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The Eighth Fibronectin Type III Domain of Protein Tyrosine Phosphatase Receptor J Influences the Formation of Protein Complexes and Cell Localization

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Regulation of receptor-type phosphatases can involve the formation of higher-order structures, but the exact role played in this process by protein domains is not well understood. In this study we show the formation of different higher-order structures of the receptor-type phosphatase PTPRJ, detected in HEK293A cells transfected with different PTPRJ expression constructs. In the plasma membrane PTPRJ forms dimers detectable by treatment with the cross-linking reagent BS^3 (*bis*[sulfosuccinimidyl]suberate). However, other PTPRJ complexes, dependent on the formation of disulfide bonds, are detected by treatment with the oxidant agent H_2O_2 or by a mutation Asp872Cys, located in the eighth fibronectin type III domain of PTPRJ. A deletion in the eighth fibronectin domain of PTPRJ impairs its dimerization in the plasma membrane and increases the formation of PTPRJ complexes dependent on disulfide bonds that remain trapped in the cytoplasm. The deletion mutant maintains the catalytic activity but is unable to carry out inhibition of proliferation on HeLa cells, achieved by the wild type form, since it does not reach the plasma membrane. Therefore, the intact structure of the eighth fibronectin domain of PTPRJ is critical for its localization in plasma membrane and biological function.

Key words: cell localization, disulfide bonds, fibronectin domain, protein complexes, protein tyrosine phosphatase.

Abbreviations: BS^3, *bis*(sulfosuccinimidyl)suberate; ECL, enhanced chemiluminescence; HA, hemagglutinin; pNPP, para-nitrophenyl phosphate; PTP, protein tyrosine phosphatase.

INTRODUCTION

The protein tyrosine phosphatases (PTPs) are key enzymes in the control of protein tyrosine phosphorylation. The so-called classical PTPs can be divided in receptor-type and cytoplasmic phosphatases (*1*). Many of them have a critical role in the process of carcinogenesis negatively or positively regulating cell growth and adhesion (*2*).

Among the receptor-type PTPs, PTPRJ/DEP-1/CD148/PTPeta was characterized for its tumour suppressor activity in different cell lineages (*3–8*). Several PTPRJ substrates play a role in cell growth and transformation and their activation is down-regulated by the phosphatase activity of PTPRJ (*9–14*). In addition, *Ptprj* is the sole gene located in the mouse *Scc1* (susceptibility colorectal cancer 1) locus (*15*), and consistently with its possible role as cancer susceptibility gene, non-synonymous polymorphisms in the extracellular region of *Ptprj* were found. The human gene, too, harbours non-synonymous polymorphisms and, on the basis of genetic studies, *PTPRJ* was proposed as a susceptibility gene for thyroid and breast cancers (*16*, *17*). PTPRJ has an extracellular region composed of eight fibronectin type III domains and, in the eighth domain, a non-synonymous polymorphism Asp/Glu is located at position 872 of PTPRJ protein sequence.

Different mechanisms of regulation for receptor type PTPs have been so far described, such as dimerization, ligand binding and oxidation (*2*). Dimerization of receptor-type PTPs has been associated with downregulation of enzyme activity (*18–21*), but the relevance of different protein domains in this process is only partially understood. Recently, mutations in the extracellular regions of some receptor type PTPs have been found in colorectal tumours (*22*), introducing the idea of a possible role played by some extracellular PTP domains in the regulation of phosphatase activity. In addition, extracellular regions of RPTPα, CD45 and Sap-1 have been implicated in the control of dimerization (*21*, *23*, *24*).

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In this study, we investigate the ability of PTPRJ to form multimeric complexes, analysing their dependence on the eighth fibronectin type III structure and the possible implications for PTPRJ function.

MATERIALS AND METHODS

Expression Vectors—Each *PTPRJ* form was cloned in pCEFL vector inserting a hemagglutinin (HA) tag at the carboxy end. We generated the Asp872Cys, and Cys1239Ser (C/S) mutants by site-specific mutagenesis using PCR amplification with mutated oligonucleotides using pCEFL-PTPRJ-HA (PTPRJ Asp872) as template (provided by Dr Stanley Lipkowitz). Deletion mutants of PTPRJ were generated by PCR with oligonucleotides complementary to the ends of the deleted regions. In Del8.1, Del8.2 mutants we deleted the regions, located in the eighth fibronectin type III domain of PTPRJ, containing amino acids from 865 to 882 and from 865 to 904, respectively. The PTPRJ Asp form and the Del8.1, the Asp872Cys and the Cys1239Ser (C/S) mutants were subcloned in pBabePuro to be used for the colony assay. pET2-Met was provided by Dr Silvia Giordano.

Cells and Transfection—HEK293A and HeLa were cultured in DMEM and RPMI with 10% of fetal bovine serum, respectively. A particular HEK293A cell clone, purchased from Qbiogene, with high adhesive properties was used for all the experiments. Transfections were made in 60 mm dishes at 70–80% of confluence with 2.5 μg of plasmid DNA using the Fugene 6 (Roche), following the manufacturer's instructions. In co-transfection experiments the proportion of PTPRJ and c-Met expression vectors was 1:1.

