Nobel Lecture
December 7, 2013

Genes and proteins that organize the secretory pathway

Randy Schekman
Department of Molecular and Cell Biology
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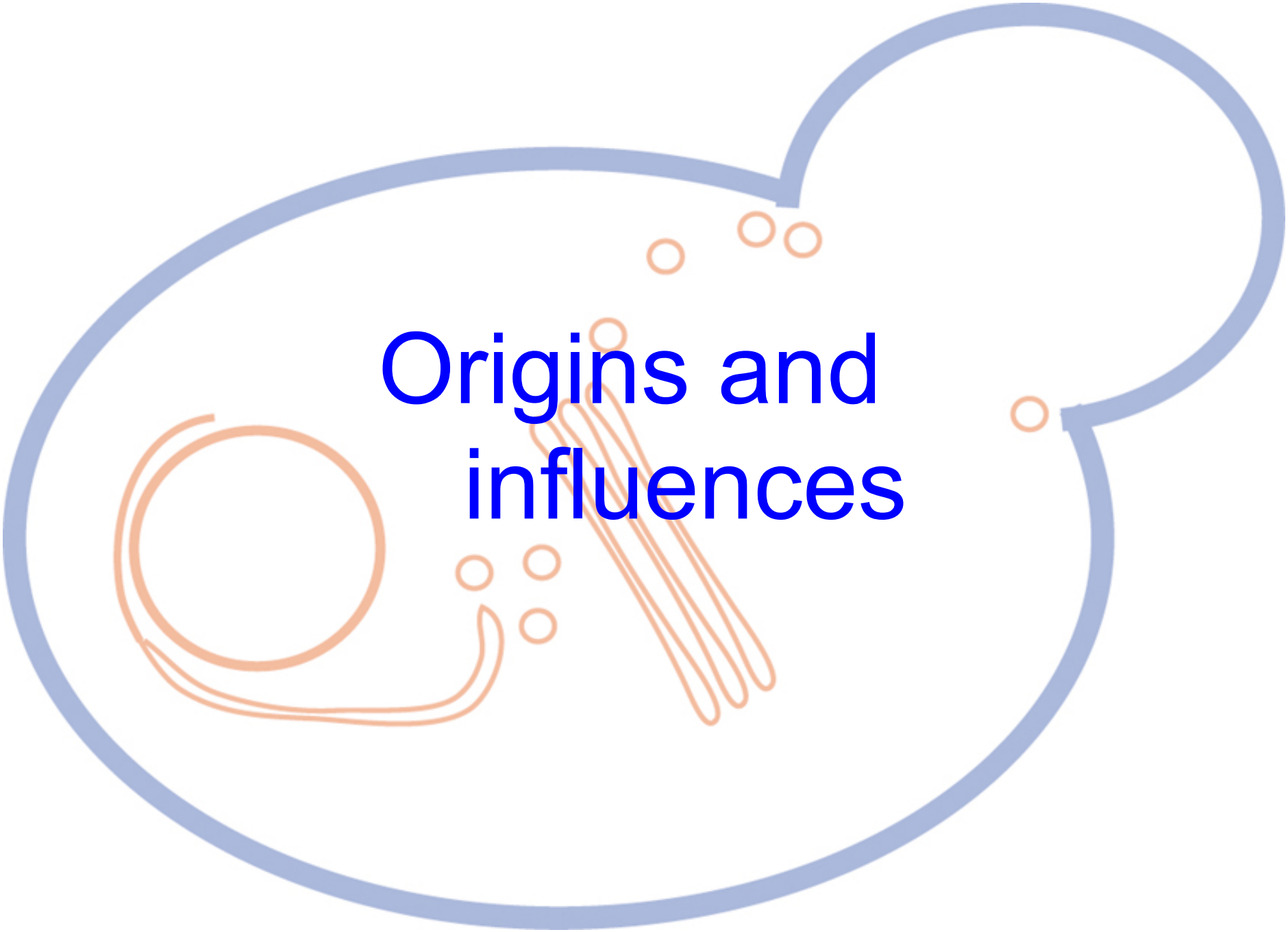
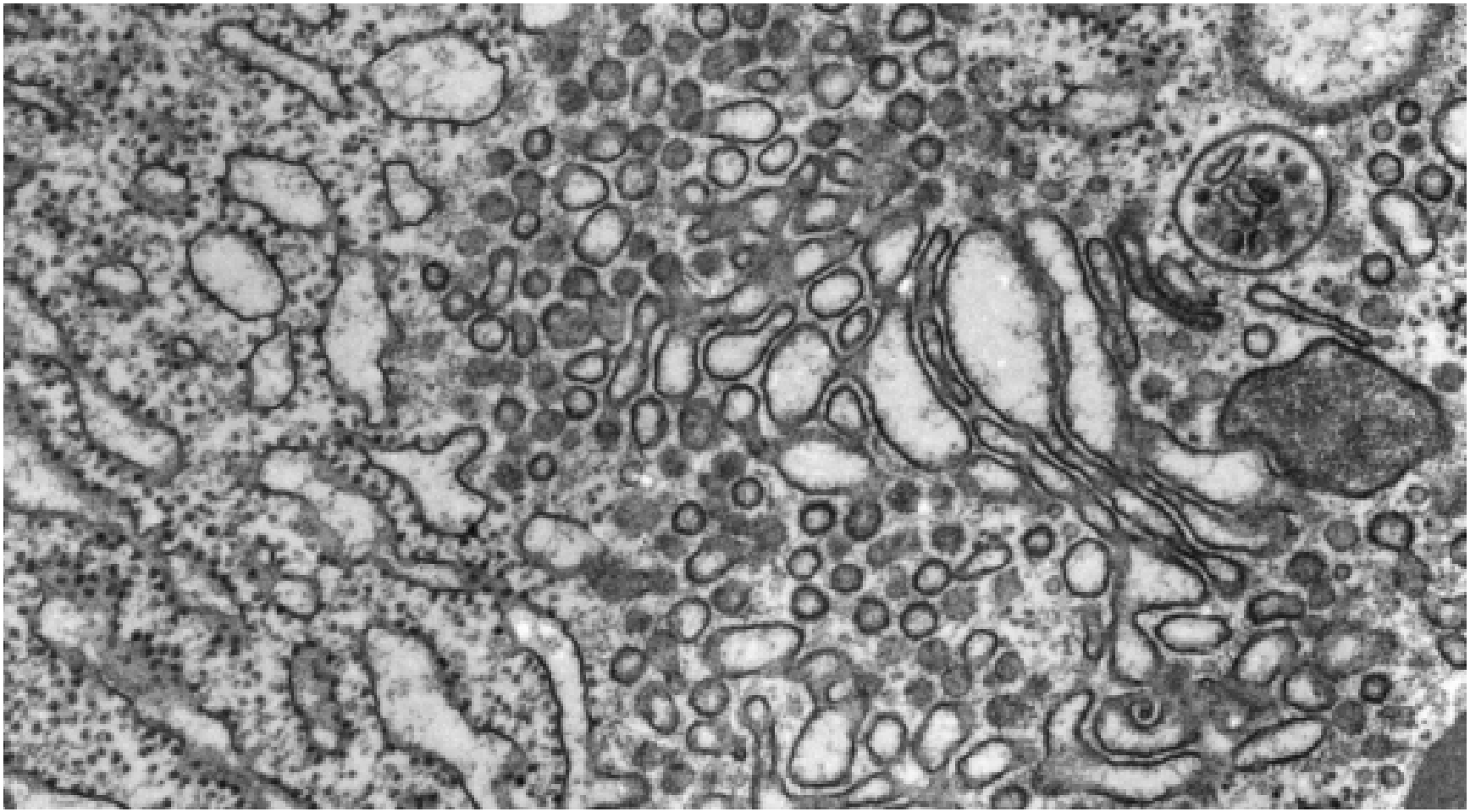
University of California, Berkeley


Origins and
influences

Pancreatic acinar cell




NEVER TURN CAM
VALVE FROM P
TO OPEN UNTIL P
LIGHT IS EXTINGU
AND
PVL GUAGE MUST
BELOW 14 TO OPEN

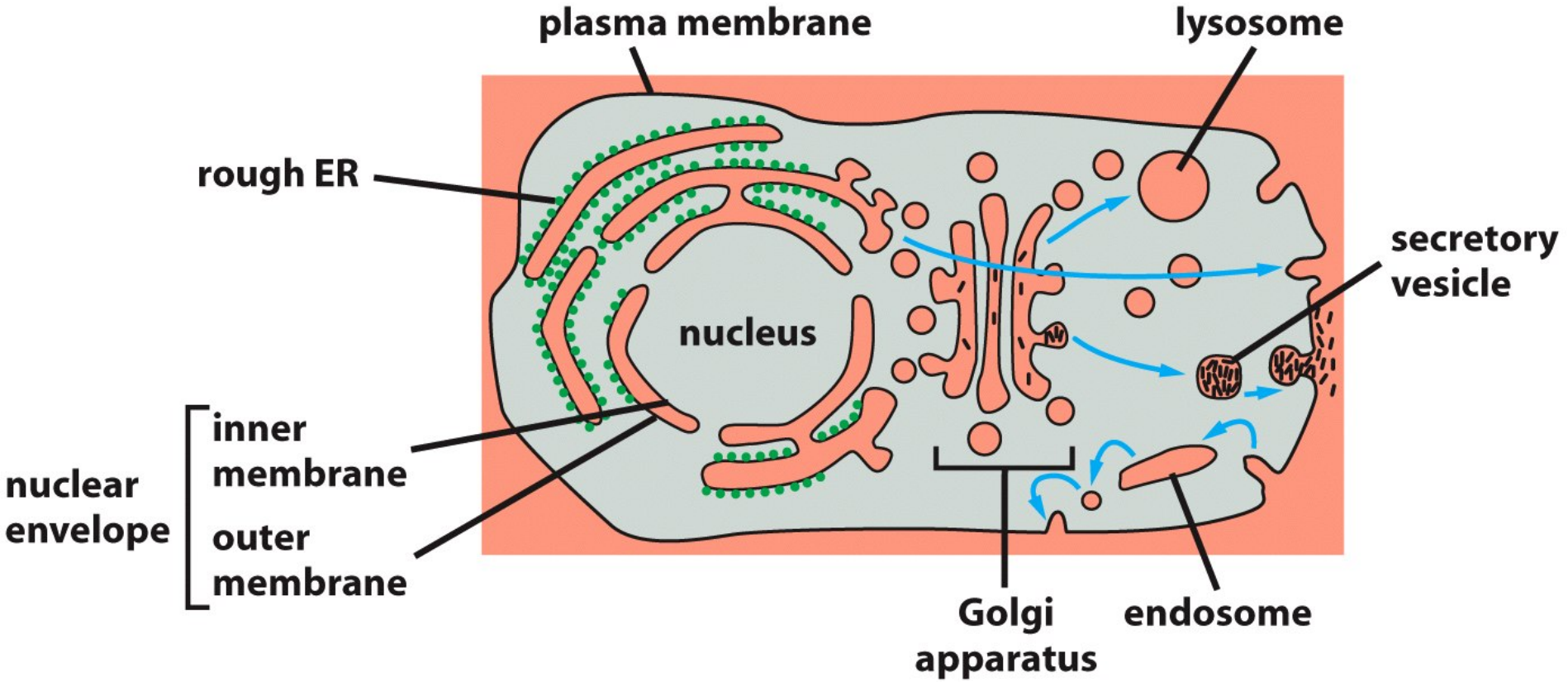
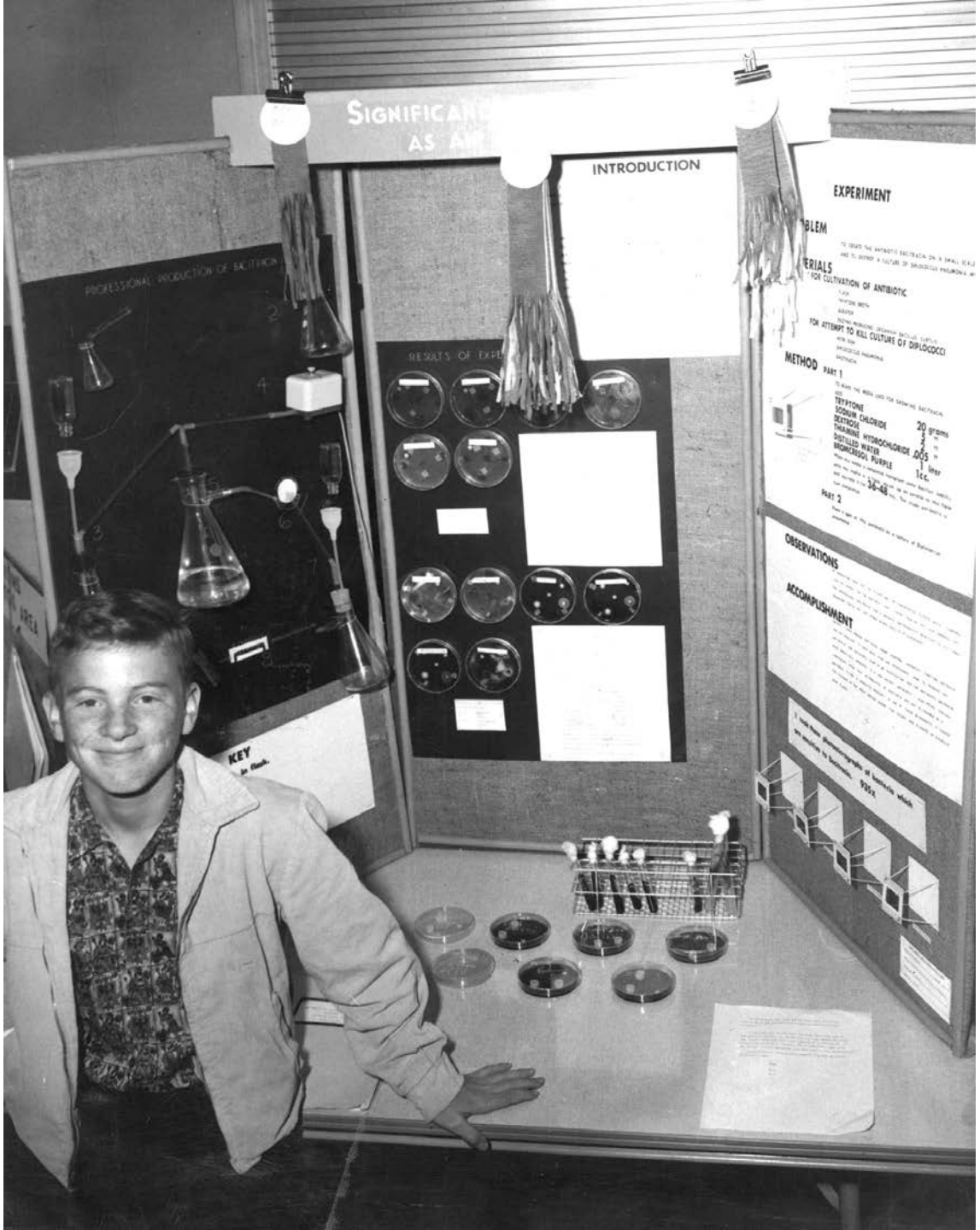


