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The Coding Sequence for the 32,000-Dalton Pulmonary Surfactant-associated Protein A Is Located on Chromosome 10 and Identifies Two Separate Restriction-Fragment-Length Polymorphisms

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SUMMARY

The primary protein component of human pulmonary surfactant is a 32,000-dalton glycoprotein called surfactant-associated protein A. This protein is important for normal lung function, and its expression is developmentally regulated. Using a mapping panel of somatic-cell hybrids, we have localized the coding sequence for pulmonary surfactant–associated protein A to chromosome 10. Additionally, this sequence identifies two separate *Msp*I restriction-fragment-length polymorphisms. Since there is a relative lack of polymorphic markers for chromosome 10, this sequence may be useful in linkage analysis.

INTRODUCTION

Pulmonary surfactant provides for the low surface tension at the air/liquid interface in the lung. Its deficiency in premature infants leads to respiratory insufficiency and hyaline membrane disease of the newborn (Clements 1965). Surfactant purified from bronchoalveolar lavage fluid is composed primarily of

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phospholipid (70% phosphatidylcholine, 10% phosphatidylglycerol, and 10% other phospholipids) and 10% protein (Hallman 1982). Under reduced conditions there are two families of surfactant-associated proteins (King 1975). One has been termed apoprotein A, although the terminology is different in different published reports (Sueishi and Benson 1981; Ng et al. 1983; Katyal and Singh 1984; Phelps et al. 1984, 1986; Floros et al. 1985; Phelps and Taeusch 1985; Whitsett et al. 1985) and is a family of glycoproteins with an approximate molecular mass of 32,000 daltons (32 kd). The other is a hydrophobic group of proteins with an apparent molecular mass of 11 kd and has been termed apoprotein B.

The 32-kd family of apoproteins is thought to be important in the formation of tubular myelin and in the facilitation of adsorption of surface-active material into the air/liquid interface (Hawgood et al. 1985; Inui et al. 1985). These apoproteins are found in the endoplasmic reticulum and lamellar bodies of lung type II epithelial cells by means of immunocytochemical analysis with polyclonal antisera (Williams and Benson 1981). In addition, excessive amounts of this family of apoproteins are found in the air spaces of patients with alveolar proteinosis, a disease of unknown pathogenesis (Whitsett et al. 1985). Recently, the human gene for the 32-kd family of apoproteins has been cloned (White et al. 1985). We therefore sought to determine its chromosomal localization, using a cDNA probe. We present evidence, derived from molecular hybridization with human-rodent somatic-cell hybrids of defined chromosomal constitution, for the assignment of the sequence encoding the 32-kd alveolar surfactant apoprotein to chromosome 10.

MATERIAL AND METHODS

Somatic-Cell Hybrids and DNA Preparation

Human/Chinese-hamster CHO-K1 cell hybrids were prepared from a series of fusions between several CHO-K1 auxotrophic mutants and different human fibroblasts or lymphocytes. The human chromosome content in the hybrids was characterized by both cytogenetic and isozyme techniques, as previously described (Cheung et al. 1984; Kao et al. 1984; Inui et al. 1985). The cytogenetic analysis included sequential staining of the same metaphase preparations with trypsin banding and Giemsa-11 differential staining in sequential steps (Morse et al. 1982). All hybrids were derived from human/CHO-K1 fusions. Certain chromosome 10–containing hybrids, such as 762-8A and 640-34p6-1C, retain chromosome 10 when grown in proline-deficient media because chromosome 10 complements a nutritional deficiency in the parental CHO cell (Jones 1975). Hybrids 836-3A, 762-8A, and 640-34p6-1C were derived from segregants of primary clone 640-34 (Jones 1975). Hybrids 640-77A, 640-63, 879-16, 836-3A, 826-26A, 762-8A, and 640-34p6-1C were all subjected to isozyme and cytogenetic analysis at the time of DNA preparation. All other hybrids were analyzed sequentially by both cytogenetic and isozyme analysis. Some cell lines have demonstrated chromosomal instability; therefore DNA was purified with one or two passages of both cytogenetic and isozyme analysis. DNA was

purified from human whole blood as previously described (Kunkel et al. 1978). DNA from hybrid cells was prepared by lysis in sodium dodecyl sulfate and proteinase K, followed by sequential phenol and chloroform extractions and ethanol precipitation (Gusella et al. 1979; Kao et al. 1982).

cDNA Probe for the 32-kd-Surfactant–Apoprotein A Gene

pHS10-5, the cDNA probe for the surfactant–apoprotein A gene, represents the entire coding sequence for the protein (White et al. 1985) and was constructed from separate clones isolated from a human lung cDNA library (White et al. 1985). This clone contains a 0.9-kb insert and has been used to express the complete human apoprotein molecule in a rodent cell line (R. T. White, personal communication).

