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Cloning of human glycosylation enzyme (GnTIV) with targeting signal to medial-Golgi stack to understand the expression pattern in the plant glycosylation pathway.

Lysosomal Storage disorders and Enzyme replacement therapy

Currently, 1 in 5,000 babies will be born with a type of lysosomal storage disorder [1]. These diseases are often neurodegenerative and can be caused by the deficiency of an enzyme, such as the fatal Wolman's disease that is caused by a complete deficiency of lysosomal acid lipase (LAL)[2]. To cure this issue, the effected individual must be treated with a recombinantly produced alternative, often made in chicken eggs(Figure 1) [3]

Figure 1: Image of the current production system for enzyme replacement therapy, chicken eggs [3]

Plant-based systems for production of recombinant enzymes

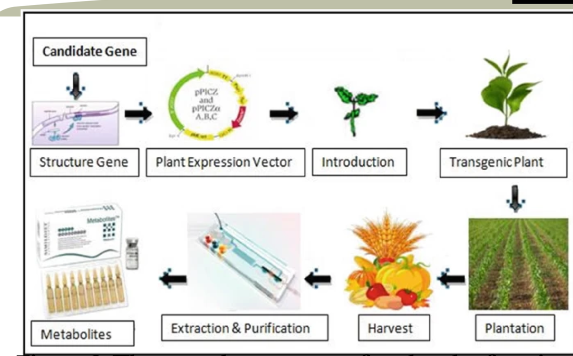

Figure 2: The complete process of molecular farming that could be used to produce a recombinant enzyme [5]

The production of recombinant enzymes within chicken eggs presents major issues with immunogenicity and sustainability [4]. Around 64% of infants had an allergic reaction, meaning that a new production system would be needed. One suggestion has been producing the enzymes within a plant system via process termed molecular farming(Figure 2) [5]

Synthetic Enginnering of Plant Glycosylation

To create a mammalian enzyme within a plant, a mammalian glycosylation profile would need to be engineered [6]. Glycosylation is a process that takes place in the Golgi bodies and modifies the function of a protein, such as an enzyme (Figure 3) [7]. The Golgi bodies in animals and plants have different enzymes, so the animal enzymes would have to be modified and put inside the plant in correct Golgi stacks to produce a fully competent human enzyme [7].The Golgi bodies are split into three stacks: the *cis,* medial and *trans* and each enzyme has to localise to the correct stack [8].

