



Nobel Lecture
December 7, 2013

Genes and proteins that organize
the secretory pathway

Randy Schekman

Department of Molecular and Cell Biology

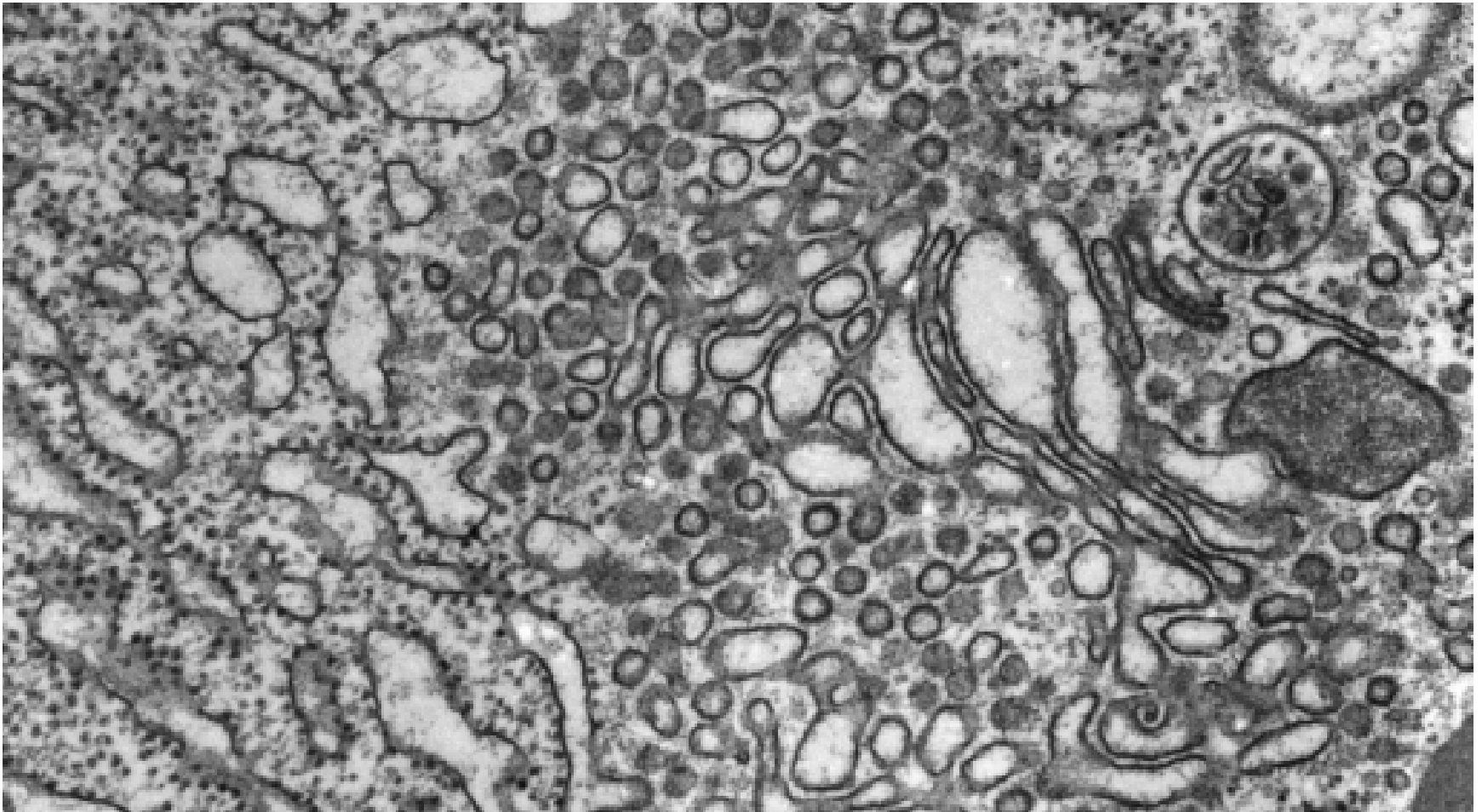
Howard Hughes Medical Institute

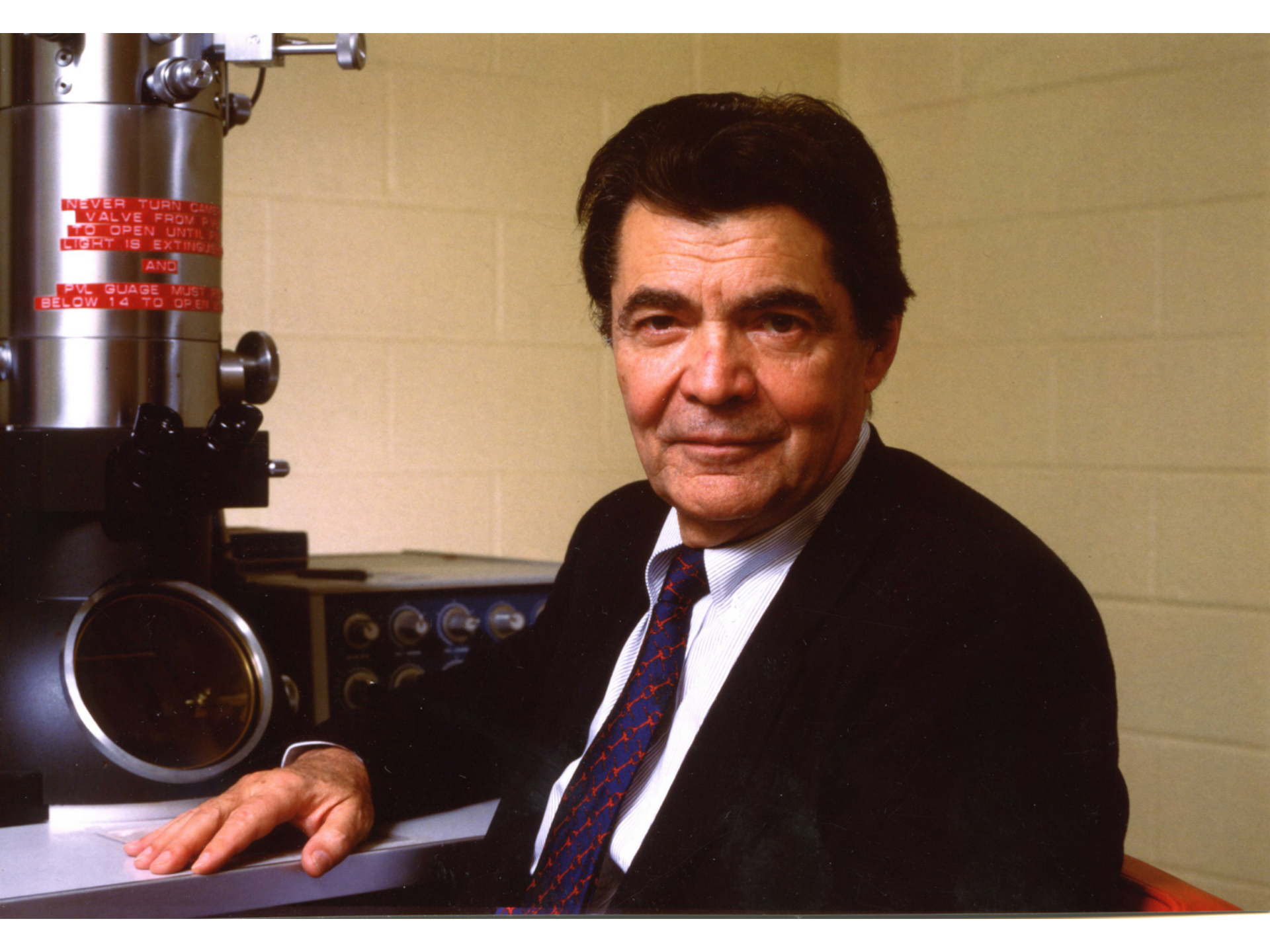
University of California, Berkeley

The image features a large, hand-drawn blue outline that resembles a cloud or a speech bubble. Inside this blue border, there are several orange-colored scribbles and shapes, including a large circle on the left, a series of parallel lines in the center, and several small circles scattered around. The text "Origins and influences" is written in a bold, blue, sans-serif font, centered within the blue border.

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

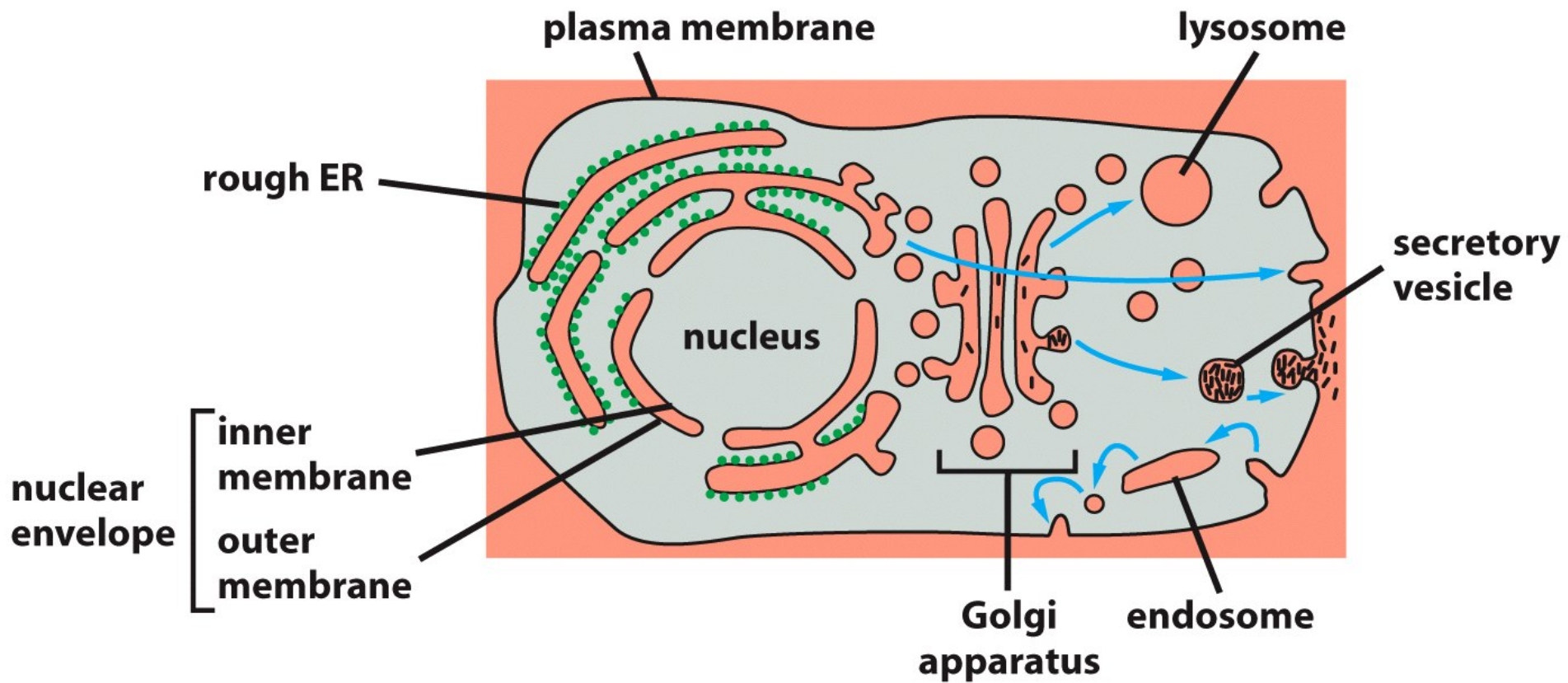
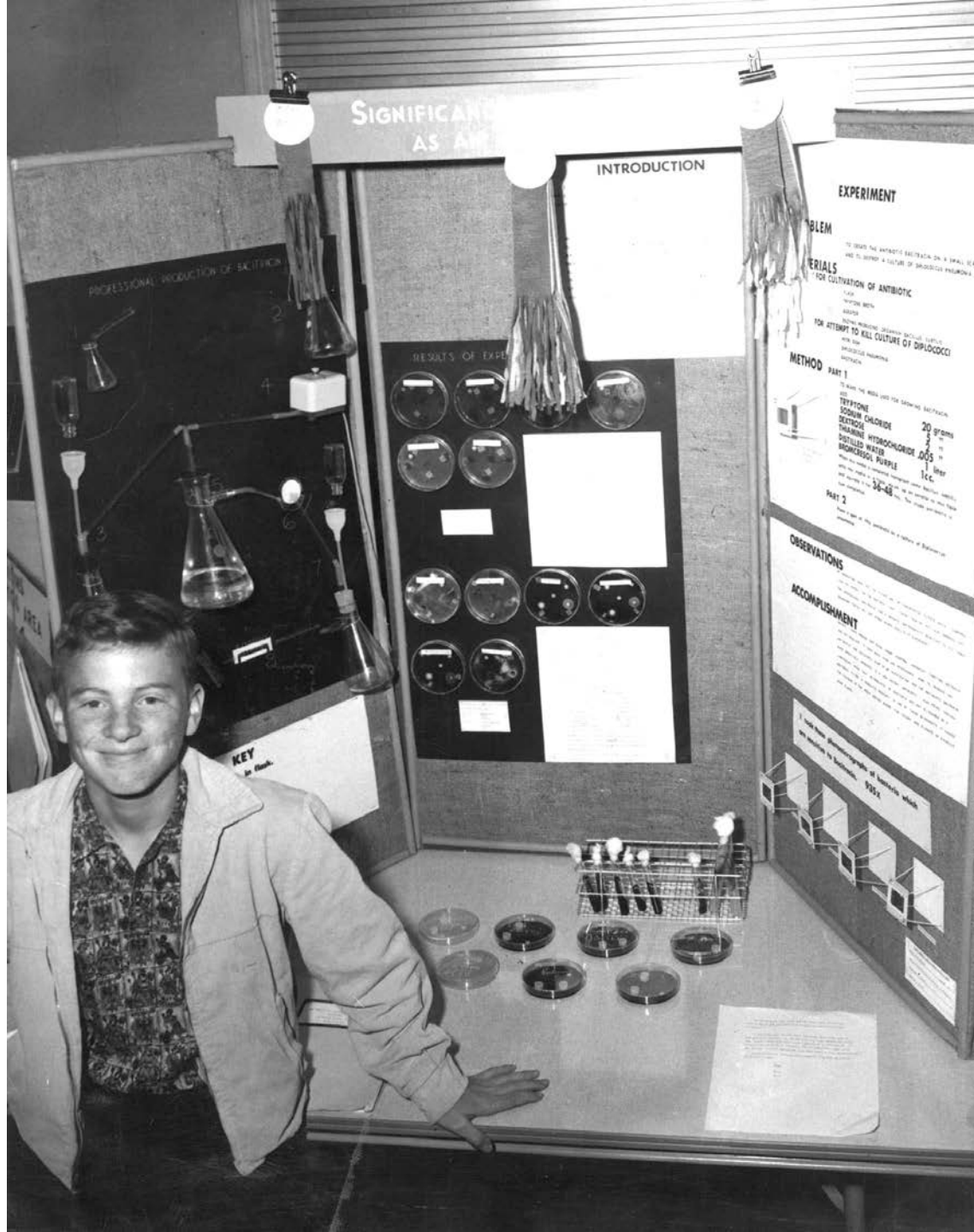


