Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 6918–6921, October 1985
Cell Biology

The cellular defect in α_1-proteinase inhibitor (α_1-PI) deficiency is expressed in human monocytes and in *Xenopus* oocytes injected with human liver mRNA

(α_1-antitrypsin/secretory defect)

DAVID H. PERLMUTTER*, ROBERT M. KAY†, F. SESSIONS COLE‡, THOMAS H. ROSSING§, DAVID VAN THIEL¶, AND HARVEY R. COLTEN||

*Divisions of Gastroenterology and Nutrition, Children's Hospital Medical Center, and Department of Pediatrics, Harvard Medical School, ‡Division of Neonatology, Children's Hospital Medical Center, and Department of Pediatrics, Harvard Medical School, §Department of Medicine, Harvard Medical School, and ||Division of Cell Biology, Childern's Hospital Medical Center, and Department of Pediatrics, Harvard Medical School, Boston, MA 02115; ¶Division of Gastroenterology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261; and †Genetics Institute, Boston, MA 02115

Communicated by Kurt J. Isselbacher, June 24, 1985

ABSTRACT **To determine the basis for low serum concentrations of α_1-proteinase inhibitor (α_1PI) in individuals with homozygous α_1PI deficiency (hereafter referred to as PiZZ), biosynthesis and secretion of α_1PI were studied in *Xenopus* oocytes microinjected with hepatic mRNA and in blood monocytes (an extrahepatic site of α_1PI gene expression). Although both the usual α_1PI (hereafter referred to as PiMM) and PiZZ α_1PI were secreted in functionally active form, the rate of secretion of α_1PI was significantly and selectively decreased in *Xenopus* oocytes injected with PiZZ liver mRNA and in monocytes from PiZZ individuals. The apparent size of α_1PI in the intracellular compartment of *Xenopus* oocytes injected with PiZZ liver mRNA was different from the corresponding intracellular PiMM α_1PI in oocytes injected with PiMM liver mRNA. There were also differences in the relative ratio of native and complexed α_1PI secreted by monocytes from individuals with PiMM and PiZZ phenotypes.**

Homozygous PiZZ α_1-proteinase inhibitor (α_1PI) deficiency is an autosomal recessive disorder resulting in low serum levels of functionally normal α_1PI. It is associated with an increased incidence of pulmonary emphysema and chronic liver disease.

Although adequately designed direct studies of α_1PI secretion in individuals homozygous for α_1PI deficiency (hereafter referred to as PiZZ) have not been reported, several lines of indirect evidence have suggested that low serum levels of α_1PI in PiZZ individuals result from a defect in secretion of α_1PI. First, inclusion bodies can be found in the endoplasmic reticulum of PiZZ hepatocytes. These inclusion bodies contain α_1PI according to immunofluorescence studies and amino acid analysis of material isolated from the inclusions (1, 2). Second, PiZZ α_1PI isolated from intrahepatocyte inclusion bodies appears to be incompletely glycosylated (3, 4). There is less sialic acid, galactose, and *N*-acetylglucosamine and more mannose in hepatic PiZZ α_1PI, suggesting that core carbohydrates cannot undergo secondary processing. Accelerated catabolism of the variant protein has been excluded as an explanation for low serum concentrations of PiZZ α_1PI (5–7).

A single amino acid substitution (8–10), encoded by a single nucleotide substitution (11), has been identified in the PiZZ α_1PI variant, but this substitution does not occupy an oligosaccharide attachment site or a site known to undergo co- or posttranslational processing. X-ray crystallography studies by Loebermann *et al.* (12) have suggested that the substitution of lysine for glutamate at residue 342 in the PiZZ α_1PI variant can affect the conformation of α_1PI. This conclusion was based on the crystal structure of α_1PI complexed with serine protease, so that the conformation of intact native, plasma, or hepatic PiZZ α_1PI can only be inferred. Furthermore, Bathurst *et al.* (4) have shown that α_1PI isolated from the liver of a PiZZ individual is functionally active, suggesting that it is in native conformation in the endoplasmic reticulum-Golgi axis.