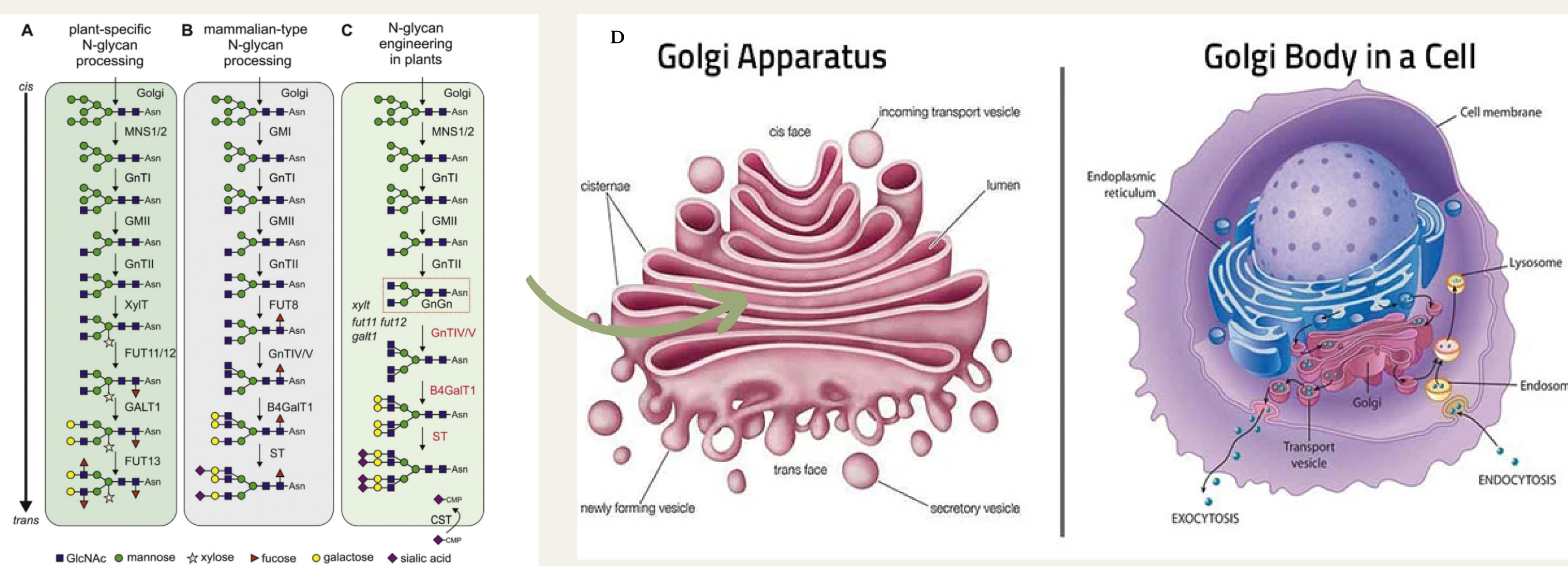

Figure 3: Diagrammatic representation of glycosylation within Golgi bodies. A.the glycosylation process enzymes in a plant. B. The glycosylation process and enzymes in an animal. C. The proposed engineered process and enzymes. D. The Golgi bodies diagram and within in the cell

Results

Confocal microscopy showed that MUR3-GnTIV-GFP was expressed within the tobacco plant and localised correctly to the Golgi bodies (Figure 6). This was established using markers that localise to the Golgi stacks. These markers are MNS1 which localises to the cis-Golgi stack, and ST which localises to the medial/trans-Golgi stack (Figure 6).

