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REVIEW

Versatile roles of lipids and carotenoids in membranes

Ildikó Domonkos*, Mihály Kis, Zoltán Gombos

Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

ABSTRACT Chemical characteristics and functions of constitutive membrane lipids, fatty acids and carotenoids, are summarized. Lipids and carotenoids are major components of biological membranes, and perform several important physiological and structural functions in all kingdoms of living organisms. Structural lipids build up and maintain the architecture of biological membrane, while active lipids originating from membranes have signaling functions. Unsaturated lipids protect photosynthetic organisms against low-temperature photoinhibition and keep necessary membrane microviscosity under various environmental conditions. Sterols are capable of controlling membrane order and segregation of lipids into rafts by intercalating between phospholipids. Carotenoids are essential structural and functional components of photosynthetic complexes and membranes in plants, algae and cyanobacteria. They have a role in light harvesting processes and the elimination of excitation energy. They can serve as protectant agents against reactive oxygen and nitrogen species. Heat dissipation is involved in protective processes. Carotenoids are effective quenchers and scavengers of singlet oxygen and other free radicals, therefore they suppress lipid peroxidation and other harmful biochemical reactions. Carotenoids are indispensable antioxidants in photosynthetic organisms and also in animals, which are not capable of synthesizing them. Both carotenoids and essential lipid species are necessary for human diet. **Acta Biol Szeged 59(Suppl.1):83-104 (2015)**

KEY WORDS

antioxidant
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Introduction

Membranes are essential components of every living organism, each cell that is alive today or has ever been alive. Early membranes had to play a crucial role in the origin of life as the structures those separated the cell interior from its external environment. During the biological evolution, the complexity of membranes increased from that of a simple surrounding plasma membrane to the complex membrane systems inside eukaryotic cells. Plasma membranes are responsible for the selective permeability of cell envelopes that enables cells to take up nutrients and exclude most harmful agents. They are playing crucial roles also in cell division and differentiation, intercellular communication and signal transduction. Huge amount of internal membranes packed tightly in eukaryotic cells providing proportional surfaces for biochemical processes. Spacious internal membrane systems can be found even in prokaryotic cells, such as the thylakoid membranes of cyanobacteria, where photosynthetic electron transport components are located.

Our idea and knowledge about membranes developed slowly during the last decades. Rapidly evolving scientific methods in the 20th century opened the door to establish realistic, working models of biological membranes. In 1935 the Danielli-Davson model described a lipid bilayer with globular proteins attached to the surface. The next model that appeared in 1957 was based on electron microscopic images. It was the Robertson' Unit Membrane model that was very similar to the previous one, but protein monolayers attached covalently to both sides of the lipid bilayer. These two early models composed a static structure that failed to take account of membrane dynamics and protein diversity. Our current concept of membrane structure is based on the Singer-Nicolson Fluid Mosaic model, published in 1972 (Singer and Nicolson 1972). This model defined a membrane as 'an oriented, 2D viscous solution of amphipathic proteins and lipids in instantaneous thermodynamic equilibrium. Proteins can be integrated or peripherals and the membrane contain also carbohydrates those attached to proteins and lipids on the outer surface. Membrane components distributed in a mosaic structure where both proteins and lipids dispersed inhomogeneously resulting in lateral and trans-membrane patches called domains. This model recognized for the first time that a biological membrane is dynamic, fluid and ever changing. However, the original Fluid Mosaic model could not explain the heterogeneous distribution of membrane lipids and proteins (Stillwell 2013).

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*Corresponding author. E-mail: domonkos.ildiko@brc.mta.hu

A new model appeared in 1997, the Simons' Lipid Raft model (Simons and Ikonen 1997). Lipid rafts are cholesterol- and sphingolipid-rich liquid-ordered microdomains that float in a non-raft liquid-disordered matrix. A liquid-ordered state has properties midway between those of a liquid-disordered (or fluid) and gel (ordered or solid) state. It is believed that rafts are held together by cholesterol. Lipid rafts have been identified and characterized in plasma membranes of mammalian cells but today it is known that lipid rafts are common features of eukaryotic cells. Many bacterial membrane proteins involved in signal transduction processes are distributed heterogeneously across the cytoplasmic membrane. These observations suggested the existence of lipid microdomains also in bacterial membranes, where they harbor and organize proteins involved in signal transduction and protein secretion (Lopez and Kolter 2010). Although, sterols and sphingolipids, dominant lipid species of eukaryotic rafts are missing from bacterial membranes, sterol functions can be replaced by hopanoids.

Biological membranes are composed of enormous variety of proteins and lipid molecules, including carotenoid subgroup of lipids that can affect structural and functional properties of membranes. Present study focuses on the most important features of lipid constituents of biological membranes.

Lipids

The classification of lipids

What are lipids?

The name lipid came from the Greek word 'lipos', meaning fat. Lipids are organic compounds that can be found in huge variety in nature. They have low solubility in water but are easily soluble in nonpolar organic solvents (*e.g.*, ether, chloroform, acetone and benzene). This definition of lipids based on solubility is rather often used; however, more specific descriptions are now also available. (http://lipidlibrary.aocs.org/Lipids/whatlip/index.htm) (Fahy et al. 2005)

The classification of lipids is difficult because of their large diversity, and accordingly there have been several systems constructed. Each classification reflects its author's point of view and has advantages and disadvantages. In this review we will consider only those lipids that have structural and/or functional roles in biological membranes. Two main categories will be applied, structural lipids that have roles in building up and maintaining of biological membranes and active lipids that derive from membranes and have signaling functions.

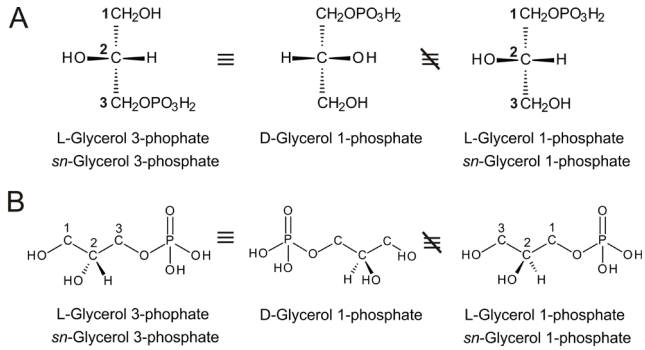

Figure 1. The compound shown on the left side can be equivalently referred to as L-glycerol-3-phosphate or D-glycerol-1-phosphate. However using stereospecific numbering (*sn*) which assigns the 2-position to the middle carbon atom of the L-glycerol derivative where a hydroxyl group is shown to the left and the carbon atom above is C1 and that below is C3, the compound is unambiguously named *sn*-glycerol-3-phophate. *sn*-glycerol 1-phosphate is the enantiomer pair. Molecules shown in Fischer projection (A), and in zigzag format (B).

Structural lipids

Glycerophospholipids (or phospholipids) are key components of the membrane bilayers of cells. The C3 (in eukaryotes and eubacteria) or the C1 (in archaebacteria) hydroxyl of glycerol is esterified to phosphate forming *sn*-glycerol 3-phosphate and *sn*-glycerol 1-phosphate (Fig. 1). Although the glycerol backbone is symmetrical, the middle carbon becomes a chiral center when the two other carbons have different substituents. Hence *sn*-glycerol 3-phosphate and *sn*-glycerol 1-phosphate are chiral molecules. Glycerol 3-phosphate shown in Fischer projection can be interpreted as L-glycerol 3-phosphate or D-glycerol 1-phosphate. To make it unambiguous the L-glycerol derivative is taken where a hydroxyl group is shown to the left of the middle carbon atom, C2, and the carbon atom above is C1 and that below is C3. The prefix "*sn*" refers to that stereospecific numbering system (http://www.chem.qmul.ac.uk/iupac/lipid/lip1n2.html).

The smallest and simplest phospholipid is the phosphatidic acid (PA) which is the precursor for other more complex phospholipids. The hydroxyl groups at C1 and C2 positions are esterified to fatty acids while the phosphoryl group to different polar groups forming a hydrophobic tail and a polar head. Glycerophospholipids can be subdivided into distinct classes, based on the nature of the polar headgroup (Fig. 2). The fatty acid moieties define individual species (http://lipidlibrary.aocs.org/Lipids/complex.html).

At physiological pH the non-esterified phosphate groups are almost totally dissociated resulting negative charges. Therefore these lipids can provide regulatable negative charge to the membrane.

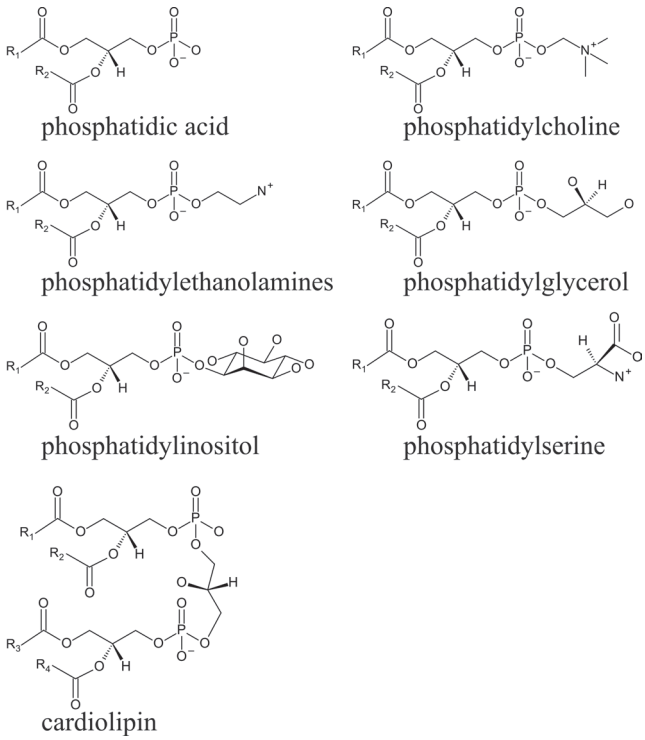

Figure 2. The general formula of the main glycerophospholipid classes. R1 and R2 are long chain hydrocarbon tails of fatty acids.

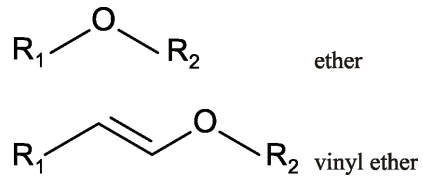

Figure 3. Illustration of ether and vinyl ether bonds, the two main types of glycerol ether bonds exist in natural lipids. The double bond in vinyl ether has *cis* configuration.

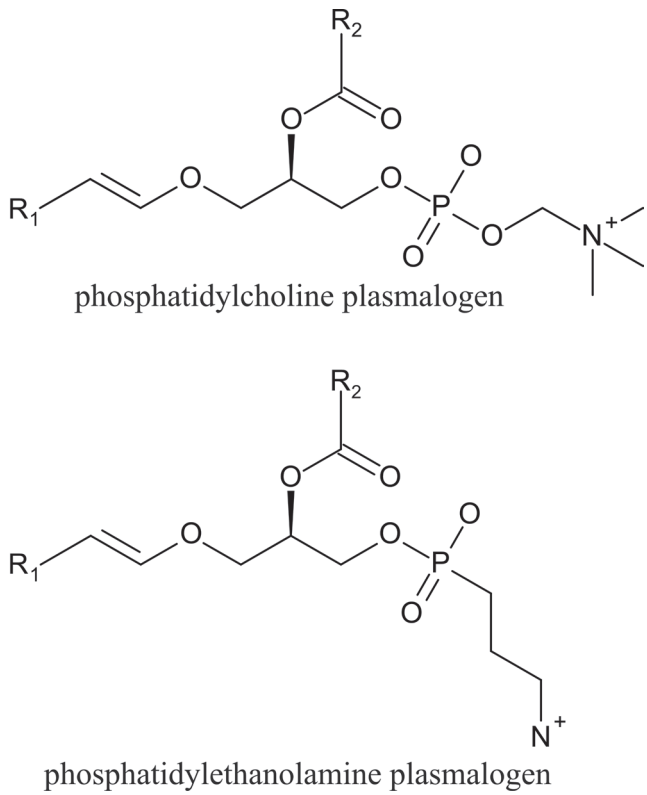

Figure 4. Plasmalogen structures.

