Lecture 14 Functional Genetics

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

A. Gene expression profile (analyses of transcriptome)

B. Reverse genetics: disrupt a particular gene or set of genes with known seq.

C. Fine structure genetics

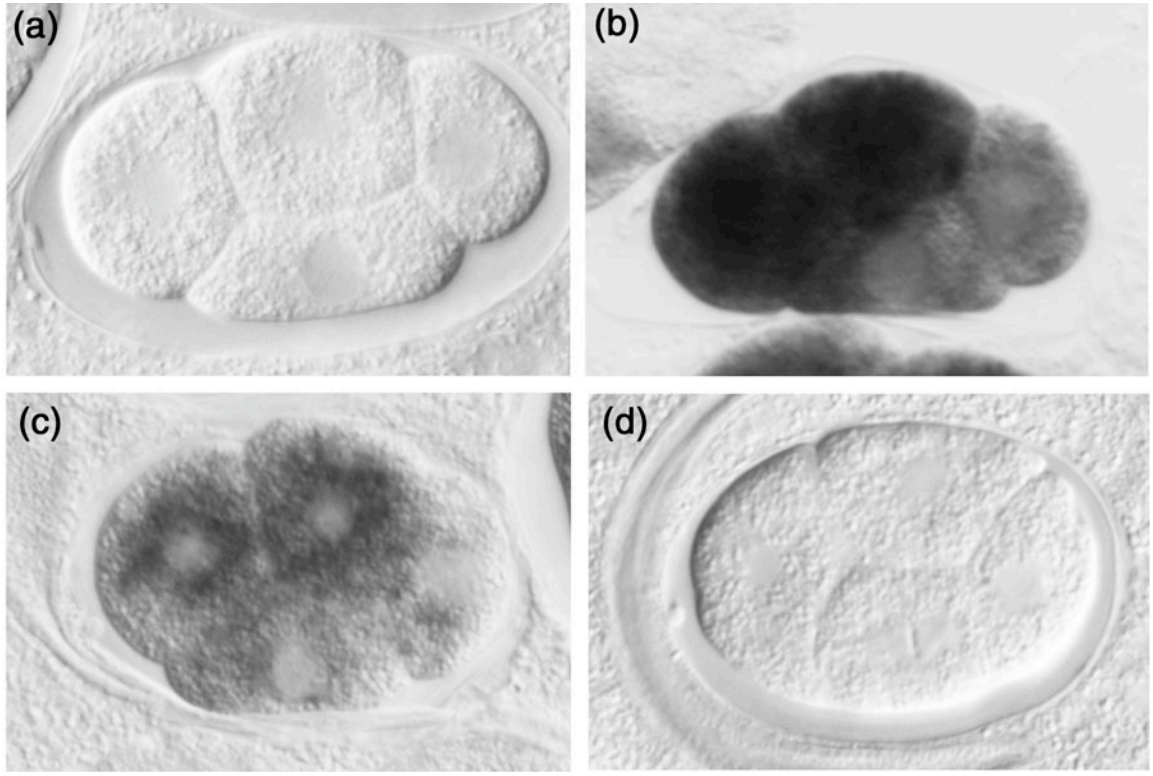
Reverse genetic methods

- **RNA interference**
- **Identify gene affected by**
  - Insertional or chemical mutagenesis
  - Then screen for the mutation
- **Delete genes by homologous recombination**
  - Can be done in yeast, mouse and flies

