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

Vertebrate Genes and Gene Products



Functional Activity:

Enzymes, hormones, structural proteins, oncogenes, etc.

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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
- $\mathsf{B}-\mathsf{MCF7}$
- C Prim. Lung Fibroblasts
- D Prim. Mammary Epithelial
- E Mammary Tumor Biopsy
- F Prim. Lung Epithelial



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





RNA Polymerase Binds the Promoter



After Initiation, RNA Polymerase Switches to the Elongation Phase



Direction of Transcription

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



Vertebrates Express Three Myb Transcription Factors



Highly Consectent ral Trans- Negative DNA Binding Altivration Dregalatory Domain

Myb Proteins Affect 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 Lymphoid Stem Cell Stem Cell c-Myb CFU-GM CFU-E **CFU-Meg CFU-Bas CFU-Eosin** Pre-B Pre-T

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

c-Myb Expression in Hematopoiesis



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



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



215 Myb Regulated Genes in MCF-7

Each Myb Protein Regulates a Different Set of Genes



Comparison of Myb Gene Activation in MCF-7



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

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



Genes activated \geq 2.5X in independent replicate assays. More than 12,000 genes tested on U95A GeneChips.

339 Myb Regulated Genes in Lung Fibroblast Cells



Genes activated \geq 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



TGFBI Gene Activation



HSPA6 Gene Activation



IL8 Gene Activation



B-Myb Gene Activation



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



Mechanisms that Could Alter the Specificity of Myb Proteins

• Tissue-Specific Combinatorial Interactions

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- 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



Context-Specific Modifications



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 activate the same reporter gene constructs

Regulatory Domain

Only v-Myb is leukemogenic

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 v-Myb_lapd c-Myb have completely different activities in human myeloid c-Mybcenesuced: CCNA1 In factQ&-MRyb and v-Myb albfost have opposite activities **IL12B** IL23A CSF2



c-Myb and v-Myb Regulated Genes



Fan Liu

Small Changes in c-Myb Completely Change its Activity



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 Activites