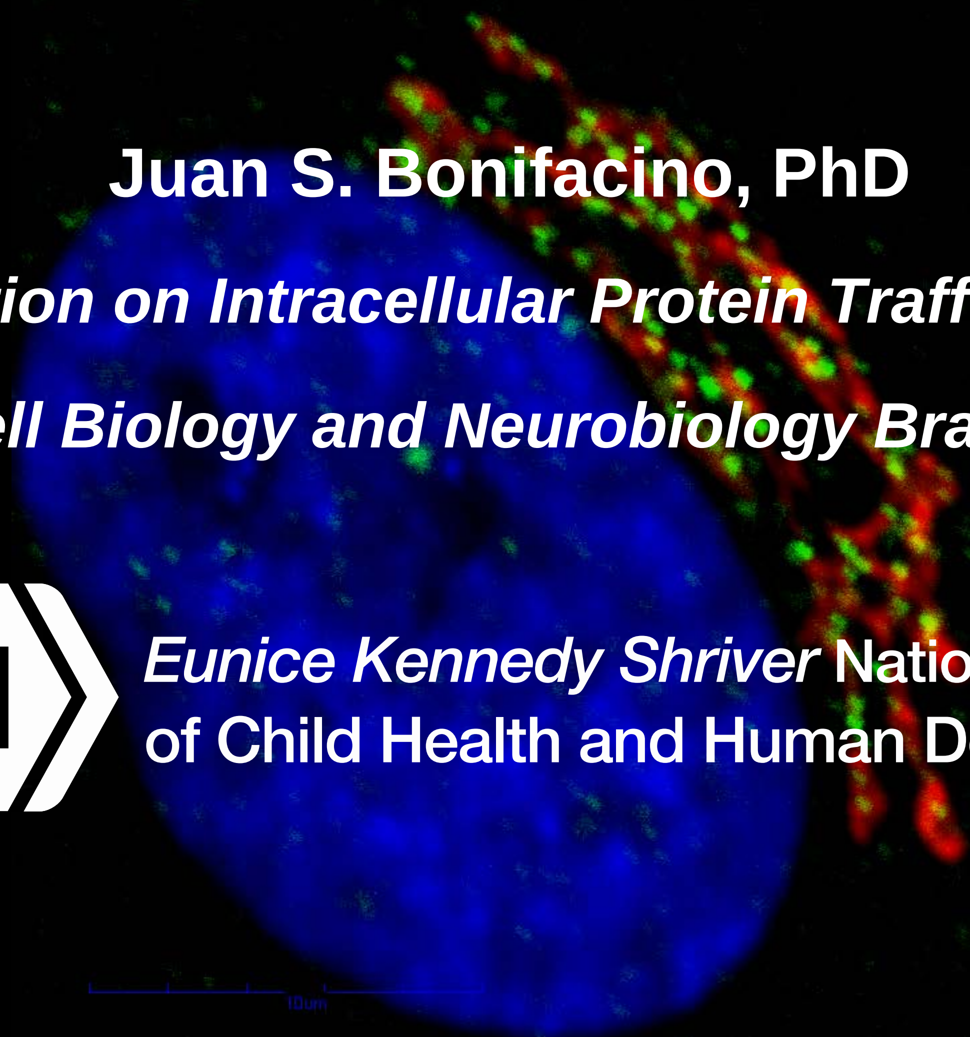
Coatopathies: Genetic Disorders of Protein Coats



Juan S. Bonifacino, PhD

Section on Intracellular Protein Trafficking

Cell Biology and Neurobiology Branch

NIH *Eunice Kennedy Shriver* National Institute of Child Health and Human Development

Section on Intracellular Protein Trafficking

Section on Intracellular Protein Trafficking

- **Molecular mechanisms of protein and organelle distribution within the cell**

Section on Intracellular Protein Trafficking

- **Molecular mechanisms of protein and organelle distribution within the cell**
- **Dysfunction in human disease**

  Hermansky-Pudlak syndrome (HPS)

  MEDNIK syndrome

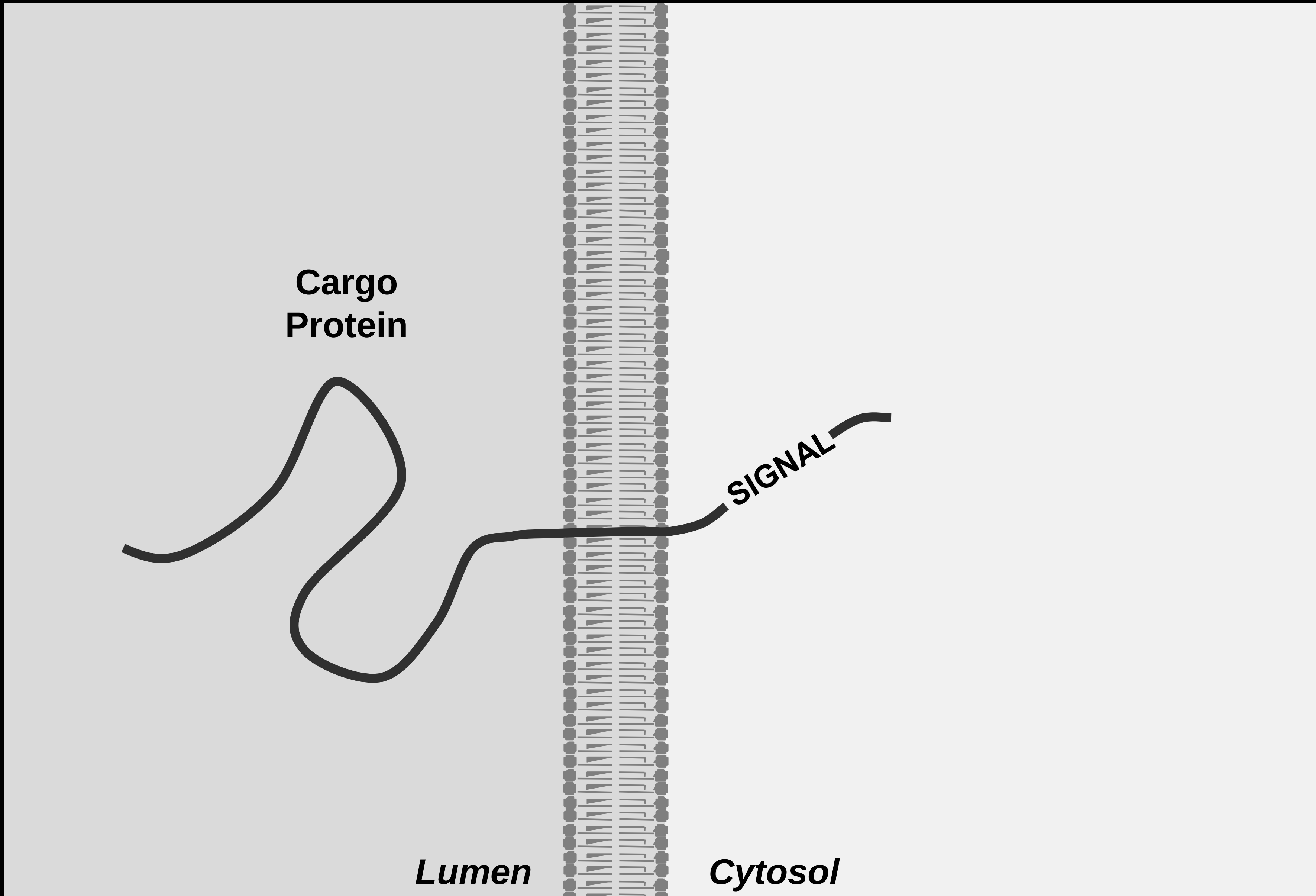
  Hereditary spastic paraplegias (HSP)

  Progressive cerebral cerebellar atrophy (PCCA)

