Human Liver Fibronectin Complementary DNAs: Identification of Two Different Messenger RNAs Possibly Encoding the α and β Subunits of Plasma Fibronectin†

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ABSTRACT: Human fibronectin polymorphism arises from variation in the C-terminal region [e.g., Sekiguchi, K., Siri, A., Zardi, L., & Hakomori, S. (1985) *J. Biol. Chem. 260*, 5105-5114]. In order to verify the chemical basis of the fibronectin polymorphism, cDNAs encoding the C-terminal region of human liver fibronectin have been isolated, sequenced, and compared with cDNAs encoding so-called "cellular fibronectin" (i.e., fibronectin produced by cultured cells in vitro). Among the five independent cDNAs thus isolated, two cDNAs, named pLF2 and pLF4, differed in the nucleotide sequence at the "type III connecting segment" (IIIcs) region. pLF4 contained 192 bases in this region whereas pLF2 completely lacked these bases. S1 mapping analysis indicated that both cDNAs with and without the 192 bases are faithful copies of two fibronectin mRNA species abundantly present in human liver. Comparison of the liver cDNAs with those coding for cellular fibronectin indicates that the latter cDNAs contain the 75-base and/or 93-base extra segments at the 5′ and 3′ boundaries of the 192-base IIIcs region. These extra segments have the consensus sequences for the 3′ splice sites at their 3′ ends, suggesting that fibronectin mRNAs with partial or complete deletion of the IIIcs sequence result from alternative splicing of a primary RNA transcript. Liver fibronectin cDNAs also lacked the 270-based "extra domain" (ED) segment present in some, but not all, cDNAs encoding cellular fibronectin. Thus, cellular fibronectin appears to have three extra peptide segments, encoded by the 75-base and 93-base segments in the IIIcs region and by the 270-base ED region, that are mostly absent in the liver fibronectin. Since plasma fibronectin is produced in the liver, pLF4 and pLF2 may encode respectively the α and β subunits of plasma fibronectin.

Fibronectin (FN)[1] is a multifunctional glycoprotein playing important roles in homeostasis of the "milieu intérieur" [for reviews, see Ruoslahti et al. (1981), Hynes and Yamada (1982), Hakomori et al. (1984), and Mosher (1984)]. There are essentially two types of FNs, i.e., "cellular FN" (cFN) and "plasma FN" (pFN). cFN is synthesized and deposited in the pericellular matrix of cultured fibroblasts or tumor cells in vitro, although FNs present in the matrix of normal tissues in vivo may not be identical with those produced by cultured cells (Matsuura & Hakomori, 1985). pFN, previously known as cold-insoluble globulin (Morrison et al., 1948), is considered to be produced mainly, like other plasma proteins, by hepatocytes (Owens & Cimino, 1982; Tamkun & Hynes, 1983).

These two types of FNs are known to differ slightly in their chemical and biological properties (see the reviews above). Our previous study with domain-specific antibodies unequivocally demonstrated that the *Hep-2* domain of cFN is apparently 20000-30000 daltons larger than that of pFN (Sekiguchi et al., 1983, 1985a). Another polymorphism has also been found in two subunit polypeptides of pFN. One subunit, α, is 10000 daltons larger than the other subunit, β. It seems that the α-subunit contains a 10000-dalton extra peptide segment located between the *Hep-2* and *Fib-2* domains (Richter et al., 1981; Hayashi & Yamada, 1983; Sekiguchi & Hakomori, 1983).

During the past few years, the primary structure of different types of FNs from various sources has been studied either by direct protein sequencing (Vibe-Pedersen et al., 1982; Skorstengaard et al., 1982, 1984; Pierschbacher et al., 1982; Petersen et al., 1983; Garcia-Pardo et al., 1983, 1985; Pande et al., 1985) or by sequencing of cDNAs (Kornblihtt et al., 1983, 1984a,b, 1985; Oldberg et al., 1983; Schwarzbauer et al., 1983; Bernard et al., 1985), although exact differences in the sequence that create human fibronectin polymorphism have not been elucidated. The presence of two or more different FN mRNA species has been demonstrated by characterization of cDNA clones from rat liver (Schwarzbauer et al., 1983) and from human tumor cells (Kornblihtt et al., 1984a,b).

