Tissue-specific Splicing Pattern of Fibronectin Messenger RNA Precursor during Development and Aging in Rat

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Abstract. Fibronectin isoforms are generated by the alternative splicing of a primary transcript derived from a single gene. In rat at least three regions of the molecule are involved: EIIIA, EIIIB, and V. This study investigated the splicing patterns of these regions during development and aging, by means of ribonuclease protection analysis. Between fetal and adult rat, the extent of inclusion of the EIIIA and/or EIIIB region in fibronectin mRNA varied according to the type of tissue analyzed; but the inclusion of the V region, and in particular the V25 alternative variant, was significantly higher in all fetal than in adult tissues. These data suggest a crucial role of the V25 variant, possibly related to its interaction with the $\alpha 4\beta 1$ integrin receptor during development. On the other hand, during aging, the only significant change observed in the splicing pattern was a decrease in the EIIIA variant in brain. The high inclusion levels of the EIIIA and EIIIB regions in young adult brain suggest that these segments may play an important role in differentiated brain tissue. The decreasing levels of inclusion of the EIIIA segment in brain fibronectin mRNA during aging may be an age-related marker with functional consequences.

CELL-to-cell and cell-to-substrate interactions play a fundamental role in cell behavior, division, and differentiation. Among the extracellular components involved in these events, fibronectin (FN)[1] is probably one of the most important and widely distributed. Fibronectin is a dimeric high molecular weight glycoprotein found in blood, lymph, and tissue fluids, as well as in association with basement membranes, connective tissue matrices and the extracellular matrix of many cells (23, 37).

The primary structure of FN has been described at both protein (human and bovine FN) and cDNA (chicken, rat, and human FN) levels (4, 13–15, 21, 26, 32, 33, 40, 41, 43, 45). The FN molecule is composed of three different kinds of repeated sequence, known as types I, II and III with a characteristic modular structure (21, 33, 40, 41, 43). These repeats are assembled into a series of structural domains, each having a distinct binding activity toward collagen, sulfated glycosaminoglycans, fibrin, and the cell surface receptors collectively termed integrins (19, 36, 49).

FN molecules are a mixture of several protein types that differ both in their primary structure, and in their posttranslational modifications. All of the sequence variations are produced by the alternative processing of a common mRNA precursor (pre-mRNA) transcribed from a single gene and differentially spliced in the various cell types (1, 18, 30, 40, 41, 45, 50).

Three sites of alternative splicing have so far been described: ED-A, ED-B, and IIICS in human FN and the corresponding sites (EIIIA, EIIIB, and V) in rat FN. EIIIA and EIIIB share a common gene structure and, in both cases, alternative splicing leads to the insertion of an additional exon coding for a type III homology domain. The V region in rat may be partially or completely excluded (depending on the splice site used) and may generate inserts of 0, 95, or 120 amino acids (45). As a result of these three alternative splicing processes, 12 potential forms of rat FN can be generated.

The expression of various forms of FN in vivo is regulated in a tissue- and cell-specific fashion during development and wound healing, thus suggesting a possibly unique role for these segments (10–12, 31, 48). At least for the V25 segment, interaction with a specific integrin receptor on different cultured cell types have been demonstrated (20, 24).

The extracellular matrix is known to undergo qualitative and quantitative changes with age (22, 27). The importance of age-associated changes in cell-to-cell and cell-to-matrix interactions has remained relatively unexplored. In this regard, fibronectins have been considered as attractive molecules to study. Recently, in an attempt to isolate antiproliferative mRNAs by differential hybridization from regenerating rat liver and early passage fibroblasts, some FN clones have been isolated (28). The expression of EIIIA FNs is asso-

1. *Abbreviation used in this paper*: FN, fibronectin.

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ciated with the synthetic or modulated phenotype of smooth muscle cells (SMCs) (16). This phenotype is observed with aging (when SMCs migrate to the progressively thickening intima, where they proliferate and secrete extracellular matrix components), as well as in atherosclerotic lesions (16). Recently, the cell type-specific fashion of the alternative splicing of rat and human FN gene transcript has been shown to be a subject for modulation during transformation both in cultured cells and in tumors (1, 2, 5, 6). To understand the expression and regulation of FN variants in vivo, the pattern of FN pre-mRNA splicing of the EIIIA, EIIIB and V regions in different rat tissues was evaluated during both development (from fetus to 5 mo of age) and aging (from 5 to 24 mo of age).

