

The franco-belgian connection

ABC, membrane and lipids

A taste of history

Giovanna CHIMINI
Brussels, October 2012

THE COMPLEXITY

In the late 80s emerged the idea of

- a large family and
- a broad conservation across evolution

Ann. Rev. Biochem. 1986, 55:397-425
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BACTERIAL PERIPLASMIC TRANSPORT SYSTEMS: STRUCTURE, MECHANISM, AND EVOLUTION

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**The yeast *STE6* gene encodes a
homologue of the mammalian
multidrug resistance P-glycoprotein**
John P. McGrath & Alexander Varshavsky
Nature **340**, 400-404 (3 August 1989)

Nature. 1985 Aug 29-Sep 4;316(6031):817-9.
**Amplification of P-glycoprotein genes in
multidrug-resistant mammalian cell lines.**
Riordan JR, Deuchars K, Kartner N, Alon N,
Trent J, Ling V.

MOLECULAR AND CELLULAR BIOLOGY, May 1986, p. 1671-1678
0270-7306/86/051671-08\$02.00/0
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Vol. 6, No. 5

Overexpression and Amplification of Five Genes in a Multidrug-Resistant Chinese Hamster Ovary Cell Line

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Received 2 December 1985/Accepted 14 February 1986

Identification of the Cystic Fibrosis Gene: Cloning and Characterization of Complementary DNA

JOHN R. RIORDAN, JOHANNA M. ROMMENS, BAT-SHEVA KEREM, NOA ALON,
RICHARD ROZMAHEL, ZBYSZKO GRZELCZAK, JULIAN ZIELENSKI, SI LOK,
NATASA PLAVSIC, JIA-LING CHOU, MITCHELL L. DRUMM, MICHAEL C. IANNUZZI,
FRANCIS S. COLLINS, LAP-CHEE TSUI

THE COMPLEXITY



➤ **Unravel complexity** degenerate PCR approach

➤ **Identify structural specificities** – subfamilies

➤ **Nomenclature committee 1999**

ABCA# ATP-binding cassette, sub-family A (ABC1),
ABCB# ATP-binding cassette, sub-family B (MDR/TAP),
ABCC# ATP-binding cassette, sub-family C (CFTR/MRP),
ABCD# ATP-binding cassette, sub-family D (ALD),
ABCE# ATP-binding cassette, sub-family E (OABP),
ABCF# ATP-binding cassette, sub-family F (GCN20),
ABCG# ATP-binding cassette, sub-family G (WHITE),

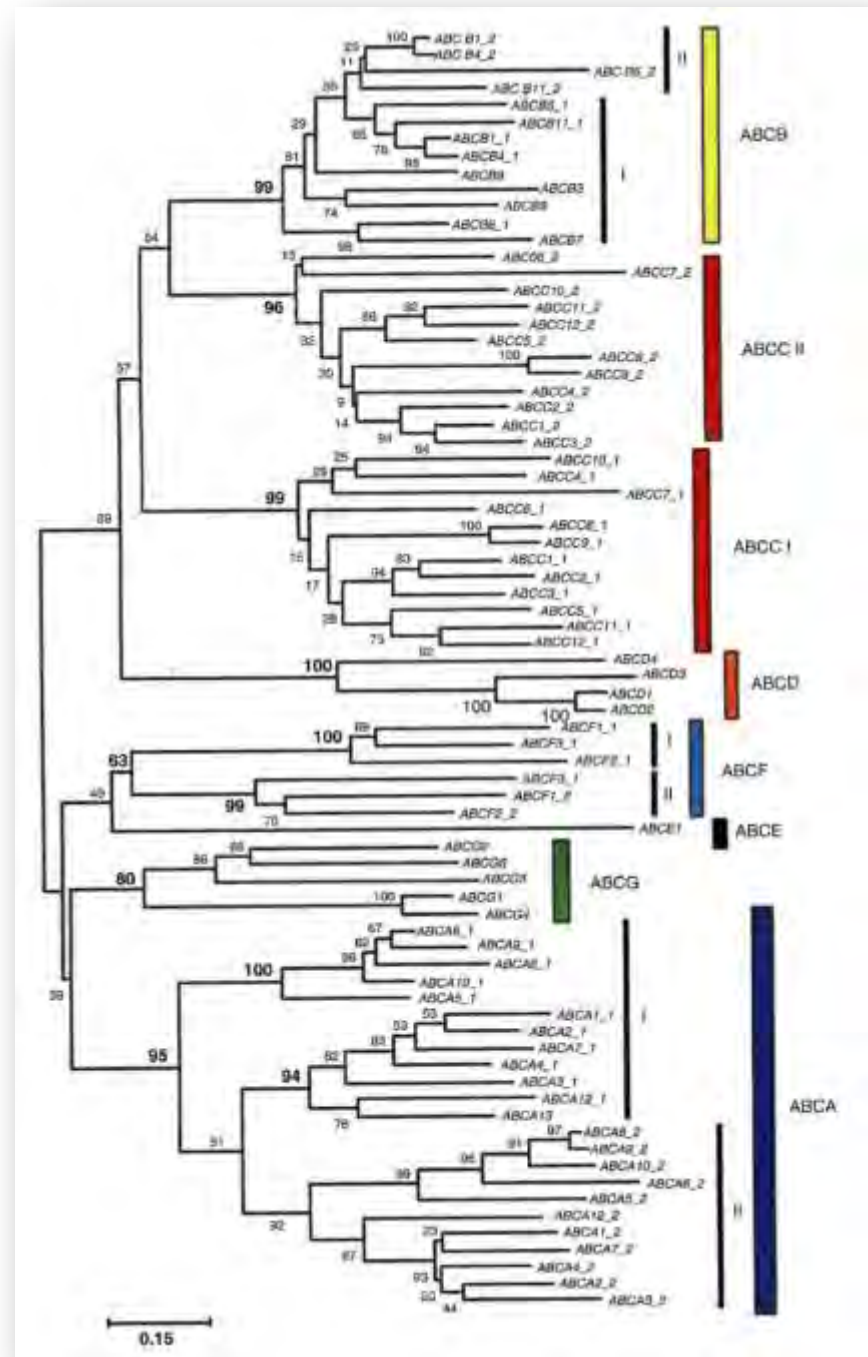
➤ **Identify target genes and suggest function**

ABCA1, ABCA2 ABCA7, ABCG1, ALDP2 etc

ABC – ATP BINDING CASSETTE-transporters in mammals

are the largest family of membrane proteins

- The complexity
- The structure
- The function



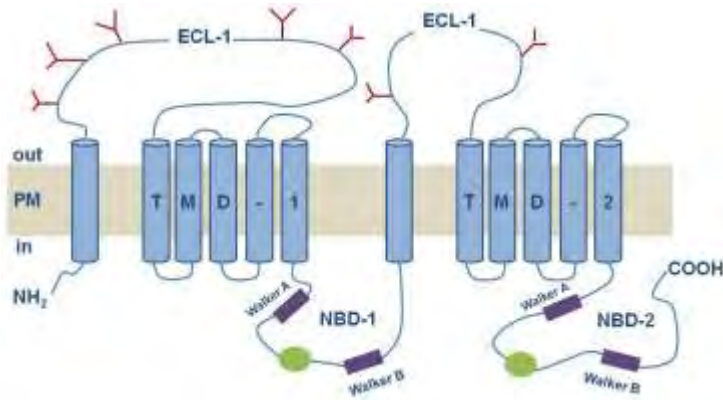
ABC A1

THE STRUCTURE

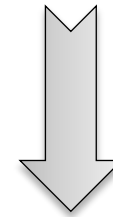
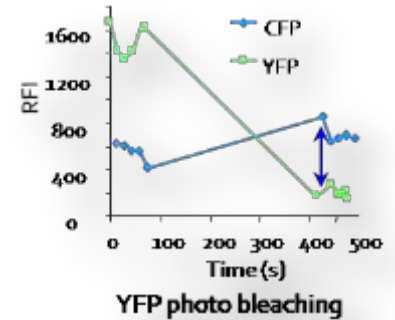
INCLUDES SYMMETRY