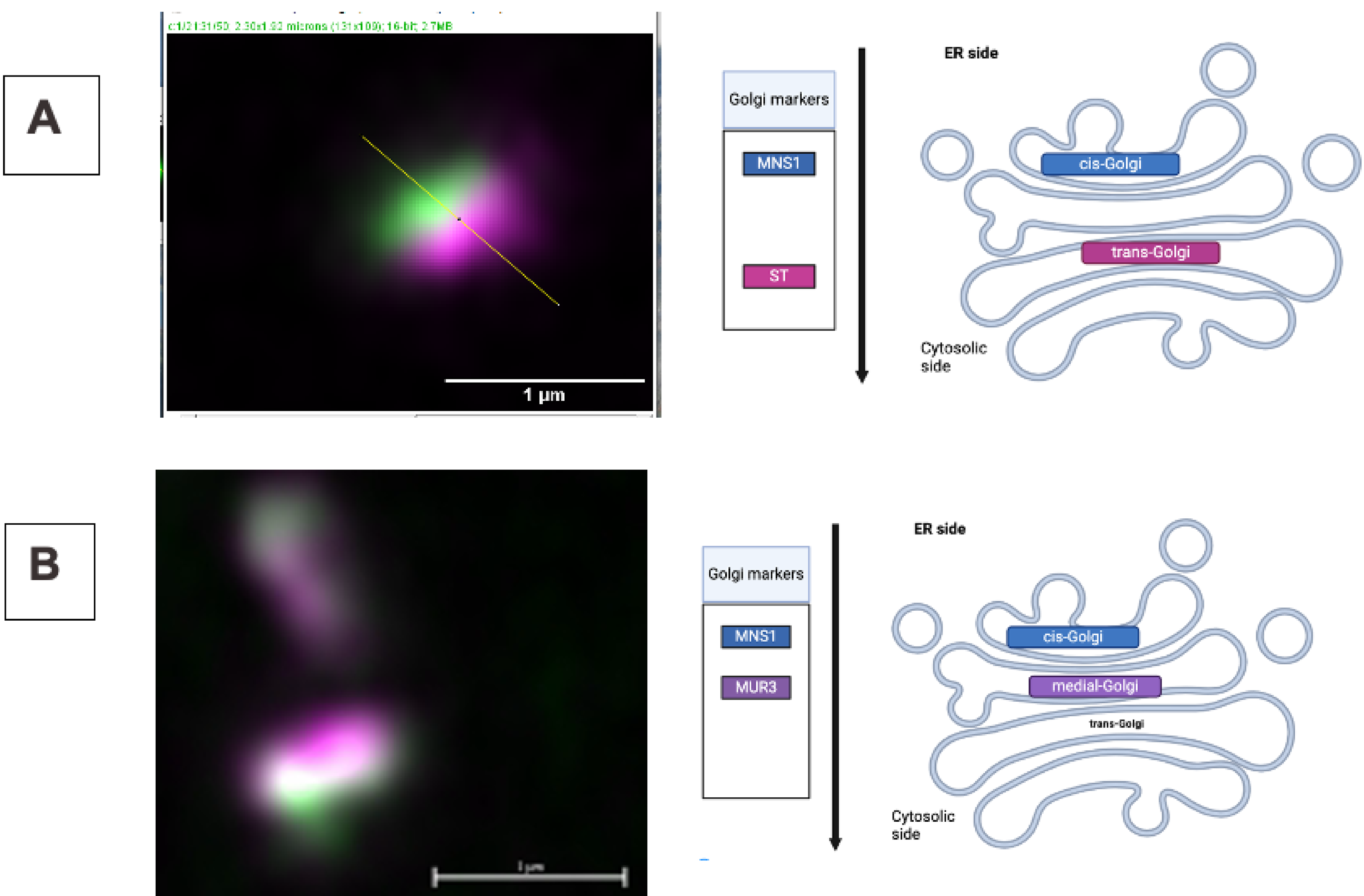

Figure 6: Confocal microscopy images of MUR3-GnTIV and markers. MNS1 and ST within the Golgi body. A. Markers MNS1, localising to the cis-Golgi stack and ST, localising to the medial/trans-Golgi stack.
B. Marker MNS1, localising to the cis-Golgi stack and MUR3-GnTIV, localising to the medial-Golgi stack.
Size bars = 1µm

Discussion

This study shows that MUR3-GnTIV can be expressed and targeted to the Golgi bodies within the tobacco plant. This can be applied to future research into engineering glycosylation within plants as it shows mammalian enzymes can be expressed within the tobacco plant. Future work could include looking at the expression of different mammalian glycosylation enzymes with in the tobacco plant to eventually look at producing the entire LAL enzyme within a tobacco plant to provide an alternative production source for enzyme replacement therapy to treat LSDs.

PROJECT AIMS

This project aims to express the human glycosylation enzyme, GnTIV to the medial-Golgi stack within *Nicotiana tabacum.* GnTIV will be fused to a fluorescent protein to allow imaging of the localisation with in the Golgi bodies. This localisation will allow further work to be completed.

METHODOLOGY

GnTIV with green fluorescent protein was cloned into a plant expression vector using gateway cloning (Figure 4). The MUR3-GnTIV-GFP destination vector was then inserted into the plant pathogen *Agrobacterium tumefaciens* and infiltrated into the tobacco plant leaf cells(Figure 5). Fluorescent microscopy (confocal) was used to see if GnTIV expressed and localised to the correct Golgi stack.