Materials and Methods

Animals and RNA Preparation

All of the animals were housed in specific pathogen-free facilities and fed ad lib. on a standard rat chow diet at the Fidia Research Laboratory (Abano Terme, Italy). Sprague-Dawley male rats of the following ages were used: 17 d (fetuses; E17), and 5, 12, 17 and 24 mo. Three of each age animals were used, with the exception of E17 and 17-mo-old rats, for which only two animals were studied. Rats were decapitated and their organs were immediately frozen in liquid nitrogen. Brain, liver, kidney, lung, and heart were obtained from rats and brain, liver, kidney, and lung from fetus. Total RNA was prepared from 0.5–2 g of frozen tissues homogenized in guanidinium thiocyanate and ultracentrifuged in cesium-chloride gradient (7).

Probe Preparation

Three sets of oligonucleotide DNA primers complementary to the published sequence of rat fibronectin (29, 40, 45) were synthesized using a DNA synthesizer (model 308A; Applied Biosystems; Foster City, CA). The sequences appear below.

EIIIA1 5′-TCA GAA CCG GAA CGG AGA AA-3′
EIIIA2 5′-ACA AGC TTC ACG GAG GTG CTG TCT GGA GAA A-3′
EIIIB1 5′-TAT CTA GAG TCA TCC CAG AGG TGC CCC A-3′
EIIIB2 5′-TAC TGC AGT CCA GAC CTG TTT TCT G-3′
VAR1 5′-ATA CTG CAG ACC ATC TAT GTC ATC GCA CTG AA-3′
VAR2 5′-ACA AGC TTA GGG CTC TTC GTC AGT GCC AA-3′

These primers spanned the EIIIA, EIIIB, and V regions of FN mRNA plus a 3′ 171 bp and 5′ 8 bp for EIIIA, 3′ 285 bp and 5′ 12 bp for EIIIB, and 3′ 102 bp and 5′ 67 bp for V. EIIIA2, EIIIB1, EIIIB2, VAR1, and VAR2 primers were modified by the addition of noncomplementary bases at the 5′ ends to create artificial recognition sites for restriction enzymes. A specific first-strand cDNA copy of the three rat FN regions was made by using one of the two oligonucleotide primers in each set that was complementary to the RNA strand and that served as a primer for reverse transcriptase. The synthesis of cDNA was carried out in a 50-μl reaction volume containing 10 μg of total rat heart RNA, 2.5 pmol of primer, and Moloney Murine Leukemia Virus reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, MD), used under the conditions suggested by the supplier with the omission of dactinomycin. Amplification of these first-strand cDNAs was performed by means of TAQ polymerase (Perkin-Elmer Cetus Corp., Norwalk, CT) according to Saiki et al. (38). 10 μl of the first strand cDNA mixture was included in a 100-μl volume with 50 pmol of each corresponding set of primers and 5 U of TAQ polymerase. 40 cycles of polymerase chain reaction (PCR) amplification were carried out, each consisting of 1 min at 93°C, 30 s at 48°C, and 3 min at 70°C. After DNA amplification, the PCR mixture was digested with 15 μg of ribonuclease A at 37°C for 30 min and then analyzed by electrophoresis on agarose gels. The amplified fragments corresponding to the EIIIA, EIIIB, and V inclusions (497, 572, and 544 bp, respectively) were electroeluted from the gel, subjected to restriction endonuclease digestions (PstI/HindIII for EIIIA and V, PstI/XbaI for EIIIB) and subcloned in pTZ19R vector (Pharmacia, Uppsala, Sweden) (39). The nucleotide sequence of these clones (pTZ-A, pTZ-B, and pTZ-V) was identical to the previously reported rat FN sequence (29, 40, 45).

Ribonuclease Protection Analysis

Single-strand RNA probes, labeled with α^{32}P-UTP and α^{32}P-CTP to a specific activity of 10^9 cpm/μg, were synthesized using a commercial transcription kit (Boehringer, Mannheim, Germany) from pTZ-A, pTZ-B, and pTZ-V clones linearized with EcoRI and then purified on 4% denaturing polyacrylamide gels (39). The resulting RNA probes of 499 bases for EIIIA, 599 bases for EIIIB and 572 bases for V had a 5′ region which was complementary to the corresponding FN mRNA (456 bases for EIIIA, 572 bases for EIIIB, and 529 bases for V) and the 3′ bases from pTZ19R vector. 10^5 cpm of single-strand RNA probe were hybridized overnight at 51°C with 20–40 μg of total RNA in 40 μl of 80% formamide, 20 mM Tris pH 7.4, 400 mM NaCl, 1 mM EDTA and 0.1% SDS. The RNA/RNA hybrids were then diluted in 350 μl of RNase solution (300 mM NaCl, 5 mM EDTA, 10 mM Tris pH 7.4, 40 μg/ml RNase A, 3 μg/ml RNase T1) and incubated at 31°C for 75 min.

