In Vitro Synthesis and Membrane Insertion of Bovine MP26, an Integral Protein from Lens Fiber Plasma Membrane

DAVID L. PAUL and DANIEL A. GOODENOUGH
Department of Anatomy, Harvard Medical School, Boston, Massachusetts 02115

ABSTRACT Synthesis of MP26, the principal protein of lens fiber plasma membranes, was directed in the reticulocyte lysate system by poly A mRNA enriched from whole bovine lens RNA using oligo (dt)-cellulose chromatography. Synthesized MP26 was enriched by immune precipitation. The in vitro-synthesized MP26 had an electrophoretic mobility indistinguishable from that of the native molecule. MP26 showed a cotranslational requirement for dog pancreas microsomes in order for membrane association to occur. Microsome-associated in vitro-synthesized MP26 showed a sensitivity to digestion with chymotrypsin which was similar to the sensitivity of native MP26 in isolated lens fiber plasma membranes, indicating correct insertion of the MP26 into the microsome. Synthesis and membrane insertion of MP26 using N-formyl-[35S]methionyl tRNA as label demonstrated that no proteolytic processing or significant glycosylation accompanied membrane insertion. Chymotryptic cleavage of membrane-inserted, N-formyl-[35S]methionine-labeled MP26 resulted in loss of label, suggesting that the N-terminal of the in vitro-synthesized MP26 faces the cytoplasm.

MP26 is a major integral membrane protein of the vertebrate eye lens. In the mature lens fiber cell, MP26 represents >50% of the total membrane protein (4). It is not detectable in early embryonic lens or in mature lens epithelial cells and thus has been used as a marker in the differentiation of epithelial cells into fiber cells (30).

MP26 has a molecular weight of ~26,000 daltons. The nature and extent of glycosylation are unclear. Wong et al. (34) found that the MP26 could be stained by the periodic acid-Schiff (PAS) reaction. Sas et al. (25) also found MP26 to be PAS positive but noted that it became positive even when periodic acid was omitted. On the other hand, Alcalá et al. (1) found MP26 to be PAS negative, and Brockhuys and Kuhlmann (8) reported no detectable glycosylation by gas chromatographic analysis of purified MP26. Serologically and biochemically related proteins have been found in fiber cells from a wide variety of sources including primate, amphibian, and avian species (28). MP26 exhibits unusual properties of stability. Since essentially no protein turnover occurs in differentiated fiber cells (35), MP26 synthesized in the prenatal lens must last the life of the organism. Breakdown of MP26 has been implicated in senility and hereditary cataract formation (29).

The functional role of MP26 remains unclear. There is no known enzymatic activity associated with this protein although some affinity for calmodulin has been demonstrated (32). Lens membranes exhibit extraordinary insulating properties more like artificial lipid bilayers than biological membranes (16). The high concentration of MP26 in lens membranes must be compatible with the maintenance of this high membrane resistance.

A possible role for MP26 is in the formation of lens fiber junctions. Lens fiber junctions are extremely abundant between fiber cells. Due to the abundance of junctions and the predominance of MP26 in the lens fiber membranes, it has been suggested that MP26 may be the major structural component of lens fiber junctions. However, MP26 displays no structural homology or serological identity with gap junction proteins isolated from liver (11, 18). Bok et al. (5) have reported an immunocytochemical localization of MP26 at the cytoplasmic surface of lens fiber junctions. However, using similar techniques, we have been unable to support their finding (20). Further investigation is required to adequately resolve this issue.

The abundance of MP26 and the relatively simple protein composition of the lens fiber plasma membrane afford an opportunity to study the in vitro synthesis and membrane insertion of this integral membrane protein. To date, a study of the early events of protein synthesis in vitro has been accomplished for only a limited number of integral plasma membrane polypeptides (33), versus a vast literature that has accumulated reporting the synthesis of intracellular membrane proteins and secretory proteins (24). A previous study of the synthesis of
MP26 (22) reported that the mRNA coding for the MP26 was preferentially associated with a lens fiber cytoskeleton-membrane complex.