Cross-Linking—HEK293A cells were washed twice with PBS and then incubated at 4°C for 1 h with a freshly prepared solution of 3 mg/ml BS^3 (*bis*[sulfosuccinimidyl]suberate) in PBS. After the incubation cells were washed once with PBS and the cross-linking reaction was quenched by incubating the cells for 20 min with a solution of Tris–HCl 50 mM in PBS.

Western Blot—Cells were harvested and lysed with a buffer containing 20 mM Tris–HCl pH 7.5, 150 mM NaCl, 1% Triton, 25 mM NaF, 1 mM Na_3VO_4 and a complete mixture of protease inhibitors (Roche). For the detection of oligomeric forms dependent on disulfide bonds, iodoacetamide at 20 mM of final concentration was added in the buffer. This alkylating agent avoids the non-specific formation of disulfide bonds during the protein extraction process. LDS loading buffer 4× (Invitrogen) was added to the protein samples and they were then heated at 70°C for 10 min. Electrophoresis of the proteins was carried out in a TAE 3–8% gradient gel (Invitrogen) for the resolution of high molecular weight complexes and non-reducing conditions were achieved avoiding the addition of reducing agent (Invitrogen) to the protein samples. SDS–PAGE at the appropriate acrylamide concentration was performed for the resolution of other protein bands. The proteins were blotted on PVDF membranes, subsequently incubated with the appropriate antibodies. Protein bands were detected using ECL (Amersham). Deglycosylation of proteins was performed by PNGase treatment following the manufacturer's instructions (New England Biolabs). Antibodies used were: rabbit polyclonal anti-HA (Upstate), mouse monoclonal anti-His6 HRP-conjugated (Qiagen), rabbit polyclonal anti c-Met (Santa Cruz), rabbit polyclonal anti phospho-Met Tyr 1234/1235 (Cell Signaling).

Immunofluorescence—Cells were grown on a cell culture chamber and transfected. Twenty-four hours after transfection cells were fixed with cold ethanol for 10 min and then permeabilized with 0.2% Triton-X100 in PBS. The slides containing the cells were incubated with an anti-HA antibody (Mouse monoclonal, Santa Cruz) in PBS containing 2% of BSA (bovine serum albumin) for 2 h, washed twice with PBS, incubated with a FITC conjugated secondary anti-mouse antibody (Molecular Probes) for 1 h and washed twice with PBS. The slides were then mounted with mowiol containing antifade reagent (Molecular Probes) and analysed by a confocal microscope (Leica).

Phosphatase Assay—The assay was performed as previously described (*6*). HEK293A transfected cells were lysed in a reaction buffer (25 mM HEPES pH 7.2, 150 mM NaCl, 2.5 mM EDTA) containing 1% NP40. Two hundred micrograms of total proteins in a final volume of 80 μl were pre-incubated at 37°C for 15 min with the serine-threonine phosphatase inhibitor NaF (25 mM). A solution containing the substrate pNPP was then added in each sample (250 μl of pNPP 1.5 mg/ml, dissolved in reaction buffer) and the reaction was then incubated for 25 min at 37°C. The reaction was stopped with 0.8 ml of 0.2 M NaOH. The assay was also performed for each sample adding vanadate as tyrosine phosphatase inhibitor, at final concentration of 1 mM. Optical density values were measured in a spectophotometer at 410 nm against a blank sample as reference.

Colony Assay—Subconfluent HeLa cells were transfected in 60 mm tissue culture dishes with the PTPRJ expression vectors. After 48 h of the transfection, cells were split in the proportion 1:3, then they were left to seed in 100 mm plates and after 24 h the selection with puromycin (3 μg/ml) was started. Cell colonies were stained with crystal violet and counted after 2 weeks of culture in the puromycin containing medium.

Statistical Analysis—Mean quantitative data were compared by two-tailed Student's *t*-test. $P < 0.05$ was considered statistically significant.

RESULTS

PTPRJ Is Able to Form Dimers on the Plasma Membrane and Other Higher-Order Complexes under Oxidant Conditions—To investigate whether PTPRJ is able to form higher-order complexes, we adopted two different strategies. Full-length PTPRJ constructs used in the experiments are shown in the Fig. 1A.

First, to detect PTPRJ multimeric forms localized in the plasma membrane, we used the cross-linking reagent BS^3, which is unable to cross the plasma membrane (Fig. 1B). In the extracts derived from the PTPRJ wild type (we considered wild type the most common Asp872 variant, named PTPRJ Asp) HEK293A transfected cells, treated with BS^3, it was detected a band higher than that corresponding to the monomeric PTPRJ band