Figure 12-5 *Molecular Biology of the Cell* (© Garland Science 2008)


INTRODUCTION
EXPERIMENT
METHOD PART 1
TRYPTONE 20 grams
SODIUM CHLORIDE 5 "
DEXTROSE 5 "
THIAMINE HYDROCHLORIDE .005 "
DISTILLED WATER 1 liter
BROMCRESOL PURPLE 1 c.c.
PART 2
OBSERVATIONS
ACCOMPLISHMENT

*ENZYMATIC SYNTHESIS OF DNA, XXIII. SYNTHESIS OF CIRCULAR REPLICATIVE FORM OF PHAGE φX174 DNA**

By Mehran Goulian† and Arthur Kornberg

DEPARTMENT OF BIOCHEMISTRY, STANFORD UNIVERSITY SCHOOL OF MEDICINE, PALO ALTO, CALIFORNIA

Communicated August 24, 1967

*ENZYMATIC SYNTHESIS OF DNA, XXIV. SYNTHESIS OF INFECTIOUS PHAGE φX174 DNA**

By Mehran Goulian,† Arthur Kornberg, and Robert L. Sinsheimer

DEPARTMENT OF BIOCHEMISTRY, STANFORD UNIVERSITY SCHOOL OF MEDICINE, PALO ALTO, AND DIVISION OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA

Communicated September 25, 1967

NATURE VOL. 224 DECEMBER 20 1969

Isolation of an *E. coli* Strain with a Mutation affecting DNA Polymerase

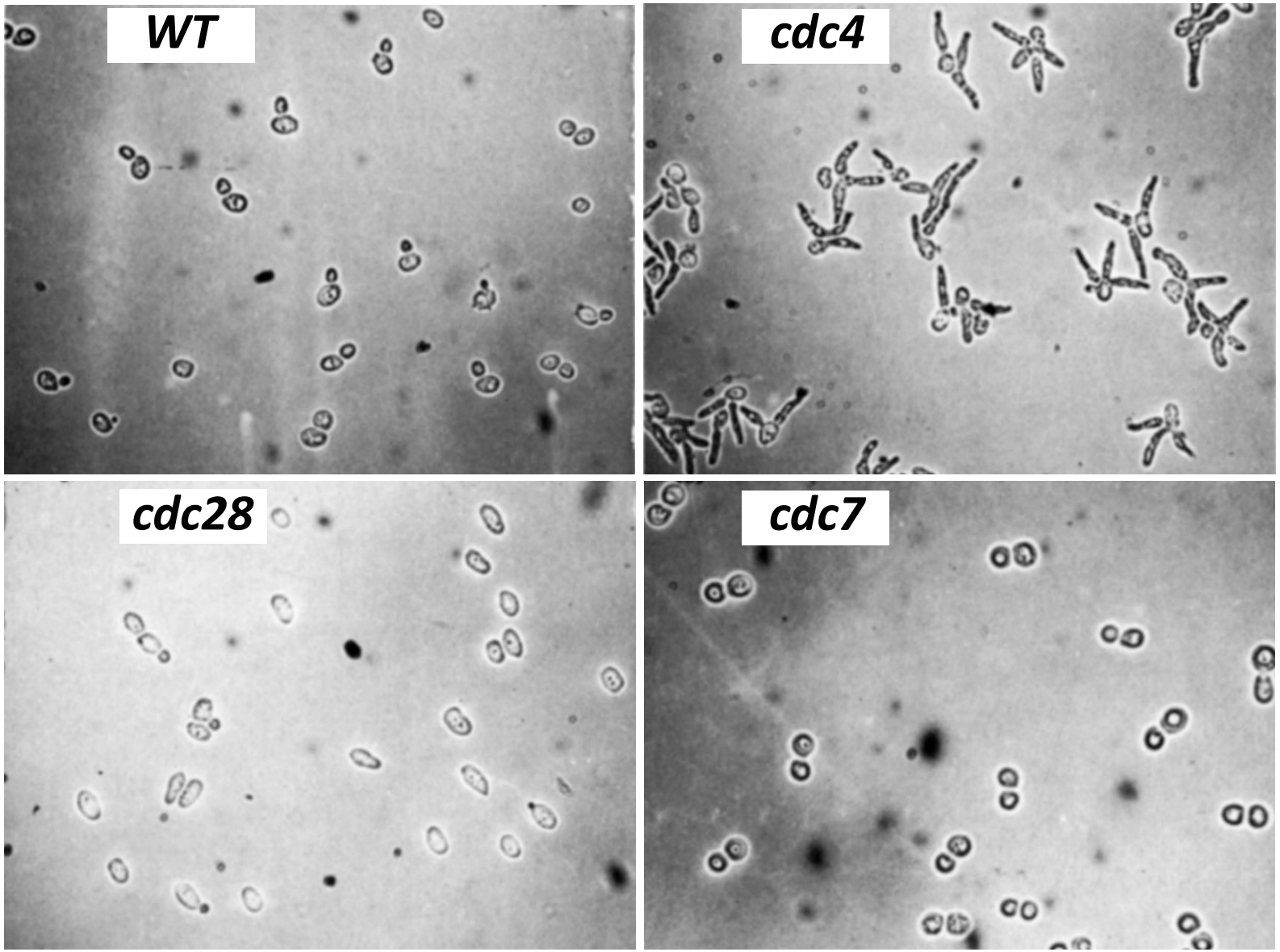
by

PAULA DE LUCIA
JOHN CAIRNS

Cold Spring Harbor Laboratory,
Cold Spring Harbor,
New York 11724

By testing indiscriminately several thousand colonies of mutagenized *E. coli*, a mutant has been isolated that on extraction proves to have less than 1 per cent of the normal level of DNA polymerase. The mutant multiplies normally but has acquired an increased sensitivity to ultraviolet light.

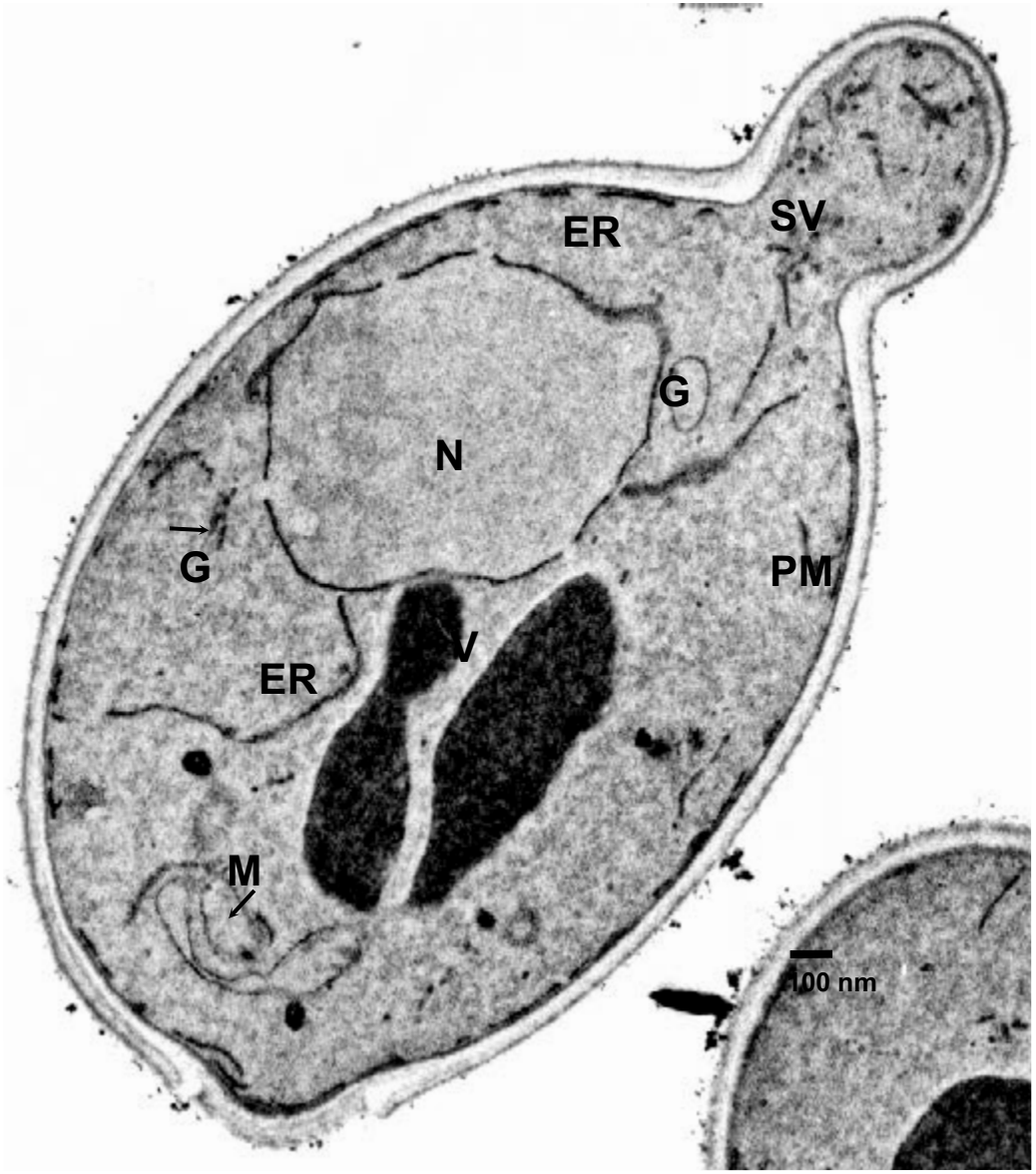
Uniform terminal morphology of temperature-sensitive cell division cycle mutants