After the addition of 30 μg of proteinase K and 20 μl of SDS 10%, the RNAs were incubated at 37°C for 15 min, extracted with phenol and then EtOH precipitated. The protected fragments were analyzed on 4% denaturing polyacrylamide gels and by subsequent autoradiography with preflashed Fuji x-ray film. In some experiments, an artifact band that was slightly smaller than the intact probe was observed. This band also appeared in the absence of cellular RNA and must derive from probe sequences that are resistant to the ribonucleases under our experimental conditions. For densitometric analysis, only signals in the linear range of film sensitivity were used.

The relative radioactivity of the ribonuclease-protected fragments was determined by means of a Zeiss Kontron Image Analyzer IBAS 2000 (Oberkochen, Germany) and expressed as a ratio between the radioactivities of the upper and lower bands. Because different amounts of radiolabeled nucleotides are incorporated according to the length of the protected fragments, this ratio is not canonical, but it is an indicator of the fluctuations of various mRNA concentrations.

Results

Expression of the EIIIA Region during Development and Aging

Alternative splicing at the EIIIA region generates two types of FN mRNA that differ in terms of the presence of the EIIIA sequence. Fig. 1 schematically shows the two FN mRNA variants, EIIIA+ and EIIIA−, and the sequences covered by the probe used to study the splicing pattern of EIIIA region by means of ribonuclease protection analysis.

Total RNAs prepared from various rat tissues at E17 and at the different months of age were analyzed by ribonuclease protection assay using the EIIIA probe. Hybridization of the RNA probe with FN mRNAs (with and without the EIIIA sequence), followed by ribonuclease digestion, yielded a 456-base and a 171-base fragment, respectively (Figs. 1, 2, and 3). Samples of the experimental results are shown in Figs. 2 and 3. Table I summarizes the EIIIA+/EIIIA− -protected fragment ratio obtained by means of densitometric analysis of the autoradiograms representing the average of three independent experiments.

During development, the highest EIIIA+/EIIIA− ratio was found in kidney with a marked decrease from 19.80 to 0.57 between E17 and 5 mo of age (Fig. 2, Table I). In the same tissue, between 5 and 24 mo of age, the EIIIA+/EIIIA− ratio remained constant at ~0.5 (Table I).

In adult liver, the EIIIA inclusion levels were very low but still detectable; in fetal liver it was clearly evident (Fig. 2). The EIIIA+/EIIIA− ratio changed from 0.14 to 0.01 between E17 and 5 mo of age (Table I). During aging, the EIIIA+/EIIIA− ratio in liver tissue remained low (Table I).

On the contrary, lung and brain tissues appeared to be relatively stable during ontogeny (Fig. 2). The EIIIA+/EIIIA−

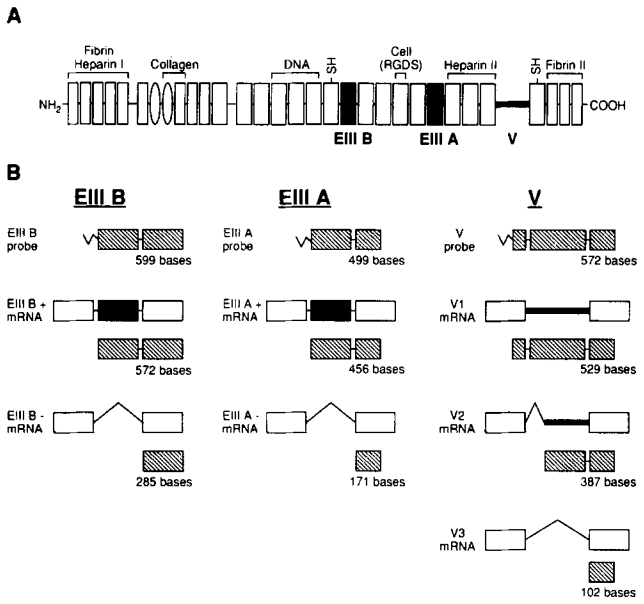

Figure 1. (*A*) Schematic map of the rat fibronectin molecule. Type I, II, and III repeats are shown as small rectangles, ovals, and large rectangles, respectively. Binding sites are indicated. Alternative spliced exons are shown in black. (*B*) Schematic representation of the three RNA probes used in RNase protection experiments and the putative fragments protected with the FN mRNA variants. The RNA probes and their protected putative fragments are shown as shaded boxes and their length is indicated. The RNA probes contain extra nucleotides derived from the cloning vector at its 3′ end (indicated by a wavy line).