In the above described lipids the fatty acyl chains are attached to glycerol by ester bonds which are easily hydrolysable chemically or enzymatically. However, there are a number of membrane lipids with the more stable ether linkages connecting one or more of the carbon atoms of glycerol to an alkyl chain. Both ether and vinyl ether bonds (Fig. 3) can be found in natural membrane lipids. Vinyl ether bonds undergo hydrolysis by general acid catalysis while ether bonds are quite resistant to both acidic and alkaline conditions.

Glycerophospholipids that contain vinyl ether linkage at *sn*-1 position are called plasmalogens. Plasmalogens are found in humans mainly in the myelin sheath, in the heart and the kidney (Gorgas et al. 2006; Moser et al. 2011). Plasmenylethanolamines with ethanolamine and plasmenylcholines with choline headgroups are the most common types (Fig. 4). Plasmalogenes appear in obligate anaerobes as well where phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine and cardiolipin are the most prevalent.

Ether lipids are particularly common in Archaea (Kates 1992). They live in extreme environments where the ester linkages of the membrane lipids would be easily opened. Typically, instead of fatty acids, in Archaea, isoprenoid side chains are ether-linked to C2 and C3 of *sn*-glycerol therefore the chirality of the glycerol moiety is the opposite of that found in Bacteria and Eucarya (Fig. 5). The alkyl chains are generally saturated. The most common core structure, archaeol (2,3-diphytanyl-O-*sn*-glycerol), contains two C_{20} isoprenoid units. Other isoprenoid groups can also be found in these positions. Another significant core lipid structures are the tetraethers. These characteristic molecules are composed of two C40 isoprenoid units bonded from position 2 to 3' and from position 3 to 2' (anti-parallel chains) of the *sn*-glycerol moieties (caldarchaeol). Isocaldarchaeol has parallel chains, accordingly with 2 to 2' and 3 to 3' linkages (Fig. 5). In some species the glycerol of core structures substituted with tetritol or nonitol. Polar lipids are synthesized from the core lipids.

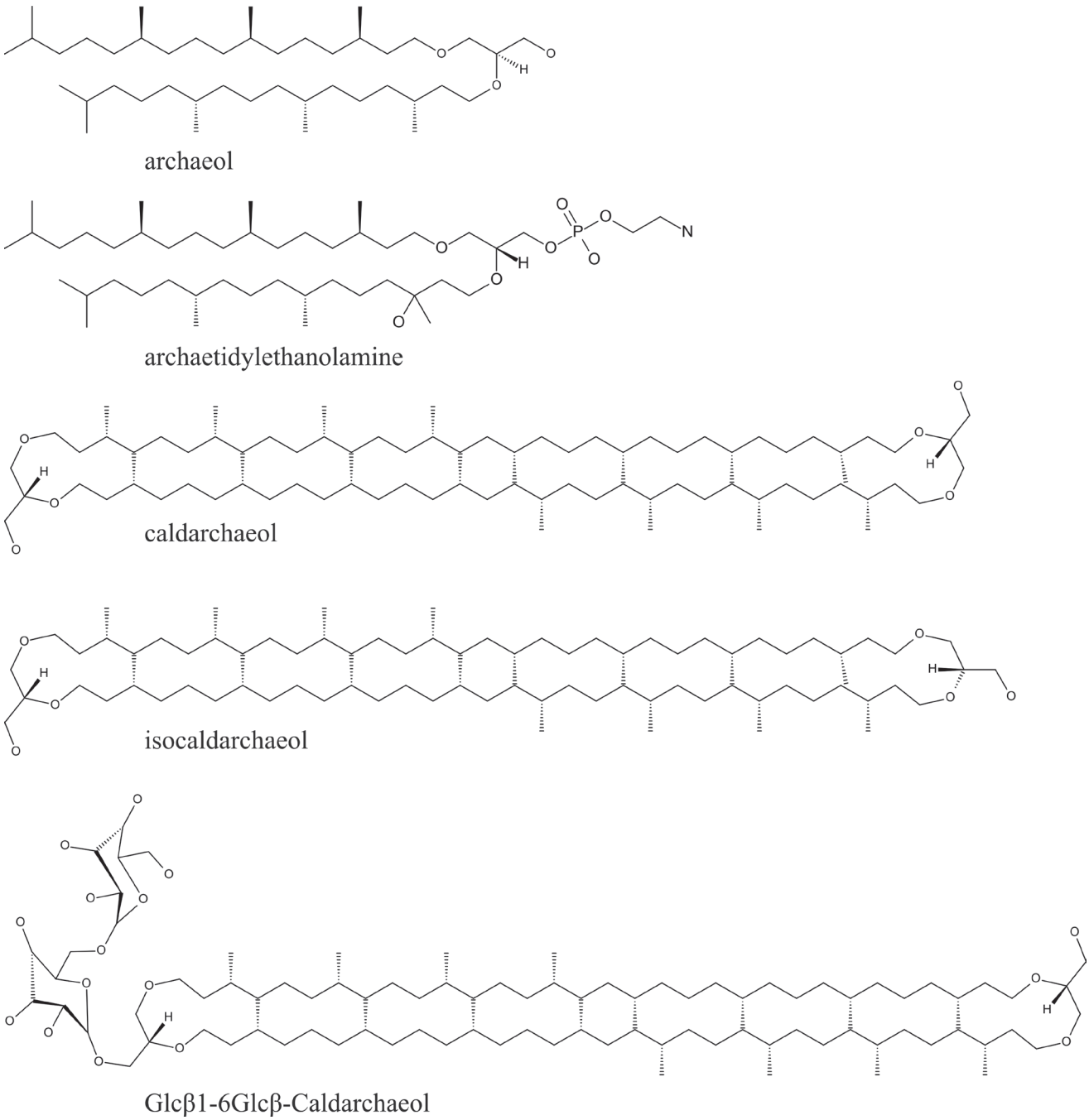

Figure 5. Structural formulas of archaeal ether lipids.

Ethanolamine, L-serine, inosine, glycerol, myo-inositol, and choline bond to glycerol in phosphodiester linkage (Fig. 5). Glycolipids include a range of sugar residues attached to glycerol, *e.g.*, glucose, mannose, galactose, gulose, N-acetylglucosamine, gentiobiose or their combinations. Tetraethers have two glycerols and this provide even more structural varieties. A polar moiety can be attached to one or both glycerols or the two attachments can be different.

Sphingolipides are derivatives of long-chain aliphatic amines (sphingoid bases) (Merrill 2008). Sphingosine, the most abundant in animals, is an 18-carbon amino alcohol with two hydroxyl groups (positions 1, 3) and a trans double bond

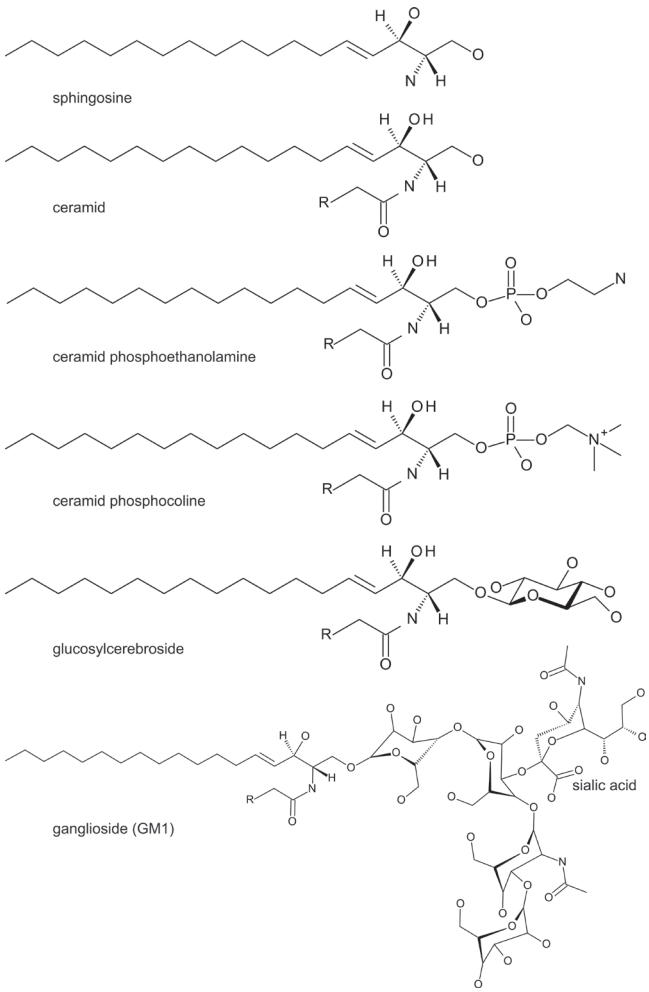

Figure 6. Examples of sphingolipids. Note the conformational resemblance to glycerophospholipids.

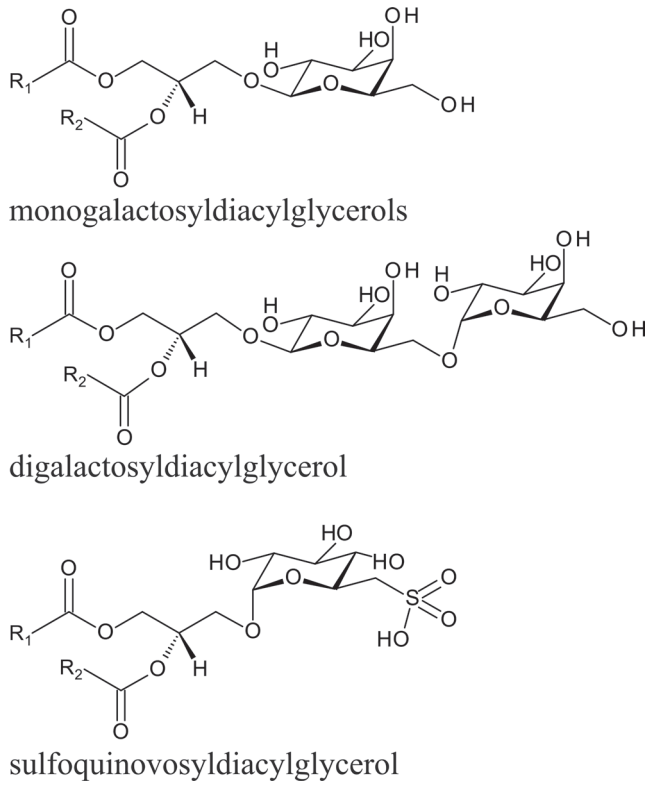

Figure 7. The main glyceroglycolipids of photosynthetic organisms.

at position 4 (Fig. 6). Variations include saturated chains, C_{16}, C_{17}, C_{18}, C_{19}, and C_{20} derivatives and trihydroxy forms. In plants phytosphingosine, a saturated C_{18} trihydroxy sphingoid and its unsaturated variants are common.

Fatty acids linked to sphingoid bases via amide bonds form ceramide (Fig. 6). Ceramides are basic building blocks of sphingolipides and generally rapidly converted to more complex forms; a wide variety of polar molecules may attach to the primary hydroxyl groups. Attachment of phosphorylcholine or, less frequently, phosphorylethanolamine creates the sphingomyelins. Ceramide phosphorylinositol with myo-inositol headgroup appears in plants or fungi. Single sugars are the head groups in cerebrosides. Galactosylceramides are important components of neuronal cells membranes while glucosylceramide are those of the plasma membrane of non-neuronal cells. Cerebrosides lack phosphate groups therefore typically are nonionic compounds. However, galactosylceramides can be sulfated to form sulfatides and become ionic. Neutral, more complex glycosphingolipids are formed by attaching two or more carbohydrate moieties to a ceramide (Fig. 6). The most common diglycosphingolipid is lactosylceramide which is the precursor of the most complex glycosphingolipids the gangliosides. The headgroups of gangliosides are oligosaccharides that contain at least one sialic acid residue (N-acetylneuraminic acid and its derivatives) (Fig. 6). The polar head groups carry a net negative charge at physiological pH and they are acidic.

