Function & biotechnology of a new glycosyltransferase for O-protein glycosylation

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Post-translational modification of proteins by glycosylation is fundamental to many biological processes, as well as human health and valuable for therapeutic treatment in disease. Glycosylation can influence protein activity and/or serum stability. Glycosylation can occur via N-linkage (at asparagine residues) or via O-linkage (at serine or threonine residues). Since O-linked glycosylation is wide-spread among human peptide hormones and blood/coagulation factors which are soluble proteins or peptides, it would be desirable to have a soluble O-based glycosylation system in *E. coli*, the workhorse for soluble protein production at an industrial scale. We showed that *Caulobacter crescentus* FlmG uses a soluble sialic acid donor molecule, pseudaminic acid (PA), to glycosylate the FljK flagellin subunit in the bacterial cytoplasm before it is assembled into the flagellum. We reconstituted glycosylation of FljK by FlmG in the cytoplasm of *E. coli* cells engineered to synthesize PA from a synthetic operon of six biosynthesis genes. Our current objectives are 1) to determine the donor promiscuity of FlmG towards sialic acids; 2) to trim the FljK acceptor sequence for use as a genetically-encoded glycosylation tag and 3) to assess acceptor promiscuity of FlmG in *E. coli* on human acceptor chimeras (i.e. peptide hormones and soluble extracellular factors).

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