Journal of Cell Science 113, 1951-1961 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
JCS0782

Talin controls the exit of the integrin $\alpha_5\beta_1$ from an early compartment of the secretory pathway

Véronique Martel, Lucile Vignoud, Sandra Dupé, Philippe Frachet, Marc R. Block* and Corinne Albigès-Rizo

Laboratoire d'Etude de la Différenciation et de l'Adhérence Cellulaires, UMR CNRS/UJF 5538, Institut Albert Bonniot, Faculté de médecine, Domaine de la Merci, 38706 La Tronche Cedex, France
*Author for correspondence (e-mail: marc-block@ujf-grenoble.fr)

Accepted 20 March; published on WWW 10 May 2000

SUMMARY

Talin is a major cytosolic protein that links the intracellular domains of β_1 and β_3 integrins to the cytoskeleton. It is required for focal adhesion assembly. However, its downregulation not only slows down cell spreading and organization of focal adhesions but also impairs the maturation of some β_1 integrins, including the fibronectin receptor $\alpha_5\beta_1$. To investigate this, we characterized the β_1 integrin synthesized in cells expressing talin anti-sense RNA (AT22 cells). We identified a large intracellular pool of β_1 integrins that is abnormally accumulated in an earlier compartment of the secretory pathway. In this report, we show that in talin-deficient AT22 cells, the aberrant glycosylation of integrin receptors is accompanied by a delay in the export of the integrin $\alpha_5\beta_1$. In normal cells, talin was found associated with β_1 integrins in an enriched membrane fraction containing Golgi and endoplasmic reticulum. Finally, microinjection of anti-talin antibodies resulted in accumulation of the integrins within the cells. These data strongly suggest that talin plays a specific role in the export of newly synthesized integrins. We propose that talin binding to the integrin may disclose a diphenylalanine export signal, which is present in the membrane-proximal GFFKR motif conserved in all integrin α chains.

Key words: Talin, Integrin, Protein transport, ER to Golgi intermediate compartment (ERGIC)

INTRODUCTION

Cell adhesion to extracellular matrix is primarily mediated by integrins, a family of heterodimeric, transmembrane receptors composed of non-covalently associated α and β subunits (Hynes, 1992). The integrin cytoplasmic domains link actin cytoskeleton via several actin-binding proteins, including α-actinin, filamin and talin (Horwitz et al., 1986; Otey et al., 1990; Pavalko and Laroche, 1993), within complex molecular structures named focal adhesions. Although little is known about talin function, its ability to interact with both structural and regulatory proteins such as vinculin (Burridge and Mangeat, 1984; Gilmore et al., 1993), FAK (Chen et al., 1995), actin (Hemmings et al., 1996; Muguruma et al., 1990) and with receptors such as integrins or layilin (Borowsky and Hynes, 1998), indicates that it is a major link between the plasma membrane and the cytoskeleton and a key element in the organization of focal adhesions.

Talin interacts with the cytoplasmic domain of the β_1 or β_3 subunits in vitro (Horwitz et al., 1986; Knezevic et al., 1996; Pfaff et al., 1998), and in vivo (Moulder et al., 1996). New findings have shown that the talin head domain is able to bind the membrane-proximal region of the integrin cytoplasmic tail (Patil et al., 1999; Calderwood et al., 1999). However, another study has suggested that both α and β subunits may participate in this interaction (Knezevic et al., 1996). The cytoplasmic domain of the β subunit contains the information necessary and sufficient to promote integrin clustering in focal adhesions (Dedhar and Hannigan, 1996; Hughes et al., 1996; LaFlamme et al., 1992). On the other hand, the α cytoplasmic domain controls ligand-dependent integrin recruitment at focal adhesions. For instance, deletion of most of the α_1 cytoplasmic domain leads to the localization of $\alpha_1\beta_1$ to focal adhesions even when the cells are spread on a substrate that is not recognized by the integrin (Briesewitz et al., 1993; Ylänne et al., 1993).

In a previous study, we showed that $\alpha_5\beta_1$ integrin underwent abnormal maturation (including a defect in glycosylation and some limited proteolysis of the N terminus of the β_1 chain) when talin was downregulated (Albigès-Rizo et al., 1995). This unexpected result pointed out that little is known about the maturation and sorting of the integrins along the exocytosis pathway. The association of the luminal domains of the precursors of β_1 with α_6 or α_5 integrin chains is mediated by a chaperone molecule named calnexin (Lenter and Vestweber, 1994; Hotchin et al., 1995). The cytoplasmic domains of α and β subunits are necessary for proper assembly and function (Briesewitz et al., 1995) and contribute to the overall stability of the $\alpha\beta$ complex. Indeed, a number of reports indicate that the conserved GFFKR motif, localized in the membrane-proximal region of all α cytoplasmic domains, is essential for conferring stability to the receptors.

With the aim of understanding the relationship between the loss of talin and the aberrant processing of integrins, we have characterized the localization and traffic of β_1 integrins synthesized in talin-deficient cells. Our results indicate that talin controls integrin exit from an early compartment of the secretory pathway.

MATERIALS AND METHODS

Antibodies

Human talin was purified from platelets as described by Turner and Burridge (1989), and polyclonal antiserum was raised in rabbits immunized against the purified protein (Vignoud et al., 1997). The anti-β_1 tail serum (anti cyto-β_1) was raised against a synthetic peptide corresponding to the cytoplasmic domain of the β_1 chain covalently coupled to Keyhole Limpet Hemocyanin.

Anti-talin monoclonal antibody (8d4) was purchased from Sigma (St Louis, MO, USA). Polyclonal calnexin antiserum, CTR433 and β-COP were generous gifts of Dr Wada (Tokyo, Japan), Dr Bornens (Paris, France) and Dr Paccaud (Geneva, Switzerland), respectively. Anti-KDEL, GM130, anti-AP1 and anti-p58K were purchased from Stressgen, Transduction Laboratories or Sigma. Anti-human β_1 subunit monoclonal antibodies, BV7, AIIB2 and 9EG7 were kindly supplied by Dr Dejana (Milano, Italy), Dr Damsky (San Francisco, USA) and Dr Vestweber (Muenster, Germany), respectively. TS2/16 and A1A5 were kindly given by Dr M. E. Hemler (Boston, MA, USA). Anti-ICAM-1 6E6 mAb and anti-MHC II were gifts from Dr Duperray (Grenoble, France) and Dr Jacquier-Sarlin (Grenoble, France), respectively. K20 was purchased from Immunotech (Marseille, France). P4C10 monoclonal antibody and anti-$\alpha_5\beta_1$ polyclonal antibody were from Gibco BRL (Gaithersburg, MD, USA). mAb13 was from Becton Dickinson. Alexa- or rhodamine-conjugated goat anti-mouse or anti-rabbit from Molecular Probes (Eugene, USA) were used as secondary antibodies in most experiments.

Cells

HeLa cells were grown in 100 mm plates to 100% confluence at 37°C in a 5% CO_2 atmosphere in α-MEM supplemented with 10% (v/v) heat inactivated fetal calf serum. The cells were harvested with phosphate-buffered saline (PBS) supplemented with 1 mM EDTA and 0.05% trypsin (w/v).

To perform cell adhesion, Petri dishes (100 mm) were coated for 2 hours with fibronectin (25 μg/ml) purified from bovine plasma according to the method of Engvall and Ruoslahti (1977). Dishes were washed three times with PBS to remove non-adsorbed fibronectin before the addition of the cells. 2 hours later, the plates were washed with PBS to remove non-adherent cells.