Electron Microscopy of a Pituitary Cell

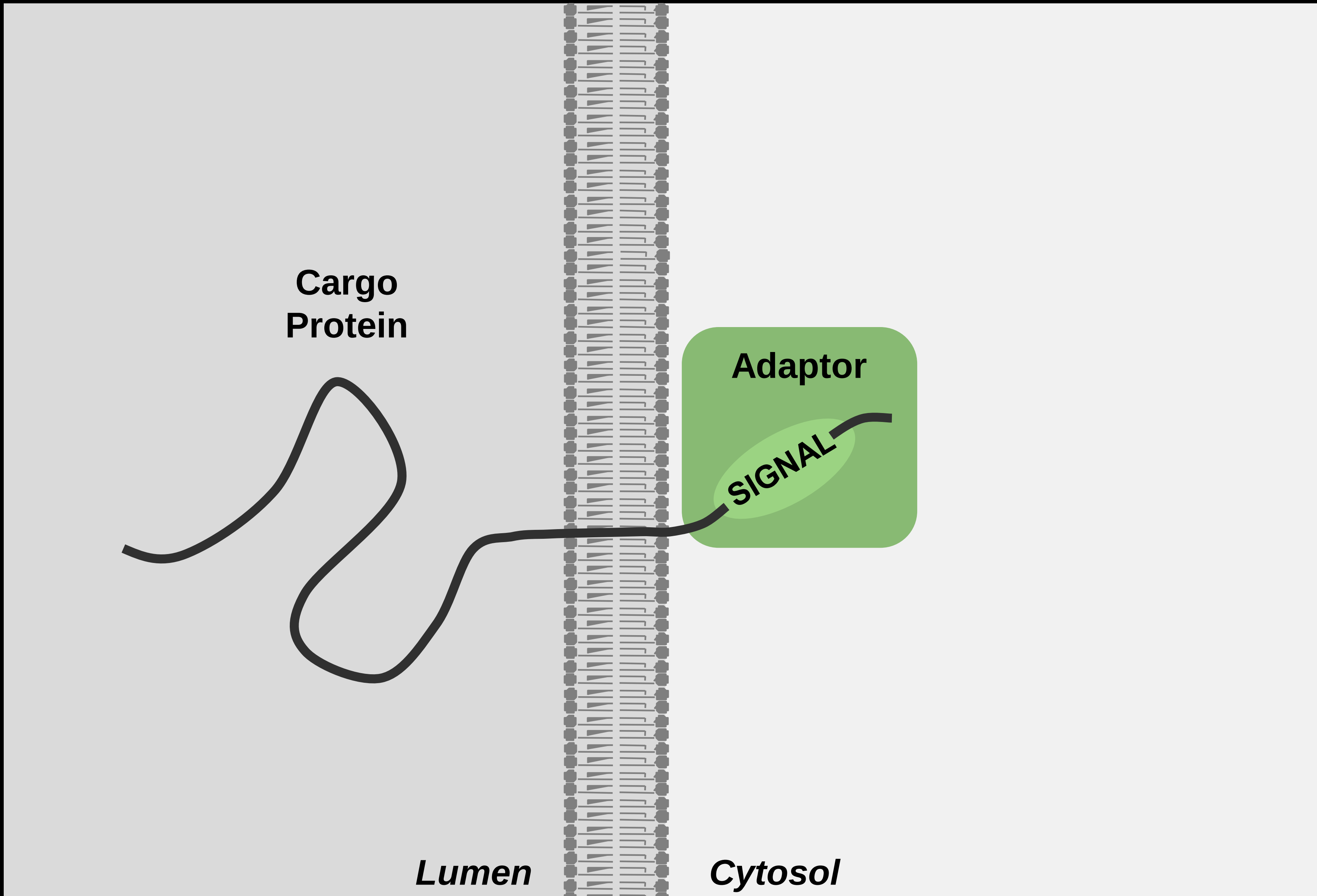
Lydia Yuan

Sorting Signals



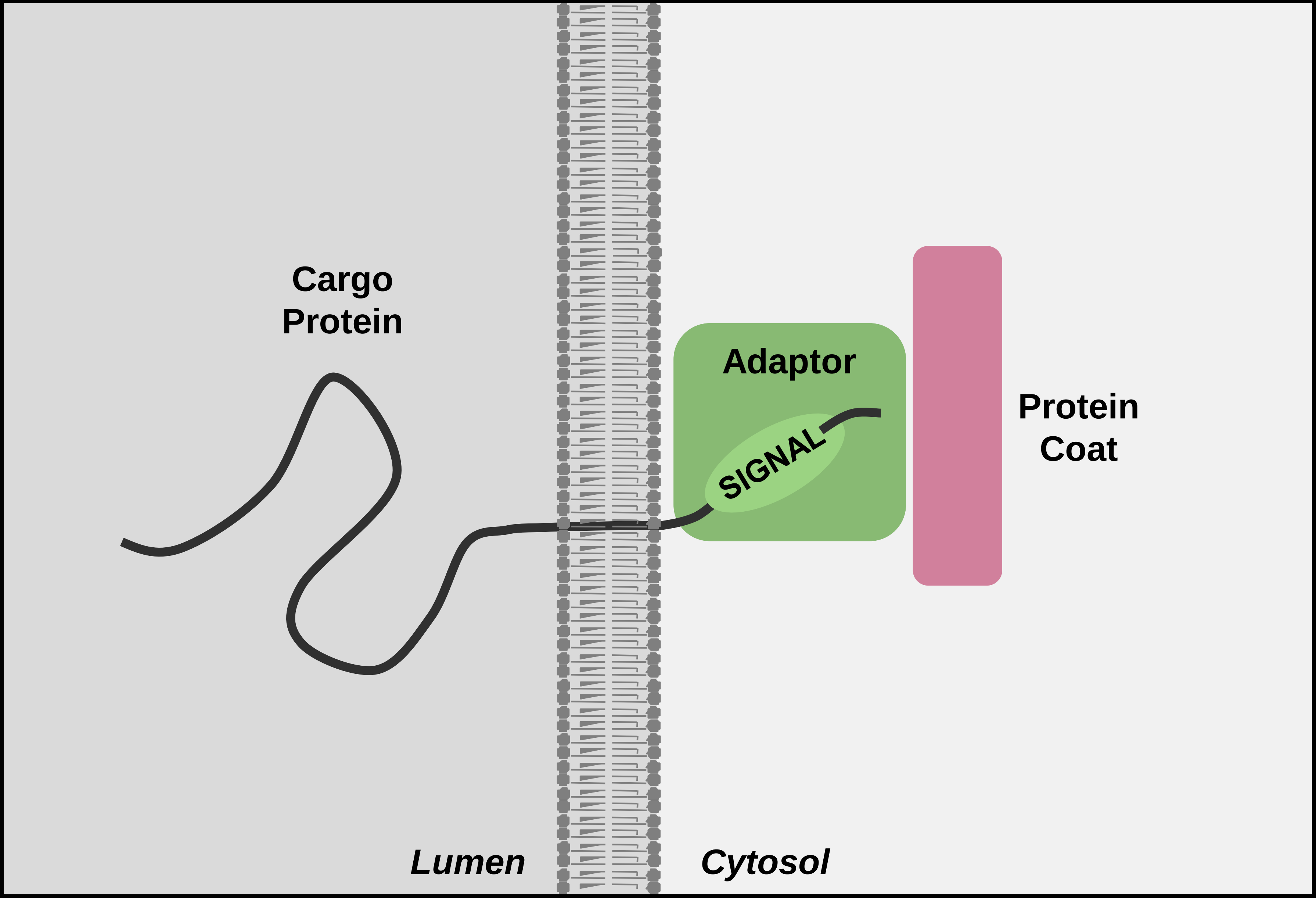
Sorting Signals

Adaptors

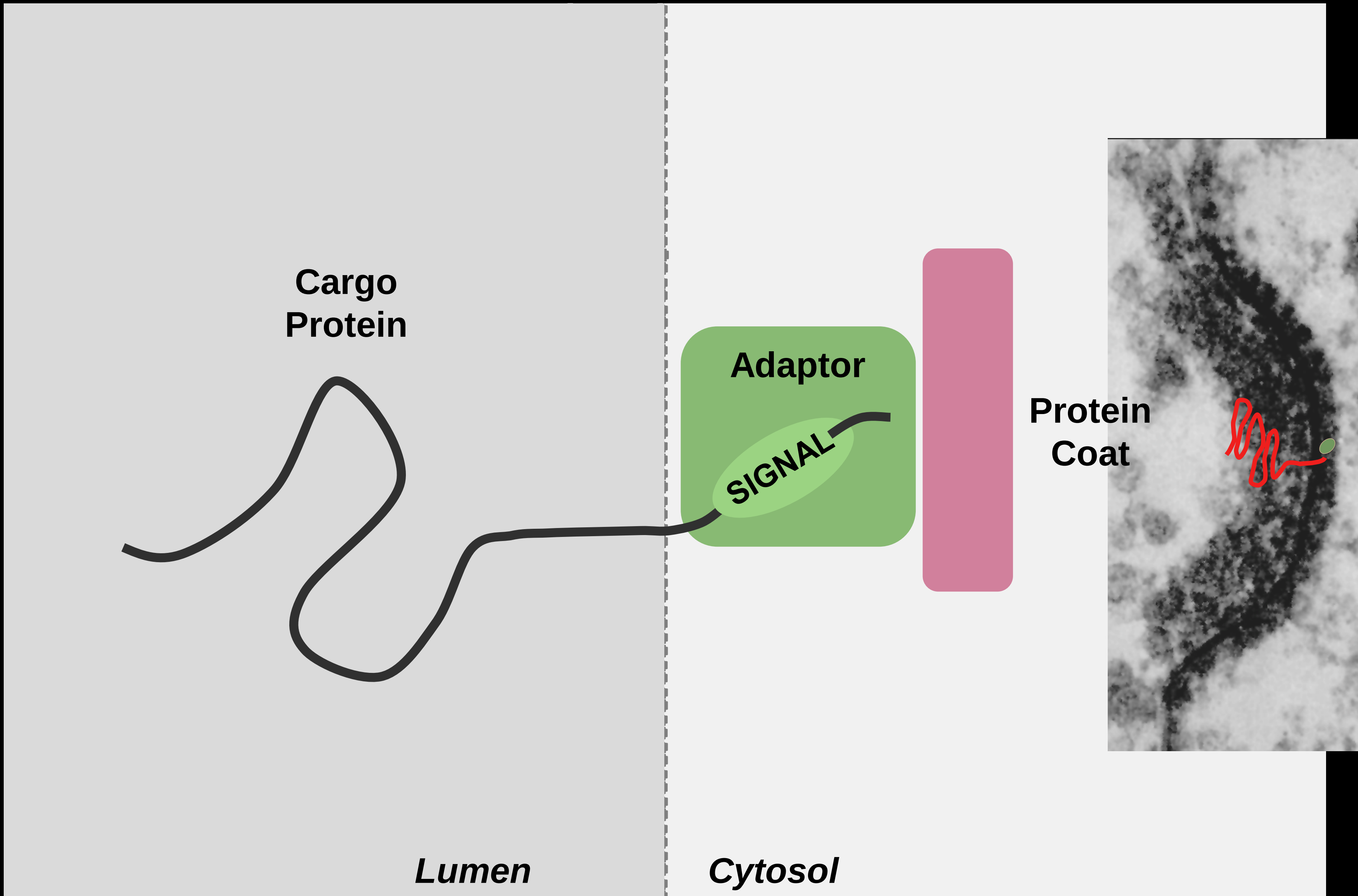


Adaptors

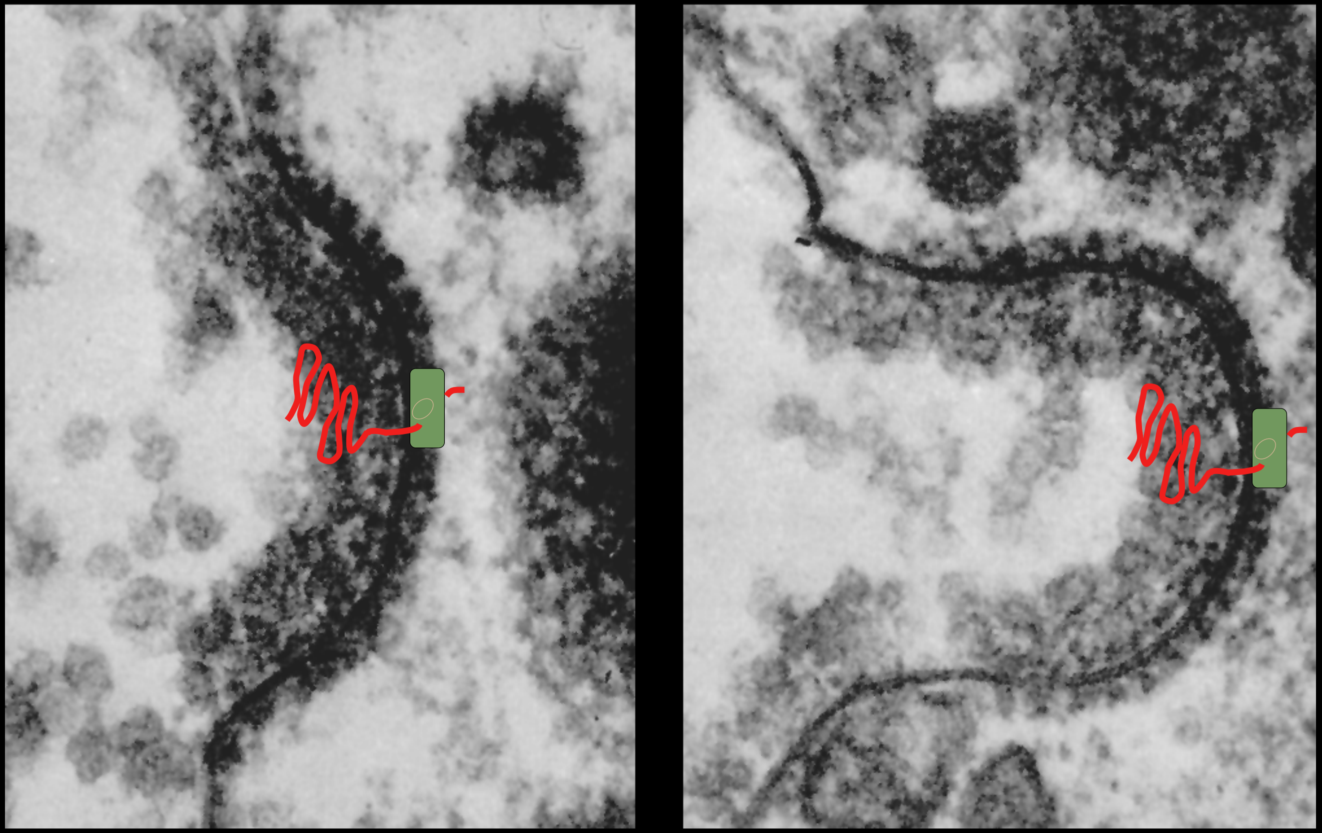
Protein Coats



Protein Coats

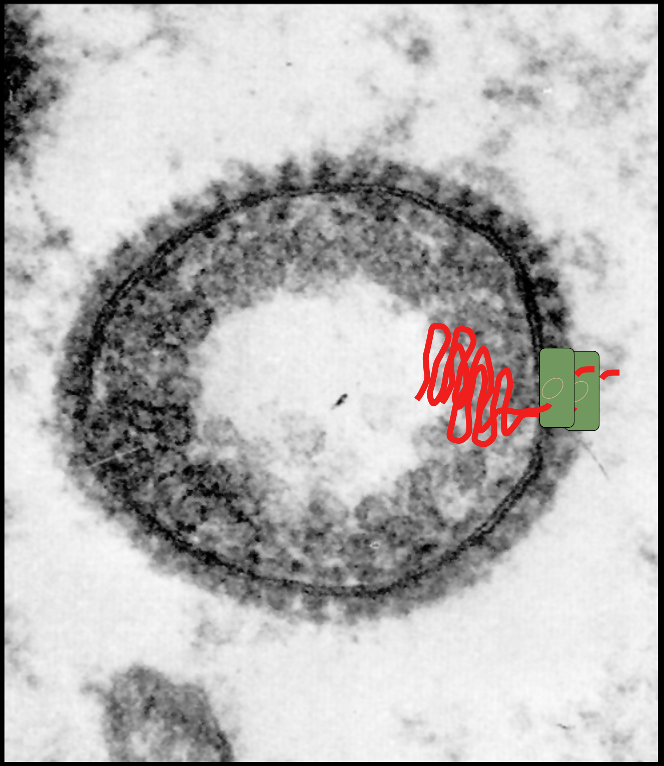


Clathrin-coated Vesicles



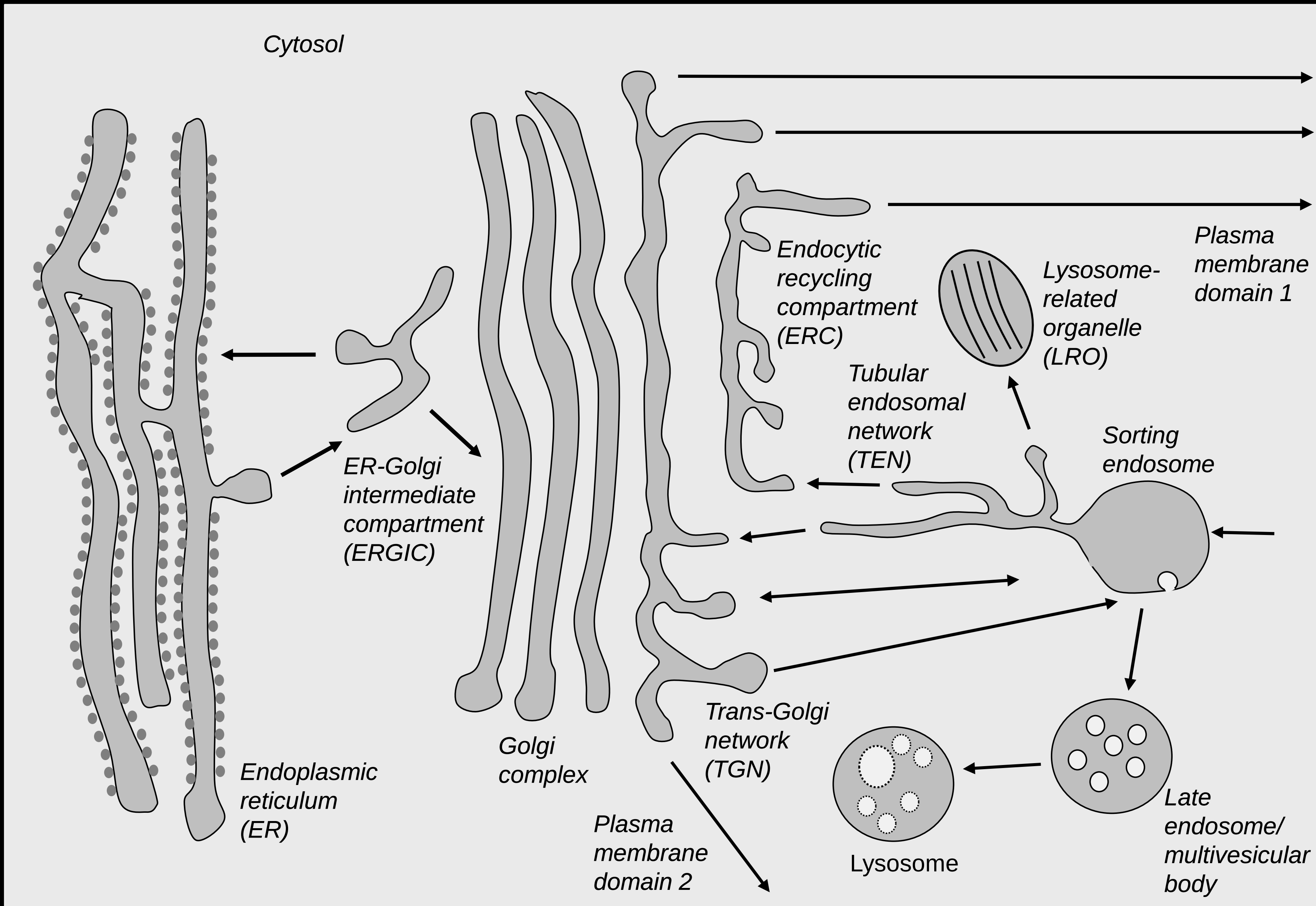
Perry & Gilbert

Clathrin-coated Vesicles

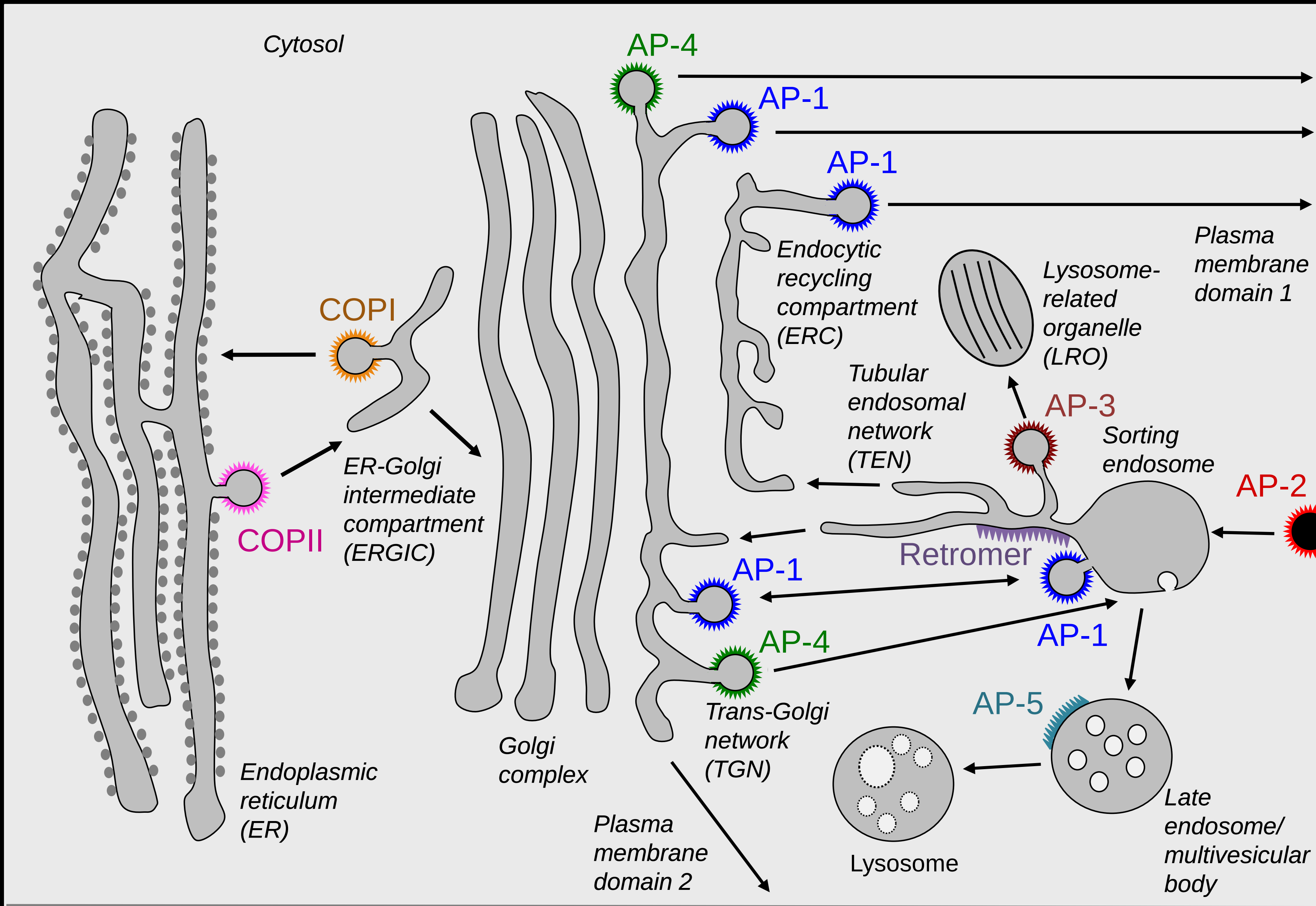


Perry & Gilbert

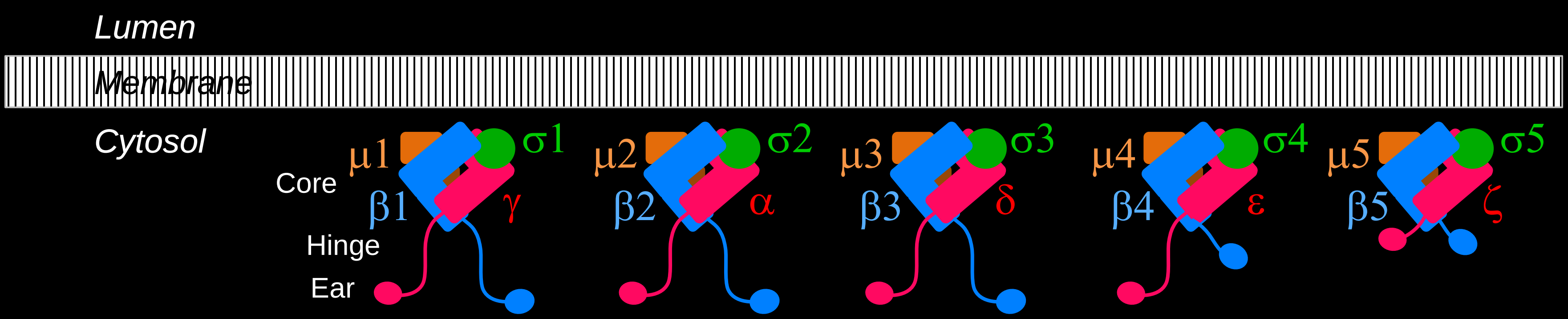
The Endomembrane System



Protein Coats in the Endomembrane System

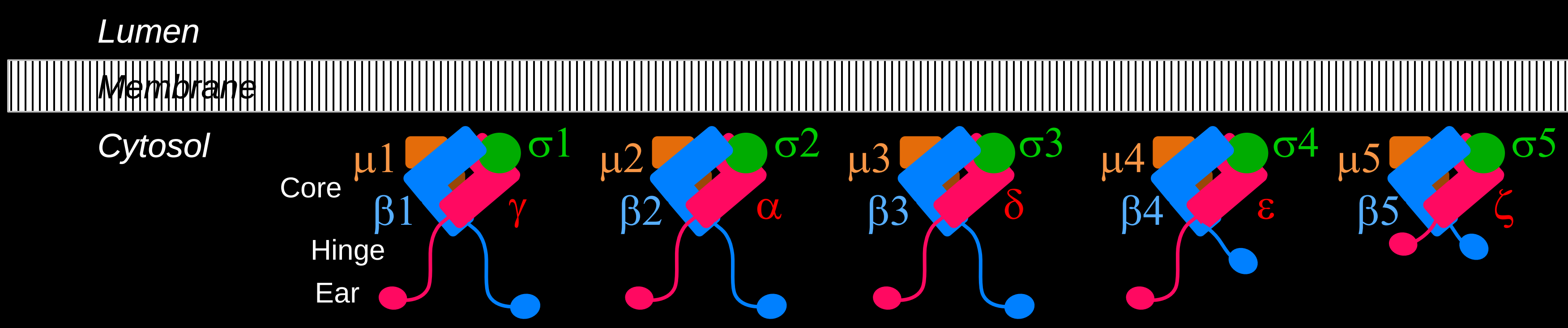


Adaptor Protein (AP) Complexes



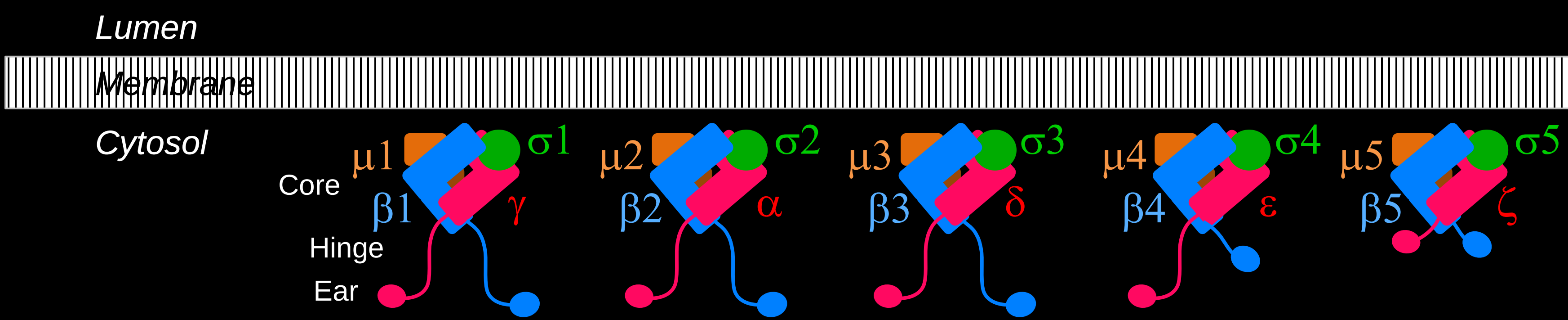
