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Comparisons of Evolutionarily Distinct Fibronectins: Evidence for the Origin of Plasma and Fibroblast Cellular Fibronectins From a Single Gene

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Plasma and fibroblast cellular fibronectins from three different species were compared for structural similarities and differences. Partial tryptic digestion of either human or chicken plasma and cellular fibronectins yields homologous protease-resistant domains within a species but few homologies between species regardless of the source. Within a species, human or chicken plasma and fibroblast cellular fibronectins are immunologically indistinguishable as determined by the ELISA technique. There is limited immunological cross-reactivity between species. Two-dimensional tryptic peptide maps of fibroblast cellular and plasma fibronectins from the same species are also very similar: 85–95% of the spots on such maps comigrate. When peptide maps from different species are compared, no more than 10% of the spots comigrate.

Three models for the genetic origin of cellular and plasma fibronectins in vertebrates are considered. A model in which both fibroblast cellular and plasma fibronectins arise from the same gene is the simplest that is consistent with the data.

Key words: fibronectin, peptide mapping, ELISA, evolution

Fibronectins are large, adhesive glycoproteins found in soluble form in plasma and in other body fluids and in insoluble form on cell surfaces and in extracellular matrices [1–9]. Fibronectin is present in all vertebrates and most invertebrates tested to date [10]. Fibronectin appears to play roles in wound healing, embryonic development, and maintaining tissue architecture. Levels of both plasma and tissue fibronectins can be altered in many disease states [8].

Plasma and fibroblast cellular fibronectins have several very similar properties, but they are not identical. Although they have nearly indistinguishable amino acid compositions [11] and immunological [12–14] and spectrophotometric properties [15–17], cellular and plasma fibronectins differ in other characteristics [18]. Fibroblast cellular fibronectin has a lower mobility than plasma fibronectin by sodium

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dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis [18–20]. Fibroblast cellular fibronectin is 50-fold more active than plasma fibronectin in restoring a more normal morphology to transformed cells and is 3 to 150-fold more active in hemagglutinating fixed erythrocytes [18,21]. Cellular fibronectin is much less soluble at neutral pH [11]. There are also differences in the carbohydrate compositions [22–24] and the isoelectric points [25] of plasma and fibroblast cellular fibronectins.

Some molecular differences between fibroblast cellular and plasma fibronectins have been identified. In chicken fibronectin, there appear to be three distinct polypeptide regions in which these molecules differ [26]. In two of these regions, fragments from plasma fibronectin are 1,000 daltons larger in apparent size than the homologous fragments from cellular fibronectin. In a third region, the fragment from cellular fibronectin is 11,000 daltons larger than its counterpart from plasma fibronectin. All three of these different regions have been localized to internal sites of the molecule rather than at the carboxy or amino terminus. One difference between plasma and cellular fibronectins from hamster has been identified using a monoclonal antibody that can distinguish between the two forms of the molecule [27,28]. This particular difference does not involve carbohydrates and is localized to a region of the cellular fibronectin molecule near the carboxy terminus but interior to the interchain disulfide bond. The results of these studies suggest that plasma and fibroblast cellular fibronectins are not produced by simple proteolytic processing of one form to the other.

The results of fibronectin gene localization studies suggest that there may be two or more independent genes for fibroblast cellular and plasma fibronectins [29–33]. In separate studies, the fibronectin gene has been assigned to chromosome 8 [29], to chromosome 11 [30,31], and to chromosome 2 [32,33]. These conflicting results could be reconciled if plasma and fibroblast cellular fibronectins were encoded by at least two separate genes.

We have tested possible models for the genetic origin of plasma and cellular fibronectins by biochemical and immunological comparisons of these two proteins from evolutionarily divergent vertebrate species. In all experiments, plasma and fibroblast cellular fibronectins from the same species are found to be more similar than fibronectins from different species, regardless of their source. These results are most consistent with the derivation of fibroblast cell surface and plasma fibronectins from a single gene.

