Vol. 160, No. 1, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1989 Pages 325-332

ISOLATION AND CHARACTERIZATION OF THE cDNA FOR PULMONARY SURFACTANT-ASSOCIATED PROTEIN-B (SP-B) IN THE RABBIT

Jiejing Xu,[1,2] Carol Richardson,[1] Carol Ford,[1]
Tina Spencer,[2] Yao Li-juan,[1] George Mackie,[2] Geoffrey Hammond,[1,2]
and Fred Possmayer[1,2]

Department of [1]Obstetrics & Gynaecology and [2]Biochemistry,
The University of Western Ontario,
London, ON, Canada N6A 5A5

Received February 23, 1989

Summary: Pulmonary surfactant contains phospholipids including dipalmitoylphosphatidylcholine and three surfactant-associated proteins designated SP-A, SP-B and SP-C. A cDNA for rabbit SP-B has been isolated from a fetal (30 days gestation) rabbit lung cDNA library constructed in λ gt11. The cDNA and deduced amino acid sequences show strong homology with the cDNAs and predicted 40 kDa proproteins for human and canine SP-B. Strong homology is also observed with the amino acid sequences directly determined for the mature 8 kDa bovine and porcine SP-B isolated from lung lavage. SP-B is remarkable for its high cysteine and proline content and for the hydrophobic nature of the organic solvent-soluble, mature protein. In vitro translation of sense but not antisense RNA transcribed from the cDNA led to the production of 40 kDa and 32 kDa proteins. These proteins were immunoprecipitated by an antibody raised against bovine SP-B. Northern blot analysis revealed the mRNA for rabbit SP-B appears in fetal rabbit lung late in gestation and falls slightly in the neonate.

Mammalian lung is stabilized by a material known as pulmonary surfactant which reduces the surface tension in the terminal airways (1-4). Pulmonary surfactant obtained through centrifugation of saline lavage from lungs contains ≈ 90% lipid and 10% protein (1-7). The major apoprotein species consists of a glycoprotein group with a molecular mass of ≈ 35,000, termed surfactant-associated protein-A (SP-A). However, lipid extracts of pulmonary surfactant lacking the 35 kDa apoprotein species retain many of the essential physiochemical characteristics of natural surfactant (2,3,5). Preparations lacking SP-A have been used clinically to prevent and/or treat the Respiratory Distress Syndrome of the neonate (2,3,6,7). It has become apparent that lipid extract surfactant contains two low molecular mass hydrophobic proteins designated SP-B and SP-C. The SP-B recovered in pulmonary surfactant, which possesses a nominal molecular mass of ≈ 15-18 kDa nonreduced or 5-8 kDa reduced, is produced by proteolytic

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; SP, surfactant-associated protein; SDS, sodium dodecyl sulphate; Sp.Act., specific activity.

0006-291X/89 $1.50

processing of a larger proprotein of ≈ 40 kDa (5). The present report describes the cloning and sequencing of a rabbit SP-B cDNA. The deduced amino acid sequence for the precursor protein in this species is compared with the deduced sequences for human (8-10) and canine (11) SP-B and also with directly determined amino acid sequences for alveolar bovine and porcine SP-B (12,13).

MATERIALS AND METHODS

Poly(A)$^+$RNA was obtained by oligo-dT affinity chromatography (14) of RNA isolated (15) from day 30 (term 31) fetal rabbit lung. The cDNA library was constructed in the EcoRI site of bacteriophage λ gt11 (16,17). Replicate nitrocellulose filter lifts of phage, plated at 5 x 10^4 plaque-forming units/150 mm plate, were screened with a human SP-B cDNA (a kind gift of Dr. J.A. Whitsett, Pediatrics, Univ. of Cincinnati). The filters were prehybridized in 50% formamide, 5 x SSPE (1 x SSPE = 150 mM NaCl, 90 mM NaH_2PO_4 and 1 mM EDTA), 5 x Denhardt's solution (14), 0.1% sodium dodecyl sulphate (SDS), and 100 μg/ml salmon sperm DNA at 42°C for 2 h. Hybridization proceeded overnight at 42°C in the same solution with the human SP-B cDNA probe labelled with ^{32}P-dCTP (Sp.Act. 3.33 x 10^8 cpm/μg) using random primed DNA labelling (Pharmacia). Filters were washed twice (15 min each) in 0.1 x SSC (SSC = 150 mM NaCl, 15 mM sodium citrate)(14) and 0.1% SDS at 50°C and exposed for autoradiography with an intensifying screen at -70°C for 18 h. Positive plaques were purified by 2 successive screenings. Phage DNA was isolated by a modified liquid lysate method (14,18). After digestion of phage DNA with EcoRI, the cDNA was analyzed by Southern blot analysis (14) using the hybridization conditions described above. The insert was further subcloned into M13mp18 and sequenced by the dideoxy sequencing method (19) with bacteriophage T7 DNA polymerase (Sequenase, United States Biochemical), using M13 "Universal" primer (Pharmacia) or appropriate synthetic oligonucleotide primers synthesized on an Applied Biosystems Model 380A DNA synthesizer.

