Ribonomics

Innovative Systems Biology

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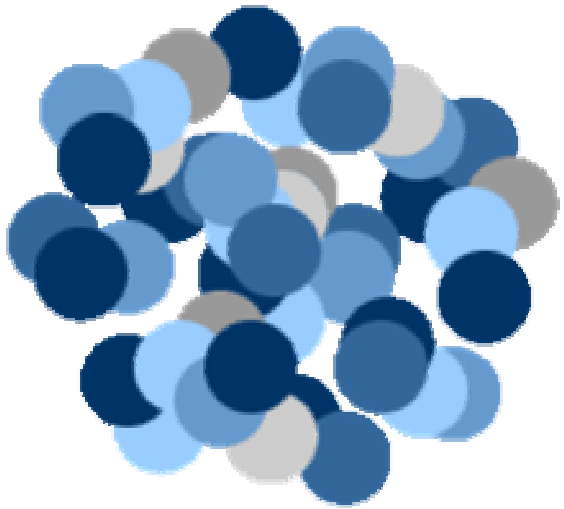
Vice President, Research & Development

RNA in Drug Development

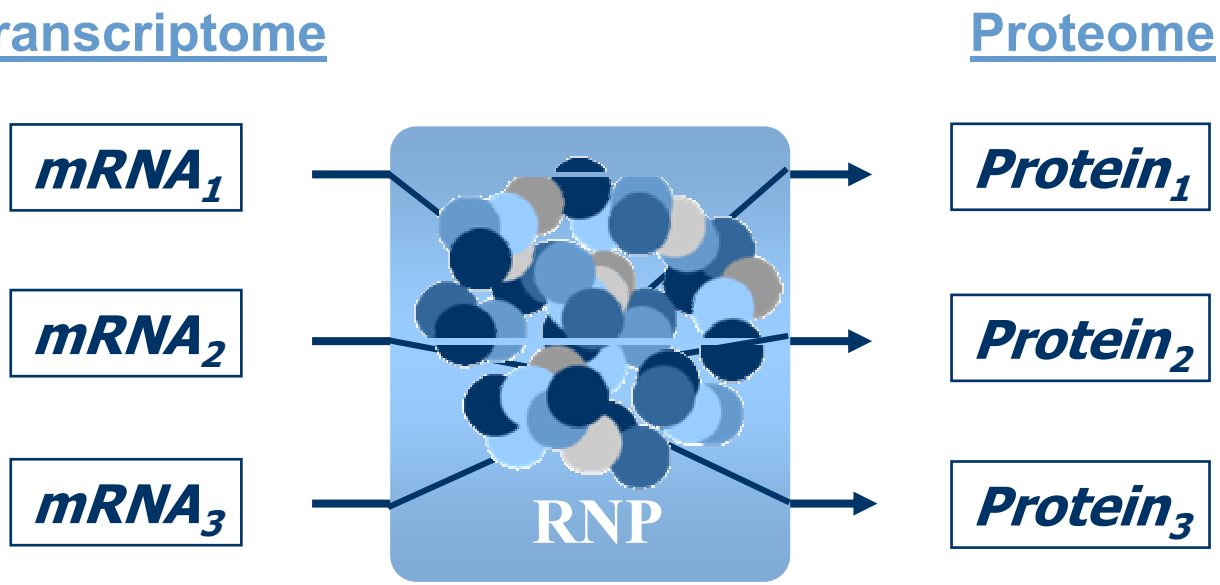
November 6-8, 2002

Ribonomics, Inc. leverages SYSTEMS BIOLOGY to discover and develop novel therapeutic opportunities



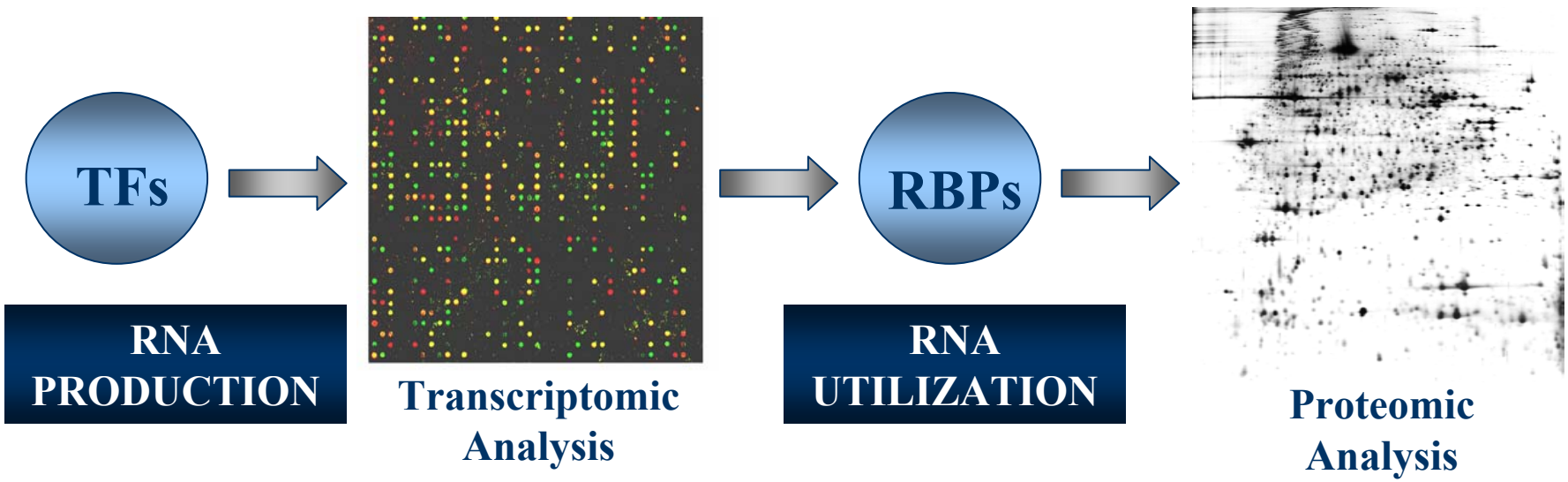
We exploit the cell's own organizational scheme for gene expression to map disease pathways

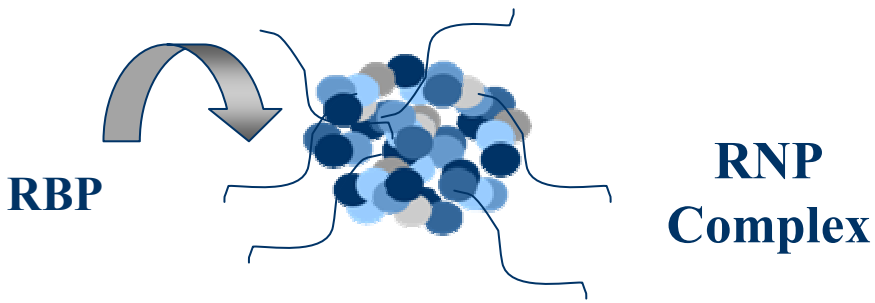
RBPs: A Systems Biology Tool



RNA Binding Proteins create functional clusters of genes

Transcription Factors and RNA Binding Proteins

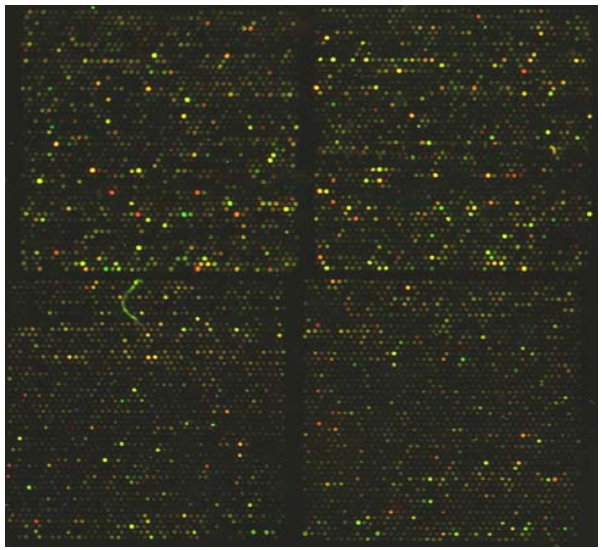




The transcriptome is an unreliable predictor of the proteome

Mol Cell Biol 19:1720, 1999; Science 292:929, 2001

Total RNA measurements



e.g. Microarray analysis

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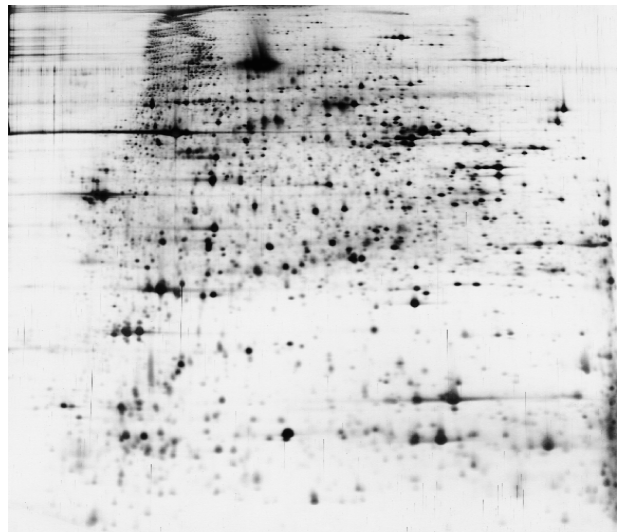
Total Protein measurements

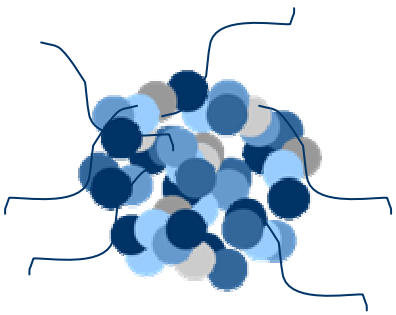


e.g. 2-D gel Proteomic analysis

Steven P. Gygi, Yvan Rochon, B. Robert Franza, and Ruedi Aebersold **Correlation between Protein and mRNA Abundance in Yeast** **Mol. Cell. Biol. 1999 19: 1720-1730.**

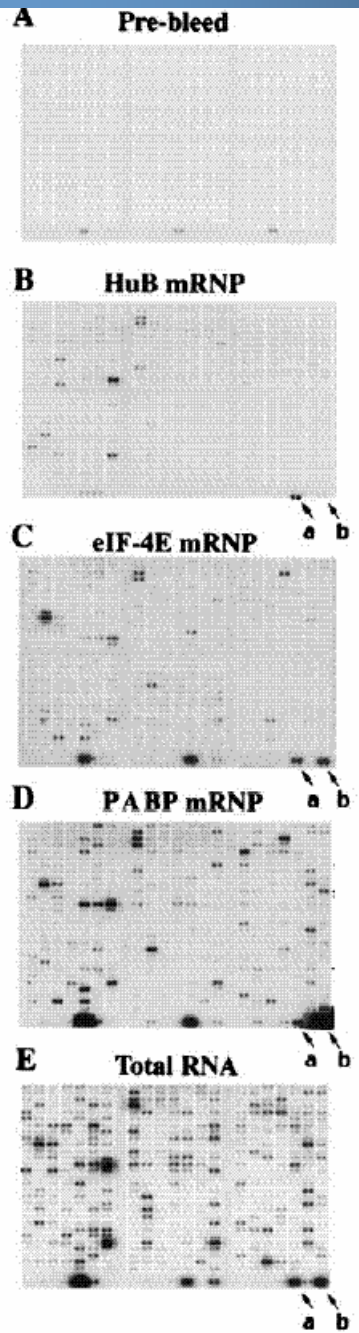
Trey Ideker, Vesteinn Thorsson, Jeffrey A. Ranish, Rowan Christmas, Jeremy Buhler, Jimmy K. Eng, Roger Bumgarner, David R. Goodlett, Ruedi Aebersold, and Leroy Hood **Integrated Genomic and Proteomic Analyses of a Systematically Perturbed Metabolic Network Science 2001 May 4; 292: 929-934.**

