Rothman J E & Lodish H F. Synchronised transmembrane insertion and glycosylation of a nascent membrane protein. *Nature* 269:775-80, 1977.
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The synthesis of the vesicular stomatitis virus glycoprotein, its insertion into endoplasmic reticulum membranes, and addition of two N-linked oligosaccharides were studied in a synchronized cell-free system. In order for the completed glycoprotein to be inserted properly, membrane addition was essential before the nascent chain was longer than ~100 amino acids. The two oligosaccharides were added in two steps when the nascent chain reached precise longer lengths. [The *SCI*® indicates that this paper has been cited in more than 440 publications.]

Travels of a Membrane Protein

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Since 1959, when as a high school student I worked with Robert Eckel at Western Reserve Medical School on potassium transport in red cells, I have been fascinated with the subject of how proteins are inserted into membranes. At that time and for many years after, there was no experimental system to study the biosynthesis of a specific membrane protein. My interests turned to the mechanism and regulation of messenger RNA translation, first using bacteriophage f_2 RNA, then globin mRNA. In 1972, David Baltimore and I began a collaborative set of studies on translation of vesicular stomatitis virus mRNAs that led us to an experimental system for studying glycoprotein synthesis and to the work described in this *Classic* paper.

Trudy Morrison, a postdoctoral fellow, and Martha Stampfer, a PhD student, set out to isolate mRNA from VSV-infected cells and translate it in a wheat germ cell-free system. They could easily detect cell-free synthesis of three viral proteins (N, NS, and M) but not of the G glycoprotein.[1] After eliminating several likely explanations, Trudy and I showed that the G mRNA was not extracted from the cells by the procedure used because it was bound to the endoplasmic reticulum (ER).[2] With Suzy Froshauer, I showed that the G mRNA was bound to microsomes by nascent G polypeptides.

The next contributions were made by two graduate students, David Knipe and Flora N. Katz. David elucidated the two forms of G protein in infected cells, one in the ER, and the other, with sialic acid, in the Golgi and on the cell surface; this was the first demonstration of the rough ER-to-Golgi-to-plasma membrane pathway for a glycoprotein.[3] Flora showed that the G protein, in the ER, was transmembrane, with a short segment at the C-terminus facing the cytoplasm, and the long N-terminus facing the ER lumen. Together with Jim Rothman and Vishu Lingappa in Gunter Blobel's lab, she showed that, when G was synthesized in a wheat germ cell-free extract in the presence of membrane vesicles derived from the rough ER of pancreas (but freed of endogenous ribosomes), this membrane protein was glycosylated and was asymmetrically inserted into the vesicle membrane, spanning the lipid bilayer, with the same orientation as the native G protein found in the rough endoplasmic reticulum in vivo.[4]

Jim E. Rothman, in this paper, was able to synchronize this system and to show that insertion and glycosylation of the G protein occurs in a series of precisely defined steps. Insertion of the nascent polypeptide must begin when the signal sequence at the N-terminus emerges from the ribosome. Addition of the two N-linked oligosaccharides occurs at precise times, apparently just after the asparagine residue can be recognized at the lumenal surface by the glycosyltransferase. The importance of this work was that it demonstrated the use of a cell-free system to study the initial steps of membrane protein synthesis. Much recent work has showed that the biogenesis of cellular plasma membrane proteins is very similar to that of the G protein. Notably, Rothman has pioneered the development of cell-free systems for the study of ER-to-Golgi-to-plasma membrane vesicular transport, again using the VSV G protein as a model. A long chapter I recently wrote in a textbook summarizes all this.[5]

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2. **Morrison T G & Lodish H F.** Site of synthesis of membrane and nonmembrane proteins of vesicular stomatitis virus. *J. Biol. Chem.* 250:6955-62, 1975. (Cited 190 times.)
3. **Knipe D M, Baltimore D & Lodish H F.** Separate pathways of maturation of the major structural proteins of vesicular stomatitis virus. *J. Virol.* 21:1128-39, 1977. (Cited 145 times.)
4. **Katz F N, Rothman J E, Lingappa V R, Blobel G & Lodish H F.** Membrane assembly in vitro: synthesis, glycosylation, and asymmetric insertion of a transmembrane protein. *Proc. Nat. Acad. Sci. USA* 74:3278-82, 1979. (Cited 370 times.)
5. **Darnell J, Lodish H & Baltimore D.** *Molecular cell biology.* New York: Scientific American Books, 1990. p. 639-80.

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