- COMMON MODULES DEFINE FAMILY FEATURES
- EXTRA MODULES DEFINE SPECIFICITIES
- QUATERNARY STRUCTURE IS BASED ON MULTIPLICATION OF SYMMETRY

THE STRUCTURE

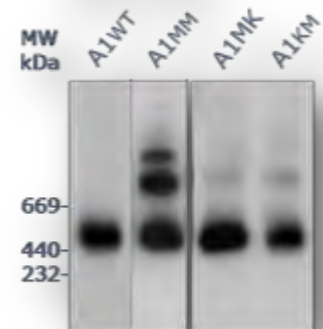


Times 2



Times 4

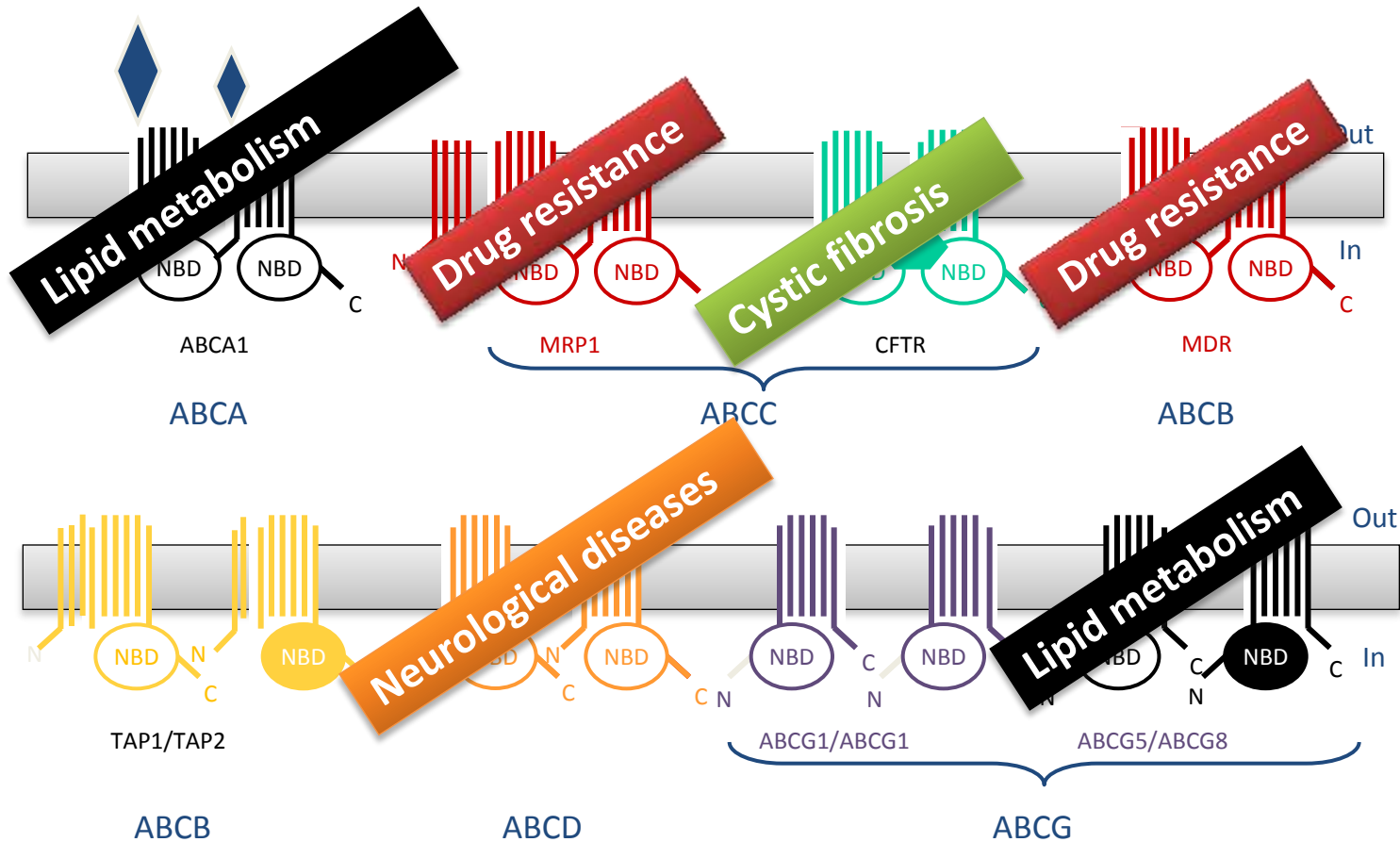
Native PAGE



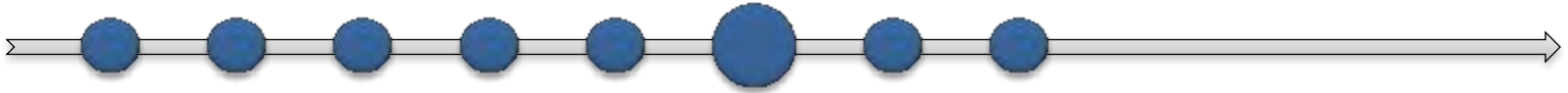
- FRET and native PAGE
- panel of ABCA1 variants tagged with multiple FP defective for trafficking , ATP ase activity or function
- home made panel of versatile monoclonal antibodies
- array of assays for ABCA1 function
flip / effluxes/ engulfment

THE FUNCTION:

DYSFUNCTION OF ABC TRANSPORTERS LEADS TO MAJOR HUMAN PATHOLOGIES



THE FUNCTION : ABC A1



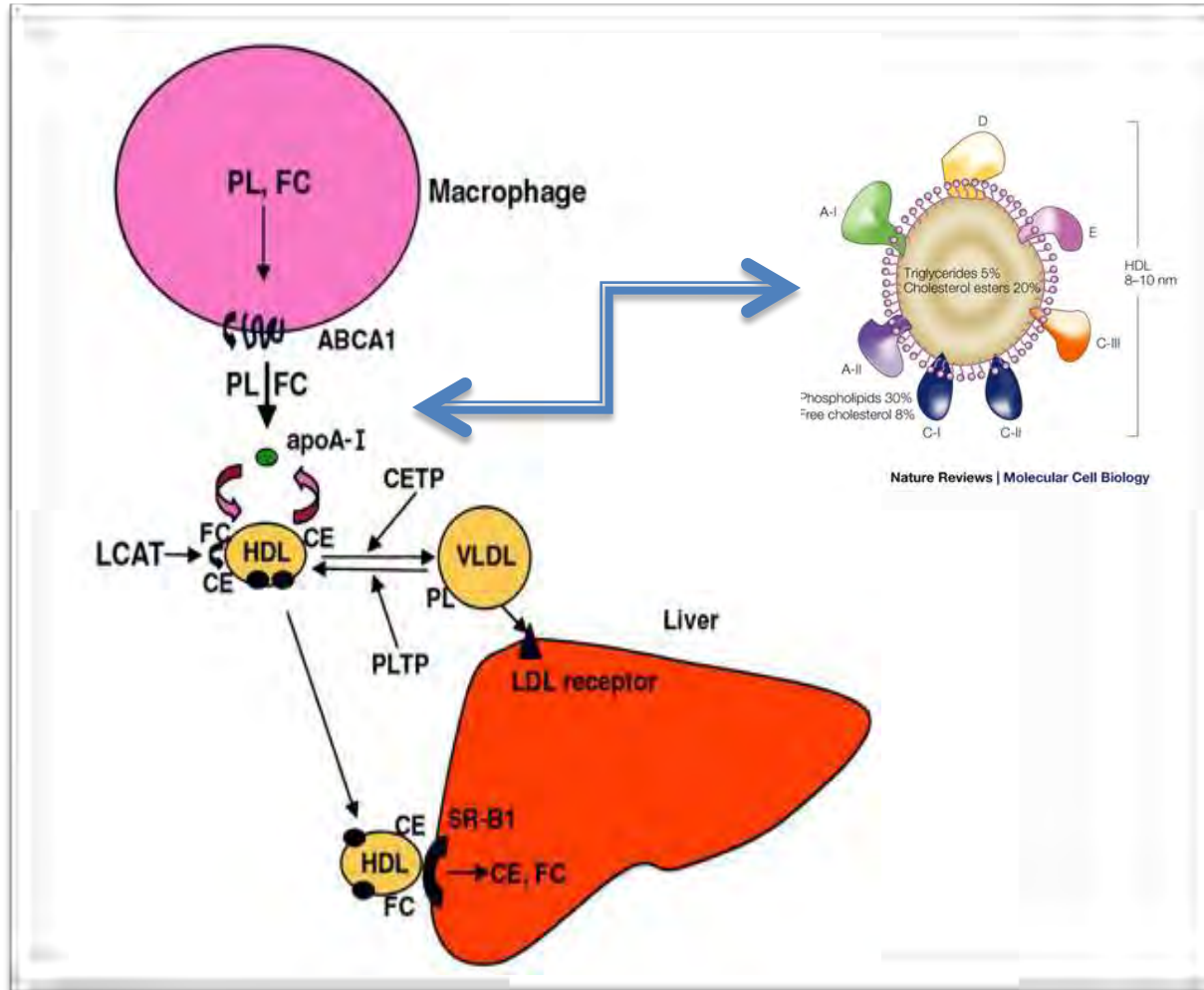
ABC A1 TRANSPORTER

Cellular Homeostasis and TANGIER Disease :

THE REVERSE CHOLESTEROL TRANSPORT PARADIGM



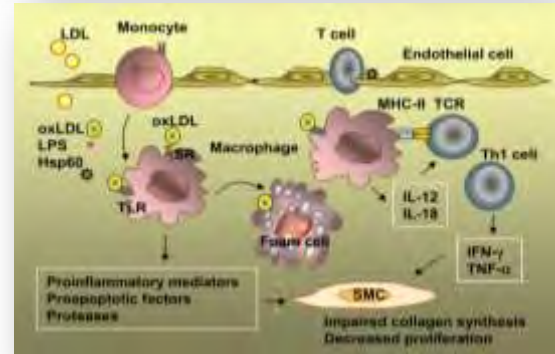
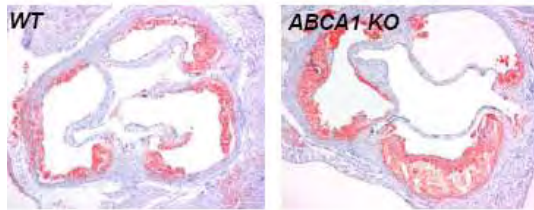
ABC A1 AND HDL FORMATION



ABCA1 INFORMS FUNCTIONALLY MΦ ACTIVATION PROGRAMS

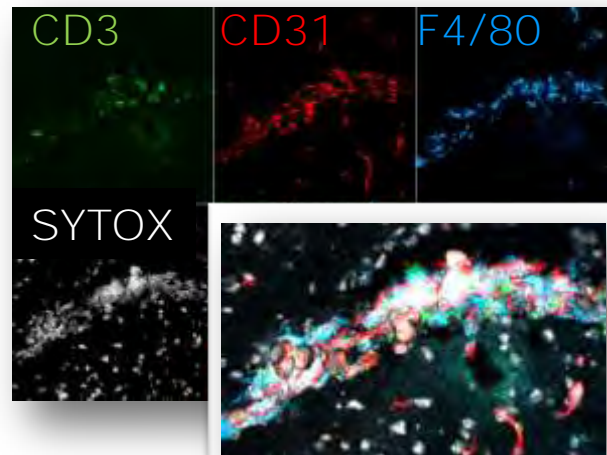
- tissue remodelling
- i.e. clearance of dead cells

- Atheroma progression
- i.e. foam cells



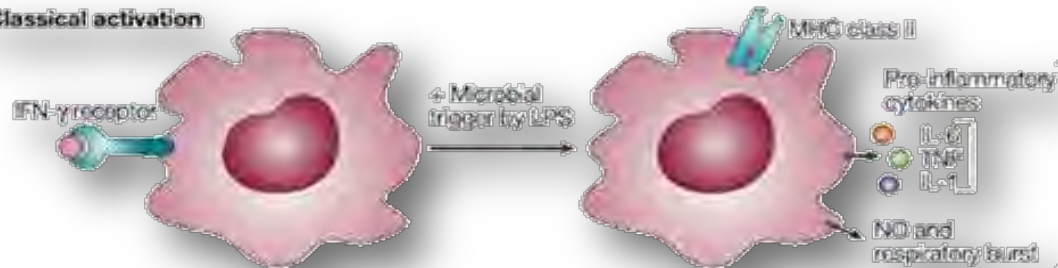
- resistance to Cerebral Malaria
In mice

Brain lesions

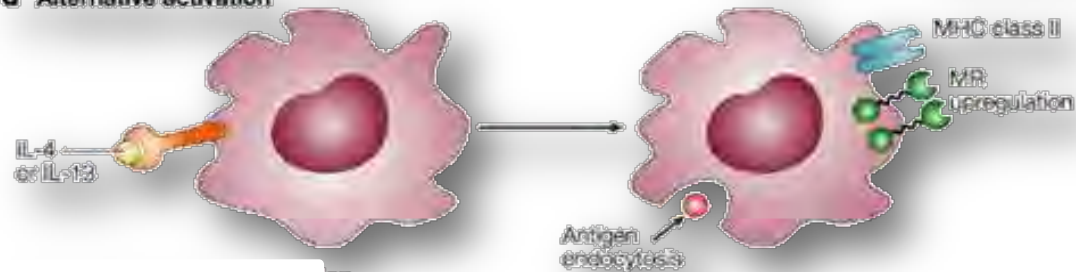


What is the incidence of ABCA1 on M ϕ reactivity to stimuli ?

c Classical activation

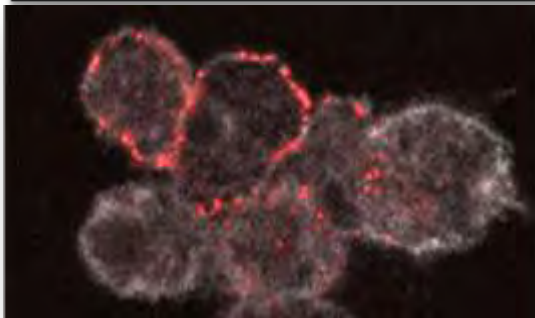


d Alternative activation

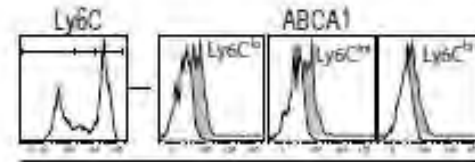


ABCA1 positive cells in tissues

mAb 1422 anti ABCA1
Extracellular epitope

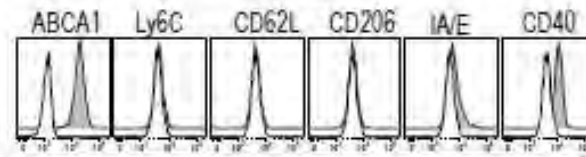


Blood



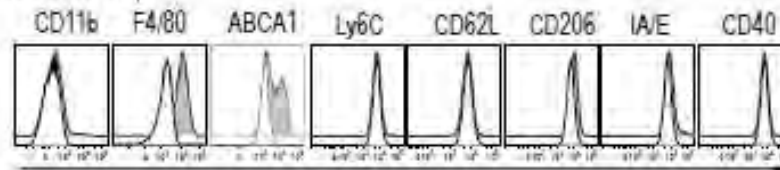
Log₁₀ Fluorescence

Peritoneal Mφ



Log₁₀ Fluorescence

Alveolar Mφ



Log₁₀ Fluorescence

.. are tissue macrophages

L. PRADEL

MYELOID CELLS IN THE SPLEEN : HETEROGENEITY

P1 ~1.5-2%
Macrophages?
-AF^{hi}
-Lin⁻
-(F4/80^{hi})