In this paper we have studied the in vitro synthesis and membrane insertion of MP26. MP26 exhibited a cotranslational requirement for the presence of dog pancreatic microsomes in order to achieve membrane insertion. In vitro membrane insertion was topographically correct as assayed by the pattern of chymotryptic cleavage products. Using N-formyl-[35S]methionine, we demonstrated that no cleavage of N-terminal sequences accompanies membrane insertion. Chymotryptic cleavage of MP26 synthesized in vitro with N-formyl-[35S]methionine and inserted into microsomes results in loss of label, indicating that the N-terminal of the MP26 may face the cytoplasmic side of the microsome.

MATERIALS AND METHODS

The preparation and characterization of antisera were described in the preceding paper (15, 20). For these experiments, affinity-purified antiMP26 antibodies (αMP26) and whole antiCT20 antiserum (αCT20) were used.

Isolation of RNA: Adult bovine lenses were decapsulated and stored at −80°C until use. Lenses were homogenized in 10 vol of 1% SDS, 50 mM Tris, pH 7.4, with 5 mM EDTA in a Dounce homogenizer with loose pestle. RNA was isolated conventionally using phenol-chloroform-isooamyl alcohol (23). Poly A RNA was extracted from total RNA by chromatography on oligo (dT)-cellulose (Clontech, Mountain View, CA) as described by Aviv and Leder (2). Poly A RNA was isolated from bovine pituitary glands by Mg2+ precipitation according to Palmer (19).

In Vitro Translation: Rabbit reticulocyte lysate was prepared and nucleate treated according to the procedure of Pelham and Jackson (21). Lysate contained 50 μM amino acids except methionine, 0.75 mM MgCl2, 100 mM KCl, 15 mM creatine phosphate and [35S]Methionine (New England Nuclear, Boston, MA). Jurkat, calf liver tRNA (Boehringer Mannheim Biochemicals, Indianapolis, IN) was at 50 μg/ml except where N-formyl-[35S]methionyl tRNA was utilized. A+ RNA was added at 0.6–1.2 A260 U/ml lysate and translated for 60 min at 30°C.

Dog pancreatic microsomes were prepared according to Shields and Blobel (26) and treated with micrococcal nuclease. Microsomes were included in translation assays at 2–4 A260 U/ml. After translation, microsomes were separated from lysates by centrifugation on a sucrose gradient. Lysates were diluted to 500 μl and layered on top of a 0.5 M/2.0 M sucrose gradient constructed in a 1.5-ml Eppendorf microcentrifuge tube. Gradients were spun at 100,000 g × 30 min in a Beckman SW-27 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, CA) using specially made adapters. Membranes are recovered at the 0.5 M/2.0 M interface.

For posttranslational assays, microsomes were added after translation had occurred for 60 min. Further elongation was inhibited with cycloheximide at 10 μg/ml. Incubation was continued for another 60 min, then the microsomes were gradient isolated as described.

For experiments involving proteolytic digestion, soluble and microsomal fractions were made 3 mM with tetracaine HC1 and 100 μg/ml with TLCK-Chymotrypsin (Sigma Chemical Co., St. Louis, MO). After 30 min at 4°C, proteolysis was terminated with 1/1,000 vol of freshly-made PMSF (17 mg/ml) in isopropanol.

In some experiments [35S]methionine was replaced with 1 mM cold methionine and N-formyl-[35S]methionyl tRNA. Calf liver tRNA was aminocylated in the presence of [35S]methionyl and formylated as described by Mitiara and Blobel (17). Charged and formylated tRNA had a specific activity of 5 × 106 cpm/μg and was included in the lysate at 1 mg/ml.