The overall structure of sphingolipids are structurally analogous to phospholipids, *i.e.* they have a polar head group and two hydrophobic tails. However, in sphingolipids the sphingoid chain is neither variable nor hydrolyzable and the fatty acid chain is attached via a nitrogen ester not an oxygen ester.

A noteworthy feature of sphingolipids is their specific fatty acids content that is very different from those of glycerolipids. Very-long-chain saturated and monoenoic fatty acids (up to 36 carbons) often with 2-*R*-hydroxy groups are typical in sphingolipids (Sandhoff 2010). They are important forming, in close associations with sterols, specific microdomains termed rafts and caveolae in the outer leaflet of the plasma membrane bilayer where sphingolipids exclusively located

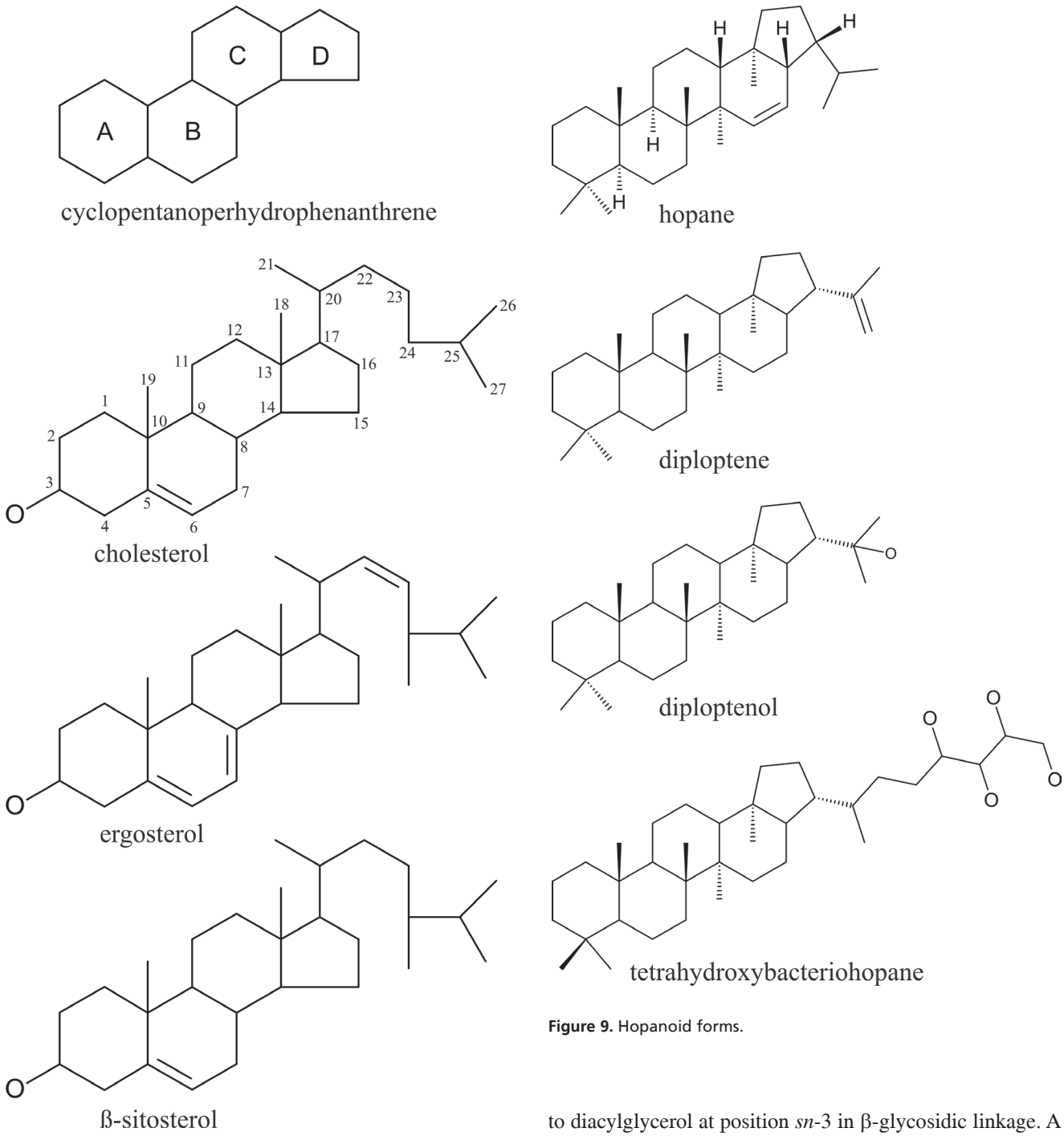

Figure 8. Sterol compounds. Cyclopentanoperhydrophenanthrene, the parent compound of sterols consists of four fused saturated rings.

Figure 9. Hopanoid forms.

(Sonnino and Prinetti 2009, 2013).

Glyceroglycolipids are the main lipid components of the photosynthetic membranes (Boudiere et al. 2014). In monogalactosyldiacylglycerols (MGDG), a galactose residue is linked to diacylglycerol at position *sn*-3 in β-glycosidic linkage. A second galactosyl unit is bound via an α-glycosidic linkage forming digalactosyldiacylglycerols (DGDG) (Fig. 7). In sulfoquinovosyldiacylglycerol (SQDG), 6-sulfoquinovose is attached in α-glycosidic linkage to diacylglycerol. Sulfoquinovose is a sulfonic acid derivative of glucose and quinovose is 6-deoxyglucose (Fig. 7). Because of the lack of phosphate they have no net charge with the exception of SQDG that is an anion at physiological pH.

Sterols are essential and one of the most abundant com-

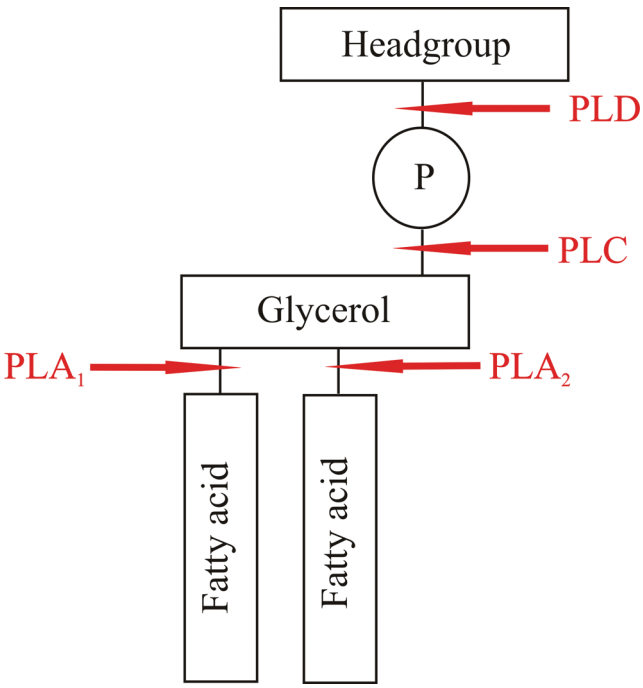

Figure 10. Sites of phospholipid hydrolysis by phospholipases. PLD, phospholipase D; PLC, phospholipase C; PLA, phospholipase A

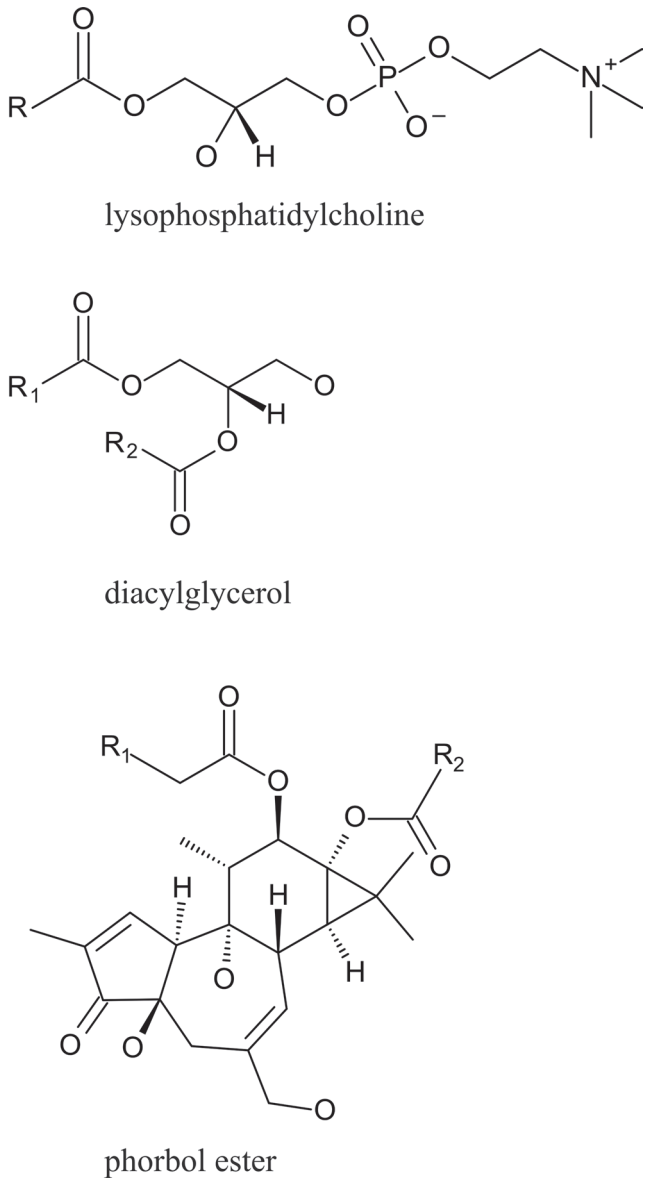

Figure 11. Lipase activity can produce second messenger lipid derivatives from membrane phospholipids. Lysophosphatidylcholine and diacylglycerol are shown as examples. Phorbol esters are diacylglycerol analogue toxic molecules.

ponents of many animal, plant, and fungal membranes (Hannich et al. 2011). The carbon skeleton is the tetracyclic cyclopentanoperhydrophenanthrene (Fig. 8). The rings are fused in the more stable *trans* configuration and two methyl groups (C-18 and C-19) and a branched carbon tail is attached (C-17). There is a double bond between carbons 5 and 6. The hydroxyl group at carbon 3 is the polar part of the molecule, while the rest is a rigid planar structure with a flexible tail and is very hydrophobic.

Cholesterol is a major component of the animal plasma membranes whereas ergosterol predominates in the membranes of lower eukaryotes (Fig. 8).

Plants have a different set of sterols from animals, the phytosterols. Major plant sterols include β-sitosterol, stigmasterol, campesterol and dihydrobrassicasterol (Fig. 8). They are structurally and functionally related to cholesterol (Dufourc 2008).

It is widely accepted that sterols are capable of controlling membrane order and segregation of lipids into rafts by intercalating between phospholipids (glycerophospholipids and sphingolipids) in lipid bilayers (Lingwood and Simons 2010; Xu and London 2000). The small structural differences of sterol molecules may be evolutionary tuned for their specific functions.

In Bacteria sterols are rarely occur but instead they contain the structurally and functionally related sterol surrogates the hopanoids (Saenz et al. 2012). Hopanoids, like sterols, are triterpene isoprenoids. They contain the pentacyclic hopane skeleton that is planar, rigid and hydrophobic. Several different modifications of this skeleton create the high diversity of natural hopanoids (Kannenberg and Poralla 1999). Diploptene, diplopterol and tetrahydroxybacteriohopane (Fig. 9) are quite common.

