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Localization of the Human Fibronectin (FN) Gene on Chromosome 8 by a Specific Enzyme Immunoassay

Stephen I. Rennard,[1] Robert L. Church,[2] David H. Rohrbach,[1,2] Dolores E. Shupp,[2] Shigeto Abe,[1] A. Tyl Hewitt,[1] J. Clifford Murray,[1] and George R. Martin[1]

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The fibronectin produced by clonal murine-human hybrid cell lines containing various complements of human chromosomes was measured. Human and murine fibronectins were assayed by specific immunoassay, and the production of human fibronectin was correlated with karyology and isozyme markers for specific human chromosomes. The data show a 100% concordance between the expression of human fibronectin and glutathione reductase, a marker for human chromosome 8, indicating that chromosome 8 codes for the fibronectin polypeptide.

KEY WORDS: fibronectin; gene mapping; ELISA; murine-human hybrids.

INTRODUCTION

Fibronectin is a large glycoprotein found on the surface of fibroblasts and in a variety of extracellular matrices (Yamada and Olden, 1978). A similar, if not identical, protein is found in plasma (Ruoslahti and Vaheri, 1975). Among other functions, fibronectin mediates the attachment of fibroblasts to collagen substrates (Klebe, 1974). Reduced amounts of fibronectin are observed on the surface of many cells after transformation (Vaheri and Ruoslahti, 1975; Hynes, 1976), and changes in expression of fibronectin are seen in several developing systems (Furcht *et al.*, 1978; Dessau *et al.*, 1978). The genetic mechanism whereby fibronectin expression is controlled is poorly understood, but would be of great interest in developmental biology and oncology.

[1] Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20205.
[2] University of Pittsburgh, School of Medicine and Eye and Ear Hospital, Pittsburgh, Pennsylvania 15213

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Murine-human cell hybrids have been extensively used to map the human genome (McKusick and Ruddle, 1977). Under selective culture conditions, these hybrid cell lines lose many human chromosomes, and cloned hybrid cells can be obtained having different complements of human chromosomes. Linkage patterns between various human gene products and human chromosomes have been established for these hybrid cell strains, and such hybrid cell strains have been used to map the chromosomal location of human genes coding for particular proteins.

Preliminary localization of the fibronectin gene to human chromosome 8 has been reported by Owerbach *et al.* (1978) using cell surface labeling with peroxidase to detect bound fibronectin. The murine parental cell line used in their study lacked cell surface fibronectin, whereas certain of the hybrids studied had the protein on their surfaces. Since transformed mammalian cells sometimes produce but do not retain fibronectin (Vaheri and Ruoslahti, 1975), it is possible that a receptor for fibronectin, rather than fibronectin itself, might have been assayed. For this reason, we have carried out related studies using immunoassays to quantitate murine and human fibronectin separately. Our results confirm Owerbach's assignment of the location of the human fibronectin gene to chromosome 8.

MATERIAL AND METHODS

Cell Lines and Culture Conditions

Hybridization between murine A9 and B82 cells and various human cell strains was carried out using β-propriolactone inactivated Sendai virus (Klebe *et al.*, 1970; Miggiano *et al.*, 1969). Hybrid line origins were as follows: ANHS hybrids from murine A9 and normal human corneal stromal fibroblasts; BNHS from murine B82 and normal human corneal stromal fibroblasts; ASCL from murine A9 and normal human scleral fibroblasts; BSCL from murine B82 and normal human scleral fibroblasts. All other hybrid strains have been previously described (SundarRaj *et al.*, 1977). Hybrid cell strains were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (GIBCO) and hypoxanthine, aminopterin, and thymidine (HAT medium) (Klebe *et al.*, 1970). Medium for fibronectin assay was removed from confluent hybrid cultures, centrifuged to remove cell debris, and frozen for later assay.

Chromosome Analysis

Confluent hybrid cell monolayers were collected and stored frozen at −80°C for later isozyme analysis. Cell extracts were prepared from these cells and

analyzed using vertical starch gel electrophoresis (Nichols and Ruddle, 1973) to differentiate between murine and human isozymes. A total of 28 different isozymes mapping to 19 different human chromosomes were analyzed (Table I). Chromosomal preparations of each hybrid line were prepared, and alkaline Giemsa (Giemsa 11) differential staining (Friend *et al.*, 1976) was carried out to distinguish human from murine chromosomes in each hybrid strain.

