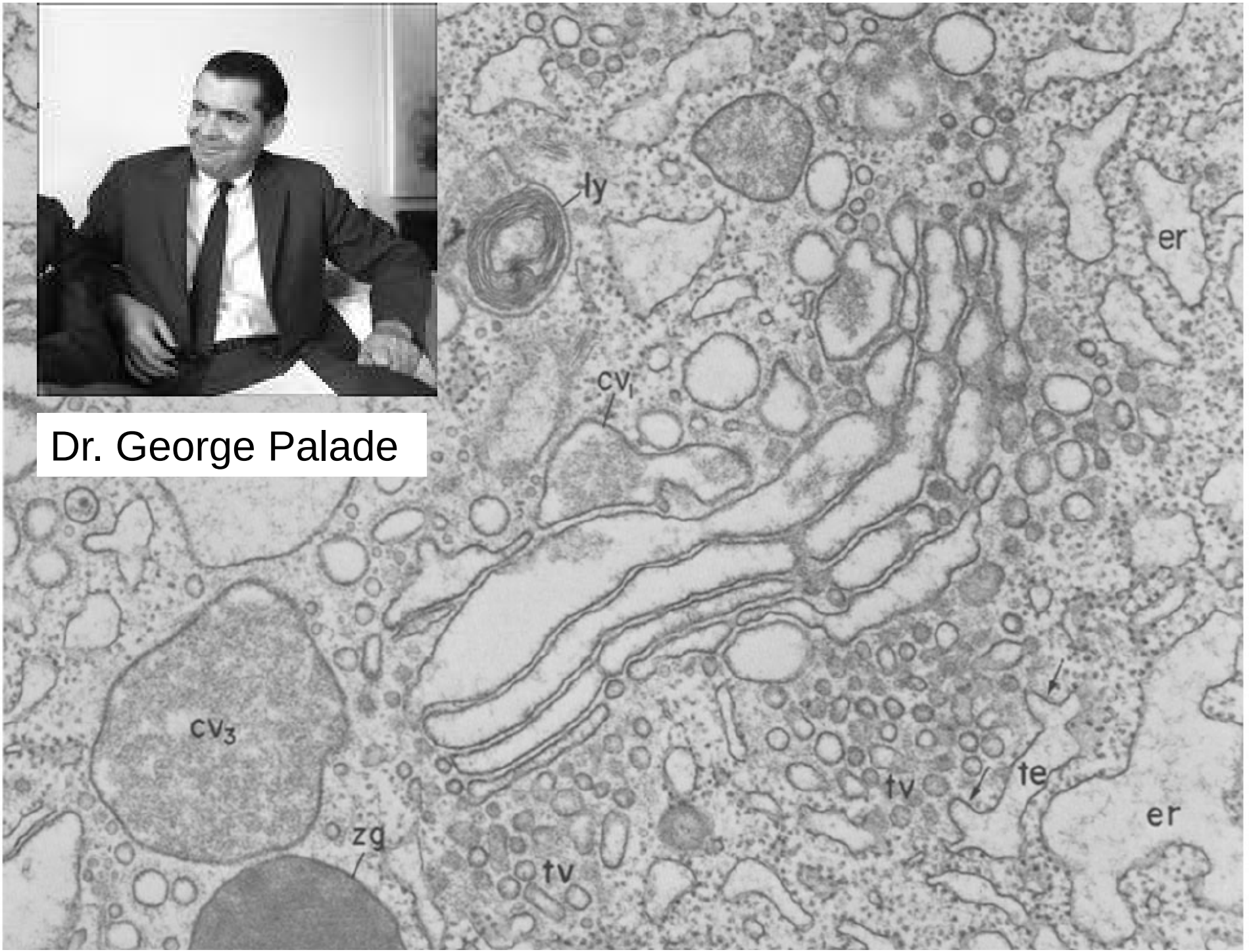
Membrane Traffic in the Secretory Pathway

Suzanne Pfeffer


Dr. George Palade
ly
cv1
er
cv3
zg
tv
tv
te
er

3D Tomogram of the Golgi

Insulin secreting HIT cell: rapid frozen, freeze substituted, cut into 400nm sections

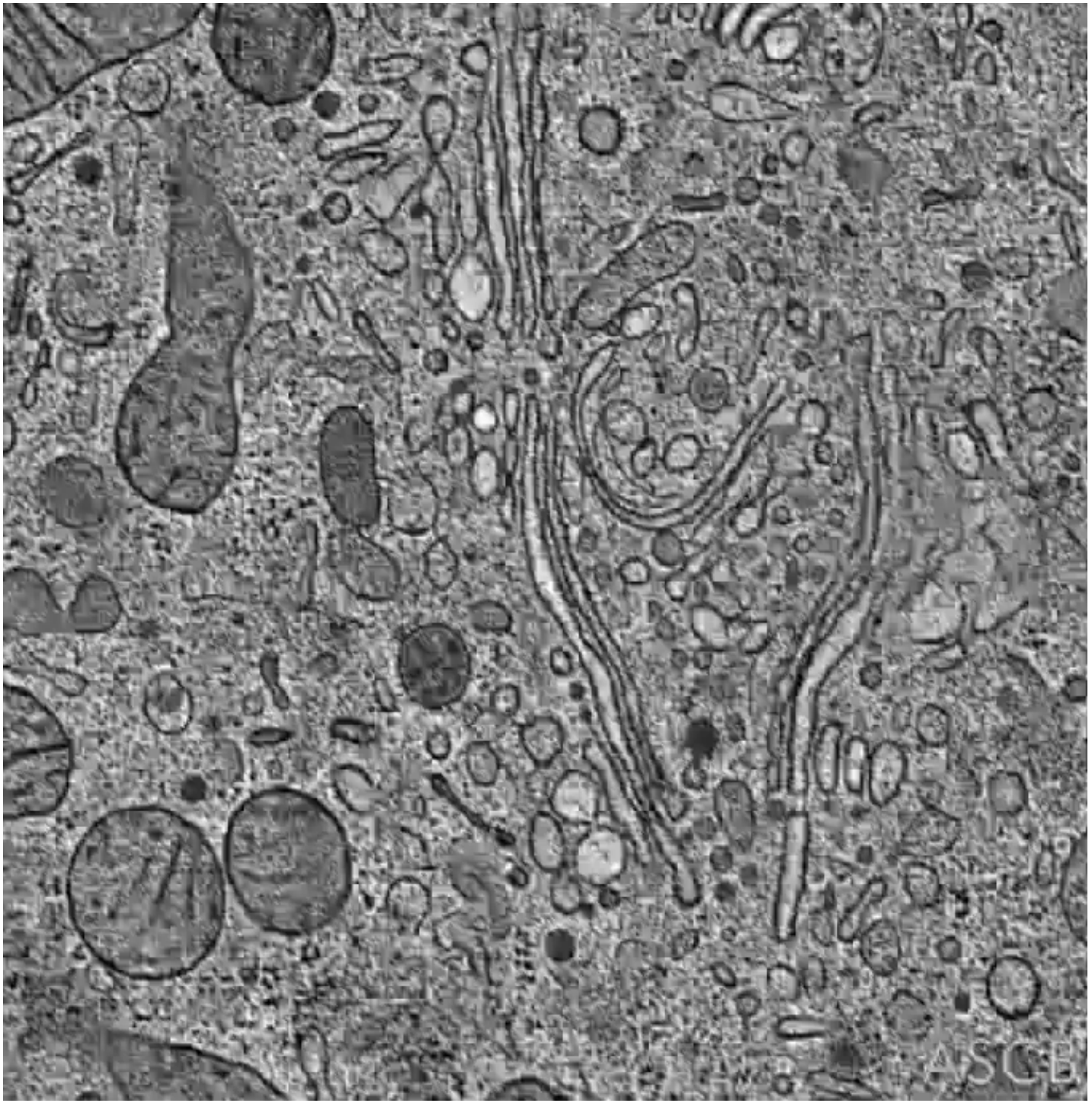
Specimen is tilted 120°, with high voltage EM images captured every 1.5°; process is repeated with 90° rotation

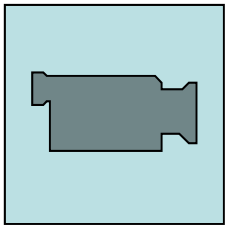
Computationally reconstructed in 3D

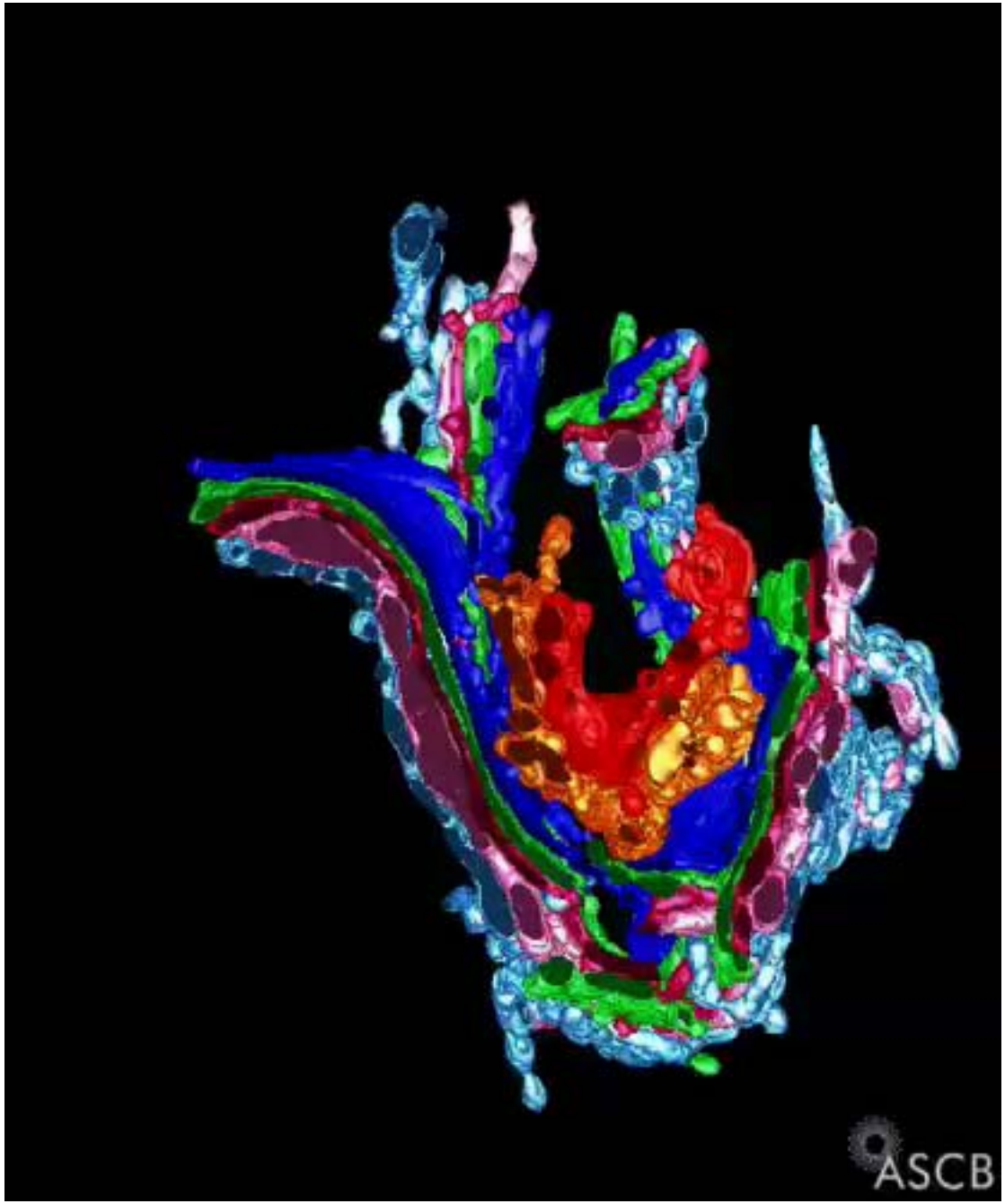
Resolution = 6-7 nm

Brad Marsh, Kathryn Howell, Dick McIntosh

University of Colorado Health Science Center (Aurora) and Boulder;
University of Queensland at Brisbane


