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Resou

Molecular Profiling of Activated Neurons by Phosphorylated Ribosome Capture

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SUMMARY

The mammalian brain is composed of thousands of interacting neural cell types. Systematic approaches to establish the molecular identity of functional populations of neurons would advance our understanding of neural mechanisms controlling behavior. Here, we show that ribosomal protein S6, a structural component of the ribosome, becomes phosphorylated in neurons activated by a wide range of stimuli. We show that these phosphorylated ribosomes can be captured from mouse brain homogenates, thereby enriching directly for the mRNAs expressed in discrete subpopulations of activated cells. We use this approach to identify neurons in the hypothalamus regulated by changes in salt balance or food availability. We show that galanin neurons are activated by fasting and that prodynorphin neurons restrain food intake during scheduled feeding. These studies identify elements of the neural circuit that controls food intake and illustrate how the activity-dependent capture of cell-type-specific transcripts can elucidate the functional organization of a complex tissue.

INTRODUCTION

A basic goal of neuroscience is to link the activity of specific neuronal cell types to the various functions of the brain. This task is complicated by the extraordinary cellular diversity of the

The ability to profile the genes uniquely expresse that respond to a stimulus would facilitate the syster ular identification of the cell types that control b molecular identification of these cells would also manipulation in vivo by using technologies that r sible to activate or inhibit neurons with light (Y 2011), generate transcriptional profiles from neurc tagged ribosomes (Heiman et al., 2008; Sanz et a label and record from neurons by using fluoresce (Gong et al., 2003). These tools achieve their selectiv ing protein expression using a promoter from a cell-t marker gene, but in many cases, the marker genes a functional population of neurons are unknown (A 2007).

Immediate early genes such as c-fos have been w visualize the neurons that respond to numerous stir and Curran, 1991). However, despite its utility in marl that have been biochemically activated, c-Fos stain reveal the genetic identity of the labeled cells. Chara coexpression of an activation marker such as c-Fos limited set of candidate genes requires processing la of histologic sections (Isogai et al., 2011). For this reas atic methods are needed to profile gene expression f subpopulations of activated neurons in the brain.

Here, we show that phosphorylation of the ribos used as a molecular tag to retrieve RNA selective vated neurons. This enables the unbiased discovery that are uniquely expressed in a functional popula rons. By quantifying in parallel the enrichment of markers, it is possible to assess the activation or numerous cell types in a complex tissue, revealing nated regulation of ensembles of neurons in resi