Characterization of *Schistosoma mansoni* fucosyltransferases for glyco-engineering of 'native' helminth N-glycan structures *in planta*

Kim van Noort[1], Ruud H.P. Wilbers[1], Kasper ter Horst[1], Lotte B. Westerhof[1], Cornelis H. Hokke[2], Geert Smant[1], Jaap Bakker[1] and Arjen Schots[1]

[1] WAGENINGEN UNIVERSITY & RESEARCH

[2] Leids Universitair Medisch Centrum

Background

Secretory glycoproteins of parasitic helminths are in the spotlight as biopharmaceuticals because of their strong, glycan-dependent immunomodulatory properties. Helminths and their secretions have been shown to dampen allergic reactions and autoimmune disorders, such as inflammatory bowel diseases, multiple sclerosis and rheumatoid arthritis. Our group has shown that plants offer a great opportunity to express helminth glycoproteins and study the role of N-glycans on protein function. However for the synthesis of highly fucosylated N-glycans from *Schistosoma mansoni* knowledge is lacking on how these specific structures are synthesized. For this purpose, we examined the function of ten selected fucosyltransferases from *S. mansoni* using transient co-expression with omega-1 as a model protein (Fig. 1). Two fucosyltransferases were identified that couple fucoses to the innermost *N*-acetyl-glucosamine residue with an α1,3 (Fig. 2) or α1,6 (Fig. 3) bond, which can be used to obtain core α1,3- and α1,6-fucosylated N-glycan structures found on native helminth secreted immunomodulatory proteins. Our results show the versatility of plants both as a production system and as a system for characterizing glycosyltransferase functionality. Further characterization of glycosyltransferases offers perspectives for the synthesis of novel complex helminth N-glycan structures in plants.

Results

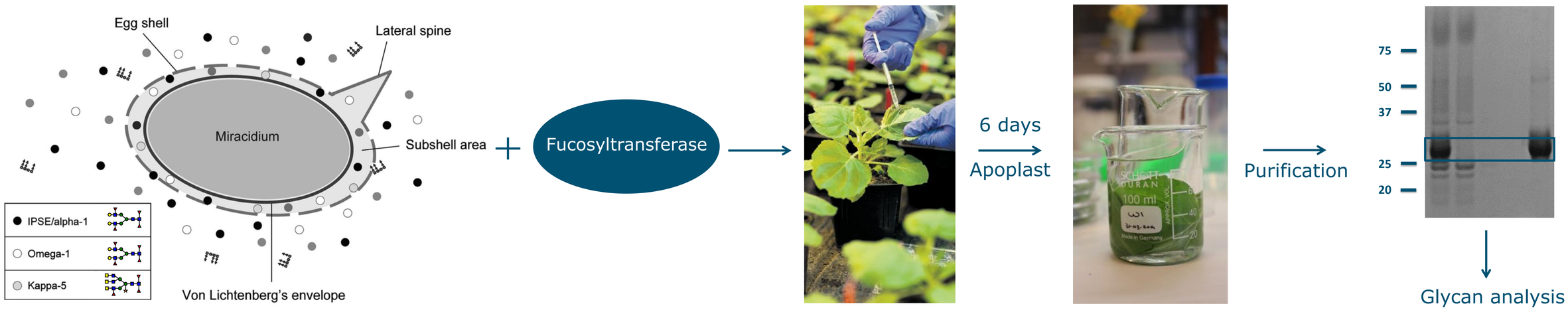

Figure 1. Characterization of *Schistosoma mansoni* fucosyltransferases by co-expression with the major *S. mansoni* egg-secreted protein omega-1 in leafs of 5-6 week old ΔFX, knock-down plants for plant fucosyltransferases and xylosyltransferase, or wild type *Nicotiana benthamiana* plants by agroinfiltration. Proteins were isolated from the extracellular space (apoplast) 6 days post infiltration. Omega-1(~27 kDa) was purified by cation exchange spin columns.

