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The GPI-anchored adhesion molecule F3 induces tyrosine phosphorylation: involvement of the FNIII repeats

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SUMMARY

The glycosyl-phosphatidylinositol (GPI)-anchored F3 molecule, a member of the Ig superfamily made up of Ig and FNIII-like domains, is involved in cell-cell adhesion, neuronal pathfinding and fasciculation. Little is known about the mechanism(s) that governs the F3-mediated cell-cell recognition. In particular, it is not known whether F3 transduces signals across the membrane.

Here we show that in F3-transfected CHO cells (1A cells) an increase in tyrosine phosphorylation occurs during F3-mediated aggregation. Moreover, under aggregation conditions F3 immunoprecipitated from ^{32}P-metabolically labeled 1A cells associated with three major phosphorylated proteins. Interestingly, genistein inhibited the F3-mediated aggregation. Increased tyrosine phosphorylation was also observed using antibody-mediated F3-cross-linking. Furtheremore, F3 expressed both in 1A cells and in post-natal mouse cerebellum forms non-covalent soluble complexes with protein tyrosine kinase(s). In cerebellum the F3-associated kinase was identified as fyn. By contrast, a truncated F3 protein, expressed in CHO cells, from which all the FN type III repeats have been deleted, does not associate with a kinase. Cross-linking of the F3-truncated form does not induce modulation of tyrosine phosphorylation. Taken together these data demonstrate that F3 is a molecule that transduces signals through both association with protein tyrosine kinase and modulation of protein tyrosine phosphorylation. The presence of FN type III domains is essential for the activation of the intracellular signaling pathway.

Key words: GPI-anchored, Adhesion molecule, F3, Protein tyrosine kinase

INTRODUCTION

A number of adhesion molecules of the immunoglobulin (Ig) superfamily anchored at the membrane by a glycosyl-phosphatidylinositol (GPI) moiety are expressed in various tissues. They are involved in cell-cell contact, cell migration and activation. These processes involve recognition events that must be translated into signals that control the cellular response. The mechanisms by which these proteins, devoid of intracellular domains, can transduce signals across the membrane remain unclear.

Data obtained essentially on various cell types of the immune system, indicated that GPI-anchored cell surface molecules transduce signals through an association with src-like protein tyrosine kinases (PTKs) (Stefanova et al., 1991; Draberova and Draber, 1993; Shenoy-Scaria et al., 1992; Thomas and Samelson, 1992; Bohuslav et al., 1993). Different studies using antibody-mediated cross-linking of GPI-anchored proteins, showed a rapid induction of tyrosine phosphorylation of several proteins (Shenoy-Scaria et al., 1992; Stefanova et al., 1991; Hsi et al., 1989), suggesting that activation of PTK(s) is part of the signaling mechanism(s). However, in other tissues, such as the nervous system, where axonal membrane GPI-anchored proteins from the Ig superfamily are specifically expressed (Furley et al., 1990; Gennarini et al., 1989; Yoshihara et al., 1994), the participation of protein tyrosine kinases in signal transduction has not been addressed. Characterization of early biochemical events associated with cell-cell recognition is critical for understanding the mechanism(s) that regulates axonal growth. In particular, axon extention depends on a complex pattern of guidance cues present in their local environment acting in spatial and temporal concert. This includes neurite growth-promoting molecules from the Ig superfamily exposed at the surface of either neuronal or non-neuronal cells that provide pathways along which axons can preferentially elongate, turn or retract.

The F3 molecule, a GPI-anchored molecule of the Ig superfamily exhibiting six Ig-like domains and four fibronectin type III (FNIII) repeats (Gennarini et al., 1989), has an expression restricted to subpopulations of neurons (Faivre-Sarrailh et al., 1992). In vitro studies using F3-transfected Chinese hamster ovary (CHO) cells, showed that it fullfils the operational criteria of a cell adhesion molecule and mediates heterophilic divalent cation-independent, temperature-dependent binding (Gennarini et al., 1991). In this model, adhesion site(s) were mapped to the FNIII repeats, while the Ig domains, in either

anchored or soluble form, were able to stimulate neurite outgrowth of sensory neurons (Durbec et al., 1993). Moreover, the axonal recognition cell suface protein F11 (Brummendorf et al., 1989), the chicken homolog of F3, undergoes a direct interaction with tenascin R (TNR) (Pesheva et al., 1993), thus inhibiting neurite extention. Interactions between F3 and its receptor(s) guide the cellular response of F3-bearing neurons through undefined signaling pathways.