ASCB




ASCB

General Pathways of Membrane Traffic

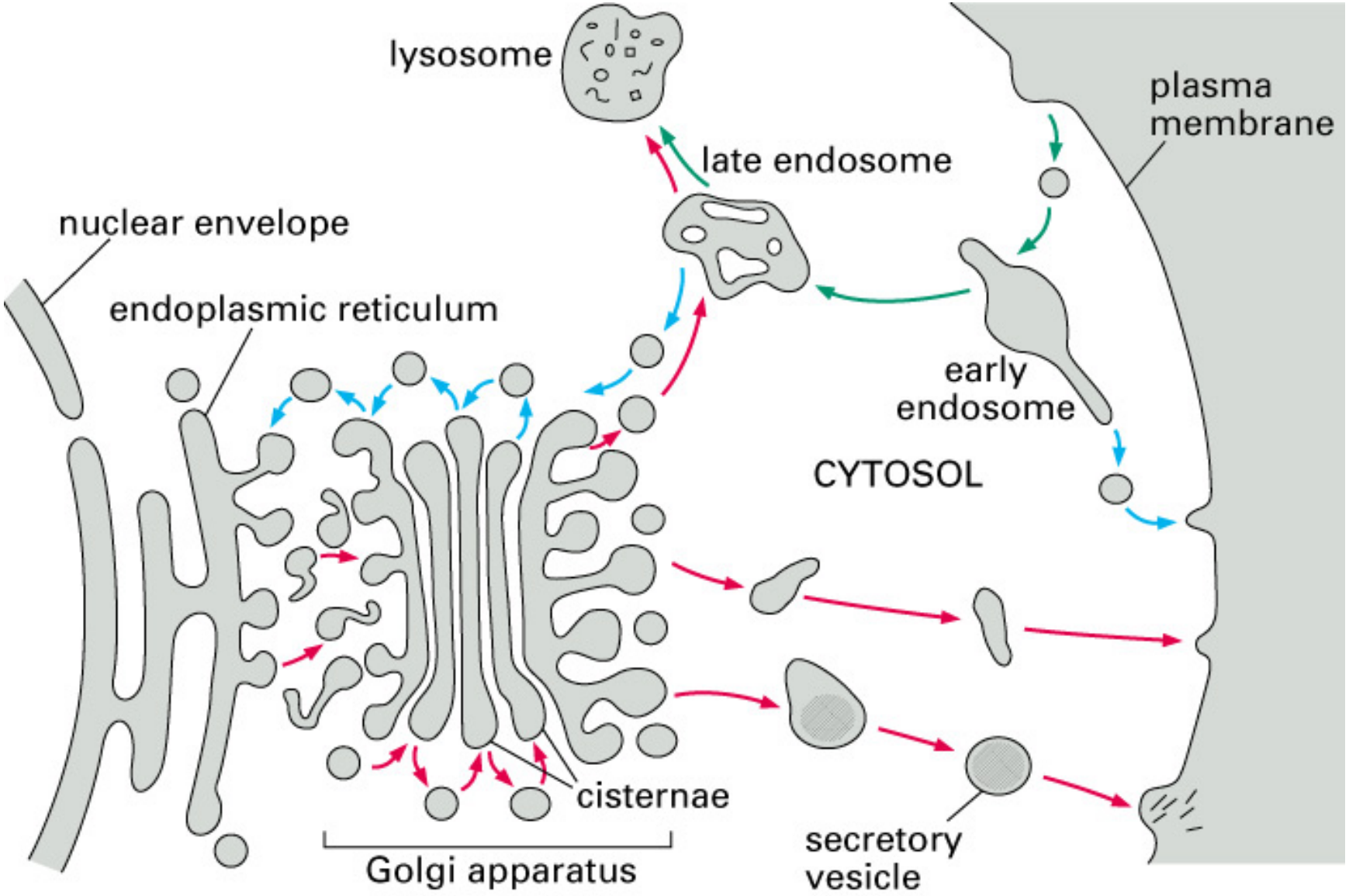


Figure 13–3. Molecular Biology of the Cell, 4th Edition.

The secretory pathway

ER -> Golgi -> Surface

HOW DO WE KNOW THIS?

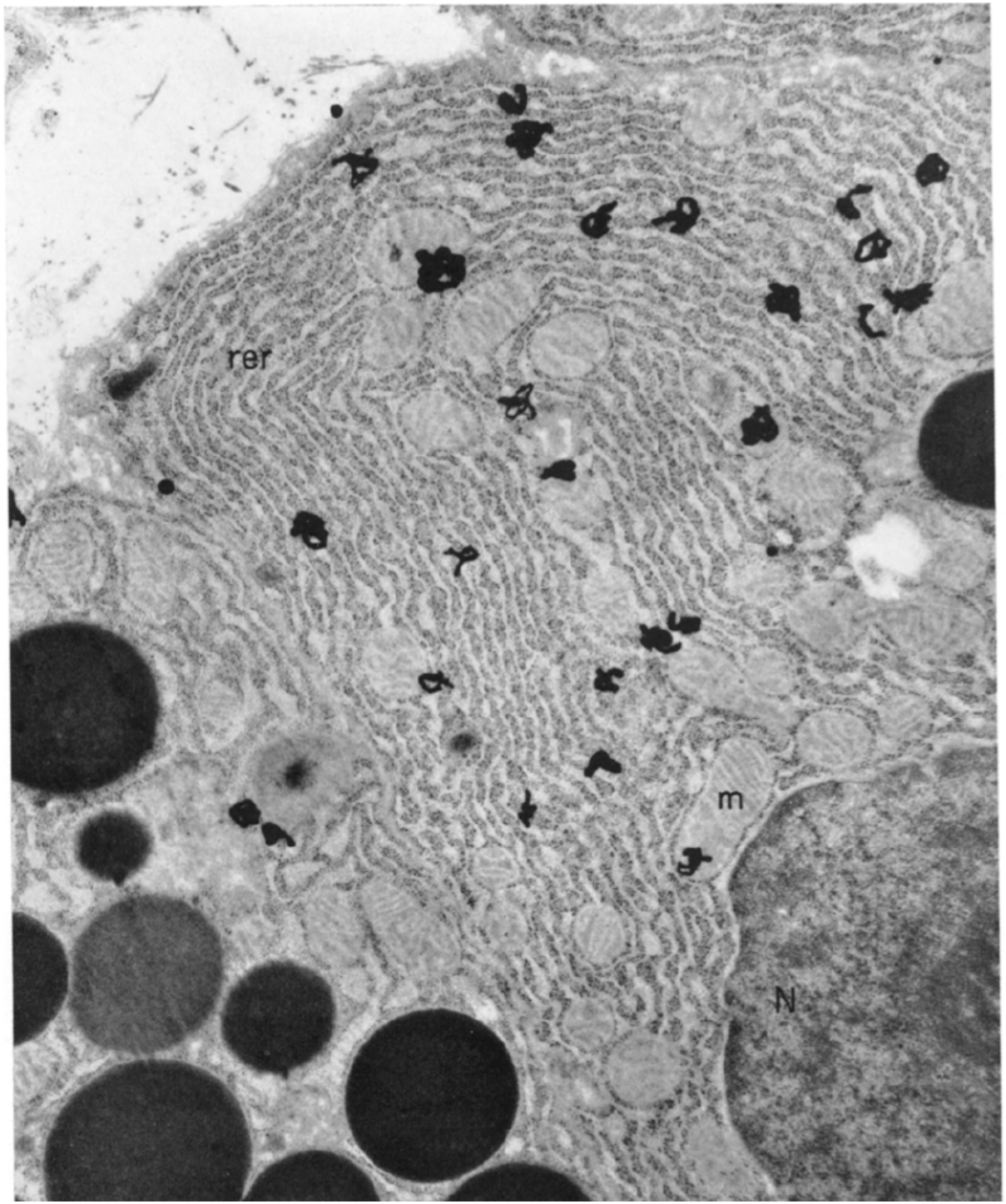
INTRACELLULAR TRANSPORT OF SECRETORY PROTEINS IN THE PANCREATIC EXOCRINE CELL

II. Transport to Condensing Vacuoles and Zymogen Granules

JAMES D. JAMIESON and GEORGE E. PALADE

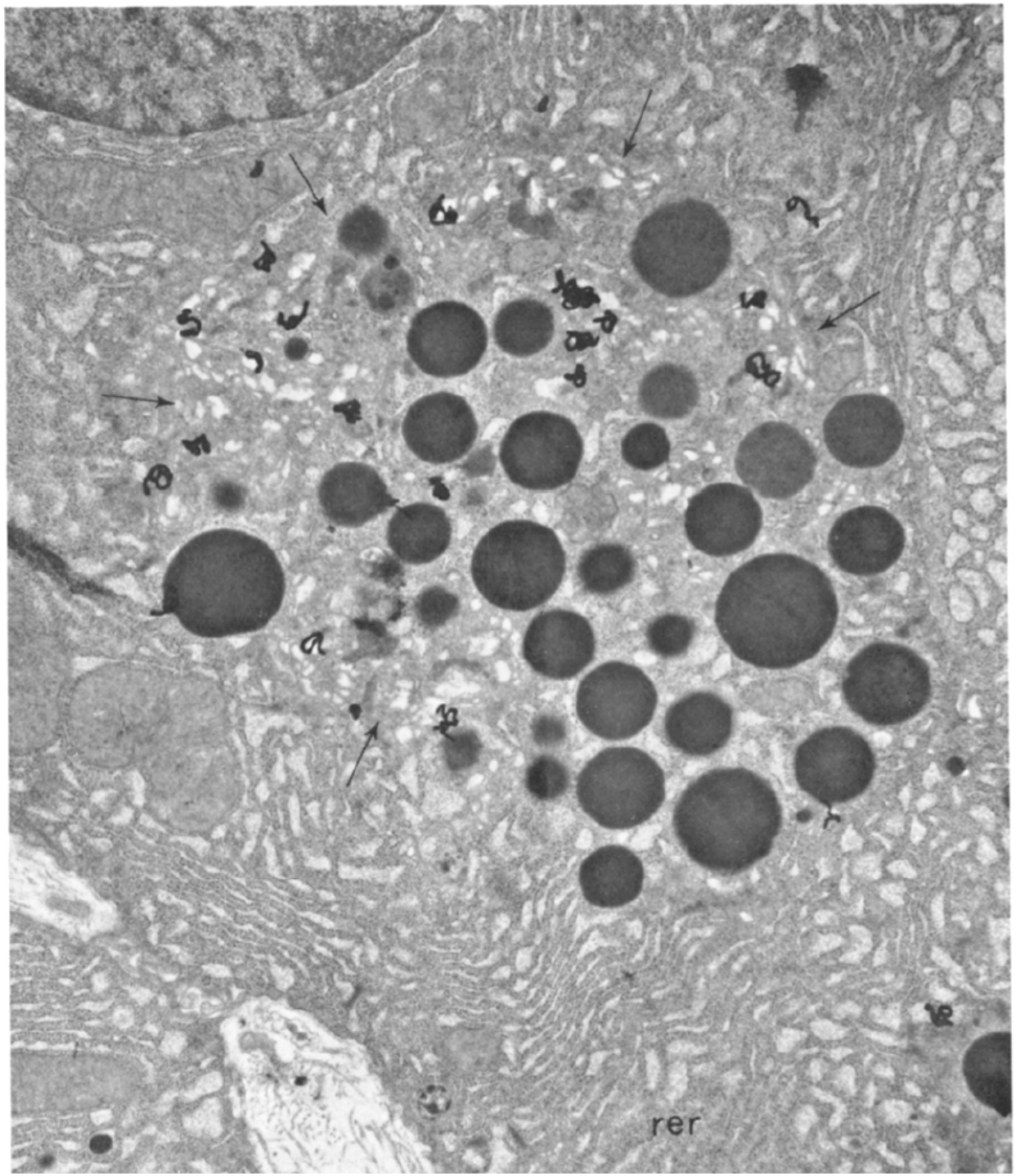
From The Rockefeller University, New York 10021