Rabbit SP-B cDNA, amplified in pUC19 and labelled with ^{32}P-dCTP as above, was used as a probe for Northern blot analysis (14). Total cellular RNA (15 μg), extracted from rabbit fetal and neonatal lung was denatured by glyoxal, size-fractionated on an agarose gel and transferred to Nytran (Schleicher and Schuell). The filters were hybridized at 42°C for 18 h in the solution described above but with 10 μg/ml poly U and 40% formamide and washed to a stringency of 1 x SSC + 0.1% SDS for 15 min twice at 30°C.

The EcoRI insert isolated from the hybrid pUC19 clone was ligated in both orientations with Bluescript.SK (Stratagene). Transcription in vitro was carried out with T7 RNA polymerase (Pharmacia) in a 100 μl reaction volume using 2 μg of cDNA template. The RNA generated was extracted with phenol and precipitated with ethanol. In vitro translation took place in a reaction volume of 10 μl containing [^{35}S]methionine + [^{35}S]cysteine (ICN), using a rabbit reticulocyte translation kit (Promega). After immunoprecipitation (20,21), the samples were electrophoresed on an 11% polyacrylamide gel containing 6M urea and 0.1% SDS (22). The dried gel was exposed to Kodak XAR-2 film at -70°C with a Cronex intensifying screen.

RESULTS AND DISCUSSION

The rabbit lung cDNA library in λ gt11 was screened with ^{32}P-labelled human SP-B cDNA. Screening a total of 5 x 10^4 plaques yielded three positive clones. Recombinant phage DNA was digested with EcoRI and examined by Southern blot hybridization. One clone was found to contain a 1.7-kb insert which hybridized with the human cDNA. The partial restriction endonuclease map and the strategy

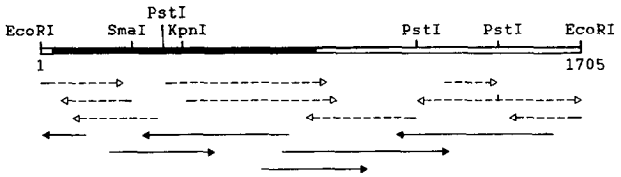

Fig. 1. Partial restriction endonuclease map of the 1.7 kilobase rabbit SP-B cDNA and strategy for determining its nucleotide sequence. Solid and open structures represent the coding and noncoding regions, respectively. Recognition sites for restriction endonucleases are indicated above the map. The arrows below the map indicate the direction and extent of sequences determined for the cDNA. Open arrows indicate sequences determined using universal primer; solid arrows indicate those sequences determined using synthetic oligonucleotide primers.

used to sequence the cDNA in both orientations are depicted in Fig. 1. The nucleotide sequence of the cDNA (Fig. 2) contains a total of 1,705 nucleotides which includes a very short 5'-untranslated region, a potential open reading frame spanning 1,111 nucleotides followed by 583 nucleotides of 3'-untranslated region. Of the two possible initiating methionines, the first at nucleotide 9 predicts a protein of 370 amino acids and a primary translation product of 40,700 daltons including a leader-like peptide, whereas the second at nucleotide 254 would produce a protein of 288 amino acids with a mass of 31,680 daltons.