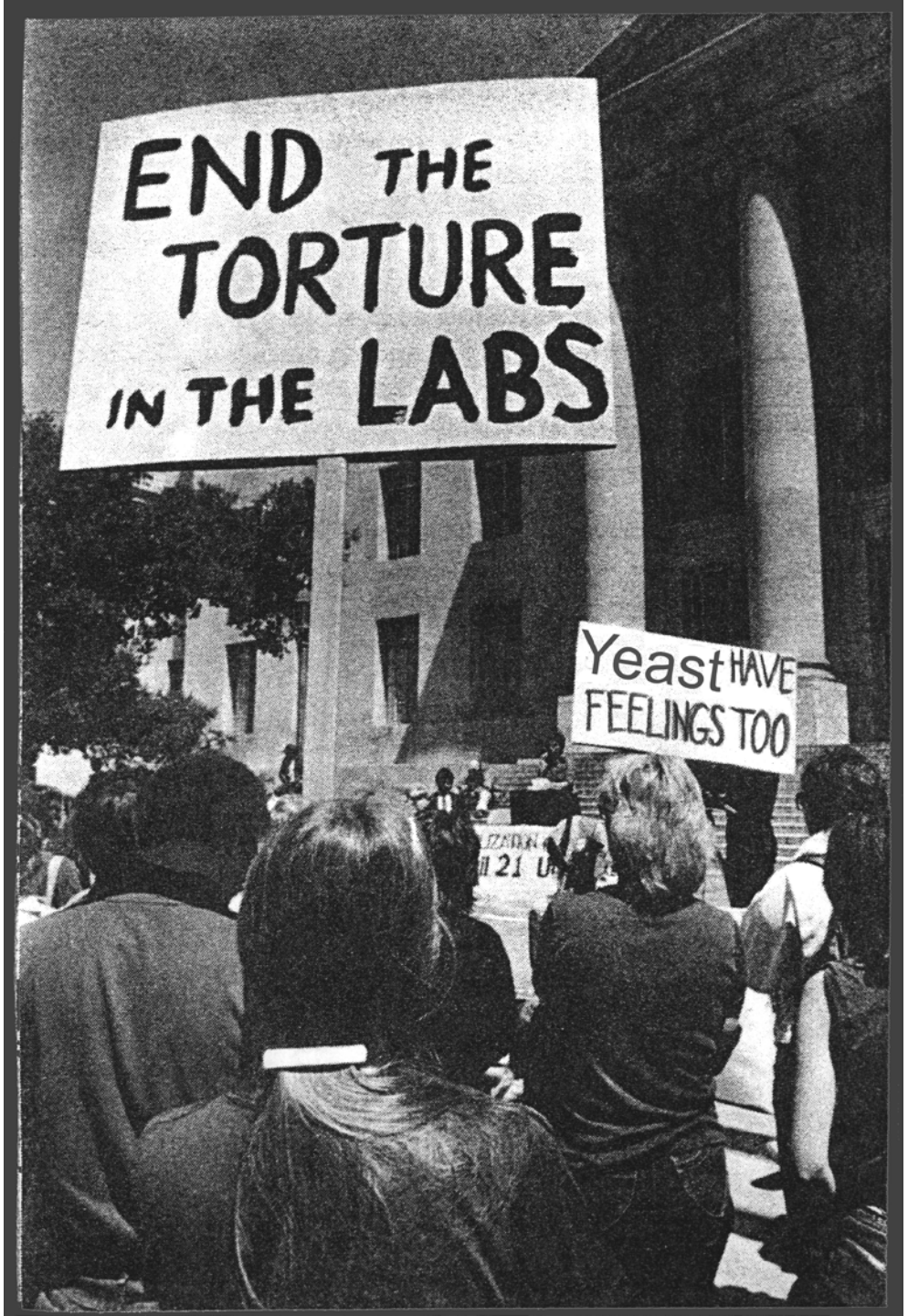


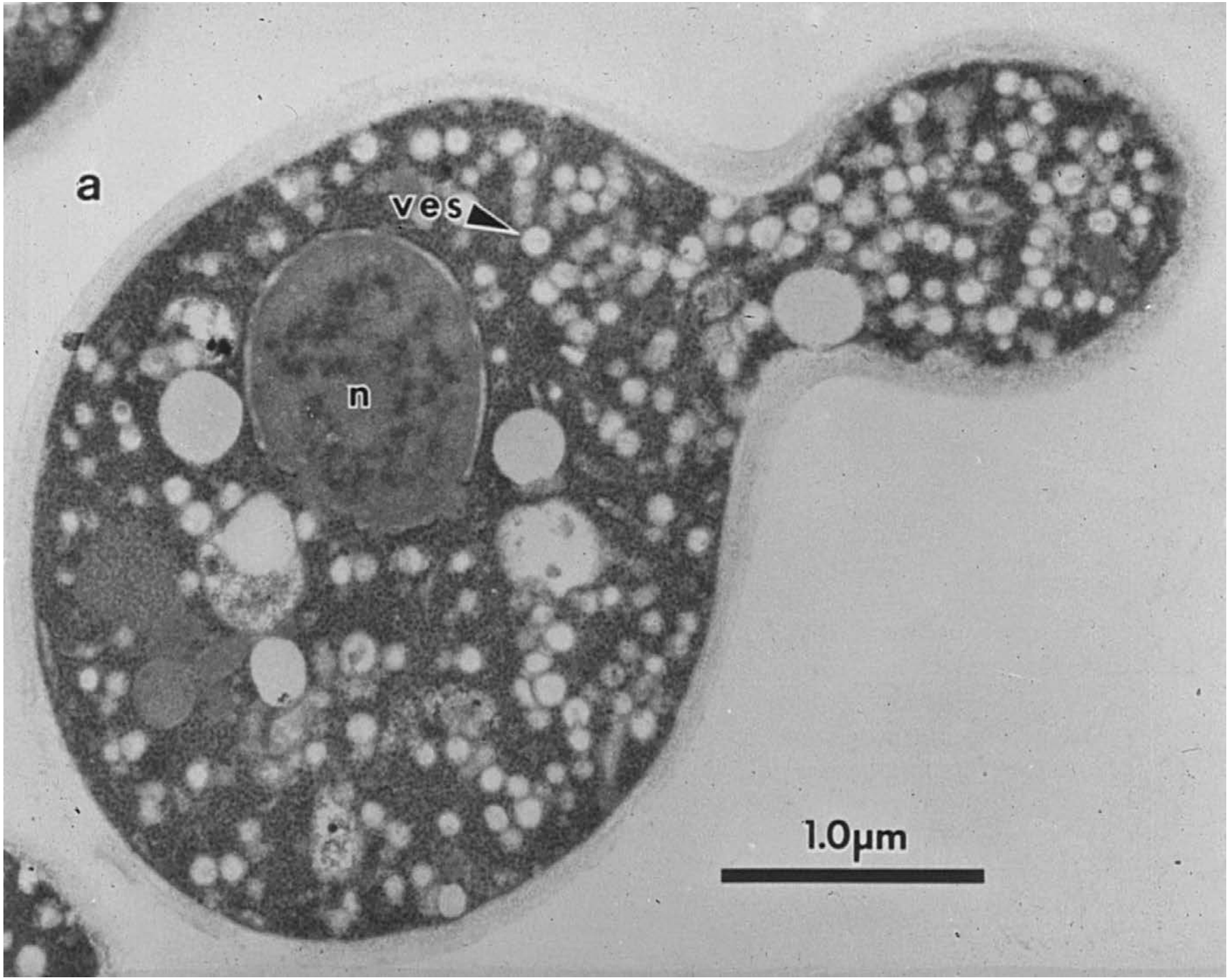
Adapted from: Hereford LM, Hartwell LH (1974) *J. Mol. Biol.* **84**: 445-461.


Berkeley, 1976

Yeast secretory organelles




END THE
TORTURE
IN THE LABS
Yeast HAVE
FEELINGS TOO


a
ves
n
1.0μm

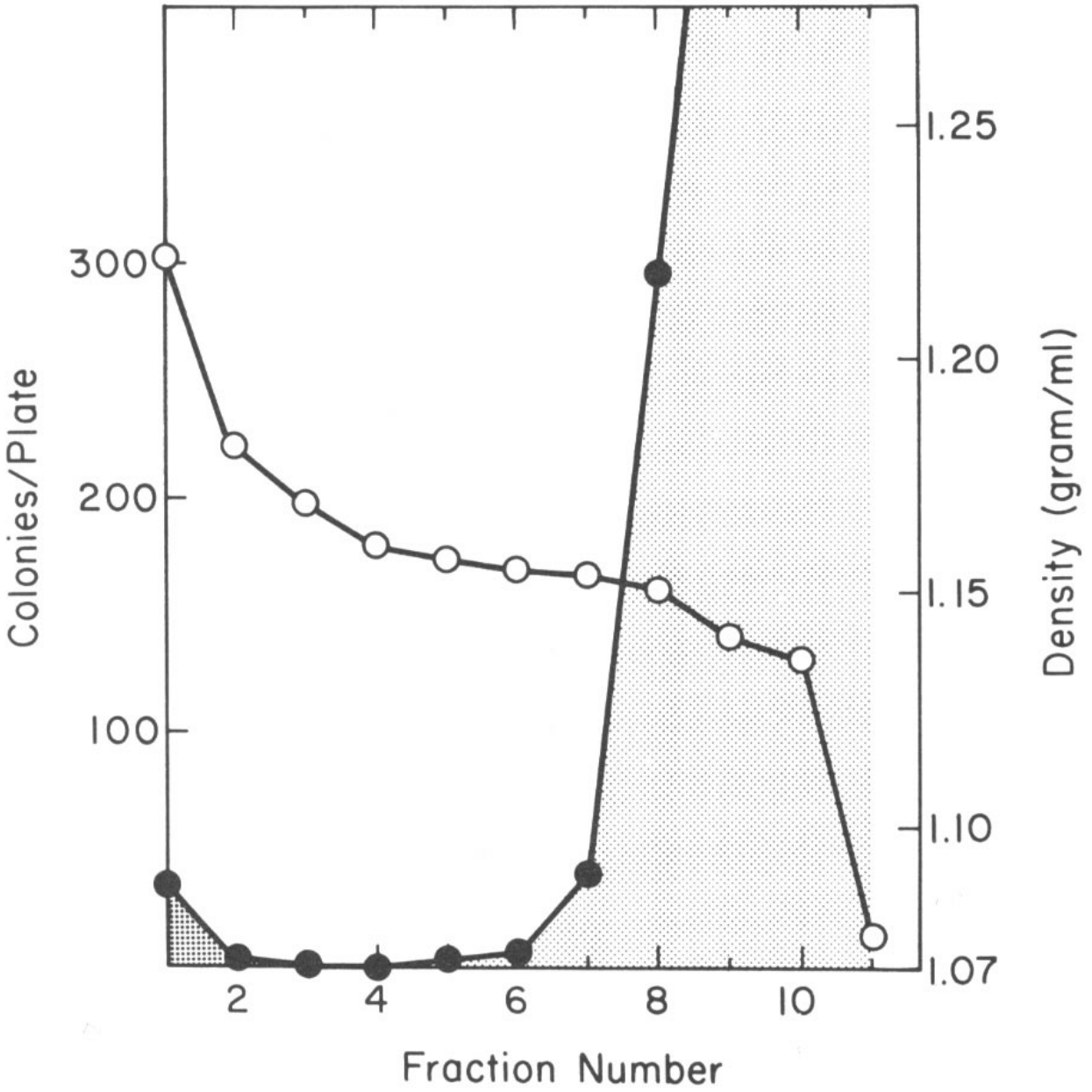
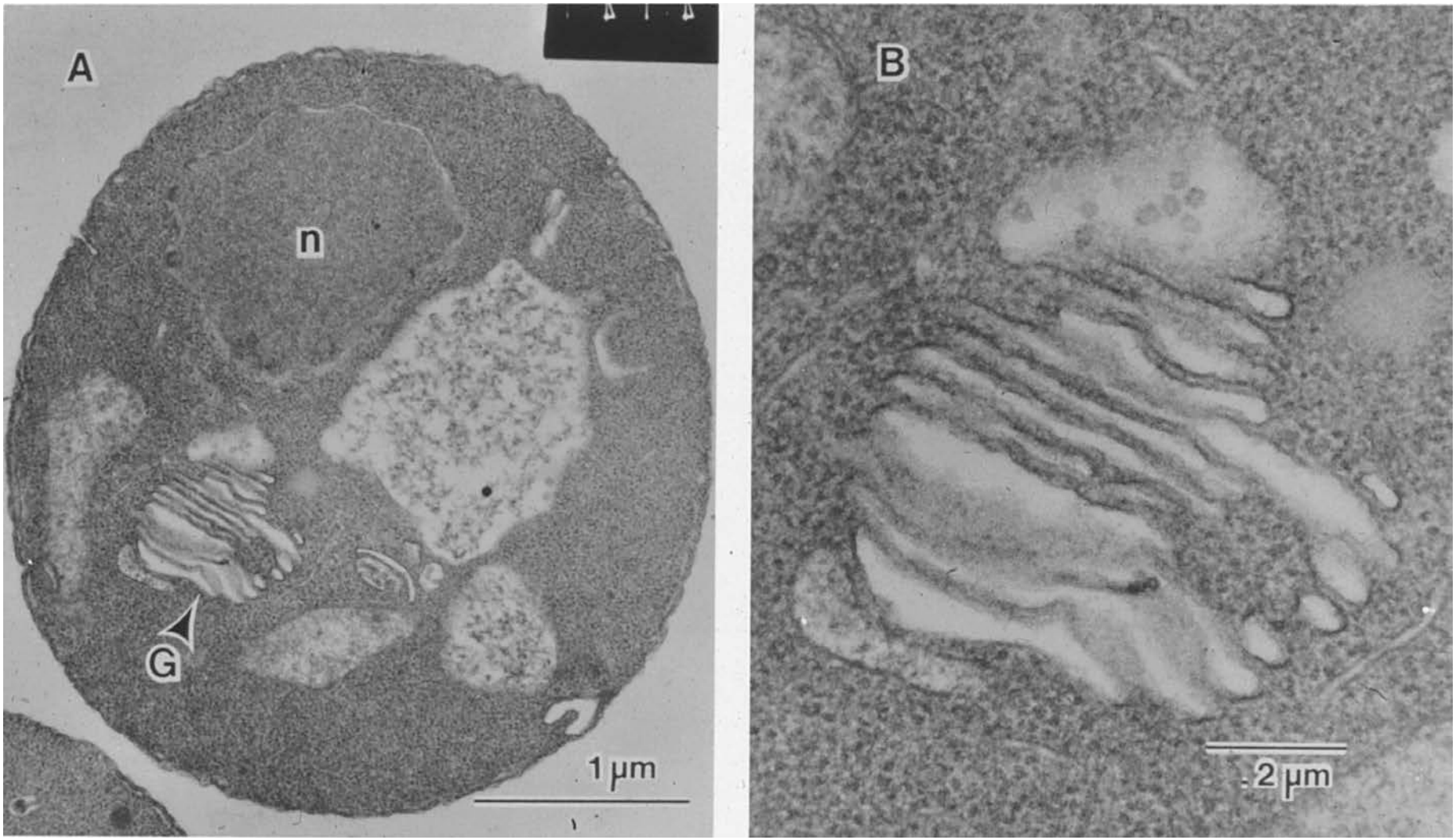
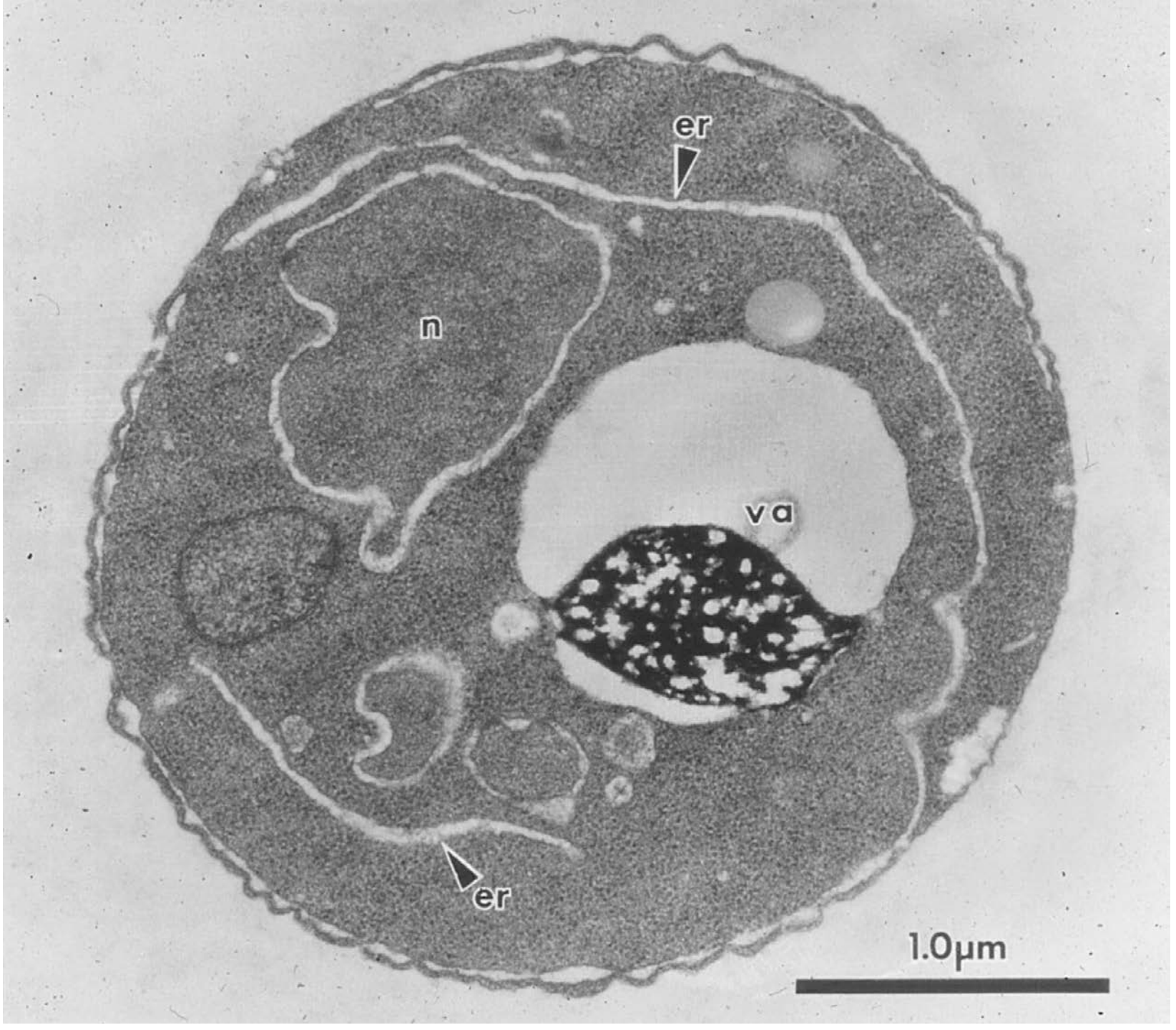
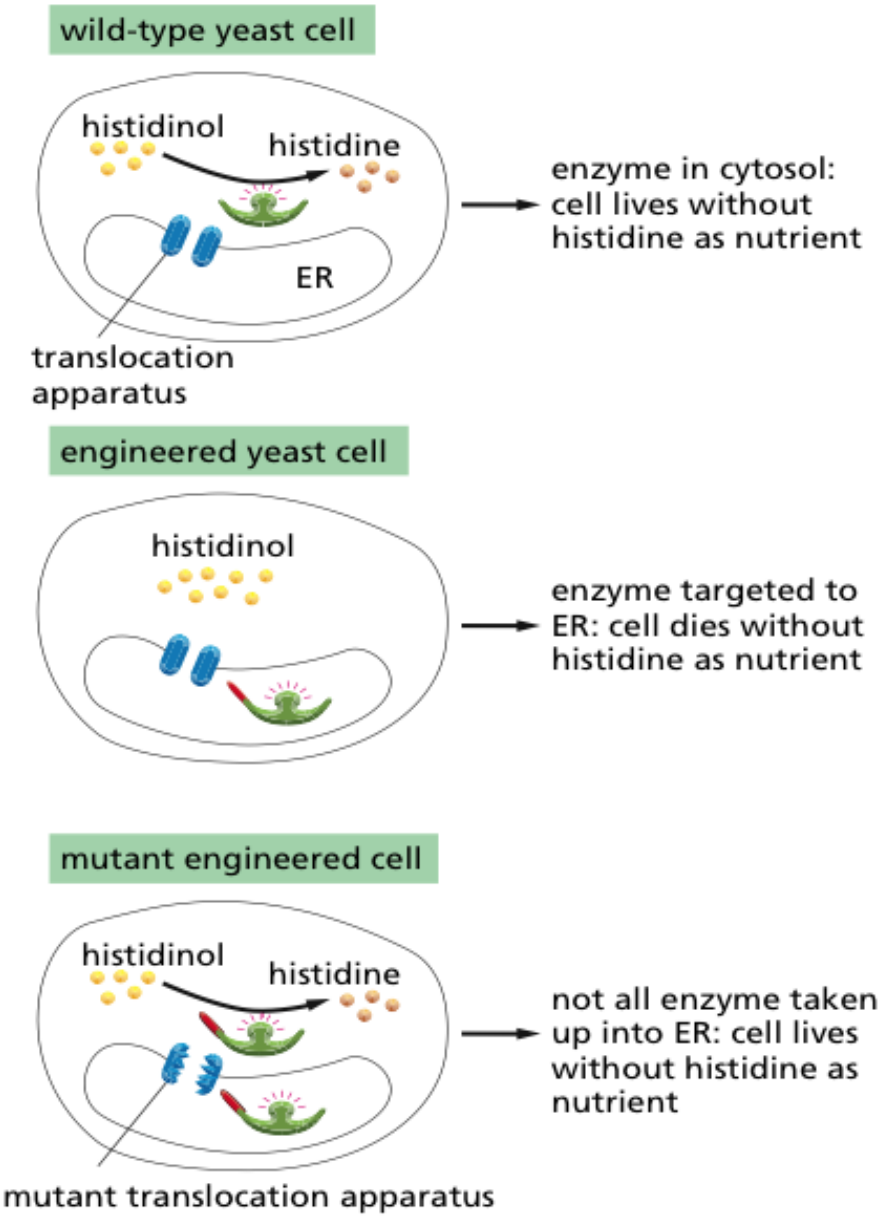