Several lines of evidence suggest that PTKs of the non-receptor class may be key components of the intracellular signaling pathways in neuronal cells. PTKs of the *src* family have been shown to be localized in developing (Manness, 1992) as well as mature neurons (Umemori et al., 1992). Using *src*- and *fyn*-minus mice it has been demonstrated that src and fyn kinases are components of the intracellular signaling pathways in L1- and NCAM-mediated axonal growth, respectively (Ignelzi et al., 1994; Beggs et al., 1994). Despite the fact that these molecules are anchored at the membrane through a transmembrane polypeptide, similar mechanisms may be used by GPI-anchored molecules.

On the other hand, studies on L1-, NCAM- and N-cadherin-mediated neurite outgrowth measured in the presence of a combination of antagonists and agonists of signaling pathways, suggest that PTK(s) act early in the processes (Doherty and Walsh, 1994). It has been shown that an unidentified erbstatin-sensitive tyrosine kinase acts early on the second messenger pathway underlying neuritic outgrowth response stimulated by these cell adhesion molecules (CAMs) (Williams et al., 1994b). Evidence exists suggesting that the tyrosine kinase receptors for fibroblast growth factors (FGFs) may be invoved in signaling from these CAMs (Williams et al., 1994a). That study reported the presence of a short sequence in the FGF receptor homologous to a specific region of NCAM and L1, suggesting that the CAM-homology domain of the FGF receptor is required for it to transmit signals that lead from CAMs to neurite outgrowth. This stretch is not present in the F3 amino acid sequence, therefore F3-signaling may use a different mechanism(s).

Little is known about the mechanisms and possible partners involved in the signal transduction pathway triggered by activation of GPI-linked neurite outgrowth-promoting molecules.

Here we used CHO cells, transfected with cDNA encoding either a whole or a truncated form of F3 deleted of the FN type III repeats, in an attempt to identify a key domain of the F3 molecule involved in signal(s) transduced after F3-mediated cell-cell recognition. We showed that F3 associated with a kinase in cerebellum, identified as fyn, and that F3-mediated signal transduction occured via association with PTK(s) and modulation of tyrosine phosphorylation. The F3-signaling pathway was disrupted when all FN type III repeats were deleted.

MATERIALS AND METHODS

Cell culture and metabolic labeling

F3-transfected CHO cell lines expressing the whole F3 molecule (1A cells) and the truncated F3 molecule (B21 cells) were obtained as described by Durbec et al. (1993). Parental and transfected cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 50 i.u./ml penicillin and 50 µg/ml streptomycin at 37°C, 5% CO_2.

For metabolic labeling, cells were washed in either phosphate- or methionine-free medium. Labeling was performed for 4 hours in 500 µl/plate with either 250 µCi of $H_3[^{32}P]O_4$ (ICN Biomedicals, France) or a mixture of L-[^{35}S]Met and L-[^{35}S]Cys (Amersham, UK). After removal of the radioactive medium, cells were used for either immunoprecipitation experiments or aggregation assays followed by immunoprecipitation, as described below.

Cell aggregation assay

For detection of the tyrosine phosphorylated proteins, aggregation assays were performed on single cells as described by Durbec et al. (1993). Briefly, the cells were incubated from 0 to 15 minutes at 37°C with or without 100 µM of activated sodium orthovanadate (Mahadevan and Bell, 1990). At different time points, cells were pelleted and lysed in Nonidet P-40 (NP-40). Lysates were subjected to immunoblotting as described below.

To evaluate the effect of tyrosine kinase inhibitors (genistein and herbimycin A) (Sigma, St Louis, MS), cells in monolayers were pretreated with different concentrations of genistein (75 and 150 µM) for 30 minutes or herbimycin A (5 and 10 µM) for 18 hours, at 37°C in a 5% CO_2 atmosphere prior to the aggregation assay. The aggregation was performed, as described by Durbec et al. (1993), in the presence of genistein or herbymicin. Samples were then withdrawn and single cells were counted with a hemocytometer. Results were expressed as the % of single cells present at the experimental time point relative to the single cells present at time zero.