In this study the hypothesis that a defect in secretion accounts for low serum concentrations of α_1PI in PiZZ individuals was directly tested by examining two cell culture systems—*Xenopus* oocytes injected with PiMM (individuals homozygous for the normal α_1PI hereafter will be referred to as PiMM) and PiZZ liver mRNA and peripheral blood monocytes from PiMM and PiZZ individuals—in which synthesis and secretion of α_1PI could be analyzed kinetically.

MATERIALS

Dulbecco's modified Eagle's medium, Dulbecco's modified Eagle medium lacking methionine, Hanks' balanced salt solution, and fetal bovine serum were purchased from GIBCO and medium 199 was from M. A. Bioproducts (Walkersville, MD). [^{35}S]Methionine (specific radioactivity, ≈1000 Ci/mol; 1 Ci = 37 GBq) and deoxycytidine 5′-[α-^{32}P]triphosphate (specific radioactivity ≈3000 Ci/mmol) were obtained from New England Nuclear and [^{14}C]methyl-labeled protein standards from Amersham. Other reagents included ethidium bromide, 2-mercaptoethanol, porcine trypsin, and porcine pancreatic elastase from Sigma; bovine α-chymotrypsin from Worthington; IgGsorb from the Enzyme Center (Cambridge, MA); leupeptin from the Peptide Institute (Osaka, Japan); Seakem agarose from FMC (Rockland, ME); 37% formaldehyde solution from Fisher; cesium chloride from Bethesda Research Laboratories; guanidine thiocyanate from Fluka; and sarcosine from ICN. Antisera included rabbit anti-human α_1PI from DAKO (Westbury, NY), goat anti-human α_1PI from Atlantic Antibodies (Westbrook, ME), rabbit anti-human α_1-antichymotrypsin from DAKO, sheep anti-human C2 from Seward Laboratories (London), goat anti-human factor B and goat anti-human lysozyme from Atlantic Antibodies.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: α_1PI, α_1-proteinase inhibitor; PiZZ, homozygous for Z-type α_1-proteinase inhibitor; PiMM, homozygous for the usual α_1-proteinase inhibitor; kb, kilobase; C2, second component of complement; C4, fourth component of complement.

METHODS

Cell Cultures. Confluent monolayers of human peripheral blood monocytes were established from eight normal PiMM volunteers and eight PiZZ individuals (with a wide range of hepatic and/or pulmonary involvement) using adherence of dextran-purified leukocytes on siliconized glass as described (13). The diagnosis of homozygous PiZZ α_1PI deficiency was based on isoelectric focusing, serum levels, and family studies.

Biosynthetic Labeling, Immunoprecipitation, and NaDodSO$_4$/PAGE. *Xenopus* oocytes were injected with 50 ng of poly(A)$^+$ mRNA per oocyte and then incubated at 20°C in Barth's medium (10 μl of medium per oocyte) for 12 hr (14). Medium was then replaced by fresh medium containing L-[^{35}S]methionine (1 mCi/ml) and left at room temperature for 12 hr (pulse period). Medium was collected, and oocytes were rinsed in phosphate-buffered saline, pH 7.2 (P_i/NaCl) and resuspended in Barth's medium containing 10 mM L-methionine. At specified time intervals the culture media were collected, and oocytes were washed in P_i/NaCl and homogenized in P_i/NaCl/1% Triton X-100 (vol/vol)/0.5% sodium deoxycholate/10 mM EDTA/2 mM phenylmethylsulfonyl fluoride/100 μg of leupeptin/ml, pH 7.6. The homogenate was clarified by centrifugation at 12,000 × *g* for 10 min. Aliquots of cell homogenates and culture media were assayed for total trichloroacetic acid (TCA)-precipitable protein as described (15). Equivalent volumes of media and homogenates from PiMM- and PiZZ-injected oocytes were incubated with the appropriate antibody and antigen–antibody complexes, isolated using IgGsorb, were analyzed on 9% NaDodSO$_4$/PAGE under reducing conditions (16) and bands were visualized by fluorography.

Confluent monolayers of monocytes were radiolabeled after 24 hr in culture. Cells to be labeled were rinsed and incubated at 37°C for 1 hr in methionine-free medium containing L-[^{35}S]methionine (500 μCi/ml). Labeled medium was removed, then monolayers were rinsed and incubated in medium containing a 200- to 1000-fold excess of unlabeled methionine for intervals up to 3 hr. Cell solubilization, clarification of cell culture fluid and lysates, immunoprecipitation, NaDodSO$_4$/PAGE, and fluorography are described above.

