Kato, A., & Nakai, S. (1980) *Biochim. Biophys. Acta 624*, 13-20.
Keen, J. M. (1985) in *Endocytosis* (Pastan, I., & Willingham, M. C., Eds.) pp 85-130, Plenum Press, New York.
Kielian, M., & Helenius, A. (1985) *J. Cell Biol. 101*, 2284-2291.
Kim, J., & Kim, H. (1986) *Biochemistry 25*, 7867-7874.
Lawaczeck, R., Gervais, M., Nandi, P. K., & Nicolau, C. (1987) *Biochim. Biophys. Acta 903*, 112-122.
Lucy, J. A. (1984) *FEBS Lett. 166*, 223-231.
Meers, P., Ernst, J. D., Düzgüneş, N., Hong, K., Fedor, J., Goldstein, I. M., & Papahadjopoulos, D. (1987) *J. Biol. Chem. 262*, 7850-7858.
Meers, P., Bentz, J., Alford, D., Nir, S., Papahadjopoulos, D., & Hong, K. (1988) *Biochemistry 27*, 4430-4439.
Monroy, A. (1985) *Eur. J. Biochem. 152*, 51-56.
Morgan, C., Willamson, H., Fuller, S., & Hudson, B. (1983) *Biochim. Biophys. Acta 732*, 668-674.
Nandi, P. K., & Edelhoch, H. (1984) *J. Biol. Chem. 259*, 11290-11296.
Nandi, P. K., Pretorius, H. T., Lippoldt, R. E., Johnson, M. L., & Edelhoch, H. (1980) *Biochemistry 19*, 5917-5921.
Olson, F., Hunt, C. A., Szoka, F. C., Vail, W. J., & Papahadjopoulos, D. (1979) *Biochim. Biophys. Acta 557*, 9-23.
Parente, R. A., Nir, S., & Szoka, F. C., Jr. (1988) *J. Biol. Chem. 263*, 4724-4730.
Pearse, B. M. F., & Crowther, R. A. (1987) *Annu. Rev. Biophys. Biophys. Chem. 16*, 49-68.
Plattner, H. (1987) in *Cell Fusion* (Sowers, A. E., Ed.) pp 69-98, Plenum Press, New York.
Schenkmann, S., Araujo, P. S., Sesso, A., Quina, F. H., & Chaimovich, H. (1981) *Chem. Phys. Lipids 28*, 165-180.
Struck, D. K., Hoekstra, D., & Pagano, R. E. (1981) *Biochemistry 20*, 4093-4099.
Stryer, L. (1968) *Science 162*, 526-533.
Szoka, F. C., & Papahadjopoulos, D. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4194-4198.
van Jaarsveld, P. P., Nandi, P. K., Lippoldt, R. E., Saroff, H., & Edelhoch, H. (1981) *Biochemistry 20*, 4129-4135.
Wakelam, M. J. O. (1985) *Biochem. J. 228*, 1-12.
White, J., Kielian, M., & Helenius, A. (1983) *Q. Rev. Biophys. 16*, 151-195.
Wilschut, J., Düzgüneş, N., Fraley, R., & Papahadjopoulos, D. (1980) *Biochemistry 19*, 6011-6021.
Yoshimura, T., & Sone, S. (1987) *J. Biol. Chem. 262*, 4597-4601.
Yoshimura, T., Maezawa, S., & Hong, K. (1987) *J. Biochem. (Tokyo) 101* 1265-1272.

Patterns of Alternative Splicing of Fibronectin Pre-mRNA in Human Adult and Fetal Tissues†

Fumitaka Oyama,‡ Yoshiharu Murata,§ Nobuhiko Suganuma,∥ Toshio Kimura,∥ Koiti Titani,‡ and Kiyotoshi Sekiguchi*,‡

Laboratory of Biomedical Polymer Science, Institute for Comprehensive Medical Science, Fujita-Gakuen Health University School of Medicine, Toyoake, Aichi 470-11, Japan, and Research Institute of Environmental Medicine and Department of Obstetrics and Gynecology, Nagoya University, Nagoya, Aichi 464, Japan

Received July 26, 1988; Revised Manuscript Received September 29, 1988

ABSTRACT: Alternative splicing of fibronectin pre-mRNA at two distinct regions, termed ED-A and IIICS, was investigated with human adult and fetal tissues by the nuclease S1 protection assay. A clear tissue specificity was observed in the splicing pattern at the ED-A region. More ED-A$^+$ than ED-A$^-$ mRNAs were identified in lung, whereas ED-A$^-$ mRNAs were predominantly expressed in liver. Endometrium contained nearly equal amounts of ED-A$^+$ and ED-A$^-$ mRNAs. The splicing pattern at the ED-A region was also different between adult and fetal liver but not between adult and fetal lung. Tissue type specific splicing was also observed at the IIICS region. Although the mRNA species containing the complete IIICS sequence comprised 40-65% of the total fibronectin mRNAs irrespective of tissue types, expression of the mRNA species lacking a part or all of the IIICS sequence was more pronounced in adult liver than in other tissues including fetal liver. These results strongly suggest that the alternative splicing of fibronectin pre-mRNA in vivo is regulated in a tissue type specific manner at both the ED-A and IIICS regions and that it is developmentally regulated in liver but not in lung. On the basis of these and other observations reported previously, a possibility that a part of the fibronectins synthesized and secreted by hepatocytes is deposited in the tissue matrix is discussed.

Fibronectins (FNs)[1] are multifunctional adhesive glycoproteins present in the extracellular matrix and plasma. FNs bind to fibroblasts and many other cell types, thereby mediating attachment and spreading of cells, and they also bind to collagens, heparin, fibrin, DNA, and certain types of bacteria [for recent reviews, see Yamada (1983), Mosher (1984), Hakomori et al. (1984), Hynes (1985), and Ruoslahti and Pierschbacher (1986)]. FNs consist of two subunits with molecular weights of about 250 000 which are connected by

† This work was supported by grants-in-aid from Fujita-Gakuen Health University, the Ministry of Science, Education, and Culture of Japan, and Uehara Memorial Foundation.