Active lipids

Major membrane lipids, besides their structural roles, are

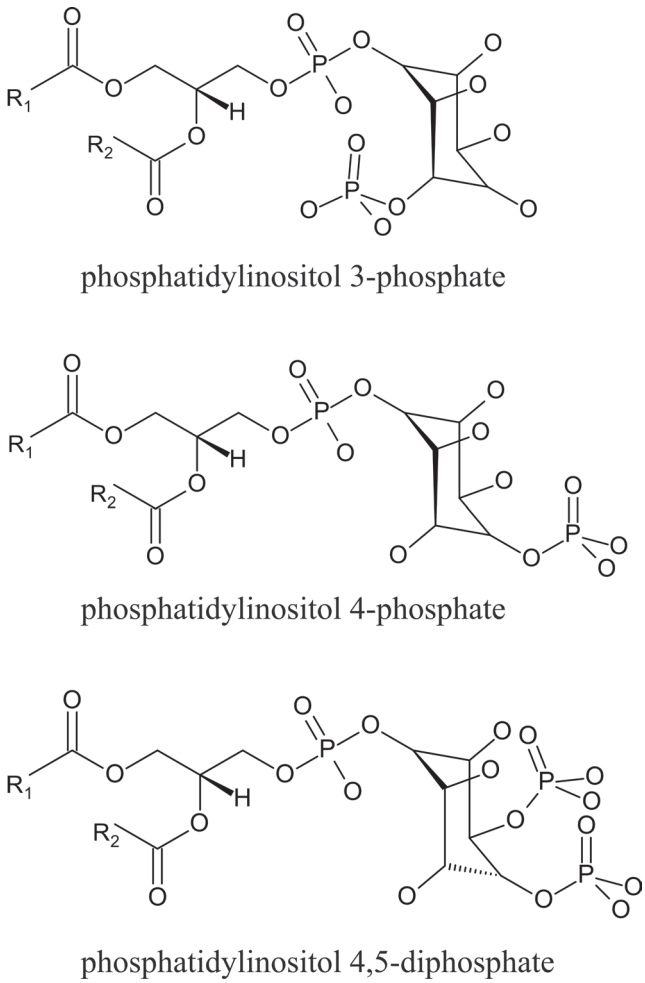

Figure 12. Structures of the most important phosphoinositides.

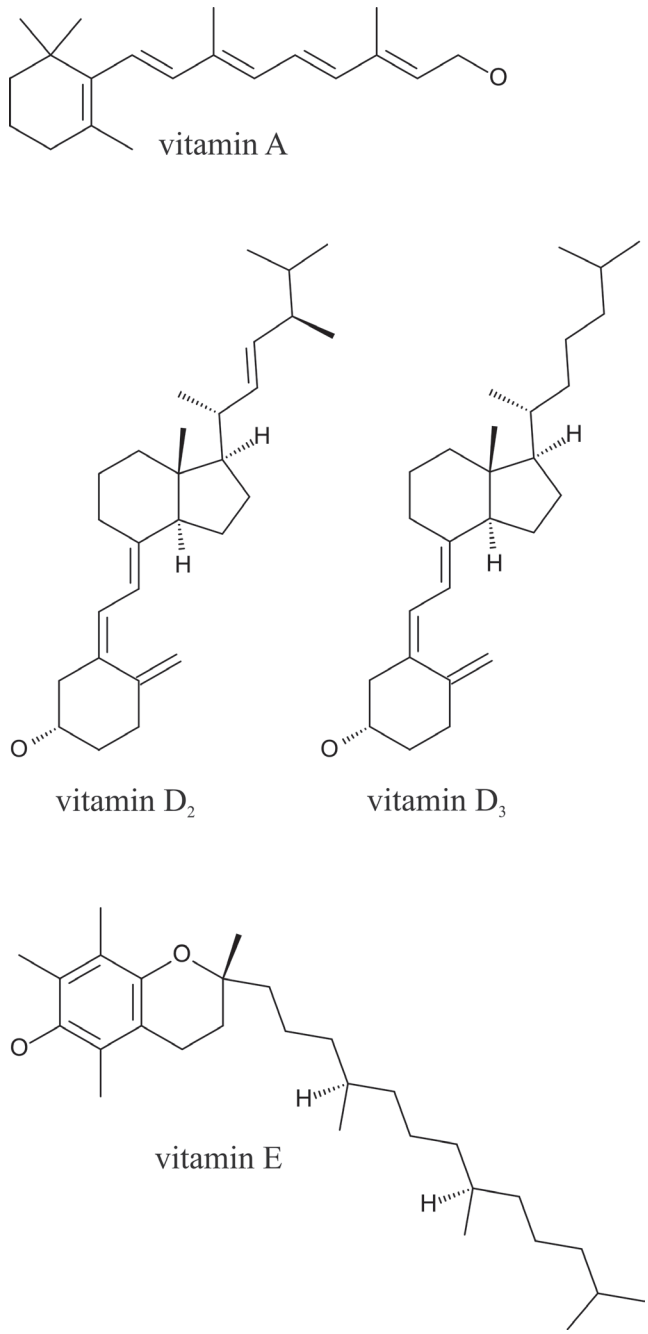

Figure 13. The lipid soluble vitamins.

substrates of lipid modification enzymes, such as lipases and phosphatases. The products of these reactions are present in low quantities however; they have very important signaling functions.

Membrane phospholipids can be hydrolyzed by different phospholipases (PLs) (Fig. 10). Phospholipase A1 and A2 cleave at positions *sn*-1 and *sn*-2, respectively, liberating the second messengers free fatty acids and lyso-phospholipids (Fig. 11) (Murakami et al. 2011). The action of phospholipase D (PLD) on structural phospholipids, such as phosphatidylcholine and phosphatidylethanolamine produces phosphatidic acid. PA has emerged as a key modulator of cellular activity in eukaryotes (*e.g.*, cytoskeletal rearrangements, secretion, endocytosis, and respiratory burst in animals; freezing, wounding, pathogen elicitation, dehydration, salt in plants). Phospholipase C (PLC) removes the phosphoryl headgroup of glycerophospholipids. The primary substrates are phosphatidylinositol and the phosphatidylinositol phosphates. The generated water soluble inositol phosphates diffuse into the cytosol and nucleus and act as cellular messengers. Diacylglycerols (DAGs) (Fig. 11), the other products, induce protein kinase C (PKC) via the increase of the concentration of calcium ions in the cell. PKC has a vital role in the regulation of cell growth and differentiation (Nishizuka 1992). Under normal conditions DAG is quickly hydrolyzed, its activation

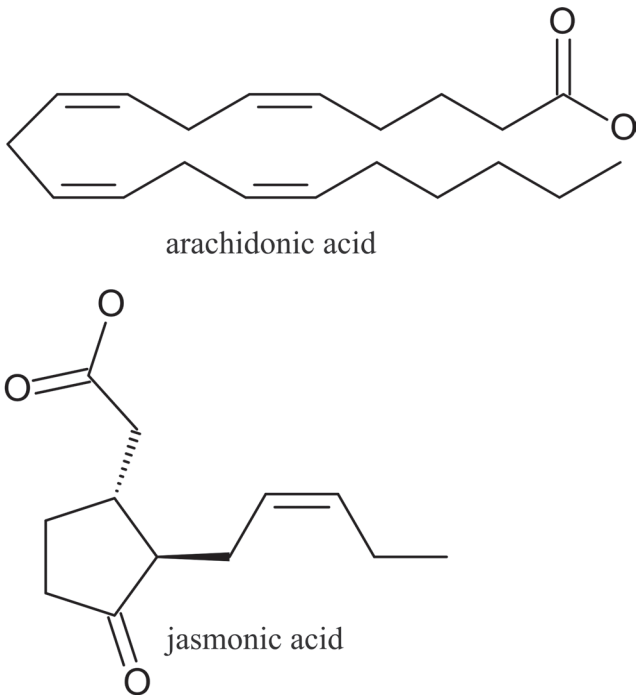

Figure 14. Arachidonic acid and jasmonic acid the initiating substrates in the biosynthesis of very important hormones.

is transient. Phorbol esters are amphiphilic molecules having a polycyclic polar head and two fatty acid residues linked by ester bonds to two neighboring hydroxyl groups (Fig. 11). Phorbol esters can act as DAG analogous but activate PKC stronger and much longer resulting tumor-promoting effect (Nishizuka 1992; Goel et al. 2007).

The membrane structure lipid phosphatidylinositol is the precursor of the phosphatidylinositol phosphates (phosphoinositides). The most important forms are phosphatidylinositol 4-phosphate, phosphatidylinositol 4,5-bisphosphate and phosphatidylinositol 3-phosphate (Fig. 12). Phosphoinositides participate in different signaling events, such as intracellular membrane trafficking, cell proliferation and other cellular process (Vanhaesebroeck et al. 2012).

Vitamins are organic compounds that are essential in the diet. They are divided into two groups, water-soluble and lipid-soluble ones. The latter in humans include vitamin A, D, E and K (Fig. 13).

Arachidonic acid (Fig. 14) is present mainly in phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositol. Phospholipase A_2 hydrolyses the fatty acids from position *sn*-2. Arachidonic acid is required for the biosynthesis of eicosanoids (prostaglandins, thromboxanes and leukotrienes). They have an incredibly wide range of actions including defense against damage and pathogens.

The plant hormones jasmonates have important signaling functions especially in plant stress responses, growth and development in higher plants (Wasternack and Hause 2013). Their biosynthesis starts with the hydrolysis of α-linolenic (Fig. 16) acid from galactolipids by the action of phospholipase A_1. Jasmonic acid (Fig. 14), a 12-carbon cyclic fatty acid is synthesized from linolenic acid via the octadecanoic pathway. Many jasmonic acid derivatives have been found in plants, such as amino acid conjugates, methylated, decarboxylated and glucosylated forms (Delker et al. 2006).

Distribution of lipids

Lipid composition of membranes can vary with different organisms, different tissues in the same organism, different compartments within a cell and even different membrane domains. Prokaryotic cells contain mostly phospholipids. Sphingolipids and sterols are missing from them. In bacterial membranes the zwitterionic phosphatidylethanolamine (PE) dominates (70-80%) and the negatively charged phosphatidylglycerol (PG) and cardiolipin (CL) are essentials. Cyanobacteria and other photosynthetic bacteria contain preferentially glycolipids. The galactose-containing MGDG and DGDG have no charge but SQDG is an anionic lipid. PG is the only phospholipid in the photosynthetic membranes with relatively low concentration and it is essential. Sterols are absent from prokaryotic cells but many bacteria and cyanobacteria produce hopanoids. Hopanoids are pentacyclic triterpenoids, the so called bacterial "sterol surrogates" that were demonstrated to perform a regulatory function in prokaryotic membranes analogous to that of sterols in eukaryotes (Saenz et al. 2012). In Archaea, membranes are built from ether lipids with very long, branched and usually fully saturated alkyl chains. Some of these lipids can span both leaflets of the bilayer while being anchored in both external and internal aqueous surfaces of the membrane. Archaea live under extreme conditions, such as high temperature, very low or high pH and high salt concentration. Their very stable ether-lipids are more resistant to hydrolysis and oxidation than ester-lipids of other organisms.

In Eukaryotes, besides of phospholipids, sphingolipids and sterols are standard lipid constituent of membranes. While phosphatidylcholine (PC) is missing from prokaryotic cells, it is the most abundant and therefore the major structural phospholipid in eukaryotic membranes. PE is the second most prevalent phospholipid in Eukaryotes and it is also an important structural lipid. Phosphatidylserine (PS) and phosphatidylinositol (PI) are less abundant but essential components of the membranes. PS is involved in activating and anchoring many proteins to membranes, such as ATPases, kinases, receptors and signal transducing proteins and is also have important roles in cell-cell recognition and communication. PIs have a central role in cell signaling and regulation. PI is often phosphorylated at position 4 and

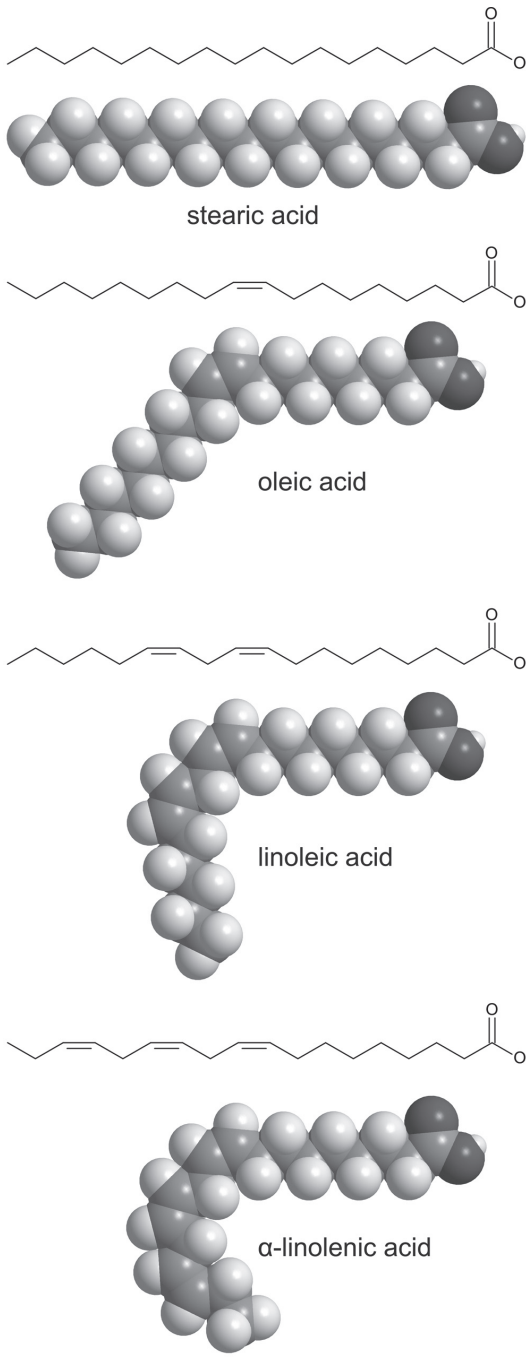

Figure 15. Increasing number of *cis* double bonds in fatty acid tails introduces more pronounced bending in the molecules. Representation of the effect of *cis* double bonds in fatty acid tails to their 3D sructures. Stearic acid, octadecanoic acid; oleic acid, 9Z-octadecenoic acid; linoleic acid, 9Z,12Z-octadecadienoic acid; α-linolenic acid, 9Z,12Z,15Z-octadecatrienoic acid.