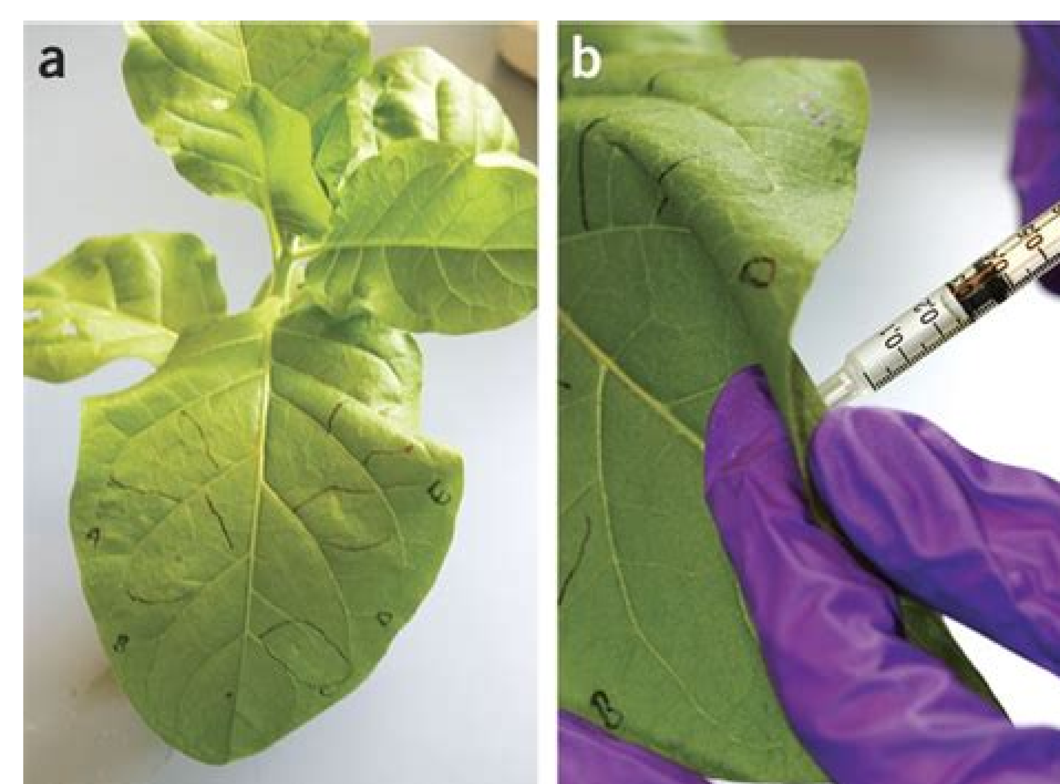

Figure 5: Syringe infiltration of MUR3-GnTIV-GFP into the tobacco plant with agrobacterium as a vector.

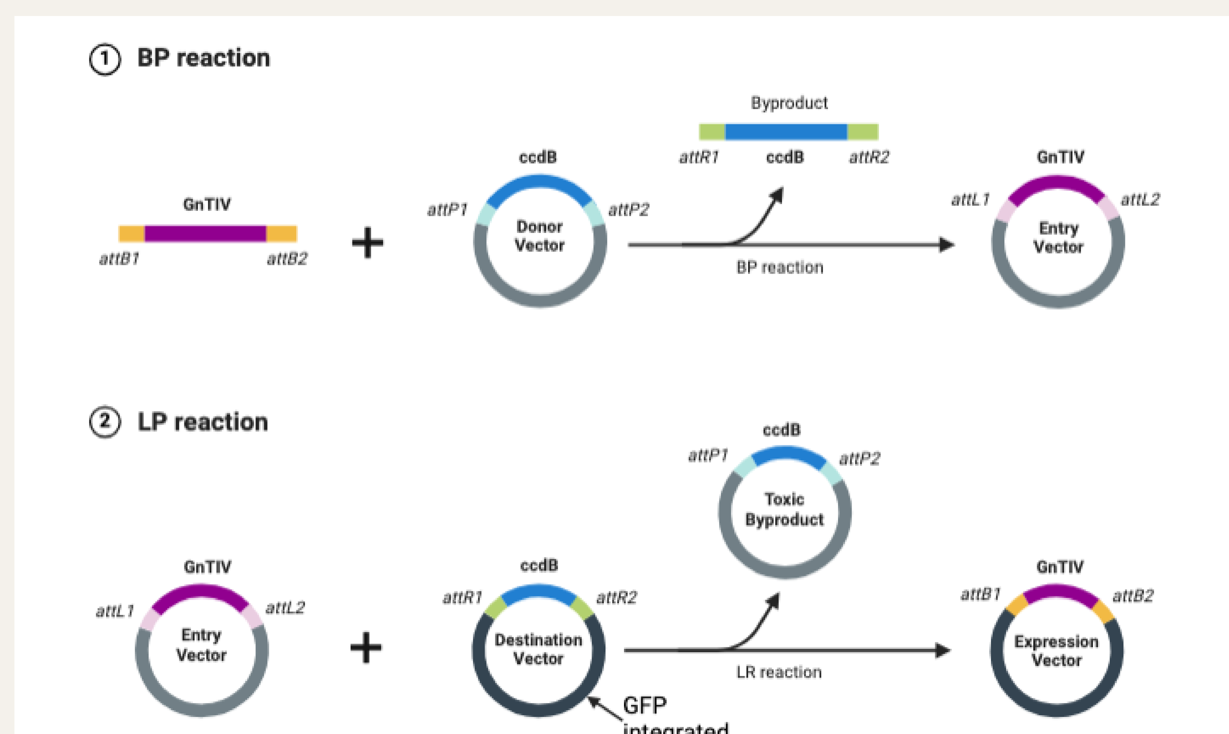

Figure 4: Methods used to express the human enzymes GnTIV within a tobacco plant. Gateway cloning reactions are taken in two steps: the BP and LR reaction.

References

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