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Immunology

# Evidence for peptide transport across microsomal membranes

**(antigen presentation/microsomes)**

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ABSTRACT **Antigenic peptides bound to class I molecules of the major histocompatibility complex (MHC) are recognized by T-cell receptors during development of an antiviral immune response. T cells respond to peptides derived from cytoplasmic viral proteins as well as viral membrane proteins, indicating that a pathway exists for the transport of proteins or peptides from the cytosol into the compartment(s) where the MHC class I molecules assemble. To investigate this pathway, we have developed an *in vitro* assay for the transport of peptides into microsomal vesicles. This assay provides evidence for the transport of chemically synthesized peptides (13–21 amino acids) containing N-linked glycosylation acceptor sequences, which serve as glycosylation substrates. Their transport results in depletion of the pool of available dolichol high-mannose oligosaccharides in the lumen of the microsomal vesicles. We have observed transport of peptides derived from antigenic human immunodeficiency virus gag and influenza B nucleoprotein sequences, but transport of a third randomly selected peptide was not detected, suggesting specificity of the transport process. We were not able to demonstrate ATP dependence of this peptide transport process by using apyrase and an ATPase inhibitor. This result was unexpected in light of the recent identification of MHC-linked genes with homology to ATP-binding cassette transporters, which have been proposed to mediate peptide transport.**

The existence of a route for transport of peptides from the cytoplasm into the secretory pathway is suggested by analysis of the immunological specificity of cytotoxic T cells. These effector cells recognize antigenic peptides bound to class I molecules of the major histocompatibility complex (MHC) (1) and have specificity for either cell-surface or cytoplasmic viral antigens. Since class I molecules associate with antigenic peptides during their biosynthetic export to the cell surface (2–4), peptides from cytoplasmic antigens must somehow be transported into the secretory pathway. Studies of class I assembly suggest that the endoplasmic reticulum (ER) is the site where peptides access class I molecules. Stable association of class I subunits for export from the ER requires the presence of peptides (5), as does the assembly of newly synthesized class I molecules in cell lysates (6, 7). Furthermore, assembly of class I subunits after *in vitro* translation and translocation into microsomes is stabilized by addition of peptides to the outside of these vesicles, implying peptide transport into the microsomal lumen (8).

The mechanism by which cytoplasmic antigenic peptides are transported into the ER is not yet established. To date, there are two known mechanisms by which proteins are transported across the ER membrane. In eukaryotic cells, nascent polypeptides containing a hydrophobic signal sequence are targeted to the membrane of the ER by the signal recognition particle (9) and are then cotranslationally transported through a proteinaceous channel in the ER membrane (10). In *Saccharomyces cerevisiae*, posttranslational transport of newly synthesized proteins into the ER has also been observed (11, 12), and it is not known whether this pathway uses the same membrane channel that is used for cotranslational translocation. Another pathway for membrane transport of peptides is mediated by membrane proteins belonging to the family of ATP-binding cassette (ABC) transporter molecules (13) that have multiple transmembrane domains attached to an ATP-binding domain. This transporter family includes the STE6 gene product in *S. cerevisiae* (14, 15) and the Opp operon gene products in bacteria (16), all implicated in transport of peptides. Another family member is the P-glycoprotein that mediates multiple drug resistance in mammalian cells (17, 18) by ATP-dependent export of small hydrophobic drugs (19, 20). Recently, two similar genes that appear to belong to the family of ABC transporters have been cloned from the class II region of the MHC in human, mouse, and rat (21–24). It has been suggested that the protein products of these genes might transport peptides into the ER, thus providing the third "subunit" needed for class I molecule assembly and rationalizing their presence in the MHC (21–25). Although these genes are required for stable class I molecule assembly (25), there is no direct evidence indicating that the MHC-linked transporter is transporting peptides and not some other necessary factor.

To study the mechanism and properties of peptide transport into the ER, we have established an assay to detect transport of peptides into microsomes. This assay takes advantage of the presence of a lumenal ER oligosaccharide transferase (26) that glycosylates the asparagine residue in the sequence NXS/T. The transported peptides contain this sequence and inhibit glycosylation reactions when present in the ER lumen. Using this assay, we have been able to study peptide import into the ER, independent of the ability of the peptide to bind to class I MHC molecules, and we have examined both the specificity and the energy requirements of the peptide transport process.

