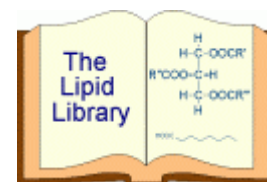


OTHER MEMBRANE-ASSOCIATED ISOPRENOIDS

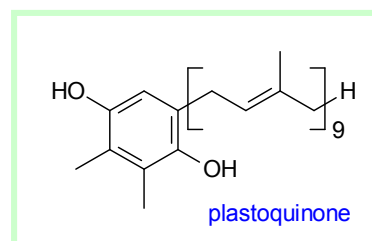


STRUCTURES, BIOCHEMISTRY AND FUNCTION

By some definitions, all isoprenoids from simple monoterpenes such as geraniol to complex polymers such as natural rubber should be classified as 'lipids'. As discussed in my web page – "[What is a lipid?](#)", I believe this goes too far. Here, only those isoprenoids that have a **functional** role in cellular membranes are discussed, including many of the fat-soluble vitamins. **Tocopherols and tocotrienols** are described in a separate document.

1. Plastoquinone

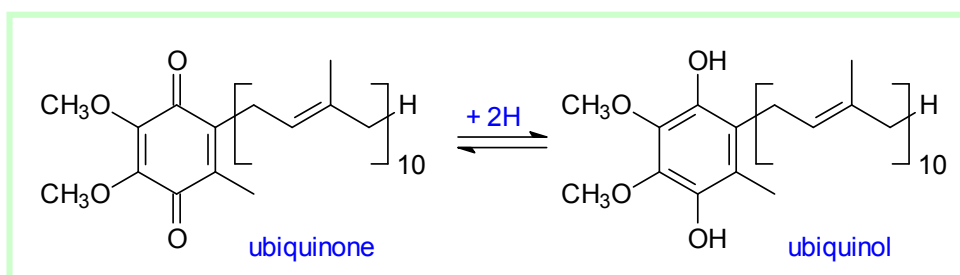
A molecule that is related to the tocopherols, **plastoquinone**, is found in plant chloroplasts and is produced by analogous biosynthetic pathways to those of the tocopherols. It is also related structurally to the isoprenoid alcohol solanesol. The molecule is sometimes designated – 'plastoquinone-n' (or PQ-n), where 'n' is the number of isoprene units, which can vary from 6 to 9.



Plastoquinone has a key role in photosynthesis, by providing an electronic connection between the two photosystems, generating an electrochemical proton gradient across the membrane. This subsequently provides energy for the synthesis of adenosine triphosphate (ATP). The reduced dihydroplastoquinone (plastoquinol) that results transfers further electrons to the photosynthesis enzymes, before being re-oxidised by a specific cytochrome complex. X-Ray crystallography studies of photosystem II from cyanobacteria show two molecules of plastoquinone forming two membrane-spanning branches.

2. Ubiquinone (Coenzyme Q)

Plastoquinone has obvious biosynthetic and functional relationships to the **ubiquinones**, which are also known as coenzyme Q or mitoquinones. These have a 2,3-dimethoxy-5-methylbenzoquinone nucleus and a side chain of six to ten isoprenoid units; the human form illustrated has ten units ('coenzyme Q10'), while that of the rat has nine. Similarly in plants, ubiquinones tend to have nine or ten isoprenoid units.



They are synthesised *de novo* in animal, plant and bacterial tissues, by a complex sequence of reactions with *p*-hydroxybenzoic acid as a primary precursor that is condensed with the polyprenyl

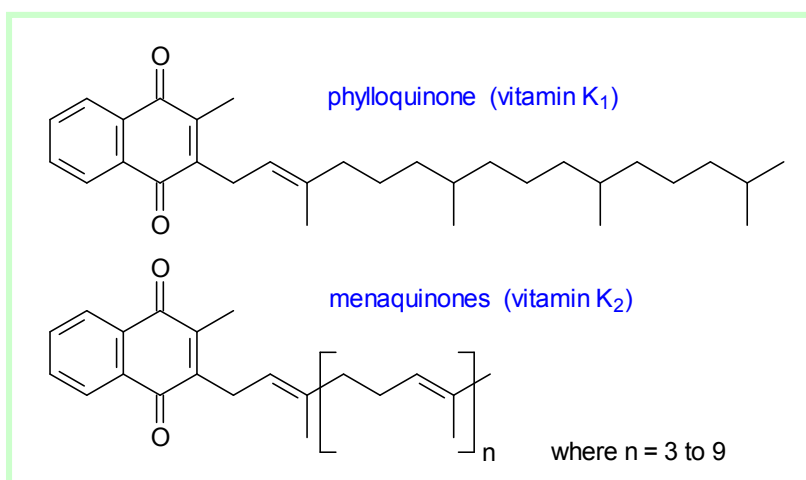
unit via a specific transferase; this is followed by decarboxylation, hydroxylation and methylation steps, depending on the specific organism. Forms with a second chromanol ring, resembling the structures of tocopherols, are also produced (ubichromanols), but not in animal tissues.

