Identification of Fusion Regulatory Protein (FRP)-1/4F2 Related Molecules: Cytoskeletal Proteins are Associated with FRP-1 Molecules that Regulate Multinucleated Giant Cell Formation of Monocytes and HIV-Induced Cell Fusion

Shigeru Suga, Masato Tsurudome, Shinji Ohgimoto, Nobutada Tabata, Noriko Watanabe, Machiko Nishio, Mitsuo Kawano, Hiroshi Komada, and Yasuhiko Ito*

Department of Microbiology, Mie University School of Medicine, 2-174, Edobashi, Tsu, Mie Prefecture 514, Japan

Key words: FRP/vimentin/actomyosin

ABSTRACT. Fusion regulatory proteins (FRPs) regulate virus-mediated cell fusion and multinucleated giant cell formation of monocytes. Anti-FRP-1 mAbs immunoprecipitated 80 kDa and 38 kDa proteins from HeLa cells. After long exposure other bands were detected, suggesting the presence of molecule(s) associated with FRP-1. To identify the molecule(s), we prepared monoclonal antibodies against immunoaffinity-purified FRP-1 complex derived from membrane fractions of HeLa cells. Immunofluorescence microscopy revealed that these monoclonal antibodies recognized the intracytoplasmic molecules in HeLa cells. Using immunoblotting, the antibodies reacted with 200 kDa, 70 kDa, 55 kDa and 35 kDa molecules, so we designated these molecules as FRP-related molecules (FRMs). Subsequently, we performed gene cloning from a HeLa ^gtll CDNA library using anti-FRM mAbs and immunoblotting analysis with either purified cytoskeletal proteins or specific antibodies against various cytoskeletal proteins. Three kinds of positive clone were obtained, which encoded partial sequences of vimentin, tropomyosin, and heat shock cognate protein 70 (hsc70). The 200 kDa molecule was expected to be a myosin heavy chain, judging from the immunoblotting pattern. Immunoblotting confirmed that these purified proteins were readily recognized by anti-FRM mAbs. Furthermore, anti-vimentin and anti-myosin mAbs reacted with the precipitates by anti-FRP-1 mAb, indicating a physical association between FRP-1 molecules and these cytoskeletal proteins. When anti-FRP-1 mAb was added to culture fluids of HeLa cells, the cell-shape and immunofluorescence-pattern stained with anti-FRM mAbs changed. Taken together, the fusion regulatory molecular complex is suggested to consist of at least FRP-1, hsc70, actomyosin and vimentin systems.

Membrane fusion is an important event in biologic phenomena. For fertilization, sperm fuse with the plasma membrane of an egg (1). Myogenesis, osteogenesis, exo- and endocytosis, organelle formation and intracellular organelle traffic are also related to membrane fusion. However, the regulatory mechanisms of membrane fusion are not well understood. We have recently isolated monoclonal antibodies (mAbs) which regulate cell fusion induced by viruses including Newcastle disease virus, measles virus and HIV (16, 21) and multinucleated giant cell formation of monocytes (26). These mAbs immunoprecipitated gp80 and gp135, which were detected on the surface of HeLa and U937 cells. These molecules were designated as fusion regulatory protein (FRP)-1 and -2, respectively. The integrins are a family of transmembrane glycoproteins, which interact with a variety of ligands including extracellular matrix glycoproteins, complement and other cells (10, 11, 27). CD98 is a disulfide-linked heterodimer and is expressed on monocytes and activated T and B cells (7) but the functions of the molecule remain unclear.

We have reported that FRP-1 and FRP-2 systems exist separately from each other and have their own signal transduction pathways (21). In addition, FRPs-mediated cell fusion was cytoskeleton dependent, suggesting a possibility of an association between FRPs and cytoskeletal components (21). The integrin cytoplasmic domains have been shown to interact with actin filament via talin, vinculin, α-actinin (3, 8, 29). On the other hand, to our knowledge, intracellular components which interact with FRP-1/CD98 molecules have not been examined. In the present study, we searched for FRP-1-related molecules (FRMs) to clarify a mecha-
nism of FRP-1-mediated cell fusion. We found that immunoprecipitation of HeLa cells by anti-FRP-1 mAb revealed the presence of FRMs. Monoclonal antibodies against immunoaffinity-purified FRP-1 complex were shown to recognize intracellular molecules, which were different from each other in their size and distribution. Further analysis of FRMs by using these mAbs identified them as vimentin, tropomyosin, myosin and hsc70. Intriguingly, we showed that the rearrangement of FRMs occurred when anti-FRP-1 mAb was added to the culture medium of HeLa cells.