Adaptors	AP-1	AP-2	AP-3	AP-4	AP-5

Coatopathies



Adaptors	AP-1	AP-2	AP-3	AP-4	AP-5
Diseases (coatopathies)	MEDNIK syndrome (σ1A) Fried/Pettigrew syndrome (σ1B) Pustular psoriasis 15 (σ1C)	Hypocalciuric hypercalcemia type III (μ2)	Hermansky-Pudlak syndrome (HPS) type 2 (β3A) and type 10 (δ) Early onset epileptic encephalo pathy (EOEE) type 48 (β3B)	Hereditary spastic paraplegia (HSP) types 47 (β4), 50 (μ4), 51 (ε) and 52 (σ4)	Hereditary spastic paraplegia type 48 (ζ)

Coatopathies



Adaptors	AP-1	AP-2	AP-3	AP-4	AP-5
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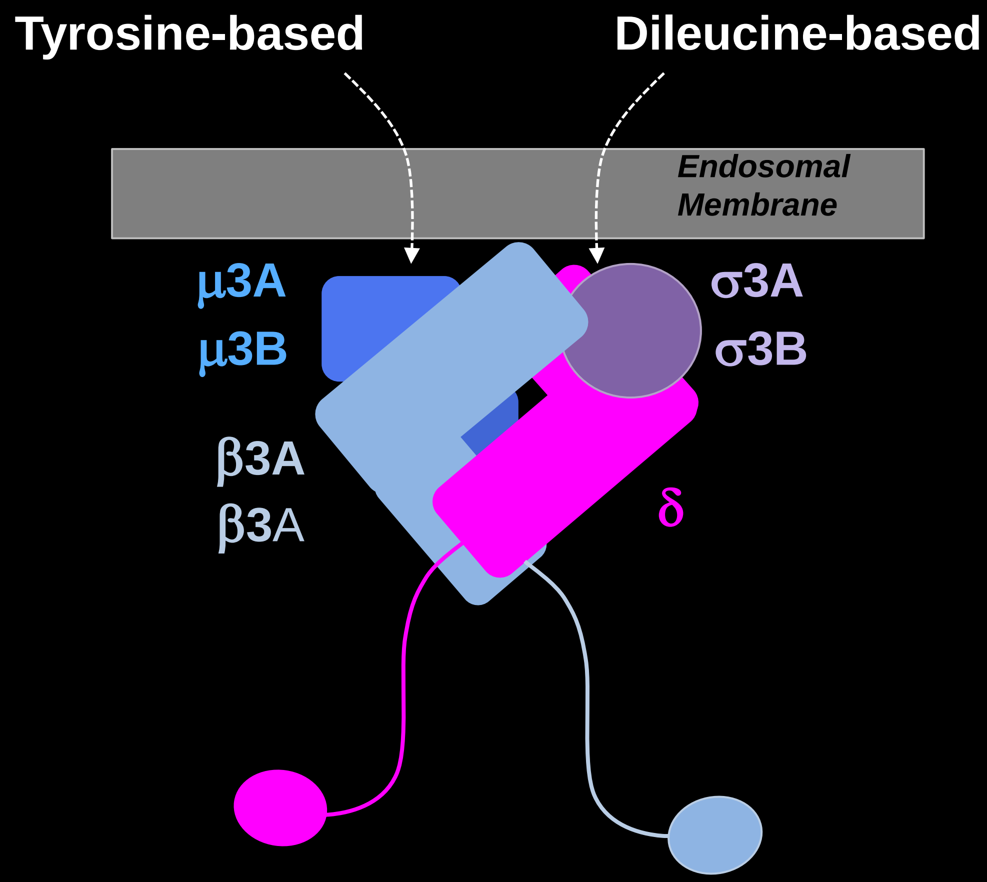
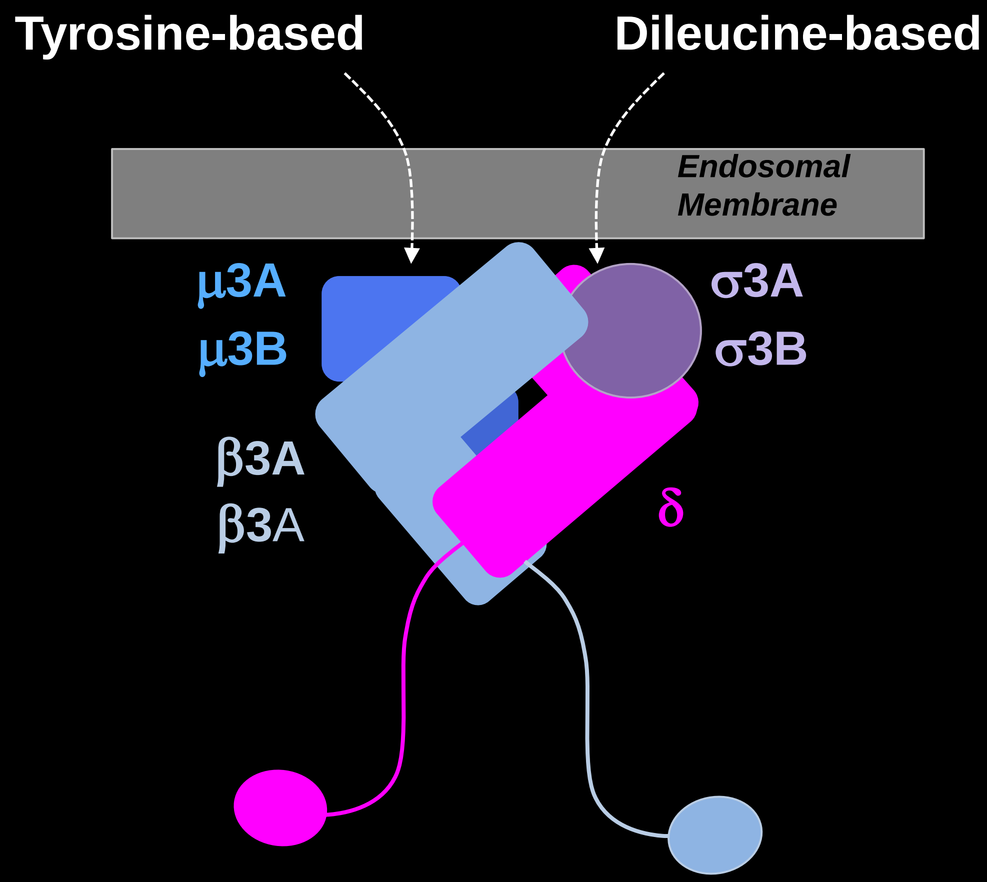
AP-3



Esteban Dell'Angelica

What is the Physiological Role of AP-3?