Southern Blot Hybridization

All digestions were done using buffers recommended by the supplier of restriction enzymes. Complete digestions were reliably obtained by using a four- to sixfold excess of enzyme. DNA (10 μg) was digested with various enzymes run through a 0.8% agarose gel, denatured, and transferred to a nitrocellulose filter (Southern 1975). The nitrocellulose filter was baked, prehybridized, and hybridized according to the methods of Wahl et al. (1979) with 10^6 cpm/ml probe, nick-translated with a commercially available kit (BRL). High-stringency washing was used as previously described (Fisher et al. 1984). Autoradiography was performed using Cronex intensifying screens and Kodak X-omat film.

RESULTS

Hybridization of the pHS10-5 Probe with Genomic DNA from Different Individuals

The clone pHS10-5 was hybridized with DNA samples (from 10 separate individuals) digested with a variety of restriction endonucleases. *Bam*HI gave a distinctive pattern with hybridization bands at ~3.46, 2.36, and 2.11 kb (fig. 1, lane 1). Southern analysis of DNA (obtained from the blood bank at the University of Colorado Health Sciences Center) from 10 randomly chosen individuals failed to reveal any evidence of restriction-fragment-length polymorphisms (RFLPs) following digestion with any of the following enzymes: *Bam*HI, *Kpn*I, *Eco*RI, *Hin*dIII, and *Sst*I. There were two RFLPs with *Msp*I (fig. 2). DNA from 30 individuals was digested with *Msp*I and probed with pHS10-5. Two *Msp*I sites appear to be commonly lost. When lost, variable site 1 generates a 6.5-kb fragment, fragment A, which is thus near one end of the gene. Variable *Msp*I site number 1 is present in 37% of chromosomes. Variable *Msp*I site 2 occurs between fragments D and E, and, when lost, generates a 2.1-kb fragment, fragment B. This site is present in 63% of chromosomes. Family studies confirming codominant transmission have not been done.

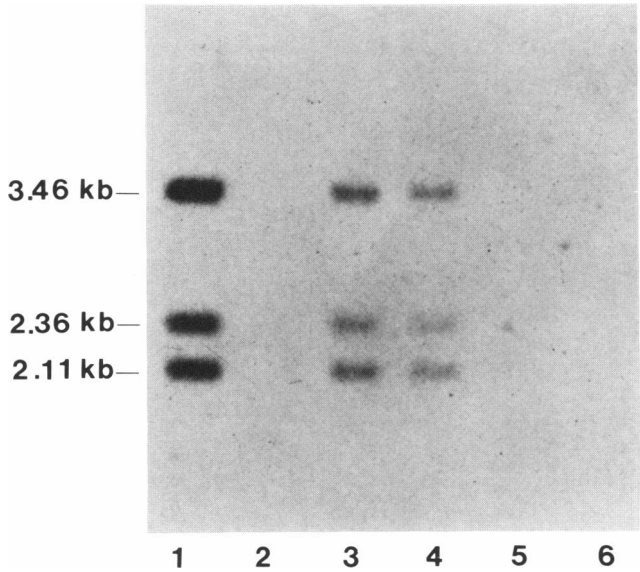

FIG. 1.—Southern blot of 10 μg genomic DNA digested with *Bam*HI and probed with pHS10-5. Lane 1, human genomic; lane 2, CHO-K1; lane 3, CP12-1; lane 4, CP26-1; lane 5, CP17-1; and lane 6, CP28-1. Hybridization bands are observed at 3.46, 2.36, and 2.11 kb in human genomic DNA and in hybrids that contain the long arm of chromosome 10. For chromosomal constitution of hybrid cells, see table 1. There is no significant cross-hybridization with hamster genomic DNA.