MATERIALS AND METHODS

Mouse and chick cellular fibronectins were purified from cultured Swiss mouse embryo fibroblasts (M. A. Bioproducts, Walkersville, MD) and chick embryo fibroblasts, respectively, as described by Yamada [34]. Human fibroblast cellular fibronectin was obtained from Bethesda Research Laboratories (Gaithersburg, MD). Mouse and chicken plasma fibronectin were purified from the respective frozen plasma (Pel Freez, Rogers, AR) exactly as described [26]. Human plasma fibronectin was purified from fresh frozen human plasma (NIH Blood Bank) by gelatin-Sepharose affinity chromatography [35]. The fibronectin was eluted from the gelatin-Sepharose affinity support with 4 M urea, 0.5 M NaCl, 50 mM 3-(cyclohexylamino)-1-propane sulfonic acid (CAPS), pH 11.0, and then dialyzed against 0.15 M NaCl, 1 mM $CaCl_2$, 10 mM

CAPS, pH 11.0 (buffer A). All fibronectins were concentrated by precipitation in 70% ammonium sulfate at 0°C followed by resuspension in buffer A and exhaustive dialysis. All fibronectins were stored at −80°C.

Partial Tryptic Digestion of Fibronectins

Chicken and human fibronectins (0.75 mg/ml) were partially digested at 30°C for 30 min with 2.25 μg/ml trypsin treated with L-1-tosylamide-2-phenyl chloromethyl ketone (TPCK-trypsin) (Worthington, Freehold, NJ, 217 units/mg) in 0.1 M NaCl, 5 mM $CaCl_2$, 50 mM Tris-HCl, pH 7.0, to generate the major protease-resistant domains. The reaction was stopped by freezing in powdered dry ice followed by boiling for 4 min in 2% SDS.

Gel Electrophoresis

SDS-polyacrylamide gel electrophoresis was performed using the Laemmli system [32]. All electrophoresis reagents were obtained from Bio Rad (Richmond, CA).

Two-Dimensional Peptide Mapping

Two-dimensional peptide mapping of ^{125}I-labeled fibronectins was performed by the method of Elder et al [37] exactly as described by Hayashi and Yamada [38]. After reduction with 0.1 M dithiothreitol (CalBiochem, La Jolla, CA) the fibronectins were further purified by SDS-polyacrylamide gel electrophoresis with a 4% stacking gel and a 6% resolving gel. The bands corresponding to fibronectin monomers were excised after staining with Coomassie blue R-250, then subjected to peptide mapping.

Enzyme-Linked Immunosorbant Assay (ELISA)

The ELISA procedures were performed essentially as described in Rennard et al [39] and by Engvall and Perlmann [40]. Wells of a microtiter assay plate (Immulon 1, Dynatech, Alexandria, VA) were coated with 50 μl of 2 μg/ml fibronectin in 0.05 M Na_2CO_3, 0.05 M $NaHCO_3$ overnight at 4°C. All other steps were performed at 23°C. After the wells were washed extensively with Dulbecco's phosphate-buffered saline (PBS) (GIBCO, Grand Island, NY), the unreacted binding sites were blocked with 0.1 ml of 3% bovine serum albumin (Fraction V, Miles Laboratories, Elkhart, IN) in PBS supplemented with 0.05% sodium azide for 30 min. The wells were then washed with PBS, and 50 μl of antifibronectin antiserum diluted in PBS as indicated was added to each well. After incubating for 1 hr, the wells were washed with PBS. Fifty microliters of horseradish peroxidase-coupled antigoat IgG (Miles Laboratories, Elkhart, IN) diluted 1:300 in PBS, plus 10% normal rabbit serum (Flow Laboratories, McLean, VA) was added to each well and incubated for 1 hr. After the wells were washed, 0.1 ml substrate solution consisting of 0.008% hydrogen peroxide and 5 mg/ml 2,2-azino-di-(3-ethylbenzthiazoline sulfonic acid) (Sigma, St. Louis, MO) in 0.1 M citrate phosphate, pH 4.0, was added to each well. After 10–15 min, the reaction was stopped by adding 0.1 ml 10% SDS to each well. The absorbance of each well was immediately measured at 410 nm using a Mini-Reader II (Dynatech, Inc.). Background levels of antibody binding were measured by omitting fibronectin coating of the wells. The antisera to chick cellular fibronectin and human plasma fibronectin

were raised in goats at the NIH Animal Farm immunized with fibronectin that had been purified by SDS-polyacrylamide gel electrophoresis. The antisera were characterized as described by Yamada [41].