The 1,705 bp cDNA was inserted into an in vitro transcription vector Bluescript.SK and T7 RNA polymerase used for transcription. Addition of 0.1 to 1.5 μg of the transcribed RNA to an in vitro translation system resulted in increasing amounts of proteins with apparent molecular masses of 40 kDa and 32 kDa (Fig. 3). These proteins were observed with the sense but not the antisense strand of the RNA. Immunoprecipitation of the translation products with a rabbit antiserum against bovine SP-B mature protein (a kind gift of Dr. J.A. Whitsett) revealed primarily the major polypeptide of 40 kDa (Fig. 3), which corresponds to the mass of the human SP-B precursor protein (8-10,20,21). A similar product could be immunoprecipitated from fetal rabbit lung poly(A)$^+$RNA (data not shown). This indicated that the rabbit SP-B cDNA clone contains the entire sequence encoding for a 40 kDa precursor. Small amounts of a 32 kDa protein were also precipitated.

Studies with human lung explants (9,20,21) and human lung adenomas (23) indicate the primary translation product is glycosylated at least once to produce a proprotein of $\approx$ 42 kDa. Further processing involves proteolytic cleavage at both the N- and the C-termini to produce the low molecular weight hydrophobic proteins of 15-18 kDa:nonreduced and 5-8 kDa:reduced which have been recovered from alveolar lavage. The nature and the cellular localization of these events must still be clarified (5,20,21,23). Comparison of the deduced amino acid sequences with canine (11) and human (9) SP-B revealed 66% of the predicted amino acids in the proprotein are conserved in all species (Fig. 4). An increasing

```
GAATTCCCATG GCC AAG TCA CAC CTG CCG CCG TGG CTG CTG CTG CTG  47
       Met Ala Lys Ser His Leu Pro Pro Trp Leu Leu Leu Leu  13

CTG CTG CCC ACA CTC TGT GGC CCA GGC ACT GCT GTC TGG GCC ACT  92
Leu Leu Pro Thr Leu Cys Gly Pro Gly Thr Ala Val Trp Ala Thr  28

TCA CCC TTG GCC TGT GCT CAG GGC CCT GAG TTT TGG TGC CAA AGC
Ser Pro Leu Ala Cys Ala Gln Gly Pro Glu Phe Trp Cys Gln Ser

CTG GAG CAA GCA TTG CAG TGC AAA GCC CTG GGA CAC TGT CTA CAG  182
Leu Glu Gln Ala Leu Gln Cys Lys Ala Leu Gly His Cys Leu Gln  58

CAA GTC TGG GGA CAC GTG GGA GCC GAT GAC CTG TGC CAG GAG TGT
Glu Val Trp Gly His Val Gly Ala Asp Asp Leu Cys Gln Glu Cys

CAG GAC ATC GTC AAC ATC CTA ACC AAG ATG ACC AAG GAG GCC ATT  272
Gln Asp Ile Val Asn Ile Leu Thr Lys Met Thr Lys Glu Ala Ile  88

TTC CAG GAC ACC ATA CGG AAG TTT CTG GAG CAT GAG TGC GAC GTT
Phe Gln Asp Thr Ile Arg Lys Phe Leu Glu His Glu Cys Asp Val

CTT CCC TTG AAG CTC CTG CTC CCC CAG TGT CAC CAC GTG CTT GAC  362
Leu Pro Leu Lys Leu Leu Val Pro Gln Cys His His Val Leu Asp  118

GTC TAC TTC CCC CTC ACC ATC ACC TAC TTC CAG AGC CAG ATT AAT
Val Tyr Phe Pro Leu Thr Ile Thr Tyr Phe Gln Ser Gln Ile Asn

GCA AAG GCC ATC TGC CAG CAC CTG GGC CTG TGC CAA CCC GGG TCA  452
