Versatile roles of lipids and carotenoids in membranes

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Significance of membranes in living cells

single cells
intracellular compartments, transport
intercellular communications
cell division, differentiation
specific membranes

Eukaryotic cells
Development of membrane models

1935. Danielli-Davson model:
- a lipid bilayer
- globular proteins attached loosely to the surface
- static!

1957. Robertson Unit Membrane Model:
- based on EM images
- a lipid bilayer
- protein monolayers attached covalently to both sides
- static!

1972. Singer-Nicolson Fluid Mosaic Model:
- an oriented, 2D viscous solution of amphipathic lipids and proteins
- carbohydrates attached to proteins and lipids on the outer surface
- components distributed in a mosaic structure (= proteins and lipids dispersed inhomogeneously) → lateral and trans-membrane patches (= domains)
- protein content of the membranes

"Cell membrane detailed diagram en" by LadyofHats Mariana Ruiz [public domain] via Wikimedia Commons
1997. Simons’ Lipid Raft Model:
- phase separated, cholesterol- and sphingolipid-rich, liquid ordered microdomains
- lipid phases in membranes
  solid gel, liquid crystalline = liquid-disordered, liquid-ordered raft

Rafts:
- lateral domains in the membranes distinguished from the bulk lipids
- diverse in their composition, lifetimes and sizes
- raft sizes: 10 – 200 nm
- several angstroms thicker than the rest of the membrane
  → atomic force microscopy
- insoluble fraction in cold non-ionic detergents (Triton X-100)
  → detergent-resistant membranes
- enriched in fatty acid- or GPI-linked proteins
- role in lipid trafficking, protein targeting, signal transduction, virus infection and budding
LIPIDS

General properties
- Amphipathic molecules: polar head group ↔ hydrophobic tail
- Spontaneous membrane organization (self-assembly process)
- Lipid polymorphism: long-range structures that amphipathic lipids can take when dispersed in water
  - Lamellar, inverted hexagonal, cubic
- Permeability
Structural lipids

Glycerolipids

- glycerol backbone
- 2 fatty acids are esterified to the sn-1 and sn-2 of glycerol → hydrophobic tail
- sn-3 is esterified to phosphate → phospholipids

Phosphatidic acid (PA)

polar head groups: choline, ethanolamine, serine, glycerol, inositol, cardiolipin

- plasmalogens
  - have an ether-linked alkenyl chain at the sn-1 and an ester-linked acyl chain at the sn-2 position
  - vertebrate heart is enriched in ether-linked lipids
  - role as a platelet activating factor
  - ↑ in cancer
Structural lipids

Glycerolipids
- glycerol backbone
- 2 fatty acids are esterified to the sn-1 and sn-2 of glycerol → hydrophobic tail
- sn-3 is bound a mono- or disaccharide → glycolipids in photosynthetic membranes!

- monogalactosyldiacylglycerol (MGDG)
- digalactosyldiacylglycerol (DGDG)
- sulfoquinovosyldiacylglycerol (SQDG)
Sphingolipids

derivatives of sphingosine (a C-18 amino alcohol)
  - C₁-C₃ part is structurally analogous to glycerol
  - C₄-C₁₈ part is a hydrophobic tail with a trans double bound

ceramide
  a fatty acid attached to sphingosine through an amide bond

Phosphosphingolipids
sphingomyelin (SM)
  - phosphocholine (or phosphoethanolamine) head group
  - fatty acyl tail is long and usually saturated

Glycosphingolipids

sphingomyelin
Glycosphingolipids

- Sugar residues always locate on the extracellular side of the plasma membranes
- Role in cell–cell recognition and immunology

**cerebroside** – a ceramide with a monosaccharide (galactose or glucose) head group
**globoside** – a ceramide with a di-, tri- or tetrascarcharide head group
  (containing glucose, galactose or N-acetyl-D-galactosamine sugars)
**ganglioside** – a ceramide with a polar head group that is a complex oligosaccharide, including at least one sialic acid (a 9-carbon anionic sugar)
Sterols

- major components of animal, plant and fungal membranes but are absent in prokaryotes (where sterol functions are replaced by hopanoids)
- structure - four rigid rings are responsible for the major functions → controlling membrane fluidity
  - a polar hydroxyl group → anchored the molecule to the aqueous interface
  - a floppy tail (function is not clear)
- in animals: cholesterol (Chol) - in vertebrates major location is the brain
  in fungi: ergosterol
  in plants: phytosterols (>250, β-sitosterol, stigmasterol)
  in plasma membranes ~ 50% of the total membrane lipids
- roles: structural - provide mechanical strength, control phase behavior, support membrane lateral organization and stability, hold cell signaling lipid rafts together
  precursor for essential biochemicals (steroid hormones, bile salts and vitamin D)
Phase behavior of lipids