THE JOURNAL OF CELL BIOLOGY · VOLUME 34, 1967



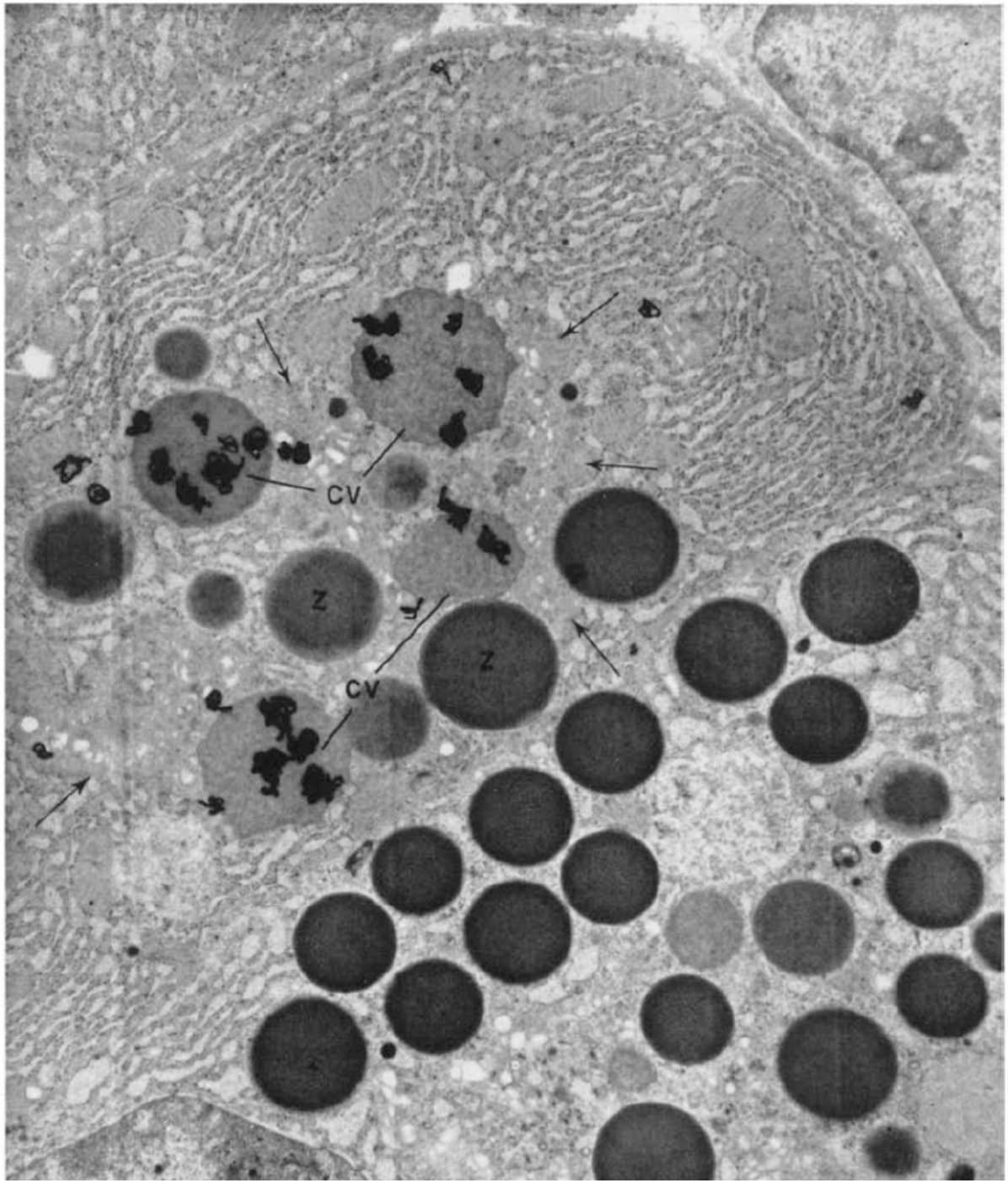
3 minutes

^{3}H-leucine



7 minute chase

^{3}H-leucine



37 minute chase

^{3}H-leucine

TABLE II

Distribution of Radioautographic Grains over Cell Components

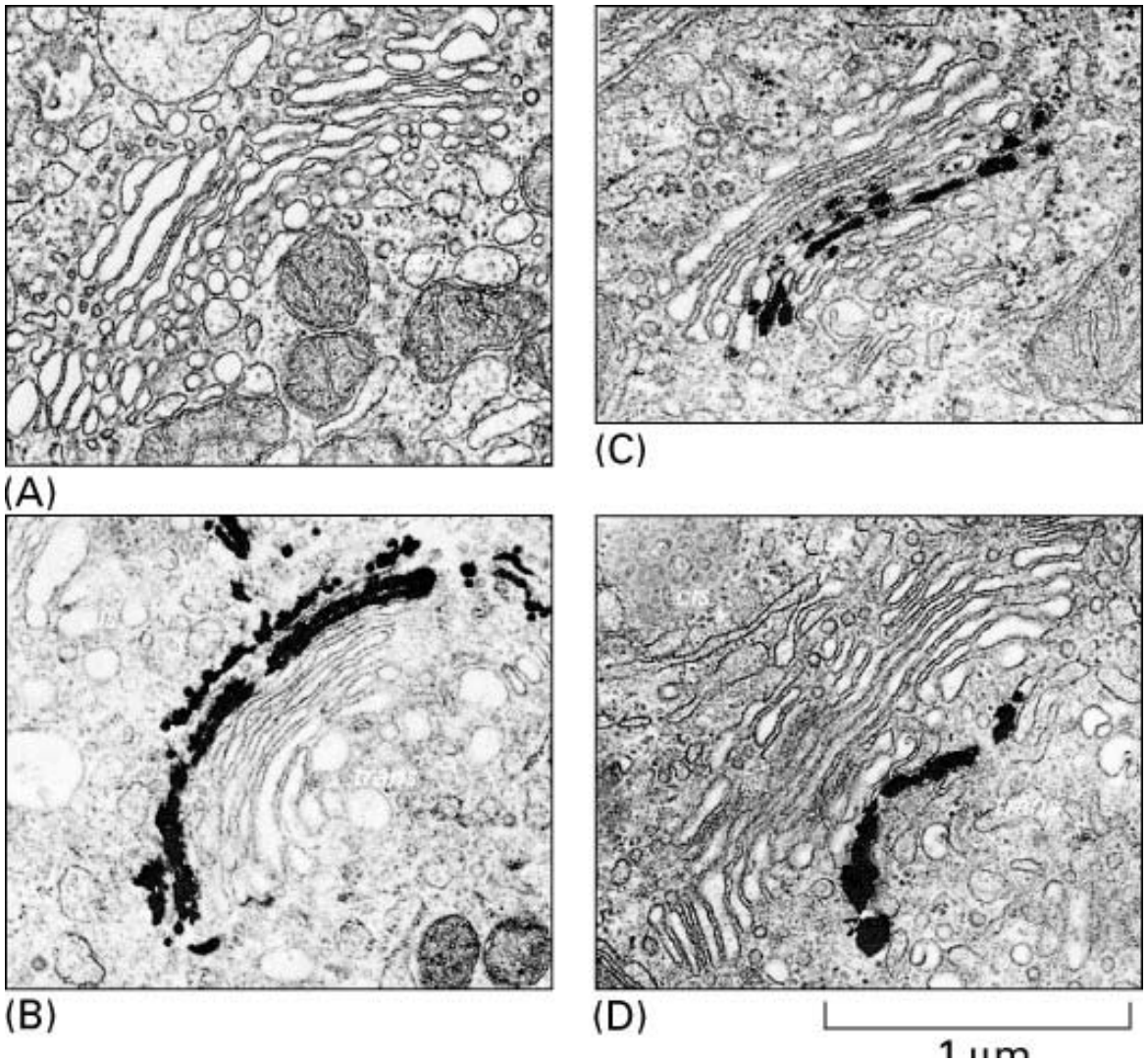
	% of radioautographic grains					
	3-min (pulse)	Chase incubation				
		+7 min	+17 min	+37 min	+57 min	+117 min
Rough endoplasmic reticulum	**86.3**	43.7	37.6	24.3	16.0	20.0
Golgi complex*						
Peripheral vesicles	2.7	**43.0**	37.5	14.9	11.0	3.6
Condensing vacuoles	1.0	3.8	19.5	**48.5**	35.8	7.5
Zymogen granules	3.0	4.6	3.1	11.3	32.9	**58.6**
Acinar lumen	0	0	0	0	2.9	7.1
Mitochondria	4.0	3.1	1.0	0.9	1.2	1.8
Nuclei	3.0	1.7	1.2	0.2	0	1.4
No. of grains counted	300	1146	587	577	960	1140

The boldfaced numbers indicate maximum accumulation of grains over the corresponding cell component.

* At no time were significant numbers of grains found in association with the flattened, piled cisternae of the complex.

Compartments had different shapes & content. Cargo seemed to pass through them.

Protein glycosylation enzymes are segregated in different Golgi compartments, consistent with entry at one face and exit at the other