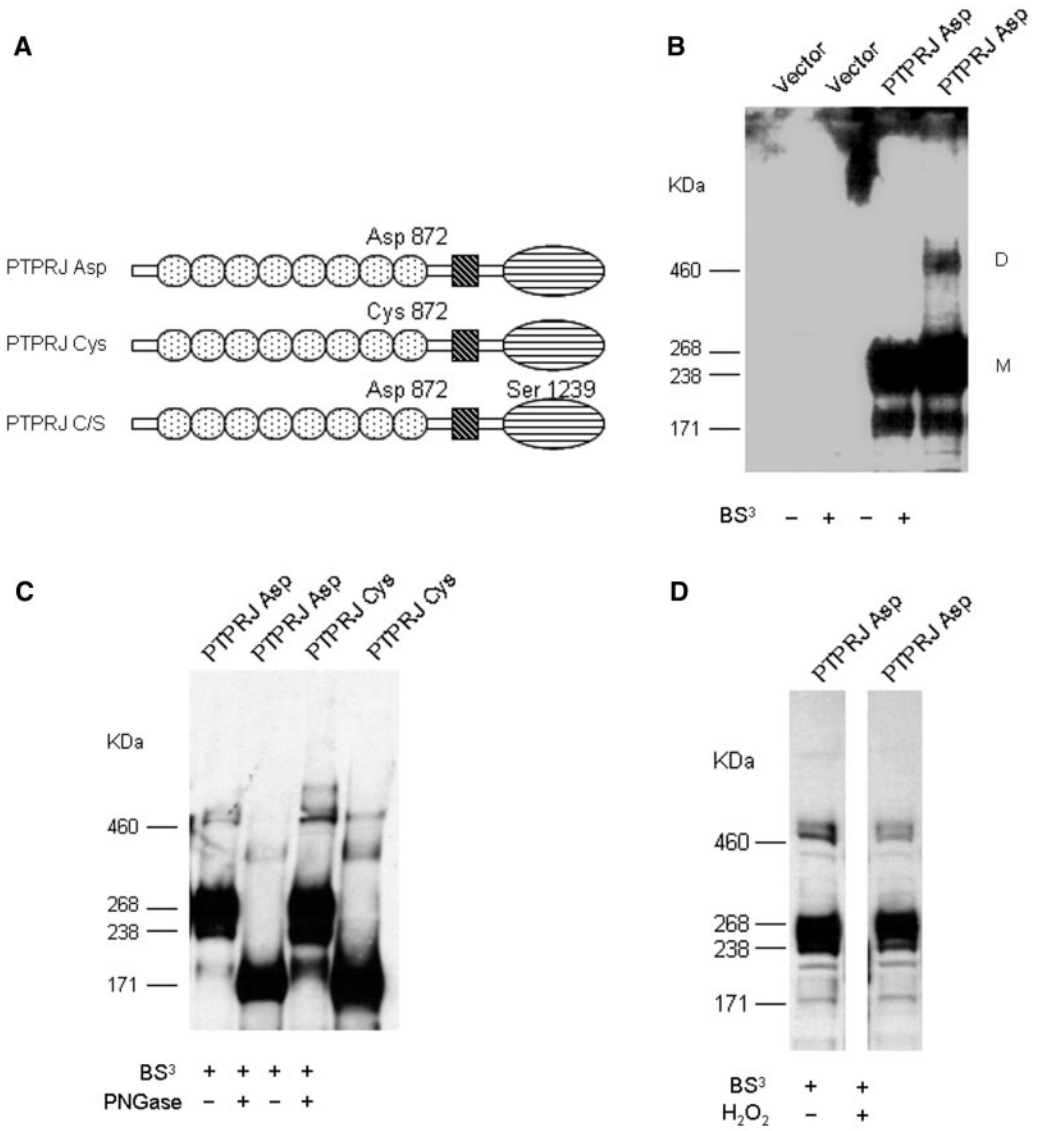

Fig. 1. **Formation of PTPRJ higher-order complexes in the plasma membrane.** (A) Schematic representation of human PTPRJ forms used. Fibronectin type III domains, transmembrane region and intracellular region are shown. All constructs were made with a HA tag to the carboxy end. We generated also the untagged version for all the constructs and a His6-tagged version for the wild type and the inactive Cys1239Ser (C/S) mutant of PTPRJ. (B) HEK293A cells were transfected with the vectors indicated and after 48 h were treated or not with the cross-linking reagent BS^3. Total proteins were extracted and 50 μg of them were subjected to immunoblotting analysis using an anti-HA antibody. D = dimer; M = monomer. (C) HEK293A cells were transfected with the vectors indicated and after 48 h were treated with the cross-linking reagent BS^3. Total proteins were extracted and deglycosylated or not by using PNGase enzyme and then subjected to immunoblotting analysis using an anti-HA antibody. (D) HEK293A transfected cells H_2O_2 treated for 10 min at 1 mM were cross-linked with BS^3. Total proteins were extracted and 50 μg of them were subjected to immunoblotting analysis using an anti-HA antibody.

and compatible with the molecular weight of a PTPRJ dimer.

To identify a possible mechanism of dimerization based on the formation of disulfide bonds, we generated a potential constitutive dimeric mutant of PTPRJ replacing the amino acidic residue in position 872, with a cysteine, a strategy already used for RPTPα (*19*). We chose this position that is located in the eighth fibronectin type III domain of PTPRJ and likely to be exposed to cell surface (*15*), because it harbours a polymorphism Asp/Glu that could be important for the susceptibility to thyroid tumours (*16*). This mutant is able to form dimers in the plasma membrane as well as the wild-type form of PTPRJ (Fig. 1C). In addition, with Asp872Cys mutant a band higher than that obtained with BS^3 treatment of PTPRJ wild-type form was detected. However, deglycosilation by treatment with PNGase of wild-type and Cys mutant complexes of PTPRJ shows that only the higher-order complexes detected either in the wild-type sample or in the mutant are compatible with the size of a PTPRJ dimer (Fig. 1C).