MATERIALS AND METHODS

Cells. HeLa cells and Human embryonic fibroblast (HEF) were cultured in Eagle's minimum essential medium (MEM) supplemented with 5% fetal calf serum.

Antibodies. Anti-FRP-1 mAb (mAb 4-5-1) has been previously described (16). Anti-vimentin antibody was purchased from Oncogene Science, Inc. (Uniondale, NY). Anti-tropomyosin antibody and anti-hsc70 antibody were purchased from Sigma Chemical Co. (St. Louis, MO). Anti-myosin antibody was purchased from Chemicon International Inc. (Temecula, CA). Rhodamine phalloidin was purchased from Cosmo Bio. (Tokyo, Japan). Anti-integrin α3 antibody and anti-CD44 (human heterotypic adhesion receptor) antibody were purchased from Oncogene Science and Telios (San Diego, CA), respectively. Anti-tubulin antibody was obtained from Amersham (Tokyo, Japan). Anti-human parainfluenza 4A virus NP mAb (3B) was used as the control antibody.

Isotopic labeling, RIPA and SDS-PAGE. Isotopic labeling of HeLa cells, radio-immunoprecipitation assay (RIPA), and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were done as described previously (14).

Western blotting. HEP lysate or purified cytoskeletal proteins were run on 10% SDS-PAGE and electroblotted on a polyvinylidine difluoride (PVDF) membrane. The blotted PVDF membrane was blocked with 5% non-fat milk in PBS containing 0.05% Tween-20 (PBST) for 1 hour and then cut into strips and incubated for 1 hour in primary antibodies. After washing for 30 minutes in PBST, the strips were incubated for 1 hour with biotinylated secondary antibody. The strips were washed as just described and incubated for 30 minutes in ABC solution (1/100 dilution of avidin and biotinylated HRP, Vector Laboratories, Inc. Burlingame, CA). Bound antibodies were visualized using 4-chloro-1-naphthol or ECL Western blotting detection kit (Amersham). Purified vimentin from bovine lens, myosin from rabbit muscle, tropomyosin from chicken gizzard and hsp70 from bovine brain were purchased from Sigma Chemical Co.

Dot blotting. One μg of purified vimentin or myosin was dotted onto nitrocellulose membrane. The membrane was processed as in Western blotting to visualize the specific binding of mAb with the dotted proteins.

Purification of FRP-1 complex. Plasma membranes were prepared from HeLa cells (2 x 10^9) by the method of Maeda et al. (19). The cell membranes were solubilized with 30 ml of 50 mM Tris-HCl, 1% Triton X-100 using an ultrasonic cell disruptor and centrifuged at 25,000 g for 20 minutes. The supernatant was passed through Sepharose 4B and control IgG (anti-parainfluenza virus type 4A mAb) column to remove nonspecifically adhering materials. The flow-through fraction was applied to an mAb 4-5-1 immunoaffinity column and the columns were washed free of unbound proteins with 10 volumes of solubilized buffer. The bound protein was sequentially eluted stepwise with LiCl in 50 mM Tris-HCl (pH 7.5). The LiCl eluate was dialyzed against NANOpure water and concentrated by using a speedvac concentrator.

Production and isolation of hybridoma cell lines. The procedures used for preparation of mAbs were similar to the method described previously (28). In brief, three mice were immunized with purified FRP-1 complex and their spleen cells were fused with SP2/0-Ag-14 myeloma cells. The culture fluids of hybridoma cells were screened by enzyme-linked immunosorbent assay (ELISA). Cultures of interest were then cloned by soft-agar colony formation and further cloned by limiting dilution.