Chromosomal Localization of pHS10-5 by Southern Blot Hybridization and Synteny Analysis

When the Chinese-hamster CHO-K1 DNA was digested with *Bam*HI, no cross-hybridizing bands were observable (fig. 1, lane 2), indicating that very little homology exists between the human pHS10-5 probe and the hamster sequence. Digested DNA from the hybrid clone panel was hybridized with pHS10-5. Positive hybridization was obtained with seven members of the clone panel, CP12-1 and CP26-1 (fig. 1, lanes 3, 4) and 640-34, 822-19-C15, 68-201-A, 762-8A, and 640-34p6-1C. All other 18 hybrids showed no detectable hybridization with the probe (for examples, see fig. 1, lanes 5, 6). The results of those hybridizations and the chromosome content of each hybrid are shown in table 1. There was 100% concordance between the presence or absence of pHS10-5 and chromosome 10. In particular, hybrid clone 640-34 contained human chromosome 10 and was positive for the pHS10-5 probe. One segregant derived from this hybrid, 836-3A, had lost chromosome 10 and was negative for the pHS10-5 probe. Two other subclones also derived from 640-34—namely, 762-8A and 640-34p6-1C—still retained chromosome 10 and were positive for pHS10-5.

Moreover, hybrid 762-8A contained human chromosome 10 and was also shown to be positive for the probe pHS10-5. Therefore, pHS10-15 can be localized to human chromosome 10. Finally, since hybrid 640-34p6-1C appeared to have a complete deletion of the short arm of chromosome 10 and was

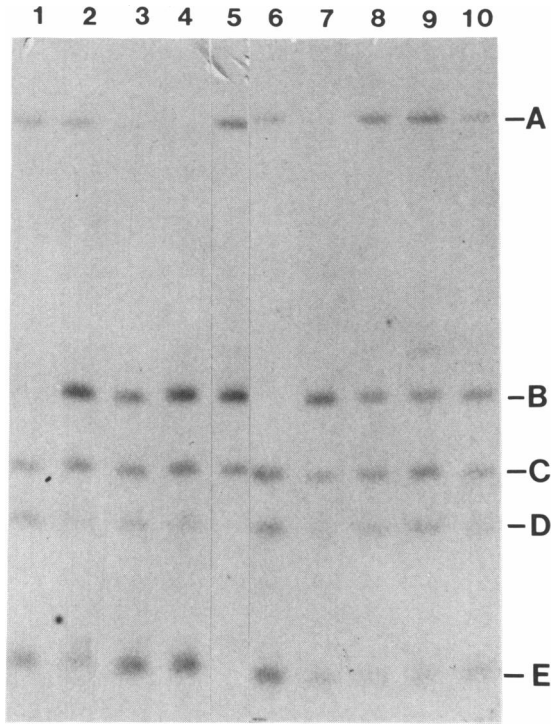

FIG. 2.—Southern blot of DNA (from 10 individuals) digested with *Msp*I and probed with pHS10-5. Approximate sizes of the bands are as follows: A, 6.5 kb; B, 2.1 kb; C, 1.85 kb; D, 1.7 kb; and E, 0.4 kb. Fragment B results from a lost *Msp*I site between fragments D and E. Fragment A probably results from a lost *Msp*I site near one end of the gene.

positive for the probe pHS10-5, it is likely that pHS10-15 maps to the long arm of chromosome 10.

DISCUSSION

Using a well-defined panel of somatic-cell hybrids, we have mapped the 32-kd apoprotein of human surfactant to chromosome 10. This hybrid panel has been used extensively for mapping other genes (Cheung et al. 1984; Kao et al. 1984; Inui et al. 1985; Jones et al. 1985; Lin-Lee et al. 1985; Nakamichi et al., in press; Natt et al., in press) and is subjected to periodic updating with isozyme and cytogenetic analysis and DNA markers. It thus appears that 100% concordance for a specific gene is highly predictive of its chromosomal localization. The fact that pHS10-5 hybridizes with clone 762-8A, which contains a single human chromosome 10, and also with 640-34-p6-1C, which contains only the long arm of chromosome 10 plus an intact chromosome 12, supports an assignment to chromosome 10, region 10q.