Since other receptor-type tyrosine phosphatases dimerize in an oxidant environment (*25*, *26*), we treated PTPRJ Asp-transfected cells with H_2O_2 and subsequently

we performed cross-linking by BS^3 to understand whether or not dimerization in the plasma membrane is influenced by oxidation. We did not detect any increase in the formation of dimers by H_2O_2 treatment (Fig. 1D) demonstrating that the amount of PTPRJ higher-order complexes in the plasma membrane is not affected by oxidation.

To investigate if PTPRJ is able to form complexes dependent on disulfide bonds but not necessarily localized in the plasma membrane, we transfected HEK293A cells with the empty vector, the PTPRJ wild type form treated or not with H_2O_2 and the Asp872Cys mutant. Total proteins were extracted in a lysis buffer containing iodoacetamide and the samples were then run on a polyacrylamide gel either in non-reducing or reducing conditions. As shown in Fig. 2A, in non-reducing conditions, it is possible to observe an upper band (of about 660 kDa), higher than the PTPRJ monomeric band (about 220 kDa), in the extracts derived from the cells transfected with the PTPRJ Asp872Cys mutant, but not in the extracts of the cells transfected with the PTPRJ wild-type form. Furthermore, the upper band is clearly detectable also in the extracts from the PTPRJ transfected cells treated with H_2O_2. The upper bands disappeared when the samples were run in reducing conditions demonstrating that disulfide bonds are necessary for the formation of PTPRJ higher-order complexes. However, the estimated molecular weight of the upper band is not compatible with the size of a PTPRJ homodimer, indicating that complexes dependent on disulfide bonds are different from those localized in the plasma membrane and detected with BS^3.

To demonstrate that the upper band observed in H_2O_2-treated cells is a complex that includes more than one PTPRJ molecule we co-transfected HA-tagged PTPRJ with His6-tagged PTPRJ and we immunoprecipitated protein extracts using an anti-HA antibody. Subsequently, we detected immunoprecipitates by western

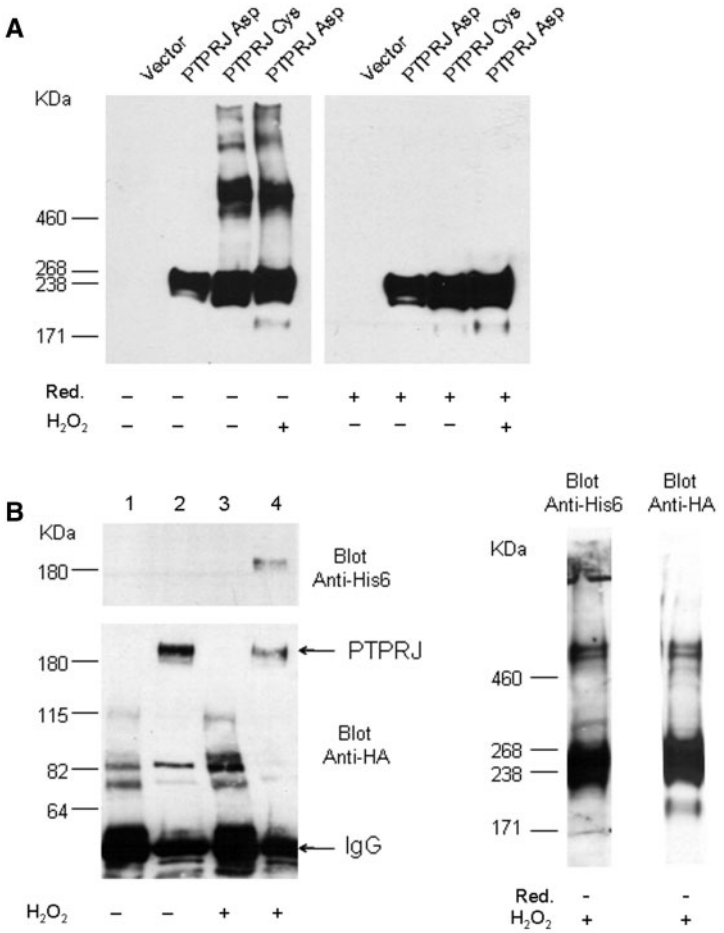

Fig. 2. **Formation of PTPRJ higher-order complexes dependent on disulfide bonds.** (A) HEK293A transfected cells were lysed in a buffer containing iodoacetamide. H_2O_2 treatment, where indicated, was done for 10 min at 1 mM. 50 μg of proteins were run in non-reducing and reducing conditions and then subjected to immunoblot analysis using an anti-HA antibody. (B) HEK293A cells were co-transfected with pCEFL-PTPRJ-HA and pCEFL-PTPRJ-His6. After 48 h cells were treated or not with 1 mM H_2O_2 for 10 min and then lysed. 500 μg of total proteins were immunoprecipitated with an anti-HA antibody or with a control IgG antibody. The immunoprecipitates were run in reducing conditions and subjected to immunoblot analysis with anti-His6 and anti-HA antibodies. 1 and 3 anti-IgG immunoprecipitates, 2 and 4 anti-HA immunoprecipitates. In the right panels, protein samples, derived from co-transfected cells treated with 1 mM H_2O_2 for 10 min, were run in non-reducing conditions and subjected to immunoblot analysis with anti-His6 and anti-HA antibodies, respectively.