Preparation/Isolation of Fibronectin

Fibronectin was prepared from normal human serum and Swiss-Webster mouse serum by affinity chromatography on collagen-Sepharose (Engvall and Ruoslahti, 1977). Material used for production of antibodies was further

Table I. Isozymes Analyzed in Hybrid Cell Lines

Enzymes	Human chromosome assignment[a,b]
Peptidase C	1
α-Fucosidase	1
Glucose dehydrogenase	1
Isocitrate dehydrogenase-1	2
Malate dehydrogenase-1	2
Phosphoglucomutase-2	4
Hexosaminidase B	5
Malic enzyme-1	6
Superoxide dismutase-2	6
β-glucuronidase	7
Uridine phosphorylase	7
Glutathione reductase	8
Glutamate oxaloacetate transaminase	10
Adenosine kinase	10
Lactate dehydrogenase A	11
Peptidase B	12
Nucleoside phosphorylase	14
Hexosaminidase A	15
Mannosephosphate isomerase	15
Adenine phosphoribosyltransferase	16
Galactokinase	17
Peptidase A	18
Glucosephosphate isomerase	19
Adenosine deaminase	20
Superoxide dismutase-1	21
Hypoxanthine-guanine phosphoribosyltransferase	X
Glucose-6-phosphate dehydrogenase	X

[a] Chromosomal assignments are according to Donald and Hamerton (1978) and McKusick and Ruddle (1977).

[b] Where relevant, chromosomal analysis was supplemented by karyology (see text).

purified by electrophoresis using 5% polyacrylamide and SDS according to the procedure described by Furthmayer and Timpl (1971). One hundred μg of protein was applied to each of 12 gels. After electrophoresis, one of the acrylamide gels was stained, and the location of the 220,000 dalton chain of fibronectin established. The appropriate segments in the other gels were removed, combined, homogenized, dialyzed briefly against PBS, and used for immunization. Material used to coat ELISA microtiter wells was obtained by affinity chromatography on collagen-Sepharose and was further purified by DEAE-cellulose chromatography in 2 M urea, 0.05 Tris-HCl, *p*H 7.4. Fibronectin was eluted from the column with linear NaCl gradient from 0.02 to 0.5 M. Material was stored in 2 M urea at −70°C until used.

Antisera

Antisera to murine and to human fibronectin were prepared by injection of 250 μg of the fibronectin preparation, suspended in complete Freund's adjuvant, subcutaneously in rabbits. After three weeks, a second injection in incomplete Freund's adjuvant was given. Two weeks later, blood was drawn from an ear vein and allowed to clot at room temperature. Affinity purified antibodies were prepared on human fibronectin-Sepharose columns. The antihuman fibronectin gave a single band by double immunodiffusion with human serum (Fig. 1a). This band showed identity with a purified fibronectin standard. Most antimurine fibronectin sera also showed a single precipitin band with both murine serum and purified fibronectin. Sera collected after a second booster injection, however, revealed a second minor band by double immunodiffusion against whole mouse serum. The minor component could be removed by incubation of a 5 ml sample of antiserum at 8°C for 24 hr with 1 ml of mouse serum previously depleted of fibronectin. The absorbed serum was centrifuged at 12,000*g* for 30 min to remove the precipitate. The supernatant fluid was collected and showed a single band in double immunodiffusion (Fig. 1b).

The specificities of antisera against murine and human fibronectin were confirmed by immunoprecipitation of conditioned media from mouse and human fibroblast cultures. Dense cultures of murine and human fibroblasts in T-75 flasks were labeled for 24 hr with 50 μCi of [U-^{14}C]-L-leucine in 10 ml of Eagle's MEM. After 24 hr, medium was harvested and dialyzed three times for 4–8 hr each against phosphate-buffered saline containing 0.04% Tween 20. Aliquots of labeled culture medium containing approximately 200,000 cpm/0.5 ml were incubated for 1 hr at 37°C with antimurine or antihuman fibronectin antisera raised in rabbits. Immune complexes were precipitated by incubating the mixture with 10% (w/v) inactivated *Staphylococcus aureus* (Pansorbin; Calbiochem, Calif.) for 2 hr at 4°C (Kessler,