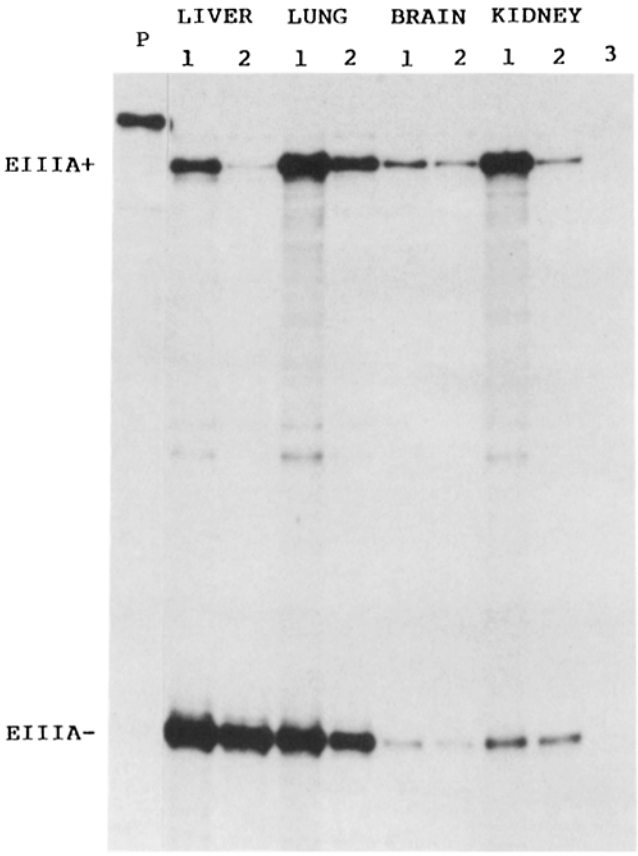

Figure 2. Ribonuclease protection experiment examining the inclusion of EIIIA in FN mRNA from various rat tissues during development. *P*, undigested probe; lane *1*, fetal day 17; lane *2*, 5 mo of age; lane *3*, yeast tRNA. Total RNAs (20 μg for liver, lung and kidney and 40 μg for brain) were hybridized with α32P labeled EIIIA probe, digested with ribonuclease and analyzed on 4% denaturing acrylamide gel as described in Materials and Methods. The protected fragments corresponding to the inclusion (*EIIIA+*) or exclusion (*EIIIA−*) of the EIIIA segment are indicated.

ratio changed between E17 and 5 mo of age from 1.4 to 0.66 in lung, and from 3.9 to 3.44 in brain. However, there was a marked decrease in the EIIIA+/EIIIA− ratio in brain tissue between E17 and 24 mo of age (Fig. 3), which became particularly acute after 12 mo when it decreased from 3.31 to 1.23 at 24 mo.

In heart tissue, the EIIIA+/EIIIA− ratio was 0.89 in the adult and did not change significantly during aging (Table I).

Expression of the EIIIB Region during Development and Aging

Alternative splicing at the EIIIB region generates two types of FN mRNA that differ in term of the presence of the EIIIB sequence (Fig. 1). Total RNAs prepared from various rat tissues at E17 and at the different months of age were analyzed by ribonuclease protection assay using the EIIIB probe. Two protected fragments were detected: a 572- and a 285-base fragment, respectively corresponding to EIIIB+ and EIIIB−. Samples of the experimental results are shown in Fig. 4, and Table I summarizes the EIIIB+/EIIIB− -protected fragment ratio obtained by means of densitometric analysis of the autoradiograms representing the average of two independent experiments.

During development, the EIIIB+/EIIIB− ratio in liver, kidney and lung tissues showed a marked decrease from fetus to 5 mo of age. At 5 mo of age, EIIIB inclusion was undetectable in liver and lung (even when the autoradiographic exposure time was increased) and was present at low levels in kidney. On the other hand, the EIIIB region in brain tissue was less regulated during development. In fact, in this tissue, the EIIIB+/EIIIB− ratio was 1.80 at E17 and 0.47 at 5 mo of age.

During aging, no significant changes in the EIIIB+/EIIIB− ratio were evident in any of the tissues analyzed.

Expression of the V Region during Development and Aging

The V region shows a more complex pattern of alternative splicing than either EIIIA or EIIIB and three different forms can be found in rat. Fig. 1 schematically shows the three FN mRNA variants (V1, V2, and V3) and the sequences covered by the probe used to study the splicing pattern of the V region by ribonuclease protection analysis.