Esteban Dell'Angelica

BLAST Search Identifies Garnet As Drosophila AP-3 δ

```
>gi|24641854|ref|NP_524785.2|  garnet CG10986-PB [Drosophila melanogaster]
     gi|22832217|gb|AAF48307.2|   CG10986-PB [Drosophila melanogaster]
                                   Length = 1034

                   Score =  912 bits (2358), Expect = 0.0
 Identities = 497/848 (58%), Positives = 609/848 (71%), Gaps = 49/848 (5%)

 Query: 1   MALKMVKGS-IDRMFDKNLQDLVRGIRNHKEDEAKYISQCIDEIKQELKQDNIAVKANAV 59
             MALK VKG+  +RMFDKNL DLVRGIRN+K++EAKYIS CI+EIKQEL+QDNI+VK NAV
 Sbjct: 1   MALKKVKGNFFERMFDKNLTDLVRGIRNNKDNEAKYISTCIEEIKQELRQDNISVKCNAV 60

Query: 60  CKLTYLQMLGYDISWAAFNIIEVMSASKFTFKRIGYLAASQSFHEGTDVIMLTTNQIRKD 119
              KLTY+QMLGYDISWA FNIIEVMS+S+FT KRIGYLAASQ FH  ++++MLTTN IRKD
Sbjct: 61  AKLTYIQMLGYDISWAGFNIIEVMSSSRFTCKRIGYLAASQCFHPDSELLMLTTNMIRKD 120

Query: 120 LSSPSQYDTGVALTGLSCFVTPDLARDLANDIMTLMSHTKPYIRKKAVLIMYKVFLKYPE 179
             L+S +QYD GVAL+GLSCF++PDL+RDLANDIMTLMS TKPY+R KAVL+MYKVFL+YPE
Sbjct: 121 LNSQNQYDAGVALSGLSCFISPDLSRDLANDIMTLMSSTKPYLRMKAVLMMYKVFLRYPE 180

Query: 180 SLRPAFPRLKEKLEDPDPGVQSAAVNVICELARRNPKNYLSLAPLFFKLMTSSTNNWVLI 239
             +LRPAFP+LKEKLEDPDPGVQSAAVNVICELAR+NPKNYL LAP+FFKLMT+STNNW+LI
Sbjct: 181 ALRPAFPKLKEKLEDPDPGVQSAAVNVICELARKNPKNYLPLAPIFFKLMTTSTNNWMLI 240

Query: 240 KIIKLFGALTPLEPRLGKKLIEPLTNLIHSTSAMSLLYECVNTVIAVLISLSSGMPNHSA 299
             KIIKLFGALTPLEPRLGKKLIEPLTNLIHSTSAMSLLYEC+NTVIAVLIS+SSGMPNHSA
Sbjct: 241 KIIKLFGALTPLEPRLGKKLIEPLTNLIHSTSAMSLLYECINTVIAVLISISSGMPNHSA 300
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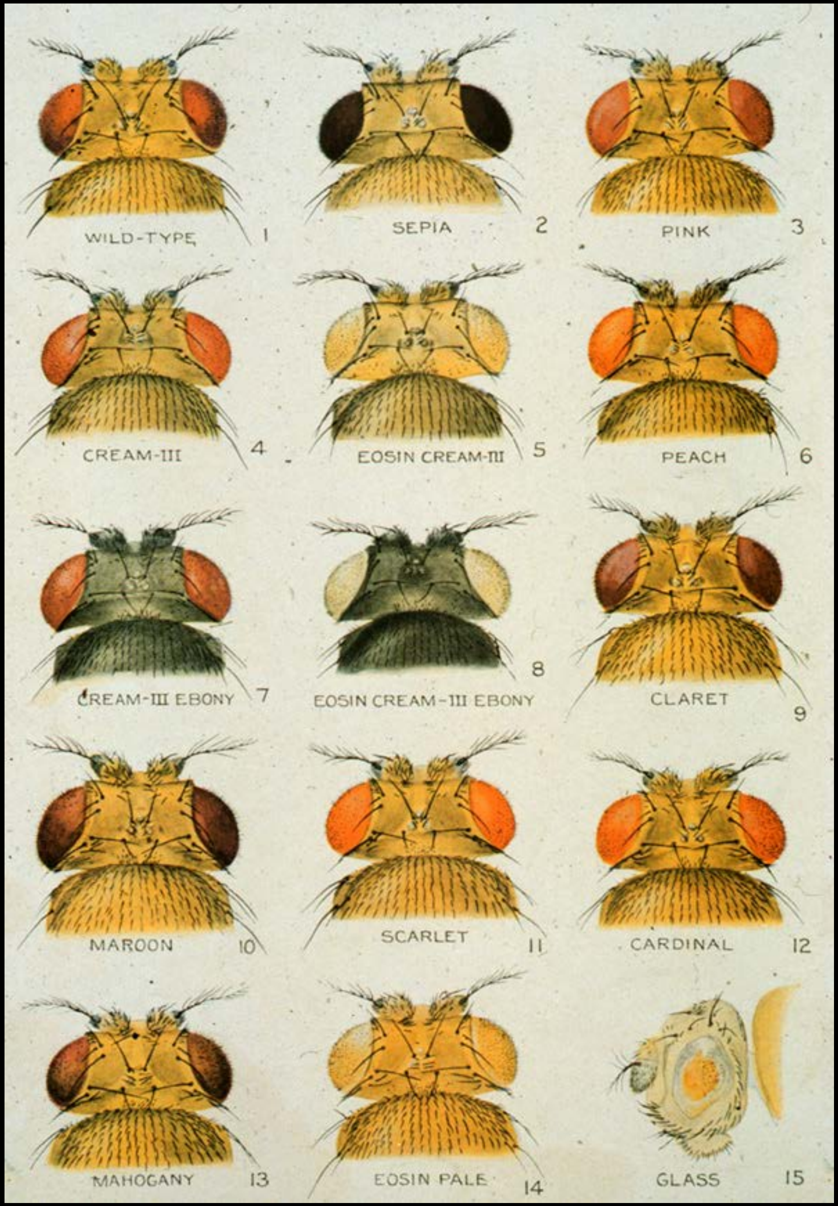
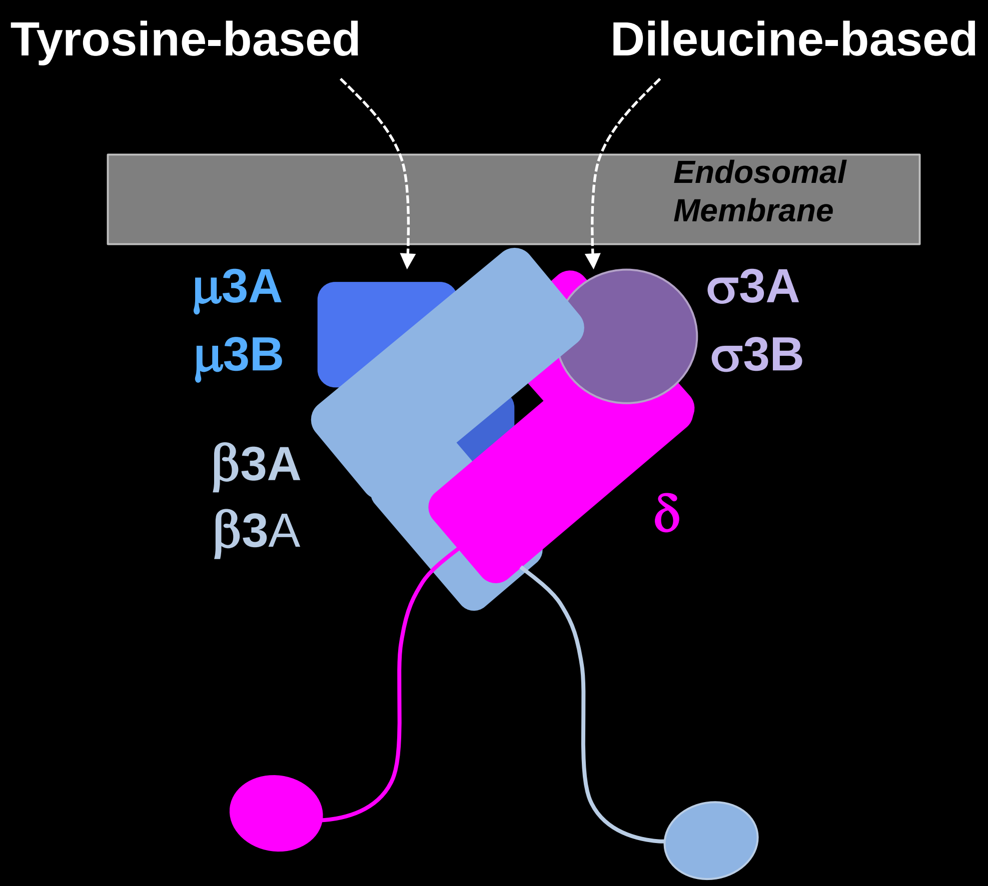
Chean Eng Ooi

Drosophila Pigmentation Mutants



Edith Wallace

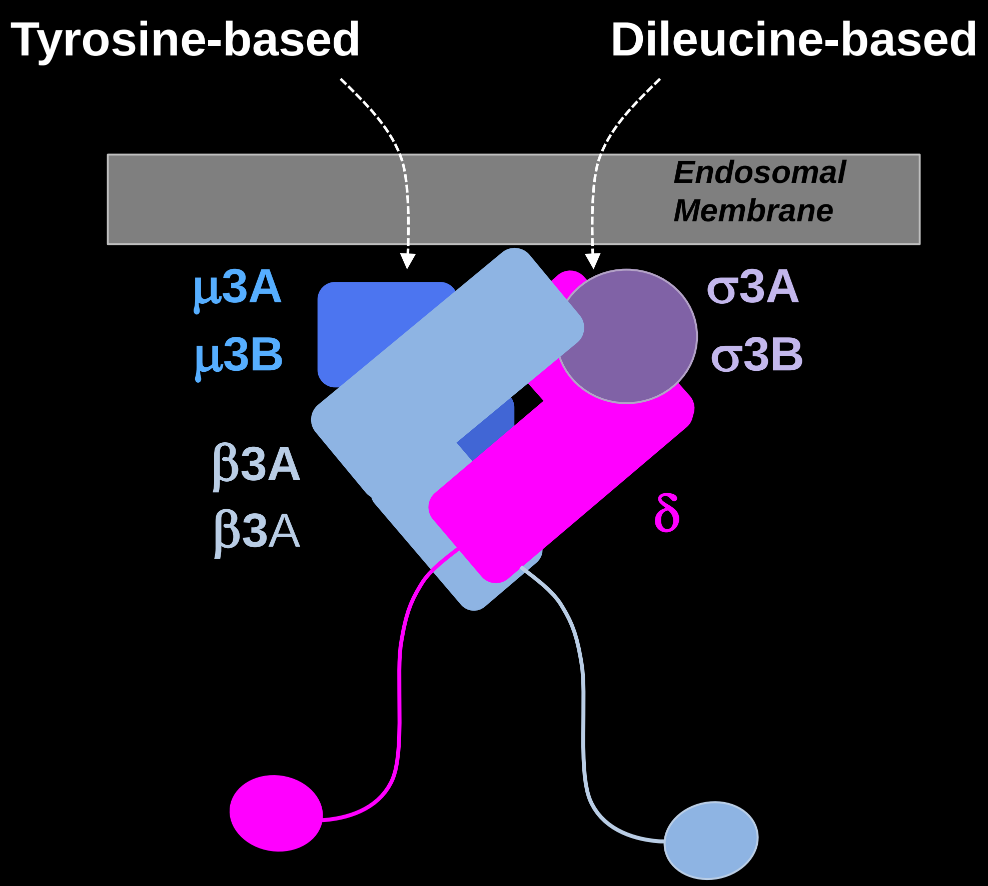
AP-3 Defects in Drosophila Pigmentation Mutants



Subunit	**Mutant**
δ	*garnet*

Chean Eng Ooi, Chris Mullins

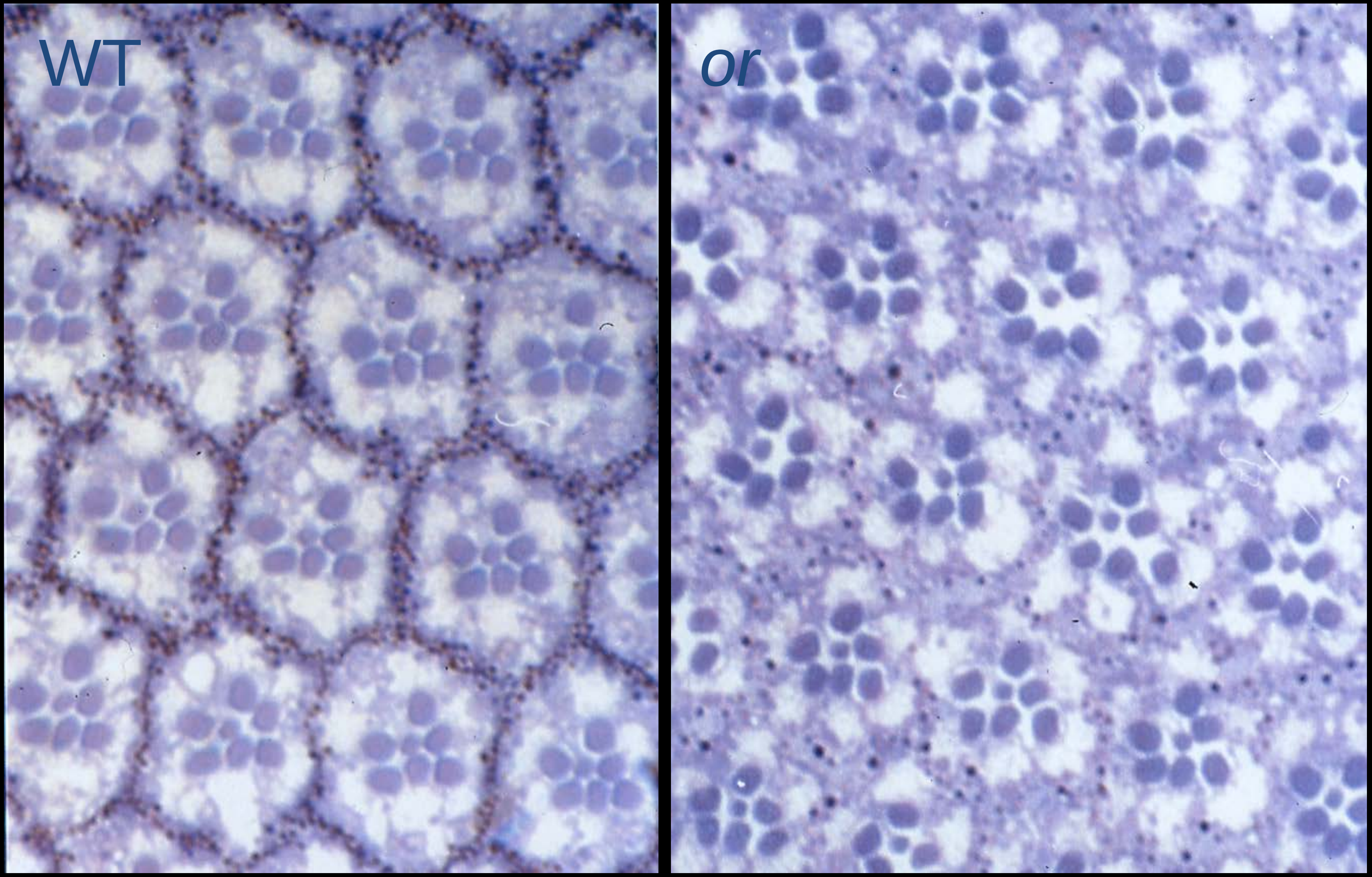
AP-3 Defects in Drosophila Pigmentation Mutants



Subunit	Mutant
δ	*garnet*
β3	*ruby*
μ3	*carmine*
σ3	*orange*

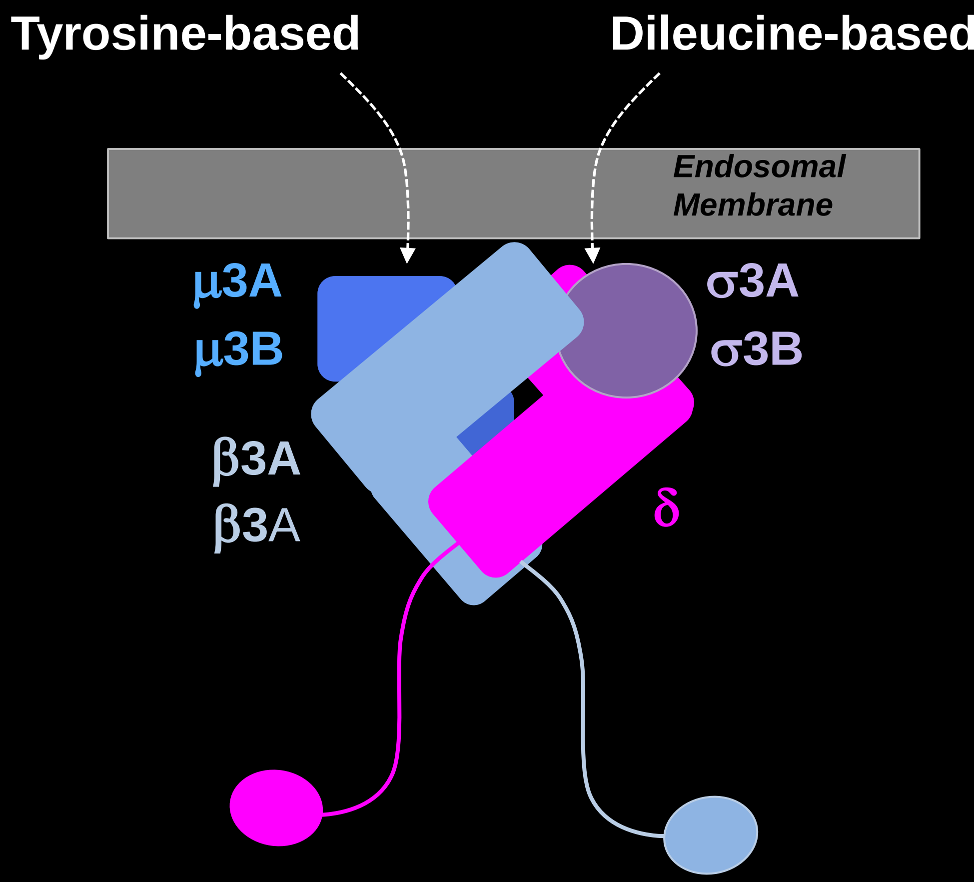
Chean Eng Ooi, Chris Mullins

Sections of Wild-Type and Orange Eyes



Lisa Hartnell

