Nobel Lecture
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Genes and proteins that organize the secretory pathway

Randy Schekman
Department of Molecular and Cell Biology
Howard Hughes Medical Institute
University of California, Berkeley
Origins and influences
Pancreatic acinar cell
ENZYMATIC SYNTHESIS OF DNA, XXIII. SYNTHESIS OF CIRCULAR REPLICATIVE FORM OF PHAGE $\phi X174$ DNA*

By Mehran Goulian† and Arthur Kornberg

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

Communicated August 24, 1967

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ENZYMATIC SYNTHESIS OF DNA, XXIV. SYNTHESIS OF INFECTIOUS PHAGE $\phi X174$ 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
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
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
- translocation apparatus

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

**mutant engineered cell**
- histidinol
- histidine
- not all enzyme taken up into ER: cell lives without histidine as nutrient
- mutant translocation apparatus
Yeast secretory pathway

Vacuole
- Vps 33, 34
- Ypt 7, 51
- 52, 53

Golgi
- Pep12
- Chc1
- Clc1
- Vps 1, 10

ER
- Sec 20, 21, 26, 27
- Ret 1, 2, 3
- Ufe 1, Arf 1
- Sec 61, 62, 63, 71, 72
- Kar2

Sec 1, 2, 3, 4, 5, 6, 8, 10, 15
- Sec 7, 14
- Pik1

Sec 12, 13, 16, 17, 18, 19, 22, 23, 24
- Sar1, Bet1, Bos1

Sec 1, 2, 3, 4, 5, 6, 8, 10, 15
- Sec 7, 14
- Pik1

Sec 12, 13, 16, 17, 18, 19, 22, 23, 24
- Sar1, Bet1, Bos1
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
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  TGGYMVMGDSFNTSL\textbf{F}KQTFQRVFTKDMHGQFKMGF
SEC23B  TGGYMVMGDSFNTSL\textbf{F}KQTFQRIFTKDFNMGDFRMAF
Sec23p  TGGVLLLTDAFSTAI\textbf{F}KQSYLRLFAKDEEGYLKMAF
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.