blotting in reducing conditions using an anti-His6 antibody. A specific His6-tagged PTPRJ band was revealed only in the H_2O_2-treated cells but not using a mouse IgG as control for immunoprecipitation. Moreover, the protein sample extracted from the co-transfected cells was run in non-reducing conditions and showed the same pattern of reactivity either with anti-HA or with anti-His6 antibodies (Fig. 2B).

The results obtained by non-reducing conditions or by cross-linking with HA-tagged PTPRJ forms were reproduced also with the untagged PTPRJ forms by using a specific anti-PTPRJ antibody in the immunoblot analysis (Supplementary Fig. 1).

Deletion of the Eighth Fibronectin Domain of PTPRJ Allows Formation of High Molecular Weight Complexes and Impairs Its Correct Localization—To better understand the role of the eighth fibronectin type III domain in the formation of PTPRJ complexes, we generated two deletion mutants in this region: Del8.1 and Del8.2 (Fig. 3A). The deleted regions do not contain any cysteine or consensus sequences for glycosylation. We performed the BS^3 treatment on the HEK293 cells transfected with the Del8.1 and Del8.2 mutants (Fig. 3B). The presence of dimers was not detected in cells transfected with the deletion mutants. It should be noted that the shift detected in the bands of the two mutant proteins is not compatible with the deletion generated in the gene constructs. In fact, the deletion mutants migrate as the lower immature band of PTPRJ detected in our experiments as well as in other studies (*5*, *8*, *27*, *28*). Probably, these mutants do not get the same post-translation modification of the wild type protein.

To find out the localization of PTPRJ an immunofluorescence experiment was performed. PTPRJ clearly localizes in the plasma membrane while both deletion mutants show intracellular localization (Fig. 3C and data not shown). These findings demonstrate that dimerization of deletion mutants are not observed with BS^3 because they are not able to localize in the plasma membrane. However, the deletion mutants show a very efficient formation of PTPRJ complexes detected by gel electrophoresis in non-reducing conditions, even in absence of H_2O_2 stimulation (Fig. 3D). These results, taken together, demonstrate a direct correlation between PTPRJ intracellular localization and the formation of multimeric complexes dependent on disulfide bonds.