There are few recombinant markers on human chromosome 10 (Human Gene Mapping 1985). The cloned DNA sequences encoding urokinase plasminogen activator (q24-qter), interleukin-2 receptor (p14-p15), glutamate dehydrogenase

TABLE 1

SYNTENY ANALYSIS USING HUMAN-RODENT HYBRID CELLS AND THE SURFACTANT-ASSOCIATED PROTEIN A PROBE FOR ASSIGNMENT TO CHROMOSOME 10

HYBRIDS	HUMAN CHROMOSOMES																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	pHS-10-5
CP3-1	−	−	−	+	+	−	−	−	−	−	+	+	−	+	−	+	+	+	+	+	+	−	+	−
CP4-1	−	+	−	+	+	−	−	+	−	−	+	−	−	+	−	−	−	−	−	−	−	+	+	−
CP5-1	+	−	−	−	+	−	−	+	+	−	−	+	−	+	+	−	+	−	+	−	+	+	−	−
CP6-1	−	−	−	+	−	−	−	−	−	−	−	+	−	+	−	−	+	+	+	+	+	+	−	−
CP12-1	−	+	−	+	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−	+	+	+
CP14-1	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	+	+	−
CP15-1	−	−	−	+	+	−	−	−	−	−	+	+	−	−	+	+	+	−	−	−	−	−	−	−
CP16-1	−	−	−	−	+	−	+	−	−	−	−	−	−	+	−	−	+	−	+	+	+	+	−	−
CP17-1	+	−	−	+	+	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−
CP18-1	+	−	−	−	−	−	−	+	−	−	+	−	−	+	+	−	+	+	+	−	−	−	−	−
CP20-1	−	+	−	−	+	−	−	−	+	−	+	−	−	−	−	−	+	−	−	−	+	−	−	−
CP26-1	+	−	−	+	+	+	+	−	+	+	+	+	+	−	−	−	+	−	−	−	−	+	−	+
CP28-1	+	−	−	+	+	−	−	+	+	−	−	−	−	−	−	−	−	+	+	+	−	−	−	−
CP29-1	−	+	+	+	+	−	−	−	−	−	+	+	−	+	+	+	−	+	+	−	+	+	+	−
640-77A	−	−	−	+	−	−	−	+	−	−	−	+	+	−	−	−	−	−	−	−	−	−	+	−
640-63	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	+	−	−	+	−	−	−
879-16	−	−	−	−	+	+	−	−	+	−	−	+	−	−	−	−	−	−	+	−	+	−	−	−
640-34	−	−	−	+	−	+	−	−	+	+	−	+	−	−	−	−	−	+	+	−	+	−	−	+
822-19-C15	+	+	+	−	+	+	−	−	−	+	+	−	−	+	+	−	+	−	+	+	+	−	−	+
836-3A	−	−	−	−	−	q	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
68-201-A	−	−	+	−	+	−	−	−	−	+	+	+	−	+	−	−	−	+	+	−	+	−	+	+
706-D-1	−	+	−	+	−	+	−	−	−	−	−	+	+	−	−	+	+	+	−	−	−	+	−	−
826-26A	−	−	−	−	+	+	+	−	+	−	+	+	−	−	−	−	−	−	+	−	+	−	−	−
762-8A	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+
640-34p6-1C	−	−	−	−	−	−	−	−	−	q	−	+	−	−	−	−	−	−	−	−	−	−	−	+
Concordant frequency (%)	64	64	76	40	36	68	68	60	56	100	56	56	68	48	60	56	40	52	48	60	48	56	60	

(q23-q24), and vimentin have all been mapped to chromosome 10. Additionally, two anonymous DNA sequences—namely, D10S1 and D10S3, which identify RFLPs—have been mapped to chromosome 10. Because there is a relative lack of polymorphic-DNA markers for the long arm of chromosome 10, the *Msp*I RFLP that we describe for the coding sequences of the 32-kd pulmonary-surfactant apoprotein should be a potentially valuable marker for linkage analysis.

Floros et al. (1986) report sequence analysis of two independent cDNA clones for the surfactant apoprotein A. An *Msp*I polymorphism occurs at nucleotide 794, where the recognition sequence for *Msp*I in clone 6A, GGCC, is altered to AGTT in clone 1A (Floros et al. 1986). Thus, clones 1A and 6A may be encoded by either (1) allelic genes on different homologues of chromosome 10 in the individual from which the cDNA library was derived or (2) duplicate genes that are nearly identical. The sequences of those two clones are sufficiently divergent to suggest the presence of two separate genes, each encoding a different protein (Floros et al. 1986). The *Bam*HI hybridization bands that we describe are not explainable on the basis of the published sequence of the apoprotein (White et al. 1985). Additionally, when *Hin*dIII digests of genomic DNA from 10 individuals were probed with pHS10-5, consistent hybridization bands were observed with masses of 5.8, 5.0, 4.4, 1.5, and 1.40 kb (data not shown). Since only three large restriction fragments (1,350, 1,360, and 1,700+ bp) have been predicted from the sequence of the genomic clone (White et al. 1985), the existence of other genomic sequences with homology to pHS10-5 is supported. It is likely that there are two separate genes that are similar but not identical. Since all specific bands observed with Southern hybridizations were syntenic with the presence of chromosome 10, it is likely that, if two separate genes exist, they are on the same chromosome.