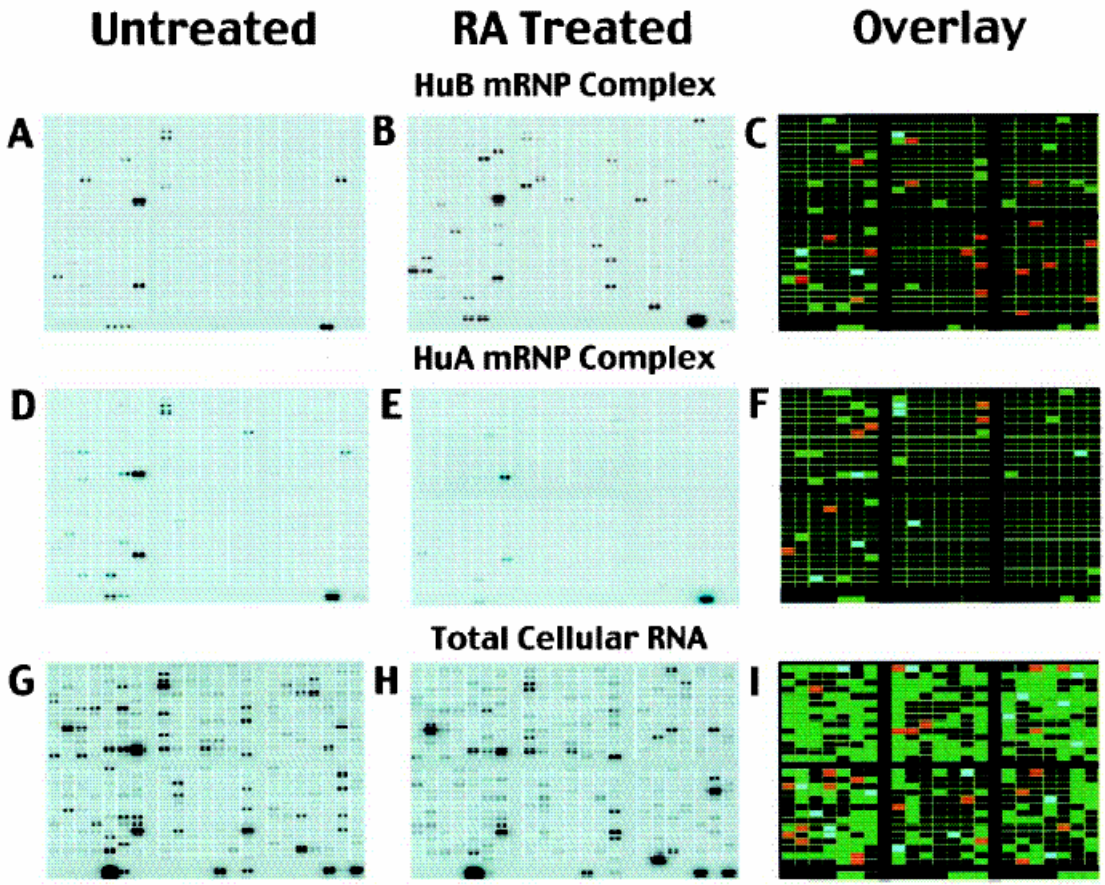
Post-Transcriptional Control of Gene Expression by RBPs



RNP

- Splicing
- Transport
- Subcellular localization
- Translation
- Stability





Tenenbaum, Carson et al., PNAS 97:14085, 2000.

Functional RNP Clusters

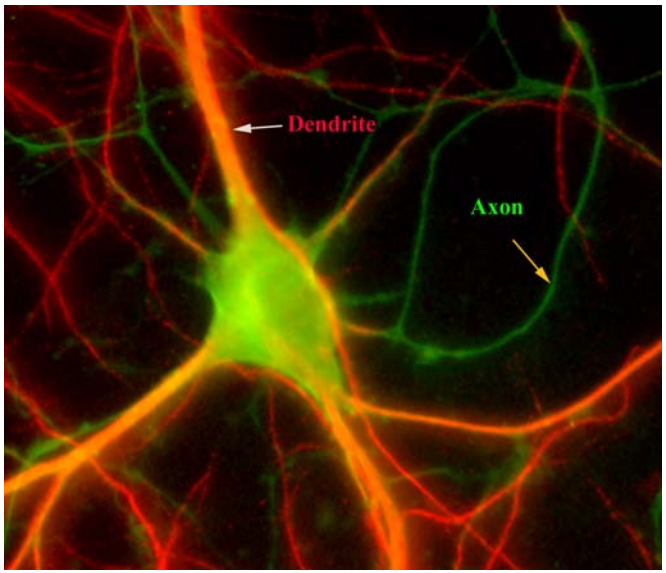
- IRON REGULATION
- HISTONES
- FRAGILE X-SYNDROME
- IMMEDIATE EARLY RESPONSE GENES
- RIBOSOMAL PROTEIN GENES
- AZOOSPERMIA

Iron Regulation
IRP Functional Cluster

GENE	Element	Function
Transferrin Receptor	3'IRE	Fe^{2+} Import
Ferritin	5'IRE	Fe^{2+} Storage
Ferroportin	3'IRE	Fe^{2+} Export
ELAS (erythroid 5-aminolevulinic acid synthetase)	5'IRE	Porphyrin biosynth
Mit Aconitase	5'IRE	Citric Acid cycle
DMT-1	3'IRE	Fe^{2+} Import

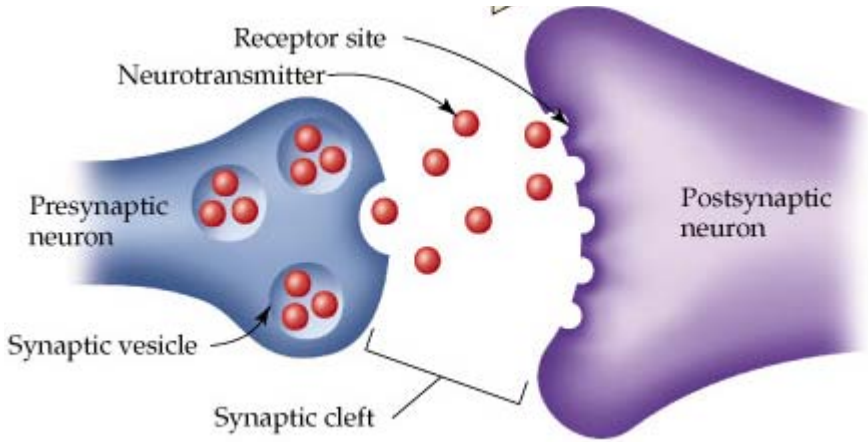
Functional Clusters Validated

RNP cluster important for neuronal differentiation



PNAS, Vol. 97, 14085-14090, 2000

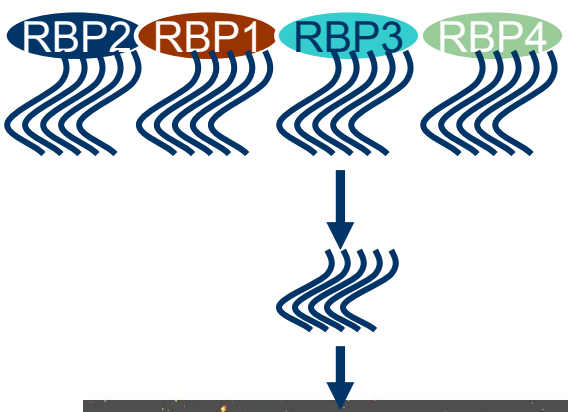
RNP cluster localizes translation for synaptic signaling

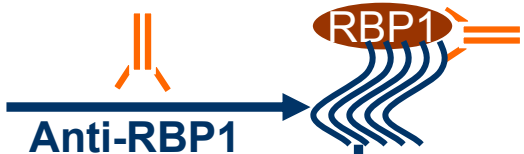


Cell, Vol. 107, 477-487, 2001

Ribonucleoprotein complexes regulate the flow of genetic information between the genome and the proteome

RAS™: Ribonomic Analysis System



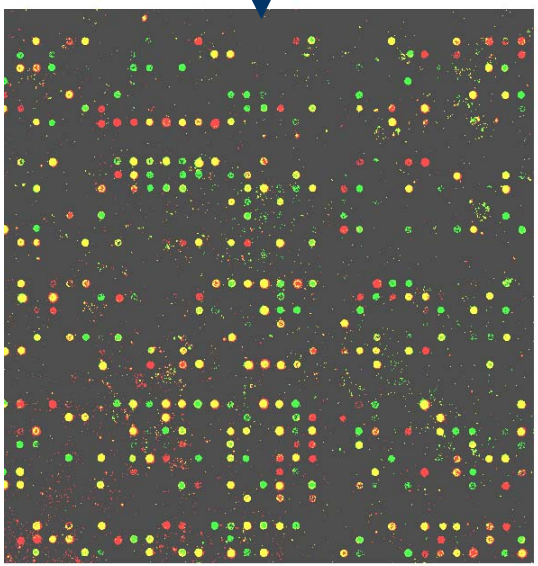


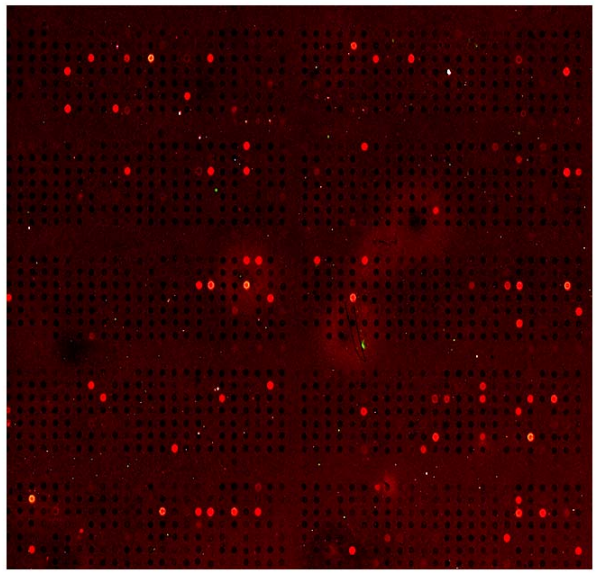
Isolated RBP1 mRNP complex

Extract mRNA

RBP1

Total RNA



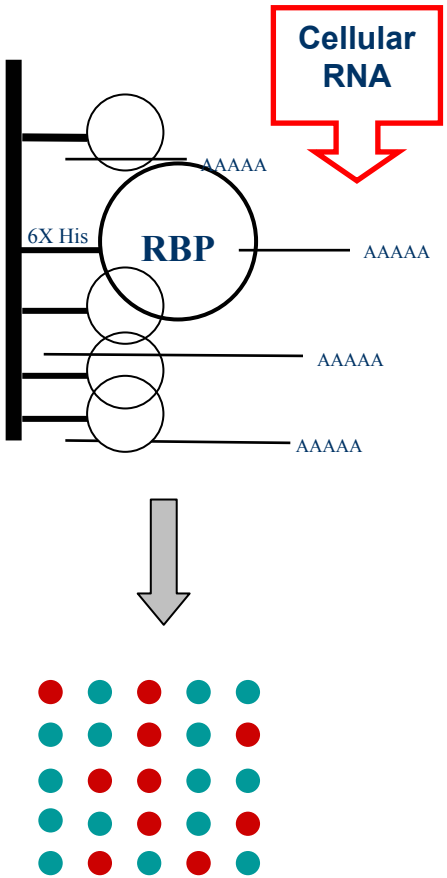


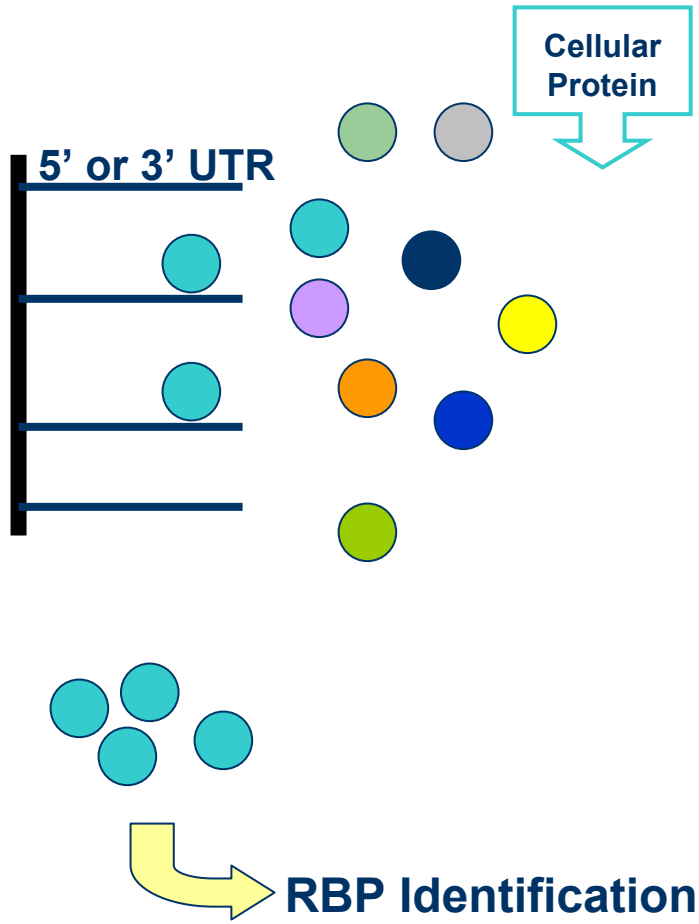
RBP1-associated mRNAs

- Global view of transcriptome
- Low sensitivity
- Complex Data Sets

- Enriched subset of mRNAs
- Higher Sensitivity
- Focus--Simplifies Data sets
- Are coordinately regulated
- Many in same pathway, i.e. ‘functionally related’
- Identify Novel Components