* To whom correspondence should be addressed.
‡ Fujita-Gakuen Health University School of Medicine.
§ Research Institute of Environmental Medicine, Nagoya University.
∥ Department of Obstetrics and Gynecology, Nagoya University.

[1] Abbreviations: FN, fibronectin; ED, extra domain; IIICS, type III connecting segment; PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); ED-A$^+$ mRNA, FN mRNA containing the ED-A sequence; ED-A$^-$ mRNA, FN mRNA lacking the ED-A sequence.

0006-2960/89/0428-1428$01.50/0

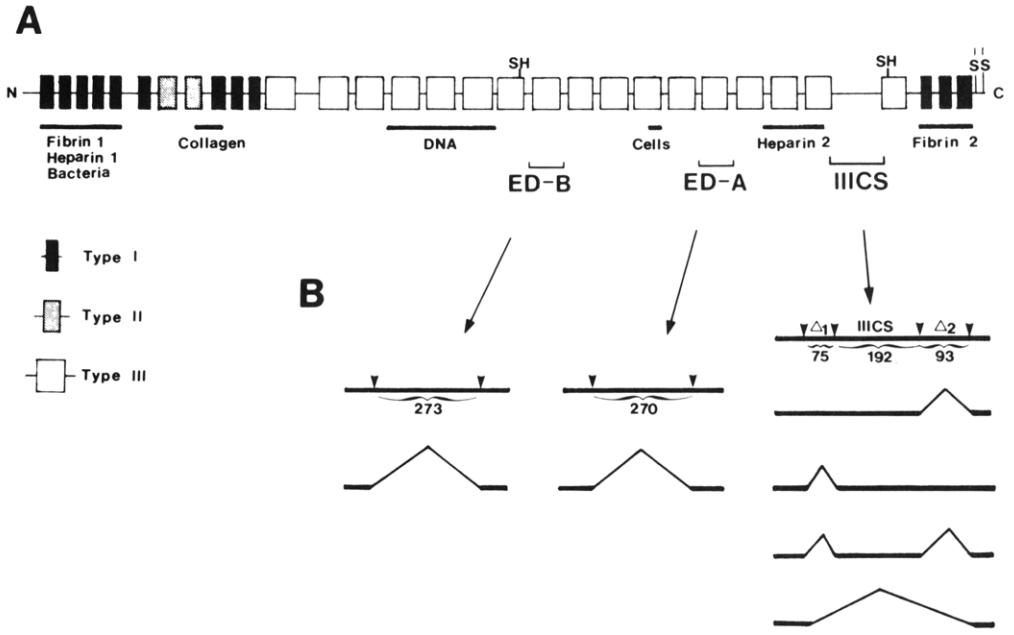

FIGURE 1: Schematic representation of FN structure and variants. (A) Modular structure of FNs. FN polypeptides are composed of three types of internal homology repeats: type I (black box), type II (shadowed box), and type III (open box). Binding sites to various ligands are indicated by bold underlines. (B) Variations of FN mRNAs. Multiple FN isoforms differing in the structure at ED-A, ED-B, and IIICS regions arise from alternative pre-mRNA splicing. Two mRNA variants resulting from "exon skipping" are identified at the ED-A and ED-B regions, whereas five variant mRNAs are generated by "exon subdivision" at the IIICS region.

disulfide bonds near the carboxyl terminus. Amino acid sequence analysis revealed that these subunits are composed of three types of internally homologous repeats (referred to as type I, II, and III homology, respectively), which are assembled into a series of globular domains with distinct biological activities (Petersen, 1985; also see Figure 1).

FNs isolated from different cell types or tissues are similar in many properties but differ slightly in their subunit size (Yamada & Kennedy, 1979; Hayashi & Yamada, 1981; Sekiguchi et al., 1985, 1986a; Paul et al., 1986; Zardi et al., 1987). Recent studies on the cloning and sequencing of FN cDNAs as well as the gene have revealed that multiple forms of FNs arise from a single gene by alternative RNA splicing at three distinct regions termed ED-A, ED-B, and IIICS (Schwarzbauer et al., 1983, 1985, 1987; Kornblihtt et al., 1984a,b, 1985; Sekiguchi et al., 1986a; Zardi et al., 1987; Gutman & Kornblihtt, 1987; also see Figure 1). The ED-A and ED-B regions, each representing an extra type III homology repeat, are individually encoded by a "cassette exon" which is alternatively excluded from the mature mRNA (Kornblihtt et al., 1984a,b; Vibe-Pedersen et al., 1984; Zardi et al., 1987; Paolella et al., 1988). The IIICS region is encoded by one of the constitutive exons which contains internal donor and acceptor sites for optional splicing within the exon (Tamkun et al., 1984; Bernard et al., 1985). Comparison of the nucleotide sequences of cDNAs possibly encoding two major FN isoforms, i.e., "plasma FN" and "cellular FN", indicated that the ED-A sequence is excluded from the mature mRNA encoding plasma FN (Kornblihtt et al., 1984b; Umezawa et al., 1985; Sekiguchi et al., 1986a). Different splicing patterns at the IIICS region were also observed between the cDNAs encoding cellular and plasma FNs (Schwarzbauer et al., 1983; Kornblihtt et al., 1984b; Bernard et al., 1985; Sekiguchi et al., 1986a; Norton & Hynes, 1987).

Despite the extensive studies on the structural differences between the two major FN isoforms at both the protein and RNA levels, little is known about the molecular structure and isoform diversity of FNs insolubilized in tissues. Previously, we have analyzed the molecular heterogeneity of FNs present in the detergent-insoluble matrix of human lung by limited proteolysis with cathepsin D (Sekiguchi et al., 1986b). It was found that the FN insolubilized in adult lung consists of subunits similar to, but not identical with, those of plasma FN, whereas the FN in fetal lung consists of larger subunits which are characteristic of cellular FN (Sekiguchi et al., 1986b), suggesting that different FN isoforms accumulate in the fetal and adult tissues.