This paper reports isolation and characterization of two distinct cDNAs possibly encoding the α and β subunits of human pFN. Comparisons of these cDNAs with those encoding cFN provide insights on the chemical and genetic basis of human FN polymorphism. A preliminary account of this study was reported elsewhere (Sekiguchi et al., 1985b).

Experimental Procedures

Materials. Restriction enzymes were purchased from Bethesda Research Laboratories (Gaithersburg, MD) and New England Biolabs (Beverly, MA) and used as recommended by the suppliers. *Escherichia coli* DNA polymerase

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[1] Abbreviations: FN, fibronectin; cFN, cellular fibronectin; pFN, plasma fibronectin; SDS, sodium dodecyl sulfate; *Hep-2*, the C-terminal heparin-binding domain; *Fib-2*, the C-terminal fibrin-binding domain; ED, extra domain; IIIcs, type III connecting segment; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane.

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was obtained from New England Nuclear (Boston, MA), nuclease S1 and calf intestine phosphatase were from Boehringer Mannheim Biochemicals (Indianapolis, IN), T4 polynucleotide kinase and Klenow fragment of *E. coli* DNA polymerase were from Bethesda Research Laboratories, and T4 DNA ligase was from New England Biolabs. Deoxyadenosine 5′-(α-[^{35}S]thiotriphosphate) was purchased from Amersham (Arlington Heights, IL). Dideoxynucleotides were from Pharmacia P-L Biochemicals (Piscataway, NJ).

Screening of the cDNA Library. A cDNA library prepared from human liver poly(A)+ RNA by using pUC13 as a vector (Kurachi et al., 1985) was initially screened with chicken genomic DNA coding for FN. The 2-kb *Eco*RI/*Hin*dIII fragment derived from the 3′ end region of chicken FN gene (Hirano et al., 1983) was a generous gift from Drs. Kenneth Yamada and Hideyasu Hirano, National Cancer Institute (Bethesda, MD). Approximately 75 000 recombinant colonies were grown on agar plates containing ampicillin (25 μg/mL), transferred to Whatman 541 filters, and incubated on agar plates containing chloramphenicol (200 μg/mL) to amplify recombinant plasmids. Colonies on the filters were then lysed with 0.5 N NaOH and prepared for hybridization as described by Wallace et al. (1981). The probe was nick-translated with [α-^{32}P]dCTP and [α^{32}P]dTTP (Rigby et al., 1977). Hybridization was carried out in 6 × SSC (1 × SSC: 150 mM NaCL/15 nM sodium citrate, pH 7.0) containing 5 × Denhardt's solution (1 × Denhardt's solution: 1% Ficoll/1% poly(vinlypyrrolidone)/1% bovine serum albumin), 0.5% SDS, 10 mM EDTA, and 0.5 × 10^6 cpm/mL of nick-translated probe at 68 °C for 16–18 h. Filters were washed with 2 × SSC containing 0.1% SDS 6 times for 15–20 min per wash at 42, 55, or 60 °C. Later, the same library was repeatedly screened for FN cDNAs with human cDNA probes, where conditions for hybridization and subsequent wash of filters were essentially the same as above except that filters were washed at 68 °C with 2 × SSC containing 0.1% SDS 4 times and then with 1 × SSC containing 0.1% SDS 2 times.

Sequence Determination. Restriction fragments were subcloned into M13 mp18 or mp19 (Yanisch-Perron et al., 1985) and sequenced by the dideoxy chain termination method (Sanger et al., 1977) with deoxyadenosine 5′-(α-[^{35}S]thiotriphosphate) and buffer gradient gels (Biggin et al., 1983).