Could Mutations in AP-3 Cause Pigmentation Defects in Humans?



Hermansky-Pudlak Syndrome (HPS)

CHAPTER 220

Albinism

Richard A. King ▪ Vincent J. Hearing
Donnell J. Creel ▪ William S. Oetting

1. Melanocytes represe
cells, yet they are exc
melanin that account
in the skin, hair, and
number of critical st
number of genes hav
processes either dire
of these genes produ
tation, such as albini
2. Melanocytes in the s
either directly, as in
keratinocytes, or ind
produced by other

Hermansky Pudlak Syndrome Gene (*HPS1*)

Hermansky-Pudlak syndrome (HPS) (MIM 203300) is a very rare type of albinism, but two isolated populations in Puerto Rico and Switzerland, provided an opportunity to map one of the HPS loci (*HPS1*) to chromosome 10q23.[60–62] Both mapping strategies were based on a common founder for the mutated gene in these two isolates. The syntenic murine region contains the *pale ear* (*ep*) and *ruby eye* (*ru*), two candidate mouse models for HPS, and *ep* is the murine homologue of *HPS1*.[63,64] The gene consists of 20 exons and spans 30.5 kb.[299] In the initial isolation of the *HPS1* gene, a 3.6-kb transcript was identified that encodes a 700-amino acid protein with a size of 79.3 kDa.[63,299] Mutations in the 3′ portion of this gene were associated with the HPS phenotype, indicating that this was the correct transcript responsible for the HPS phenotype.[63] A second 1.5-kb transcript of the *HPS1* gene that encodes a 324-amino acid protein was also identified.[67] The two cDNA transcripts are from the same gene and result from alternative splicing.[67] Both transcripts are polyadenylated, and contain transmembrane domains and a putative melanosomal localization signal. The 3.6-kb transcript codes for a transmembrane protein containing two transmembrane domains, where the 1.5-kb transcript is missing the transmembrane domain in the carboxy end of the protein. There are no homologies between the putative *HPS1* gene product and known proteins and its function are currently unknown. Initial analysis using confocal immunofluorescence has shown a cytoplasmic and membrane-associated distribution of the protein, suggesting a soluble and nonsoluble

