The Challenges of Genes, Gene Expression and Genomics

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Genes and Gene Products

Chromosome:

Gene:

Spliced mRNA:

Encoded Protein:

Functional Activity: Enzymes, hormones, structural proteins, oncogenes, etc.
Genes and Gene Products

Chromosome:

Gene:

Spliced mRNA:

Encoded Protein: Activated Oncogene

Functional Activity: Enzymes, hormones, structural proteins, oncogenes, etc.

e.g. p53, ras, myb
Results of the Human Genome Project

- Humans have at least 50,000 genes
  - Genes encode functional RNAs, proteins
  - Many genes encode multiple products
  - At least 200,000 different gene products

- Genetic Differences = Diversity
  - At least 1,000 allelic differences per person
Genomics I: Measuring Differences in Gene Expression Profiles

- Different cell types (e.g. liver vs. kidney, normal vs. tumor) express different genes
- Gene expression patterns can distinguish between different cell or tumor types (classification)
- Differences in gene expression can identify novel targets for drug development
Comparison of Gene Expression Profiles

Cells or Tissue
e.g. Normal vs. Tumor

Purify RNAs,
label with fluorescent “tags”

“Hybridize” to microarray,
detect fluorescence
Affymetrix® GeneChip

GeneChip Probe Array

1.28cm

Human Genome Array > 50,000 genes
> 1,200,000 features
Genomics Research at UNM SOM

• Human Genomics
  • Cancer Biology – Novel Targets, Diagnostic Tools
  • Asthma, Diabetes, Drug Screening, Schizophrenia
  • Epidemiology – Risk Detection and Prediction

• Genomics in Animal Models
  • Stroke, Neurological Studies, Toxicology

• Microbial Genomics
  • DNA Repair and Mutagenesis
  • Cell Cycle Control, Gene Regulation
  • Biosensor, Antibiotic, Diagnostics Development
  • Hantavirus Biology
  • Host-Pathogen Interactions (Cystic Fibrosis)
Example: Lymphoma Gene Profiling - Willman Lab

392 Genes

142 Patients
Gene Expression in PKD Samples

Under-expressed in disease samples
Gene Expression in PKD Samples

Over-expressed in disease samples
Vertebrates Express Three Myb Transcription Factors

- **c-Myb**
  - Central Trans-Activation Domain
  - DNA Binding Domain
  - Negative Regulatory Domain

- **A-Myb**
  - Central Trans-Activation Domain
  - DNA Binding Domain
  - Negative Regulatory Domain

- **B-Myb**
  - Central Trans-Activation Domain
  - DNA Binding Domain
  - Negative Regulatory Domain
Myb Proteins Affect Development

B-Myb knockout blocks cell proliferation

c-Myb knockout blocks definitive hematopoiesis

A-Myb knockout affects testis, mammary gland development
Myb Proteins are Transcription Factors

Myb Proteins Control Cell Fate by Regulating the Expression of Other Genes

Myb Proteins

Target Genes

Regulate Differentiation
Regulate Apoptosis
Regulate Proliferation
c-Myb Expression in Hematopoiesis

Hematopoietic Stem Cell

Myeloid/Erythroid Stem Cell

Lymphoid Stem Cell

CFU-E
CFU-Meg
CFU-Bas
CFU-Eosin
CFU-GM
Pre-B
Pre-T

Erythrocyte  Megakaryocyte  Basophil  Eosinophil  Neutrophil  Monocyte  B-Cell  T-Cell
c-Myb Expression in Hematopoiesis

Hematopoietic Stem Cell

Myeloid/Erythroid Stem Cell

CFU-E
CFU-Meg
CFU-Bas

Lymphoid Stem Cell

CFU-Eosin
CFU-GM
Pre-B
Pre-T

v-Myb

Erythrocyte
Megakaryocyte
Basophil
Eosinophil
Neutrophil
Monocyte
B-Cell
T-Cell
v-Myb Induces Myeloid Leukemias

