Diversity and Asymmetry of Membrane Phospholipids

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2024/11/11　OIST

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Institute of Microbial Chemistry

Director General

COI
Ono Pharmaceutical Company.
Shimadzu Coop.

4 Major functions of lipids

- **Major component of biological membranes (Origin of life)**
- **Most efficient energy source (triglycerols, cholesterols)**
- **Regulation of inflammation, immune responses etc by lipid mediators (prostaglandins, S1P, cannabinoids etc)**
- **Insulators as skin barrier, myeline sheath etc. (ceramide, sphingomyelin…)**

Lipid research-charm and risk

- Essential for life (such as membrane components, efficient energy source)
- Not directly coded by genes, thus, structure unpredictable

  →More unknown lipids and novel functions
- Related to various diseases (inflammation, immune disorders, liver cancer, colon cancer, atherosclerosis etc.)
- Opportunity for drug development; EPA/DHA, statin, sphingolimod, prostaglandin analogues, enzyme inhibitors, receptor antagonists,,,,,
- Difficult to amplify, metabolically and chemically unstable

  →Many artefacts and errors in published articles (including top journals!)
- Knowledge and reliable techniques of chemistry, biochemistry and biophysics are necessary for lipid research

Agenda of my talk

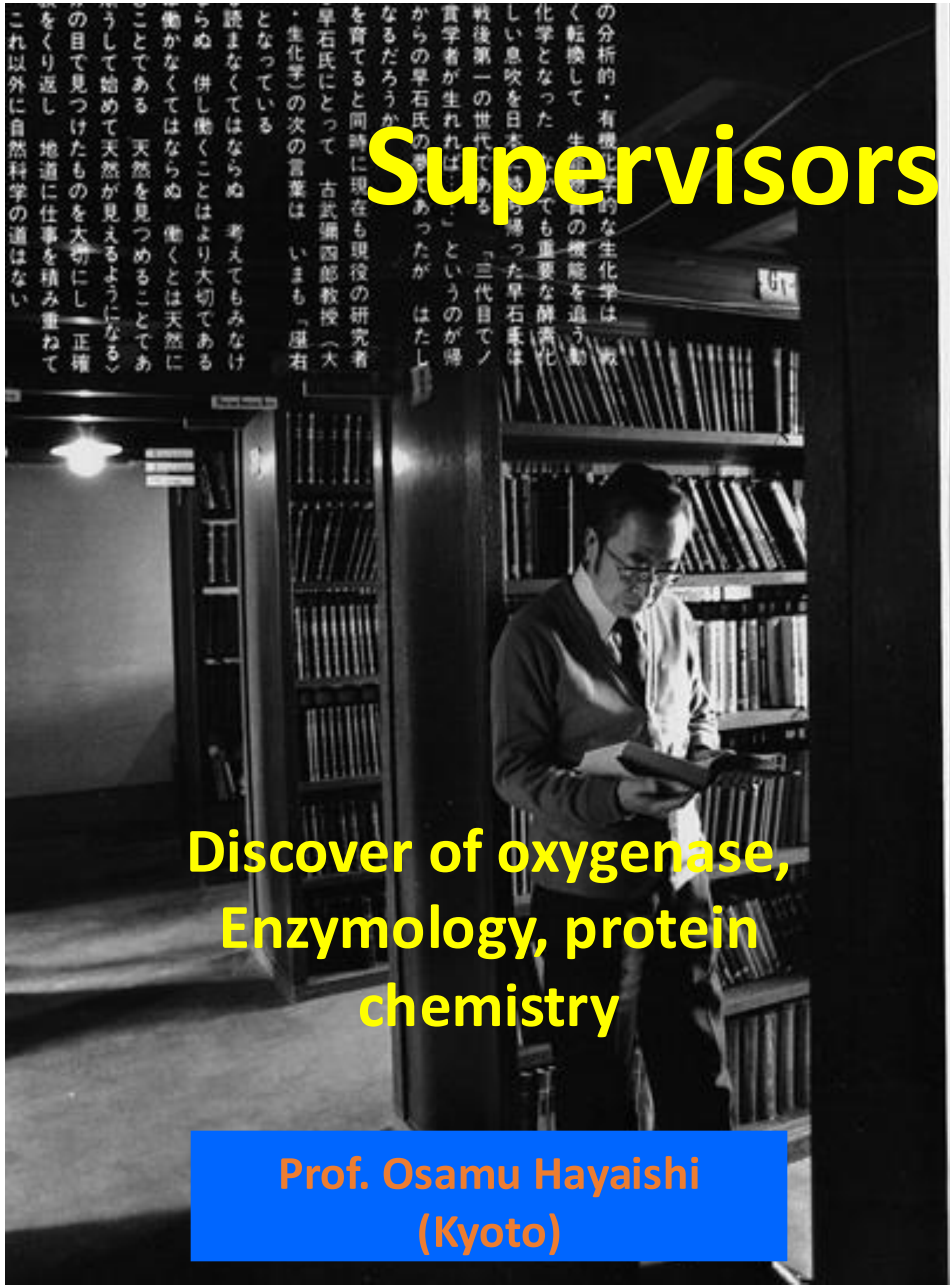
INTRODUCTION:

ENZYMES AND RECEPTORS OF LIPID MEDIATORS

LYSOPHOSPHOLIPID ACYLTRANSFERASES FOR MEMBRANE DIVERSITY

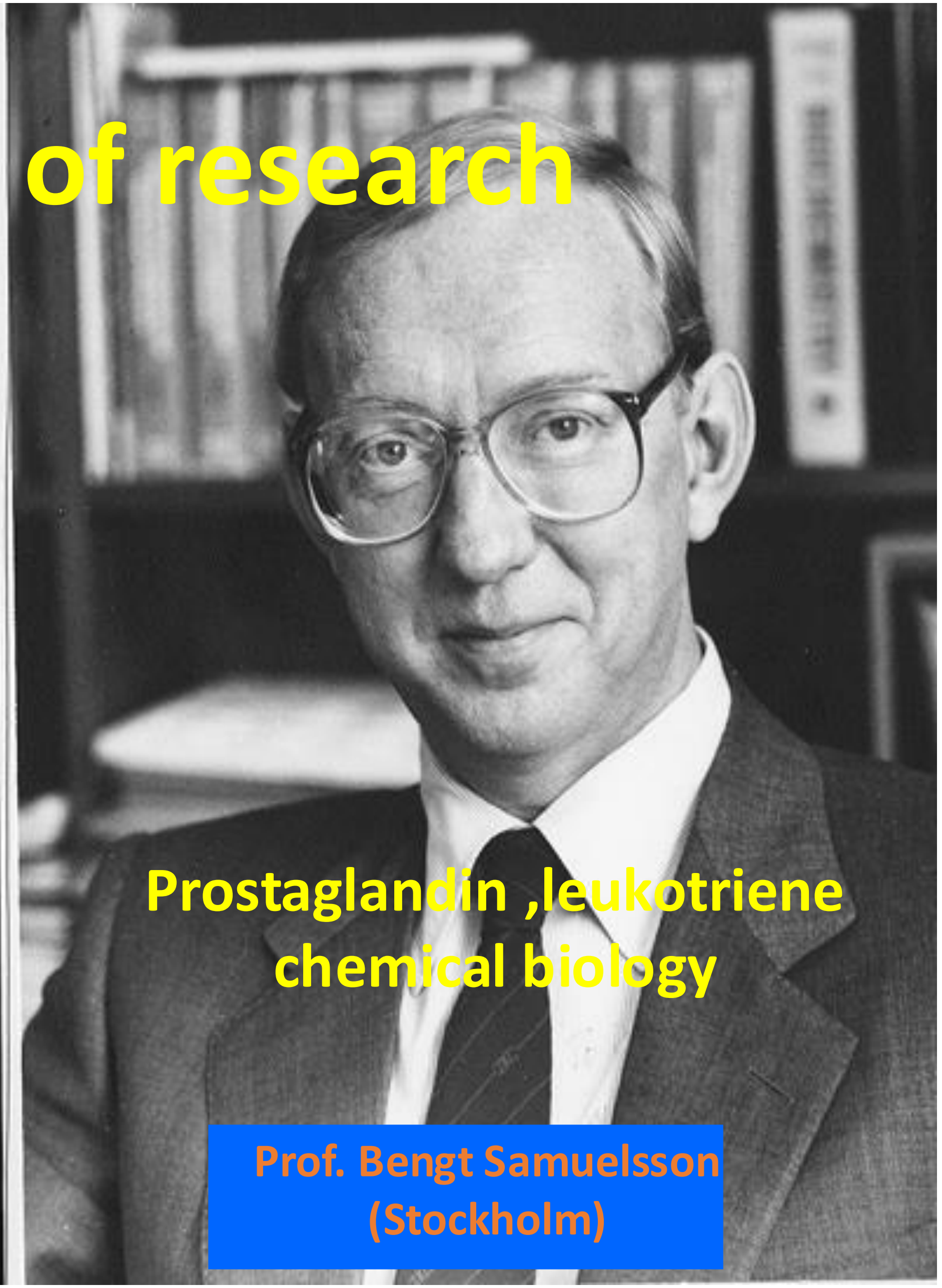
PERSPECTIVE

Supervisors of research



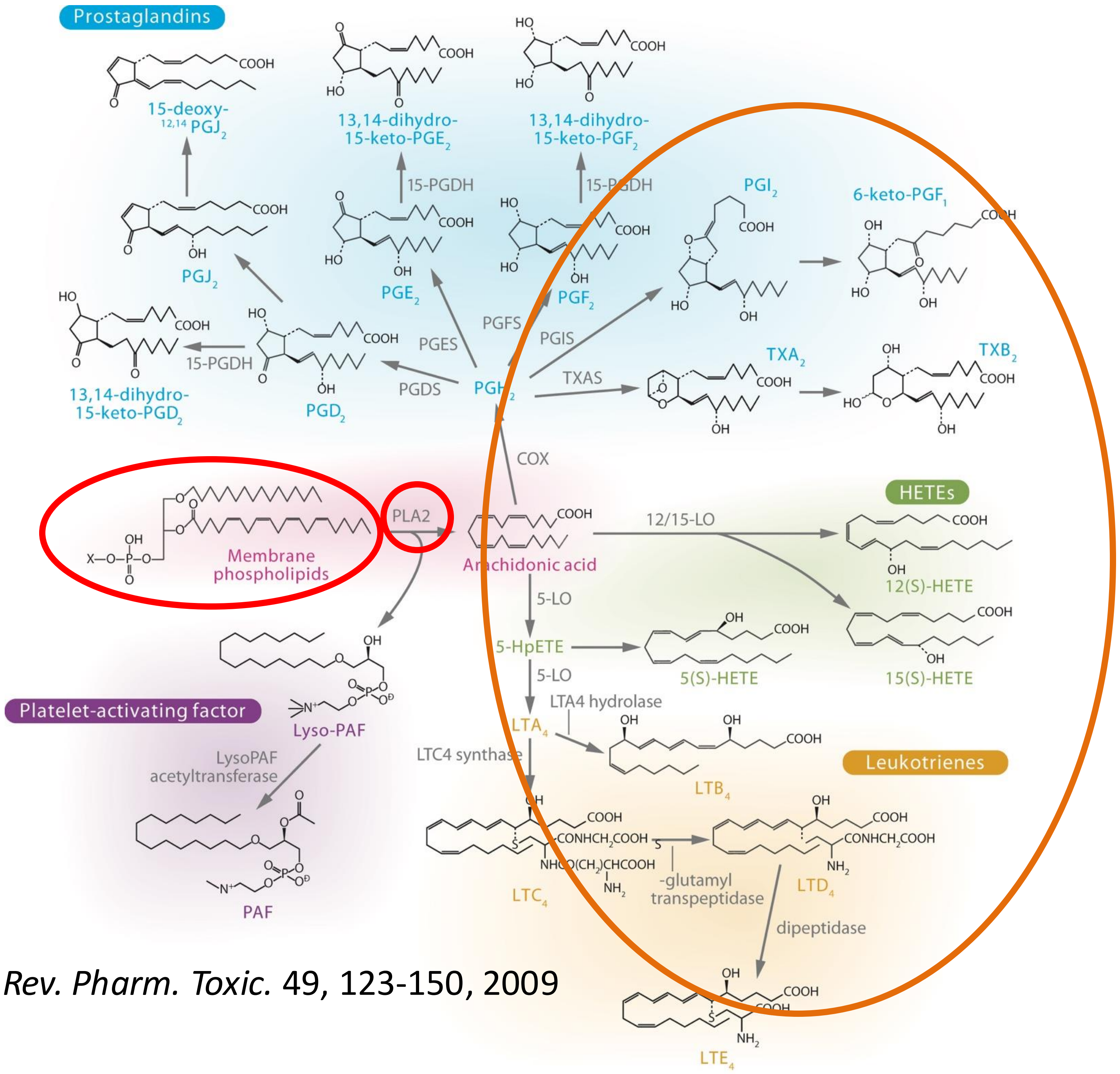
Discover of oxygenase, Enzymology, protein chemistry

Prof. Osamu Hayaishi (Kyoto)



Prostaglandin ,leukotriene chemical biology

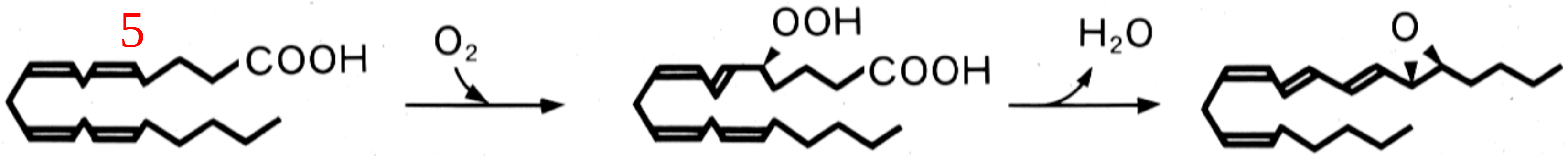
Prof. Bengt Samuelsson (Stockholm)



Shimizu, T. *Annu. Rev. Pharm. Toxic.* 49, 123-150, 2009

Arachidonate 5-lipoxygenase

Dual activities to produce LTA4 from arachidonic acid



Arachidonic acid **5-HPETE** **LTA4**

Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 689–693, February 1984
Biochemistry

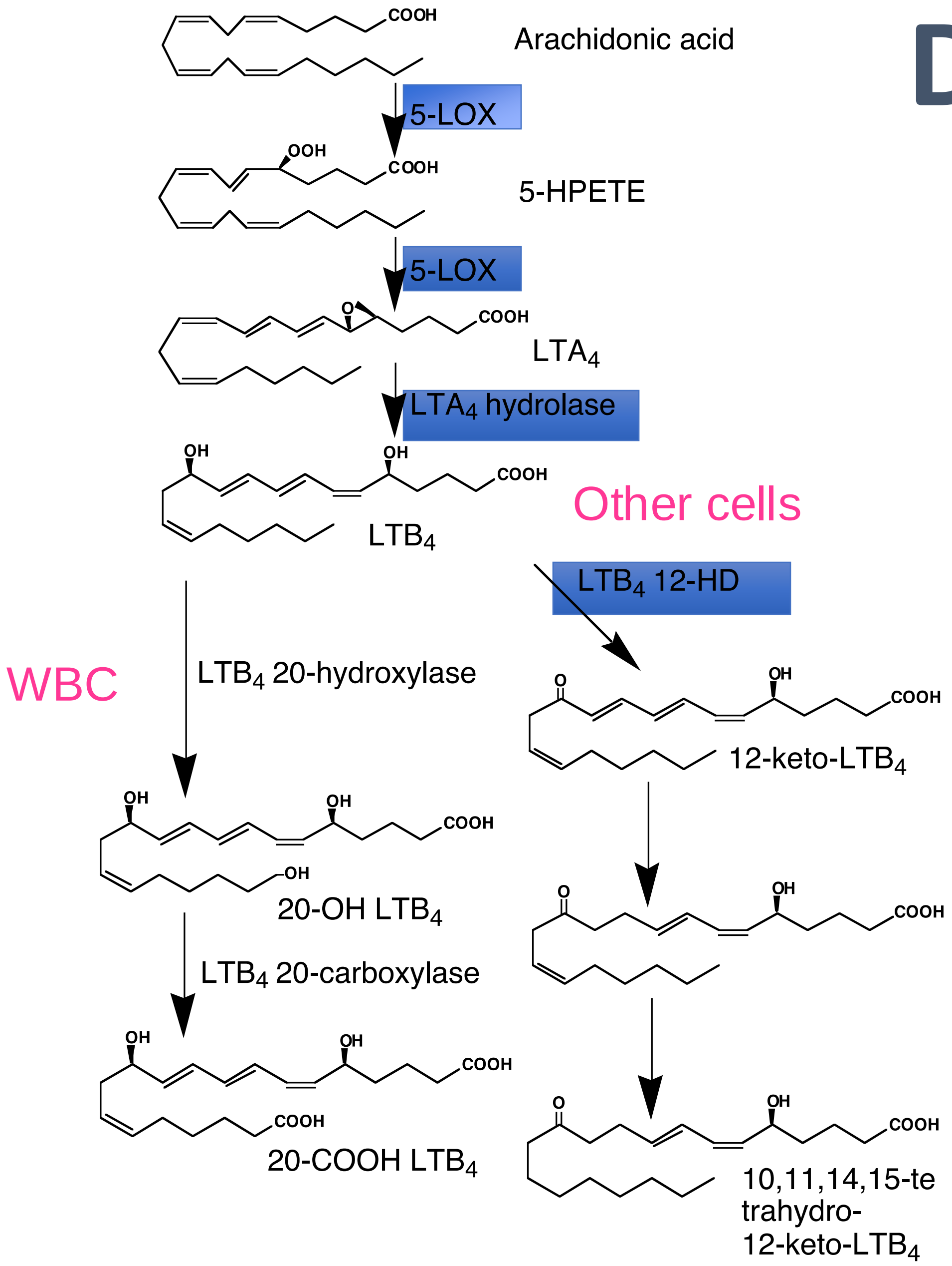
Enzyme with dual lipoxygenase activities catalyzes leukotriene A_4 synthesis from arachidonic acid

(potato lipoxygenase/bishomo-γ-linolenic acid/8-lipoxygenase/*D*-hydrogen/5-hydroperoxyicosatetraenoic acid)

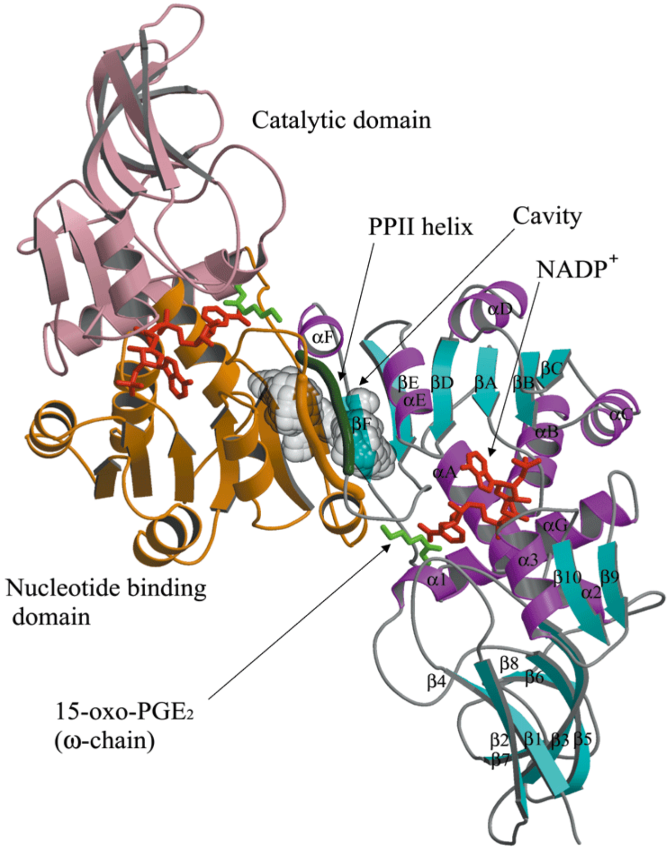
Takao Shimizu*, Olof Rådmark, and Bengt Samuelsson†

Department of Physiological Chemistry, Karolinska Institutet, S-104 01 Stockholm, Sweden

Drawing a map

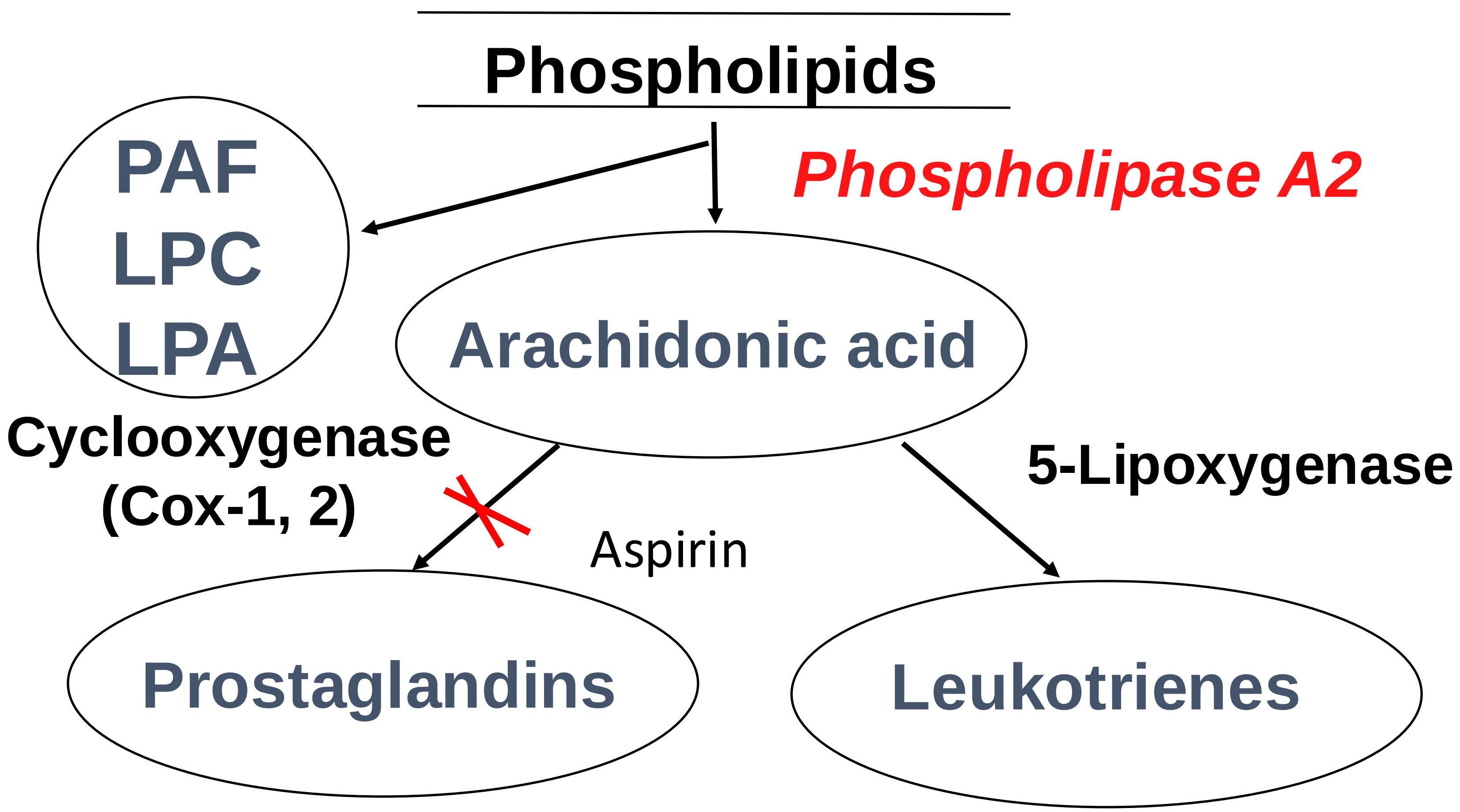


LTB4HD/PGKR

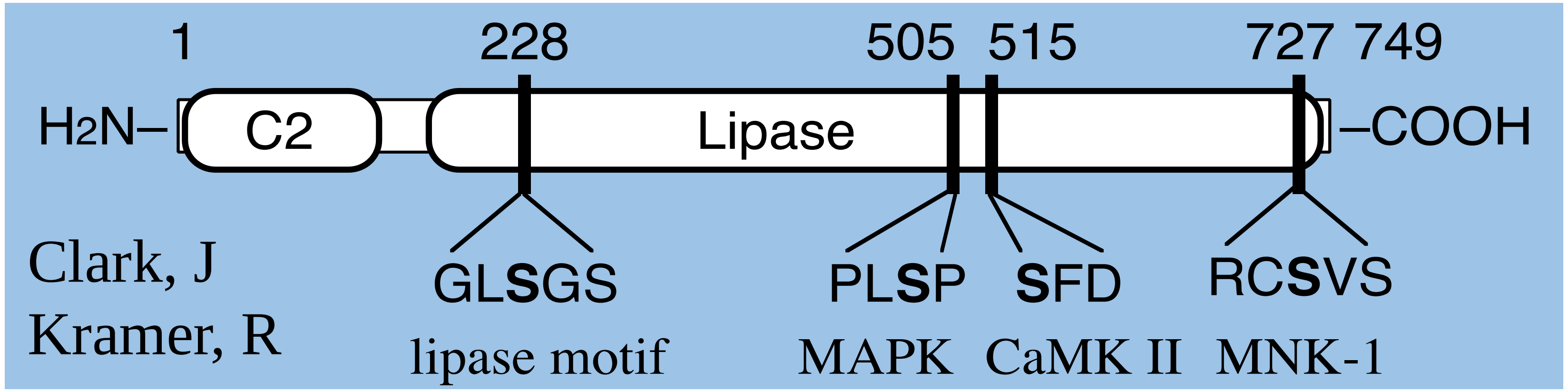


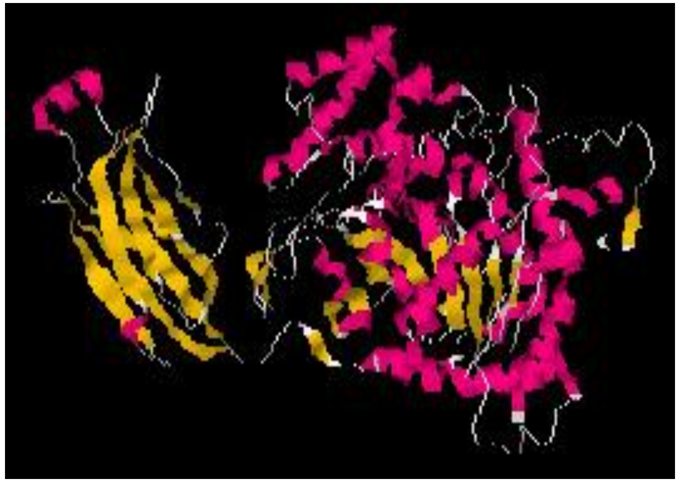
Hori, Yokomizo et al. *JBC* 2004

Phospholipase A_2 is a key molecule to produce lipid mediators

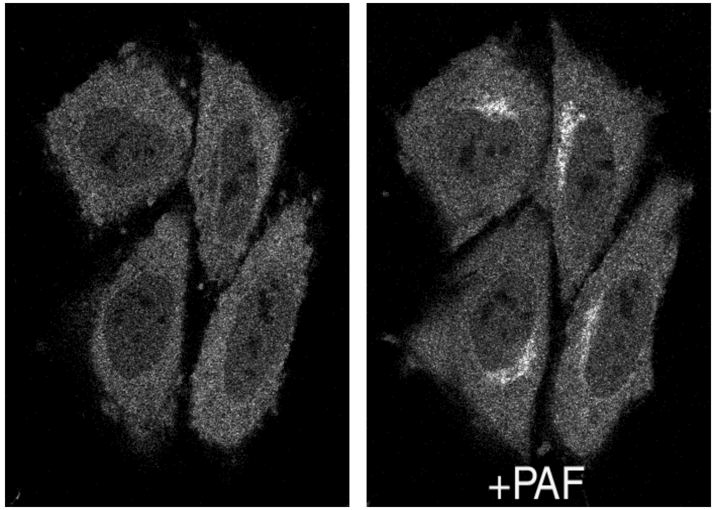


Structure and redistribution of cytosolic phospholipase A2α





Dessen et al. 1999

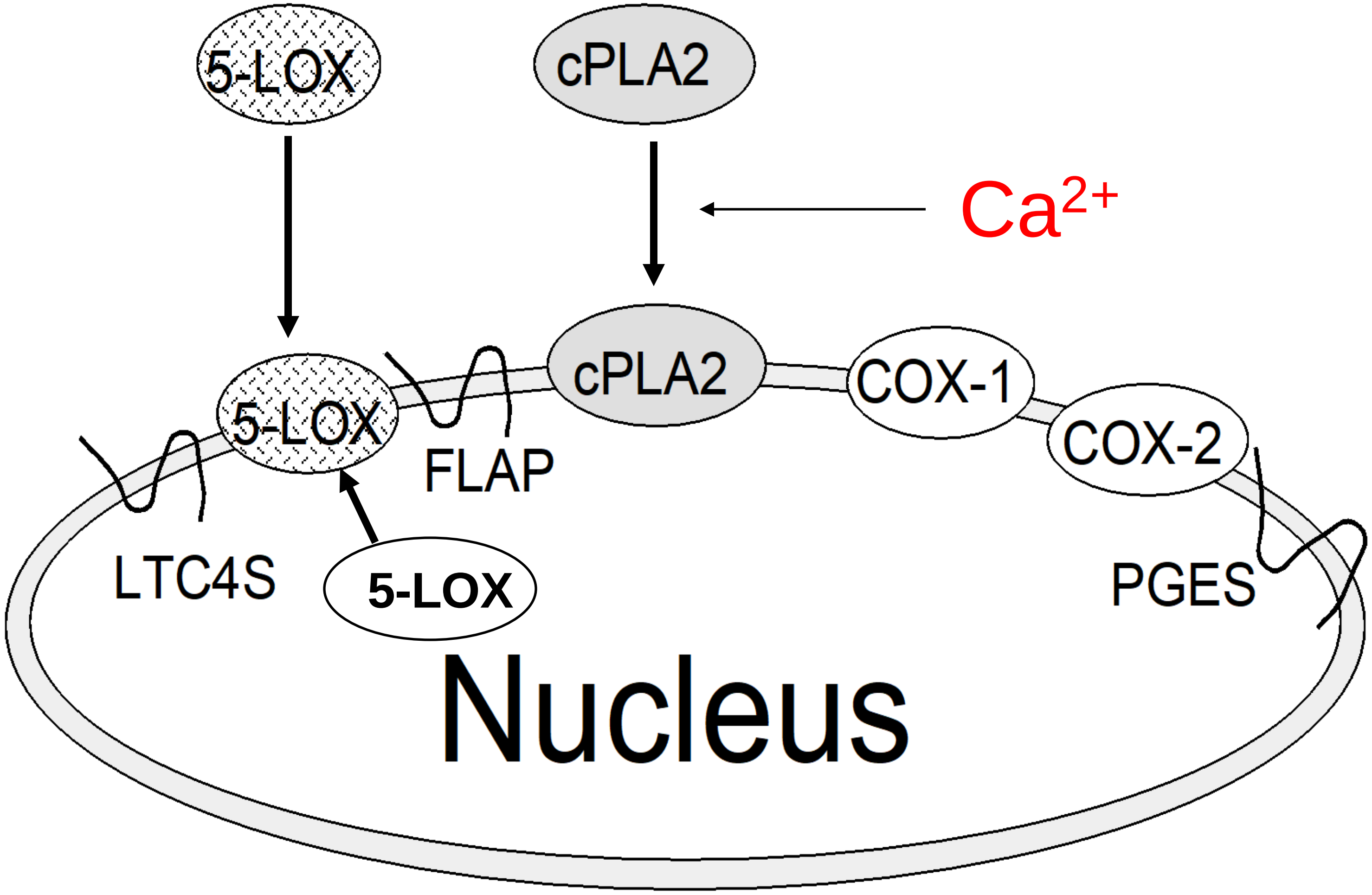


Hirabayashi et al. 1999

Properties of cPLA2α

- **Expressed in almost all tissues and cells**
- **Preferential liberation of arachidonic acid among various fatty acids**
- **Translocation from cytosol to Golg-ER by Ca increase to meet downstream enzymes (5-Lox, Cox-1, 2 etc)**
- **Activation by phosphorylation with mapk and p38 kinases**

