Bi/Ch 113: Biochemistry of the Cell, Spring 2017

- protein and membrane trafficking

www.its.caltech.edu/~bich113

Goals:

- To present our current understanding of these topics, with an emphasis on the underlying molecular mechanisms.
- To illustrate the experimental strategies biologists use to analyze cell biological processes.
- To read research papers in detail and to discuss them critically.

Bi/Ch 113: Biochemistry of the Cell, Spring 2017

Lectures: Tuesday and Thursday 11-11:55 am, Broad 100

Section attendance is mandatory

Teaching assistants

Amanda Mock, jmock@caltech.edu
Greg Varuzhanyan, gvaruzhanyan@caltech.edu
Ruohan Wang, rwwang@caltech.edu

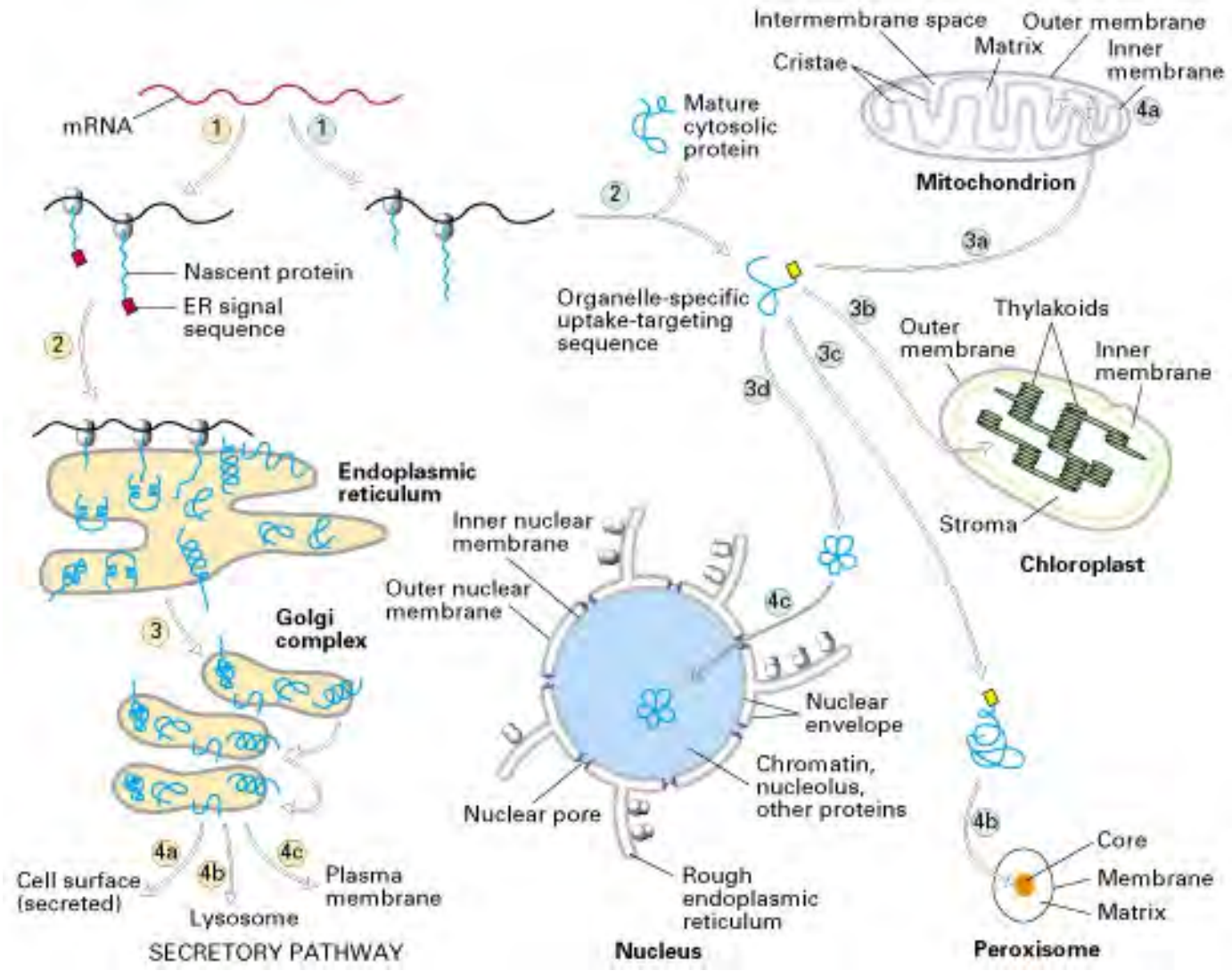
Section 01: Wednesday 2-3:55 pm, Broad 200 (Ruohan)
Section 02: Thursday 7-8:55 pm, Kerckhoff 101 (Greg)

Grading

Midterm: due at the beginning of class, May 9.
Final: due June 8 for seniors and graduate students; due June 15 for others.
Course grade is determined by: section performance, 1/3; midterm, 1/3; final exam 1/3.
No extensions are allowed for paper summaries, the midterm, or the final.

Protein and membrane trafficking

- How do proteins get to their destination?



Lodish et al, 2000

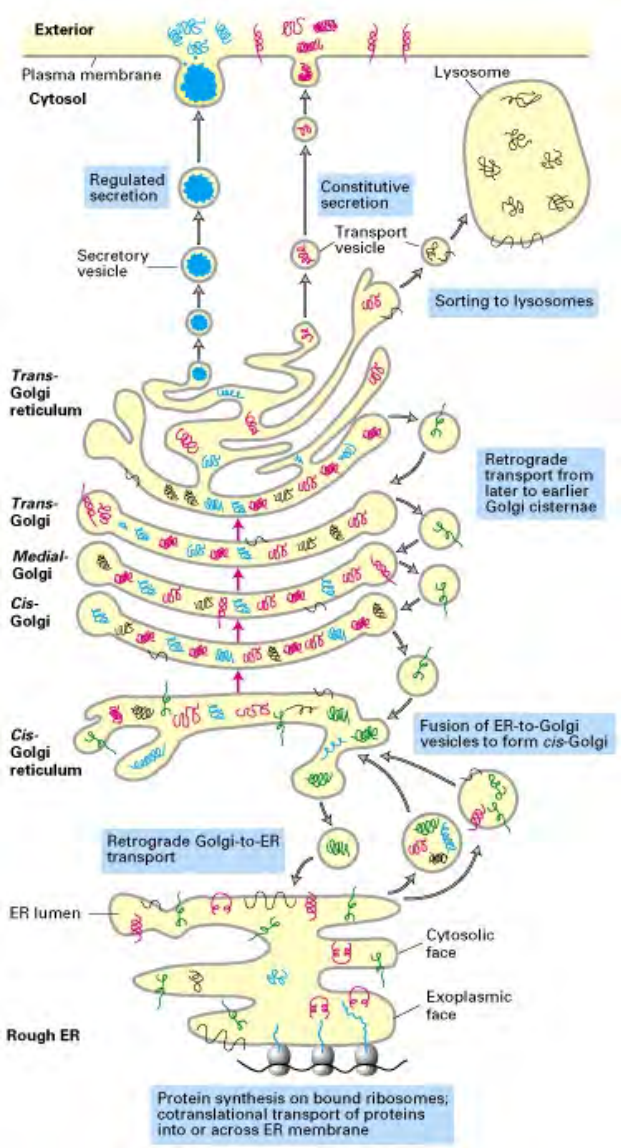
Protein and membrane trafficking

Endocytosis

Secretion

Vesicle fusion

Protein translocation



How are proteins, lipids, and organelles sorted to the correct compartment?

Model systems:

ER: translocation, secretion, morphology

Viruses

Endocytosis

Nucleus structure and import

Autophagy

Apoptosis

Mitochondria

Relation to disease

Oncogenesis

Lodish et al, 2000

Guidelines for reading and discussing papers

- 8 research papers will be presented and critiqued in section.

Sections of paper

Title

Abstract

Introduction

Materials and methods

Results (Figures/Tables)

Discussion

Reading and discussing research papers

1. What is the overall question the authors are addressing? Is it an important question; does it address a gap in knowledge?
2. What experimental strategy do the authors use to answer this question?
3. Are the experiments appropriate for the question, and are they well-done? Are the data convincing?
4. Are the experiments properly controlled?
5. How strongly do the data support the conclusions? That is, do you believe their conclusions?
6. Are the findings interesting and important?
7. Where does one go from here?
   -What additional studies can be done to test the conclusions?
   -Is a new line of research suggested by these findings?

Presenting research papers in section

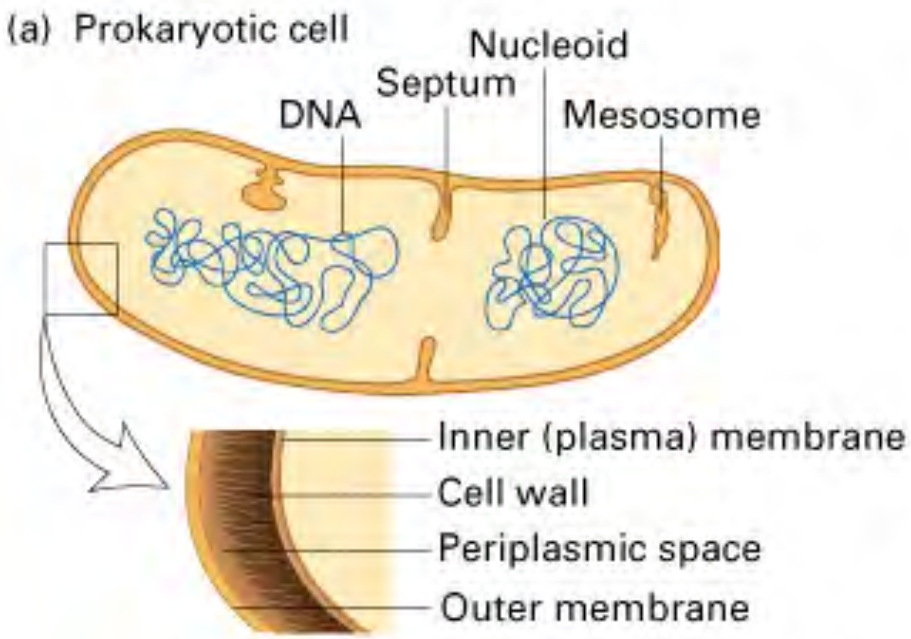
TAs will provide guidance on presentations

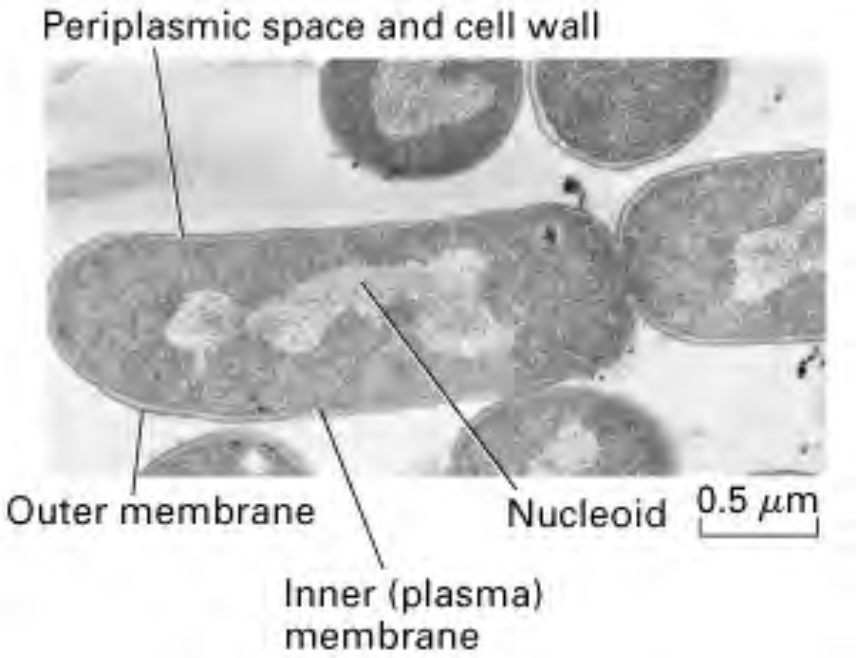
1) Provide **introduction** giving background material and describing the state of knowledge at the time the experiment was done. How does this work fit into the overall state of the field?

2) Analyze the **individual figures/tables** in the paper. What is the figure purporting to demonstrate, and what method is used? Are the data convincing? Which are the most critical pieces of data?

3) **Overall evaluation/conclusions**: does the paper accomplish what it claims to do? Are the results important? What are the directions for future work?