In the present investigation, we analyzed the isoform diversity of FNs synthesized in various human tissues at the level of mRNA in order for understanding expression and regulation of the FN polymorphism in vivo. The results presented herein indicate that the alternative splicing at the ED-A and IIICS regions is regulated in a tissue type specific manner and is modulated differently during ontogeny in liver but not in lung.

Experimental Procedures

Materials. Nuclease S1 was purchased from Boehringer Mannheim Yamanouchi (Tokyo, Japan), pGEM3 from Promega (Madison, WI), [α-^{32}P]dCTP from Amersham Japan (Tokyo, Japan), and the Klenow fragment of DNA polymerase I from Takara Shuzo (Kyoto, Japan). Restriction enzymes were purchased from New England Biolabs (Beverly, MA; *Hpa*II), Nippon Gene (Toyama, Japan; *Ava*II), and Toyobo (Osaka, Japan; all other enzymes).

Cells and Tissues. Human fetal lung fibroblast IMR-90 was supplied by the Japanese Cancer Research Resources Bank (Tokyo, Japan) and grown in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum. Normal adult liver and lung tissues were obtained from surgically dissected specimens of hepatocellular carcinomas and lung adenocarcinomas, respectively. Endometrial tissues were obtained by gynecological scrapings. Fetal liver and lung tissues were obtained from fetuses of 16–27-week gestation at autopsy after therapeutic abortion. The tissues were immediately frozen in liquid nitrogen or in some cases at −85 °C after surgical operation. The number of samples examined for each tissue was as follows: normal lung, 3; normal liver, 3; fetal lung, 1; fetal liver, 5; endometrium, 5.

Nuclease S1 Protection Analysis. Total RNA was prepared from 0.5–2 g of freshly frozen tissues or from two confluent monolayer cultures of human fibroblasts using guanidine isothiocyanate as a denaturing agent of ribonuclease as previously described (Chirgwin et al., 1979; Chomczynski & Sacchi, 1987). The integrity of the RNAs was routinely verified by Northern blot analysis with cDNA probes encoding fibronectin and actin. Nuclease S1 protection analysis was performed as described by Berk and Sharp (1977). The probe DNA for the ED-A region was prepared from the FN cDNA clone pFH111, which was kindly provided by Dr. A. R. Kornblihtt (Instituto de Investigaciones en Ingenieria Genetica y Biologia Molecular, Buenos Aires, Argentina) (Kornblihtt et al., 1984a). The 752-base *Acc*I–*Eco*RV fragment of pFH111, covering the entire ED-A region, was subcloned into the *Acc*I and *Sma*I sites of pGEM3. The resulting plasmid was digested with *Ava*II and *Pvu*II to yield the 541-base fragment, the ED-A probe, of which the 5′ 481 bases spanning the ED-A region were derived from pFH111 and the 3′ 60 bases were from pGEM3. The probe DNA for the IIICS region was prepared from another FN cDNA clone, pFH1, which was kindly provided by Dr. F. E. Baralle (Sir William Dunn School of Pathology, University of Oxford, Oxford, England) (Kornblihtt et al., 1983). The 610-base *Taq*I–*Rsa*I fragment covering the 267-base IIICS region was subcloned into the *Acc*I and *Sma*I sites of pGEM3. The resulting plasmid was digested with *Hpa*II and *Eco*RI to yield the 421-base fragment, the IIICS probe, of which 14 bases at the 3′ end were derived from pGEM3. Both probe DNAs were labeled at the 3′ end by the end-filling reaction with Klenow fragment as described (Oyama et al., 1988) and subjected to strand separation as described by Maxam and Gilbert (1980).

The 3′ end-labeled, single-stranded probe DNAs were hybridized with 5–10 μg of RNAs in 30 μL of 40 mM PIPES buffer, pH 6.4, containing 1 mM EDTA, 0.4 M NaCl, and 80% formamide, at 53 °C for 18 h. The DNA/RNA hybrids were then diluted with 300 μL of ice-cold "S1 solution" (50 mM sodium acetate containing 0.28 M NaCl, 4.5 mM $ZnSO_4$, and 600 units of nuclease S1, pH 4.6) and incubated at 37 °C for 30 min to digest the single-stranded portion of the DNA/RNA hybrids. After addition of 50 μL of 4 M ammonium acetate/0.1 M EDTA to inactivate the enzyme, the DNAs were extracted with phenol and then precipitated with 2-propanol. The nuclease-resistant fragments were analyzed on 6% polyacrylamide sequencing gels containing 7 M urea and by subsequent autoradiography. The relative radioactivities of the nuclease-resistant fragments were determined by using a Fujix Bio-Image Analyzer BA 100 (Fuji Photo Film Co., Kanagawa, Japan).

Results

Expression of the ED-A Region. Alternative splicing at the ED-A region generates two types of FN mRNAs, one with and the other without the ED-A sequence (see Figure 1). In order to study the regulation of alternative splicing at the ED-A region in vivo, total RNAs extracted from various human tissues as well as from cultured human lung fibroblasts were analyzed by the nuclease S1 protection assay using the antisense strand DNA covering the entire ED-A sequence as a probe (Figure 2). The probe DNA, 541 nucleotides long, contains an extra 60 bases derived from the cloning vector at its 5′ end (Figure 2A). Hybridization of the probe DNA with FN mRNAs with and without the ED-A sequence (i.e., "ED-A$^+$" and "ED-A$^-$" mRNAs) and subsequent nuclease S1 digestion should yield a 481-base and a 105-base fragment, respectively, retaining the 3′ end ^{32}P label (Figure 2A).

