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Membrane glycoprotein gp130 of *Dictyostelium discoideum* is lipid-linked and its fate altered in the presence of tunicamycin

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We are studying the structure and role in phagocytosis of gp130, a glycoprotein located on the extracellular surface of *D. discoideum* amoebae. Predictions from the protein sequence of gp130 deduced from the cDNA indicate its attachment to the membrane via a glycosylphosphatidylinositol (GPI)-anchor. This was confirmed when radiolabeled palmitic acid was incorporated into gp130 of axenically grown cells, and when nitrous acid deamination released gp130 from purified membranes. The GPI-anchor of gp130 resisted cleavage by bacterial and mammalian phosphoinositol-specific phospholipases. However, in the presence of protease inhibitors, we detected *in vitro* a time-dependent cleavage of gp130 that was effectively inhibited by ZnCl₂. This activity and its inhibition were analogous to the release documented for two known GPI-linked proteins, gp80 of *D. discoideum* and VSG of *Trypanosoma brucei*. Cells treated with tunicamycin (0.5 µg/ml; TM), an inhibitor of N-linked glycosylation, produced an immunoreactive 85 kDa version of gp130 that was apparently unanchored and soluble rather than membrane-associated. The 85 kDa species did not bind Concanavalin A, and thus presumed to be devoid of (N-linked) carbohydrate. Also detected in TM-treated cells but absent in untreated, control cells were small quantities of an 81 kDa species that was either a second unglycosylated form of gp130 or a proteolytic product. TM-treated cells had two-fold less mature gp130 than control cells, as judged by densitometric analyses of immunoblots of cell lysates and sucrose gradient-purified plasma membranes. The totaled signals from the gp130-immunoreactive species of TM-treated cells were comparable to the gp130 signal of control cells. We conclude that the TM-inhibited glycosylation interfered with the attachment of the GPI-anchor to gp130. Consequently, a soluble and unglycosylated form of gp130 accumulated, presumably in the lumen of the ER/Golgi network.