The surfactant apoprotein is a clinically important, developmentally regulated gene (Katyal et al. 1984). It is interesting to note that respiratory symptoms are absent in the few patients who have been described with either 10q-monosomy (Grouchy and Turleau 1977; Borgaonkar 1984) or 10q-trisomy syndromes (Grouchy and Turleau 1977; Borgaonkar 1984). A haploid dose of this particular gene may provide sufficient surfactant apoprotein to prevent respiratory failure. It is also possible that a 50% excess of this gene does not lead to respiratory symptoms. Thus, it remains to be determined whether the lack of symptomatology in these patients is due to a refined gene regulation or to the fact that the levels of surfactant apoprotein in the alveolar surface fluid are not crucial in surfactant function.

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REFERENCES

Borgaonkar, D. S. 1984. Chromosomal variation in man. 2d ed. Alan R. Liss, New York.

Cheung, P., F. T. Kao, M. C. Law, C. Jones, T. T. Puck, and L. Chan. 1984. Localization of the structural gene for human apolipoprotein A-1 on the long arm of human chromosome 11. Proc. Natl. Acad. Sci. USA **81**:508–511.

Clements, J. A., and D. F. Tierney. 1965. Alveolar instability associated with altered tension. Pp. 1565–1583 *in* W. O. Fenn and H. Rahn, eds. Handbook of physiology. Vol. **31**. American Physiological Society, Washington, D.C.

Fisher, J. H., Y. E. Miller, R. S. Sparkes, J. B. Bateman, K. A. Kimmel, T. E. Carey, T. Rodell, S. A. Shoemaker, and C. H. Scoggin. 1984. Wilms' tumor-aniridia association: segregation of affected chromosome in somatic cell hybrids, identification of cell surface antigen associated with deleted area, and regional mapping of c-Ha-ras-1 oncogene, insulin gene, and beta-globin gene. Somatic Cell Mol. Genet. **10**:455–464.

Floros, J., D. S. Phelps, and W. H. Taeusch. 1985. Biosynthesis and in vitro translation of the major-surfactant associated protein from human lung. J. Biol. Chem. **260**:495–500.

Floros, J., R. Steinbrink, K. Jacobs, D. S. Phelps, R. Krit, M. Recny, E. Stultzman, S. Jones, H. W. Taeusch, H. A. Frank, and E. F. Fritsch. 1986. Isolation and characterization of cDNA clones for the 35K Da pulmonary surfactant-associated protein. J. Biol. Chem. **19**:9029–9033.

Grouchy, J., and C. Turleau. 1977. Chromosome 13. Pp. 126–141 *in* Clinical atlas of human chromosomes. Wiley, New York.

Gusella, J., A. Varsanyi-Breiner, F. T. Kao, C. Jones, T. T. Puck, C. Keys, S. Orkin, and D. Houseman. 1979. Precise location of human beta-globin gene complex on chromosome 11. Proc. Natl. Acad. Sci. USA **76**:5239–5243.

Hallman, M., R. Spragg, J. H. Harrell, K. M. Moser, and L. Gluck. 1982. Evidence of lung surfactant abnormality in respiratory failure: study of bronchalveolar lavage phospholipids, surface activity, phospholipase activity, and plasma myoinositol. J. Clin. Invest. **70**:673–683.

Hawgood, S., B. J. Benson, and R. L. Hamilton. 1985. Effects of surfactant-associated protein and calcium ions on the structure and surface activity of lung surfactant lipids. Biochemistry **24**:184–190.

Human Gene Mapping 8. 1985. Cytogenet. Cell Genet. **40**:1–823.

Inui, K., F. T. Kao, S. Fujibayashi, C. Jones, H. G. Morse, M. L. Law, and D. A. Wenger. 1985. The gene coding for sphingolipid activator protein, SAP-1, is on human chromosome 10. Hum. Genet. **69**:197–200.

Jones, C. 1975. Synteny between the pro^+ marker and human glutamate oxaloacetate transaminase. Somatic Cell Genet. **1**:345–354.