In order to induce the expression of talin antisense RNA, cells were treated with 10 μM cadmium for 1-2 hours.

FACS analysis

FACS analysis was performed as previously described (Albigès-Rizo et al., 1995) using a FACScan flow cytometer (Becton Dickinson) and the FACStar research software program (Lysis II). Background fluorescence was determined with a non-immune mouse IgG control. The data were displayed as cell number (ordinate) versus log fluorescence (abscissa).

Immunofluorescence microscopy

For immunofluorescence or confocal microscopy, paraformaldehyde-fixed cells were extracted with 0.2% Triton X-100 in PBS for 5 minutes, and non-specific protein binding sites were blocked by a 1 hour incubation with 10% goat serum in PBS at room temperature. Subsequent treatment of the cells was performed as described previously (Vignoud et al., 1997). Dilutions of the primary antibodies used were: anti-cyto β_1, 1:100; anti-ICAM1 6E6, 1:200 from a 20 μg/ml solution stock solution; anti-MHC, 1:10; anti-β_1 TS2/16, 1:100 from a 1 mg/ml purified stock solution; anti-β_1 9EG7, from cell culture supernatant; anti-58K, 1:100 from a commercial stock solution (Sigma); antibody CTR433, 1:20. The cells were mounted in mowiol solution and viewed using a confocal laser scanning microscope (Zeiss LSM 410) or a conventional epifluorescence microscope (Olympus Provis AX 70).

Integrin exocytosis assay

Parental HeLa cells or talin-deficient AT22 cells were grown in a Petri dish (100 mm diameter) to 50% confluence. They were harvested with PBS supplemented with 1 mM EDTA and 0.05% trypsin (w/v). They were pelleted by a centrifugation at 1500 rpm for 5 minutes, then washed once with PBS at 4°C, and finally resuspended in 5 ml of PBS at 4°C supplemented with 10 mM DTT, 1 mM EDTA and 1 mM EGTA. A sample was treated with Proteinase K (500 μg/ml) for 30 minutes on ice. The reaction was stopped with 2 mM PMSF and the incubation was followed on ice for 5 minutes. Then, the cells were washed in α-MEM and were either plated on dishes coated with fibronectin at 37°C, or further treated for immunofluorescence studies as described above with the 9EG7 anti-β_1 monoclonal antibody (in the presence of 1 mM Mn^{2+}) or with anti-ICAM-1 antibody after different incubation times at 37°C in α-MEM.

Subcellular fractionation, sucrose density gradients and solubilization

Subcellular fractionation was performed using a modification of a previously described method (Ray, 1970). Cells were broken in 10 mM Tris, pH 7.5, containing 69% sucrose (0.2 ml per 0.1 ml cell pellet) and protease inhibitors in a 2 ml Dounce homogenizer. A discontinuous density gradient was prepared with about 2 ml of the previous homogenate placed in the bottom of the tube followed by 2.9 ml of 45% sucrose, 3.6 ml of 41% sucrose and 3 ml of 37% sucrose buffered with 10 mM Tris, pH 7.5, respectively. The membrane fractions were floated up by centrifugation in a TST 41-14 rotor (Kontron) at 30000 rpm for 2 hours at 4°C with braking. After centrifugation, three bands were resolved from the top to the bottom; the first one was enriched in plasma membranes, the second was mainly microsomes and the heaviest band mitochondria. Fractions were removed from the top of the gradient, diluted with sucrose-free buffer and centrifuged at 80000 rpm for 15 minutes at 4°C. The final pellet was resuspended in the NP-40/glycerol buffer containing 20 mM Tris, pH 8, 137 mM NaCl, 1% NP-40, 10% glycerol, 1 mM Na_3VO_4, 1 mM PMSF, 10 μM pepstatin, 10 μg/ml leupeptin. Protein concentration was estimated by the micro BCA protein assay (Pierce).

Cell surface biotinylation, lysis, western blot and immunoprecipitation

Cell surface proteins were labeled with biotin using the Amersham kit and immunoprecipitated as described earlier (Albigès-Rizo et al., 1995). For western blotting experiments, proteins were detected with the appropriate monoclonal or polyclonal antibodies, followed by an incubation with an anti-mouse or anti-rabbit IgG antibody coupled to horse radish peroxidase (BioRad). Detection was achieved using a chemiluminescent probe (ECL, Amersham).

Microinjection

HeLa cells were plated on fibronectin-coated glass coverslips and were microinjected with a polyclonal anti-talin antibodies. Then the cells were returned to the incubator for 3 hours before paraformaldehyde fixation and 0.2% Triton X-100 permeabilization. The cells were stained with rhodamine-conjugated anti-mouse or anti-rabbit secondary antibodies to allow the identification of the microinjected cells. Localization of the β_1 integrin was performed with the monoclonal antibody TS2/16 followed by a detection with Alexa-conjugated anti-mouse secondary antibodies. All injections

were carried out with the aid of a micromanipulator 5171 connected to an Eppendorf microinjector unit (Transjector 5246). Microinjected cells were observed using a fluorescence microscope (Olympus Provis AX70) equipped with a Plan apo ×63 oil immersion NA 1.40 objective lens.

RESULTS

Characterization of the integrin $\alpha_5\beta_1$ in talin-deficient cells (AT22 cells)

HeLa cells were stably transfected with the talin antisense RNA under the transcriptional control of the inducible metallothionin promotor (Albigès-Rizo et al., 1995). A cell line, designated AT22, was generated and used for the present study. Induced AT22 cells acquired a round morphology and led to aberrant maturation of $\alpha_5\beta_1$ integrin. Until recently, the intracellular localization of $\alpha_5\beta_1$ integrins could not be determined by immunofluorescence because of the lack of antibodies able to recognize the $\alpha_5\beta_1$ integrin in AT22 cells. To characterize the integrin expressed in AT22 cells, we localized the epitopes present on the β_1 subunit using flow cytometry. As summarized in Table 1, all the anti-β_1 antibodies tested reacted with the β_1 chain in wild-type or mock-transfected HeLa cells, but only the monoclonal antibody 9EG7 and polyclonal antibodies recognizing either the $\alpha_5\beta_1$ complex or the cytoplasmic domain of the β_1 subunit were able to detect the integrin expressed in AT22 cells. These three antibodies were used in the following experiments.