Bilayer-forming lipids
  cylindrical shape: PC, PS, PA, PI, SM, CL, DGDG, SGDG, cerebrosides
  → lamellar phase

Non-bilayer lipids
  cone: MGDG, PE, Chol, CL+Ca^{2+} → inverted hexagonal phase (H_{II})
  wedge (inverted cone): gangliosides, lyso-PC → micelle

Source: CC BY / Juliette Joutret
in Frontier of Plant Science 03 December 2013
Active lipids

derivatives of membrane lipids, at very low amounts

- free fatty acids and lyso-phospholipids
  - hydrolyzed by phospholipases A1 and A2
  - harmful to membranes (detergent effect)
- DAG – hydrolyzed by phospholipase C
  - activator of several membrane proteins
- AA → eicosanoids (prostaglandins, thromboxans, leukotrienes)
  - act similarly to hormones
  - roles in inflammation processes
- α-linolenic acid → jasmonates (and other oxylipins) in plants
  - hormones with signaling functions
- lipid-soluble vitamins A, D, E and K
- PI derivatives – PI → PI-4,5-bisphosphate → IP3 and DAG
  - release of Ca\(^{2+}\) from ER

Source: RoadnotTaken under GFDL via Wikimedia Common
Fatty acid composition of structural lipids

- mono-carboxylic acids with an unbranched hydrocarbon tail
- chain length: 14-24 carbons (even numbered ← result of biosynthesis from 2-carbon acetyl units)
- acyl: general term meaning an esterified fatty acid of unspecified chain length

- saturated
- unsaturated: 1-6 double bonds (methylene interrupted) primarily cis configuration

Nomenclature

- systematic name: cis-9-octadecanoic; trans-9-octadecanoic
- trivial name: oleic; elaidic
- structure: 18:1(Δ9); 18:1(Δ9t)

MUFA: monounsaturated fatty acid
PUFA: polyunsaturated fatty acid
## Most abundant fatty acids

<table>
<thead>
<tr>
<th>Systematic name</th>
<th>Trivial name</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexadecanoic</td>
<td>palmitic</td>
<td>16:0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16:1</td>
</tr>
<tr>
<td>Octadecanoic</td>
<td>stearic</td>
<td>18:0</td>
</tr>
<tr>
<td>9-octadecanoic</td>
<td>oleic</td>
<td>18:1(Δ9)</td>
</tr>
<tr>
<td>trans-9-octadecanoic</td>
<td>elaidic</td>
<td>18:1(Δ9t)</td>
</tr>
<tr>
<td>9,12-octadecadienoic</td>
<td>linoleic</td>
<td>18:2(Δ9,12)</td>
</tr>
<tr>
<td>9,12,15-octadecatrienoic</td>
<td>α-linolenic</td>
<td>18:3(Δ9,12,15) or (ω-3)</td>
</tr>
<tr>
<td>6,9,12-octadecatrienoic</td>
<td>γ-linolenic</td>
<td>18:3(Δ6,9,12) or (ω-6)</td>
</tr>
<tr>
<td>5,8,11,14-eicosatetraenoic</td>
<td>arachidonic</td>
<td>20:4(Δ5,8,11,14) or (ω-6)</td>
</tr>
<tr>
<td>5,8,11,14,17-eicosapentaenoic</td>
<td>EPA</td>
<td>20:5(Δ5,8,11,14,17) or (ω-3)</td>
</tr>
<tr>
<td>7,10,13,16,19-docosapentaenoic</td>
<td>DPA</td>
<td>22:5(Δ7,10,13,16,19) or (ω-3)</td>
</tr>
<tr>
<td>4,7,10,13,16,19-docosahexaenoic</td>
<td>DHA</td>
<td>22:6(Δ4,7,10,13,16,19) or (ω-3)</td>
</tr>
</tbody>
</table>
Melting point (Tm) of fatty acids increase with chain length and decrease with higher degree of unsaturation

**Homeoviscous adaptation:**

- efforts to maintain proper membrane properties
  - acyl chain length
  - acyl chain double bonds
  - phospholipid head groups
  - sterols: cholesterol
  - isoprene lipids (vitamin E and D)