Immunoprecipitation: Samples were prepared for immunoprecipitation by diluting to 1 ml to final concentrations of 0.15 M NaCl, 50 mM Tris pH 8.2, 5 mM EDTA, 0.05% NP40, 0.05% SDS, and 1 mM fresh PMSF (NET buffer). 2.5 μl of affinity-purified antibody (0.7 mg/ml) or antisera was added and incubated at 4°C for 1 h, 100 μl of IgSorb (New England Enzyme Center, Boston, MA) was added and incubation continued for 30 min. IgSorb was pelleted (5,000 g × 5 min, Beckman JS-13 rotor), then washed twice in 1 ml NET buffer, and once in 1 ml 50 mM Tris pH 7.6. Pellets were resuspended in 15–25 μl sample of dissolving buffer for SDS PAGE and incubated at 37°C for 10 min. Gels were prepared as described in the preceding paper (13, 20) and fluorographed according to Bonner and Laskey (6). To serve as standards, MP26 and a chymotrypsin fragment called CT20 (20,000 Mw) were isolated from adult lens membranes (20) and 14C-labeled according to Dottavio-Martin and Ravel (9), except that all buffers contained 0.1% SDS.

RESULTS

In Vitro Translation

Lens mRNA was translated in vitro, immunoprecipitated with αMP26, electrophoresed on an SDS gel, and fluorographed. Fig. 1 shows the immunoprecipitation with affinity-purified antisera against MP26 (αMP26) (lane B) and preimmune IgG (lane C). Lane A contains a 14C-labeled MP26 standard and lane D contains the MP26 standard and a 20,000 dalton chymotryptic fragment (CT20) standard. αMP26 specifically precipitated a band that had an apparent mobility indistinguishable from that of labeled MP26 standard. This suggested that MP26 was synthesized without a cleavable leader sequence of the type found in many membrane proteins and nearly all secreted proteins (24, 33).

Membrane Insertion

For study of the association of newly synthesized protein with membranes, translation was performed in the presence of dog pancreatic microsomes. These membranes have been shown to support in vitro processing and insertion of membrane and secreted proteins from an enormous variety of sources (3). After translation, the microsomes were separated from the soluble components of the lysate on a sucrose gradient, and the two fractions were immunoprecipitated. Fig. 2 shows the soluble (lane B) and microsomal (lane C) fractions precipitated with αMP26. MP26 standard is contained in lane A.

Under these conditions of translation, nearly all of the newly synthesized MP26 became associated with the microsomes. If microsomes were added to the lysate posttranslationally, most of the newly synthesized MP26 remained in the soluble fraction (lane D). Only a trace amount was found associated with the microsomes (lane E).

When MP26 is synthesized and sedimented on a sucrose gradient without any addition of microsomes, most of it stays in the soluble supernatant but a very small fraction is recovered at the 0.5/2.0 M interface (data not shown). Therefore, the trace amount of MP26 in Fig. 2, lane E can be considered “background,” and not indicative of posttranslational membrane integration. The background in the precipitations involving membrane additions (Fig. 2) is less than that observed in Fig. 1, because insoluble material in the lysate that contributes to the background is separated out on the sucrose gradient.

In these experiments, equal volumes of lysate were immunoprecipitated. Total protein synthesis was actually inhibited ~50% by microsomes. This is reflected in the difference in background between the cotranslated soluble fraction (lane B) and posttranslated soluble fraction (lane D). We observed an apparent stimulation of MP26 synthesis in the presence of microsomal membranes (lane C).

These results suggest that the MP26 exhibited a cotranslational requirement for microsomal membranes in order to become associated with them. As found in the translation experiments without microsomes, there was no reproducibly detectable difference in SDS mobility between the MP26 standard and the MP26 synthesized in vitro with microsomes. Due to limitations in the resolution of the SDS gels, these data did not completely rule out the possibility of a cleaved leader sequence. The amount of cleavage could have been too small to detect by this method (perhaps five to six amino acids).
FIGURE 1 In vitro synthesis of lens MP26. Immunoprecipitated MP26 was analyzed by SDS PAGE and fluorography (see Materials and Methods). Lanes A and D contain authentic MP26 and CT20 prepared from isolated plasma membrane and labeled with [14C]-formaldehyde. Lane B: Immunoprecipitation with affinity-purified aMP26. Lane C: Immunoprecipitation with preimmune IgG.