Because of their hydrophobic properties, ubiquinones are located entirely in membrane bilayers. They are essential components of the electron transport system in mitochondria, taking part in the oxidation of succinate or NADH via the cytochrome system, reactions that are coupled to ATP synthesis. In this process, coenzyme Q transfers electrons from the primary substrates to the oxidase system while simultaneously transferring protons to the outside of the mitochondrial membrane, resulting in a proton gradient across the membrane. It is reduced to ubiquinol as a consequence. Mitochondrial coenzyme Q is also implicated in the production of reactive oxygen species by a mechanism involving the formation of superoxide from ubisemiquinone radicals, and in this way is responsible for causing some of the oxidative damage behind many degenerative diseases. In this action, it is a pro-oxidant.

In complete contrast, in its reduced form (ubiquinol), it acts as an endogenous antioxidant, inhibiting lipid peroxidation in biological membranes and serum low-density lipoproteins. It may also protect mitochondrial membrane proteins and DNA against oxidative damage. Although it only has about one tenth of the antioxidant activity of vitamin E (α -tocopherol), it is able to stimulate the effects of the latter by regenerating it from its oxidized form. However, ubiquinones and tocopherols appear to exhibit both cooperative and competitive effects under different conditions. There are also suggestions that coenzyme Q may be involved in redox control of cell signalling and gene expression. Dietary ubiquinone, i.e. that in food or dietary supplements, leads to elevated levels in blood, enhancing protection against lipid peroxidation with apparent beneficial effects to health.

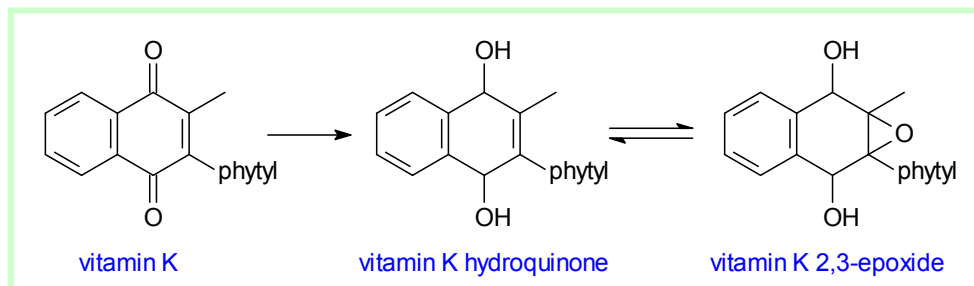
3. Phylloquinone (Vitamin K)

Phylloquinone or 2-methyl-3-phytyl-1,4-naphthoquinone is synthesised in the chloroplasts of plants where it is a key component of the photosystem I complex and serves as an electron acceptor. In an obvious parallel to the plastoquinones (above), two molecules of phylloquinone form two membrane-spanning branches, as demonstrated by X-ray crystallography studies of photosystem I from cyanobacteria. Both phylloquinone cofactors pass on electrons to an iron-sulfur centre in the complex. The menaquinones are related bacterial products with a variable number (4 to 10) of unsaturated isoprenoid units in the tail, sometimes designated 'MK-4' to 'MK-10'.



Phylloquinone is an essential component of the diet of animals and has been termed 'vitamin K₁', and it must be supplied by green plant tissues or seed oils. In animal tissues, the only known function of vitamin K is to act as a cofactor specific to the vitamin K-dependent enzyme γ -glutamyl

carboxylase and the formation of γ -carboxyglutamic acid, which is involved in the activation of the prothrombin and other proteins involved in blood clotting. Vitamin K must first be converted to the reduced form, vitamin K hydroquinone, which is the actual cofactor for the enzyme, and during the reaction this is converted to vitamin K 2,3-epoxide. A further enzyme system regenerates the hydroquinone form by reduction of the epoxide so that the former can be re-utilized many times. Warfarin, the rodenticide, prevents blood clotting by interfering with vitamin K metabolism.