The integrin $\alpha_5\beta_1$ is accumulated within AT22 cells

The availability of antibodies recognizing β_1 integrins expressed in AT22 cells allowed us to study the distribution of these integrins in talin-deficient cells. Cells were cultured for 20 minutes on fibronectin-coated coverslips, fixed, permeabilized, labeled with the anti-cyto-β_1 antibody and examined by confocal microscopy. This short time of spreading allowed us to obtain relatively round cells, which facilitated our estimation of the amount of receptors expressed at the cell surface as compared to a potential pool retained within the cells. Wild-type HeLa cells exhibited a localization of the β_1 integrin chain mainly at the plasma membrane (Fig. 1A), whereas with AT22 cells, the same immunofluorescence revealed a much lower surface expression together with a large intracellular pool of the integrin (Fig. 1D). Such a pattern was also observed in AT21 cells, another independent clone expressing talin anti-sense RNA (not shown). As already mentioned in our previous work (Albigès-Rizo et al., 1995), after immunoprecipitation of cell-surface-labeled proteins in AT22 cells, α_5 and β_1 subunits were characterized by a shift in their molecular mass due to an aberrant glycosylation and a limited proteolysis of the β_1 chain (Fig. 1G). Another integrin $\alpha_{IIb}\beta_3$ ectopically expressed in AT22 cells showed a similar aberrant maturation (unpublished). Conversely, the export of other receptors to the cell surface such as

Table 1. The reactivity of antibodies to human β_1 chain expressed in wild-type HeLa cells (WT) and talin antisense-expressed HeLa cells (AT22)

mAb	HeLa WT or Mock	HeLa AT22
mAb K20	+	–
mAb BV7	+	–
mAb TS2/16	+	–
mAb P4C10	+	–
mAb AIIB2	+	–
mAb 13	+	–
mAb 9EG7	+	+
polyAb cyto β1	+	+
polyAb α5β1	+	+

+ represents positive reactivity of the β_1 integrin to the antibodies.

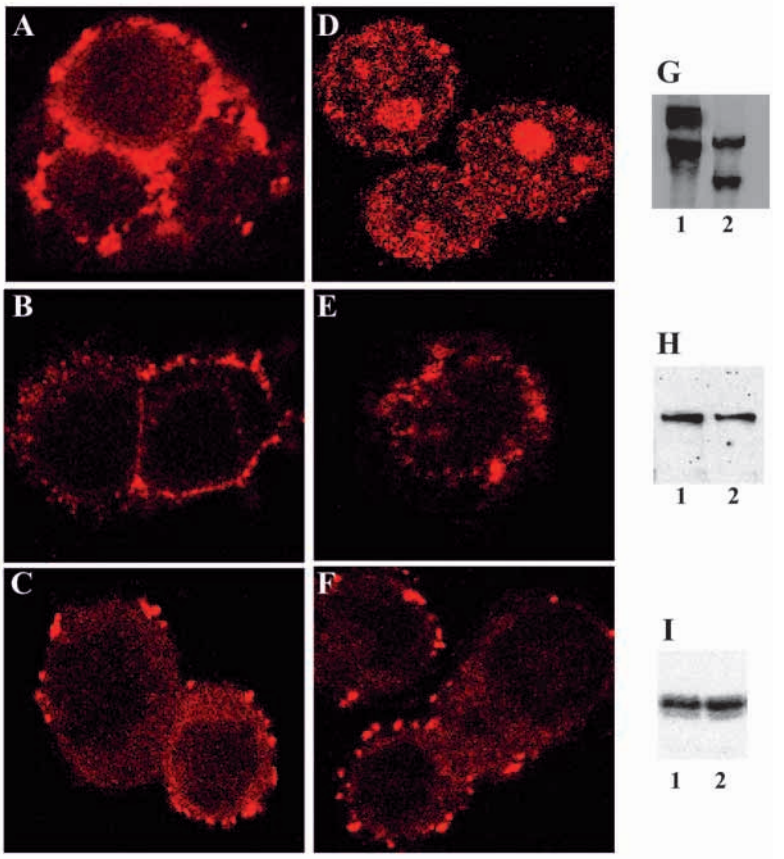


Fig. 1. Localization of the β1 integrin subunit in AT22 and HeLa cells. Mock HeLa cells (A-C) and AT22 cells (D-F) were allowed to attach to fibronectin-coated coverslips for 20 minutes at 37°C before paraformaldehyde fixation, permeabilization and staining with primary antibodies anti cyto-β_1 (A,D), or anti-ICAM-1 (B,E) or anti MHC-II (C,F). Optical sections of the stained cells were obtained with a Zeiss confocal laser scanning microscope. Note the large intracellular pool of β_1 integrins and the scarce signal at the cell periphery in AT22 cells (D) as compared to mock transfected HeLa cells (A). Other receptors such as ICAM-1 (B,E) or MHC II (C,F) are exported to the cell surface in identical ways in mock transfected cells (B,C) and in the AT22 cells (E,F). Immunoprecipitation of surface biotinylated receptors was performed to examine the potential shift in their molecular mass: $\alpha_5\beta_1$ integrin (G), ICAM-1 (H) and MHC II (I) in mock transfected HeLa cells (lane 1) and in talin antisense AT22 cells (lane 2). Note the significant shift in the pattern of $\alpha_5\beta_1$ integrin expressed in AT22 cells (G, lane 2).

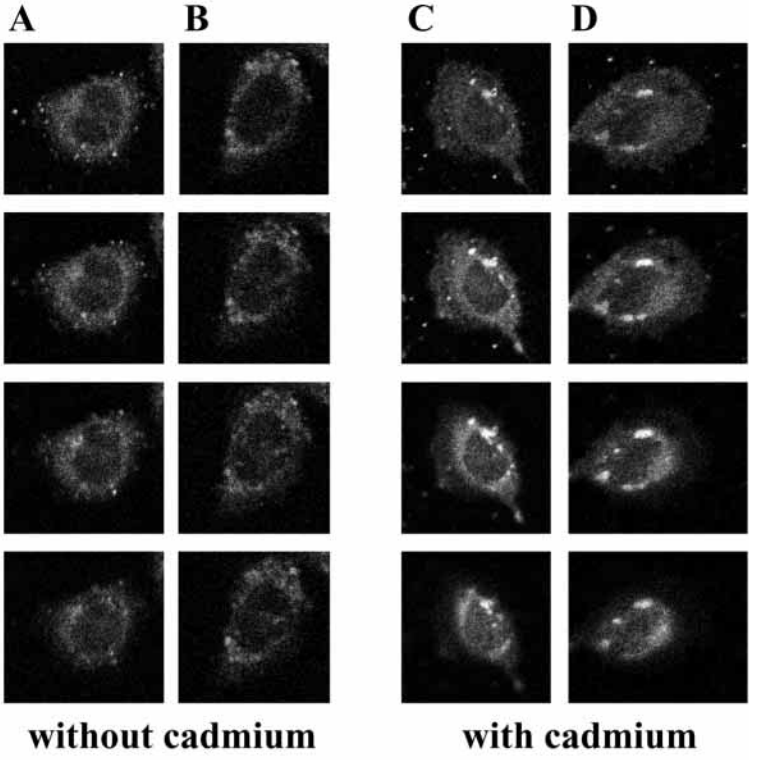
ICAM-1 (Fig. 1B,E) or MHC class II (Fig. 1C,F) was unchanged in AT22 cells as compared to the mock transfected cells. Furthermore, immunoprecipitations of cell-surface-labeled proteins with the appropriate antibodies revealed that the molecular masses of ICAM-1 (Fig. 1H) and MHC II (Fig. 1I) were unmodified in both cell types. These results suggested that the defects in transport and maturation observed in talin-deficient cells were specific to integrins.

A further decrease in talin expression can be obtained by the induction of talin antisense RNA with cadmium (Albigès-Rizo et al., 1995). In order to test the relationship between the decrease of talin expression and the intracellular β_1 accumulation in AT22 cells, induced cells were processed for immunofluorescence with antibodies against the β_1 cytoplasmic domain. When AT22 cells were induced with cadmium, we observed by confocal microscopy an increased accumulation of β_1 integrins to the perinuclear region (Fig. 2), compared to a weaker accumulation in AT22 cells without induction. This increased defect was correlated with the decrease in talin expression (Fig. 2E,F). This accumulation of β_1 integrins was never observed in mock transfected cells induced or not by cadmium (not shown).