Double-stranded RNA-induced RNA interference causes destruction of a specific mRNA in *C. elegans*

uninjected, no probe

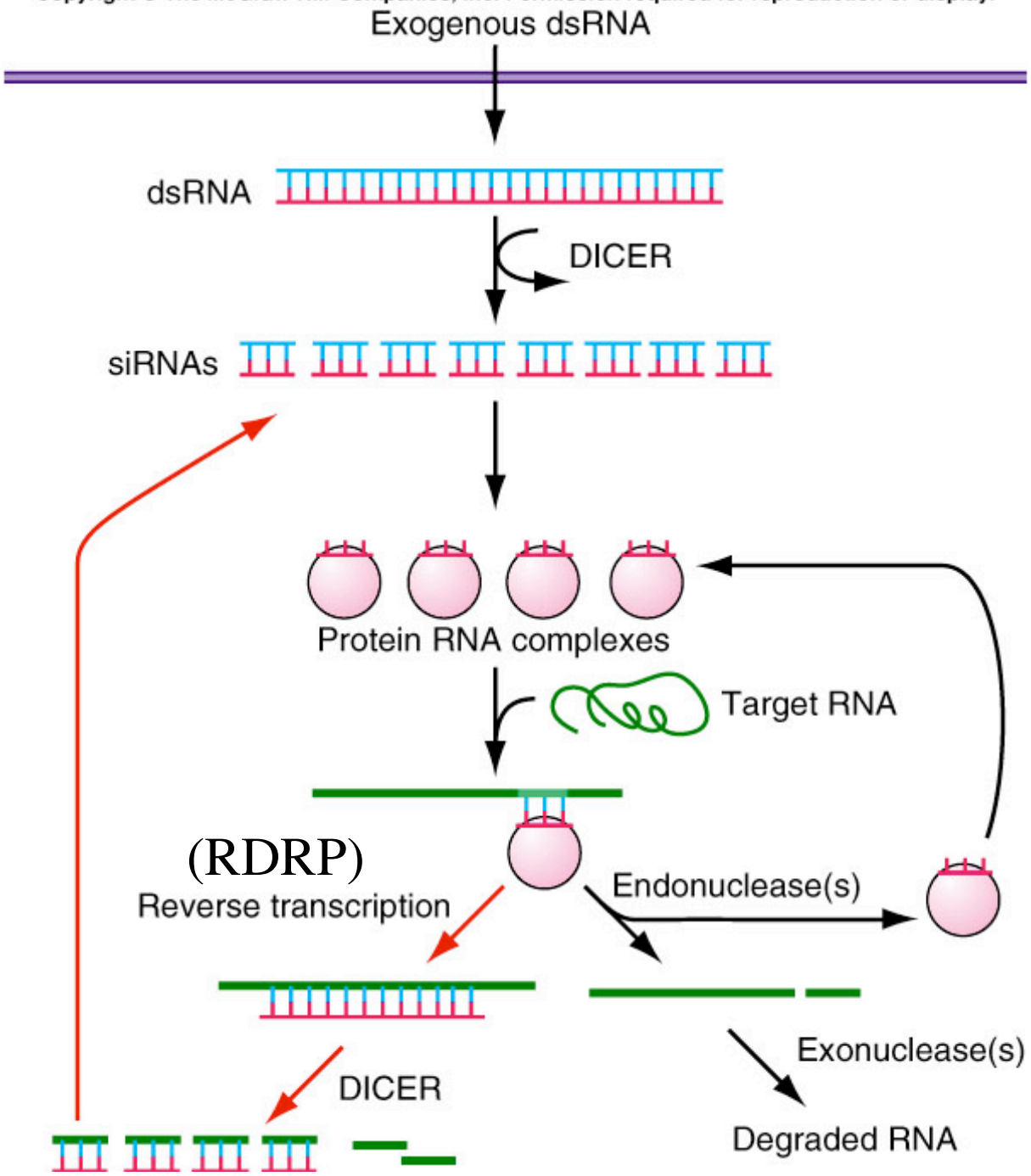
uninjected, mex-3 probe



antisense mex-3 RNA, mex-3 probe

double-stranded mex-3 RNA injected, mex-3 probe

Guo, S. and Kemphues, K. J. *Cell* 81, 611-620 (1995)
Fire, A. et al. *Nature* 391, 809 (1998)