In separate experiments, aliquots of medium and cell lysates from PiZZ monocytes, PiMM monocytes, PiZZ liver mRNA-injected oocytes, and PiMM liver mRNA-injected oocytes, of equal volume or of equal total TCA-precipitable protein, were subjected to NaDodSO$_4$/PAGE. Radiolabeled bands were cut from gels, solubilized in 15% H_2O_2, and subjected to scintillation counting in Scientific Scintiverse solution (Fisher).

Isolation of RNA, RNA Blot Analysis, and *in Vitro* Translation. Livers were obtained from three PiMM human adults at autopsy and from three PiZZ patients with severe hepatic dysfunction who underwent liver transplantation. Severe hepatic dysfunction was the indication for liver transplantation in the three PiZZ patients. Total cellular RNA was isolated using guanidine hydrochloride extraction and ethanol precipitation (17). Poly(A)$^+$ mRNA was purified by affinity chromatography on oligo(dT)cellulose (18). The preparation of the human liver cDNA library (19) and isolation of cDNA clones for α_1PI (20), the second component of complement (C2) (21), the fourth component of complement (C4) (22), and factor B (19) have been described. Agarose-formaldehyde electrophoresis, transfer to nitrocellulose filters, and hybridization followed methods described (23). Blots were washed, autoradiographed, and then boiled in 10 mM Tris·HCl/1 mM EDTA, pH 7.6, for 10 min to remove labeled probes in preparation for hybridization with other radiolabeled cDNA probes. Densitometry was performed on the Helena (Beaumont, TX) Quick scanner with integrator.

A rabbit reticulocyte lysate system was used for cell-free translation (24) and specific products were immunoprecipitated for analysis as described above.

RESULTS

Detection of α_1PI RNA in PiMM and PiZZ Livers. An α_1PI-specific [^{32}P]cDNA probe bound predominantly to a ≈1.4 kilobase (kb) mRNA from PiMM or PiZZ liver (Fig. 1, lanes 2–6). Another, less abundant, ≈3.2 kb α_1PI mRNA was also detected. The α_1PI mRNA content was lower in PiZZ than PiMM livers but the reduction was comparable to the reduction in mRNA content for three other glycoproteins, C4, factor B (Fig. 1, lanes 8–12), and C2, and in ribosomal RNA content (data not shown). Therefore, the reduction in α_1PI mRNA content was a nonspecific effect probably related to liver disease.

Cell-free translation of RNA from PiMM and PiZZ livers results in a single 47-kDa α_1PI primary translation product (data not shown). The amount of the primary translation product from the PiZZ liver was reduced relative to that from the PiMM liver mRNA, but was again comparable to the relative amounts of C2 and factor B primary translation products in PiZZ and PiMM livers. These results are consistent with those of Bathurst *et al.* (26) demonstrating no differences in the products of cell-free biosynthesis programmed with PiMM and PiZZ liver mRNA.