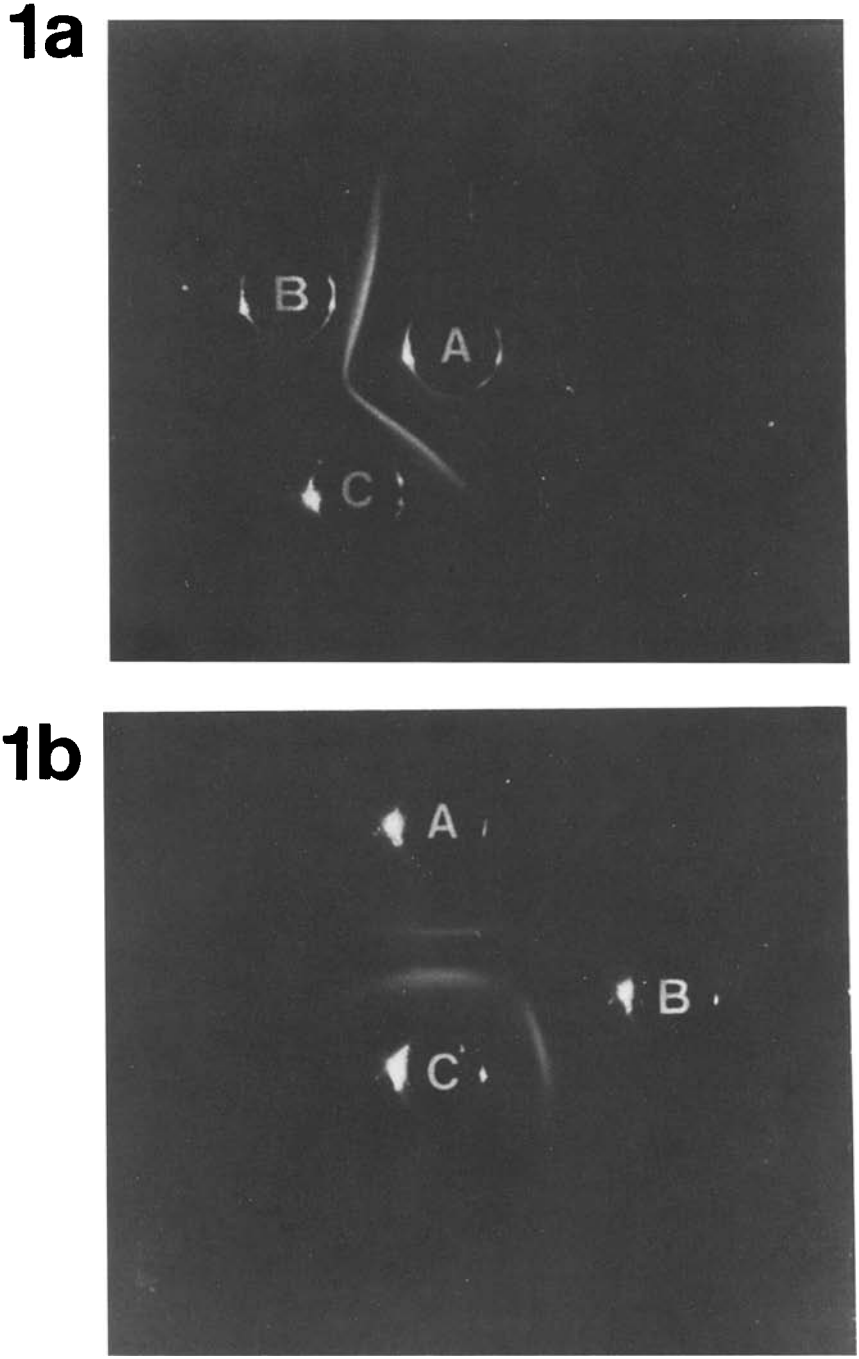


Fig. 1. (a) Immunodiffusion of antifibronectin antisera. Antihuman antiserum: wells filled with (A) antihuman fibronectin, (B) whole human serum, and (C) purified human fibronectin. A single line of identity is apparent. (b) Immunodiffusion of antimurine fibronectin. Wells filled with (A) antimurine fibronectin (whole serum); (B) antimurine fibronectin (serum adsorbed with fibronectin free serum), and (C) whole murine serum. A single precipitation band is present.

1975). After washing the precipitate four times with 0.15 M NaCl, 0.05 M Tris HCl, *p*H 7.4, labeled materials were eluted from the *S. aureus* suspension by boiling in 0.1 ml of 0.05 M Tris HCl, *p*H 8.0, plus 2% SDS. Samples were diluted twofold with sample buffer for gel electrophoresis and portions were electrophoresed on 5% polyacrylamide gels by the method of Laemmli (Laemmli, 1970). Samples of whole labeled media were run alongside of the immunoprecipitates. Each antiserum precipitated a single species characteristic of fibronectin having a molecular weight of 220,000 daltons after reduction (Fig. 2).