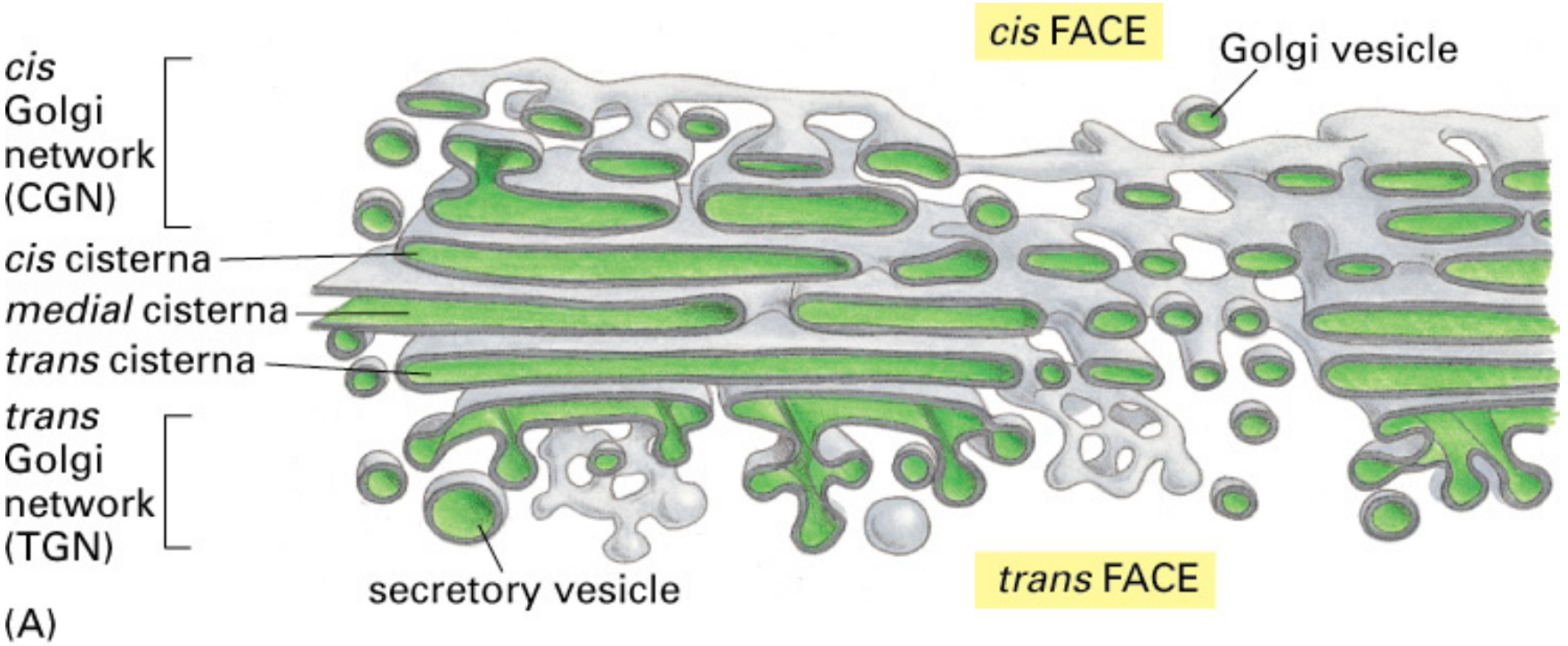


Figure 13–22 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

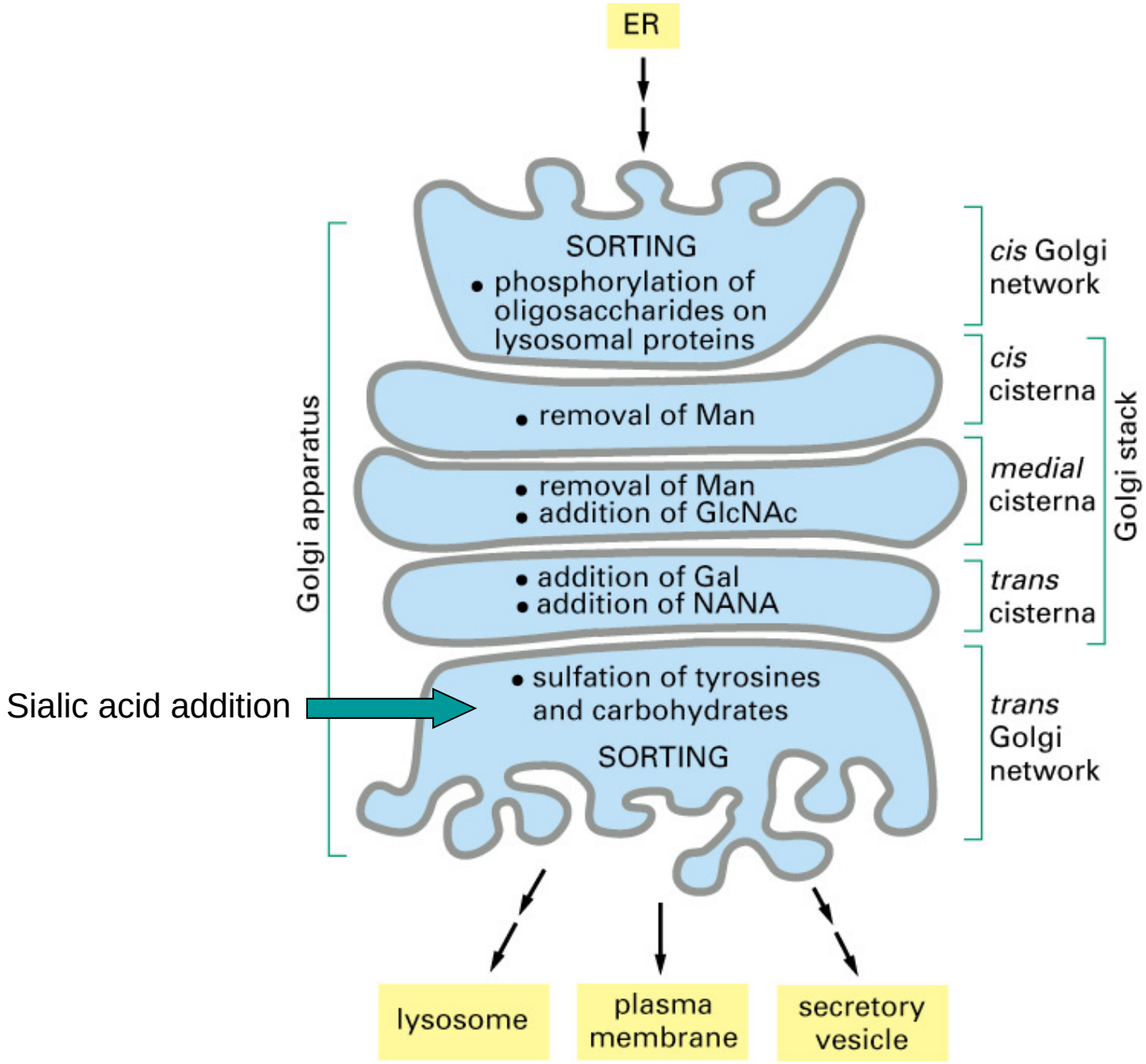
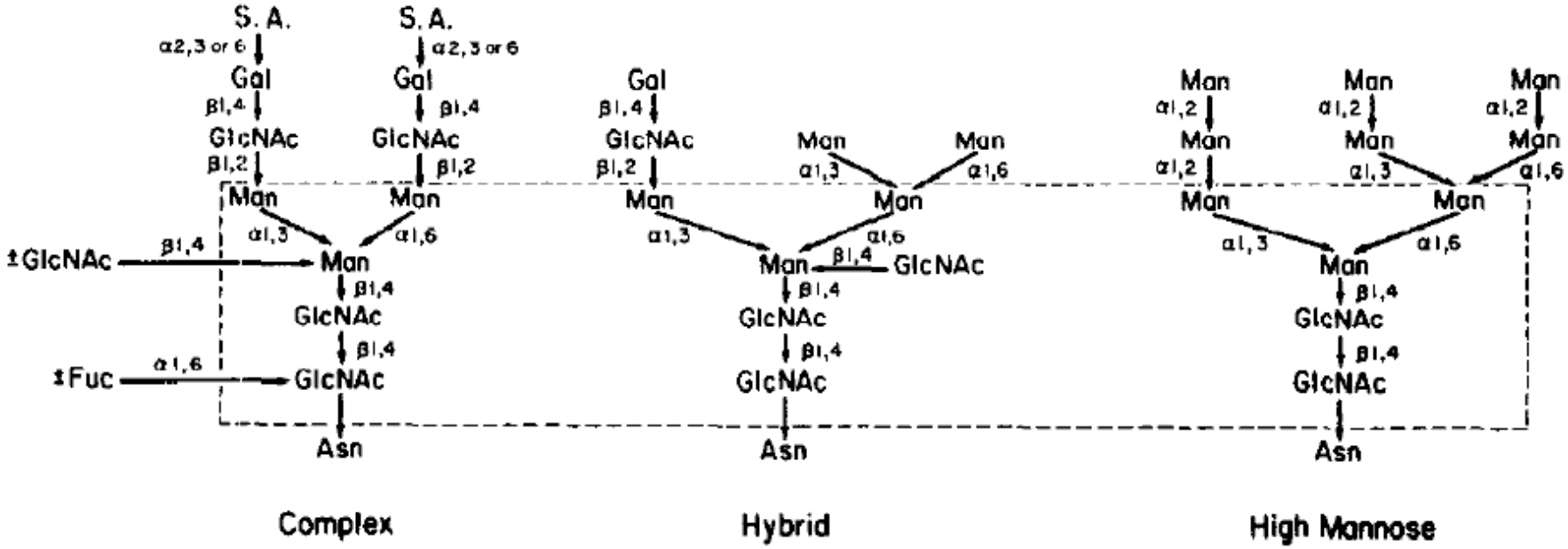


Figure 13–29. Molecular Biology of the Cell, 4th Edition.


S. A.
α2,3 or 6
Gal
β1,4
GlcNAc
β1,2
Man
α1,3
α1,6
±GlcNAc
β1,4
Man
β1,4
GlcNAc
±Fuc
α1,6
GlcNAc
Asn
Complex
Gal
β1,4
GlcNAc
β1,2
Man
α1,3
Man
α1,6
Man
β1,4
GlcNAc
GlcNAc
GlcNAc
Asn
Hybrid
Man
α1,2
Man
α1,2
Man
α1,3
Man
α1,6
Man
α1,3
Man
β1,4
GlcNAc
GlcNAc
GlcNAc
Asn
High Mannose

Yeast revealed molecules needed for the secretory pathway

Cell, Vol. 21, 205–215, August 1980, Copyright © 1980 by MIT

Identification of 23 Complementation Groups Required for Post-translational Events in the Yeast Secretory Pathway

Peter Novick, Charles Field and Randy Schekman*
Department of Biochemistry
University of California, Berkeley
Berkeley, California 94720

In 1977, Susan Henry (Albert Einstein) showed a tight coupling of net cell surface growth with inositol metabolism. Starve cells for inositol, and they became very dense.

Novick and Schekman (1980) showed that a Sec1 mutant they thought was secretion deficient was also very dense in a gradient of Ludox--a commercial floor polish that is now improved as Percoll. They, thus enriched for conditional mutant cells by density gradient.

Ludox gradient of a mix of wild type and sec1 cells

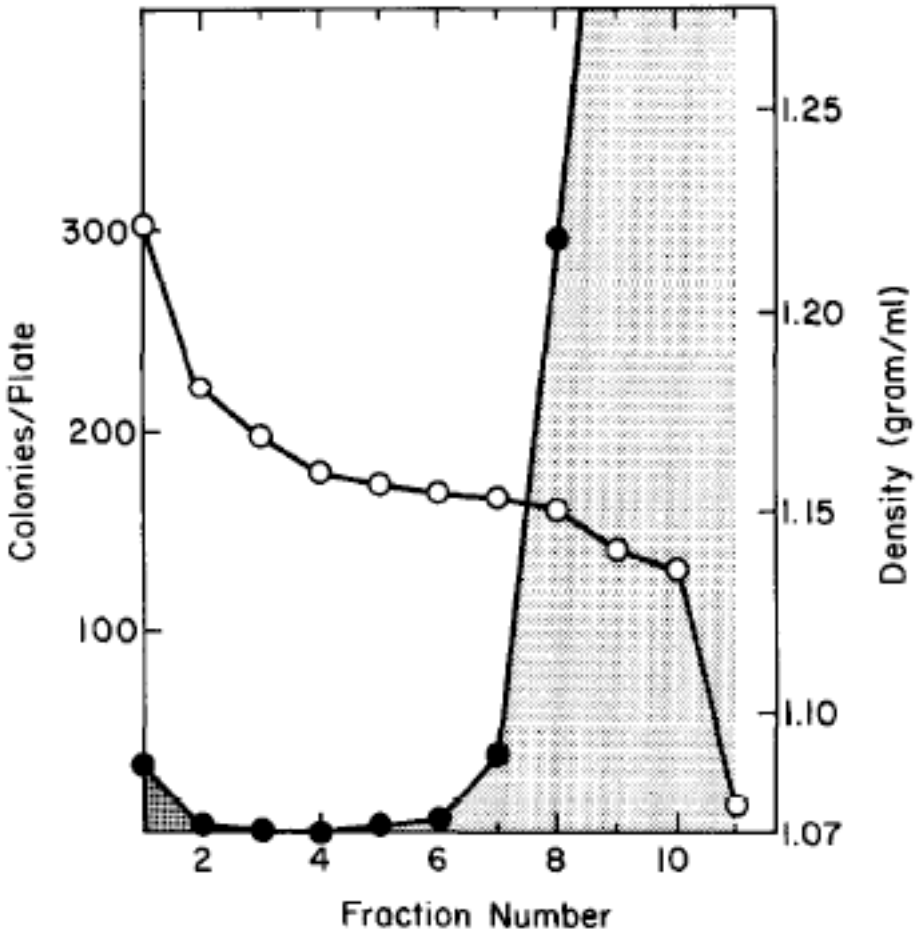


Figure 1. Density Gradient Separation of *sec*1-1 and X2180 Cells

SF150-5C and X2180-1A cells were grown in YPD medium at 25°C. After 3 hr at 37°C, the cells were sedimented, washed and resuspended in 1 ml of water. Cell aliquots were mixed (49.5 A_{600} units of X2180 and 0.5 A_{600} unit of SF150-5C) and sedimented on a Ludox gradient. Fractions were collected and diluted 10^4 fold, and 0.1 ml

Novick and Schekman sought yeast mutants that were temperature sensitive for growth but continued protein and lipid synthesis for at least 3 hours.

- shift cells to 37° for 3 hours
- enrich on a density gradient
- screen colonies for acid phosphatase and invertase secretion