RESULTS

Comparison of Protease-Resistant Fragments

Limited comparisons between the protease-resistant fragments of fibronectins have been described elsewhere [26,42,43]. Chicken plasma and fibroblast cellular fibronectins yield homologous fragments after limited thermolysin digestion [26]. Fibroblast cellular fibronectins from human and chicken yield unrelated proteolytic fragments [44]. When the protease-resistant fragments generated by partial trypsin digestion of human and chicken plasma and fibroblast cellular fibronectin under identical conditions are compared, the pattern of major fragments from human plasma fibronectin is very similar to that from human cellular fibronectin (data not shown). Likewise, chicken plasma and fibroblast cellular fibronectins yield very similar partial tryptic patterns (data not shown) [26]. However, when human cellular and chicken cellular fibronectins are compared or when human plasma and chicken plasma fibronectins are compared, there are relatively few fragments of similar size. These results are summarized in Table I, showing that fibronectins from the same species have more similarities in the size of protease-resistant domains than fibronectins of the same type from different species.

Immunological Comparisons

To examine the immunological relationships among fibronectins from different sources and different species, we tested the reactivities of two antisera against fibronectins, one each specific for human plasma and chick fibroblast cellular fibronectin. Figure 1A shows that both chicken plasma and fibroblast cellular fibronectins show reactivities of high affinity with antiserum against chick cellular fibronectin, as indicated by the relatively large amount of enzymatic reaction product observed even at high antiserum dilution. In contrast, both human cellular and plasma fibronectins require over 100-fold higher concentrations of these antibodies to reach the same level of binding. In the complementary experiment (Fig. 1B), both human plasma and fibroblast cellular fibronectins react with high dilutions of antiserum against human plasma fibronectin, whereas both chick cellular and plasma fibronectins require approximately 30-fold higher concentrations of these antibodies to achieve the same level of binding. The slight apparent difference in reactivity of human plasma and

TABLE I. Comparisons of Homologous Protease-Resistant Fragments of Human and Chicken Fibronectins*

Comparison	Major homologous fragments (kD)
Human cellular and plasma fibronectins	84, 63, 52, 30.5, 29
Chicken cellular and plasma fibronectins	76, 33, 24, 23
Human and chicken plasma fibronectins	63.5, 24

*Chicken and human plasma and fibroblast cellular fibronectins were partially digested with trypsin as described in Materials and Methods. The resulting fragments were compared by SDS-polyacrylamide gel electrophoresis using a 4% stacking gel and a 10% resolving gel.

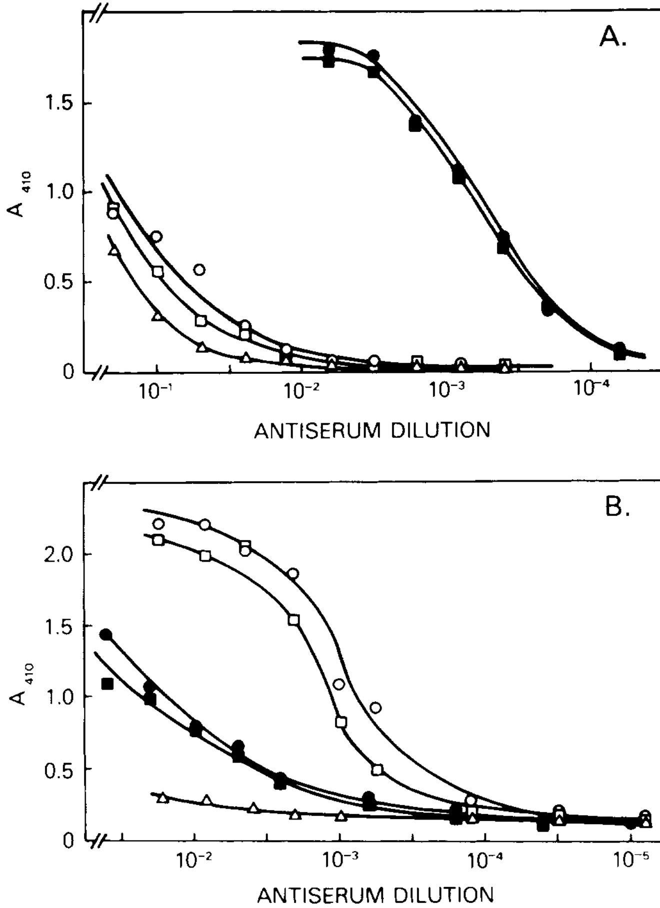

Fig. 1. The binding of antibodies to human and chicken fibronectins as measured by ELISA. Human plasma (○—○) and fibroblast cellular (□—□) fibronectins and chicken plasma (●—●) and fibroblast cellular (■—■) fibronectins were adsorbed to wells of a microtiter plate and reacted with antiserum raised against chick fibroblast cellular fibronectin (A) or with antiserum raised against human plasma fibronectin (B) as described in Materials and Methods. The extent of binding was quantitated by adding excess horseradish peroxidase-coupled antiimmunoglobulin followed by enzyme substrate and measuring the extent of enzyme-catalyzed reaction at 410 nm. Levels of background binding are also indicated (△—△).