Figure 1. Density Gradient Separation of *sec*1-1 and X2180 Cells


A
n
G
1 µm
B
2 µm


er
n
va
er
1.0μm

GENETIC APPROACHES FOR STUDYING THE MECHANISM OF PROTEIN TRANSLOCATION



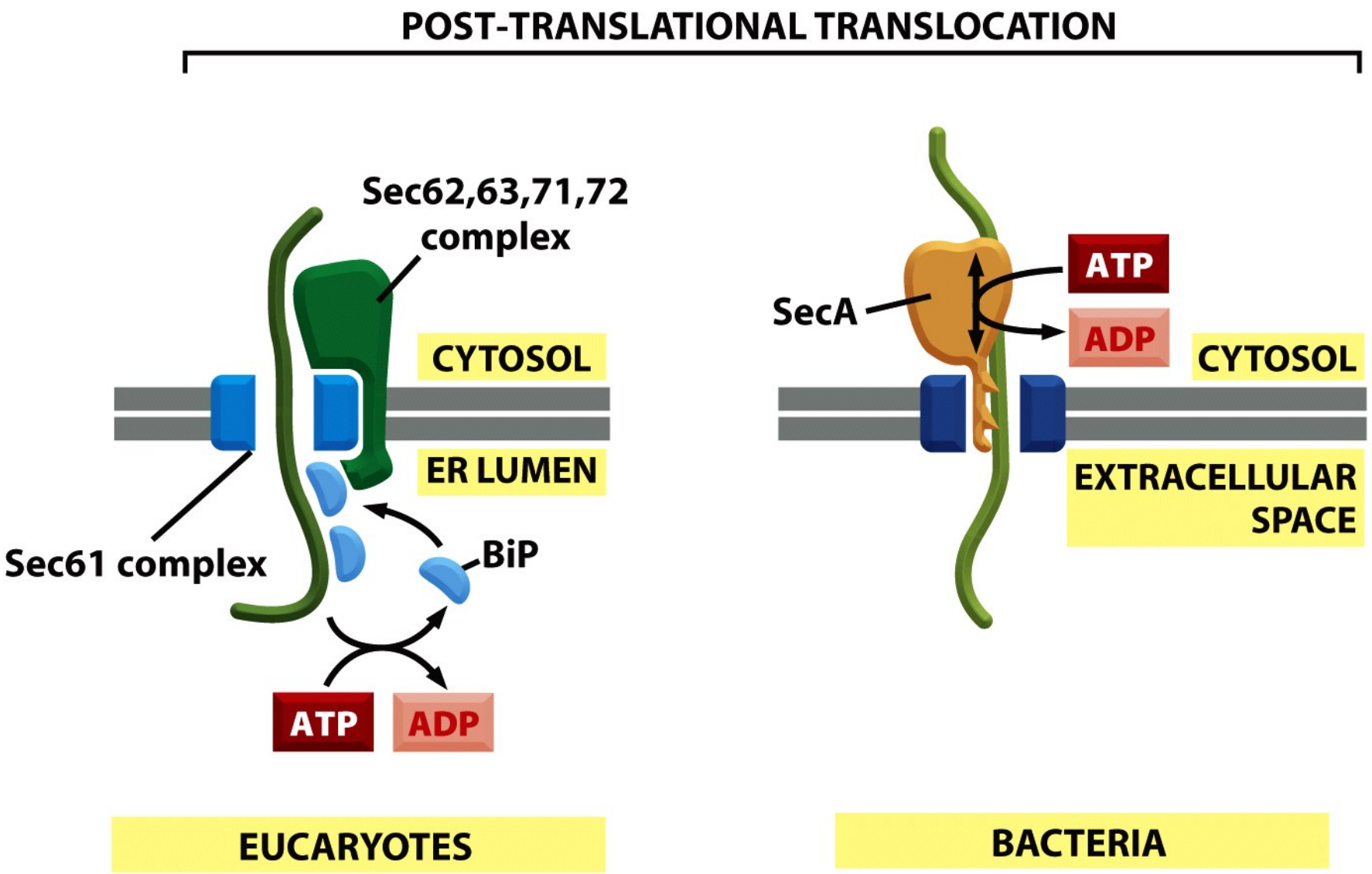
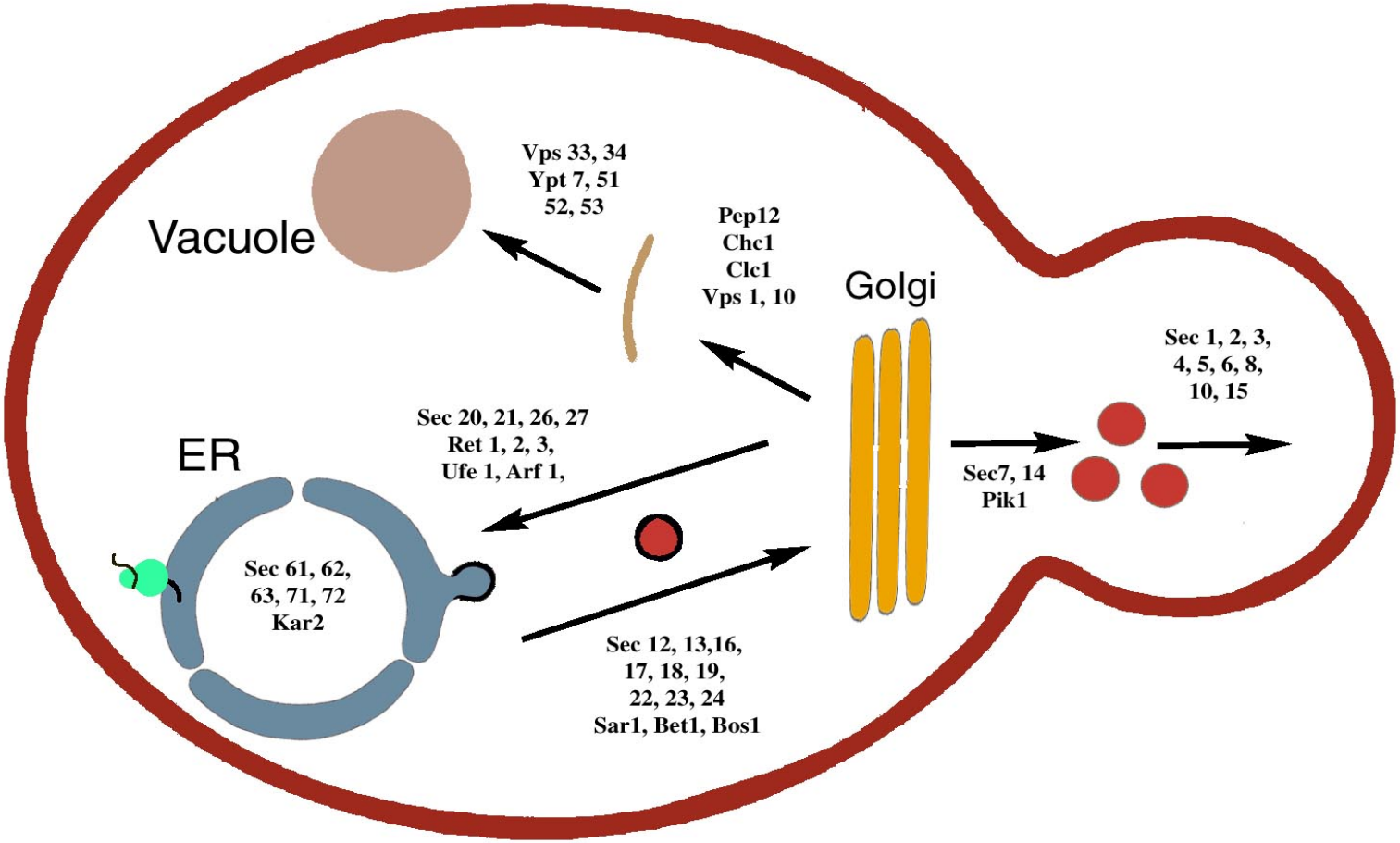
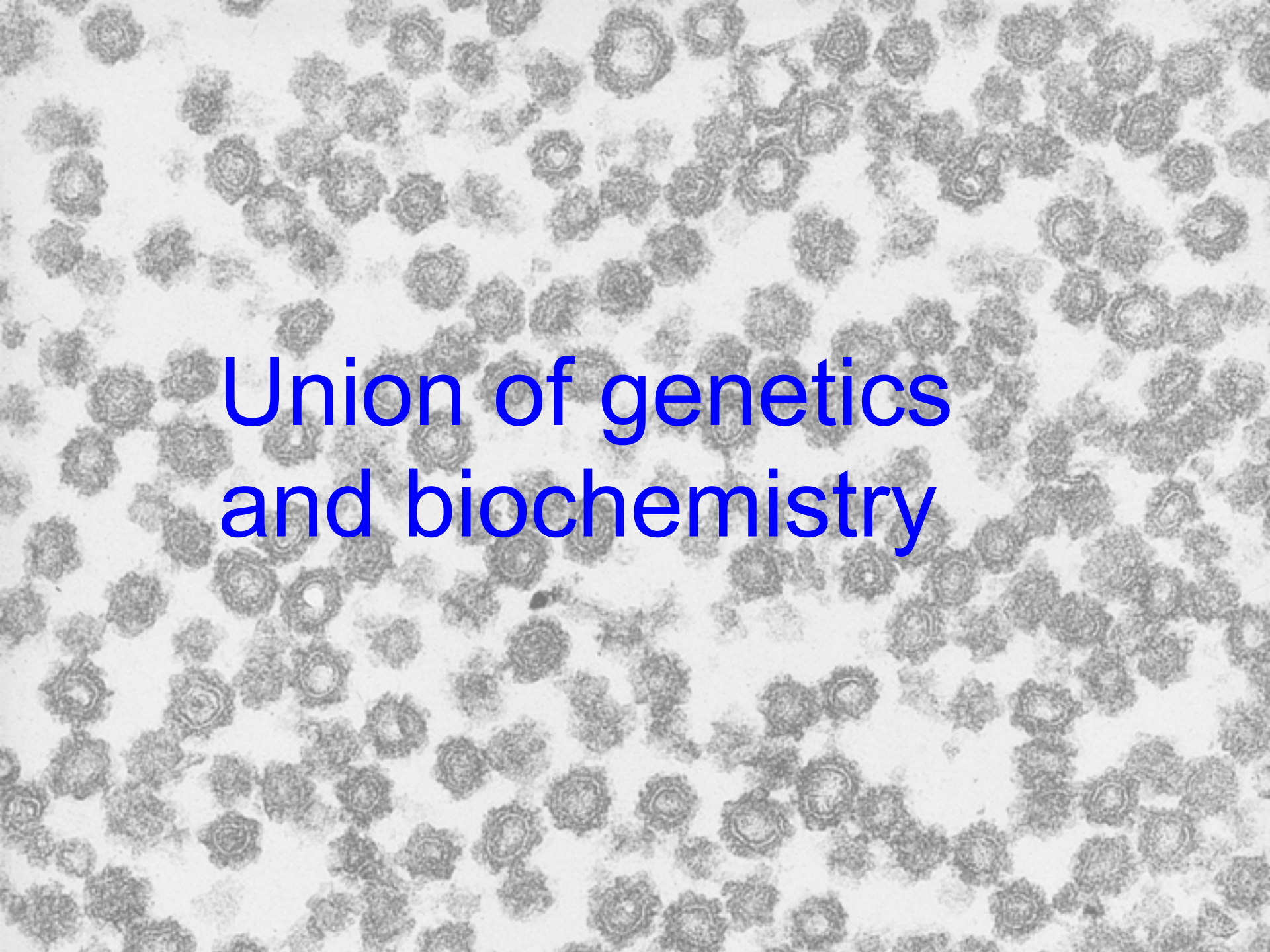