S1 Mapping Analysis. S1 mapping analysis of mRNA/cDNA hybrids was performed by the method of Berk and Sharp (1977) with the following modification made by Paul Labhard (Fred Hutchinson Cancer Research Center, Seattle, WA). Human liver poly(A)+ RNA was prepared from freshly frozen liver (2 g) with guanidine isothiocyanate as a denaturing agent of ribonuclease (Maniatis et al., 1982). The *Hpa*II/*Rsa*I fragments covering the entire IIIcs region were isolated from pLF4 and pLF2 and used as probes. The 331-base *Hpa*II/*Rsa*I fragment of pLF4, including the 192 bases deleted in pLF2, was labeled at the 5′ end by T4 polynucleotide kinase (Maniatis et al., 1982). The 139-base *Hpa*II/*Rsa*I fragment derived from pLF2 was labeled at the 3′ end by adding [^{32}P]dCTP with Klenow fragment of *E. coli* DNA polymerase (Maniatis et al., 1982). Strand separation was carried out as described by Maxam and Gilbert (1980). The 5′ end or 3′ end labeled probe (5 × 10^4 cpm) was hybridized with 15 μg of liver poly(A)+ RNA (or yeast tRNA as a control) in 30 μL of 10 mM Tris-HCl (pH 7.5)/1 mM EDTA/0.3 M NaCl for 16–20 h at 65 °C with a cover of paraffin oil. The hybrids were digested with nuclease S1 at 42 °C for 1 h after dilution with 270 μL of S1 buffer (30 mM sodium acetate/1 mM $ZnSO_4$/250 mM NaCl, pH 4.5) containing 200 units of nuclease S1. S1-resistant fragments were analyzed on a 6% urea–acrylamide gel. The size of the fragments was determined by comparison with DNA sequencing ladders.

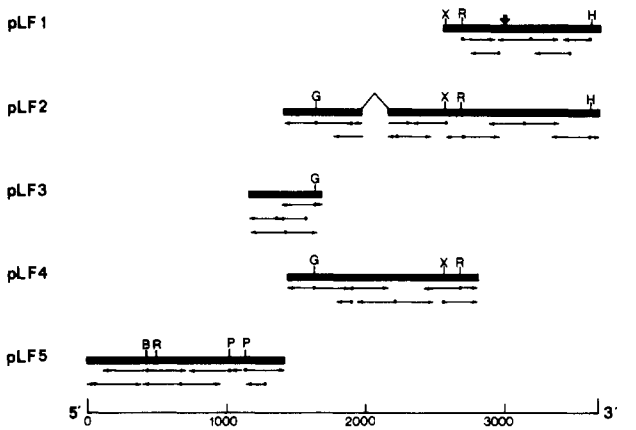

FIGURE 1: Alignment and partial restriction maps of the five selected cDNAs coding for human liver fibronectin. Restriction sites for *Bam*HI (B), *Eco*RI (R), *Pst*I (P), *Bgl*II (G), *Xba*I (X), and *Hin*dIII (H) are marked. The extent and direction of each nucleotide sequence analysis are shown by horizontal arrows below each cDNA. A gap of 192 bases between *Bgl*II (G) and *Xba*I (X) sites in pLF2 is shown by a bent line. The position of the termination codon is marked by a bold arrow.

RESULTS

Isolation of FN cDNA. A human liver cDNA library was screened for cDNAs coding for FN. Initially, 75 000 colonies were screened by using as a probe a 2-kb *Eco*RI/*Hin*dIII chicken genomic DNA fragment derived from the 3′ end region of FN gene. This fragment was shown to contain the terminal 800-base exon located at the 3′ end (Hirano et al., 1983). Hybridization was performed at a low stringency condition, since the sequence homology between human and chicken FN gene was not known. Five positive clones were identified. One of these clones, which gave the strongest signal, named pLF1, was purified and characterized by partial nucleotide sequencing. pLF1 contained a 1.3-kb insert whose nucleotide sequence was found to be identical with the sequence reported for the 3′ region of the FN cDNAs (Kornblihtt et al., 1983, 1984a; Bernard et al., 1985).

A 130-base *Eco*RI/*Bgl*I fragment derived from near the 5′ end of pLF1 was used as a probe for the rescreening of the same library. A clone containing a 2.3-kb insert, named pLF2, was thus isolated. In a similar manner, repeated screening of the same library with human cDNA probes resulted in the isolation of three additional clones, i.e., pLF3, pLF4, and pLF5, which extend further to the 5′ end.

Alignment and partial restriction maps of these cDNA clones are shown in Figure 1. Interestingly, two partially overlapping clones, pLF2 and pLF4, were found to differ in the length of their *Bgl*II/*Xba*I fragments. pLF2 lacked 192 bases present between the *Bgl*II and *Xba*I sites in pLF4. This was further confirmed by nucleotide sequencing of these clones (see below).