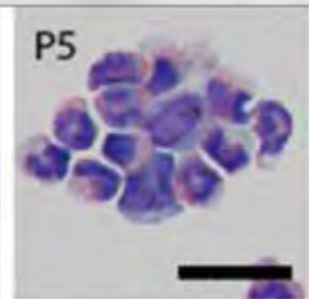
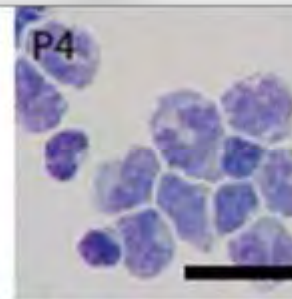
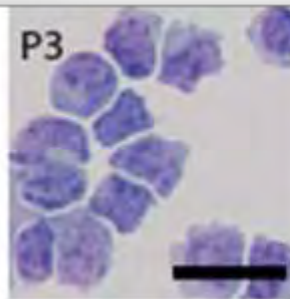
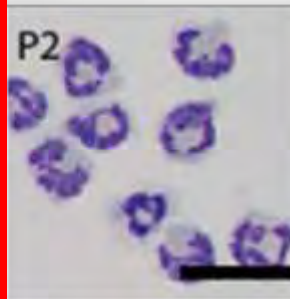
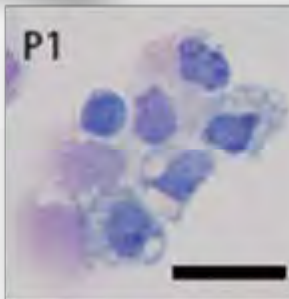
ABCA₁

P2 ~1-2%
Neutrophils?
-AF^{int/lo}
-Lin⁻
-CD11b^{hi}
-Ly6G^{hi}

P3 ~2-3%
DC?
-AF^{int/lo}
-Lin⁻
-CD11c^{hi}

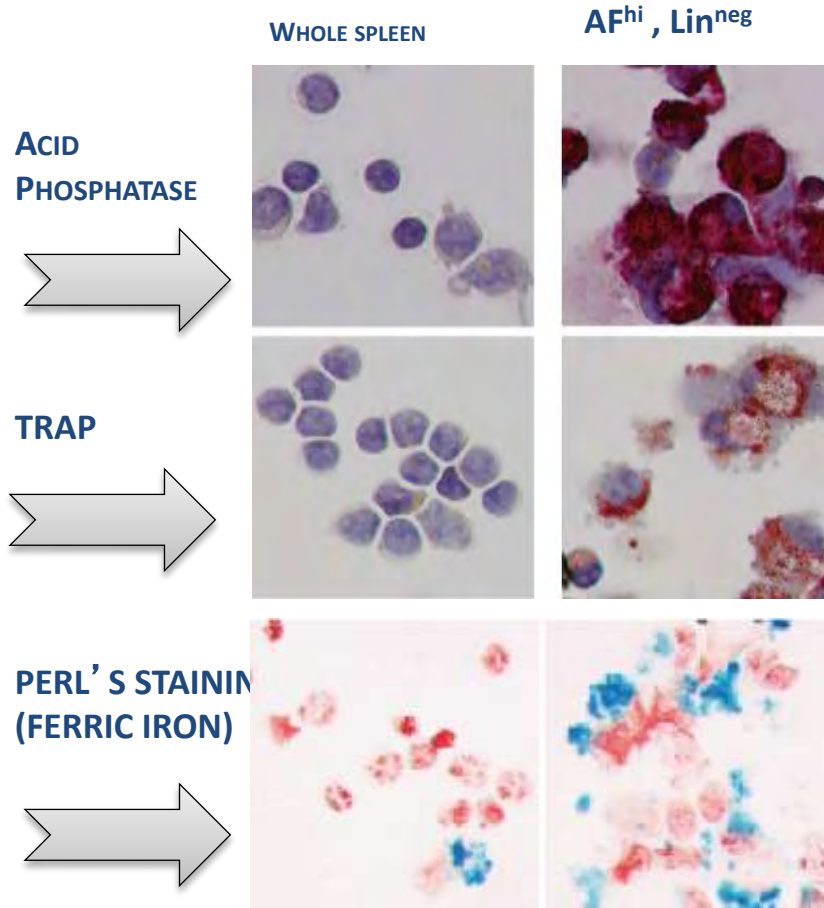
P4 ~1-2%
Monocytes?
-AF^{int/lo}
-Lin⁻
-CD11b^{hi}
-CD11c^{lo}
-SSC^{lo}