Figure 12-44b,c *Molecular Biology of the Cell* (© Garland Science 2008)

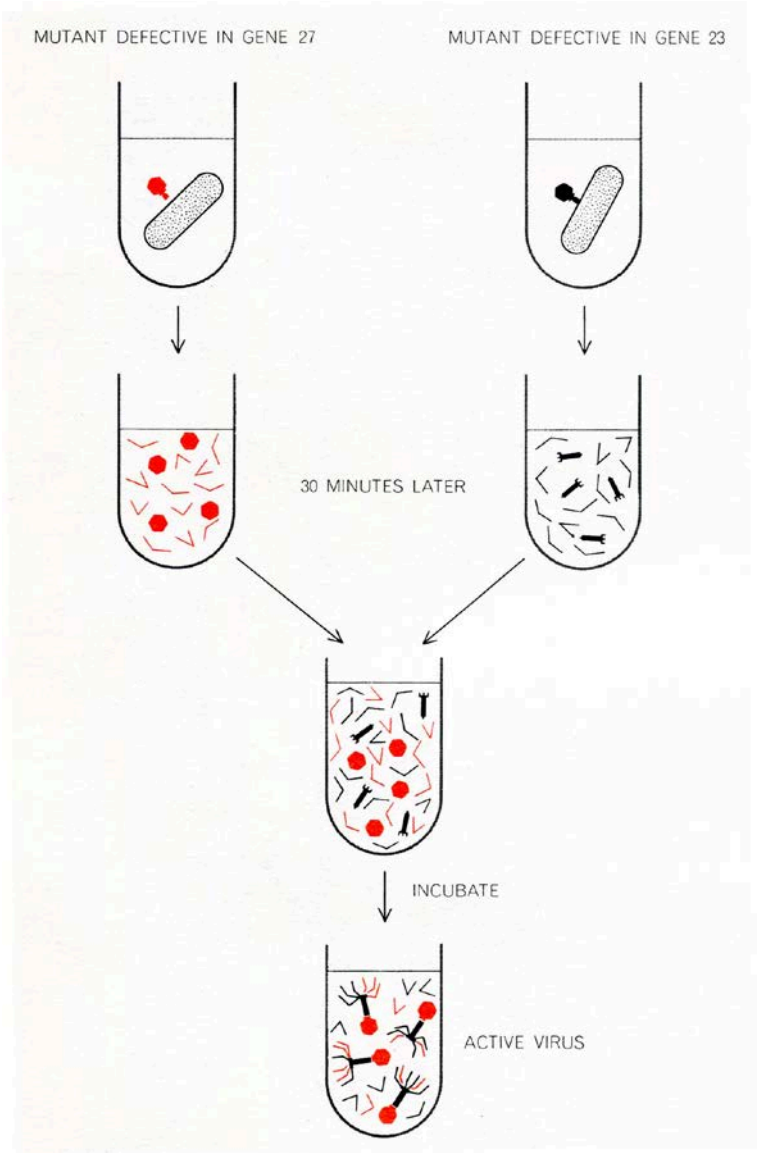
Yeast secretory pathway

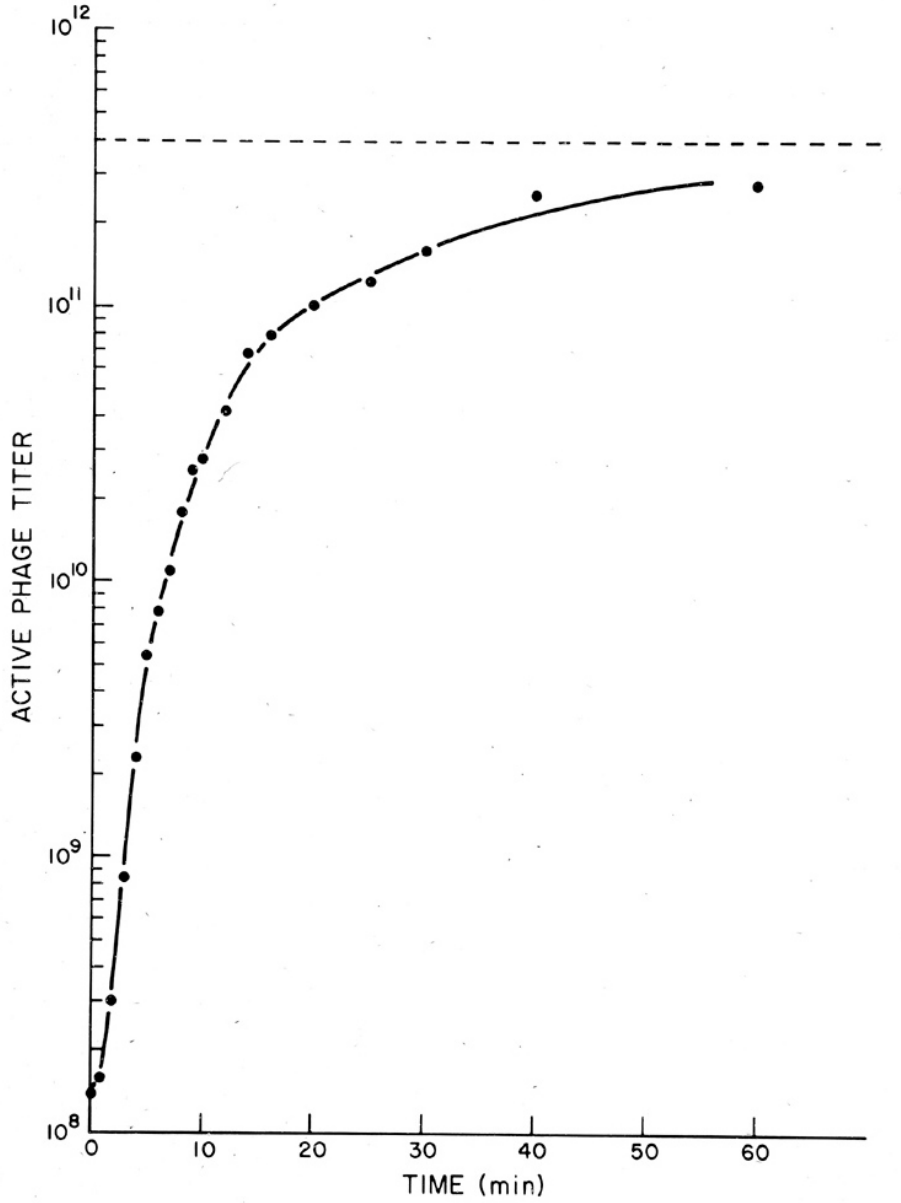



Union of genetics
and biochemistry

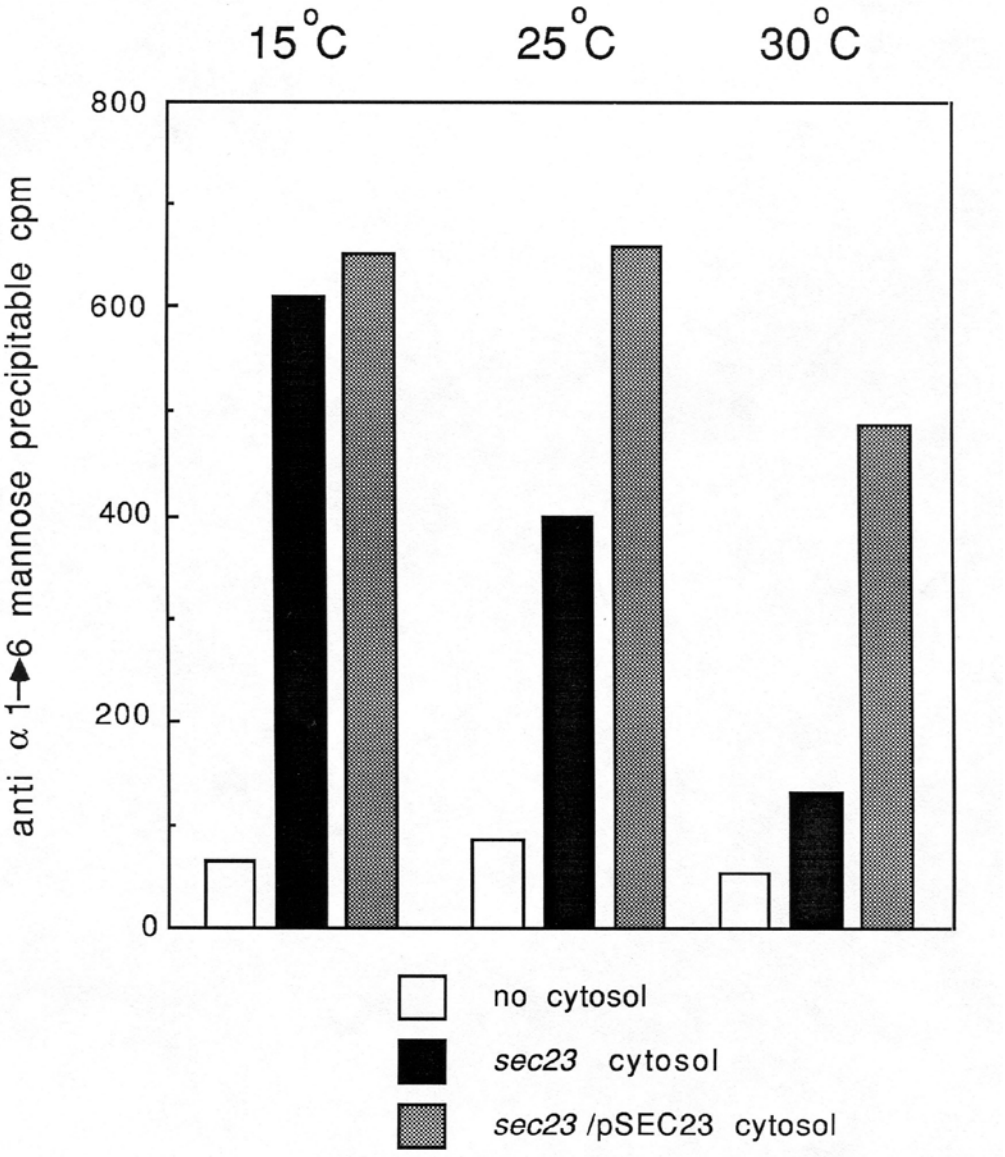
William Wood and Robert Edgar, 1965

Biochemical complementation in lysates of mutant bacteriophage infected cells

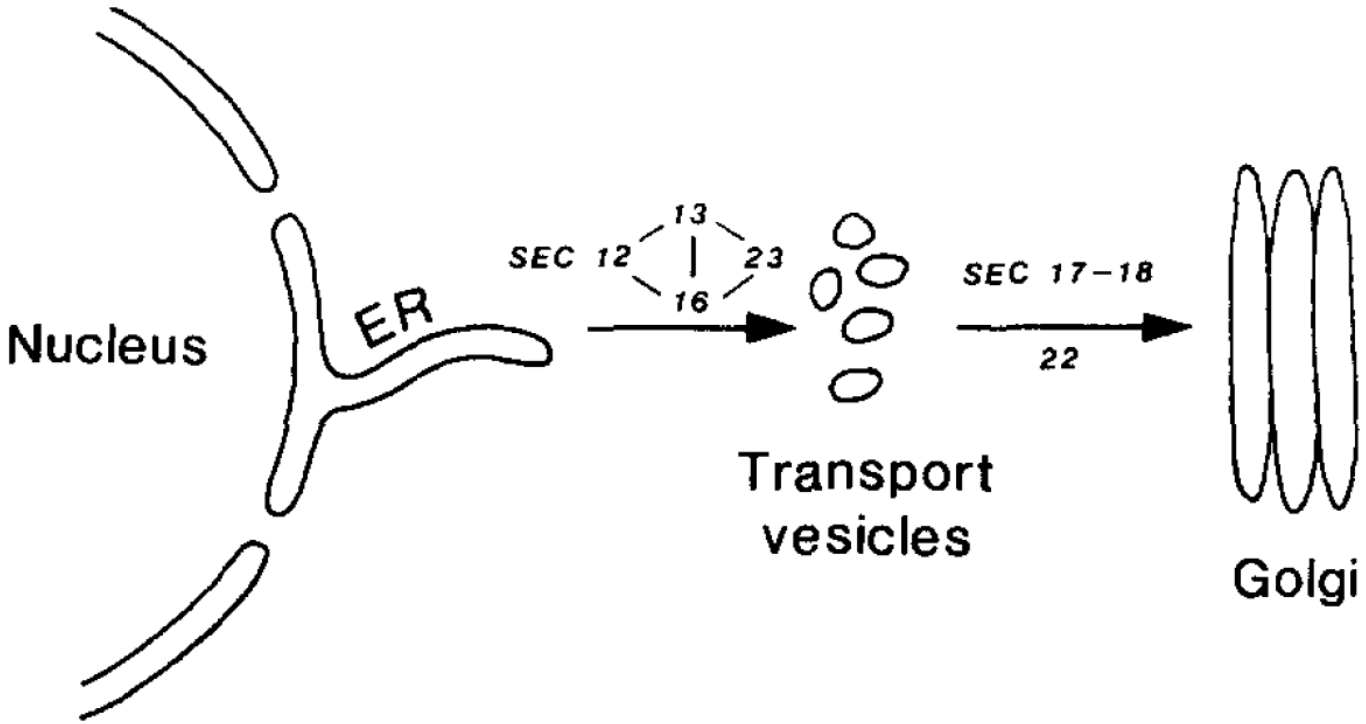




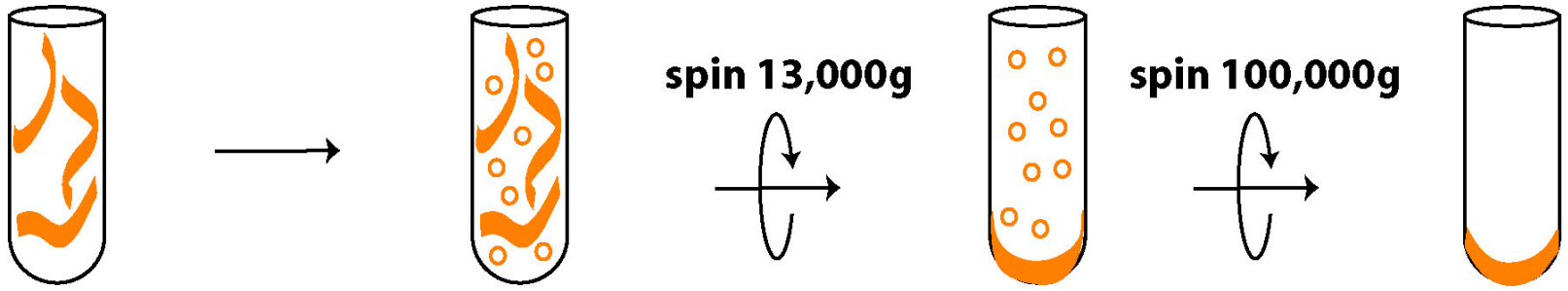
Mutant sec23 complementation in vitro