Ribo Tags and Traps: *In Vitro*

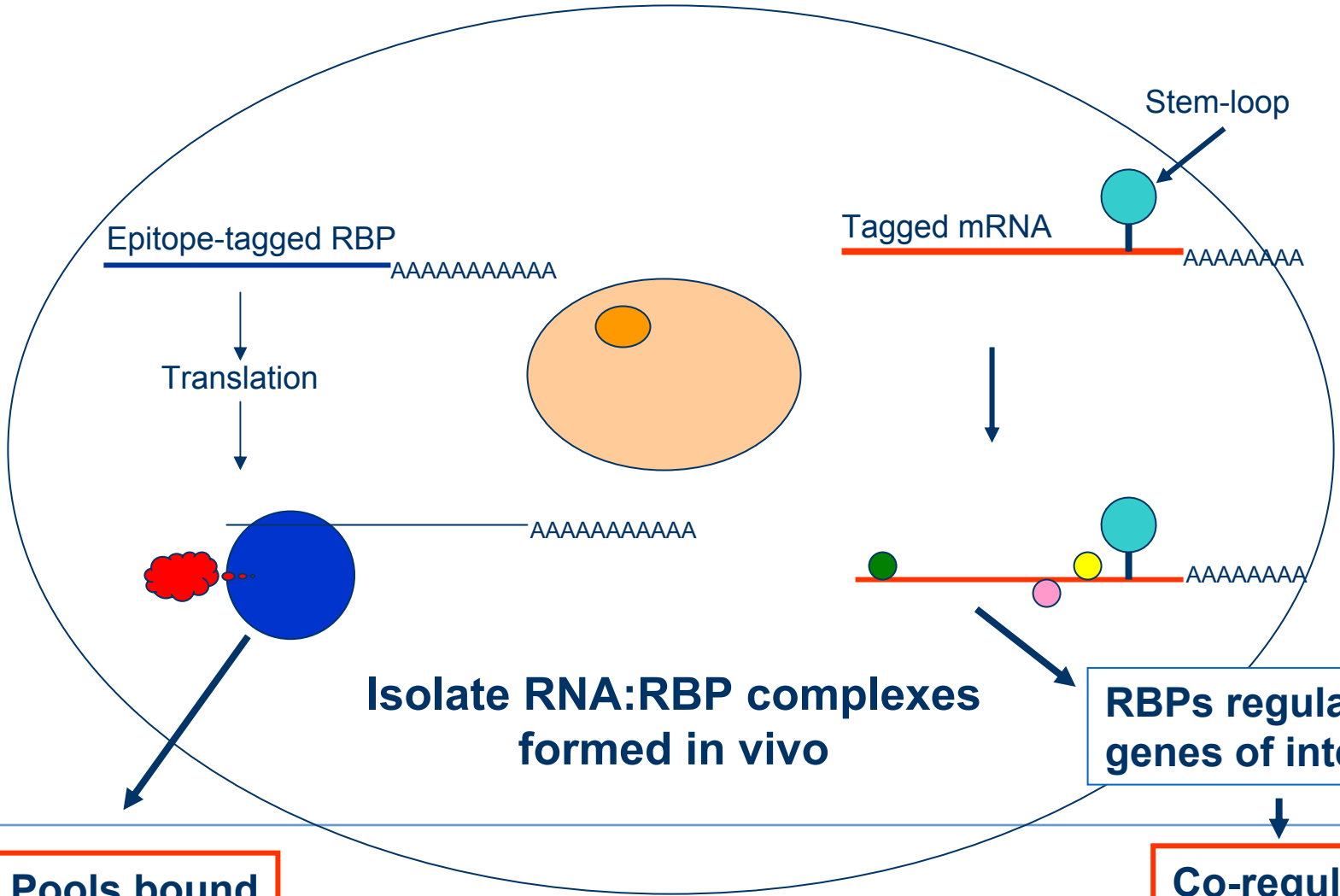




Tagged RBP Traps the mRNA pool

Tagged RNA Traps the RBP

Ribo Tags and Traps: *In Vivo*



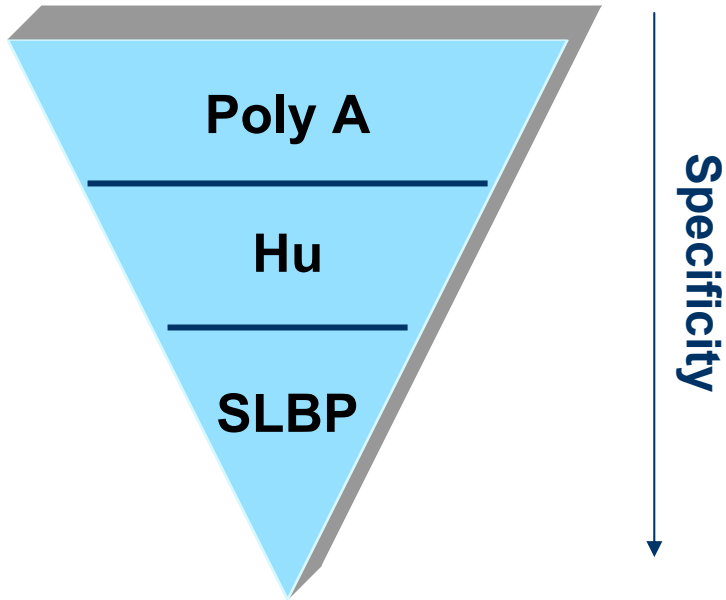
Largest Gene Families

Nature 409:860, 2001

Family	Human	Fly	Worm
Ig domains	765 (1)	140 (9)	64 (34)
C2H2 ZF	706 (2)	357 (1)	151 (10)
Pr. Kinase	575 (3)	319 (2)	437 (2)
Rho-like GPCR	569 (4)	97 (14)	358 (3)
P-Loop	433 (5)	198 (4)	183 (7)
Rev Transc	350 (6)	10 (65)	50 (41)
RRM domain	**300 (7)**	**157 (6)**	**96 (21)**
G-Pro WD-40	277 (8)	162 (5)	102 (19)
Ankyrin	276 (9)	105 (13)	107 (17)
Homeobox	267 (10)	148 (7)	109 (15)

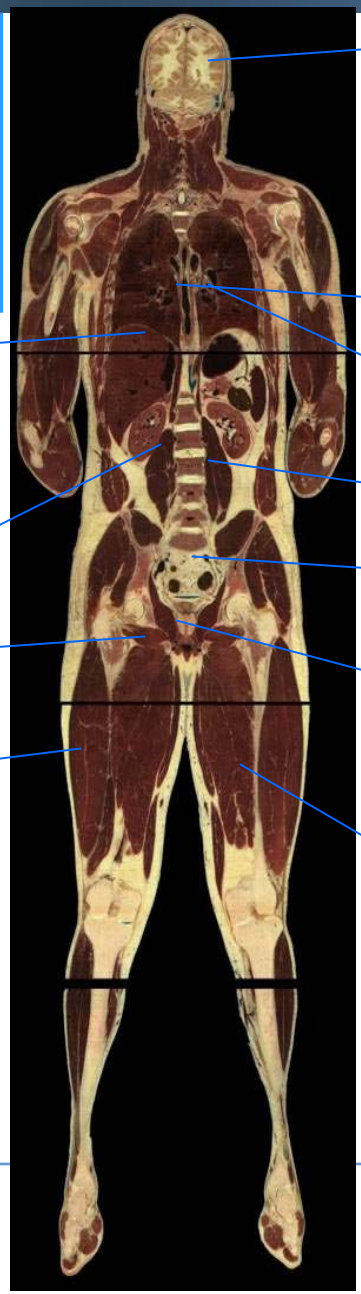
Overview of mRNA Binding Proteins

- Types
  - Global
    - PolyA binding protein
  - Group
    - Hu Family
  - Type
    - Histone SLBP
- Examples of RNA binding motifs
  - RNA Recognition Motif (RRM)
  - hnRNP K Homology Domain (KH Domain)
  - Arginine-Glycine Domain (RRG)
  - Pumilio Domain
  - Helix-Loop-Helix Zinc Binding Domain