Antibody-mediated F3 cross-linking

Cells (2×10^5 to 2.5×10^5 cells) were washed with ice-cold DMEM and incubated on ice for 30 minutes with a 1:50 dilution of either the polyclonal anti-F3 antibody (1 mg/ml) (anti-F3 serum 24III directed against Ig-like domains; Gennarini et al., 1989) or normal rabbit serum (NRS). Cells were washed three times with ice-cold DMEM and then incubated with a 1:50 dilution of goat anti-rabbit IgG (Sigma, St Louis, M) for 10 minutes at 37°C in the presence of 100 µM sodium orthovanadate. After three washes on ice, cells were lysed with 100 µl of NP-40 lysis buffer (50 mM Tris-HCI, pH 7.5, 150 mM NaCl, 5 mM ethylene diamine tetraacetic acid (EDTA), 1% NP-40, 5 mM iodoacetamide, 10 mM sodium fluoride, 100 µM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 µg/ml leupeptin, 10 µg/ml aprotinin) at room temperature for 15-20 minutes. Lysates were centrifuged at 12,000 ***g*** for 10 minutes at 4°C. Protein concentration of supernatants was determined with a Bio-Rad protein assay kit (Bio-Rad Laboratories) according to the manufacturer's recommendations, using bovine serum albumin (BSA) as a standard.

Gel electrophoresis and immunoblotting

A 20-40 µg sample of proteins from each supernatant (obtained from cross-linking experiments and aggregation assays) was mixed with reducing 3× Laemmli sample buffer and subjected to sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli (1970). Proteins were electrophoretically transferred to a nitrocellulose membrane (Amersham) using a semi-dry fast blot apparatus (Biometra), detected using a monoclonal anti-phosphotyrosine antibody (Sigma, St Louis, MS; 1:2,000 dilution) and visualized using an ECL detection kit (Amersham Corp, Arlington Heights, IL) according to the manufacturer's instructions.

Immunoprecipitations and immuno-complex kinase assays

CHO, 1A, and B21 cells (1×10^7) and cerebellum from 5-day postnatal mice were solubilized in 1 ml of 1% NP-40 or 1% Brij 96 lysis buffer containing 2 mg/ml BSA, for 15-20 minutes at room temperature. The insoluble material was removed by centrifugation at 300 ***g*** for 10 minutes at 4°C. Lysates were pre-cleared by incubation with 7-10 µl of NRS and 100 µl of 50% Protein A-Sepharose CL4B (Pharmacia) for 1 hour at room temperature, under shaking con-

ditions. A second pre-clearing step was performed by incubating the lysates with 50 µl of 50% Protein A-Sepharose CL4B for 30-45 minutes at room temperature. After removal of the beads, supernatants were incubated with 7-10 µl of anti-F3 polyclonal antibody and 75 µl of 50% Protein A-Sepharose CL4B overnight at 4°C. Immunoprecipitates were washed once in lysis buffer, twice in lysis buffer without EDTA, and twice in kinase buffer (50 mM Hepes, pH 7.2, 100 mM NaCl, 5 mM $MnCl_2$, 0.1% NP-40, 100 µM sodium orthovanadate). Immunocomplexes were used for the kinase reaction, which was carried out using either a non-radioactive or a radioactive tyrosine kinase assay. For the non-radioactive kinase assay, an enzyme immunoassay kit (Boehringer Mannheim) was used according to the manufacturer's recommendations.

For the radioactive assay, immunocomplexes were incubated in the presence of kinase buffer supplemented with 5 µCi of [γ-^{32}P]ATP (Amersham), 1 µM ATP, 5 mM $MgCl_2$, for 5 minutes at 20°C, then 20 minutes at 37°C. The samples were washed and subjected to a 10% SDS-PAGE. Labeled proteins were visualized by autoradiography.