SEC genes required for budding and targeting vesicles from the ER to the Golgi



Vesicle budding assay

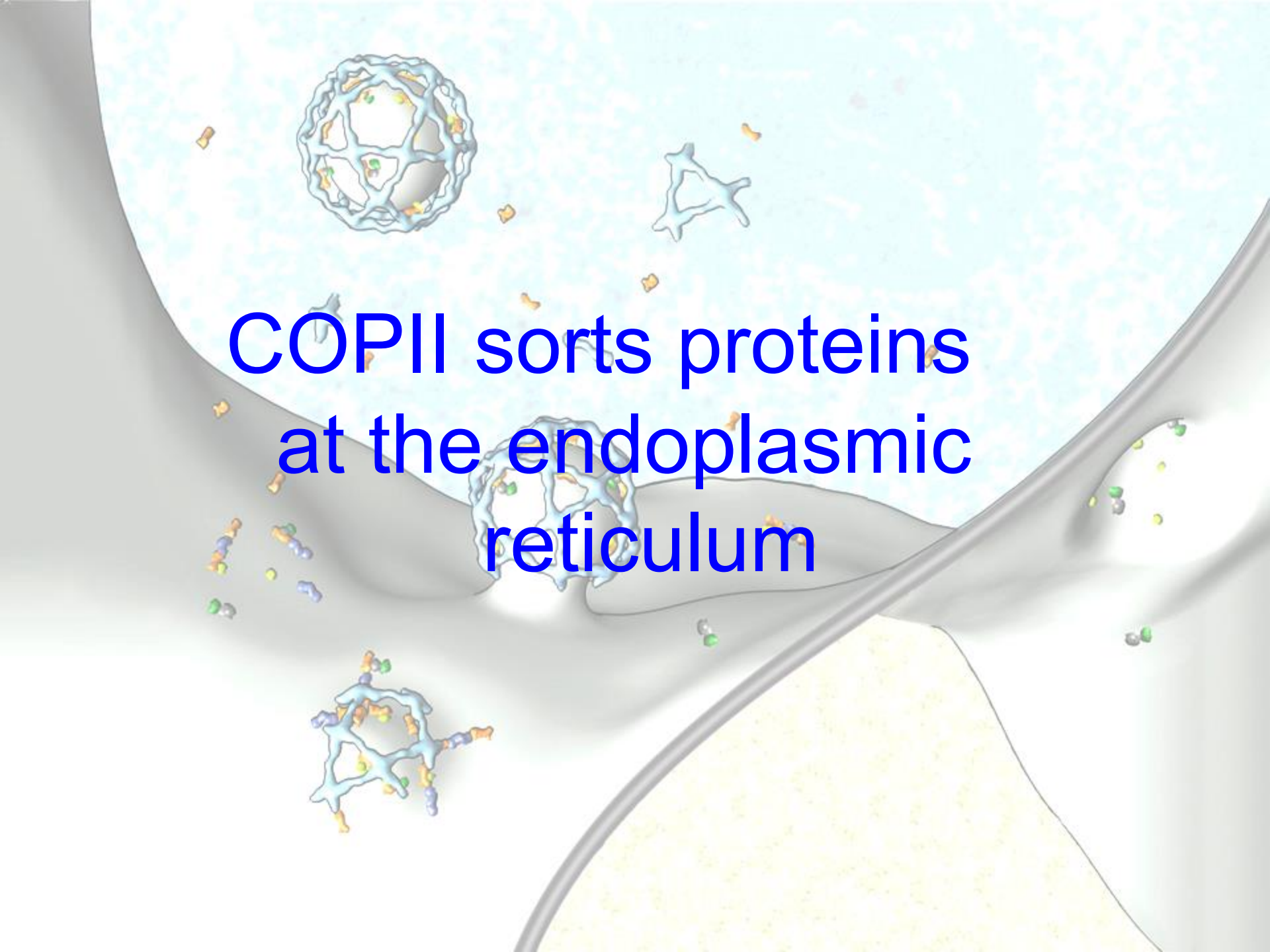


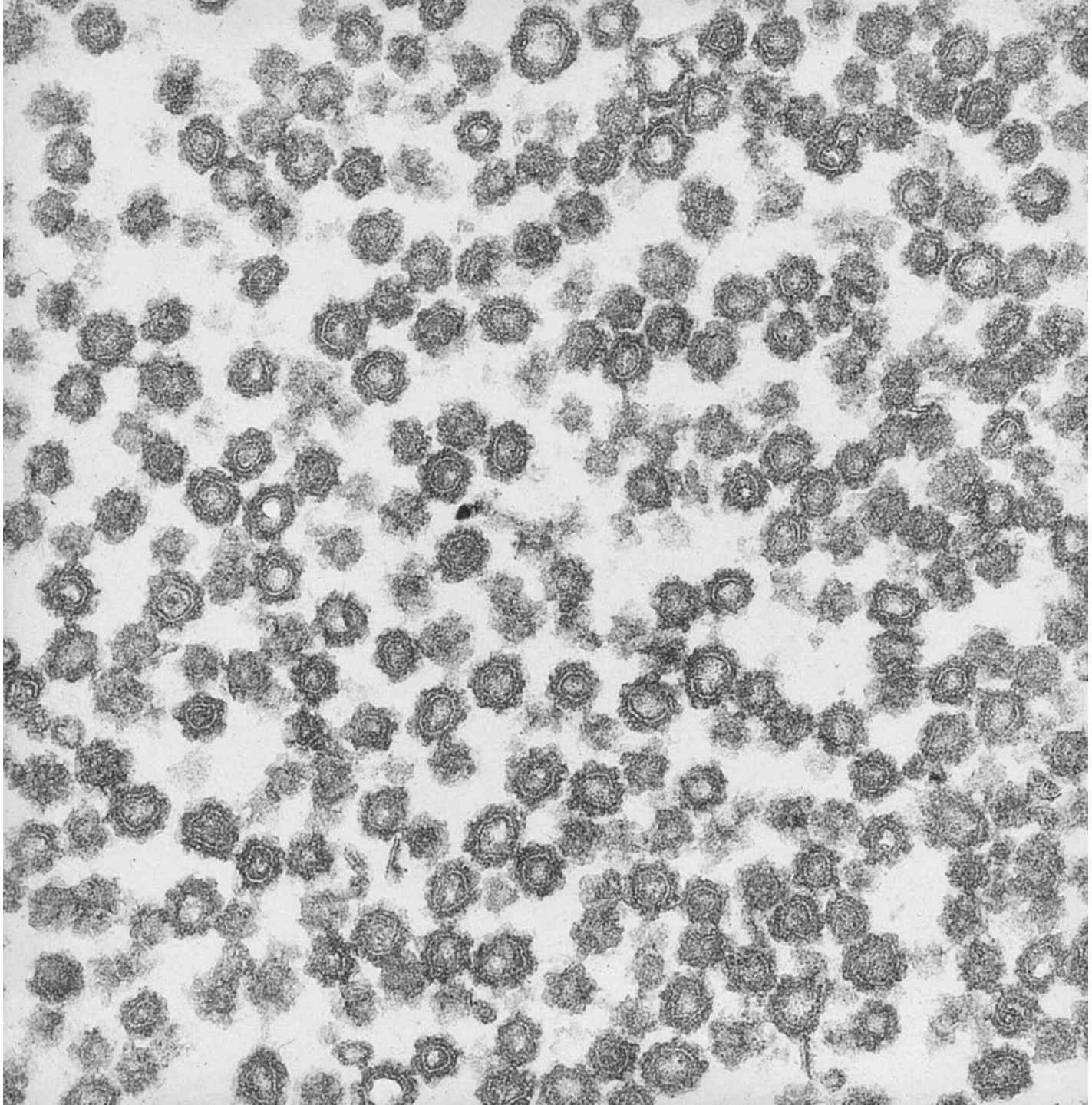
Donor membranes (microsomes or semi-intact cells)

Donor membranes + COPII proteins + nucleotide

Vesicles in supernatant

Vesicles in pellet


COPII sorts proteins
at the endoplasmic
reticulum

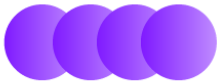


The Players…

COPII Subunits:

Sar1p

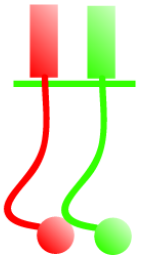
Sec23/24p



Sec13/31p

Others:

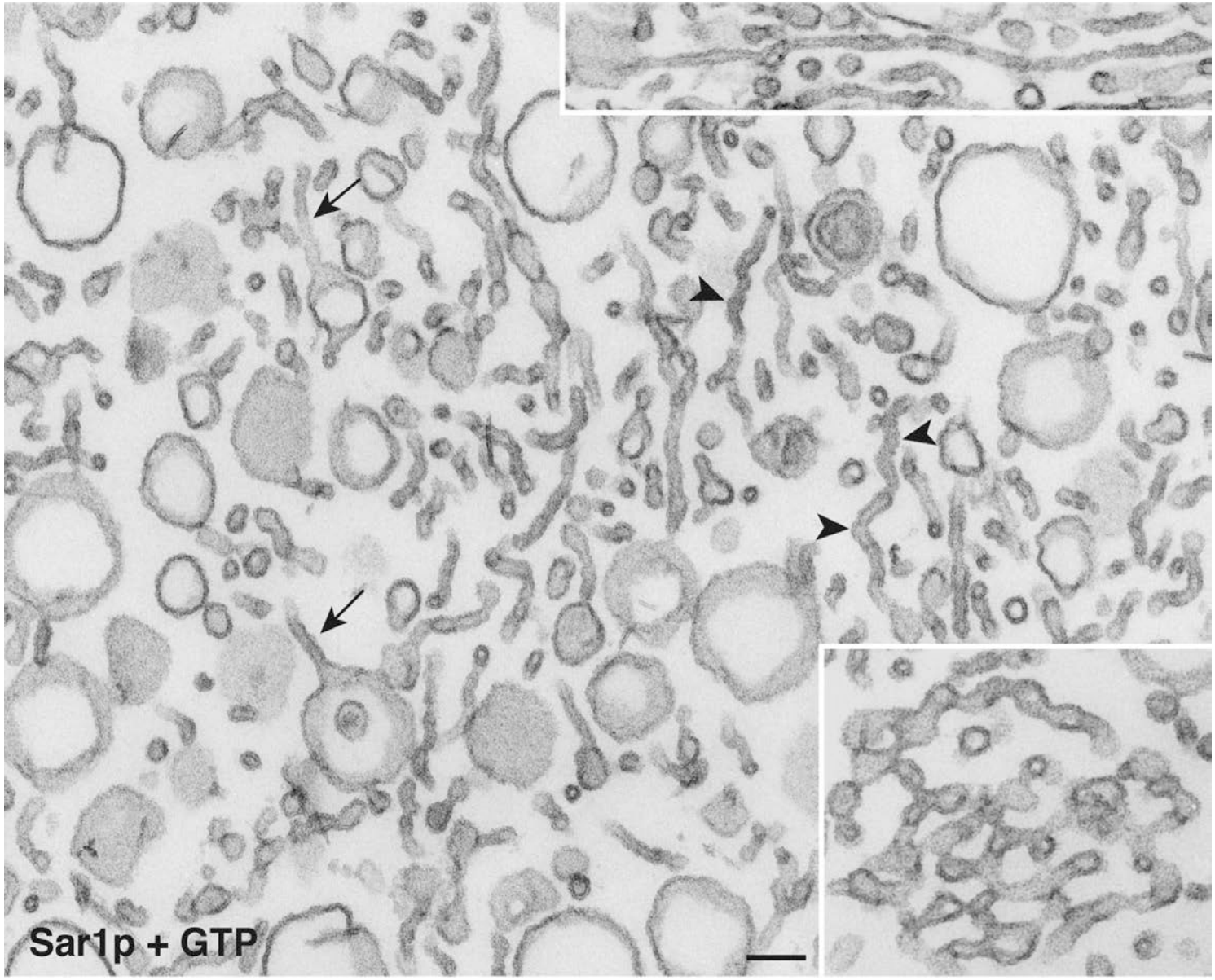
Sec12p



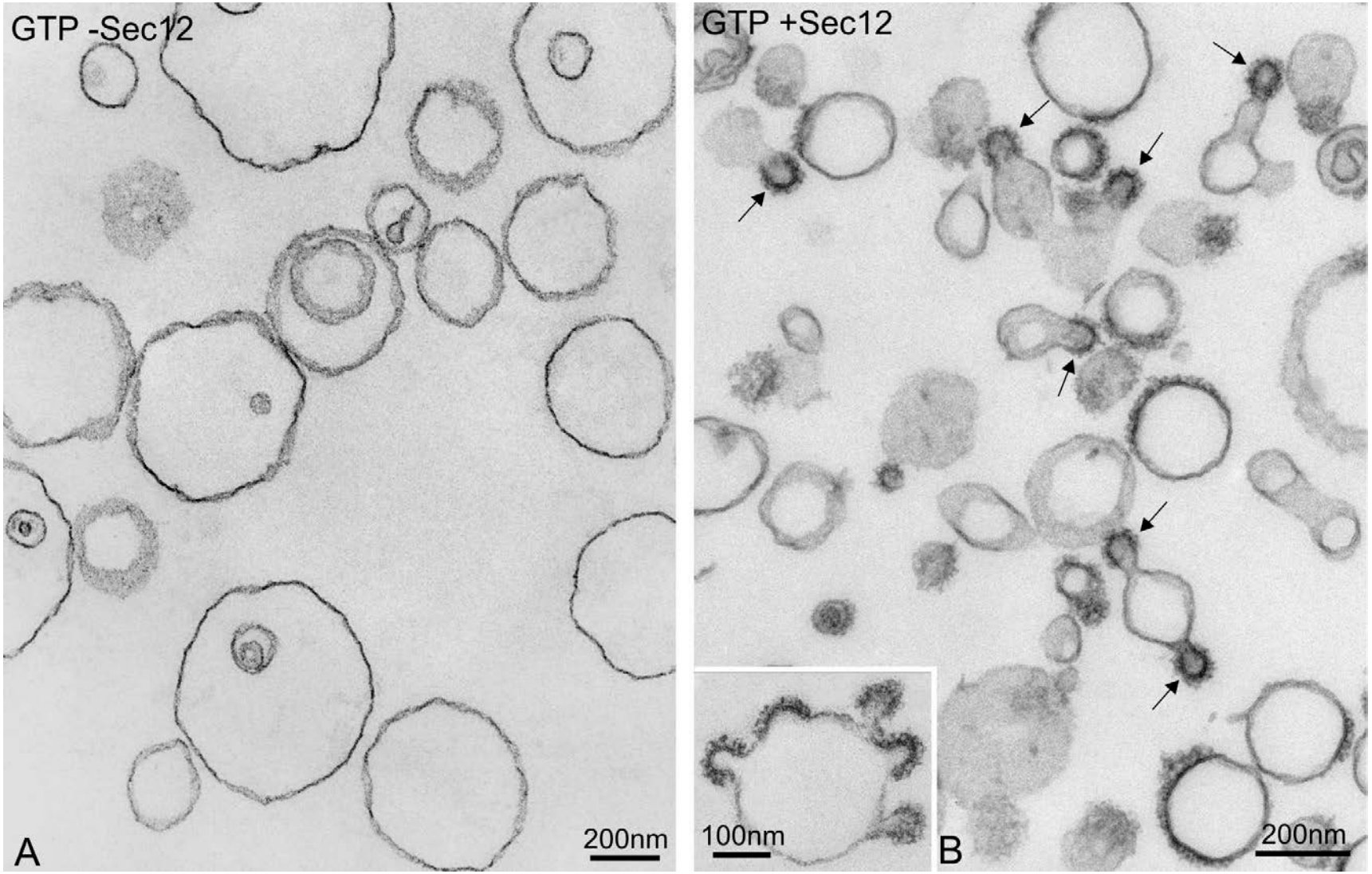
Cargo Molecules

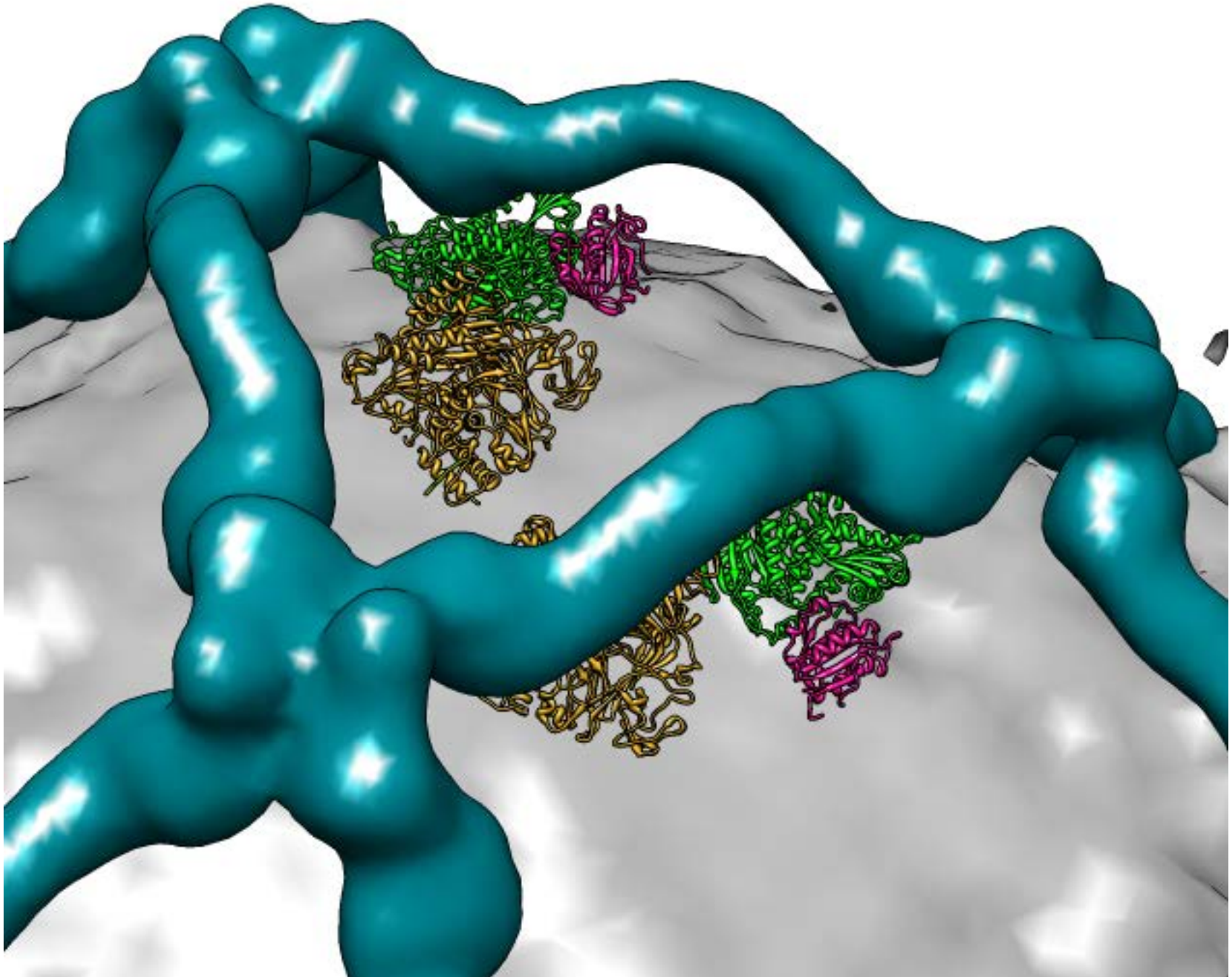

D
Sec12p

Sar1p deforms membranes in a nucleotide-dependent manner

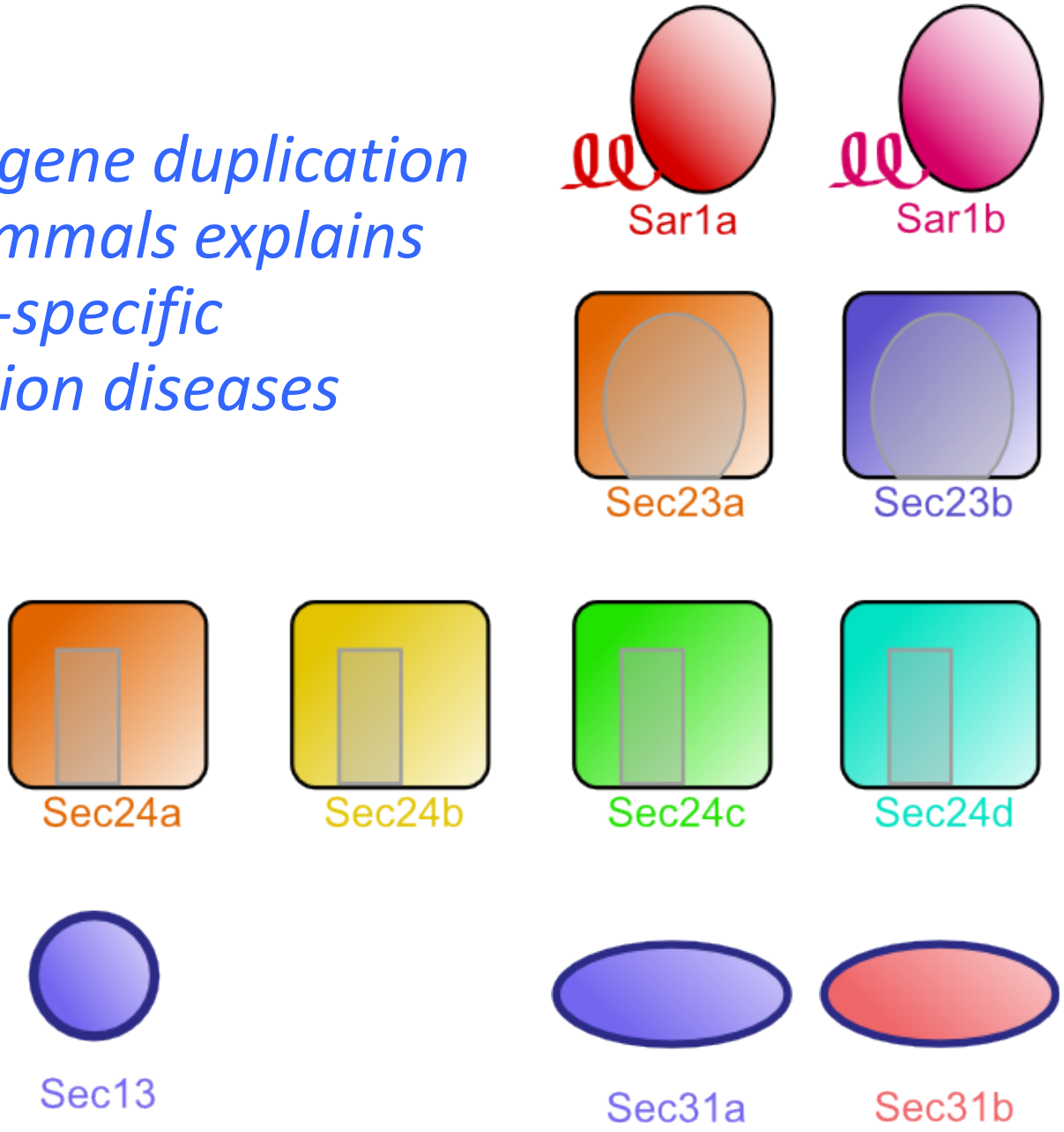


Sec12p enables COPII bud formation on synthetic liposomes





COPII gene duplication in mammals explains tissue-specific secretion diseases

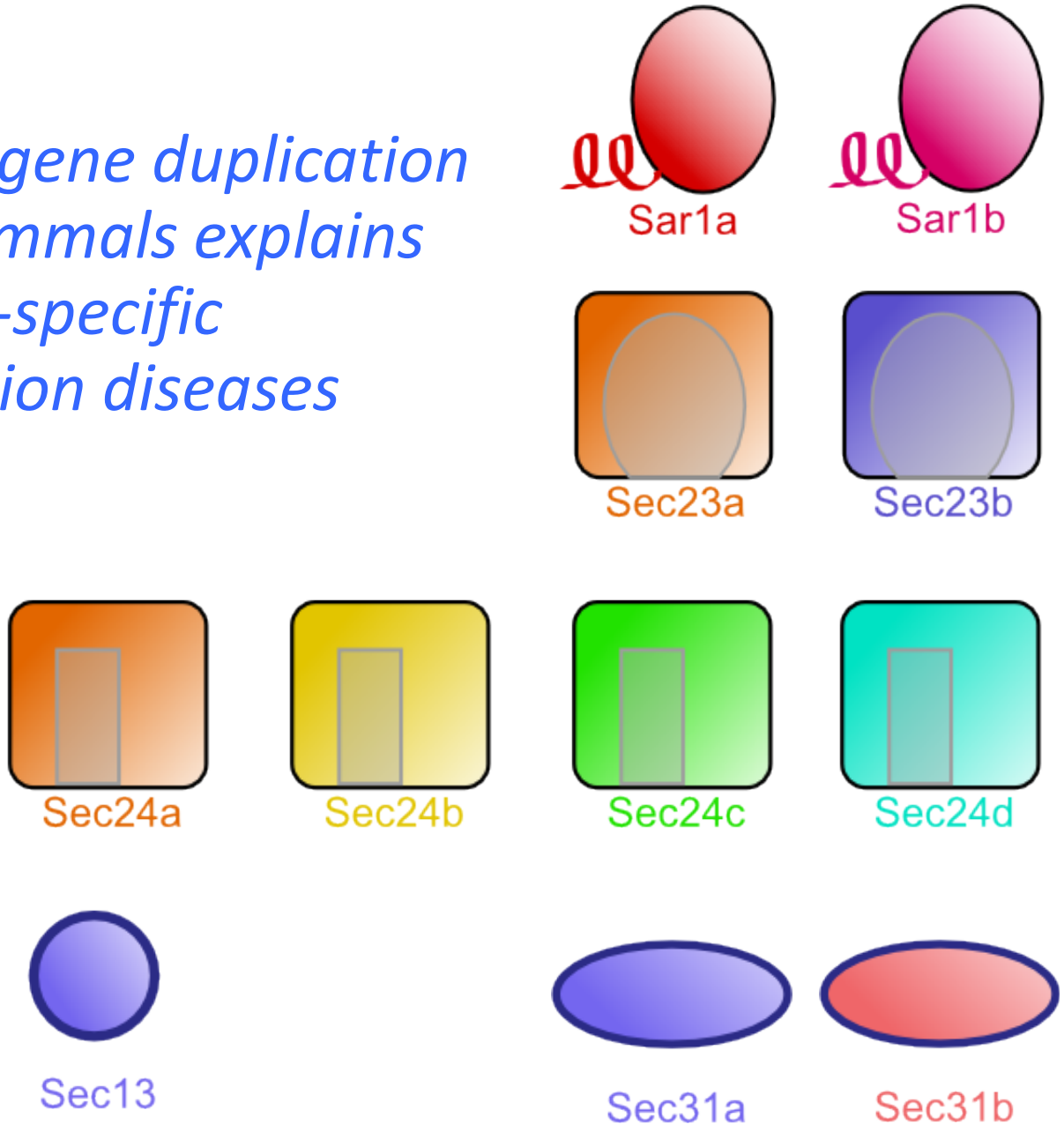


Mutations in a Sar1 GTPase of COPII vesicles are associated with lipid absorption disorders