Nucleotide Sequence and Predicted Amino Acids. The nucleotide sequences of the five cDNAs, except pLF1, were determined completely. These cDNAs altogether cover 3670 bases from the poly(A) addition site, comprising nearly 46% of 7.9-kb human FN mRNA (Kornblihtt et al., 1983). The sequence consists of a 692-base noncoding region and a 2978-base coding region. Overlapping sequences among these cDNAs were exactly identical except that pLF2 lacked 192

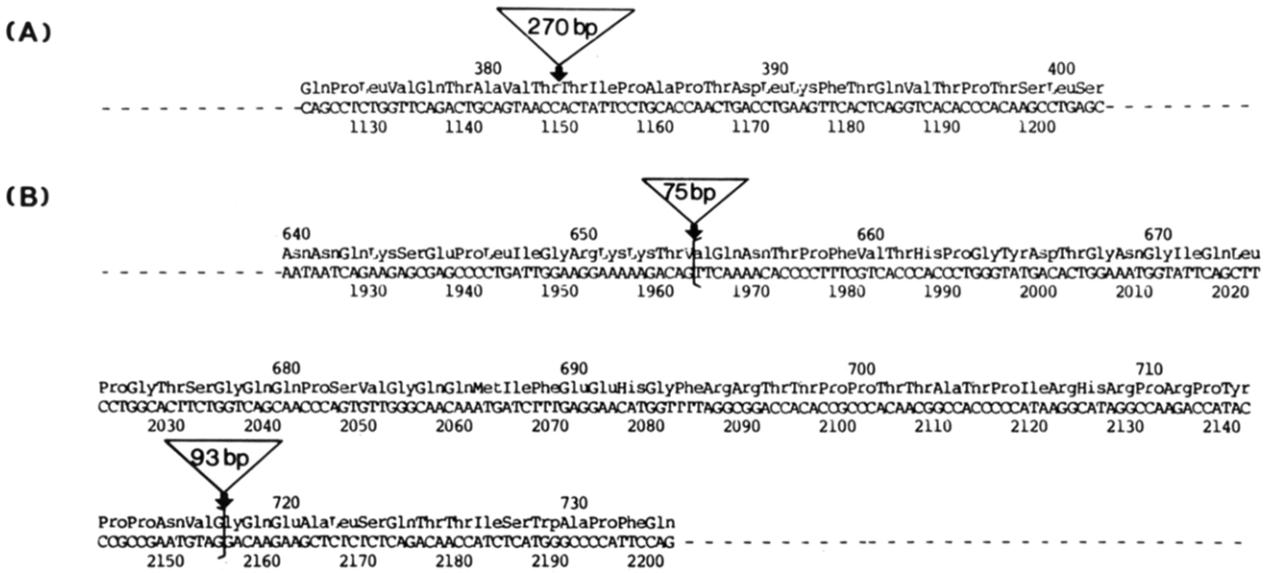

FIGURE 2: Nucleotide sequence and predicted amino acids of the human liver FN cDNAs. Only a part of the sequence containing the two variations regions, i.e., the ED and its flanking region (A) and the IIIcs and its flanking region (B), is shown here. The rest of the sequence is identical with the sequence reported for the cDNAs prepared from cultured cells (Kornblihtt et al., 1984a; Bernard et al., 1985). The nucleotide sequence of the liver FN cDNA begins at nucleotide number 850 (i.e., ACACTGTCAA...) of the sequence reported by Kornblihtt et al. (1984a). The 192-base segment absent in pLF2 is marked by brackets. The arrows indicate the positions of the extra nucleotide segments present in cDNAs prepared from the cultured cells. Nucleotides and amino acids are numbered below and above the sequence, respectively.

bases between positions 1963 and 2154 (Figure 2B). Although the overall nucleotide sequence thus determined is mostly identical with the sequence of cDNAs obtained from human cultured cells (Kornblihtt et al., 1984a; Bernard et al., 1985), there are the following differences: cDNAs obtained from the liver did not contain the 270-base "extra domain" (ED) sequence inserted between positions 1148 and 1149 in some cDNAs obtained from cultured cells (Figure 2A). Liver cDNAs also lacked the 75-base and 93-base segments present between positions 1962 and 1963 and positions 2154 and 2155, respectively, in the cDNAs obtained from culture cells (Figure 2B). The 192-base segment (positions 1963–2154) and the 75- and 93-base flanking segments are collectively referred to as the "type III connecting segment" (IIIcs) region (Kornblihtt et al., 1984a).