cellular fibronectins in Figure 1B is not reproducible in multiple experiments and the differences between the human fibronectins were always substantially smaller than the differences between chicken and human fibronectins. These data suggest that plasma and fibroblast cellular fibronectins from the same species are immunologically very similar, whereas cellular or plasma fibronectins from different species have much less immunological cross-reactivity.

Comparisons of Two-Dimensional Peptide Maps

To test whether or not corresponding relationships exist in the primary structures of fibronectins from different sources and different species, cellular and plasma

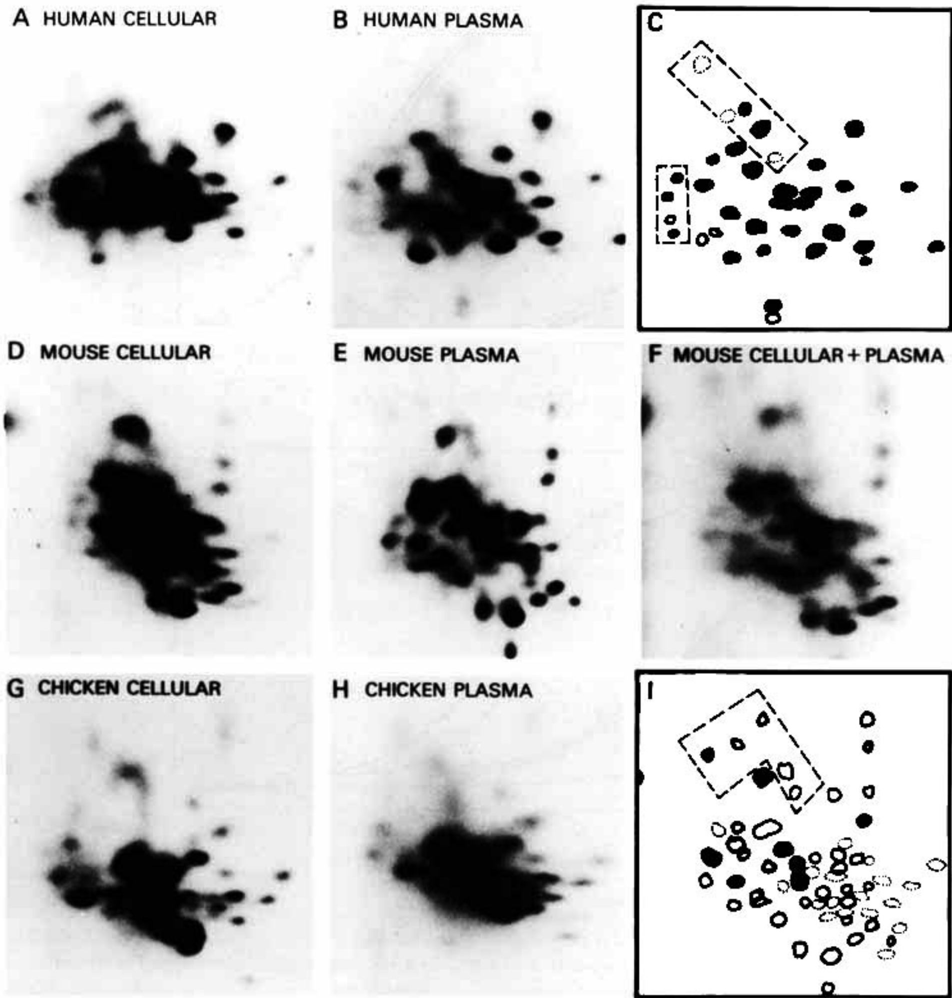

Fig. 2. Two-dimensional tryptic peptide mapping of human, mouse, and chicken fibronectins. Cellular and plasma fibronectins purified in SDS-polyacrylamide gels were radiolabeled, cleaved exhaustively by trypsin, and electrophoretically separated and chromatographed on cellulose thin-layer chromatography plates. The origin is at the lower left-hand corner of each plate; electrophoresis is from left (+) to right (−), and chromatography from bottom to top. A) map of human cellular fibronectin. B) map of human plasma fibronectin. C) analysis of A and B for similarities. (●) Superimposible spots present in A and B; (○), spots present in A but not in B; and (⬭), spots present in B but not in A. The spots in the boxed areas are uncertain because of high background and variable migrations and intensities in multiple runs and therefore cannot be used reliably for analysis. D) map of mouse cellular fibronectin. E) map of mouse plasma fibronectin. F) map of mouse plasma and cellular fibronectin peptides mixed and analyzed. G) map of chick cellular fibronectin. H) map of chicken plasma fibronectin. I) analysis of G and H for similarities. (●) Superimposible spots present in both G and H; (○), spots present in G but not in H; (⬭), spots present in H but not in G. The spots in the boxed areas are uncertain because of high background and variable migration and intensities in multiple analyses and therefore cannot be reliably interpreted. The results shown in panels C and I represent the average positions of the spots from repeated mappings.

TABLE II. Comparisons of Peptide Maps of Fibronectins*

Comparison	Major spots in common/ total spots
Human cellular: human plasma	0.85
Mouse cellular: mouse plasma	0.95
Chicken cellular: chicken plasma	0.94
Human plasma: mouse plasma	0.09
Human plasma: chicken plasma	0.10
Mouse plasma: chicken plasma	0.09

*The major spots of the two-dimensional peptide maps of the fibronectins were counted and compared for comigration. The results were obtained by comparing multiple mappings and confirmed by mapping mixtures of the fibronectins on the same plate.

