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The choice of mammalian cell host and possibilities for glycosylation engineering

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Non-human mammalian cells such as CHO have been used predominantly for the production of biopharmaceuticals including monoclonal antibodies (Mabs). Although the glycosylation profile of these products is 'human-like' there is still the possibility of immunogenic epitopes such as α-Gal and Neu5Gc. Human cell lines have now been designed for high productivity of recombinant proteins and ensuring authentic glycosylation patterns. The control of glycosylation on such proteins is important for the efficacy of recombinant biopharmaceuticals as well as the immunogenic properties of viral vaccines such as influenza. We are now starting to understand some of the relationships between the structure of glycans and the function bestowed on the associated protein. This has promoted cell culture technologies for the targeted control of glycosylation to produce pre-determined glycan profiles of secreted products.

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Introduction

Glycosylation has a substantial influence on the physical and functional properties of recombinant glycoproteins, and is one of the most important critical quality attributes for optimal efficacy and safety of a biopharmaceutical [1]. Numerous factors affect cellular glycosylation, including the host cell line, the protein structure itself, media components and the culture conditions. A targeted approach in biomanufacturing involves the identification of the critical quality attributes of the glycoprotein molecule that maximizes the desired function. This drives the design of the bioprocess to ensure both batch to batch consistency and a product with maximal targeted clinical function. Appropriate glycosylation is central to this process of 'Quality by Design (QBD)' to ensure consistent and targeted glycan micro-heterogeneity and macro-heterogeneity [2,3].

Glycosylation of proteins and lipids involves a complex metabolic network of over 250 gene products that are likely to vary between cell lines (Figure 1) [4]. The complexity and variability of glycosylation is affected further by environmental factors such as substrate availability, hormonal regulation of enzyme activity and mediation by epigenetics [5].

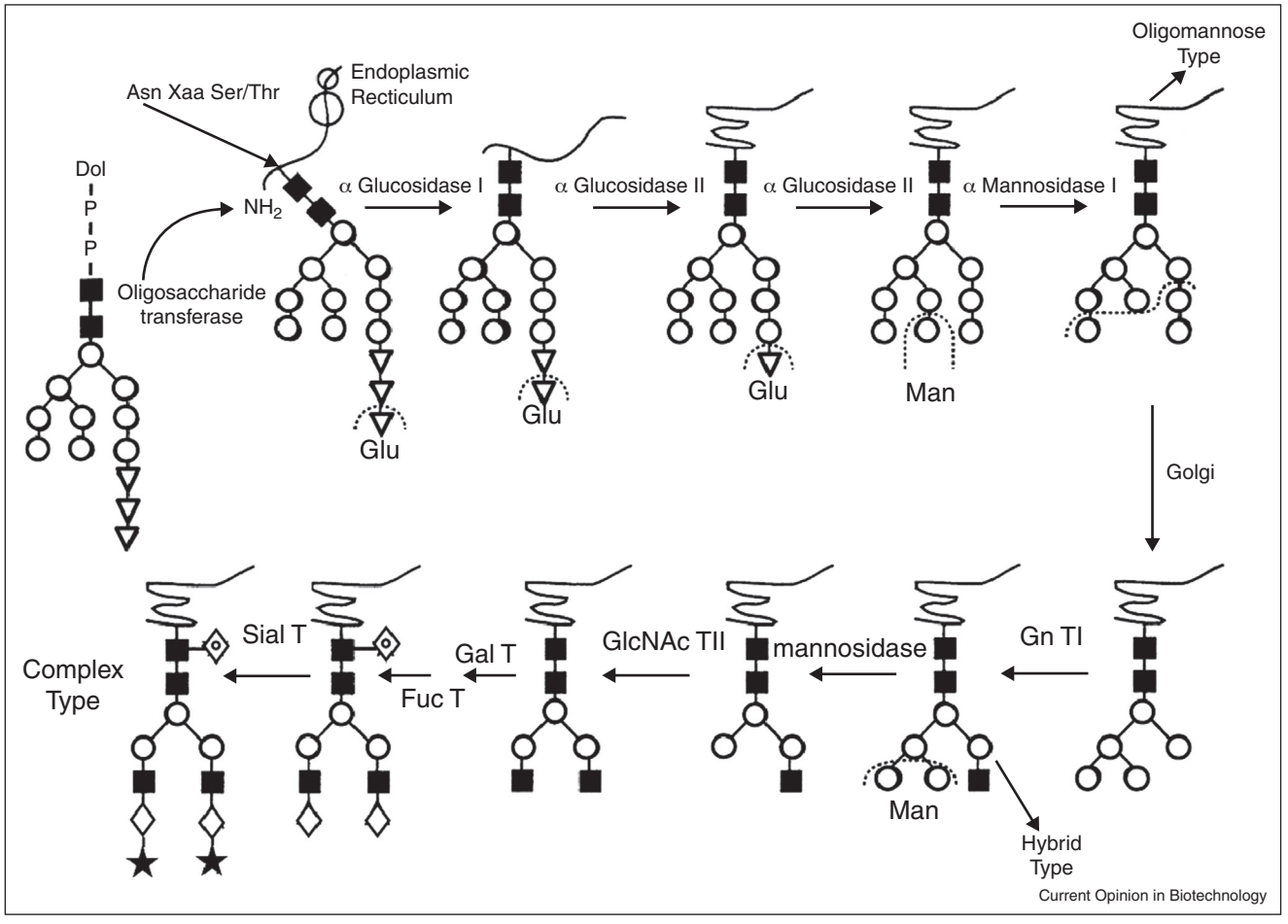
In this article we consider the choice of host cell line as well as the possibility of glycoengineering to obtain a targeted glycoform from the culture bioprocess. Controlling these factors serves to reduce glycosylation heterogeneity or produce a defined and restricted glycan profile.

