major oligomannoside (Serafini-Cessi *et al.,* Biosci. Rep. 4, 269 – 274, 1984). The other giycans in mature T-H appear to be processed mainly to polyantennary chains and to a minor extent to diantennary species. Our data allowed us to calculate that all eight potential *N*-glycosylation sites predicted by T-H sequence are actually glycosylated. In T-H expressed in HeLa cells the vast majority of the protein was membrane bound and only 10% was secreted in the culture medium. Supported by A.I.R.C and CNR Progetto finalizzato ACRO.

S6.9

The Role of Glycosylation in the Biosynthesis and Secretion of β-Amyloid Precursor Protein

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The senile plaques seen in the brain of patients with Alzheimer's disease contain β-amyloid (βA4). This peptide is derived by proteolytic cleavage from β-amyloid precursor protein (βAPP), a heavily glycosylated transmembrane glycoprotein. Since normal cells produce and secrete βA4, and since the βAPP sequence is normal in most Alzheimer's disease patients, it is important to understand how changes in βAPP metabolism lead to increased βA4 production and deposition. To study the role of glycosylation in this process, the 695 amino acid human βAPP isoform (βAPP_{695}) was transfected into wild-type and glycosylation-deficient Chinese hamster ovary (CHO) cells. Although βAPP has two potential *N*-glycosylation sites, using site-directed mutagenesis of βAPP_{695} we showed that only Asn_{467}, but not Asn_{496}, is glycosylated. Metabolically labeled, immunoprecipitated, non-mutated mature βAPP_{695} was resistant to Endoglycosidase H, suggesting that the *N*-glycan is of complex type. The synthesis and secretion of βAPP_{695} were decreased and delayed when transfected CHO cells were cultured in the presence of tunicamycin. Similar results were seen when non-mutated βAPP_{695} was expressed in the presence of castanospermine, which blocks *N*-glycan processing. Similarly, βAPP_{695} synthesis and secretion were decreased and delayed in transfected Lec 8 CHO cells. Lec 8 cells have a defect resulting in premature termination of *O*-glycans. Thus, alterations which prevent *N*-linked core glycosylation, block *N*-glycan processing, or interfere with *O*-glycosylation, perturb the metabolism of βAPP_{695}. Future studies will examine whether these types of metabolic perturbations lead to increased production and deposition of βA4.

S6.10

Core Glycosylation and N-Glycan Processing of Transmembrane and Secreted Forms of Rabies Virus Glycoprotein (RGP)

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RGP is a 505 amino acid Type I membrane glycoprotein with three potential *N*-linked glycosylation sites (sequons). Mutant forms of RGP deleting one or more sequons were generated by site-directed mutagenesis. Previous studies showed that 1) Asn_{37} was inefficiently core glycosylated, 2) Asn_{247} and Asn_{319} were efficiently core glycosylated, 3) glycosylation at any one sequon was required for RGP cell surface expression, and 4) core glycosylation efficiency at each sequon was unaffected by glycosylation at other sites. In the current study, wild-type and mutant RGP cDNAs were transfected into wild-type CHO cells. The *N*-glycans at Asn_{247} and Asn_{319} were 100% and 50% Endo H sensitive, respectively, when each was the only sequon on mutant RGP. In contrast, when both sequons were present, the *N*-glycans were almost completely Endo H resistant. This shows that *N*-glycan processing at individual RGP sequons is influenced by glycosylation at other sites. To compare glycosylation of secreted and transmembrane forms of RGP, a stop codon was inserted just upstream of the transmembrane domain creating a 434 amino acid truncated protein (RGP434); RGP434 variants deleting one or more sequons were also generated. Using a cell-free system transcription/translation/glycosylation system, core glycosylation efficiency at each RGP434 sequon was identical to that found with wild-type RGP. Large amounts of RGP434 (containing all three sequons) were found in the conditioned medium of transfected CHO cells; the *N*-glycans on the secreted protein were Endo H resistant. Although castanospermine (CS) did not affect the rate of RGP434 biosynthesis in transfected CHO cells, it markedly inhibited RGP434 secretion. In contrast, CS did not affect the biosynthetic and degradation rates of the transmembrane form of RGP. This suggests that *N*-glycan processing is important in the secretion of a truncated form of this protein.

S.7 BIOLOGICAL ROLE OF PROTEIN AND LIPID GLYCOSYLATION

S7.1

Topology and Regulation of Glycolipid Metabolism

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Glycosphingolipids are components of the outer leaflet of plasma membranes where they form patterns which are characteristic for individual cell types, stages of cell differentiation and oncogenic transformation. They are essential for cellular growth[1], stabilize cellular membranes and keep them impermeable to protons even at low pH[2]. Glycolipids have been identified as binding sites for toxins, viruses and bacteria[3]. Their implication in cell adhesion processes as ligands for selectins as well as their effects on growth factor receptors have also been described[4]. Synthesis of appropriate glycolipid and ganglioside derivatives allowed the investigation of uptake, intracellular transport and topology of ganglioside metabolism[5]. Biosynthesis of gangliosides, sialic acid containing glycolipids, starts with the formation of dihydroceramide (*N*-acylsphinganine) at the cytoplasmic surface of the endoplasmic reticulum. Subsequent glycosylation steps are catalyzed by membrane-bound transferases most of which are localized on the luminal surfaces of Golgi membranes[6]. Competition experiments with