Ala Lys Ala Ile Cys Gln His Leu Gly Leu Cys Gln Pro Gly Ser  148

CCA GAG CCT CCG CTG GAC CCT CTG CCT GAC AAG CTG GTC CTC CCC
Pro Glu Pro Pro Leu Asp Pro Leu Pro Asp Lys Leu Val Leu Pro

ACA CTG CTG GGG GCC CTC CCA GCA AAG CCT GGG CCC CAC ACG CAG  542
Thr Leu Leu Gly Ala Leu Pro Ala Lys Pro Gly Pro His Thr Gln  178

GAT CTG TCG GCG CAG CCG TTC CCC ATC CCC CTG CCC TTG TGC TGG
Asp Leu Ser Ala Gln Arg Phe Pro Ile Pro Leu Pro Leu Cys Trp

CTC TGC AGG ACT CTC CTC AAG CGG ATC CAG GCC ATG ATT CCC AAG  632
Leu Cys Arg Thr Leu Leu Lys Arg Ile Gln Ala Met Ile Pro Lys  208

GGT GTC CTG GCC ATG GCT GTG GCA CAG GTG TGC CAC GTG GTA CCC
Gly Val Leu Ala Met Ala Val Ala Gln Val Cys His Val Val Pro

CTG GTG GTG GGC GGT ATC TGT CAG TGC CTG GCC GAA CGC TAC ACT  722
Leu Val Val Gly Gly Ile Cys Gln Cys Leu Ala Glu Arg Tyr Thr  238

GTC ATT CTG CTG GAG GTG CTA CTG GGC CAT GTG CTG CCC CAG CTG
Val Ile Leu Leu Glu Val Leu Leu Gly His Val Leu Pro Gln Leu

GTC TGT GGC CTT GTC CTC CGG TGC TCC AGC GTG GAC AGC ATT GGC  812
Val Cys Gly Leu Val Leu Arg Cys Ser Ser Val Asp Ser Ile Gly  268

CAA GTC CCG CCC ACT CTG GAA GCC CTG CCA GGG GAG TGG CTG CCG
Gln Val Pro Pro Thr Leu Glu Ala Leu Pro Gly Glu Trp Leu Pro

CAA GAC CCA GAG TGC CGC CTT TGC ATG TCT GTG ACC ACC CAG GCC  902
Gln Asp Pro Glu Cys Arg Leu Cys Met Ser Val Thr Thr Gln Ala  298

AGG AAC ATC AGT GAG CAG ACC AGG CCG CAG GCA GTG TAC CAC GCC
Arg Asn Ile Ser Glu Gln Thr Arg Pro Ala Ala Val Tyr His Ala
                *
TGC CTC AGC TCC CAG CTG GAC AAG CAA GAG TGT GAA CAA TTT GTG  992
Cys Leu Ser Ser Gln Leu Asp Lys Gln Glu Cys Glu Gln Phe Val  328

GCT GCA CAC GCC CCA GCT GCT GAG CCT GCT GTC CAG GGG TTG GGA
Ala Ala His Ala Pro Ala Ala Glu Pro Ala Val Gln Gly Leu Gly

TGC CCG CGC AAT CTG CCA GGC CCT GAG GGG CGT GTA GTG GCC ACG 1082
Cys Pro Arg Asn Leu Pro Gly Pro Glu Gly Arg Val Val Ala Thr  358

CTC AGC CCT CTC CAG TGC ATC CAA AGC CCT CAC TTC TGA GGACTCA
Leu Ser Pro Leu Gln Cys Ile Gln Ser Pro His Phe End          370

GCTGTCCAGCCTGAATCACAGCCCAACACCAGAGCCACTTCTGTCCTGTCCGGCAAGCA 1187
GCAAGAGCCGTGTGCCCCACAGCAGGTCTGCAGAGAGACCCCACAGCCTTCACTCCAGC 1246
AGAGCCACACGAGGGCTCCCACAGCCTCCCAGGGGCGCAGGCTGACCAAATACTGCCTCCC 1305
CACCCCCACCCCCACCTCACTCCAGAGACTGGCTTTACCGCTACTTGCTCACAGTCTAC 1364
GAAGTTCAAAATACAGAAGAGAGAGGCTGGTGCTGTGACCCAGCAGGTTGGCCACCGCC 1423
TGCAGTGCCGGCATGCCATGTGGGCACCAGTTTGAGTTCCAGCTGCTCCACTCCTGATC 1482
CAGCTCCCTCGTAACGCGCCTGGGAAAGCAACAGAAGATGGTCCAAGTGTTTGGACCCC 1541
TGCCACCCATGTGGAGATCCAGAAGAAGCTCCTGGCTCCTGGCTGGCCCAGCCCTGGCC 1592
ATTGCAGCCATTTGGGGAGTGAACCAGCAGACGGAAGAGCTCAATCTCTAACTCTTACA 1659
AATAAATAAAATAAATCTTTAAAAAATAAATACATCAAGGGAATTC               1705
```

Fig. 2. Nucleotide and deduced amino acid sequences for the 1.7-kb SP-B cDNA. The putative start of the active fragment of SP-B protein is underlined. The potential glycosylation site is indicated by an asterisk.