Most enzymes on eicosanoid biosynthesis assemble at perinuclear membrane



Phenotypes of $cPLA_2\alpha$ (-/-) mice

1. Reduced symptom of bronchial asthma (*Nature*,1997).
2. Decreased mortality & symptoms of ARDS (*Nature Immunol*, 2001).
3. Milder symptoms in bleomycin-induced fibrosis (*Nature Med*, 2002)
4. Reduced mortality due to thromboembolism （*J. Exp. Med*, 2002, *Blood* 2009)
5. Marked reduction of collagen-induced arthritis (*J. Exp. Med*, 2003).
6. Milder symptoms in inflammatory bone resorption (*J. Exp. Med*, 2003*)*
7. Milder symptoms in allergic encephalomyelitis (*J. Exp. Med* 2005, *PNAS*, 2010)
8. Prevention from atherosclerosis (*Amer. J. Physiol.* 2012*)*
9. Impairment of synaptic plasticity and delivery (*PNAS*, 2010)
10. Protection of intestinal polyposis (*J. Exp. Med*, 2015; *PNAS*, 2017)

Most of phenotypes are explained by the deficiency of downstream lipid mediators

Failure of cPLA2α inhibitor for clinical use

- Collaboration with A company for 12 years
- Screening out a potent and selective inhibitor (20 mg per day, po, good PK and PD)
- Rheumatoid arthritis, bronchial asthma, osteoporosis
- Stop development recently, because of adverse effects at high doses during phase III clinical trial in US
- Potential use for Covid-19-induced ARDS

Expression cloning of PAF receptor
the 1st example of lipid GPCRs (1991)

Cloning by functional expression of platelet-activating factor receptor from guinea-pig lung

Zen-ichiro Honda*†, Motonao Nakamura*, Ichiro Miki*, Michiko Minami*, Tsuyoshi Watanabe*, Yousuke Seyama*, Haruo Okado‡, Hiroyuki Toh§, Kohji Ito||, Terumasa Miyamoto|| & Takao Shimizu*¶

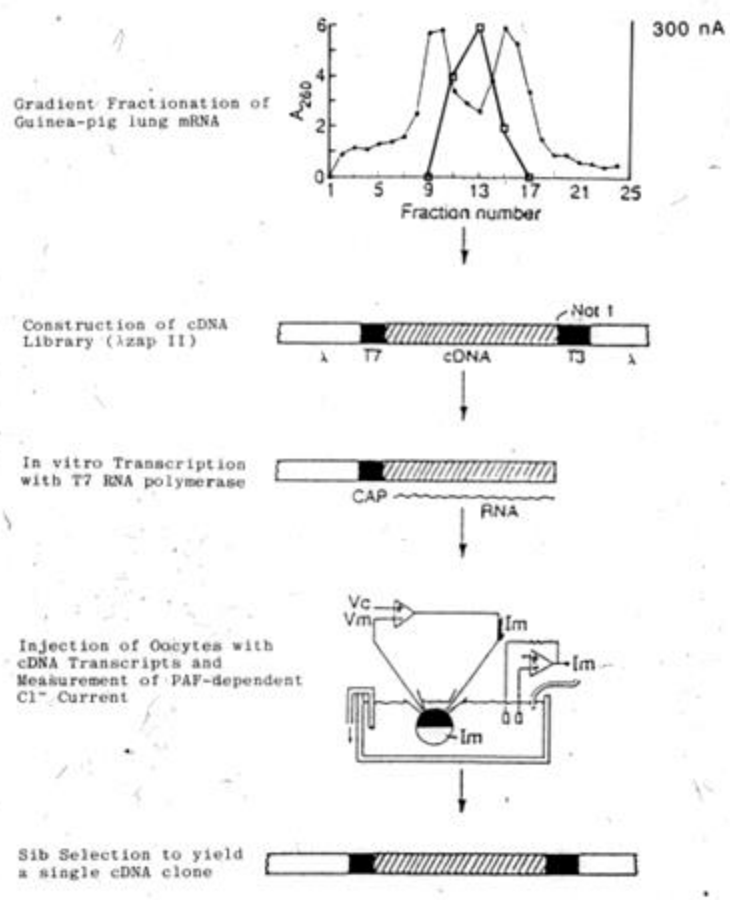
* Department of Physiological Chemistry and Nutrition,
‡ Department of Neurobiology, Institute of Brain Research, and
|| Department of Internal Medicine and Physical Therapy,
Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan
§ Protein Engineering Research Institute, 6-2-3 Furuedai, Suita, Osaka 565, Japan

PLATELET-activating factor (PAF), a unique phospholipid mediator, possesses potent proinflammatory, smooth-muscle contractile and hypotensive activities, and appears to be crucial in the pathogenesis of bronchial asthma and in the lethality of endotoxin and anaphylactic shock[1–3]. Despite this, little is known of the molecular properties of the PAF receptor and related signal transduction systems. Although several lines of evidence suggest that activation of the PAF receptor stimulates phospholipase C and subsequent inositol trisphosphate formation through G protein(s)[4,5], the PAF receptor and calcium channel are reported to show a close relation[2,6]. As a first approach to cloning lipid autacoid

† On leave from Department of Internal Medicine and Physical Therapy, Faculty of Medicine, University of Tokyo, Japan.
¶ To whom correspondence should be addressed.

NATURE · VOL 349 · 24 JANUARY 1991

Cloning Strategy of PAF Receptor cDNA



PAF Receptor cDNA

Receptors

PAF receptor, *Nature* 1991, *Neuron*, 1992
LTB4 receptors (BLT1, 2), *Nature* 1997,
J. Exp. Med., 2000, *J. Exp. Med*, 2005;
Nature CB, 2018
Non-edg LPA4 and LPA6, *JBC*,2003, *JBC*,2009
JCI Insight, 2018; *Human Mol. Genetics*, 2022

Z. Honda, I. Miki
(Firenze, 1991)

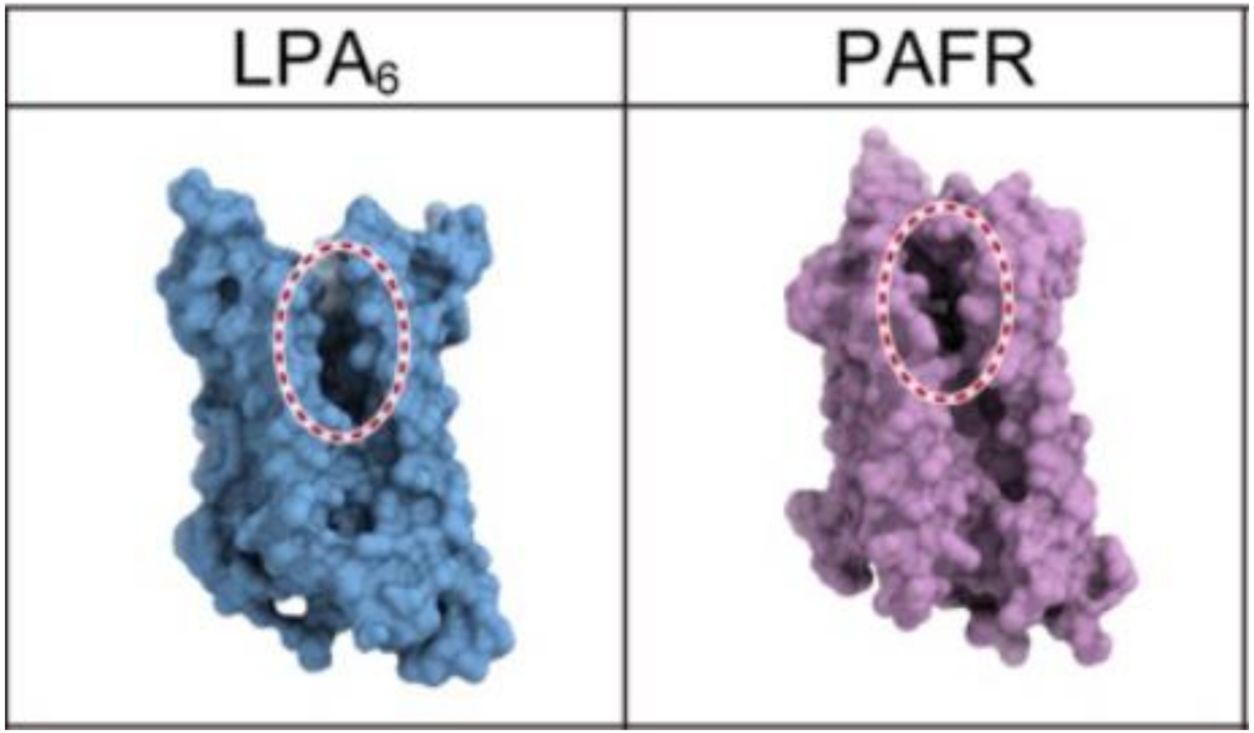
S. Ishii, K. Yanagida, K. Noguchi
Snowmass, 2005

Takehiko Yokomizo,
Boston,1999

Recent hot topics on PAFR, LPA6

Rupadatine, a PAF receptor antagonist approved by FDA, now on market over 90 countries.

The drug is now used for adverse effects after SARS-Covid-19 vaccination



Cleft between TM4 and 5, suggesting lateral movement of ligand
Taniguchi, Nature 2017; Cao, et al. *Nature SMB*. 2018

Summary-1 (Mediators)

- Arachidonate 5-lipoxygenase catalyzes LTA4 formation by its 8-lipoxygenase activity (both potato and mammals)
- LTA4 hydrolase was cDNA cloned, which has dual activities of epoxide hydrolase (LTB4 formation) and Zn-aminopeptidase.
- By knockout mice studies, cPLA2a plays important roles in health and diseases through productions of eicosanoids and PAF.
- Like catecholamines or peptides, most lipid mediators also exert their biological activities by GPCR activations (PAF, LTB4, LPA etc)

Agenda of my talk

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PERSPECTIVE

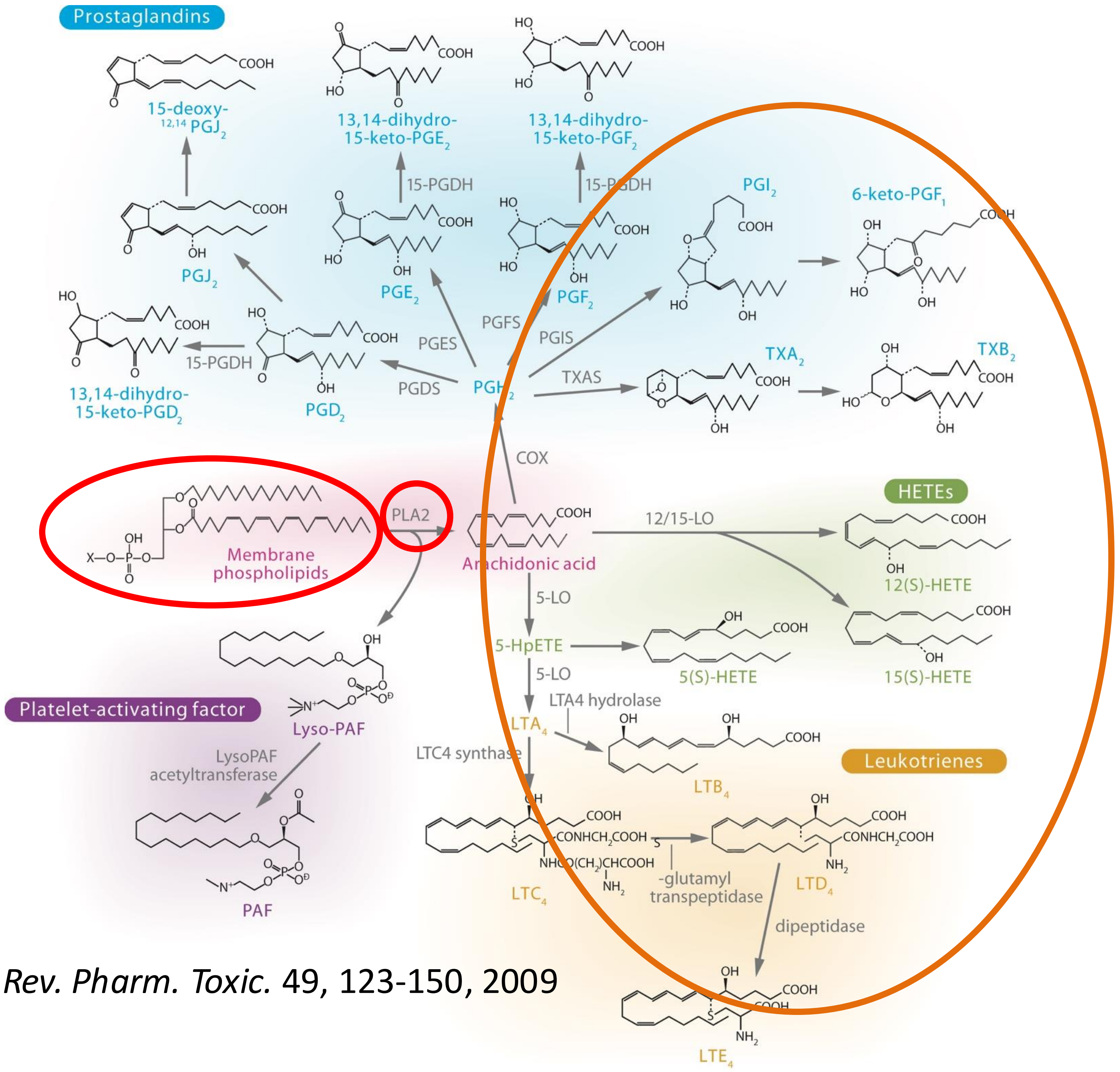
Turning point in 2003

a year of whole human genome was sequenced,
and 10 years before my formal retirement

- **How arachidonate is located at *sn*-2 position, and how membrane diversity is made?**

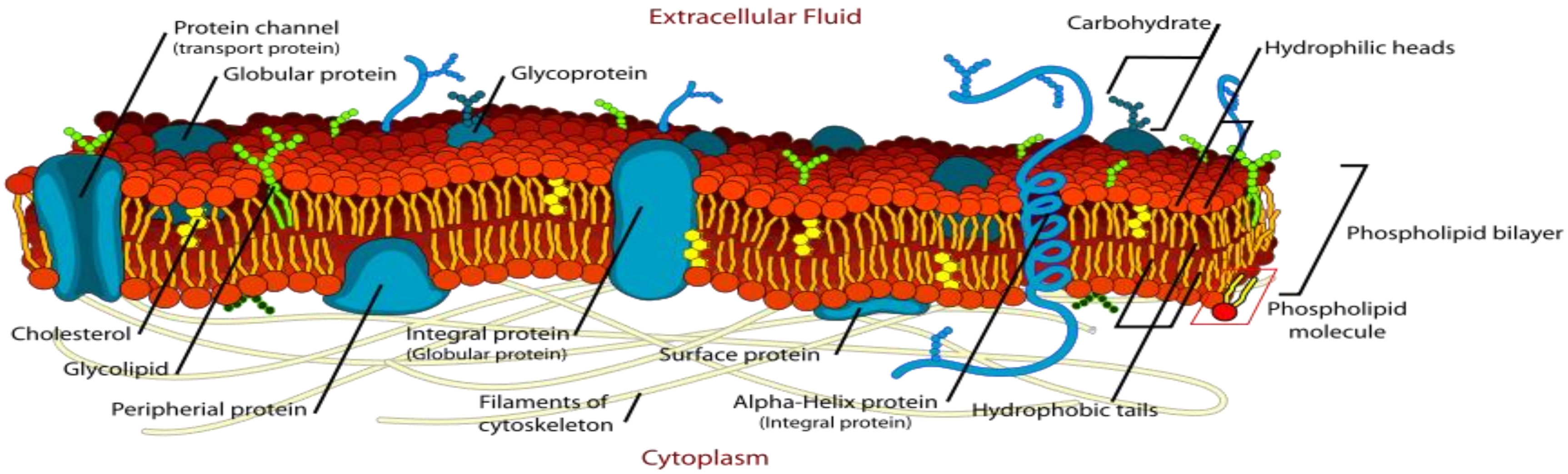
- **Need development of comprehensive lipidomics techniques**

(Ono Pharmaceutical and Shimadzu supported establishment of a metabolome laboratory at U-Tokyo.)

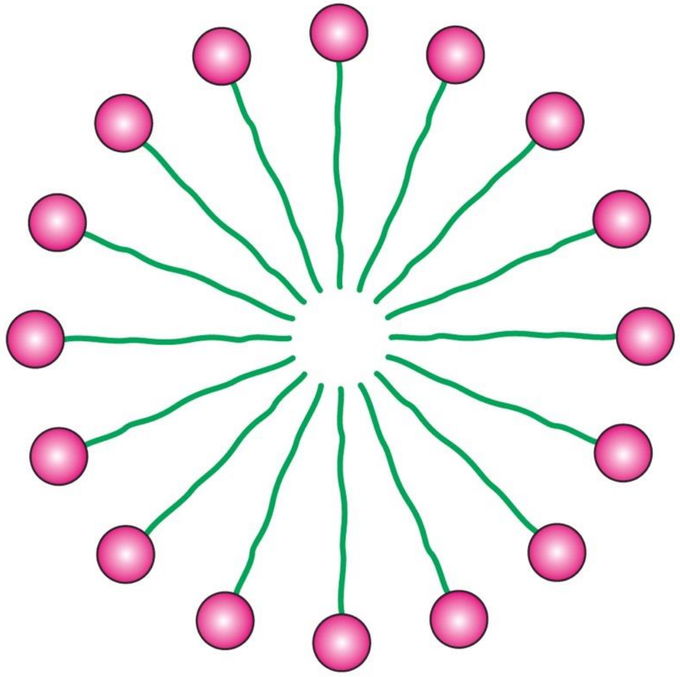


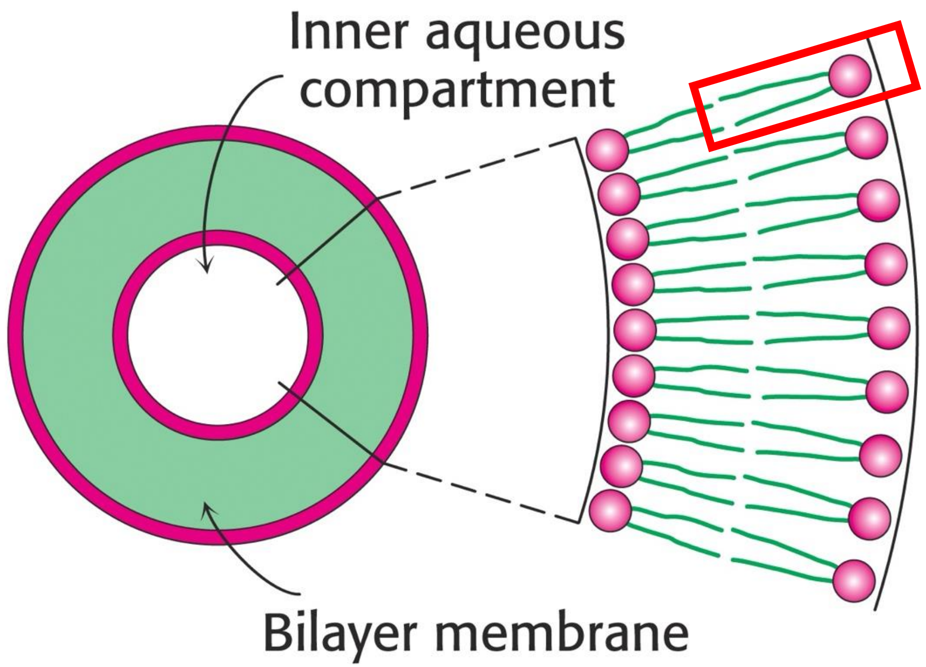
Shimizu, T. *Annu. Rev. Pharm. Toxic.* 49, 123-150, 2009