Figure 12-5 *Molecular Biology of the Cell* (© Garland Science 2008)



SIGNIFICANCE OF ANTIBIOTICS AS ANTI-ANTIBIOTICS

INTRODUCTION

EXPERIMENT

PROBLEM

TO DETERMINE THE ANTIBIOTIC EFFECTIVENESS ON A SMALL SCALE AND TO DETERMINE A CULTURE OF BACTERIAL INFECTIONS IN

MATERIALS

FOR CULTIVATION OF ANTIBIOTIC

1. LAMP

2. INOCULUM BATH

3. BODIBATH

FOR ATTEMPT TO KILL CULTURE OF DIPLODOCOS

4. BODIBATH

5. BODIBATH

6. BODIBATH

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*ENZYMATIC SYNTHESIS OF DNA, XXIII. SYNTHESIS
OF CIRCULAR REPLICATIVE FORM OF PHAGE ϕ X174 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 ϕ X174 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

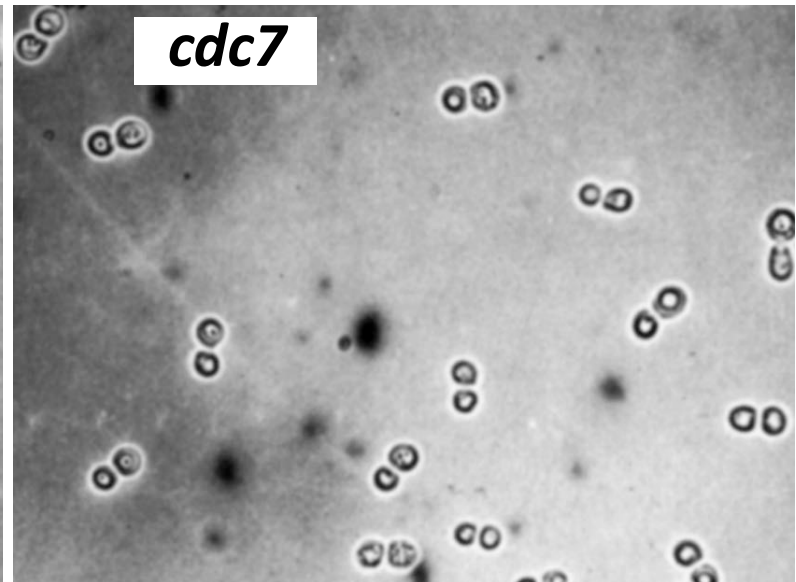
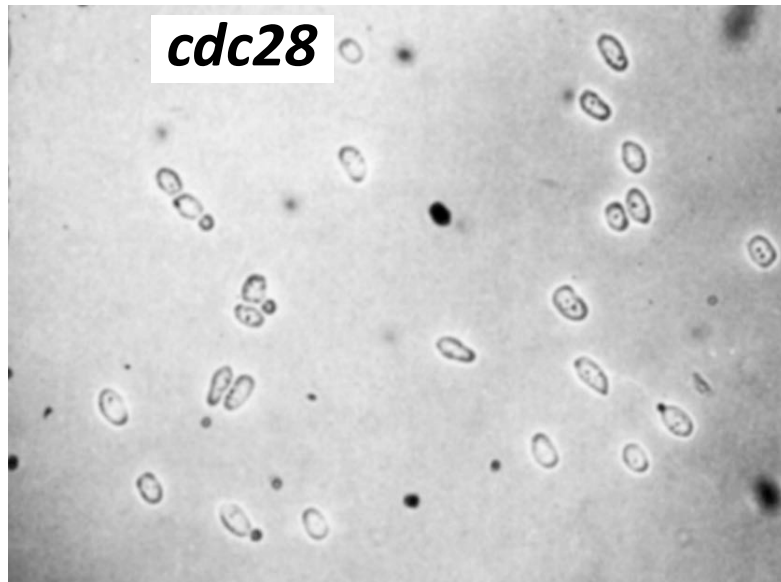
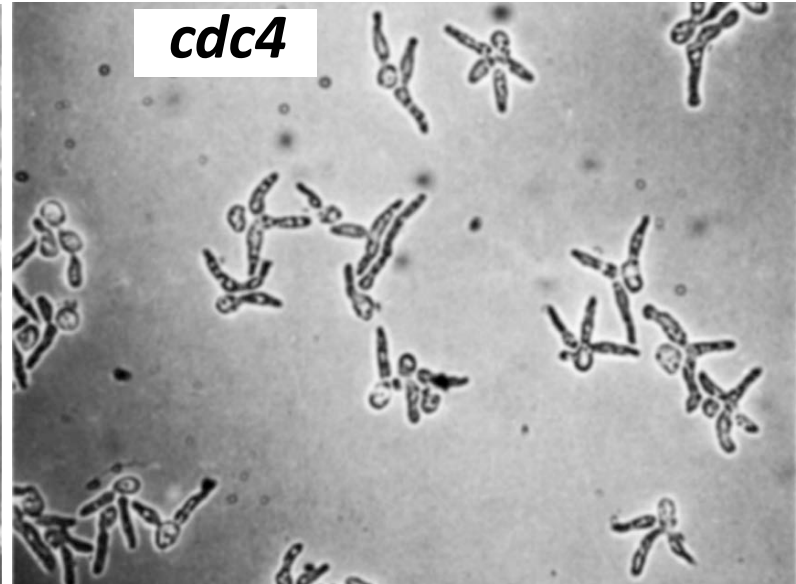
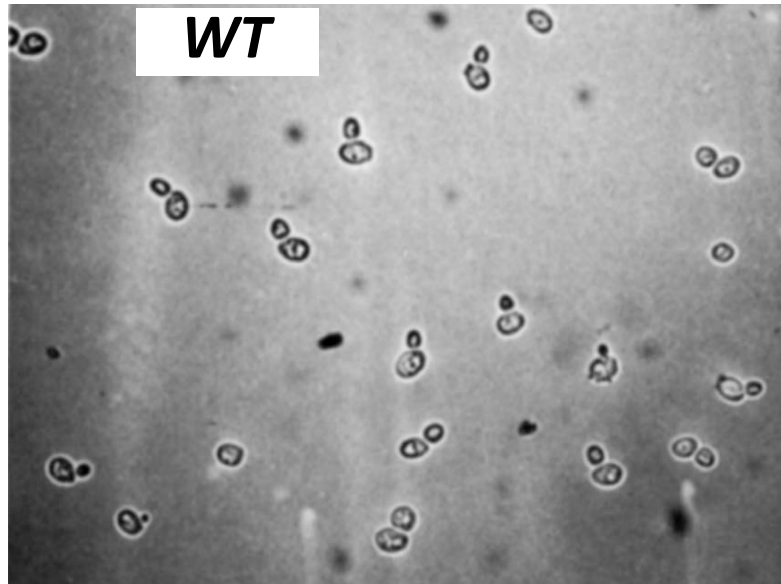
by

PAULA DE LUCIA
JOHN CAIRNS

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

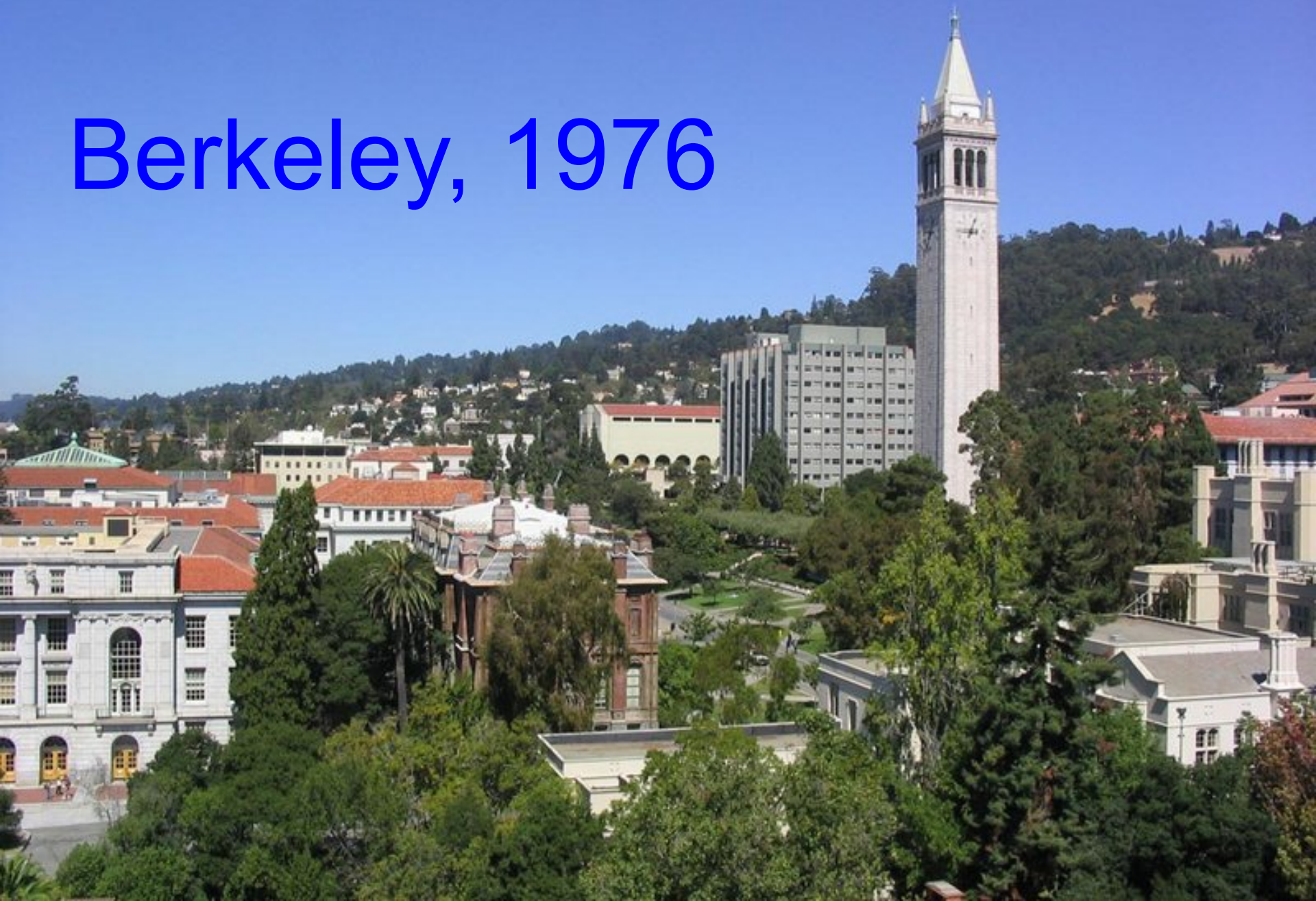
By testing indiscriminately several thousand colonies of mutagenized *E. coli*, a mutant has been isolated that on extraction proves to have less than 1 per cent of the normal level of DNA polymerase. The mutant multiplies normally but has acquired an increased sensitivity to ultraviolet light.