Enzyme-linked immunosorbent assay (ELISA). ELISA was performed as described previously (22) except that purified FRP-1 complex and peroxidase-conjugated goat antimouse immunoglobulin (diluted 1:2000, Cappel Laboratories, Durham, NC) were used as the coating antigen and the secondary serum, respectively.

cDNA library screening and nucleotide sequencing. A HeLa Sgr11 cDNA library was obtained from Clontech Laboratories (Palo Alto, CA). The clones were immunoscreened using anti-FRM mAbs according to the manufacturer’s protocol (Clontech Laboratories). Positive clones were subcloned into pBluescript II SK(-) and sequenced by the dideoxy chain termination method (Sanger et al., 1977). Nucleotide and amino acid sequence analyses were aided by GENETYX-MAC/CD computer programs (Software Development, Tokyo, Japan).

Indirect immunofluorescence. HeLa cells were fixed with 3% formalin, treated with 0.05% Triton X-100, and stained with hybridoma supernatant or purified antibody and FITC-conjugated anti-mouse Ig antiserum or FITC-conjugated anti-mouse IgM antiserum, according to the method described previously (15). When we examined surface fluorescence, non-permeabilized HeLa cells were used.

RESULTS

Localization of FRP-1 molecule on the surface of
FRP-1 and cytoskeletal proteins

Fig. 1.
**HeLa cells.** HeLa cells were cultured for 24 hours in the presence of 5% FCS, and then the cells were fixed with 3% formalin. Subsequently non-permeabilized cells were stained with anti-FRP-1 mAb (4-5-1), anti-integrin α3 mAb, anti-CD44 mAb and FITC-conjugated anti-mouse Ig antiserum. As shown in Fig. 1, FRP-1 molecules were detected as clusters on the cell surface, indicating that the immunofluorescence pattern is similar to those of adhesion molecules such as integrin α3 or CD44. At higher magnification, FRP-1 molecules seemed to be fine granules on the cell surface (Fig. 1D).

**Detection of molecules associated with FRP-1 molecule.** We performed immunoprecipitation using anti-FRP-1 mAb (4-5-1) to detect molecule(s) associated with FRP-1 molecule. HeLa cells were labeled metabolically with [35S]-methionine, and the cell lysates were immunoprecipitated by anti-FRP-1 mAb. Two bands of 80 kDa and 38 kDa, namely, heavy chain and light chain of CD98, were detected under reducing conditions after 12 hours exposure (Fig. 2A). No specific band was found for control antibody. However, after being exposed for 4 days, a band around 55 kDa appeared in addition to the band of FRP-1, suggesting that 55 kDa molecule is associated with FRP-1, forming a molecular complex (Fig. 2B).

**Purification of FRP-1 complex.** To examine molecules which associate with FRP-1, we purified FRP-1 complex by immunoaffinity chromatography. The affinity-purified preparation was analyzed by SDS-PAGE (Fig. 3). Eighty kDa and 38 kDa molecules were visualized by staining with Coomassie brilliant blue. No other bands were evident under our purification and staining conditions. This result indicated that the purified preparation actually contained FRP-1 and that other associated proteins, if any, were of much smaller amounts than FRP-1.

![Fig. 2. The co-immunoprecipitation of 55 kDa molecule with FRP-1. (A and B) The cells were labeled with [35S]-methionine for 3 days, then the lysates were immunoprecipitated by control antibody (Lane 1) or anti-FRP-1 mAb (Lane 2) followed by SDS-PAGE. The dried gel was processed and exposed to x-ray film. The approximate molecular weights are indicated in kilodaltons on the left. (A) After 12 hours exposure, only a heavy chain and a light chain of CD98 were evident in Lane 2. (B) After being exposed for 4 days, a band around 55 kDa appeared only in Lane 2 (arrow).](image1)

![Fig. 3. Detection of FRP-1 molecules in immunoaffinity purified preparation. The eluate from anti-FRP-1 mAb immunoaffinity column was analyzed by SDS-PAGE and stained with Coomassie brilliant blue. Only 80 kDa and 38 kDa, which correspond to the heavy chain and light chain of CD98/4F2, were evident.](image2)