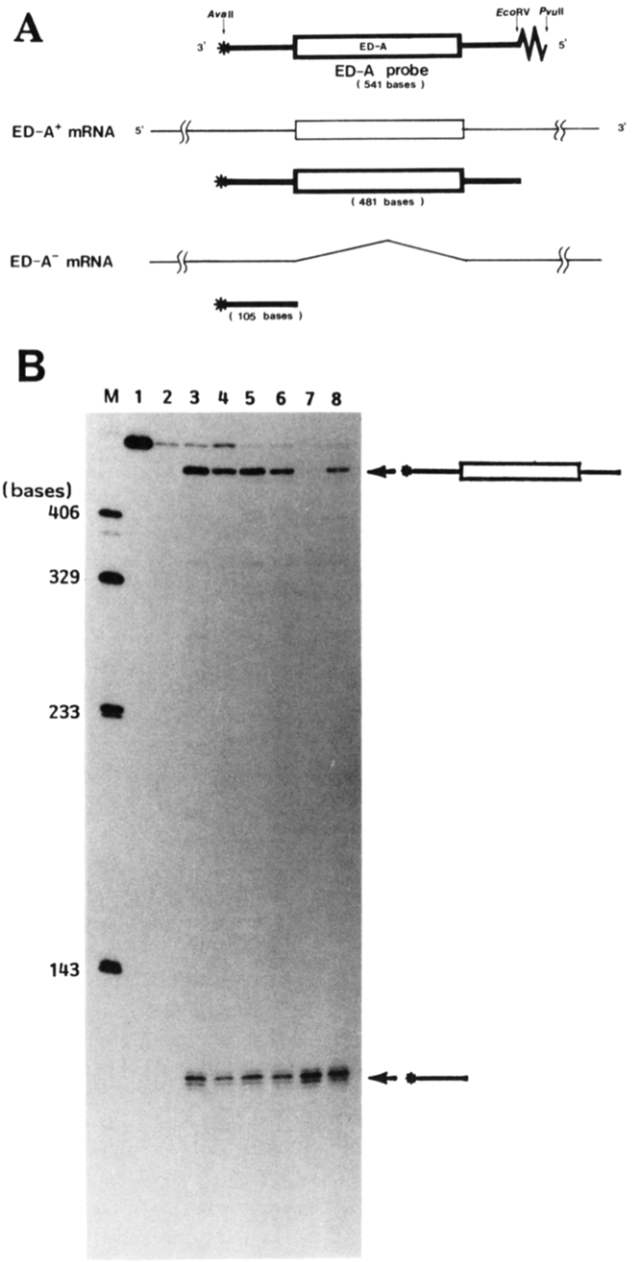

FIGURE 2: Expression of the ED-A sequence in human tissues. (A) Schematic representation of the probe DNA and the putative fragments protected with FN mRNA variants. The probe DNA contains extra nucleotides derived from the cloning vector at the 5′ end (indicated by a wavy line). The probe DNA and its putative fragments protected with the ED-A$^+$ and ED-A$^-$ mRNAs are drawn by bold lines. (B) Nuclease S1 protection analysis. The single-strand, ^{32}P-labeled probe DNA was hybridized with RNAs isolated from different sources and digested with nuclease S1 as described under Experimental Procedures. Lane 1, the undigested probe DNA. Lanes 2–8, the nuclease digests after hybridization with the following RNAs: lane 2, rat pituitary RNA; lane 3, RNA from human lung fibroblast IMR-90; lanes 4–8 are RNAs from human tissues—adult lung (lane 4), fetal lung (lane 5), endometrium (lane 6), adult liver (lane 7), and fetal liver (lane 8); lane M, molecular weight markers (the *Taq*I digest of *φX174* DNA). The numbers indicated at the left of the panel are the sizes (in nucleotides) of the molecular weight markers. Positions of the expected fragments are indicated by arrows at the right of the panel.

Upon protection with the RNA obtained from cultured lung fibroblasts, the probe DNA gave rise to both the 481-base ED-A$^+$ and the 105-base ED-A$^-$ fragments, of which the ED-A$^+$ fragment gave a stronger signal than the ED-A$^-$ fragment (Figure 2B, lane 3). A small amount of the probe DNA remained intact without losing the vector-derived se-

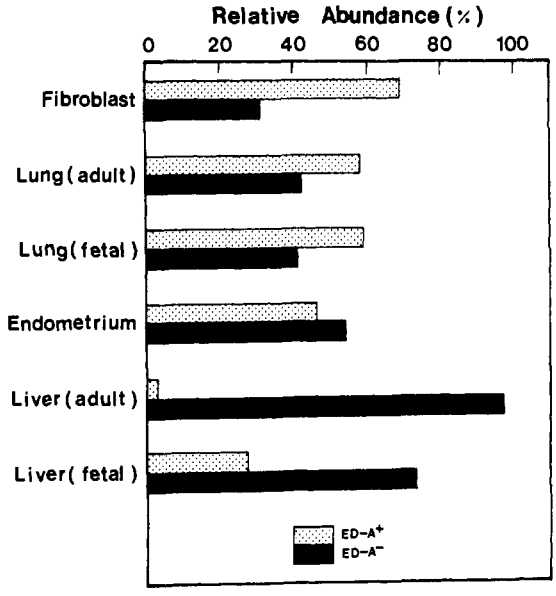

FIGURE 3: Relative abundance of the ED-A$^+$ and ED-A$^-$ mRNA species in human tissues. Relative radioactivities of the ED-A$^+$ and ED-A$^-$ fragments shown in Figure 2B were determined with a Fujix Bio-Image Analyzer BA 100 and expressed as percentage of the total radioactivities recovered in both fragments. Dotted bars, ED-A$^+$ mRNA; black bars, ED-A$^-$ mRNA.

quence in this and other nuclease digests (Figure 2B, lanes 2–8). This could be due to a contamination of the sense strand DNA in the probe, possibly resulting from incomplete strand separation during preparation of the probe DNA or due to the contaminating nuclease inhibitor present in the RNAs. Digestion with higher concentrations of nuclease S1 gave essentially the identical protection profile (data not shown). Hybridization with the rat RNA gave neither the ED-A$^+$ nor the ED-A$^-$ fragment but preserved a small amount of the intact probe DNA (Figure 2B, lane 2). Relative radioactivities of the protected fragments indicated that nearly two-thirds of the FN mRNAs present in cultured fibroblasts is the ED-A$^+$ species (Figure 3), being consistent with the previous observations (Kornblihtt et al., 1984a,b; Norton & Hynes, 1987).