Phospholipids as major components of biological membrane



Lipid bilayer is made with phospholipids with amphipathic properties



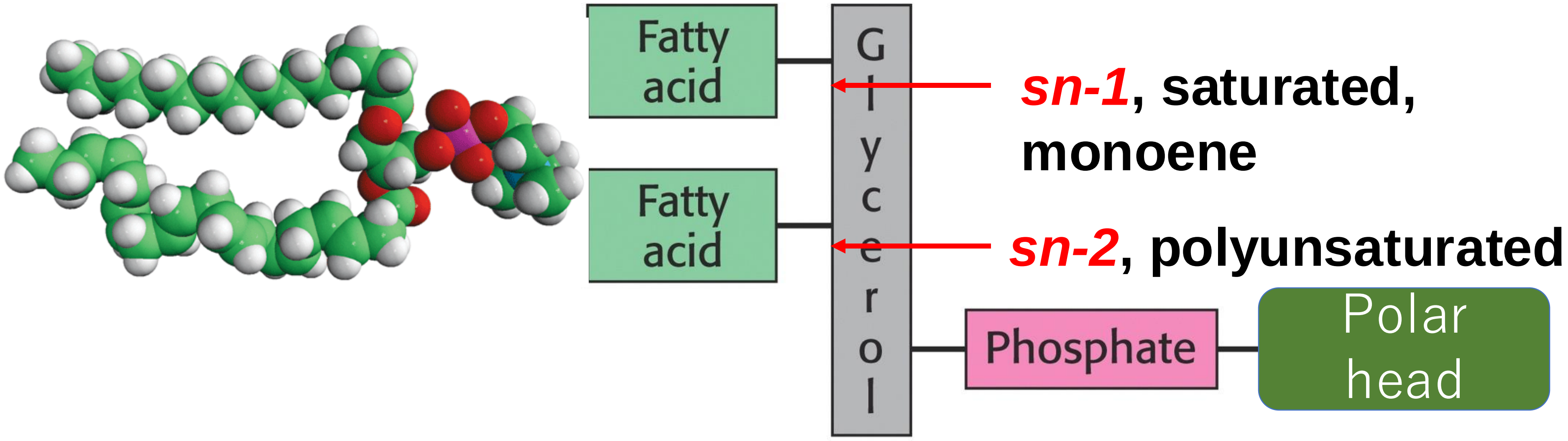


Stryer, 8th ed

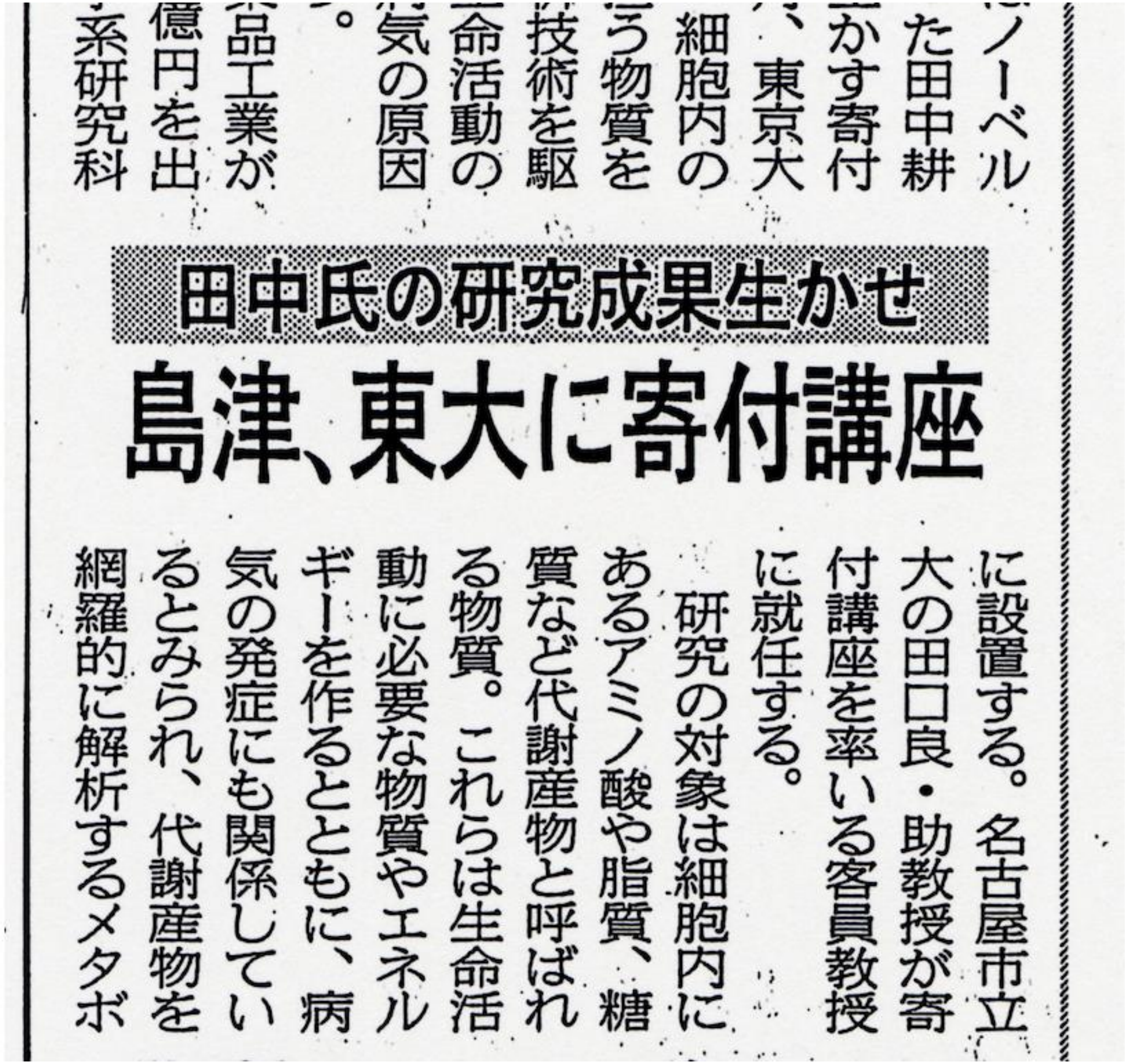
Micelle

Liposome

Fatty acyl diversity and asymmetry of glycerophospholipids



- ***How?   Biochemical mechanisms***
- ***So what?   Biological consequence***



はノーベル
た田中耕
生かす寄付
、東京大
細胞内の
う物質を
技術を駆
生命活動の
病気の原因
る。
品工業が
億円を出
系研究科

田中氏の研究成果生かせ

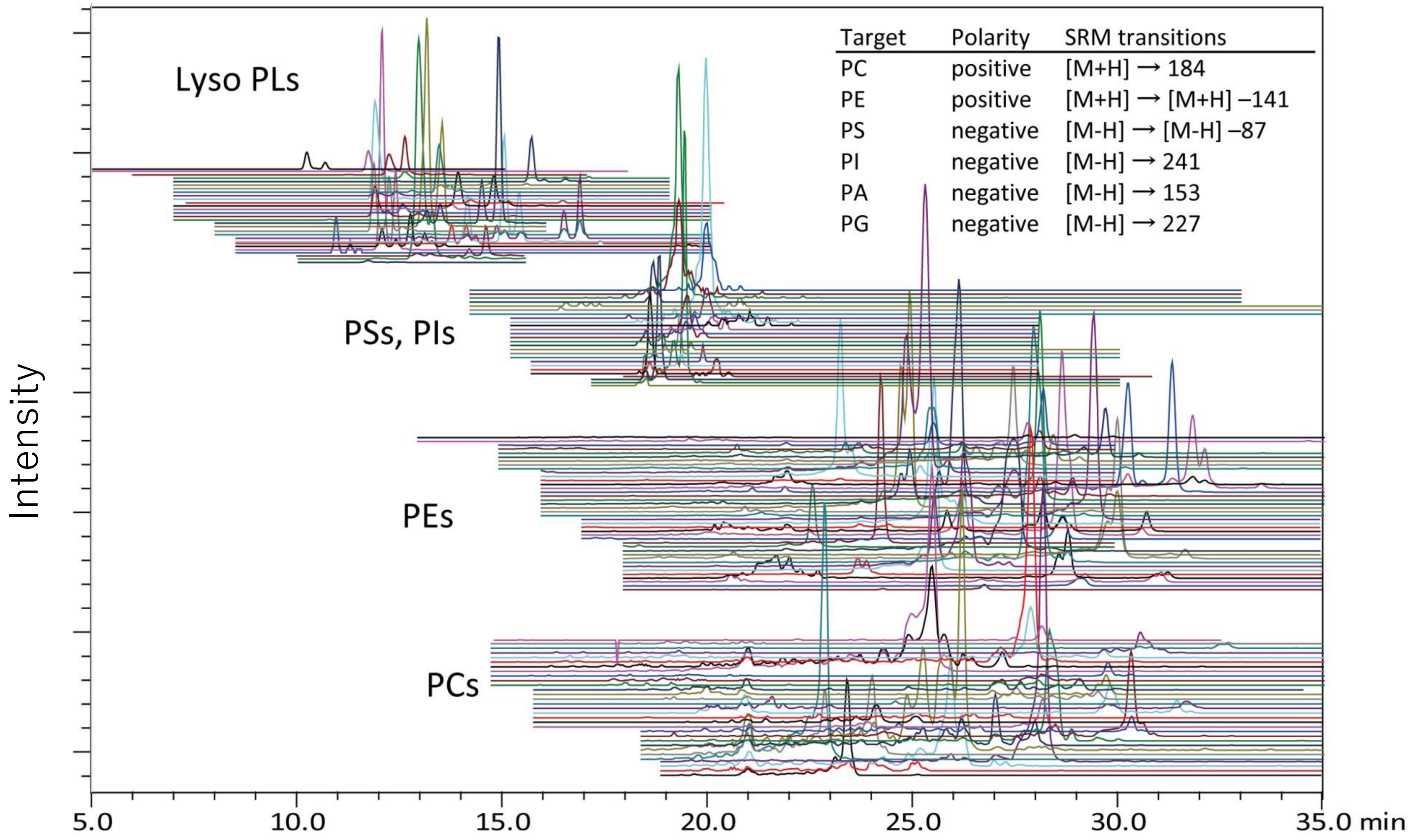
島津、東大に寄付講座

に設置する。名古屋市立大の田口良・助教授が寄付講座を率いる客員教授に就任する。

研究の対象は細胞内にあるアミノ酸や脂質、糖質など代謝産物と呼ばれる物質。これらは生命活動に必要な物質やエネルギーを作るとともに、病気の発症にも関係しているとみられ、代謝産物を網羅的に解析するメタボ

2002.10.7　日経新聞

A typical SRM of brain phospholipids



Tokuoka, Kita et al.
Lipid extract from mouse brain

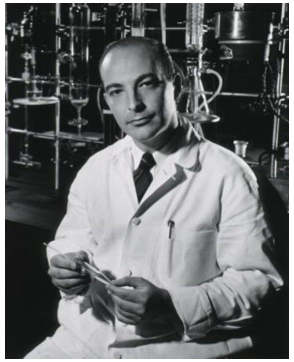
First concept by Kornberg

1. Fatty acid activation（acyl-CoA ligase, ACL）

R-COOH + CoASH +ATP →R-COCoA + AMP+PPi

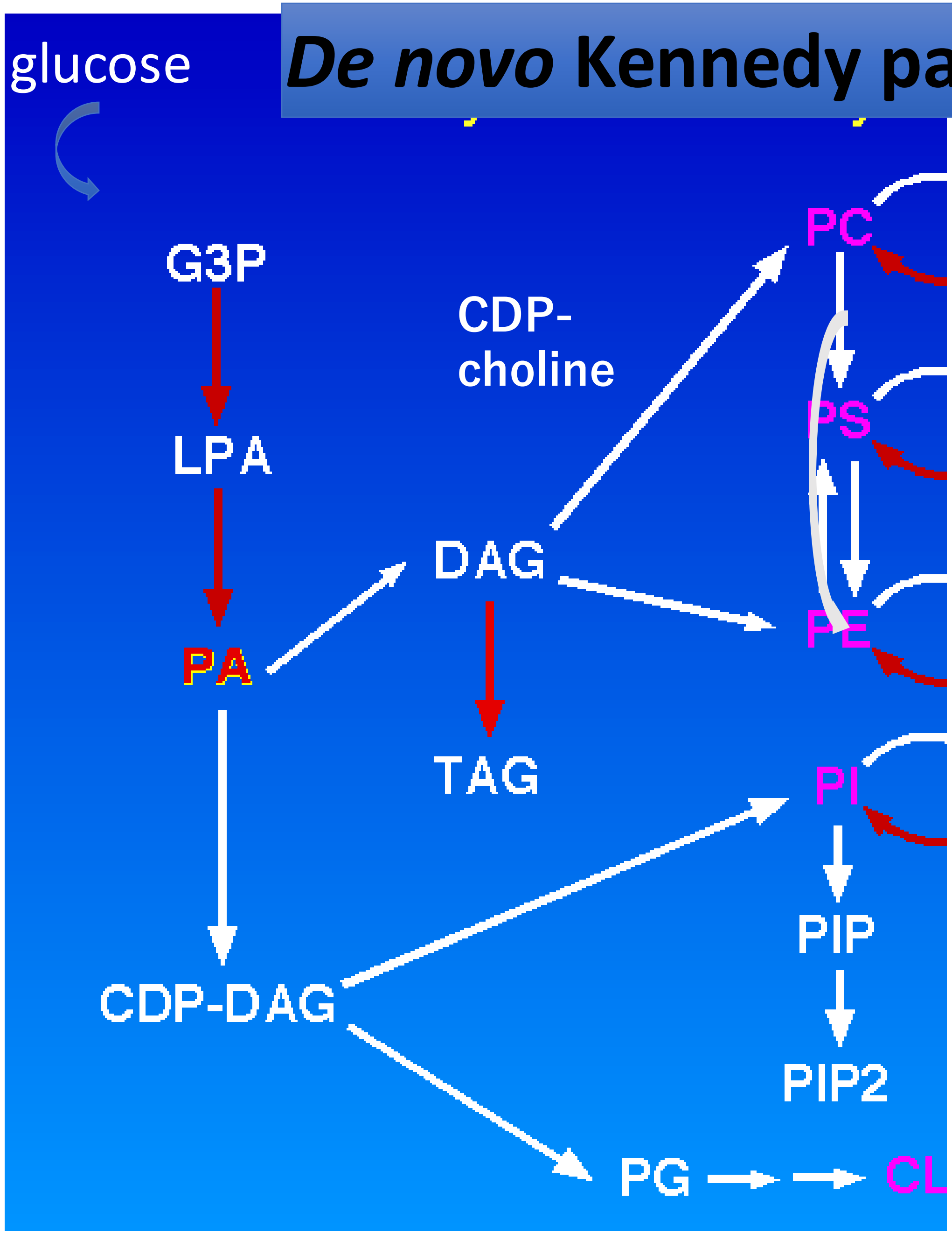
2. Transfer of acyl-CoA（acyltransferase）

R-COCoA + Acceptor(-OH) → Phospholipids



Pricer and Kornberg, J. Biol Chem. 1950

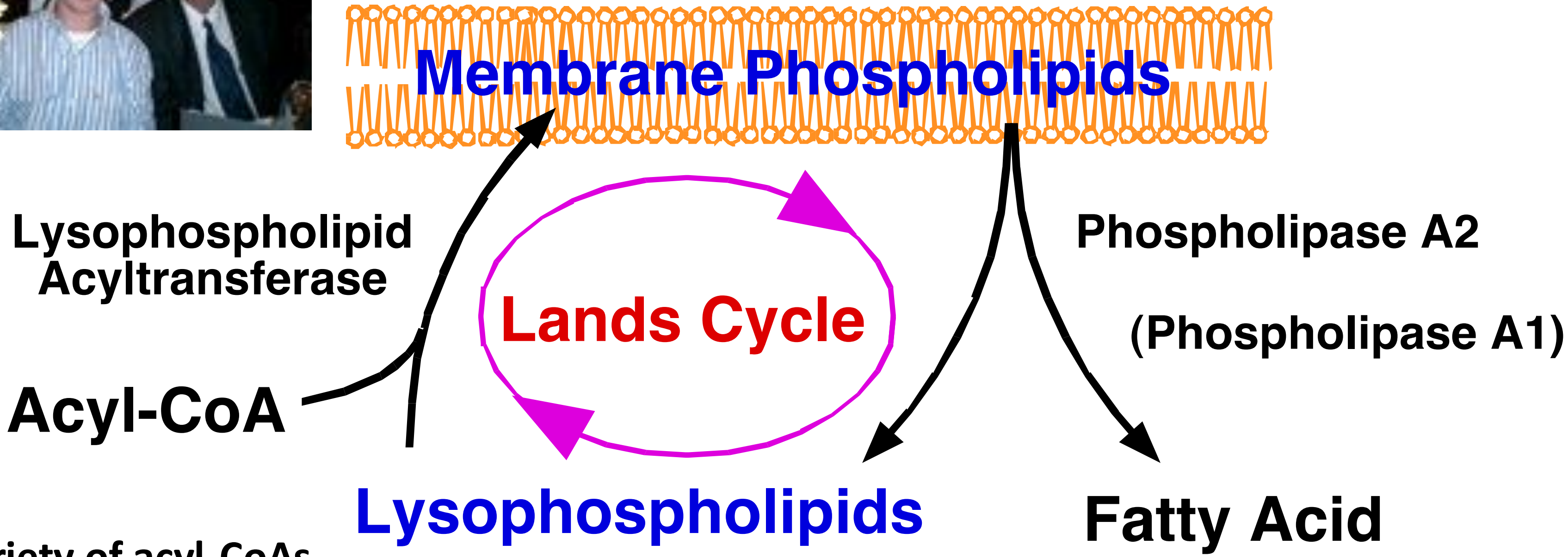
De novo Kennedy pathway



Eugene P. Kennedy

1919-2011

Remodeling pathway to make mature membrane with diversity



A variety of acyl-CoAs including PUFA's

Lands WEM et al., 1960

Earlier study on lipid acyltransferases

Characterization of *sn-Glycerol 3-Phosphate Acyltransferase from Guinea Pig Harderian Gland Microsomes*[1]
Kazuhiko KUME, Takao SHIMIZU and Yousuke SEYAMA
J. Biochem. 101, 653-660, 1987

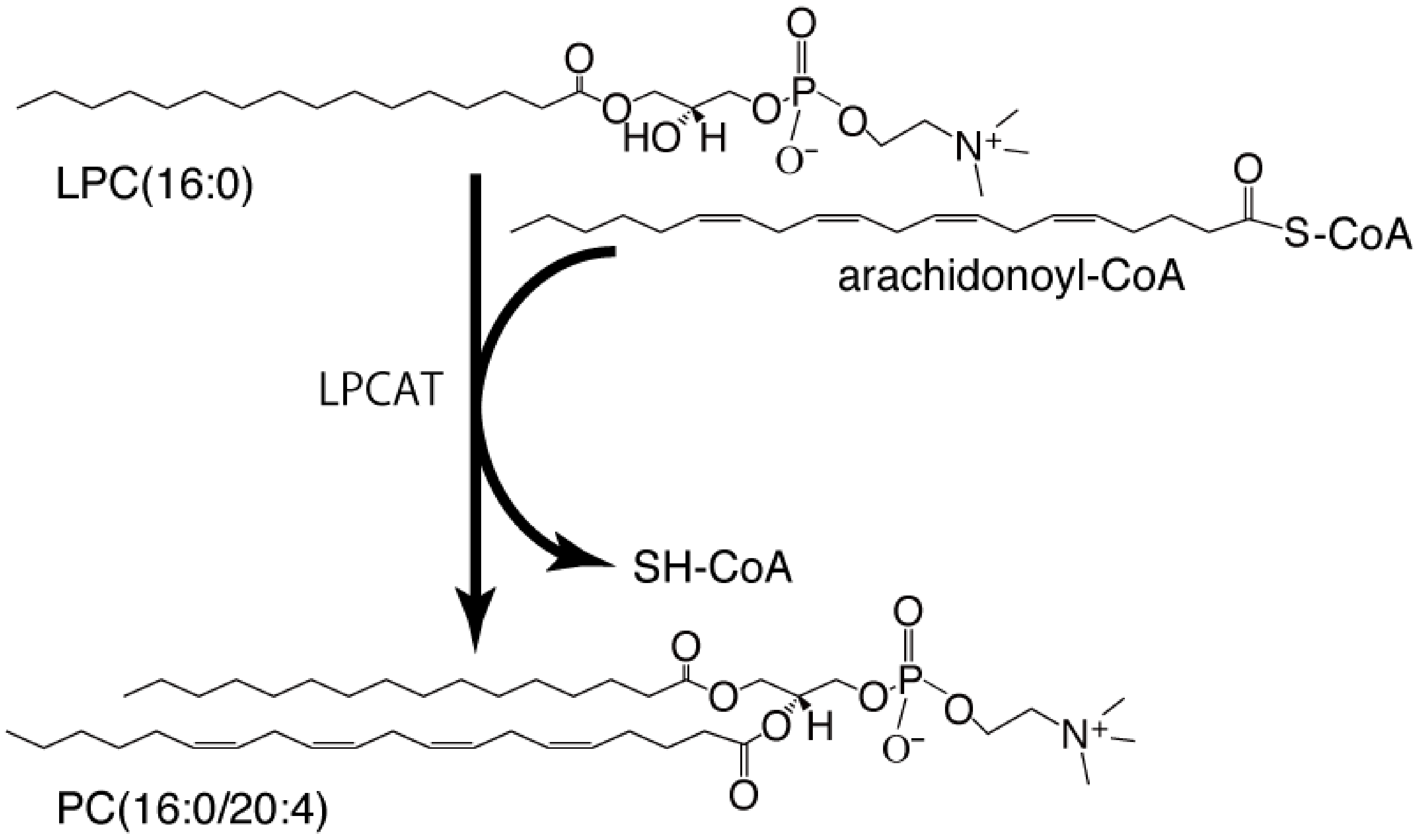
粂和彦

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **237**, 663–666 (1997)
ARTICLE NO. RC977214

cDNA Cloning and Expression of Murine 1-Acyl-*sn*-glycerol-3-phosphate Acyltransferase

Kazuhiko Kume and Takao Shimizu *1997*
Department of Biochemistry and Molecular Biology, Faculty of Medicine, The University of Tokyo, Hongo, Bunkyo, Tokyo 113, Japan

Lysophosphatidylcholine (LPC) acyltransferases (LPCAT)



Discovery of the first LPCAT (LPCAT1)

Nakanishi et al., *J. Biol. Chem*. 2006

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Human LPCAT1    1  MRLRGCGPRAAPASSAGASDARLLAPPGRNPFVHELRLSALQKAQVALMTLTLFPVRLLV   60
Mouse LPCAT1    1  MRLRGRGPRAAPSSSSGAGDARRLTPPGRNPFVHELRLSALQKAQVAFMTLTLFPIRLLF   60
  Rat LPCAT1    1  MRLRGRGPRAAPSSSSGAGDARRLAPPGRNPFVHELRLSALQKAQVAFMTLTLFPIRLLF   60
                   *****.******.**.**.***.*.**********************.*******.***.

Human LPCAT1   61  AAAMMLLAWPLALVASLGSAEKEPEQPPALWRKVVDFLLKAIMRTMWFAGGFHRVAVKGR  120
Mouse LPCAT1   61  AAFMMLLAWPFALLASLGPPDKEPEQPLALWRKVVDFLLKAIMRTMWFAGGFHRVAVKGR  120
  Rat LPCAT1   61  AAFMMLLAWPFALVASLGPPDKEPEQPLALWRKVVDFLLKAIMRTMWFAGGFHRVAVKGR  120
                   **.*******.**.****  .******.********************************
                                 motif 1                      motif 2
Human LPCAT1  121  QALPTEAAILTLAPHSSYFDAIPVTMTMSSIVMKAESRDIPIWGTLIQYIRPVFVSRSDQ  180
Mouse LPCAT1  121  QALPTEAAILTLAPHSSYFDAIPVTMTMSSIVMKAESRDIPIWGTLIRYIRPVFVSRSDQ  180
  Rat LPCAT1  121  QALPTEAAILTLAPHSSYFDAIPVTMTMSSIVMKAESRDIPIWGTLIRYIRPVFVSRSDQ  180
                   ***********************************************.************
                                            motif 3
Human LPCAT1  181  DSRRKTVEEIKRRAQSNGKWPQIMIFPEGTCTNRTCLITFKPGAFIPGAPVQPVVLRYPN  240
Mouse LPCAT1  181  DSRRKTVEEIKRRAQSNGKWPQIMIFPEGTCTNRTCLITFKPGAFIPGVPVQPVVLRYPN  240
  Rat LPCAT1  181  DSRRKTVEEIKRRAQSNGKWPQIMIFPEGTCTNRTCLITFKPGAFIPGVPVQPVVLRYPN  240
                   ************************************************.***********

Human LPCAT1  241  KLDTITWTWQGPGALEILWLTLCQFHNQVEIEFLPVYSPSEEEKRNPALYASNVRRVMAE  300
Mouse LPCAT1  241  KLDTITWTWQGPGALKILWLTLCQFQNQVEIEFLPVYCPSEEEKRNPALYASNVRRVMAK  300
  Rat LPCAT1  241  KLDTITWTWQGPGALKILWLTLCQFQNQVEIEFLPVYCPSEEEKRNPALYASNVRRVMAK  300
                   ***************.*********.***********.*********************.

Human LPCAT1  301  ALGVSVTDYTFEDCQLALAEGQLRLPADTCLLEFARLVRGLGLKPEKLEKDLDRYSERAR  360
Mouse LPCAT1  301  ALGVSVTDYTFEDCQLALAEGQLRLPADTCLLEFARLVRGLGLKPENLEKDLDKYSESAR  360
  Rat LPCAT1  301  ALGVSVTDYTFEDCQLALAEGQLRLPADTCLLEFARLVRGLGLKPENLEKDLDKYSESAR  360
                   **********************************************.******.***.**

Human LPCAT1  361  MKGGEKIGIAEFAASLEVPVSDLLEDMFSLFDESGSGEVDLRECVVALSVVCRPARTLDT  420
Mouse LPCAT1  361  MKRGEKIRLPEFAAYLEVPVSDALEDMFSLFDESGGGEIDLREYVVALSVVCRPSQTLAT  420
  Rat LPCAT1  361  MKRGEKIRLPEFAAYLEVPVSDALEDMFSLFDESGGGEIDLREYVVALSVVCRPSQTLAT  420
                   **.****...****.*******.************.**.****.**********..**.*

Human LPCAT1  421  IQLAFKMYGAQEDGSVGEGDLSCILKTALGVAELTVTDLFRAIDQEEKGKITFADFHRFA  480
Mouse LPCAT1  421  IQLAFKMYGSPEDGSIDEANLSCILKTALGVSELTVTDLFQAIDQEDKGRITFDDFCGFA  480
  Rat LPCAT1  421  IQLAFKMYGSPEDGSIDEADLSCILKTALGISELTVTDLFQAIDQEERGRITFDDFCGFA  480
                   *********..****..*..**********..********.*****..*.***.**..**

Human LPCAT1  481  EMYPAFAEEYLYPDQTHFESCAETSPAPIPNGFCADFSPENSDAGRKPVRKKLD        534
Mouse LPCAT1  481  EMYPDYAEDYLYPDQTHFDSCAQTPPAPTPNGFCIDFSPENSDFGRKNSCKKAD        534
  Rat LPCAT1  481  EMYPDFAEDYLYPDQTHSDSCAQTPPAPTPNGFCIDFSPEHSDFGRKNSCKKVD        534
                   ****..**.********..***.*.***.*****.*****.**.***...** *
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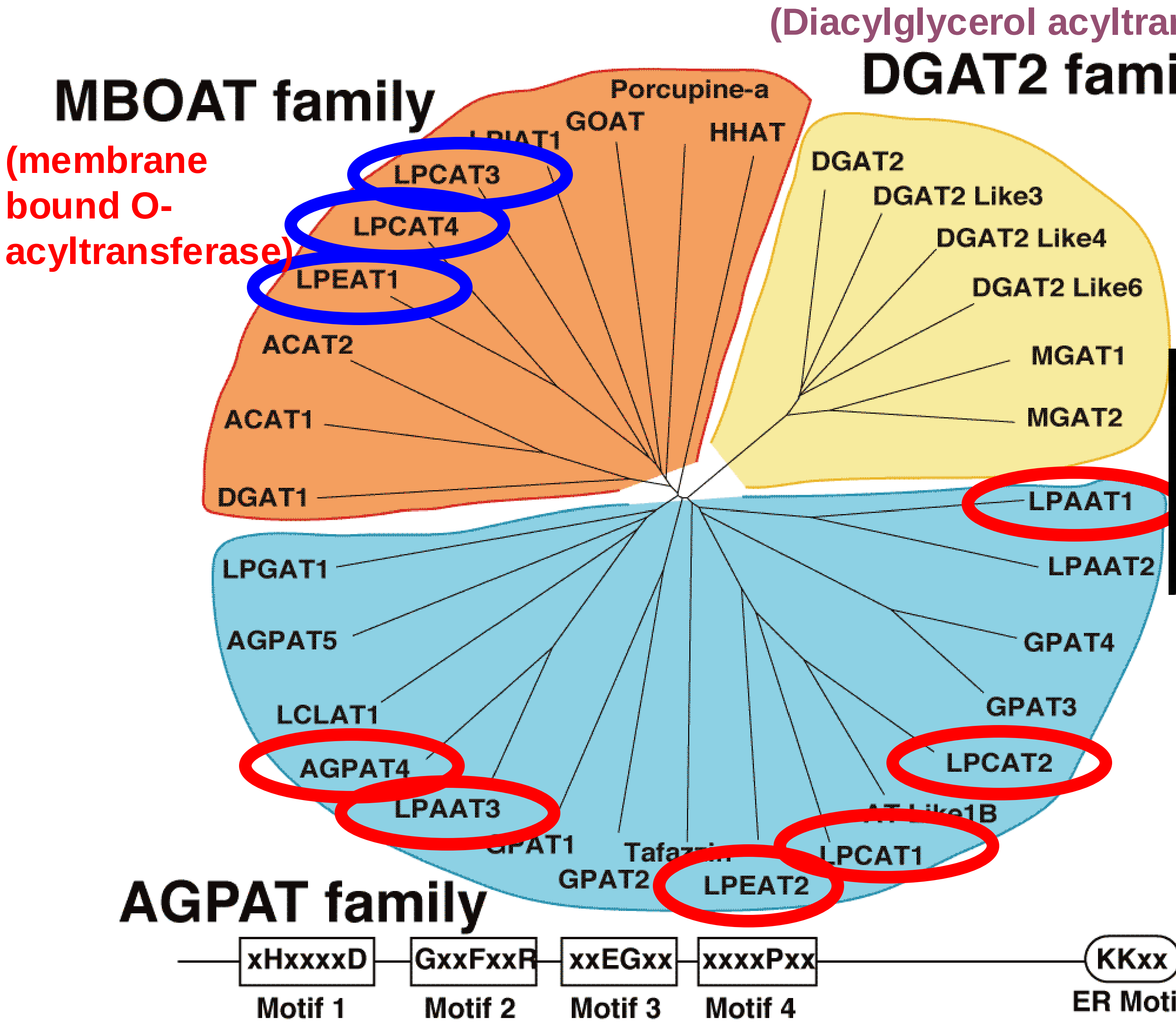
Ref. Chen, X et al. *PNAS*, 2006

Acyltransferase Family



Conversations with Lipid Leaders: Dr. Bruno Antonny

Posted on September 02, 2021

What do you consider the greatest breakthrough in lipid research in recent years?

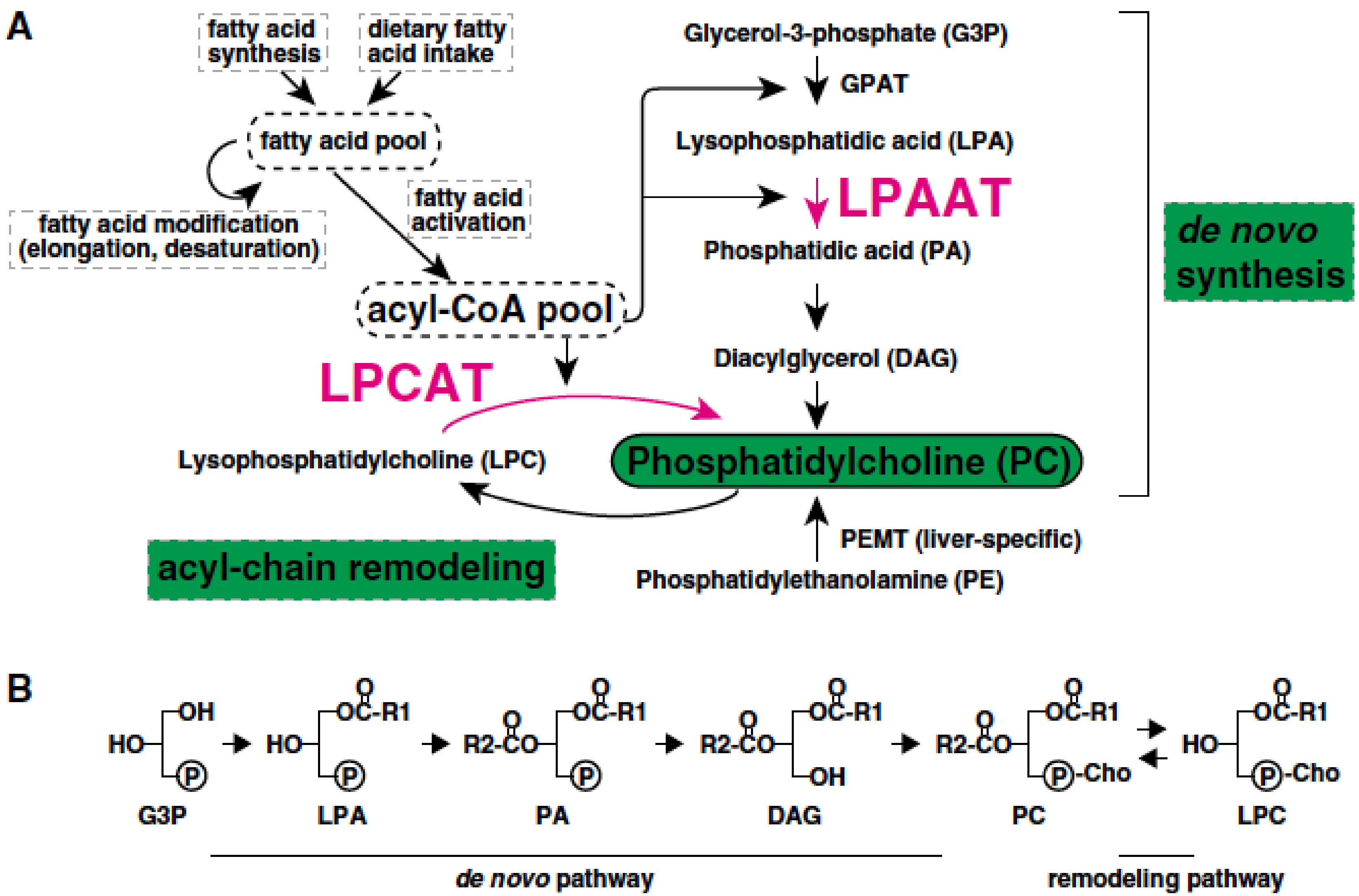
The discovery of lipid remodeling enzymes, notably by the Shimizu lab in Japan because it opens an avenue for understanding how and why cells in real tissues control so well the acyl chain profiles their organelles. Classical cell lines used by cell biologists are very rudimentary in this respect.