RBPMap

Identification of Tissue and Disease Specific RNPs



Brain RBPs

Heart RBPs

Breast RBPs

Pancreas RBPs

Colon RBPs

Testicular (Ovarian) RBPs

Bone RBPs

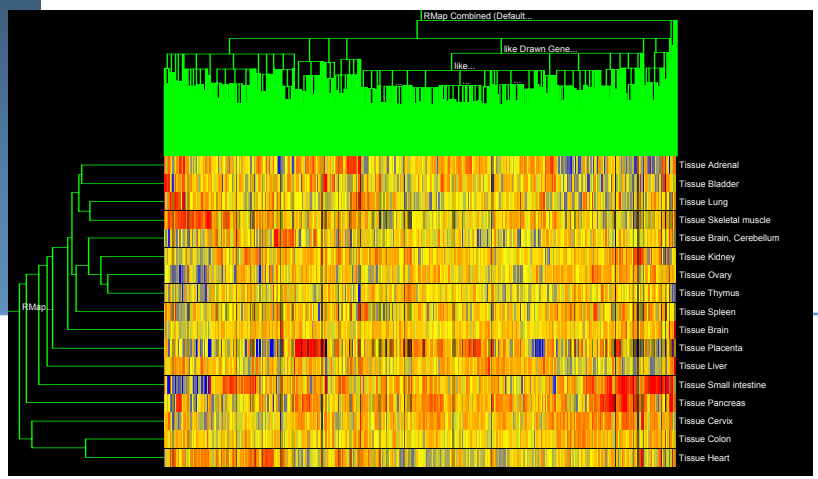
Lung RBPs

Hematopoietic RBPs

Liver RBPs

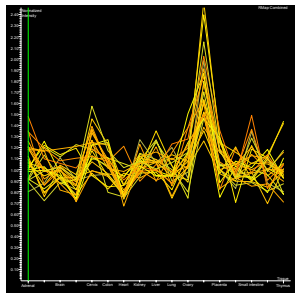
Prostate RBPs

Muscle RBPs



Tissue and Disease Specific RNPs

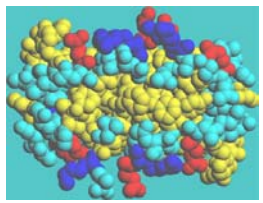
INTERNAL APPLICATIONS



Target Discovery

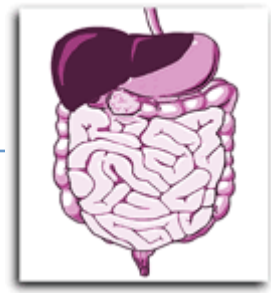
Toxicogenomics

Diabetes Glucose Response Pathways
Insulin Secretion



Obesity Satiety
Adipocyte Differentiation and
insulin response pathways

- RNP Sentinels
  e.g. Liver Toxicity
- Drug Screening



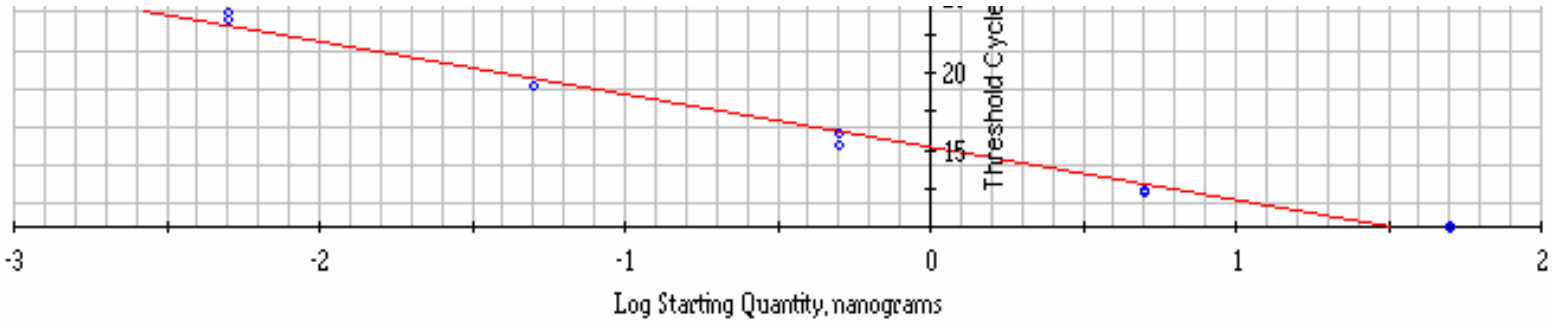
Tissue and Disease Specific RBPs

- Expression Mapping
  - Quantitative PCR
  - Microarray Analysis
  - Northern Analysis
  - Western Analysis
- Normal and Disease Tissues

Gene Expression Analysis using Real-Time Quantitative PCR

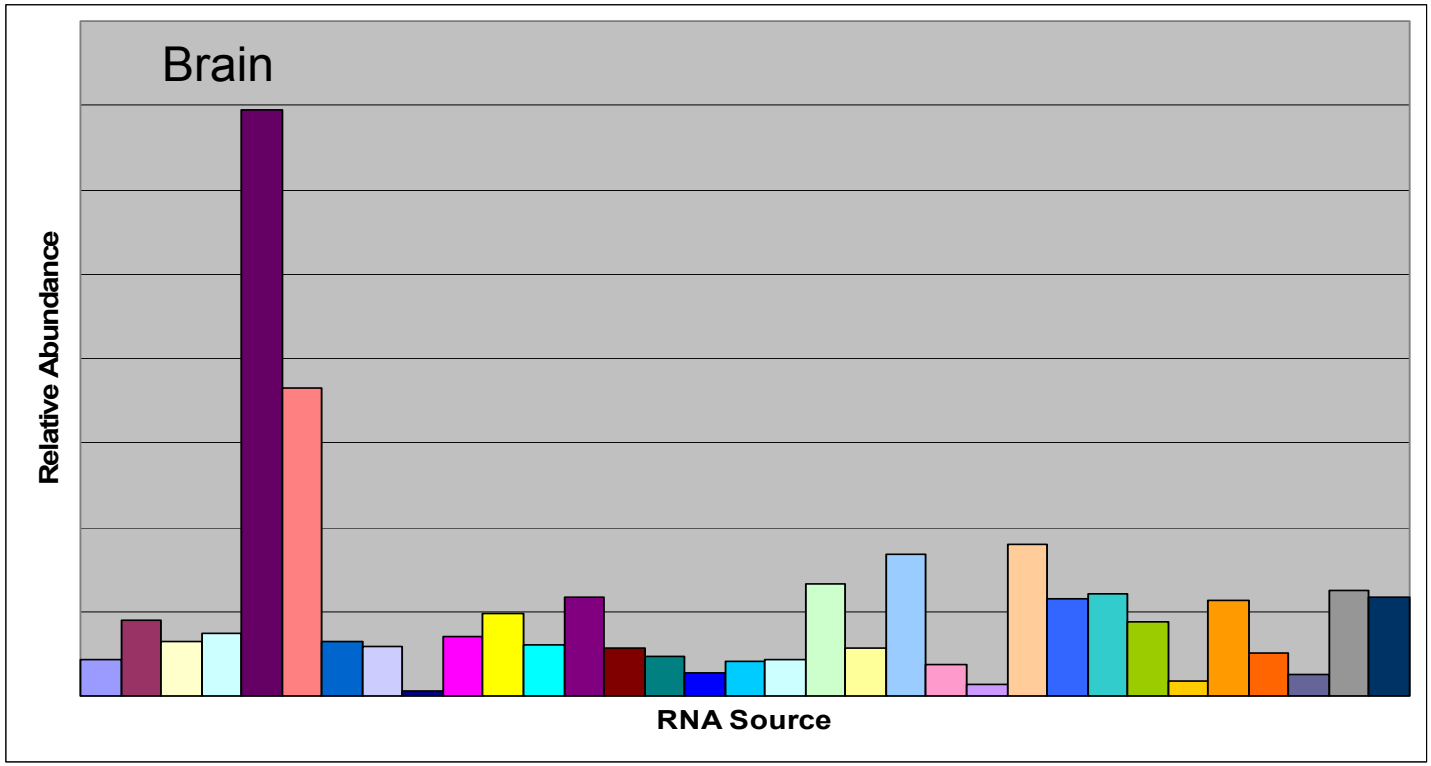
- Gold standard for quantification of nucleic acid
  - Basis is exponential amplification of nucleic acid
    - Sensitive to 1 copy
    - Highly specific for mRNA(s) of interest
    - Quantitative
    - Robust
  - Properties enable robust quantitative comparison of mRNA content across biological samples

The Same Data:
Linear Quantification Over 5 Orders of Magnitude



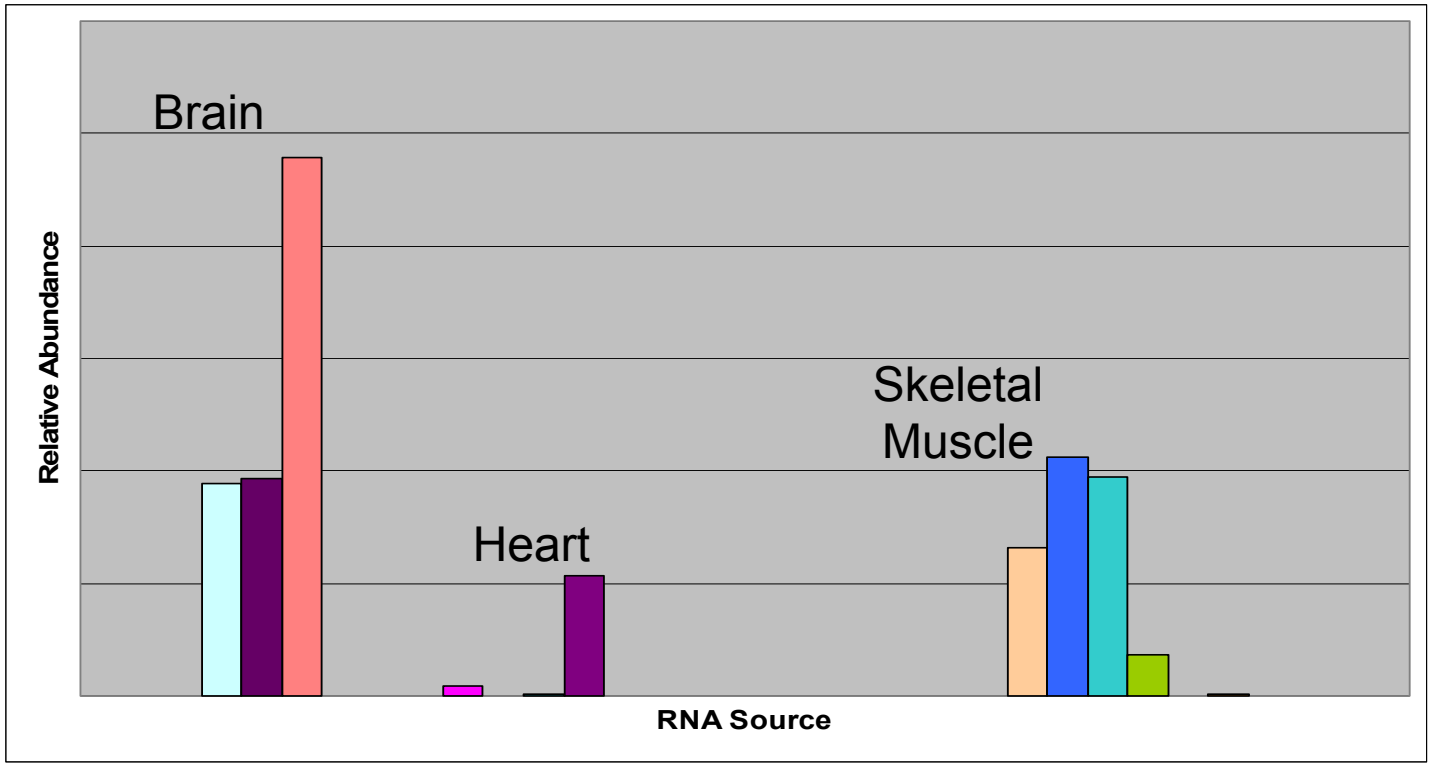
PCR Standard Curve: 8-3 GAPD Validation of Screening plate 3 01-Oct-01 1035.opd