Uniform terminal morphology of temperature-sensitive cell division cycle mutants

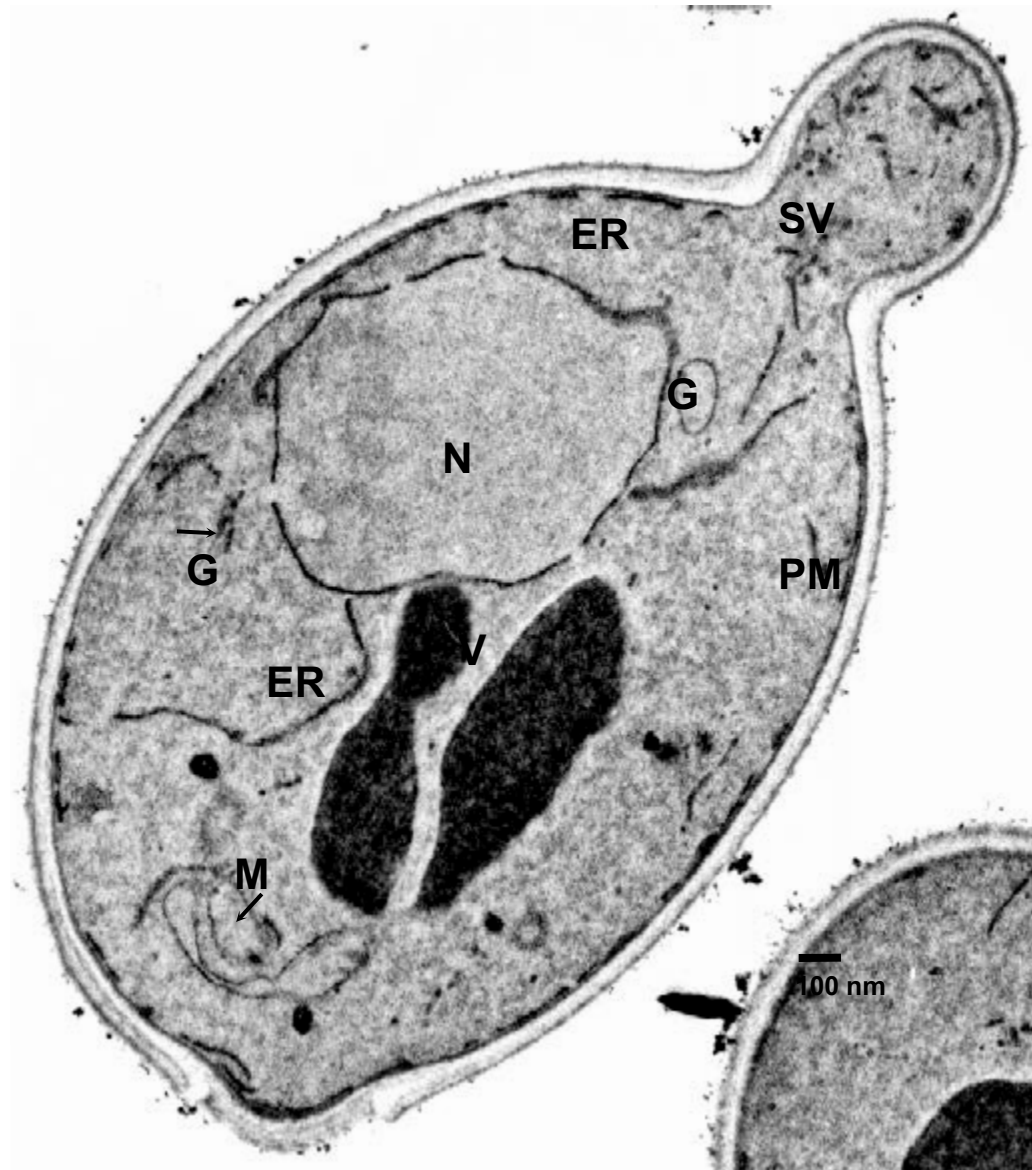


Adapted from: Hereford LM, Hartwell LH (1974) *J. Mol. Biol.* **84**: 445-461.