## MATERIAL AND METHODS

**Reagents.** Oligomycin (mixture of oligomycins A, B, and C), Con A-Sepharose 4B, and apyrase were from Sigma. cDNA encoding pre-pro-$\alpha$ factor from *S. cerevisiae* (pDJ100 gift of D. Julius, University of California, San Francisco) was subcloned into the pSP64 plasmid (27). The following peptides were synthesized: human immunodeficiency virus gag residues 265–279 (KRWIILGLNKIVRMYC), NYS gag derivative (Ac-NYSGGKRWIILGLNKIVRMYC), influenza strain B nucleoprotein (NP) residues 85–94 (KLGEFYNQMM), NYS-NP derivative (Ac-NYSKLGEFYNQMM), RG4 peptide (CLQVEQLLYESPERYS), and NYS-RG4 derivative (Ac-NYSLQVEQLLYESPERYS) were derived

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Abbreviations: MHC, major histocompatibility complex; ER, endoplasmic reticulum; ABC, ATP-binding cassette; NP, nucleoprotein.

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from residues 739–753 of the RING4 gene product (21). N termini of the NYS-containing peptides were acetylated.

**Preparation of Microsomal Vesicles.** Microsomal vesicles were prepared from canine pancreas (28), and polysomes removed by a salt wash [1 M KOAc/50 mM triethanolamine/2 mM $Mg(OAc)_2$/1 mM dithiothreitol (DTT)/1 mM EDTA was added to microsomes suspended in buffer A (50 mM triethanolamine, pH 7.5/250 mM sucrose/1 mM DTT)]. Unless otherwise indicated, the salt-washed microsomal vesicles (20 $\mu$l at 2 equivalents per $\mu$l) were incubated 1 hr at 25°C in buffer A containing peptides and then washed three times. Apyrase treatment of microsomal vesicles was in buffer A (pH 6.5) for 10 min at 30°C followed by three washes.

**Translation and Translocation Assays.** Translation and translocation of pre-pro-$\alpha$ factor mRNA were in 20-$\mu$l wheat germ extracts (29) with 25 $\mu$Ci (1 Ci = 37 GBq) of [$^{35}$S]methionine, microsomal vesicles (4 equivalents), and 1.25 mM ATP at 26°C (29). One-half of the reaction volume was digested with protease K (30) and samples were analyzed by SDS/PAGE (31) and autoradiography.

**Detection of Glycopeptide Formation in Partially Extracted Microsomes.** Microsomal vesicles (20 $\mu$l) were partially extracted with 0.05% Nikkol detergent (octaethylene glycol *n*-dodecylether; Nikko Chemicals, Tokyo)/1 mM $MnCl_2$/1 mM $CaCl_2$/0.1 M NaCl for 5 min at 4°C (49). Radioiodinated peptide (0.25 $\mu$Ci; specific activity, 10 Ci/mmol) was incubated with extracted microsomes at 25°C for 1 hr, and then vesicles were completely solubilized in 1% Triton X-100/0.5 M NaCl/1 mM $MnCl_2$/1 mM $CaCl_2$/0.2 M Hepes, pH 7.4 (binding buffer), with or without 0.4 M methyl $\alpha$-D-mannopyranoside or 0.4 M D-galactose. Samples were incubated with Con A-Sepharose 4B beads and washed three times with binding buffer (32) before cpm bound was measured. Peptide [0.5 mg/ml in 100 $\mu$l of 150 mM phosphate (pH 7.4)] was iodinated with 1 mCi of $Na^{125}I$ (Amersham IMS.30) using one Iodo-Bead (Pierce) and purified by gel filtration (Sephadex G-10).