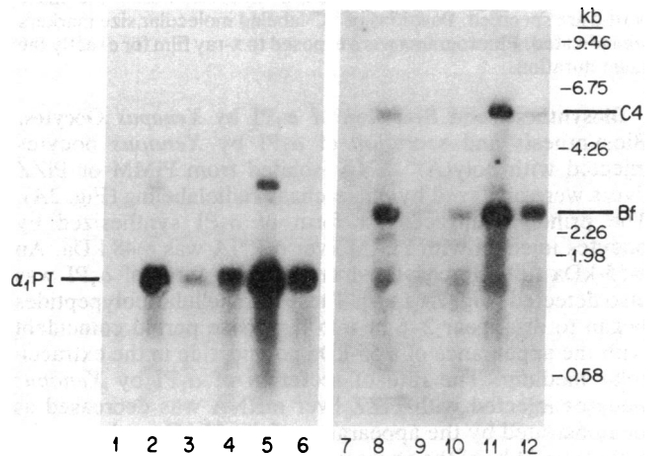

FIG. 1. Detection of PiMM and PiZZ liver α_1PI mRNA, and comparison with PiMM and PiZZ liver C4 and factor B mRNA by RNA hybridization blot analysis. Lanes 1–6, α_1PI-specific [^{32}P]-cDNA; lanes 7–12, C4-specific and factor B-specific [^{32}P]cDNA. Lanes: 1 and 7, U937 cells unstimulated, total cellular RNA = 20 μg; 2 and 8, PiMM liver total cellular RNA = 5 μg; 3 and 9, PiZZ liver total cellular RNA = 5 μg; 4 and 10, PiZZ liver total cellular RNA = 20 μg; 5 and 11, PiMM liver poly(A)$^+$ = 1 μg; 6 and 12, PiZZ liver poly(A)$^+$ = 1 μg. Apparent size markers are *Hin*dIII fragments of λ phage DNA. Densitometry was performed on the Helena Quick scanner with integrator using 1.4-kb band for α_1PI, 2.5 kb for factor B (Bf) and 5 kb for C4. Relative densitometric values for α_1PI were as follows: lane 1, 0; lane 2, 12.7; lane 3, 2.5; lane 4, 8.3; lane 5, 23.9; lane 6, 15.6. Relative densitometric values for Bf were as follows: lane 7, 0; lane 8, 10.3; lane 9, 1.2; lane 10, 4.8; lane 11, 25.7; lane 12, 10.5. Relative densitometric values for C4 were as follows: lane 7, 0; lane 8, 4.0; lane 9, 0.3; lane 10, 0.5; lane 11, 13.7; lane 12, 3.2. Although it has been previously suggested that α_1PI is produced by U937 cells as detected by immunoelectrophoresis (25), α_1PI RNA was not detected in U937 cells by RNA hybridization blot analysis or biosynthetic labeling in these studies. Differences in state of activation of U937 cells have been described and may explain this discrepancy.

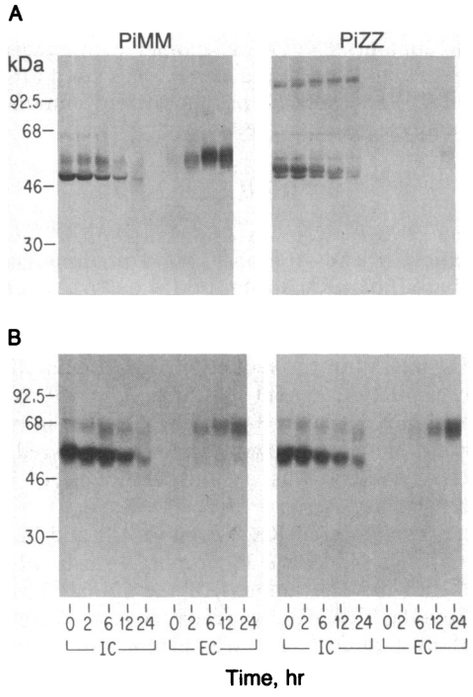

FIG. 2. Rate of synthesis and secretion of α_1PI (*A*) and α_1-antichymotrypsin (*B*) by *Xenopus* oocytes microinjected with mRNA from PiMM and PiZZ livers. Intracellular lysates (IC) and extracellular media (EC) from PiMM (*Left*) and PiZZ (*Right*) liver mRNA-injected oocytes are indicated, and time intervals during the chase period are specified. Positions of ^{14}C-labeled molecular size markers are indicated. Fluorograms were exposed to x-ray film for exactly the same duration.