Cellular dimensions- prokaryotes

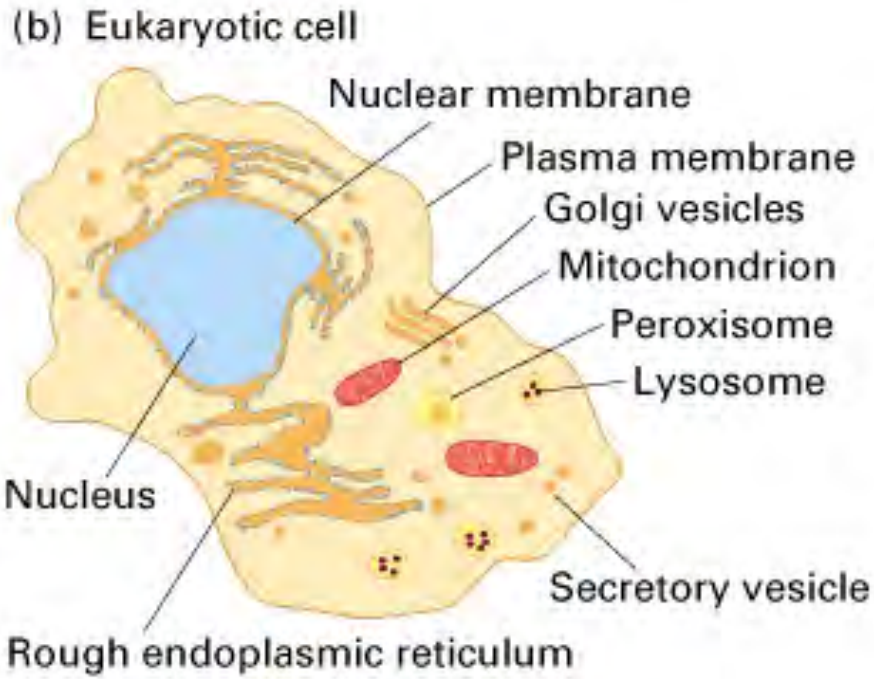


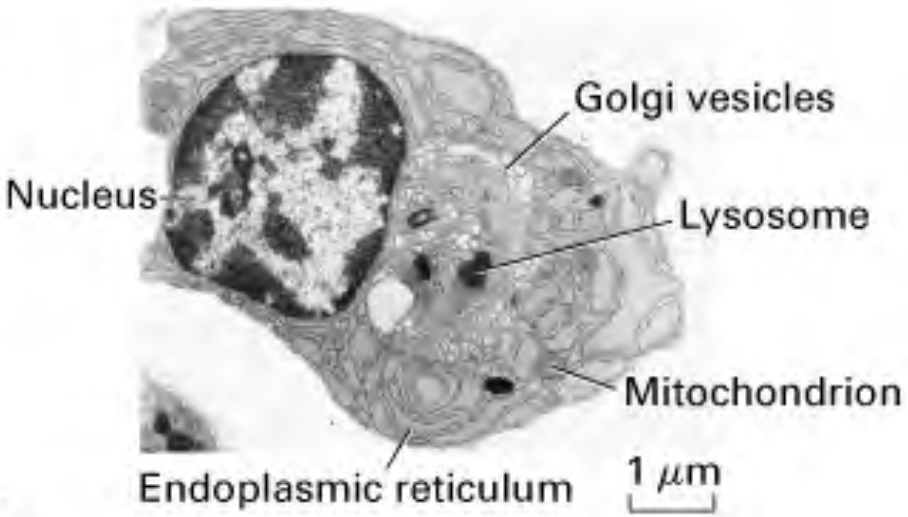


Bacteria: 0.5 μm diameter

Lodish et al, 2000

Cellular dimensions- eukaryotes





Mammalian cell: 10-30 μm

Nucleus: 3-10 μm

Lipid bilayer: ~4 nm

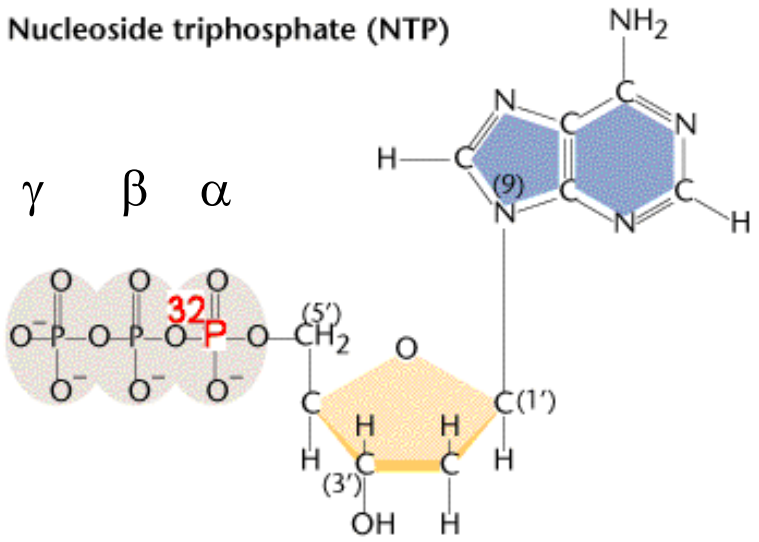
Vesicles: >50 nm

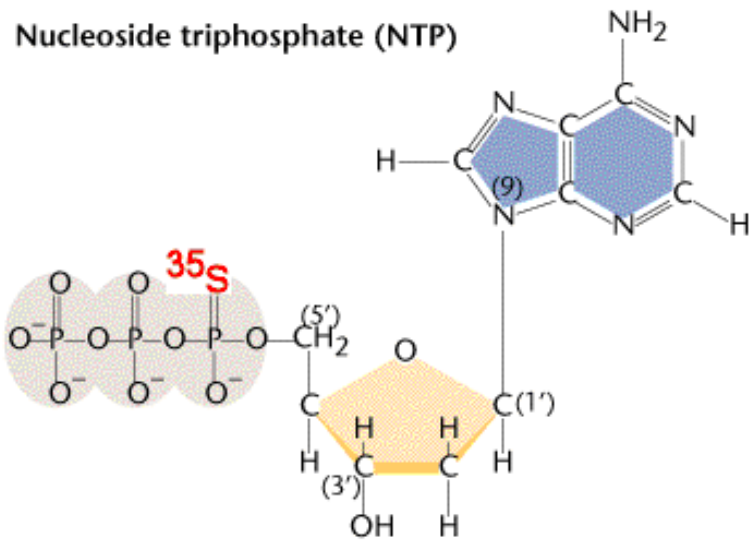
Mitochondria: 0.4 μm diameter

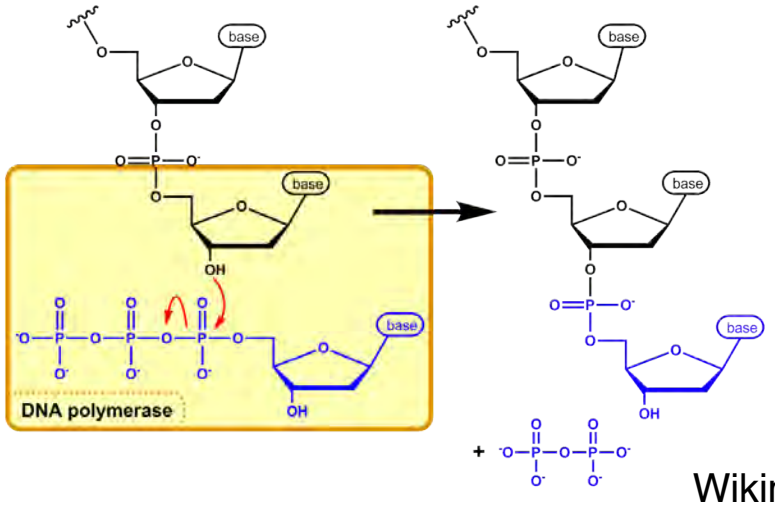
α-helix: 11 Å diameter; 1.5 Å translation per residue

Lodish et al, 2000

Labeling nucleic acids with radioactive isotopes



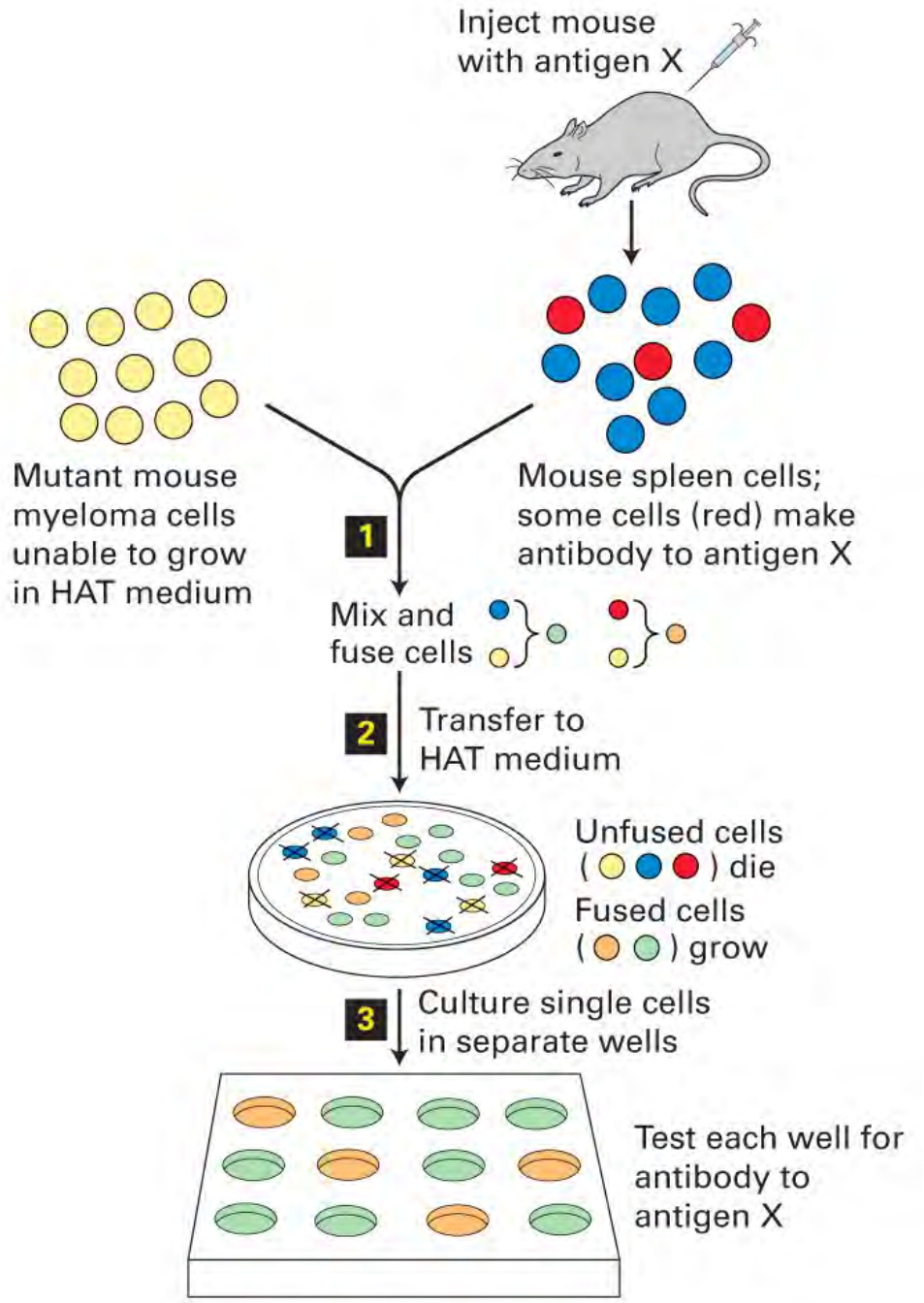




During DNA synthesis the α-phosphate group of the free nucleotide is added to the 3' OH of the growing chain.

Wikimedia.org

Monoclonal antibodies



- Polyclonal antibodies recognize multiple epitopes.
- A monoclonal antibody recognizes a single epitope.
- Monoclonal antibody is a defined, renewable reagent.
- Specificity versus sensitivity.

Lodish et al, 2004

Assigning gene function

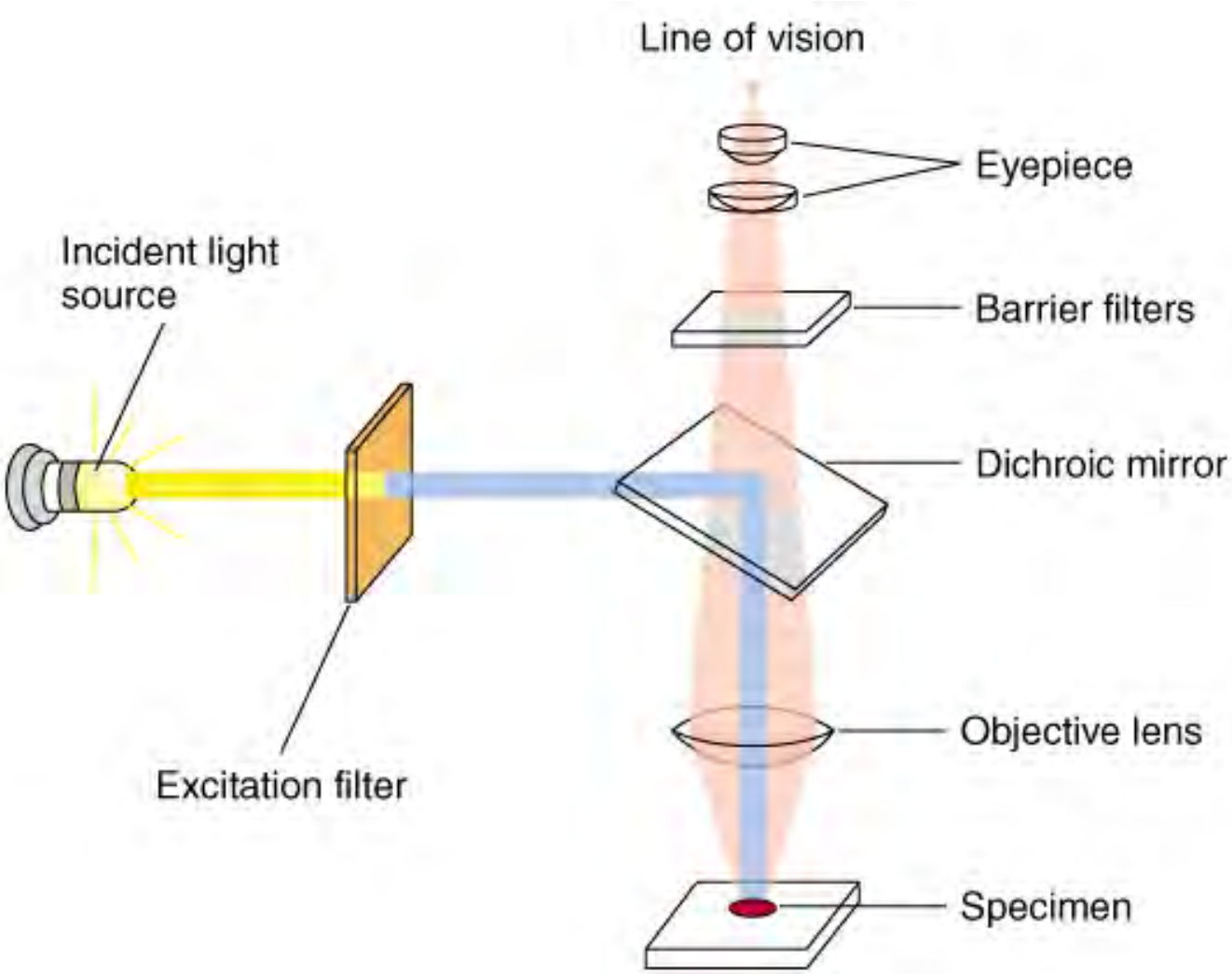
A. Temporal and spatial correlations-- limitations

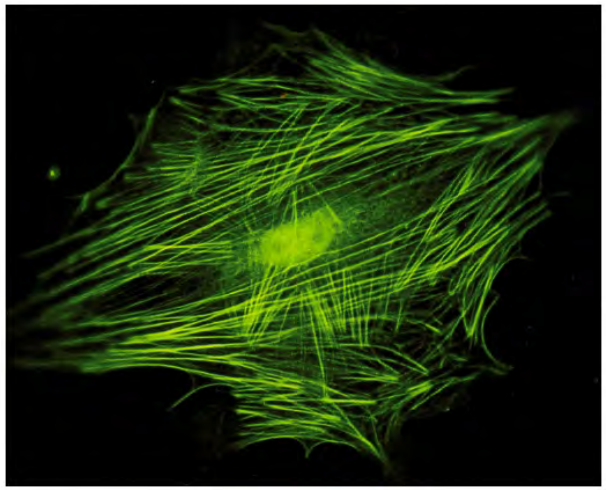
1. RNA localization- Northern blot analysis, RT-PCR, in situ hybridization
2. Protein localization- Western blot analysis, immunofluorescence, epitope tagging, chimeric GFP