Permissive and non-permissive temperatures: *sec15-1*

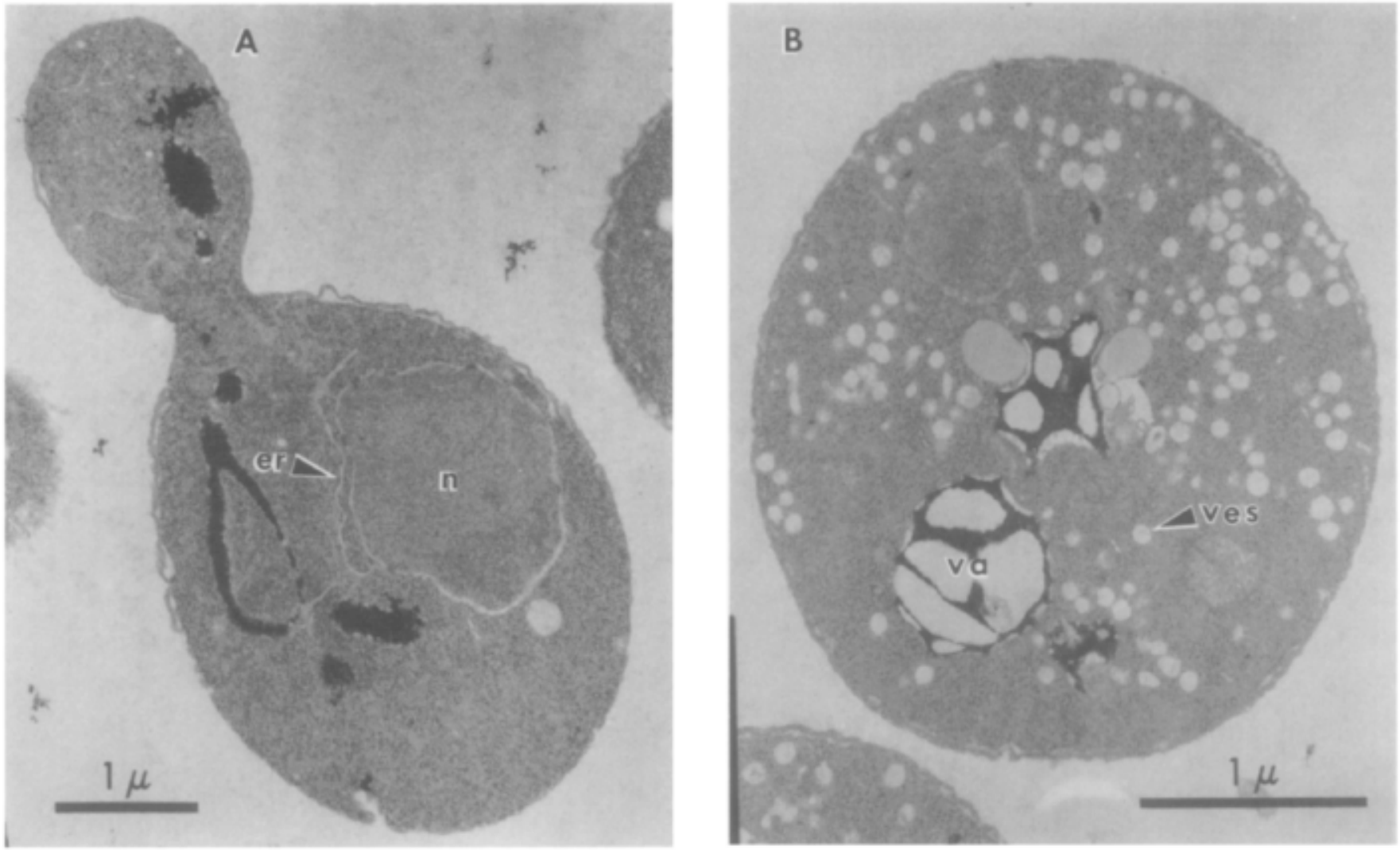


Figure 2. Thin Section Electron Micrographs of Cells Grown in YPD Medium

(A) HMSF 13 (*sec4-2*) grown at 25°C; (B) HMSF 171 (*sec15-1*) incubated at 37°C for 2 hr. Symbols: (n) nucleus; (va) vacuole; (er) endoplasmic reticulum; (ves) vesicles.

Table 5. Organelles Accumulated in the *sec* Strains

Strain (HMSF)	*sec*	Structure(s)
1	1-1	vesicles, Berkeley bodies
47	2-7	vesicles
3	3-1	vesicles
13	4-2	vesicles
81	5-8	vesicles
12	6-1	vesicles
6	7-1, -2	Berkeley bodies
93	8-4	vesicles
89	9-3	vesicles, Berkeley bodies
147	10-2	vesicles
154	11-7	
162	12-4	ER
163	13-1	ER
169	14-3	Berkeley bodies, vesicles
171	15-1	vesicles
174	16-2	ER
175	17-1	ER, small vesicles
176	18-1	ER, small vesicles
178	19-1	vesicles, Berkeley bodies, ER
179	20-1	ER
180	21-1	ER
183	22-3	ER, small vesicles
190	23-1	ER

Cell, Vol. 61, 723–733, May 18, 1990,

Distinct Sets of *SEC* Genes Govern Transport Vesicle Formation and Fusion Early in the Secretory Pathway

Chris A. Kaiser and Randy Schekman
Department of Molecular and Cell Biology
Division of Biochemistry and Molecular Biology
University of California
Berkeley, California 94720

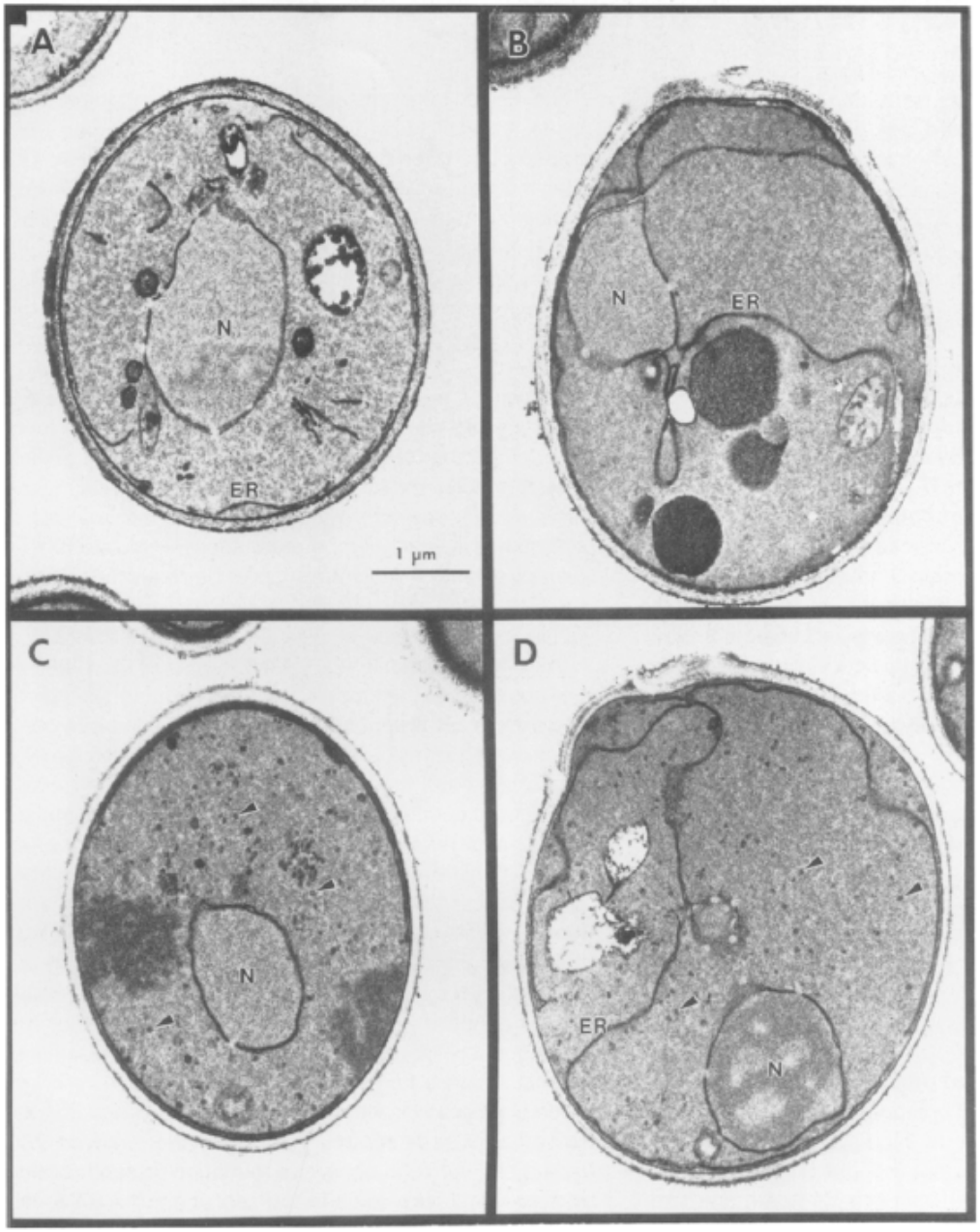


Figure 1. Thin-Section Electron Micrographs of Yeast Cells Fixed with Potassium Permanganate

(A) Wild type (RSY255) grown at 30°C. (B) *sec12-4* (RSY263) grown at 24°C and then for 1 hr at 37°C. (C) *sec17-1* (RSY269) grown at 17°C and then for 1 hr at 37°C. (D) *sec22-3* (RSY279) grown at 17°C and then for 1 hr at 37°C. N: nucleus. ER: endoplasmic reticulum. Arrows in (C) and (D) point to representative 50 nm vesicles

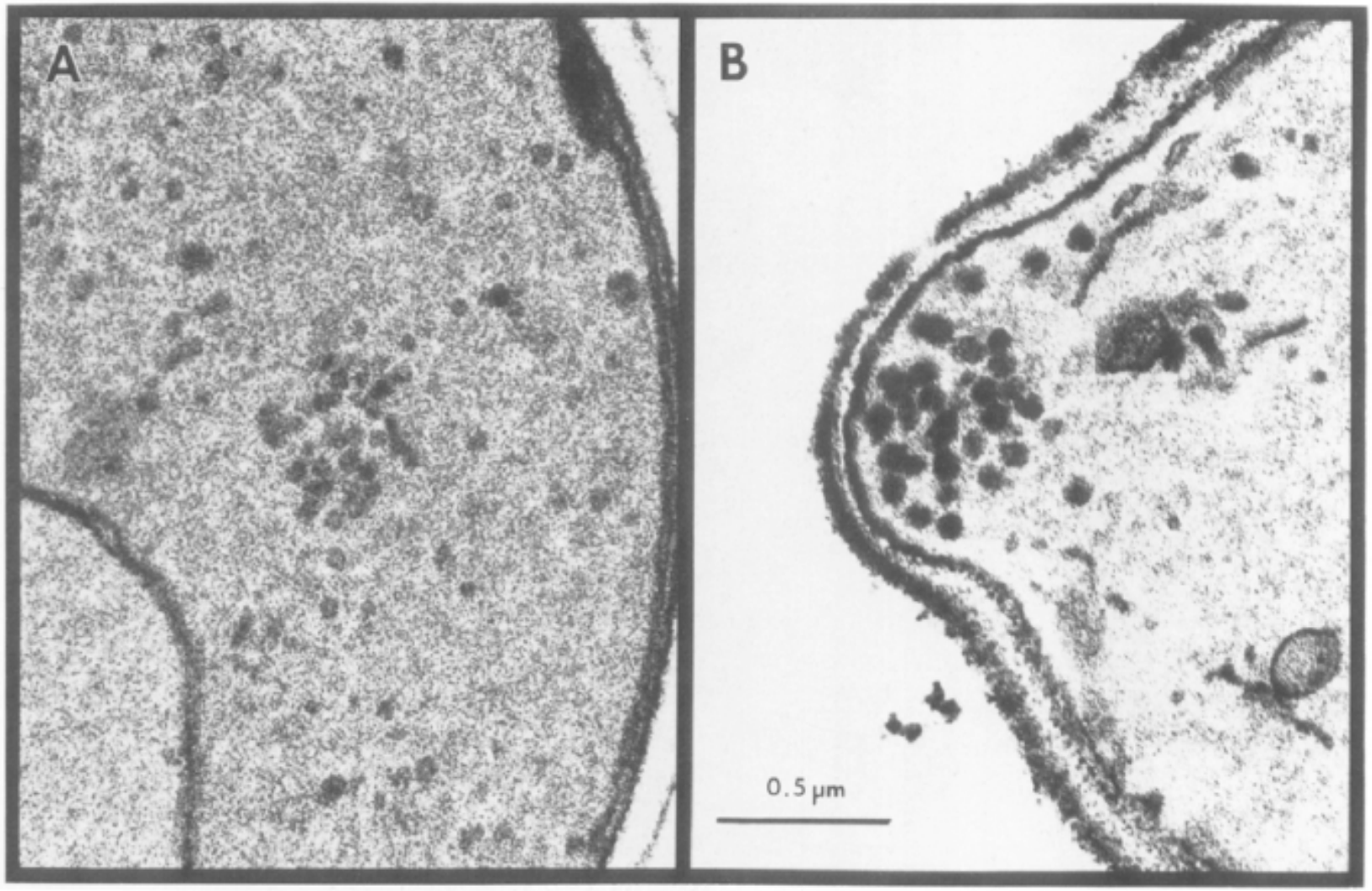


Figure 2. Thin-Section Electron Micrographs of Yeast Cells Illustrating the Difference in Morphology between 50 nm Vesicles and Mature Secretory Vesicles