Localization of $\alpha_5\beta_1$ intracellular pool in the ERGIC

To further characterize the intracellular compartment in which β_1 integrin was accumulated in talin-deficient cells, we visualized the submembranous structures of the endoplasmic reticulum (ER) and the Golgi apparatus. Protein transport from the rough ER to the Golgi apparatus involves complex membrane structures whose organization remains controversial. In mammalian cells, the Golgi complex has a ribbon-like structure composed of stacks of tightly opposed flattened cisternae with extensive vesicular and tubular profiles associated with the rims of the stacks. The stacks are functionally and morphologically polarized and contain pleiomorphic tubulovesicular structures on the incoming cis-face and the outgoing trans-face. The tubulovesicular structures near the cis-Golgi have been called the cis-Golgi network, ER to Golgi intermediate compartment (ERGIC) or vesicular tubular clusters (VTCs) (Hauri and Schweitzer, 1992; Farquhar and Palade, 1998). The exact identity and fate of these structures is still being debated, but it appears that ERGIC represents transport intermediates ferrying cargo from the ER to the Golgi (Presley et al., 1997; Scales et al., 1997), specialized for sorting and retrieval of resident ER proteins. The membranes of the ERGIC are enriched in the 58 kDa membrane protein ERGIC-53/p58 (Bannykh et al., 1998).

As shown in Fig. 2, immunofluorescence with anti-cyto β_1 antibody revealed perinuclear structures with a Golgi-like structures. Similar patterns were observed using another



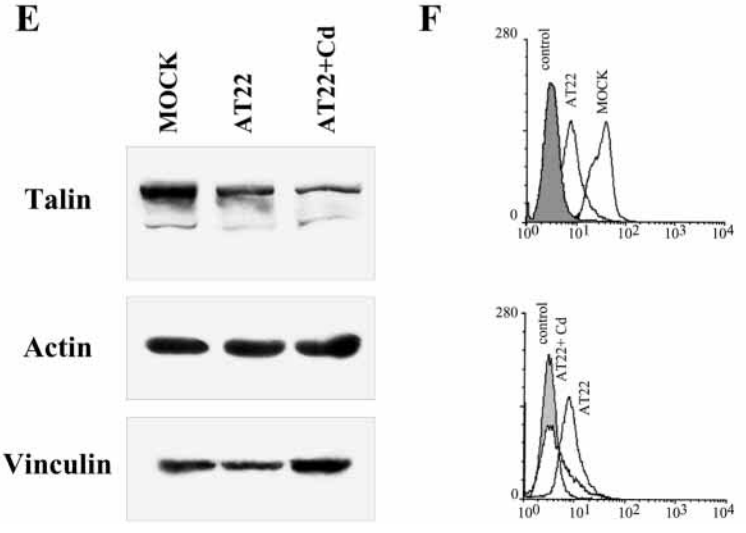


Fig. 2. Immunolocalization of the β1 integrin to the perinuclear area increased in presence of cadmium in AT22 cells. AT22 cells were treated with 10 μM of cadmium for 1-2 hours to induce the expression of talin anti-sense RNA, fixed, permeabilized and processed for immunofluorescence with antibody against the β1 cytoplasmic domain. Detection was done with 568 Alexa anti-rabbit secondary antibodies. Each column of images represents optical sections (0.5 μm) of the same cell or group of cells without induction (A,B) or with induction (C,D). Notice the increased accumulation of β1 integrin to the perinuclear region upon cadmium induction as compared to the weaker accumulation of β1 observed in cells without induction. All images were obtained with the same settings. Western blots with antibodies raised against talin (clone 8d4), actin C-terminal fragment and vinculin (clone V284) (E) and flow cytometry analysis of talin downregulation in AT22 cells (F) in the absence or presence of 10 μM cadmium, as compared with mock transfected cells.

Table 2. Distribution of the β1 subunit in talin-deficient cells on structure labeled with markers of the secretory pathway

Target	Compartment stained	Clone number	Colocalization with β1
KDEL	ER	10C3	+
58K	ERGIC	59k-9	+++
β COP	cis-Golgi	CM1A10	–
GM130	cis-Golgi	35	–
CTR 433	median-Golgi	-	–
AP-1	TGN	100/3	–

monoclonal antibody (9EG7) directed against the human β_1 chain (not shown). To determine whether the intracellular concentration of integrin in AT22 cells was associated with intracellular organelles involved in the secretory pathway, the cells were double-immunostained with the anti-cyto β_1 polyclonal antibody and monoclonal antibodies directed against ER (anti-KDEL), ERGIC (anti-58K), cis-Golgi (βCOP, GM130), median-Golgi (CTR433) (Jasmin et al., 1989) or trans-Golgi (AP1), and analyzed by confocal microscopy (Fig. 3). Confocal analysis of AT22 cells induced with cadmium and stained with anti-ERGIC 58K antibodies showed focal concentrations of fluorescence that were very similar in appearance and localization to those observed for intracellular β_1 staining (Fig. 3B). The pattern of β_1 integrin in cadmium-induced AT22 cells overlapped almost completely with the p58K and partially with the KDEL staining (Fig. 3A). To better investigate the distribution of β1 integrins in talin-deficient cells, we used other markers of the Golgi apparatus, but no colocalization of βCOP, GM130, CTR433 or AP1, with the β1-containing structures was observed (Table 2). These results clearly demonstrated that the intracellular pool of integrin in AT22 cells was essentially associated with the ERGIC marker corresponding to an early compartment of the secretory pathway.

Delay in the translocation of $\alpha_5\beta_1$ integrin to the plasma membrane

Since $\alpha_5\beta_1$ was accumulated within talin-deficient cells, one could suspect that the abnormal maturation of the integrin might result from a defect in the secretory pathway that would allow long-term exposure of the integrin precursors to the intracellular compartment environment. To test this hypothesis, direct measurements of the export of newly synthesized $\alpha_5\beta_1$ complexes to the plasma membrane were performed on parental HeLa and AT22 cells. Briefly, the cells were harvested and stripped with proteinase K at 4°C for 30 minutes as described under Materials and Methods. After inactivation of the protease by PMSF and one wash, the cells were placed at 37°C and the appearance of newly β_1 integrins at the cell surface was monitored either by following cell adhesion on fibronectin or by immunofluorescence microscopy on nonpermeabilized cells after staining with the 9EG7 monoclonal antibody in the presence of 1 mM Mn^{2+}, an integrin activating ion that fully disclosed the 9EG7 epitope (Lenter et al., 1993). Although HeLa cells used to spread rapidly on fibronectin, Fig. 4A shows that cell spreading of WT HeLa cells on fibronectin was fully abolished by protease treatment. After a lag period of 1 hour, the cells slowly recovered and reached full spreading after a 7 hour incubation at 37°C. This recovery was due to the export of newly synthesized externalized receptors, since it could be completely abolished by cycloheximide. Externalized β_1 integrins at the cell surface were followed by immunofluorescence using the anti-β_1 9EG7 antibody on both parental HeLa and AT22 cells in presence of Mn^{2+} (Fig. 4B). While almost no staining remained after Proteinase K treatment in both cell lines (emphasising the efficiency of cell stripping), the fluorescence level in HeLa cells was almost fully recovered after 5 hours at 37°C. This result was fairly consistent with those of the spreading assay experiments on fibronectin. Conversely, in AT22 cells, 9EG7 staining recovery was strongly delayed since only 40% of the control levels were obtained after the same incubation period (Fig. 4B,C).