Berkeley, 1976

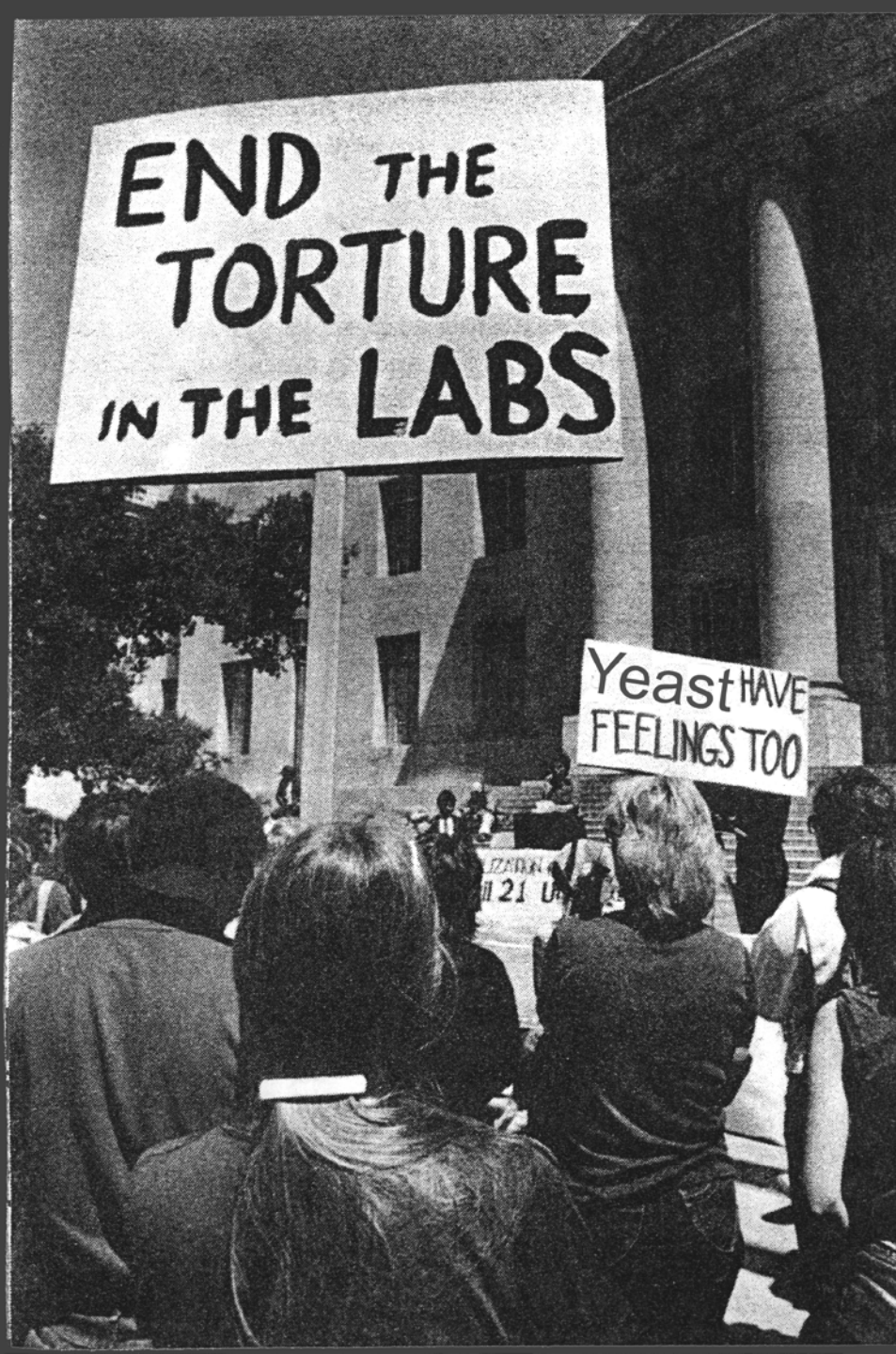


Yeast secretory organelles

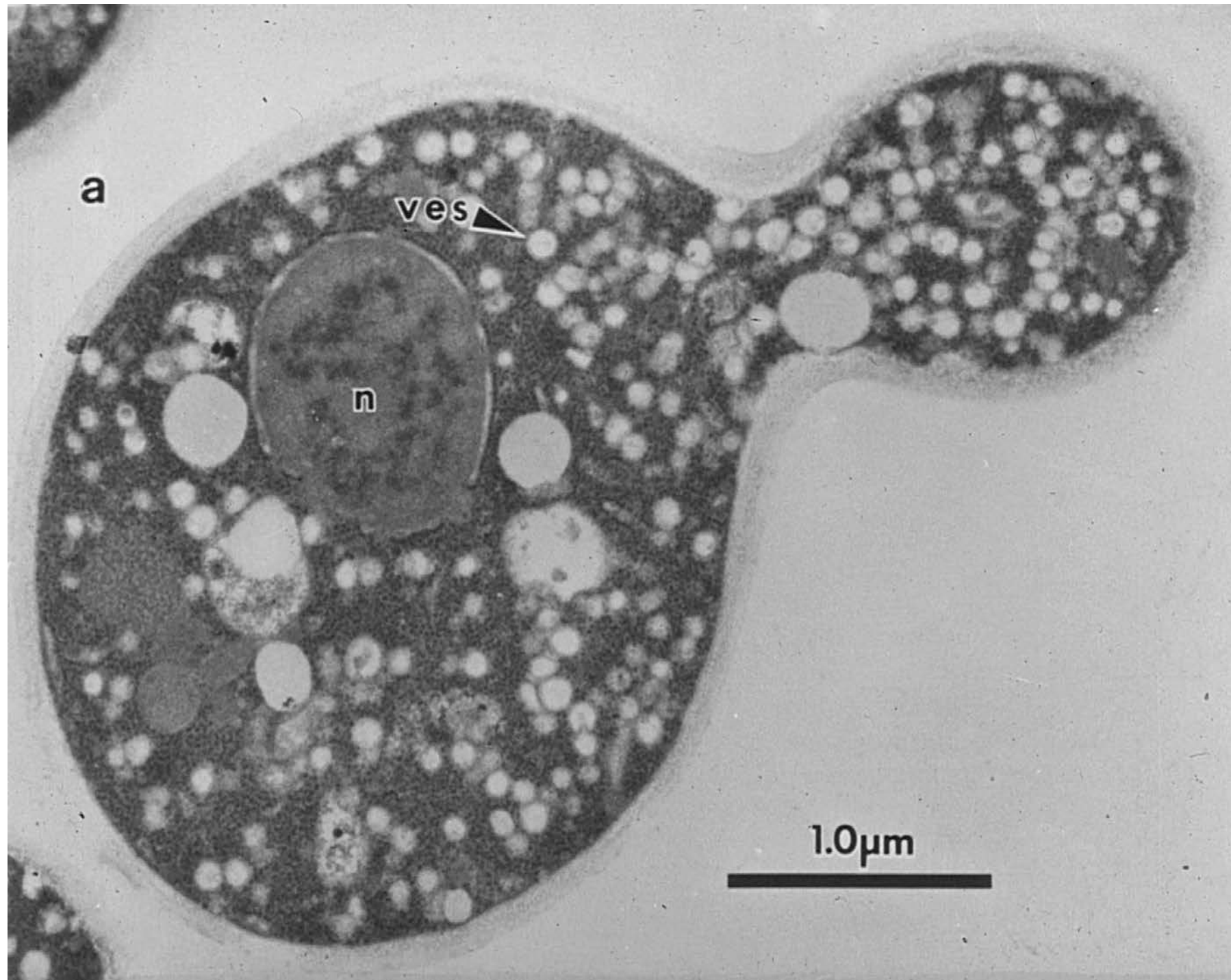


END THE
TORTURE
IN THE LABS

Yeast HAVE
FEELINGS TOO







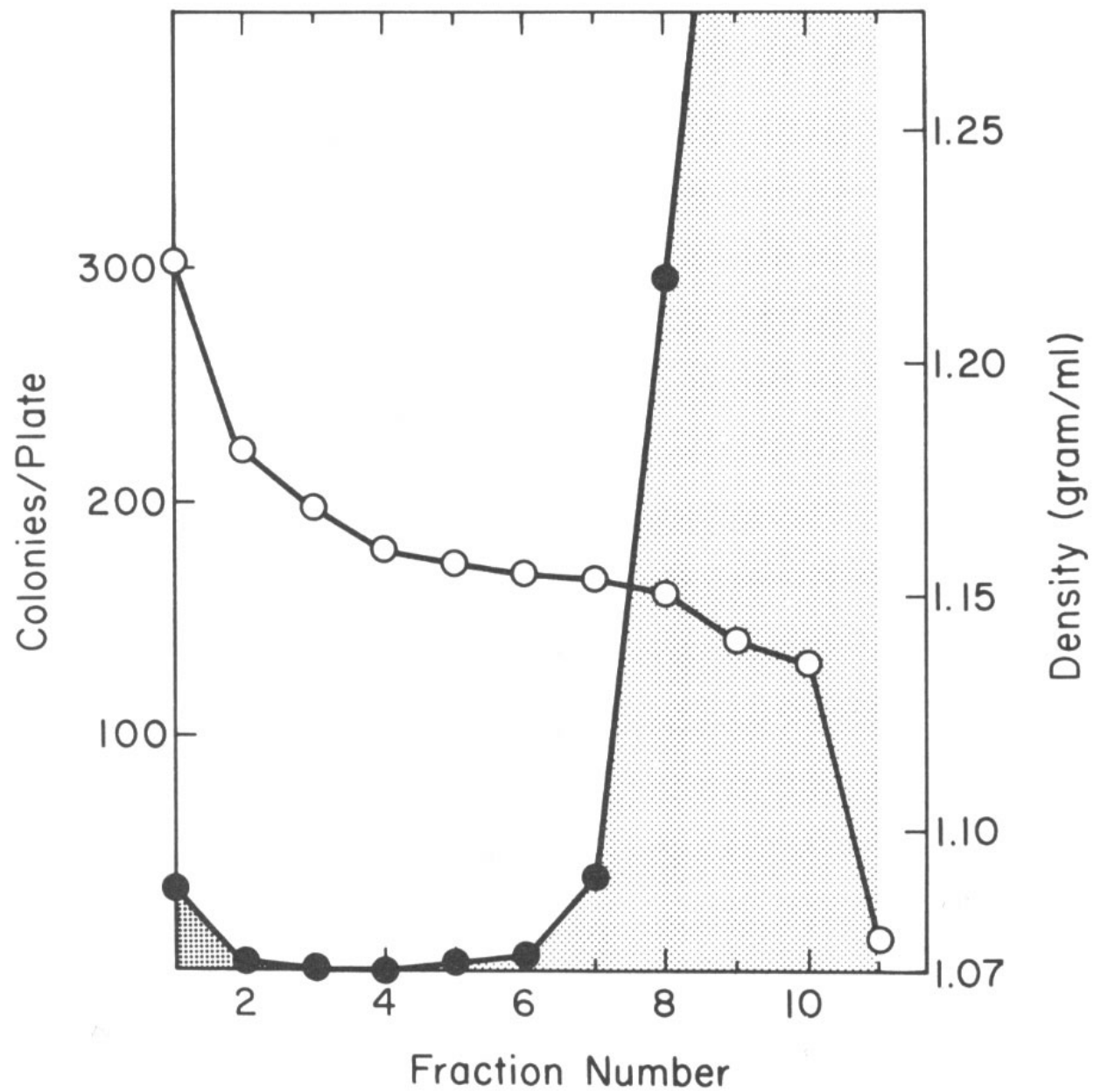
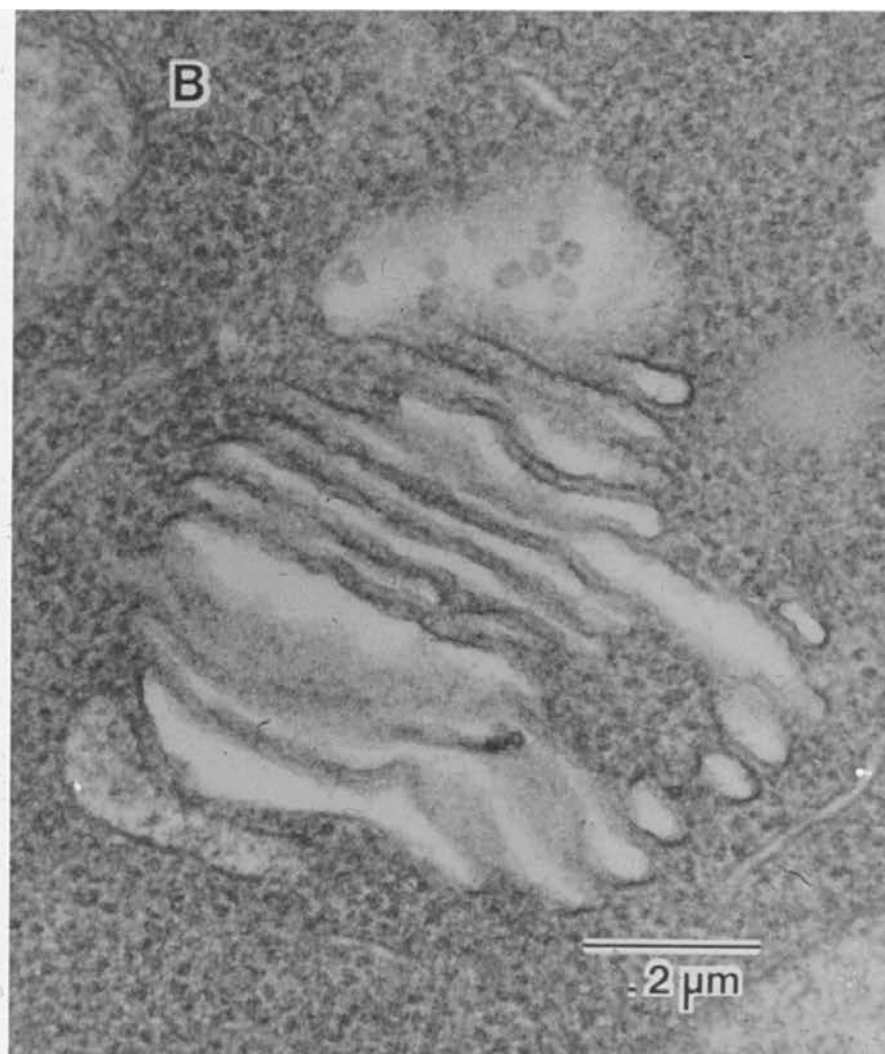
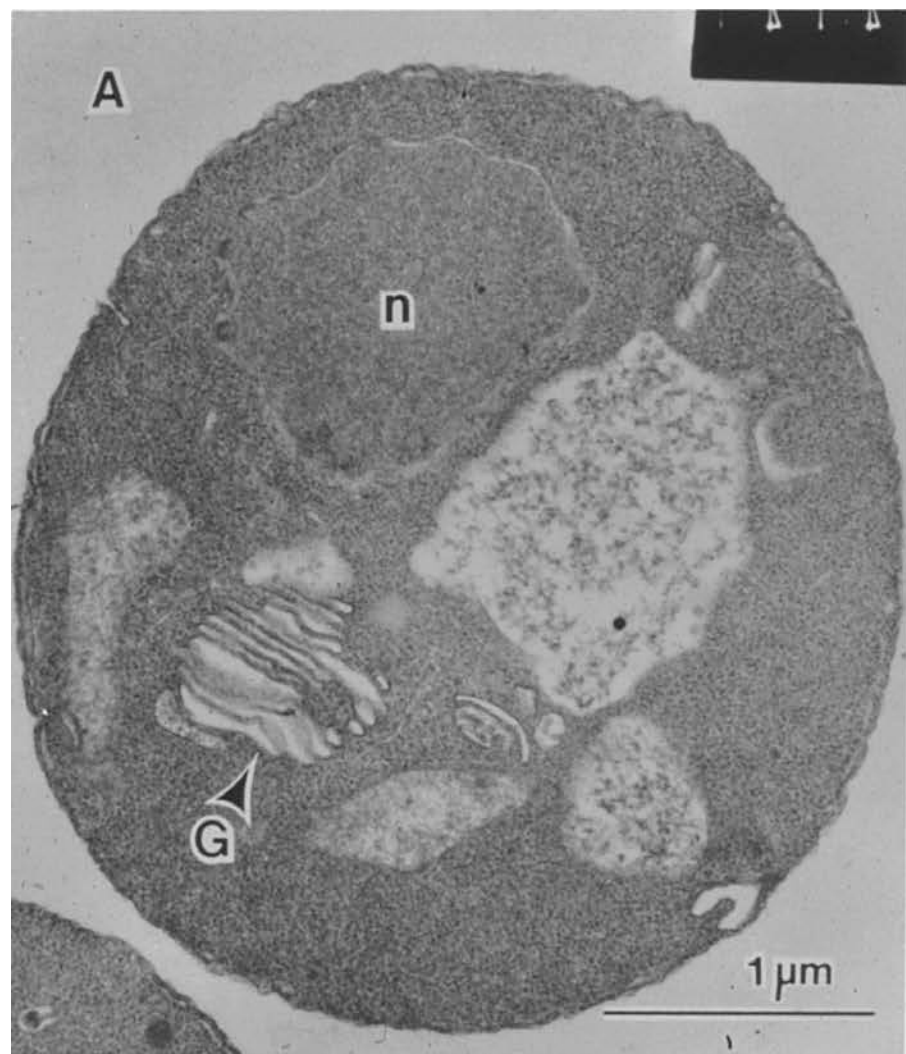
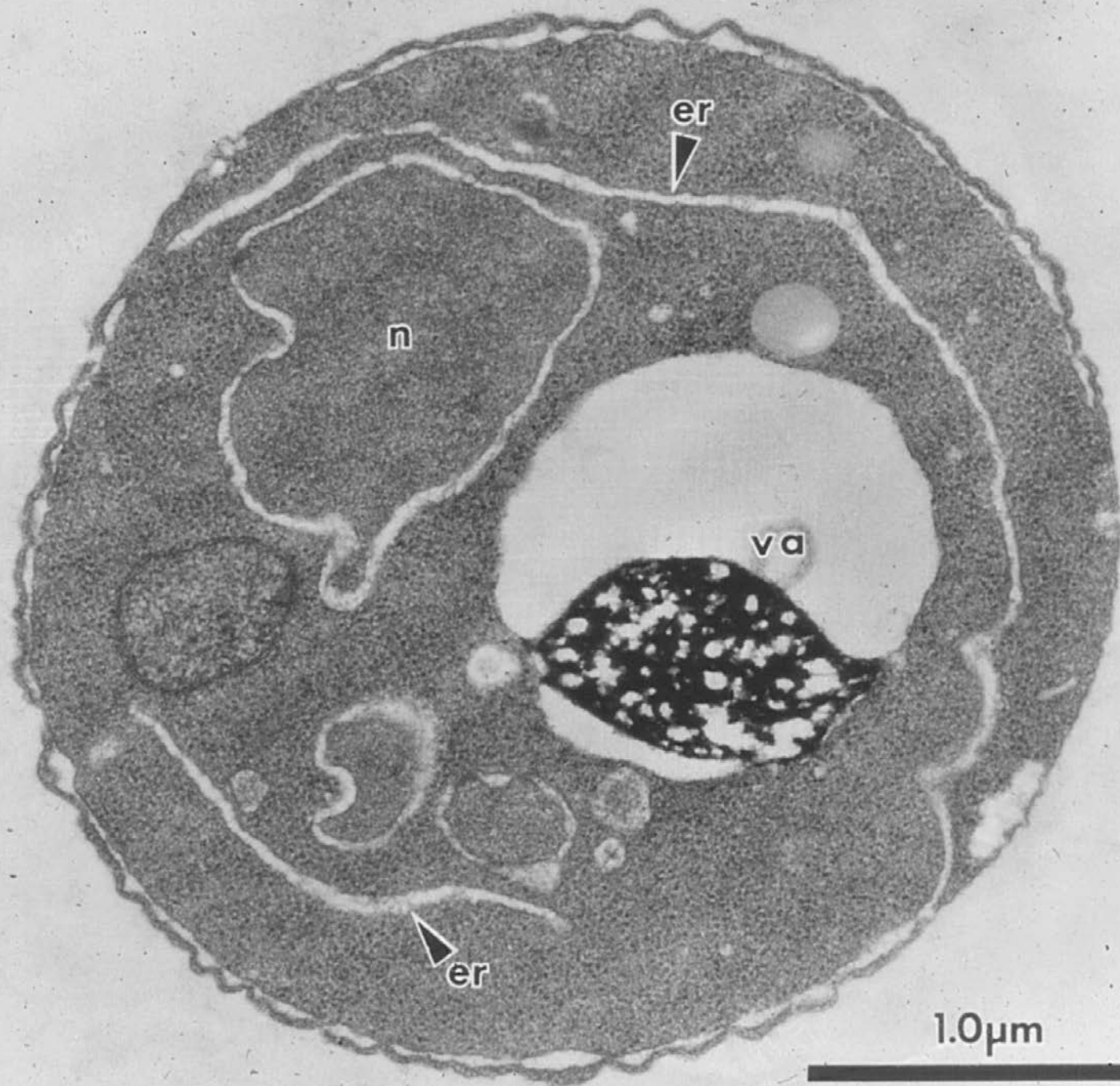


