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Localization of the cellular-fibronectin-specific epitope recognized by the monoclonal antibody IST-9 using fusion proteins expressed in *E. coli*

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Here we report on a monoclonal antibody (IST-9) which distinguishes between human cellular and plasma fibronectin. Using β-galactosidase-fibronectin fusion proteins expressed in *E. coli* we have demonstrated that this monoclonal antibody is specific for a fibronectin segment (ED) which can be included or omitted from the molecule depending on the pattern of splicing of the mRNA precursors. Furthermore, using the same fusion proteins we have been able to localize precisely the epitopes of two other monoclonal antibodies (IST-1 and IST-2), specific for the heparin-binding domain 5 of fibronectin.

Fibronectin; Monoclonal antibody; Fusion protein; Splicing

1. INTRODUCTION

Fibronectins (FNs) are high-molecular-mass glycoproteins present in a soluble form in plasma and other body fluids, and in an insoluble form in the extracellular matrices and basement membranes. FN molecules act as bridges between the cell surface and extracellular material. In fact, the FN molecules contain a cell-binding site and binding sites for collagen, heparin, gangliosides and fibrin. Due to their multiple interactions FNs play an important role in diverse biological phenomena including cell adhesion, cell migration, hemostasis and thrombosis, wound healing and the ability to induce a more normal phenotype in transformed cells [1-5].

It has been demonstrated that FN polymorphism may be at least partially due to alternative splicing schemes in two regions (ED and IIICS) since as many as ten different mRNAs may originate from the primary transcript of a single gene [6-13] localized on chromosome two [14,15].

Monoclonal antibodies (Mabs) specific to sequences the expression of which is regulated by the alternative splicing of pre-mRNA may represent useful tools for studying in vivo the distribution and structure-function relationships of the various FN isoforms. While Mabs able to distinguish between the plasma (plFN) and cellular (cFN) forms of FN have been reported [16-18], the exact localization within the FN molecule of their epitopes has never been possible.

The advent of recombinant DNA technology has offered a powerful new tool both for the preparation of specific antisera and for the localization of epitopes recognized by Mabs. In fact, a recent report [19] describes the preparation of a rabbit antiserum to rat ED sequence [8,9] using a fusion protein as antigen. Here we report on a Mab (IST-9) which is specific for cFN and, using β-galactosidase-FN fusion proteins expressed in *E. coli*, show that the epitope it recognizes is localized within the ED sequence [8,9].

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2. MATERIALS AND METHODS

Cultured normal human fibroblast (GM-5659) and human fibrosarcoma cell lines (HT-1080) were grown in Eagle's minimum essential medium supplemented with 10% fetal calf serum (Flow Laboratories, Irvine, Scotland), previously depleted of bovine FN by passage through a large capacity gelatin-Sepharose column.

FN was purified from human plasma and from the conditioned media of the cell lines as in [20,21]. Thermolysin (Protease type X, Sigma, St. Louis, MO, USA) digestion of FN was performed according to [20] using 5 μg/mg of FN for 2 h at 22°C. SDS-PAGE and immunoblotting were carried out as in [23].

The Mabs IST-1 and IST-2, specific for both plFN and cFN, were obtained using plFN as antigen; they have been shown to react with the heparin-binding domain 5 of FN [21,22]. The Mab IST-9, specific for cFN only, was obtained using as antigen cFN from the SV40-transformed WI38-VA13 cell line using the immunization, hybridization and cloning procedures described [28]. Construction of recombinant plasmids and expression of β-galactosidase-FN fusion proteins was carried out as described in [24].

3. RESULTS

Fig.1 shows a schematic representation of the domain structure of human FN and of the β-galactosidase-FN fusion proteins used in this study. The fusion protein pXFN-111 contains the ED sequence plus 129 amino acids of the cell-binding domain 4 at its amino-terminal and 158 amino acids of the heparin-binding domain 5 at its carboxy-terminal. The fusion protein pXFN-154 is identical to pXFN-111 except that it lacks the entire ED sequence. The fusion protein pXFN-4 contains part of the gelatin-binding domain 2. Fig.2A shows the polypeptide patterns on SDS-PAGE of undigested and thermolysin-digested FNs from human plasma (plFN) and from the tissue culture medium of the human fibrosarcoma cell line HT-1080 (cFN), and of the three β-galactosidase fusion proteins. Fig.2B–D shows immunoblots of SDS-PAGE similar to that shown in fig.1A, and probed with the Mabs IST-9, IST-2 and IST-1, respectively. The Mab IST-9 (fig.2B) reacts only