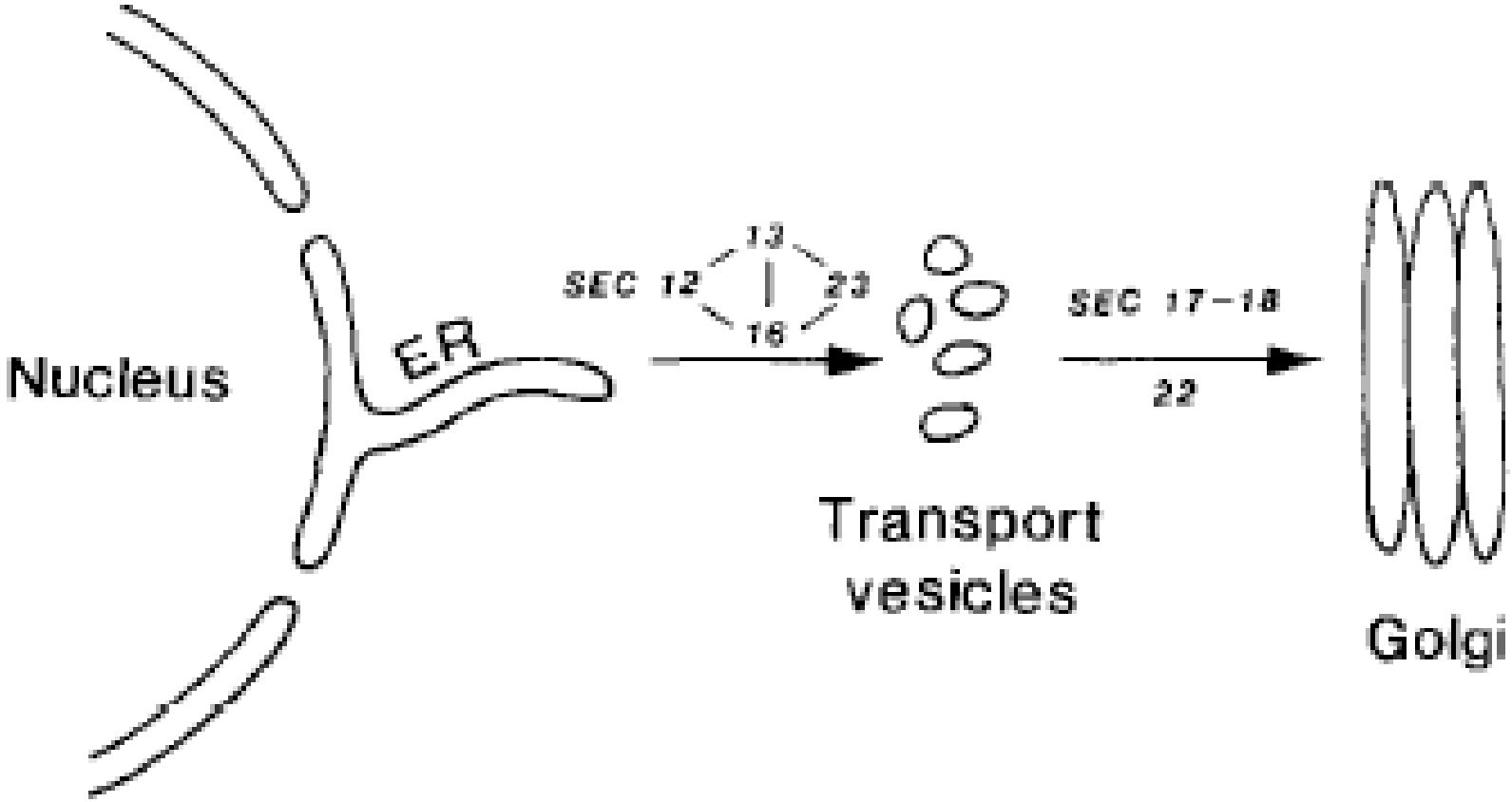
(A) Enlarged view of 50 nm vesicles in the *sec17-1* cell shown in Figure 1C.

(B) Late secretory vesicles in the growing bud of a wild-type cell (RSY255) grown at 30°C.

Table 1. 50 nm Vesicle Accumulation after 1 hr at 37°C

Strain	Allele	Vesicles per μm^3 of Cell Volume
RSY269	*sec17-1* (37°C)	61.9 ± 1.8
RSY269	*sec17-1* (17°C)	13.0 ± 0.8
RSY387	*sec17-2* (37°C)	58.5 ± 3.4
RSY271	*sec18-1* (37°C)	47.9 ± 3.4
RSY319	*sec18-2* (37°C)	41.0 ± 2.5
RSY319	*sec18-2* (17°C)	12.2 ± 1.6
RSY321	*sec22-1* (37°C)	38.5 ± 2.6
RSY279	*sec22-3* (37°C)	55.3 ± 3.0
RSY277	*sec21-1* (37°C)	22.2 ± 1.5
RSY275	*sec20-1* (37°C)	17.4 ± 1.2
RSY255	*SEC*$^+$ (37°C)	7.7 ± 0.7
RSY255	*SEC*$^+$ (17°C)	11.3 ± 0.8
RSY309	*sec12-1* (37°C)	5.9 ± 0.6
RSY263	*sec12-4* (37°C)	7.3 ± 0.9
RSY265	*sec13-1* (37°C)	7.8 ± 0.7
RSY313	*sec13-3* (37°C)	6.4 ± 0.8
RSY317	*sec16-1* (37°C)	9.8 ± 1.1
RSY281	*sec23-1* (37°C)	11.6 ± 1.2

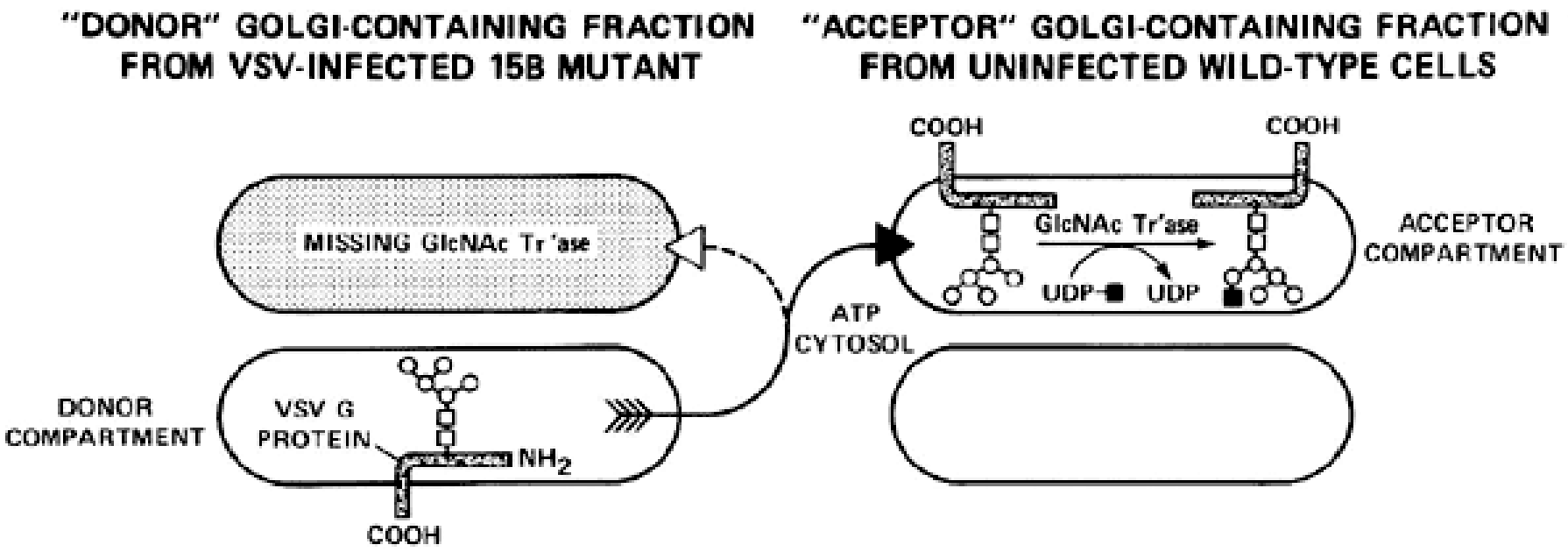
Vesicle density values are given as mean ± standard error of the mean. The standard error is the sample standard deviation divided by the square root of the sample size ($n = 32$).


Nucleus
ER
SEC 12
13
23
16
Transport
vesicles
SEC 17–18
22
Golgi

Transport Vesicles are proven intermediates

- How do vesicles form?
- How do vesicles find their targets?
- What proteins mediate their fusion?

PERHAPS THESE GENE PRODUCTS HOLD THE ANSWERS! Biochemical approaches came too…



See Balch et al. (1984) Reconstitution of Transport of protein between successive compartments of the Golgi…Cell 39, 405-416

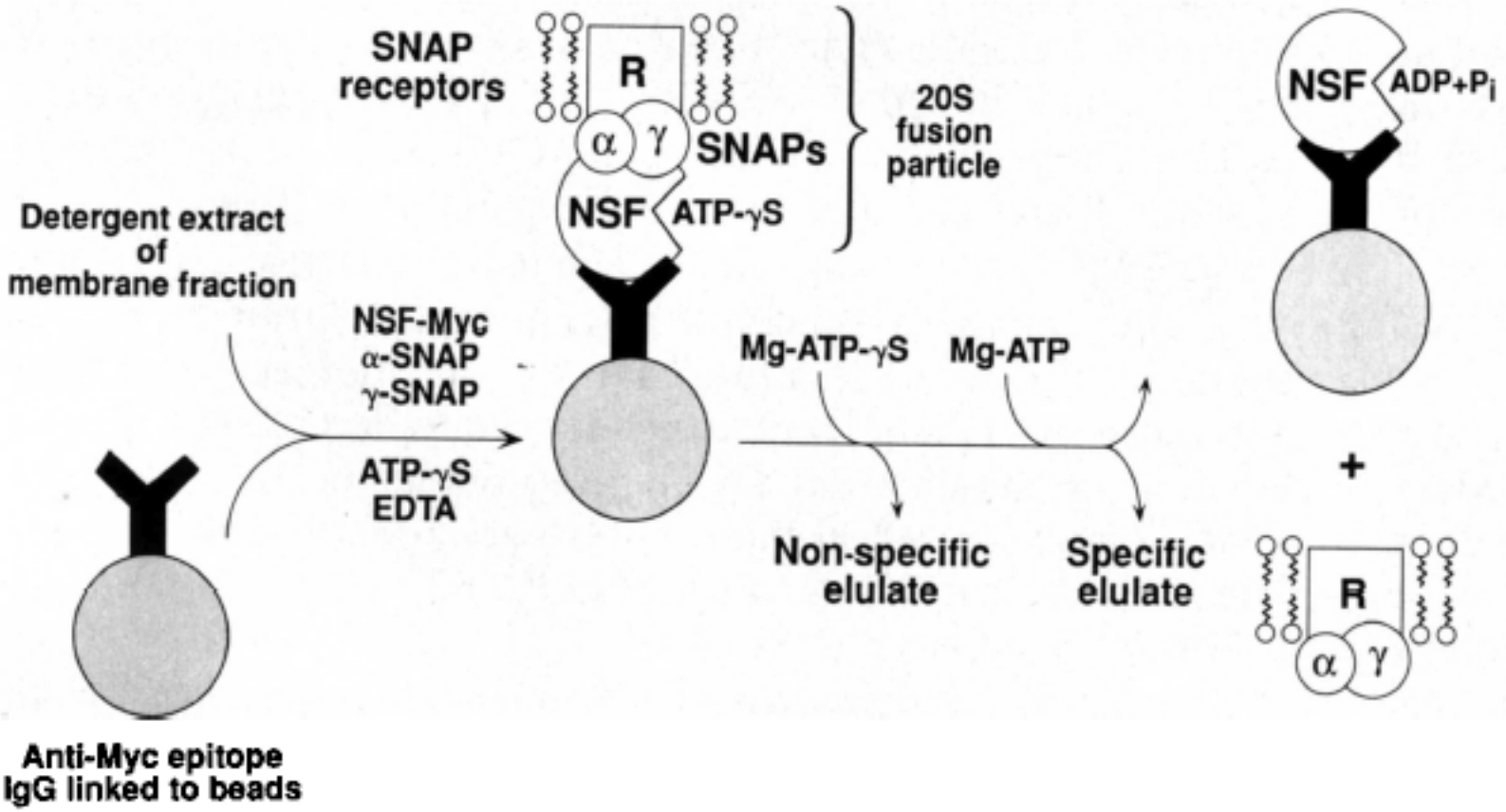
Transport required cytosolic proteins and ATP

To purify the cytosolic factors, Rothman and colleagues used selective inhibition with N-ethylmaleimide to selectively inhibit (and then purify) enzymes that had an active-site thiol.