The nucleotide sequence thus determined encodes the C-terminal 992 amino acids that comprise 42% of the FN polypeptide. There are two types of internal homology, i.e., type I and type III homology, in this region (Petersen et al., 1983; Schwarzbauer et al., 1983; Kornblihtt et al., 1984). The 64 amino acids encoded by the 192 bases deleted in pLF2 are not related to either type I or type III homology. These amino acids contain two putative tryptic cleavage sites, i.e., Arg-Arg-Thr (positions 695–697) and Arg-His-Arg (positions 708–710). This indicates that the subunit with the extra 64 amino acids can be cleaved at the junction between the *Hep-2* and *Fib-2* domains whereas the other subunit lacking the extra peptide, i.e., the subunit encoded by pLF2, may be resistant to trypsin at the corresponding junction (see Discussion).

S1 Mapping Analysis. The presence of two cDNA clones with and without the 192-base extra sequence indicates that there are at least two FN mRNAs differing in the nucleotide sequence at the IIIcs region in the human liver. Whether both pLF2 and pLF4 are the faithful copies of mRNA species present in the liver was tested by an S1 nuclease protection assay (Berk & Sharp, 1977).

Two probes, with and without the 192-base extra segment, were prepared from pLF4 and pLF2, respectively. One probe was the 331-base *Hpa*II/*Rsa*I fragment derived from pLF4 (Figure 3A). This fragment covers the 192-base IIIcs region

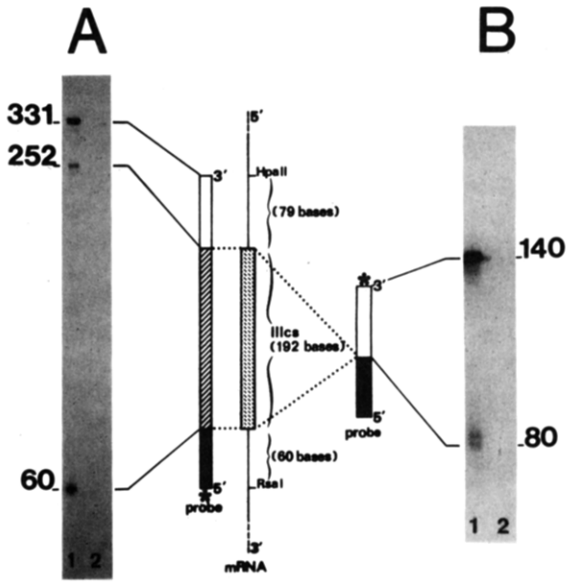

FIGURE 3: S1 mapping analysis of RNA/cDNA hybrids. RNAs (15 μg) were hybridized with single-stranded, ^{32}P-labeled probes and digested with nuclease S1 as described under Experimental Procedures. The probes used are the 5′ end labeled 331-base *Hpa*II/*Rsa*I fragment prepared from pLF4 (A) and the 3′ end labeled 140-base *Hpa*II/*Rsa*I fragment prepared from pLF2 (B). Lane 1, poly(A)+ RNA from human liver; lane 2, yeast tRNA. The size of fragments produced upon S1 nuclease digestion is shown in the margin. The asterisk marks the position of the ^{32}P label in the probe.

and its 5′ and 3′ flanking regions. Upon hybridization with the poly(A)+ RNA obtained from liver and subsequent S1 nuclease digestion, this probe gave three fragments retaining the 5′ end label (Figure 3A). The largest fragment with 331 bases was the fully protected probe. This confirms the presence of the mRNA species containing the 192 bases. The presence of smaller fragments (i.e., 252-base and 60-base fragments) indicates that there also exist other FN mRNA species with sequence mismatches at positions 252 or 60 relative to the 5′ end of the probe. The mismatch giving rise to the 60-base fragment could be due to the presence of either the mRNA