Figure 1. Density Gradient Separation of sec1-1 and X2180 Cells

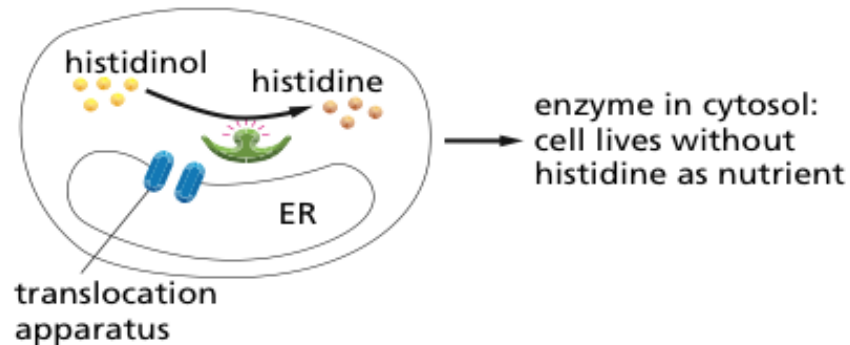






GENETIC APPROACHES FOR STUDYING THE MECHANISM OF PROTEIN TRANSLOCATION

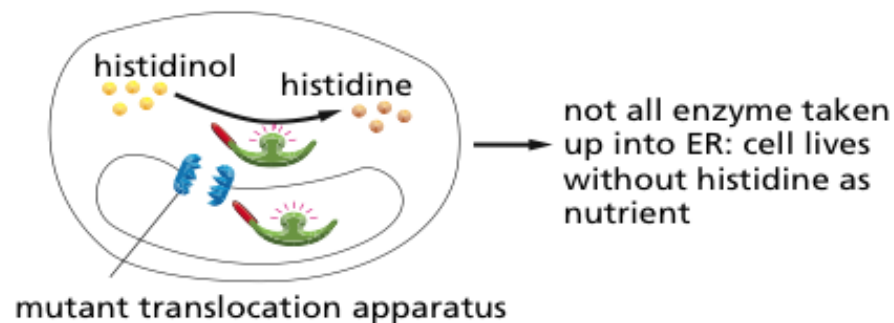
wild-type yeast cell



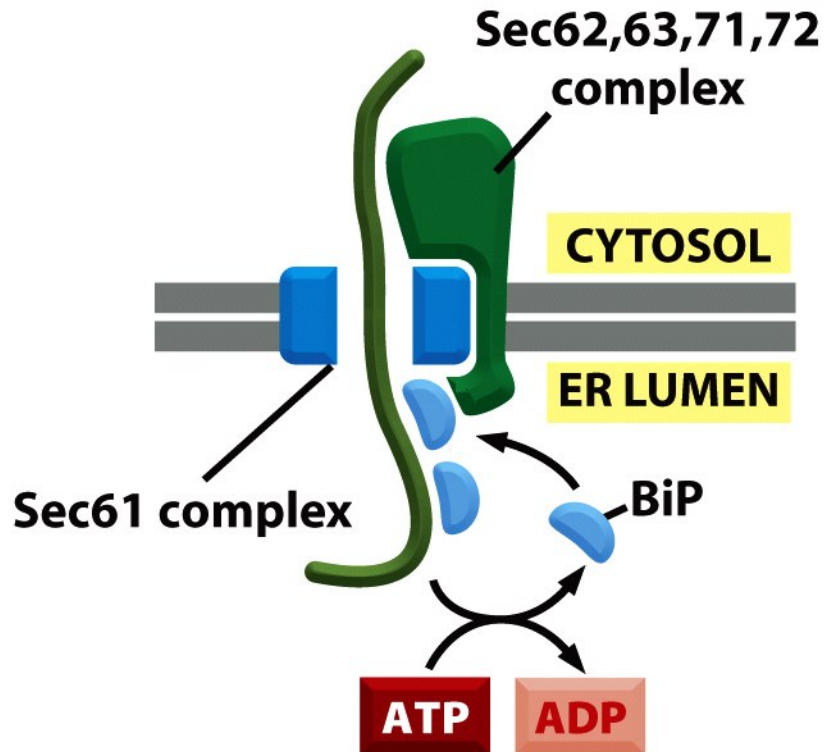
engineered yeast cell



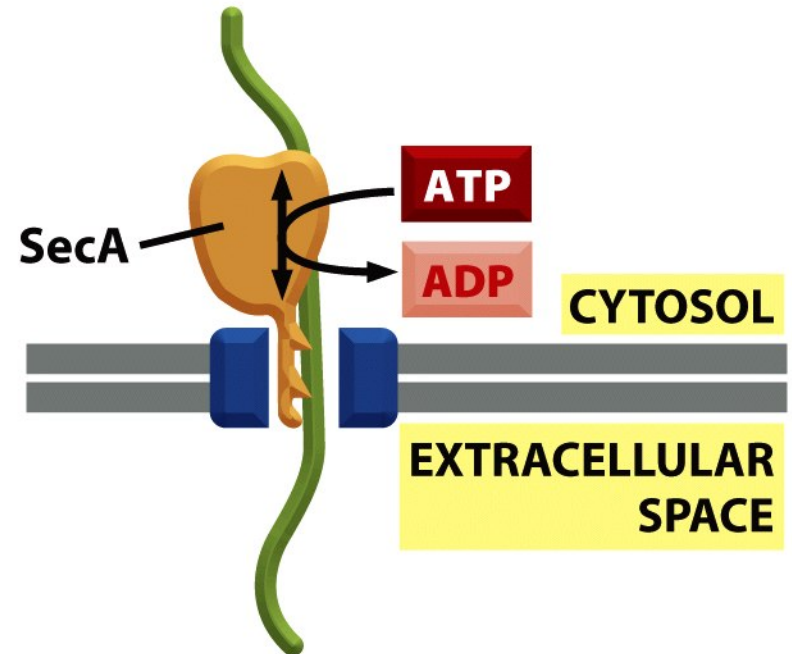
mutant engineered cell



POST-TRANSLATIONAL TRANSLOCATION

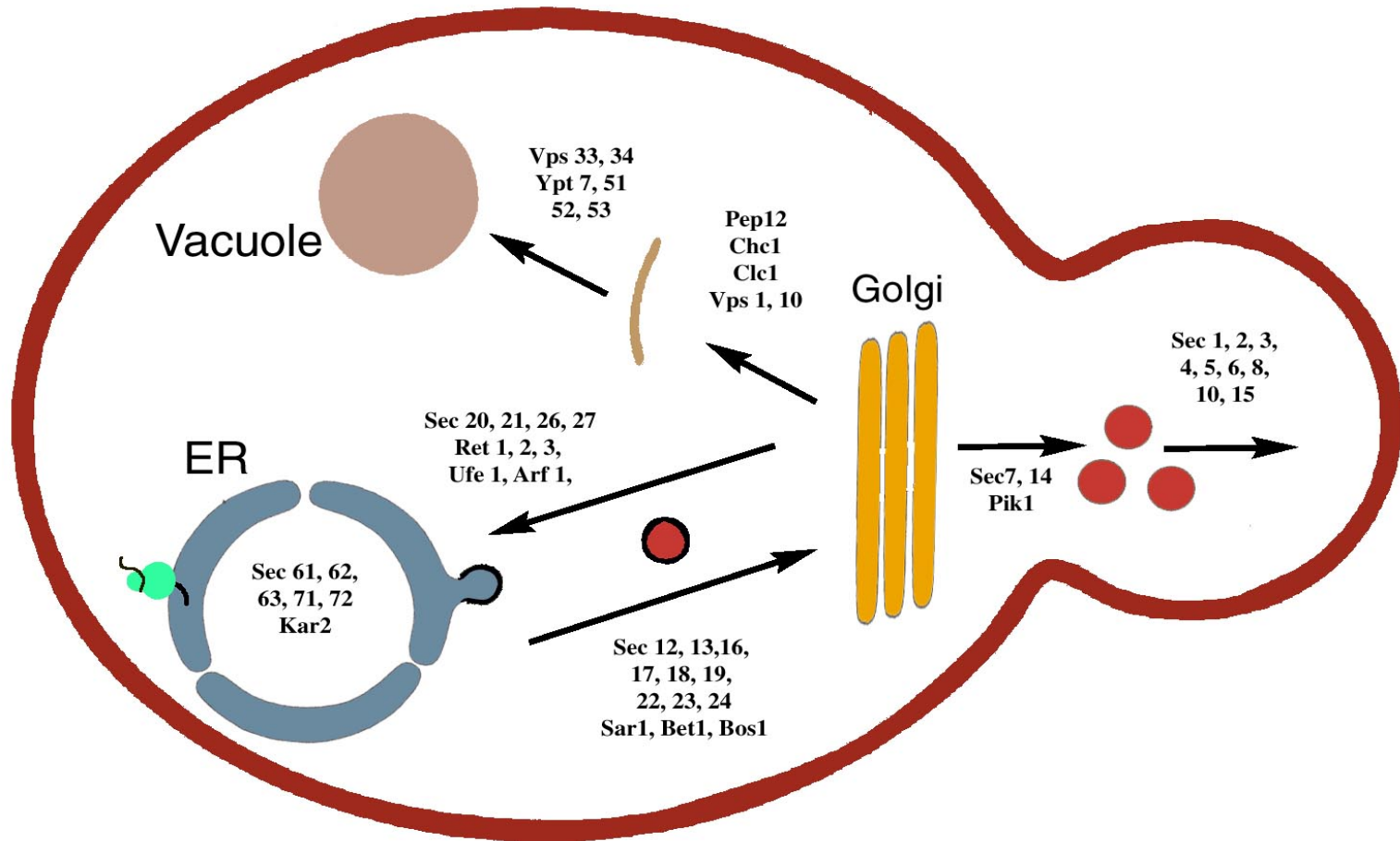


EUCARYOTES



BACTERIA

Yeast secretory pathway



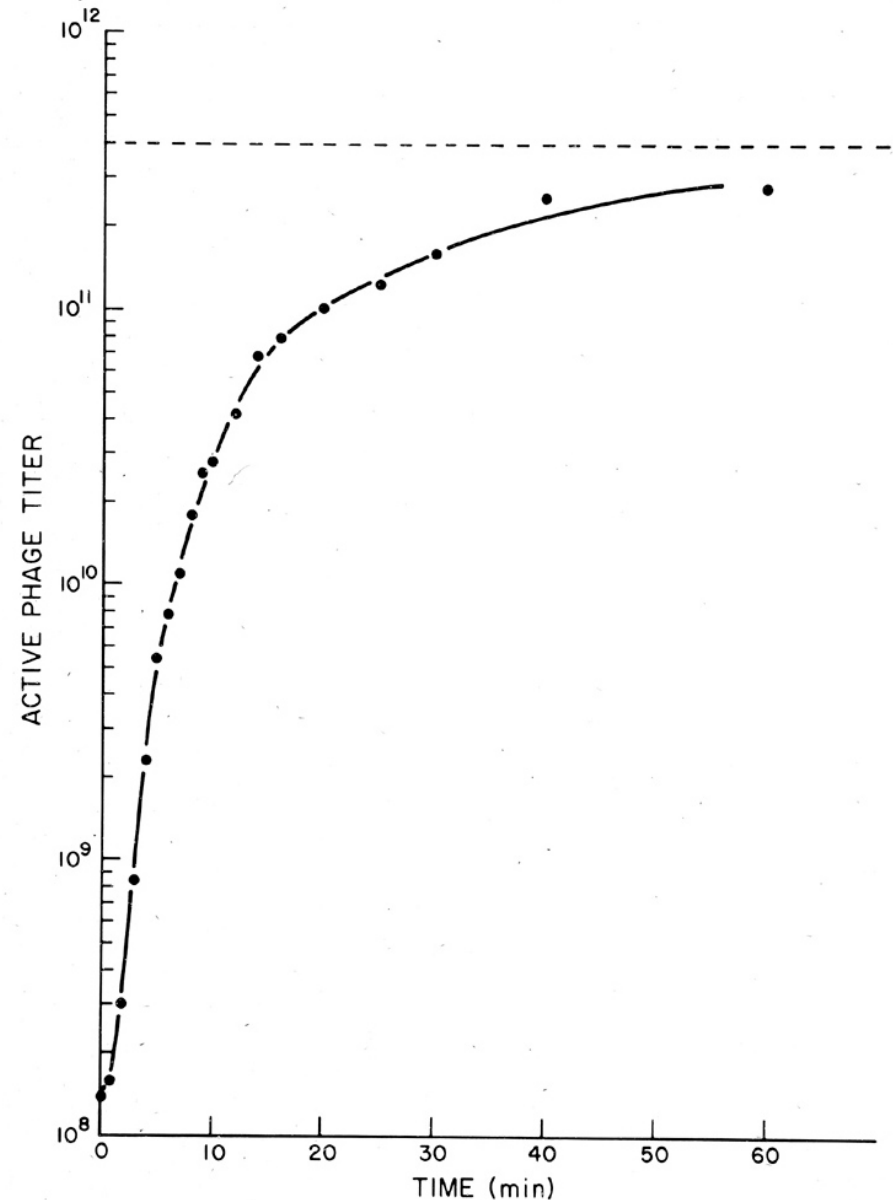
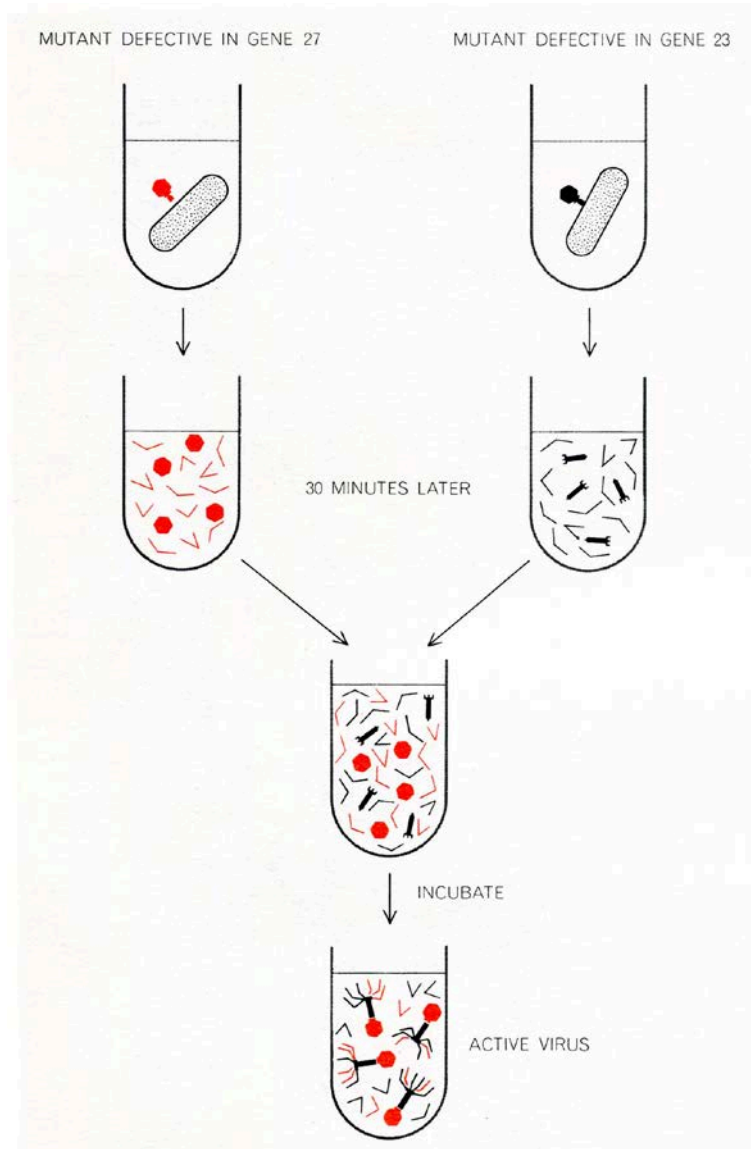