Classical idea for membrane diversity and asymmetry

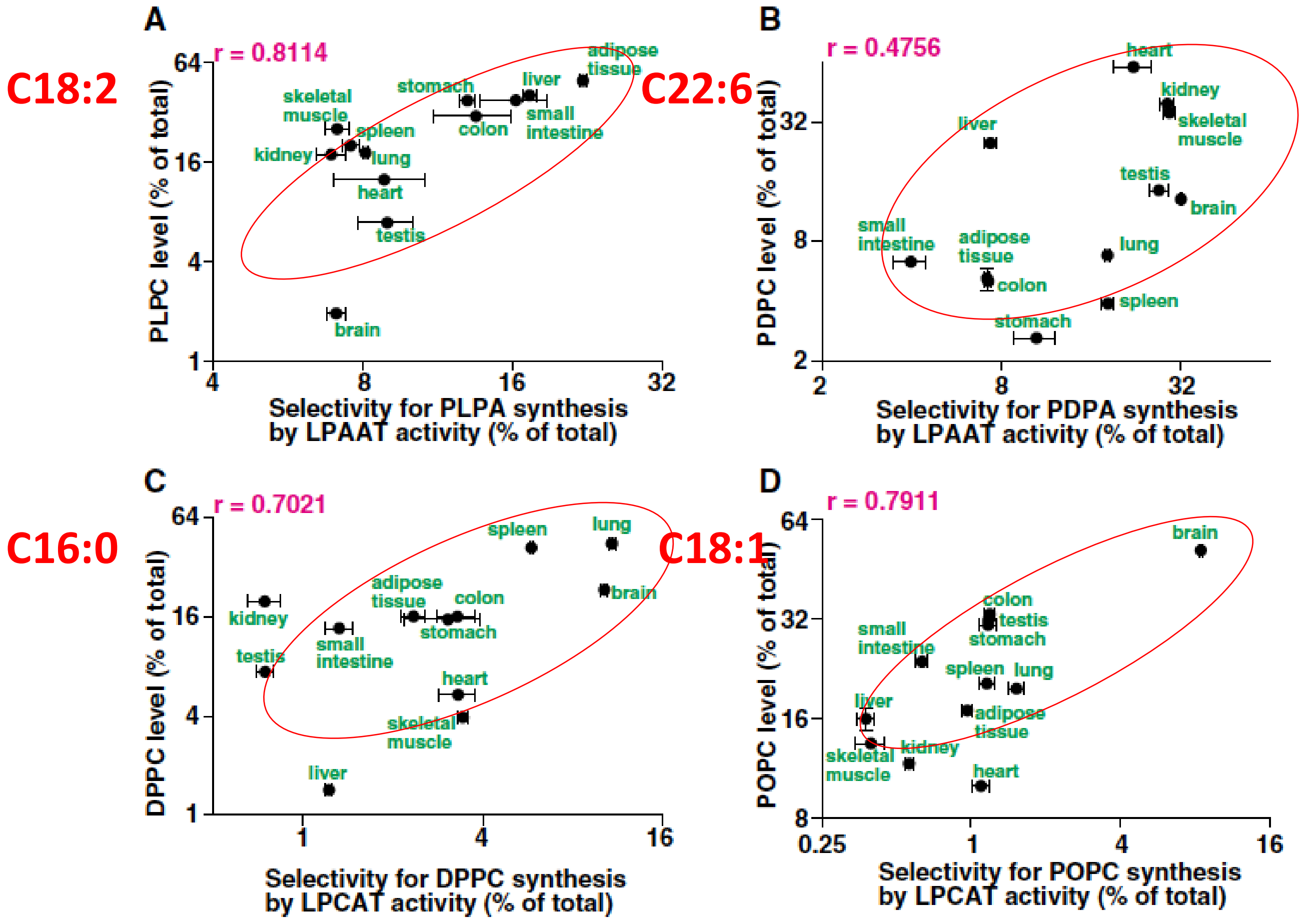
- **Kennedy pathway (*de novo* pathway)** prefers saturated fatty acids, and no diversity is made.

- **Lands' cycle** matures membrane phospholipids with *sn-1* saturated and *sn-2* PUFA by the action of phospholipase A2 and lysophospholipid acyltransferases.

2 Steps to determine fatty acid at sn-2 position



Correlation of tissue phospholipid contents and enzyme activities



De novo pathway

Remodeling pathway

PLPC, palmitoic/linoleic acid; PDPC, P/DHA; DPPC, di-p; POPC, p/oleic acid

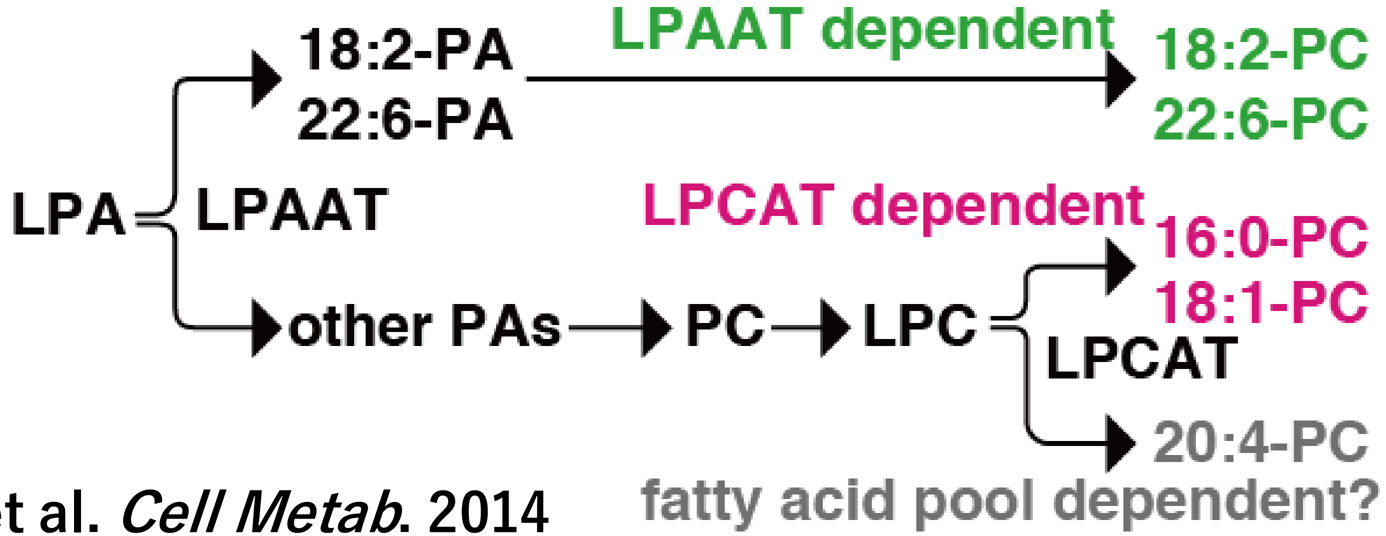
Impact of enzymes (de novo *vs* remodeling) on phospholipid compositions

E

DPPC 16:0/16:0
POPC 16:0/18:1
PLPC 16:0/18:2
PAPC 16:0/20:4
PDPC 16:0/22:6

	LPAAT	LPCAT
DPPC	0.4609	0.7021
POPC	-0.2937	0.7911
PLPC	0.8114	0.4595
PAPC	0.2393	0.3416
PDPC	0.4756	-0.1646

Pearson's correlation coefficient



Kennedy

Lands

Harayama, T. et al. *Cell Metab*. 2014

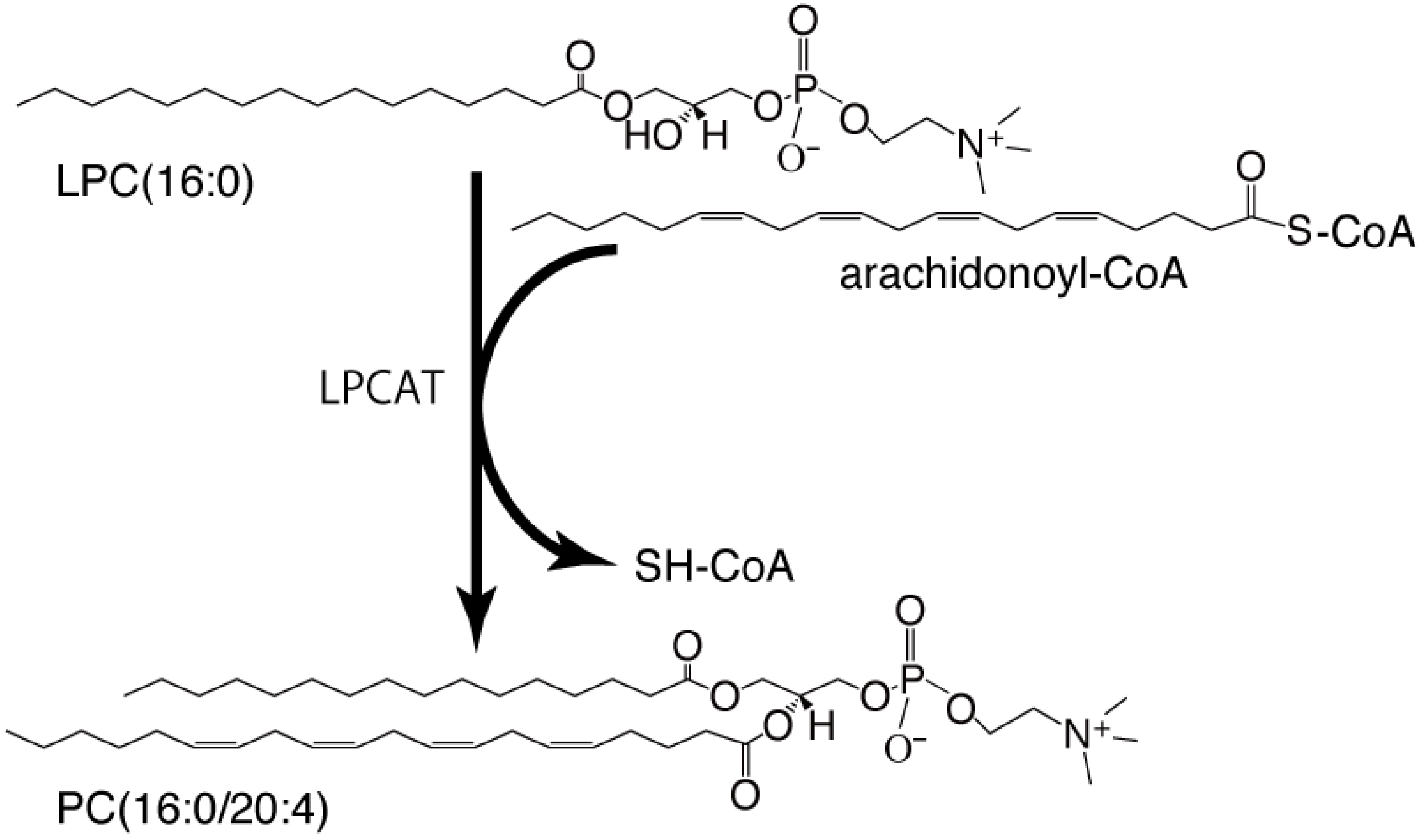
A proposed revised model for fatty acid incorporation to PLs

- **Palmitic acid (16:0), oleic acid (18:1) and arachidonic acid (20:4) are incorporated by LPC acyltransferases in Lands' cycle.**

- **Linoleic acid (18:2) and DHA (22:6) are incorporated by LPA acyltransferases in *de novo* pathway.**

Harayama, T. et al. *Cell Metabolism*, 2014, 2016

Lysophosphatidylcholine (LPC) acyltransferases (LPCAT), in Lands' cycle



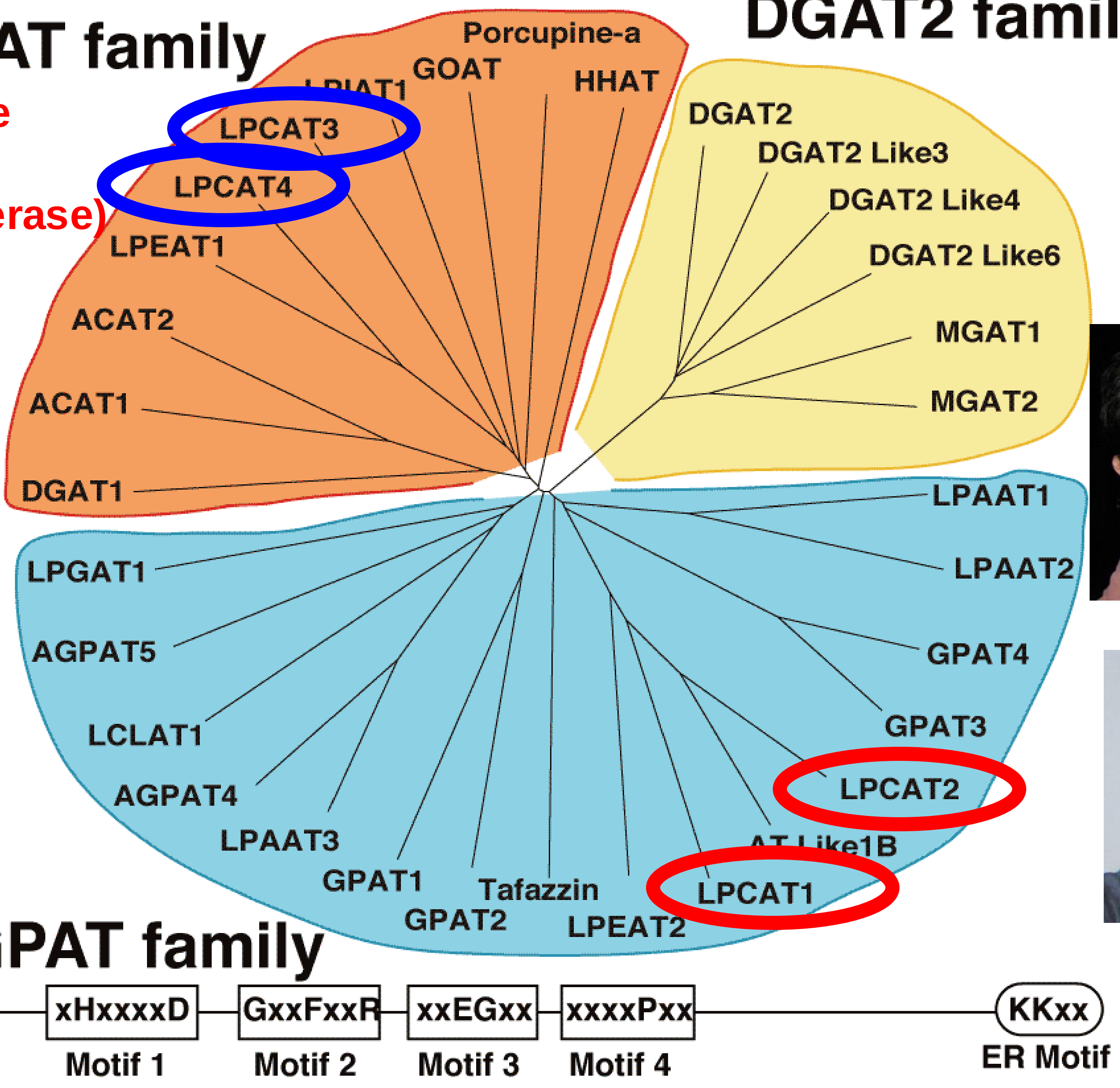
Acyltransferase Family

(Diacylglycerol acyltransferase 2)

DGAT2 family

MBOAT family

(membrane bound O-acyltransferase)



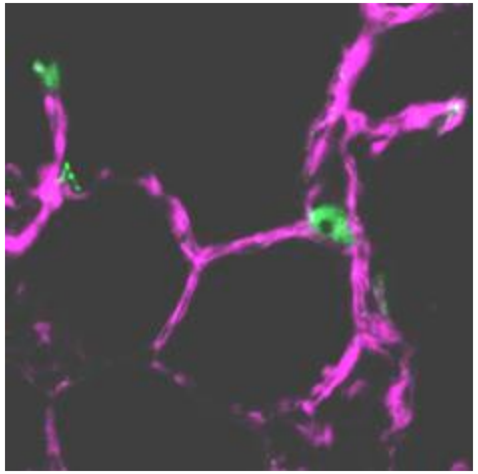
AGPAT family

Hishikawa et al., *PNAS*, 2008

LPCAT1 (discovered in 2006)

Nakanishi et al. *JBC*, 2006
Chen et al. *PNAS*, 2006

- Produce PC and PG with saturated fatty acids (C16:0)
- Rich in lung (alveolar type 2 cells) and retina
- Knockout mice survive, but are blind, and more sensitive to acute lung injury



Important work from other laboratories

Overexpression in cancer and related to prognosis (Bi, J.. Cravatt, BF; *Cell Metabolism* 2019)

Over 100 publications on oncogenic properties, both in animal and human studies.

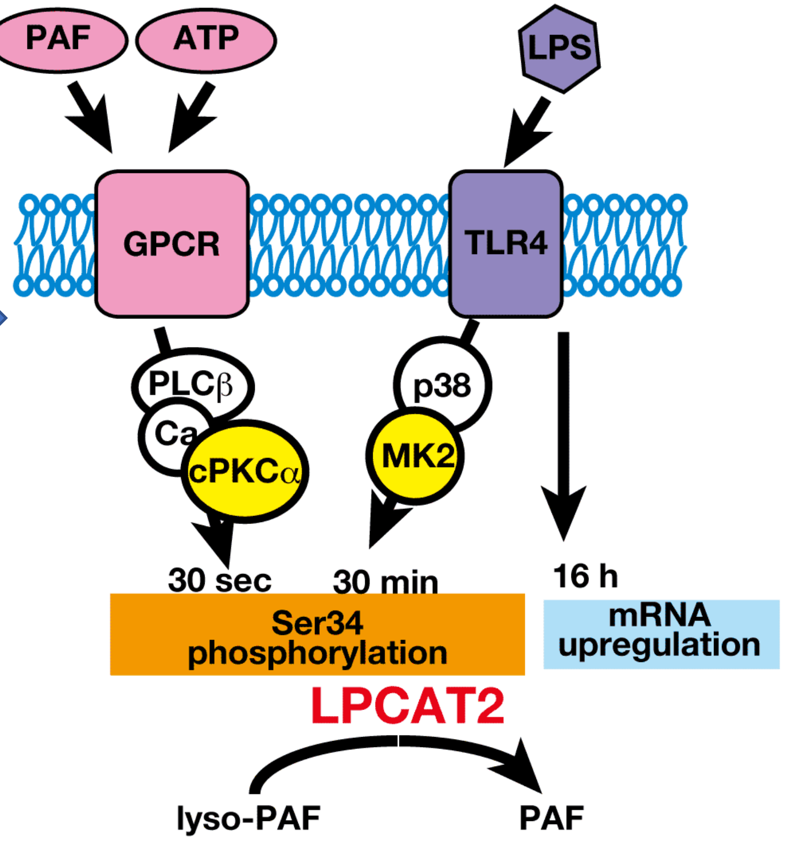
300,000 low birth-weight infants are rescued by surfactant replenishment world-wide every year

Prof. Tetsuo Fujiwara
Dept. Pediatrics,
Iwate Medical School

First clinical trial in 1979 at
Akita University

LPCAT2=lysoPAF acetyltransferase!

- Cloned by homology to LPCAT1 (48%)
- Incorporates acetyl-CoA to produce PAF
- Enzyme regulated in three pathways
- Highly expressed in macrophages and other immune cells.
- KO mice or use of LPCAT2 inhibitor (TSI-01) ameliorates neuropathic pain and allergic reactions.



Shindou et al. *J. Immunol*. 2005; *JBC*, 2007; Morimoto et al. *JBC*, 2010; 2014

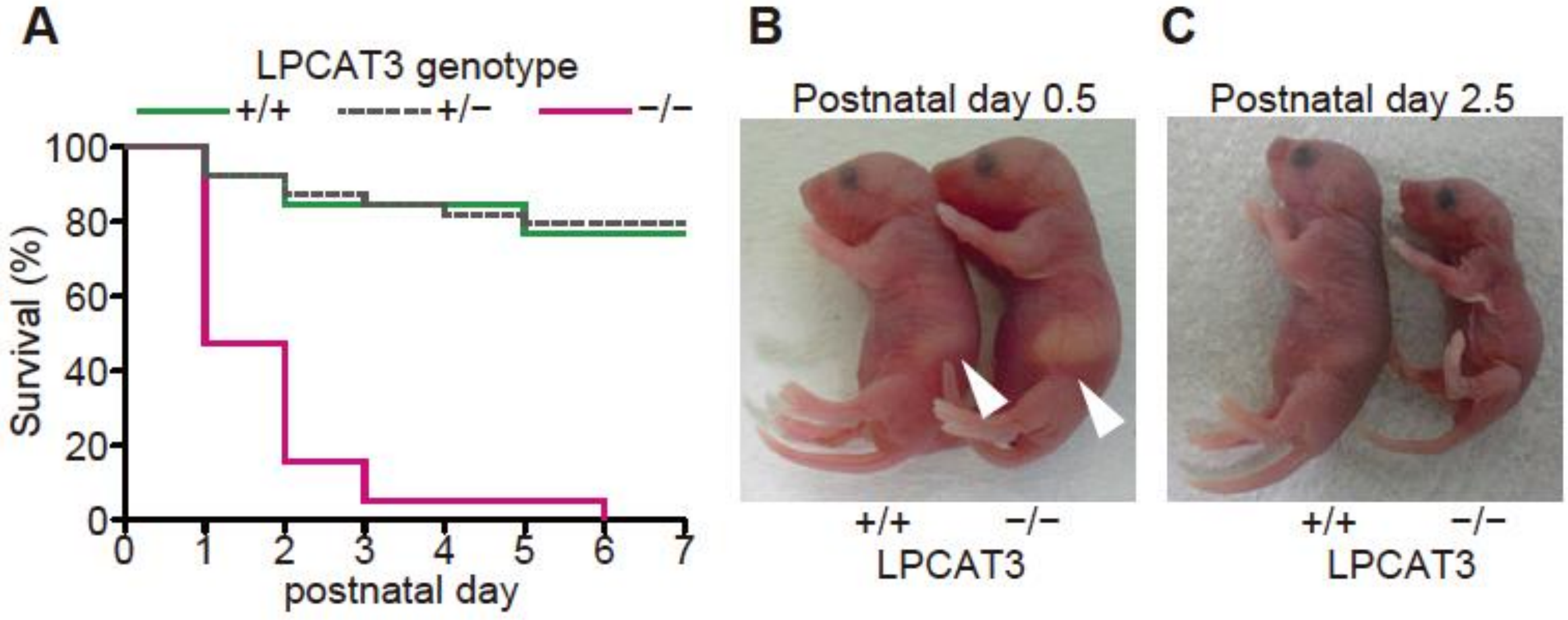
LPCAT3, a major lysoPL acyltransferase

- **Enzyme discovered in 2008 by three groups including us**

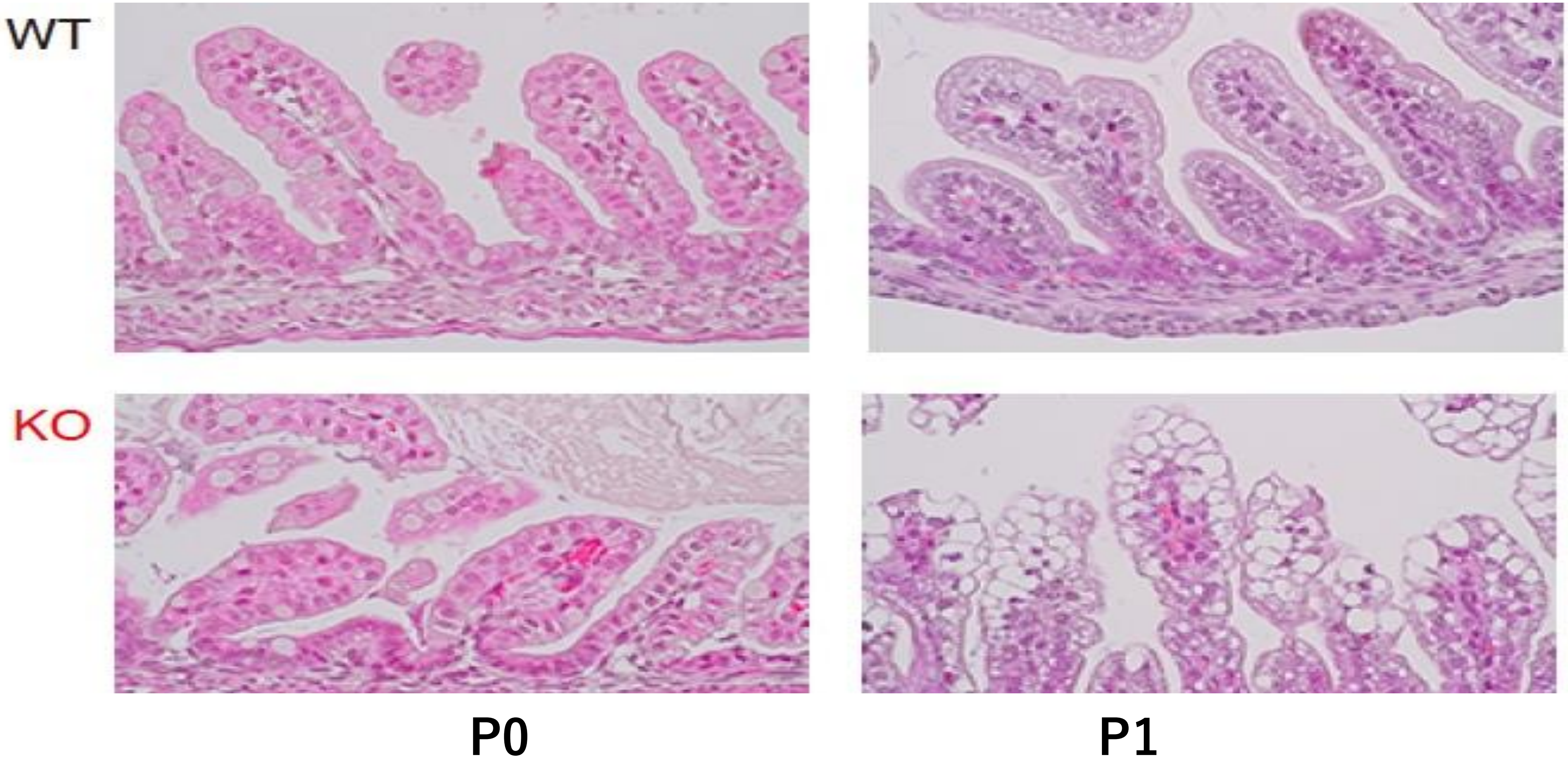
(Hishikawa, *PNAS;* Gion, *JBC*, Matsuda, *Genes to Cells*)

- **Incorporates C18:2 << C20:4 to lysoPC and lysoPS**
- **KO mice are neonatally lethal by malnutrition and hypoglycemia due to fatty degeneration of intestinal cells.**
- **AA-containing phospholipids are important for triglyceride transport and lipoprotein productions.**
- **Phenotypes are independent of eicosanoid productions.**