Bethan Jones, Emma L. Jones, Stephanie A. Bonney, Hetal N. Patel, Arjen R. Mensenkamp, Sophie Eichenbaum-Voline, Mats Rudling, Urban Myrdal, Grazia Annesi, Sandhia Naik, Nigel Meadows, Aldo Quattrone, Suhail A. Islam, Rossitza P. Naoumova, Bo Angelin, Recaredo Infante, Emile Levy, Claude C. Roy, Paul S. Freemont, James Scott, & Carol C. Shoulders

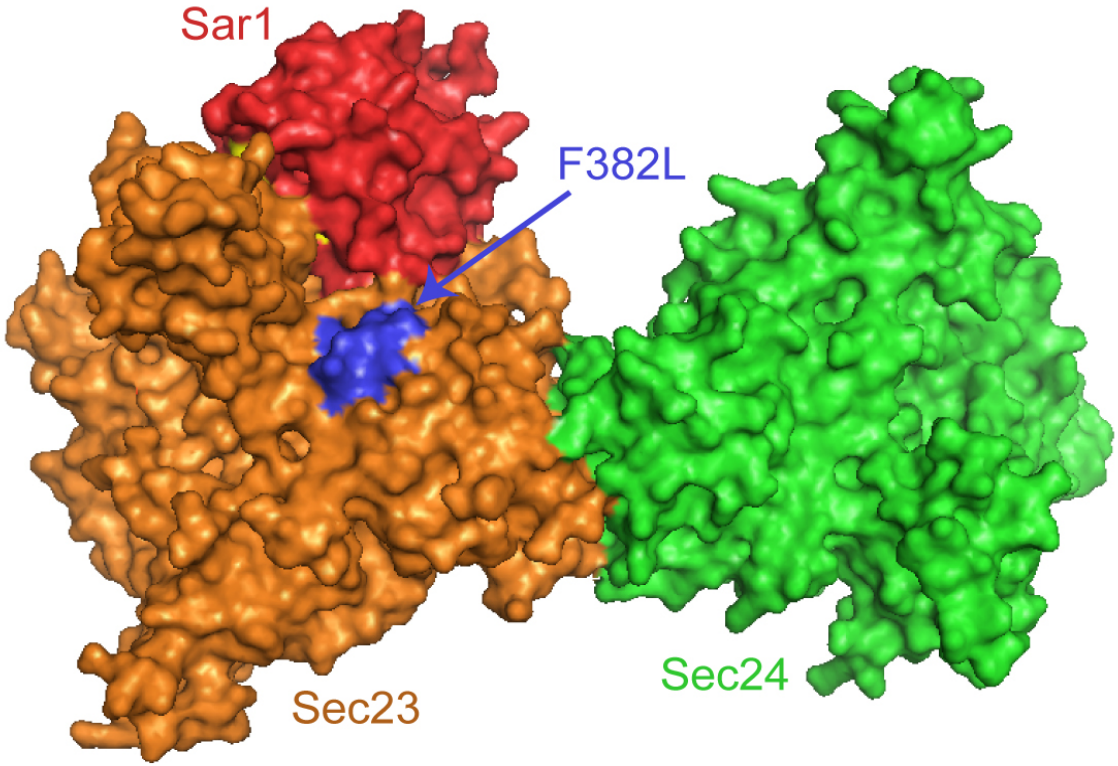
Dietary fat is an important source of nutrition. Here we identify eight mutations in *SARA2* that are associated with three severe disorders of fat malabsorption. The Sar1 family of proteins initiates the intracellular transport of proteins in COPII (coat protein)-coated vesicles. Our data suggest that chylomicrons, which vastly exceed the size of typical COPII vesicles, are selectively recruited by the COPII machinery for transport through the secretory pathways of the cell.

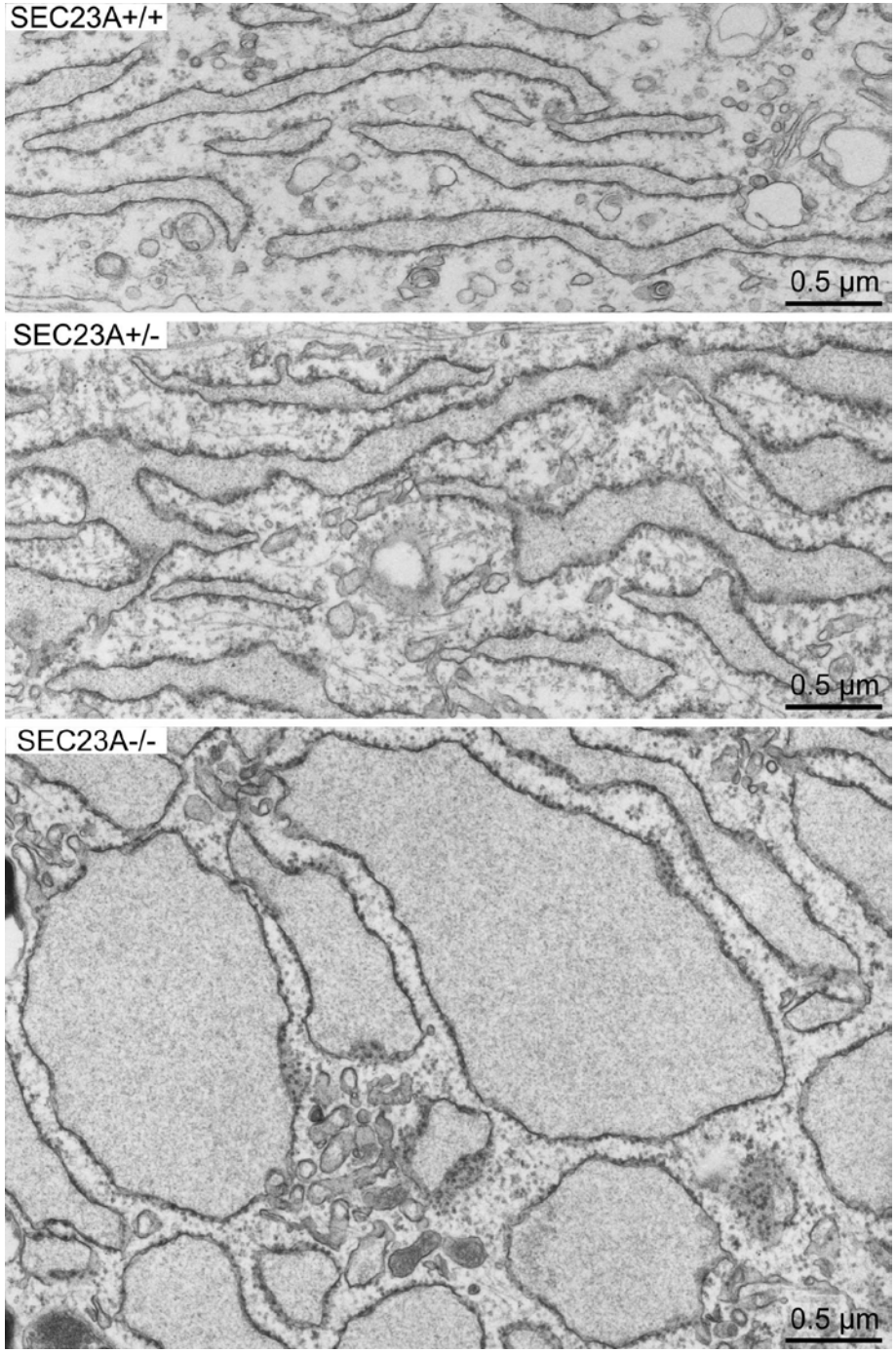
COPII gene duplication in mammals explains tissue-specific secretion diseases



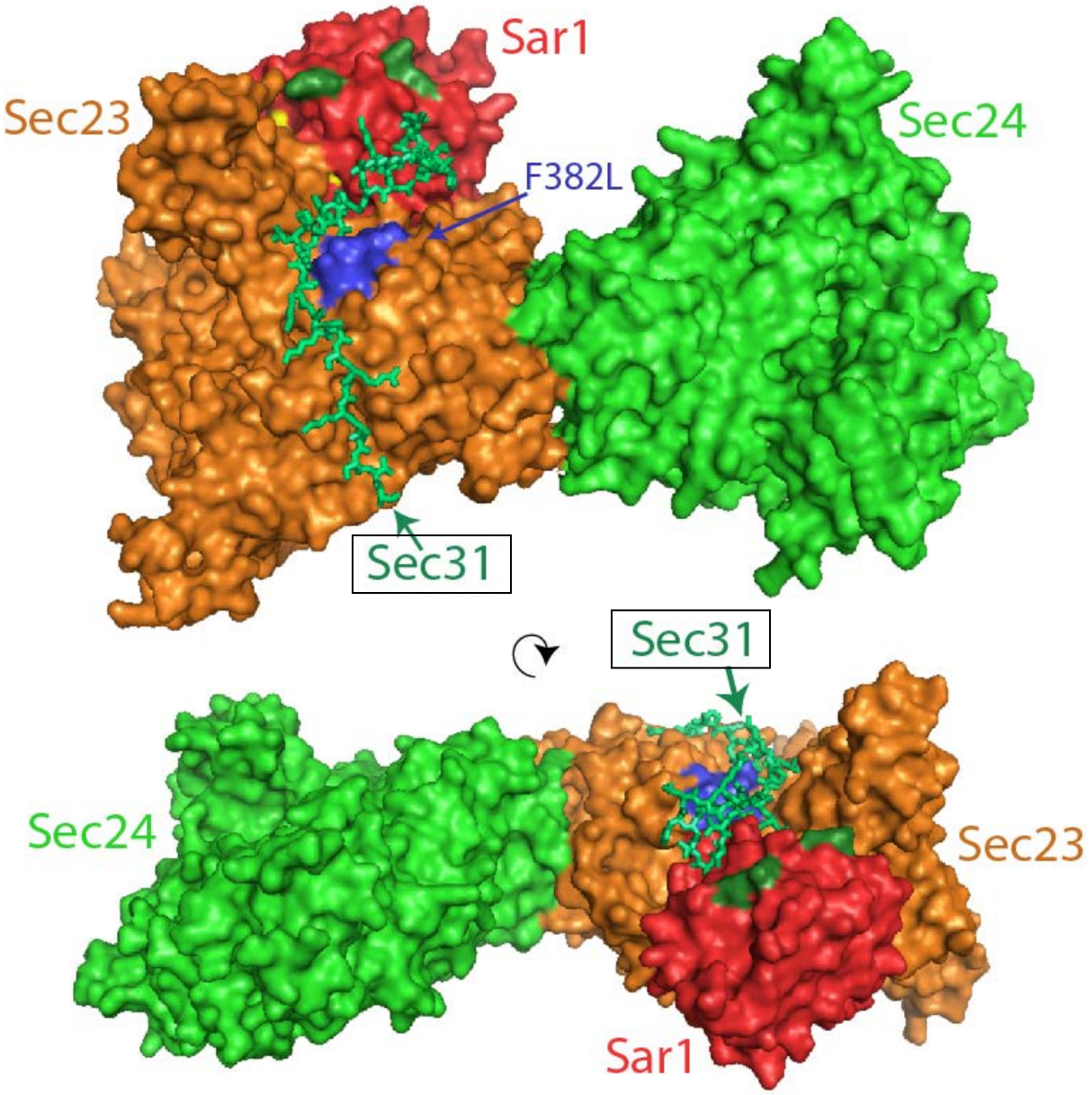
CLSD mutation: Alignment with yeast sequence and structure

```
SEC23A   TGGYMVMGDSFNTSLFKQTFQRVFTKDMHGQFKMGF
SEC23B   TGGYMVMGDSFNTSLFKQTFQRIFTKDFNGDFRMAF
Sec23p   TGGVLLLTDAFSTAIFKQSYLRLFAKDEEGYLKMAF
```




SEC23A+/+
0.5 µm
SEC23A+/-
0.5 µm
SEC23A-/-
0.5 µm

The Sec31 binding site on Sar1 and Sec23



Xiping Bi, Jonathan Goldberg, Dev. Cell, 2007

Major conclusions

1. Secretion and plasma membrane assembly are physically and functionally linked through a series of obligate organelle intermediates.

2. Polypeptide translocation and vesicular traffic machinery conserved over a billion years of evolution.

3. COPII coat sorts cargo molecules by recognition of transport signals and physically deforms the ER membrane to create budded vesicles.

October 7, 2013