Fibronectin undergoes a variety of posttranslational modifications including glycosylation, proteolysis, cross-linking, and polymerization. Absence of an enzyme carrying out such a modification could alter the antigenicity of the molecule. We wished to measure the presence of the actual gene product, the polypeptide. For this reason, we investigated the nature of

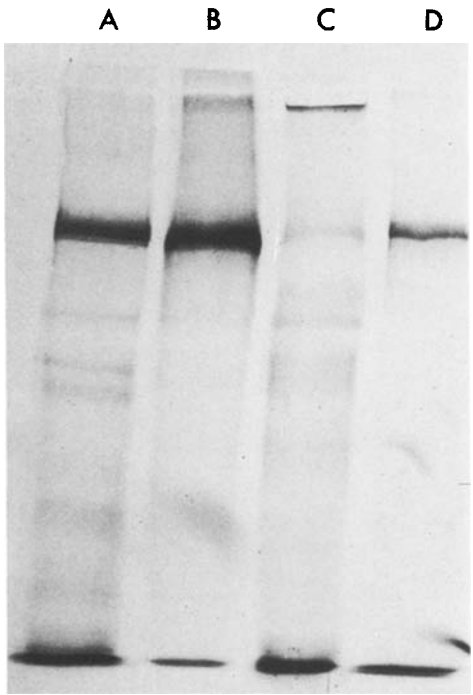


Fig. 2. Autoradiogram of SDS-PAGE of (A) labeled protein secreted into the media by human fibroblasts, (B) immunoprecipitation of labeled medium protein by antihuman fibronectin, (C) whole murine tissue culture medium labeled with C^{14}-leucine and (D) immunoprecipitation of C^{14}-leucine labeled medium with antimurine fibronectin.

Table II. Sensitivity of the Antigenic Site in Fibronectin to Various Treatments[a]

Treatment	% Antigen destroyed
None	0
60°C for 30 min	89
100°C for 5 min	98
Dithiolthreitol (DTT)	0
DTT + iodoacetate	48
Trypsin	9
Pepsin	67
DTT + iodoacetate + trypsin	87
DTT + iodoacetate + pepsin	96

[a]Human serum fibronectin in a 10 μg/ml solution was incubated as described. Reduction with dithiothreitol (DTT) was overnight in the dark at 4°C. In some cases, iodoacetate was added and allowed to react for 16 hr. Samples were treated with pepsin (1:20 wt:wt) for 16 hr at *p*H 2.0 and stopped by neutralization to *p*H 7.6. Samples treated with trypsin were incubated with 250 units/ml for 15 min at 37°C and stopped with a two-fold excess of soybean trypsin inhibitor.

the antigenic site in fibronectin (Table II) reacting with our antisera. These studies showed that antigenic activity was largely destroyed by heating fibronectin, as expected for a protein antigen. Reduction and alkylation destroyed about half the antigenic activity. Trypsin had little effect on the antigenicity of the native protein, but almost completely destroyed the antigenicity of the reduced and alkylated protein. Pepsin treatment produced similar effects. These studies indicate that the antigenic determinants in the fibronectin molecule reside in the polypeptide chain and that some of the determinants are conformational and some sequential. These studies indicate that our antibody to human fibronectin is directed against the primary gene product.

RESULTS

Using the ELISA assay, it is possible to carry out quantitative species-specific immunoassays for fibronectin production by murine-human somatic cell hybrids. Figure 3a shows an ELISA assay for human fibronectin at 4°C. Human serum fibronectin can be detected at a concentration of 10 ng/ml. Murine serum fibronectin interacts much less avidly with the antibody and is able to compete for binding sites only about one ten-thousandth as effectively. However, because of the large amounts of fibronectin in serum, there is some inhibition by murine serum, as shown in Fig. 3a. As the temperature of the reaction is increased, the antigen-antibody interaction becomes weaker (Figs.