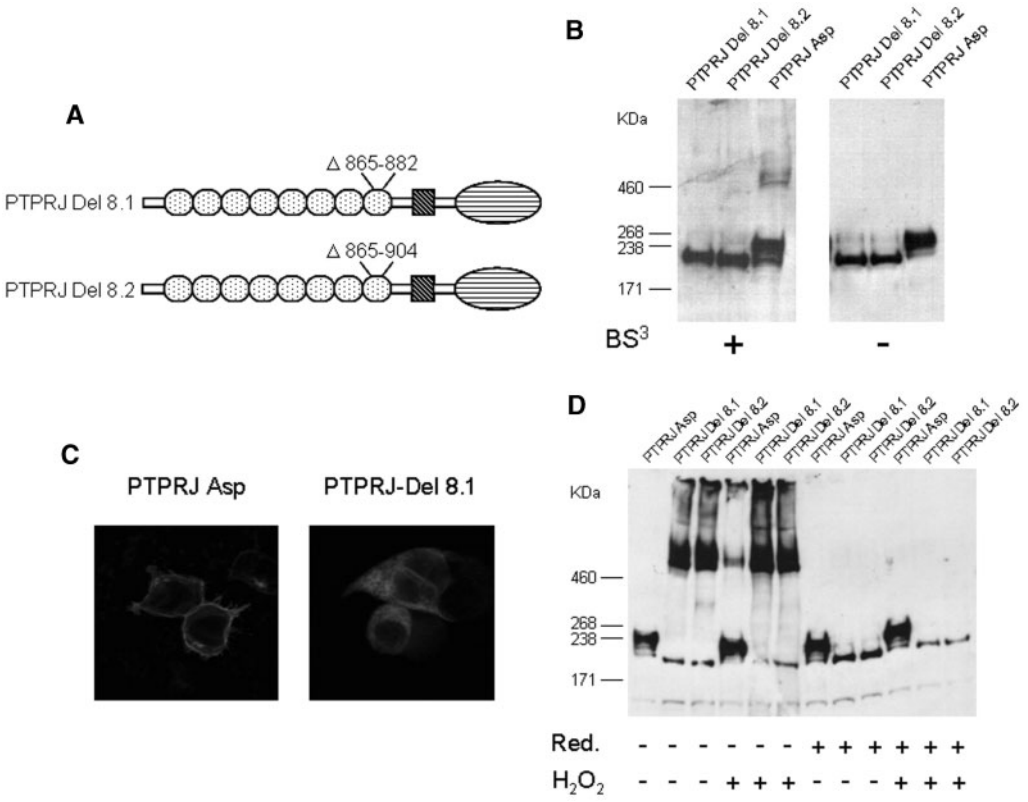

Fig. 3. **PTPRJ deletion mutants of the FNIII8 domain form higher-order complexes that do not localize in the plasma membrane.** (A) Schematic representation of PTPRJ deletion mutants used. Amino acids deleted were indicated. All constructs were made with a HA tag to the carboxy end. (B) HEK293A cells were transfected with the vectors indicated and after 48 h were treated or not with the cross-linking reagent BS^3. Total proteins were extracted and 50 μg of them were subjected to immunoblotting analysis using an anti-HA antibody. (C) Fixed and permeabilized HEK293A transfected cells were incubated with an anti-HA antibody. The primary antibodies were detected with a FITC-conjugated secondary antibody. The pictures were obtained by a confocal microscopy. (D) HEK293A transfected cells were lysed in a buffer containing iodoacetamide. H_2O_2 treatment, where indicated, was done for 10 min at 1 mM. 50 μg of proteins were run in non-reducing and reducing conditions and then subjected to immunoblot analysis using an anti-HA antibody.

A PTPRJ deletion mutant in the fourth fibronectin type III domain maintains the ability to reach the plasma membrane and it is not able to form with high efficiency higher-order complexes in non-reducing conditions (Fig. 4).

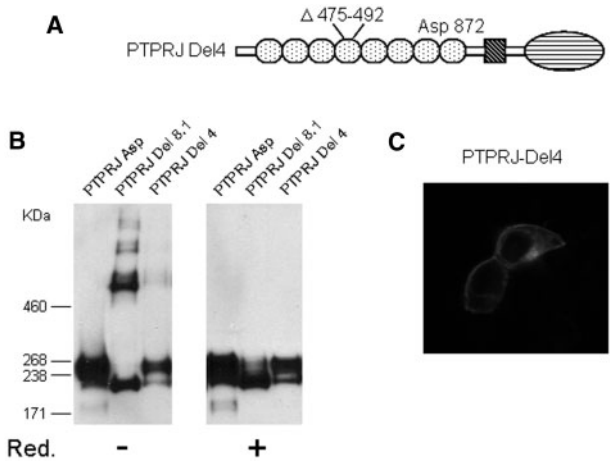

Fig. 4. **PTPRJ complexes and cell localization of the of the FNIII4 domain deletion mutant.** (A) Schematic representation of PTPRJ deletion mutant of the fourth fibronectin type III domain. Amino acids deleted were indicated. The construct was generated with a HA tag to the carboxy end. (B) HEK293A transfected cells were lysed in a buffer containing iodoacetamide. Fifty micrograms of proteins were run in non-reducing and reducing conditions and then subjected to immunoblot analysis using an anti-HA antibody. (C) Fixed and permeabilized HEK293A transfected cells were incubated with an anti-HA antibody. The primary antibodies were detected with a FITC-conjugated secondary antibody.

Plasma Membrane Localization Is Required for the PTPRJ Function—The next step of our work was the evaluation of the activity of different PTPRJ constructs. Therefore, we measured the phosphatase activity of the different PTPRJ forms by co-transfection in HEK293 cells with its substrate c-Met (*12*) and subsequent detection of the c-Met phosphorylation levels by phosphospecific antibodies (Fig. 5A). Two protein bands corresponding to c-Met are detectable: the upper band is the immature uncleaved protein and the lower band is the mature Met protein (*29*). For unknown reasons the amount of immature c-Met is higher when the protein is hyperphosphorylated. The phosphorylation levels of the mature transmembrane form of c-Met are significantly decreased in the cells transfected with the PTPRJ wild-type form and poorly reduced in the Del8.1 transfected cells. Interestingly, the cytoplasmic immature uncleaved form of c-Met was dephosphorylated efficiently either by PTPRJ or Del8.1 but not by the catalytically inactive mutant Cys1239Ser (C/S). This result suggests that PTPRJ deletion mutant does not dephosphorylate efficiently c-Met mature form because it becomes mislocalized. This hypothesis was confirmed evaluating the activity of the PTPRJ deletion mutant using an *in vitro* phosphatase assay. In fact, as shown in Fig. 5B, the Del8.1 mutant displays a significant catalytic activity.