Example of Specific Expression



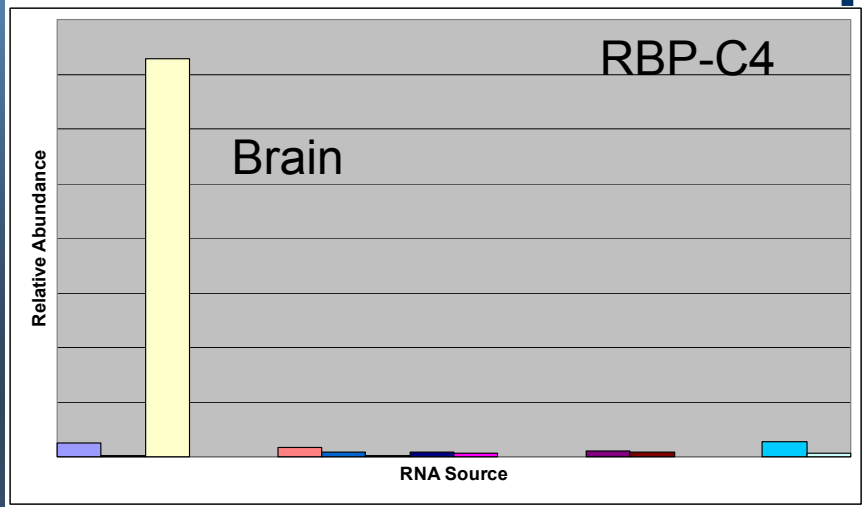
Q-PCR Screening

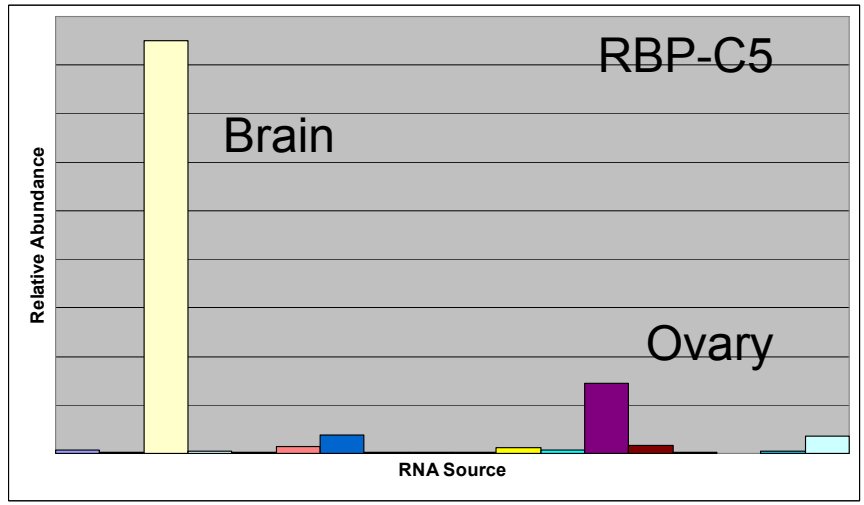
Example of Selective Expression

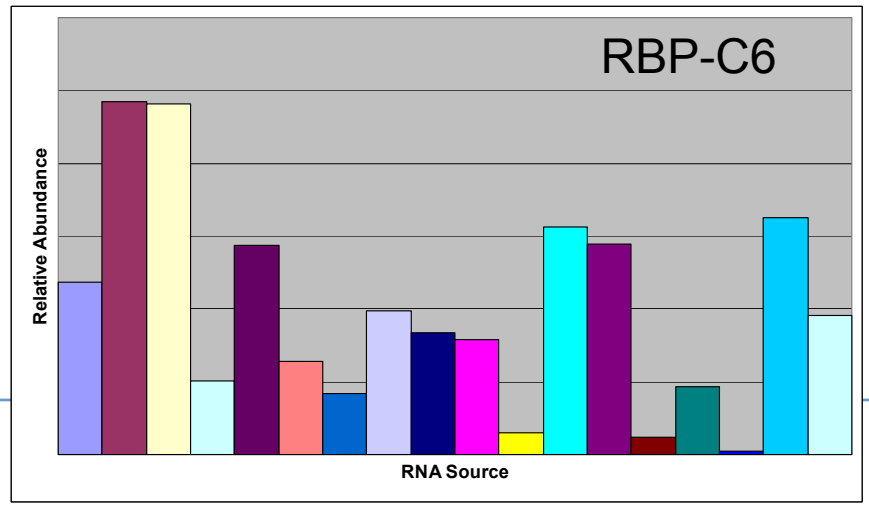


Q-PCR Screening

Members of RBP Families Exhibit Distinct Expression Patterns







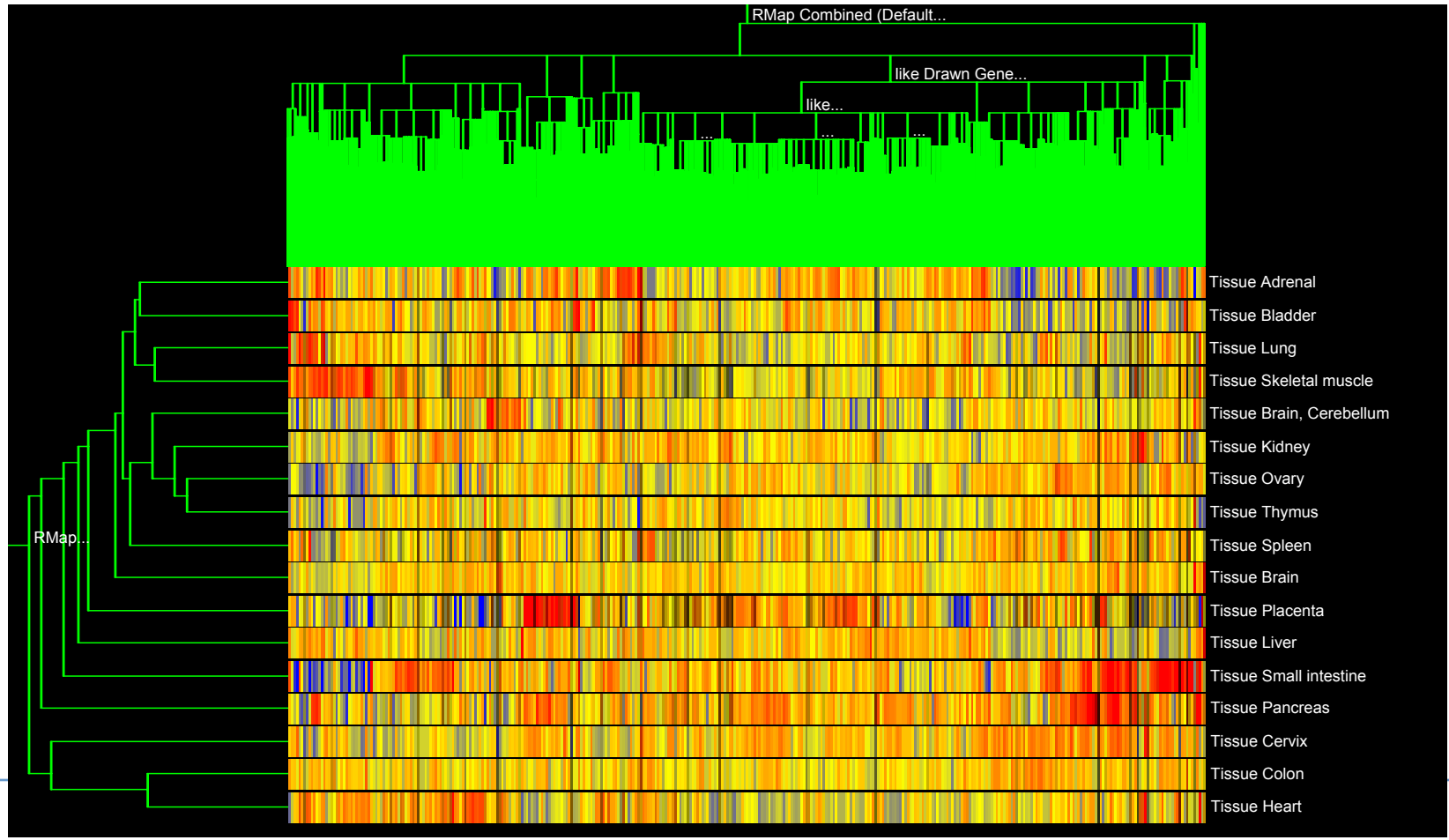
Q-PCR Screening

RBP Gene List Fabrication

- Extraction from public resources
  - Known (annotated) RBPs
    - GenBank, LocusLink, UniGene, GO (Gene Ontology)
  - Homology-based identification
    - pFAM, SCOPES, BLAST
  - Literature Mining
- Cross-referencing and family expansion
  - Unknowns, splice variants, etc.
- Homolog Extraction
- RiboChip v.1
  - Human (1469 features)
  - Mouse (1302 features)
  - Rat (712)

Many RBP genes not present or not annotated on commercial arrays

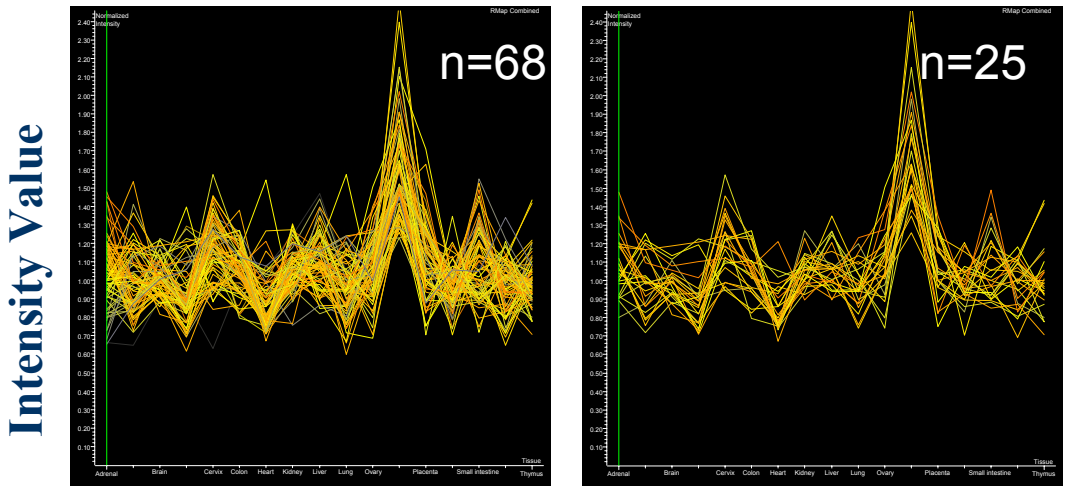
RBP$_{MAP}$
Tissue Distribution of RNA Binding Proteins



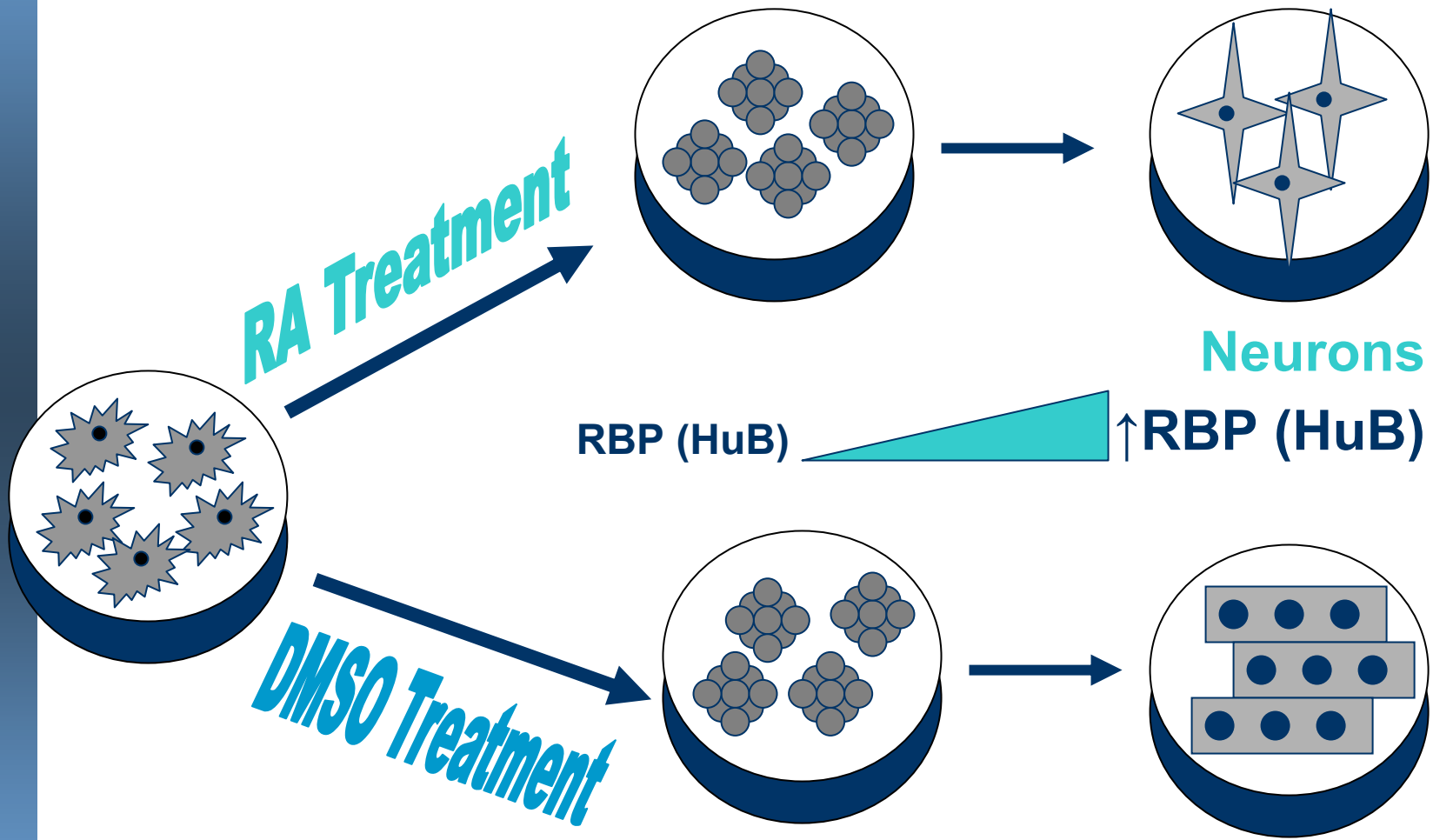
Pancreas-Specific RBP Expression

Correlation Coefficient

0.98 0.985



P19 Neuronal Differentiation Model



HuB is required for neuronal development:
J. Cell Sci. 109: 579, 1996; PNAS 96:9885, 1999

