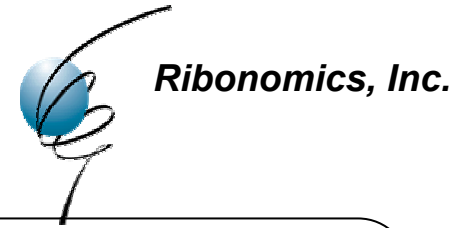


A Ribonomic Analysis of Adipocytes: A Systems Biology Tool

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ABSTRACT

Adipocytes have long been considered a primary location for glucose disposal and energy storage in the form of triglycerides. In recent years, a broader view has emerged which characterizes adipocytes as much more than a simple warehouse for lipids. There is growing evidence that the adipocytes comprise a critical endocrine tissue that not only responds to insulin through glucose uptake and lipogenesis, but also synthesizes and secretes a variety of signaling molecules involved in systemic energy homeostasis. In an effort to better understand adipocyte function we have utilized a novel systems biological approach that focuses on RNA-binding proteins (RBPs) and their role in gene expression. All mRNAs in cells are bound by one or more RBPs via specific cis-acting sequences and/or secondary structural elements within the mRNA. The RBPs organize mRNAs into distinct clusters and coordinately regulates the utilization of the bound mRNAs (i.e. controlling the flow of information from the transcriptome to the proteome). The Ribonomics™ Technology is the isolation of specific ribonucleoprotein (RNP) clusters and identification of the associated mRNAs. As a first step for a ribonomic analysis it is necessary to identify RBPs that are expressed in the particular cell or tissue-type of interest. In the current study we have examined two criteria for identifying RBPs that may be involved in adipocyte function: 1) RBPs that are enriched in mature adipocytes vs. pre-adipocytes, and 2) RBPs in adipocytes whose expression is altered by treatment with insulin. RBP expression profiling was accomplished using our current version of a proprietary microarray chip (RiboChip™ V.1 array) containing approximately 1400 features representing distinct RBPs. By comparing the expression pattern of RBPs in pre-adipocytes vs mature adipocytes from three lean patients (BMI <24) we have identified a list of 11 RBPs that are enriched (> 2-fold) in the mature adipocytes. In contrast to the adipocytes from lean individuals, adipocytes from obese patients (BMI >30) showed a significantly altered pattern of RBP expression. For example, only 4 of the 11 RBPs enriched in lean adipocytes were present in adipocytes from obese individuals. In addition, in mature adipocytes from lean individuals, approximately 50 RBP genes were responsive to insulin (either up- or down-regulated). These data provide a refined list of candidate RBPs for further validation in adipocyte function and for the isolation of specific RNPs and identification of the associated genes.

INTRODUCTION

Large-scale gene expression analysis technologies offer tremendous potential to obtain global perspectives on biological systems. However, pharmaceutical and biotechnology companies are struggling to effectively incorporate these technologies into their drug discovery pipelines. The major problems are: 1) too many candidate targets, and 2) the emerging candidates are poorly correlated with disease requiring extensive downstream validation. Together, these two factors have created a significant bottleneck in the genomics-based discovery pipelines.

Ribonomics, Inc. was founded on a novel approach to gene expression analysis. Our approach relies on interrogation of subsets of mRNA formed within a cell through interactions with RNA binding proteins. By focusing on clusters of mRNAs that are co-regulated by a specific mRNA binding protein, we address the two major hurdles facing genomics-based discovery programs. First, attention is focused on a smaller group of mRNAs, greatly simplifying analysis. And second, because this collection of mRNAs is regulated as a group by a common RNA binding protein they are by definition "functionally-related" providing an often lacking context for biological validation.

In these studies, we have begun to explore the potential of the Ribonomics™ Technology in the mapping of regulatory and metabolic pathways in human adipocytes. Novel insights from such studies will reveal a new generation of therapeutic targets and diagnostic biomarkers for obesity and type 2 diabetes.

RBP MAP

The first stage of the Ribonomics™ Technology Platform is prioritization of RNA binding proteins for study in particular tissues or diseases (Figure 3). Starting with a proprietary collection of more than 1400 RNA binding proteins, we identify those RNA binding proteins that are tissue or cell-type specific or that are deregulated in disease. This serves as a prioritization of RNA binding proteins for subsequent investigation.

RAS™

Prioritized RNA binding proteins move into the second stage of analysis known as RAS™, the Ribonomic Analysis System (Figure 4). RAS™ is the affinity isolation and characterization of in vivo formed complexes of mRNA and RNA binding proteins. Antibodies specific to the RNA binding protein of interest are used to co-immunoprecipitate the RNA binding protein and the associated subset of mRNAs. The mRNA content is interrogated using standard microarray technology.

CONCLUSION

The Ribonomics™ Technology Platform offers a significant advance over existing expression analysis platforms. By relying on the cell's own organization scheme for gene expression we are able to:

- Simplify Gene Expression Data Sets
- Identify Novel Expression Information
- Uncover Relationships Within and Between Biological Pathways and Processes
- Detect Rare mRNAs
- Elucidate Novel Components of Regulatory Networks

Ribonomics, Inc. is utilizing this strategy for discovery and validation of therapeutic targets and diagnostic biomarkers in metabolic disease. The data presented here illustrates the capability of the technology to simplify gene expression information, provided novel expression information and elucidate relationships within and between biological pathways and processes.

