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Review

At the membrane frontier: A prospectus on the remarkable evolutionary conservation of polyprenols and polyprenyl-phosphates

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ABSTRACT

Long-chain polyprenols and polyprenyl-phosphates are ubiquitous and essential components of cellular membranes throughout all domains of life. Polyprenyl-phosphates, which include undecaprenyl-phosphate in bacteria and the dolichyl-phosphates in archaea and eukaryotes, serve as specific membrane-bound carriers in glycan biosynthetic pathways responsible for the production of cellular structures such as N-linked protein glycans and bacterial peptidoglycan. Polyprenyl-phosphates are the only form of polyprenols with a biochemically-defined role; however, unmodified or esterified polyprenols often comprise significant percentages of the cellular polyprenol pool. The strong evolutionary conservation of unmodified polyprenols as membrane constituents and polyprenyl-phosphates as preferred glycan carriers in biosynthetic pathways is poorly understood. This review surveys the available research to explore why unmodified polyprenols have been conserved in evolution and why polyprenyl-phosphates are universally and specifically utilized for membrane-bound glycan assembly.

Introduction

The long-chain polyisoprenoid alcohols, including polyprenols and dolichols, are a unique class of secondary metabolites within the isoprenoid natural product family (Fig. 1). These polyprenols and the corresponding phosphorylated derivatives comprise a small percentage of the total glycerophospholipid content in cellular membranes of bacteria (~1%) [1,2] and eukaryotes (~0.1%) [3–5]. Polyprenyl-phosphates act as oligosaccharide carriers during glycan biosynthesis, which is essential to many conserved cellular processes including N-linked protein glycosylation, C- and O-protein mannosylation, and bacterial cell wall biosynthesis. Despite extensive research on the enzymes that utilize polyprenols in glycan assembly pathways, relatively little is known about why unmodified polyprenols populate cellular membranes and why polyprenyl-phosphates prevail as the most common membrane-bound glycan carriers in nature. This review begins with an overview of polyprenol structure and polyprenyl-phosphate dependent processes in biology. While providing essential background on the "what" and "where" of polyprenols, we will also address the more interesting question of "why polyprenols in the first place?"

Structural features of long-chain polyprenols

The linear polyprenols (or polyisoprenols) contain 7–24 isoprene units in either the *trans* or *cis* configuration and can broadly be separated into two subclasses [5–8]. The first class includes undecaprenol and related homologs and contains only unsaturated isoprene units, whereas the second class, known as dolichols, is distinguished by the presence of a single saturated isoprene unit in the α-position (Fig. 1). In both classes, α refers to the unit closest to the hydroxyl moiety and ω is used to designate the terminal isoprene unit in the linear structure. A typical polyprenol structure contains an ω isoprene unit followed by two or three *trans* isoprene units, and anywhere from three to 17 *cis* isoprene units (Fig. 1). Very recently, a third class of linear polyprenols termed 'alloprenols' has been identified in several plant species. Alloprenols possess an unsaturated structure as in the first class of polyprenols, but contain an unusual *trans* (*E*) isoprene unit at the α-position [9,10].

Many studies have focused on elucidating the process of polyprenol biosynthesis, which involves the enzyme-catalyzed condensation of dimethylallylpyrophosphate (DMAPP)[1] with multiple units of isopentenylpyrophosphate (IPP). The biosynthesis of isoprenoid alcohols depicted in Fig. 2 has been reviewed recently [7,8,11–13]. This general pathway is responsible for the production of a wide range

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[1] *Abbreviations used:* CDG, congenital glycosylation disorder; CHO, Chinese hamster ovary; diNAcBac, di-*N*-acetylbacillosamine; Dol-P, dolichyl-phosphate; Dol-P-Man, dolichyl-phosphate mannose; Dol-PP, dolichyl-diphosphate; DMAP, dimethylallylpyrophosphate; ER, endoplasmic reticulum; GalNAc, *N*-acetylgalactosamine; Glc, glucose; GlcNAc, *N*-acetylglucosamine; IPP, isopentenylpyrophosphate; Lipid I, Und-PP-MurNAc(pentapeptide); Lipid II, Und-PP-MurNAc(pentapeptide)GlcNAc; Man, mannose; MurNAc, *N*-acetylmuramic acid; NDP, nucleotide diphosphate; UDP, uridine diphosphate; Und-P, undecaprenyl-phosphate; Und-PP, undecaprenyl-diphosphate.