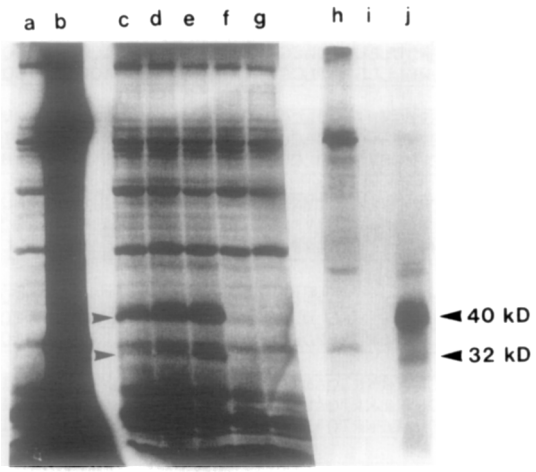

Fig. 3. Immunological identification of translation products of rabbit SP-B mRNA produced by in vitro transcription. Rabbit antibovine SP-B antiserum was used to immunoprecipitate the in vitro translation products. The positions of the SP-B mRNA dependent proteins are indicated by arrows. Lanes a, b: translation products without exogenous RNA and with control Brome Mosaic Virus (BMV) mRNA, respectively. Lanes c to e: translation products of the rabbit SP-B mRNA (0.5, 1.0 and 1.5 μg respectively). Lanes f and g: translation products of the antisense rabbit SP-B RNA (0.5 and 1.0 μg). Immunoprecipitations of translated protein from samples with BMV RNA, no exogenous RNA and rabbit sense RNA are shown in lanes h, i and j respectively.

homology of 84% is observed in the putative mature protein region. Many of the replacements are conservative, particularly within the hydrophobic active region. Deletions required to maintain alignment are observed in all sequences but these are 5′ and 3′ to the coding region for the mature polypeptide. Rabbit SP-B contains 37 prolines and 23 cysteines within the 370 amino acid proprotein. Some 27/37 of the prolines and 22/23 of the cysteines are also present in the same position in either, and usually both, the human and canine sequences. Three domains found in the human sequence (24), each with sequence $CX_2CX_{23-27}CX_{9-11}CX_{24}CX_5CX_{13-26}$ (where X is any amino acid) are retained in the rabbit sequence. One significant difference is the absence of the potential glycosylation site in the N- part of the rabbit protein sequence, a feature also reported for one of the human cDNA clones (8). Comparison of the predicted amino acid sequences for rabbit SP-B with the sequences directly determined for bovine (12) and porcine (13) SP-B confirmed the highly conserved nature of the mature active polypeptide.