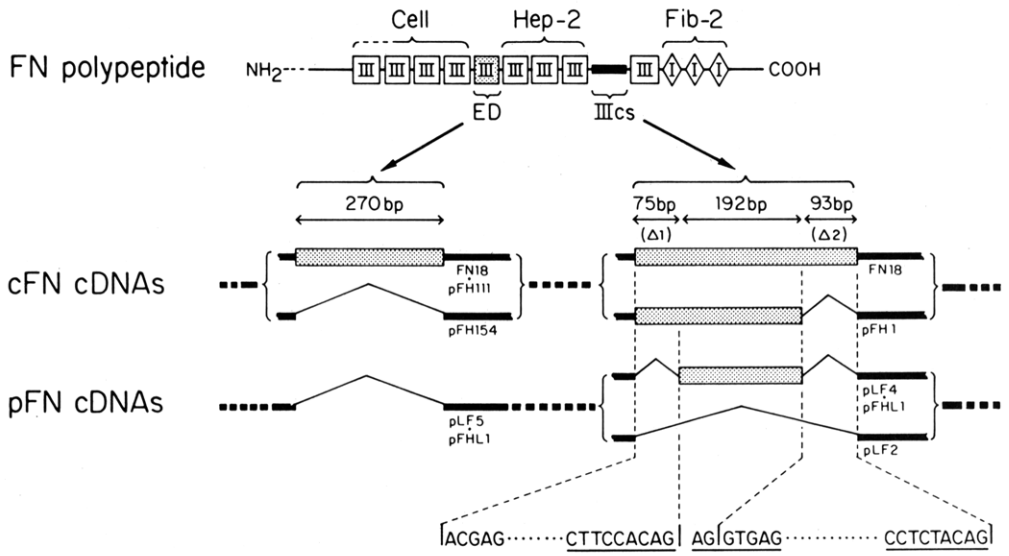

FIGURE 4: Comparison of the FN cDNAs coding for human cFN and pFN. The nucleotide sequence of the liver FN cDNAs (pFN cDNAs) was compared with the sequence of the cDNAs obtained from cultured cells (cFN cDNAs) at the two variation regions, i.e., the ED and the IIIcs regions. The cDNAs coding for cFN have two variations at the ED region: one with the ED [e.g., FN18 and pFH111; Kornblihtt et al. (1984b) and Bernard et al. (1985)] and the other without the ED (e.g., pFH154; Kornblihtt et al., 1984b). In contrast, all of the liver FN cDNAs so far obtained lack the ED sequence [Umezawa et al. (1985) and this report]. There are four variations in the IIIcs region: FN18 (Bernard et al., 1985) contains 360 bases in the IIIcs region including both the 75-base (Δ_1) and 93-base (Δ_2) extra segments flanking the central 192 bases; pFH1 (Kornblihtt et al., 1984a) lacks the 3′ flanking sequence (Δ_2); pLF4 lacks both the 5′ and 3′ flanking sequences (Δ_1 and Δ_2); pLF2 lacks the entire 360 bases. The partial nucleotide sequence at the 5′ and 3′ boundaries of the Δ_1 and Δ_2 segments are shown below the diagram. Underlined are the consensus sequences for the 5′ splice sites (i.e., GTGAG) and for the 3′ splice sites (i.e., YYYYYYNAG, where Y and N represent pyrimidine and any base, respectively).

species lacking the entire 192-base IIIcs segment (e.g., pLF2) or the mRNA species containing the extra nucleotides at the 3' boundary of the 192 bases (e.g., the FN cDNA clone, FN18, prepared from normal human fibroblasts; Bernard et al., 1985). The mismatch giving rise to the 252-base fragment appears to be due to the presence of another mRNA species containing an extra 75 bases at the 5′ boundary of the 192-base IIIcs sequence (e.g., the cDNA clone, pFH1, prepared from human tumor cells; Kornblihtt et al., 1983, 1984a).

In contrast, the other probe, a 139-base *Hpa*II/*Rsa*I fragment derived from pLF2, gave upon S1 nuclease digestion two major products. One of these was the fully protected probe, confirming the presence of the mRNA lacking the entire IIIcs region. The other product was a group of fragments protected for the 3′ end 84–80 bases, being consistent with the presence of the mRNA species containing the 192 bases in the IIIcs region. Since the mRNA species containing the extra nucleotides at both the 5′ and 3′ boundaries of the 192 bases (e.g., FN18) would also give rise to the same partially protected probe, these results cannot exclude the presence, nor estimate the relative abundance, of such a mRNA species. However, the presence of a considerable amount of the fully protected probes suggests that both mRNA species corresponding to pLF4 and pLF2 are abundantly present in the liver.