Alternatively, a more extensive cleavage could have been balanced by a substantial glycosylation.

For a critical demonstration of the lack of N-terminal protein cleavage associated with membrane insertion, translation was performed using N-formyl-[35S]methionyl tRNA as a source of label. With this label, newly synthesized proteins can become labeled only at their N termini. A control experiment is shown in Fig. 3, lanes A–D. Bovine anterior pituitary mRNA was translated in normal lysate with (lane B) and without (lane A) microsomes. Preprolactin (Ppl) and pregrowth hormone (Pgh) were processed by microsomes to yield prolactin (pl) and growth hormone (gh). When translated with N-formyl-[35S]methionyl tRNA, the preproteins were labeled (lane C) but processed proteins, which lose their N termini, were not visible (lane D).

MP26 was synthesized in the presence of microsomes with N-formyl-[35S]methionyl tRNA (Fig. 3). The microsomes were sucrose gradient isolated and the membrane (lane E) and soluble fractions (lane F) were separately immunoprecipitated.
FIGURE 3 In vitro translation using N-formyl-[35S]methionyl-tRNA. Control experiment using bovine pituitary mRNA is shown in lanes A–D. Arrows indicate preprolactin (Ppl), pregrowth hormone (Pgh), prolactin (pl), and growth hormone (gh). Lanes A and B: Pituitary mRNA is translated without and with microsomes, respectively, using [35S]methionine. Lanes C and D: Pituitary mRNA is translated without and with microsomes, respectively, using N-formyl-[35S]methionyl-tRNA. In lanes E and F, lens mRNA is translated in the presence of microsomes, using N-formyl-[35S]methionyl-tRNA. Microsomes are separated from remainder of lysate and separately immunoprecipitated with αMP26 (see Fig. 2, lanes B and C). Lane E: Immunoprecipitation of microsomes. Lane F: Immunoprecipitation of lysate. Lane G contains standards as in Fig. 1.

The membrane fraction (lane E) contained the majority of MP26 with a small amount visible in the soluble fraction (lane F). The presence of radiolabeled membrane-associated MP26 demonstrates that membrane association occurred without cleaving an N terminal sequence and without significant glycosylation.

Orientation of MP26 in the Membrane

The topology of the membrane association produced in vitro was investigated in the following experiments. In isolated adult lens membranes, MP26 was shown to yield a 20,000 Mr fragment called CT20 upon exhaustive digestion with chymotrypsin (20). If MP26 was inserted into microsomal membranes with the same orientation as found in vivo, then chymotryptic digestion of MP26 synthesized in vitro in the presence of microsomes would be expected to yield the CT20. Since the antigenic sites recognized by the antibody used in the previous experiments were destroyed by chymotryptic digestion, a new antiserum (αCT20) directed against the CT20 was prepared (20).

Lens A+ mRNA was cotranslated in the presence of microsomes. Following sucrose gradient enrichment, the microsomes were then digested with chymotrypsin and precipitated with αCT20. Fig. 4 (lane B) is a fluorograph of this precipitate. Lane A contains MP26 and CT20 standards. The major species present in lane B has a mobility indistinguishable from that of authentic CT20. The immunoprecipitate also contains a small amount of a peptide of ~24,000 Mr. A species with a similar
mobility is sometimes detectable in digests of isolated plasma membrane, but is quantitatively less significant (see Discussion). Immunoprecipitation of posttranslationally incubated microsomes or cotranslationally inserted microsomes digested in the presence of 1% NP40 yielded blank lanes (data not shown).