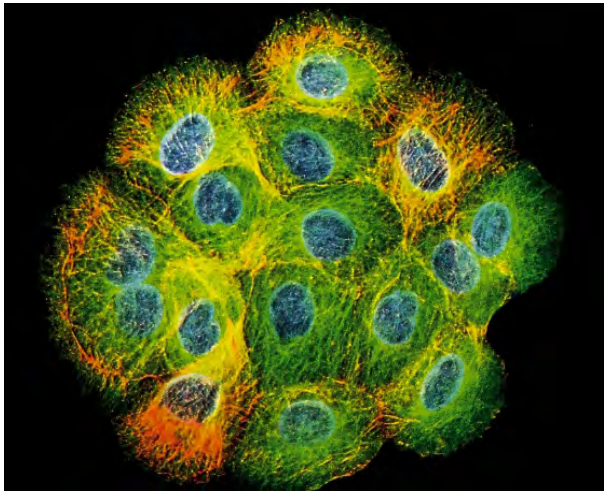
B. Functional approaches

1. Overexpression- transfection, viral infection-- limitations
2. Misexpression- viral infection, oocyte injection
3. Gene disruption
   - -Yeast
   - -homologous recombination in mice- standard and conditional
   - -RNA interference
   - -dominant negative alleles
4. Mutational studies

The fluorescent microscope

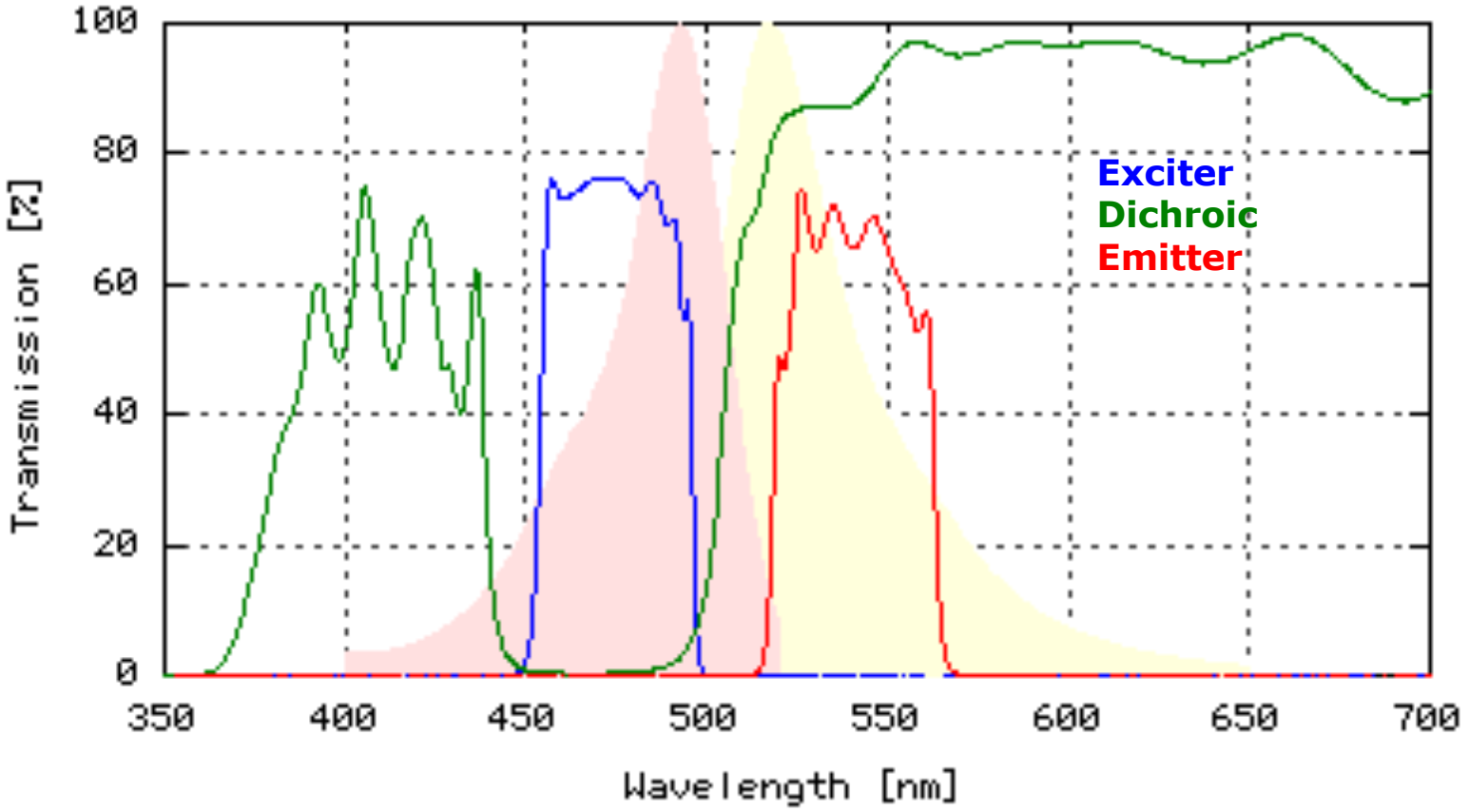






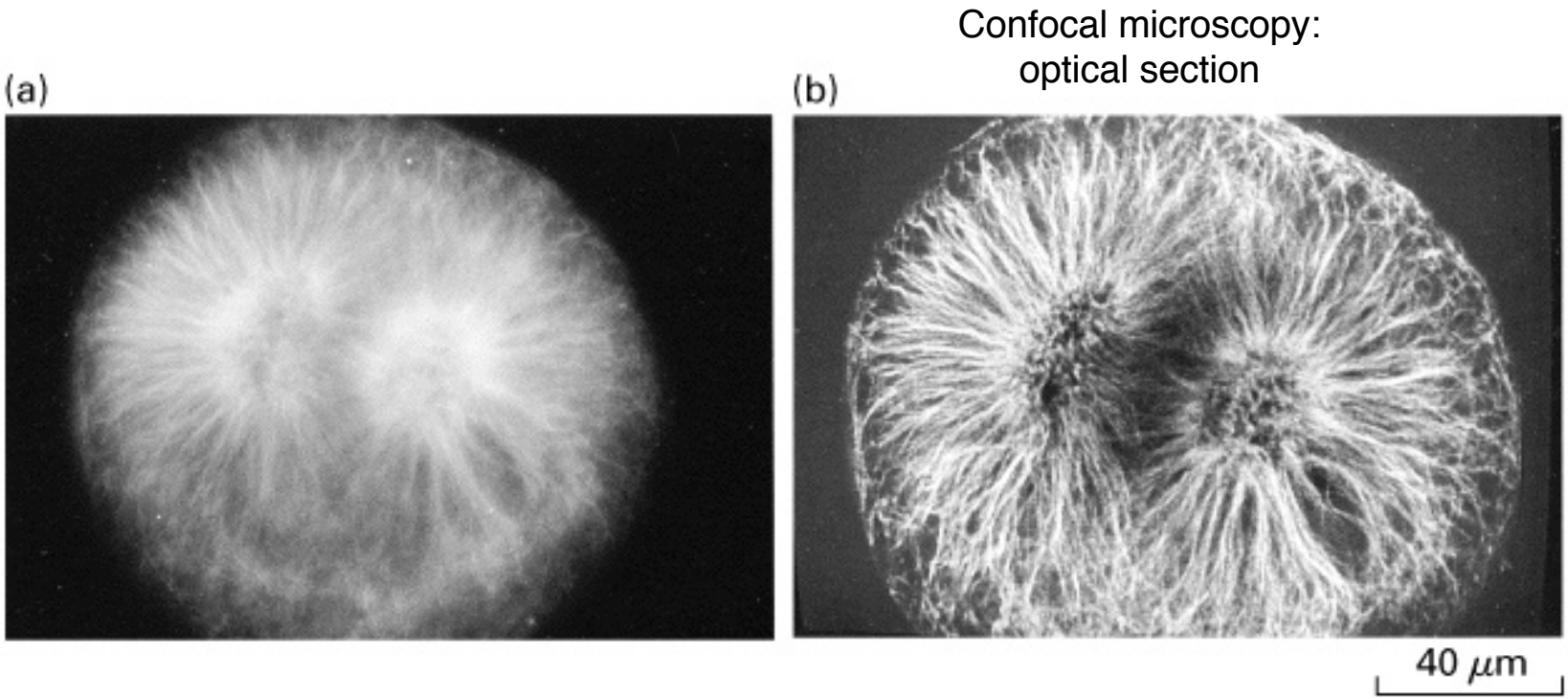
Lodish et al, 2000

Fluorescence filter set for fluorescein (FITC)



Exciter filter: allows excitation (shorter wavelength) light to pass
Dichroic filter: deflects excitation light and allows emitted (longer wavelength) light to pass
Emitter filter: allows emitted light to pass