More ED-A$^+$ than ED-A$^-$ fragment was also obtained by protection with the RNAs extracted from the adult and fetal lung, although the difference in the signal strength between these two fragments was less pronounced than that obtained with the fibroblast RNA (Figure 2B, lanes 4 and 5; also see Figure 3). There was no significant difference in the splicing pattern between adult and fetal lung, in both of which the ED-A$^+$ mRNA comprised about 60% of the total FN mRNA (Figure 3). It should be noted that dominant expression of the ED-A$^+$ mRNA in adult lung apparently contradicts our previous observation (Sekiguchi et al., 1986b) that the major FN isoforms insolubilized in adult lung were those of plasma FN, which should not contain the ED-A region (see Discussion). Another adult tissue, endometrium, was found to contain nearly equal amounts of the ED-A$^+$ and ED-A$^-$ mRNAs (Figure 2B, lane 6, and Figure 3).

In contrast, the RNAs extracted from liver gave a distinct nuclease protection pattern. More ED-A$^-$ than ED-A$^+$ fragment was obtained by protection with the RNAs extracted from both adult and fetal liver (Figure 2B, lanes 7 and 8). Relative radioactivities of these fragments indicated that the ED-A$^-$ mRNA comprised 97% and 73% of the total FN messages in adult and fetal liver, respectively (Figure 3). A significant increase of the ED-A$^+$ mRNA in the fetal liver suggests that regulation of the alternative splicing at the ED-A region is altered during ontogeny in liver. Furthermore, striking differences in the relative expression of the ED-A$^+$ and ED-A$^-$ mRNAs among liver, lung, and endometrium indicate that alternative splicing at the ED-A region is regulated in a tissue-specific manner.

Expression of the IIICS Region. Alternative splicing at the IIICS region is more complex than at the ED-A region and generates five possible mRNA variants (Bernard et al., 1985; Kornblihtt et al., 1985; Sekiguchi et al., 1986a; also see Figure 1). Whether alternative splicing at the IIICS region is also regulated in a tissue-specific manner was also examined by nuclease S1 protection assay using a single-strand probe DNA prepared from the variant 2 cDNA (Figure 4). The probe DNA and its putative fragments protected with five variant mRNAs are schematically illustrated in Figure 4A. Since the probe DNA lacks the 5′ 93 bases within the 360-base IIICS sequence, referred to as "Δ_2" in this paper, hybridization of this probe DNA with the variant 1 mRNA containing the complete IIICS sequence results in a partial protection of the 3′ 345 bases due to the mismatching at the boundary between the central 192 bases and the adjacent Δ_2 sequence. The variant 2 mRNA should fully protect the probe DNA except the 5′ 14 bases derived from the cloning vector, yielding a 407-base fragment. Three other mRNAs, the variants 3, 4, and 5, should protect only the 3′ 78 bases common to all FN mRNA species (see Figure 4A).

Hybridization of the IIICS probe DNA with RNAs prepared from cultured lung fibroblasts (Figure 4B, lane 3), adult and fetal lung (Figure 4B, lanes 4 and 5), and endometrium (Figure 4B, lane 6) yielded all of the three expected fragments with more or less similar proportions. The rat RNA failed to protect any of these fragments (Figure 4B, lane 2). Although a variable amount of the probe DNA remained undigested in these and other nuclease digests (Figure 4B, lanes 2–8), this could be either due to the contamination of the sense strand DNA in the probe or due to the unknown nuclease inhibitor(s) derived from the tissues or cells during extraction of the RNAs.

Relative radioactivities of the protected fragments, summarized in Figure 5, indicate that (a) the variant 1 mRNA was most abundant in adult and fetal lung, endometrium, and cultured fibroblasts, comprising a half to two-thirds of the total FN mRNAs, (b) the variant 3, 4, and 5 mRNAs altogether accounted for only 15–20% of the total FN mRNAs in fetal lung, endometrium, and cultured cells and slightly higher (i.e., 32%) in adult lung, and (c) the variant 2 mRNA comprised 20–25% of the total FN mRNAs in these tissues and cells.

In contrast, hybridization with the RNA from adult liver gave a significantly different protection pattern (Figure 4B, lane 7). Adult liver contained much less variant 1 and 2 mRNAs but relatively more variant 3, 4, and 5 mRNAs than lung, endometrium, or cultured cells. Relative radioactivities of the protected fragments indicated that the variant 3, 4, and 5 mRNAs altogether comprised 53% of the total FN mRNAs whereas the variant 1 and 2 mRNAs accounted for 41% and 6%, respectively (Figure 5). Interestingly, the protection profile obtained with the fetal liver RNA was different from that with the adult liver RNA, but similar to those obtained with the RNAs from lung or endometrium (Figure 4B, lane 8, and also Figure 5). These results indicate that the in vivo splicing at the IIICS region is regulated in a tissue-specific manner and that it is also developmentally regulated in liver but probably not in lung.

