Cell-type specific mRNA analysis by translating ribosome affinity purification (TRAP)

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Technical Journal Club

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Bulk vs cell-specific mRNA analyses

Limitations in studies on bulk mRNA from whole tissue/organ:

- Upregulation of a transcript in a cell type can be masked by downregulation in a different cell type

- Changes in rare cell types are difficult to detect

→ Changes in specific cell types are not analysed
Cell-specific mRNA analysis: current approaches

In situ hybridization:

Limitations:
- Not quantitative
- Identity of cells expressing the transcript not always evident
- Genome-wide studies are difficult and restricted to reference samples (Allen Brain Atlas, Eurexpress)

Lein ES Nature 2007
The Allen Brain Atlas
Cell-specific mRNA analysis: current approaches

Laser capture microdissection

Microdissection & FACS

Limitations:
- Fixation required
- Laborious
- Special instruments required
- Cell projections not captured

Limitations:
- Artefacts from sample processing

Schütze Nat Biotech 1998

Lobo Nat Neurosci 2006
mRNA tagging in *C. elegans*

(a) **myo-3p**

ATG - flag - Stop

Poly(A)-binding protein (*pab-1*)

1 kb

(b) Muscle mRNA co-immunoprecipitated

No mRNA co-immunoprecipitated

Roy Nature 2002
A Translational Profiling Approach for the Molecular Characterization of CNS Cell Types

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BAC Transgenic mice and the GENSAT Database

Gong Nature 2003
Schmidt Cold Spring Harb Protoc 2013
TRAP strategy: screens for ribosome tagging

Screening of candidate ribosomal proteins:
→ L10a

Screening of affinity tags
→ EGFP
Generation of BAC-TRAP transgenic mouse lines

*Drd2* bac-TRAP line  
*Drd1a* bac-TRAP line

**Striatopallidal neurons:**
- Dorsal and ventral striatum
- Olfactory tubercle
- Hippocampus
- Substantia nigra pars compacta
- Ventral tegmental area

**Striatonigral neurons:**
- Dorsal and ventral striatum
- Olfactory tubercle
- Olfactory bulbe
- Cortical layers 5 and 6
Protein and mRNA purification from bac-TRAP lines

Optimization of purification protocol:

- Rapid homogenization of fresh tissue
- Mg$^2+$ and cycloheximide in lysis buffer
- Inhibition of endogenous Rnase
- Solubilization of RER-bound polysomes
- High-affinity anti-EGFP antibodies
- High-salt washes after
Translational profiling with TRAP

Replication of known enriched transcript (in blue)
Identification of ≈70 striatopallidal and ≈150 striatonigral-enriched transcripts
Generalization of TRAP methodology

**Chat bac-TRAP line**

**Pcp2 bac-TRAP line**

**Cholinergic motor neurons:**
- Dorsal and ventral striatum
- Basal forebrain
- Brain stem
- Spinal chord
- Medial habenula

**Purkinje neurons:**
- Cerebellum
Generalization of TRAP methodology
Summary

- Establishment and proof-of-principle of TRAP technology

- Generation of 4 bac-TRAP lines

- Identification of novel physiological differences between striatopallidal and striatonigral neurons, at steady state and after cocaine treatment (not shown)
Application of a Translational Profiling Approach for the Comparative Analysis of CNS Cell Types

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DOI 10.1016/j.cell.2008.10.029
Generation of novel bac-TRAP lines

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In depth-characterization of bac-TRAP lines
Measurement of mRNA enrichment by TRAP
Identification of known cell-specific markers
Identification of novel cell-specific markers by TRAP

Cerebellar Golgi cells
Validation of quantitative aspects of TRAP

qRT-PCR for known and novel genes

- **Chat** bac-TRAP
- **Pcp2** bac-TRAP
Comparative analysis of TRAP microarray data

A Hierarchical clustering of cell types and tissue samples.

- Cerebellar Astroglia
- Bergmann Glia
- Cortical Astrocytes
- Basal Forebrain Cholinergic Neurons
- Corpus Striatum Cholinergic Neurons
- Brain Stem Motor Neurons
- Spinal Cord Motor Neurons
- Cck Positive Neurons
- Pnoc Positive Neurons
- Granule Cell Layer Interneurons
- Layer 5b Corticospinal, Corticospinal Pyramidal Neurons
- Layer 6 Corticostriatal Pyramidal Neurons
- Layer 5a Corticostriatal Pyramidal Neurons
- Cort Positive Interneurons
- Whole Tissue, Cortex
- Whole Tissue, Forebrain
- Whole Tissue, Corpus Striatum
- Whole Tissue, Brain Stem
- Whole Tissue, Spinal Cord
- Whole Tissue, Cerebellum
- Drd1 Positive Medium Spiny Neurons
- Drd2 Positive Medium Spiny Neurons
- Mature Oligodendrocytes from Cerebellum
- Mature Oligodendrocytes from Cortex
- Mixed Oligodendroglia from Cerebellum
- Mixed Oligodendroglia from Cortex
- Granule Cells
- Stellate and Basket Cells
- Unipolar Brush Cells
- Purkinje Cells