Normal Chicken Blood

v-Myb-Induced Leukemia
Myb Protein Comparisons

- A-, B- and c-Myb proteins have distinct expression patterns
  - A-Myb: specialized epithelial, hematopoietic cells
  - B-Myb: all dividing cells
  - c-Myb: immature hematopoietic, epithelial
- v-Myb: oncogenic, induces leukemia
- Each protein has a unique biological activity
- All the Myb proteins have similar structures
Test the Activities of Myb Proteins on Endogenous Human Genes

Myb Expression Vector

Recombinant Adenoviruses

Use Affymetrix GeneChips to measure changes in endogenous gene expression

Human Cells
MCF-7: Mammary Epithelial Cells

- Estrogen-responsive mammary carcinoma cells
- Common model for estrogen-responsive breast cancer
- Express A-Myb, B-Myb and c-Myb during cell cycle, in response to estrogen
Adenovirus Expression Vectors

c-Myb DNA Binding Domain

Engrailed Repressor Domain
Filtering Microarray Data to Identify Myb Regulated Genes

Affymetrix U95A, U95B, U95C, U95D, U95E arrays

- 60,000 genes (100%)
- 18,063 genes (30%)
- 215 genes (3.5%)
215 Myb Regulated Genes in MCF-7

Each Myb Protein Regulates a Different Set of Genes

- Genes Repressed by A-Myb
- Genes activated by c-Myb
- Genes activated by A-Myb
- Genes activated by B-Myb
Comparison of Myb Gene Activation in MCF-7

A-Myb Activated (119)
- 61 genes
- 8 genes
- 30 genes

B-Myb Activated (100)
- 46 genes
- 1 gene

c-Myb Activated (43)
- 49 genes
- 4 genes

Genes activated ≥ 2.5X in independent replicate assays. More than 12,000 genes tested on U95A GeneChips.
Activation of Hep27 and DSIPI

Affymetrix GeneChip Data
Myb Binding Sites in the Hep27 and DSIPI Gene Promoters

Hep27 Promoter 1
-1500 NFIL6 Myb Ets Ets Myb

DSIPI Promoter
-1114 YY1 Myb YY1 SP1
Swap Domains Between c-Myb and A-Myb

A-Myb

Conserved Restriction Sites

CHA

AHC

c-Myb

DNA Binding Domain

Transcriptional Activation Domain
A-Myb and c-Myb Specificity in Human Cells

Activated by A-Myb and c-Myb
Activated by the c-Myb transactivation specific for the A-Myb DNA binding domain
A-Myb and c-Myb Deletions

A-Myb

ΔAP

ΔAB

ΔAN

c-Myb

CCA

ΔCB

ΔCN
Domains that Affect Gene Activation

The ability to activate many genes is determined by the C-terminal domains of c-Myb and A-Myb
Structure of the Human c-myb Gene

Alternative splicing can yield > 64 different c-myb mRNAs

Myb proteins with alternative C-termini are likely to have different specificities and to activate different genes
Alternative c-myb Gene Exons Expressed in Normal Cells

Exons 8A, 9A, 9B, 10A and 13A are all detected
Alternative c-*myb* Gene Exons in Leukemia Samples

Leukemias Express Additional c-*myb* Transcripts
c-Myb Alternative Splice Products

Alternative C-termini = Alternative Activities
Compare the Effects of Myb Proteins in Different Cell Types

Myb-Expressing Adenoviruses

Do Myb proteins activate the same genes in different cell types?
75 Myb Regulated Genes in Lung Epithelial Cells

A-Myb
31 genes

B-Myb
28 genes

c-Myb
55 genes

Genes activated ≥ 2.5X in independent replicate assays. More than 12,000 genes tested on U95A GeneChips.
339 Myb Regulated Genes in Lung Fibroblast Cells