Discussion

Molecular diversity of FNs in various cell types and tissues

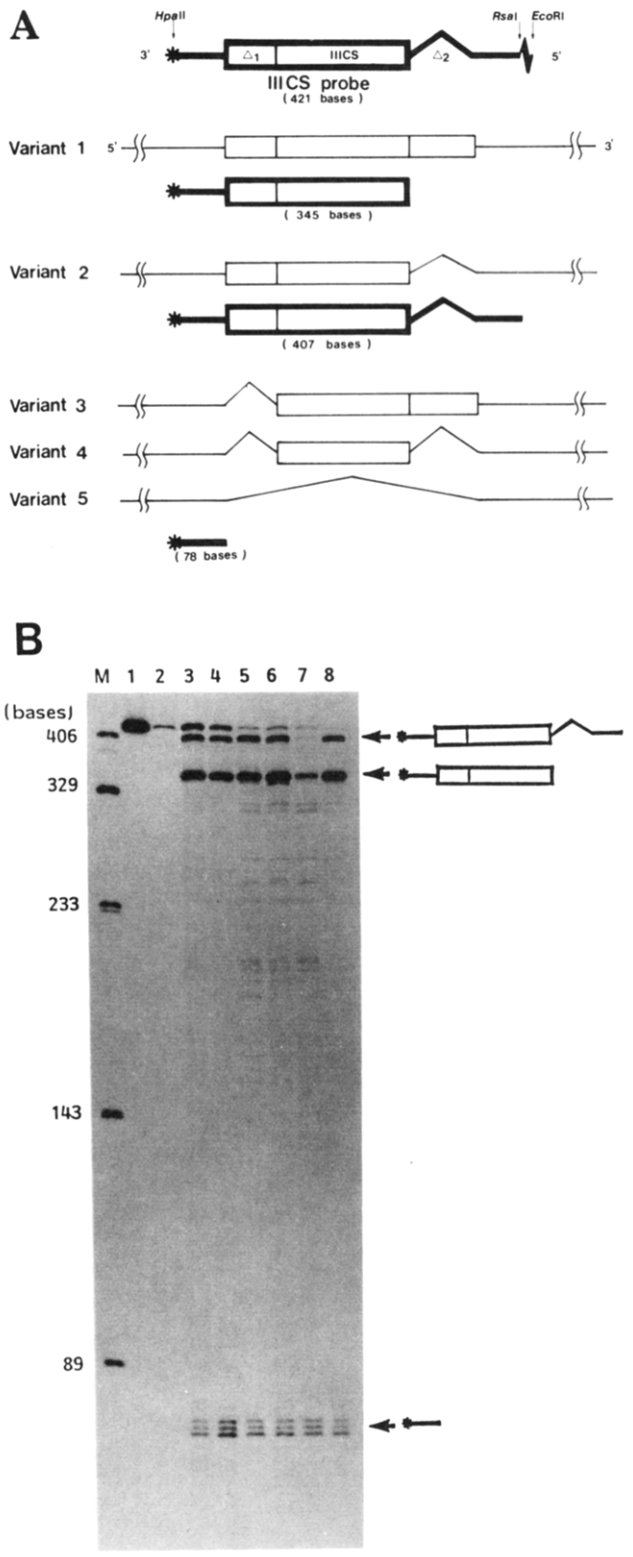

FIGURE 4: Expression of the IIICS sequence in human tissues. (A) Schematic representation of the probe DNA and the putative fragments (drawn by bold lines) protected by FN mRNA variants (drawn by skinny lines). The probe DNA contains extra nucleotides derived from the cloning vector at the 5′ end (indicated by the wavy line). (B) Nuclease S1 protection analysis. The analysis was performed essentially as described in the legend for Figure 2B using the IIICS probe DNA. Lane 1, the undigested probe DNA; lane 2, rat pituitary RNA; lane 3, RNA from human lung fibroblast IMR-90; lanes 4–8 are RNAs from human tissues—adult lung (lane 4), fetal lung (lane 5), endometrium (lane 6), adult liver (lane 7), and fetal liver (lane 8); lane M, molecular weight markers (the *Taq*I digest of $\phi X174$ DNA).

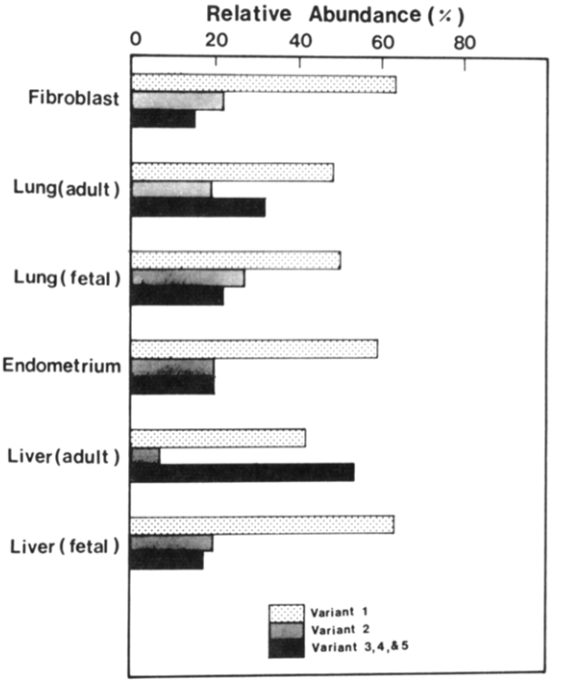

FIGURE 5: Relative abundance of the IIICS variant mRNAs in human tissues. The relative radioactivities of the differentially protected fragments (i.e., the 407-base, 345-base, and 78-base fragments) were determined with a Fujix Bio-Image Analyzer BA 100 and expressed as a percentage of the total radioactivities recovered in these fragments. Dotted bars, variant 1 mRNA; hatched bars, variant 2 mRNA; black bars, variant 3, 4, and 5 mRNAs.

is now considered to arise from alternative pre-mRNA splicing at three specific regions of the molecule, termed ED-A, ED-B, and IIICS (Hynes, 1985; Gutman & Kornblihtt, 1987; Schwarzbauer et al., 1987; Zardi et al., 1987). Posttranslational modifications such as glycosylation, sulfation, and phosphorylation may also contribute to increase the diversity (Paul & Hynes, 1984; Liu et al., 1985; Nichols et al., 1986). It is not well understood, however, how alternative splicing at these regions is regulated in different tissues and why it is regulated in such a manner. Previously, we analyzed FN isoforms insolubilized in adult and fetal lung by limited proteolysis and found that plasma FN is the major isoform in adult lung, whereas cellular FN is predominantly expressed in fetal lung (Sekiguchi et al., 1986b). Thus, we assumed that the major FN isoform insolubilized in tissues switches from the cellular to the plasma form during ontogeny. The experiments described herein were initiated in order to confirm these observations at the level of mRNA.