5 or 3, the major phosphoinositide is PI 4,5-bisphosphate (PIP_2). PIs are storage forms of the signaling second messenger molecules, membrane-bound DAG and water-soluble inositol phosphates. PG is found in eukaryotic membranes in low amounts and it supports precursors for CL synthesis in mitochondria. 20% of mitochondrial inner membrane lipids are CL, which is closely linked with electron transport and oxidative phosphorylation (Stillwell 2013).

The lipid composition of plant membranes is generally similar to membranes of other eukaryotic cells with exception of photosynthetic membranes in chloroplasts. Chloroplasts, as a result of their cyanobacterial origin, contain a thylakoid membrane system built up mostly from glycolipids (MGDG, DGDG and SQDG) instead of phospholipids.

Eukaryotic cells generally contain various sphingolipids preferentially in the outer leaflet of their plasma membranes. Sphingomyelin, galactosyl ceramides and gangliosides are typical mammalian sphingolipids that have not been found in plant cells. In plants, glucosylceramides and glycosyl inositol phosphorylceramides (GIPCs or phytoglycolipids) are important components of the membranes. Most fungi contain cerebrosides with glucosyl or galactosyl residues and GIPC with mannosyl residues (Sperling and Heinz 2003).

Sterols are major components of eukaryotic membranes. In animals the prevalent sterol is cholesterol (often more than 50% of the total lipids in plasma membranes), whereas in fungi ergosterol is dominant. In plants, more than 250 different phytosterols were identified. Sterols are involved in providing mechanical strength, controlling phase behavior and supporting membrane lateral organization. They are molecular glues that hold lipid rafts together.

Eukaryotic cells contain various membrane-surrounding compartments. Their lipid composition depends on the sites of lipid biosynthesis, lipid trafficking inside the cell and the function of the compartment. Endoplasmic reticulum is the main site of lipid synthesis, from which most of the lipids are trafficked to other organelles and the plasma membrane. Golgi apparatus is a lipid-based sorting station. It specializes in sphingolipid synthesis and export to plasma membrane. Plasma membranes are enriched in sphingolipids and sterols which are packed at high density and resist mechanical stress. Endosomes are originated from plasma membrane but their lipid composition is changing during maturation to late endosomes. Mitochondria have bacterial origin, accordingly their inner membranes are cholesterol-free and 45% of their phospholipids are autonomously synthesized by the organelles. Presence of 20-25% CL in their inner membrane is also unique to eukaryotic membranes. A detailed discussion has published by Gerrit van Meer and coworkers about the lipids of membranes and the spatial organization of their metabolism in mammalian cells and yeast (van Meer et al. 2008).

Fatty acid constituents of lipid molecules

Fatty acids (FAs) are mono-carboxylic acids with usually

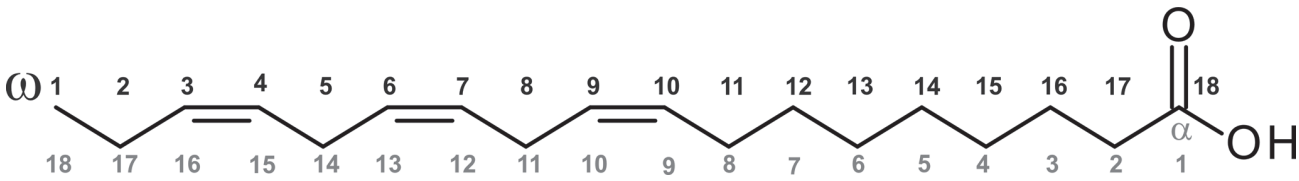

Figure 16. Numbering of carbon atoms in α-linolenic acid.

unbranched aliphatic tails which may be saturated or unsaturated (Fig. 15). They are synthesized from 2-carbon acetyl units; hence even numbers of carbon atoms are typical. Odd numbered FAs have also been detected but they are far less abundant. FAs differ from one another in the lengths of their hydrocarbon tails and the number and position of the double bonds in the chain (Gunstone et al. 2007). Fatty acids with a chain of 2 to 36 carbons occur in nature; the most abundant are 14, 16 and 18 carbons saturated and 16 or 18 carbons monounsaturated fatty acids. All higher organisms contain considerable amount of polyunsaturated fatty acids (PUFAs) with two or more double bonds up to six in their membrane lipids (Aguilar and de Mendoza 2006). Double bonds in fatty acids are almost always in *cis*-configuration and almost never conjugated. A *cis* double bond introduces a kink into the chain because a double bond cannot rotate. More double bonds increase the number of kinks in the hydrophobic chains of membrane phospholipids (Fig. 15). This bending limits the ability of fatty acids to be closely packed. Chain length also affects the melting point; longer hydrocarbon chains can form stronger associations than shorter ones. Summing up, short chain length (Table 1 A), unsaturation (Table 1 C) and *cis* double bond (Table 1 B) enhance the fluidity of fatty acids and of lipids derived from them. Consequently, these factors can regulate the membrane order (Holthuis and Menon 2014).

Nomenclature of fatty acids

The nomenclature of fatty acids can be confusing because several different naming systems are used:
Trivial names: Most common fatty acids have historical names that are often used in the literature *e.g.*, α-linolenic acid (Fig. 16).
Delta (Δ) nomenclature: number of carbon atoms:number of double bonds Δnumber of carbons from the carboxylic acid (alpha) end to the first carbon in the double bonds (often in superscript) c (*cis*) or t (*trans*) *e.g.*, 18:3 ^{9c,12c,15c} or 18:3 Δ9c, Δ12c, Δ15c (Fig. 16).

Omega (ω) nomenclature: number of carbon atoms:number of double bonds ω-number of carbons from the methyl end (ω end) to the first carbon in the double bond closest to the methyl end. Instead of omega, the prefix n is frequently used *e.g.*, 18:3 ω-3 or 18:3 n-3. This means that the first double bond is the third C-C bond from the terminal methyl (ω) end. Double bonds are in *cis* conformation and separated by a single methylene group unless otherwise noted (Fig. 16).

Systematic nomenclature: They are named from the parent hydrocarbon with the same number of carbon atoms + oic (saturated), +enoic (monounsaturated), +dienoic (with two double bonds), +trienoic (with three double bonds). Counting begins from the carboxylic end and double bonds are labeled with cis-/trans- (or E-/Z) notation (IUPAC-IUB CBN 1977) e.g. cis-9,12,15-octadecatrienoate (Fig. 16).

Essential fatty acids

There are two fatty acids that mammals cannot produce: linoleic acid (18:2 ω-6), abbreviated LA and α-linolenic acid (18:3 ω-3) abbreviated ALA (Fig. 15). Humans lack the desaturase enzymes required for their production. Therefore they are essential FAs and must get them from diet (Das 2006). Fortunately, many plant and animal foods contain LA and ALA. They are substrates of metabolic pathways that produce important long-chain polyunsaturated fatty acids the so-called ω-3 and ω-6 FAs such as, eicosapentaenoic acid (EPA, 20:5 ω-3), docosahexaenoic acid (DHA, 22:6 ω-3), gamma-linolenic acid (GLA, 18:3 ω-6) or arachidonic acid (AA, 20:4 ω-6) (Calder 2012).

Modulation of membrane microviscosity

Lipid, fatty acid desaturation can occur both eukaryotic and prokaryotic ways. The first mainly use acyl-CoA as substrate, while the other one can use glycerol backbone and called acyl-lipid desaturase. The first gene encoding acyl-lipid desaturase was identified and sequenced from a cyanobacterial strain in 1990 (Wada et al. 1990). That was the start point for identification of other lipid desaturases and encoding genes of cyanobacteria and later on the base of homologue sequence search for that of higher plants, as well (Mcconn et al. 1994; Napier et al. 1999; Wallis and Browse 2002).

The past few years have witnessed a major upsurge in research towards the goal of modifying the lipid composition of plants. When with known genes together with developing transformation techniques the physiological importance of unsaturated lipids could be studied with well-defined mutant

Table 1. Melting points of some saturated and unsaturated fatty acids.

Chain	Common name (acid)*	Systematic name (acid)	Melting points (°C) (Knothe and Dunn 2009)
A			
8:0	caprylic	octanoic	15.41
10:0	capric	decanoic	30.80
12:0	lauric	dodecanoic	43.29
14:0	myristic	tetradecanoic	53.47
16:0	palmitic	hexadecanoic	62.20
18:0	stearic	octadecanoic	69.29
20:0	arachidic	eicosanoateic	74.76
22:0	behenic	docosanoic	79.54
24:0	lignoceric	tetracosanoic	83.82
B			
16:1 Δ9c	palmitoleic	*cis*-9-hexadecenoic	1.22
16:1 Δ9t	palmitelaidic	*trans*-9-hexadecenoic	32.22
18:1 Δ6c	petroselinic	*cis*-6-octadecenoic	29.11
18:1 Δ6t	petroselaidic	*trans*-6-octadecenoic	52.38
18:1 Δ9c	oleic	*cis*-9-octadecenoic	12.82
18:1 Δ9t	elaidic	*trans*-9-octadecenoic	43.35
18:1 Δ11c	asclepic	*cis*-11-octadecenoic	15.40
18:1 Δ11t	vaccenic	*trans*-11-octadecenoic	43.37
20:1 Δ5c		*cis*-5-eicosenoic	26.6
20:1 Δ11c	gondoic	*cis*-11-eicosenoic	23.37
20:1 Δ11t		*trans*-11-eicosenoic	51.94
22:1 Δ13c	erucic	*cis*-13-docosenoic	32.18
22:1 Δ13t	brassidic	*trans*-13-docosenoic	59.16
24:1 Δ15c	nervonic	*cis*-15-tetracosenoic	42.87
C			
14:1 Δ9c	myristoleic	*cis*-9-tetradecenoic	-3.91
16:1 Δ9c	palmitoleic	*cis*-9-hexadecenoic	1.22
18:1 Δ9 c	oleic	*cis*-9-octadecenoic	12.82
18:2 Δ9c, Δ12c	linoleic	cis,cis-9,12-octadecadienoate	-7.15
18:3 Δ9c, Δ12c, Δ15c	linolenic	cis-9,12,15-octadecatrienoate	-11.58a

http://www.aocs.org/Membership/content.cfm?ItemNumber=1068

(Amiri et al. 2010). Lipid desaturation can manipulate membrane microviscosity. With desaturase deficient mutants a rigidifying effect would be demonstrated, while overexpressing genes encoding various desaturases a fluidizing effect was observed (Murata and Wada 1995). Moreover, the lipid desaturation was demonstrated as one of key factors for protection of photosynthetic apparatus to low-temperature and high-light stresses. Regulation of fatty-acid desaturation is an effective tool for manipulating membrane microviscosity. State of membranes can determine that how a photosynthetic organism can protect itself to low-temperature stresses (Gombos et al. 1994). Transformable cyanobacterial strains as model systems demonstrated that dienoic lipids are crucial parts in the protection. Trienoic fatty acids were found as non-efficient protectants, which are not needed for preserving working photosynthesis even in stress conditions. Earlier it was demonstrated that complete elimination of polyunsaturated lipids resulted in a low-temperature sensitive mutant strain, which was not able to overtake low-temperature photoinhibition. Photoinhibition affects the level of D1 protein. This protein is a key factor in photoinhibitory process. Its level depends on balance between degradation and recovery processes. Degraded products can induce resynthesis via induction of gene expression. Unsaturated lipids can modulate degradation by blocking a C- terminal proteinase, which is responsible for D1 degradation (Gombos et al. 1997). When the membranes do not contain unsaturated lipids the cleavage sides of proteins can be blocked for proteinases, consequently the recovery process is blocked, as well (Gombos et al. 1997).