For the identification of the kinase from cerebellum extracts, samples from the first round of immunoprecipitation, with an anti-fyn polyclonal antibody (UBI, Lake placid NY, USA) or an anti-F3 polyclonal antibody, were incubated with 1% SDS for 5 minutes at 95°C. Supernatants were diluted 1:10 in lysis buffer and subjected to a second round of immunoprecipitation overnight with an anti-fyn or an anti-src antibody (Biomol, Plymouth Meeting, PA, USA). Phosphorylated proteins were detected as described above.

RESULTS

F3 is a neural-associated protein expressed at high level in post-natal cerebellum. In order to investigate the possibility of a kinase associated with F3 from neuronal sources, we used 5-day-old post-natal mouse cerebellum extracts. A kinase assay performed on immunoprecipitates from this tissue showed the presence of a phosphorylated protein with a molecular mass of 60 kDa that co-immunoprecipitated with F3 (Fig. 1A). This assay does not distinguish between phosphorylation events occurring on serine/threonine or tyrosine residues. Similar results were obtained using two different detergents (Brij 96 or NP-40) to solubilize the proteins. NP-40 is known to disrupt some protein-protein interactions, whereas Brij 96 is used as milder detergent. Results obtained with the two detergents suggest that strong interactions exist among the complexed proteins.

To establish the identity of the 60 kDa protein co-immunoprecipitated with F3, we re-precipitated phosphorylated proteins from the first immunoprecipitation, after their dissociation with 1% SDS, with either an anti-src or an anti-fyn antibody. Under these conditions the 60 kDa protein was immunoprecipitated only with the anti-fyn antibody (Fig. 1A). No protein was detected with the anti-*src* antibody. During the second round of immunoprecipitation some of the fyn protein was lost. A similar decrease in the amount of fyn from the first immunoprecipitated material was observed after a second round of immunoprecipitation with the same antibody (Fig. 1B). This result indicates that the fyn kinase is associated with F3 in cerebellum.

CHO-transfected cells were used in this study in order to investigate the signal(s) transduced by the GPI-anchored adhesion molecule F3, and the involvement of specific domains of the molecule. We first analyzed whether modulation of protein tyrosine phosphorylation was induced after F3-