Hybridization of the RNA probe with the FN mRNAs in the three different forms, followed by ribonuclease digestion, yielded a 529-, a 387-, and a 102-base fragment, respectively corresponding to V1, V2, and V3 (Fig. 1). The low intensity of the V3 protected band in most tissues required longer exposures before it could be visualized. This led to the linear

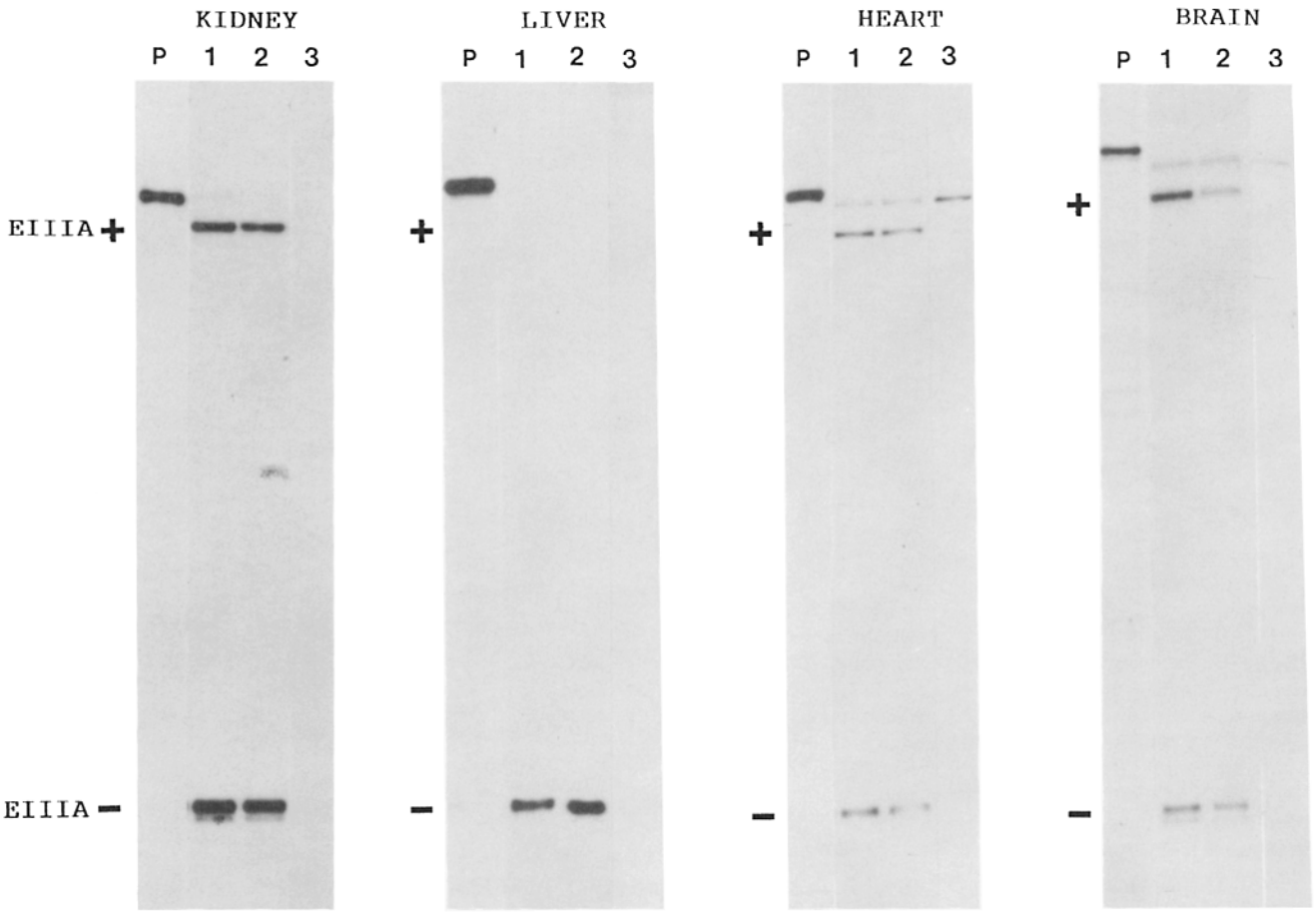

Figure 3. Ribonuclease protection experiments examining the inclusion of EIIIA in FN mRNA from various tissues as a function of age. *P*, probe; lane *1*, 5 mo of age; lane *2*, 24 mo of age; lane *3*, yeast tRNA. Total RNAs (20 μg for kidney, liver, and heart and 40 μg for brain) were hybridized with α32P-labeled EIIIA probe, digested with ribonuclease and analyzed on 4% denaturing acrylamide gel as described in Materials and Methods. The protected fragments corresponding to the inclusion (+) or exclusion (−) of the EIIIA segment are indicated.

range of film sensitivity of the higher V1 and V2 bands being lost. Hence, in this study only the V1 and V2 bands were considered for densitometric analysis.