## RESULTS

To study processes that might be involved in import of antigenic peptides into the ER, we have developed an assay measuring the transport of peptides across microsomal membranes. Glycosylation of peptides bearing the NXS/T acceptor sequence is an indication of their access to the lumen of microsomal vesicles (33) and forms the basis of our transport assay. Three peptides were chemically synthesized containing or lacking an N-terminal glycosylation acceptor sequence, acetyl-NYS. The core sequences for two of the peptides (gag 265–279 and NP 85–94) were derived from cytosolic viral proteins and have been shown to bind to class I HLA molecules (human MHC) (34, 35). The third peptide sequence [RG4 739–753 (21)], to which an acceptor sequence was added, was randomly selected. To determine whether the synthetic peptides with acceptor sequences (acceptor peptides) could be glycosylated, they were iodinated and incubated in the presence of partially extracted canine pancreas microsomal vesicles, through which peptides have direct access to lumenal glycosyltransferase and carbohydrates. Then the microsomes were completely solubilized in detergent and glycosylation of peptides was demonstrated by their binding to Con A coupled to Sepharose (Fig. 1). The Con A binding of acceptor peptides, NYS-RG4, NYS-NP, and NYS-gag, was dependent on their exposure to microsomes and was blocked by competition with methyl $\alpha$-D-mannopyranoside but not galactose, as expected for the carbohydrate specificity of Con A. In contrast, no specific binding to Con A was observed for the nonacceptor peptides RG4, NP, and gag after exposure to extracted microsomes. This experiment suggests that all three acceptor peptides can serve as specific substrates for glycosylation in the lumen of the ER.

An initial experiment indicated that when the iodinated NYS-NP peptide was incubated with intact microsomes, the amount of peptide glycosylated after transport was too low to detect by direct binding to Con A. Thus, we tested the ability of acceptor peptides imported into the ER to inhibit glycosylation of a "readout" protein (Fig. 2). Inhibition of glycosylation depended on the ability of acceptor peptides to deplete the pool of dolichol high-mannose oligosaccharide, which can be transferred to the sequence NXS/T (36). After preincubation of canine pancreas microsomes with acceptor or nonacceptor peptides, the microsomes were tested in a translation/translocation reaction with mRNA for yeast pre-pro-$\alpha$ factor, which is normally modified by the addition of three high-mannose oligosaccharide side chains (37). Inhibition of glycosylation of pre-pro-$\alpha$ factor was then monitored by electrophoretic analysis.

When pre-pro-$\alpha$ factor mRNA was translated in the absence of microsomes, a polypeptide of 18.5 kDa was produced and was susceptible to degradation after exposure to protease K (Fig. 3*A*). When the mRNA was translated in the presence of microsomes, pre-pro-$\alpha$ factor molecules receiving three, two, one, or zero high-mannose sugars were produced. All of these species were located inside the lumen of the microsomal vesicles and were protected from degradation by protease K added to the reaction mixture. Consistent with previous reports, the translocated form of pre-pro-$\alpha$ factor without any high-mannose modification migrated slightly slower than the untranslocated form. This has been attributed to anomalous mobility in SDS/PAGE after signal sequence cleavage (38). When pre-pro-$\alpha$ factor was translated in the presence of microsomes preincubated (60 min) with two acceptor peptides, NYS-gag and NYS-NP, there was a substantial inhibition of pre-pro-$\alpha$ factor glycosylation. There was a concomitant increase in the amount of translocated but unglycosylated species. The appearance of this latter species was the most diagnostic indicator of inhibition of glycosylation. After preincubation of microsomes with the

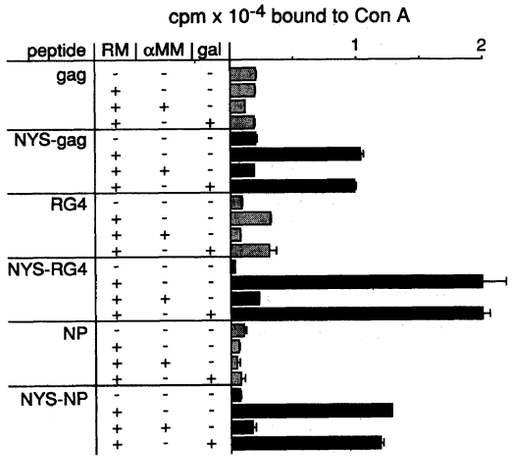


FIG. 1. Glycosylation of peptides in partially extracted microsomes. Iodinated peptides were incubated for 1 hr with microsomes (RM) partially extracted with detergent. The microsomes were then completely solubilized in detergent and the lysate was incubated with Con A beads. Binding was carried out in the absence of competing carbohydrate or in the presence of methyl $\alpha$-D-mannopyranoside ($\alpha$MM) or D-galactose (gal). Iodinated peptides gag, NYS-gag, RG4, and NYS-RG4 were adjusted to equivalent specific activities. The specific activities of iodinated NP and NYS-NP peptides were equivalent but lower than that of the other peptides. Glycopeptide formation is indicated by cpm bound to Con A beads. The small amount of RG4 binding that can be blocked by competition with $\alpha$MM is most likely due to a low level of nonspecific binding of this peptide to glycolipid or glycoprotein in the microsomal extract that is sticking to the Con A beads.