![Fig. 4. Immunoblotting of HEF lysates with mAbs against immuno-affinity-purified FRP-1 complex or cytoskeletal proteins. (A) Lane 1: mAb 3; Lane 2: mAb 8; Lane 3: mAb 16 and Lane 4: mAb 57. The mAbs 9, 11, 12, 14, 17, 24, and 124 showed same reactivity as mAb 8 (data not shown). (B) Lane 1: anti-tropomyosin mAb; Lane 2: anti-vimentin mAb; Lane 3: anti-hsp70 mAb and Lane 4: anti-myosin mAb. The approximate relative molecular masses of the immunoreactive components are shown in kilodaltons.](image3)
Production and characterization of mAbs against immuno-affinity-purified FRP-1 complex. We raised monoclonal antibodies against purified FRP-1 complex described above. Eleven mAbs (mAb 3, 8, 9, 11, 12, 14, 16, 17, 24, 57 and 124) were obtained, and then characterized by immunoblotting and indirect immunofluorescence. Immunoblotting of these mAbs using HEF lysate revealed reactivity with specific bands (Fig. 4A). The mAbs 8, 9, 11, 12, 14, 17, 24, 57 and 124 recognized the 55 kDa molecule (Fig. 4A, lane 2 and data not shown).

Fig. 5. Indirect immunofluorescent staining of HeLa cells (A, B) or HEF (C, D, E, F) with mAb 3(A), 12(B), 16(C), 57(E) or rhodamine phalloidin (D, F). Bar: 10 μm.
The mAbs 3 and 16 recognized 70 kDa and 200 kDa molecule, respectively. The mAb 57 detected three different bands, 35 kDa, 55 kDa and 200 kDa molecules, the 55 kDa and 200 kDa bands recognized by mAb 57 seemed to be identical with bands by mAbs 3 and 16, respectively. These mAbs reacted with the lysates of murine L929 cells, baby hamster kidney (BHK) cells as well as HEF lysate (data not shown). mAbs directed against 80 kDa heavy chain or 38 kDa light chain of CD98 could not be obtained, probably due to antigenic instability of FRP-1 (data not shown).

Subsequently, indirect immunofluorescence was performed using these antibodies. As shown in Fig. 5, although staining patterns varied, each antibody recognized intracytoplasmic components, not cell surface proteins. When HeLa cells were fixed and immunostained with the mAb 3, fine granular structures were diffusely stained (Fig. 5A). Using the mAbs which recognize the 55 kDa molecule, filamentous structures, resembling the cytoskeleton, were stained (Fig. 5B). The mAbs 16 and 57 stained fine granular structures. Unlike the mAb 3, the fine granular structures mainly colocalized with actin filaments (Fig. 5C, D, E, F). As summarized in Table I, the mAbs recognized FRP-1-related molecules, and FRMs consisted of at least four molecules differing in size and intracellular localization. Furthermore, immunofluorescence staining suggested some FRMs could be cytoskeletal or cytoskeleton-associated proteins.

**Isolation and sequencing of cDNA encoding FRMs.** Using anti-FRM mAbs, we screened about $1 \times 10^5$ plaques from a HeLa $\lambda gt11$ cDNA library and cloned three positive phage recombinant, that is, F3 (271 bp) was reacted with mAb 3; F39 (383 bp) was reacted with mAbs 12, 17, 57 and 124; F57 (1,000 bp) was reacted with mAb 57. The inserts were subcloned into pBluescript II SK(-) and then sequenced. Nucleotide sequences of F3, F39, and F57 were completely identical with those of hsc70, vimentin, and tropomyosin, respectively (Fig. 6).

