Nobel Lecture
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Genes and proteins that organize the secretory pathway

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Origins and influences
Pancreatic acinar cell
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,
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Communicated August 24, 1967

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

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AND DIVISION OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA

Communicated September 25, 1967
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

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

- histidinol
- histidine

enzyme in cytosol:
cell lives without histidine as nutrient

engineered yeast cell

- enzyme targeted to ER:
cell dies without histidine as nutrient

mutant engineered cell

- not all enzyme taken up into ER:
cell lives without histidine as nutrient

mutant translocation apparatus
POST-TRANSLATIONAL TRANSLOCATION

Sec62,63,71,72 complex
CYTOSOL
ER LUMEN
Sec61 complex
BiP
ATP
ADP

EUCARYOTES

BACTERIA

ATP
SecA
ADP

CYTOSOL
EXTRACELLULAR SPACE

Figure 12-44b,c  Molecular Biology of the Cell (© Garland Science 2008)
Yeast secretory pathway
Union of genetics and biochemistry
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

spin 13,000g

spin 100,000g
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


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  TGGYVMVGDSFNTSL**F**KQTFQRVFHTKDMHGQFKMGF
SEC23B  TGGYVMVGDSFNTSL**F**KQTFQRIFHTKDFNGDFRMAF
Sec23p  TGGVLLLLTDASFSTAI**F**KQSYLRLFAKDEEGYLMKF

Sar1

F382L

Sec23

Sec24
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.