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Review

Structure and synthesis of polyisoprenoids used in N-glycosylation across the three domains of life

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ABSTRACT

N-linked protein glycosylation was originally thought to be specific to eukaryotes, but evidence of this post-translational modification has now been discovered across all domains of life: Eucarya, Bacteria, and Archaea. In all cases, the glycans are first assembled in a step-wise manner on a polyisoprenoid carrier lipid. At some stage of lipid-linked oligosaccharide synthesis, the glycan is flipped across a membrane. Subsequently, the completed glycan is transferred to specific asparagine residues on the protein of interest. Interestingly, though the N-glycosylation pathway seems to be conserved, the biosynthetic pathways of the polyisoprenoid carriers, the specific structures of the carriers, and the glycan residues added to the carriers vary widely. In this review we will elucidate how organisms in each basic domain of life synthesize the polyisoprenoids that they utilize for N-linked glycosylation and briefly discuss the subsequent modifications of the lipid to generate a lipid-linked oligosaccharide.

1. Introduction

Historically, it was thought that N-glycosylation was unique to eukaryotic organisms and that a single, defined pathway led to the biosynthesis of the polyisoprenoids that act as the lipid-linked oligosaccharide (LLO) donors in N-glycosylation. In the last few decades, however, both of these ideas have been disproven, evoking questions about how N-glycosylation occurs in different organisms, including what type of carrier lipids are used and how they are synthesized.

In 1976, Mescher and Strominger reported a protein from the cell envelope in *Halobacterium salinarium* that contained glycans covalently linked to asparagine residues [1]. This marked the first observation of N-glycosylation in what would later become the domain Archaea. With this discovery, the idea that N-linked glycosylation occurred only in eukaryotes was dispelled. More recently, evidence of N-glycosylation in bacteria was discovered by Szymanski et al. in the Gram-negative bacteria *Campylobacter jejuni* [2]. N-glycosylation is now thought to be a protein modification that is conserved across all three major domains of life: Eucarya, Bacteria, and Archaea [2,3].

The N-glycosylation pathways in each domain are similar in that they include the step-wise assembly of sugars, donated by nucleotide-activated sugars or activated lipids, onto a polyisoprenoid carrier by specific glycosyltransferases to form a LLO [4,5]. At some point during synthesis, the LLO is flipped across a membrane, and the oligosaccharide is transferred from the carrier lipid onto the protein by an oligosaccharide transferase (OST) enzyme or enzyme complex [4,5]. The glycan is ultimately bound to an asparagine residue in the protein via a β-glycosylamide linkage [4].

Although eukaryotes, bacteria, and archaea all seem to have certain characteristics of the N-glycosylation pathway in common, the identity of the lipid oligosaccharide carrier, its method of biosynthesis, and the structure of the attached glycans are sometimes strikingly different. This review will focus primarily on the synthesis of the polyisoprenoid alcohols that provide the carrier lipid portion of the LLO. Polyisoprenoid

Abbreviations: LLO, lipid-linked oligosaccharide; OST, oligosaccharide transferase; IPP, isopentenyl pyrophosphate; DMAPP, dimethylallyl pyrophosphate; MVA, mevalonate; DOXP, 1-deoxy-D-xylulose-5-phosphate; HMG-CoA, 3-hydroxy-3methylglutaryl-CoA; HMGS, HMG-CoA synthase; HMGR, HMG-CoA reductase; MPPD, MVA-5-pyrophosphate decarboxylase; Dxs, DOXP synthase; IspC, DOXP reductase; MEP, 2-C-methyl-D-erythritol,4-phosphate; CTP, cytidine triphosphate; CDP-ME, 4-pyrophosphocytidyl-2-C-methyl-D-erythritol; IspD, CDP-ME synthase; IspE, 4-pyrophosphocytidyl-2C-methyl-D-erythritol kinase; CDP-MEP, CDP-ME-2-phosphate; MECPP, 2C-Methyl-D-erythritol-2,4-cyclopyrophosphate; IspF, MECPP synthase; CMP, cytidine monophosphate; HMBPP, 1-hydroxy-2-methyl-2-(E)-butenyl-4-pyrophosphate; GPP, geranyl pyrophosphate; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; FGPP, farnesylgeranyl pyrophosphate; Poly-PP, polyprenyl pyrophosphates; Poly-P, polyprenyl phosphate; Dol-P, dolichol phosphate; Dol-PP, dolichol pyrophosphate; ER, endoplasmic reticulum; UDP-GlcNAc, uridine diphosphate N-acetylglucosamine; GDP-Man, guanosine diphosphate mannose; Glc, glucose; Oligo-PP, oligoprenyl pyrophosphate; UPPS, undecaprenyl pyrophosphate synthase; Und-PP, undecaprenyl pyrophosphate; Bacillosamine, Bac, 2,4-diacetamido-2,4,6-trideoxyglucopyranose; GalNAc, N-acetylgalactosamine; IP, isopentenyl phosphate