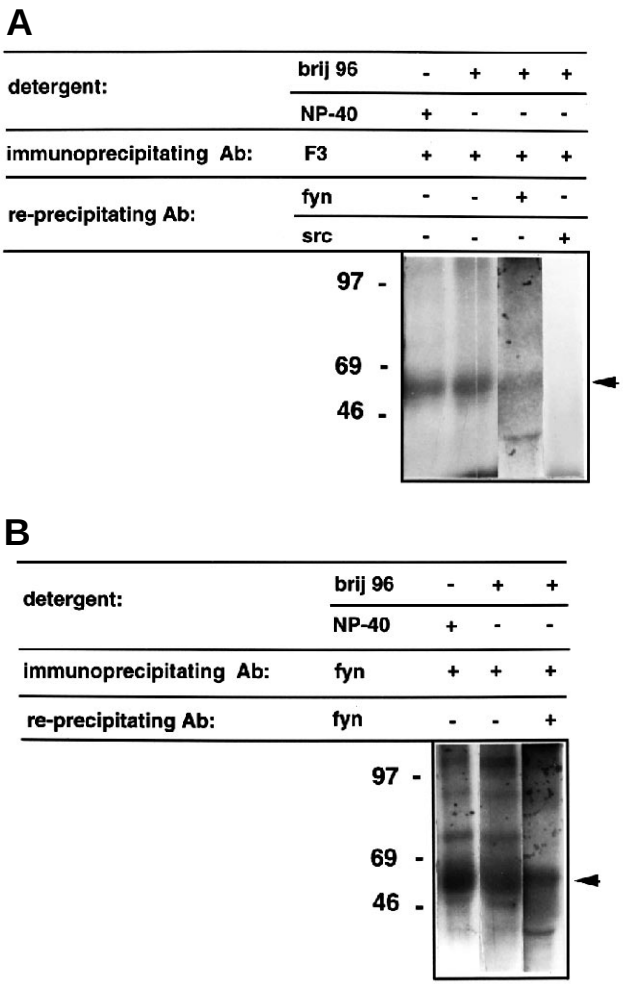

Fig. 1. Characterization of the kinase activity associated with F3 in mouse cerebellum tissue. NP-40 and Brij 96 extracts from mouse cerebellum tissue were immunoprecipitated with the anti-F3 antibody (A) or with an anti-fyn antibody (B). Immunoprecitated complexes were subjected to a radioactive in vitro kinase assay. (A-B) After the kinase assay, F3 and fyn immunoprecipitates from Brij 96 extracts were dissociated and subjected to a second round of immunoprecipitation with the indicated antibody. Labeled proteins were separated on a 10% SDS-PAGE gel. The gel was then dried and autoradiographed at −80°C for different times: 48 hours for the detection of co-immunoprecipitated kinase with F3; 72 hours for the re-immunoprecipitation with the fyn and src antibodies and 12 hours for the detection of fyn kinase. Arrows indicate the position of fyn kinase. Molecular mass is on the left in kDa in Figs 1, 2, 3, 5, 6, 7.

mediated cell aggregation. We used a short-term aggregation assay where untransfected CHO cells, bearing several CAMs, do not show significant aggregation, while F3-mediated aggregation of CHO cells transfected with a cDNA encoding the entire F3 (1A cells) is appreciable (Durbec et al., 1993). No modulation of protein tyrosine phosphorylation was observed during the aggregation of 1A cells when the assay was perfomed in the absence of an inhibitor of protein tyrosine phosphatases (PTPs) (Fig. 2). In contrast, in the presence of vanadate, tyrosine phosphorylation increased in a time-dependent manner on a few major proteins, with molecular masses ranging from about 50 to 100 kDa. To analyze whether

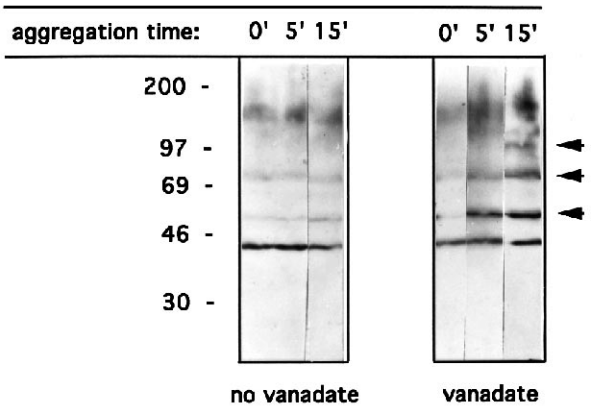

Fig. 2. Effect on tyrosine phoshorylation of F3-mediated aggregation. 1A cells were used in an aggregation assay for the indicated times in the absence or in the presence of 100 μM sodium orthovanadate. After F3-mediated aggregation NP-40 lysates were separated on a 10% SDS-PAGE gel and subjected to immunoblotting using an anti-phosphotyrosine antibody. Antibody binding was visualized by a chemiluminescence detection system. Arrows indicate the major substrates for tyrosine phosphorylation.

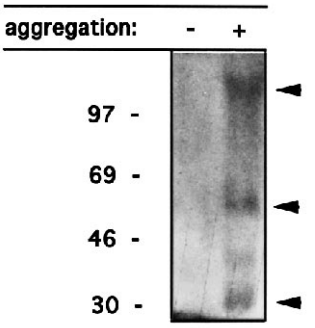

Fig. 3. Detection of phosphorylated proteins associated with F3 molecule after F3-mediated aggregation. ^{32}P-metabolically labeled 1A cells were either allowed to aggregate for 20 minutes (+) or used as single cells (–). NP-40 lysates were immunoprecipitated with an anti-F3 antibody. Labeled proteins were separated on a 10% SDS-PAGE gel. The gel was then dried and autoradiographed for 48 hours at –80°C. Arrows indicate the positions of the major phosphorylated proteins co-immunoprecipitated with F3.

the phosphorylated proteins induced after aggregation were associated with F3, we used immunoprecitates from single or aggregated ^{32}P-labeled 1A cells. F3, immunoprecipitated from ^{32}P-metabolically labeled 1A single cells, was not associated with phosphorylated proteins (Fig. 3). In contrast, after 20 minutes of aggregation three major phosphorylated proteins with molecular masses of about 35, 60 and 110 kDa, respectively, were co-immunoprecipitated with F3 (Fig. 3). Interestingly, in the presence of genistein, a PTK inhibitor, the degree of F3-mediated aggregation decreased in a dose- and time-dependent manner (Fig. 4). The same phenomenon was observed when the aggregation assay was performed in the presence of herbimycin A, another PTK inhibitor (result not shown). These results indicate that F3-mediated aggregation activates protein tyrosine kinase(s) (PTKs).