**Desaturase enzymes**

1. Acyl-CoA desaturases (yeast, fungi and animals)
   \[18:0-\text{CoA} \rightarrow 18:1(\Delta 9)-\text{CoA} \rightarrow ? 18:2(\Delta 6,9)\]
2. Acyl-ACP desaturases (plant chloroplasts)
   \[18:0-\text{ACP} \rightarrow 18:1(\Delta 9)-\text{ACP}\]
3. Acyl-lipid desaturases (cyanobacteria and plants)

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>Tm (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>palmitic</td>
<td>16:0</td>
<td>63.1</td>
</tr>
<tr>
<td>stearic</td>
<td>18:0</td>
<td>69.6</td>
</tr>
<tr>
<td>oleic</td>
<td>18:1</td>
<td>16.2</td>
</tr>
<tr>
<td>elaidic</td>
<td>18:1(t)</td>
<td>43.7</td>
</tr>
<tr>
<td>linoleic</td>
<td>18:2</td>
<td>-5</td>
</tr>
<tr>
<td>α-linolenic</td>
<td>18:3</td>
<td>-11</td>
</tr>
<tr>
<td>arachidic</td>
<td>20:0</td>
<td>75.3</td>
</tr>
<tr>
<td>EPA</td>
<td>20:5</td>
<td>-54</td>
</tr>
<tr>
<td>DHA</td>
<td>22:6</td>
<td>-44</td>
</tr>
</tbody>
</table>
Synechocystis sp. PCC 6803

Desaturase

Δ9  desC
Δ12  desA
Δ6  desD
ω3 (Δ15)  desB
Distribution of lipids

Prokaryotes

Bacteria: PE (70-80%), PG (20-25%), CL (5%), hopanoids
Cyanobacteria: MGDG (55%), DGDG (25%), SQDG (20%), PG (10%), hopanoids
Archaea: ether lipids - the non-polar chains are joined to a glycerol backbone by ether linkages → resistant to hydrolysis
- the alkyl chains are very long, saturated and branched → resistant to oxidation
they live under extreme conditions (high temp., high salt, low pH)

Eukaryotes

Plants: PC > PE > PS, PI, phytosterols (β-sitosterol, stigmasterol)
chloroplast: MGDG, DGDG, SQDG, PG
Yeasts and fungi: PC > PE, PS, PI, PA, DAG > PG, CL
ergosterol, SLs (Cer-derivatives)
Mammals: PC > PE > PI > PS > CL > PG > PA
SM and Chol
Compartments of mammal cells

ER (endoplasmic reticulum)
- the main site of lipid synthesis: PLs, Chol → transported to other organelles
- Cer, GalCer → precursors of complex SPs
- it has low concentration of Chol and complex GSLs → loose packing of mbr lipids
  → insert of newly synthesized lipids and proteins
- minor lipids (PA, DAG, lysophospholipids, dolichol)

Golgi apparatus
- a lipid-based sorting station
- specializes in SL synthesis (SM, GlcCer, GalCer, complex GSLs)
  → export to plasma mbr

Plasma membrane
- SLs and Chol, packed at a high density → resists mechanical stress
  signaling and recognition lipids (<1% of total PLs)

Endosomes
- early endosomes (similar to plasma mbr)
  → late endosomes: ↓Chol, ↓PtdSer, ↑BMP (bis-monoacylglycerophosphate)
  → multivesicular body generation, fusion processes
- specific phosphoinositides
  → identify endocytic mbrs
  → to recruit proteins from the cytosol

Mitochondria
- have bacterial lipids – oxidative phosphorylation
- lipid synthesis (45% of PLs): PA, LPA, PtdEtn, PG → CL (unique!)
Lipid asymmetry

first established in the plasma membrane of erythrocytes

inner leaflet: PE and PS (amine lipids)
    PA, PI and PI4,5-(bis)phosphate
outer leaflet: PC and SM
    gangliosides
    Chol (but rapid transmembrane flip-flop!)
CL equally distributed between leaflets

Courtesy: National Science Foundation
Lipid-protein interactions

Structural and functional role of phosphatidylglycerol in photosystem II
yellow, PG; red, D1 protein; blue, D2 protein; greens, α-helixes of inner antennas CP43 and CP47