14,000 genes on Chip

10,000 genes expressed

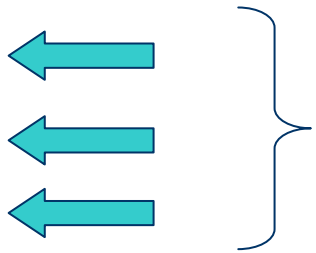
1,072 genes in mRNP pellet

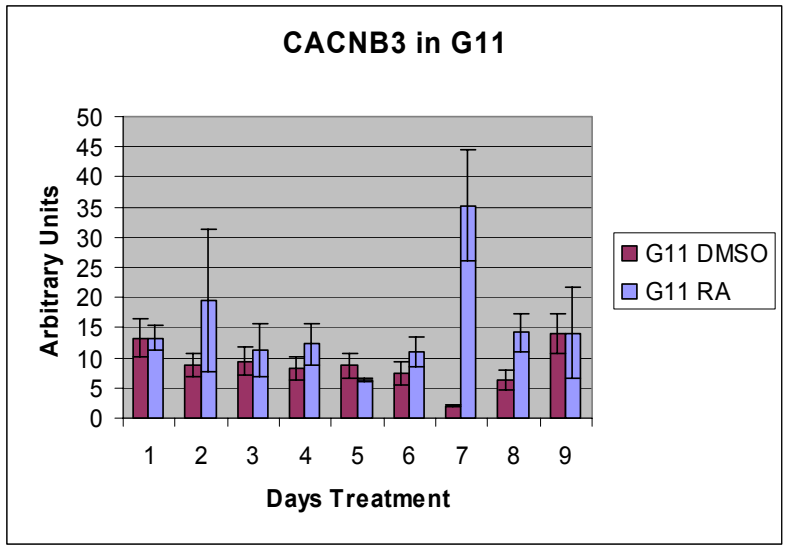
Gene Class	Number Detected in Total RNA	Number Detected in HuB Complex
Kinases	275	27
Phosphatases	148	43
Proteases	137	14
Receptors	87	14
Cytokines	143	39
Growth Factors	87	20

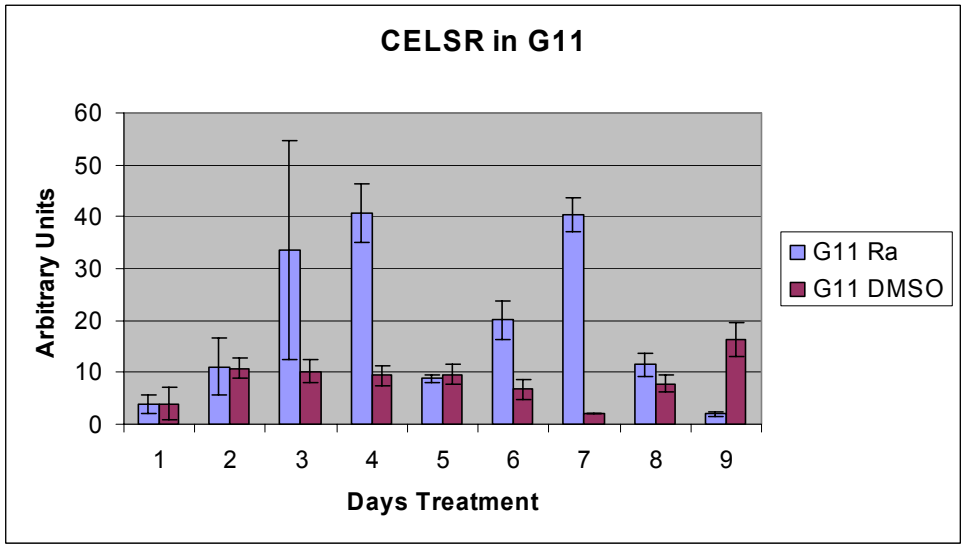
Simplifying Gene Expression Data

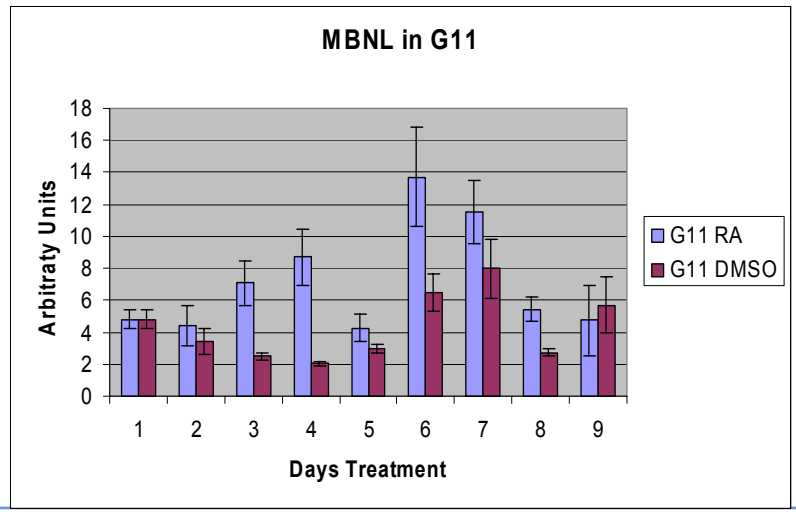
Genes upregulated in RNP w/o change in Totals (n=20)

RA/Undiff RNP	RA/Undiff Total RNA	Protein Name
↑17x	nc	EST
↑15x	↑1.9x	EST
↑15x	↑1.9x	Signaling Protein
↑14x	nc	Ion Channel
↑9x	nc	Receptor
↑9x	nc	RBP
↑8x	nc	Steriod regulated protein
↑7x	nc	TCA cycle
↑6x	nc	Motility receptor