According to these measurements the authors should conclude that protein degradation and re-synthesis can be the most crucial processes in low- temperature photoinhibition. A cyanobacterial mutant with additionally added extra desaturase was more resistant to low temperature stress than the wild type (Gombos et al. 1994). It was a direct evidence for role of desaturated lipids against low-temperature stresses.

Unsaturated lipids are indispensable components of membranes not only in photosynthetic organisms but also in mammalians, as well. Fatty acid desaturases play important roles in controlling the physical properties of membranes and

in the synthesis of signal molecules such as prostaglandins and pheromones. Lipid desaturases control the fatty acid composition of both plant membranes and plant storage lipids, which account for 30% of the calories in the human diet (Somerville and Browse 1996). While the plants are able to produce polyunsaturated fatty acids, mammalian organisms need to eat plants and use these fatty acids for their diet, which provides the importance of plants in human diet.

Earlier the regulation of acyl-lipid desaturases was thought to strictly depend on the environmental temperature and expression of their genes can be induced by low temperature. Recently however, it was demonstrated that not only the temperature but the light is another important factor, which can modulate lipid desaturase related genes (Kis et al. 1998).

Phase behavior of lipids

One of the most important properties of a lipid bilayer is the relative mobility (fluidity) of the individual lipid molecules and how this mobility changes with temperature. This response is known as the phase behavior of the bilayer. Broadly, at a given temperature a lipid bilayer can exist in either a liquid or a solid phase. The solid phase is commonly referred to as a "gel" phase. All lipids have a characteristic temperature at which they undergo a transition (melt) from the gel to liquid phase. In both phases the lipid molecules are constrained to the two dimensional plane of the membrane, but in liquid phase bilayers the molecules diffuse freely within this plane. Usually the liquid crystalline phase provides a natural medium for biochemical and physiological processes where the requested substrates could move easily to meet enzymes imbedded in membranes. Thus, in a liquid bilayer a given lipid will rapidly exchange locations with its neighbor millions of times a second and will, through the process of a random walk, migrate over long distances.

Membrane structures were modeled and Fluid-Mosaic Model phospholipid was first proposed by S.J. Singer in 1971 as a lipid-protein model and extended to include the fluid character in a publication with G.L. Nicolson in "Science" (Singer and Nicolson 1972). Simons and Vanmeer hypothesized the existence of lipid rafts in 1988 (Simons and Vanmeer 1988). "Rafts" are specialized membrane domains enriched in certain lipids, cholesterol and proteins. They suggested a homeoviscous adaptation as a regulating force in biological membrane related processes.

Photosynthetic membranes of plants and other photosynthetic organisms contain high amounts of galactolipids (MGDG and DGDG) that are indispensable for the efficiency of photosynthetic light reactions. Besides these typical nonpolar lipids there are two anionic lipids in these membranes. One of the anionic lipids is a sulfolipid and the other one is a phospholipid, SQDG and PG, accordingly. DGDG, SQDG and PG are cylindrical, bilayer forming lipids, while MGDG is a conical, inverse hexagonal forming lipid, which is the main constituent of photosynthetic membranes and provides an ideal matrix to accommodate proteins involved in photosynthetic apparatus. These membrane lipids are esterified with enormously high amount unsaturated fatty acids, which provides a functionally necessary background for photosynthetic processes. The anionic lipids play an important role in the binding to, and subsequent movement of charged protein groups in lipid membranes.

Lipid-protein interactions

Membranes are composed primarily from lipids and proteins. Protein content of membranes varies from 20% to 80% according to their biochemical roles. The very low 20% protein is found in myelin sheath that serves as an electrical insulator around nerve fibers, energy generating mitochondrial and thylakoid membranes contain 75-80% proteins, while mammalian plasma membranes approximately 50% proteins. It is obvious that integral proteins must be solvated by lipids in the surrounding bilayer. These lipids are known as annular or boundary lipids and can be distinguished from the bulk lipids of the bilayer. The nature of protein interactions with annular lipids has been studied using electron spin resonance, fluorescence quenching and energy transfer, and molecular dynamics simulations methods. It was concluded that association of annular lipids with integral proteins must be transient and very weak (Stillwell 2013; Luckey 2014).

There is a third class of lipids, which are tightly bound to integral proteins and required for their activity. These lipids are called non-annular or co-factor lipids. One of the well-known examples for co-factor lipid is phosphatidylserin that is an essential co-factor specifically binds to and activates protein kinase C (Johnson et al. 1998). In photosynthetic organisms the anionic lipid phosphatidylglycerol is the only phospholipid in thylakoid membranes. Its concentration is relatively low but it is essential for the cells as it was demonstrated in several studies (Wada and Murata 2007; Domonkos et al. 2008). After removing PG molecules from the cells, the function of photosystem II was significantly disturbed. X-ray crystallography of cyanobacterial PSII confirmed the presence of five PG molecules at functionally important locations inside the complex (Umena et al. 2011).

Dietary lipids and human health

Essential polyunsaturated fatty acids commonly found in human body are: LA (18:2 ω-6), ALA (18:3 ω-3), AA (20:4 ω-6), EPA (20:5 ω-3) and DHA (22:6 ω-3). Since human cells cannot produce LA and ALA, polyunsaturated fatty acids must be primarily obtained from diet.

LA (18:2 ω-6) and ALA (18:3 ω-3) are common components of plants and they have been part of our diet for

thousands of years. Before the agricultural revolution 10,000 years ago, the ratio of consumed ω-6 to ω-3 fatty acids was approximately 1:1, as it was estimated by studying present hunter-gatherer populations. Pre-agricultural humans ate fish, lean meat, and enormous variety of wild green leafy vegetables, fruits, berries, nuts and honey. Today less than 20% of plant species provide 90% of the world's food supply, with the greatest percentage of three cereal grains, wheat, maize and rice. Cereal grains are high in carbohydrates and ω-6 fatty acids, but low in ω-3 fatty acids and antioxidants. In the past 150 years the balance of ω-6 to ω-3 fatty acids has been changed dramatically. Today, in Western cultures this ratio is 10-20:1 instead of 1-4:1 (Simopoulos 1999). Longer-chain polyunsaturated fatty acids can be produced by the transformation of both ω-6 LA and ω-3 ALA, but since both ω-6 and ω-3 PUFA are metabolized by the same desaturation/elongation pathway, a competition exists between these two types of fatty acids (Burdge and Calder 2005). Higher concentration of LA than ALA results in greater conversion of ω-6 PUFA. Why does it cause health risk? Both the ω-6 and ω-3 PUFAs are the parent molecules for the production of eicosanoids. Eicosanoids are bioactive molecules, hormone-like activators of the immune system including leukotrienes, prostaglandins and tromboxans. Eicosanoids derived from ω-6 PUFAs have generally proinflammatory and proaggregatory effects, while those derived from ω-3 PUFAs are predominantly anti-inflammatory and inhibit platelet aggregation (DeFilippis and Sperling 2006). Beside the high ratio of LA to ALA, we ingest considerable amount of ω-6 PUFA arachidonic acid from animal food source. The eicosanoids derived from AA are biologically active in very small quantities and, if they are formed in large amount, they increase the formation of thrombi and atheromas, leads to allergic and inflammatory disorders and to proliferation of cells. Experimental studies provided evidence that many of the chronic conditions, cardiovascular disease, diabetes, cancer, obesity, autoimmune diseases, asthma and depression, are associated with increased production of ω-6 derived eicosanoids (Simopoulos 2002). Epidemiological and experimental studies showed that increased intake of ω-3 PUFAs (EPA and DHA) decreases the risk of chronic diseases. Furthermore, the presence of DHA in the neuronal membranes is critical in the function of the central nervous system. It modulates the activities of several signaling pathways in the brain and is critical for optimal retinal function (Burdge and Calder 2005). Traditionally the primary source of DHA in the human diet is oily, cold-water fish such as mackerel, salmon or tuna. The best plant sources of ALA are linseed and canola oils.

Trans-fatty acids (TFAs) have been closely linked to coronary heart disease and arteriosclerosis, since the 1950s. Numerous epidemiology studies supported this observation and also revealed that TFAs are carcinogenic and affect normal brain function. A common TFA, elaidic acid (18:1Δ9t), is naturally present in the fat, meat and milk of ruminant animals and in the dairy products, therefore it is also found in human membranes. Elaidic acid that has been in the human diet for thousands of years cannot cause the large increase in heart disease observed in the last hundred years. An industrial process was developed at the beginning of 20th century to decrease susceptibility of plant oils to oxidation and open the way to their application in food processing. Partial hydrogenation of polyunsaturated plant lipids produces various TFAs, the results of *cis* double bond reduction and migration up and down the chain and partial conversion of *cis* to deleterious *trans* double bonds. Partial hydrogenation results in a wide range of geometric and positional TFA isomers, which ingesting in the diet can incorporate into membrane phospholipids thus altering the structure and function of the membranes (Stillwell 2013). For example, if dietary TFAs replace natural DHAs in the brain phospholipids, it is likely that electrical properties of neurons and hence brain function will be affected. In recent years, the increase of heart disease has resulted in a demand on elimination of *trans*-fatty acids from products of the food industry.

Carotenoids

Synthesis and chemical structure of carotenoids

Carotenoids (CARs) are a large group of natural pigment molecules that perform important physiological functions in all kingdoms of living organisms. They provide fruits, flowers, birds and insects with beautiful variations of the warm colors of yellow, orange and red.

CARs are isoprenoid pigments and synthesized by all photosynthetic organisms, aphids, some bacteria and fungi. Therefore animals must get them in diet. CARs are composed of eight condensed C5 isoprene precursors that generate a C40 linear backbone (Fig. 17) from which all carotenoid species are derived. The parent skeleton is desaturated, and then become the subject of various modifications at the ends: cyclization and addition of oxygen-containing functional groups. CARs can be divided into the class of hydrocarbons, the carotenes and their oxygenated derivatives, the xanthophylls. Carotenes are very hydrophobic while the oxygenated ends in xanthophylls enhance their water solubility.

The most characteristic features of CARs are the core conjugated double bond system which allows electrons in the double bonds to easily delocalize. This electron-rich polyene structure is responsible for the antioxidant capability of the carotenoids (Britton 1995 ; Carocho and Ferreira 2013). The configurations of the double bonds in carotenoids are generally the energetically favorable *trans* form. The molecules have

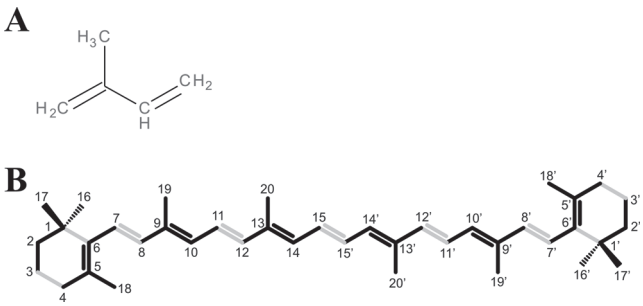

Figure 17. Isoprenoids, including carotenoids, are derived from isoprene, 2-methyl-1,3-butadiene (A). β-Carotene is composed of eight five-carbon isoprenoid units (B).

a linear, rigid shape. However, if one or more double bonds have *cis* configurations the molecules shape are no linear. The decreased possibility of associations of the molecules results better solubility and transportability. The sizes, compositions and shapes of the end groups are also important factors to define the conformations and functionality of carotenoids (Britton 1995; Landrum et al. 2010).