This led to the purification of NEM-sensitive factor (NSF) which is the same as Sec18p

Alpha SNAP (Sec17p) is needed for membrane association of NSF

SNAREs were identified by a search for the Alpha SNAP receptor on brain membranes…



Söllner, T. et al. (1993) SNAP receptors implicated in vesicle targeting and fusion. Nature 362, 318-324.

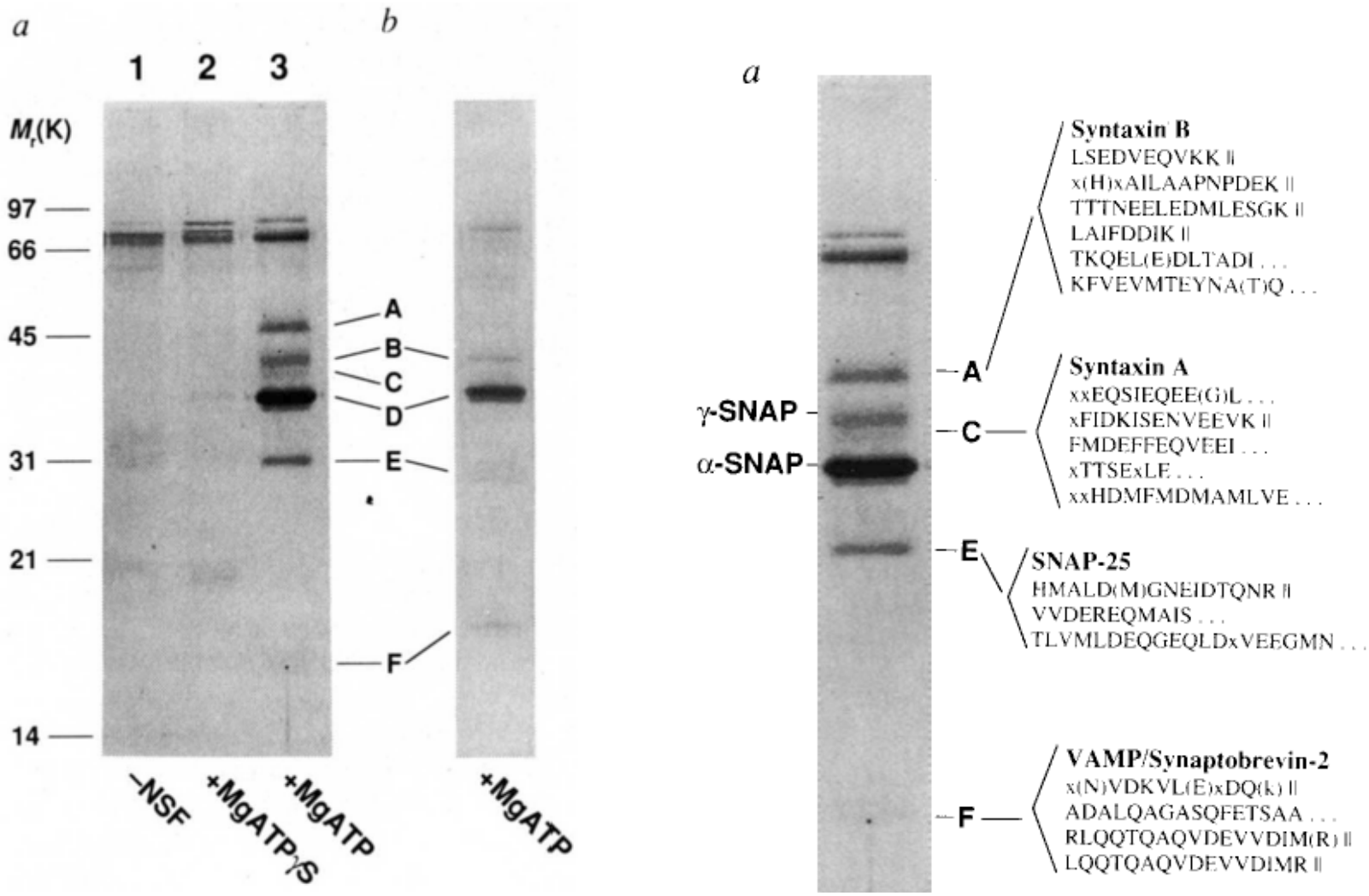
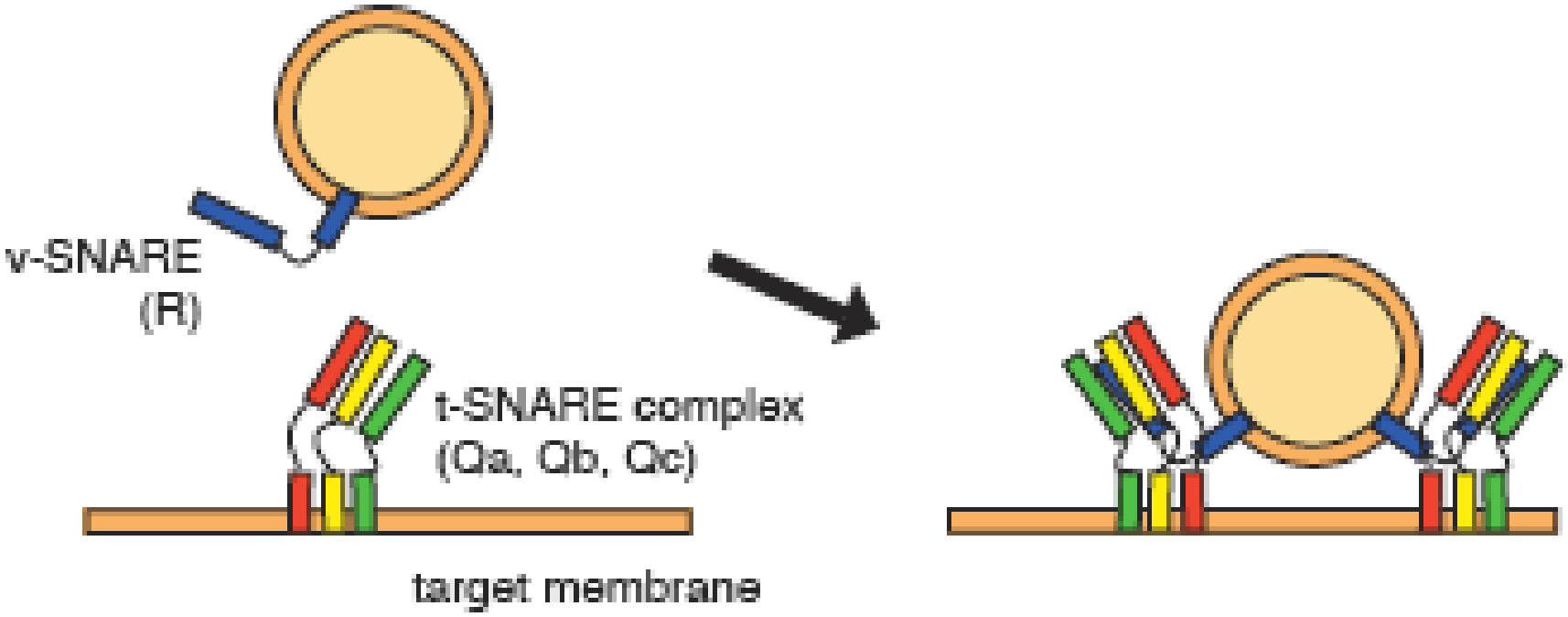
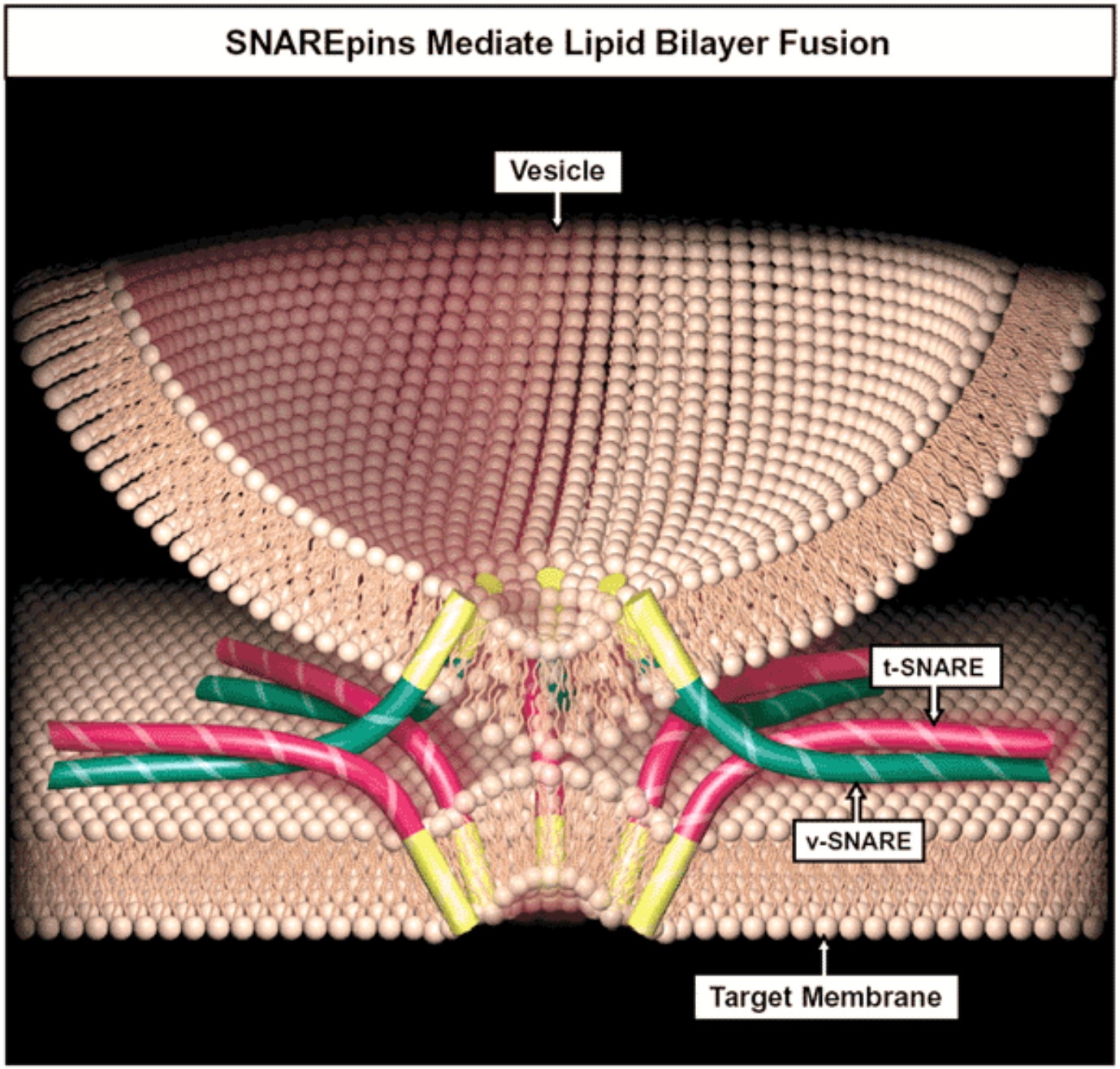


FIG. 2 Identification of proteins released from NSF after ATP hydrolysis. *a*, Polyacrylamide gel stained with Coomassie blue. Lane 1, control, Mg–ATP eluate of control binding reaction in the absence of NSF; lane 2, 'nonspecific' eluate from complete binding reaction with NSF–Myc and Mg–ATP-γS; lane 3, 'specific' eluate of the same column as for lane 2 following the exchange of ATP for ATP–γS (in the presence of EDTA) and addition of Mg^{2+} to allow ATP hydrolysis (Fig. 1). *b*, Silver-stained Laemmli gel of the specific (Mg–ATP) eluate.