P5 ~0.5%
Eosinophils?
-AF^{int/lo}
-Lin⁻
-CD11b^{hi}
-CD11c^{lo}
-SSC^{hi}

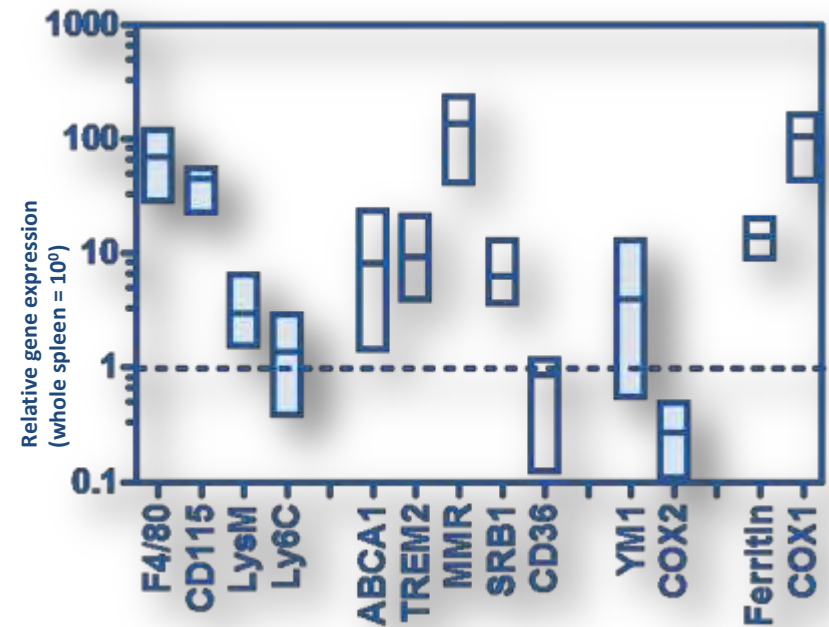


AUTOFLUORESCENCE DISGUISED

ABCA1^{pos} CELLS IN THE SPLEEN ARE RED PULP MΦ

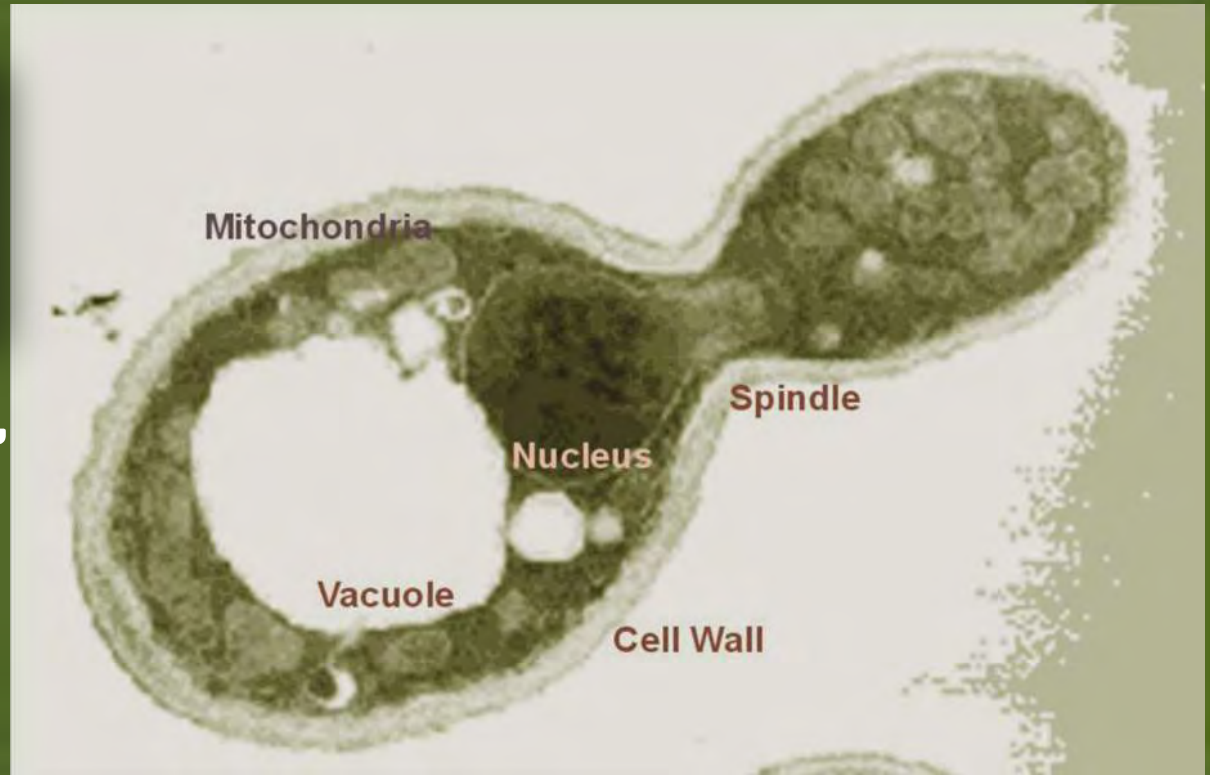


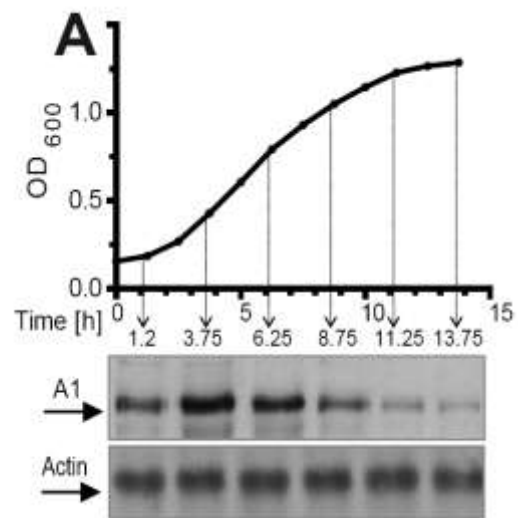
ABCA1 SORTED CELLS: GENE EXPRESSION BY QRT PCR





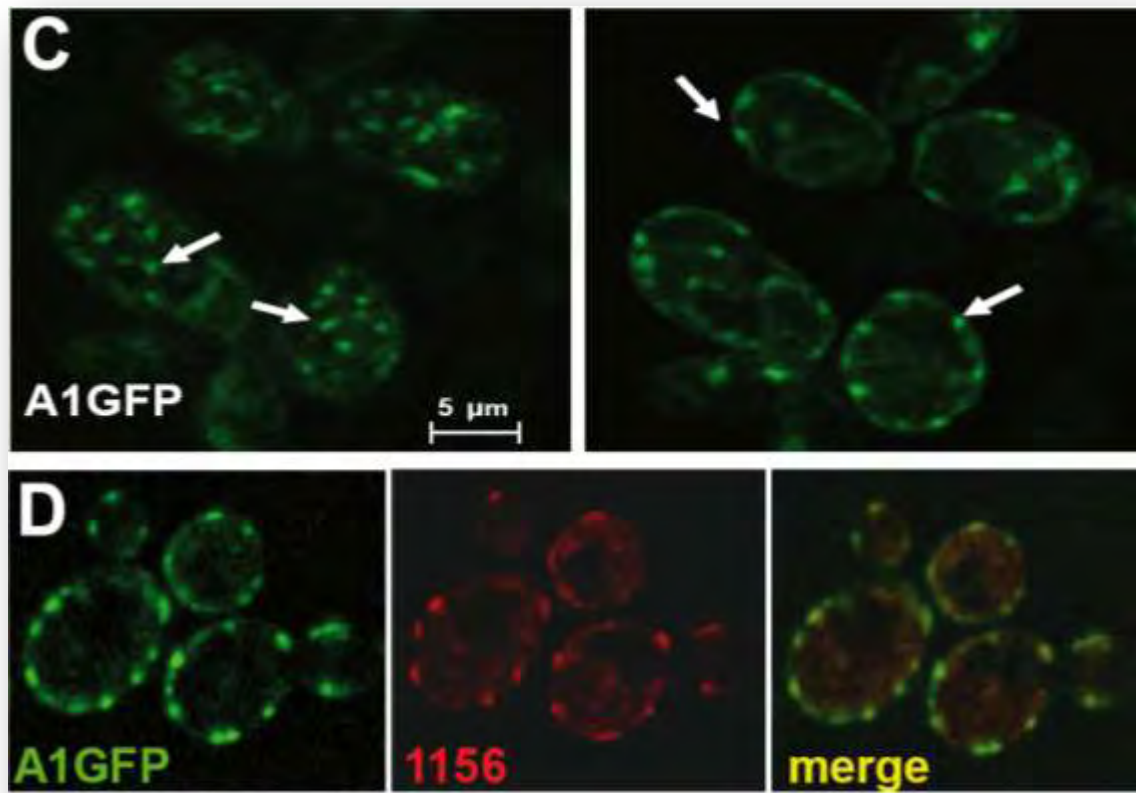
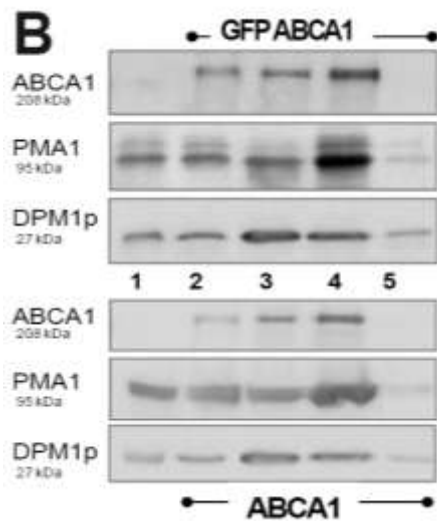
**Andre GOFFEAU,
me and
YEAST**

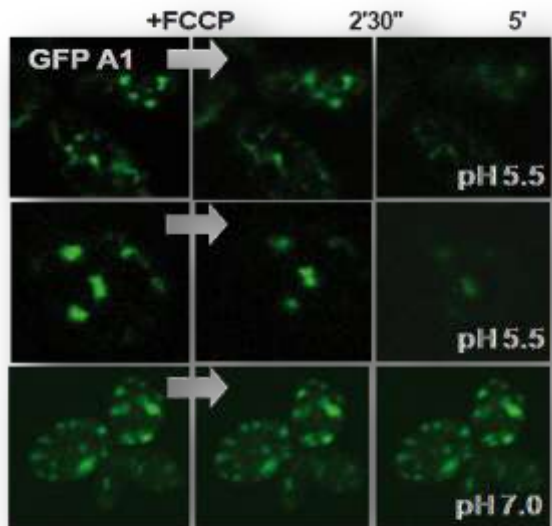
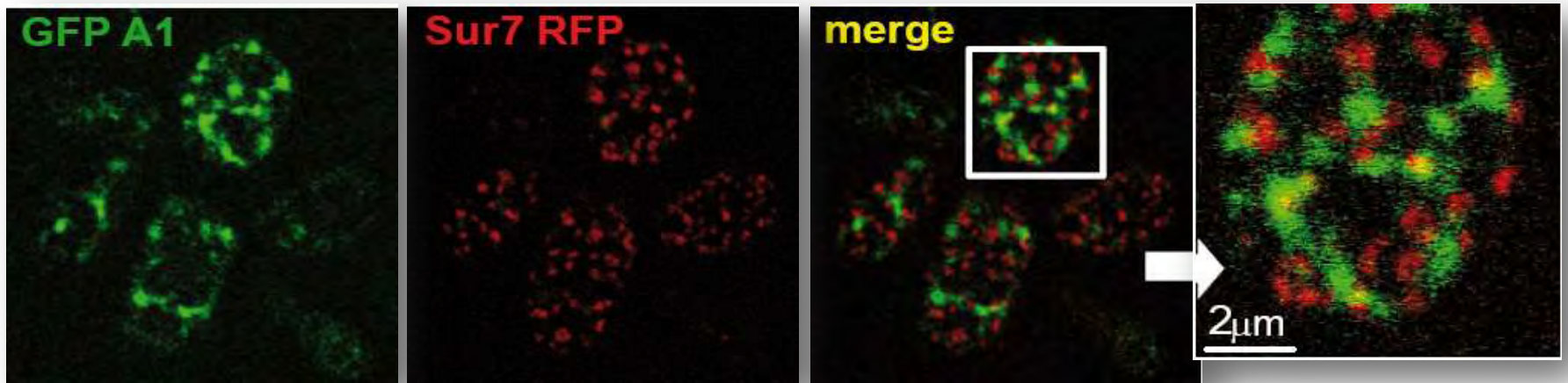