**Comparison of immunoblotting patterns of anti-FRM mAbs to anti-cytoskeletal protein antibodies.** As described above, immunofluorescent staining using anti-FRM mAbs showed cytoskeleton-like patterns. Then, we compared immunoblotting patterns of anti-FRM mAbs with those of anti-cytoskeletal protein, including vimentin and tropomyosin, mAbs and anti-hsp70 mAb. HEF lysates were electrophoresed and blotted on PVDF membrane. Anti-vimentin mAb reacted with the 55 kDa antigen (Fig. 4B, lane 2). This band has the same molecular mass as the one identified by anti-FRM mAbs 8, 9, 11, 12, 14, 17, 24, 57 and 124 (Fig. 4A, lane 2 and data not shown). Anti-tropomyosin mAb and anti-hsc70 mAb reacted with 35 kDa and 70 kDa proteins, respectively, which were identical with the bands identified by anti-FRM mAbs 57 and 3 (Fig. 4A, lanes 1, 4 and Fig. 4B, lanes 3, 4). Other antibodies against cytoskeletal proteins, such as desmin, cytokera- tin, glial fibrillary acidic protein, tubulin, vinculin and $\alpha$-actinin, showed reactivity with quite different bands from FRMs (data not shown). Interestingly, an mAb directed against myosin demonstrated reactivity with the same size band as identified by anti-FRM mAbs 16 and 57 (Fig. 4A, lanes 3, 4 and Fig. 4B, lane 4). These data indicated a strong possibility that the 35 kDa, 55 kDa, 70 kDa, and 200 kDa molecules identified by anti-FRM mAbs were tropomyosin, vimentin, hsc70, and myosin, respectively.

**Immunoreactivities of anti-FRM mAbs with vimentin, tropomyosin, myosin, and hsp70.** We performed immunoblotting of purified vimentin, tropomyosin, myosin, and hsp70 using anti-FRM mAbs. Anti-FRM mAbs 8, 9, 11, 12, 14, 17, 24, 57 and 124 equally reacted with...
FRP-1 and cytoskeletal proteins

Fig. 7. Immunoreactivities of anti-FRM mAbs with vimentin, tropomyosin, myosin, and hsp70. Purified tropomyosin (lanes 1 and 2), vimentin (lanes 3 and 4), myosin (lanes 5 and 6), or hsp70 (lanes 7 and 8) were separated by SDS-PAGE, transferred to PVDF membrane, and probed with mAb 57 (lanes 1, 3, and 5), anti-tropomyosin mAb (lane 2), anti-vimentin mAb (lane 4), mAb 16 (lane 6), mAb 3 (lane 7), or anti-hsp70 mAb (lane 8). The approximate relative molecular masses of the immunoreactive components are shown in kilodaltons.

with the 55 kDa vimentin band (Fig. 7 and data not shown). Anti-FRM mAb 57 showed cross-reactivity with tropomyosin and myosin, which was recognized by anti-FRM mAb 16, and anti-FRM mAb 3 recognized purified hsc70 (Fig. 7). These results showed that FRMs consist of vimentin, hsc70, and myosin. As to tropomyosin, because of the cross-reactivity of anti-FRM mAb 57, the possibility remained that the protein was not really one of the FRMs. To exclude the possibility, ELISA using purified FRP-1 complex and anti-tropomyosin mAb was done. Anti-tropomyosin mAb reacted with the purified FRP-1 complex, suggesting that tropomyosin is certainly contained in the complex (data not shown). In addition, anti-hsc70, -vimentin and -myosin mAbs also reacted with the purified FRP-1 complex in ELISA (data not shown).

Coprecipitation of FRMs with anti FRP-1 mAb. To confirm the physical association between FRP-1 and FRMs, we immunoprecipitated the membrane fraction of HeLa cells with anti-FRP-1 mAb and then probed blots of the precipitates with the anti-vimentin, anti-myosin, anti-tropomyosin and anti-hsc70 mAbs. As shown in Fig. 8, anti-vimentin and anti-myosin mAbs specifically reacted with the precipitates by anti-FRP-1 mAb. Other mAbs, anti-tropomyosin and anti-hsc70 mAbs did not react with any of the bands. To exclude the possibility that anti-FRP-1 mAb cross-reacted with vimentin or myosin, we performed dot blotting. No cross-reactivity of the mAb with these proteins was recognized (data not shown). These data clearly supported the association between FRP-1 and two cytoskeletal proteins, vimentin and myosin.