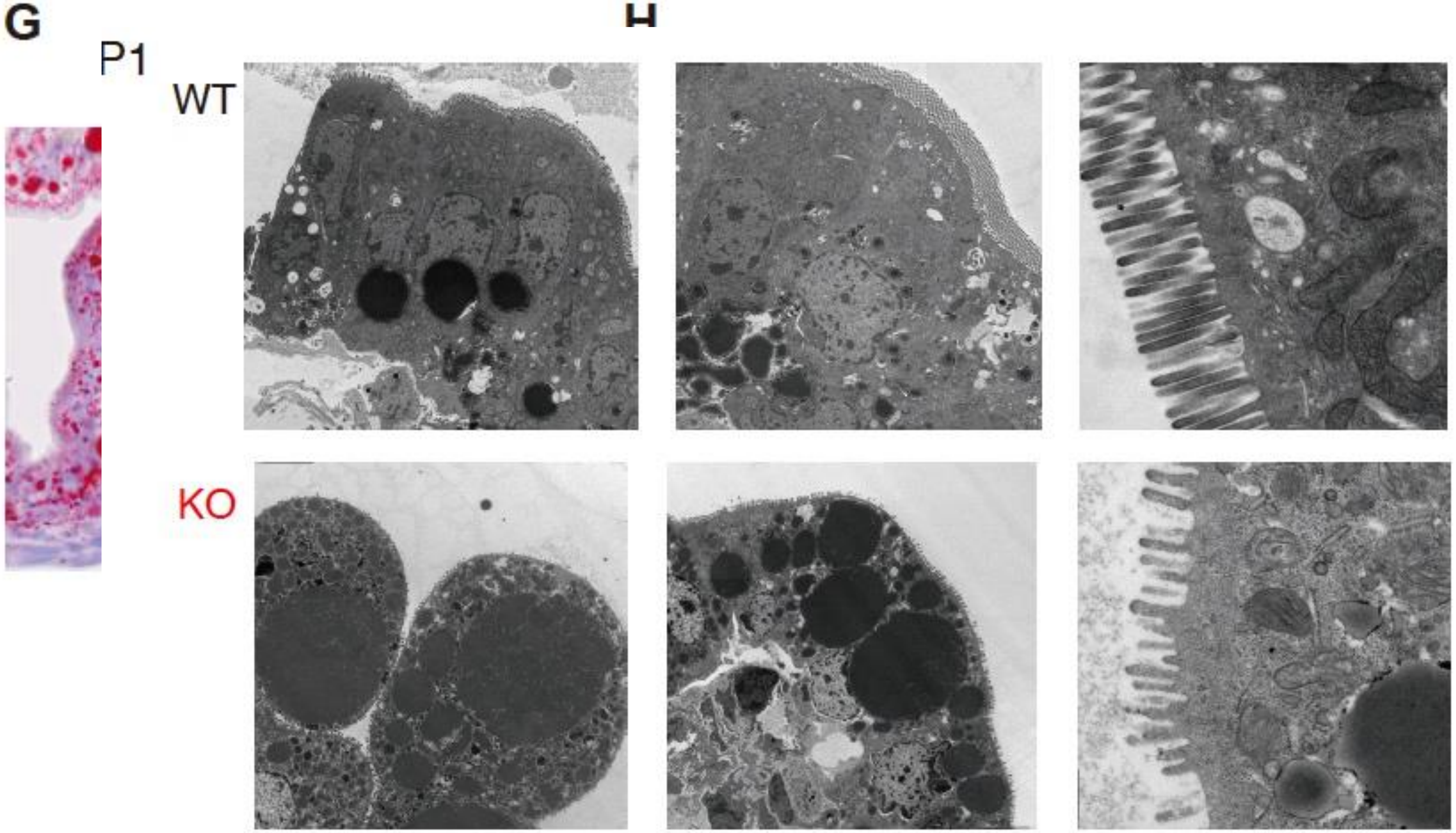
LPCAT3 KO mice are neonatally lethal



Degeneration of intestinal epithelial cells by LPCAT3 deficiency

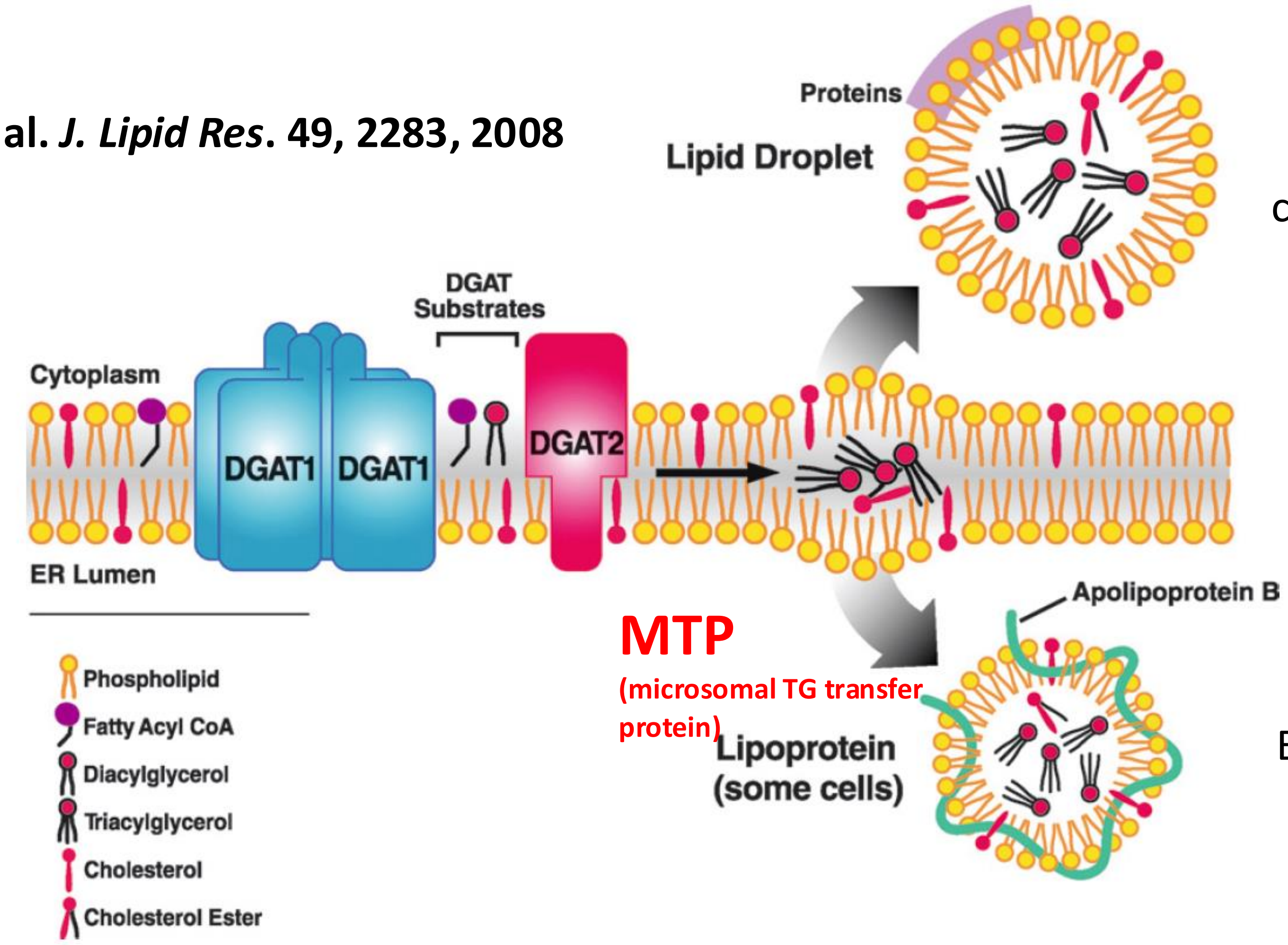


Accumulation of lipid droplets in KO intestine



TG transport from ER membrane

Yen, C-L.E et al. *J. Lipid Res*. 49, 2283, 2008



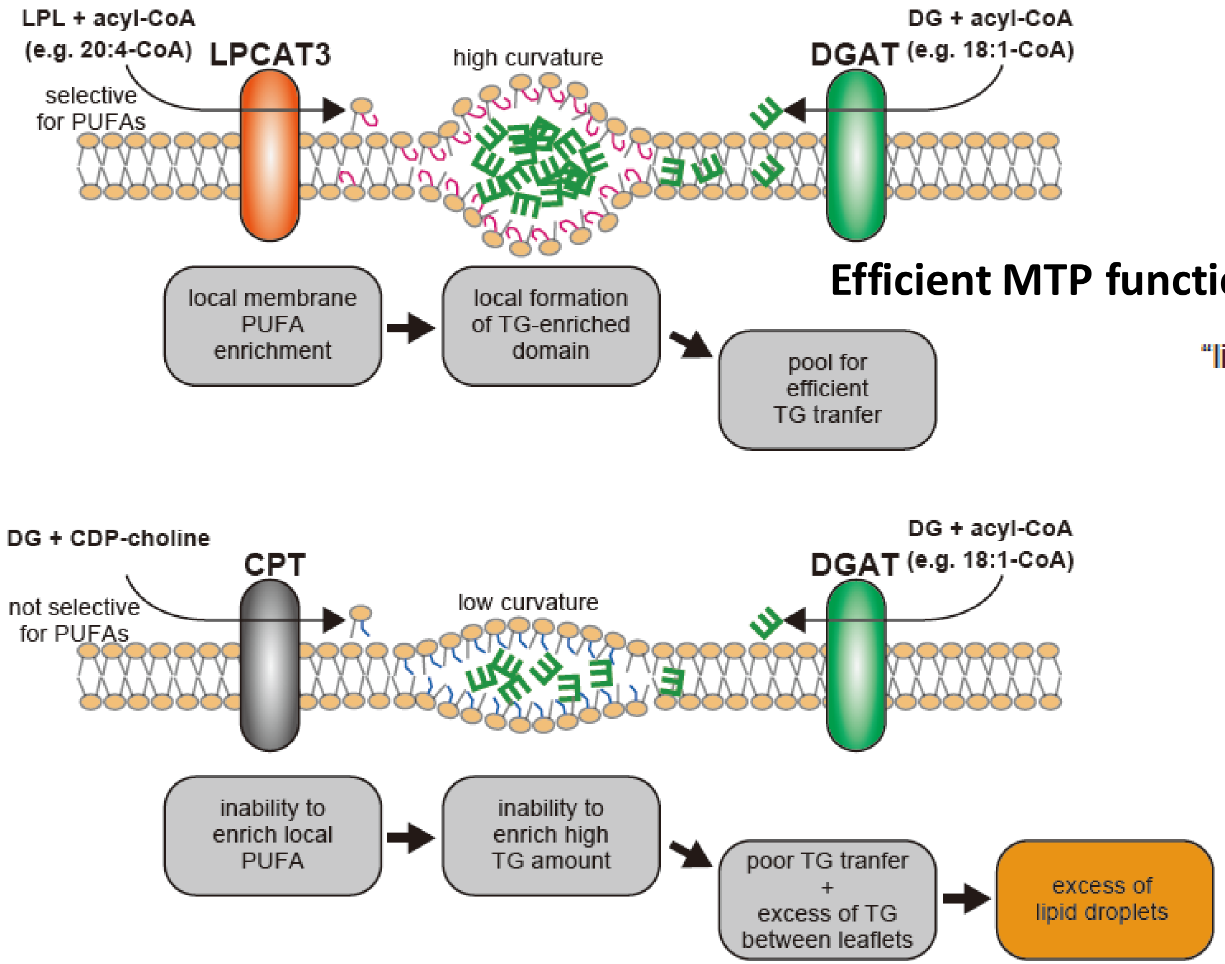
cytosol

MTP
(microsomal TG transfer protein)

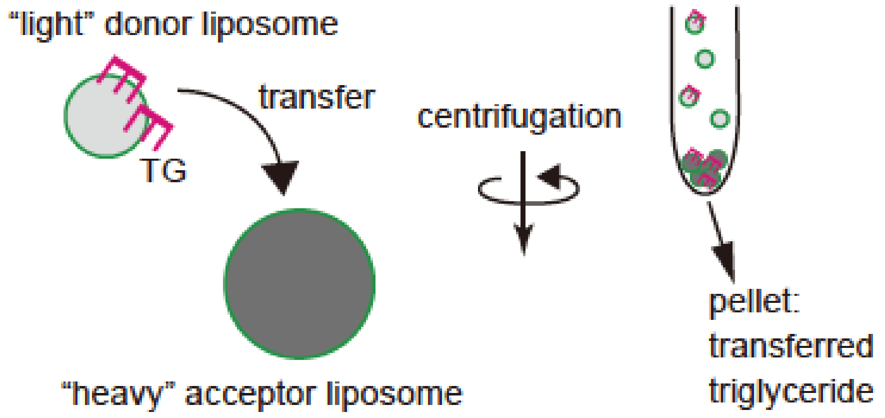
ER lumen

Ref. Raabe et al. *PNAS*, 1998

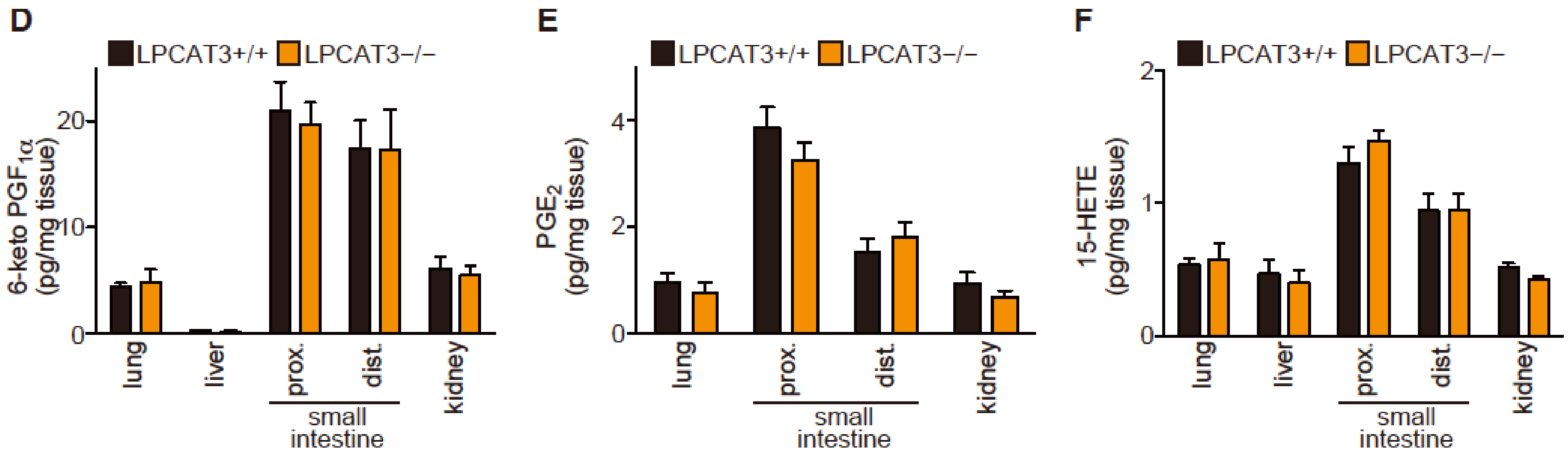
A proposed model of 20:4-induced TG transfer



Efficient MTP function



No change in arachidonate-derived eicosanoid levels in LPCAT3 KO mice



eLIFE
elifesciences.org

Fatty acid remodeling by LPCAT3 enriches arachidonate in phospholipid membranes and regulates triglyceride transport

Tomomi Hashidate-Yoshida[1†], Takeshi Harayama[1†], Daisuke Hishikawa[1], Ryo Morimoto[2‡], Fumie Hamano[2,3], Suzumi M Tokuoka[2], Miki Eto[1,2], Miwa Tamura-Nakano[4], Rieko Yanobu-Takanashi[5], Yoshiko Mukumoto[6], Hiroshi Kiyonari[6], Tadashi Okamura[5,7], Yoshihiro Kita[2,3], Hideo Shindou[1,8], Takao Shimizu[1,2]*

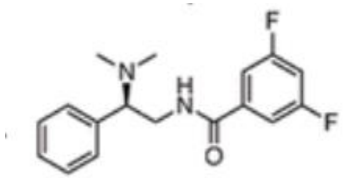
Lpcat3-dependent production of arachidonoyl phospholipids is a key determinant of triglyceride secretion

Xin Rong[1], Bo Wang[1], Merlow M Dunham[2,3], Per Niklas Hedde[4,5], Jinny S Wong[6], Enrico Gratton[4,5], Stephen G Young[7], David A Ford[2,3], Peter Tontonoz[1]*

Important contributions from other laboratories

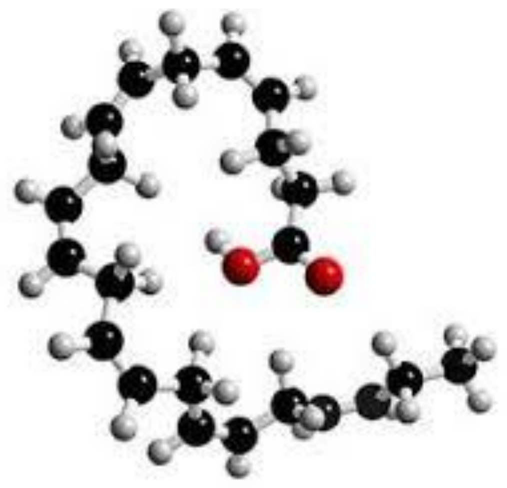
- **Regulation of LPCAT3 by LXR (Demeure et al. *Gene*, 2011; Wang and Tontonoz, *Nature Rev. Endocr.* 2018)**
- **Intestinal stemness and tumorigenesis. (Wang et al. *Cell Stem Cell*, 2018)**
- **LPCAT3 deficiency promotes atherosclerosis (Thomas et al. *Atherosclerosis*, 2018)**
- **Auditory dysfunction and brain microgliosis. (Ichu et al. *Biochemistry*, 2020)**
- **Insulin sensitivity in skeletal muscle (Ferrara et al. *JCI,* 2021)**
- **Structure revealed by X-ray and cryoEM (Zhang et al. *Nature Commun*. 2021)**
- **LPCAT3 inhibitors protect cells from ferroptosis**

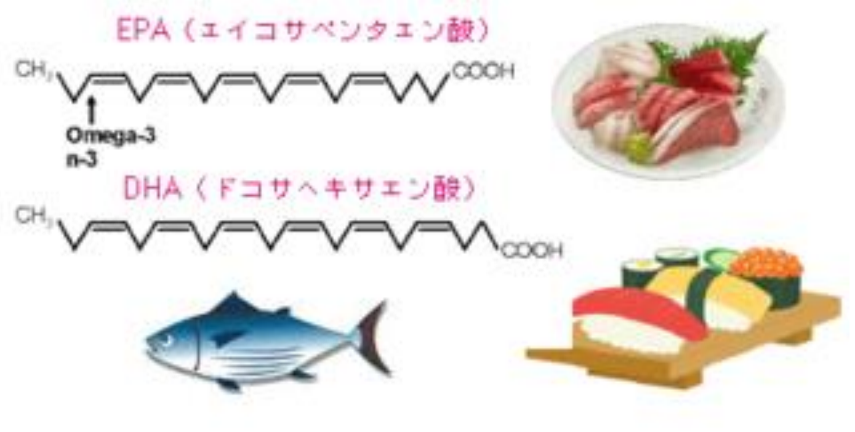
  (Reed et al. *ACS Chem. Biol*. 2022)



(*R*)-HTS-3

DHA=docosahexaenoic acid　C22:6





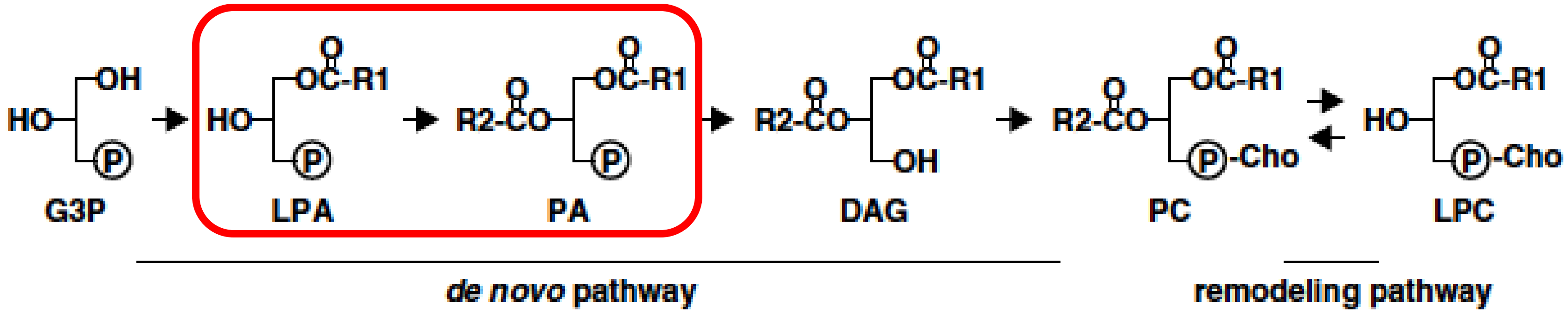
http://blog.goo.ne.jp/kfukuda_ginzaclinic/e/e86064c12994eb521a8297254575c286

ω３ essential fatty acid, rich in testis, brain, retina, heart, muscle

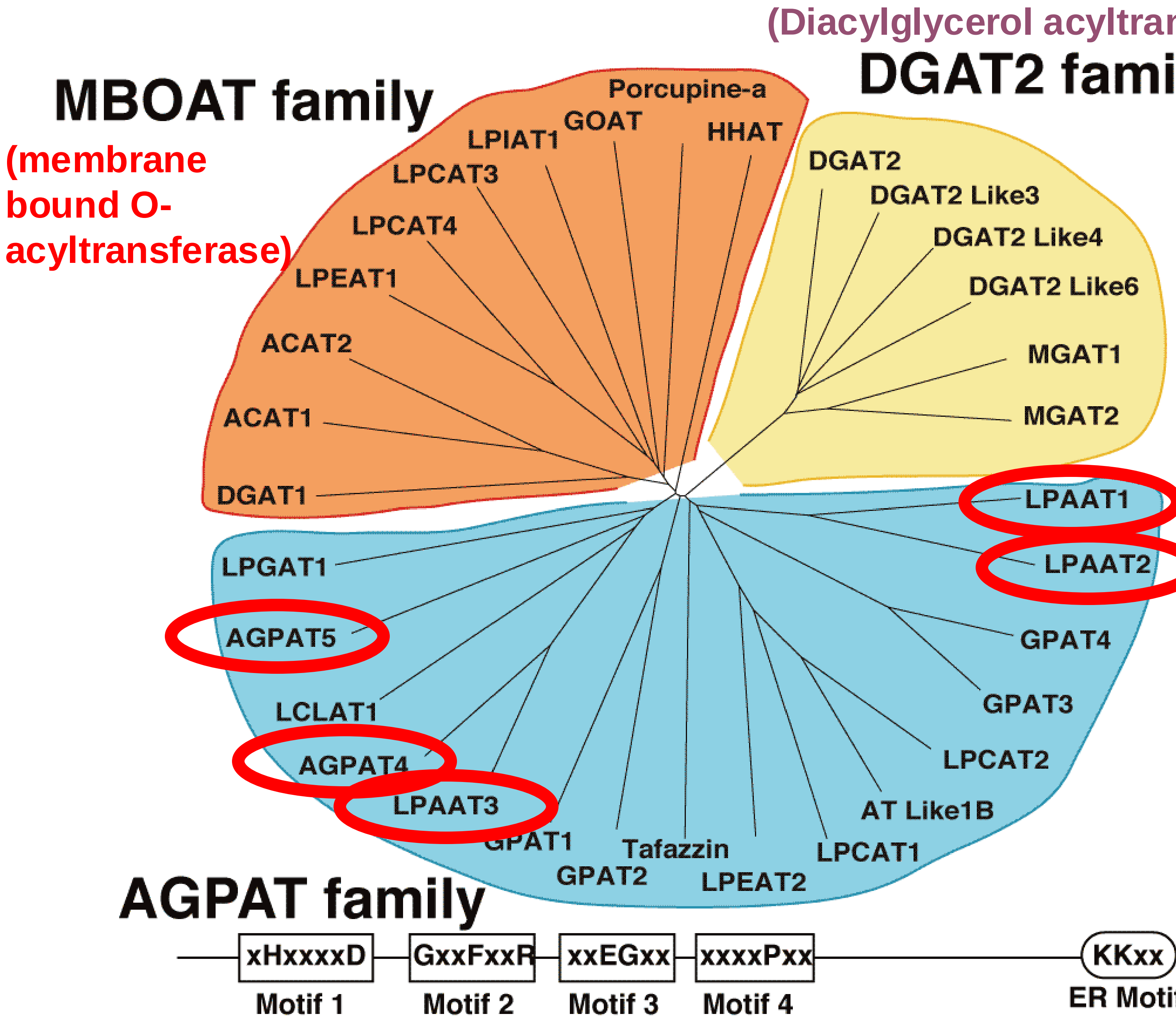
About 60% Japanese have lower FADS1 (Δ5-desaturase) activity (Nakayama et al. ***Human Genet,*** 2010)

LPAAT in *de novo* pathway

Lysophosphatidic acid (LPA) acyltransferase



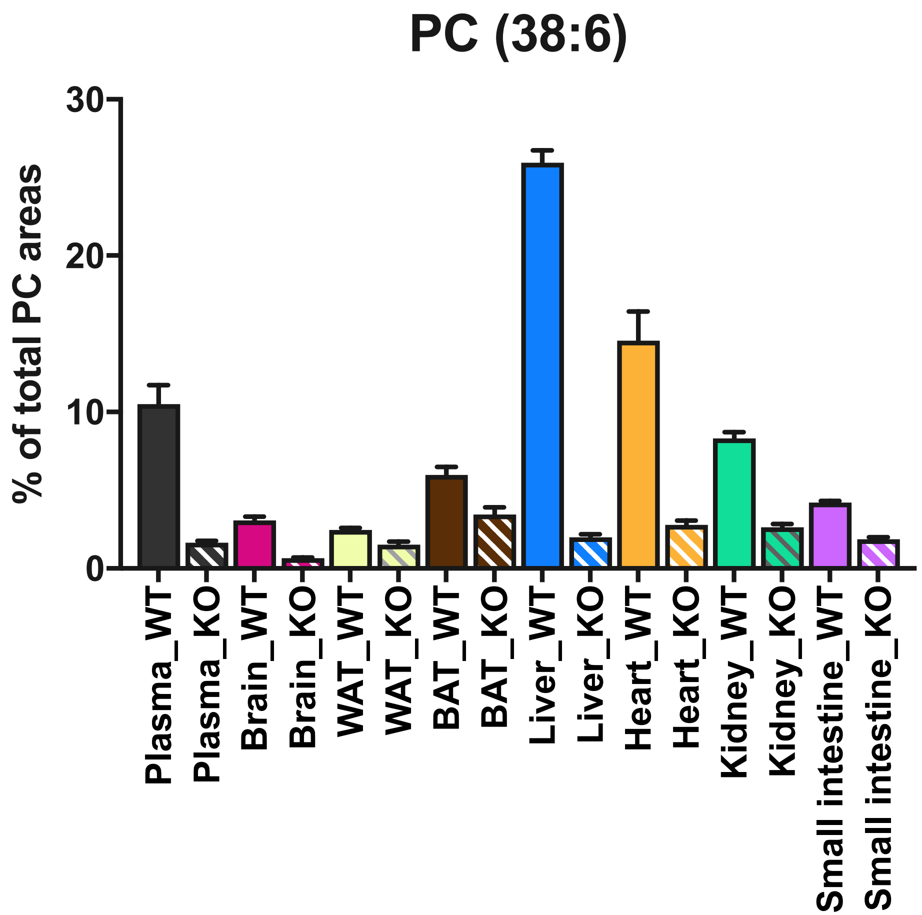
Acyltransferase Family

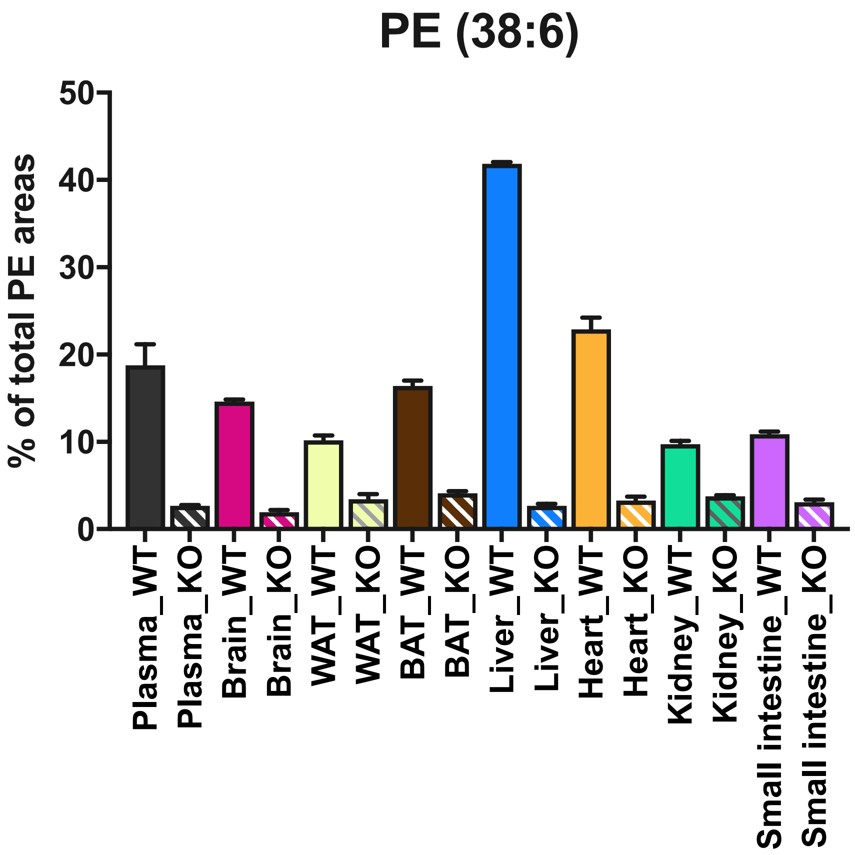


5 Different LPAATs (LysoPA→PA)

- **LPAAT1**; no preference for acyl-CoA
  ubiquitous expression
- **LPAAT2**; prefers C18:2-CoA, adipocytes
- **LPAAT3 (AGPAT3)**; prefers C22:6-CoA, testis, brain, retina, and muscle
- **LPAAT4(AGPAT4)**; prefers 22:6-CoA, brain
- **LPAAT5(AGPAT5)**; n.d. oleic acid?