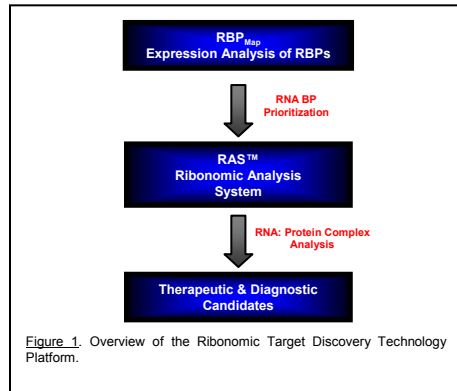


Figure 1. Overview of the Ribonomic Target Discovery Technology Platform.

Largest Gene Families

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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)

Figure 2. RRM (RNA Recognition Motif) –containing proteins are the 7th largest family in the human genome. Several other RNA binding motifs have been described e.g. KH domain, pumilio.

RBPMap

Identification of Tissue and Disease Specific RBPs

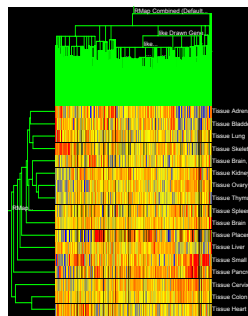
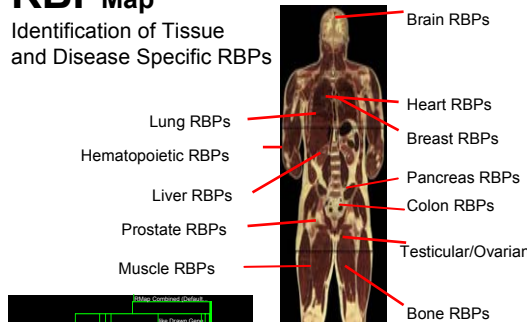


Figure 3. Microarray analysis of 1400 RBP genes by proprietary RiboChip™. A sample of the > 0.5 million data points are shown.

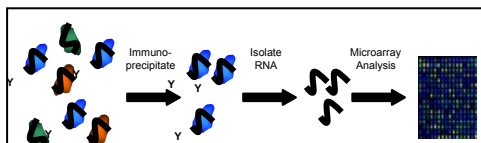


Figure 4. Schematic overview of RAS™. Messenger RNA subsets are isolated as complexes with specific RNA binding proteins by immunoprecipitation. The mRNA is extracted and interrogated by standard microarray techniques.

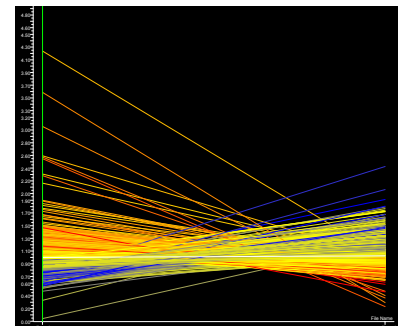


Figure 6. An example of a microarray expression analysis of ~1400 RBP genes (RiboChip™) during differentiation in culture of human primary adipocytes derived from a single patient.

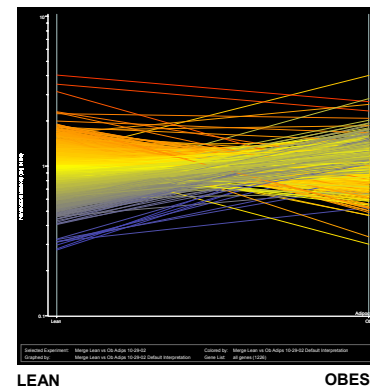


Figure 7. Average expression of >1400 RBP genes in adipocytes from 3 lean and 3 obese patients

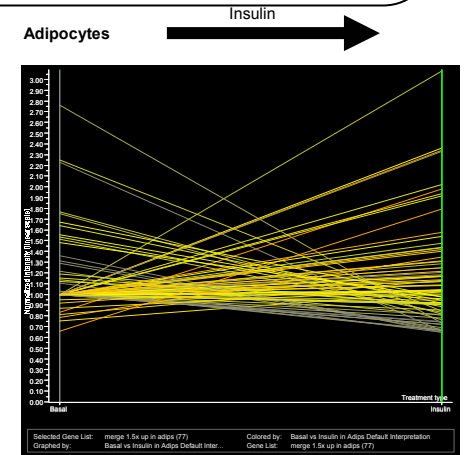


Figure 8. Microarray analysis of RBP gene expression following treatment of differentiated human adipocytes with 100 nM insulin for 2 hrs.

Summary of Expression Data

- > 11 RBP genes are induced during adipocyte differentiation in 3 lean patients > 2X
- > 4/11 are also induced in adipocytes derived from 3 obese patients
- > 7 RBP genes are aberrantly regulated in association with obesity and not induced in adipocytes from obese patients
- > Approximately 50 RBP genes are induced >1.5 X in adipocytes from lean patients following treatment with insulin