Unexpectedly, there was no clear difference in the splicing patterns at the ED-A and the IIICS regions between fetal and adult lung. The ED-A^+ mRNA was more abundant than the ED-A^- mRNA in both fetal and adult lung. Since the presence of the ED-A extra type III homology is a major distinction for the cellular FN (Kornblihtt et al., 1984b; Paul et al., 1986; Peters et al., 1986; Borsi et al., 1987), these results strongly suggest tht the cellular FN is the major isoform(s) synthesized in adult lung, contradicting our previous observations obtained at the protein level. Although it is not yet known so far why the ED-A^- FN, instead of the ED-A^+ FN, is predominantly found in the tissue matrix of adult lung, it is tempting to speculate that the majority of the FNs insolubilized in adult lung are derived from circulating plasma. Deposition of plasma FN in connective tissue matrix was previously demonstrated by Oh et al. (1981). Thus, FNs insolubilized in tissues can be a mixture of those synthesized in situ and those deposited from plasma, the proportion of which may well be

greatly affected by cell differentiation, proliferation, and developmental stages. It could not be ruled out, however, that the ED-A$^+$ FN isoforms are selectively degraded or removed from the extracellular matrix of adult tissues by an unknown mechanism. Neither could it be ruled out that the ED-A$^+$ messages are less efficiently translated into proteins than the ED-A$^-$ messages in adult lung.

Several lines of evidence have indicated that the expression of the ED-A region is regulated in a cell type specific manner (Kornblihtt et al., 1984b; Paul et al., 1986; Borsi et al., 1987). Liver, considered as the source of plasma FN, predominantly produces the ED-A$^-$ mRNA, whereas fibroblasts and other cell types grown in vitro express more the ED-A$^+$ than the ED-A$^-$ mRNA (Kornblihtt et al., 1984b; Norton & Hynes, 1987). Similar observations also have been made at the level of protein by utilizing specific antibodies raised against the ED-A peptide segment (Paul et al., 1986; Peters et al., 1986; Borsi et al., 1987; Vartio et al., 1987). However, it is not yet clear whether any human tissue other than liver synthesizes as much the ED-A$^+$ mRNAs as cultured fibroblasts. Our results clearly indicate that both adult lung and fetal lung do express more ED-A$^+$ than ED-A$^-$ mRNAs, confirming that in vivo alternative splicing at the ED-A region is indeed regulated in a tissue-specific manner. Although no specific function has emerged for the ED-A peptide segment, it may modulate the assembly of FNs into the extracellular matrix by modifying the protein conformation or the interaction with the FN receptor molecules mediated by the Arg-Gly-Asp cell adhesion signal.

Tissue-specific regulation of alternative splicing is observable not only in the ED-A but also in the IIICS region. Expression of the variant 3, 4, and 5 mRNAs was significantly increased in adult liver when compared with adult lung and endometrium. Conversely, expression of the variant 1 mRNA was lower in adult liver than in other tissues. It should be noted, however, that nearly 40% of the FN messages in adult liver were still the variant 1 mRNA, potentially controversial to the previous observation that the Δ_2 peptide is absent in the major IIICS-containing tryptic fragment of plasma FN (Garcia-Pardo et al., 1985). Our preliminary study with antibodies specifically recognizing the Δ_2 peptide also indicated that only a minor population of plasma FN contains the Δ_2 peptide (K. Sekiguchi, unpublished observation). Given the proposed role of liver as a major source of plasma FN (Owens & Cimino, 1982; Tamkun & Hynes, 1983), these observations at the protein level are not consistent with our data obtained at the mRNA level. The reason for this discrepancy is not well understood, but the following possibilities should be considered: The variant 1 mRNA could be unstable and only poorly translated into the protein in adult liver. Alternatively, the FN isoform with the complete IIICS sequence is synthesized and secreted by hepatocytes, but it may be quickly removed from circulating plasma through direct or indirect binding to the blood vessel wall or to the connective tissue matrix. Selective interaction of this isoform with certain plasma protein(s) and subsequent pinocytosis by macrophages may not be ruled out.

Our results also suggest that alternative splicing at both the ED-A and IIICS regions is developmentally regulated in liver but probably not in lung. Matsuura and Hakomori (1985) have isolated a monoclonal antibody that specifically recognizes FNs present in fetal and cancer tissues but absent from normal adult tissues. This oncofetal epitope has now been identified as a novel glycopeptide, of which both peptide and carbohydrate portions are required for the antigenicity (Matsuura et al., 1988). Our results, however, indicate that the alternatively spliced peptide segments per se could be a differentiation marker in liver. Whether in vivo splicing at the ED-A and IIICS regions is altered in hepatoma and other cancer tissues remains to be determined.

ACKNOWLEDGMENTS

We thank Drs. Alberto R. Kornblihtt and Francisco E. Baralle for providing the FN cDNA clones and Drs. Setsuo Hirohashi, Kohei Shiota, and Toshio Adachi for kindly allowing us to examine their human tissue specimens.