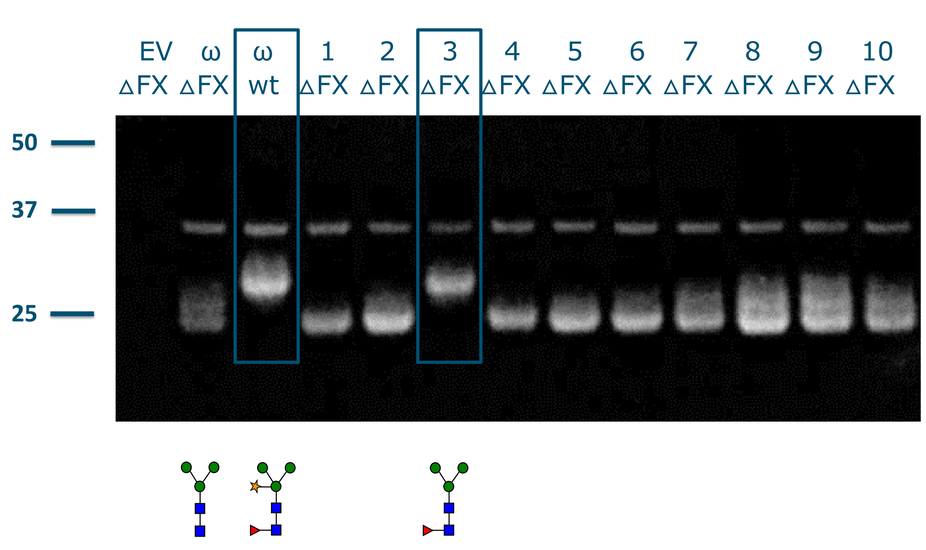

Figure 2. N-glycan analysis of omega-1 (ω) co-expressed with various *Schistosoma mansoni* fucosyltransferases (number 1-10) in ΔFX or wild type (wt) *Nicotiana benthamiana* plants. 1.25ug omega-1 treated with PNGaseF was analyzed on a SDS-PAGE gel, stained with oriole. PNGase F digestion is inhibited when a core α1,3-fucose is present, which can be seen for omega-1 produced in wild type plants (ω wt) and omega-1 co-expressed with fucosyltransferase number 3 in ΔFX plants (3 ΔFX). Below the gel picture expected glycan structures are shown schematically.

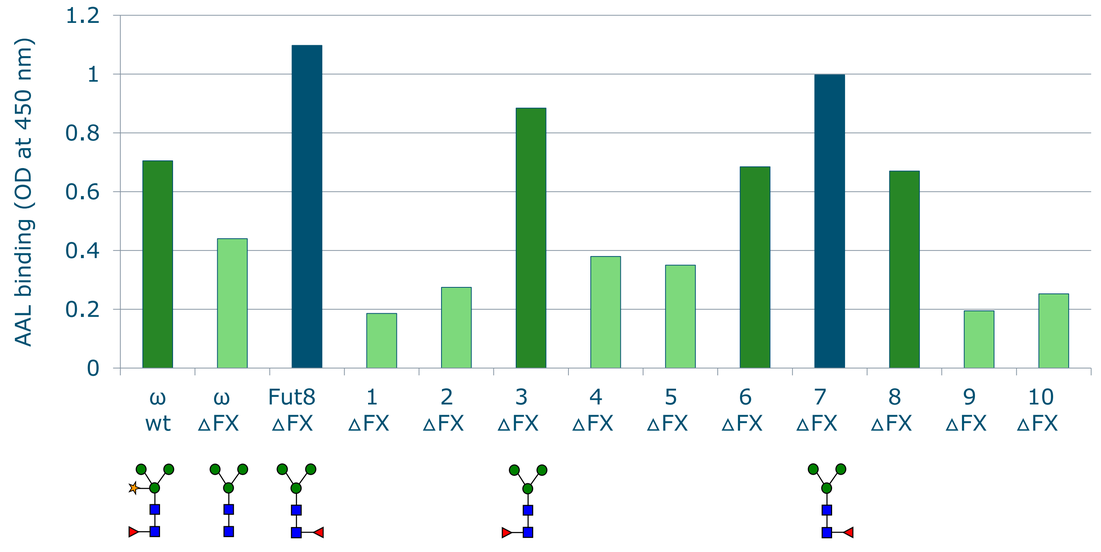

Figure 3. Binding of biotinylated *Aleuria aurantia* lectin (AAL) to omega-1 (ω) co-expressed with various fucosyltransferases (number 1-10) of *Schistosoma mansoni* and *Drosophila melanogaster (*Fut8, positive control) in ΔFX or wild type (wt) *Nicotiana benthamiana* plants. Omega-1 was coated on an ELISA plate at a concentration of 1 µg/mL and binding of AAL to core α1,6-fucose on the N-glycans of omega-1 was determined. Fucosyltransferase number 7 seems responsible for the addition of core α1,6-fucose to the N-glycans of omega-1. However, AAL also seems to bind to N-glycans on omega-1 that carry core α1,3-fucose. Below the graph expected glycan structures are shown schematically.

Conclusions

- Fucosyltransferase number 3 adds a core α1,3-fucose to the N-glycans of omega-1.
- Fucosyltransferase number 7 seems to add a core α1,6-fucose to the N-glycans of omega-1.
- *Aleuria aurantia* lectin does not specifically bind to core α1,6-fucose.
- Fucosyltransferases number 6 and 8 seem to add a, yet undetermined, fucose to the N-glycans of omega-1.
- Plants are an excellent system for functional characterization of fucosyltransferases.

Acknowledgments

We would like to thank Prof. Herta Steinkellner for providing us with ΔFX *Nicotiana benthamiana* plants.

Laboratory of Nematology
Plant Sciences Group, Wageningen University & Research
P.O. Box 8123, 6700 ES Wageningen, The Netherlands
Contact: kim.vannoort@wur.nl
T: +31 (0)317-485261