fibronectins from three divergent species (human, mouse, and chicken) were reduced, denatured in SDS, and further purified on SDS-polyacrylamide gels, then labeled with ^{125}I and subjected to two-dimensional tryptic peptide mapping (Fig. 2). Fibronectins from the same species yield very similar peptide maps in Figure 2 (compare panel A with B, panel D with E, and panel G with H). In Figure 2F, the peptides from mouse cellular and plasma fibronectins were mixed and analyzed. Virtually all of the spots from mouse cellular and plasma fibronectins comigrate. Similar results were obtained when human fibroblast cellular and plasma fibronectins and chicken fibroblast cellular and plasma fibronectins were mixed and analyzed (not shown). When major spots are counted, peptide maps of plasma and cellular fibronectins from the same species have at least 85% of the spots in common (Table II). Either fibroblast cellular fibronectins (Fig. 2, panels A, D, and G) or plasma fibronectins (Fig. 2, panels B, E, and H) from different species have maps that appear to differ substantially from each other. When peptide samples from plasma fibronectins of different species are mixed pairwise and analyzed (results not shown) they have no more than 10% major spots in common (Fig. 2I and Table II). These results from peptide mapping analyses suggest that the primary structure of fibroblast cellular and plasma fibronectins from the same species are very similar (almost identical) whereas substantial differences exist among fibronectins from different animal species, even when comparing fibronectins of the same type.

DISCUSSION

The major conclusions of this study are: 1) plasma fibronectin and fibroblast cellular fibronectin from the same species show substantial structural and immunological similarities regardless of the animal species of origin; and 2) when comparing plasma fibronectins or fibroblast cellular fibronectins from different species, some limited similarities are found, but these molecules show substantial differences. The same conclusions are obtained whether one compares the gross structural features of the protease-resistant domains, the smaller regions that define antibody-binding sites, or the fine-scale differences in primary structure detectable by two-dimensional peptide maps.

The results of comparisons of protease-resistant fragments (Table I) are consistent with other studies. There are substantial homologies between fragments generated

from plasma and fibroblast or amniotic cell fibronectins generated with a series of proteases with different specificities [26,43]. However, the patterns of protease-resistant fragments from chicken and human fibronectins were found to be consistently different in this study. Hamster and human plasma fibronectins were found to give more similar patterns of trypsin-generated fragments except for the presence of one major 37 kilodalton (kD) fragment from human fibronectin [45]. The reasons for these differing results are not clear. One possible explanation might be the closer relationship of hamsters and humans in evolution.