Union of genetics and biochemistry

William Wood and Robert Edgar, 1965

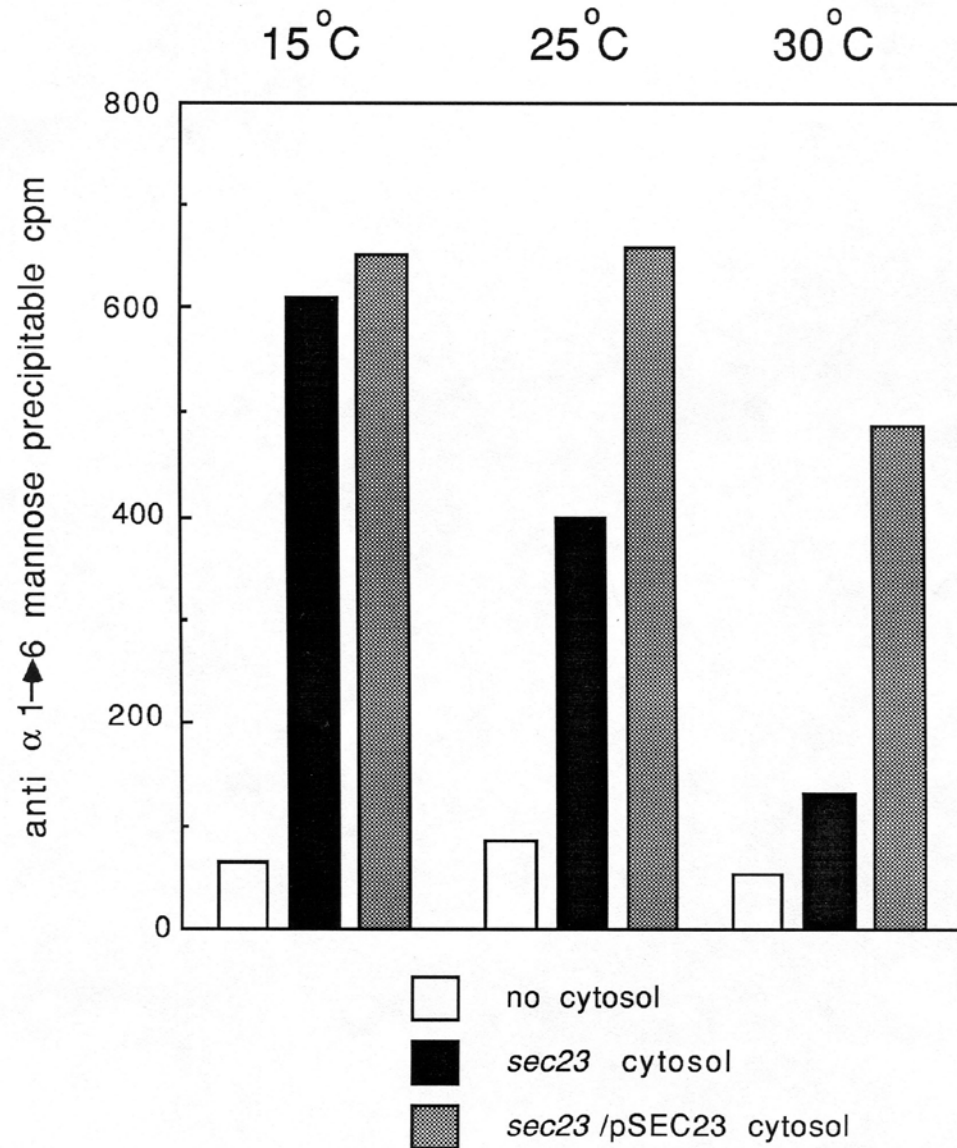


Biochemical complementation in lysates of mutant bacteriophage infected cells

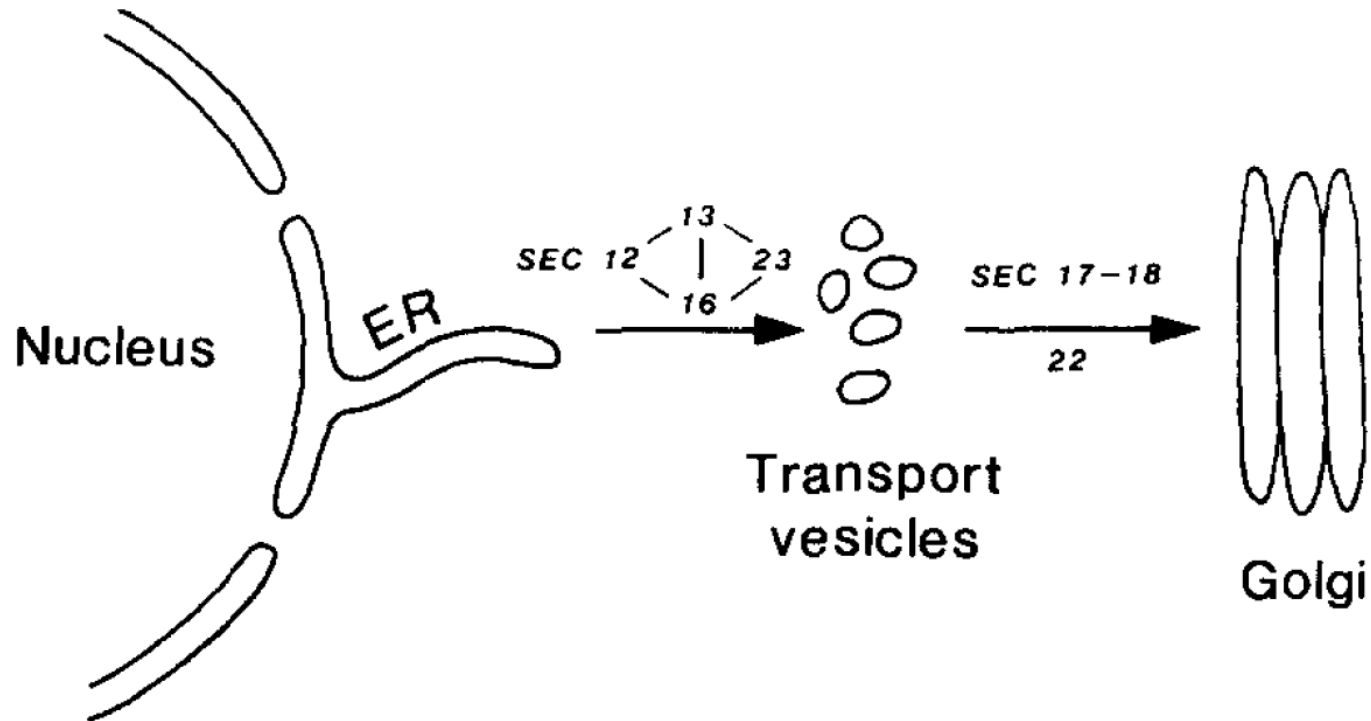




Mutant *sec23* complementation in vitro



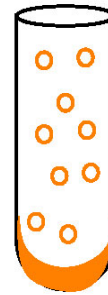
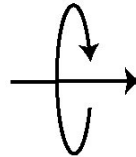
SEC genes required for budding and targeting vesicles from the ER to the Golgi



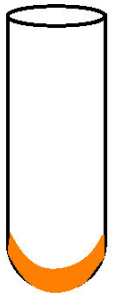
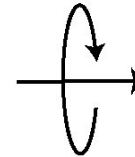
Vesicle budding assay



spin 13,000g



spin 100,000g



**Donor membranes
(microsomes or
semi-intact cells)**

**Donor membranes
+ COPII proteins
+ nucleotide**

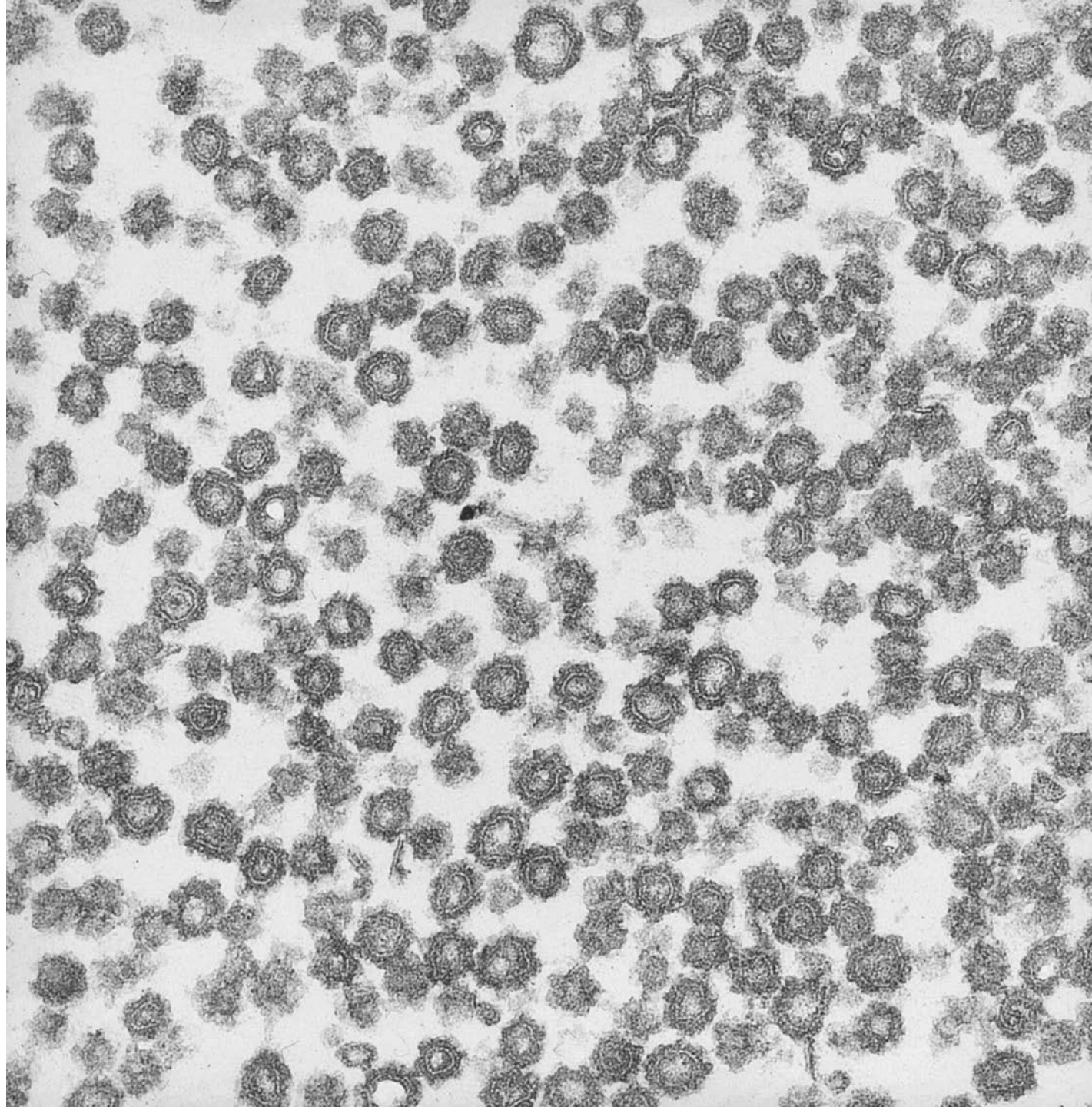
**Vesicles in
supernatant**

**Vesicles in
pellet**



The diagram illustrates the sorting of proteins at the endoplasmic reticulum (ER) exit site. A large, light blue, spherical structure with a cage-like surface, representing a COPII coat, is shown in the upper left. It is surrounded by various small, colorful protein molecules (orange, yellow, green, blue, and grey). In the center, a grey, tubular structure represents the ERGIC (Endoplasmic Reticulum-Golgi Intermediate Compartment). A COPII coat is shown budding from the ERGIC, with several small protein molecules being packaged into it. To the right, a yellow, textured structure represents the Golgi apparatus. A COPII coat is shown budding from the ERGIC, with several small protein molecules being packaged into it. The text "COPII sorts proteins at the endoplasmic reticulum" is overlaid in the center in blue.