Confocal and deconvolution microscopy create sharper images

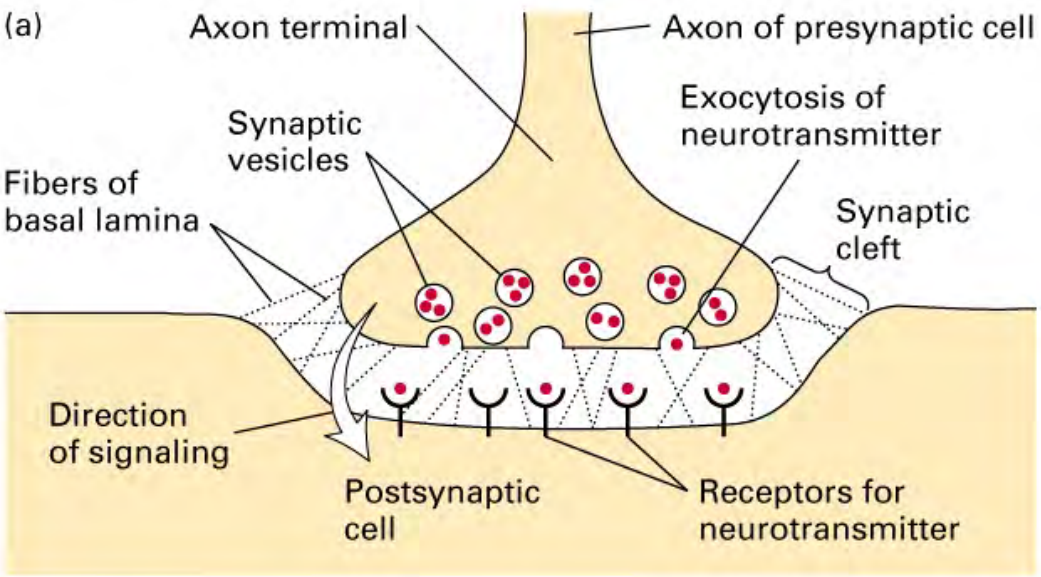


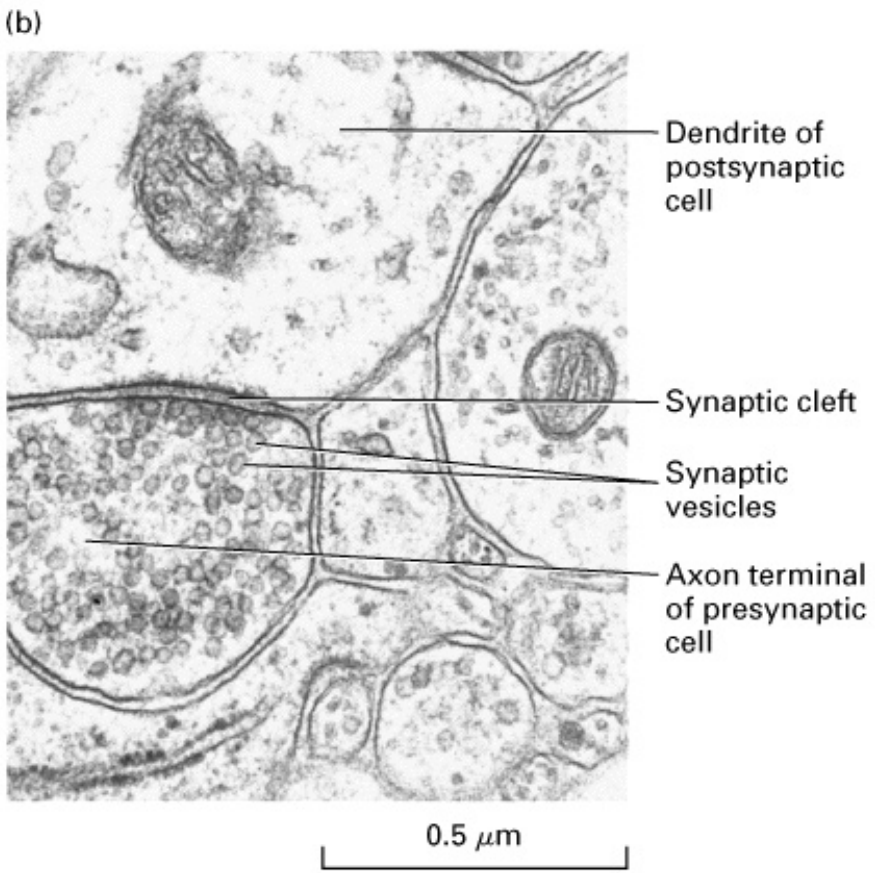
conventional

confocal

Lodish et al, 2000

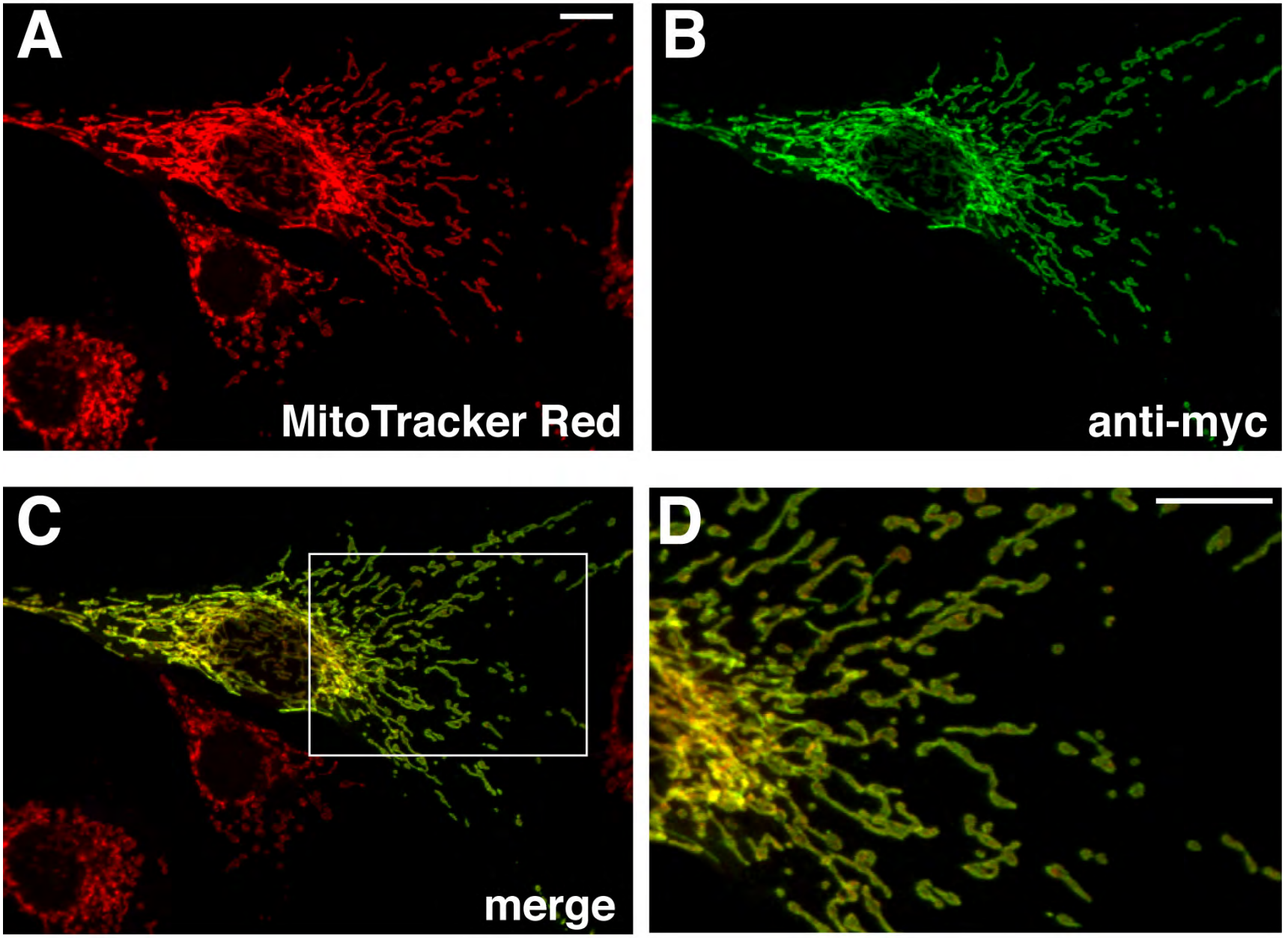
Electron microscopy



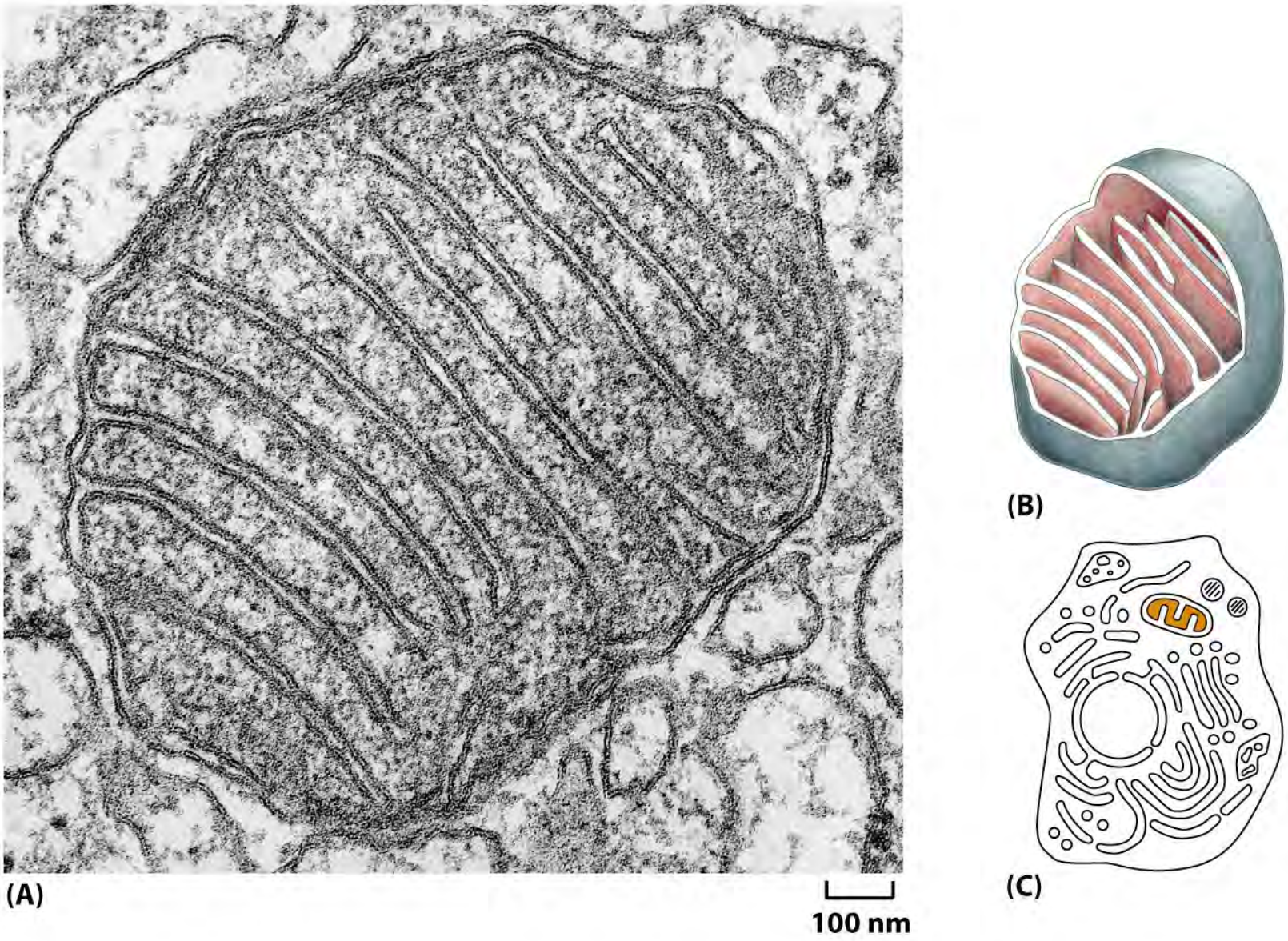


Lodish et al, 2000

Mitofusins localize to mitochondria

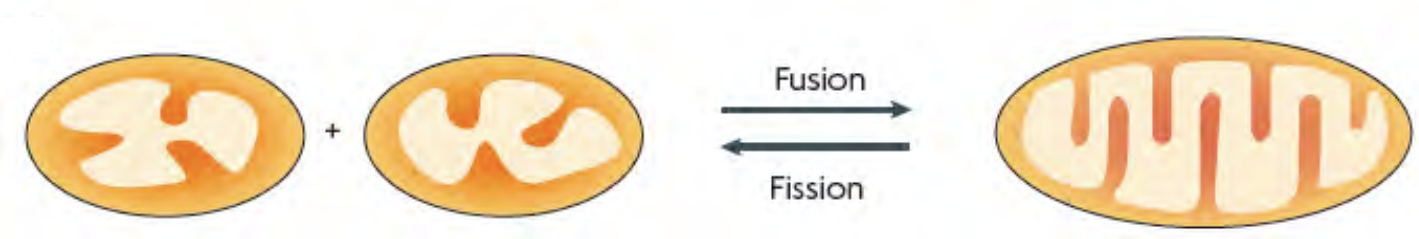


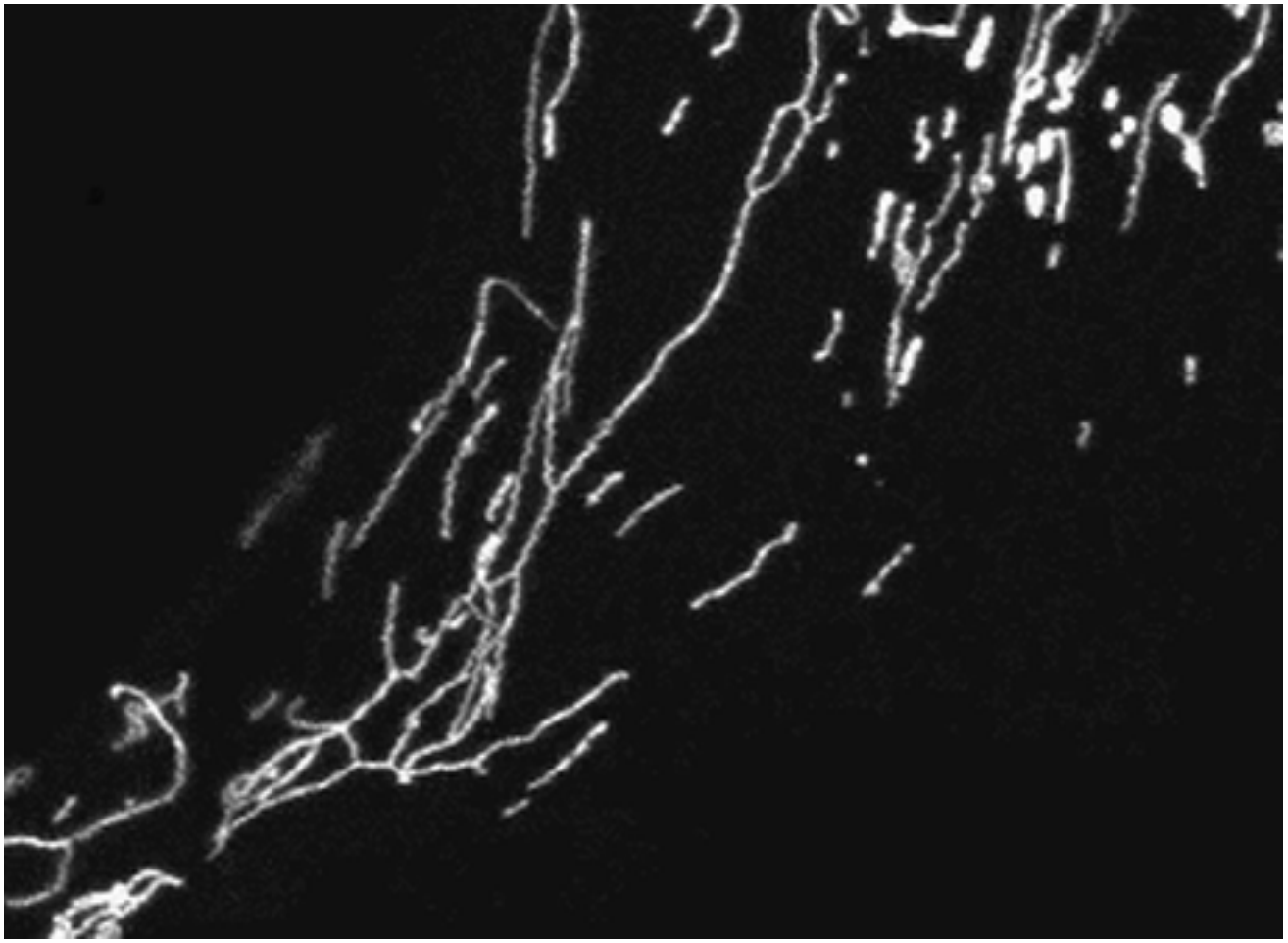
Structure of mitochondria



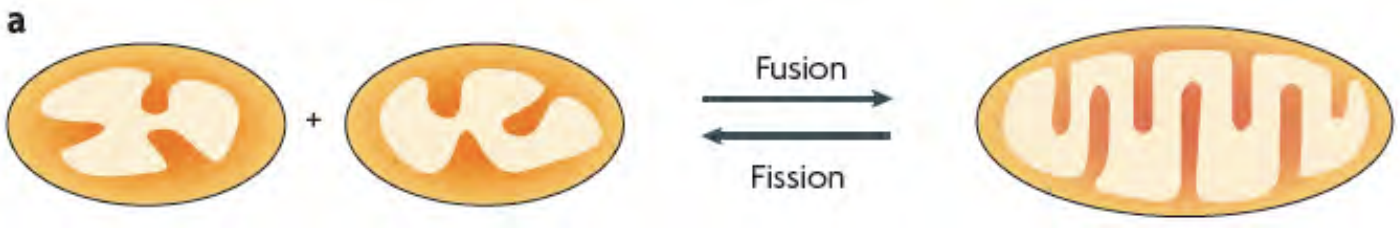
Molecular Biology of the Cell, Fifth Edition (© Garland Science 2008)