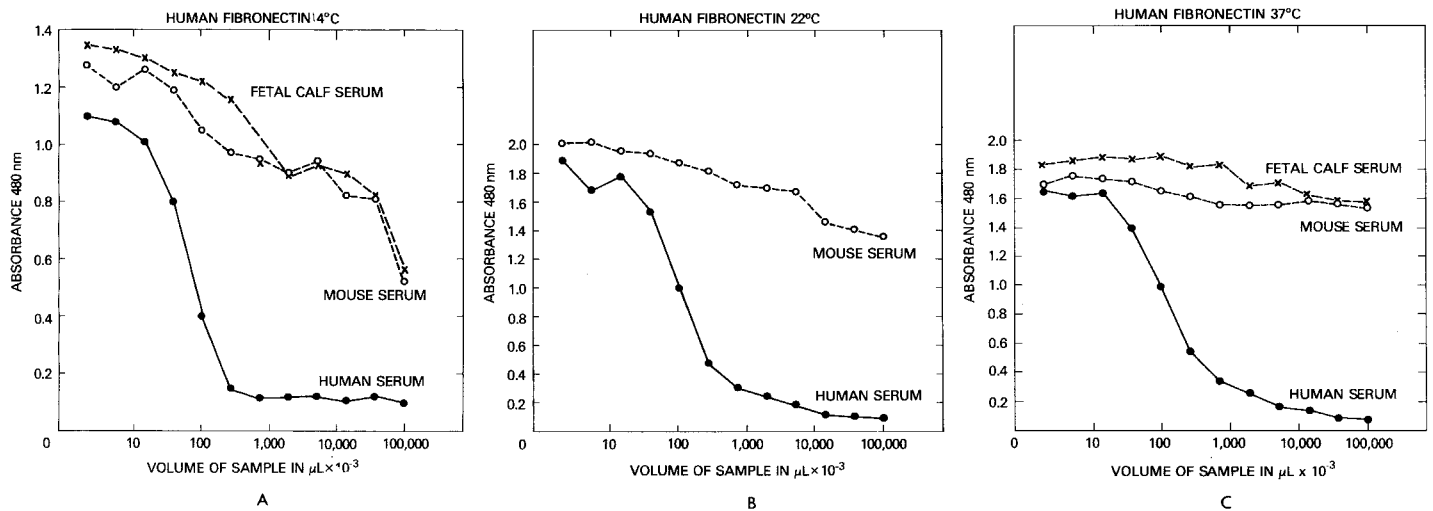


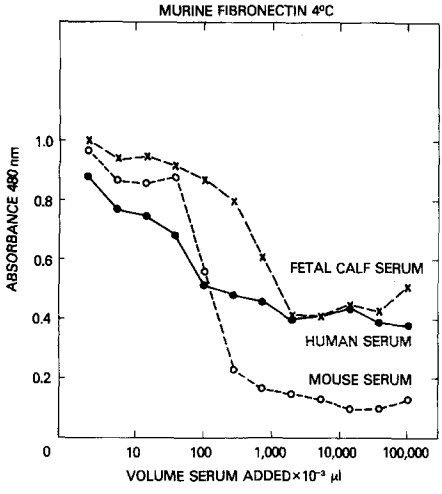
Fig. 3. ELISA inhibition tests for human fibronectin. Shown are inhibition of binding of rabbit antihuman fibronectin to solid phase human fibronectin inhibited by human, murine, and fetal bovine serum. Panels: (a) inhibition at 4°C, (b) inhibition at 22°C, (c) inhibition at 37°C.

3b and c), producing a less sensitive test for human fibronectin. However, the effect is much greater for the reaction with murine fibronectin, and this increases even further the specificity of the test for the human protein. We have obtained identical results using affinity purified antihuman antibodies, although this reagent gives a test with somewhat lower sensitivity. The lower sensitivity with the affinity purified antibodies would be expected if the antibodies with the highest affinity for antigen are not recovered from the affinity column. The murine ELISA also shows an increased species specificity with increasing temperature (Fig. 4). The antisera used, however, did not provide a specific test even at 37°C (Fig. 4c), and it was necessary to increase the amount of antigen coating each microtiter well from 200 to 500 ng. Although these conditions resulted in a loss of sensitivity for murine fibronectin (Fig. 4d), the test became species-specific as human fibronectin no longer was able to compete effectively for binding sites in the murine assay.