According to the quantitative ELISA technique (Fig. 1), antichick cellular fibronectin antibodies bind to both plasma and fibroblast cellular fibronectins from chickens with 100-fold higher affinities than to either plasma or cellular fibronectins from humans. Conversely, antihuman plasma fibronectin antibodies bind with at least 30-fold higher affinity to either type of human fibronectin than to either type of chicken fibronectin. Thus, plasma and fibroblast cellular fibronectins are immunologically indistinguishable, and fibronectins from different species show only limited immunological similarities. Similar conclusions are also suggested by studies with monoclonal antibodies to fibronectins. Although a monoclonal antibody that shows a markedly higher affinity to cellular fibronectin than to plasma fibronectin has been described [27], there have been no reports yet of any monoclonal antibody that binds exclusively to cellular fibronectin and not to plasma fibronectin. On the other hand, there are several monoclonal antibodies that appear to show absolute species specificity [46–48].

The peptide maps (Fig. 2 and Table II) demonstrate that the primary structures of plasma and cellular fibronectins from the same species show strong similarities whereas fibronectins from different species, even those of the same type (ie, cellular or plasma fibronectins from different species), show substantial differences. The technique of two-dimensional peptide mapping is very sensitive and can detect even single amino acid differences between peptides [49]. The maps shown here have few similarities to previously published two-dimensional maps of cell-surface iodinated fibronectins from human, hamster, and chicken cells [50,51]. This discrepancy could be the result of differential labeling caused by the conformation of the surface-iodinated molecules in the earlier studies. The maps presented here should be free of such potential artefacts.

One-dimensional tryptic or cyanogen bromide maps for human fibronectin shed into medium by fibroblasts and plasma fibronectin were reported to be identical [52]. However, the molecular weights of these two forms of fibronectin were also stated to be identical. In contrast, fibronectin isolated directly from fibroblast monolayers consistently migrates more slowly on SDS-polyacrylamide gels than does plasma fibronectin from the same species [18–20,26; this study, data not shown]. These apparent conflicts may be explained by the finding that fibronectin secreted into medium by these human foreskin fibroblasts has a migration rate intermediate to those of plasma and cell-surface fibronectins which obscures differences between plasma and secreted cellular fibronectins (Akiyama and Yamada, unpublished data).

The data presented here indicate that interspecies differences for the same types of fibronectins are much greater than differences between fibronectins of different types isolated from the same species. Examples of proteins showing closer structural relationships between each type from different species than between different types from the same species include spectrins from chickens but not from mammals (Glenney and Glenney, manuscript in preparation), the globins [54] and collagens [55].

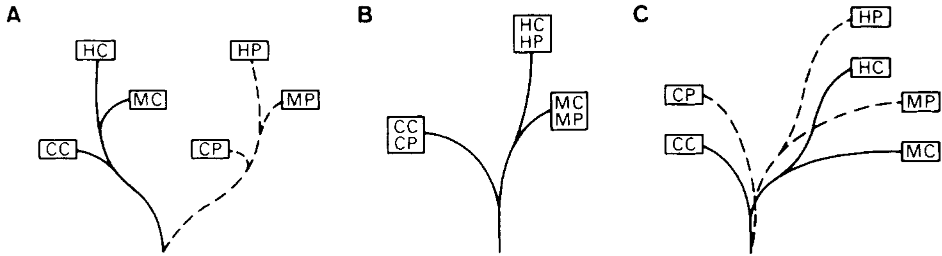

Fig. 3. Schematic evolutionary trees for plasma and fibroblast cellular fibronectins in chicken, mouse and human species. Three models depicting the evolution of human plasma (HP) and fibroblast cellular (HC) fibronectins, mouse plasma (MP) and fibroblast cellular (MC) fibronectins, and chicken plasma (CP) and cellular (CC) fibronectins are shown. A) model for evolution assuming separate genes for fibroblast cellular and plasma fibronectins and independent evolution. B) model for evolution assuming a single gene for both fibroblast cellular and plasma fibronectins. C) model for evolution assuming separate genes for cellular and plasma fibronectin accompanied by a mechanism which maintains marked sequence homology.

There are several possible models for the genetic origin of plasma and cellular fibronectins. In the simplest cases, cellular and plasma fibronectins could arise either from two different genes, or from a single gene. If derived from a single gene, the biological and physical differences between plasma and cellular fibronectins can still be explained by differential messenger RNA splicing or some form of complex posttranslational modifications. The evolution and relatedness of fibronectins as predicted by the two-gene and one-gene models are shown schematically in Figure 3 for the three vertebrate species examined. In Figure 3A plasma and cellular fibronectins are encoded by two separate genes which diverged in evolution, eg, at some time before the divergence of birds and mammals. Cellular and plasma fibronectins would be expected to evolve separately and independently, and differences between these forms should be as large as for fibronectins from different species. This model is inconsistent with the data.