Total RNAs, prepared from the various rat tissues at E17 and at the different months of age, were analyzed by ribonuclease protection assay using the V probe. Fig. 5 shows the different splicing patterns of this variable region in the different tissues between E17 and 5 mo of age. Table I summarizes the V1/V2 mRNA ratio revealed by means of densitometric analysis of the autoradiograms obtained from ribonuclease protection assays, representing the average of three independent experiments.

All of the tissues examined showed a marked decrease in the presence of the V1 form between E17 and 5 mo of age (Fig. 5, Table I). On the contrary, despite increased exposure time, no difference in band intensity was apparent for the V3 band (data not shown).

In adult tissues, the highest V1/V2 mRNA ratio was observed in kidney (2.71) and the lowest in liver (0.7). During aging, neither the V1/V2 mRNA ratio nor the intensity of the V3 band changed significantly in any of the analyzed tissues.

Discussion

The alternative splicings of FN pre-mRNA and hence their possible FN isoform translations seem to be regulated by nuclear *trans*-acting factors that in turn may be modulated by the environment in which the cell is present. There are two levels of regulation: the stable program that can be seen in tissue culture lines and adult organs, and the reprogramming induced by external agents (growth factors, transforming agents, physical stimuli, position in the embryo, et cetera). The present study considered the changes occurring in FN pre-mRNA processing in various rat tissues during the lifespan of the animal: after 17 d of gestation (fetal) and at 5 mo (the developmental period), and at 12, 17, and 24 mo (aging).

During development (from fetus to adult age), the three alternatively spliced regions of FN have a tissue-specific regulation in which three different patterns of expression can be observed. The first is in kidney and liver tissues, where all of the three variants (EIIIA, EIIIB, and V) are developmentally regulated (Figs. 2, 4, and 5; Table I). The second pattern is observed in lung, where only the EIIIB and V variants

Table I. Fibronectin mRNA Ratios for EIIIA, EIIIB, and V Alternatively Spliced Regions in Rat Tissues as a Function of Age Expressed in Months

mRNA ratio	Tissue	Age (*mo*)				
		E17	5	12	17	24
EIIIA+/EIIIA−	Liver	0.14	0.01	0.01	0.01	0.01
	Kidney	19.80	0.57	0.57	0.48	0.55
	Brain	3.90	3.44	3.31	2.60	1.23
	Lung	1.40	0.66	0.51	ND	ND
	Heart	ND	0.89	0.85	0.90	0.70
EIIIB+/EIIIB−	Liver	0.10	<0.01	<0.01	<0.01	<0.01
	Kidney	15.00	0.03	0.03	0.05	0.04
	Brain	1.80	0.47	0.55	0.60	0.42
	Lung	1.01	<0.01	<0.01	ND	ND
V1/V2	Liver	10.00	0.70	0.87	0.91	0.95
	Kidney	14.40	2.71	3.35	ND	2.50
	Brain	11.20	2.13	2.22	2.00	1.80
	Lung	15.00	1.16	1.18	ND	ND
	Heart	ND	1.52	1.30	0.90	1.41

Data obtained by densitometric analysis of autoradiograms deriving from ribonuclease protection experiments represent the average of three independent experiments for the EIIIA+/EIIIA− and V1/V2 mRNA ratios and the average of two independent experiments for the EIIIB+/EIIIB− mRNA ratio. *E17* = fetal d 17; <0.01 means that the high molecular weight protected band was undetectable even when the exposure time of autoradiographic films was increased.

show changes, and where the EIIIA appears to be less closely regulated, as shown by the EIIIA+/EIIIA− mRNA ratio between E17 and 5 mo of age (1.40 vs. 0.66). The third pattern can be seen in brain, where only the V25 variant is developmentally regulated. In this tissue, between E17 and 5 mo of age, the EIIIB+/EIIIB− mRNA ratio slightly decreased (1.80 vs. 0.47) and the EIIIA+/EIIIA− mRNA ratio did not change (3.90 vs. 3.44).

As far as the EIIIA and EIIIB regions are concerned, developmental changes in the relative inclusion of these segments in FN mature transcripts have been previously reported in some human and chicken tissues (10, 11, 31). Oyama et al. (37) have shown that EDA in humans is developmentally regulated in liver but not in lung; we observed the same thing in the corresponding rat tissues. During chicken development, ffrench-Constant et al. (10, 11) have reported the presence of all of the three alternatively spliced forms in early embryo. However, at later embryonic stages (16 d), the EIIIA and EIIIB regions were virtually absent in liver and in the other tissues, the amount of EIIIB was less than that of EIIIA (which was still present in large amounts at this time). In fetal rat liver, both EIIIA and EIIIB were clearly detectable in FN mRNA at E17 but had disappeared from the livers of 5-mo-old rats. Both of these variants were also the predominant forms in fetal rat kidney (Table I). In rat brain, there was only a slight decrease in the inclusion of the EIIIB region between the ages of E17 and 5 mo, whereas there was no change in the EIIIA region. Some of these differences may be due to the species-specific timing of alternative splicing during fetal development.