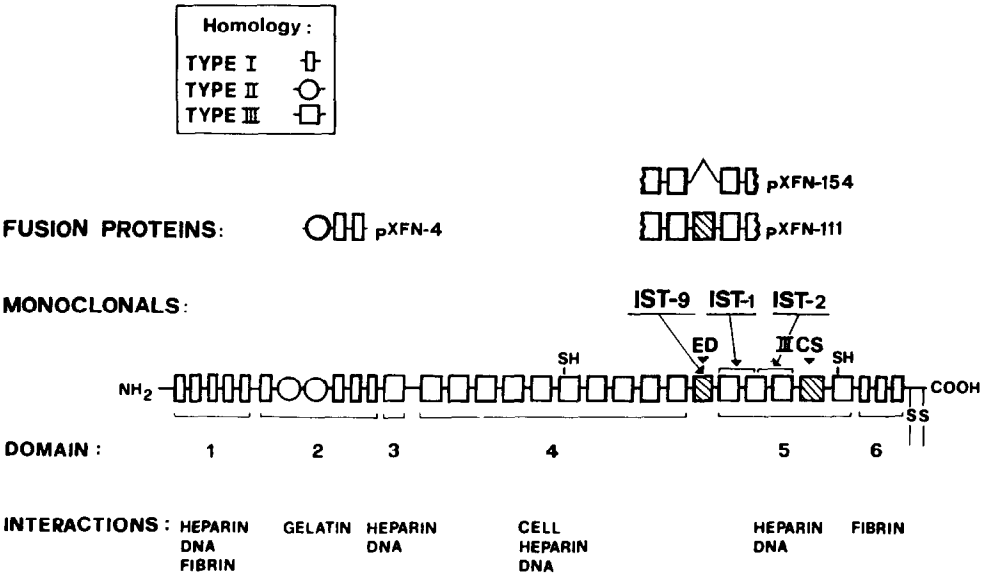

Fig.1. Schematic representation of the structure of human FN and of the β-galactosidase-FN fusion proteins used in this study. ED and IIICS represent the two known regions of variability due to alternative splicing of mRNA precursors. The site of specificity of the three Mabs used in this paper are indicated. Also indicated are the three types of internal homology [26].