The menaquinones also have vitamin K activity and are termed ‘vitamin K₂’, while a synthetic saturated form of this, which is used in animal feeds, is known as ‘vitamin K₃’. A deficiency in vitamin K results in inhibition of blood clotting and can lead to haemorrhaging, though this has not been defined in humans, presumably because intestinal bacteria produce sufficient for our needs.

4. Retinol (Vitamin A)

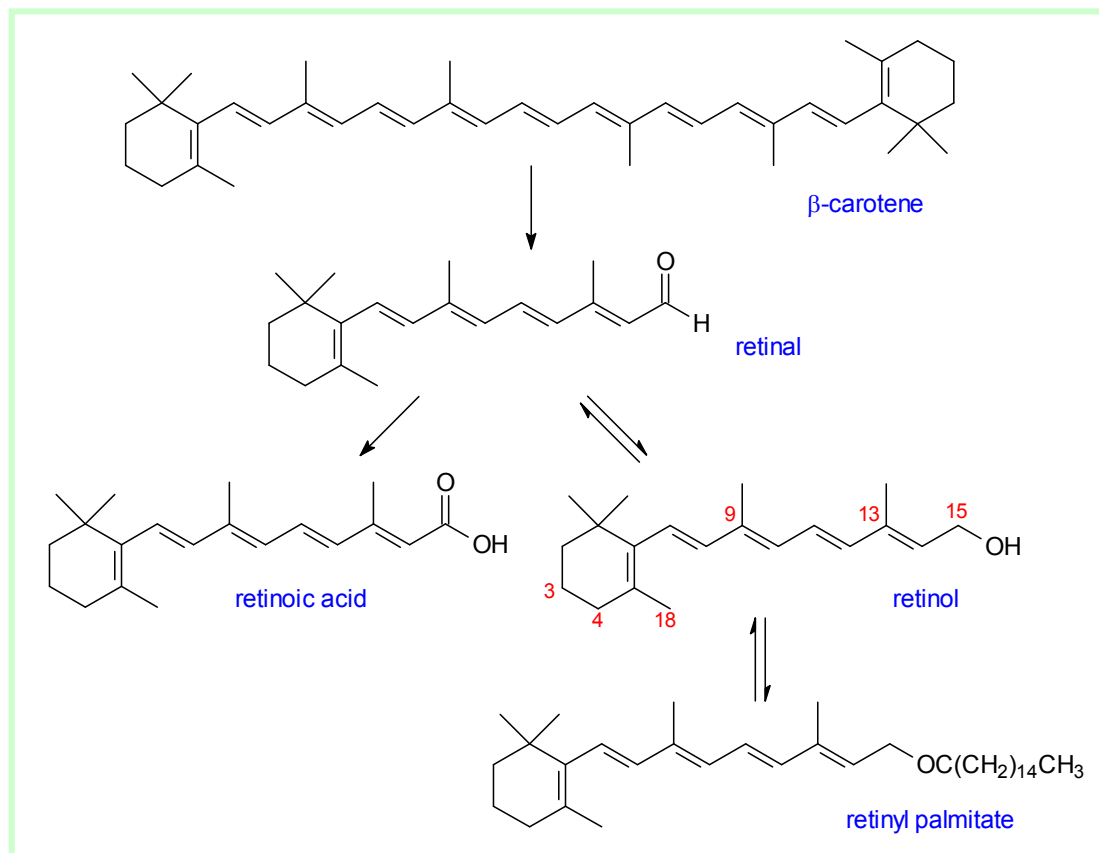
The term ‘vitamin A’ is used to denote **retinol** or all-*trans*-retinol and a family of biologically active retinoids derived from this. These are only found in animal tissues, where they are essential to innumerable biochemical processes. However, the biosynthetic precursors of these compounds are plant carotenoids (provitamin A) of which β -carotene is most efficient, and occurs in the green parts of plants and seed oils. In the human diet, plant sources tend to be less important than those from dairy products, meat, fish oils and margarine. In the U.K., for example, all margarine must be supplemented with the same level of vitamin A (synthetic retinol or β -carotene) as is found in butter.