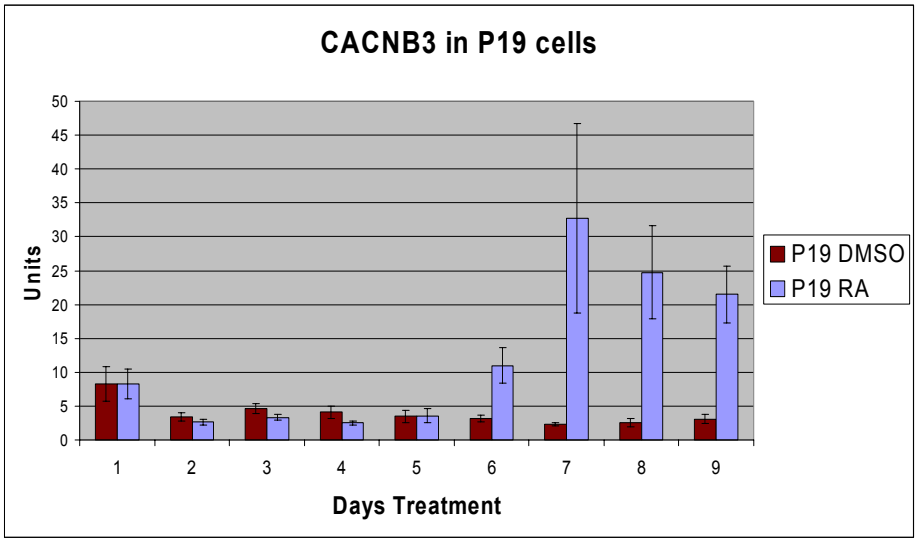


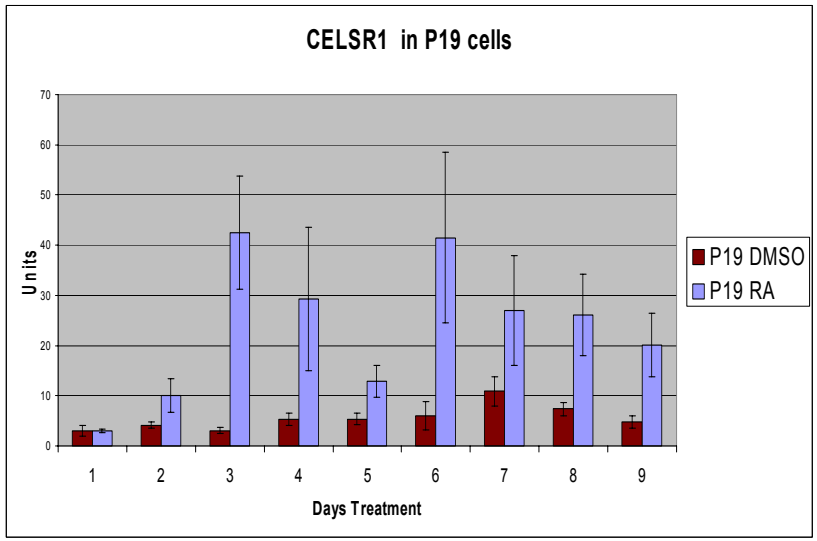


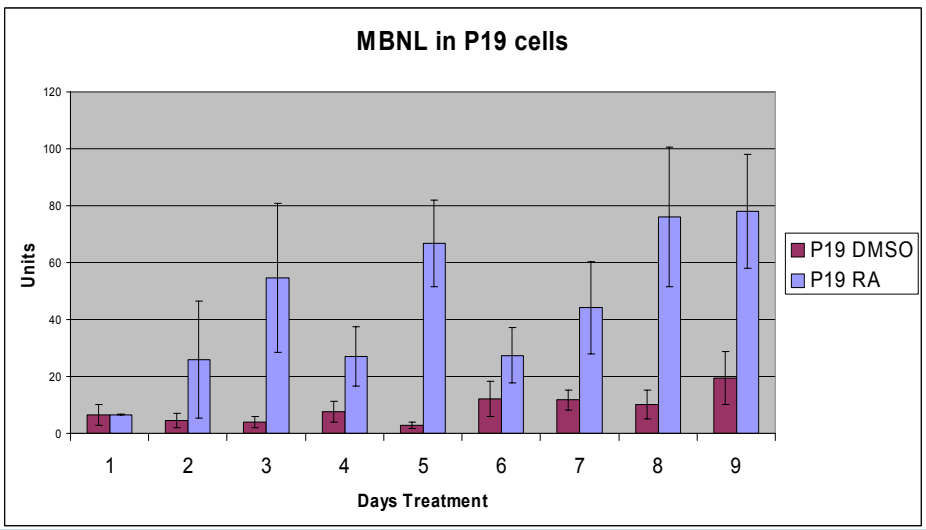
RA neuronal differentiation

DMSO muscle differentiation

QPCR Analysis During Neuronal and Muscle Differentiation in G11 cells





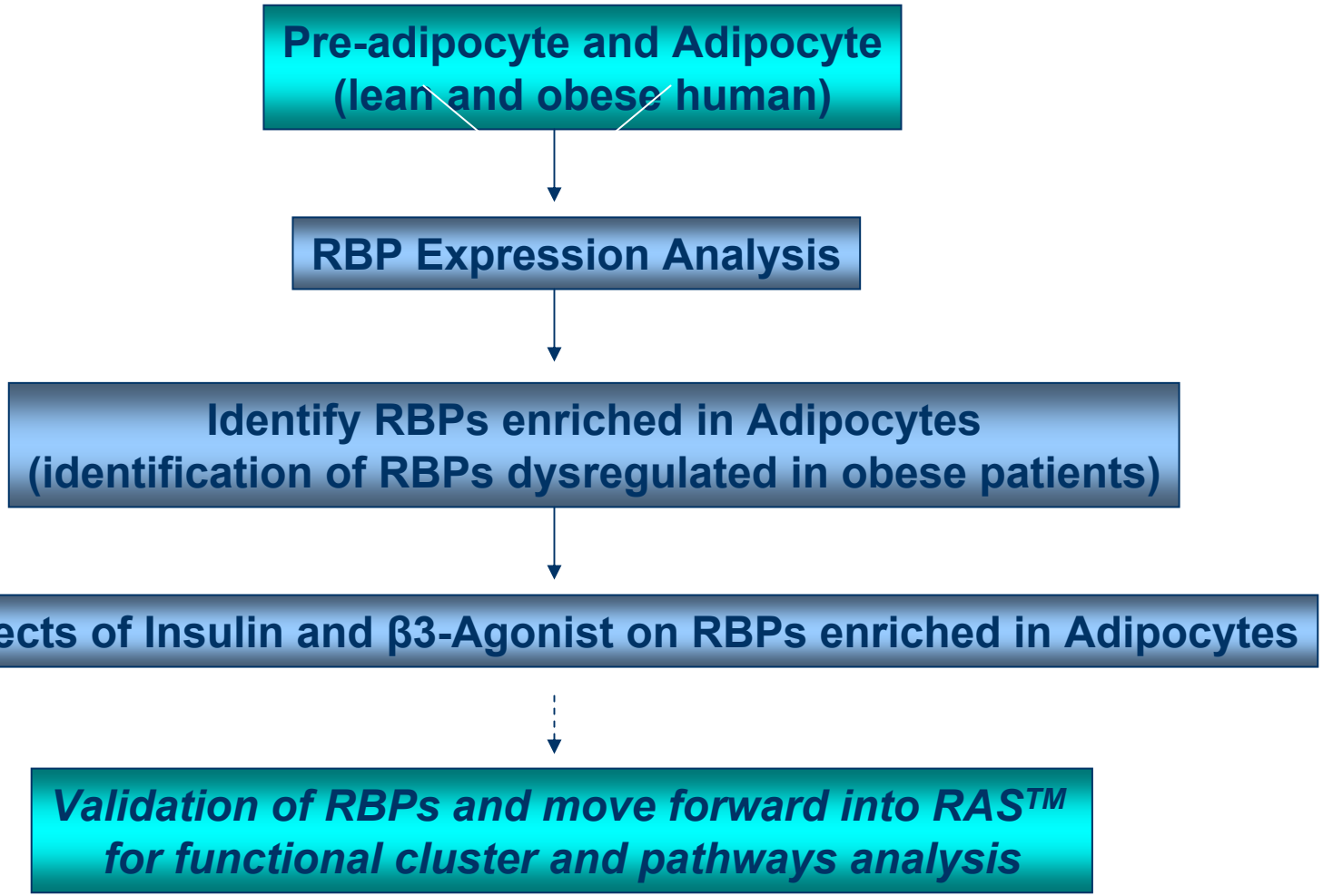


RA neuronal differentiation

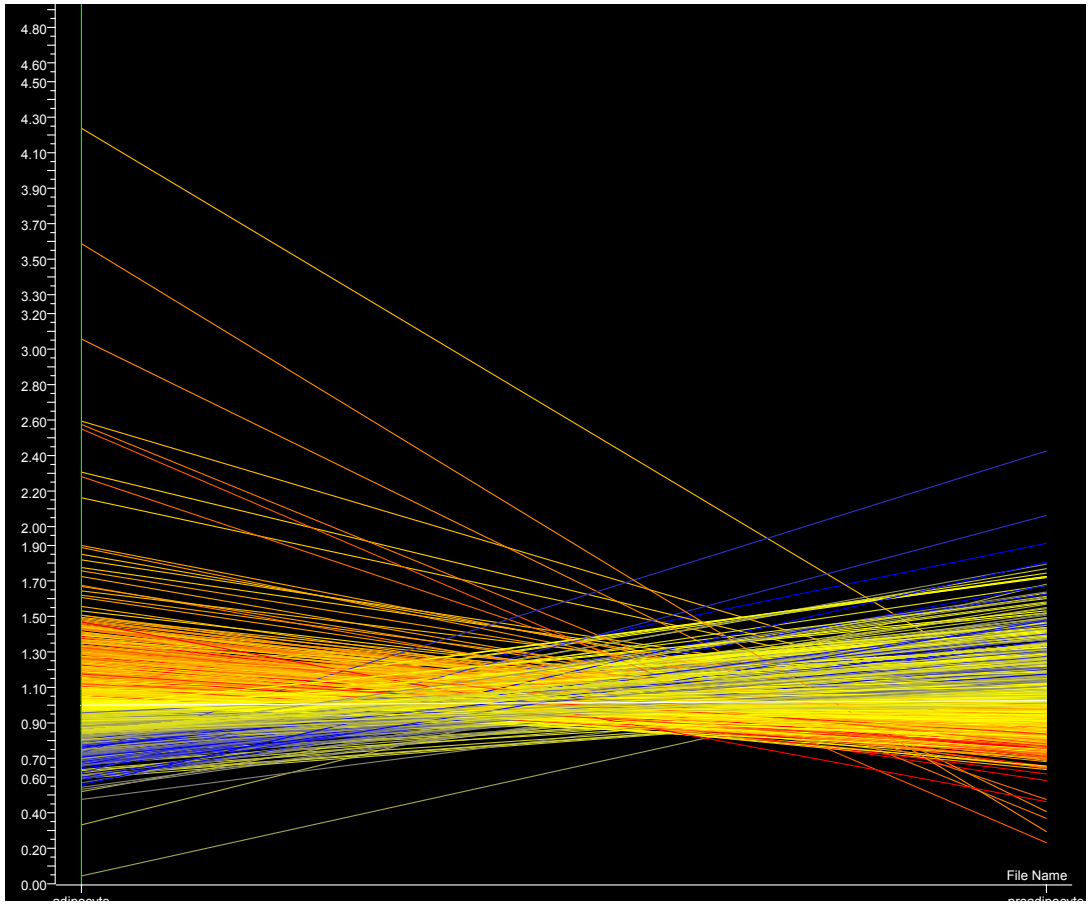
DMSO muscle differentiation

QPCR Analysis During Neuronal and Muscle Differentiation in P19 Parental Cells

Obesity Target Discovery in Primary Adipocytes



RBPs in Differentiating Human Adipocytes

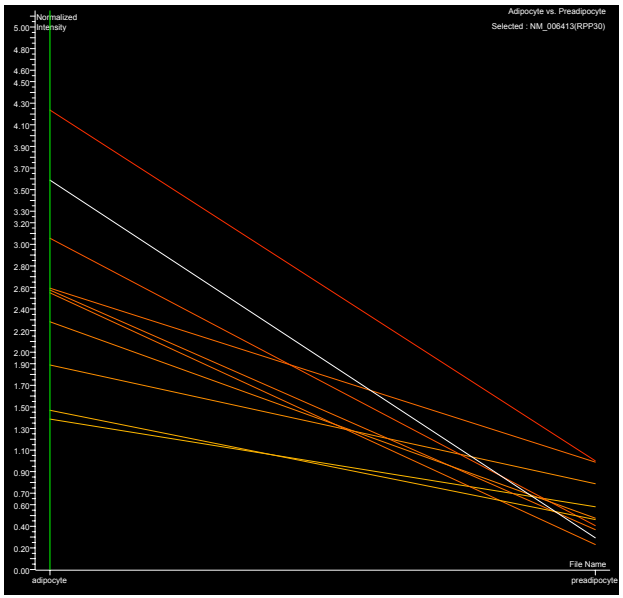


Adipocyte

Pre-Adipocyte

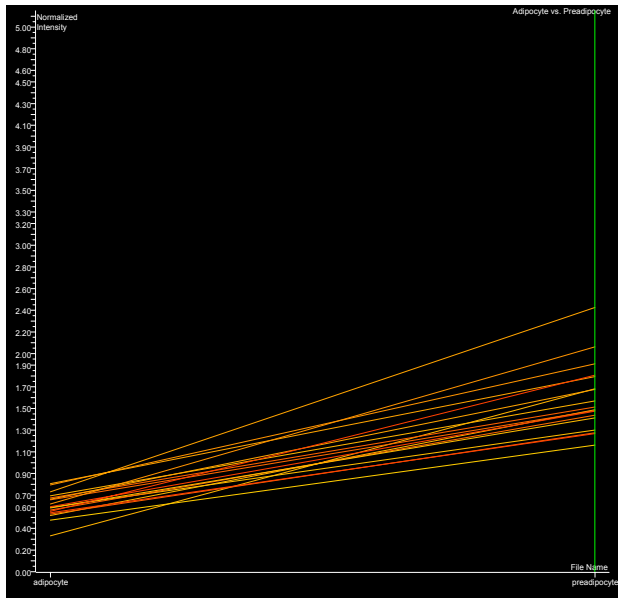
RBPs in Differentiating Human Adipocytes