Use of fluorescent proteins to track organelle dynamics in live cells



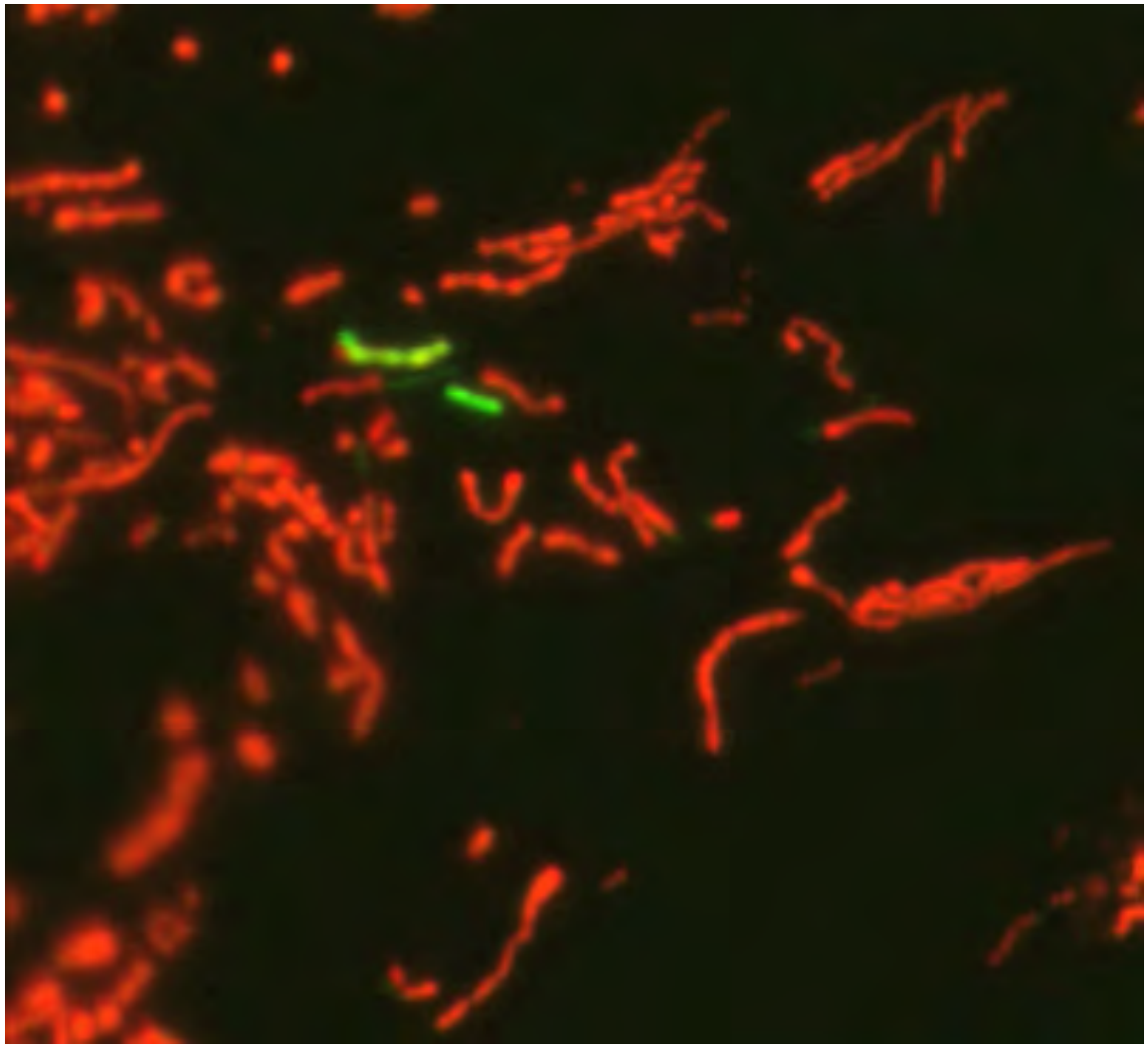


Tracking individual mitochondria by photoactivation

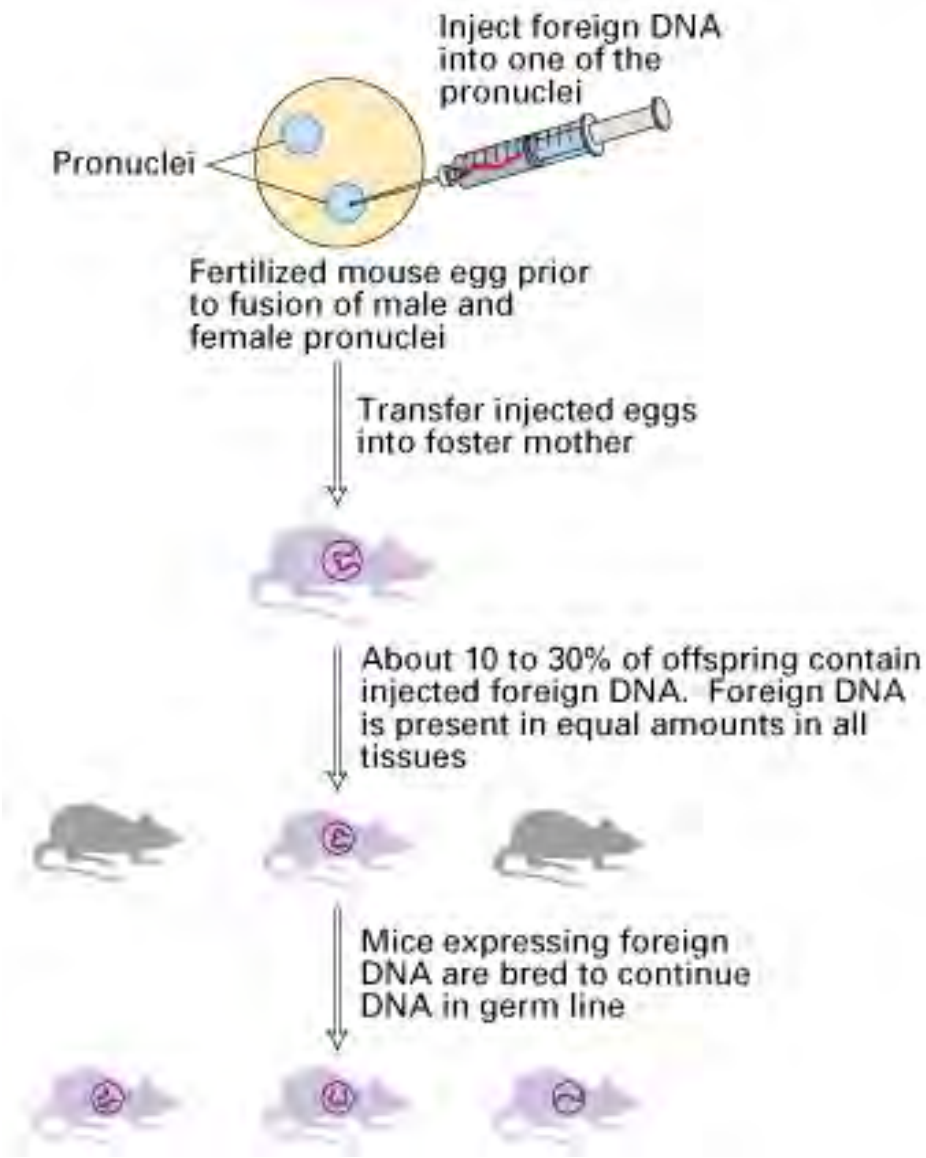


Mito-DsRed

Mito-PA-GFP

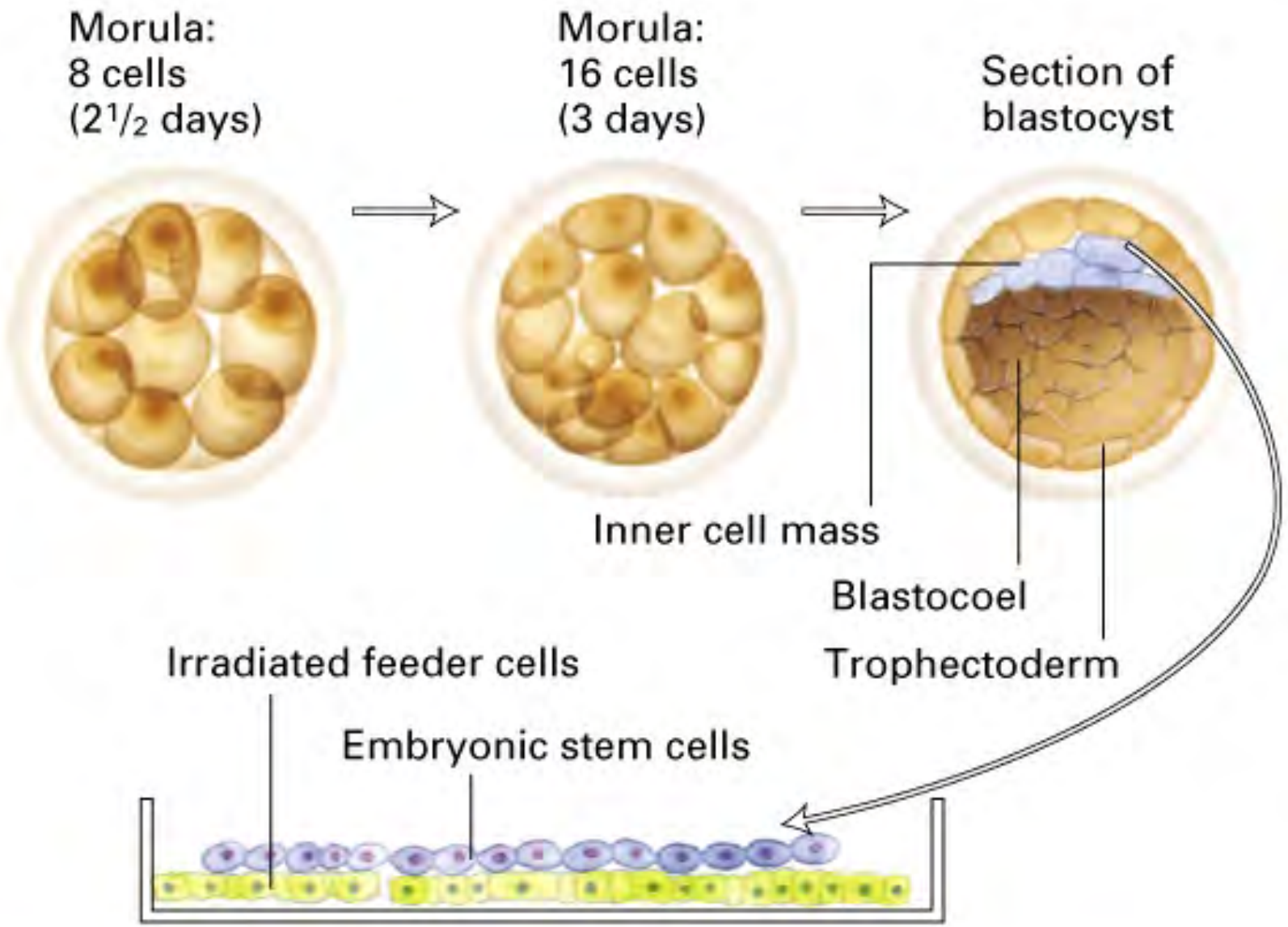


Production of transgenic mice

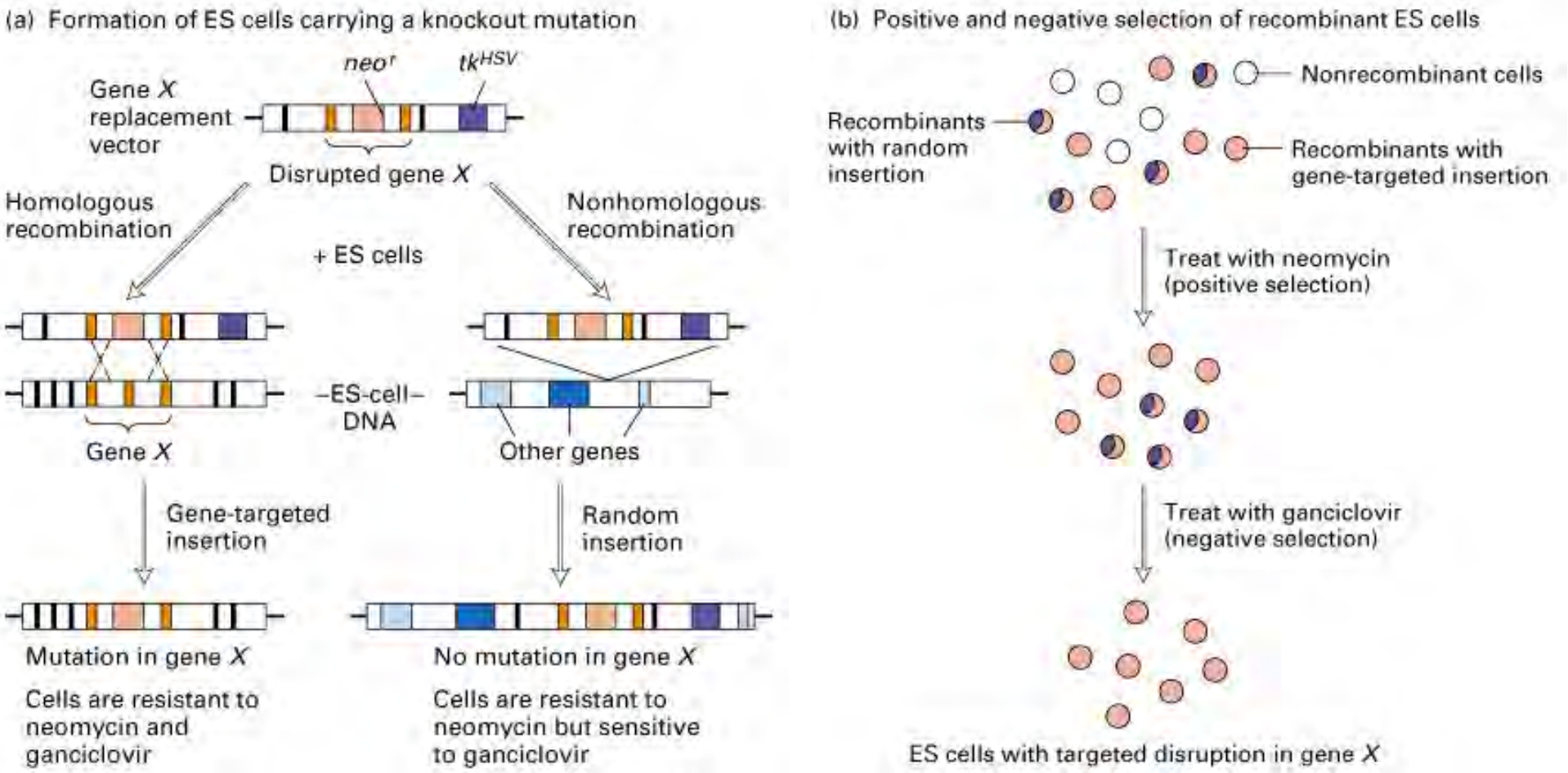


Lodish et al, 2000

Embryonic stem cells are totipotential

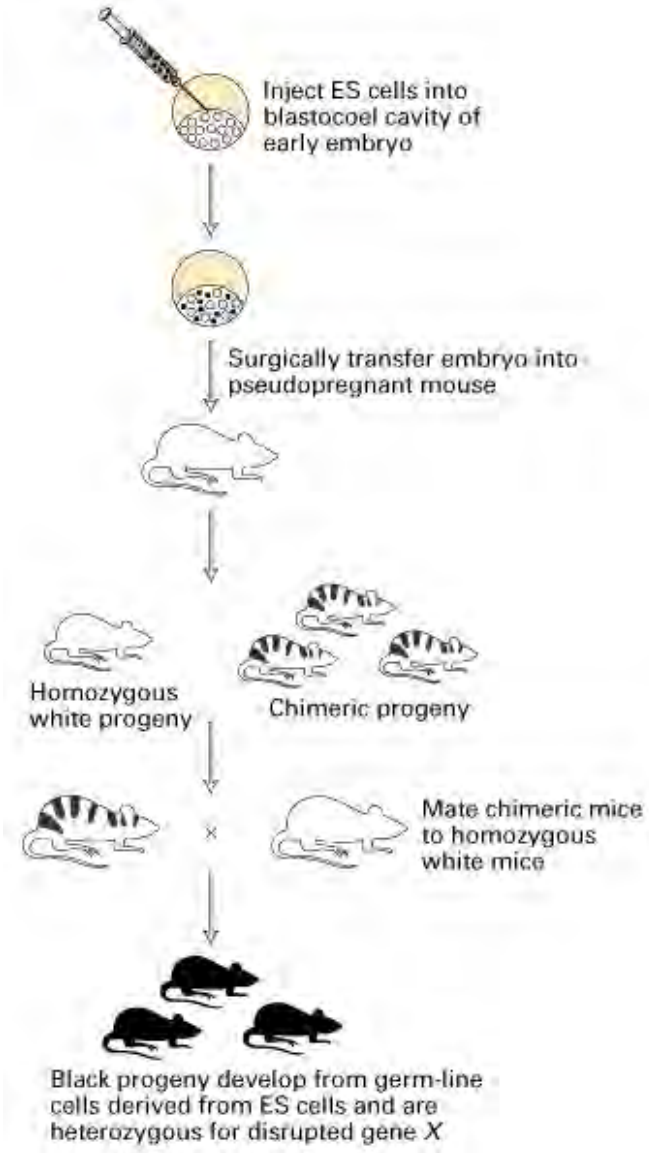


Creation of mice ES cells carrying a knockout mutation



