Regulating the Specificity of Transcription Factors

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Results of the Human Genome Project

- Humans have at least 25,000 genes
  - Genes encode functional RNAs, proteins
  - Many genes (70%) encode multiple products
  - At least 200,000 different gene products

- Genetic Differences = Diversity
  - At least 1,000 allelic differences per person
Gene:
Spliced mRNA:
Functional Activity: Enzymes, hormones, structural proteins, oncogenes, etc.

Vertebrate Genes and Gene Products

Chromosome:
Gene:
Spliced mRNA:
Encoded Protein:

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

Human Genome Array > 50,000 gene probes
> 1,200,000 features
Gene Expression Profiles

A – 293 Fibroblasts
B – MCF7
C – Prim. Lung Fibroblasts
D – Prim. Mammary Epithelial
E – Mammary Tumor Biopsy
F – Prim. Lung Epithelial
Genomics: 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
Genes and Promoters

Chromosome:

Gene:

Promoter Region

Coding Region
Basic Gene and Promoter Structure

Start Site

5’-end of RNA

Promoter Region

Binds Regulators
Controls Gene Expression

May extend >20 kb upstream

Coding Region

Encodes RNAs or Proteins

Extends many kb downstream
RNA Polymerase Binds the Promoter
After Initiation, RNA Polymerase Switches to the Elongation Phase
Repression is the Simplest Type of Promoter Regulation

Operator = Repressor Binding Site
A Bound Repressor Can Block Access by RNA Polymerase
Promoters Bind Multiple Regulators
Promoters Bind Multiple Regulators
Activators Increase Gene Expression

Activators Interact with RNA Polymerase to Increase its Binding or to Increase its Activity
Different Levels of Gene Expression

- **No Expression**
  - Activator and Repressor active
  - RNA Polymerase bound to DNA

- **Weak Expression**
  - Activator dominates
  - RNA Polymerase bound to DNA

- **High Expression**
  - Activator dominates
  - RNA Polymerase bound to DNA
Vertebrates Express Three Myb Transcription Factors

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

Highly Conserved DNA Binding Domain
Central Trans-Activation 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
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

Erythrocyte
Megakaryocyte
Basophil
Eosinophil
Neutrophil
Monocyte
B-Cell
T-Cell

v-Myb
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

A-Myb

B-Myb

MybEng

c-Myb

Engrailed Repressor Domain
215 Myb Regulated Genes in MCF-7

Each Myb Protein Regulates a Different Set of Genes

- Genes Repressed by A-Myb
- Genes activated by A-Myb
- Genes activated by B-Myb
- Genes activated by c-Myb

genes activated by A-Myb
genes activated by B-Myb
genes activated by c-Myb
genes repressed by A-Myb

Combined
smooth
Colored
by:
MCF7 expressed genes
Default Interpretation

Gene List:
Combined
2.5X up or down (320)
Comparison of Myb Gene Activation in MCF-7

A-Myb Activated (119)

B-Myb Activated (100)

c-Myb Activated (43)

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

Legend
- Hep27
- DSIPI
Compare the Effects of Myb Proteins in Different Cell Types

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

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

Legend
A-Myb
B-Myb
c-Myb

Fold Activation

MCF-7
Lung Epith
Lung Fibro
HSPA6 Gene Activation

Legend
- A-Myb
- B-Myb
- c-Myb
IL8 Gene Activation

![Graph showing IL8 gene activation with legend for A-Myb, B-Myb, and c-Myb. The x-axis represents MCF-7, Lung Epith, and Lung Fibro, while the y-axis represents 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
  • Myb proteins cooperate with other transcription factors
  • 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

![Diagram showing modifications of c-Myb](image-url)
Context-Specific Modifications

A

Gene 1

Gene 2

Myb

B

Gene 1

Gene 2
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

- Both bind the same sites in DNA
- Both activate the same reporter gene constructs
- Only v-Myb is leukemogenic

C-Terminal Deletion Removes Negative Regulatory Domain
Compare c-Myb and v-Myb

- c-Myb and v-Myb have completely different transcriptional activities
- v-Myb differences are due mostly to changes outside the DNA binding domain

Fan Liu
Transcriptional Activities of c-Myb and v-Myb

Isolate primary human monocytes from Buffy coat preps

Infect with modified adenoviruses expressing GFP only, c-Myb or v-Myb

Identify genes that are up or down regulated in replicate samples

v-Myb Induced:
- Rab11
- LIPA
- MINA

v-Myb Induced:
- CCNA1
- OXTR
- KLF5
- IL6
- IL12B
- IL23A
- CSF2

Fan Liu

v-Myb and c-Myb have completely different activities in human myeloid cells

In fact, c-Myb and v-Myb almost have opposite activities
c-Myb and v-Myb Regulated Genes

Real Time PCR: Human Monocytes

Legend
- Control
- cMyb
- vMyb

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<th>LipA</th>
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Small Changes in c-Myb Completely Change its Activity

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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
c-Myb Alternative Splice Products

Alternative C-termini = Alternative Activities
Conclusions

- Very Small Changes (1 amino acid) in c-Myb Change its Transcriptional Activity

- Cells Co-Express Multiple Forms of c-Myb, Many in Sub-Stoichiometric Ratios

- Myb Proteins (and other transcription factors) Have Multiple Activities and Regulate Different Genes in Different Situations

- New Methodologies Will Be Required to Follow the Activities of Sub-Stoichiometric Fractions of Transcription Factors with Different Activities