Subsequently, we evaluated PTPRJ function at a cellular level, analysing the inhibitory activity of the different PTPRJ mutants in a colony formation assay in HeLa cells. We found a significant cell growth inhibitory effect of the PTPRJ wild type, but not of the catalytically inactive (Cys1239Ser) or the deleted (Del8.1) mutant (Fig. 6A). Since the intracellular PTPRJ Del8.1 mutant is not active in the cell colony inhibition, we deduced that

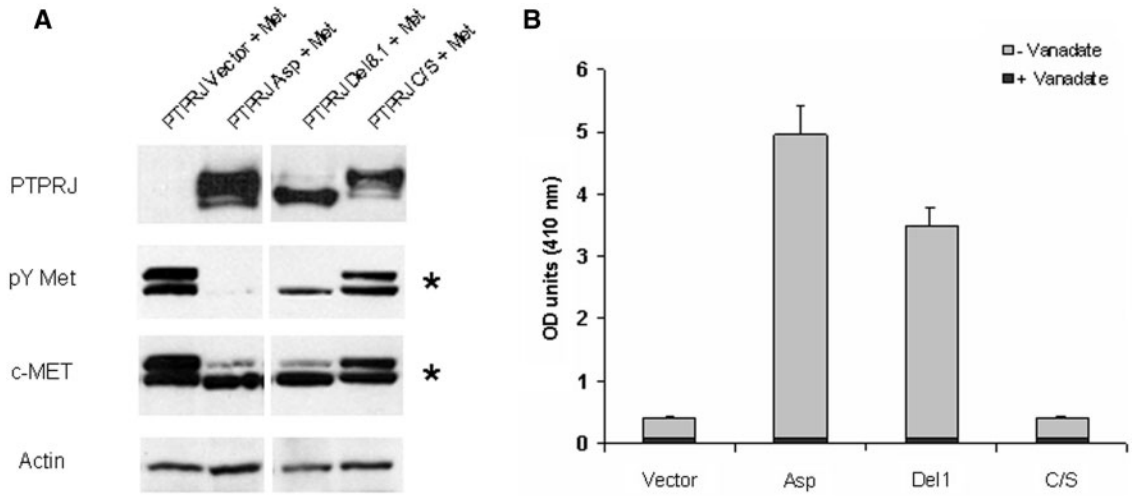

Fig. 5. **Phosphatase activity of different PTPRJ forms.** (A) HEK293A cells were co-transfected with pET2-Met plasmid and the PTPRJ vectors indicated. Cells were lysed after 48 h and 50 μg of total proteins were subjected to immunoblotting analysis by using specific anti-HA, anti-pY-Met (Tyr 1234/1235) and anti-cMet antibodies. The c-Met bands are indicated with a star, being the upper one the uncleaved immature form and the lower one the cleaved mature form. For unknown reasons the amount of immature c-Met is higher when the protein is hyperphosphorylated. (B) HEK293A transfected cells were lysed and 200 μg of total proteins were incubated in a buffer containing the serine-threonine phosphatase inhibitor NaF (25 mM) and the substrate pNPP. The assay was also performed for each sample adding 1 mM of vanadate, as tyrosine phosphatase inhibitor. OD values were measured in a spectophotometer and put in the graphic. Averages and standard deviations were calculated on the basis of two experiments performed in triplicate. PTPRJ Asp *vs*. Vec: $P < 0.0001$; Del1 *vs*. Vec: $P < 0.0001$; PTPRJ C/S *vs*. Vec: not significant (two-tailed Student's *t*-test).

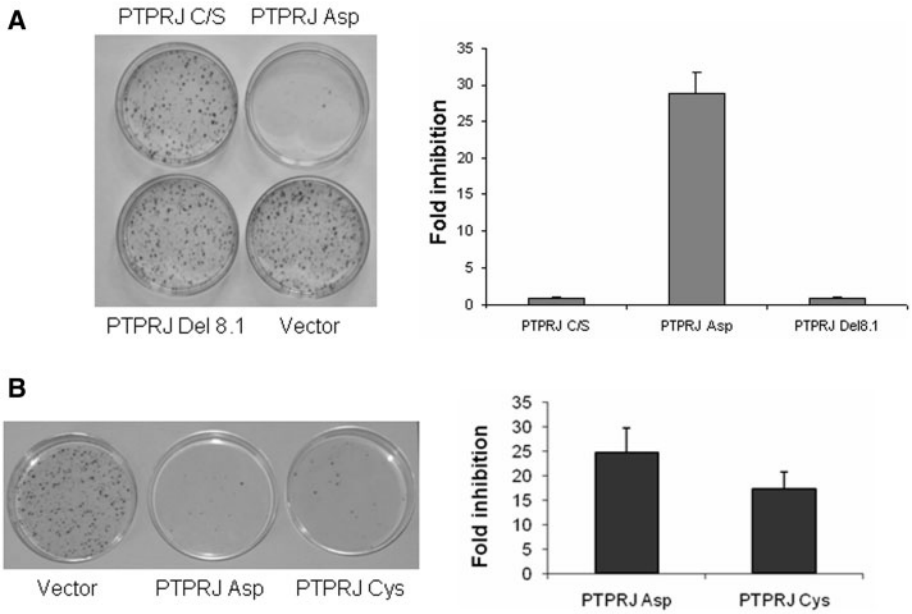

Fig. 6. **PTPRJ inhibitory activity on HeLa cell proliferation depends on its ability to get plasma membrane.** (A) HeLa cells were transfected with the vectors indicated. Cell colonies were obtained by puromycin selection and stained with crystal violet. Fold inhibition was calculated as the ratio of colonies obtained with the empty vector transfection to colonies counted in the samples indicated. Averages and standard deviations were calculated on the basis of two experiments performed in triplicate. PTPRJ Asp *vs*. Vec: $P < 0.00001$; Del1 *vs*. Vec: not significant; PTPRJ C/S *vs*. Vec: not significant (two-tailed Student's *t*-test). (B) The experiment was performed as described in 'A' for the expression vectors indicated. PTPRJ Asp *vs*. Vec: $P < 0.00001$; PTPRJ Cys *vs*. Vec: $P < 0.00001$; PTPRJ Asp *vs*. PTPRJ Cys: not significant.

the plasma membrane localization of PTPRJ is required to obtain cell proliferation inhibition in HeLa cells. Moreover, the mutant Asp872Cys, which is able to get the plasma membrane, possesses an almost intact inhibitory activity, detected by colony assay, on the proliferation of HeLa cells (Fig. 6B), confirming that the cell localization of PTPRJ is essential for its biological function.