Cardiolipin in mitochondrial inner membrane

"Proton trap" by Rochellehx
- modified based on FEBS Letters 528 35-39
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Dietary lipids and human health

essential lipids

$\omega$-3 PUFAs

- $\alpha$-linolenic acid  \(18:3(\Delta 9,12,15)\)
- eicosapentaenoic acid (EPA)  \(20:5(\Delta 5,8,11,14,17)\)
- docosahexaenoic acid (DHA)  \(22:6(\Delta 4,7,10,13,16,19)\)

$\alpha$-linolenic acid

$\omega$-6 PUFA: arachidonic acid (AA)  \(20:4(\Delta 5,8,11,14)\) → precursor for eicosanoids
(prostaglandins, thromboxans, prostacyclins, leukotrienes)

ratio of $\omega$-6 and $\omega$-3 PUFAs !

$\textit{trans}$-fatty acids
Dietary lipids and human health

lipid-soluble vitamins

- vitamin A – derived from β-carotene
  - oxidation states: retinol – antioxidant
  - retinal – essential for color vision
  - retinoic acid – gene control
  - complex roles in signaling, growth and differentiation
- vitamin D – derived from cholesterol
  - converted to a hormone (1,25-dihydroxycholecalciferol)
  - regulates Ca^{2+} uptake and levels in kidney and bones
- vitamin E – α-tocopherol prevents lipid peroxidation in membranes
CAROTENOIDs

- lipophilic pigment molecules
- C5 isoprene precursor $\times 8 \rightarrow C40$
- long polyene chain $\rightarrow$ conjugated double bonds
- synthesized in bacteria, algae, plants and fungi
- in animals incorporated from diet

C5 $\rightarrow$ C10 $\rightarrow$ C15 $\rightarrow$ C20 + C20

\[\downarrow\]

C40 15-cis-phytoene

\[\downarrow\]

all-trans-lycopene

\[\downarrow\]

cyclase

carotenes

\[\downarrow\]

hydroxylase, ketolase, epoxylase

oxanthophylls

zeaxanthin

lutein

echinonene

myxol-2'-glycoside
Antioxidant properties of carotenoids

- photoprotective roles by quenching triplet or singlet chlorophylls and singlet oxygen
- scavenge free radicals (reactive oxygen species, ROS; reactive nitrogen species, RNS)

\[
\begin{align*}
O_2^{•−} \text{ (superoxide anion)} & \rightarrow \text{ secondary ROS} \\
•\text{OH} \text{ (hydroxyl radical)} & \rightarrow \text{ high reactivity! } \rightarrow \text{ damage in DNA, proteins, membranes} \\
\text{ROO•} \text{ (peroxyl radicals)} & \rightarrow \text{ lipid peroxidation} \\
\text{NO•} \text{ (nitric oxide)} & \rightarrow \text{ signaling } \rightarrow \text{ nitrosative stress} \\
\text{ONOO•} \text{ (peroxynitrite anion)} & \rightarrow \text{ DNA fragmentation, lipid peroxidation}
\end{align*}
\]

- interact synergistically with other antioxidants (vitamin E and C)

- antioxidants $\leftrightarrow$ prooxidants?
Structural roles of carotenoids

- modulation of membrane viscosity

- constituents of pigment-protein complexes

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http://www.sciencedirect.com/science/journal/01637827
Functional roles

In photosynthetic organisms
- light harvesting pigment at 450-570 nm
- photoprotection by quenching chlorophyll triplet state
- singlet oxygen scavenging
- thermal dissipation of excess energy
- xanthophyll cycle in plants
- stabilization of pigment-protein complexes
In photosynthetic organisms

- xanthophyll cycle in plants
- thermal dissipation of excess energy
- stabilization of pigment-protein complexes
In animals

- ~600 carotenoids in nature, only ~20 in human plasma and tissues
  - lycopene > β-carotene, zeaxanthin, cryptoxanthin, lutein, α-carotene

- lycopene
  - sources: tomato products, watermelon, pink grapefruit, papaya
  - scavenges singlet oxygen and peroxyl radicals
  - induces antioxidant enzymes (superoxide dismutase, glutation reductase, glutation peroxidase)
  - reduces the risk of cancer and cardiovascular disease
In animals (cont.)

- β-carotene → vitamin A
  - retinol – antioxidant
  - retinal – essential for color vision
  - retinoic acid – gene control
  - complex roles in signaling, growth and differentiation

forms of vitamin A

- age-related macular degeneration – lutein and zeaxanthin

*Rhodopsin-transducin* by Dpyran
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