Fig. 4 (lanes C and D) shows an experiment designed to determine the location of the MP26 N-terminus. MP26 was synthesized with microsomes using N-formyl-[35S]methionyl-tRNA as a source of label. The microsomes were separated from the soluble fraction on a sucrose gradient; half were precipitated with aCT20 (lane C) and half were digested with chymotrypsin and precipitated with aCT20 (lane D). Gel loading was adjusted so that bands corresponding to MP26 and CT20 would have equal intensity if synthesized conventionally with [35S]methionine. The disappearance of the CT20 in lane D indicates that the chymotrypsin removes the N-terminus. Control experiments using microsomes to process in vitro translated prolactin and growth hormones indicate that the microsomal vesicle protects 90% of the processed hormone from digestion with exogenous proteases (data not shown).

DISCUSSION

Our data indicate that MP26 is synthesized without a cleavable leader sequence and inserts into membranes cotranslationally. In vitro-synthesized MP26 shows no detectable difference in SDS gel mobility from native MP26. Insertion of in vitro-synthesized MP26 into microsomal membranes also produces no detectable alteration in SDS gel mobility. No known cleaved leader sequence is smaller than ~1,500 daltons, a difference that would be easily detected in the gel system used here. The appearance of membrane-inserted MP26 with an N-formyl-[35S]methionine-labeled N-terminal amino acid residue rules out the possibility that the cleavage of a leader sequence is balanced by glycosylation concomitant with membrane insertion. These data support the finding of several groups (1, 8) that native MP26 is not substantially glycosylated. If MP26 does not undergo asparagine-linked core glycosylation, a direct insertion into plasma membrane is at least theoretically possible, as reported for the large nonglycosylated subunit of Na+, K, ATPase (24). However, we found that neither co- nor post-translational incubation of MP26 with isolated lens fiber plasma membrane vesicles resulted in membrane insertion of the MP26 (data not shown).

We have used proteolytic digestion of synthesized MP26 to probe the nature of its in vitro membrane insertion. Both in isolated lens fiber plasma membranes and in microsomes with MP26 inserted in vitro, the major product of chymotryptic digestion is a 20,000 Mₐ, fragment termed CT20. We have interpreted this to indicate that insertion of MP26 into microsomal membrane substantially resembles that found in plasma membranes in vivo. However, immunoprecipitation of chymotrypsin-digested microsomes reveals a significant amount of a species of ~24,000 Mₐ. Thus, MP26 in microsomal membranes is slightly less sensitive to proteolysis than MP26 in isolated plasma membranes. A similar differential sensitivity to proteolysis was observed for opsin by Goldman and Blobel (10). While the significance of the finding is unclear, it could imply that important posttranslational processing steps, which could change sensitivity to proteolysis, accompany MP26 assembly in the lens fiber plasma membrane.

Experiments with proteolytic digestion of N-formyl-[35S]methionine-labeled MP26 suggest that the N terminal is exposed at the cytoplasmic surface of microsomal vesicles. By analogy with respect to other membrane proteins whose in vitro membrane insertion has been studied, this result suggests that the N-terminus of plasma membrane MP26 faces the cytoplasm. However, it cannot be rigorously excluded that the N-terminus is on the luminal side of the microsome, and that only the cleavage site is exposed on the cytoplasmic surface. Further, the disposition of the C-terminus is not known, and some C-terminal as well as N-terminal residues may be removed by chymotrypsin.

Initially, proteins with intracellularly oriented N-termini, such as the erythrocyte plasma membrane Band 3 (27), were difficult to reconcile with the simple signal hypothesis model. Recent modifications of this hypothesis (3, 12, 14, 24) allow for variations in the orientation of proteins in the membrane by postulating internal sequences rather than N-terminal sequences to specify membrane insertion. For example, Braell and Lodish (7) have shown that for Band 3, about half of the protein can be synthesized before the ability to become membrane inserted is lost. They postulate an internal signal sequence 450 amino acid residues away from the N-terminus.

The apparent stimulation of MP26 synthesis in the presence of microsomes is puzzling. Recently, Walter et al. (31) have described a complex of proteins called signal recognition protein (SRP). SRP was shown to specifically bind polysomes synthesizing signal peptide-containing proteins and to inhibit their synthesis in the absence of membranes. In light of this finding, it is possible that the synthesis of MP26 is actually being inhibited in the absence of membranes by SRP-like components of the lysate. However, it is not known whether "internal" signal sequence proteins such as erythrocyte Band 3 or MP26 utilize an SRP-dependent mechanism.