Being terpenoids CARs are synthesized by successive condensations of the two active isoprene forms: isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP). These basic C5-terpenoid precursors are synthesized via the so-called methylerythritol phosphate (MEP) pathway (Lichtenthaler 1999; Kuzuyama and Seto 2012) that, in eukaryotic phototrophs, localized in the chloroplasts. The starting substrates are pyruvate and D-glyceraldehyde 3-phosphate. The polymerization of the isoprenoid units generate geranylgeranyl diphosphate (GGPP, C_{20}) (Bouvier et al. 2005).

The head-to-head condensation of two molecules of GGPP, the committed step in the CAR biosynthetic pathway, forms 15-*cis* phytoene, the first C_{40} carotene (Fig. 18) (Misawa et al. 1994). The generation of the conjugated polyene core of CARs requires four sequential desaturation steps and isomerisations producing all-*trans*-lycopene (Sandmann 2009; Masamoto et al. 2001) (Fig. 18). As the number of the conjugated double bonds in the hydrocarbon backbone increases the whole absorption spectrum shifts to longer wavelengths. This determines the color of CARs. Phytoene with three conjugated double bonds absorbs ultraviolet light, while lycopene that contains eleven conjugated double bonds has red color.

Carotenoid biosynthesis bifurcates after lycopene. The introductions of terminal ε- and β-ionone end groups by the cyclization of isoprene groups of lycopene generates β,ε-(α-carotene) and β,β-carotenes (β-carotene) (Pecker et al. 1996) (Fig. 19). They are the typical dicyclic CARs in land

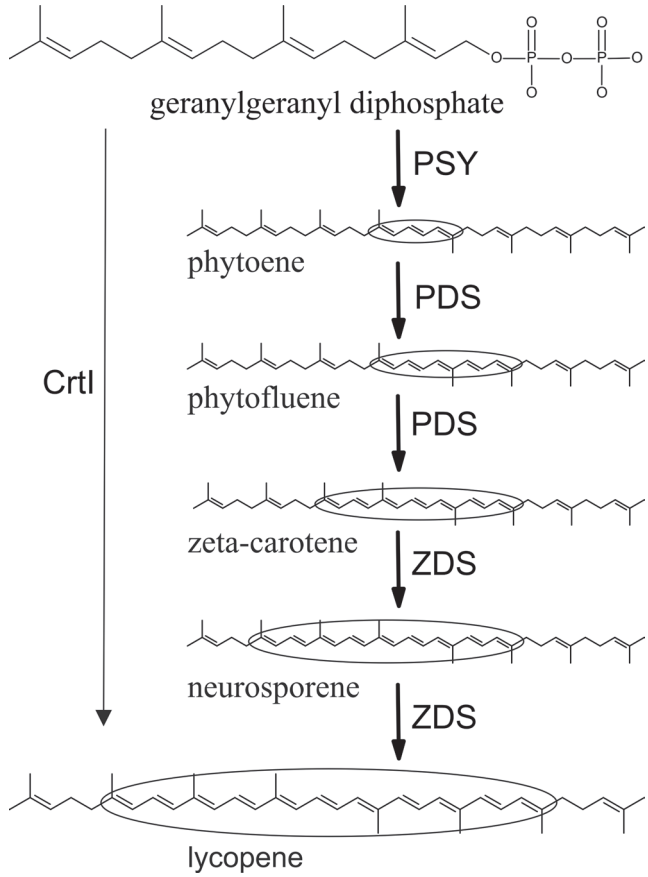

Figure 18. The biosynthesis of all-trans-lycopene from geranylgeranyl diphosphate. In non-photosynthetic bacteria and fungi a single enzyme, CrtI, carries out the whole series of desaturation reactions. However, in the plant pathway a series of four desaturation reactions are carried out by two related enzymes, phytoene desaturase and ζ-carotene desaturase. PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, ζ-carotene desaturase; CrtI, bacterial type phytoene desaturase.

plants. Algae and cyanobacteria contain mainly β-carotene and its derivatives, but a few phyla of algae and some of the cyanobacterial species also synthesize α-carotene (Takaichi and Mochimaru 2007).

The various end group modifications of carotenes produces a large variety of CARs with different physical properties. Hydroxylation of each ring on α- and β-carotene produces lutein or zeaxanthin, respectively (Kim and DellaPenna 2006). Epoxidation of zeaxanthin, forms antheraxanthin then violaxanthin. The sequential removal of the two epoxy groups can regenerate zeaxanthin and forms the xanthophyll cycle. (Goss and Jakob 2010) (Fig. 19). Violaxanthin can be further converted to neoxanthin.

In cyanobacteria the typical CARs are β-carotene, its hydroxylated and ketolated xanthophyll derivatives, and the

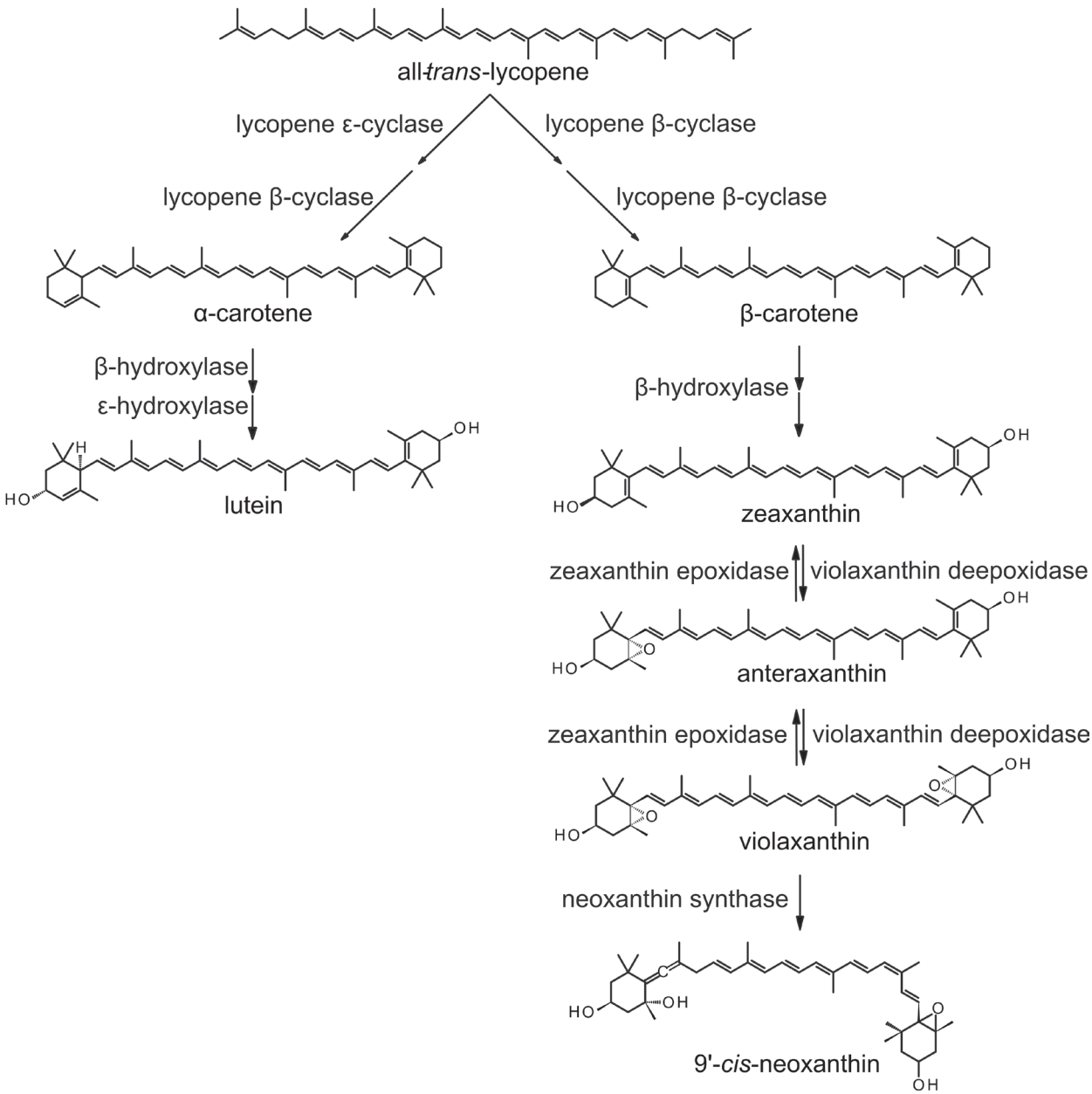

Figure 19. The typical carotenoid biosynthetic pathway of higher plants.

CAR glycosides (Fig. 20) (Takaichi and Mochimaru 2007).

Role of carotenoids as biological antioxidants

Biological antioxidants are compounds that protect living organisms against the potentially harmful effects of processes or reactions that can cause destructive oxidation, ones that lead to a complete collapse of photosynthesis. Several mechanisms have been suggested for explaining the protective function of CARs in biological systems. These include the deactivation of free radicals, including reactive singlet oxygen and peroxyl or alkoxyl radicals, which can be generated within cells and might initiate harmful oxidative reactions. Oxidized lipid derivatives were shown to be seriously destructive in homogeneous solutions, liposomes, isolated membranes, and intact cells. The antioxidant activity of CARs originates from their chemical structure and it has been reported to be especially effective at low oxygenic environment. The antioxidant

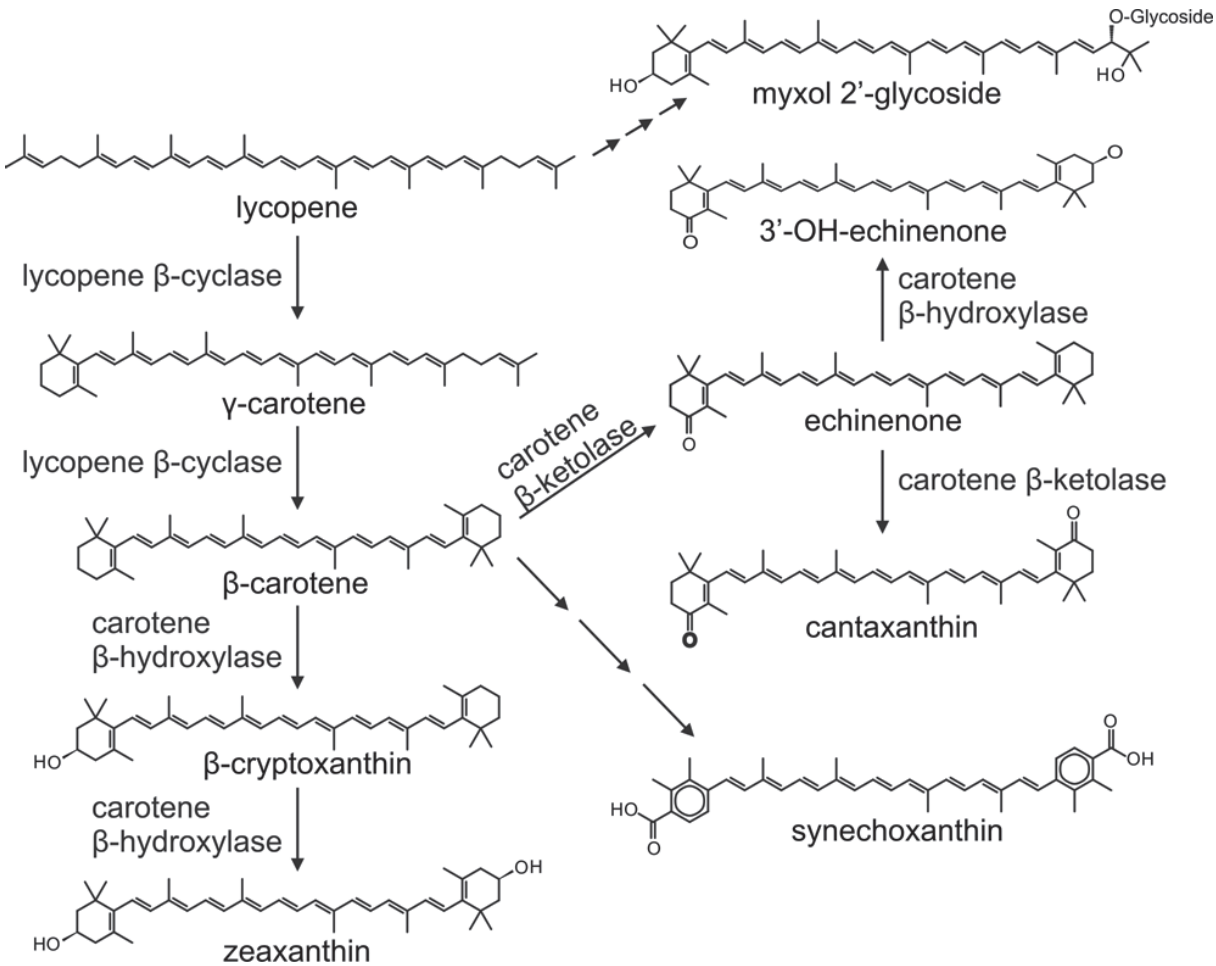

Figure 20. The typical main carotenoids and their biosynthetic pathways in cyanobacteria.

properties of these molecules and their structural basis has been reviewed by Jomova and Valko (2013) and Palozza and Krinsky (1992).