DISCUSSION

In this article we showed that PTPRJ is able to form different oligomeric complexes and we studied their dependence on the structure of eighth fibronectin type III domain. Protein bands compatible with a PTPRJ homodimer were detectable by using BS^3, a crosslinking agent unable to cross plasma membrane. To look into the mechanism of molecular aggregation of PTPRJ, we also used a strategy, identical to that adopted by others for RPTPα (*19*), which consists in the mutation to cysteine of an extracellular amino acidic residue. This mutation generates dimers in the case of RPTPα by the formation of disulfide bonds. We chose to mutate the position 872, located in the eighth fibronectin type III domain of PTPRJ, which harbours a polymorphism Asp/Glu potentially associated with cancer susceptibility in thyroid tumours (*16*). This mutation generates high molecular complexes, detected in non-reducing conditions, similar to those obtained treating the cells with the oxidant agent H_2O_2. These complexes, as it occurs also for other receptor-type tyrosine phosphatases (*21*, *25*, *26*), depend on the presence of disulfide bonds but in contrast with that observed for the other phosphatases they have a molecular weight higher than a dimer, suggesting that disulfide bonds are not relevant in the formation of PTPRJ dimers located in the plasma membrane. The complexes dependent on disulfide bonds are formed by at least two molecules of PTPRJ (experiment shown in Fig. 2B) and have a molecular weight compatible with a PTPRJ trimer, however, we cannot reject the hypothesis of a complex formed by two molecules of PTPRJ plus other interacting proteins. Without H_2O_2 stimulation, we did not detect any disulfide bond-dependent complexes in PTPRJ wild-type transfected cells and H_2O_2 stimulation did not increase the amount of PTPRJ complexes detected by BS^3 cross-linking; these are further proofs demonstrating that PTPRJ complexes found in non-reducing conditions are different from those located in the plasma membrane and detected by BS^3 cross-linking. Remarkably, the mechanism of PTPRJ dimerization differs from that reported for Sap-1 by Walchli *et al.* (*21*), in which high molecular complexes were detected either in non-reducing conditions or with BS^3 treatment. Since we can exclude that dimers localized in the plasma membrane are dependent on disulfide bonds, our findings are in agreement with the hypotheses of a dimerization based on PTPRJ transmembrane interaction independent from disulfide bridges (*30*) or a dimer formation by catalytic domain interaction as demonstrated for rPTPeta, the PTPRJ rat homologous protein (*31*).

PTPRJ deletion mutants of the eighth fibronectin domain type III form large amounts of disulfide-dependent higher-order complexes that are retained in the cytoplasm. Conversely, a deletion in the fourth

fibronectin III domain does not increase significantly the formation of PTPRJ complexes dependent on disulfide bonds and does not affect PTPRJ localization.

We wondered what is the sense of PTPRJ complexes. Deletions in the eighth fibronectin III domain could generate a misfolded protein with the consequent formation of PTPRJ aggregates, a phenomenon already described for other proteins (*32*). The eighth fibronectin domain thus could play a key role in the folding control of PTPRJ.

Retention of PTPRJ complexes could occur in the endoplasmic reticulum by a cysteine-dependent mechanism (*33*, *34*). The same mechanism has been implicated in the intracellular trimer formation and subsequent degradation of a deletion mutant of Collagen X (*35*). Interestingly, in this case, the deletion hits a sequence very similar to that surrounding the position 872 considered in our PTPRJ Del8 mutants.

Since in oxidant conditions we detected PTPRJ complexes similar to those observed for PTPRJ mutants retained in the cytoplasm, it is possible to speculate that oxidative stress could lead to formation of PTPRJ aggregates. In this scenario the structure of the eighth fibronectin type III domain may be necessary to inhibit the formation of such complexes allowing the maturation and the correct positioning of PTPRJ in the plasma membrane.

Finally, we demonstrated that the activity of PTPRJ depends on its localization. In fact, the Del8.1 mutant possesses a catalytic activity but does not dephosphorylate efficiently the mature form of its substrate c-Met, and more importantly it does not inhibit the proliferation of HeLa cells. Conversely, PTPRJ wild type form and Asp872Cys mutant that localize in the plasma membrane are efficient in dephosphorylation of c-Met and HeLa cell proliferation inhibition.

SUPPLMENTARY DATA

Supplementary data are available at *JB* online.

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CONFLICT OF INTEREST

None declared.

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