Biosynthesis and Secretion of α_1PI by *Xenopus* Oocytes. Biosynthesis and secretion of α_1PI by *Xenopus* oocytes injected with poly(A)$^+$ RNA isolated from PiMM or PiZZ livers were analyzed by pulse–chase radiolabeling (Fig. 2*A*). The principal intracellular form of α_1PI synthesized by oocytes injected with PiMM liver mRNA was ≈48 kDa. An ≈55-kDa fully glycosylated intracellular form of α_1PI was also detected (Fig. 2*A Left*). These intracellular polypeptides began to disappear 2–6 hr into the chase period coincident with the appearance of a 55-kDa polypeptide in the extracellular medium. The rate of secretion of α_1PI by *Xenopus* oocytes injected with PiZZ liver mRNA was decreased as demonstrated by the appearance of the 55-kDa polypeptide, only after 24 hr of the chase period (Fig. 2*A Right*). Although α_1PI was also synthesized by oocytes injected with PiZZ liver mRNA as an ≈48-kDa polypeptide, an additional ≈52-kDa radiolabeled polypeptide was identified in the intracellular lysates of the oocytes injected with PiZZ liver mRNA (Fig. 2*A Right*). The 48-, 52-, and 55-kDa polypeptides were blocked in immunoprecipitation by unlabeled purified α_1PI (a gift of C.-B. Laurell, Malmo, Sweden). A band with apparent size >92.5 kDa was present in the intracellular lysates of oocytes injected with PiZZ liver mRNA but was a nonspecific product of immunoprecipitation as determined by blocking experiments with unlabeled purified α_1PI. Phenotypes of the secreted peptides corresponded to the phenotype of the liver RNA donor as determined by isoelectric focusing in polyacrylamide gels (data not shown). A shift in apparent size of the radiolabeled extracellular PiMM and PiZZ α_1PI from ≈55 kDa to ≈66 kDa after incubation with unlabeled α-chymotrypsin demonstrated that α_1PI secreted by injected oocytes was able to complex with protease (27, 28).

The decrease in rate of secretion of PiZZ α_1PI was shown to be specific in that the rate of secretion of three other liver-derived glycoproteins, α_1-antichymotrypsin (Fig. 2*B*), C2, and factor B (data not shown), was similar in *Xenopus* oocytes injected with PiZZ and PiMM liver mRNA. The specific decrease in rate of secretion of PiZZ α_1PI was confirmed by scintillation counting of radiolabeled bands excised from the gels (Fig. 3).

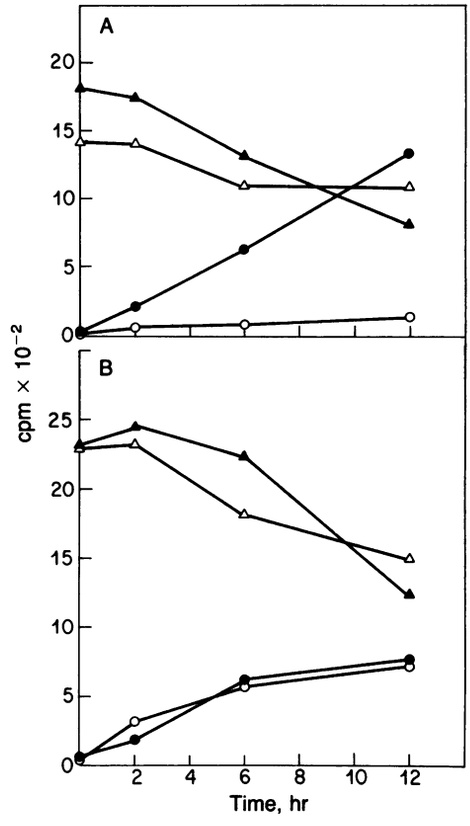

FIG. 3. Rate of synthesis and secretion of α_1PI (*A*) and α_1-antichymotrypsin (*B*) by *Xenopus* oocytes injected with mRNA from PiMM (▲ and ●) and PiZZ (△ and ○) livers as determined by excising radiolabeled bands from the gel. Intracellular lysates (▲ and △) and extracellular media (● and ○) are indicated. Only the specific bands—48, 52, and 55 kDa for α_1PI; 58 and 68 kDa for α_1-antichymotrypsin—were excised.

Biosynthesis and Secretion of α_1PI by Peripheral Blood Monocytes. To exclude the possibility that the defect in secretion of PiZZ α_1PI was limited to the *Xenopus* oocyte, a primary culture system was needed. Since the *α_1PI* gene is expressed in human mononuclear phagocytes (13), secretion of α_1PI could be studied in primary cultures of PiMM and PiZZ human peripheral blood monocytes. Two intracellular α_1PI polypeptides, ≈48 and ≈52 kDa, were synthesized by PiMM and PiZZ peripheral blood monocytes (Fig. 4*A*). The kinetics of secretion of α_1PI were decreased in monocytes from PiZZ when compared to those of PiMM individuals. Native, ≈55 kDa α_1PI, and ≈66- and ≈75-kDa α_1PI–proteinase complexes (1) were secreted within 30 min of the chase period by PiMM monocytes (Fig. 4 *Left*) but not until 120 min of the chase period by PiZZ monocytes (Fig. 4 *Right*). The ratio of native to complexed forms of α_1PI secreted by PiZZ monocytes was greater than that secreted by PiMM monocytes (Fig. 4*A*). The decrease in rate of secretion of α_1PI by PiZZ monocytes was shown to be specific in that the rate of secretion of other monocyte secretory products, such as lysozyme (Fig. 4*B*), the third component of complement, and α_1-antichymotrypsin (data not shown), was similar in PiZZ and PiMM monocytes. In *Xenopus* oocytes injected with liver mRNA and in monocytes the apparent recovery of radiolabeled PiMM α_1PI was greater than that of PiZZ α_1PI, suggesting that, in tissue culture, the rate of catabolism of PiZZ α_1PI is greater than that of PiMM α_1PI.