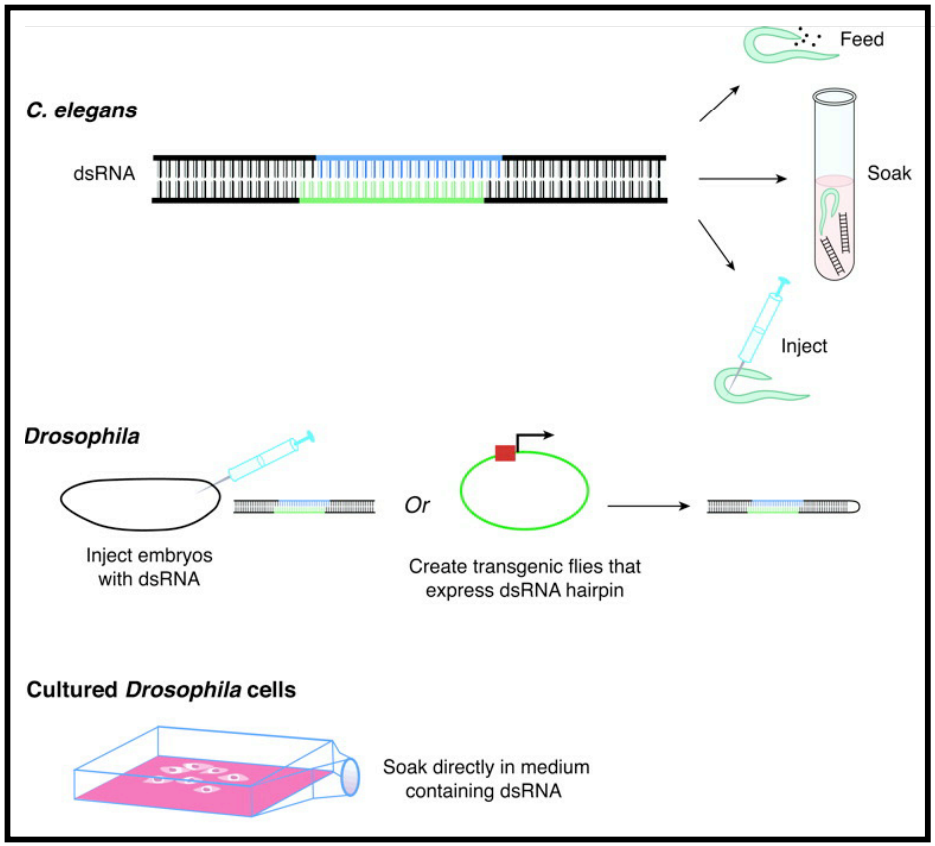
RNA interference

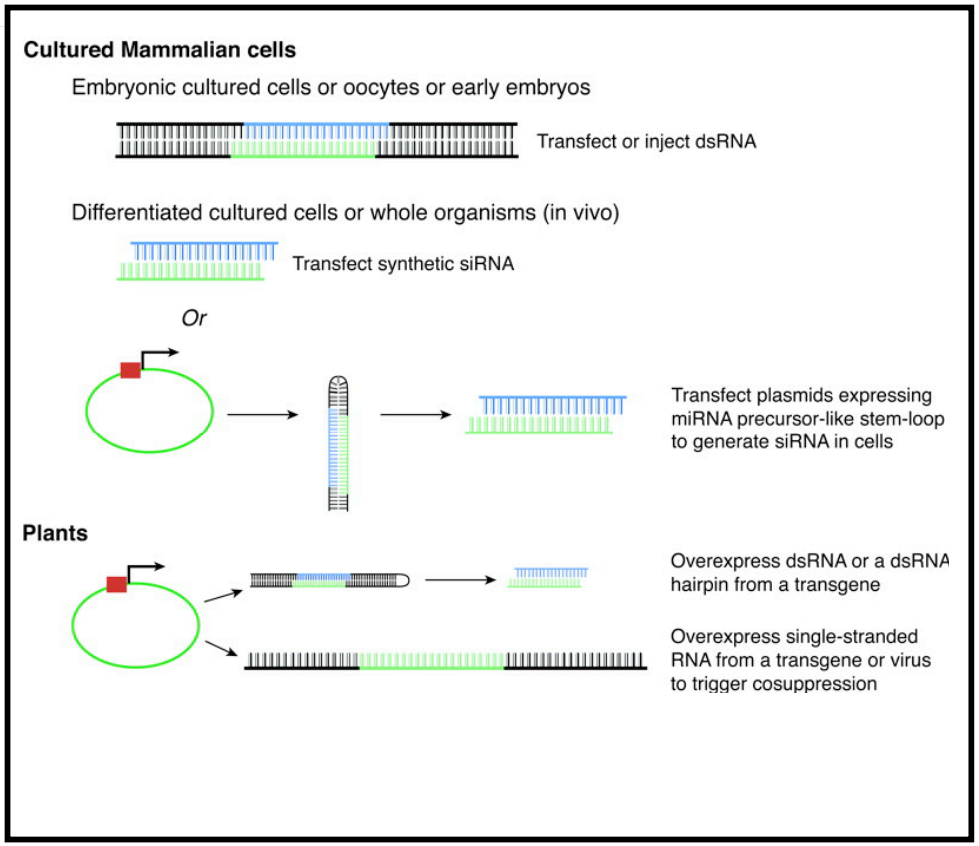
RNAi movie www.nature.com/focus/rnai/animations/index.html