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alcohols are hydrophobic polymers generated by the condensation of isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), the universal 5-carbon precursors of all isoprenoids [6–8]. A schematic diagram of how polyisoprenoid alcohols are formed and utilized in N-glycosylation is shown in Fig. 1. The review will begin with an overview of the two possible pathways for generating the IPP and DMAPP building blocks. It will then discuss the formation of the polyisoprenoid alcohols, dolichol and polyprenol, from IPP and DMAPP. Next, the review will identify the polyisoprenoid alcohols known to be used in N-glycosylation for organisms in each of the three domains of life and will determine which biosynthetic pathways are responsible for providing the IPP and DMAPP building blocks in each case. Finally, the sugar modifications that result in generation of a completed LLO will be described briefly for each domain.

2. Isoprenoid biosynthesis

Originally, there was thought to be a single pathway responsible for the biosynthesis of IPP and DMAPP, the universal 5-carbon precursors of all isoprenoids [6–8]. This was called the mevalonate (MVA) pathway and it involves the formation of IPP from acetyl-CoA with mevalonate as an intermediate (Fig. 2). It was widely accepted that all organisms form DMAPP and IPP only through this pathway [7]. However, in 1993, Rohmer et al. provided evidence for an alternate pathway for DMAPP and IPP synthesis that did not involve mevalonate (Fig. 3) [9,10]. This work began with the observation of unexpected labeling patterns in bacterial hapanoids using ^{13}C-labeled acetate [11]. Independently, Arigoni et al. discovered the existence of a mevalonate-independent pathway in the plant, *Ginkgo biloba*, as well as in *E. coli* [12–14]. Since then, this pathway, called the 1-deoxy-D-xylulose-5 phosphate (DOXP) pathway, has been further elucidated and has proven to be an alterative to the MVA pathway for IPP and DMAPP synthesis [8,15,16].

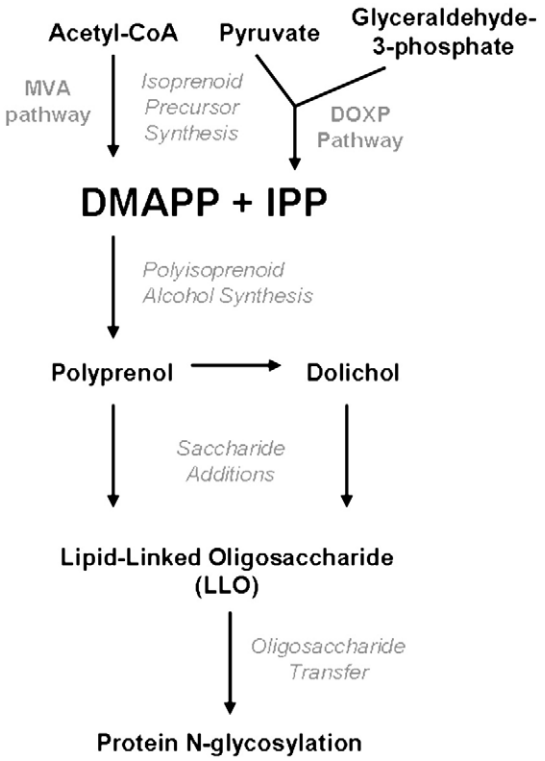

Fig. 1. Schematic overview of polyisoprenoid alcohol formation and utilization in N-glycosylation. The MVA and DOXP pathways provide the isoprenoid precursors DMAPP and IPP, which are used to synthesize dolichol and polyprenol. Dolichol and polyprenol are modified by sugar additions into a completed LLO, then the oligosaccharide is transferred off of the carrier lipid and onto asparagine residues on the protein.