Effects of anti-FRP-1 mAb on intracellular distribution of FRMs. To examine whether FRMs functionally related to FRP-1, indirect immunofluorescence of HeLa cells with anti-FRM mAbs after anti-FRP-1 mAb treatment was carried out. When HeLa cells were cultured in the presence of 5 μg/ml of anti-FRP-1 mAb (4-5-1), at various periods the cells were fixed and then were stained with Giemsa’s solution or anti-FRM mAbs. The cell morphology was changed from polygonal shape to spindle shape through incubation of HeLa cells with anti-FRP-1 mAb (4-5-1) (Fig. 9A, B, C). Furthermore, treatment of HeLa cells by anti-FRP-1 mAb induced drastic changes in immunofluorescence pat-
Fig. 9.
FRP-1 and cytoskeletal proteins

terns stained by anti-FRM mAbs which recognized vimentin (Fig. 9E, F, G, H). The filamentous stains were no longer present, but fine granules and some spheroidal bodies were stained. No other anti-FRM mAbs showed any significant change by anti-FRP-1 mAb treatment. Microtubules and microfilaments also retained the original structures throughout the observation (data not shown).

**DISCUSSION**

We have recently reported that cytochalasin B (anti-microfilament agent) completely blocks the cell agglutination and cell fusion of U2ME-7 cells induced by anti-FRP-1 mAb. Intriguingly, cytochalasin B inhibits the cell fusion whenever it is added during incubation (even after cell agglutination has already taken place), although existing cell aggregation is not loosened by cytochalasin B. In addition, vinblastine (anti-microtubules agent) inhibits FRP-1-mediated polykaryocyte formation of U2ME-7 cells, but has no effect on cell agglutination. These findings indicated that FRP-1-mediated cell fusion is cytoskeleton dependent and that there is the possibility of an association between FRP-1 molecule and cytoskeletal components.

In the present study, we have shown that FRP-1 molecules are associated with cytoskeletal components, including vimentin, tropomyosin, myosin and hsc70. Vimentin is one of the intermediate filaments (IFs), which is relatively well characterized in terms of its molecular structure and conformation (24, 25). IFs are fibrous proteins forming prominent components of the cytoskeleton of most eukaryotic cells, although their precise roles are unclear. There is abundant experimental evidence that IFs interact with the plasma membrane. For example, an interaction between vimentin and peripheral membrane protein ankyrin has been described (5). Desmosomes and hemidesmosomes act as attachment points for IF at cell-cell and cell substrate borders, respectively.

We demonstrated that 55 kDa molecule was coprecipitated by anti-FRP-1 mAb. In addition, among anti-FRM mAbs, mAbs 8, 9, 11, 12, 14, 17, 24, 57 and 124 were found to recognize vimentin by gene cloning and immunoblotting. These results suggested an association between FRP-1 and vimentin. Intriguingly, indirect immunofluorescence by mAb 57 showed a different staining pattern from vimentin filaments and colocalization with actin filaments. This observation could be explained by its cross-reactivity with myosin and tropomyosin.

Myosin and tropomyosin are well-known actin binding proteins. Therefore, our finding that they are included in FRMs suggest an association between FRP-1 and actin filament. Nonmuscle myosins, like muscle myosins, consist of six polypeptides: two apparently identical heavy chains of 200 kDa and two pairs of light chains of 20 kDa and 16 kDa (17). Immunoblotting showed anti-FRM mAbs 16 and 57 reacted with 200 kDa mass of purified myosin, suggesting that the antibodies recognized epitope(s) on the heavy chains of myosin. Interestingly, mAb 57 cross-reacted with tropomyosin. A monoclonal antibody which recognizes both vimentin and tropomyosin was reported (2). However, it is unique that a monoclonal antibody such as our mAb 57 cross-react with three different cytoskeletal proteins, vimentin, tropomyosin and myosin.

The heat shock proteins of the 70 kDa class were first identified in stressed cells (18). Later it became clear that they also play important roles in unstressed cells. They form a conserved protein family including constitutively expressed hsc70 as well as stress-inducible hsp70 isoforms. The result of gene cloning and the fact that mAb 3 recognizes 70 kDa molecules of unstressed HEF and HeLa cells indicates that FRMs contain hsc70 among their protein family. Hsp70/72 was reported to mediate an association between the plasma membrane glycoprotein and the cell cytoskeleton (9). Hsc70 was shown in Dictostelium to affect actin polymerization through interacting with cap32/34, actin capping proteins (6). Therefore, hsc70 could act as one of the FRMs through connecting vimentin and/or actin to FRP-1.