CARs are a class of abundant pigments in nature, which provide the familiar orange-red to yellow color of fruits, vegetables, and flowers. Ample scientific evidence indicates that CARs scavenge and deactivate free radicals, and they may act as antioxidants both in food systems (*in vitro*) and in the human organism (*in vivo*). Overall, medical data link higher CAR intake and tissue concentration to reduced cancer and cardiovascular disease risk. However, various experiments have also demonstrated that CARs, despite their antioxidant capacity, can obtain prooxidant character depending on the biological environment. A summary of the antioxidant potential of natural CARs, both in oil model systems and *in vivo*, is presented in the review of Palozza and Krinsky (1992). CAR pigments are widely distributed in nature, where they play an important role in protecting cells and organisms against photosensitized oxidation. They have indispensible role as biological antioxidants in radical-initiated processes, both *in vitro* and *in vivo*.

Reactive oxygen species (ROS) can attack not only membrane components, such as pigments, proteins and lipids, but they can also damage DNA. CARs and sterols, as antioxidants, are capable of protecting these important compounds (Di Mascio et al. 1991).

Structural and functional roles of carotenoids in photosynthetic organisms

CARs are essential for the survival of photosynthetic organisms under normal and stress conditions. They function as light-harvesting molecules and have antioxidant characters providing photoprotection, which can protect photosynthetic organisms from various combinations of light and temperature stress. The behavior of CARs in the reaction centers

and light-harvesting complexes of photosynthetic bacteria is similar to those observed in experiments using isolated CARs as *in vitro* model systems.

In *Synechocystis* sp. PCC 6803, one of the best-studied cyanobacterial strain, the major CARs are β-carotene and its hydrophilic xanthophyll derivatives containing oxygen in addition carbon and hydrogen, such as the hydroxy-derivative zeaxanthin, keto-derivatives echinenone and 3'-hydroxyechinenone, and the carotenoid glycoside myxoxanthophyll. Recent X-ray structural analyses revealed β-carotene as the only CAR present in the photosynthetic complexes of cyanobacteria. Hydrophobic β-carotene can resist detergent treatment, which is used during protein crystallization, which is required for the X-ray crystallographic analyses employed to determine the detailed structure of photosynthetic reaction centers. Usually this detergent treatment can remove more hydrophilic compounds from the reaction centers, that is why CARs remain detectable in the vicinity of the reaction centers. Besides β-carotene, all other CARs of cyanobacteria are found in the thylakoid membrane, cytoplasmic membrane and the cytosol. The level of CARs is increased under various stress conditions. The substitutions of a CAR molecule determine its protective properties against photooxidative stress. In general, hydroxy-carotenoids can inactivate peroxy radicals, whereas keto-derivatives are more efficient in quenching of reactive singlet oxygen and have the best stability against peroxy radicals and photooxidative processes. X-ray crystallography revealed twelve CARs in monomeric PSII complex of *Thermosynechococcus elongatus* (Guskov et al. 2009). PSII is the most vulnerable photosynthetic complex to photoinhibition. Earlier studies suggested that β-carotene is indispensable for the accumulation of the D1 protein of PSII in the green alga *Chlamydomonas reinhardtii* and the cyanobacterium *Synechocystis* PCC 6803, demonstrating an evolutionarily conserved protective role of β-carotene. Therefore, the availability of CARs is a major factor in the assembly of functional PSII (Sozer et al. 2010). In the structure of monomeric PSI of *T. elongatus* 22 CARs have been identified *(*Jordan et al. 2001*)*. PSI is generally less sensitive to light stress than PSII, and its CAR-based photoprotection is not indispensable. PSI is enzymatically protected against oxidative stress. The enzymes, such as superoxide dismutase, catalase and peroxidase are localized to the vicinity of PSI reaction centers.

CARs are prevalent in the outer membrane of the cell, and are also present in thylakoids. The localization of CARs in intact cells have been determined by analyzing Raman signals and very weak fluorescence (Vermaas et al. 2008).

Functional roles of carotenoids in animals

More than 700 CARs have been described in nature, many of them are found in animals. Red and yellow colors of birds, insects and marine invertebrates are due to the presence of CARs. Animals are not able to synthesize CARs therefore they have to get them in diet and modify them through metabolic processes. In human diet there are about 40 CARs but only 24 was found in human blood and tissues (Khachik et al. 1995). Among of them only 6 CARs dominate in the human plasma: lycopene, β-carotene, α-carotene, β-cryptoxanthin, zeaxanthin and lutein (Rao and Rao 2007).

Most well-established role of CARs in animal organisms is their provitamin A effect. In vertebrates, retinal form of vitamin A is indispensable for vision, and retinoic acid form for gene regulation of growth, development and immune response. Only 10% of CARs show provitamin A activity. Vitamin A precursors contain at least one β-ionone ring without oxygenated functional groups. β-carotene is the most important precursor but α-carotene and β-cryptoxanthin are also eligible. The first step of metabolic processes for conversion of β-carotene into vitamin A is an oxidation process with central cleavage of the carotene resulting two all-*trans*-retinal molecules. Herbivores and omnivores (such as humans) metabolize the absorbed provitamins in the intestinal epithelium cells and the resulted retinyl esters packed into chylomicrons and secreted into lymph. The most of the circulating retinyl esters get to the liver where they can be stored or secreted to the blood in protein-bound retinol form. After the all-*trans*-retinol absorbed by the eyes, it must be converted to 11-*cis*-retinal, the active chromophore of the light-absorbing protein rhodopsin, which is essential for both low-light and color vision (von Lintig 2012). Blindness of children caused by an inadequate vitamin A supply is a major health problem in developing countries. In human diet the sources of CARs with provitamin A activity are vegetables and fruits. The best sources of β-carotene are carrots, red bell peppers, pumpkins, broccoli and green vegetables, while α-carotene is found in carrots and pumpkins. β-cryptoxanthin is one of the major pigments in red peppers and some tropical fruits, like papaya (Fernandez-Garcia et al. 2012). The carotenoid-cleaving oxygenase enzymes are missing from carnivores and they cannot convert any CARs to retinal, therefore they support all their vitamin A need from animal food. Humans also can absorb preformed vitamin A from animal food sources (mostly from liver and egg yolk).

Lycopene is the most dominant CAR in the human blood. It is transferred in LDL and VLDL fraction of the serum (Stahl and Sies 1996). Lycopene is an open chain hydrocarbon without β-ionone rings; therefore it has no provitamin A activity. Probably, its antioxidant property is the main mechanism of its beneficial effects. Lycopene was found the most effective CAR in the scavenging of reactive oxygen species such as singlet oxygen and peroxyl radicals. ROS are generated endogenously in the organisms through normal metabolic functions and react with lipids, proteins and DNA resulted in their oxidative damage. Chronic degenerative disorders

such as cancer, cardiovascular diseases, stroke, immunosenescence, age-related degeneration or osteoporosis thought to be associated with oxidative damage. *In vitro* tests and experiments on animal models showed that principally lycopene and in lesser extent also some other CARs such as α-carotene, lutein and β-cryptoxanthin can decrease the risk of mammary gland, liver, lung, colon and skin cancers (Nishino et al. 2009). The best demonstrated effect of lycopene was the prevention of prostate cancer (Giovannucci 1999). Human epidemiological studies also proved the effectiveness of high lycopene intake in the prevention of several chronic diseases (Tapiero et al. 2004).

Oxidation of circulating LDL that carries cholesterol is thought to play a key role in the initiation of atherosclerosis (Witztum 1994). Since lycopene is transported with LDL in the blood, it seems obvious that it can protect LDL from oxidation. It was shown that lycopene can reduce the level of oxidized LDL and also the serum cholesterol level therefore it can decrease the risk of cardiovascular disease (Fuhrman et al. 1997; Agarwal and Rao 1998). The antioxidant capacity of blood plasma is due to the total concentration of antioxidant compounds such as CARs, vitamin C, vitamin E and their synergism. Studies on the antioxidant status of blood in rats showed that also antioxidant enzymes such as superoxide dismutase, glutathione reductase and glutathione peroxidase can be induced by lycopene (Breinholt et al. 2000).

Beside their antioxidant properties, CARs can affect via other mechanisms that include gap junctional communication, gene regulation and immune or hormonal regulation (Tapiero et al. 2004; Rao and Rao 2007). Gap junctions are cell-to-cell channels that allow ions and signaling molecules to pass between cells. Most tumor cells have dysfunctional gap junction communication. Cell culture experiments revealed that some cleavage products of CARs activate gap junctional communications. Lycopene also enhanced the expression of connexin 43 gene encoding the main gap junction protein (Krutovskikh et al. 1997). *In vivo* metabolism of lycopene is hardly known, only some cleavage products and oxidation products were identified but it seems that combinational administration of multiple CARs and antioxidant vitamins could be useful in the prevention of cancer and chronic diseases.

Two CARs, lutein and zeaxanthin are found in high concentration in the macula lutea of the eyes. Due to their antioxidant properties these pigments protect the retina against photooxidative damages. Age-related macular degeneration is the major cause for irreversible blindness among the elderly people in the world. Studies show that increased intake of macular pigments lutein and zeaxanthin together with ω-3 fatty acids resulted in decreased risk of age-related macular degeneration (Lim et al. 2012).

Vegetables and fruits are rich sources of carotenoids (Fraser and Bramley 2004) but the bioavailability of these pigments not only depends on their amount in the food. CARs are lipophilic compounds, they must to be solubilized from the food matrix and incorporated into micelles making them accessible for intestinal epithelium cells. Food processing such as mechanical homogenization, heat treatment and addition of vegetable oils increases the efficiency of digestion and absorption. Mediterranean diet seems very effective in CAR intake due to its high content of fresh and processed fruits, vegetables and olive oil (Fernandez-Garcia et al. 2012). Tomato and tomato products (juice, ketchup, sauce, and soup) are the most important sources of lycopene. Beside of tomato, only watermelon, pink grapefruit and guava contain considerable amount of lycopene.

Since vitamin A deficiency causes serious health problems in the developing countries and epidemiological evidences indicate that high lycopene intake reduces the risk of chronic diseases, various strategies were developed for enhancing CAR levels in crop plants (Fraser and Bramley 2004). Conventional plant breeding has resulted in tomato and pepper varieties with different CAR profiles. Genetic engineering of CAR biosynthesis in crop plants is a faster and promising method for increasing the CAR levels in food. The humanitarian project for 'Golden Rice' received the highest publicity among these projects. In Asia, vitamin A deficiency is associated with the predominant consumption of rice that lacks provitamin A in the grain. Golden Rice is the name of the genetically modified rice that produces CARs, preferentially β-carotene in the endosperm of the grain, giving a characteristic yellow color for it (Paine et al. 2005). Researchers make an effort to produce genetically modified crop plants with fortified CAR content in spite of the lack of consumer acceptance of genetically modified foods, at present.

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