DHA-containing PC/PE are decreased in almost all tissues of AGPAT3 KO mice





AGPAT3 is expressed in photoreceptor cells

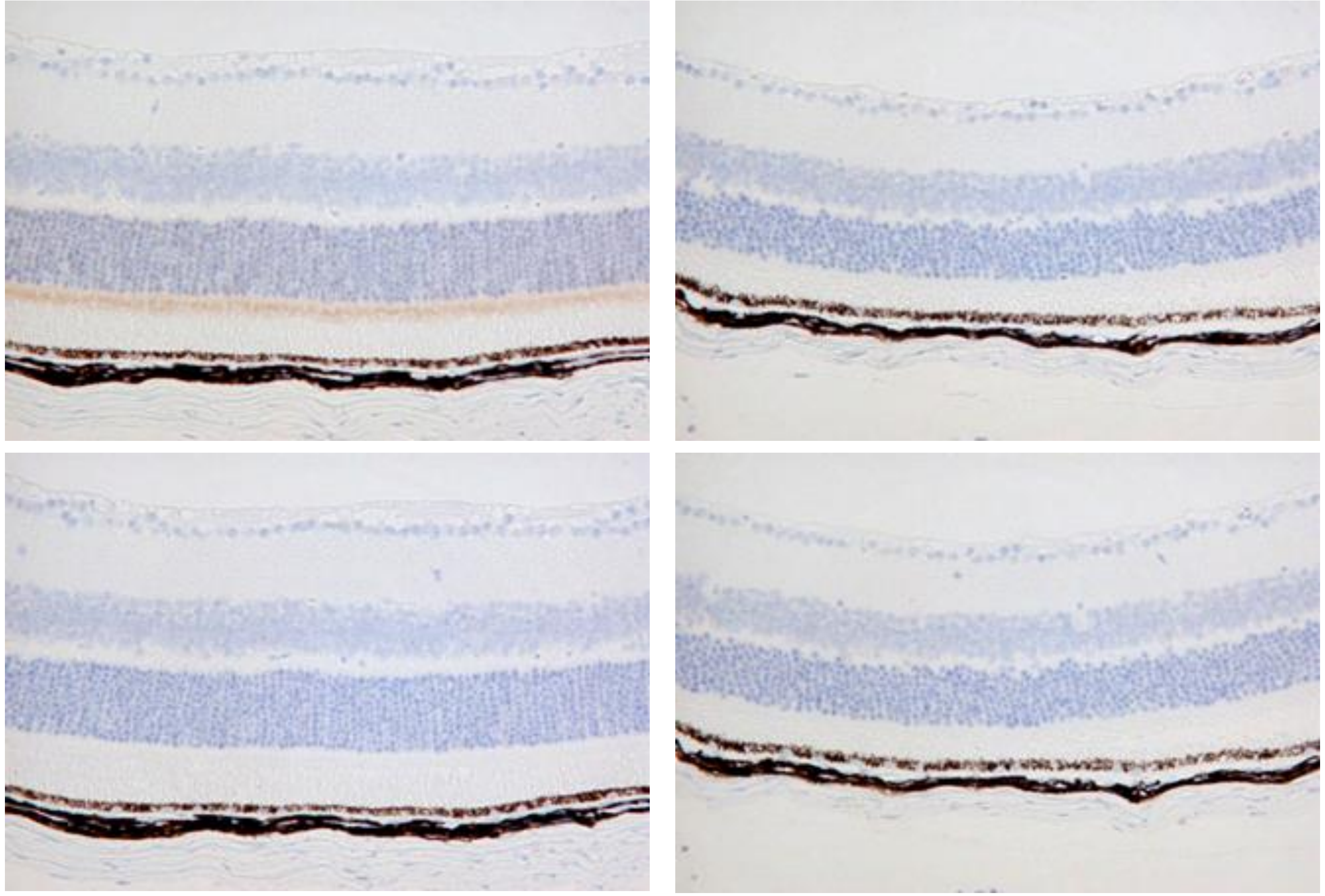
Fig.1

×200

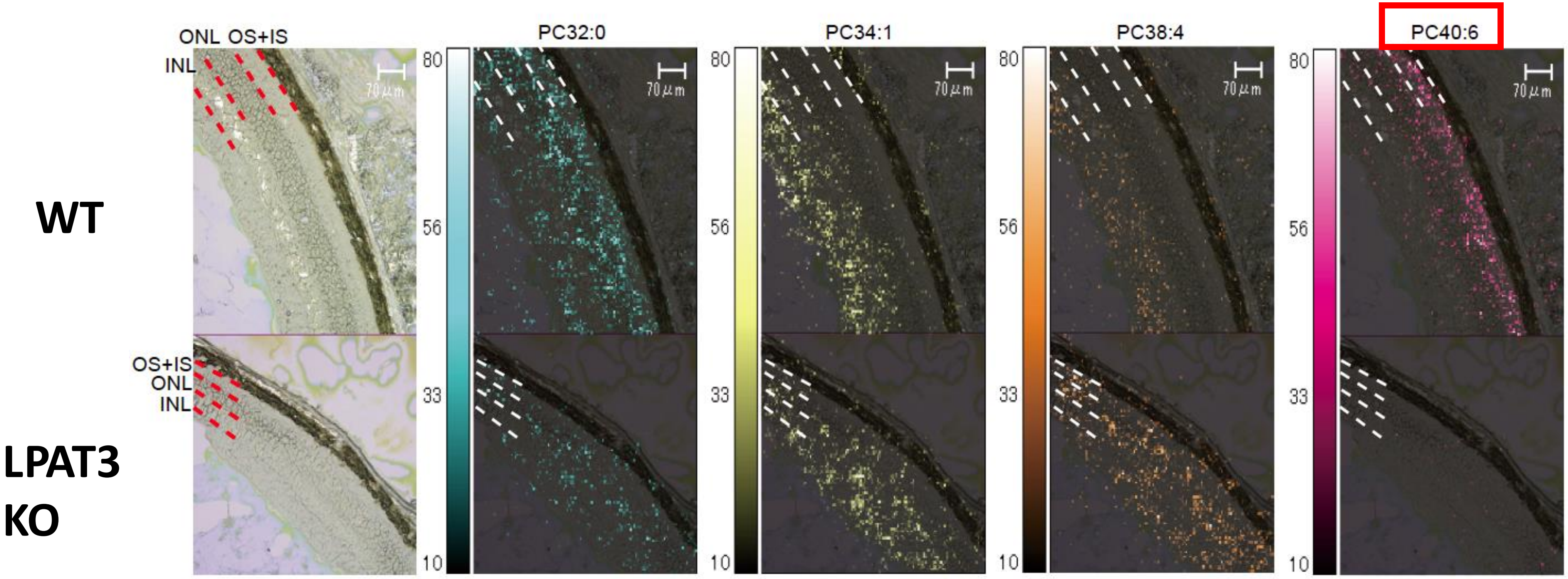
WT | **AGPAT3 KO**

AGPAT3 Ab

Control Ab

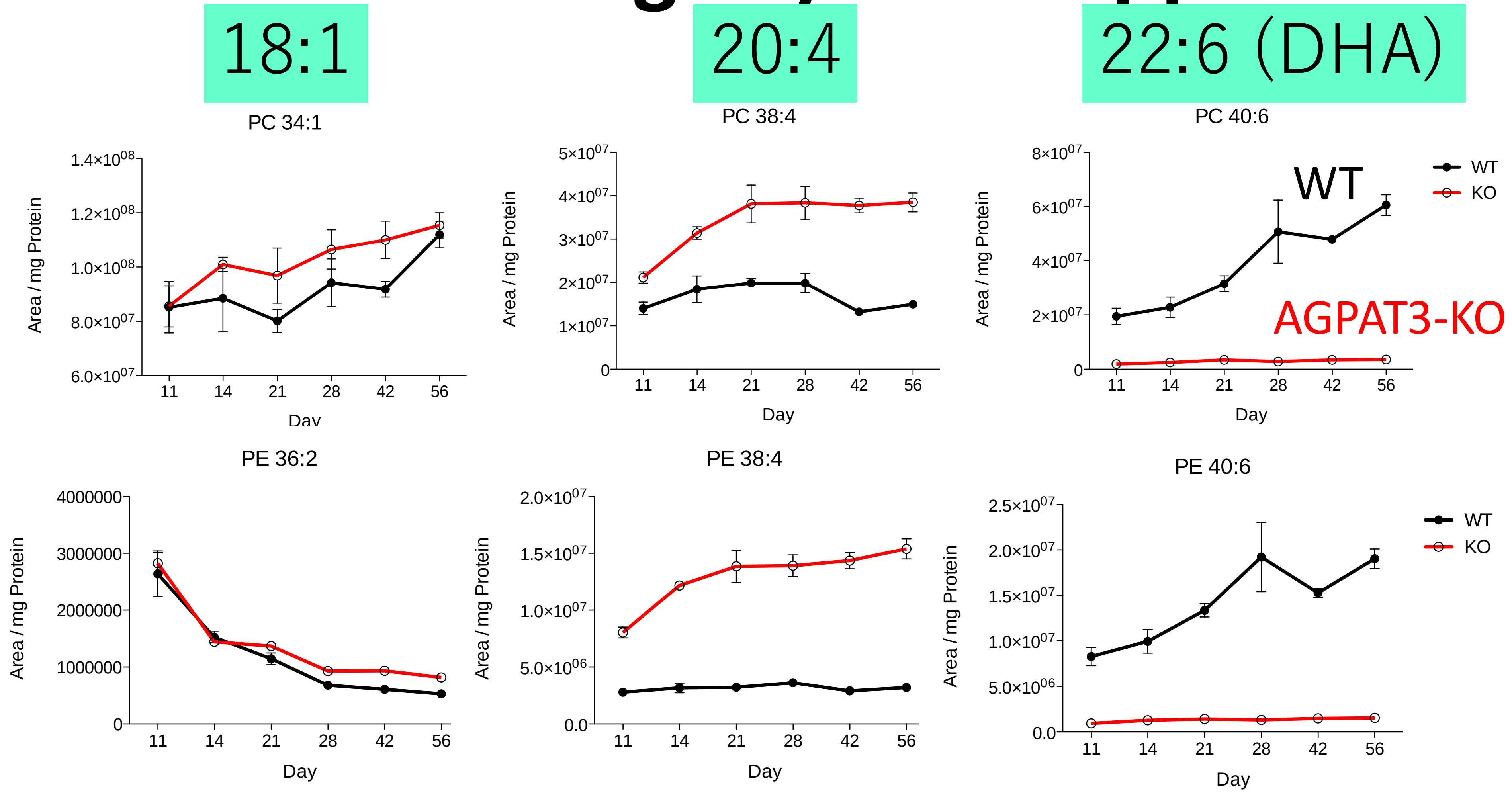


Imaging mass spectrometry

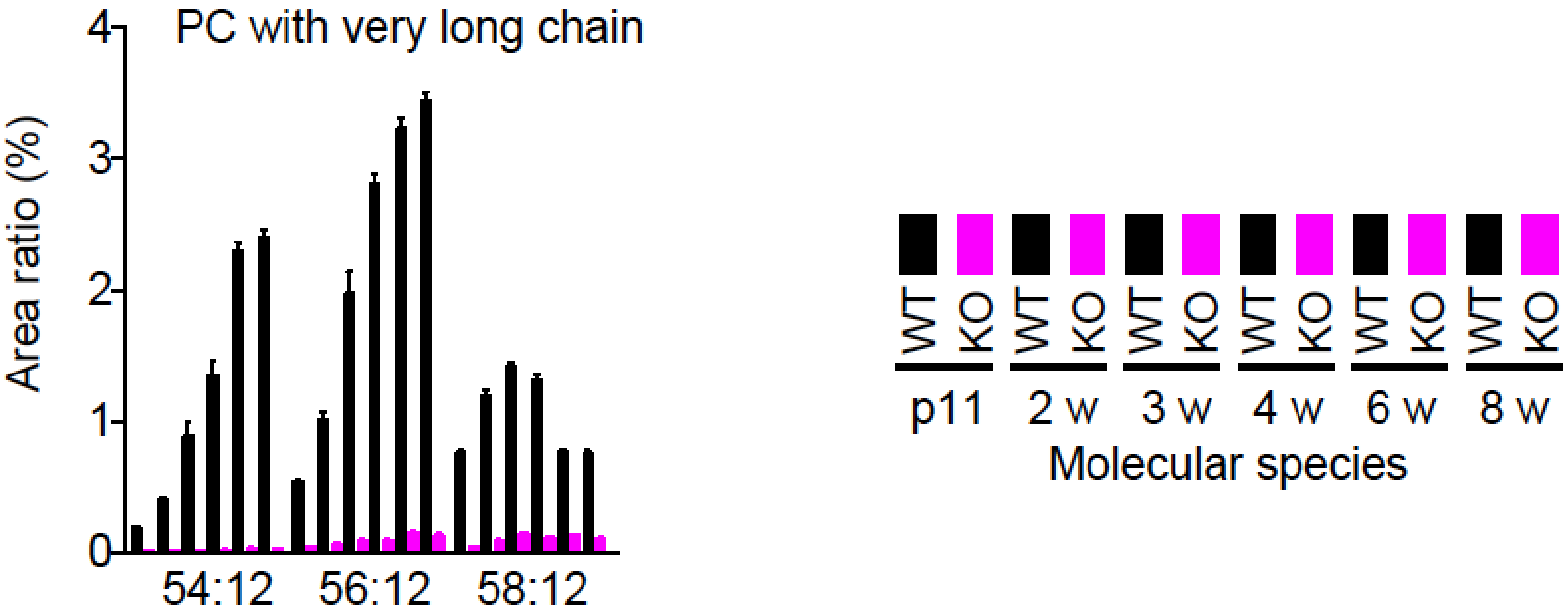


In collaboration with Shimadzu, Co.

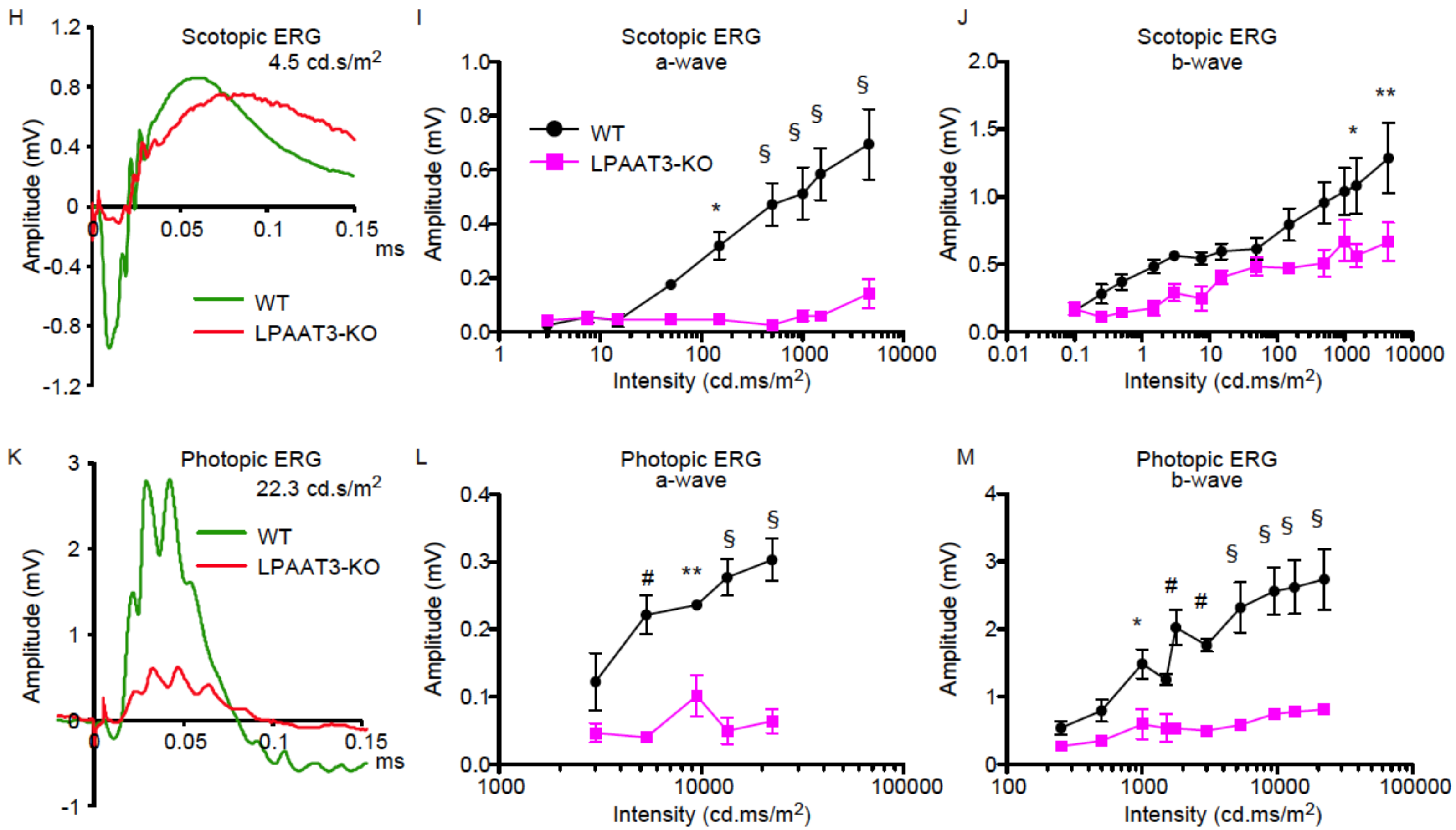
DHA-containing PC/PE disappeared



PC with very long chain fatty acids (C22:6, C32:6, C34:6, C38:6 etc) are also missing

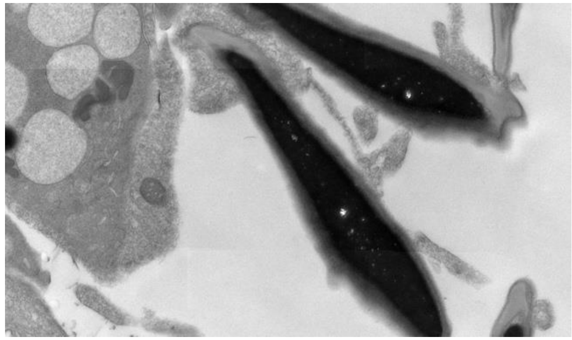


Loss of α-wave determined by ERG

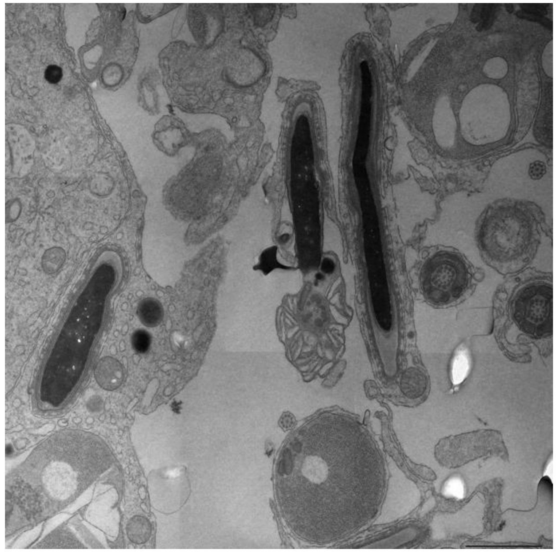


AGPAT3 KO mice are male infertile and blind

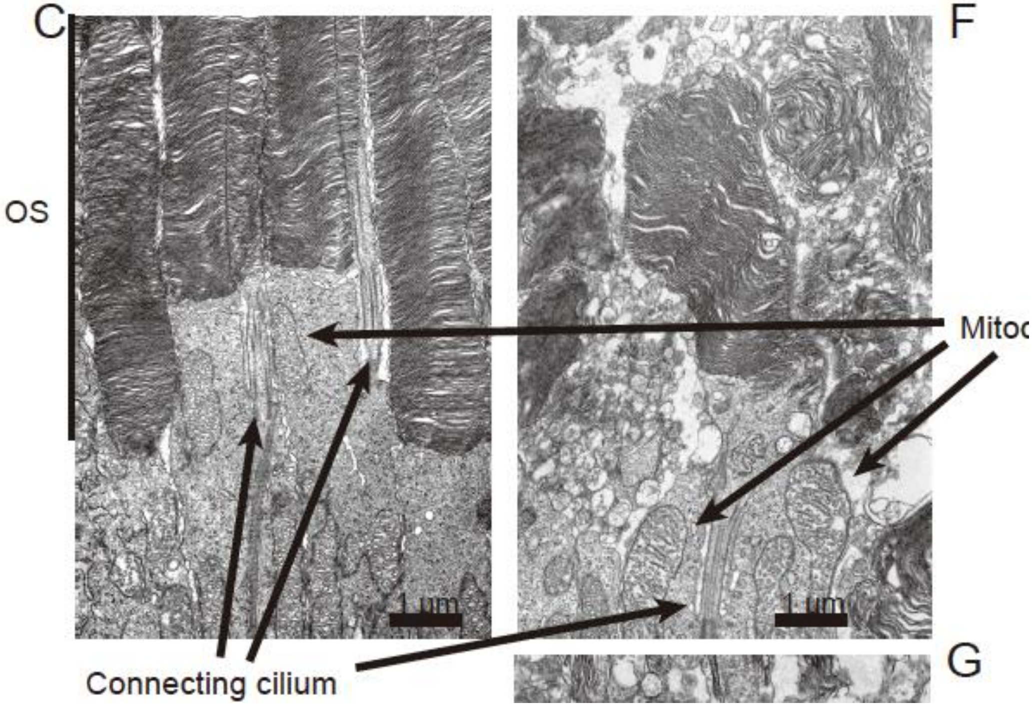
WT



KO



WT **KO**



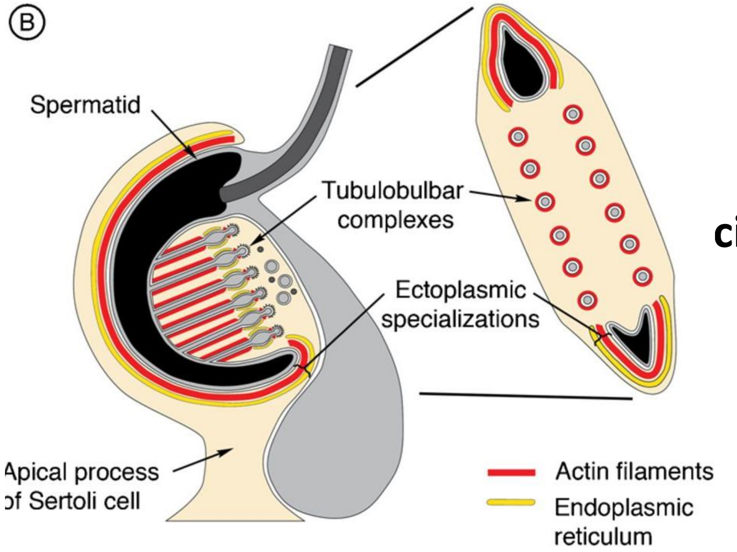
KO sperm contains excess cytoplasm

Outer segment disorganized in KO retina

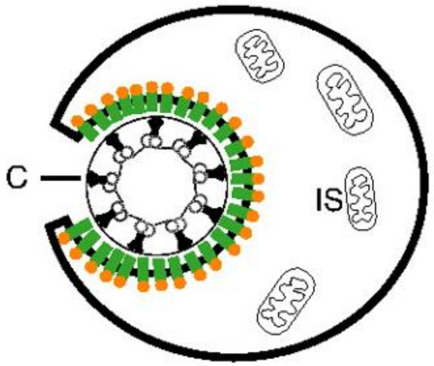
Similar machinery for spermiogenesis and photocell transport

- **Tubulobulbar complex (TBC) in Sertoli cells and connecting cilia (CC) in inner segment**
- **Small vesicle transport through 50-300 nm actin/microtubule-bundles nanotubes**

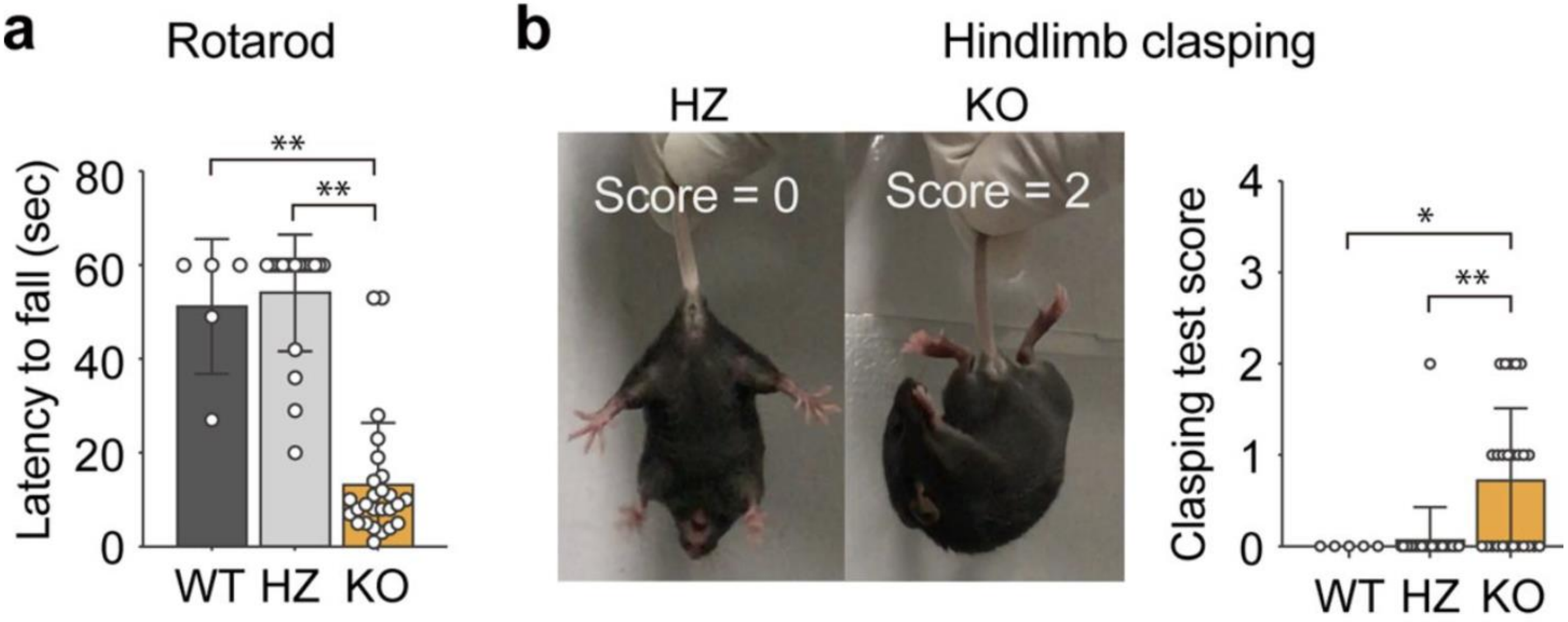
TBC in Sertoli cells



cilia



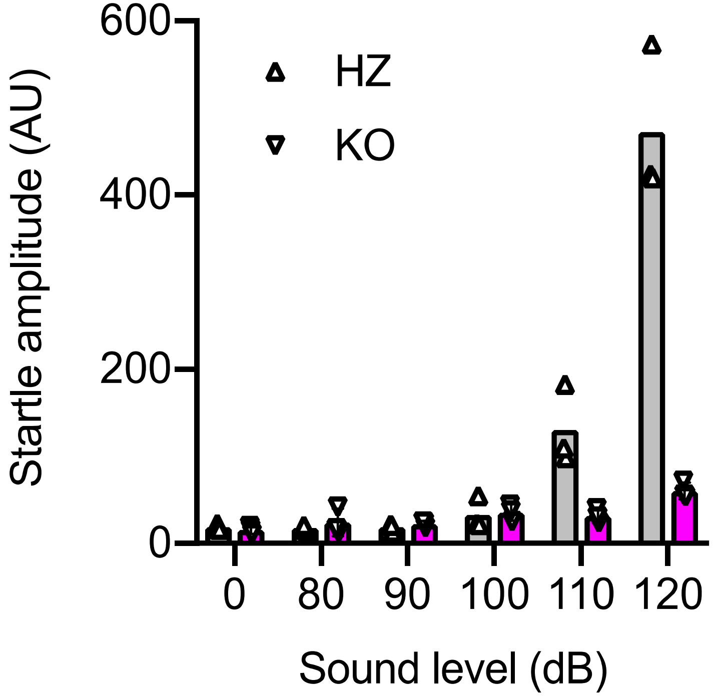
How about brain functions?