The delay in the export to the cell surface was restricted to integrins since there was no difference in ICAM1 export expressed in talin anti-sense cells as compared to the HeLa cells (Fig. 5). These experiments clearly demonstrated that the exocytosis of β_1 integrins was altered in talin-deficient cells.

Talin interacts with $\alpha_5\beta_1$ integrin in microsomal preparations

The accumulation of integrins in the early secretory pathway when talin levels were reduced suggested that an early interaction between both proteins may occur. This putative early interaction was substantiated by the experiment shown in Fig. 6. Microsomal membranes from HeLa cells were solubilized in NP-40/glycerol buffer, as described under Materials and Methods, and immunoprecipitation was achieved with an anti-$\alpha_5\beta_1$ complex polyclonal antibody. The immune complexes were resolved by SDS-PAGE, transferred onto a nitrocellulose membrane, and immunoblotted with the anti-talin monoclonal antibody. Talin was specifically detected in the anti $\alpha_5\beta_1$ integrin immunoprecipitate (Fig. 6A, lane 2) but not in complexes precipitated with non-immune polyclonal antiserum, demonstrating the specific association between talin and the $\alpha_5\beta_1$ integrin (Fig. 6A, lane 4). The anti-talin monoclonal antibody also reacted with a lower band, which probably represented some proteolytic degradation of the protein (Bolton et al., 1997). Since there have been reports that integrin occupancy by extracellular matrix components such as fibronectin allows the recruitment of talin (Miyamoto et al., 1995; Vignoud et al., 1997), we tried to determine whether the interaction of talin with $\alpha_5\beta_1$ could be detected on whole cell lysate made from HeLa cells spread for 60 minutes on plastic or fibronectin-coated dishes. After lysis in NP40/glycerol buffer and immunoprecipitation with the anti-$\alpha_5\beta_1$, the presence of talin was analyzed by western blotting as described above. Under these experimental conditions, we were unable to detect talin (not shown). Indeed, the strong association of talin with $\alpha_5\beta_1$ was specific for the microsomal integrin.

Previous immunoprecipitation experiments of calnexin followed by immunoblotting with anti-β_1 antibodies revealed the association of calnexin with the immature high mannose precursor of integrin $\alpha_6\beta_1$ (Lenter and Vestweber, 1994) and $\alpha_5\beta_1$ (Hotchin et al., 1995). Therefore, we considered the possibility that talin might bind to the calnexin-integrin complex within the endoplasmic reticulum. However, in calnexin immune complexes performed with the microsomal fraction, the integrin precursor (Fig. 6B, lane 4) but not talin (Fig. 6B, lane 3) was detected. Thus, it appeared that the

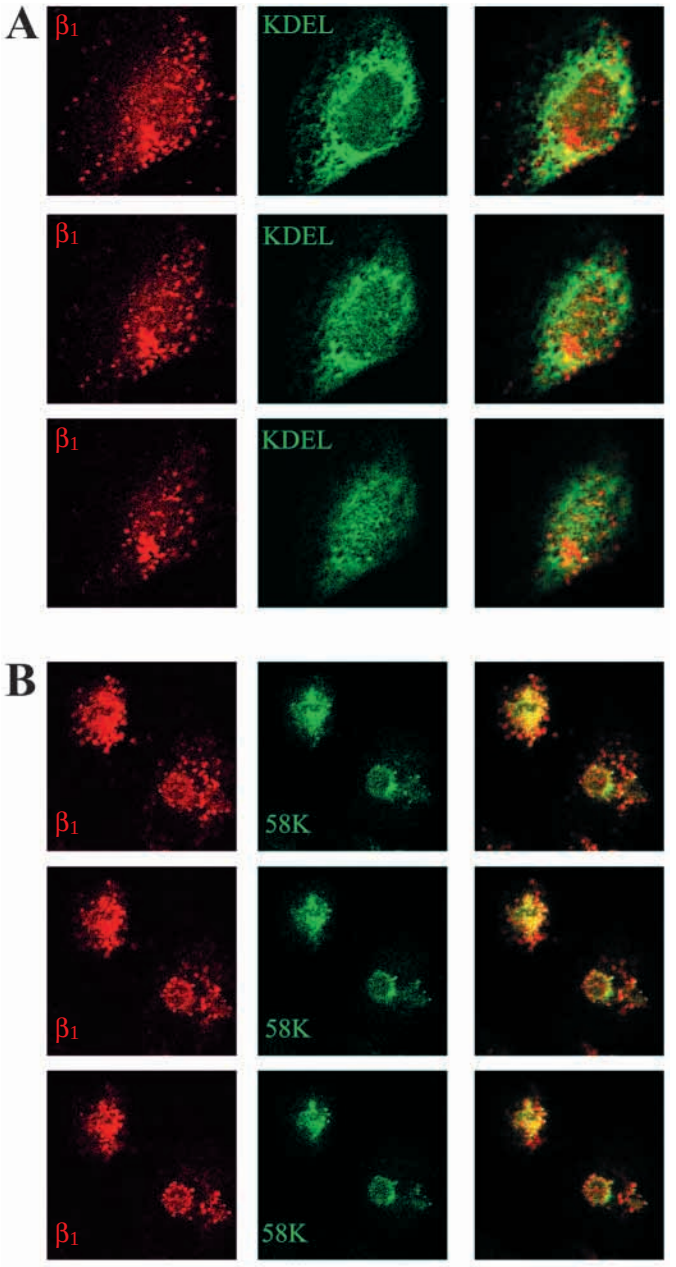


Fig. 3. The intracellular pool of β_1 subunits in AT22 cells colocalizes with the earlier secretory pathway. The subcellular localization of β_1 subunit was compared with that of KDEL and p58K in AT22 cells induced with 10 μm cadmium. β1 cytoplasmic polyclonal antibody was used to label human β1 integrin and detection was done with Alexa 568 goat anti-rabbit IgG secondary antibodies. All other marker proteins were counterstained using 488 Alexa-conjugated anti-mouse IgG secondary antibody. Confocal micrographs of immunolabeled proteins are shown with the respective colors in the corners of the images. Composite images were generated by superimposition of the green and the red signals with areas of overlap appearing yellow. Each panel of images in (A) and (B) represents different optical sections (0.5 μm) of the same cell or group of cells. Notice the relative weak colocalization of β_1 integrin with the ER marker (KDEL) in (A). β_1 integrin colocalizes much better with ERGIC p58 (58K), a resident protein of the intermediate compartment (B).

formation of a ternary complex between calnexin, integrin and talin was unlikely. Finally, the monoclonal antibody A1A5, known to recognize the mature and trimmed forms, but not the high mannose precursor of the human β_1 subunit (Hemler et al., 1983; Heino et al., 1989), was found to immunoprecipitate talin/integrin complexes (not shown). All together, these results suggested that talin was not associated with the high mannose precursor of the β_1 chain but with the trimmed β_1 form.