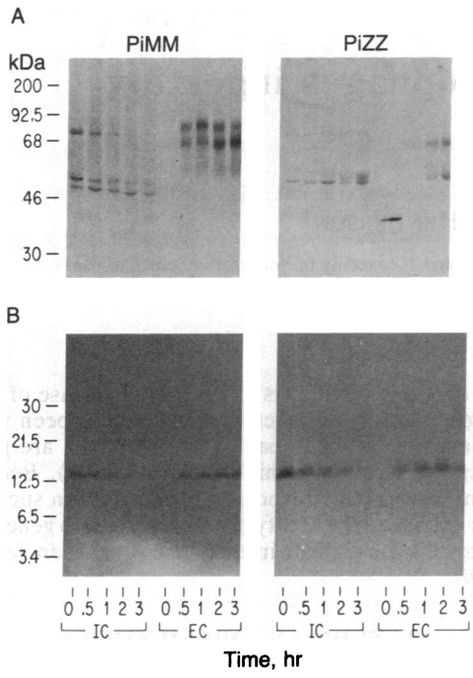

FIG. 4. Rate of synthesis and secretion of α_1PI (*A*) and lysozyme (*B*) by human peripheral blood monocytes from PiMM and PiZZ individuals. Intracellular lysates (IC) and extracellular media (EC) from monocytes of PiMM (*Left*) and PiZZ (*Right*) individuals are indicated, and time intervals during the chase period are specified. ^{14}C-labeled molecular size markers are included. Fluorograms in the two panels were exposed to x-ray film for exactly the same duration.

DISCUSSION

In previous studies a secretory abnormality in PiZZ α_1PI deficiency has been inferred from analyses that allowed only static observations—i.e., immunocytochemistry, x-ray crystallography, and structural analysis of material isolated from liver inclusions (1–4, 12). Technical limitations in biosynthetic labeling and analytical methods in several recent studies examining biosynthesis and secretion of α_1PI (25, 29) have prevented definitive identification of the mechanism accounting for low serum concentrations in deficient individuals. The results of our study indicate that α_1PI is synthesized and secreted by *Xenopus* oocytes injected with PiZZ liver mRNA and by peripheral blood monocytes from PiZZ individuals but secretion occurs at a reduced rate when compared to that of PiMM α_1PI. The expression of this defect by PiZZ monocytes in primary cell culture as well as by *Xenopus* oocytes injected with PiZZ liver mRNA demonstrates that the defect is not the result of the surrogate cell and that the defect is not tissue specific. Differences in the form of intracellular PiZZ α_1PI and in the relative amount of proteinase complexing by secreted PiZZ α_1PI may be related to the defect in secretion or may result from the defect in secretion.

Several possible mechanisms could explain this defect in secretion. Since the amino acid substitution does not occupy an oligosaccharide attachment site or a site known to undergo posttranslation processing, it is most likely that it alters the conformation of α_1PI during or immediately after translation. An alteration in conformation could result in diminished solubility, increased degradation, interference with oligosaccharide processing, interference with the recognition of α_1PI by a receptor responsible for its transport (30, 31), or a change in the interaction of this inhibitor with another product ordinarily involved in its posttranslational processing/transport. Alternatively, the defect in secretion may be unrelated to the single amino acid substitution, resulting instead from an abnormality in posttranslational processing/transport encoded by a cosegregating allele. In either case, α_1PI secretion by *Xenopus* oocytes injected with PiZZ liver mRNA and by mononuclear phagocytes from PiZZ individuals provides an appropriate system for further investigation of the defect and of mechanisms ordinarily involved in the posttranslational processing and transport of proteins destined for secretion.

