1.6.1 Precursor Synthesis

First the six-carbon substrate enters into the Entner–Douderoff pathway also known as the KDPG pathway, where pyruvate is formed, which is then channeled to the tricarboxylic acid (TCA) cycle, while oxaloacetate from the TCA cycle is converted to fructose-6-phosphate via gluconeogenesis. The fructose-6-phosphate is converted to mannose-6-phosphate by the phosphomannose isomerase (PMI) activity of the bifunctional protein AlgA (PMI-GMP). Mannose-6-phosphate is directly converted to its isomer form, mannose-1-phosphate, by AlgC (phosphomannomutase). The activated mannose-1-phosphate is converted to GDP-mannose with the hydrolysis of GTP by the GDP-mannose pyrophosphorylase (GMP) activity of AlgA (PMI-GMP). The GMP activity of this enzyme favors the reverse reaction, but AlgD (GDP-mannose-dehydrogenase) constantly converts GDP-mannose to GDP-mannuronic acid, and the reaction is shifted toward GDP-mannuronic acid and ALG production. This AlgD catalyzed reaction is essentially irreversible and provides the direct precursor for polymerization, GDP-mannuronic acid. The high intracellular levels of GDP-mannose indicate that this AlgD catalyzed step is a limiting step and/or is an important kinetic control point in ALG biosynthesis [27].

The two genes, *algA* and *algD*, are found on the ALG operon, whereas *algC* is located elsewhere in the genome at PA5322 [28]. AlgC plays an important role in general exopolysaccharide biosynthesis, i.e., not only ALG biosynthesis but it is also required for precursor synthesis of Psl, as well as LPS and rhamnolipids [29, 30]. The crystal structures of these two enzymes, AlgD and AlgC, have been determined [31, 32]. A common structural feature of enzymes involved in nucleotide binding, such as in the generation of activated sugars, is the presence of at least one β/α/β nucleotide binding domain. This domain is known as a Rossmann fold, which has a secondary structure consisting of alternating β-strands and α-helices arranged such that they form a central six-stranded parallel β-sheet linked to five surrounding α-helices. An example of many variations of the "classical" Rossmann fold or nucleotide binding domain is AlgD. This protein forms a dimer with each individual subunit containing one complete N-terminal nucleotide binding domain and a C-terminal nucleotide-like binding domain, which lacks the third β-strand and final α-helix of this motif [32]. In spacerhelix, the two nucleotide binding domains are separated by a long 33 residue α-helix.

Interestingly, the protein forms a domain-swapped dimer, whereby the N-terminal nucleotide binding domain of one subunit interacts with the C-terminal nucleotide binding domain of the second subunit. The interface