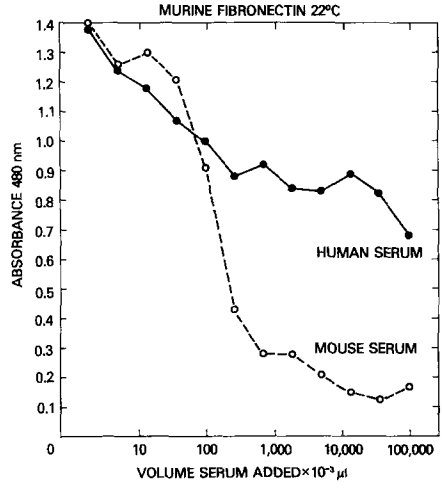
Using the modifications of the assay outlined above, it was possible to measure the fibronectin accumulation in the media of human, murine, and murine-human hybrid cell strains. Control human cell strains accumulated 1–2 μg/ml of fibronectin in the culture medium under the conditions of growth. This amount of fibronectin inhibited antibody binding in the ELISA test completely, and so the medium was diluted 10-to-20-fold prior to assay. Undiluted culture medium containing 10% fetal calf serum inhibited the ELISA test slightly (Fig. 3c), and thus gave a mean background of about 40 ng/ml for the test serum. Murine cell strains secreted 2–3 μg/ml of fibronectin into the media.

Two groups of hybrid cell lines were found (Fig. 5). The first group had a mean human fibronectin content that did not differ significantly from the reaction found with media containing 10% fetal calf serum alone (43.29 ± 7.26 ng/ml). Values obtained with the second group had a mean of 152.18 ± 24.28 ng/ml of human fibronectin in the culture medium during the incubation period. Although this is less than control human cell strains, which accumulated 1–2 μg/ml, it represents greater than 50% inhibition of antibody binding, an amount of inhibition impossible for nonhuman fibronectin. Murine cell lines, as expected, did not produce human fibronectin. With a single exception, ANOM-1, all hybrid lines produced murine fibronectin in amounts that varied from 0.5 to 3 μg/ml, and were comparable to amounts produced by control murine cells. Further studies of ANOM-1, which appears to have lost the murine fibronectin gene, are in progress.

A total of thirty-two separate mouse-human hybrid clones from at least nine independent hybridization events were examined for assignment of the human fibronectin gene. Table III shows the human chromosomal complement of each hybrid clone tested correlated with the presence of human

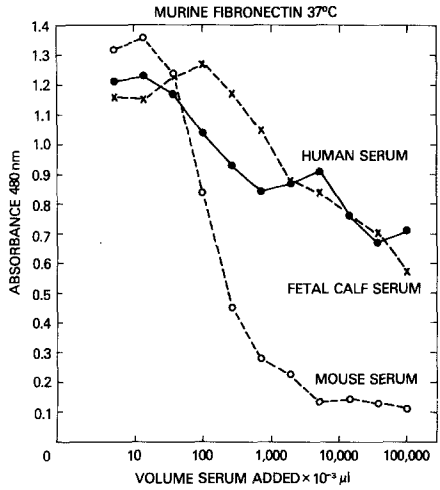


A

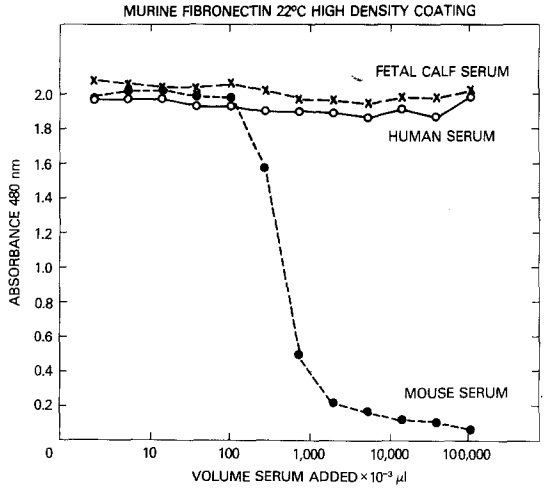


B

Fig. 4. ELISA inhibition tests for murine fibronectin. Shown are inhibition of binding of rabbit antimurine fibronectin to 200 μg insolubilized fibronectin inhibited by human, murine, and fetal bovine serum at (a) 4°C, (b) 22°C, (c) 37°C. In panel (d), inhibition of binding of antimurine fibronectin to 500 μg insolubilized fibronectin at 22°C is shown.



C



D

Fig. 4. Continued.