This study would not have been possible without generous contributions of time, materials, and expertise from many quarters. We thank Dr. Joan Ruderman and Mr. Eric Rosenthal for help in preparing mRNA and lysate; Dr. Dan Perlman for his gift of microsomes; Drs. Tom Rajbhandary, Herb Weissbach, and Nick Robakis for help in preparing tRNA; and Drs. Harvey Lodish and D. Biswas for helpful discussions.

This work was supported by grants #EY 02430 and GM 18974 from the National Institutes of Health.

Received for publication 25 June 1982, and in revised form 15 November 1982.

REFERENCES

1. Alcalà, J., N. Lieska, and H. Maisel. 1975. Protein composition of bovine lens cortical fiber cell membranes. Exp. Eye Res. 21:581–595.
2. Aviss, H., and P. Leder. 1972. Purification of biologically active globin mRNA by chromatography on oligothymidylic acid cellulose. Proc. Natl. Acad. Sci. USA. 69:1408–1412.
3. Blobel, G. 1980. Intracellular protein topogenesis. Proc. Natl. Acad. Sci. USA. 77:1496–1500.
4. Bloemendahl, H., A. Zweers, F. Vermocken, I. Dunia, and E. L. Beneditti. 1972. The plasma membrane of eye lens fibers. Biochemical and structural characterization. Cell Differ. 1:91–106.
5. Bok, D., J. Dockstader, and J. Horowitz. 1982. Immunocytochemical localization of the lens membrane intrinsic polypeptide (MP26) in communicating junctions. J. Cell Biol. 92:213–220.
6. Bonner, W. M., and R. A. Laskey. 1974. A film detection method for Tritium labelled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. 46:83–88.
7. Braelli, W. A., and H. F. Lodish. 1982. The erythrocyte anion transport protein is cotranslationally inserted into microsomes. Cell 30:33–31.
8. Brockley, R. E., and D. D. Kuhlmarm. 1978. Lens membranes. IV. Preparative isolation and characterization of membranes and various membrane proteins from calf lens. Exp. Eye Res. 26:305.
9. Dottiasso-Martin, D., and J. M. Ravel. 1978. Radiolabeling of proteins by reduction with 3H formaldehyde and sodium cyanoborohydride. Anal. Biochem. 87:562–572.
10. Goldman, B. M., and G. Blobel. 1981. In vitro biosynthesis, core glycosylation and membrane integration of opsin. J. Cell Biol. 90:236–242.
11. Hertzberg, E. L., D. J. Anderson, M. Friedlander, and N. B. Gihila. 1982. Comparative analysis of the major polypeptides from liver gap junctions and lens fiber junctions. J. Cell Biol. 92:33-39.

12. Jackson, R. C., P. Walter, and G. Blobel. 1980. Secretion requires a cytoplasmically disposed sulphydryl of the RER membrane. Nature (Lond.) 284:174-176.

13. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.) 227:680-686.

14. Lodish, H. F., W. A. Brasell, A. L. Schwartz, G. J. A. M. Strons, and A. Zilberstein. 1981. Synthesis and assembly of membrane organelle proteins. Int. Rev. CytoL. (Suppl.) 12:247-307.

15. March, S. C., L. Parikh, and P. Cnatrecasas. 1974. A simplified method for cyanogen bromide activation of agarose for affinity chromatography. Anal. Biochem. 60:449-452.

16. Mathies, R. T., J. L. Rae, and R. S. Eisenberg. 1979. Electrical properties of structural components of the crystalline lens. Biophys. J. 25:181-201.

17. Mihara, K., and G. Blobel. 1980. The four cytoplasmically made subunits of yeast mitochondrial cytochrome C oxidase are synthesized individually and not as a polypeptide. Proc. Natl. Acad. Sci. U.S.A. 77:4610-4616.