REFERENCES

Berk, A., & Sharp, P. A. (1977) *Cell 12*, 721–732.
Bernard, M. P., Kolbe, M., Weil, D., & Chu, M.-L. (1985) *Biochemistry 24*, 2698–2704.
Borsi, L., Carnemolla, B., Castellani, P., Rosellini, C., Vecchio, D., Allemanni, G., Chang, S. E., Taylor-Papadimitriou, J., Pande, H., & Zardi, L. (1987) *J. Cell Biol. 104*, 595–600.
Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., & Rutter, W. J. (1979) *Biochemistry 18*, 5294–5299.
Chomczynski, P., & Sacchi, N. (1987) *Anal. Biochem. 162*, 156–159.
Garcia-Pardo, A., Pearlstein, E., & Frangione, B. (1985) *J. Biol. Chem. 258*, 12670–12674.
Gutman, A., & Kornblihtt, A. R. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 7179–7182.
Hakomori, S., Fukuda, M., Sekiguchi, K., & Carter, W. G. (1984) in *Extracellular Matrix Biochemistry* (Piez, K., & Reddi, A. H., Eds.) pp 229–275, Elsevier, New York.
Hayashi, M., & Yamada, K. M. (1981) *J. Biol. Chem. 256*, 11292–11300.
Hynes, R. O. (1985) *Annu. Rev. Cell Biol. 1*, 67–90.
Kornblihtt, A. R., Vibe-Pedersen, K., & Baralle, F. E. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 3218–3222.
Kornblihtt, A. R., Vibe-Pedersen, K., & Baralle, F. E. (1984a) *EMBO J. 3*, 221–226.
Kornblihtt, A. R., Vibe-Pedersen, K., & Baralle, F. E. (1984b) *Nucleic Acids Res. 12*, 5853–5868.
Kornblihtt, A. R., Umezawa, K., Vibe-Pedersen, K., & Baralle, F. E. (1985) *EMBO J. 4*, 1755–1759.
Liu, M. C., Yu, S., Sy, J., Redman, C. M., & Lipmann, F. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 7160–7164.
Matsuura, H., & Hakomori, S. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 6517–6521.
Matsuura, H., Takio, K., Titani, K., Greene, T., Levery, S. B., Salyan, M. E., & Hakomori, S. (1988) *J. Biol. Chem. 263*, 3314–3322.
Maxam, A. M., & Gilbert, W. (1980) *Methods Enzymol. 65*, 499–580.
Mosher, D. F. (1984) *Annu. Rev. Med. 35*, 561–575.
Nichols, E. J., Fenderson, B. A., Carter, W. G., & Hakomori, S. (1986) *J. Biol. Chem. 261*, 11295–11301.
Norton, P. A., & Hynes, R. O. (1987) *Mol. Cell. Biol. 7*, 4297–4307.
Oh, E., Pierschbacher, M. D., & Ruoslahti, E. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 3718–3721.
Owens, M. R., & Cimino, C. D. (1982) *Blood 59*, 1305–1309.
Oyama, F., Kikuchi, R., Omori, A., & Uchida, T. (1988) *Anal. Biochem. 172*, 444–450.
Paolella, G., Henchcliffe, C., Sebastio, G., & Baralle, F. E. (1988) *Nucleic Acids Res. 16*, 3545–3557.
Paul, J. I., & Hynes, R. O. (1984) *J. Biol. Chem. 259*, 13477–13487.
Paul, J. I., Schwarzbauer, J. E., Tamkun, J. W., & Hynes, R. O. (1986) *J. Biol. Chem. 261*, 12258–12265.

Peters, J. H., Ginsburg, M. H., Bohl, B. P., Sklar, L. A., & Cochrane, C. G. (1986) *J. Clin. Invest. 78*, 1596–1603.

Petersen, T. E. (1985) in *Plasma Fibronectin* (McDonagh, J., Ed.) pp 7–30, Marcel Dekker, New York.

Ruoslahti, E., & Pierschbacher, M. D. (1986) *Cell 44*, 517–518.

Schwarzbauer, J. E., Tamkun, J. W., Lemischka, I. R., & Hynes, R. O. (1983) *Cell 35*, 421–431.

Schwarzbauer, J. E., Paul, J. I., & Hynes, R. O. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 1424–1428.

Schwarzbauer, J. E., Patel, R. S., Fonda, D., & Hynes, R. O. (1987) *EMBO J. 6*, 2573–2580.

Sekiguchi, K., Siri, A., Zardi, L., & Hakomori, S. (1985) *J. Biol. Chem. 260*, 5105–5114.

Sekiguchi, K., Klos, A. M., Kurachi, K., Yoshitake, S., & Hakomori, S. (1986a) *Biochemistry 25*, 4936–4941.

Sekiguchi, K., Klos, A. M., Hirohashi, S., & Hakomori, S. (1986b) *Biochem. Biophys. Res. Commun. 141*, 1012–1017.

Tamkun, J. W., & Hynes, R. O. (1983) *J. Biol. Chem. 258*, 4641–4647.

Tamkun, J. W., Schwarzbauer, J. E., & Hynes, R. O. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 5140–5144.

Umezawa, K., Kornblihtt, A. R., & Baralle, F. E. (1985) *FEBS Lett. 186*, 31–34.

Vartio, T., Laitinen, L., Narvanen, O., Cutolo, M., Thornell, L.-E., Zardi, L., & Virtanen, I. (1987) *J. Cell. Sci. 88*, 419–430.

Vibe-Pedersen, K., Kornblihtt, A. R., & Baralle, F. E. (1984) *EMBO J. 3*, 2511–2516.

Yamada, K. M. (1983) *Annu. Rev. Biochem. 52*, 761–799.

Yamada, K. M., & Kennedy, D. W. (1979) *J. Cell Biol. 80*, 492–498.

Zardi, L., Carnemolla, B., Siri, A., Petersen, T. E., Paolella, G., Sebastio, G., & Baralle, F. E. (1987) *EMBO J. 6*, 2337–2342.