Retinol is the main form of the vitamin that is transported in blood, bound mainly to retinol-binding protein (with some directly from the diet in the chylomicrons and their remnants), from which it can be taken up from tissues by means of a specific receptor. Retinol esters (principally retinyl palmitate) are the main storage form, occurring chiefly but not exclusively in the liver, with over 90% in the form of lipid droplets in hepatic stellate cells. In addition, specialized cells in the eye store retinoids in the form of lipid droplets. A relatively small proportion of the cellular retinoids is located in membranes.

The biosynthesis of carotenoids has much in common with that of cholesterol (see the appropriate web page), but this is too specialized a topic for discussion here. In animals, dietary β -carotene is subjected to oxidative cleavage at its centre to yield two molecules of all-*trans*-retinal, which is reversibly reduced to retinol and then esterified to form retinyl palmitate by transfer of fatty acids from the position *sn*-1 of phosphatidylcholine via the action of a lecithin:retinol acyltransferase. Activation of the retinol pathway, involves first mobilization of the ester, followed by hydrolysis and reversible oxidation of retinol to retinal. The last is then oxidized irreversibly to retinoic acid. Both retinol and retinoic acid are precursors of a number of metabolites (retinoids), which are required for specific purposes in tissues, by various desaturation, hydroxylation and oxidation reactions.

It has long been known that retinoids are essential for vision, and there is a good appreciation of how this works at the molecular level (see below). It is now realized that they also have essential roles in growth and development, reproduction and resistance to infection. They are particularly important for the function of epithelial cells in the digestive tract, lungs, nervous system, immune

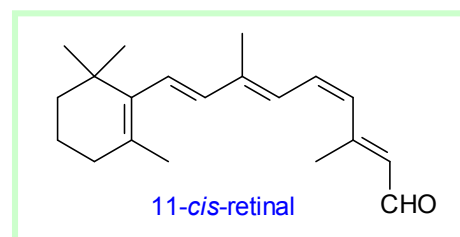
system, skin and bone at all stages of life. For example, they are required for the regeneration of damaged tissues. They appear to have some potential as chemo-preventive agents for cancer and for the treatment of skin diseases such as acne.



More recently, it has been recognized that many of the retinol metabolites function as ligands to activate specific transcription factors for particular receptors in the nucleus of the cell, and thus they control the expression of a large number of genes, including those essential to the maintenance of normal cell proliferation and differentiation, for a healthy immune system, and for male and female reproduction. **Retinoic acid** is especially important in this context. It has also become evident that many of the functions of retinoids are mediated via the action of specific binding proteins, which control their metabolism *in vivo* by reducing the effective or free retinoid concentrations, by protecting them from unwanted chemical attack, and by presenting them to enzyme systems in an appropriate conformation. For example, a specific retinol-binding protein secreted by adipose tissue (RBP4) is involved in the development of insulin resistance and type 2 diabetes, possibly by affecting glucose utilization by muscle tissue.

Vitamin A deficiency in children and adult patients is usually accompanied by impairment of the immune system, leading to a greater susceptibility to infection and an increased mortality rate. Thus it is not always easy to distinguish between these effects and primary defects of retinoid signalling.

However, one of the primary effects of vitamin A deficiency in malnourished children, and seen too often in the underdeveloped world, is blindness. This is doubly tragic in that it is so easily prevented. Retinal rod and cone cells in the eye contain membranous vesicles that serve as light receptors. Roughly a half of the proteins in these vesicles consist of the protein conjugate, rhodopsin, which consists of a protein – opsin – and **11-cis-retinal**. When this is activated