Taken together, the experimental data which support the physical association between FRP-1 and FRMs are as follows: (1) The immunoaffinity purified material actually contained FRP-1, while comparable amounts of any other molecules were not present. (2) Anti-FRM mAbs, which were raised against the FRP-1 complex, recognized vimentin, myosin, tropomyosin and hsc70. (3) Immunoprecipitation using [35S]-methionine labeled HeLa cells showed anti-FRM-1 mAb coprecipitated 55 kDa molecules. (4) The precipitates with anti-FRP-1 mAb were probed with anti-vimentin and anti-myosin mAb in immunoblotting, indicating that 55 kDa vimentin and 200 kDa myosin were coprecipitated with FRP-1. (5) Anti-vimentin, -myosin, -hsc70 and -tropomyosin mAbs reacted with the purified FRP-1 complex in ELISA. (6) Dot blotting showed no cross-reactivity of anti-FRP-1 mAb with FRMs.

Some questions against our data may be raised. As to why the affinity-purified FRP-1 complex elicited anti-FRP-1 mAbs, we can conjecture that FRP-1 may be too quickly lost its immunogenicity in LiCl solution to elicit the antibodies, although it has dialyzed against NANOpure water soon after the elution. ELISA data that affinity-purified FRP-1 did not react with anti-FRP-1 mAb in a short time after the purification suggested the antigenic unstability of FRP-1 (data not shown). As to why did not successfully coprecipitate FRP-1 molecule with anti-FRM mAbs (data not shown).
shown), may be that only a small fraction of cytoskeletal proteins associate FRP-1 and almost anti-FRM mAb precipitate free cytoskeletons.

Although the precise molecular mechanisms of cell fusion are still unclear, some important findings were obtained through analyses of FRP-1 and FRMs. When HeLa cells were cultured in the presence of anti-FRP-1 mAb, the cell morphology was clearly altered. The morphological change from polygonal shape to spindle shape would indicate that the adhesion of cells and/or organization of cytoskeletons are affected by anti-FRP-1 mAb treatment. The functions of FRP-1/CD98 molecule are not well understood, however, and immunofluorescent microscopy with anti-FRP-1 mAb suggests the possibility that FRP-1 has the character of an adhesion molecule. Therefore, it is likely that the addition of anti-FRP-1 mAb to culture fluids loosens cell-to-cell and/or cell-to-culture plate attachment, resulting in the formation of the spindle-shaped cells. It is another interesting finding that anti-FRP-1 mAb treatment induced the remarkable change of intracellular distribution of FRMs. Among eleven anti-FRM mAbs, only anti-vimentin mAbs showed disassembly of the orginal structure. Furthermore, the distribution of microtubules and microfilaments did not seem to be affected by anti-FRP-1 mAb. These results indicate that vimentin filaments are functionally related to FRP-1 and their morphological change is induced by an interaction between FRP-1 molecule and anti-FRP-1 mAb (or its ligand).

It has been reported that phosphorylation of vimentin by A kinase, C kinase or p34cdc2 induces disassembly of the filament structure (4, 12, 13). In our previous study, anti-FRP-1 mAb-induced cell fusion is inhibited by both C kinase inhibitor (21) and A kinase inhibitor (unpublished data). From these data, we could speculate that the phosphorylation of vimentin followed by its disassembly is involved in anti-FRP-1 mAb induced cell fusion.

In summary, we have found that there are at least two fusion regulatory systems, FRP-1 and FRP-2 systems. FRP-2 system is the integrin systems as previously reported (21). It is well-known that integrins are associated with cytoskeletal components through some molecules such as talin, vinculin and α-actinin. The FRP-1 system is found to consist of FRP-1/CD98 molecule and other intracytoplasmic component such as hsc70, IF and actomyosin. Therefore, it is inferred that the outside-in signals produced by the interaction of FRP-1/CD98 molecules with anti-FRP-1 mAb is transmitted via these molecules and other cytoskeletal components.

Acknowledgments. We are very grateful to Mr. Morihiro Ito for his capable technical assistance. This work was supported by a Grant-in Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan and by the Mie Medical Research Fund.

REFERENCES

FRP-1 and cytoskeletal proteins


(Received for publication, October 16, 1995 and revised form, November 7, 1995)