Based on 20% of genes with highest coefficient of variation
Summary

- Confirmation and extension of TRAP technology
- Generation of 12 additional bac-TRAP lines (16 in total)
- Comparative microarray analysis of different CNS cell types (incl. data not shown)
  $\rightarrow$ All plasmids and mouse lines are available upon request

Advantages:
- *In situ*, avoiding artefacts from dissociation or tissue fixation
- EGFP tagging allows visualization of cells under study (e.g. electrophysiology)
- Translatome, rather than transcriptome
- Relatively rapid
- Does not require special instruments or reagents (besides bac-TRAP lines)

Limitations:
- A genetic element to drive cell-type specific expression of TRAP transgene is needed
- Specific bac-TRAP lines for the cell of interest have to be generated
- Low mRNA yield
- Translatome, rather than transcriptome
**TRAP strategy**

**bacTRAP transgenic mouse brain**

Dissection of brain region of interest containing cells expressing EGFP-tagged L10a ribosomal protein

**Tissue Lysis**

Stabilization of ribosome/mRNA complexes with cycloheximide

**Ribosome/mRNA complexes (polysomes)**

**Purification of EGFP-L10a and associated mRNA with EGFP antibody-coated magnetic beads**

**Bound fraction**

**Unbound fraction**

**RNA purification**

Gene expression analysis

**Comparative analysis of mRNA profiles between:**

- Bound versus unbound fractions
- Different bacTRAP mouse lines
- Different experimental conditions

Emery & Barres Cell 2008
Interrogating translational efficiency and lineage-specific transcriptomes using ribosome affinity purification

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Generation of a Cre-activated TRAP mouse line

**A**
- Cytoplasmic RNA
- GFP
- Ribosome-associated ("TRAP") RNA
- α-GFP beads
- RNA purification

**B**
- CAG
- Neo
- pA
- GFP-L10
- BirA
- pA

**C**
- Sucrose Gradient - RNA
- Fraction
- GFP-L10a
Ubiquitous Cre-mediated activation of TRAP transgene

Rosa26<sup>CAG-TRAP</sup> adult mice

IB: GFP

Coomassie

Br  He  Ki  Li  Lu  Sk
Cardiomyocyte-restricted activation of TRAP transgene
Endothelial cell-restricted activation of TRAP transgene
Summary

- Establishment and proof-of-principle of Cre-conditional TRAP transgene

- Study of endothelial-cell enriched/specific genes in heart

- Study of translational modifications in cardiomyocytes after aortic banding (not shown)

→ Rosa26\(^{fsTRAP}\) and Rosa26\(^{CAG-TRAP}\) lines are available at JAX laboratories
Evaluation of TRAP-sequencing technology with a versatile conditional mouse model

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Generation of a Cre-activated TRAP mouse line

All epiblast derivatives

Endothelial cells

Dorsal telencephalon
Summary

- Establishment and proof-of-principle of an additional Cre-conditional TRAP transgene

- Study of endothelial-cell enriched/specific genes in brain and kidney (data not shown)

- Study of differences in transcriptome and translatome in different organs (not shown)
A Versatile Method for Cell-Specific Profiling of Translated mRNAs in Drosophila


Cell Type–Specific Translational Profiling in the Xenopus laevis Retina


Development of Translating Ribosome Affinity Purification for Zebrafish


Robert C. Tryon, Nilambari Pisat, Stephen L. Johnson, and Joseph D. Dougherty
Improved protocol, yielding higher RNA amount upon TRAP purification
- Two days from sample preparation to RNA purification
- Freshly isolated samples give higher RNA yields
- Compatible with Northern blotting, qPCR, microarray and RNA-Seq
Summary

- Allows cell-specific studies of translatome

Advantages:
- *In situ*, avoiding artefacts from dissociation or tissue fixation
- EGFP or mCherry tagging allows visualization of cells under study (e.g. electrophysiology)
- Translatome, rather than transcriptome
- Relatively rapid
- Does not require special instruments or reagents (besides bac-TRAP or conditional TRAP lines)

Limitations:
- A genetic element to drive cell-type specific expression of TRAP transgene is needed
- Limited temporal control of TRAP transgene expression
- Translatome, rather than transcriptome