18. Nicholau, B., M. W. Hunkapiller, L. E. Hood, and J. P. Revel. 1980. Partial sequencing of the gap junctional protein from rat lens and liver. J. Cell Biol. 97:200a. (Abstr.)

19. Palmiter, R. D. 1974. Magnesium precipitation of ribonucleoprotein complexes. Expedient techniques for the isolation of undegraded polysomes and messenger ribonucleic acid. Biochemistry. 13:3606-3615.

20. Paul, D. L., and D. A. Goodenough. 1983. Preparation, characterization, and localization of antisera against bovine MP26, an integral protein from lens fiber plasma membranes. J. Cell Biol. 96:625-632.

21. Pelham, R. B., and B. J. Jackson. 1976. An efficient mRNA dependent translation system from reticulocyte lysates. Eur. J. Biochem. 67:247-256.

22. Ramakers, F. C. S., A. M. E. Selten-Versteegen, E. L. Benedetti, I. Dunia, H. Bloemendahl. 1980. In vitro synthesis of the major lens membrane proteins. Proc. Natl. Acad. Sci. U.S.A. 77:225-229.

23. Ruderman, J. V., and M. L. Parduie. 1978. A portion of all major classes of histone messenger RNA in amphibian oocytes in polyadenylated. J. Biol. Chem. 253:2018-2025.

24. Sabatini, D. D., G. Krebich, T. Morimoto, and M. Adesnik. 1982. Mechanisms for the incorporation of proteins in membranes and organelles. J. Cell Biol. 92:1-22.

25. Saz, D., P. Koenig, K. Johnson, and R. Johnson. 1980. Characterizing gap junctions from cell lens: questions of glycosylation and protease sensitivity. J. Cell Biol. 87:96a. (Abstr.)

26. Shields, D., and G. Blobel. 1978. Efficient cleavage and segregation of nascent presecretory proteins in a reticulocyte lysate supplemented with microsomal membranes. J. Biol. Chem. 253:3753-3756.

27. Steck, T. L. 1978. The band 3 protein of the human red cell membrane. A review. J. Supramol. Struct. 9:311-324.

28. Takemoto, I. J., J. S. Hansen, and J. Horowitz, 1981. Intraspacess conservation of the main intrinsic protein (MIP) of the lens membrane. Comp. Biochem. Physiol. B Comp. Biochem. 68:101-106.

29. Tanaka, M., P. Russel, S. Smith, S. Uga, T. Kubawara, and J. H. Kinoshita. 1980. Membrane alterations during cataract development in the Nakano mouse lens. Invest. Ophthalmol. 19:619-629.

30. Vermorken, A. J. M., J. M. C. Hilderick, I. Dunia, E. L. Benedetti, and H. Bloemendahl. 1977. Changes in membrane protein pattern in relation to lens cell differentiation. FEBS Lett. 72:301-306.

31. Walter, P., I. Ibrahim, and G. Blobel. 1981. Translocation of proteins across the endoplasmic reticulum. I. Signal recognition protein (SRP) binds to in vitro assembled polyribosomes synthesizing secretory protein. J. Cell Biol. 91:543-550.

32. Welsh, M., J. Aster, M. Ireland, J. Alcala, and H. Maisel. 1982. Calmodulin binds to chick lens gap junction protein in a calcium-independent manner. Science (Wash. DC). 216:642-644.

33. Wickner, W. 1980. Assembly of proteins into membranes. Sciaman (Wash. DC). 210:861-868.

34. Wong, M. M., N. P. Robertson, and J. Horwitz. 1978. Heat induced aggregation of the SDS solubilized main intrinsic polypeptide isolated from bovine lens plasma membrane. Biochem. Biophys. Res. Commun. 84:158-165.

35. Young, R. W., and H. W. Fulhorst. 1966. Regional differences in protein synthesis within the lens of the rat. Invest. Ophthalmol. 5:288-297.