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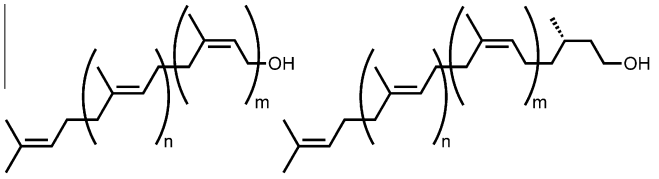

Fig. 1. Structures of fully unsaturated polyprenols and dolichols. The length of the polyprenol and the number of *cis* and *trans* isoprene units (*m* and *n*, respectively) is species dependent. (Left) Fully unsaturated polyprenols contain all *trans* isoprene units or a mixture of *trans* and *cis* units as shown. (Right) Dolichols are distinguished by a single saturated α-isoprene unit.

of polyprenol structures, which vary in length across organisms, ranging from an average of C55 polyprenols in bacteria and C95 dolichols in mammals to C200 polyprenols in plants (Table 1). Bacteria typically exploit undecaprenol, a C55 unsaturated polyprenol [14], while archaeal organisms typically contain C55–C65 dolichols [15], *Saccharomyces cerevisiae* (yeast) contain C70–C80 dolichols [16] and mammalian tissues contain C90–C100 dolichols (Table 1) [17]. In addition, unusual polyprenols that include additional saturated isoprene units at the ω-terminus have been identified in *Mycobacterium* [18,19] as well as the archaeal species, *Haloferex volcanii* [20] and *Sulfolobus acidocaldarious* [21].

Plants exhibit the greatest polyprenol diversity, as both unsaturated polyprenols and dolichols with a wide range of lengths have been characterized [22–24]. The lipid bilayers of most plants contain C55 polyprenols, although in contrast to undecaprenol, these polyprenols contain three, rather than two, *trans* isoprene units, due to small differences at the beginning of the biosynthetic pathway [11]. Plants in the gymnosperm family contain C80–C100 polyprenols [23], while plants in the genus *Potentilla* contain mixtures of polyprenols with the longest possessing up to 200 carbons [25]. It is interesting to note the wide diversity of linear polyprenol architectures that has evolved amongst the three kingdoms of life, as the known functions of these molecules are very similar in all organisms.

To date, the question of why different organisms utilize polyprenols of different sizes and degrees of unsaturation has principally included studies on the biophysical effects of selected polyprenols in model lipid bilayers and the analysis of enzyme specificity for polyprenyl-phosphate substrates. However, neither of these methods can explain the evolutionary driving force behind the varied lengths of polyprenols and it remains an important unanswered question in the field. Some speculation has focused on the origin of the α-isoprene subunit saturation. It has been suggested that α-saturation lends greater stability relative to the corresponding unsaturated polyprenol derivatives, which might be an advantage for archaeal organisms that live in extreme conditions. However, many bacteria, which almost exclusively feature fully unsaturated polyprenols, survive in harsh conditions, and thus this argument is not convincing. As will be discussed later, it is well established that reduction of the α-isoprene subunit in dolichol biosynthesis (Fig. 2) is an important regulatory point in the eukaryotic pathway, and it may be that this element of structural diversity engenders greater biosynthetic complexity and allows for more precise regulatory control of the many processes reliant on dolichyl-phosphate.