A-Myb 225 genes
B-Myb 142 genes

c-Myb 170 genes

Genes activated ≥ 2.5X in independent replicate assays. More than 12,000 genes tested on U95A GeneChips.
Myb Activities are Context-Specific

Almost no overlap in Myb activated genes in different cell types
Unique Activities in Each Cell Type

- MCF-7: 199 genes
- LE: 75 genes
- LF: 339 genes

Three Myb proteins, three cell types, little overlap amongst activated genes.
MSI1 Gene Activation

Legend
- A-Myb
- B-Myb
- c-Myb

Fold Activation

MCF-7  Lung Epith  Lung Fibro

0  1  2  3  4  5  6  7
TGFB1 Gene Activation

Legend
- A-Myb
- B-Myb
- c-Myb

Fold Activation

MCF-7 | Lung Epith | Lung Fibro
--- | --- | ---
4.0 | 1.0 | 2.5
4.5 | 1.5 | 3.0
HSPA6 Gene Activation

Legend
- A-Myb
- B-Myb
- c-Myb

Fold Activation

MCF-7  Lung Epith  Lung Fibro
IL8 Gene Activation

Legend
- A-Myb
- B-Myb
- c-Myb

MCF-7 Lung Epith Lung Fibro

Fold Activation
B-Myb Gene Activation

Legend

A-Myb
B-Myb
c-Myb

Fold Activation

MCF-7  Lung Epith  Lung Fibro
Mechanisms that Could Alter the Specificity of Myb Proteins

- **Tissue-Specific Combinatorial Interactions**
  - Myb proteins cooperate with other transcription factors
  - Unique domains in Myb protein interact with different factors
  - Different cell types have different cooperating factors

![Diagram](image-url)
Mechanisms that Could Alter the Specificity of Myb Proteins

• Tissue-Specific Combinatorial Interactions
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  • Unique domains in Myb protein interact with different factors
  • Different cell types have different cooperating factors

• Context-Specific Post-Translational Modifications
  • Myb proteins are modified at numerous sites
  • Modifications may alter Myb activity or promote specific interactions
  • Different cell types have different modifying enzymes
Myb Proteins are Subject to Multiple Modifications

Myb proteins are modified by X*-ylation

Do Differences in Modifications Alter Interactions with Cellular Co-Factors?

X* – Insert your favorite modification here
Multiple Modifications of c-Myb

- CKII
- Pim-1
- CDK6
- MAPK

p300/CBP
Context-Specific Modifications

A
Gene 1
Gene 2

Myb

B
Gene 1
Gene 2

Myb
Mutations Unmask the Oncogenic Potential of c-Myb

AMV v-Myb is a mutated, oncogenic version of c-Myb
The v-Myb Mutations Could Deregulate c-Myb

C-Terminal Deletion Removes Negative Regulatory Domain
AMV v-Myb is a mutated, oncogenic version of c-Myb
Test the Activities of Myb Proteins on Endogenous Human Genes

Myb Expression Vector

Recombinant Adenoviruses

Use Affymetrix GeneChips to measure changes in endogenous gene expression

Human Cells
Comparing A-Myb, c-Myb, v-Myb

The DBD mutations do not affect some genes.

The v-Myb DBD mutations affect a subset of the c-Myb regulated genes.

The activity of v-Myb is the most unique.

Some genes are preferentially activated by MutMyb or v-Myb.
Unique Specificity of v-Myb

Minor differences in Myb proteins cause dramatic changes in gene expression

Northern Blot

Con  C  Mut  V  A  AHC  CHA

DSIPI

TGFBI

Hep27

actin
The v-Myb Protein Has a Unique Transcriptional Activity

The mutations in v-Myb make it qualitatively different from c-Myb
Ness Lab: Adenoviruses, Genomics: John Rushton
Wanli Lei
Fan Liu
Lisa Davis

Hematopoiesis, Lentiviruses: John O’Rourke

Pim-1 Activity: Louise Winn (Queen’s U., Kingston)

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My Backyard