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Title: Dissection of Hexosyl- and Sialyltransferase Domains in the Bifunctional Capsule Polymerases from *Neisseria meningitidis* Wand Y Defines a New Sialyltransferase Family

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Abstract: Background: Capsule polymerases of *Neisseria meningitidis* serogroups W and Y comprise hexosyl- and sialyltransferase activity. Results: Hexosyltransferase activity is encoded by the predicted N-terminal GT-B fold. Sialyltransferase activity requires 168 additional amino acids upstream of the predicted C-terminal GT-B fold. Conclusion: The sialyltransferase domains of NmW/Y define a new glycosyltransferase (CAZy) family. Significance: The new CAZy family comprises sequences from distantly related species.

Crucial virulence determinants of disease causing *Neisseria meningitidis* species are their extracellular polysaccharide capsules. In the serogroups W and Y, these are heteropolymers of the repeating units (6)-d-Gal-(14)-Neu5Ac-(2)(n) in NmW and (6)-d-Glc-(14)-Neu5Ac-(2)(n) in NmY. The capsule polymerases, SiaD(W) and SiaD(Y), which synthesize these highly unusual polymers, are composed of two predicted GT-B fold domains separated by a large stretch of amino acids (aa 399-762). We recently showed that residues critical to the hexosyl- and sialyltransferase activity are found in the predicted N-terminal (aa 1-398) and C-terminal (aa 763-1037) GT-B fold domains, respectively. Here we use a mutational approach and synthetic fluorescent substrates to define the boundaries of the hexosyl- and sialyltransferase domains. Our results reveal that the active sialyltransferase domain extends well beyond the predicted C-terminal GT-B domain and defines a new glycosyltransferase family, GT97, in CAZy (Carbohydrate-Active enZYmes Database).

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