Polyprenyl-phosphate as glycan carriers in biosynthetic pathways

Polyprenyl-phosphates are essential substrates for critical cellular functions in both eukaryotes and prokaryotes. These roles include N- and O-linked protein glycosylation in eukaryotes, archaea and bacteria and the biosynthesis of central structural components in bacteria such as peptidoglycan and O-antigen. Of these biosynthetic processes, N-linked protein glycosylation may be the most well known and the role of polyprenols in this process has been recently reviewed by Krag and coworkers [8]. It involves the stepwise assembly of an oligosaccharide on polyprenyl-diphosphate or polyprenyl-phosphate and occurs in eukaryotes, archaea, and several bacterial species (Fig. 3) [8,26,27]. In eukaryotes, a conserved dolichyl-diphosphate (Dol-PP) heptasaccharide ($GlcNAc_2Man_5$) is assembled on the cytoplasmic face of the endoplasmic reticulum (ER) membrane (Fig. 4) [28–30]. The Dol-PP-glycan intermediate is then translocated across the lipid bilayer to the ER lumen, where it is further glycosylated to form a $GlcNAc_2Man_9Glc_3$ tetradecasaccharide that is transferred to the nitrogen in the primary amide side chain of asparagine residues in nascent proteins (Fig. 4).

To date, characterization of N-linked glycosylation in archaea is limited, but these pathways are known to involve assembly of

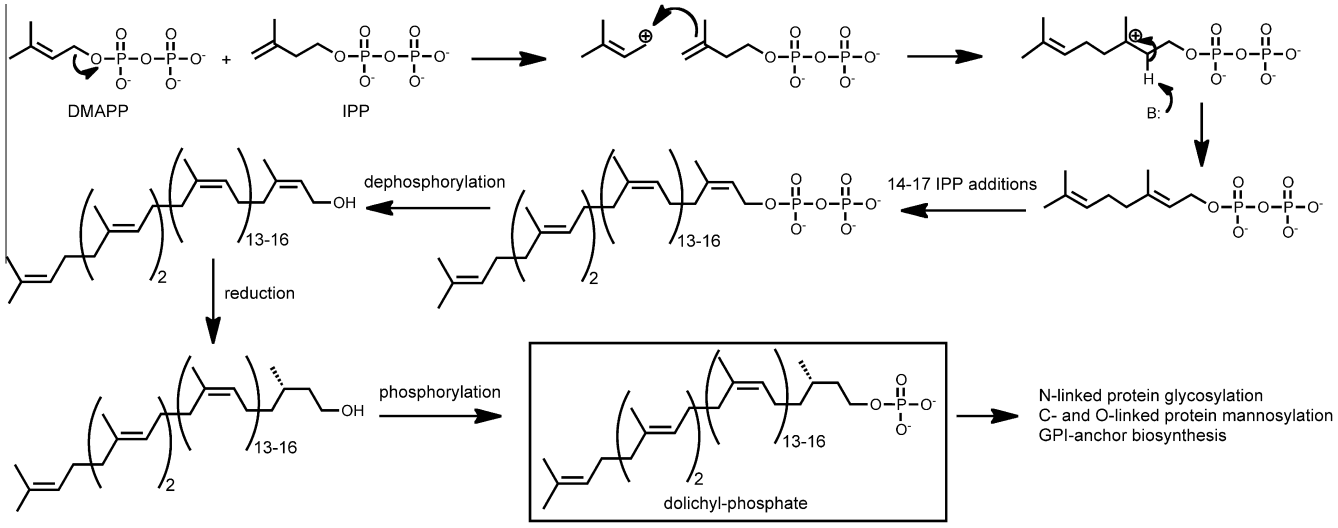

Fig. 2. Polyprenol biosynthesis from DMAPP and IPP. Prenyltransferases catalyze the condensation of DMAPP with IPP to form long polyprenyl-diphosphate molecules and direct which stereoisomer (*cis* or *trans*) is produced in each reaction. In eukaryotes, the final steps of dolichol biosynthesis involve enzyme-catalyzed reduction of the α-isoprene subunit, which is thought to occur on the unmodified polyprenol, followed by phosphorylation. Specific phosphatases responsible for the hydrolysis of dolichyl-diphosphate have not yet been identified. Bacteria do not require reduction at the α-subunit, and thus their biosynthetic pathways terminate with a single hydrolysis reaction to generate undecaprenyl-phosphate.