticity.

Upon transcription, nascent mRNAs associate with several factors that form messenger ribonucleoprotein particles (mRNP) (3). Along the way from nuclear export to dendritic anchoring, proteins are added or removed from the mRNP complexes in a dynamic way. These proteins finely control the successive steps that insure proper expression of mRNA at specific times and space. Staufen2 (Stau2), a protein mainly expressed in brain is a well accepted player for mRNA localization (4-9). In brain, Stau2 is expressed in several cell types including neurons and oligodendrocytes. Four Stau2 protein isoforms are generated by differential splicing. Stau2 isoforms bind double-stranded RNAs, are incorporated into mRNP and display a microtubule-dependent somatodendritic distribution in neurons. In oligodendrocytes, Stau2-containing mRNPs are found in both the cell bodies and myelinating processes. Interestingly, over-expression of Stau2 in dendrites increases the amount of dendritic mRNAs showing the importance of Stau2 for mRNA transport (5). Neurons in which Stau2 has been down-regulated by RNAi show a reduced density of dendritic spines, associated with a change in their morphology (10). These phenotypes result in reduced amplitude of the miniature excitatory postsynaptic currents, a measure of synaptic transmission.

In the somatodendritic compartment, Stau2 associates with the two main types of cytoplasmic mRNA/protein complexes involved in transport: mRNA granules and mRNA transport particles (4, 11). Whereas mRNA particles are devoid of ribosomes, mRNA granules form bigger complexes that are associated with ribosomes. It was suggested that RNA particles might represent the observed transport mRNPs (12). While the distribution and functions of Stau2 in brain cells begin to be elucidated, the identity of transported mRNAs is still unknown, preventing in-depth analyses of the molecular mechanisms involved in specific phenotypes. In this paper, we immunoprecipitated Stau2-containing mRNPs from embryonic rat brains and used a genome wide approach to identify Stau2-associated mRNAs. Several mRNAs coding for proteins mainly involved in protein modification, translation and intracellular trafficking were identified.

RESULTS

Identification of mRNAs in Stau2-containing mRNPs in embryonic brains

In order to identify the mRNA content of Stau2-containing RNP, extracts of embryonic rat brains were prepared and en-
dogenous Stau2 was immunoprecipitated using polyclonal anti-Stau2 antibodies and the pre-immune serum as control. To minimize the identification of non-specific mRNAs, two different polyclonal anti-Stau2 antibodies were used in parallel experiments (Fig. 1A). Co-immunoprecipitated mRNAs were purified. Commercially available rat DNA oligonucleotide microarrays from Illumina containing 22,226 probe sets were hybridized with cRNAs derived from Stau2-dependent IPs and controls (Fig. 1B). A total of 12 microarrays were hybridized: four with cRNA probes generated from mRNAs isolated with the anti-Stau2 L1 antibody, two with the anti-Stau2 L2 antibody and six with their respective pre-immune serum. To estimate the validity of these hybridization data, we first generated a PCA plot that translates into a particular space on the plot each measurement and structure of one gene expression data array (13). The PCA plot from the hybridization data of the twelve arrays shows that hybridization data are grouped together according to the antibody used for immunoprecipitation (L1, L2 or PI) (Fig. 1C) indicating reproducible hybridization data. It also indicates that both Stau2-derived hybridizations are different from those of the pre-immune arrays and that anti-Stau2 L1 and L2 antibodies are not perfectly identical. To identify Stau2-associated mRNAs, data were analyzed with the FlexArray 1.2 software (14). We selected mRNAs that were enriched at least 8 fold in both Stau2-dependent IPs (to minimize non-specific hybridization) as compared to controls (P value < 0.002). Using these criteria, 1780 mRNAs were identified (Supplemental Table S1).

**RT-PCR validation of identified mRNAs**

Several mRNAs identified in the microarray experiments as being enriched in Stau2 mRNPs were validated by RT-PCR experiments. Genes that were enriched less than 6 fold were used as negative controls. Stau2-containing mRNPs were immunoprecipitated from embryonic rat brain extracts using anti-Stau2 antibodies and a pre-immune serum as control. Co-immunoprecipitated mRNA was isolated and RT-PCR amplified using specific primers (Fig. 2). Consistent with the microarray data, several mRNAs were enriched in Stau2-containing complexes as compared to the pre-immune IP. Enrichment was observed with the two different anti-Stau2 antibodies. In contrast, control mRNAs were not enriched in Stau2-dependent immunoprecipitates as compared to those of the pre-immune serum indicating that they were absent from Stau2-containing complexes, as expected from the microarray data. Serial dilutions of the control mRNAs before RT-PCR amplification confirmed that the amplifications were in the linear range (Fig. 2B).

**Gene ontology (GO)**

Next, we identified pathways in which Stau2-associated mRNAs are involved as a mean to understand the biological functions of Stau2 in brain cells. Probe set lists have been analyzed with the BABELOMICS functional annotation tool (15) to