Changes in EIIIA and/or EIIIB inclusion have been previously observed not only during development but also in experimental conditions of induced FN reprogramming, such as wound healing (12) and the malignant transformation of cells (2, 5). These changes have been interpreted as indicating that these segments (in particular the EIIIB segment) play

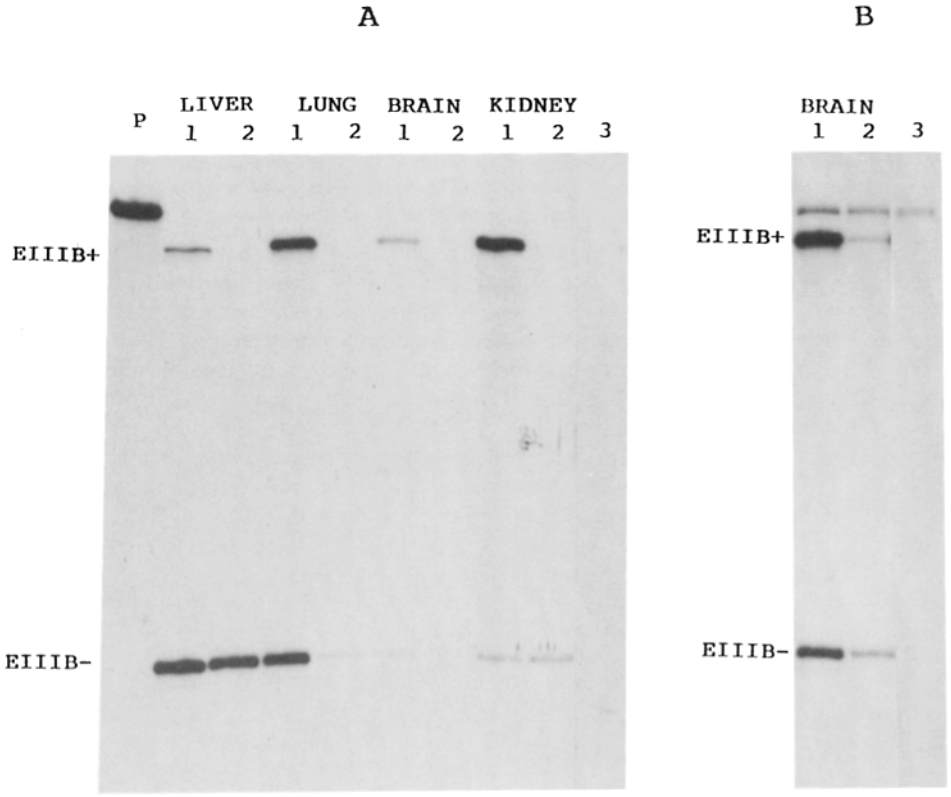

Figure 4. (*A*) Ribonuclease protection experiment examining the inclusion of EIIIB in FN mRNA from different rat tissues during development. *P*, undigested probe; lane *1*, fetal day 17; lane *2*, 5 mo of age; lane *3*, yeast tRNA. Total RNAs (20 μg for liver, lung, and kidney and 40 μg for brain) were hybridized with α32P-labeled EIIIB probe, digested with ribonuclease and analyzed on 4% denaturing acrylamide gel as described in Materials and Methods. The protected fragments corresponding to the inclusion (*EIIIB+*) or exclusion (*EIIIB−*) of the EIIIB segment are indicated. In *B*, the same lanes for brain and yeast tRNA exposed for a longer time.