Non-human mammalian host cell systems

Mammalian cell lines have been chosen for the production of most glycoproteins destined to be used as biopharmaceuticals because the resulting glycan profile is most similar to that of human proteins, at least compared to alternative plant, insect, bacterial or fungal cell platforms. Chinese hamster ovary (CHO) cell lines have become the predominant mammalian cell host used for commercial production for several reasons. They were the host cells used for the first approved recombinant biopharmaceutical — tissue plasminogen activator (t-PA) in 1986. This laid the ground for subsequent bioprocesses of recombinant therapeutics to be developed using these cells, thus minimizing the risk of delay of regulatory approval that could occur by introducing novel cell hosts. At least 70% of recombinant protein therapeutics are now produced from CHO cells [6]. The cells are well characterized and there are undoubtedly technical advantages in using them in commercial bioprocesses [7]. They are capable of growth in suspension, can be adapted to chemically defined media and are not receptive to human viruses. Several gene amplification systems have been established with CHO cells that allow high gene copy number and subsequent high specific protein productivity [8].

Baby hamster kidney cells (BHK21) and murine myeloma cells are alternative cell hosts. BHK21 have been used for the production of the coagulation factors, Factor VIIa and Factor VIII [9]. These are highly complex recombinant proteins requiring post-translational modification that can be provided by these cells of kidney origin [10–12].

Figure 1



N-glycosylation pathway in the endoplasmic reticulum and Golgi of mammalian cells. The upper part of the figure shows the consensus series of reactions that occurs in the endoplasmic reticulum of mammalian cells as well as lower eukaryotes. This starts with the transfer of the 14 unit oligomer glycan from the dolichol phosphate (Dol-P-P-) to the protein as it is synthesized. The lower part of the figure is a representation of some of the enzymic reactions occurring uniquely in the Golgi of mammalian cells. Although the reactions are shown in a linear sequence, this is for simplicity of presentation. In reality this is a network of competing reactions that gives rise to the heterogeneity of glycans found in the glycoproteins synthesized from mammalian cells. Key to symbols: ■ = GlcNAc, ○ = mannose, ◇ = galactose, ⟐ = fucose, ★ = neuraminic acid, ▽ = glucose.

Murine myeloma cells (NS0 and Sp2/0) have been used to produce a number of monoclonal antibodies (Mabs) as they were derived from a non-differentiated B cell and capable of high levels of immunoglobulin synthesis [13,14].

Potential immunogenicity of non-human mammalian cell products

There are significant variations in the capacity for glycosylation between different eukaryotes (animal, insect and fungi) but also between different mammalian species [15]. Hence recombinant glycoproteins produced in non-human mammalian cell lines may contain glycosylated structures that are antigenic in humans [8]. There are two critical, well-documented glycan epitopes that could elicit adverse immunological reactions. These are the Galα1,3-Gal residues (alpha-Gal) and N-glycolylneuraminic acid (Neu5Gc) epitopes (Figure 2), both of which can be expressed as terminal units on glycans [16].

Mouse cells have an α1,3-galactosyltransferase enzyme that produces glycans containing alpha-Gal [17]. This enzyme is inactive in humans, apes and old world monkeys but present in most other mammalian species [16]. The second potential immunogenic epitope is Neu5Gc, a terminal neuraminic acid, that is common in all non-primate mammalian cells [18••]. This is formed by the hydroxylation of the common human form of sialic acid N-acetylneuraminic acid (Neu5Ac) through an enzyme, CMP-Neu5Ac hydroxylase which is not expressed in humans. Murine cells express considerably higher levels of both of these epitopes compared to hamster (including CHO and BHK) and therefore recombinant products from murine cells are more likely to be immunogenic [19].