and has been identified as a lysosomal trafficking regulator (LYST). This would explain the abnormal trafficking of melanogenic proteins found in the melanocytes. Several mutations in the mouse have been described.[304] The bg^{11} is a 5-kb deletion at the 3′ end of the gene that disrupts three exons, which would result in a truncated protein with probable splicing abnormalities as well. The bg^{2J} mutation has a drastically reduced level of transcription, due to a 116-bp insertion of a LINE1 sequence in the coding region producing a truncated protein.[303] The bg^{8J} allele has a nucleotide substitution of a C to T at bp 2027, producing a nonsense mutation that is predicted to lack 1442 amino acids.[304]

Ocular Albinism (OA1) (MIM 300500)

The ocular albinism type 1 gene (OA1; Nettleship-Falls X-linked OA) maps to chromosome Xp22.[305,306] Both the human (*OA1*) and the murine (*Moa1*) genes have been isolated.[307,308] In humans, the *OA1* gene is divided into 9 exons within a 40-kb region.[307] The gene codes for a protein of 424 amino acids that contains several putative transmembrane regions.[307,309,310] The amino acid sequence does not share identity with any known proteins and its function is unknown. The gene is expressed almost exclusively in the retinal pigment epithelium[307] and cutaneous melanocytes,[310] and at a much lower level in the brain and adrenal tissues.[307] Although the clinical manifestations involve primarily the eye, the protein is a membrane glycoprotein localized to the melanosome in ocular and cutaneous melanocytes, indicating OA1 is really a type of OCA with changes in the eye and skin melanocytes.[310]

The Metabolic and Molecular Bases of Inherited Disease

Hermansky-Pudlak Syndrome (HPS)

Hermansky-Pudlak Syndrome (HPS)

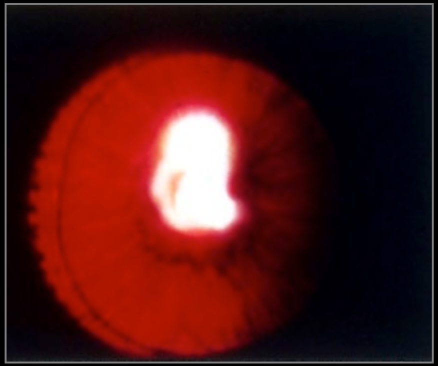
- **Autosomal recessive disorder**

Hermansky-Pudlak Syndrome (HPS)

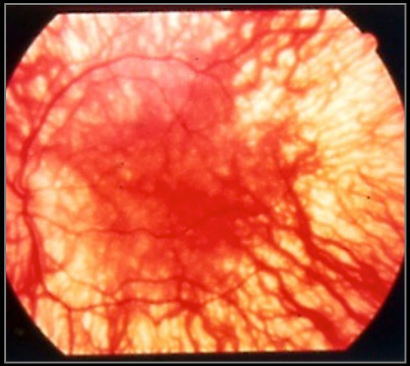
- **Autosomal recessive disorder**
- **Oculocutaneous albinism**

Hermansky-Pudlak Syndrome (HPS)

Hair and skin



Iris



Retina

Hermansky-Pudlak Syndrome (HPS)

- **Autosomal recessive disorder**
- **Oculocutaneous albinism**

  Abnormal melanosomes