Discussion

Comparisons of FN domain fragments released by limited proteolysis and chemical cleavage have suggested that FN polymorphism (e.g., pFN vs. cFN, α vs. β subunits, as well as species differences) arises from variation in the C-terminal region (Richter et al., 1983; Hayashi & Yamada, 1983; Sekiguchi et al., 1985a). This was unequivocally demonstrated by the studies with domain-specific monoclonal or polyclonal antibodies (Sekiguchi et al., 1983; Sekiguchi et al., 1985a). To elucidate the exact sequence variation of the C-terminal region of human FNs, we isolated five independent cDNAs encoding human liver FN and compared their sequences with other FN cDNAs isolated from normal and tumor cells. Among these, two overlapping cDNAs, pLF2 and pLF4, are of particular interest. Both cDNAs have identical nucleotide sequences, except that pLF4 contains an extra 192-base sequence in the IIIcs region whereas pLF2 lacks all these bases. FN cDNAs containing the same 192 bases in the IIIcs region were also recently isolated from a human liver cDNA library (Umezawa et al., 1985). However, no human cDNA comparable to pLF2 has been described thus far.

S1 mapping analysis of cDNA/RNA hybrids indicated that pLF4 and pLF2 represent two FN mRNAs abundantly present in human liver, although mRNAs containing longer IIIcs sequences may also exist. Since liver is considered to be the major source of pFN (Owens & Cimino, 1982; Tamkun & Hynes, 1983), pLF4 and pLF2 may encode the α and β subunits of pFN, respectively. This is further supported by the following observations: (i) The α subunit is approximately 10000 daltons larger than the β subunit. This difference has been mapped to the junction between the *Hep-2* and *Fib*-2 domains (Hayashi & Yamada, 1983; Sekiguchi & Hakomori, 1983). This is consistent with the presence of the extra 64 amino acids in the subunit encoded by pLF4. This extra peptide segment is located betwen the *Hep-2* and *Fib-2* domains (see Figure 4) and has an approximate M_r of 7000. The extra peptide segments and the O-linked carbohydrate unit(s) attached to this segment (Petersen & Skorstengaard, 1985) may well explain the apparent 10000-dalton difference between the α and β subunits. (ii) The *Fib-2* domain of the α subunit, but not of the β subunit, can be released as a M_r 31000–32000 fragment upon mild trypsin digestion (Smith et al., 1982; Hayashi & Yamada, 1983; Sekiguchi & Hakomori, 1983; Garcia-Pardo et al., 1985). The complete amino acid sequence of this tryptic fragment was recently determined (Garcia-Pardo et al., 1985). The extra 64 amino acids encoded by pLF4 contain two tryptic cleavage sites, Arg-Arg-Thr (positions 695–697) and Arg-His-Arg (positions 708–710). The sequence of the tryptic fragment starts from the His

residue of the second site and is identical with the sequence predicted by pLF4. This strongly indicated that the α subunit is encoded by pLF4. In contrast, the β subunit is not susceptible to trypsin at the junction between the *Hep-2* and *Fib-2* domain, being consistent with the absence of the putative tryptic cleavage sites in the subunit encoded by pLF2. (iii) FN cDNAs with and without the IIIcs sequence were previously obtained from rat liver (Schwarzbauer et al., 1983). The antibodies directed to the peptide segments encoded by the IIIcs sequence were recently shown to react with the large, but not with the small, subunit of rat pFN (Schwarzbauer et al., 1985). This indicates that the cDNA containing the IIIcs sequence encodes the large (i.e., α) subunit and the cDNA lacking it encodes the small (i.e., β) subunit.

Recently, cDNAs coding for cFN were isolated from human carcinosarcoma cells (Kornblihtt et al., 1983, 1984a) and from normal firbroblasts (Bernard et al., 1985). These cDNAs contain longer nucleotide segments in the IIIcs region than the liver FN cDNA. Thus, pFH1, isolated from the tumor cells, contains an extra 75 bases at the 5′ boundary of the 192 bases, and FN18, isolated from normal fibroblasts, contains an extra 75 and 93 bases at both the 5′ and 3′ boundaries of the 192 bases, (Figure 4). Comparison of the nucleotide sequence among the cDNAs from liver and cultured cells indicates that a part, or all, of the 360 bases present in the IIIcs region of FN18 is differentially deleted in other cDNAs (Figure 4). The 93 bases at the 3′ boundary of the 192 bases (marked as Δ_2 in Figure 4) are absent in pFH1, pLF4, and pLF2. The 75 bases at the 5′ boundary (marked as Δ_1 in Figure 4) are deleted in pLF4 and pLF2. The entire 360 bases are deleted in pLF2. Similar differential deletion of the IIIcs sequence was previously reported in rat liver FN cDNAs (Schwarzbauer et al., 1983), except that the 93 bases at the 3′ boundary were not deleted in the rat cDNAs.