In contrast, cellular and plasma fibronectins are very similar in the one-gene model (Fig. 3B). There would, however, be evolutionary divergence among fibronectins from different species. This model is entirely consistent with the data. The two-gene model can, however, be modified to fit the data. In the version depicted in Figure 3C, plasma and cellular fibronectins are assumed to be encoded by separate genes. Some powerful mechanism would prevent divergence between fibronectins in the same species, even though substantial drift would occur in different species. One other improbable model could involve the simultaneous divergence of cell surface and plasma fibronectins in all three species in the recent past. There are also some unusual mechanisms such as gene conversion [53] that could maintain strong sequence homology between two genes. The experiments performed in the present study cannot exclude such a possibility. Thus, the data presented here strongly imply that, excluding a gene conversion mechanism that would involve virtually the entire gene, there is only a single gene for both plasma and cellular fibronectins in birds and mammals.

Our conclusions are consistent with results from fibronectin gene cloning experiments. During the screening of clones containing overlapping segments of genomic DNA spanning the entire chick cellular fibronectin gene, there was no evidence for two genes for chicken fibronectin [56]. Southern blot analyses also show no evidence for two fibronectin genes [57,58]. Furthermore, several different messenger RNA species have been detected [59,60]. Although it is not yet possible by these criteria to

exclude the presence of two genes with identical sequences or which do not hybridize, the combination of these hybridization results and the protein data of this study strongly support the hypothesis of the existence of only a single fibronectin gene. Our results are not consistent with the presence of two or more fibronectin genes suggested by the conflicting chromosomal mapping studies, which might require more careful reexamination. Complete sequencing of cellular and plasma fibronectins or their cDNAs from the same and different species will probably be required to resolve these questions definitively.

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REFERENCES

1. Mosesson MW, Amrani DL: Blood 56:145–158, 1980.
2. Mosher DF: Prog Hemostas Thromb 5:111–151, 1980.
3. Pearlstein E, Gold LI, Garcia-Pardo A: Mol Cell Biochem 29:103–128, 1980.
4. Akiyama SK, Yamada KM, Hayashi M: J Supramolec Struct Cell Biochem 16:345–358, 1981.
5. Ruoslahti E, Engvall E, Hayman EG: Colloid Res 1:95–128, 1980.
6. Hörmann H: Klin Wochenschr 60:1265–1277, 1982.
7. Hynes RO: In Hay ED (ed): "Cell Biology of the Extracellular Matrix." New York: Plenum Press, 1982, pp 295–333.
8. Akiyama SK, Yamada KM: In Wagner B, Fleischmajer R, Kaufman N (eds): "Connective Tissue Diseases." Baltimore: Williams and Wilkens, 1983, pp 55–96.
9. Yamada KM: Ann Rev Biochem 52:761–799, 1983.
10. Akiyama SK, Johnson MD: Comp Biochem Physiol 76B:687–694, 1983.
11. Yamada KM, Schlesinger DH, Kennedy DW, Pastan I: Biochemistry 16:5552–5559, 1977.
12. Ruoslahti E, Vaheri A: J Exp Med 141:497–501, 1975.
13. Mosesson MW: Thromb Haemostas 38:742–750, 1977.
14. Chen LB, Burridge K, Murray A, Walsh ML, Copple CD, Bushnell A, McDougall JK, Gallimore PH: Ann NY Acad Sci 312:366–381, 1978.
15. Alexander SS Jr, Colonna G, Yamada KM, Pastan I, Edelhoch H: J Biol Chem 253:5820–5824, 1978.
16. Colonna G, Alexander SS Jr, Yamada KM, Pastan I, Edelhoch H: J Biol Chem 253:7787–7790, 1978.
17. Alexander SS Jr, Colonna G, Edelhoch H: J Biol Chem 254:1501–1505, 1979.
18. Yamada KM, Kennedy DW: J Cell Biol 80:492–498, 1979.
19. Hynes RO, Destree AT, Mautner V: J Supramolec Struct 9:189–202, 1976.
20. Mosher DF: Biochim Biophys Acta 491:205–210, 1977.
21. Hynes RO, Ali IU, Destree AT, Mautner V, Perkins ME, Senger, DR, Wagner DD, Smith KK: Ann NY Acad Sci 312:317–342, 1978.
22. Fukuda M, Hakomori S: J Biol Chem 254:5451–5457, 1979.
23. Takasaki S, Yamashita K, Suzuki K, Iwanaga S, Kobata A: J Biol Chem 254:8548–8553, 1979.
24. Ruoslahti E, Engvall E, Hayman EG, Spiro RG: Biochem J 193:295–299, 1981.
25. Tamkun JW, Hynes RO: J Biol Chem 257:4641–4647, 1982.
26. Hayashi M, Yamada KM: J Biol Chem 256:11292–11300, 1981.
27. Atherton BT, Hynes RO: Cell 25:133–141, 1981.
28. Atherton BT, Taylor DM, Hynes RO: J Supramolec Struct Cell Biochem 17:153–161, 1981.
29. Owerbach D, Doyle D, Shows TB: Proc Natl Acad Sci USA 75:5640–5644, 1978.
30. Eun CK, Klinger HP: Cytogenet Cell Genet 27:57–65, 1980.
31. Klinger HP, Ruoslahti E: Cytogenet Cell Genet 28:271–279, 1980.

