EXPLORING THE N-GLYCOSYLATION MACHINERY IN *CHLOROVIRUSES*

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N-glycosylation has been extended from living forms to non-living forms with the discovery that giant viruses have an autonomous glycosylation system[1]. The complex mechanism by which giant viruses produce N-glycans is completely unknown and represents a new frontier to be crossed. In this study, we investigate how the N-glycosylation process occurs in Paramecium Bursaria Chlorella Virus-1 (PBCV-1), the prototype of the family Phycodnaviridae, for which the structure of the N-glycans and some of the Glycosyl transferases (GTs) involved in the assembly of the oligosaccharide are known [1]. In detail, we want to address three fundamental questions: 1) Does the virus follow the same pathway as eukaryotes and prokaryotes? 2) Does the virus have a soluble GT that binds the first sugar directly to the protein moiety? 3) Is there a mechanism other than those currently known? A comprehensive bioinformatics study was carried out to identify potential GTs involved in this process. PBCV-1 encodes 416 proteins, of which 60 are of unknown function, forming the narrow set for GT identification. This set of proteins was screened using several approaches: 1) refinement of the annotation of protein functions using the HHpred tool; 2) evaluation of the degree of conservation of the target protein in chloroviruses using tBlastn (https://blast.ncbi.nlm.nih.gov); 3) phylogenetic relationships with proteins known to be involved in the N-glycosylation process. As results, three putative glucosyltransferases have emerged: A473L, A219/222/226R and A301L. Among these, A473L represents a very good candidate for several reasons: 1) it is conserved in all chloroviruses sharing the same core region of the N-glycan; 2) it is very well expressed at the early stage of the infection cycle; 3) all residues involved in UDP-glucose binding and catalysis are conserved, as demonstrated by modelling (alpha fold) and structural multiple alignment studies. From the structural point of view, A473L is predicted to be a GT-2 membrane protein by alpha fold and it resembles the cellulose synthase of *Rhodobacter sphaeroides-4HG6* (Dali server comparison). Indeed, like 4HG6, A473L has an extramembrane catalytic domain with the typical GT-A fold (98-346 aa) linked to three membrane interface helices (IFH), and a transmembrane domain (TM) that, unlike 4HG6, consists of 6 TMHs instead of 8. In addition, A473L has a structural similarity to the dolichol phosphate (Dol-P) mannosyltransferase from *Pyrobaculum calidifontis* (6YV8), which has a minimal cellulose synthase-like fold. In addition to the GT-A domains, the IFHs of A473L overlaps well with that of 6YV8. These data are of great interest because it has been known that a common feature of the enzymes that recognise the Dol-P-like is the presence of two or three IFHs rich in hydrophobic residues that can interact with the dolichol molecules. A473L could therefore be involved in the binding of UDP-Glc to a Dol-P-like. Based on the above results, work is in progress the express A473L in order to experimentally validate its function.

References:

1. I. Speciale, A. Notaro, C. Abergel, R. Lanzetta, T. L. Lowary, A. Molinaro, M. Tonetti, J. Van Etten, C. De Castro. *Chem. Rev.* **2022**, *122,* 15717-15766.