ABCA1 CAN BE EXPRESSED IN YEAST

... And goes where it should

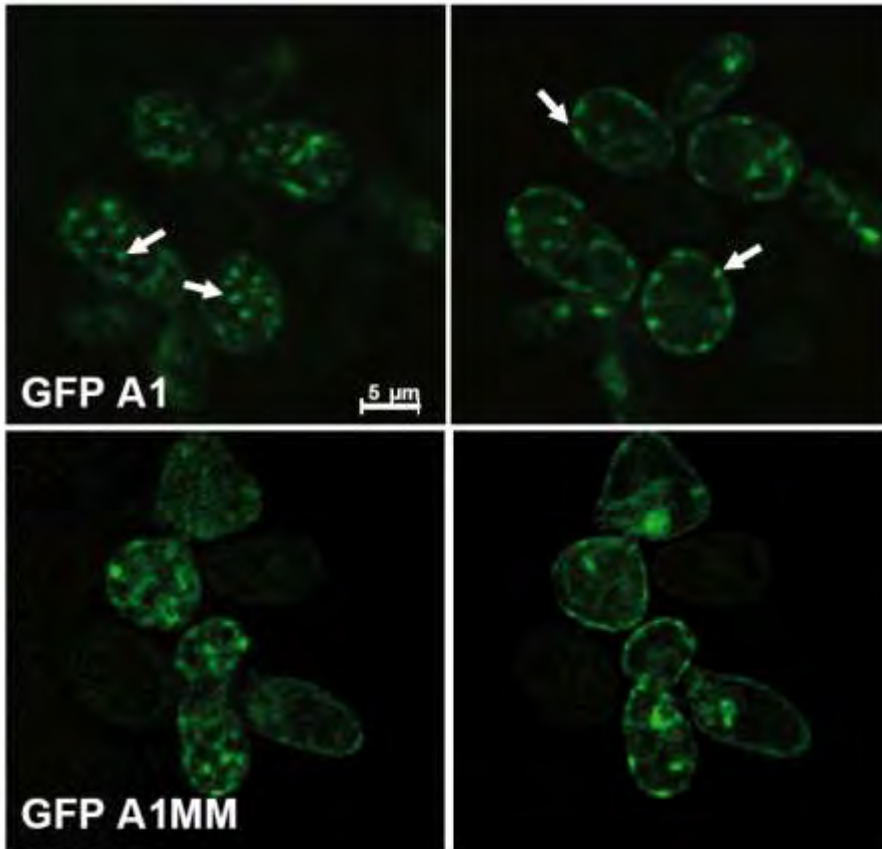




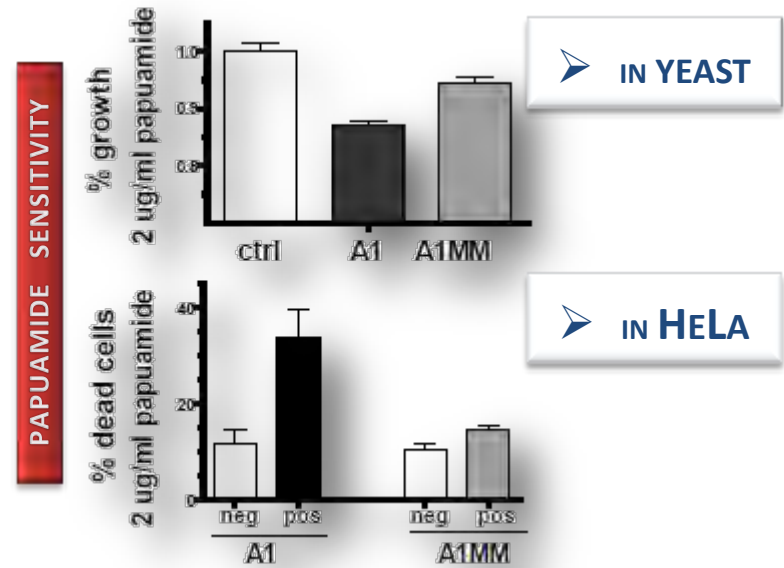
**ABCA1 GENERATES
«LIPID SENSITIVE AND VISIBLE» DOMAINS IN YEAST**

ABCA1 IS FUNCTIONAL IN YEAST

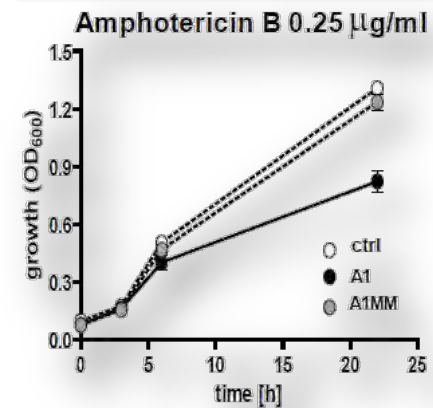
1. DEAD MUTANT LOSES SURFACE CLUSTERING



2. PS EXPOSURE IS PRESERVED

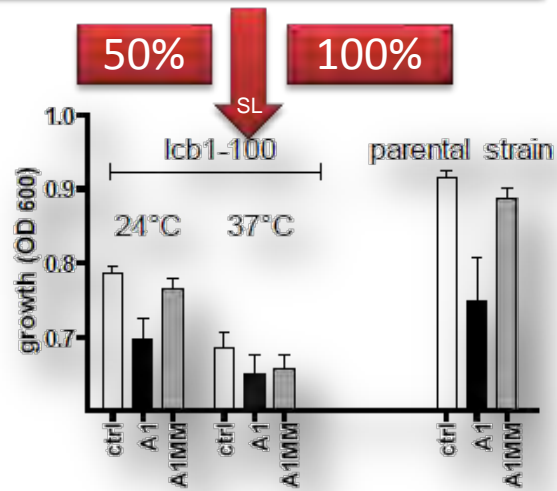
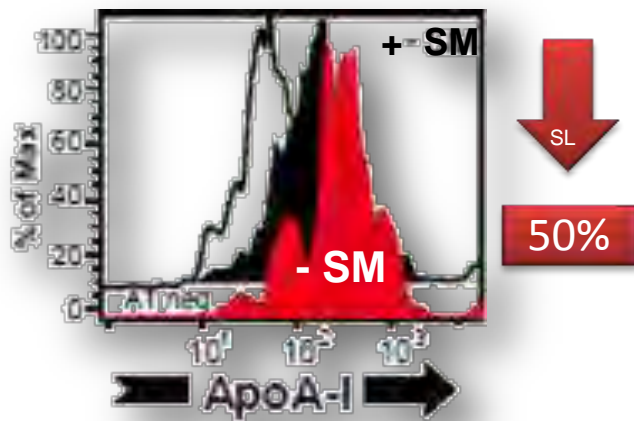
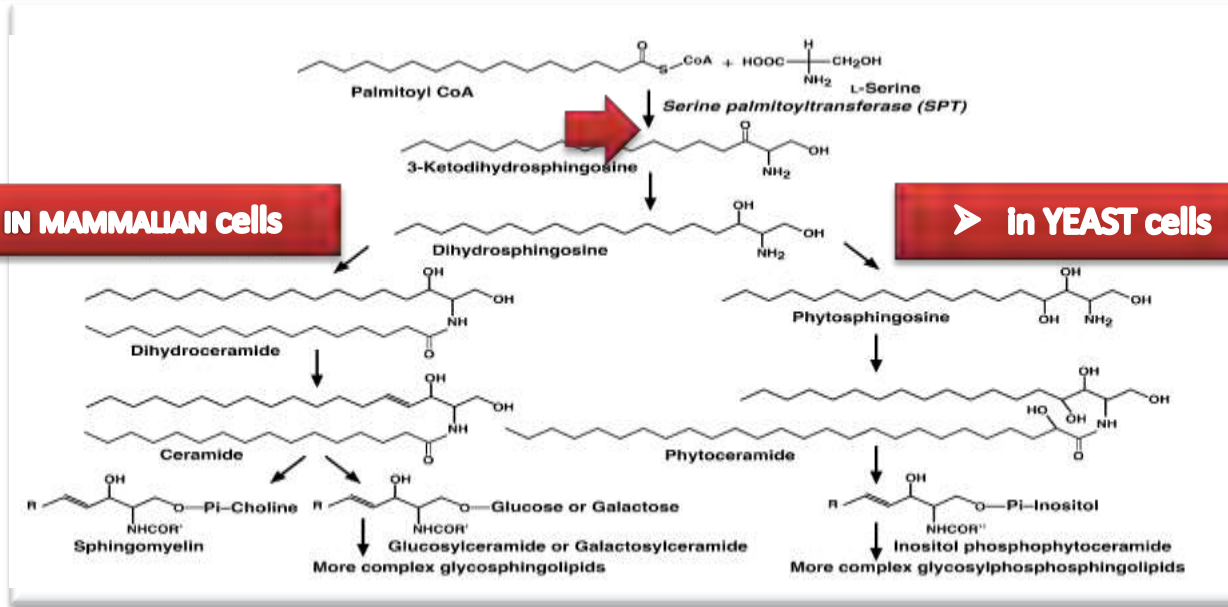