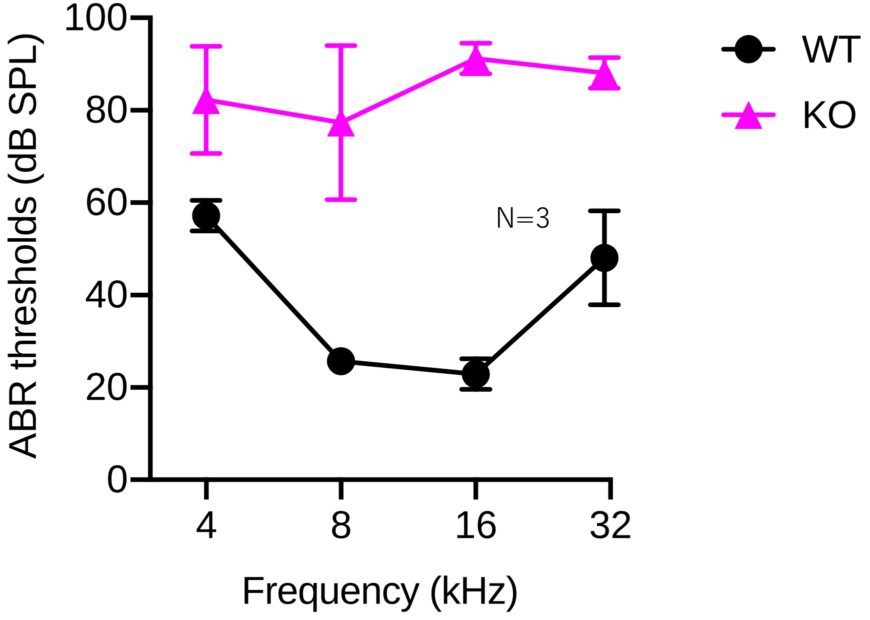
Yanagida, K et al. unpublished

Hearing deficit of AGPAT3 KO mice

Startle response



Auditory brainstem response



Teramura, K. et al. unpublished

A family with loss of function in AGPAT3

ARTICLE

Check for updates

A loss of function variant in *AGPAT3* underlies intellectual disability and retinitis pigmentosa (IDRP) syndrome

Madiha Amin Malik[1,2], Muhammad Arif Nadeem Saqib[3], Edwin Mientjes[2], Anushree Acharya[4], Muhammad Rizwan Alam[1], Ilse Wallaard[2], Isabelle Schrauwen[4], Michael J. Bamshad[5,6], Regie Lyn P. Santos-Cortez[7], Ype Elgersma[2], Suzanne M. Leal[4,8]✉ and Muhammad Ansar[1]✉

Eur Journal of Human Genetics, 10.1038/s41431-23-01475, 2023

Validation of DOHaD

- **Purpose:**

Kanatani, Yanagida, ***Nature Commun***, 2024

To determine impact of perinatal maternal DHA-phospholipid synthesis on offspring growth and brain functions

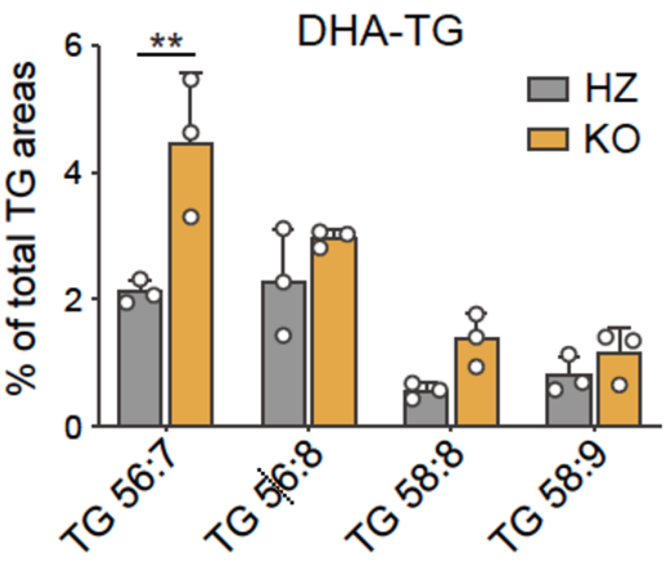
- **Methods:**

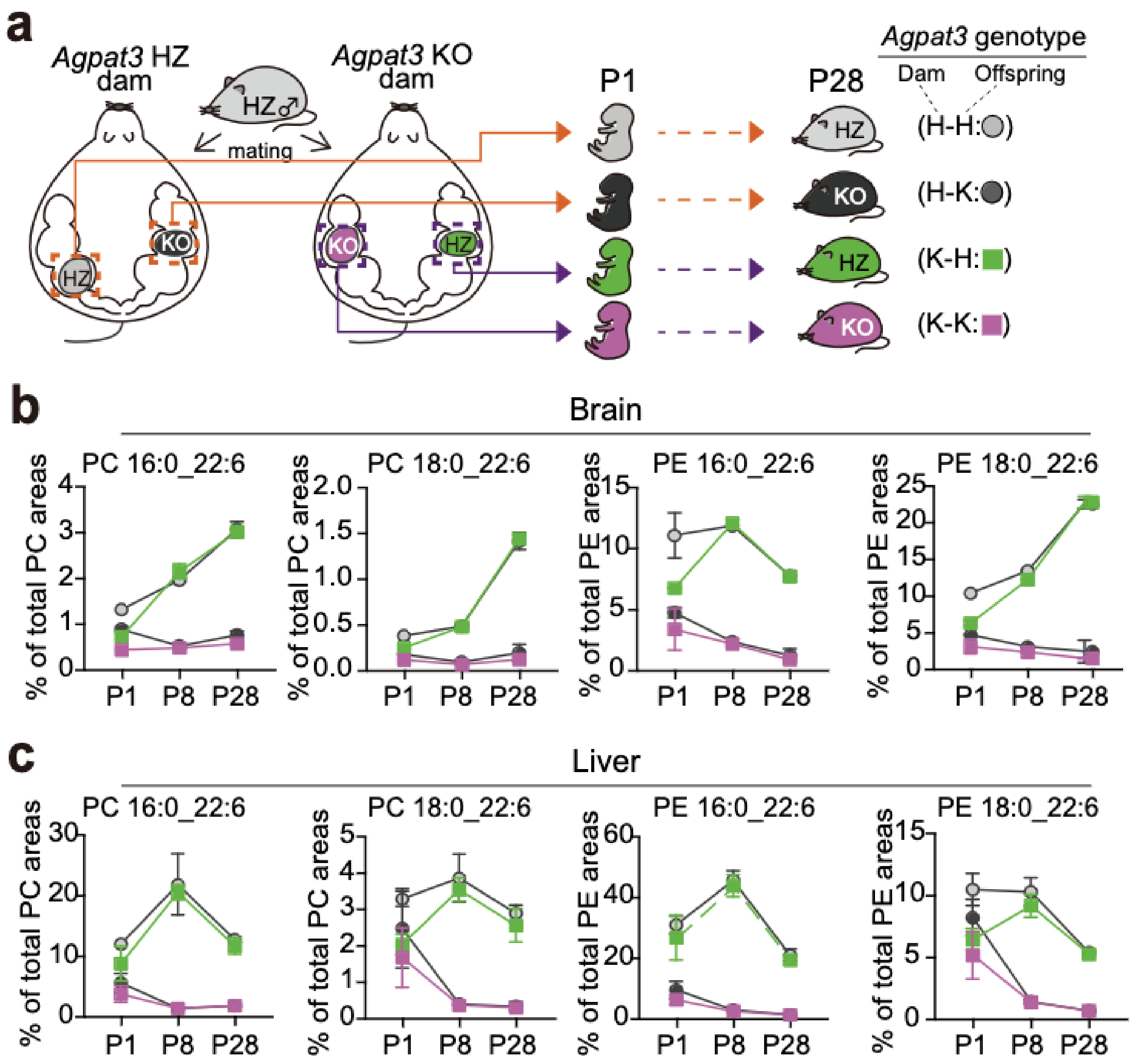
Selective modulation of DHA-phospholipid by cross breeding of AGPAT3 hetero and KO mice

- **Conclusion:**

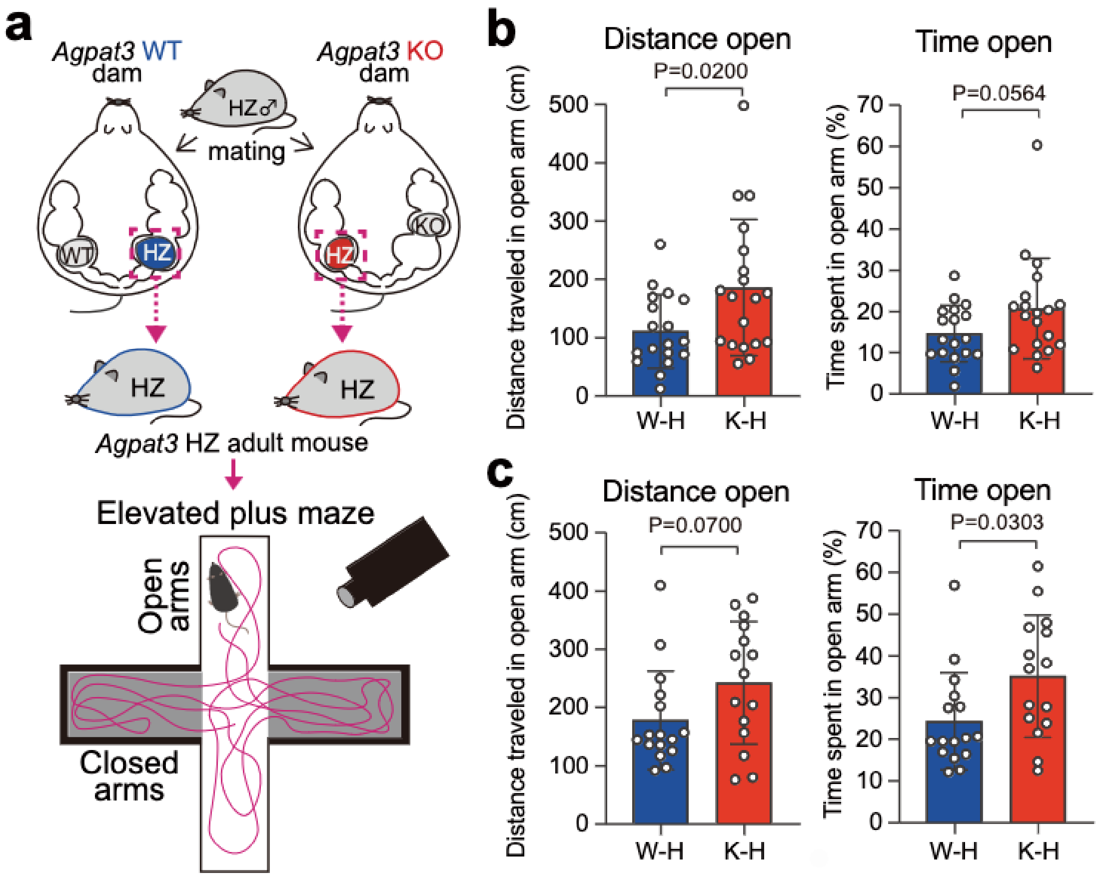
Maternal DHA deficiency causes various neurological and psychiatric abnormalities, even DHA is fully given after birth.

Loss of DHA-PL during fetal period is restored by 4 weeks after birth





Fetal stage-specific DHA deficiency causes anxiety (fear)-related behavior of adult mice



Kanatani et al. *Nature Commun*, 2024

Summary-2 (Membrane)

- **From AGPAT and MBOAT family, 9 lysophospholipid acyltransferases are identified, each has different but overlapping substrate specificities to make over 1,000 phospholipid species.**
- ***De novo*** **pathways contribute to enrichment of C18:2 and C22:6-phospholipids, while Lands cycle yields C16:0, C18:1 and C20:4-phospholipids.**
- **C20:4 in phospholipids not only plays as precursors of eicosanoids, but also maintains membrane fluidity/curvature for proper cellular functions.**
- **C22:6 phospholipids are important for spermatogenesis, photoreceptor arrangements, and possibly neuronal functions.**

Agenda of my talk

INTRODUCTION:

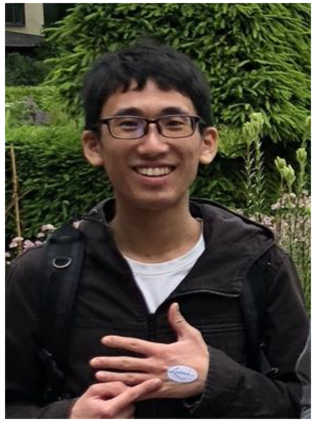
ENZYMES AND RECEPTORS OF LIPID MEDIATORS

LYSOPHOSPHOLIPID ACYLTRANSFERASES FOR MEMBRANE DIVERSITY

PERSPECTIVE

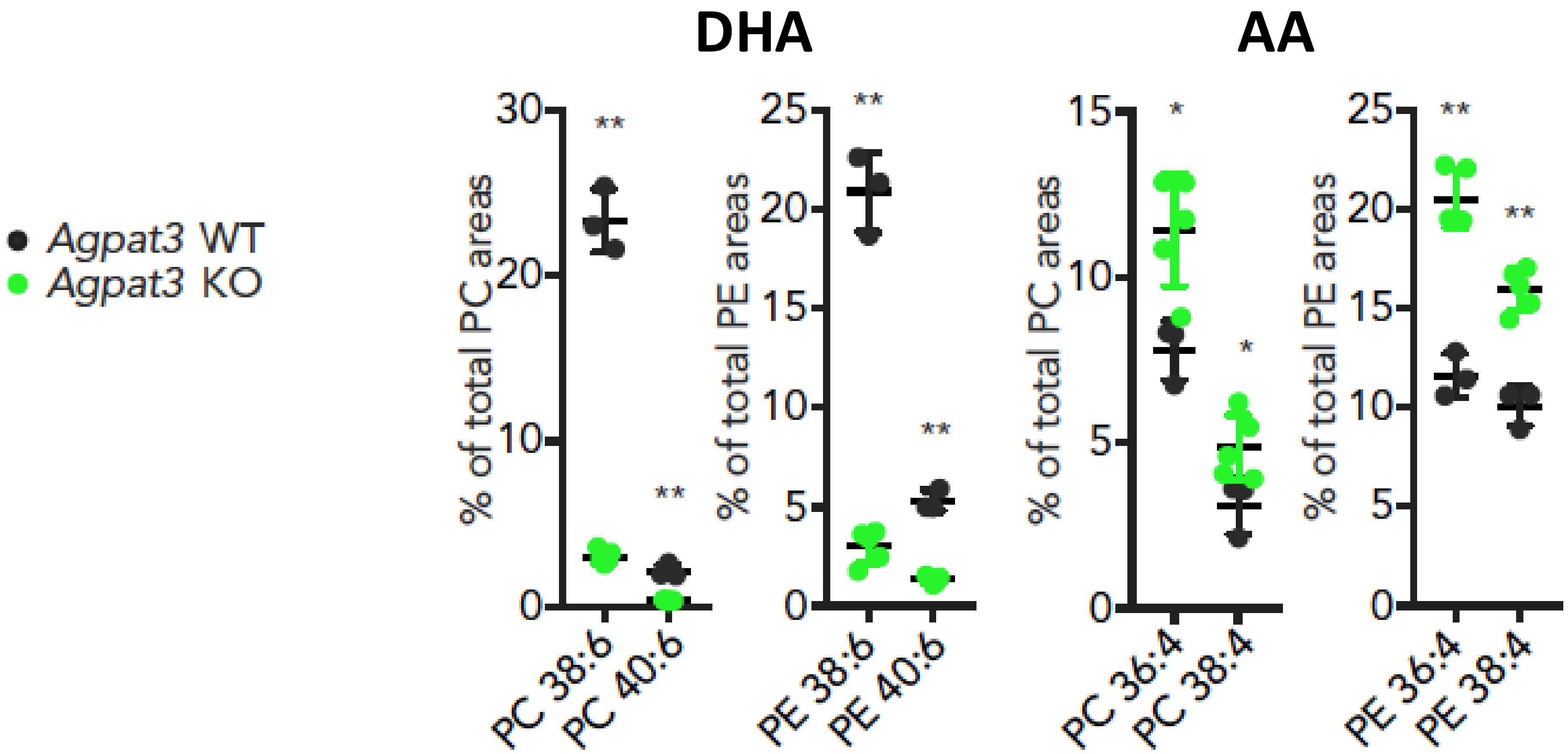
Perspective

- **Transport of PUFAs from liver to brain, retina, testis, and fetus.**
- **Molecular mechanism of cellular functions with phospholipid diversity (mediators? Biophysical properties, protein interaction?)**
- **Subμm localization and dynamic movement of phospholipid species.**

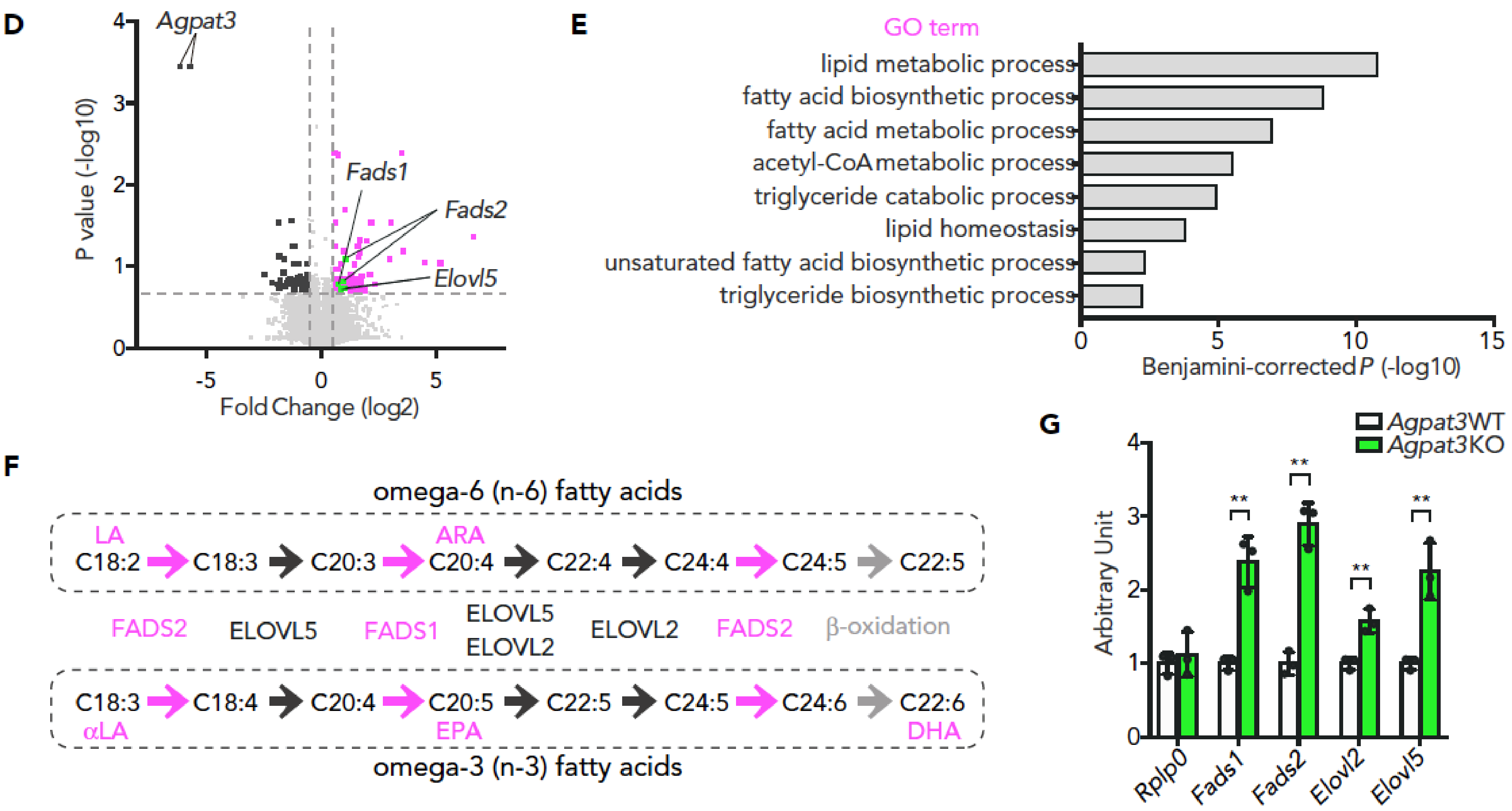


Uematsu, M et al. *FASEB J*. 2020:
Uematsu, M. and Shimizu, T. *Commun Biol*. 2021

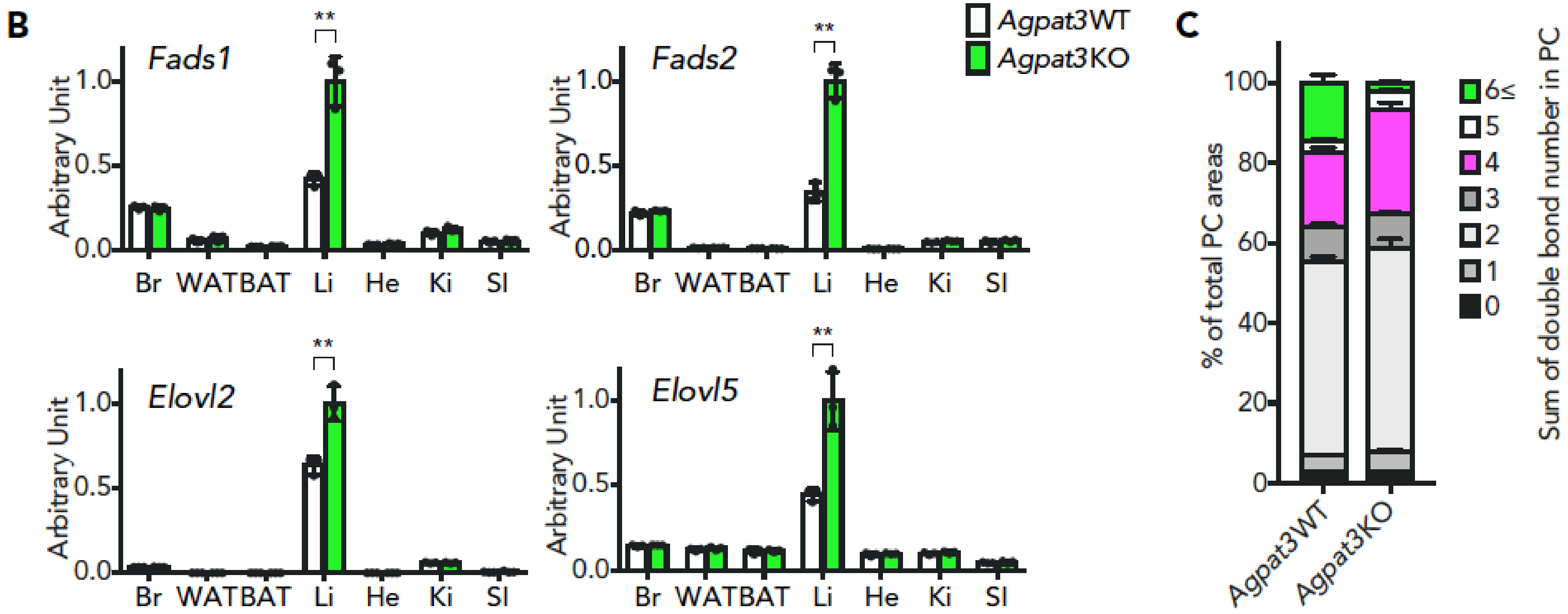
Decrease in DHA-phospholipids, but increase in AA-phospholipids in LPAAT3 (AGPAT3) KO mice liver