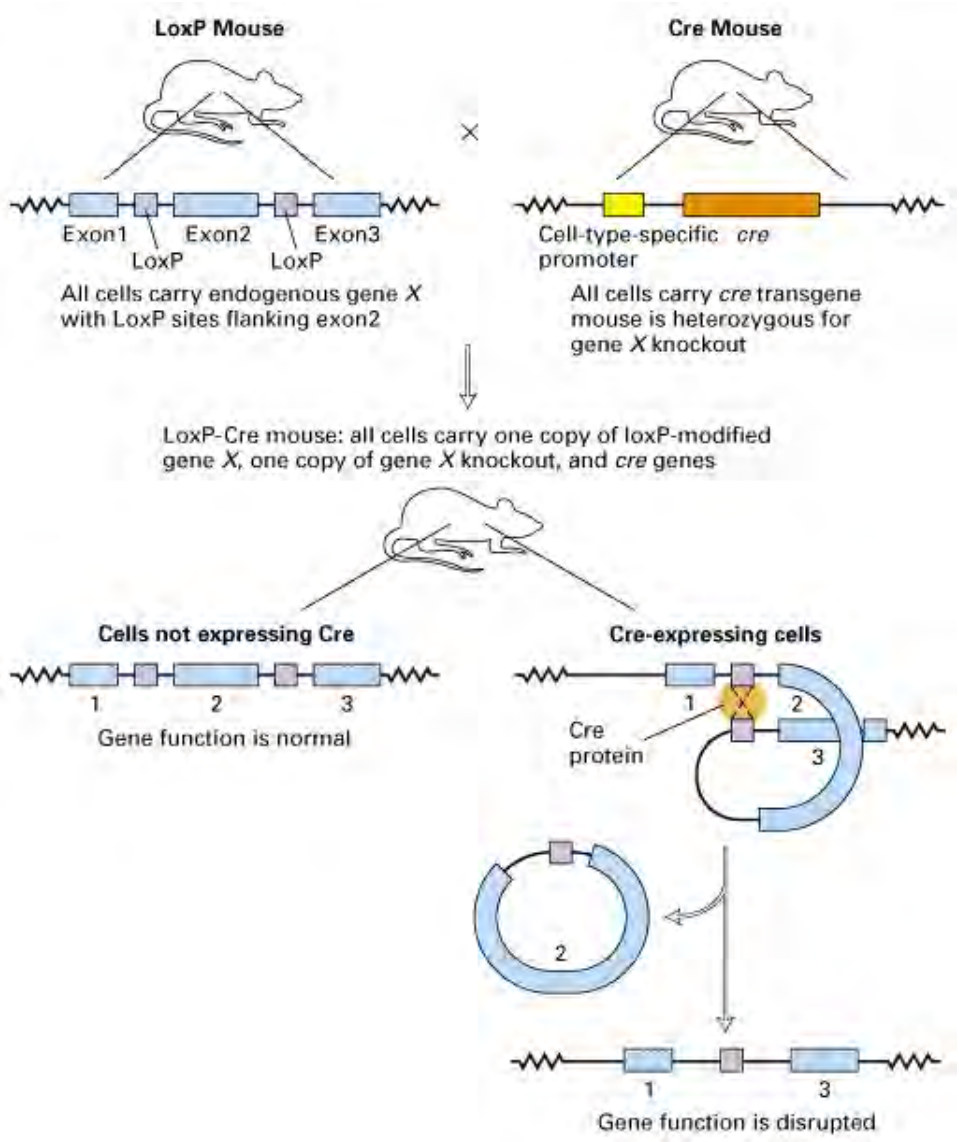
Lodish et al, 2000

Gene knockout in mice



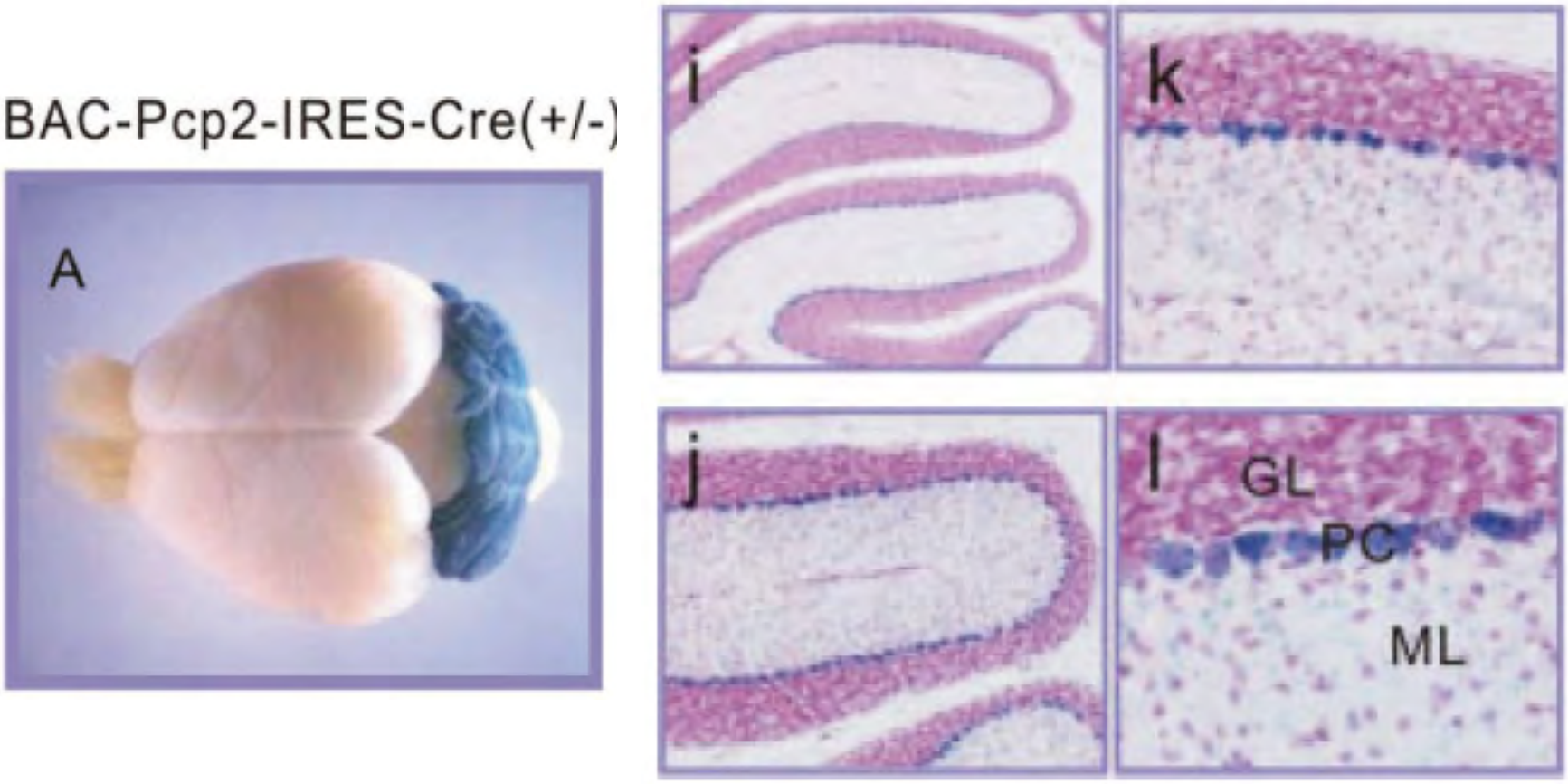
Lodish et al, 2000

Cell-type-specific (conditional) gene knockouts in mice



Lodish et al, 2000

The *cre-loxP* system allows temporal and spatial control of gene disruption

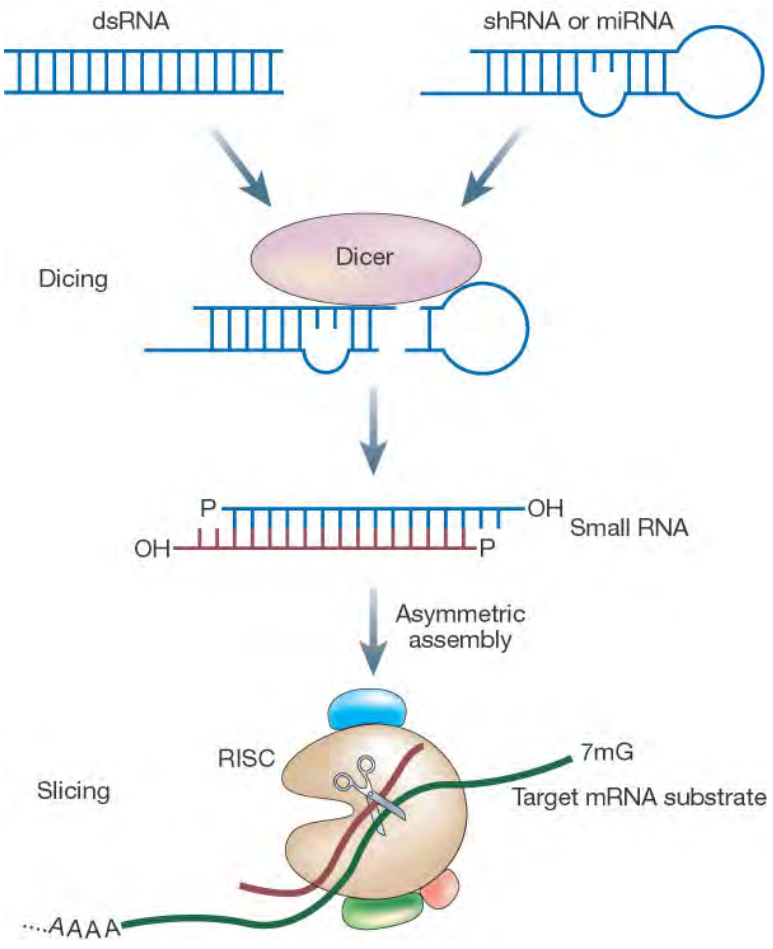


specific expression of Cre recombinase
inPurkinje cells of cerebellum

Zhang et al (2004) *Genesis*

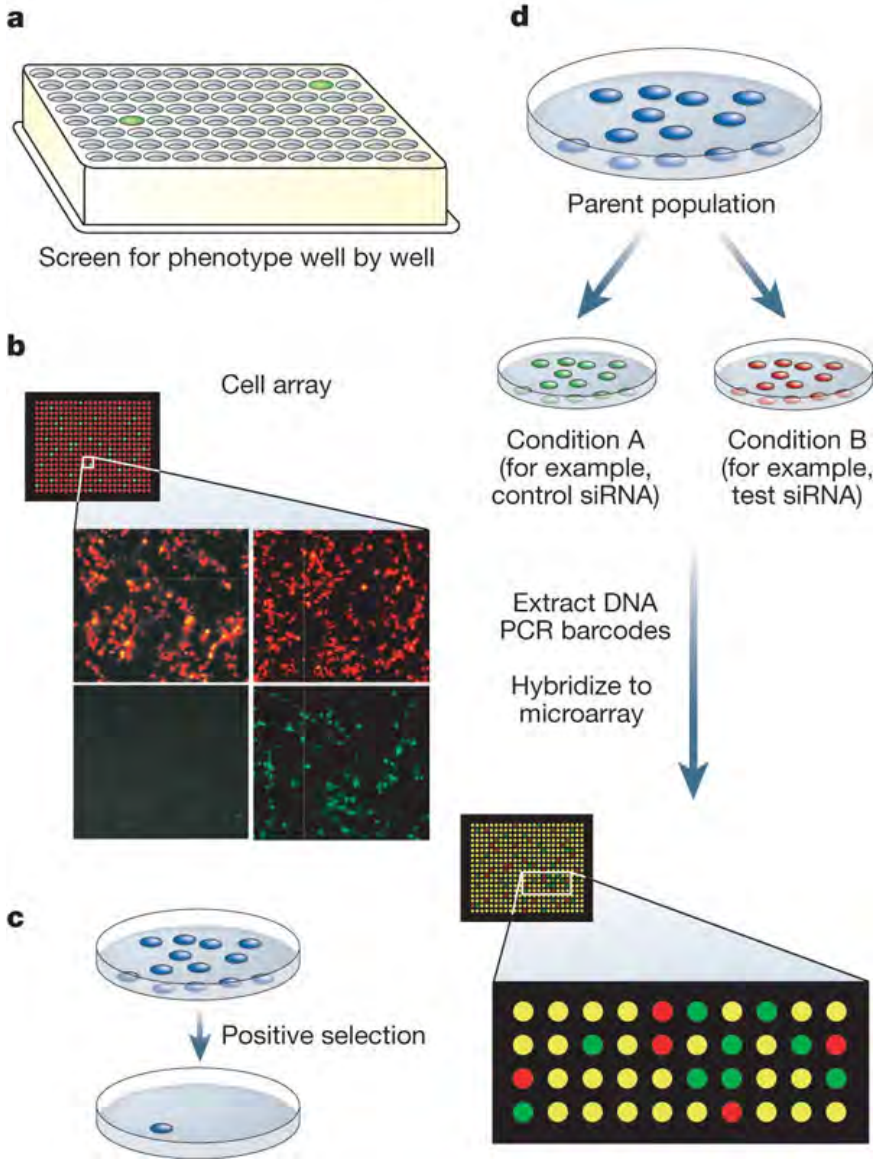
Using RNA interference to disrupt gene function