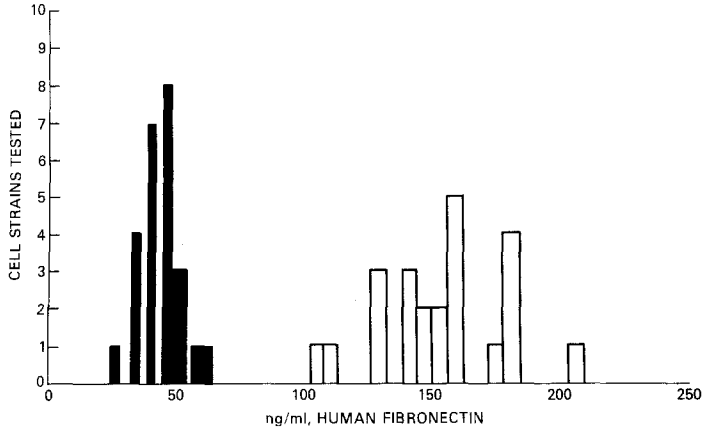


Fig. 5. Accumulation of human fibronectin by human-mouse hybrids. Solid bars represent cell strains that do not contain human chromosome 8, while open bars represent cell strains positive for chromosome 8. All cell strains were assayed 2–3 times.

fibronectin production. Table IV demonstrates that complete concordancy exists with the presence of human chromosome 8 and human fibronectin production. As seen in Fig. 5, all human fibronectin positive lines contained human chromosome 8 and produced between 110 and 210 ng/ml of human fibronectin.

DISCUSSION

The expression of the fibronectin gene product has been related to both transformation (Vaheri and Ruoslahti, 1975; Hynes, 1976) and differentiation (Dessau *et al.*, 1978; Furcht *et al.*, 1978). Fibronectin has separate binding sites for collagen and for cell surfaces, and appears crucial in the interaction between cells and the extracellular matrix (Klebe, 1974). As such, it appears to be involved in determining the morphology of some normal and transformed cells. Thus the study of the molecular regulation of fibronectin expression is of great interest.

Previous gene localization experiments with murine-human hybrids have used surface labeling to detect the expression of fibronectin (Owerbach *et al.*, 1978). These studies have used a transformed line of murine cells to form the hybrid strains, and have found concordance of fibronectin surface expression with human chromosome 8. The decrease in fibronectin on the surface of transformed cells can be due to decreased synthesis, increased degradation, and increased release into the medium of surface associated material (Yamada and Olden, 1978; Vaheri and Ruoslahti, 1975). Thus it is impossi-

Table III. Human Chromosomes Present in Each Human-Mouse Hybrid Correlated with the Production of Human Fibronectin

Cloned hybrid lines	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Human fibronectin production
ANHS17b	–	–	+	–	–	–	+	–	–	–	–	–	+	+	–	–	–	–	+	+	+	+	+	–
ANHS17I	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	+	+	–	+	–
ANHS17J	–	–	–	–	–	–	–	–	+	–	–	–	+	+	–	–	–	–	–	+	+	–	+	–
ANHS96B-1	–	–	–	–	+	–	–	–	–	–	–	–	–	+	+	–	–	–	+	–	–	–	+	–
ANHS960-1	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	+	+	–	–	–	+	–
ANHS96C-3	–	–	–	–	+	+	+	–	–	+	–	+	–	+	+	–	–	–	+	+	–	–	+	–
ANHS96C-4	–	–	–	+	–	–	–	–	–	+	–	+	–	–	–	–	–	+	+	–	–	–	+	–
ANHS95A-2	–	–	–	–	+	–	–	–	–	–	+	–	–	+	+	–	–	–	+	+	–	–	+	–
ANHS95B-2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–
ANHS95C-2	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	+	–
ANHS96A-1	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	–	–	+	–	–	–	+	–
BNHS2	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–
ANHS1	–	+	–	+	–	+	–	–	–	+	–	+	–	+	+	–	–	+	+	–	–	–	+	–
ANHS13	+	–	+	+	+	+	+	+	+	+	–	+	–	+	+	–	–	+	+	+	+	–	+	+
ANHS15	–	–	–	+	–	–	+	+	–	–	–	–	–	+	+	–	–	+	+	+	+	–	+	+
ANHS18	+	+	–	–	–	–	–	+	–	–	–	+	–	+	–	–	–	–	–	+	+	–	+	+
ANHS3	–	–	–	–	–	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	+	–
ANOM3	–	+	–	+	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	+	–	+	+
ANOM7	–	–	–	+	–	–	–	+	–	–	–	+	–	+	–	–	–	–	–	–	–	+	+	+
ANOM11	–	+	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+
J1OH7	+	–	+	–	–	+	+	–	–	+	+	–	–	–	–	+	+	+	–	–	–	–	+	–
J1OH12	+	–	+	–	–	+	+	–	–	+	+	+	–	–	–	+	+	+	–	+	–	–	–	–
WAIa	–	–	–	+	–	–	+	+	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	+
WALLa	–	–	+	+	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–	+	–	–	–
WAV	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+	–	–
BSCL8	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–
BSCL9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–
BSCL15	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–	–	+	–	–	–	+	–	+	–
BSCL16	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–	+	–	–	–	+	–	–	–
ASCL4	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–
BSCL1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–
BSCL16	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–