Microinjection of antibodies directed against talin mimics the blockade of $\alpha_5\beta_1$ transport observed in AT22 cells

Microinjection of anti-talin antibodies was studied previously in terms of cell spreading and focal adhesion disruption. It was found that the effect depended on how recently the cells had been plated (Nuckolls et al., 1992): cells that had newly developed focal adhesions on fibronectin were induced to round up due to the disassembly of adhesive structures. However, if fibroblasts were allowed to spread completely prior to microinjection, cell spreading was unaffected. Keeping in mind that talin was involved in integrin transport, it became interesting to check out whether microinjection of anti-talin antibodies had any effect on integrin trafficking. We performed the microinjection of the anti-talin polyclonal antibody under conditions where HeLa cells were fully spread on fibronectin. The cells were fixed and permeabilized 3 hours later. The microinjected cells were discriminated by their staining with a rhodamine-conjugated anti-rabbit secondary antibody, whereas distribution of the β_1 chain was observed with the monoclonal antibody TS2/16 followed by Alexa-conjugated anti-mouse secondary antibody. We confirmed that the microinjection did not induce a disassembly of focal adhesions. We first observed that 3 hours after injection, anti-talin antibodies were predominantly localized around the nucleus, suggesting a perinuclear localization of talin (Fig. 7A). This pattern was correlated with a large accumulation of β_1 integrin in a perinuclear area (Fig. 7B), whereas microinjection of a non-immune serum (Fig. 7C) had no effect on integrin retention within the cells (Fig. 7D). These results provided evidence that anti-talin antibodies could hamper integrin transport in a way that was similar to that observed in talin-deficient cells.

DISCUSSION

In this study, we have shown that the decrease in talin availability, either by downregulation with anti-sense RNA expression or by blocking antibodies, induces intracellular accumulation of β_1 integrins. According to β1 colocalization with specific markers, this blockade takes place at the exit of an early compartment of the secretory pathway. This result is confirmed by the observation in wild-type cells that talin strongly and specifically interacts with β_1 integrins located in the microsomal fraction. Our data raised two questions. (1) How does talin interact with components of the secretory pathway? (2) Why and how talin can affect export of newly synthesized $\alpha_5\beta_1$ integrin?

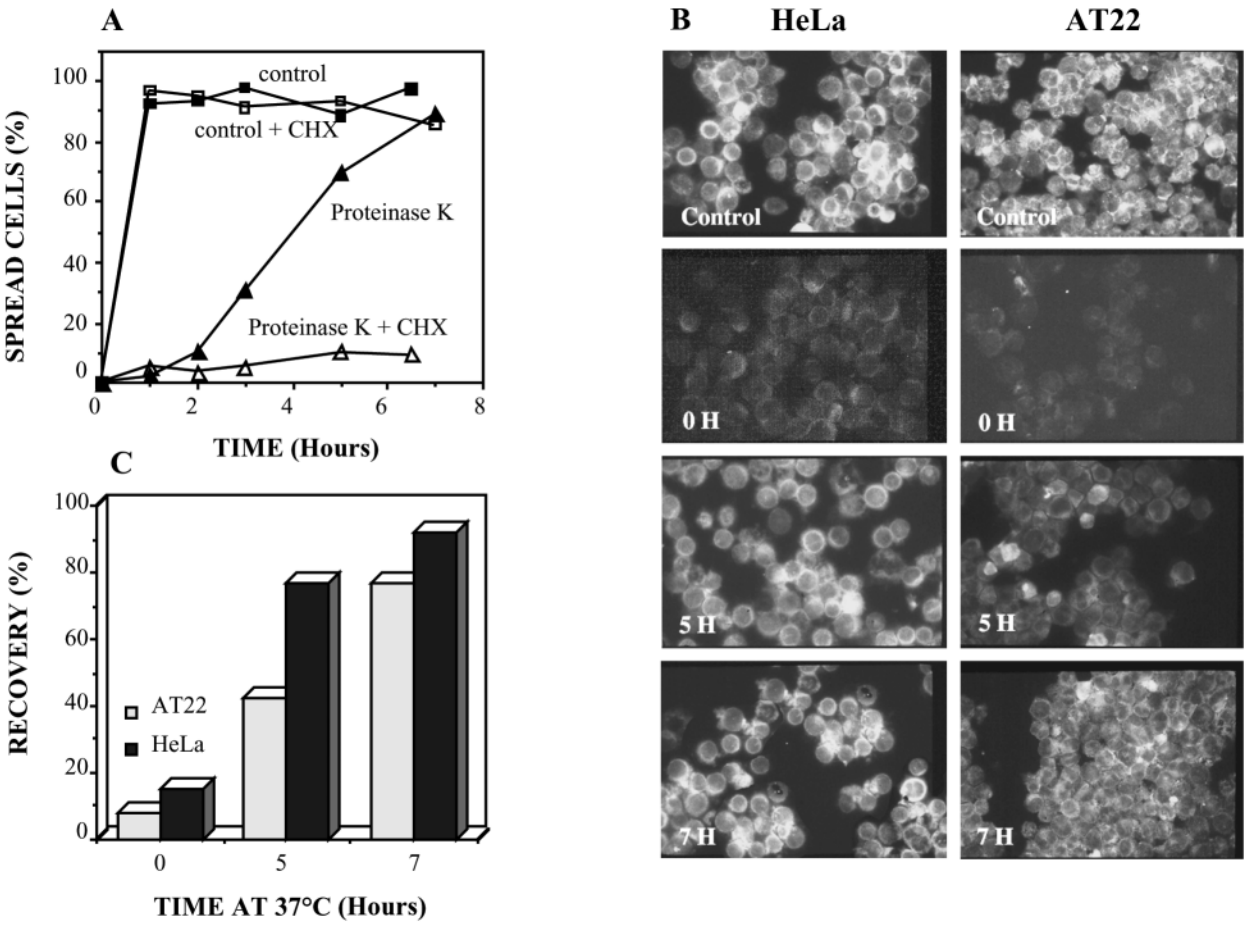


Fig. 4. Exocytosis of $\alpha_5\beta_1$ in HeLa and AT22 cells. (A) The kinetics of exocytosis of the $\alpha_5\beta_1$ fibronectin receptors in HeLa cells were monitored by the recovery of cell spreading on fibronectin-coated plastic (5 μg/ml, overnight) after plasma membrane stripping of the cells with Proteinase K. Membrane stripping was performed as described under Materials and Methods. The percentage of spread cells was determined visually under a microscope from a sample of 200 cells after different incubation times at 37°C. When indicated (+CHX), the cells were pretreated with cycloheximide (100 μg/ml) for 15 minutes at room temperature, and the cycloheximide concentration was maintained throughout the experiment. (B) Exocytosis of $\alpha_5\beta_1$ integrins is delayed in AT22 cells as compared to parental HeLa cells. Control or stripped cells were incubated at 37°C in suspension in α-MEM medium for various indicated times. The cells were then cytospinned and fixed with paraformaldehyde without permeabilization. Immunofluorescence labeling of cell surface integrins was achieved with the anti-β_1 9EG7 monoclonal antibody, followed by rhodamine-conjugated secondary antibody. (C) Quantification of surface expression of β_1 integrins after membrane stripping. The recovery was estimated according to the following formula: Surface fluorescence at time t – non-specific surface fluorescence / Surface fluorescence of control – non-specific fluorescence.