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- **Autosomal recessive disorder**
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  Abnormal melanosomes

- **Prolonged bleeding**

  Absence of platelet dense granules

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    Absence of platelet dense granules

- **Fibrosis of the lungs, inflammatory colitis**

Hermansky-Pudlak Syndrome (HPS)

- **Autosomal recessive disorder**
- **Oculocutaneous albinism**

  Abnormal melanosomes
- **Prolonged bleeding**

  Absence of platelet dense granules
- **Fibrosis of the lungs, inflammatory colitis**

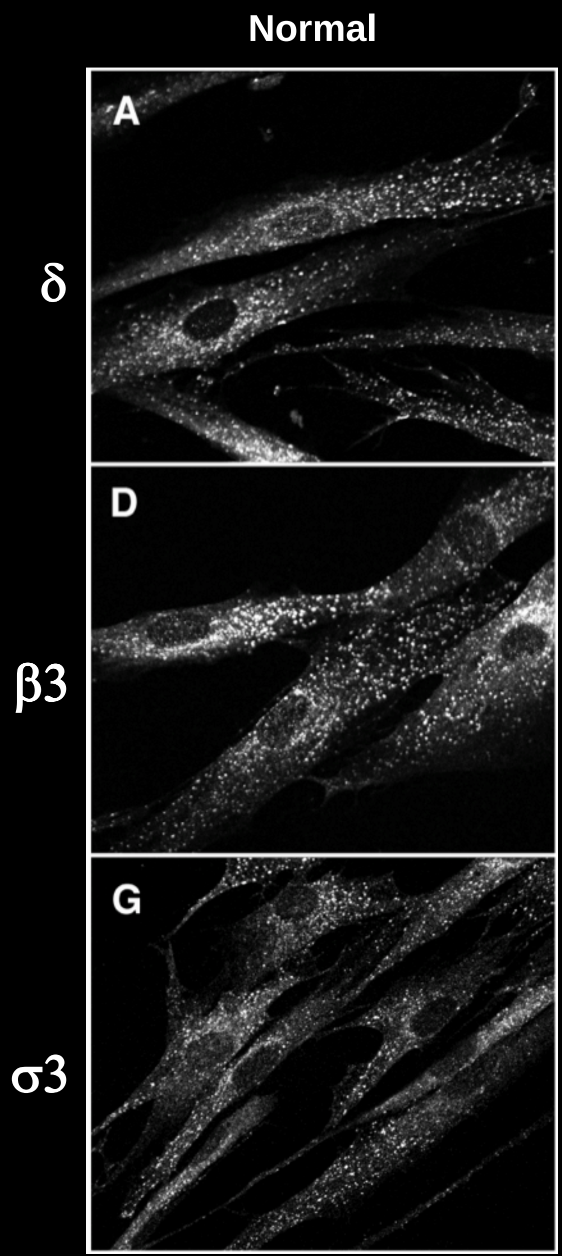
  Abnormal lung lamellar bodies, ceroid lipofucsin in macrophages

William Gahl and Colleagues (NICHD/NHGRI)

HPS Patients

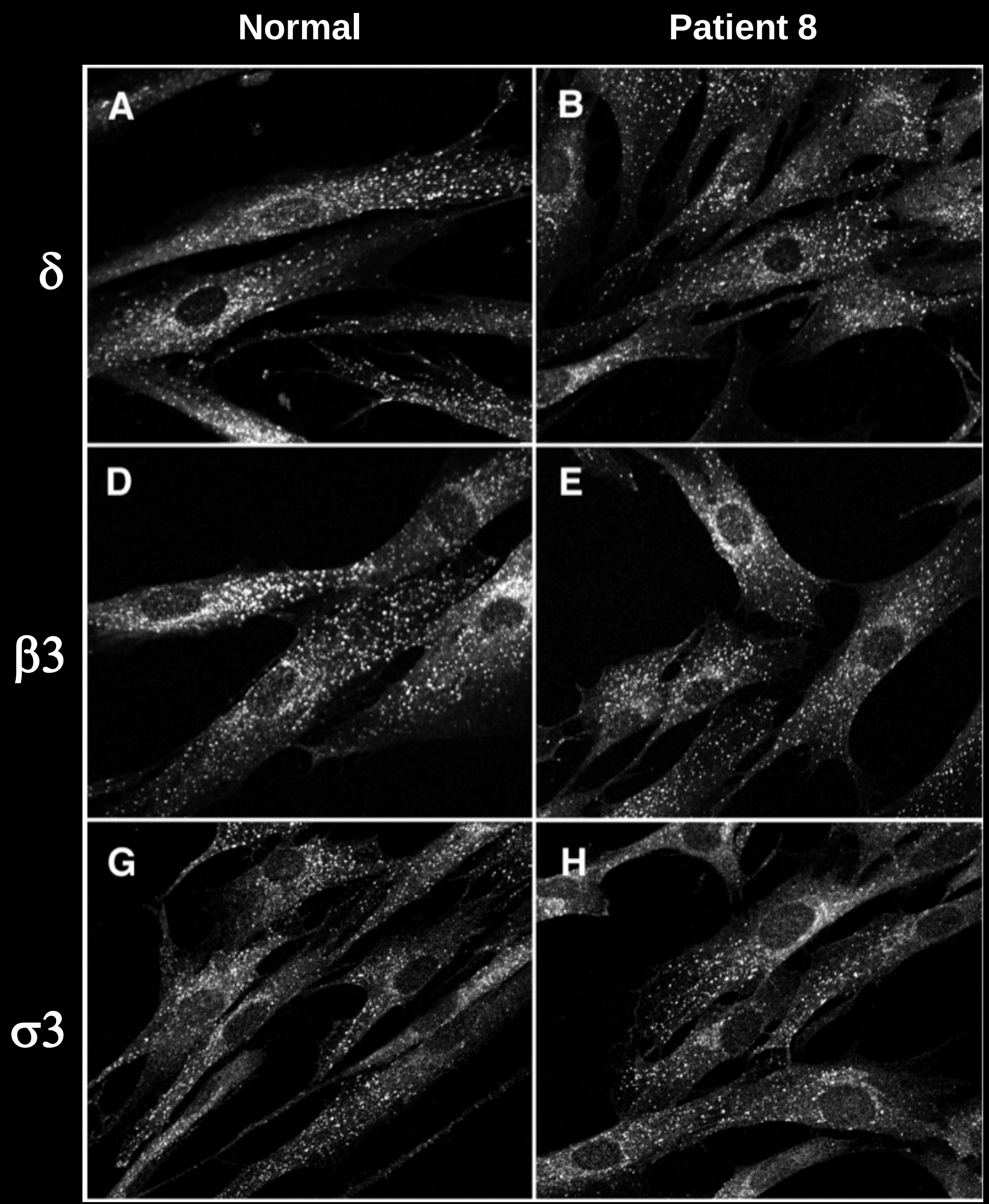
Photos Courtesy of Bill Gahl

AP-3 Defects in HPS Fibroblasts



Esteban Dell'Angelica

AP-3 Defects in HPS Fibroblasts



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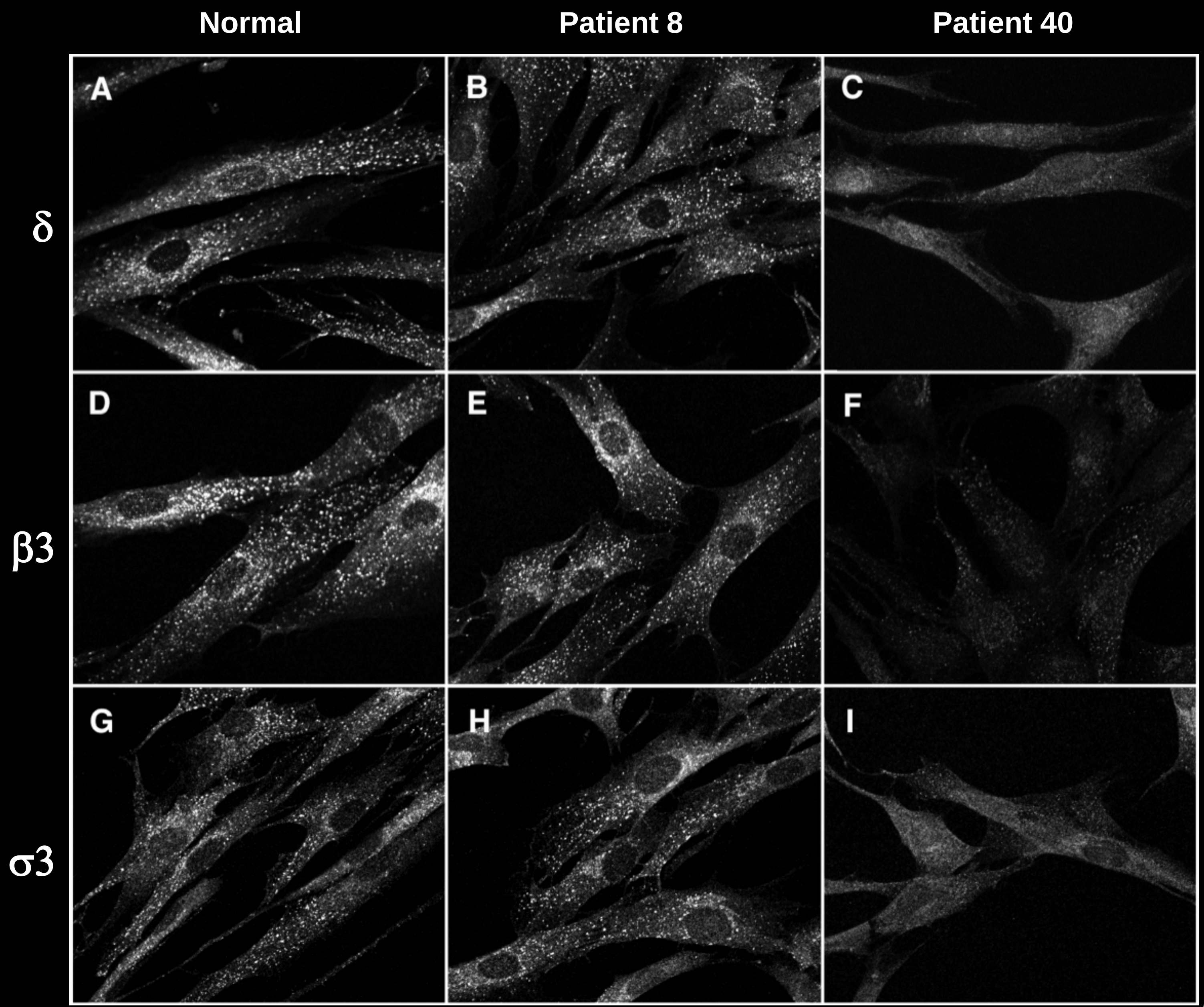
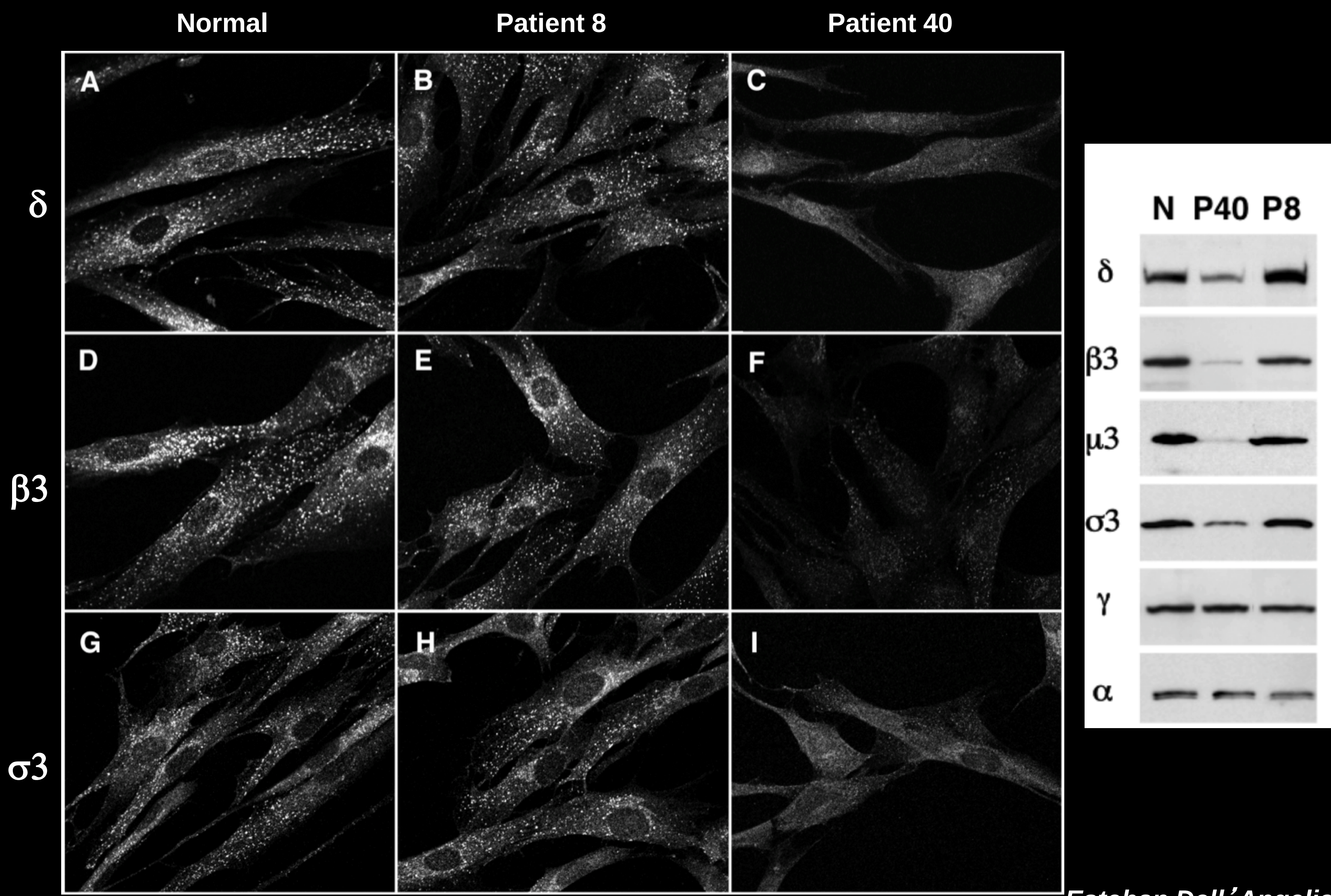


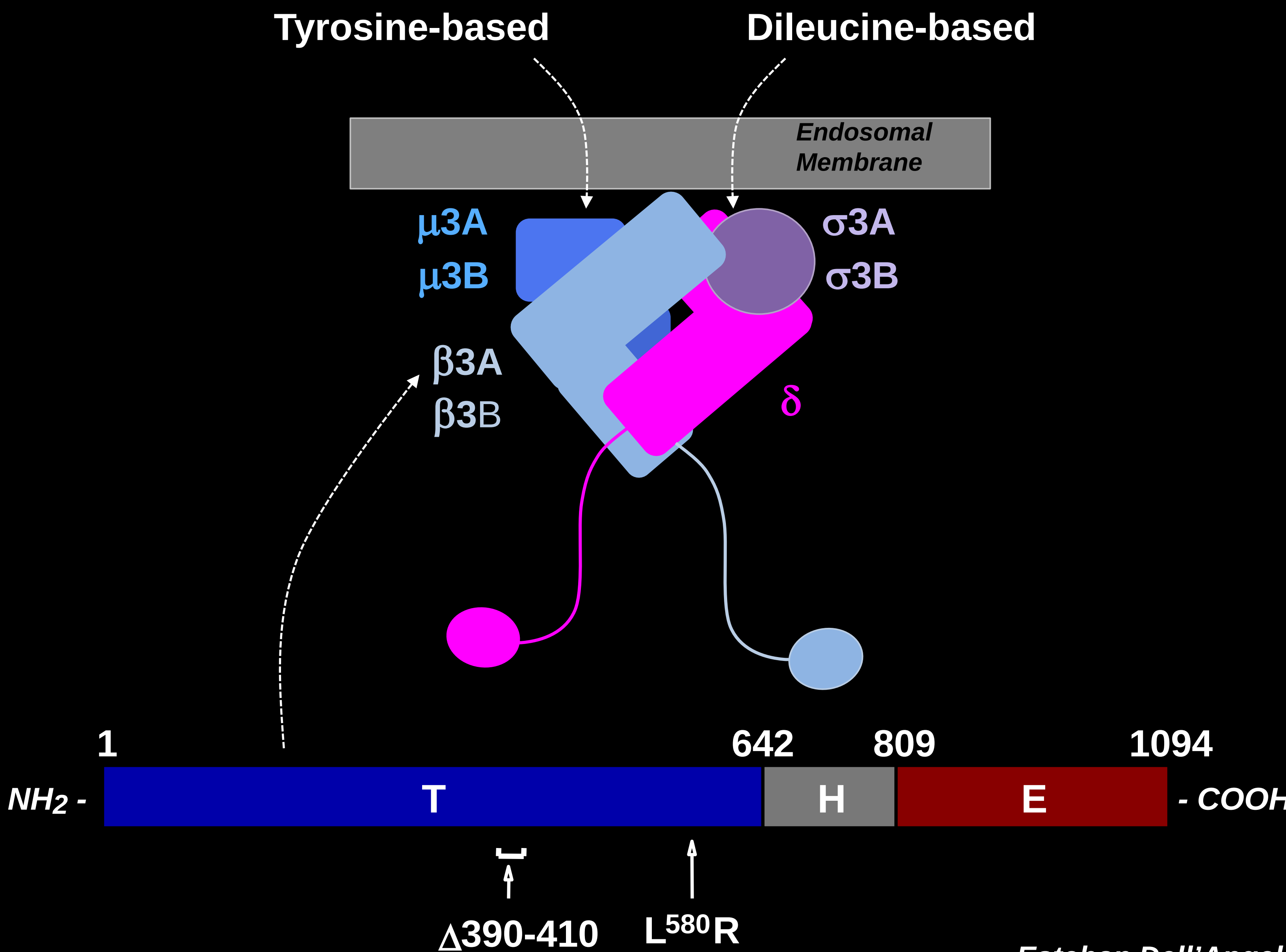
Esteban Dell'Angelica

AP-3 Defects in HPS Fibroblasts



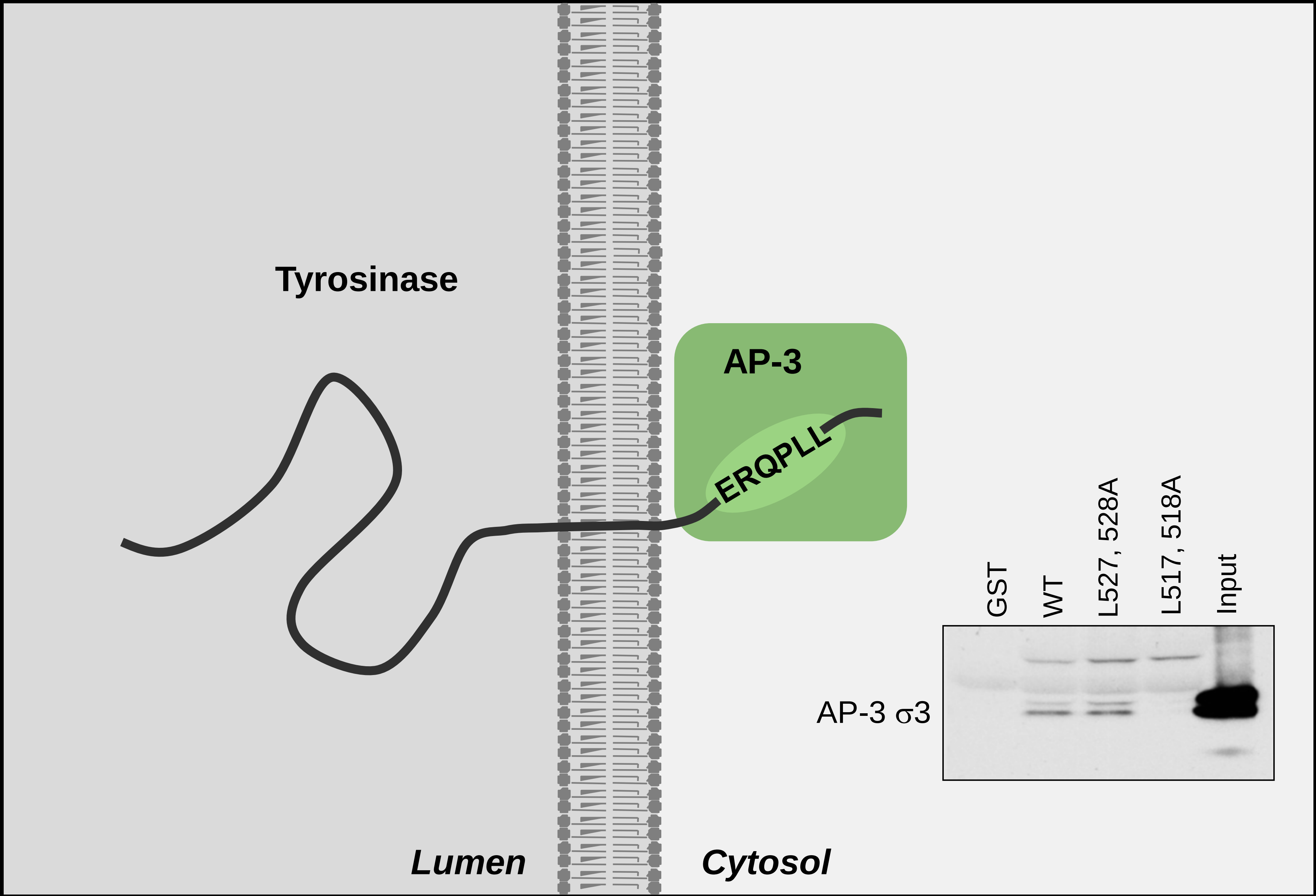
Esteban Dell’Angelica

Mutations in β3A in HPS-2



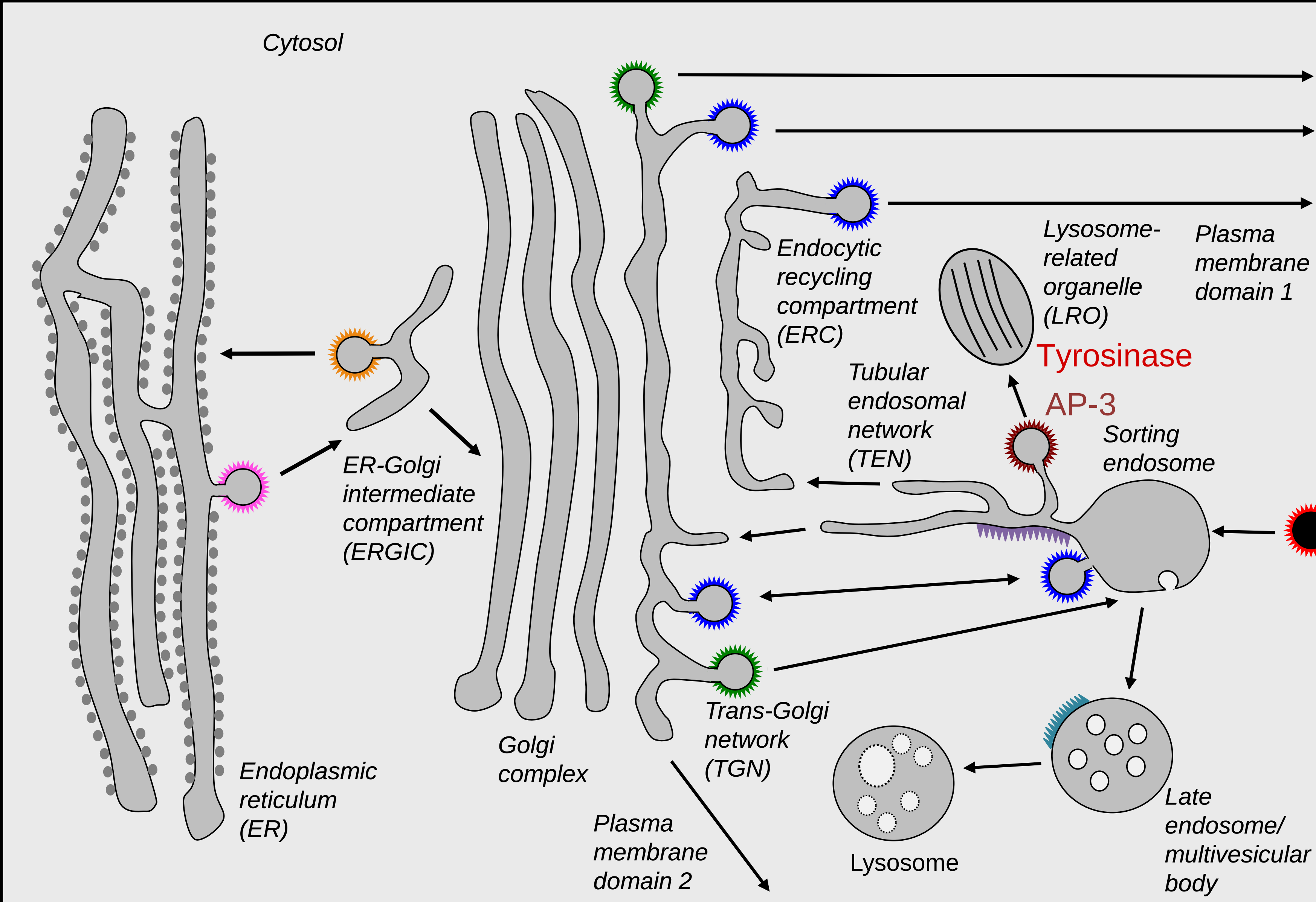
Esteban Dell'Angelica

Signal-Mediated Sorting of Tyrosinase by AP-3



José Martina, collaboration with Mickey Marks (U. Penn)

AP-3 Sorts Tyrosinase to Melanosomes



Collaboration with Mickey Marks (U. Penn)

Heterogeneity of HPS

Photos Courtesy of Bill Gahl

Human HPS Types

Photo Courtesy of Bill Gahl

HPS1

HPS2

HPS3

HPS4

HPS5

HPS6

HPS7

HPS8

HPS9

HPS10

Human and Mouse HPS Types

Photo Courtesy of Bill Gahl

HPS1	Pale ear
HPS2	Pearl
HPS3	Cocoa
HPS4	Light ear
HPS5	Ruby eye-2
HPS6	Ruby eye
HPS7	Sandy
HPS8	Reduced pigmentation
HPS9	Muted
HPS10	Mocha
	Cappuccino
	Pallid
	Subtle gray
	Gunmetal

Biogenesis of Lysosome-Related Organelles Complexes (BLOCs)

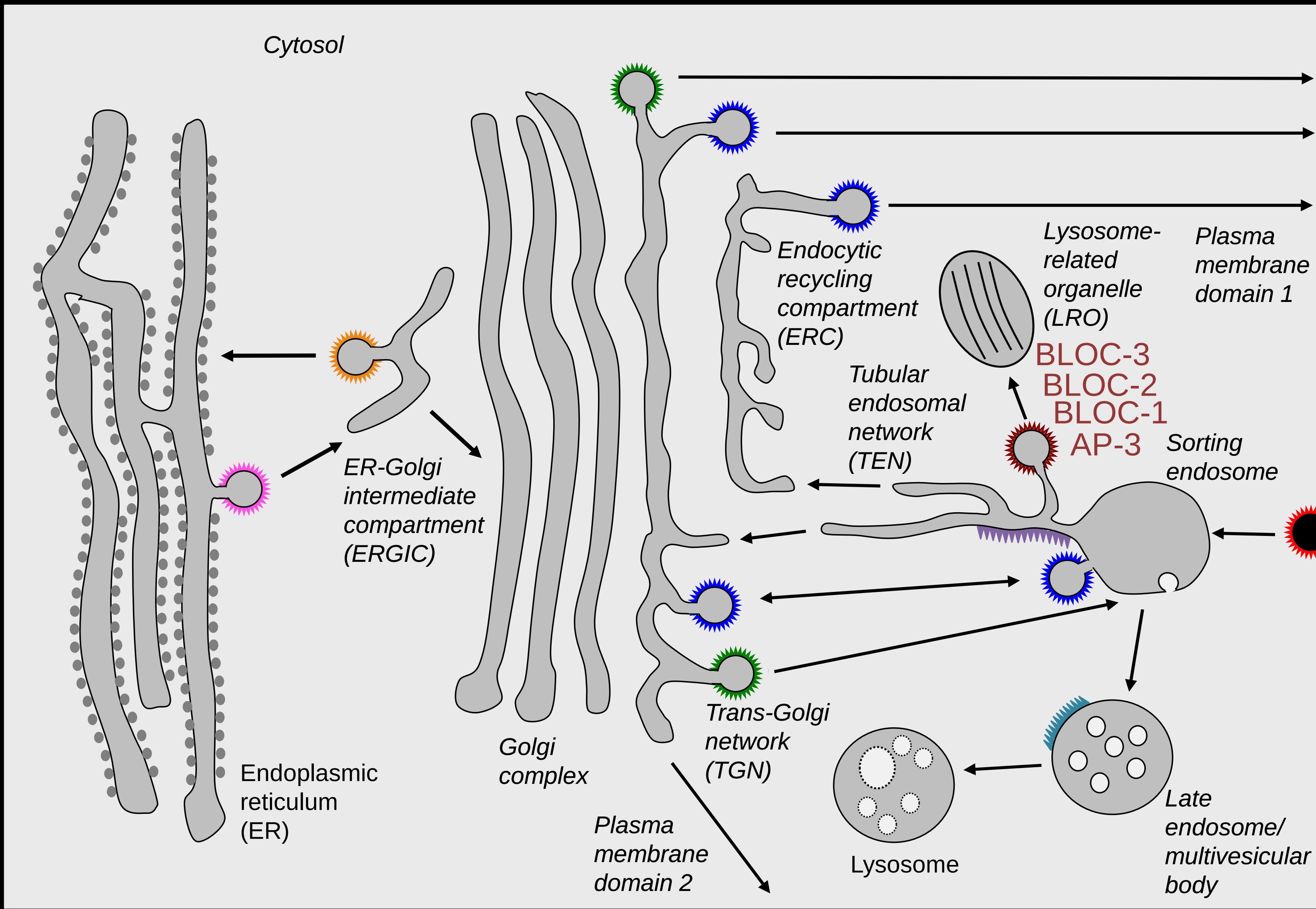
BLOC-1
- BLOS1
- BLOS2
- Snapin
- Dysbindin
- Pallidin
- Muted
- Cappuccino
- BLOS3

BLOC-2
- HPS3
- HPS5
- HPS6

BLOC-3
- HPS1
- HPS4

Kengo Moriyama, José Martina, Dell'Angelica lab, Swank lab, Spritz lab