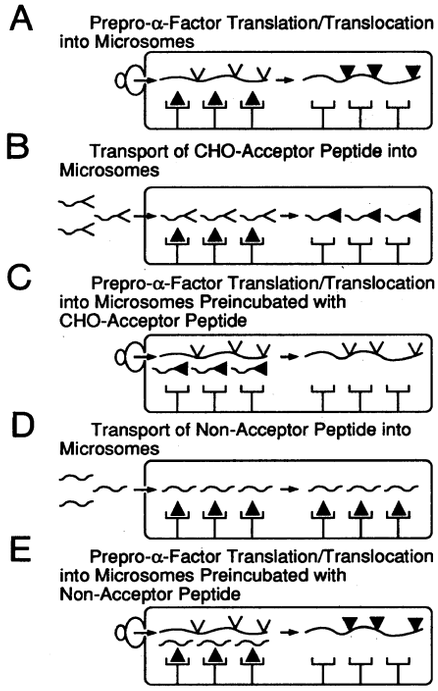


FIG. 2. Assay to measure transport of peptides into microsomes. (*A*) Yeast pre-pro-α factor mRNA is translated, translocated, and glycosylated at three sites. (*B*) Peptide with the NYS sequence [carbohydrate (CHO)-acceptor peptide] is transported into microsomes and glycosylated, depleting the pool of available lipid-linked carbohydrate. (*C*) Yeast pre-pro-α factor translocated into microsomes preincubated with acceptor peptides is not glycosylated. (*D*) Nonacceptor peptides are translocated but do not affect the donor CHO pool. (*E*) Yeast pre-pro-α factor translocated into microsomes preincubated with nonacceptor peptides is glycosylated normally.

nonacceptor peptides gag and NP, the translocated, unglycosylated molecules were not produced, and the pattern of pre-pro-α factor glycosylation was the same as that found in the absence of preincubating peptide.

A third acceptor peptide tested, NYS-RG4 (18 residues), did not inhibit pre-pro-α factor glycosylation (Fig. 3*B*). NYS-RG4 can be glycosylated (Fig. 1), so lack of inhibitory activity suggests that it was present at considerably lower levels in the microsomal vesicles. Although intermediate in length, it appears to be transported to a lesser extent than the 13-residue NYS-NP or the 20-residue NYS-gag peptides. This selective transport demonstrates that peptide transport into microsomal vesicles was not due to diffusion or leakage.

Kinetic properties of peptide transport into microsomes were analyzed by varying the peptide concentration, as well as the time and temperature of preincubation, prior to assaying inhibition of pre-pro-α factor glycosylation (Fig. 4). Inhibition was concentration dependent and increased from 32% after preincubation of microsomes with 100 μM NYS-NP to 67% with 375 μM peptide. Percentage inhibition was determined by quantitative analysis of autoradiograms, expressing inhibition as a ratio of translocated, unglycosylated pre-pro-α factor to total translocated pre-pro-α factor (Fig. 4*A*). Increasing the time of preincubation with 660 μM peptide at 25°C indicated accumulation of transported peptide during the preincubation period. After 15 min of preincubation with peptide, 40% of the translocated pre-pro-α factor was unglycosylated, while after 60 min of preincubation, the unglycosylated species increased to 73% of total translocated pre-pro-α factor (Fig. 4*B*). At 4°C, 26% of the pre-pro-α factor was unglycosylated after 15 min of preincubation and there was no apparent increase in peptide transport over time. These results revealed a temperature-