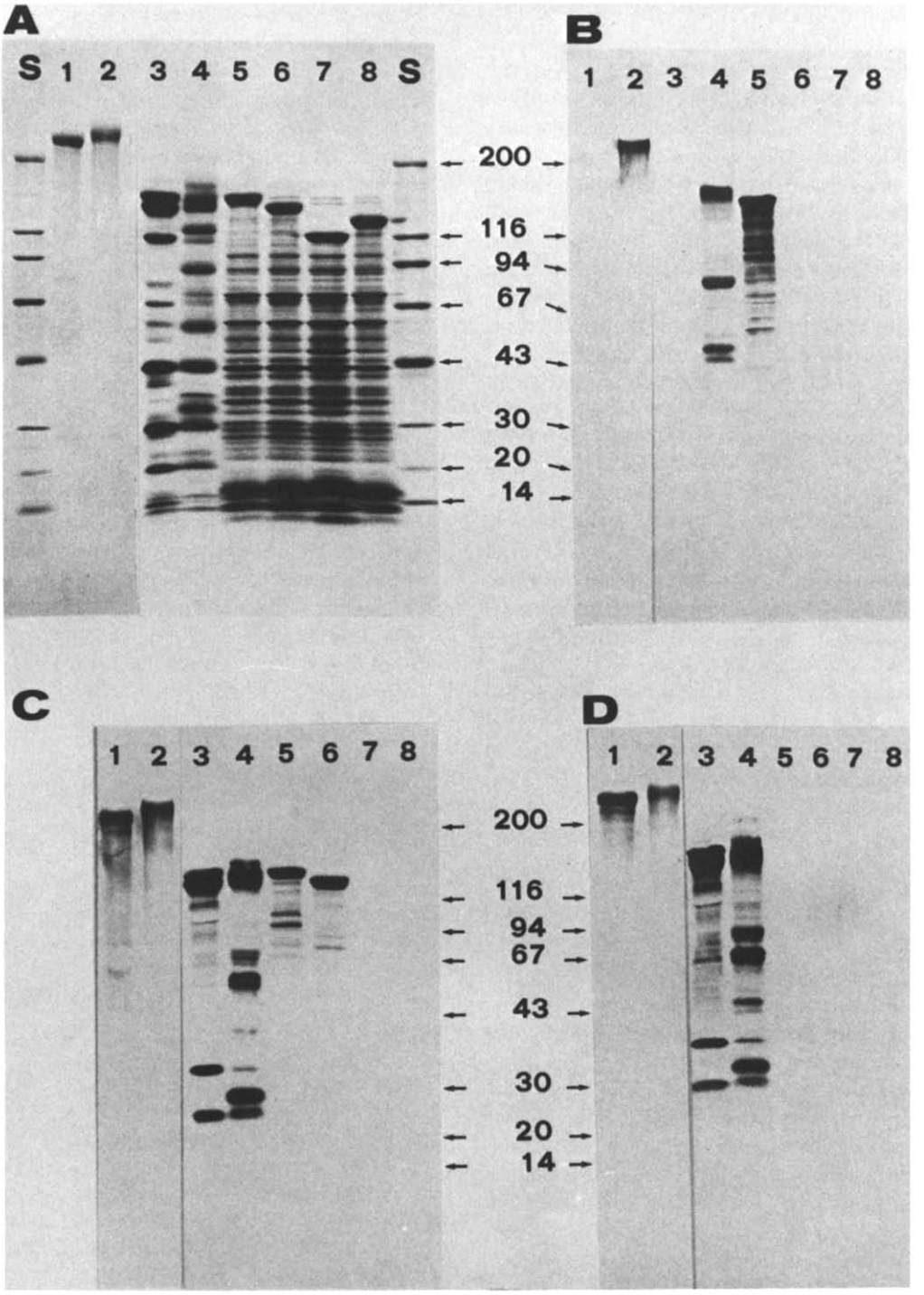

Fig.2. (A) 4–18% SDS-PAGE gradient of purified plFN (lane 1); purified cFN (HT1080) (2); plFN digested by thermolysin (3); cFN digested by thermolysin (4); β-galactosidase fusion proteins pXFN-111 (5); pXFN-154 (6); β-galactosidase expressed by the pEX2 vector (7) and fusion protein pXFN-4 (8). Proteins in lanes 7 and 8 were used as negative controls in immunoblot experiments. The values on the right are the molecular masses (in kDa) of the standards (S). (B–D) Immunoblot analysis of a 4–18% SDS-PAGE as in A using the Mabs IST-9 (B), IST-1 (C) and IST-2 (D). In all experiments an immunoglobulin concentration of 10 μg/ml was used.

with cFN and with the fusion protein pXFN-111, which contains the ED sequence. This Mab reacted with cFN at concentrations as low as 1 μg/ml, but not with plFN at any concentration tested (from 0.5 to 500 μg/ml). The Mab IST-2 (fig.2C) reacts only with cFN and plFN, but not with any of the fusion proteins tested, even at an immunoglobulin concentration as high as 500 μg/ml. The Mab IST-1 (fig.2D) reacts with plFN, cFN and fusion proteins pXFN-111 and pXFN-154. In the reaction of Mabs with β-galactosidase-FN fusion proteins it is possible to observe a major high-molecular-mass protein, which represents the entire fusion protein, plus a number of lower molecular-mass polypeptides which are degradation products due to the presence of proteolytic enzymes in *E. coli*.

4. DISCUSSION

The precise localization of the epitopes recognized by Mabs within molecules is often very difficult. The use of recombinant fusion proteins expressed in bacteria may greatly simplify this localization.

Here we report on the localization within the ED sequence of human FN of the epitope recognized by the Mab IST-9 using β-galactosidase-FN fusion proteins expressed in *E. coli*. This Mab has been shown to be specific for cFN, but not for plFN, both in radioimmunoassay and immunoblot experiments [29]. It also reacts with the fusion protein pXFN-111 which contains the ED sequence but does not react with the fusion protein pXFN-154 which is identical to pXFN-111 except lacking the ED sequence. This result clearly demonstrates that the epitope recognized by the Mab IST-9 is localized within the ED sequence. These data and the observation that this Mab reacts only with cFN and not with plFN are consistent with primary structure studies showing that the ED sequence is absent in human plFN [22], and with experiments showing that the ED segment is absent in liver mRNA which is the source of plFN [8,27].

These fusion proteins also gave us the opportunity to more precisely define the epitopes recognized by the Mabs IST-1 and IST-2, previously localized within the first three type III homology repeats of the heparin-binding domain 5 (fig.1) [20]. Only the first and part of the second type III homology repeat of domain 5 are present in the fusion proteins pXFN-111 and pXFN-154, and both these proteins react with IST-1 but not with IST-2. Thus, the epitope recognized by IST-1 is within the first 158 amino acids of domain 5, while that recognized by IST-2 should be within the remaining 112 amino acids. While these two Mabs (IST-1 and IST-2) may be useful in the precise localization of the heparin-binding site, the Mab IST-9 may represent a powerful reagent to study the structure-function relationship of the different FN isoforms as well as their production by different cell types.

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