**COPII sorts proteins
at the endoplasmic
reticulum**





The Players...

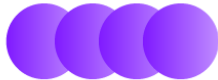
COPII Subunits:



Sar1p



Sec23/24p

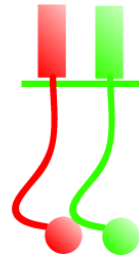


Sec13/31p

Others:



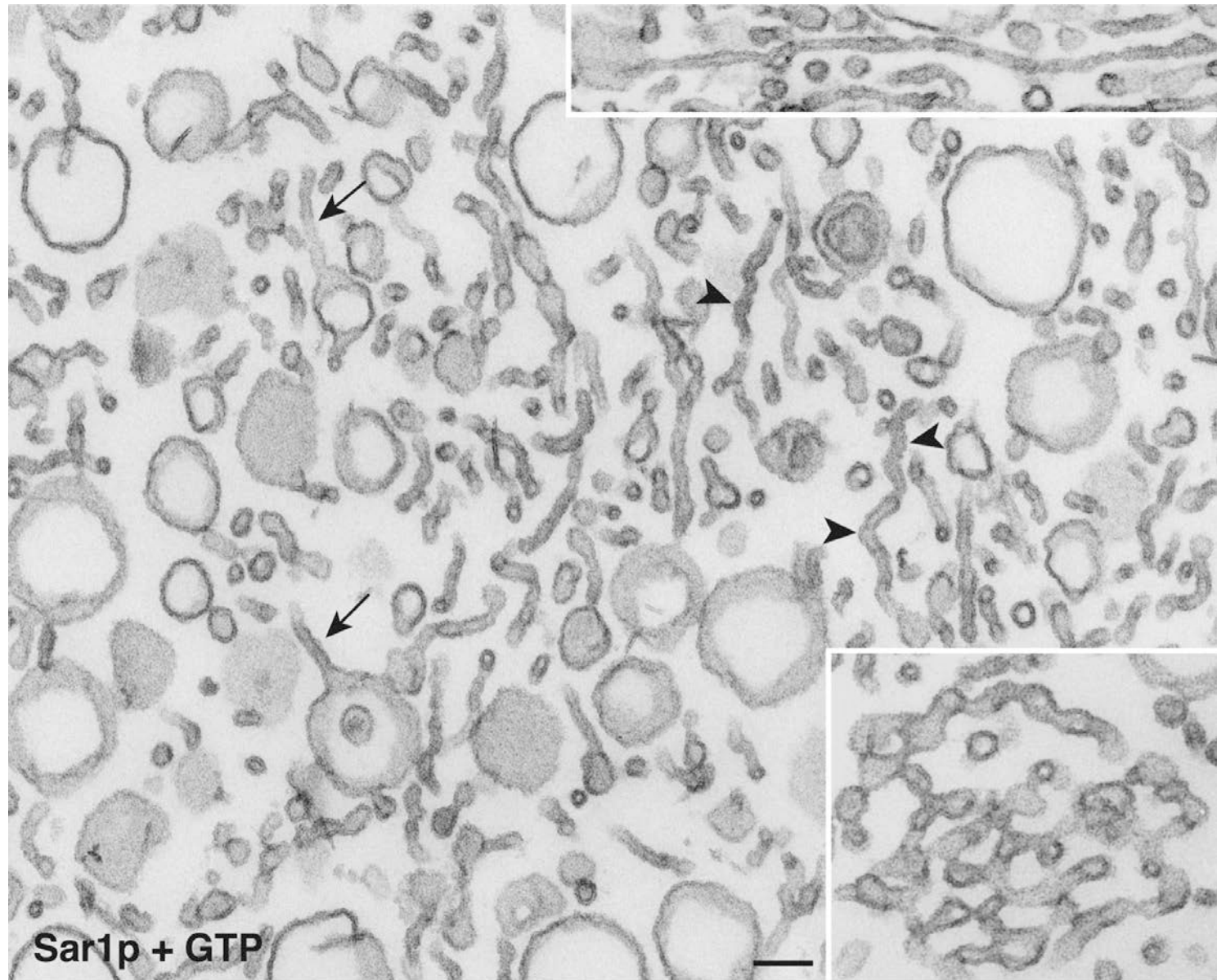
Sec12p



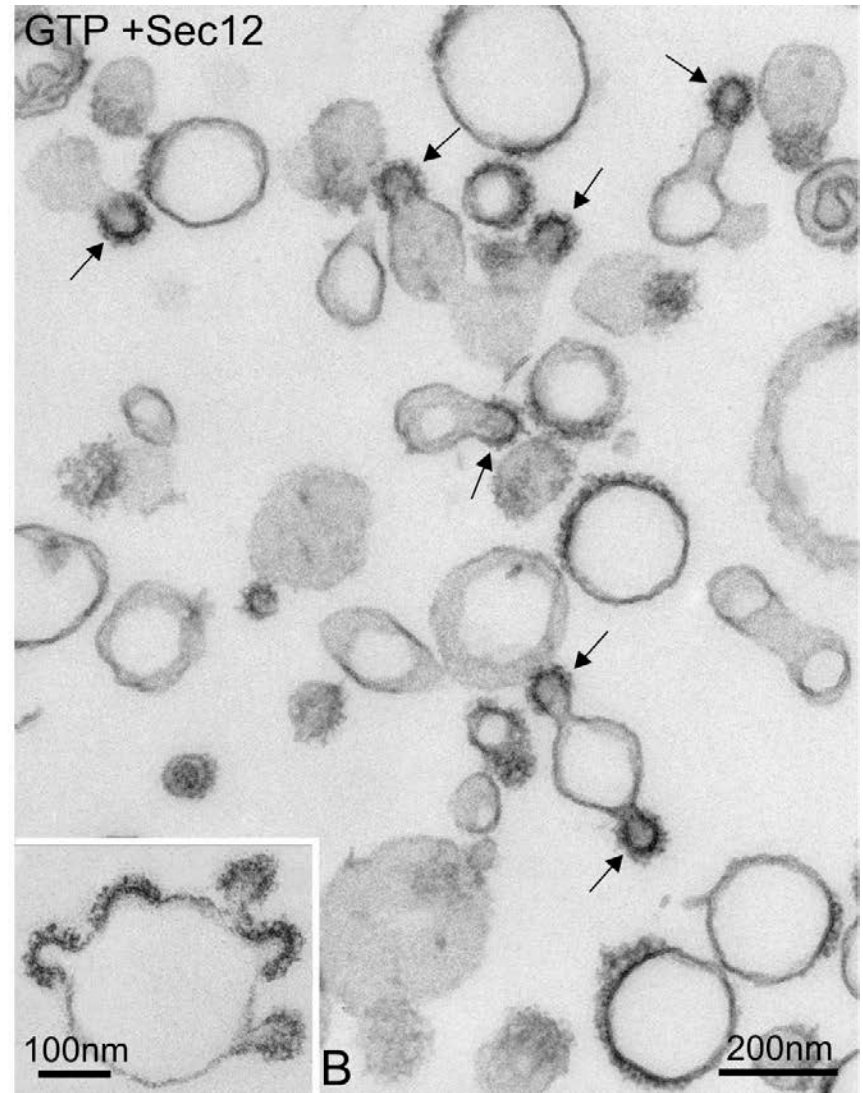
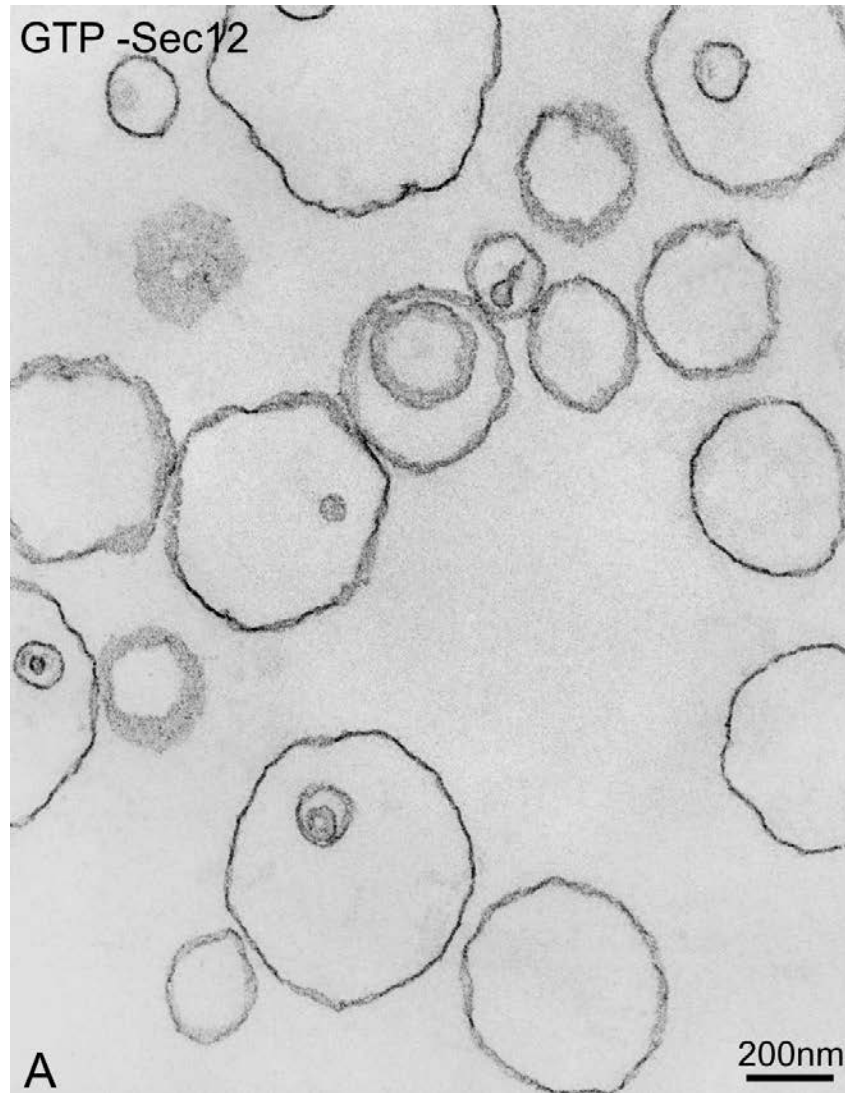
Cargo Molecules