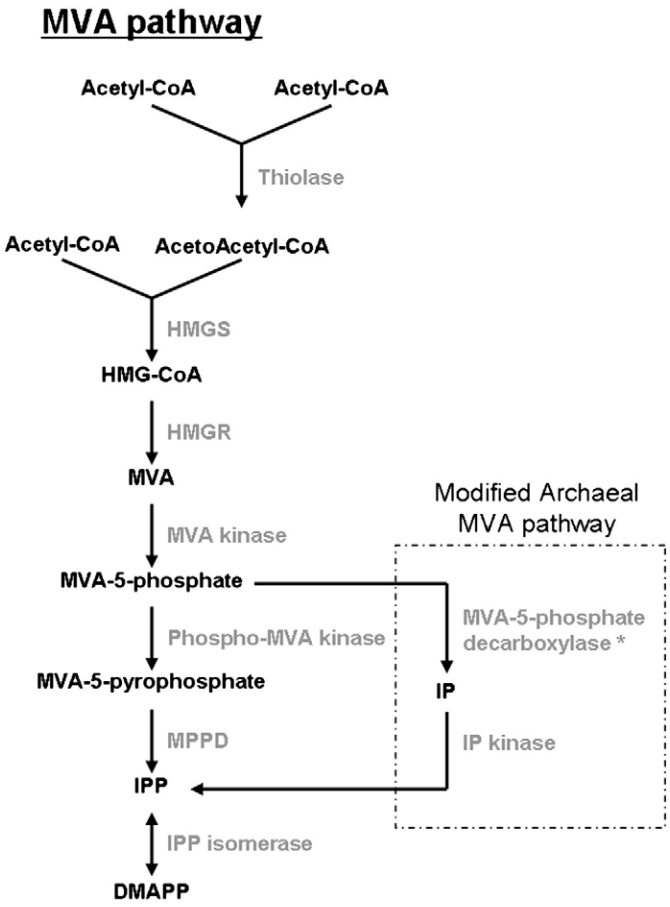

Fig. 2. The MVA biosynthetic pathway for IPP and DMAPP. Acetyl-CoA is used to synthesize IPP and DMAPP with MVA as an intermediate. In the modified archaeal pathway, MVA-5-phosphate is first decarboxylated then phosphorylated to form IPP, the opposite of what occurs in the traditional MVA pathway. *Enzyme is theoretical and has yet to be discovered. For a review of the MVA pathway with chemical structures see [108].

2.1. The MVA pathway

The MVA pathway (Fig. 2) begins with the conversion of two molecules of acetyl-CoA to acetoacetyl-CoA by the enzyme acetyl-CoA acetyltransferase (also called thiolase) [6,17–19]. A third molecule of acetyl-CoA and the acetoacetyl-CoA are then condensed to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) via HMG-CoA synthase (HMGS) [6,17,20]. HMG-CoA is reduced to mevalonate by HMG-CoA reductase (HMGR), in a NADPH-dependent reaction. HMGR is the key regulatory enzyme in the MVA pathway [21–23]. In the next step, MVA is phosphorylated by MVA kinase to form MVA-5-phosphate, which is further phosphorylated by phospho-MVA kinase to form MVA-5-pyrophosphate [24,25]. Both of these phosphorylation reactions are ATP dependent [26,27]. MVA-5-pyrophosphate undergoes a decarboxylation reaction via MVA-5-pyrophosphate decarboxylase (MPPD) to produce IPP [6,28]. In the final step of this pathway, some of the IPP is converted to DMAPP by IPP isomerase [27,29]. IPP isomerase is essential for the production of DMAPP via the MVA pathway [8].

2.2. The DOXP pathway

The DOXP pathway (Fig. 3, for review see [6]) does not require acetyl-CoA, but rather begins with the condensation of pyruvate and glyceraldehyde 3-phosphate into DOXP by DOXP synthase (Dxs) [30,31]. This reaction requires thiamine pyrophosphate as a cofactor [6]. DOXP reductase (IspC) converts DOXP to 2-C-methyl-D-erythritol