To analyze developmental changes in the levels of hybridizable SP-B mRNA in rabbit, total RNA from lung tissue of 19 to 30-day gestational age fetal rabbit and from neonates (3 day) was subjected to Northern blot analysis (Fig. 5). A single RNA species of 1.9-kb, not detected in fetal RNA at 24 days gestation, was apparent in lung tissues of 27-day gestational age. This corresponds in size to the 2.0-kb mRNA identified in adult human lung (8,9). The relative proportion of SP-B mRNA increased on day 30, but declined slightly

```
canine    1      LLWLLLLPTLCGLGAADWSAPSLACARGPAFWCQSLEQALQCR
rabbit    1 MAKSHLLPWLLLLLLPTLCGPGTAVWATSPLACAQGPEFWCQSLEQALQCK
human     1 MAESHLLQWLLLLL-PTLCGPGTAAWTTSSLACAQGPEFWCQSLEQALQCR

canine   34 ALGHCLQEVWGNR-ADDLCQECQDIVRILTKMTKEAIFQSMVRKFLEHECD
rabbit   53 ALGHCLQEVWGHVGADDLCQECQDIVNILTKMTKEAIFQDTIRKFLEHECD
human    52 ALGHCLQEVWGHVGADDLCQECEDIVHILNKMAKEAIFQDTMRKFLEQECN

canine   84 VLPLKLLTPQCHHMLGTYFHVVVDYFQSQINPKIICKHLGLCKPGLPEPEQ
rabbit  104 VLPLKLLVPQCHHVLDVYFPLTITYFQSQINAKAICQHLGLCQPGSPEP--
human   103 VLPLKLLMPQCNQVLDDYFPLVID-FQNQTDSNGICMHLGLCKSRQPEPEQ
                                    *                    ▼
canine  135 QESE----------LSDPLLDKLILPELPGALQVT-GPHTQDLSEQQLPI
rabbit  153 ----------PLLDPLPDKLVLPTLLGALPAKPGPHTQDLSEQQFPI
human   153 EPGMSDPLPKPLRDPLPDPLLDKLVLPVLPGALQARPGPHTQDLSEQQFPI

canine  184 PLPYCWLCRTLIKRIQAMIPKGVLAVTVGQVCHVVPLVVGGICQCLGERYT
rabbit  189 PLPLCWLCRTLLKRIQAMIPKGVLAMAVAQVCHVVPLVVGGICQCLAERYT
human   204 PLPYCWLCRALIKRIQAMIPKGALAVACAQVCRVVPLVAGGICQCLAERYS
                                    ▼
canine  234 VLLLDALLGRMLPQLVCGLVLRCSHEDSAG---PALASLPSEWSPQESKCQ
rabbit  240 VILLEVLLGHVLPQLVCGLVLRCSSVDSTGQVPPTLEALPGEWLPQDPECR
human   255 VILLDTLLGRMLPQLVCRLVLRCSMDDSAGPRSPT-----GEWLPRDSECH

canine  283 LCMEVTTQAGNHSEQATPQAIRQACLSSWLDRQKCEQFVEQHMPRLQTLAS
rabbit  291 LCMSVTTQAGNISEQTRPQAVYHACLSSQLDKQECEQFVAAHAPAAEPAV-
human   301 LCMSVTTQAGNSSEQAIPQAMLQACVGSWLDREKCKQFVEQHTPQLLTLVP
                    *
canine  334 GGRDAHTTCQALGACRT----------TFS-PLQCIHIPHF
rabbit  342 -----------QGLGCPRNLPGPEGRVVATLS-PLQCIQSPHF
human   352 RGWDAHTTCQALG-----------VCGTMSSPLQCIHSPDL
```

Fig. 4. Comparison of the deduced amino acid sequences for rabbit, dog, and human SP-B. Amino acids identical to that of human SP-B are boxed. Amino acids of each protein are numbered on the left. Asterisks indicate the potential glycosylation sites. The arrowheads indicate the putative start and end of the active fragment of SP-B.

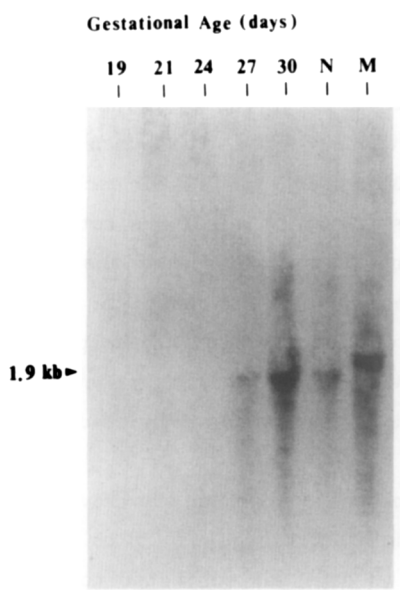

Fig. 5. Developmental changes in the levels of SP-B mRNA by Northern blot analysis. Total RNA isolated from 19 to 30 day fetal, 3 day neonatal (N) and maternal (M) rabbit lung was size-fractionated by agarose gel electrophoresis, transferred to Nytran, and then hybridized with ^{32}P-labelled rabbit SP-B cDNA as described in Materials and Methods. The position of the SP-B mRNA is indicated by the arrow.

after birth. These observations are consistent with the rapid induction of mRNA for SP-B as well as SP-A (5,24) in late gestation during the period in which surfactant phospholipids accumulate in preparation for birth.

Studies from a number of laboratories have demonstrated that reconstituted surfactant containing SP-B plus appropriate phospholipids can reduce the surface tension of a pulsating bubble to near 0 mN/m (10,26,27). The availability of cDNA clones for SP-B should facilitate studies on the structure-function relationships of this protein and could eventually lead to the production of artificial surfactants employing low molecular weight hydrophobic proteins derived through biotechnology.