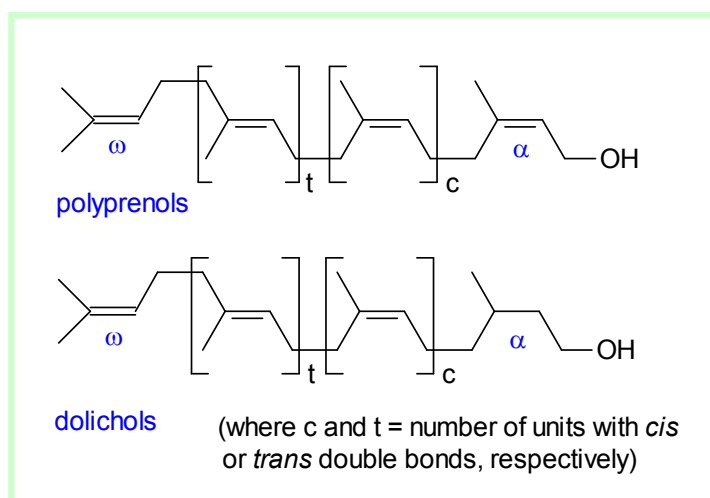


by light, the *cis*-double bond is isomerized non-enzymatically to the 11-*trans* form with a change of conformation that in turn affects the permeability of the membrane and influences calcium transport. This results in further molecular changes that culminate in the release of opsin and all-*trans*-retinal, which are the trigger that sets off the nerve impulse so the light is perceived by the brain. The all-*trans*-retinal is converted back to 11-*cis*-retinal by a various enzymatic reactions, so that the rhodopsin can be regenerated. As a side-reaction, some of the all-*trans*-retinal condenses with **phosphatidylethanolamine** to produce troublesome *bis*-retinoid products.

Retinyl- β -D-glucoside, retinyl- β -D-glucuronide, and retinoyl- β -D-glucuronide are naturally occurring and biologically active metabolites of vitamin A, which are found in fish and mammals. Indeed, the last has similar activity to all-*trans*-retinoic acid without any of the unwanted side effects in some circumstances. In addition, retinoic acid can undergo metabolism into 4-hydroxy-, 4-oxo- and 18-hydroxy-retinoic acids, as part of the process of degradation and elimination.

5. Dolichol and Polyprenols

Polyisoprenoid alcohols, such as dolichols, are ubiquitous if minor components, relative to the glycerolipids, of membranes of most living organisms from bacteria to mammals. They are hydrophobic linear polymers, consisting of up to twenty isoprene residues or a hundred carbon atoms (or many more in plants especially), linked head-to-tail, with a hydroxy group at one end (α -residue) and a hydrogen atom at the other (ω -end). In **dolichols** (or dihydropolyprenols), the double bond in the α -residue is hydrogenated, and this distinguishes them from the **polyprenols** with a double bond in the α -residue.



Polyisoprenoid alcohols are further differentiated by the geometrical configuration of the double bonds into three subgroups, i.e. di-*trans*-poly-*cis*, tri-*trans*-poly-*cis*, and all-*trans*. For many years, it was assumed that polyprenols were only present in bacteria and plants, especially photosynthetic tissues, while dolichols were found in mammals or yeasts, but it is now known that dolichols can also occur at low levels in bacteria and plants and polyprenols in animal cells. Within a given species, components of one chain-length may predominate, but other homologues are usually present. The chain length of the main polyisoprenoid alcohols varies from 11 isoprene units in eubacteria, to 16 or 17 in *Drosophila*, 15 and 16 in yeasts, 19 in hamsters and 20 in pigs and humans. In plants, the range is from 8 to 22 units, but some species of plant have an additional class of polyprenols with up to 40 units. In tissues, polyisoprenoid alcohols can be present in the free form, esterified with acetate or fatty acids, phosphorylated or monoglycosylated phosphorylated (various forms), depending on species and tissue. Polyisoprenoid alcohols *per se* do not form bilayers in aqueous solution, but rather a type of lamellar structure. However, they are found in most membranes, especially the plasma membrane of liver cells and the chloroplasts of plants.

Dolichoic acids, i.e. related molecules with a terminal carboxyl group and containing 14–20 isoprene units, have been isolated from the substantia nigra of the human brain. However, they were barely detectable in pig brain.

Biosynthesis of the basic building block of dolichols, i.e. isopentenyl diphosphate, follows either the mevalonate pathway discussed in relation to **cholesterol** biosynthesis elsewhere on this site, or a more recently described methylerythritol phosphate pathway, depending on the nature of the organism. Subsequent formation of the linear prenyl chain is accomplished by prenyl transferases that catalyse the condensation of isopentenyl diphosphate and the allylic prenyl diphosphate. The end products are polyprenyl pyrophosphates, which are dephosphorylated first to polyprenols phosphate and thence to the free alcohol.

Although polyprenols and dolichols were first considered to be simply secondary metabolites, they are now known to have important biological functions. In particular, glycosylated phosphopolyisoprenoid alcohols serve as carriers of oligosaccharide units for transfer to proteins and as glycosyl donors, i.e. substrates for glycosyl transferases for the biosynthesis of glycans in a similar manner to the cytosolic sugar nucleotides. They differ from the latter in their intracellular location, with the lipid portion in the membrane of the endoplasmic reticulum and the oligosaccharide portion specifically located either on the cytosolic or luminal face of the membrane. For this purpose, dolichol and the polyprenols are first phosphorylated by specific kinases, which are important in that they determine the size of the pool available for use in *N*-glycosylation. The degree of unsaturation and chain-length of the product are important for recognition by the enzymes in the next stage of the pathway.