RNA-induced silencing complex



Hannon and Rossi (2004) *Nature 431, 371-378*

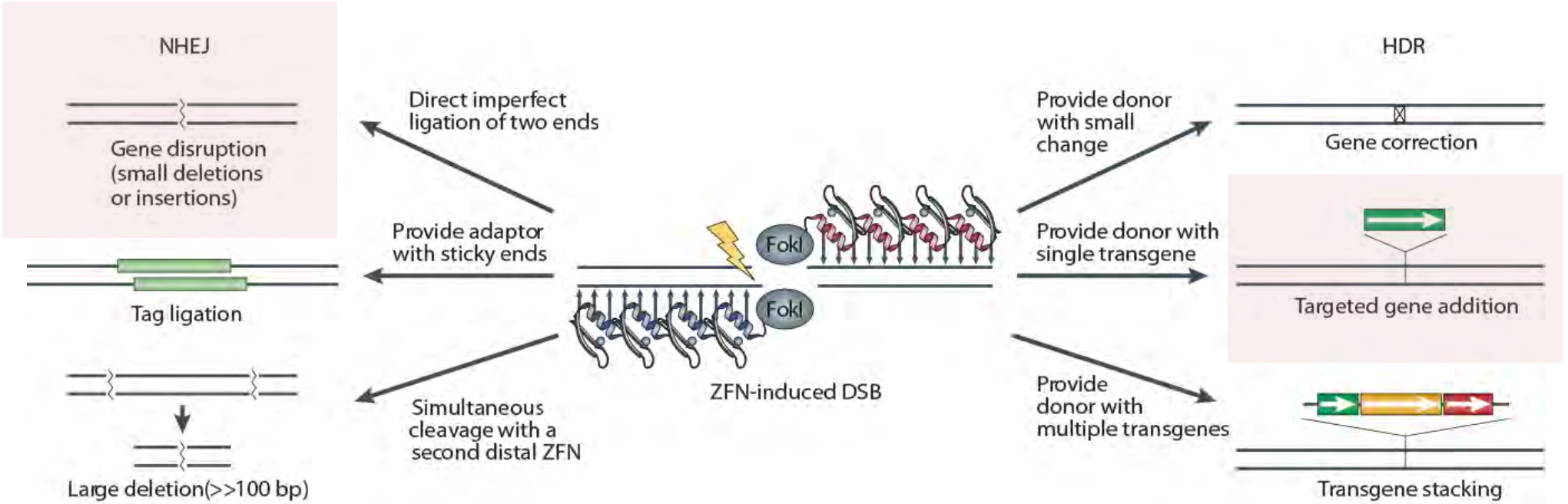
RNAi screens to discover new genes



Hannon and Rossi (2004) *Nature 431, 371-378*

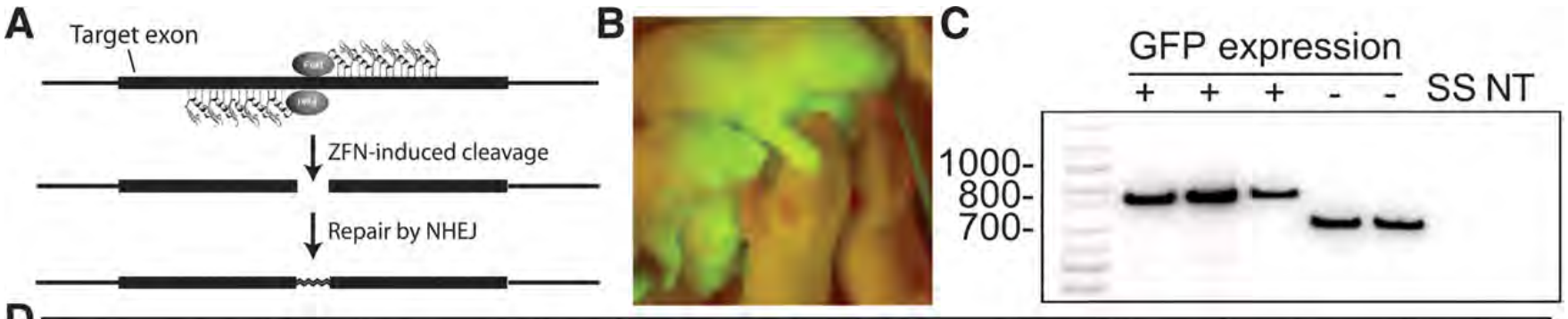
Genome editing with engineered zinc finger nucleases

Fyodor D. Urnov, Edward J. Rebar, Michael C. Holmes, H. Steve Zhang and Philip D. Gregory



Urnov et al. Nature Reviews Genetics 2010

ZFN-mediated gene disruption in rat embryos



D

Strain	Target/ Construct	Route/ Dose (ng/ul)	Injected/ Transferred (%)	Founders (%)*	Mutants (%)†	Bred/ F1 inheritance
SS	GFP/mRNA	PNI/1.5	36/25 (69)	5 (20)	2 (40)	1/1
SD	IgM/Plasmid	PNI/10	609/493 (81)	54 (11)	6 (11)	4/3
SD	IgM/Plasmid	PNI/2	605/468 (77)	82 (18)	8 (10)	ND
SD	IgM/Plasmid	PNI/0.4	511/423 (83)	62 (15)	4 (6)	ND
SD	IgM/mRNA	PNI/10	186/104 (56)	14 (13)	4 (29)	ND
SD	IgM/mRNA	PNI/2	230/142 (62)	21 (15)	4 (19)	ND
SD	IgM/mRNA	PNI/0.4	183/118 (64)	19 (16)	1 (5)	ND
SD	IgM/mRNA	IC/10	272/197 (72)	4 (2)	3 (75)	ND
SD	IgM/mRNA	IC/2	197/134 (68)	17 (13)	2 (12)	ND
FHH	Rab38/mRNA	IC/2	91/83 (91)	17 (20)	1 (6)	ND

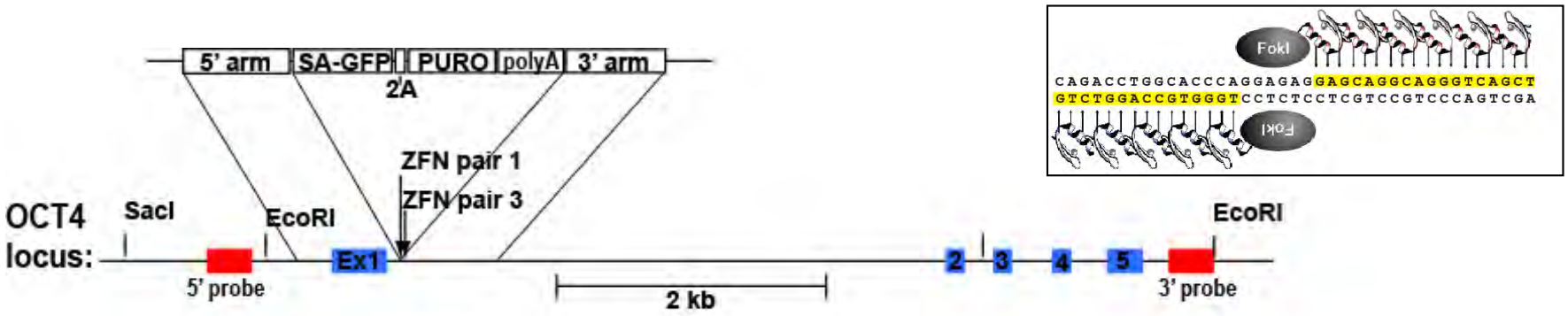
PNI - pronuclear injection; IC - intracytoplasmic; * - of transferred; † - of liveborn founders; ND - not determined

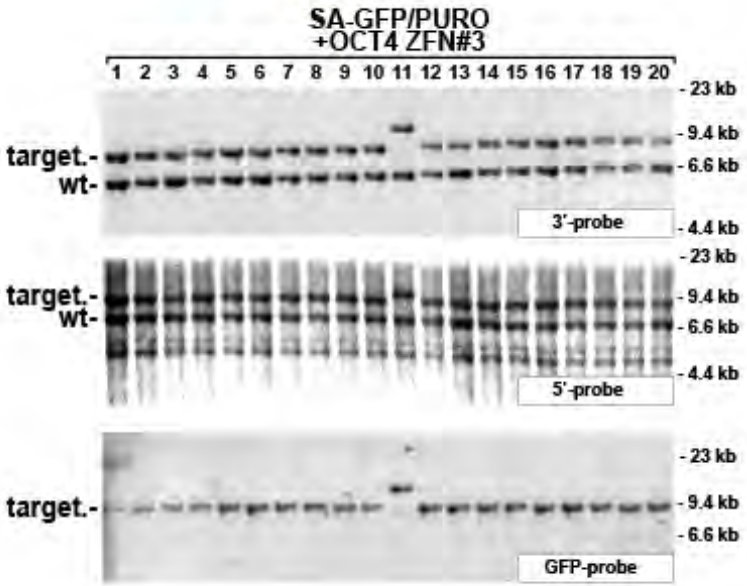
A M Geurts et al. Science 2009;325:433-433

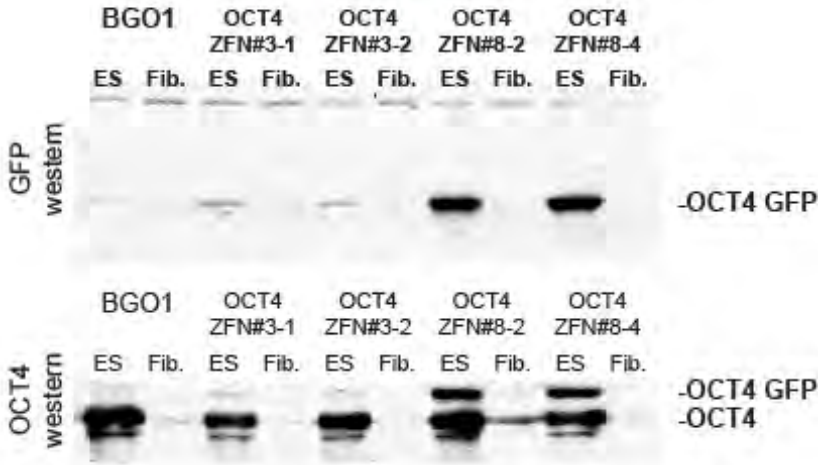
ZFN targeted against GFP transgene in transgenic rats

Published by AAAS

Human ES cells and iPS cells: Endogenous Gene → Reporter at 95% Efficiency







Hockemeyer et al. *Nature Biotechnology* 2009

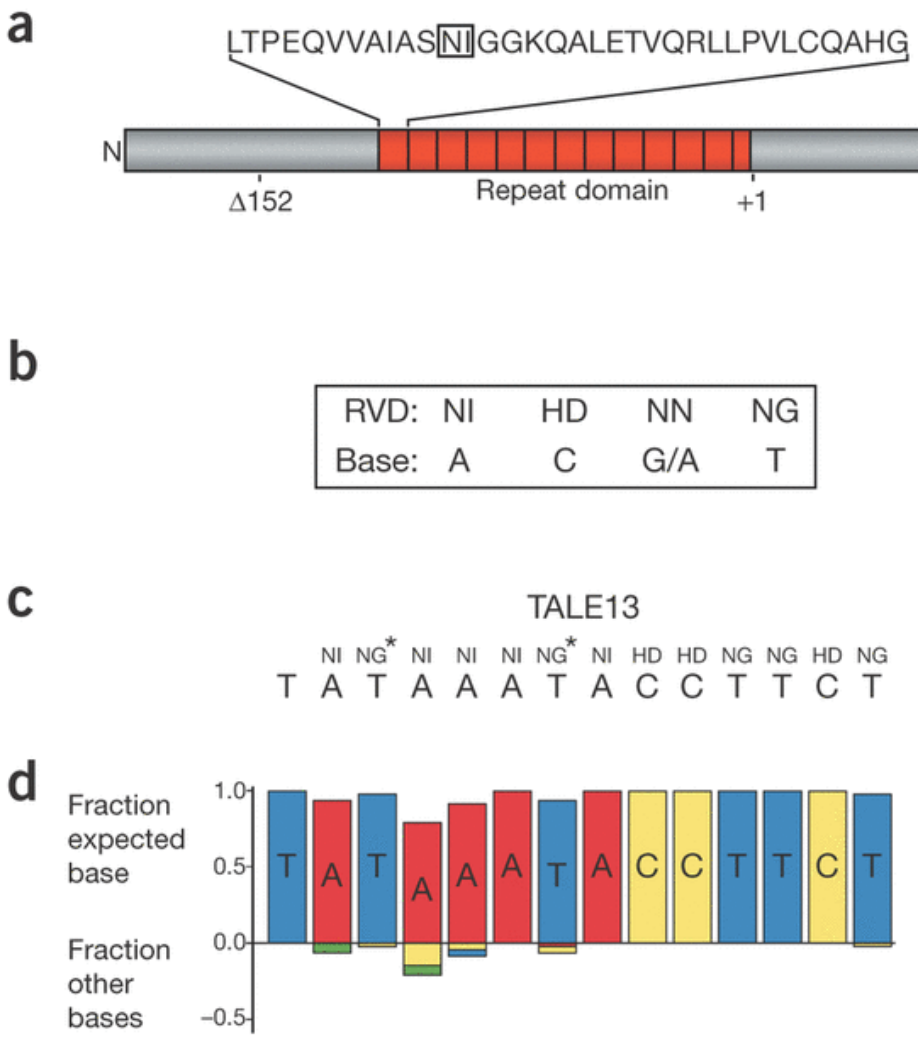
TALE: transcription activator–like effector proteins from Xanthomonas

•**TAL effectors:** virulence factors produced by a genus of plant pathogens, Xanthomonas spp.