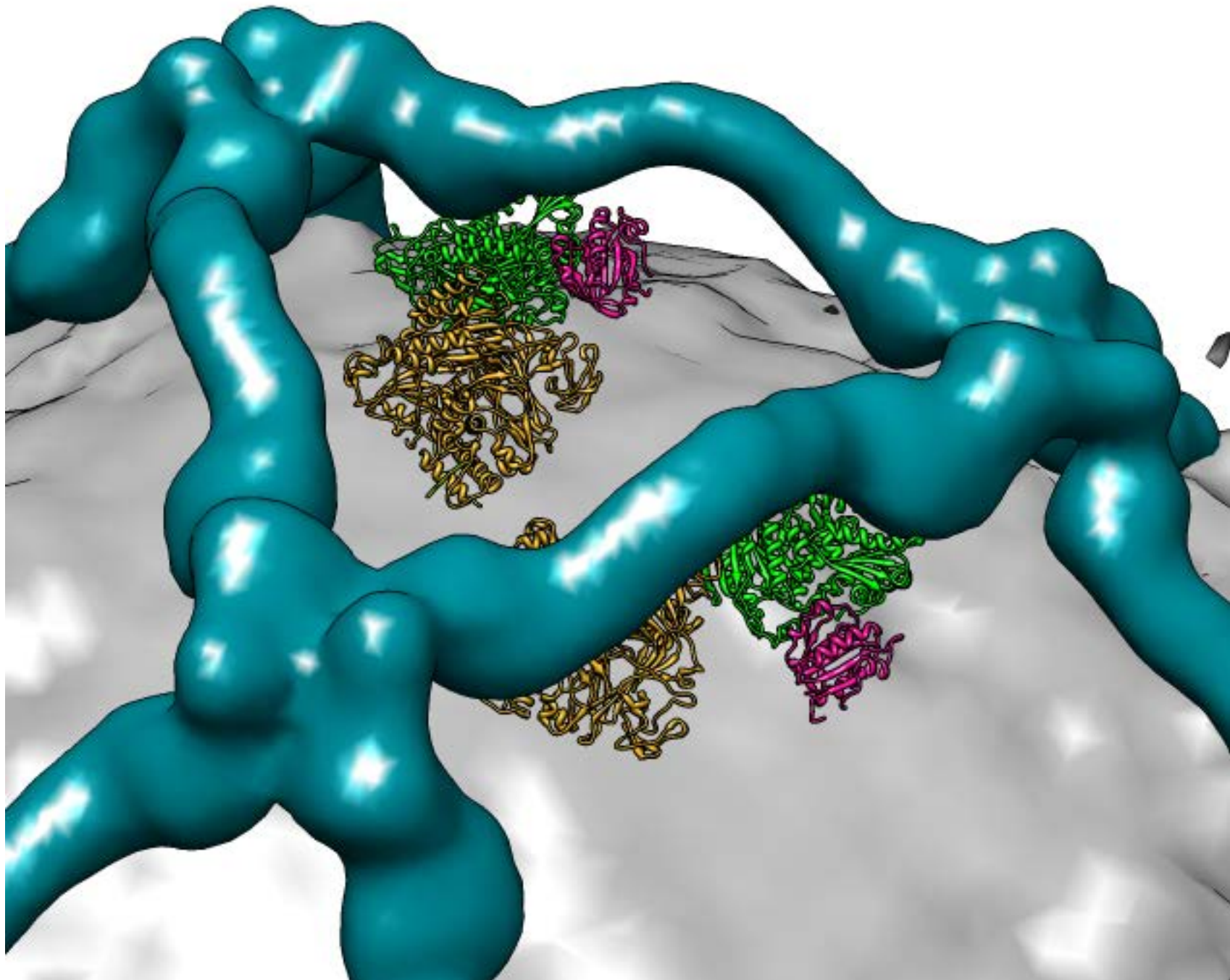


Sar1p deforms membranes in a nucleotide-dependent manner



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

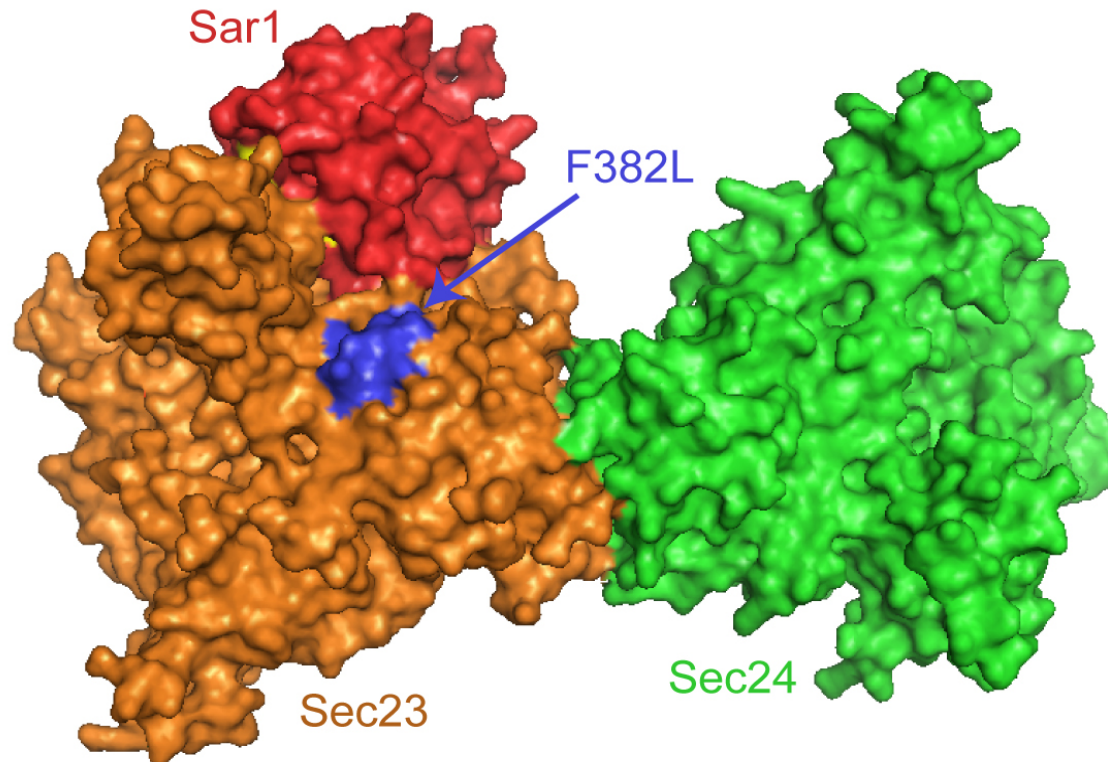
Dietary fat is an important source of nutrition. Here we identify eight mutations in *SARA2* that are associated with three severe disorders of fat malabsorption. The Sar1 family of proteins initiates the intracellular transport of proteins in COPII (coat protein)-coated vesicles. Our data suggest that 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*

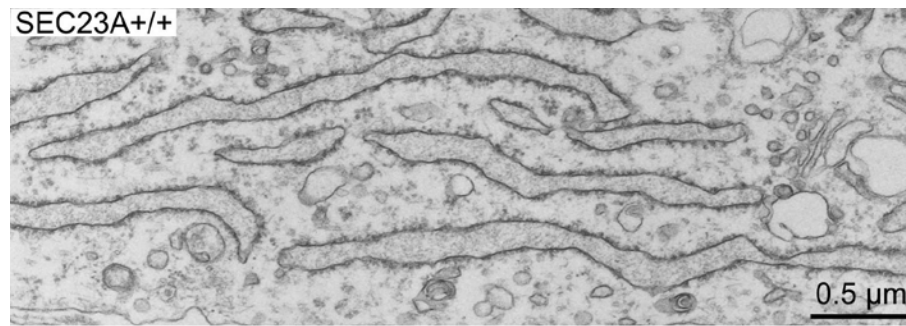


CLSD mutation: Alignment with yeast sequence and structure

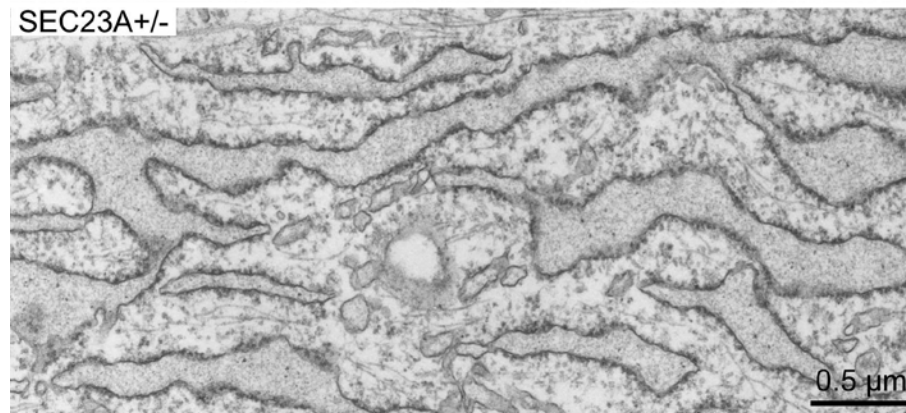
SEC23A	TGGYMVMGDSFNTSL F KQTFQRVFTKDMHGQFKMGF
SEC23B	TGGYMVMGDSFNTSL F KQTFQRIFTKDFNGDFRMAF
Sec23p	TGGVLLLTDAFSTAI F KQSYLRLFAKDEEGYLKMAF



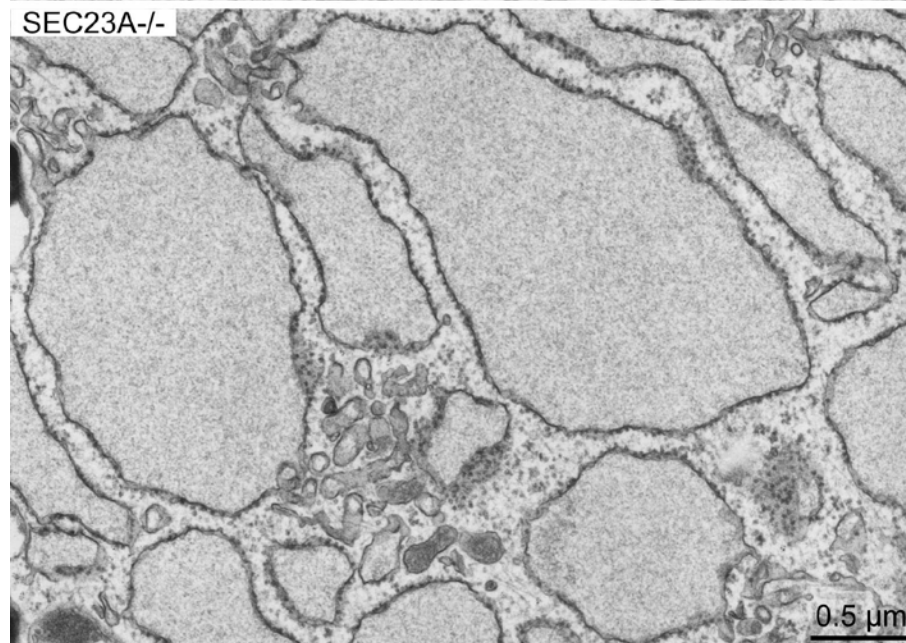
SEC23A^{+/+}



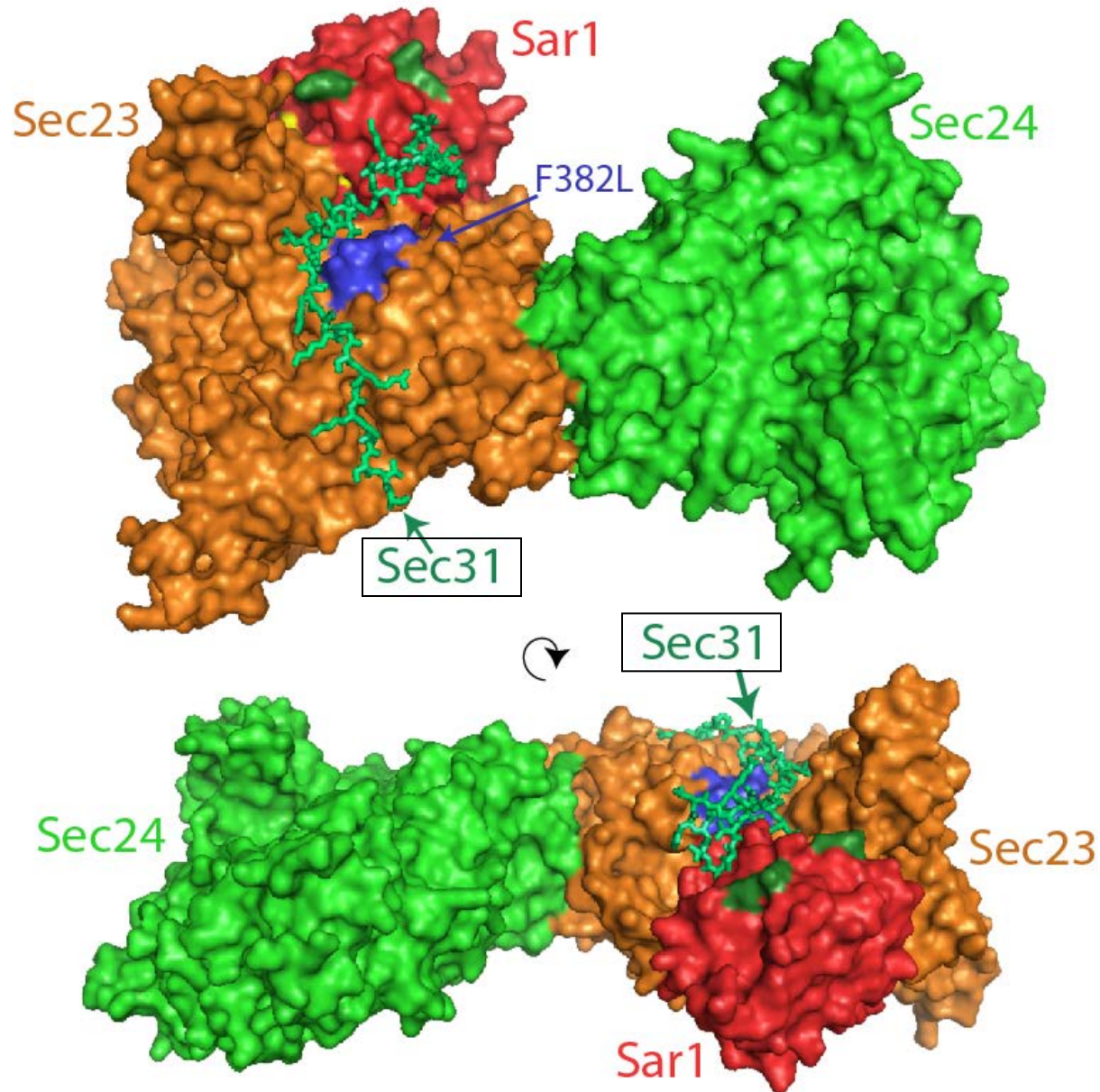
SEC23A^{+/-}



SEC23A^{-/-}



The Sec31 binding site on Sar1 and Sec23



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