Jones, C., H. G. Morse, F. T. Kao, A. Carbone, and E. Palmer. 1985. Human T-cell receptor alpha-chain genes: location on chromosome 14. Science **228**:83–85.

Kao, F. T., J. A. Hartz, M. L. Law, and J. N. Davidson. 1982. Isolation and chromosomal localization of unique DNA sequences from a human genomic library. Proc. Natl. Acad. Sci. USA **79**:865–869.

Kao, F. T., H. G. Morse, M. C. Law, A. Lidsky, T. Chandree, and S. L. C. Woo. 1984. Genetic mapping of the structural gene for antithrombin III to human chromosome 1. Hum. Genet. **67**:34–36.

Katyal, S. L., J. S. Amenta, G. Singh, and J. A. Silverman. 1984. Lung surfactant apoprotein and fetal lung maturity. Prog. Respir. Rev. **18**:106–111.

Katyal, S. L., and G. Singh. 1984. Analysis of pulmonary surfactant apoproteins by isoelectric focusing. Biochem. Biophys. Acta **794**:411–418.

King, R. J., J. Ruch, E. G. Gikas, A. C. G. Platzker, and R. K. Creasy. 1975. Appearance of apoproteins of pulmonary surfactant in human amniotic fluid. J. Appl. Physiol. **39**:735–741.

Kunkel, L. M., K. D. Smith, S. H. Boyer, D. S. Borgaonkar, S. S. Wechtel, D. J. Miller, W. R. Breg, H. W. Jones, and S. M. Rary. 1978. Analysis of human Y-chromosome specific reiterated DNA in chromosome variants. Proc. Natl. Acad. Sci. USA **74**:1245–1249.

Lin-Lee, Y. C., F. T. Kao, P. Cheung, and L. Chan. 1985. Apolipoprotein E gene mapping and expression: localization of the structural gene to human chromosome 19 and expression of apoE mRNA in lipoprotein and non-lipoprotein-producing tissues. Biochemistry **24**:3751–3756.

Morse, H. G., P. Patterson, and C. Jones. 1982. Giemsa-11 technique: applications in basic research. Mammalian Chromosomes Newsletter **23**:127–133.

Nakamichi, N. N., F. T. Kao, J. Wasmuth, and D. J. Roufa. Twelve ribosomal protein gene sequences map to human chromosome 5, 8, 17, 22 and X. Somatic Cell Mol. Genet. (in press).

Natt, E., F. T. Kao, R. Rettenmeier, and G. Scherer. 1986. Assignment of the human tryosine aminotransferase gene to chromosome 16. Hum. Genet. **75**:225–228.

Ng, V., V. Herndon, C. Mendelson, and J. Snyder. 1983. Characterization of rabbit surfactant-associated proteins. Biochem. Biophys. Acta **754**:218–226.

Phelps, D. S., J. Floros, and H. W. Taeusch. 1986. Post translational modification of the major human surfactant associated proteins. Biochemical J. **237**:373–377.

Phelps, D. S., and H. W. Taeusch. 1985. A comparison of the major surfactant-associated proteins in different species. Comp. Biochem. Physiol. **82B**:441–446.

Phelps, D. S., W. H. Taeusch, B. Benson, and S. Hawgood. 1984. An electrophoretic immunochemical characterization of human surfactant associated proteins. Biochim. Biophys. Acta **791**:226–238.

Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.

Sueishi, K., and B. J. Benson. 1981. Isolation of a major apolipoprotein of canine and murine pulmonary surfactant: biochemical and immunochemical characteristics. Biochim. Biophys. Acta **665**:442–453.

Wahl, G. M., M. Stern, and G. R. Stark. 1979. The efficient transfer of large DNA fragments from agarose gel to diazobenzyloxymethyl paper and rapid hybridization using dextran sulfate. Proc. Natl. Acad. Sci. USA **76**:3683–3687.

White, R. T., D. Damm, J. Miller, K. Spratt, J. Schilling, S. Hawgood, B. Benson, and B. Cordell. 1985. Isolation and characterization of the human pulmonary surfactant apoprotein gene. Nature **317**:361–363.

Whitsett, J. A., W. Hull, G. Ross, and T. Weaver. 1985. Characteristics of human surfactant-associated glycoproteins A. Pediatr. Res. **195**:501–508.

Williams, M. C., and B. J. Benson. 1981. Immunocytochemical localization and identification of the major surfactant protein in adult rat lung. J. Histochem. Cytochem. **29**:291–305.