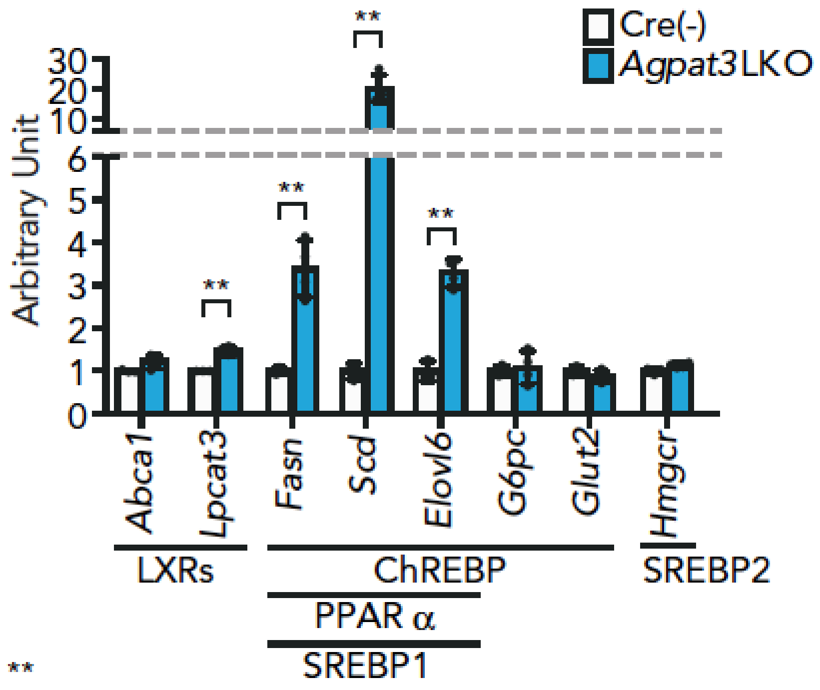
Transcriptomic analyses of liver genes

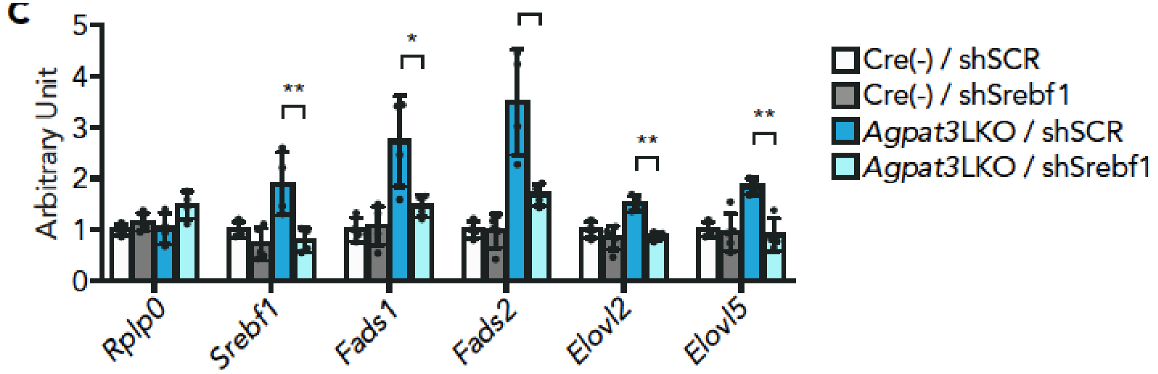


Liver-specific induction of genes for PUFA synthesis by DHA-deficiency



SREBP1, the most likely master gene

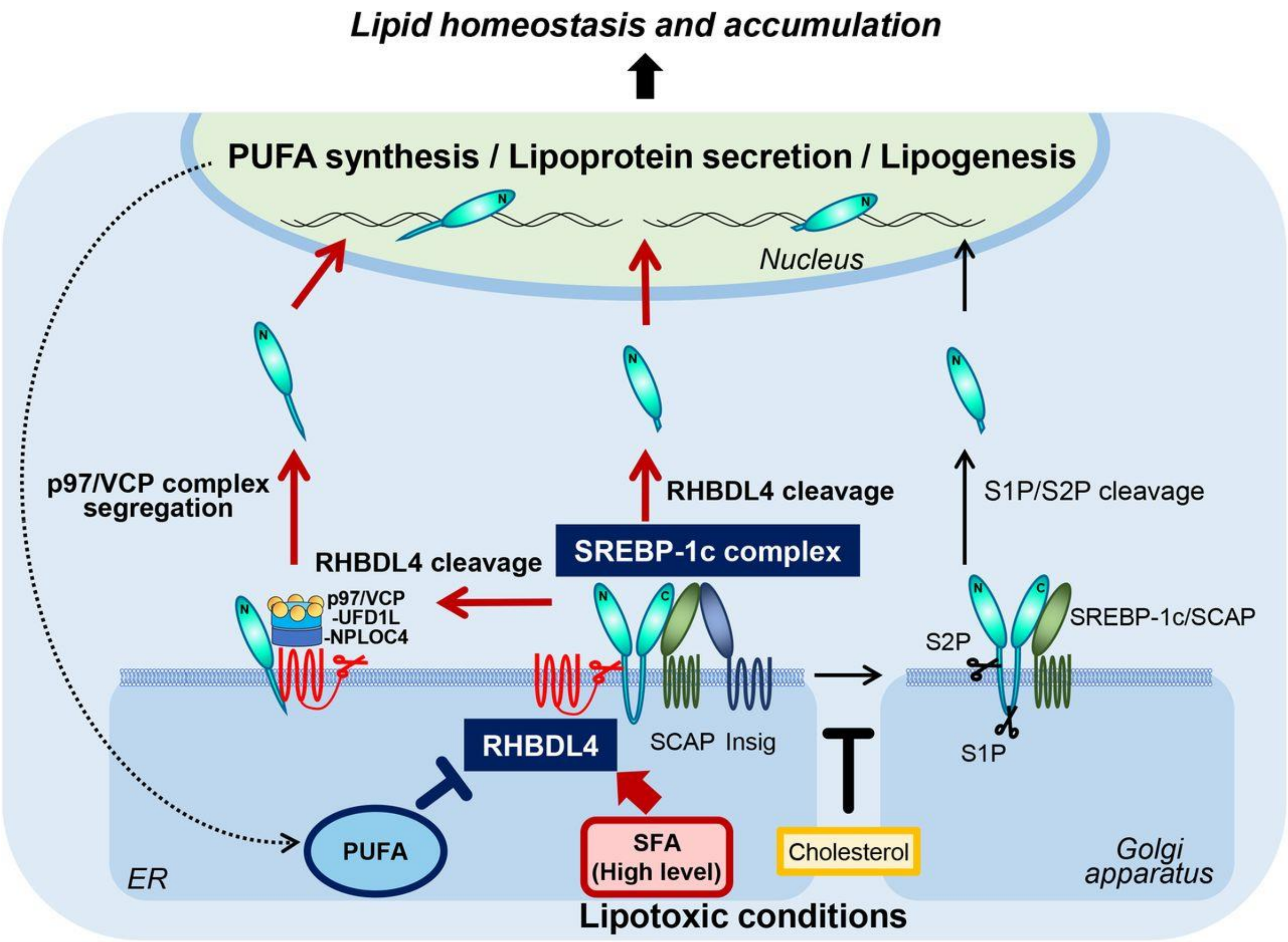




Induction of genes (blue) are suppressed by Srebf1 silencing (adenovirus)

Hishikawa et al. *iScience* 2021

A possible mechanism how PUFAs (DHA) regulates SREBP1c; Roles of RHBLD4



Han, S-I et al. 2023

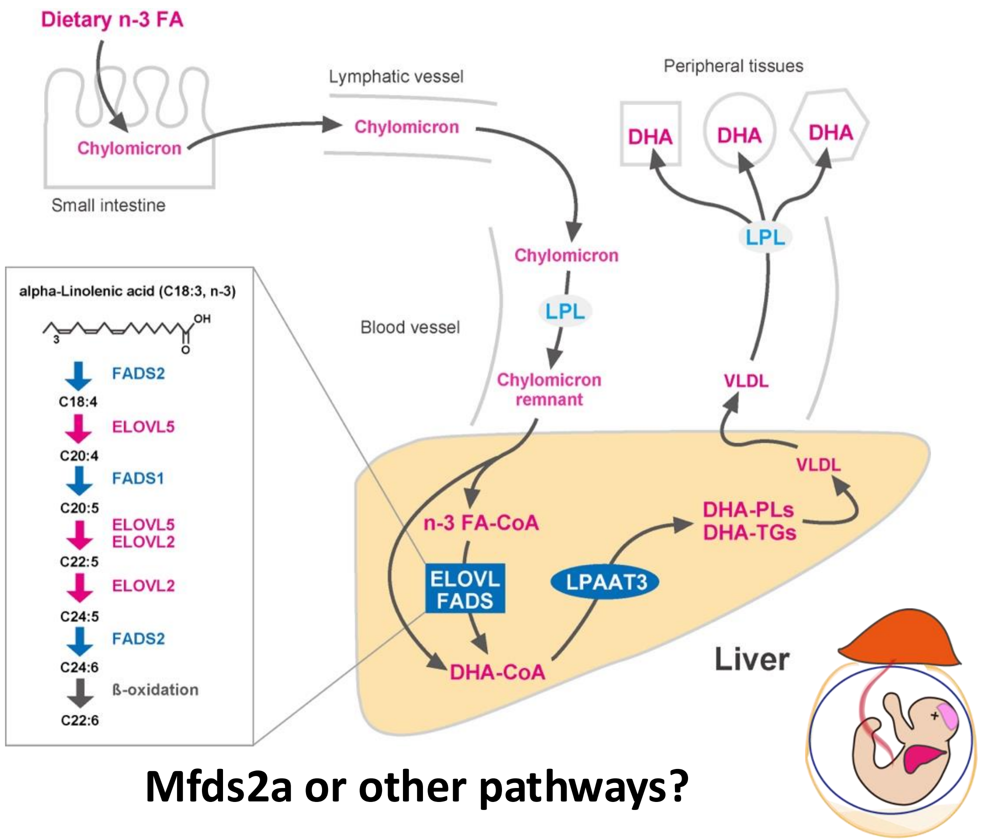
PNAS nexus

PUFA production in liver and transport to brain, retina, and fetus

Hishikawa

SREBP1c by RHBLD4?

Hishikawa et al. *iScience*, 2021
Han, SI et al. *PNAS Nexus, 2023*

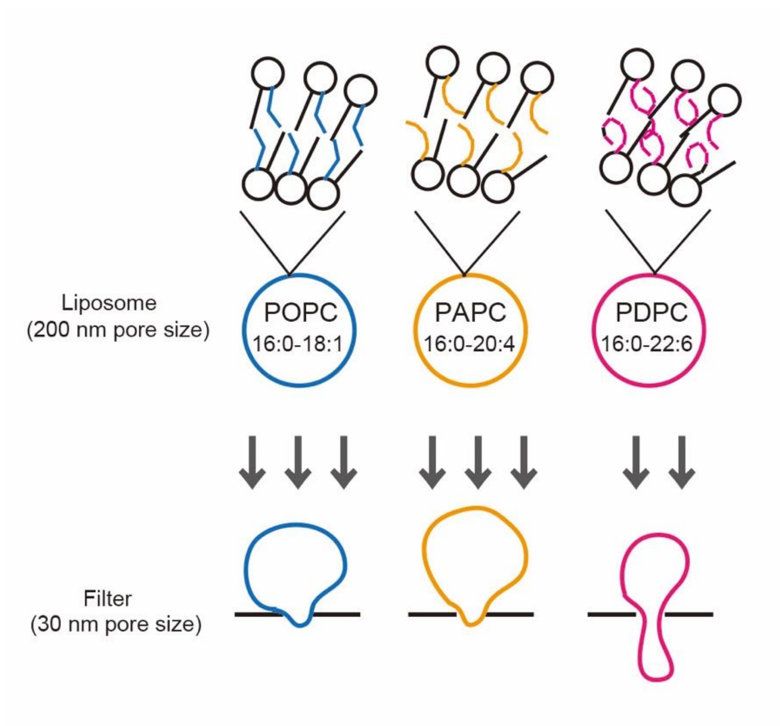


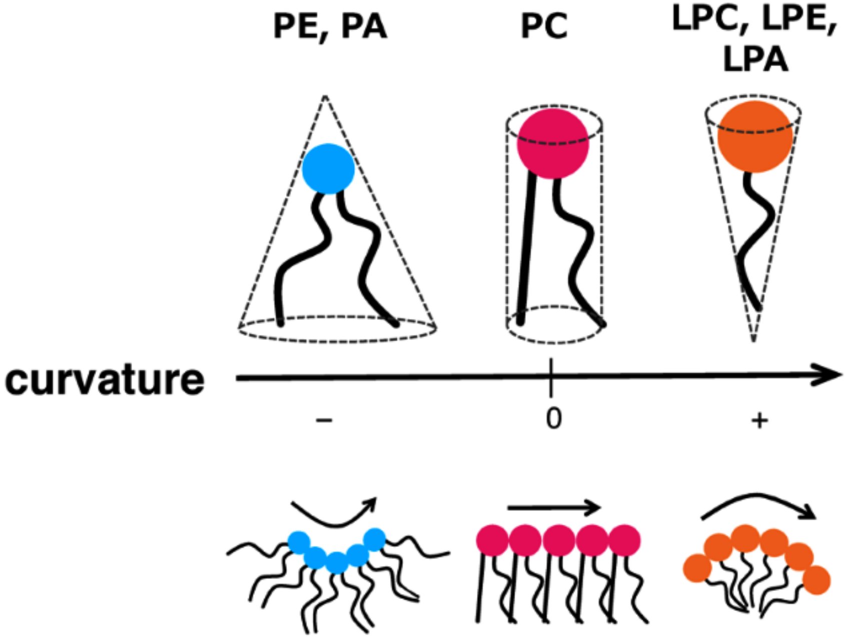
Mfds2a or other pathways?

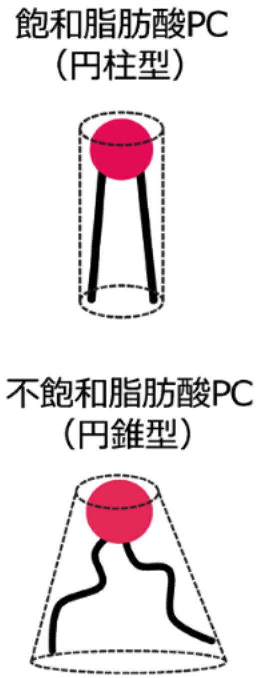
Yanagida

Kanatani

PUFAs modulate cellular functions, not only by production of lipid mediators, but change in membrane curvature, flexibility etc.








R
RENTAL

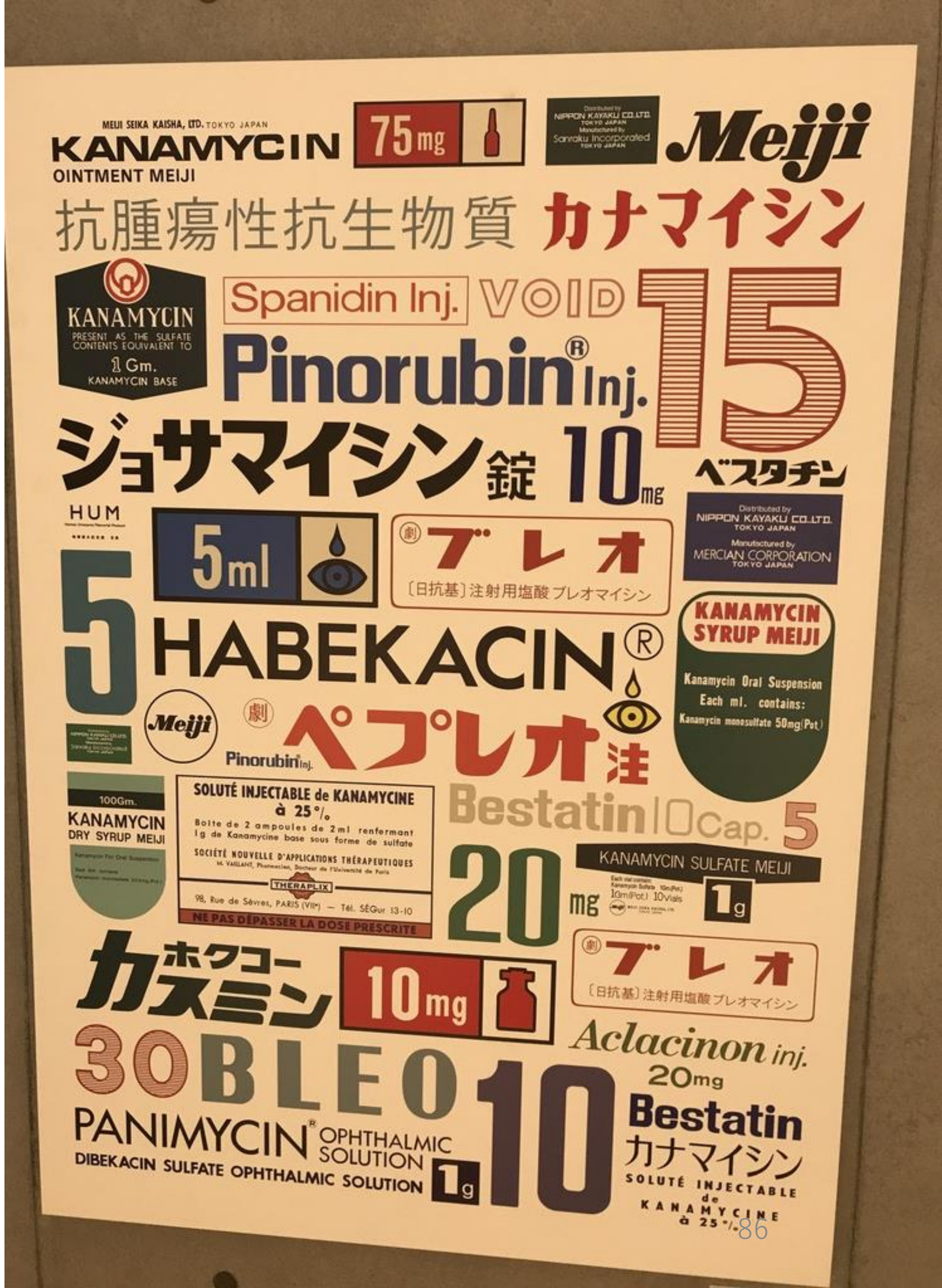
Institute of Microbial Chemistry, Tokyo

Dr. Hamao Umezawa, 1914-1986, University of Tokyo

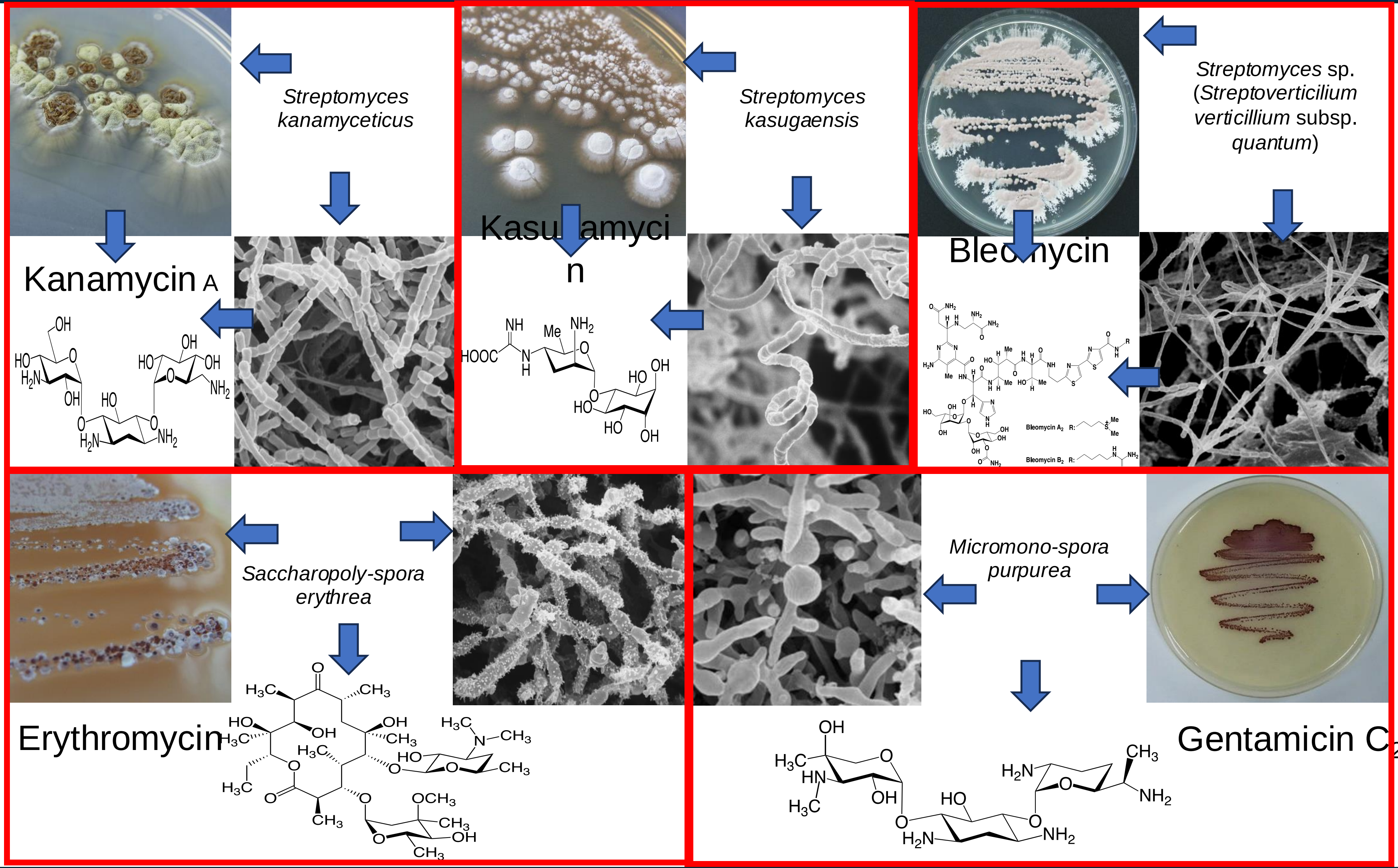
- Established in 1958 with the royalties of Kanamycin (anti-tuberculosis drug)
- Discovery and marketing of kasugamycin (against rice blast disease), bleomycin for squamous cell cancer, josamycin etc from *Striptomyces, fungi etc.*
- Protease inhibitors (leupeptin, pepstatin, chymostatin etc) on Market

Challenge of IMC

- Rich in library of bacteria (over 45,000 strains), fungi and their culture media
- Well trained researchers and technicians of culture, isolation and purification of natural products including middle-sized compounds.
- Structure biology (X-ray, EM, cryo-EM, NMR)
- Molecular structure of natural compounds (NMR, LC-MS, X-ray, EM)
- Organic chemistry
- Animal facilities, pharmacodynamics, toxicity determination, in vivo tumor growth etc



Example of a natural resource 1



Example of a natural resource 2

Caterpillar fungus (Cordyceps sinensis)

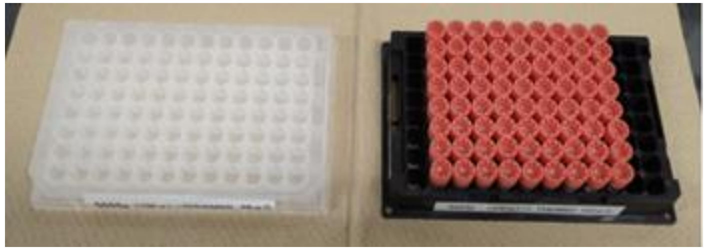
Broth library & Database

Broth preparation using a variety of culture methods

Liquid culture

Solid (wheat) culture

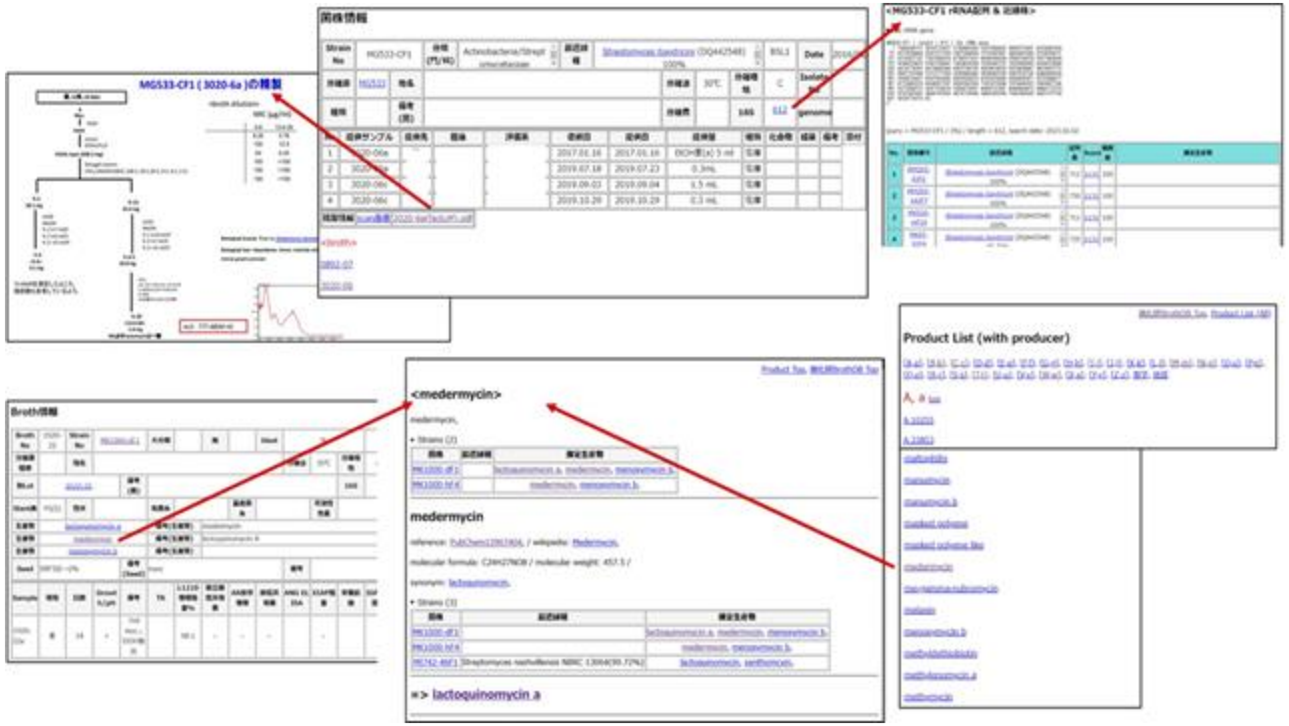
Dispense into assay plates



96-well plate for primary screening

cluster tube for confirmation

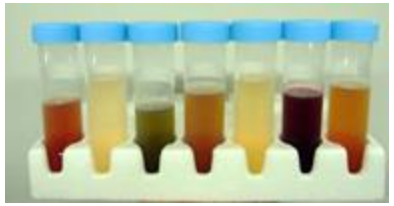
Enrichment of database



More than 20,000 samples available

From Bacteria to Drug Discovery

Extraction

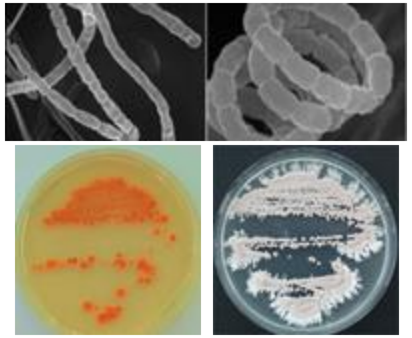


Culture

Liquid culture

Solid culture

Isolation of bacteria



Collection of natural resources

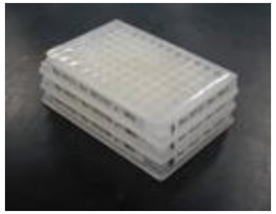
Soil

Insect Symbiosis Actinomycetes

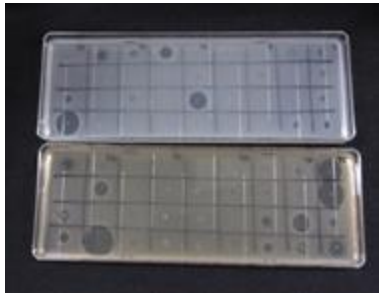
Deep Sea & Hydrosphere

Actinomycetes from litter

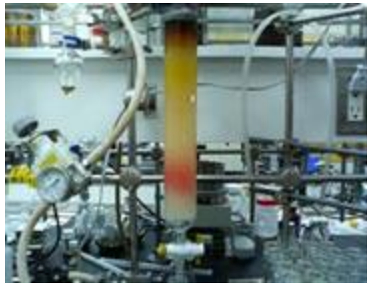
Dispense to plates



Screening



Isolation and structural determination of active substances



- Structural optimization
- Mechanism elucidation
- Pharmacological studies
- Safety studies
- Pharmacokinetic studies

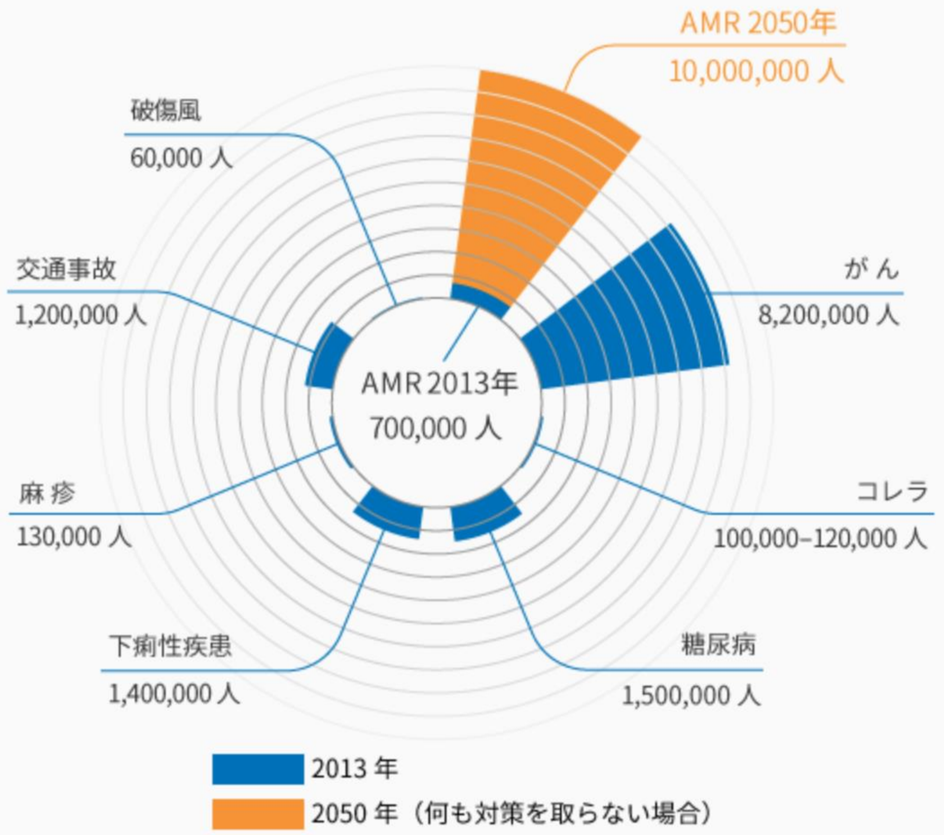
My next challenge, crossing a bridge between IMC and NCGM (JIHS)

- Finding good seeds, and good partners for drug developments

- Clinical trials at NCGM and National Infection Institute (merged to JIHS, 2025)

迫るAMR(anti-microbial resistance)

図1　薬剤耐性（AMR）に起因する死亡者数の推定



Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations The Review on Antimicrobial Resistance Chaired by Jim O'Neill December 2014 を改変

個々の製薬の力の限界

↓

国、民間、財団などの支援必須

Delink model導入

Thank you for your attention!

You can reach me by Facebook

or

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