Our current model for SNARE-mediated membrane fusion




SNAREpins Mediate Lipid Bilayer Fusion
Vesicle
t-SNARE
v-SNARE
Target Membrane

NSF and alpha SNAP untangle SNARE pairs post-fusion

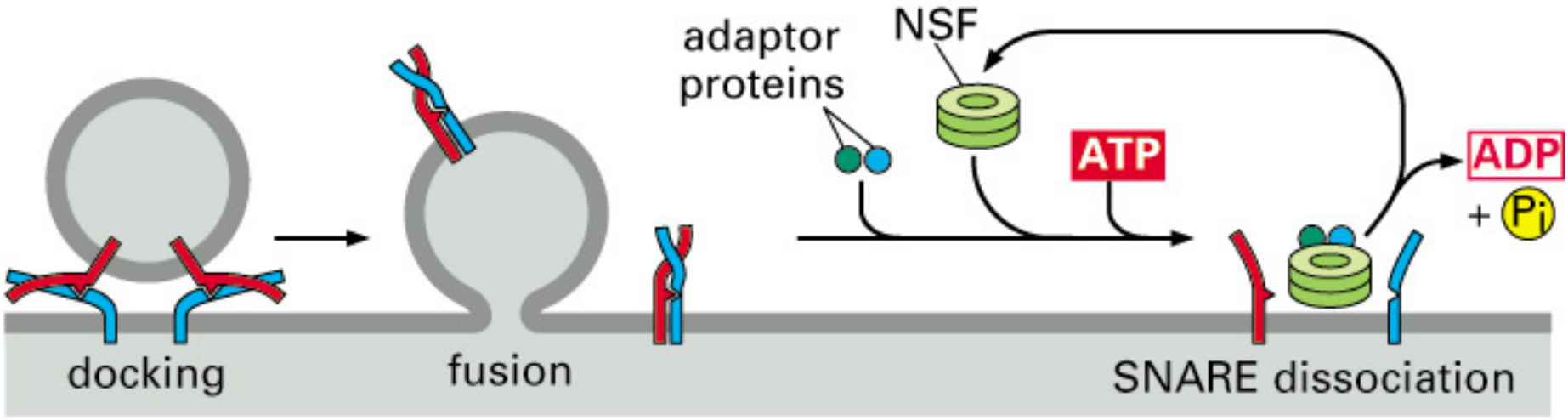
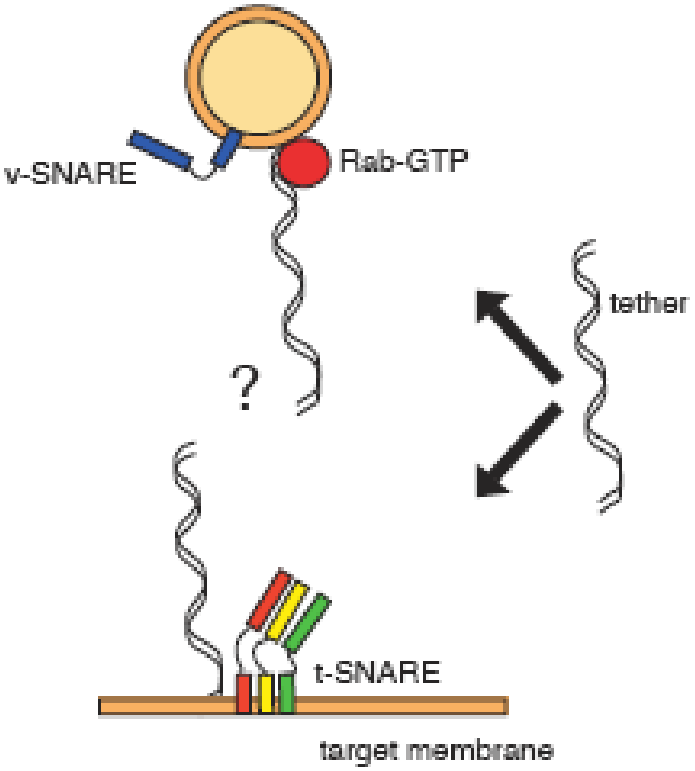


Figure 13–13. Molecular Biology of the Cell, 4th Edition.

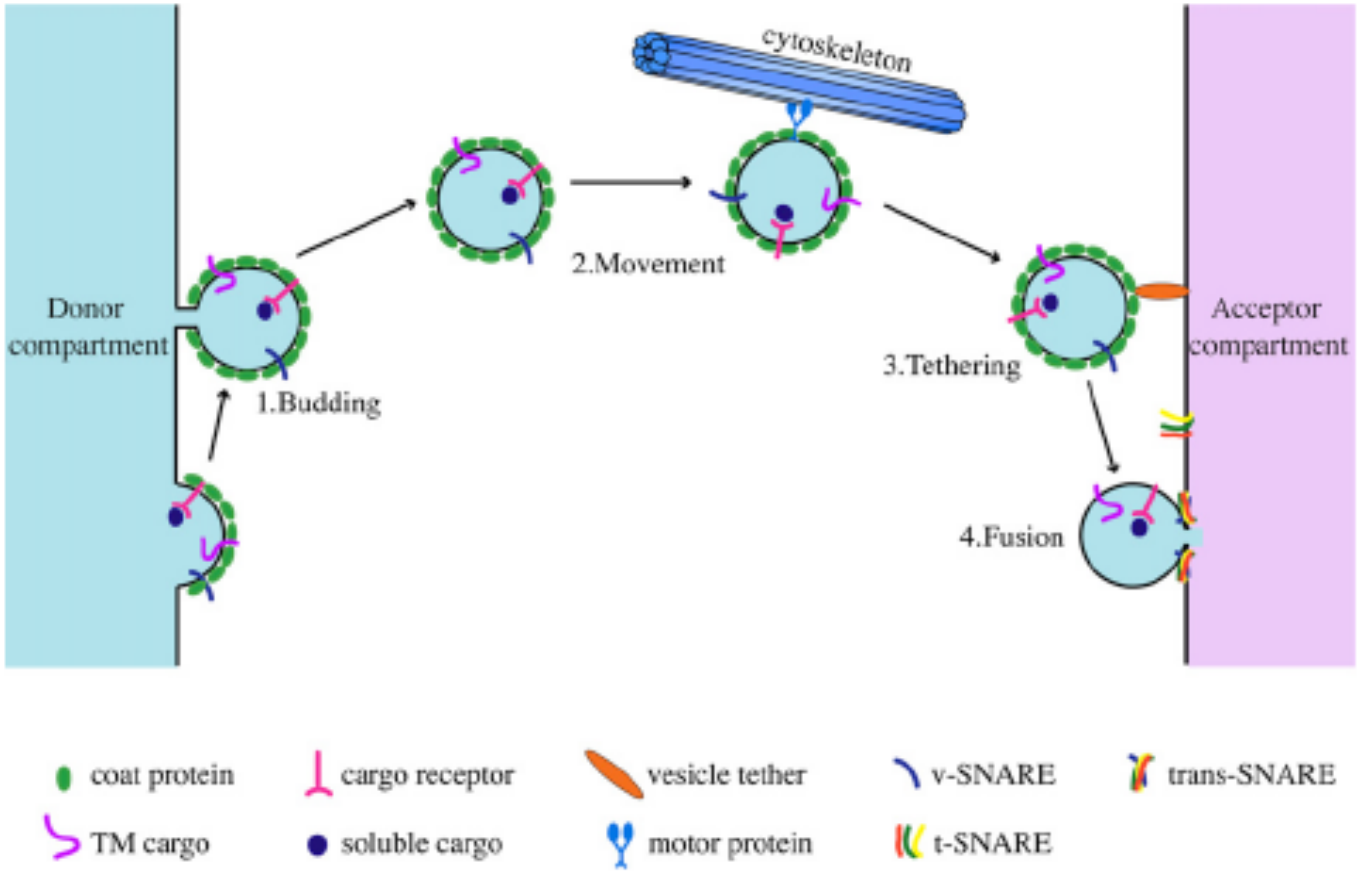
Tethers help SNAREs pair



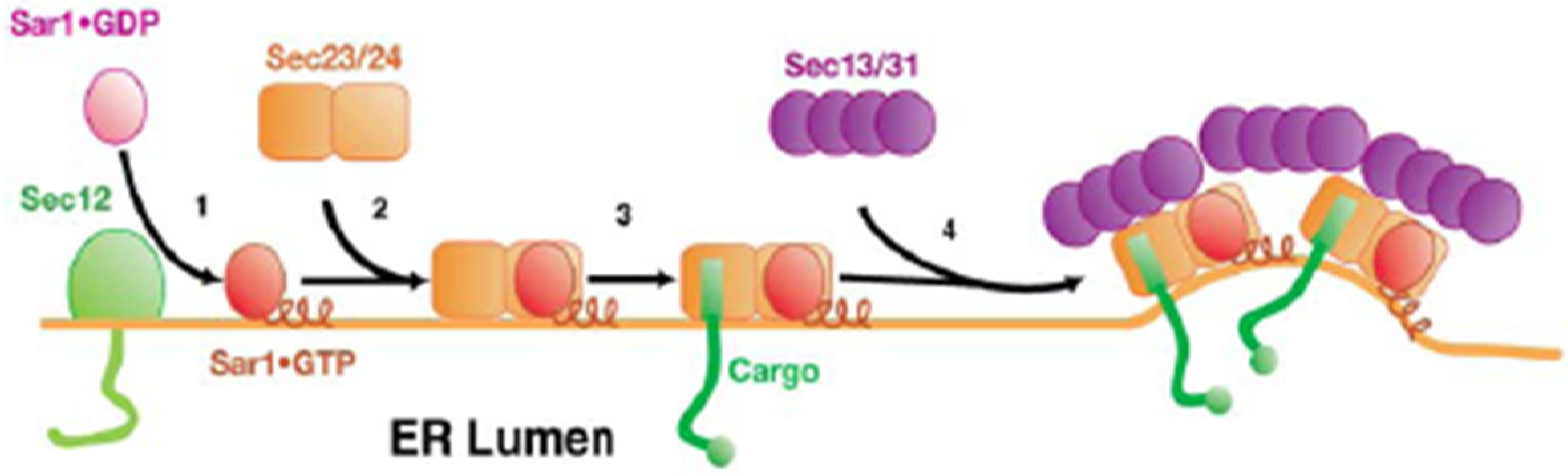
Developmental Cell
Review
Volume 12, Issue 5, 8 May 2007, Pages 671-682

Coats, Tethers, Rabs, and SNAREs Work Together to Mediate the Intracellular Destination of a Transport Vesicle

Huaqing Cai,[1] Karin Reinisch,[2] and Susan Ferro-Novick[1,2,*]
[1] Howard Hughes Medical Institute



Vesicle budding from the ER



FROM: Lee MC, Miller EA, Goldberg J, Orci L, Schekman R. (2004) Bi-directional protein transport between the ER and Golgi. Annu Rev Cell Dev Biol. 20:87-123

Table 1. 50 nm Vesicle Accumulation after 1 hr at 37°C

Strain	Allele	Vesicles per μm^3 of Cell Volume
RSY269	*sec17-1* (37°C)	61.9 ± 1.8
RSY269	*sec17-1* (17°C)	13.0 ± 0.8
RSY387	*sec17-2* (37°C)	58.5 ± 3.4
RSY271	*sec18-1* (37°C)	47.9 ± 3.4
RSY319	*sec18-2* (37°C)	41.0 ± 2.5
RSY319	*sec18-2* (17°C)	12.2 ± 1.6
RSY321	*sec22-1* (37°C)	38.5 ± 2.6
RSY279	*sec22-3* (37°C)	55.3 ± 3.0
RSY277	*sec21-1* (37°C)	22.2 ± 1.5
RSY275	*sec20-1* (37°C)	17.4 ± 1.2
RSY255	*SEC*$^+$ (37°C)	7.7 ± 0.7
RSY255	*SEC*$^+$ (17°C)	11.3 ± 0.8
RSY309	*sec12-1* (37°C)	5.9 ± 0.6
RSY263	*sec12-4* (37°C)	7.3 ± 0.9
RSY265	*sec13-1* (37°C)	7.8 ± 0.7
RSY313	*sec13-3* (37°C)	6.4 ± 0.8
RSY317	*sec16-1* (37°C)	9.8 ± 1.1
RSY281	*sec23-1* (37°C)	11.6 ± 1.2

Vesicle density values are given as mean ± standard error of the mean. The standard error is the sample standard deviation divided by the square root of the sample size ($n = 32$).

α-SNAP for SNARE recycling

NSF for SNARE recycling

V-SNARE

Sar1 GTPase GEF

COP-II coat

ER exit site scaffold

Sar1GAP, COP-II coat

What we have learned and how:

Compartment identities and routes

Morphology

Enzyme markers and localization

Transport experiments to determine continuity and routes

Live cell microscopy and EM to determine continuity and routes

Mutations in the pathway

Drugs? Protein blocks and Temperature blocks

What is required to set these compartments and pathways?

- Compartment identity determinants
- Retention/retrieval/signals/receptors
- Compartment recognition during transport and for maintenance

What don’t we know?

Why is the Golgi cisternal?
How is Golgi polarity is established?
How do you make an ER or Golgi and retain identity
Why do SNAREs only work in one direction--how are they regulated?