Lecture 17 Functional Genetics III

Functional genomics: Identify the function of each and every gene in the genome. Since the characterization of the function of a protein domain in one organism generally provides hint to its function in another organism, the first goal of functional genomics is to identify as many genes as possible in major model organisms

Basic Approaches

Gene expression profile (discussed already lectures 15-16)
Protein-protein and protein-DNA interactions (lectures 15-16)
Reverse genetics: disrupt a particular gene or set of genes with known seq.
Reverse genetic methods

• RNA interference
• Delete genes by homologous recombination (yeast, moss, mouse and flies)
• Insertional or chemical mutagenesis
• Activation tagging and enhancer trap

(most can be performed in high throughput manner)
Double-stranded RNA-induced RNA interference causes destruction of a specific mRNA in *C. elegans*

RNA interference

RNAi movie www.nature.com/focus/rnai/animations/index.html

• Initially characterized in:
  - C. elegans
    • Double-stranded RNA injection-named RNAi
  - Plants
    • Resistance to spread of virus
    • Suppression of transgene expression

• Function of RNAi likely used to detect:
  - genome-invading transposable genetic elements and double-stranded (ds) RNA viruses
  - Other abnormal gene expression
Diverse organisms display RNAi

- Model animals (*Drosophila*, *C. elegans*, mouse, etc.)
- Non-model animals (cnidaria, beetles, crickets, crustaceans)
- Protozoa (e.g. *Tetrahymena*)
- Dictyostelium
- Plants (e.g. *Arabidopsis*, maize)
- Fungi (e.g. *Neurospora*)
RNAi works in other organisms

silencing of GFP in leaf veins

depletion of ORC6 results in multinucleated HeLa cells

silencing of GFP in C. elegans nuclei

depletion of White results in unpigmented Drosophila eyes

Potential Practical Applications of RNA Interference

- Control virus infection
- Analysis of cell biology by silencing specific gene
- Target validation for drug development
- Potentially new therapeutic approaches to treating diseases - a new approach to antisense and new possibilities for gene therapy
Systematic RNAi screens in *C. elegans* and mammalian cells

- In the nematode, *C. elegans*, RNAi is easy to do
  - Inject dsRNA
  - Feed bacteria expressing dsRNA
  - Or soak in solution of dsRNA
- Makes systematic RNAi screens possible
  - Fraser, 2000—Chromosome I—feeding
  - Gonczy, 2000—Chromosome III—injection
  - Kamath, 2003—Genome-wide feeding
  - Sonnichsen, 2005—Genome-wide injection
Genome screen by feeding worms with dsRNA expressing *E. coli*

Tuschl 2003 *Nature*
Homologous recombination and gene knock-out in yeast and mouse
Pluripotent, embryonic stem cells originate as inner mass cells within a blastocyst. The stem cells can become any tissue in the body, excluding a placenta.
Knockout Mice Targeting Constructs for Homologous Recombination

Exons of gene X

Targeting vector

Homologous recombination

Gene X in ES cell

Targeted gene insertion

Mutation in gene X; ES cell resistant to neomycin and insensitive to ganciclovir

Nonhomologous recombination

Unrelated gene in ES cell

Random insertion

No mutation in gene X; ES cell resistant to neomycin and sensitive to ganciclovir
Transgenic Mice

~Generation of Knockouts~

- Transfect targeting construct into ES cells from mouse with dominant coat color
- Neomycin treatment (positive selection)
- Ganciclovir treatment (negative selection)
- Inject ES cells with targeted mutation into mouse blastocyst
- Implant blastocyst into pseudopregnant female mouse
- Chose offspring with chimeric coat color partly derived from ES cells and breed to achieve germline transmission
Identify gene function by insertional or chemical mutagenesis

1) T-DNA or transposon insertions and PCR-based screens

2) Arabidopsis Tilling project
1. Screen for T-DNA (or Ds) insertion in specific genes

Gene X

PCR products:  

Screening pools (p1-p5)

1kb ladder

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Data-base searches for T-DNA insertions in the genes of interests

Salk Institute Genomic Group (http://signal.salk.edu/cgi-bin/tdnaexpress)

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SEU=At1g43850