ACKNOWLEDGMENTS

The authors would like to thank Dr. J.A. Whitsett and his coworkers (Pediatrics, Univ. of Cincinnati) for the human cDNA for SP-B, the antibovine SP-B antisera, and for helpful suggestions. We thank Ms. Barbara McDougall for excellent editorial services. These studies were supported by grants from the Medical Research Council of Canada, the Ontario Thoracic Society, and by a Natural Sciences and Engineering Research Council of Canada Strategic grant.

REFERENCES

1. Goerke, J.and Clements J.A. (1986) In Macklem, P.T., Mead, J., eds. Handbook of Physiology. Am. Physiol. Soc., Bethesda, MD, pp. 247-261.
2. Notter, R.H. (1988) Sem. Perinatol. 12, 180-212.
3. Possmayer, F., Yu, S.-H., Weber, M. and Harding, P.G.R. (1984) Can. J. Biochem. Cell Biol. 62, 1121-1131.
4. Wright, J.R. and Clements, J.A. (1987) Am. Rev. Resp. Dis. 135, 426-444.
5. Possmayer, F. (1988) Am. Rev. Respir. Dis. 138, 990-998.
6. Jobe, A. and Ikegami, M. (1987) Am. Rev. Respir. Dis. 136, 1256-1275.
7. Robertson, B. and Lachman, B. (1988) Exp. Lung Res. 14, 279-310.
8. Glasser, S.W., Korfhagen, T.R., Weaver, T., Pilot-Matias, T., Fox, J.L. and Whitsett, J.A. (1987) Proc. Natl. Acad. Sci. USA 84, 4007-4011.
9. Jacobs, K.A., Phelps, D.S., Steinbrink, R., Fisch, J., Kriz, R., Mitsock, L., Dougherty, J.P., Taeusch, H.W. and Floros, J. (1987) J. Biol. Chem. 262, 9808-9811.
10. Revak, S.D., Merritt, T.A., Degryse, E., Stefani, L., Courtney, M., Hallman, M. and Cochrane, C.G. (1988) J. Clin. Invest. 81, 826-833.
11. Hawgood, S., Benson, B.J., Schilling, J., Damm, D. and Clements, A. (1987) Proc. Natl. Acad. Sci. USA 84, 66-70.
12. Olafson, R.N., Rink, U., Kielland, S., Yu, S.-H., Chung, J., Harding, P.G.R. and Possmayer, F. (1987) Biochim. Biophys. Res. Commun. 148, 1406-1411.
13. Curstedt, T., Johansson, J., Barros-Söderling, J., Robertson, B., Nilsson, G., Westberg, M. and Jörnvall, H. (1988) Eur. J. Biochem. 172, 521-525.
14. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) In Molecular Cloning, a Laboratory Manual. Cold Spring Harbor Laboratory, NY.
15. Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294-5299.
16. Huynh, T.V., Young, R.A. and Davis, R.W. (1985) In Glover, D.M., ed., DNA Cloning: A Practical Approach, Vol. 1, IRL Press, Oxford, pp. 49-78.
17. Gubler, U. and Hoffman, B.J. (1983) Gene (Aust.) 25, 263-269.
18. Leder, P., Tiemeier, D. and Enquist, L. (1977) Science 196, 175.
19. Biggin, M.D., Gibson, T.J. and Hong, G.F. (1983) Proc. Natl. Acad. Sci. USA 80:3963-3965.

20. Whitsett, J.A., Weaver, T.E., Clark, J.C., Sawtell, N., Glasser, S.W., Korfhagen, T.R. and Hull, W.M. (1987) J. Biol. Chem. 262, 15618-15623.
21. Weaver, T., Sarin, V.K., Hull, W.M. and Whitsett, J.A. (1988) J. Appl. Physiol. 65, 982-987.
22. Laemmli, U. (1970) Nature Lond. 227, 680-685.
23. O'Reilly, M.A., Gazdar, A., Morris, R.E. and Whitsett, J.A. (1988) Biochim. Biophys. Acta 970, 194-204.
24. Hawgood, S. and Benson, B.J. (1989) In Molecular Biology of Surfactant Protein in Lung Cells: Structure, Biology and Function (Massero, D., ed.), Marcel Dekker, New York, pp. 701-734.
25. Yu, S.-H. and Possmayer, F. (1988) Biochim. Biophys. Acta 961, 337-350.
26. Curstedt, T., Jörnvall, H., Robertson, B., Bergman, T. and Berggren, P. (1987) Eur. J. Biochem. 168, 255-267.