Targeting of talin and other cytoskeletal-associated proteins to the structures related to the secretory pathway

Our work indicates that talin can localize at the cell membrane and at the level of structures associated to the secretory pathway. This dual localization at the plasma membrane and around the nucleus seems to be shared by a number of actin-binding proteins that have been shown to be localized in the Golgi apparatus (Stow et al., 1998). For instance, myosin I, which supports transport of vesicles along actin filaments towards the barbed membrane attached end (Fath et al., 1994), and paxillin, a focal adhesion component (Mazaki et al., 1998). In addition, isoforms of spectrin and ankyrin have been proposed to be involved in tethering Golgi to microtubules, while a family of large coiled-coil proteins of unknown function often appears as Golgi autoantigens in autoimmune responses. Bearing in mind that each isoform might be restricted to different localizations as was shown for paxillin (Mazaki et al., 1998), spectrin (Beck et al., 1994) and ankyrin (Beck et al., 1997), the recent identification of a novel talin isoform may have a functional significance (McCann and Craig, 1998). The precise role of all these proteins associated with Golgi is unclear, but presumably they may either be responsible for regulating events in the Golgi itself or stored on Golgi for further release to sites elsewhere in the cell.

Obviously, some proteins peripherally associated with Golgi membranes can bind specifically to the cytoplasmic tails of Golgi-localized membrane proteins. The N-terminal head of talin has been recently reported to encompass the major binding site for the β_1 and β_3 integrin heterodimers (Patil et al., 1999; Calderwood et al., 1999). However, this interaction depends on integrin occupancy by a matrix substrate (Miyamoto et al., 1995; Vignoud et al., 1997). Conversely, the talin carboxy-terminal rod can bind the β_1 and β_3 cytoplasmic

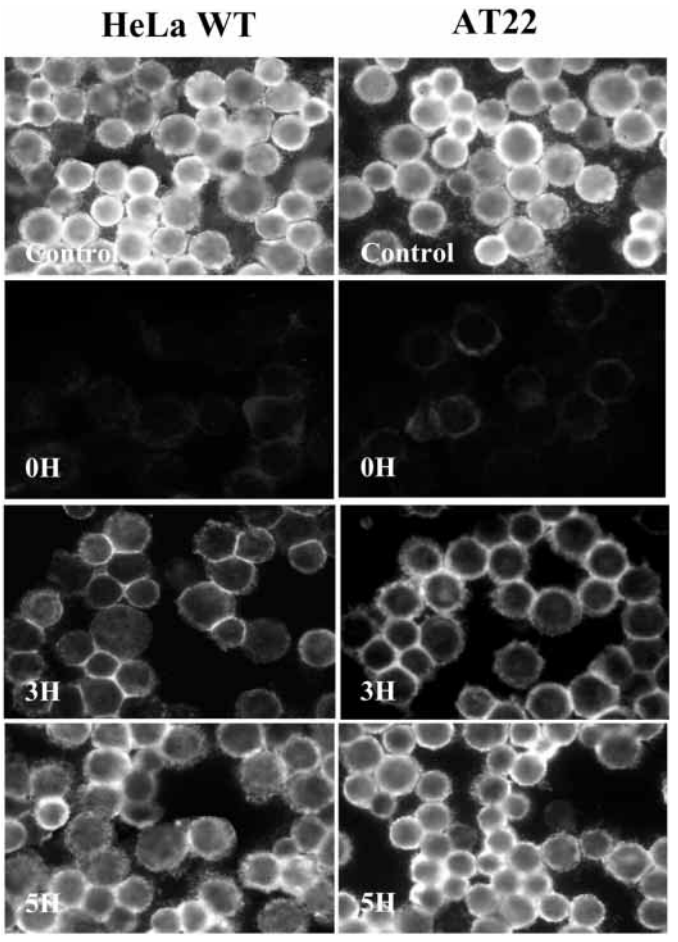


Fig. 5. Exocytosis of the ICAM1 receptor in HeLa and AT22 cells. The kinetics of exocytosis of the ICAM-1 receptors in HeLa cells and AT22 cells were monitored by the recovery of anti-ICAM-1 monoclonal antibody at the cell surface after plasma membrane stripping of the cells with Proteinase K. Membrane stripping was performed as described in Materials and Methods. Stripped cells were incubated at 37°C in suspension in α-MEM medium for various indicated times. The cells were cytospinned and fixed with paraformaldehyde without permeabilization. Immunofluorescence labeling of cell surface ICAM1 was achieved with the anti-ICAM-1 monoclonal antibody, followed with rhodamine-conjugated secondary antibody. There was no difference between ICAM-1 export in HeLa cells and in AT22 cells.

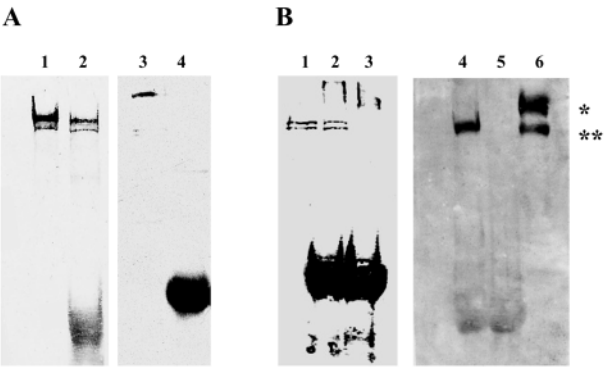


Fig. 6. Interaction of $\alpha_5\beta_1$ with the Golgi/ER compartment. (A) Coimmunoprecipitation of talin with the integrin $\alpha_5\beta_1$ located in the microsomal fraction of HeLa cells. Microsomal preparations from HeLa cells were resuspended in NP40/glycerol buffer. About 300-500 μg of proteins were mixed with antiserum raised against $\alpha_5\beta_1$. The precipitated material was separated by SDS-PAGE under reducing conditions, transferred to a nitrocellulose membrane and probed with anti-talin monoclonal antibody (lane 2) followed by ECL detection of an anti-mouse IgG secondary antibody coupled to HRP. As a control, talin was also detected directly from 30 μg of microsomal membranes on the same gel (lane 1). Talin present in the endoplasmic fraction (lane 3) was not detected in the immune complex precipitated with non-immune serum (lane 4). (B) Lack of ternary complex between $\alpha_5\beta_1$ talin and calnexin. Lane 1 shows western blots of talin from about 30 μg of microsomal fraction. The presence of talin was also detected in immune complexes carried out with antisera directed against $\alpha_5\beta_1$ (lane 2) but not in those obtained with anti-calnexin (lane 3). Conversely, in anti-calnexin immune complexes, the immature form of the human subunit β_1 was detected with the monoclonal antibody TS2/16 (lane 4) whereas no signal was detected after immunoprecipitation with a non-immune serum (lane 5). Lane 6 shows the position of the immature and mature forms of β_1 subunit of HeLa cells detected with the TS2/16 monoclonal antibody. The asterisk and double asterisk indicate the position of mature and immature forms of β_1 integrin, respectively.

domains (Horwitz et al., 1986). Talin was recently shown to bind the cytoplasmic tail of unliganded β_2 integrins (Sampath et al., 1998). It is tempting to hypothesize that this kind of interaction occurs in the Golgi apparatus since early interaction of talin with $\alpha_5\beta_1$ may involve the fibronectin-free integrin. On the other hand, for other Golgi-associated proteins, the targeting region is a sequence at the N terminus that includes sites for lipid modification (Munro, 1998). Indeed, the N terminus of talin has been reported to bind phospholipids (Niggli et al., 1994). This type of interaction might be important or might stabilize talin/integrin interaction within the Golgi apparatus.