To investigate the role played by the different domains of F3 (i.e. Ig versus FNIII) in F3-signaling through PTK, we used

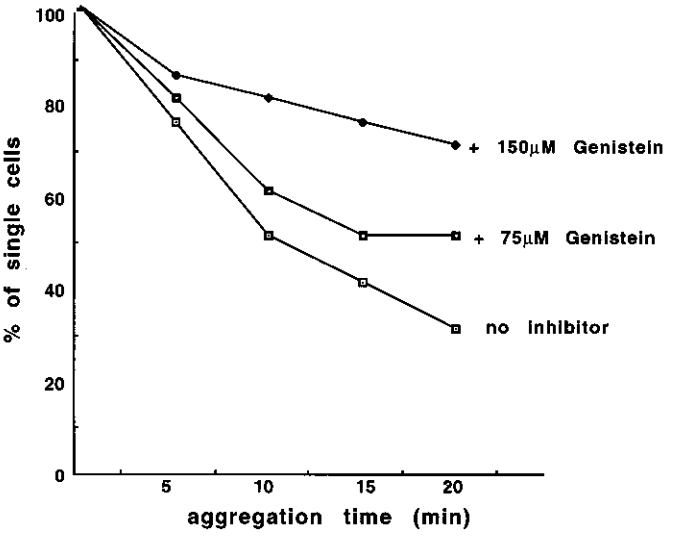

Fig. 4. Effect of genistein on short-term aggregation of 1A cells. Cells treated with different concentrations of genistein, as described in Materials and Methods, were used in an aggregation assay. At different time points, samples were withdrawn and single cells were counted in a hemocytometer. Results are expressed as the % of single cells at time 0. The mean of the number of single cells in at least 5 randomly chosen fields is shown.

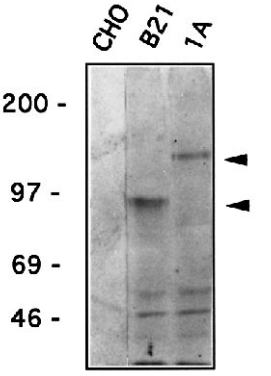

Fig. 5. Immunoprecipitation of F3 from F3-transfected cells and untransfected cells. NP40 lysates from ^{35}S-metabolically labeled CHO, B21, and 1A cells were immunoprecipitated with a polyclonal anti-F3 antibody. Immunoprecipitated proteins were separated on a 7.5% SDS-PAGE gel. The gel was then dried and autoradiographed for 24 hours at –80°C. Arrows indicate the position of the truncated form (92 kDa) and the entire F3 molecule (135 kDa) in B21 and 1A cells, respectively.

cells expressing a truncated form of F3 (B21 cells), where all the FNIII domains have been deleted. These domains are responsible for the adhesive function of F3 (Durbec et al., 1993), therefore modulation of tyrosine phosphorylation cannot be followed under an aggregation assay. Immunoprecipitation with the anti-F3 antibody from lysates of ^{35}S-labeled 1A and B21 cells showed that equivalent amounts of the two forms were immunoprecipitated (Fig. 5).