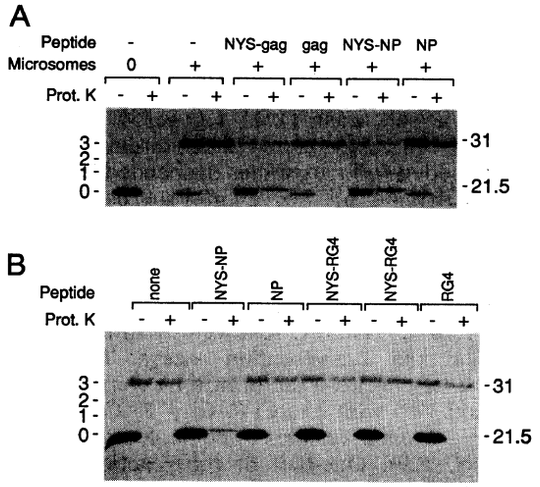


FIG. 3. Differential effect of peptides containing N-linked glycosylation acceptor sequences on depletion of high-mannose oligosaccharides from microsomal vesicles. (*A*) Microsomal vesicles were preincubated for 1 hr at 25°C with peptides (375 μM) containing or lacking the N-terminal extension NYS. The vesicles were subsequently washed extensively and used in *in vitro* translation/translocation assays for synthesis of pre-pro-α factor. Bands show synthesized, translocated, and glycosylated pre-pro-α factor. Protease K was added to the indicated reaction mixtures to digest untranslocated protein. Note the prominent band at ≈20 kDa generated after preincubation with acceptor peptides that is protease K resistant. (*B*) NYS-RG4 does not deplete the available pool of high-mannose oligosaccharide. Two separate stock solutions of NYS-RG4 (375 μM) were incubated with microsomes as described in *A*. Then vesicles were assayed for glycosylation of pre-pro-α factor. Note the difference between the effects of NYS-NP and NYS-RG4. Numbers on the right are molecular mass markers (kDa). Numbers on the left indicate the number of high-mannose oligosaccharide side-chain modifications of pre-pro-α factor.

sensitive component to peptide transport across microsomal membranes that mediates a time-dependent increase in transported peptide. The low level of time-independent peptide

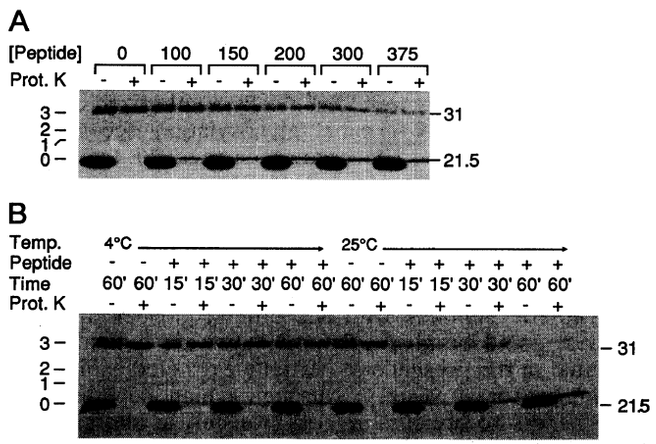


FIG. 4. Peptide-mediated depletion of microsomal high-mannose sugars is dependent on concentration, time, and temperature. (*A*) Increasing concentrations (μM) of NYS-NP peptide were preincubated at 25°C with microsomal vesicles for 1 hr. The vesicles were subsequently washed and used in an *in vitro* translation/translocation assay for the synthesis of pre-pro-α factor. (*B*) NYS-NP (660 μM) was incubated at either 4°C or 25°C with microsomal vesicles for increasing periods of time. The microsomes were extensively washed and assayed for glycosylation of pre-pro-α factor. Numbers on the right are molecular mass markers (kDa). Numbers on the left indicate the number of high-mannose oligosaccharide side-chain modifications of pre-pro-α factor.

transport, observed after preincubation with peptide at 4°C, could result from peptides bound to the surface of microsomes at 4°C that are then translocated into microsomes when translation of yeast pre-pro-α factor is initiated at 26°C.

Energy requirements for peptide transport were examined by carrying out the preincubation of microsomal vesicles with peptide in the presence or absence of ATP and an ATP-regenerating system (Fig. 5*A*). There was no change in peptide-mediated inhibition of pre-pro-α factor glycosylation under either condition. To ensure the absence of endogenous ATP, microsomal vesicles were incubated with apyrase (39) for 10 min, washed before incubation with peptides, and washed again before use in the translation of pre-pro-α factor. Pretreatment with apyrase did not affect peptide-mediated inhibition of pre-pro-α factor glycosylation (Fig. 5*B*).