RBPs Up-regulated in Human Adipocytes



Adipocyte Pre-Adipocyte

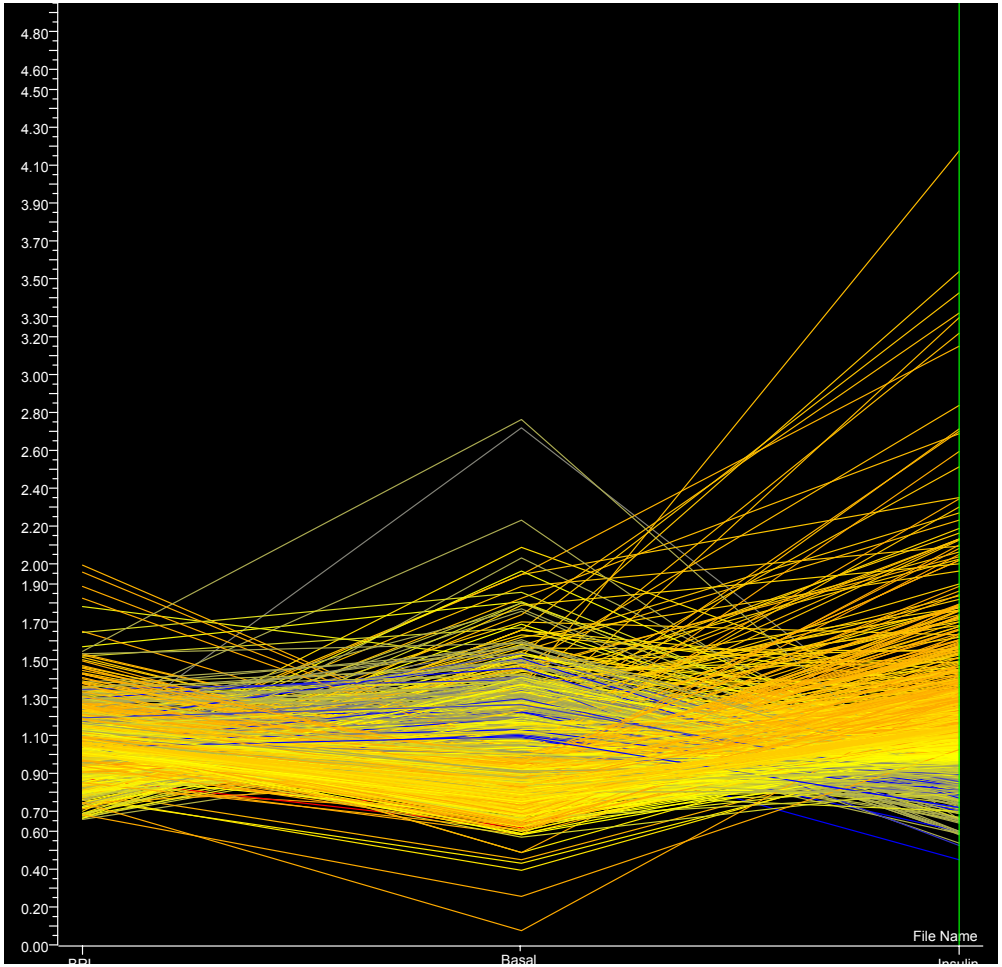
RBPs Down-regulated in Human Adipocytes



Adipocyte Pre-Adipocyte

RBP Expression in Human Adipocytes

-Effects of Insulin and the β3 Agonist BRL-37344-



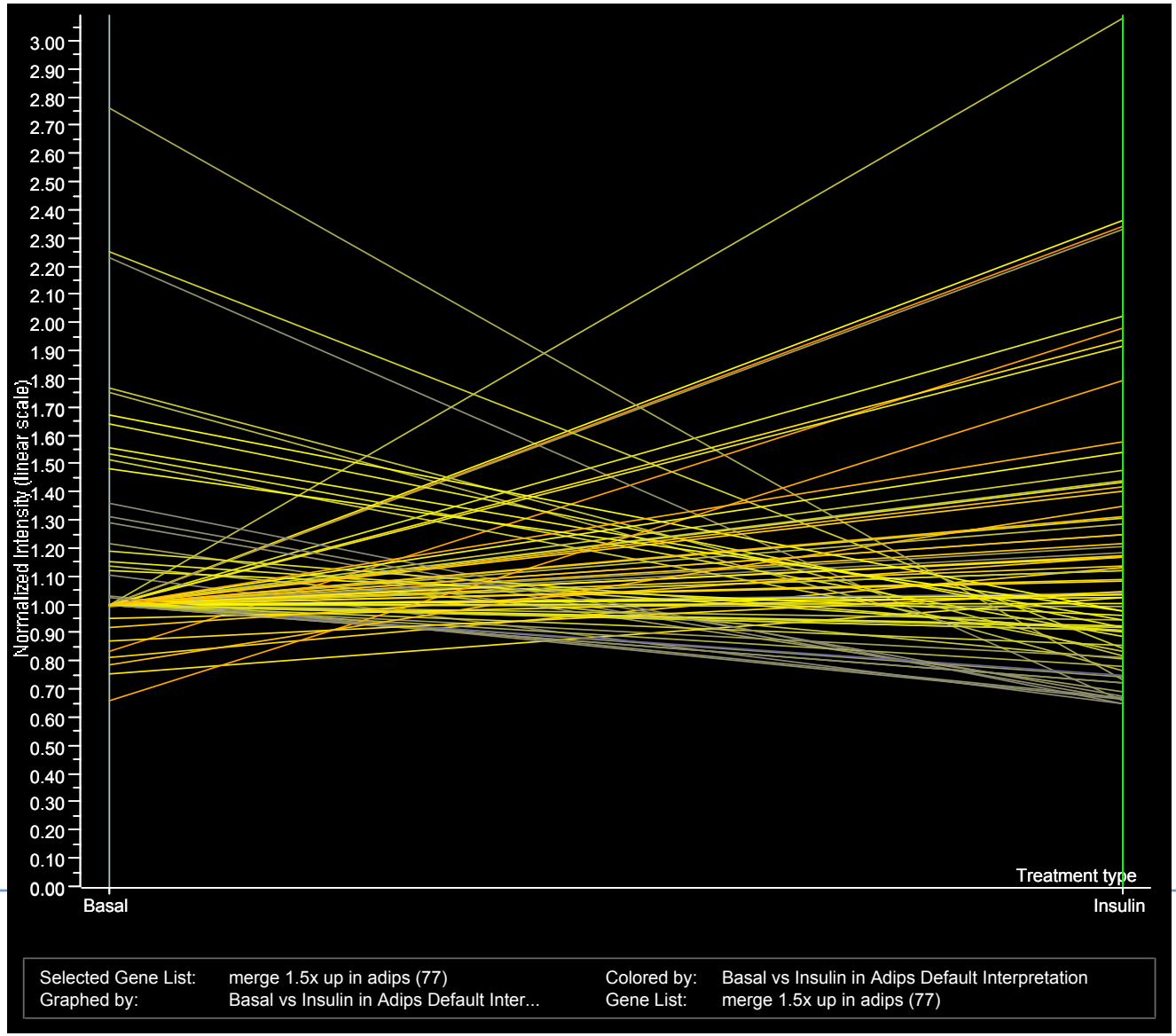
BRL-37344
1 μM

Basal

Insulin
100 nM

Effect of Insulin on RBPs in Adipocytes from a Lean Patient

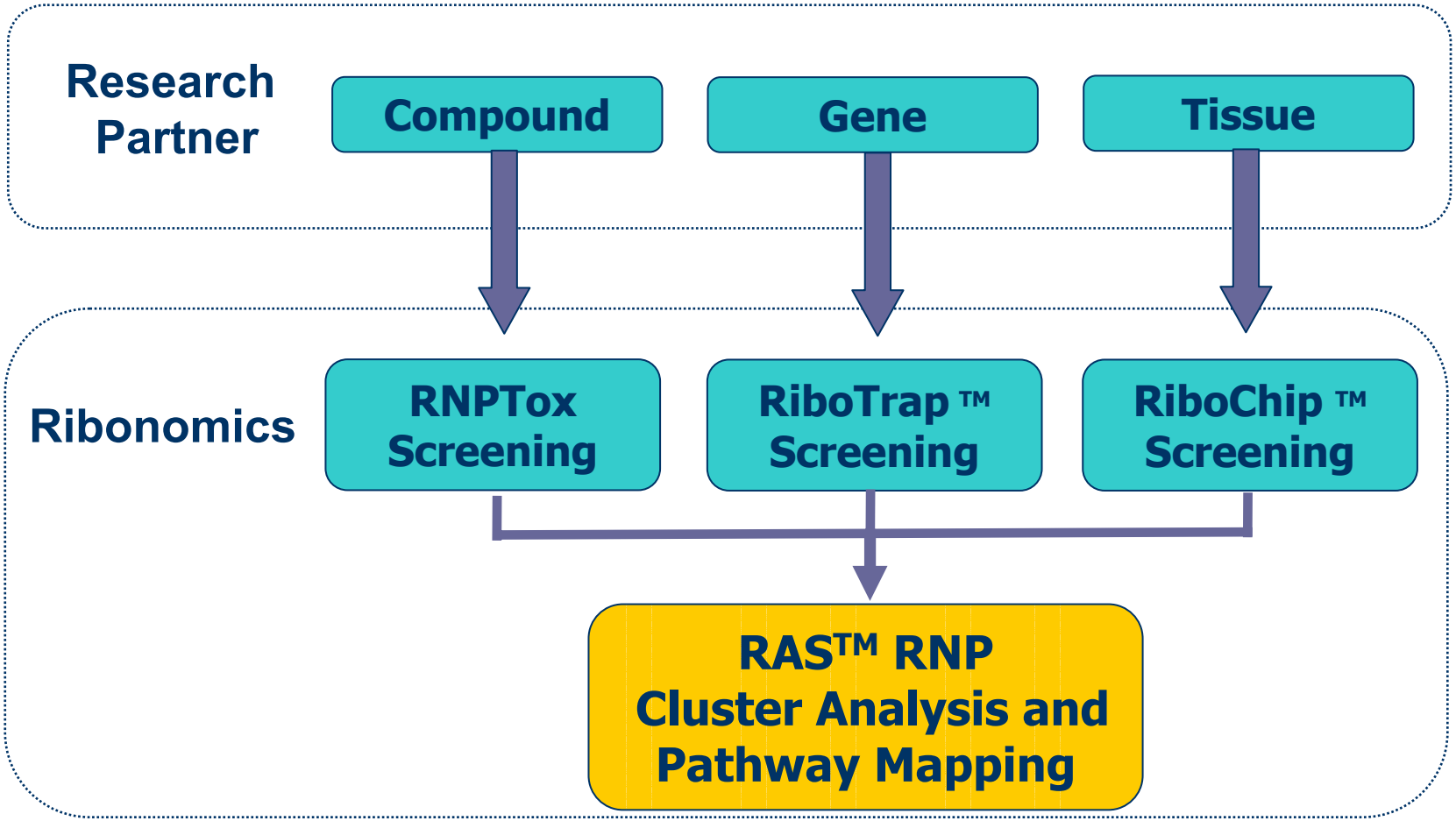
-RBPs from 1.5x up in Adipocytes-



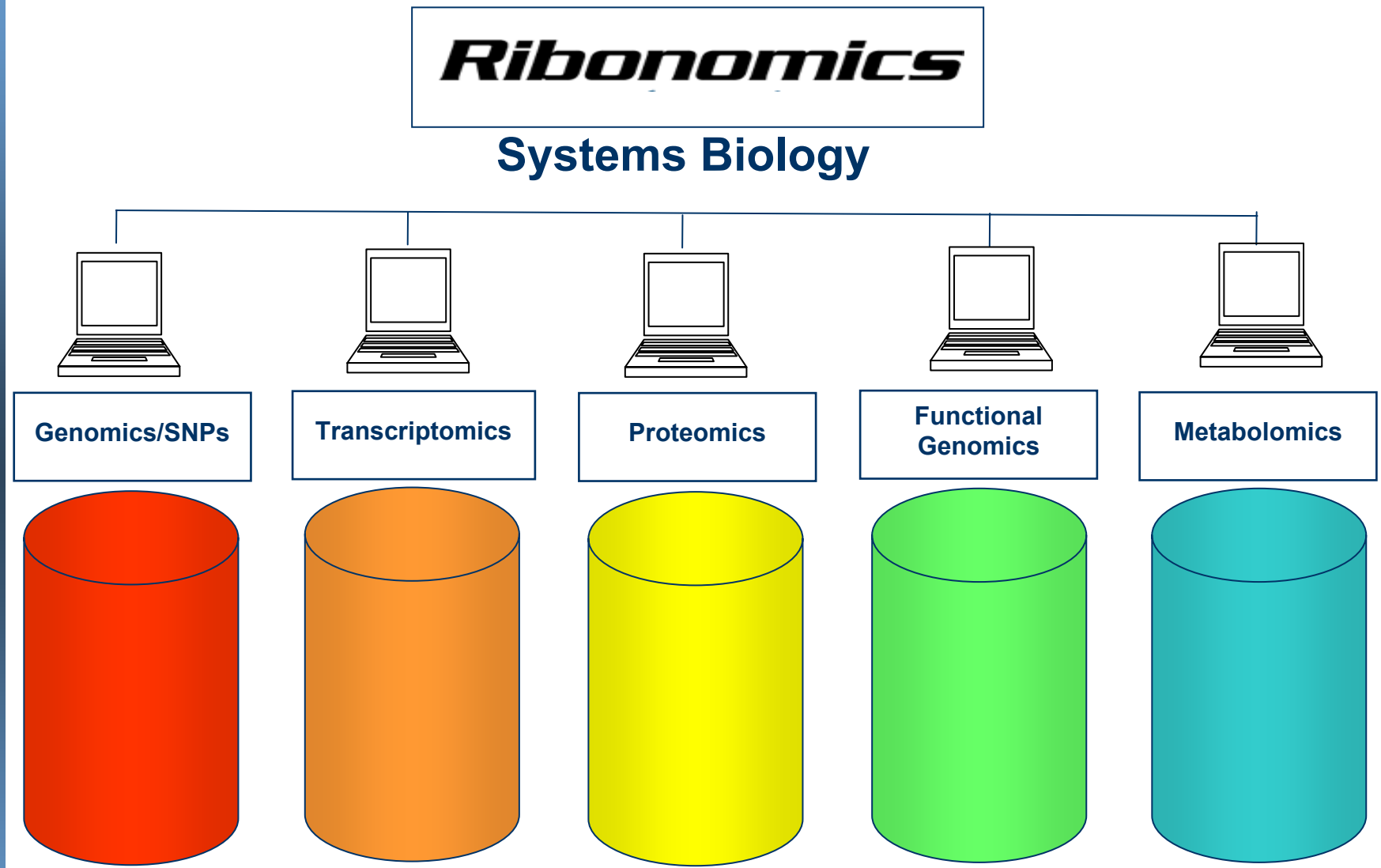
Differentiation of Adipocytes
Lean vs Obese

- Human preadipocytes harvested from elective liposuction (3 lean & 3 obese)
- Differentiated in culture to adipocytes
- Determined RBP gene expression with RiboChip™
- 12 RBP genes consistently induced > 2X in all lean patients; 5 similarly induced in obese patient adipocytes

Research Partnering Process



We provide RNP cluster analysis for partners



A truly biological approach to Systems Biology