We compared the effect of antibody-mediated F3 cross-linking on the modulation of tyrosine phosphorylation in both 1A and B21 cells. F3 cross-linking on 1A cells, in the presence of vanadate, induced tyrosine phosphorylation of a protein with

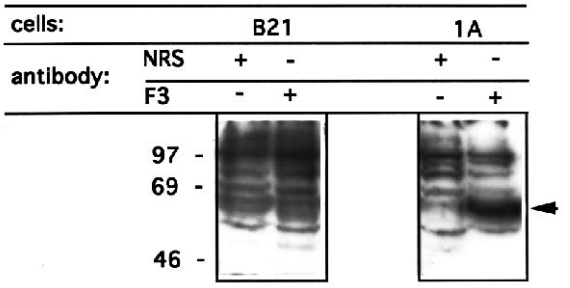

Fig. 6. Effect on tyrosine phoshorylation of antibody-mediated F3 cross-linking. 1A and B21 cells were used for antibody-mediated F3 cross-linking with either anti-F3 (F3) or normal rabbit serum (NRS). After F3 cross-linking, NP-40 lysates were treated as described in Fig. 1. The arrow indicates the major substrate for tyrosine phosphorylation.

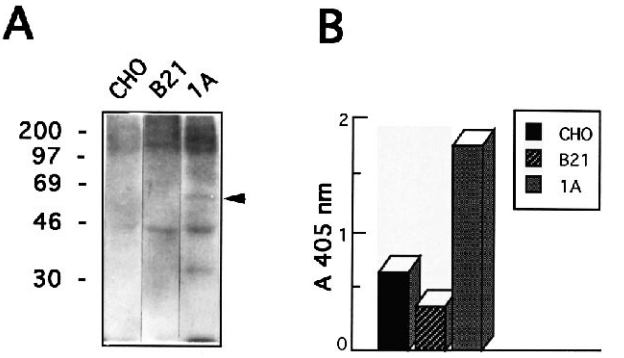

Fig. 7. Co-immunoprecipitation of protein kinase activity with F3. NP-40 lysates from CHO, 1A, and B21 cells were immunoprecipitated with an anti-F3 polyclonal antibody. (A) Immunoprecipitated complexes were subjected to a radioactive in vitro kinase assay. Labeled proteins were separated on a 9% SDS-PAGE gel. The gel was then dried and autoradiographed for 48 hours at –80°C. An arrow indicates the position of the major phosphorylated proteins co-immunoprecipitated with F3. (B) Immuno-complexes were used in a non-radioactive PTK assay as described in Materials and Methods. Results are expressed as the absorbance measured at 405 nm after 45 minutes of substrate development reaction.

an apparent molecular mass of 60 kDa (Fig. 6). In contrast, no modulation in protein tyrosine phosphorylation was observed when the cross-linking experiment was performed on B21 cells (Fig. 6). FACS analysis had already showed that 1A and B21 cells express at the cell surface the same level of the two F3 forms that are equally recognized by the anti-F3 antibody (Durbec et al., 1993). These results indicate that the differences observed in the modulation of phosphoryation were specifically related to the absence of the FNIII repeats.

We next investigated whether F3 is associated with a protein kinase in transfected CHO cells. For this purpose, an in vitro radioactive kinase assay was performed directly on proteins immunoprecipitated with the anti-F3 antibody from 1A and B21 cells, respectively. Phosphorylated proteins with molecular masses of about 60 and 35 kDa, respectively, were detected in immunoprecipitates from 1A cells (Fig. 7A). A protein with a 35 kDa molecular mass was also observed in the reimmunoprecipitation of fyn in the cerebellum, suggesting that this protein can represent a degradation product. In addition, using an in vitro immunoassay developed to detect receptor(s) and src-like PTKs, the kinase(s) associated with F3 was identified as a member of the PTK family (Fig. 7B). In contrast, no specific phosphorylated protein (Fig. 7A) or PTK activity (Fig. 7B) was co-immunoprecipitated with F3 from B21 cells, indicating that differences observed in the co-immunoprecipitation of the kinase(s) reflect the fact that the FNIII-like domains play an active role in the association of F3 with the PTK(s).

DISCUSSION

Many neuronal molecules from the Ig superfamily have been classified as adhesion molecules. Although the way they act in aggregation experiments may be rather passive they have an active role in signaling, in particular during cell migration and axonal guidance. In this study, we investigated whether the axon-associated GPI-anchored adhesion molecule F3, a protein of the Ig superfamily, transduces signals across the membrane and whether specific domains of the molecule were involved during this phenomenon. For this purpose, we took advantage of the availability of well characterized transfectants expressing at their surface similar amounts of either the whole F3 molecule or a truncated form with the FN type III domains deleted (Durbec et al., 1993).