To investigate whether molecules with properties of the ABC transporter family are involved in peptide transport across microsomal membranes, peptide transport was tested after exposure to oligomycin. This noncompetitive inhibitor of ATPases (40, 41) has been shown to inhibit drug transport activity of the P-glycoprotein when present at 10 μM (42). To maximize the potential effect of oligomycin, the inhibitor was incubated with microsomes in the presence of the lowest concentration of peptide (200 μM) that induced a detectable effect on glycosylation. Microsomes were then extensively washed and used in the pre-pro-α factor translation/translocation reaction. There was no substantial effect of up to 250 μM oligomycin on peptide-mediated inhibition of pre-pro-α factor glycosylation (Fig. 6). Increasing the oligomycin concentration to 650 μM or adding the oligomycin to microsomes 30 min before incubation with peptide also had no effect (data not shown).

## DISCUSSION

We have provided evidence for transport of peptides with a glycosylation acceptor sequence into microsomal vesicles. Our assay measures the effect of transported peptides on glycosylation events inside the vesicle lumen (33). Previously, Kvist and Hamann (8) demonstrated that peptides incubated with microsomes could facilitate the association of MHC class I subunits, suggesting that microsomal vesicles could import MHC-binding peptides (8). Here we were not restricted to studying transport of MHC-binding peptides. We found that two MHC-binding peptides with glycosylation acceptor sequences were transported, but a third randomly chosen acceptor peptide was not. The peptide selectivity in our assay suggests that the import of peptides into microsomes is not simply due to diffusion, although transport measured did not appear to be ATP dependent. During the course of our studies, we learned that Levy *et al.* (43) measured the import of MHC-binding peptides into microsomes both by MHC binding and by BiP-binding, and also found the transport process to be ATP independent.

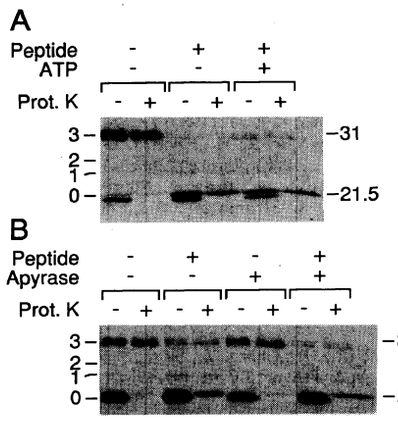


FIG. 5. Peptide-mediated inhibition of glycosylation is not affected by addition or depletion of ATP during preincubation of microsomes with peptides. (*A*) NYS-gag (375 μM) was incubated at 25°C with microsomes in the absence or presence of an ATP regenerating buffer (1.6 mM ATP, 15–20 units of creatine phosphokinase, and 16 mM creatine phosphate). The microsomes were extensively washed and used for *in vitro* translation and translocation of pre-pro-α factor. (*B*) Microsomal vesicles were treated with 0.6 unit of apyrase for 10 min or were mock-treated by incubation in the same buffer without apyrase, after which they were washed extensively and incubated in the presence or absence of 375 μM NYS-NP for 1 hr at 25°C. The vesicles were pelleted and used for *in vitro* translation and translocation of pre-pro-α factor. Numbers on right are molecular mass markers (kDa). Numbers on left indicate the number of high-mannose oligosaccharide side-chain modifications of pre-pro-α factor.

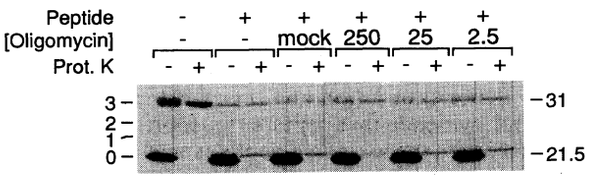


FIG. 6. Peptide-mediated inhibition of glycosylation is not affected by oligomycin. Microsomal vesicles were untreated, mock-treated, or incubated with oligomycin (concentrations shown are μM) in the presence or absence of 200 μM NYS-NP. The microsomes were extensively washed and used for *in vitro* translation and translocation of pre-pro-α factor. Numbers on right are molecular mass standards (kDa). Numbers on left indicate the number of high-mannose oligosaccharide side-chain modifications of pre-pro-α factor.

The measurement of peptide import into microsomal vesicles in our assay, as in the assays carried out by Levy *et al.* (43), is indirect since these assays depend on peptide interaction with other elements in the vesicle lumen. Thus, the question of whether we are actually measuring transport should be considered. In our assay, we see effects that are specific to the peptides with acceptor sequences, implying that their effect is due to their glycosylation in the lumen of the microsome and subsequent depletion of carbohydrate available to glycosylate pre-pro-α factor. It would be difficult to explain inhibition of glycosylation of pre-pro-α factor by peptides binding to the outside of microsomes. Although such binding could conceivably affect the cytoplasmic domain of the ribophorins, which have been reported to have oligosaccharyltransferase activity (R. Gilmore, personal communication), this mechanism would not account for the specificity of inhibition observed only with acceptor peptides.