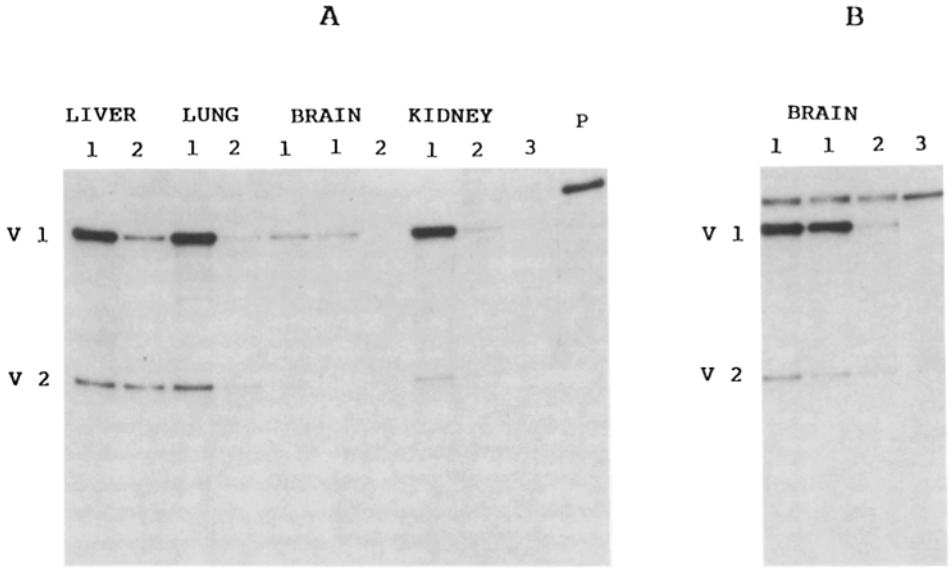

Figure 5. (*A*) Ribonuclease protection experiment examining the inclusion of the V region in FN mRNA from different rat tissues during development. *P*, undigested probe; lane *1*, fetal day 17; lane *2*, 5 mo of age; lane *3*, yeast tRNA. Total RNAs (20 μg for liver, lung, and kidney and 40 μg for brain) were hybridized with α32P-labeled V probe, digested with ribonuclease and analyzed on 4% denaturing acrylamide gel as described in Materials and Methods. The protected fragments corresponding to the inclusion (VI) or exclusion (V2) of the V25 segment are indicated. The autoradiography, obtained at nonsaturating exposure time, do not show the V3 protected fragment. In *B*, the same lanes for brain and yeast tRNA exposed for a longer time.

a major role in the migration and/or proliferation of cells. However, under these experimental conditions, no different expression in the V region has been clearly demonstrated. The vast majority of previous studies did not test for a selective specific exclusion of the V25 peptide (21, 40). The only study that specifically analyzes the V region in detail did not detect a differential expression during embryonic development in chicken (11), although it must be said that the alternative splicing of FN in embryos was not compared with that in adult chicken tissues. Furthermore, unlike in rat, chicken FN at the V region showed only two alternatively spliced forms (26). The V region of the chicken is only 50% homologous with the amino acid residues of the corresponding region in mammals, which suggests that in avian species the V region may not possess a similar cell binding function.

In our study, the V1/V2 ratio represents the presence of the V25 peptide in mature protein, if we assume that the mRNA forms correspond to the FN isoforms previously described (3, 35, 41, 44, 45). This peptide can be selectively spliced out independently of the rest of the V region (11, 40) and is identical in human, rat, and cow fibronectins (34). This region (also called CS-1 in human FN) interacts with a specific integrin receptor localized on the surface of some transformed cells (20, 24). In neural avian crest cells in vitro, this is the only segment which promotes attachment and, in cooperation with the RGDS cell binding site and the synergic site domain of FN, it is the segment responsible for migration (9). The high level of inclusion of the V25 variant in all of the fetal tissues analyzed suggests that it plays a major role during fetal development, which is possibly related to an interaction with the $\alpha 4\beta 1$ integrin receptor (20, 34).

The alternative splicing of FN pre-mRNA was also investigated in aging animals. Perhaps the most striking result observed was the change in FN mRNA in the EIIIA region of the aging brain (Fig. 3, Table I). This is the first report of tissue-specific alterations in FN pre-mRNA processing in vivo during aging. The observed change in FN pre-mRNA alternative splicing in brain may be due to the occurrence of a cell specific alteration of the genetic program during aging (17, 42), age-related modifications in brain cell populations (8, 46), or even a combination of both.

The cell type localization of FN synthesis in the brain is not known. Neural avian crest cells and their derivatives do not synthesize FN, as detected by in situ hybridization (10, 25), but they appear to be very responsive to surrounding FN and in vitro migrate on layers of the molecule (47). On the contrary, rat astrocytes have been shown to synthesize FN in vitro (35) and this FN includes all of the three alternative spliced segments (41). The high level of inclusion of EIIIA and EIIIB, as expressed by the corresponding mRNA ratios observed in adult rat brain (Table I) indicates the important role of these segments in differentiated brain tissue. Previous studies using specific mAbs have failed to identify the EDA and EDB segments in adult human brain tissue (which respectively correspond to rat EIIIA and EIIIB) at protein level (2, 5). It should be noted that overall FN expression in low in adult rat brain tissue and that immunohistochemical assays are probably less sensitive in detecting low quantities of FN isoforms than RNase protection techniques. However, it cannot be excluded that rat and human brain tissue may behave differently in this respect and that the alternatively spliced forms of FN pre-mRNA might be differently translated in this tissue. To understand the functional role of selective fibronectin changes in aging brain, we are currently performing further studies to examine cell localization and regulation of pre-mRNA alternative splicing in this tissue.

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