Here we demonstrate that F3-mediated aggregation in CHO-transfected cells (1A cells) induced tyrosine phosphorylation of a limited number of cellular proteins, in the presence of vanadate (a PTP inhibitor). Moreover, some phosphorylated proteins associate with F3 during F3-mediated cell recognition. These results indicate that during F3-signaling activated PTK(s) generate phosphorylation of proteins that are either recruited and/or activated into a complex. The participation of signaling molecules (PTKs), during F3-mediated cell-cell recognition, was also demonstrated by the inhibitory effect of F3-mediated aggregation of two inhibitors of PTK(s), i.e. genistein (Fig. 4) and herbimycin A (not shown). This implies that at least one step, probably early in the process, involves the activation of PTK(s) whose role in F3-mediated adhesion to axon guidance remains to be determined.

Transfectants expressing the truncated form of F3 do not aggregate (Durbec et al., 1993). We therefore studied the role played by Ig domains versus FNIII using antibody-mediated clustering of F3 to mimic the events occurring during F3 activation. The mechanism by which antibodies act remains unclear, although several studies on GPI-anchored proteins from lymphocytes point out that modulation of phosphorylated proteins was revealed using this treatment (Hsi et al., 1989; Shenoy-Scaria et al., 1992; Stefanova et al., 1991). Antibody-mediated clustering of F3 in 1A cells resulted in the tyrosine phosphorylation of an undetermined protein, indicating that F3 transduced a signal across the membrane through the activation of a PTK. In contrast, no modulation of phosphorylation was observed with B21 cells, demonstrating that FN type III plays an important role in F3-signaling. This hypothesis is strongly supported by the results obtained after an in vitro kinase assay performed on immunocomplexes co-immunoprecipitated with F3 from both transfectants. An association between F3 and PTK(s) was detected only in anti-F3-immunoprecipitates from cells expressing the whole molecule, while anti-F3-immunoprecipitates from cells expressing the truncated form did not

demonstrate the associated kinase(s). Together the results obtained with cells expressing the entire F3 or the truncated F3 molecule show that the presence of fibronectin type III domains of the F3 molecule is necessary for the correct function and/or association with a PTK. It is likely that the absence of FNIII repeats modifies the tertiary structure of F3 and, as a consequence, the membrane organization, preventing the association or functional activity and impeding the transfer of signaling information. Alternatively, the transmission of signaling information could occur via the FNIII repeats from the Ig domains. Finally, either the FNIII repeats or the Ig domains may interact with an 'adaptor' responsible for the transmission of the signal. These first and last hypotheses would explain the absence of both signal transduction and association of kinase activity with the truncated form of F3.

The F3-associated kinase has been characterized as fyn, a src family kinase, using F3 immunoprecipitates form a neuronal source (cerebellum tissue) known to express endogenously high levels of F3. The exposure time for the autoradiograms that was needed to detect about half the amount of F3-associated fyn kinase versus the total fyn kinase in the cerebellum was four times longer, suggesting that only a small fraction of the total kinase is associated with F3. fyn kinase association with diverse receptors has been shown to regulate pathways of signal transduction in different model systems (Shenoy-Scaria et al., 1992; Thomas and Samelson, 1992), and fyn kinase has also been demonstrated to be involved in NCAM-mediated neurite outgrowth of cerebellar cells (Beggs et al., 1994). Although NCAM is a transmembrane protein it is possible that different CAMs use similar mechanisms to activate cellular responses. However, for GPI-anchored molecules devoid of intracellular domains, the mechanism by which the signals are transmitted across the membrane remains undetermined.

Results presented in this study strongly suggest that the GPI-anchored adhesion molecule F3 is able to transduce signals across the cell membrane through FNIII repeats. Various partners may be activated after cell-cell contact, including different receptors activating the kinase co-immunoprecipitated with F3, leading to the regulation of axon guidance. Further experiments need to be done to elucidate the role of fyn kinase in the elongation and/or retraction of F3-bearing neurons, and to identify the partner(s) linking the signaling molecule(s) and F3.

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