of these two domains forms the active site; the location of which was verified by the structure of AlgD in complex with its substrate, NAD(H) and product GDP-mannuronate (ManUA). Two dimers of AlgD likely interact to form a tetrameric structure in the cell cytoplasm, creating the GDP-ManUA product, which is the irreversible step in ALG precursor formation.

The second enzyme, which is involved in the production of ALG precursor, is magnesium-dependent mutase, AlgC (PDB ID:3CO4). The structure of AlgC has been determined. It shows specificity for both phosphomannose and phosphoglucose substrates [31]. This protein contains four domains, which are approximately of equal size. The first three domains share a common topological core consisting of a four-stranded β-sheet sandwiched between 2 α-helices, while the fourth domain is a member of the TATA-box binding protein-like fold superfamily. This domain consists of a four-stranded antiparallel β-sheet, flanked by two α-helices and two short β-strands. All of the four domain residues help in the formation of a large active site cleft at the center of this "heart"-shaped molecule. The specificity for glucose vs mannose in this class of enzymes is thought to be determined by a conserved sequence motif GEMS(G/A) found in domain 3, which has been postulated to act as the sugar binding loop.

While the structure for AlgA has not been determined, it is predicted by structural modeling to have extensive similarity to other proteins with GMP activity, such as the *Thermotoga maritima* guanosine-diphospho-D-mannose pyrophosphorylase (PDB ID:2X65) and a putative mannose-1-phosphate guanyltransferase from *Thermus thermophilus* (PDB ID:2CU2). Both of these proteins contain Rossmann-like β/α/β nucleotide binding domains characteristic of proteins that generate or bind sugar-nucleotide precursors [33].

1.6.2 Polymerization and Cytoplasmic Membrane Transfer

In the periplasm, the activity of AlgI, AlgJ, and AlgF alters the obtained polymer through selective O-acetylation, and epimerization is carried out by AlgG (Figure 1.5) [34, 35]. At the polymer level, D-mannuronic acid residues can be converted to L-guluronic acid by AlgG, and acetylation can occur at the hydroxyl groups of either the C2 or C3 position; ALG can have somewhat random structure. This random structure distinguishes ALG from capsular polysaccharides of many of the *Escherichia coli* and from Psl, as these polymers are composed of regular repeating subunits.

It has been studied that another crucial role in the formation of polymerase complex is of Alg8 and Alg44. Some kind of periplasmic scaffold is formed to guide and protect the nascent ALG chain from degrading from lyase and is considered to be provided by AlgG, AlgK, and AlgX, along with the outer