Biogenesis of Lysosome-Related Organelles Complexes (BLOCs)

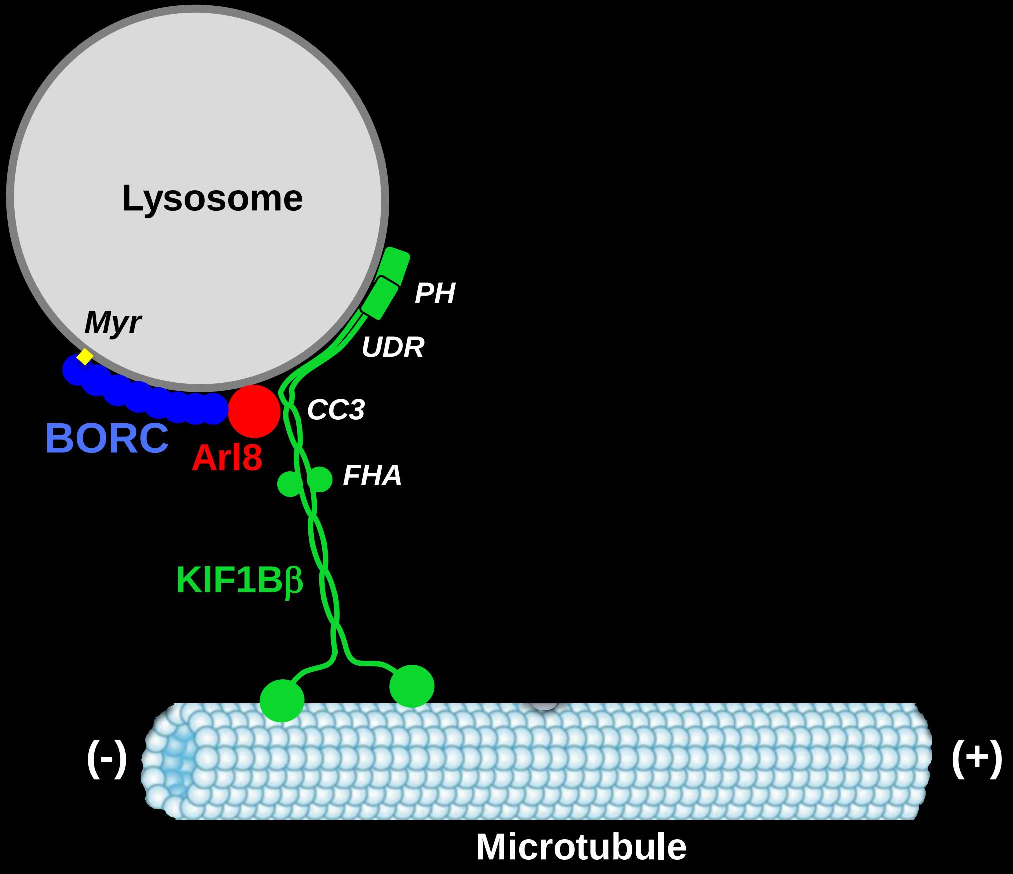


BLOC-One-Related Complex (BORC)

BLOC-1	BLOC-2	BORC
BLOS1	HPS3	BLOS1
BLOS2	HPS5	BLOS2
Snapin	HPS6	Snapin
Dysbindin	**BLOC-3**	Myrlysin
Pallidin	HPS1	Lyspersin
Muted	HPS4	Diaskedin
Cappuccino		MEF2BNB
BLOS3		KXD1

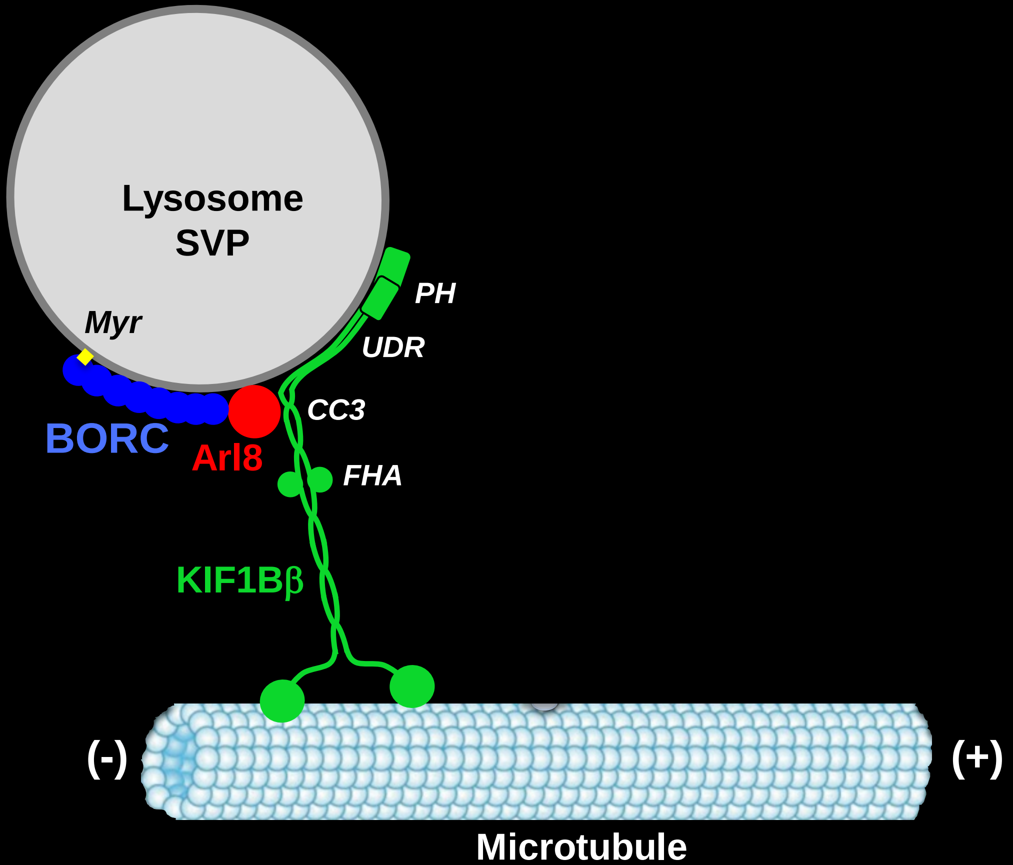
Chris Schindler, Jing Pu

BORC Couples Lysosomes and SVPs to Kinesins



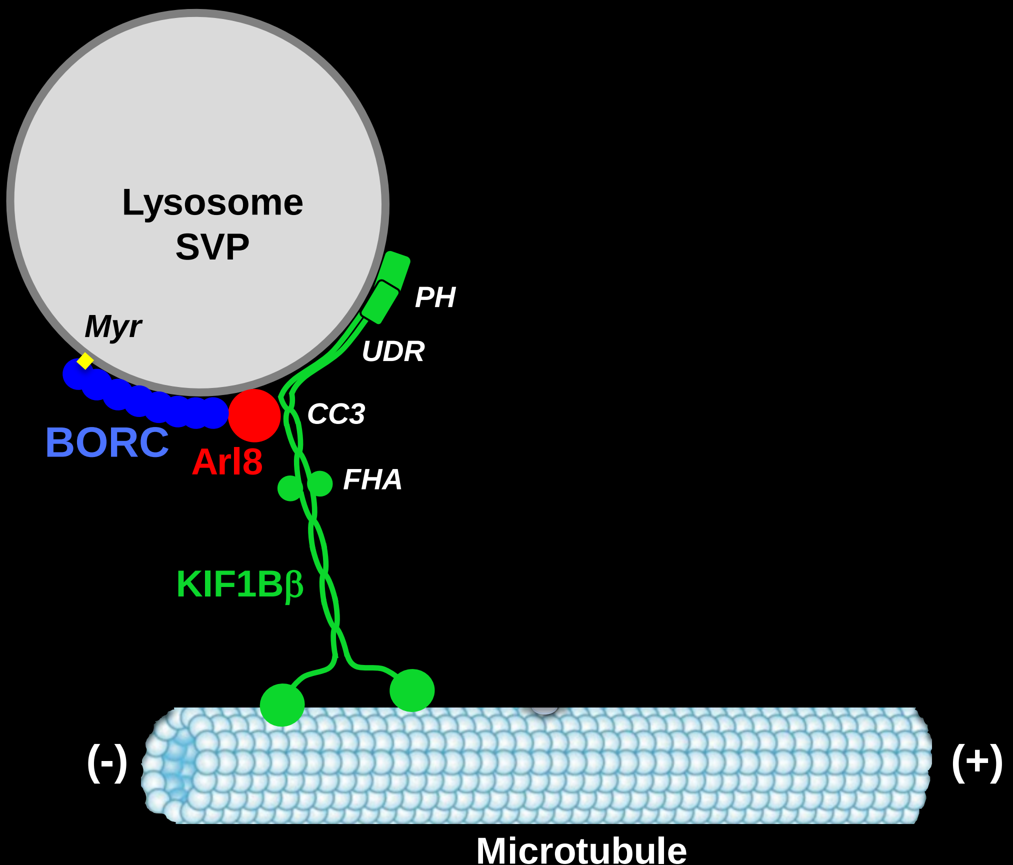
Jing Pu, Charly Guardia

BORC Couples Lysosomes and SVPs to Kinesins



Jing Pu, Charly Guardia; Kang Shen's lab at Stanford

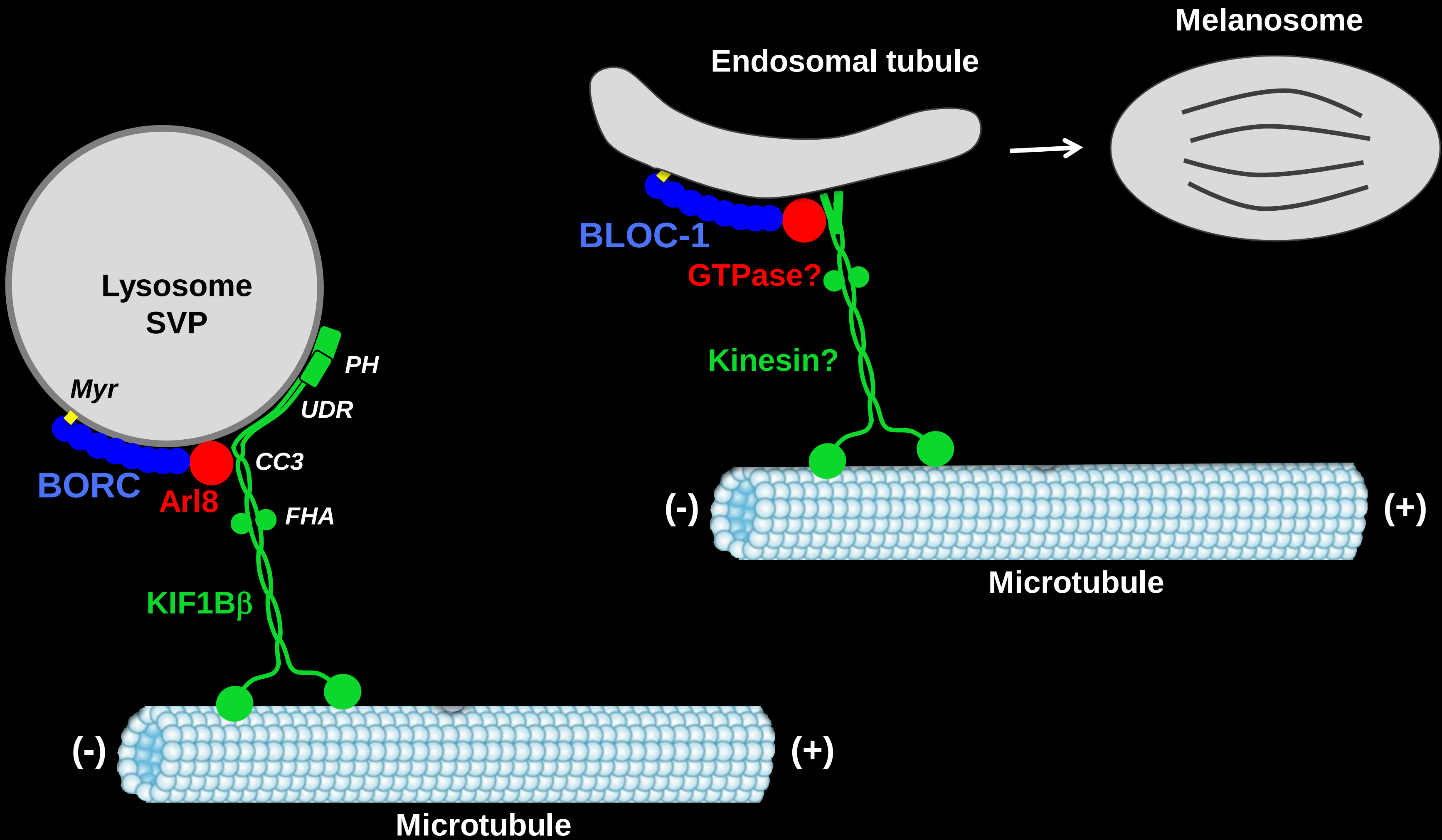
BORC Couples Lysosomes and SVPs to Kinesins



BORCS7/Diaskedin: major schizophrenia susceptibility gene

Jing Pu, Charly Guardia; Kang Shen's lab at Stanford

Does BLOC-1 Couple Melanosomal Carriers to Kinesin?



Jing Pu, Charly Guardia; Kang Shen's lab at Stanford

Graça Raposo, Mickey Marks

Conclusions

AP-3 is involved in the biogenesis of lysosome-related organelles in flies, mice and humans

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Mutations in AP-3 are the cause of Hermansky-Pudlak syndrome type 2

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AP-3 mediates the sorting of tyrosinase from endosomes to melanosomes by recognition of a dileucine sorting signal

Conclusions

AP-3 is involved in the biogenesis of lysosome-related organelles in flies, mice and humans

Mutations in AP-3 are the cause of Hermansky-Pudlak syndrome type 2

AP-3 mediates the sorting of tyrosinase from endosomes to melanosomes by recognition of a dileucine sorting signal

BLOC-1, BLOC-2 and BLOC-3 are novel components of a molecular machinery for the biogenesis of lysosome-related organelles

Conclusions

BORC couples lysosomes and SVPs to kinesins

BLOC-1 may act in a similar manner to couple melanosome-bound carriers to kinesins

Thanks!

Esteban Dell'Angelica
Chean Eng Ooi
Chris Mullins
Kengo Moriyama
José Martina
Lisa Hartnell
Chris Schindler
Jing Pu
Charly Guardia
(CBMB, NICHD)

Bill Gahl
(NICHD/NHGRI)

Mickey Marks
(U. Pennsylvania)

Graça Raposo
(Curie Institute)

NIH *Eunice Kennedy Shriver* National Institute of Child Health and Human Development