We thank Claudia S. Leonard for technical assistance, Dr. Gabriel Goldberger for advice, and Helen Hourihan for secretarial assistance. The study was supported in part by Public Health Service Grants IF32AM07114, KO8HD00584, HL32361, and AI20959; and American Liver Foundation Postdoctoral Fellowship Award; and a United Cerebral Palsy Grant R335.

1. Eriksson, S. & Larsson, C. (1975) *N. Engl. J. Med.* **292,** 176–180.
2. Jepsson, J.-O., Larsson, C. & Eriksson, S. (1975) *N. Engl. J. Med.* **293,** 576–579.
3. Hercz, A., Katona, E., Cutz, E., Wilson, J. R. & Barton, M. (1978) *Science* **201,** 1229–1232.
4. Bathurst, I. C., Travis, J., George, P. M. & Carrell, R. W. (1984) *FEBS Lett.* **177,** 179–183.
5. Makino, S. & Reed, C. E. (1970) *J. Lab. Clin. Med.* **75,** 742–746.
6. Laurell, C. B., Nosslin, B. & Jepsson, J.-O. (1977) *Clin. Sci. Mol. Med.* **52,** 457–461.
7. Jepsson, J.-O., Laurell, C. B., Nosslin, B. & Cox, D. W. (1978) *Clin. Sci. Mol. Med.* **55,** 103–107.
8. Yoshida, A., Lieberman, J., Gaidulis, L. & Ewing, C. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1324–1328.
9. Jepsson, J.-O. (1976) *FEBS Lett.* **65,** 195–197.
10. Owen, M. C. & Carrell, R. W. (1977) *FEBS Lett.* **79,** 245–247.
11. Kidd, V. J., Wallace, R. B., Itakura, K. & Woo, S. L. C. (1983) *Nature (London)* **304,** 230–234.
12. Loebermann, H., Tokuoka, R., Diesenhofer, J. & Huber, R. (1984) *J. Mol. Biol.* **177,** 531–556.
13. Perlmutter, D. H., Cole, F. S., Kilbridge, P., Rossing, T. H. & Colten, H. R. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 795–799.
14. Lane, C. D., Marbaix, G. & Gurdon, J. B. (1971) *J. Mol. Biol.* **61,** 73–81.
15. Roberts, B. E. & Paterson, B. M. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 2330–2335.
16. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
17. Chirgwin, T. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1978) *Biochemistry* **18,** 5294–5301.
18. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
19. Woods, D. E., Markham, A. F., Ricker, A. T., Goldberger, G. & Colten, H. R. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 5661–5665.
20. Rogers, J., Kalsheker, N., Wallis, S., Speer, A., Coutelle, C. H., Woods, D. E. & Humphries, S. E. (1983) *Biochem. Biophys. Res. Commun.* **116,** 375–382.
21. Woods, D. E., Edge, M. D. & Colten, H. R. (1984) *J. Clin. Invest.* **74,** 634–638.
22. Whitehead, A. S., Goldberger, G., Woods, D. E., Markham, A. F. & Colten, H. R. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5387–5391.
23. Thomas, P. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
24. Pelham, H. R. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67,** 247–256.
25. Van Furth, R., Kramps, J. A. & Diesselhof-DenDulk, M. M. C. (1983) *Clin. Exp. Immunol.* **51,** 551–557.
26. Bathurst, I. C., Stenflo, J., Errington, D. M. & Carrell, R. W. (1983) *FEBS Lett.* **153,** 270–274.
27. Travis, J. & Salvesen, G. S. (1983) *Annu. Rev. Biochem.* **52,** 655–709.
28. Morii, M., Odani, S. & Ikenaka, T. (1979) *J. Biochem. (Tokyo)* **86,** 915–921.
29. Foreman, R. C., Judah, J. D. & Colman, A. (1984) *FEBS Lett.* **168,** 84–88.
30. Lodish, H. F. & Kong, N. (1984) *J. Cell Biol.* **98,** 1720–1729.
31. Yeo, T. K., Yeo, K. T., Parent, J. B. & Olden, K. (1985) *J. Biol. Chem.* **260,** 2565–2569.