- **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

RNAi works in other organisms



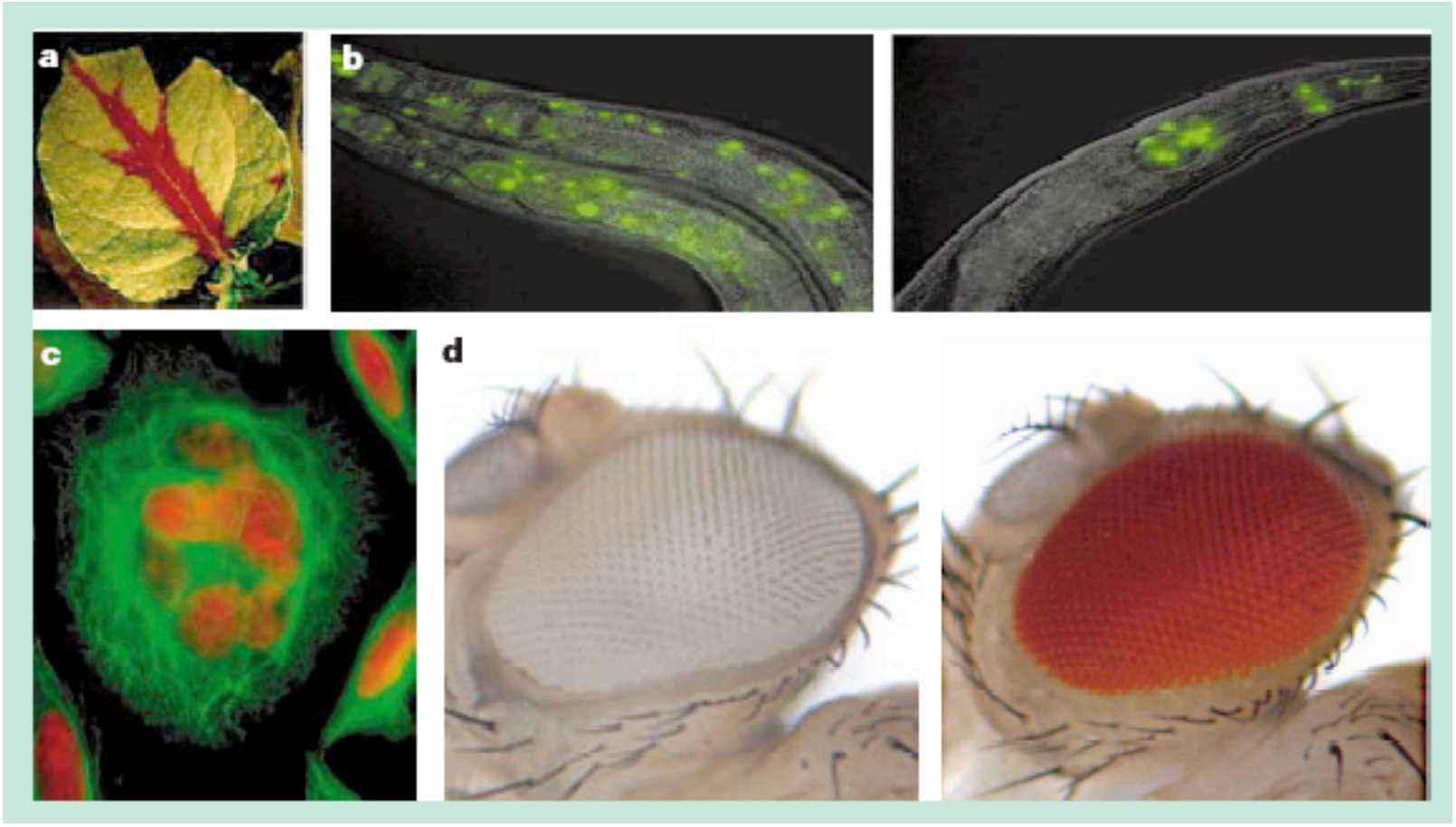


Zamore, P. D. *Science* 296, 1265-1269 (2002)

RNAi works in other organisms

silencing of GFP in leaf veins

silencing of GFP in *C. elegans* nuclei



depletion of ORC6 results in multinucleated HeLa cells

depletion of White results in unpigmented Drosophila eyes

Hannon, G. J. *Nature* 418, 244-251 (2002)

Elimination of SIN3 by RNAi in *Drosophila* tissue culture cells

Synthesize ~800 bp dsRNA in vitro

↓

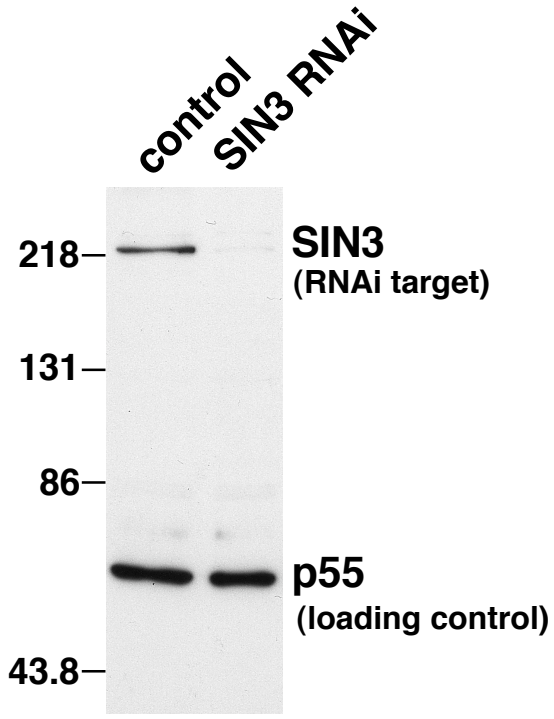
Add ~50μg of dsRNA to 2X10^6 cells

↓

Incubate at room temperature ~3 days

↓

Harvest cells for protein, RNA, or FACs analysis



Western blot

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*)

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

Challenges for siRNAs as a therapeutic agent

Cellular uptake in tissue under physiological conditions

a) **Chemical modifications to improve**
   1) Stability in blood
   2) Tissue redistribution and cellular transport
   3) Efficient silencing of gene

b) **Specific target site delivery**
   Examples, eye and CNS

c) **Delivery as a complex**

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-Chromsome III-injection

  -Kamath, 2003-Genome-wide feeding

  -Sonnichensen, 2005-Genome-wide injection

Identify gene function by insertional or chemical mutagenesis

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

2) Arabidopsis Tilling project