• bind to specific host promoter sequences

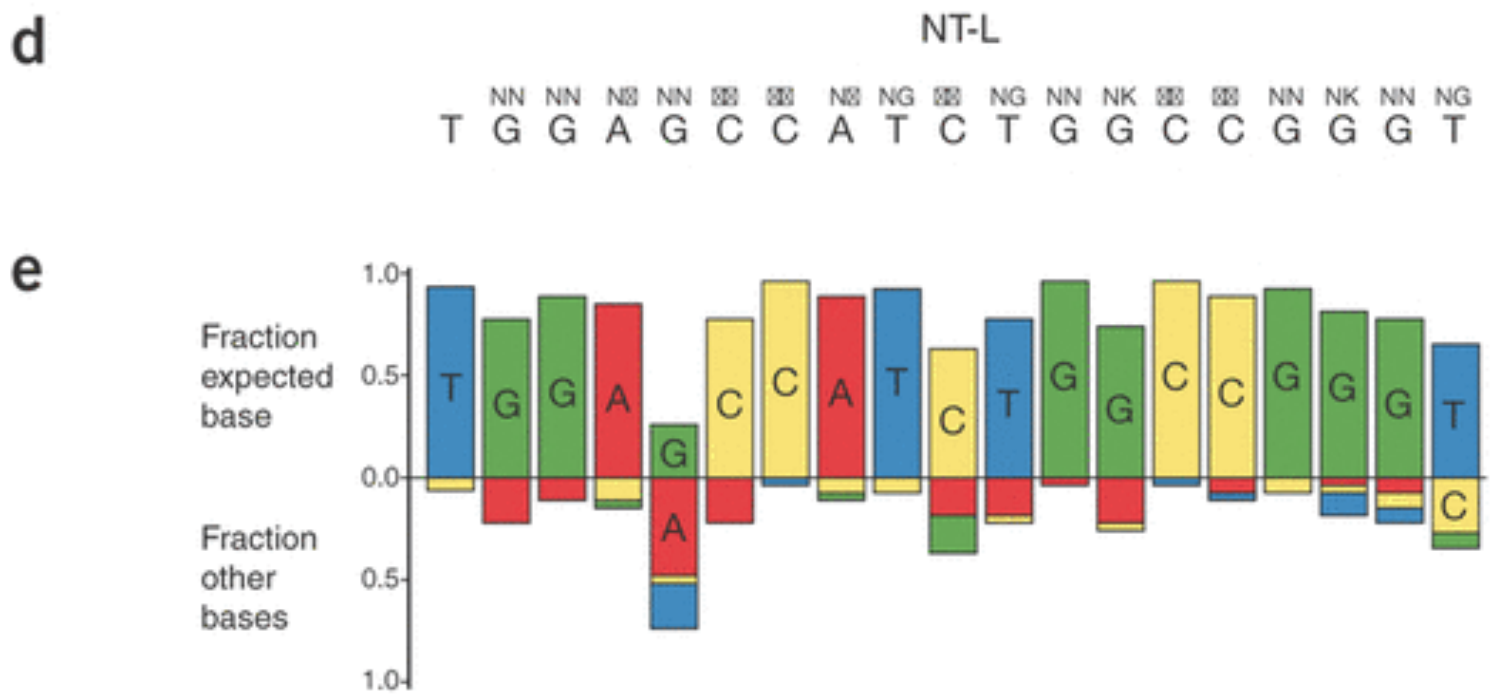
• contain 17–18 repeats of 34 amino acids.

• DNA binding specificity determined by positions 12 and 13



Miller et al., Nat Biotech 2010

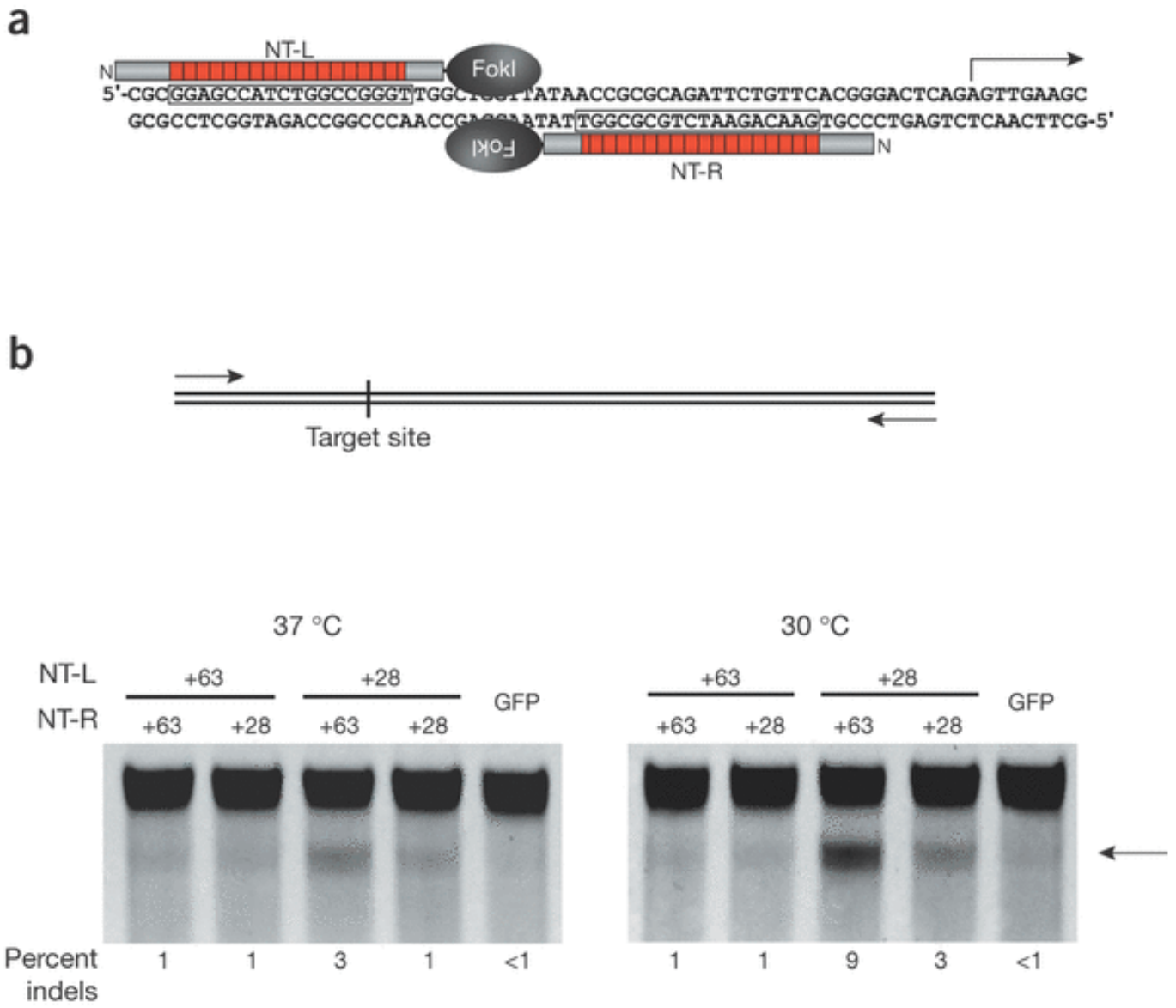
Engineering TALEs using a simple code



Miller et al., Nat Biotech 2010

TAL effector nucleases (TALENs)

- Fok1 endonuclease is an obligate dimer



Miller et al., Nat Biotech 2010

TAL effector nucleases (TALENs) induce indels

hey2:

#1297/ #1257 Mutations in 12 of 110 sequences: ~11%

```
GCTCTTCCGTTTCCACATCCACCACATCCCAACAGAGCAGCGGGAGCAGCAGTAAACC  WT

<----------------------------------------GAGCAGCAGTAAACC  Δ142
GCTCTTCCGTTTCCACATCCACC--------------CAGCGGGAGCAGCAGTAAACC  Δ14
GCTCTTCCGTTTCCACATCCACC-------------ACAGCGGGAGCAACAGTAAACC  Δ13
GCTCTTCCGTTTCCACATCCAC-----------AGAGCAGCGGGAGCAGCAGTAAACC  Δ11              [2x]
GCTCTTCCGTTTCCACATCCACCAC--------AGAGCAGCGGGAGCAGCAGTAAACC  Δ8               [3x]
GCTCTTCCGTTTCCACATCCACCACAT------tGAGCAGCGGGAGCAACAGTAAACC  Δ6 (Δ7 and +1)
GCTCTTCCGTTTCCACATCCACCACATC--AACAGAGCAGCGGGAGCAGCAGTAAACC  Δ2
GCTCTTCCGTTTCCACATCCACCACATaaaccaccacACAGAGCAGCGGGAGCAGCAG  +6 (Δ4 and +10)

ACCTTCCCTCTATCATT<----------/ /--------->TCTGGGAAGAAAAGAAA  Δ303
```

gria3a:

#1258/ #1260 Mutations in 13 of 89 sequences: ~15%

```
GGAGTCGTCCAATAGCTTCTCAGTCACGCACGCCTGTGAGTTTCTGCTCTTTATCTT  WT

GGAGTCGTCCAATAGCTTC----------GCCTGTGAGTTTCTGCTCTTTATCTT  Δ12
GGAGTCGTCCAATAGCTTCTCA---------GCCTGTGAGTTTCTGCTCTTTATCTT  Δ9
GGAGTCGTCCAATAGCTTCTCAGT---------CTGTGAGTTTCTGCTCTTTATCTT  Δ9
GGAGTCGTCCAATAGCTTCTCAG---------CCTGTGAGTTTCTGCTCTTTATCTT  Δ9               [2x]
GGAGTCGTCCAATAGCTTCTCAGTCA-----GCCTGTGAGTTTCTGCTCTTTATCTT  Δ5
GGAGTCGTCCAATAGCTTCTCAGTCAgaaa--CCTGTGAGTTTCTGCTCTTTATCTT  Δ2 (Δ6 and +4)
GGAGTCGTCCAATAGCTTCTCAGTCtcagtCGCCTGTGAGTTTCTGCTCTTTATCTT  +0 (Δ5 and +5)
GGAGTCGTCCAATAGCTTCTCAGTCacgcACGCACGCCTGTGAGTTTCTGCTCTTTA  +4               [3x]
GGAGTCGTCCAATAGCTTCTCAGctgtgcctgtaACGCCTGTGAGTTTCTGCTCTTT  +5 (Δ6 and +11) [2x]
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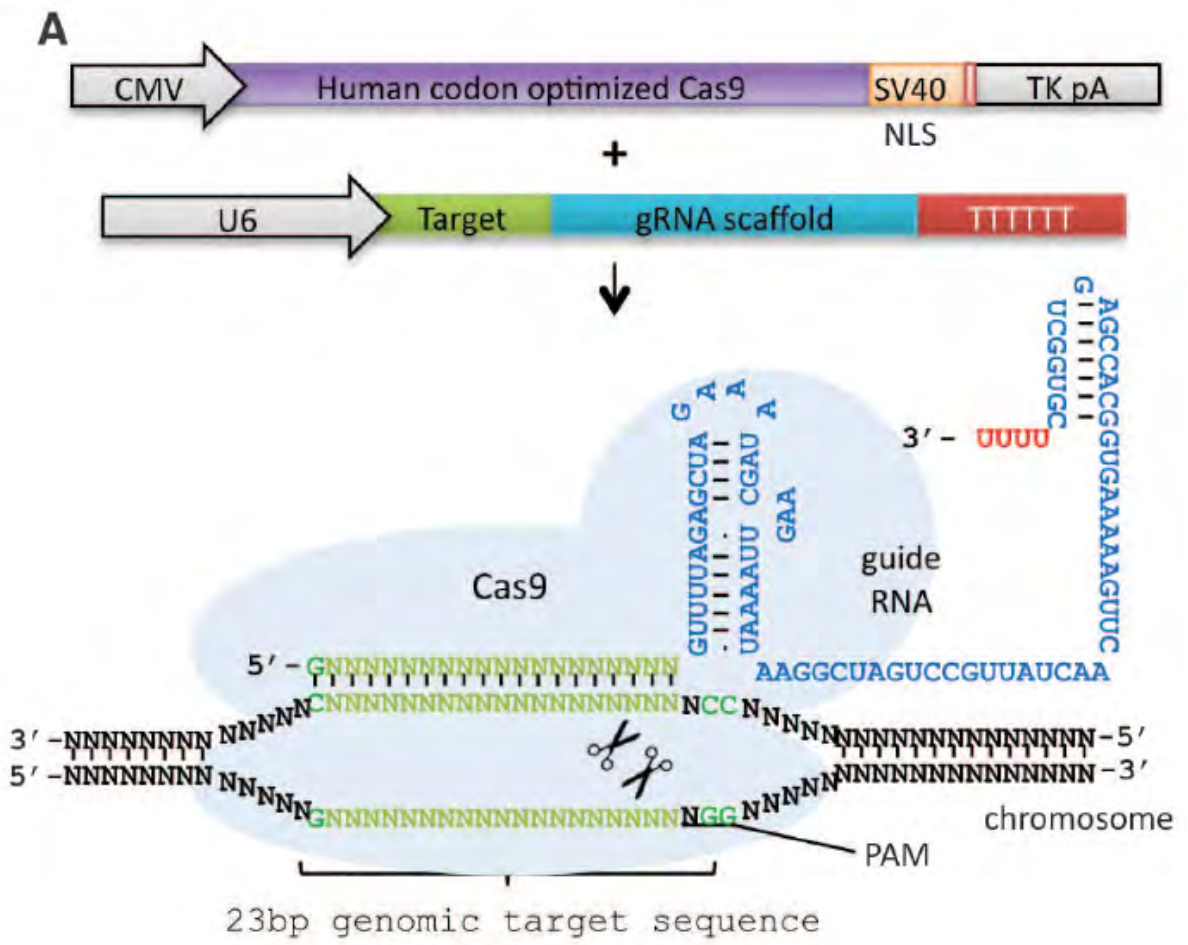
Sander et al., Nat Biotech 2011

CRISPR-mediated genome editing



- CRISPR: clustered regularly spaced short palindromic repeat system
- Found in archaea and bacteria
- Key components consists of Cas9 nuclease and guide RNA
- By engineering the guide RNA, Cas9 cleavage can be targeted to any desired site.

Assigning gene function

A. Temporal and spatial correlations-- limitations

1. RNA localization- Northern blot analysis, RT-PCR, in situ hybridization
2. Protein localization- Western blot analysis, immunofluorescence, epitope tagging, chimeric GFP

B. Functional approaches

1. Overexpression- transfection, viral infection-- limitations
2. Misexpression- viral infection, oocyte injection
3. Gene disruption
   - -Yeast
   - -homologous recombination in mice- standard and conditional
   - -RNA interference
   - -dominant negative alleles
4. Mutational studies