Our conclusion that the peptide transport we detect is ATP independent rests on the assumption that transport takes place during preincubation of peptides with microsomes. We found that addition of apyrase or oligomycin during this stage of the assay does not alter the inhibitory effect of the peptides on pre-pro-α factor glycosylation. We cannot completely rule out the alternative possibility that peptides are not transported during the preincubation step but merely bind to the outside of the vesicles and are then translocated during the synthesis of yeast pre-pro-α factor, a reaction involving ATP. However, we believe that transport occurs during preincubation of peptides with microsomes since increasing the preincubation time increases the inhibition of yeast pre-pro-α factor glycosylation, implying increased peptide transport during the preincubation step (Fig. 4). Using a newly developed filtration assay that measures the association of radioactive peptides with microsomes on their surface and in the lumen, we have recently established that increasing the time of incubation of peptides with microsomes does not increase the amount of peptide bound to the surface of microsomes but it does increase the amount of peptides transported into the lumen of microsomes (data not shown). This further suggests

that the apparent increase in peptide transport is not a function of increased surface binding over time and it seems to occur by an accumulation of transported peptide before pre-pro-$\alpha$ factor translation and the translocation reaction.

Given our evidence that peptide transport into the ER may be selective, then the antigenic peptides that can bind to class I MHC molecules would undergo initial selection at the transport stage before their binding to the MHC molecules, a step that is also selective. With regard to predicting antigenic determinants, it will be of interest to define the features of peptides that allow them to be transported into the ER. These characteristics may determine an initial requirement for antigenicity before the MHC binding step. The lack of detectable transport of NYS-RG4 cannot be attributed to its size since it is intermediate in length between two peptides that are transported, so selection must be due to another physical parameter such as sequence, charge, or hydrophobicity. Peptides eluted from the cleft of class I molecules have been identified as octamers or nanomers (44, 45) and we have demonstrated the transport of peptides ranging from 13 to 21 residues. This would suggest that initial proteolytic processing of peptides may occur in the cytosol, but that subsequent processing could be completed in the ER (46).

The recent cloning of two MHC genes with predicted sequence homology to ABC transporters (21–24) and their requirement for class molecule assembly (25) has raised speculation that these gene products may be responsible for transport of MHC-binding peptides into the ER. Cells that are missing genes encoding subunits of the MHC-linked transporter are defective in class I molecule assembly (25, 47) and appear to have only a limited number of peptides available to bind MHC molecules (5, 25). The fact that these cells can assemble and export some MHC molecules suggests that they are missing an efficient peptide transport system, rather than defective in coassembly of class I molecules with available peptides (48). Based on activity of characterized members of the ABC transporter family (19, 20), it would be expected that the function mediated by the MHC gene products would be ATP dependent. There are several explanations for our inability to measure ATP dependence of the peptide transport process. First, the MHC-linked ABC transporters may not be involved in peptide import but they may mediate ATP-dependent supply of other molecules, such as ions that are required for class I molecule assembly. This seems unlikely based on the fact that some MHC molecules can assemble with peptides in cells defective in transporter gene expression. Second, the MHC-linked transporters may be different from other members of the transport family and may not require ATP for their transport activity. Third, the concentrations of peptides that are required to detect a transport signal in our assay may be higher than the concentration of peptides in the cytoplasm. Perhaps at high peptide concentrations ATP dependence of transport, which would be evident at lower concentrations, can be overcome. Last, the expression of MHC-linked transporters appears to be low in many tissues, sensitive to interferon regulation, and increased in transformed lymphoid cells (21). If levels of the MHC-linked transporter are very low in pancreatic microsomes, we may be measuring a peptide transport process that is not a function of the MHC gene products but reflects another, less efficient, peptide import pathway. For example, peptides may be imported at a low level through the same channel in the ER membranes that translocates nascent polypeptides bearing signal sequences (10). The relevance of a possible second pathway in supplying peptides for MHC class I molecule assembly will then have to be considered.

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