Multiple FN mRNAs are considered to arise from a single gene by alternative splicing of a primary RNA transcript (Tamkun et al., 1984; Hynes, 1985). In support of this, the extra 75-base and 93-base segments in the IIIcs region contain the consensus sequences for the 3′ splice site at their 3′ ends (Figure 4). The 93-base segment also contains the 5′ splice site at the 5′ end. Differential splicing among these internal splice sites as well as those present in the adjacent intron results in the deletion of the extra 75 and 93 bases or the entire 360 bases. Little is known about the mechanisms of the differential splicing of FN mRNAs. Since cFN, probed with a specific monoclonal antibody, appears to be absent in normal adult tissues but present in fetal and cancer tissues in vivo (Matsuura & Hakomori, 1985), the splicing of FN mRNAs may be oncodevelopmentally regulated.

The ED region, which has been shown to be present in some of the FN cDNAs (Kornblihtt et al., 1984a,b; Bernard et al., 1985), is absent in liver FN cDNAs [Umezawa et al. (1985) and this study]. This is consistent with the previous observation (Kornblihtt et al., 1984a) that S1 mapping analysis failed to detect the ED sequence in the total liver RNA. Thus, the 90 amino acids encoded by the ED appear to be absent in liver FN.

The extra 25 and 31 amino acids present in the IIIcs region, i.e., Δ_1 and Δ_2 in Figure 4, may also be specifically present in human cFN, although a minor species of pFN may contain either the Δ_1 or Δ_2 peptide segment. S1 mapping experiments suggested that an mRNA species encoding the Δ_1 as well as the central 64 amino acids was present in human liver. It is known whether other types of FNs, e.g., those present in normal and malignant tissues, also contain the Δ_1, Δ_2, or ED segments. Specific antibodies against these peptide segments will provide tools for understanding the regulation of the expression of these segments in various types of FNs.

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Raman Spectroscopy of Oxidized and Reduced Nicotinamide Adenine Dinucleotides†

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ABSTRACT: We have measured the Raman spectra of oxidized nicotinamide adenine dinucleotide, NAD^+, and its reduced form, NADH, as well as a series of fragments and analogues of NAD^+ and NADH. In addition, we have studied the effects of pH as well as deuteration of the exchangable protons on the Raman spectra of these molecules. In comparing the positions and intensities of the peaks in the fragment and analogue spectra with those of NADH and NAD^+, we find that it is useful to consider these large molecules as consisting of component parts, namely, adenine, two ribose groups, two phosphate groups, and nicotinamide, for the purpose of assigning their spectral features. The Raman bands of NADH and NAD^+ are found generally to arise from molecular motions in one or another of these molecular moieties, although some peaks are not quite so easily identified in this way. This type of assignment is the first step in a detailed understanding of the Raman spectra of NAD^+ and NADH. This is needed to understand the binding properties of NADH and NAD^+ acting as coenzymes with the NAD-linked dehydrogenases as deduced recently by using Raman spectroscopy.

It is well-known that nicotinamide adenine dinucleotide, NAD^+,[1] and its reduced form, NADH, are essential in many oxidation–reduction reactions. The NAD-linked dehydrogenases, a class of more than 300 enzymes, use these molecules as coenzymes. The enzymatic reactions of many NAD-linked dehydrogenases are strictly ordered, with the formation of the binary product of enzyme and coenzyme as the first and last steps (Theorell & Chance, 1951; Nygaard & Theorell, 1955; Wratten & Cleland, 1963). A clear understanding of how NAD^+/NADH binds at the active sites of these dehydrogenases is necessary to understanding their enzymatic behavior. Various techniques have been used to study these interactions, including circular polarization

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[1] Abbreviations: NAD^+, oxidized β-nicotinamide adenine dinucleotide; NADH, reduced β-nicotinamide adenine dinucleotide; 9EtAd, 9-ethyladenine; AMP, adenosine 5′-monophosphate; ADP, adenosine 5′-diphosphate; ADPR, adenosine 5′-diphosphoribose; NMN^+, oxidized β-nicotinamide mononucleotide; NMNH, reduced β-nicotinamide mononucleotide; 1-MN^+, 1-methylnicotinamide; 3-$PAAD^+$, 3-pyridinealdehyde adenine dinucleotide (oxidized).

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