32. Koch GA, Schoen RC, Klebe RJ, Shows TB: Exp Cell Res 141:293–302, 1982.
33. Zardi L, Cianfriglia M, Balza E, Carnemolla B, Siri A, Croce CM: EMBO J 1:929–933, 1982.
34. Yamada KM: In Furthmayer H (ed): "Immunochemistry of the Extracellular Matrix, Vol. I." Boca Raton: CRC Press, 1983, pp 111–123.
35. Engvall E, Ruoslahti E: Int J Cancer 20:1–5, 1977.
36. Laemmli UK: Nature 227:680–685, 1970.
37. Elder JM, Picket RA II, Hampton J, Lerner RA: J Biol Chem 252:6510–6515, 1977.
38. Hayashi M, Yamada KM: J Biol Chem 258:3332–3340, 1983.
39. Rennard SI, Martin GR, Crystal RG: In Furthmayer H (ed): "Immunochemistry of the Extracellular Matrix, Vol. I." Boca Raton: CRC Press, 1983, pp 238–252.
40. Engvall E, Perlmann P: J Immunol 109:129–135, 1972.
41. Yamada KM: J Cell Biol 78:520–541, 1978.
42. Balian G, Click EM, Crouch E, Davidson JM, Bornstein P: J Biol Chem 254:1429–1432, 1970.
43. Balian G, Crouch E, Click EM, Carter WG, Bornstein P: J Supramolec Struct 12:505–516, 1979.
44. Bernard BA, Akiyama SK, Newton SA, Yamada KM, Olden K: J Biol Chem 259:9899–9905, 1984.
45. Sekiguchi K, Hakomori S: J Biol Chem 258:3967–3973, 1983.
46. Schoen RC, Bentley KL, Klebe RJ: Hybridoma 1:99–108, 1982.
47. Koch GA, Schoen RC, Klebe RJ, Shows TB: Exp Cell Res 141:293–302, 1982.
48. Zardi L, Cianfriglia M, Balza E, Carnemolla B, Siri A, Croce CM: EMBO J 1:923–933, 1982.
49. Bond MW, Chiu NY, Cooperman BS: Biochemistry 19:94–102, 1980.
50. Nairn R, Hughes RC: Biochem Soc Trans 4:165–167, 1976.
51. Hughes RC, Nairn R: Ann NY Acad Sci 312:192–206, 1978.
52. Vuento M, Wrann M, Ruoslahti E: FEBS Lett 82:227–231, 1977.
53. Baltimore D: Cell 24:592–594, 1981.
54. Dayhoff MO: "Atlas of Protein Sequence and Structure." Silver Spring, Maryland: National Biomedical Research Foundation, 1972.
55. Bornstein P, Traub W: In Neurath M and Hill RL (eds): "The Proteins" 3rd Ed. NY: Academic Press, 1979 pp 411–532.
56. Hirano H, Yamada Y, Sullivan M, de Crombrugghe B, Pastan I, Yamada KM: Proc Natl Acad Sci USA 80:46–50, 1983.
57. Tyagi JS, Hirano H, Merlino GT, Pastan I: J Biol Chem 258:5787–5793, 1983.
58. Kornblihtt AR, Vibe-Petersen K, Baralle FE: Proc Natl Acad Sci USA 80:3218–3222, 1983.
59. Schwarzbauer JE, Tamkun JW, Lemischka IR, Hynes RO: Cell 35:421–431, 1983.
60. Kornblihtt AR, Vibe-Petersen K, Baralle FE: EMBO J 3:221–226, 1984.