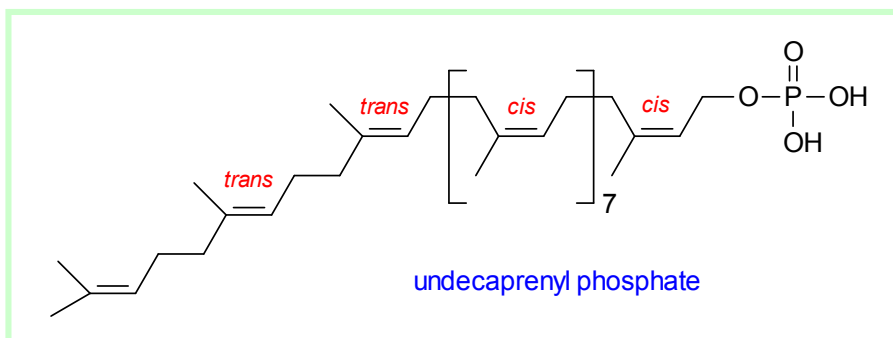
In eukaryotes, *N*-glycosylation begins on the cytoplasmic side of the endoplasmic reticulum with the transfer of carbohydrate moieties from nucleotide-activated sugar donors, such as uridine diphosphate *N*-acetylglucosamine, onto dolichol phosphate. Then, *N*-acetylglucosamine phosphate is added to give dolichol-pyrophosphate linked to *N*-acetylglucosamine, to which a further *N*-acetylglucosamine unit is added followed by five mannose units. The resulting dolichol-pyrophosphate-heptasaccharide is then flipped across the endoplasmic reticulum membrane to the luminal face [with the aid of a “flippase.” Four further mannose and three glucose residues are added to the oligosaccharide chain by means of glycosyltransferases, which utilise as donors dolichol-phospho-mannose and dolichol-phospho-glucose, which are also synthesised on the cytosolic face of the membrane and flipped across to the luminal face. The final lipid product is a dolichol pyrophosphate-linked tetradecasaccharide, the oligosaccharide unit of which is transferred from the dolichol carrier onto specific asparagine residues on a developing polypeptide in the membrane. The carrier dolichol-pyrophosphate is dephosphorylated to dolichol-phosphate then diffuses or is flipped back across the endoplasmic reticulum to the cytoplasmic face.

Most bacteria use undecaprenyl phosphate as a glycosylation agent in a similar way (next section), but the Archaea use dolichol in their synthesis of lipid-linked oligosaccharide donors with both dolichol phosphate and pyrophosphate as carriers. Archaea of course use isoprenyl ethers linked to glycerol as major membrane lipid components (see the appropriate web page).

6. Undecaprenyl Phosphate and Lipid II

Undecaprenyl phosphate (a C_{55} isoprenoid), also referred to as bactoprenol, is a lipid intermediate that is essential for the biosynthesis of peptidoglycan and many other cell-wall polysaccharides, and for *N*-linked protein glycosylation in prokaryotes (both in Gram-negative and Gram-positive bacteria). It is synthesised by the addition of eight units of isopentenyl pyrophosphate to farnesyl pyrophosphate, a reaction catalysed by undecaprenyl pyrophosphate synthase, followed by the removal of a phosphate group. Undecaprenyl phosphate is required for the synthesis and transport

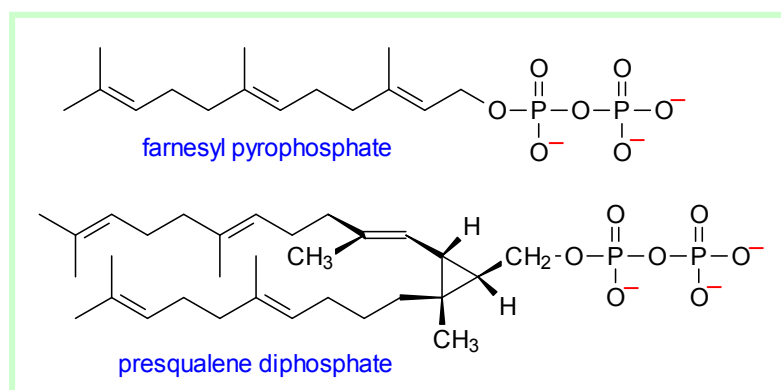
of hydrophilic GlcNAc-MurNAc-peptide monomers across the cytoplasmic membrane to external sites for polymer formation.