Table IV. Correlation of the Production of Human Fibronectin with Individual Human Chromosomes in Human-Mouse Hybrids

Human chromosome	Human fibronectin/chromosome[a]				Concordant clones (+/+ and −/−)	Discordant clones (+/− and −/+)
	+/+	+/−	−/+	−/−		
1	2	5	2	23	25	7
2	3	4	1	24	27	5
3	1	6	4	21	22	10
4	6	1	4	21	27	5
5	1	6	5	20	21	11
6	1	6	5	20	21	11
7	4	3	7	18	22	10
8	7	0	0	25	32	0
9	1	6	1	24	25	7
10	1	6	7	18	19	13
11	0	7	5	20	20	12
12	3	4	6	19	22	10
13	0	7	3	22	22	10
14	5	2	11	14	19	13
15	2	5	7	18	20	12
16	0	7	4	21	21	11
17	0	7	9	16	16	16
18	2	5	5	20	22	10
19	3	4	9	16	19	13
20	4	3	7	18	22	10
21	4	3	7	18	22	10
22	10	6	2	23	27	5
X	6	1	16	9	15	17

[a] +/+, human fibronectin positive/chromosome positive; +/−, human fibronectin positive/chromosome negative; −/+, human fibronectin negative/chromosome positive; −/−, human fibronectin negative/chromosome negative.

ble to assess the location of the fibronectin gene from surface labeling experiments alone. A gene that alters the expression of fibronectin receptors could be carried on a human chromosome and allow incorporation of murine fibronectin onto the cell surface. The effects of several possible genes in regulating fibronectin expression may account for some of the difficulty others have had with this technique. To alleviate this problem, we developed a species-specific quantitative fibronectin assay. This assay allowed us to measure fibronectin accumulation in the conditioned medium of a variety of hybrid cell clones.

Using these clones, we found that hybrids produced both human and murine fibronectin, although in most cases the human fibronectin was quantitatively less than the murine. Murine fibronectin production resembled that of control murine cells. The loss of human fibronectin production seemed

to parallel the loss of genetic material, since human chromosomes were preferentially lost from most hybrid lines. Whether this reflects the loss of regulatory genes, however, will remain for further studies to determine. The production of human fibronectin did show absolute concordance with human chromosome 8, confirming earlier results (Owerbach *et al.*, 1978).

Since fibronectin has a complex molecular structure and undergoes several posttranslational modifications, it is potentially a complex antigen. It is subject to proteolytic cleavage, glycosylation, and cross-linking by at least two mechanisms (Mosher *et al.*, 1979). If these posttranslational modifications were antigenically important, then the gene product mapped by the immunoassay could correlate with the enzymes responsible for these changes. To determine the chemical nature of the antigen, we subjected fibronectin to a variety of proteolytic and denaturing conditions. Since the antigenic determinants measured in our ELISA are sensitive to both heat and extensive protease digestion, both conformational and sequential antigens are present, and both of these are determined by the primary fibronectin polypeptide sequence. Therefore, it is the primary fibronectin polypeptide that correlates with chromosome 8.

It is of interest that all of our murine-human hybrids were obtained from human fibroblast sources. In addition, we have tested over 20 murine-human hybrids, in which the human parent was obtained from lens epithelium. All of these lines were completely negative for human fibronectin production, using our human fibronectin antibody. Human chromosome 8 was present in several of these hybrid lines, indicating that these epithelial cells may have a mechanism for regulation of fibronectin expression distinct from fibroblasts.

The use of species-specific quantitative assays for fibronectin should prove a powerful technique in the study of fibronectin genetics. Although we studied the secreted product, the cell surface material can be studied in a similar manner. The study of fibronectin expression in the conditioned media and at the cell surface can also be studied in systems where fibronectin production can be modulated. Such systems offer promise for the delineation of the controlling mechanism for fibronectin expression. Lastly, the assay for fibronectin is relatively simple, and may prove to be more practicable than the conventional enzyme markers used to detect chromosome 8.

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