3. LIPID SENSITIVE GROWTH PHENOTYPE



IN YEAST

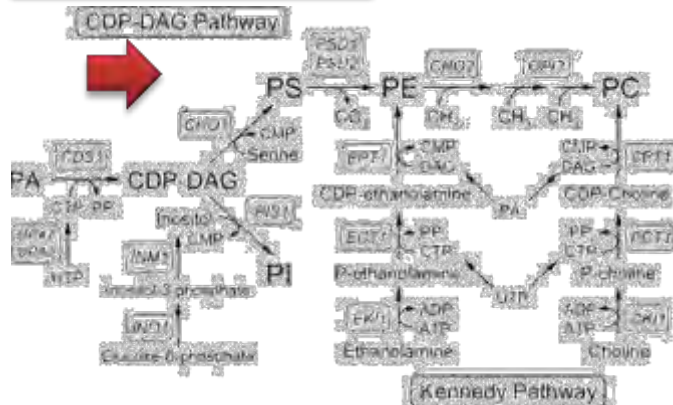
MEMBRANE LIPID CONTENT AFFECTS ABCA1 - THE CASE OF SLs



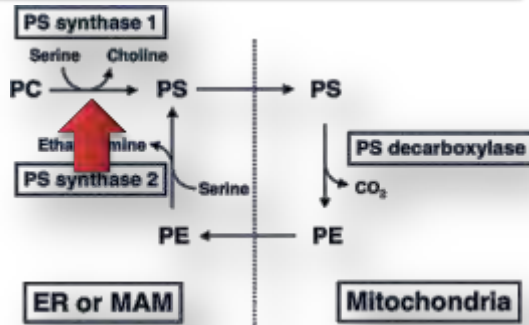
AMPH B SENSITIVITY

MEMBRANE LIPID CONTENT AFFECTS ABCA1 - THE CASE OF PS

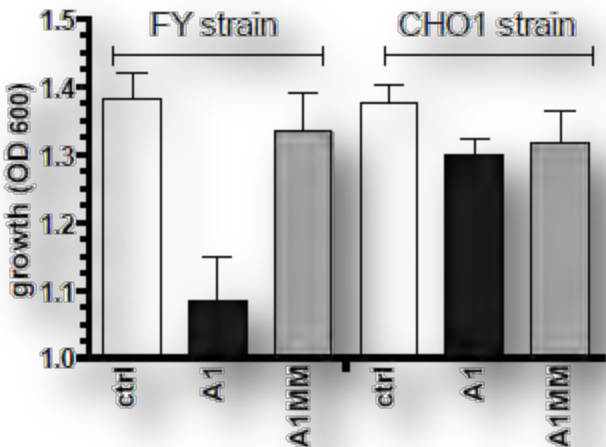
➤ In YEAST cells



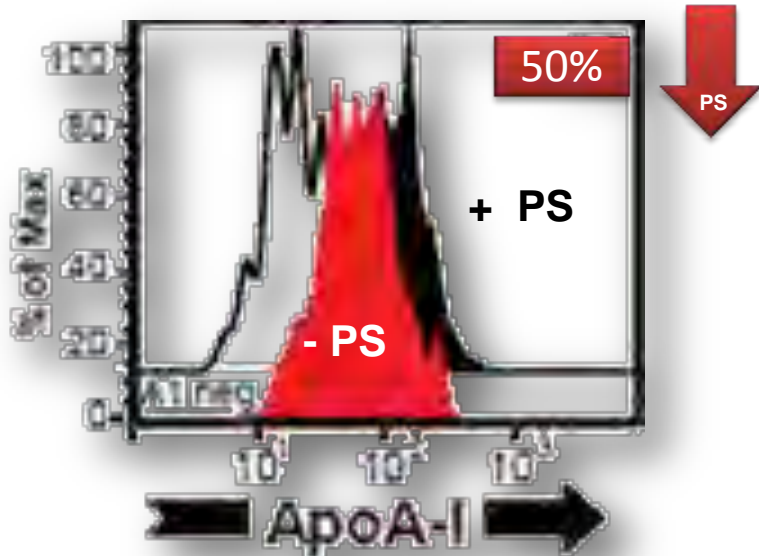
➤ In MAMMALIAN cells



100%
PS



AMPH B SENSITIVITY



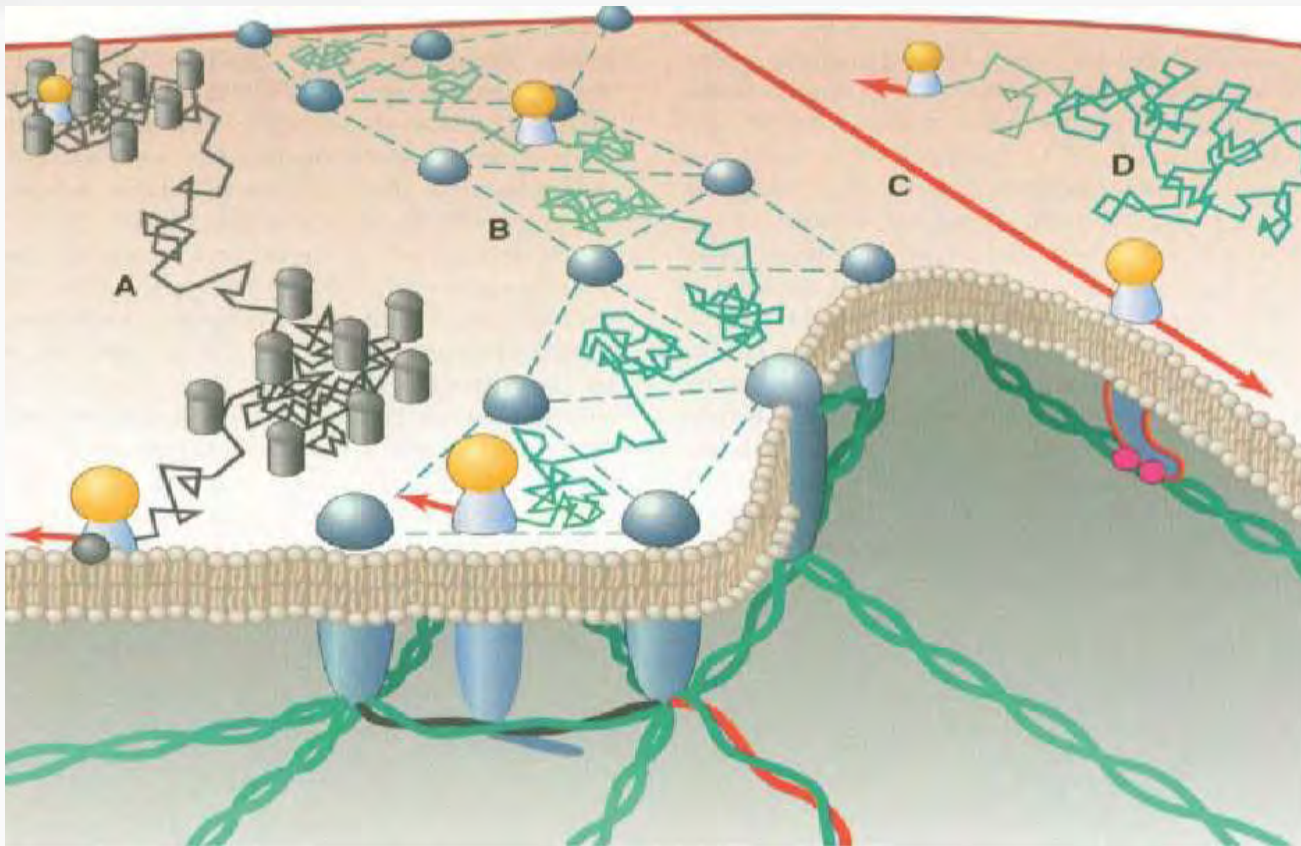
➤ **ABCA1 REQUIRES PS TO FUNCTION**