Undecaprenyl diphosphate-MurNAc-pentapeptide-GlcNAc, sometimes termed lipid II, is the last significant lipid intermediate in this process, and it has only recently been identified as a normal constituent *in vivo* of the membranes of *Escherichia coli*, by the application of modern mass spectrometric methods. This molecule must be translocated by an as yet unknown mechanism from the cytosolic to the exterior membrane of the organism, where it yields up the MurNAc-pentapeptide-GlcNAc monomer to form the complex peptidoglycan polymer that provides strength and shape to bacteria. Synthesis and transport of lipid II is now considered an important target for antibiotics. In Gram-negative bacteria, undecaprenyl phosphate is also required for the biosyntheses of lipid A and of the O-antigen. There appear to be parallels with the involvement of glycosylated phosphopolyisoprenoid alcohols as carriers of oligosaccharide units for transfer to proteins and as glycosyl donors in higher organisms (see above).

7. Farnesyl Pyrophosphate and Related Compounds

Farnesyl pyrophosphate is a key intermediate in the biosynthesis of sterols such as **cholesterol** and it is the donor of the farnesyl group for isoprenylation of many proteins, but it is also known to mediate various biological reactions via interaction with a specific receptor. It is synthesised by two successive phosphorylation reactions of farnesol.



Presqualene diphosphate is unique among the isoprenoid phosphates in that it contains a cyclopropylcarbinyl ring. In addition to being a biosynthetic precursor of squalene, and thence of cholesterol, it is a natural anti-inflammatory agent, which functions by inhibiting the activity of phospholipase D and the generation of superoxide anions in neutrophils.

Recommended Reading

- o Booth, S.L. and Saltzman, E. **Vitamin K: structure and function**. In: *Encyclopedia of Life Sciences*. John Wiley & Sons, Ltd: Chichester – <http://www.els.net/> (doi: 10.1038/npg.els.0001411) (2001).
- o Bouhss, A., Trunkfield, A.E., Bugg, T.D.H. and Mengin-Lecreulx, D. **The biosynthesis of peptidoglycan lipid-linked intermediates**. *FEMS Microbiol. Rev.*, **32**, 208-233 (2008).
- o de Kruijff, B., van Dam, V. and Breukink, E. **Lipid II: A central component in bacterial cell wall synthesis and a target for antibiotics**. *Prostaglandins, Leukotrienes Essential Fatty Acids*, **79**, 117-121 (2008).
- o DellaPenna, D. and Pogson, B.J. **Vitamin synthesis in plants: tocopherols and carotenoids**. *Annu. Rev. Plant Biol.*, **57**, 711-738 (2006).
- o James, A.M., Smith, R.A.J. and Murphy, M.P. **Antioxidant and prooxidant properties of mitochondrial Coenzyme Q**. *Arch. Biochem. Biophys.*, **423**, 47-56 (2004).
- o Jones, M.B., Rosenberg, J.N., Betenbaugh, M.J. and Krag, S.S. **Structure and synthesis of polyisoprenoids used in N-glycosylation across the three domains of life**. *Biochim. Biophys. Acta*, **1790**, 485-494 (2009).
- o Krag, S.S. **The importance of being dolichol**. *Biochem. Biophys. Res. Commun.*, **243**, 1-5 (1998).
- o Meissburger, B. and Wolfrum, C. **The role of retinoids and their receptors in metabolic disorders**. *Eur. J. Lipid Sci. Technol.*, **110**, 191-205 (2008).
- o Swiezewska, E. and Danikiewicz, W. **Polyisoprenoids: structure, biosynthesis and function**. *Prog. Lipid Res.*, **44**, 235-258 (2005).
- o Turunen, M., Olsson, J. and Dallner, G. **Metabolism and function of coenzyme Q**. *Biochim. Biophys. Acta*, **1660**, 171-199 (2004).

William W. Christie

Scottish Crop Research Institute (and Mylnfield Lipid Analysis), Invergowrie,
Dundee (DD2 5DA), Scotland

Last updated: Jan. 18th, 2010