LIPID CONTENT



ABCA1 (FRAP)

HOW DOES ABCA1 FUNCTION AFFECT THE MEMBRANE ?

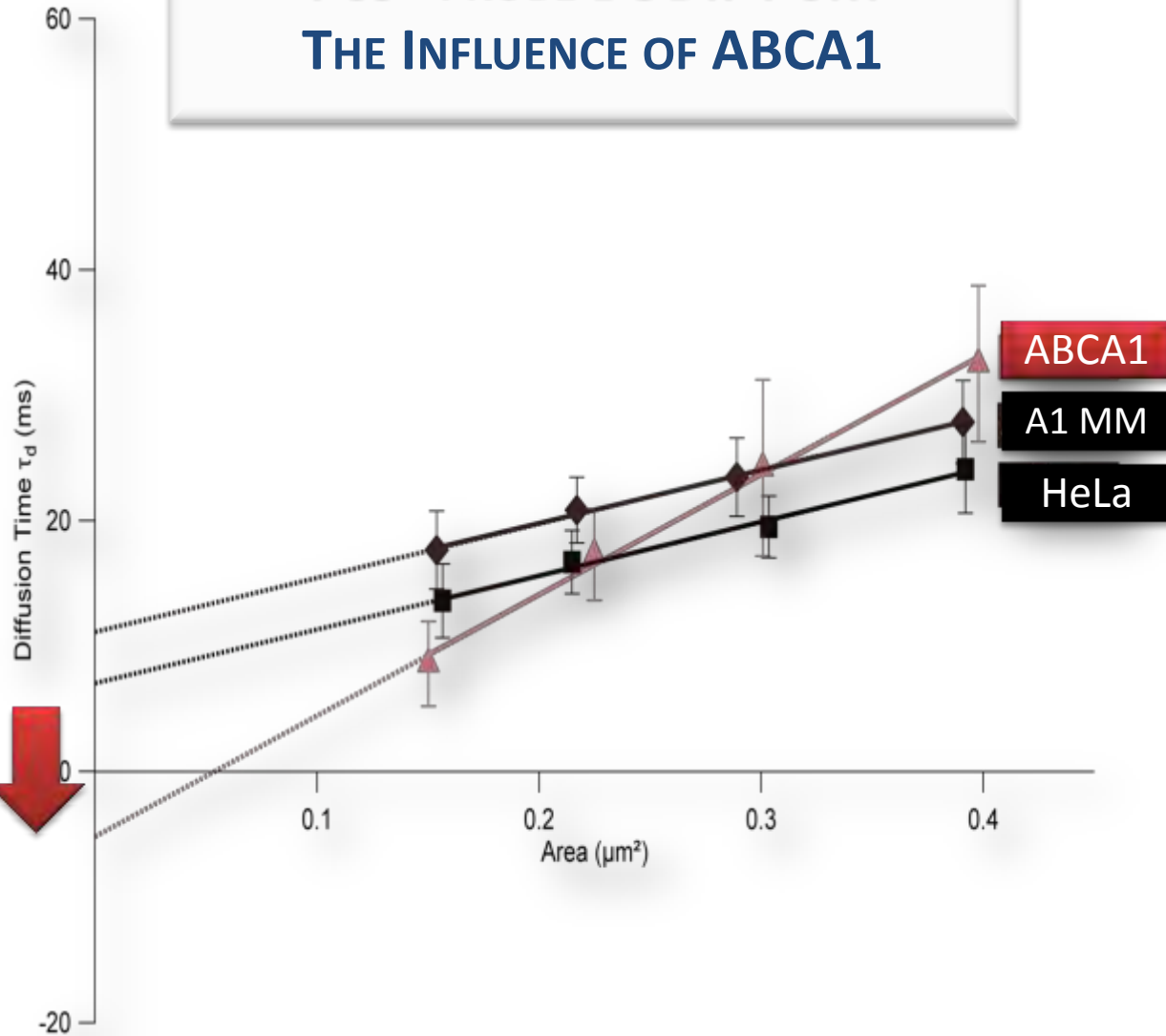


ABC A1

- EXPANDS *non raft* DOMAINS
- HAS PROFOUND EFFECTS ON LIPID MICROENVIRONMENT

~ BIOCHEMISTRY , CATIONIC PROBES, FLIM, FRAP, MEMBRANE GUVS ~

FCS PROBE BODIPY SM THE INFLUENCE OF ABCA1

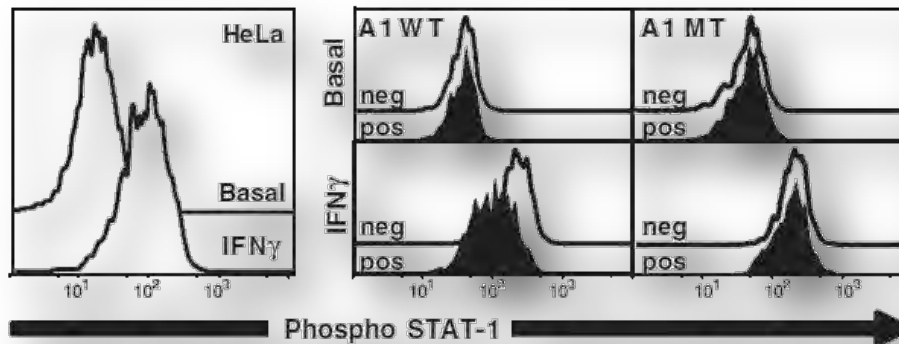
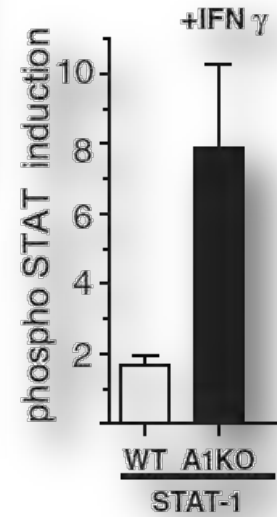
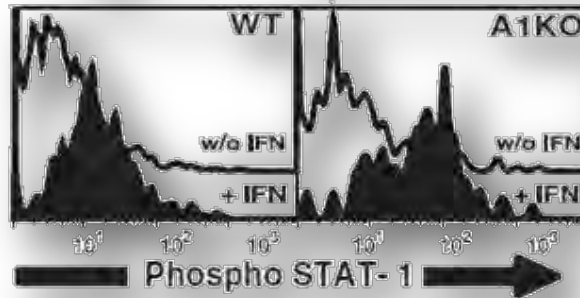


Ana Z.

Didier M.
Tomasz T.



ABCA1



THANKS
TO EVERYBODY