How does talin control the exit of $\alpha_5\beta_1$ from ERGIC?

Talin was discovered as a component of focal contacts at the leading edge of migrating cells (Burridge and Connell, 1983). It belongs to the FERM domain family, which includes proteins such as ezrin, moesin, radixin, band 4.1, protein tyrosine phosphatase PTPD1, etc. This family is characterized by the presence of a unique module involved in the linkage of cytoplasmic proteins to the membrane (Rees et al., 1990). The conservation of this domain suggests that some of the partners or functions might also be conserved. To date, proteins interacting with the FERM domain have been ill characterized. However, using the FERM domain of the protein-tyrosine phosphatase PTPD1 as a bait in a yeast two-hybrid screen, the kinesin-like protein KIF1C has been recently identified (Dorner et al., 1998). Although the evaluation of the physiological relevance of this interaction is still in progress, the association of a kinesin-like motor protein with a FERM domain is reminiscent of the involvement of talin in integrin trafficking. Interestingly, unconventional myosin VIIa and myosin X also belong to the FERM protein family. These actin-based motors are now known to be intimately involved in vesicle trafficking (Titus, 1997; Mermall et al., 1998). Therefore, talin may link integrin-containing cargo vesicles to molecular motors involved in vesicle transport along the cytoskeleton network. At present, we cannot rule out an

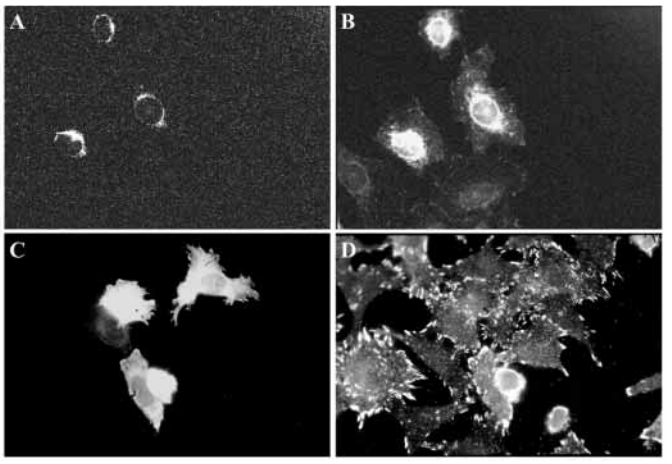


Fig. 7. Accumulation of intracellular β_1 integrins after microinjection of antibodies directed against talin. HeLa cells were microinjected with anti-talin polyclonal antibodies (A,B) or with non-immune serum (C,D). Cells were fixed 3 hours after microinjection and stained either with rhodamine-conjugated goat anti-rabbit to aid identification of microinjected cells (A,C) or with TS2/16 monoclonal antiboby followed by Alexa-conjugated goat anti-mouse to visualize localisation of β_1 integrin (B,D). Microinjection of anti-talin polyclonal antibodies (A) generates accumulation of β_1 integrin around the nucleus (B) whereas there is no intracellular pool of β_1 integrin accumulated in non-immune serum-microinjected cells (D).

alternative possibility that talin itself contains membrane targeting information.

Generally, truncation of both α and β cytoplasmic domains results in both inefficient endoplasmic reticulum exit and reduced or complete loss of cell surface delivery that could be ascribed to some misfolding of the receptor. One can imagine that talin would act as a folding factor for newly synthesized integrins. However, deletion of only the cytoplasmic domain of the β_1 subunit still allows integrins to reach the plasma membrane normally, while the interaction of talin with integrin is likely to be dramatically impaired (Retta et al., 1998). This result strongly suggests that talin itself may not play a direct role in integrin transport. Another explanation is that the early interaction of talin with integrins would disclose a targeting signal on the cytoplasmic tail of the α subunit. In ERGIC, several targeting signals have been described to date. The sequences KKXX-COOH or KXKXX-COOH are retrieval signals of escaped resident proteins of ER (Cosson and Letourneur, 1994; Jackson et al., 1990). Conversely, a diphenylalanine motif close to the transmembrane segment followed by two or three basic residues is found in the p24 family of cargo receptors and was suggested to promote efficient anterograde transport (Fiedler et al., 1996). Finally, a DXE export motif has been described as an export signal from ER (Nishimura and Balch, 1997). The sequence analysis of the integrin cytoplasmic domains revealed neither a retrieval dilysine sequence, nor diacidic export signals. However, the membrane-proximal domain of the integrin α subunit contains a motif of five amino acid residues, GFFKR, conserved in all α chains described to date. This motif has already been shown to be involved in the efficient formation and the stability of the heterodimer (Pardi et al., 1995; DeMelker and Sonnenberg, 1996). However, when the cytoplasmic domain of the β subunit is deleted, the heterodimer αβ reaches the cell surface solely when the deletion of the α cytoplasmic domain preserves this conserved GFFKR motif (Briesewitz et al., 1993; Peyruchaud et al., 1998). In that case, due to the lack of the β cytoplasmic tail, the sequence GFFKR is totally unmasked and does not require further talin interaction for disclosure. Besides its role in integrin heterodimerization, the sequence GFFKR may act as an integrin export signal from the ERGIC. This could explain why deletion or point mutations in this sequence prevent the export of the integrins (Pardi et al., 1995; DeMelker and Sonnenberg, 1996; Peyruchaud et al., 1998). Thus, we suggest that binding of talin to integrin discloses an export signal within the GFFKR conserved sequence that would promote the interaction with coatomer and selective packaging of the integrins into distinct COP-I anterograde vesicles. In this regard, it has recently been reported that the talin head could bind the membrane-proximal region of the integrin cytoplasmic domain (Patil et al., 1999; Calderwood et al., 1999), inducing a conformational change, which in turn may expose the GFFKR motif as an export signal. The availibility of talin for binding to integrin in ER could be a way to regulate integrin entry into the secretory pathway and, therefore, the amount of integrin available for adhesion functions at the cell surface.

Finally, control of the exit of plasma membrane receptors from ER or ERGIC by cytoplasmic proteins interacting with them may not be restricted to integrins. For instance, β-catenin, a cytoplasmic protein known to interact with its receptor cadherin, has been proposed to act as a chauffeur, to facilitate transport of E-cadherin out of the ER (Chen et al., 1999).

We are grateful to Dr M. Bornens and Dr J. Salamero for helpful comments. We thank Geneviève Tavernier and Brigitte Peyrusse for technical assistance. This work was supported by the Centre National de la Recherche Scientifique (CNRS), a grant from the Ligue Nationale Contre le Cancer (FNCLCC), the Association Contre le Cancer (ARC), the Association Espoir and the Fondation pour la Recherche Médicale. V.M. is supported by a fellowship from the Ministère de la Recherche et de l'Enseignement Supérieur.

REFERENCES

Albigès-Rizo, C., Frachet, P. and Block, M. R. (1995). Down regulation of talin alters cell adhesion and the processing of the $\alpha_5\beta_1$ integrin. *J. Cell Sci.* **108**, 3317-3329.

Bannykh S. I., Nishimura N. and Balch, W. E. (1998). Getting into the Golgi. *Trends Cell Biol.* **8**, 21-25.

Beck, K. A., Buchanan, J. A., Malhotra, V. and Nelson, W. J. (1994). Golgi spectrin: identification of an erythroid beta-spectrin homolog associated with the Golgi complex. *J. Cell Biol.* **127**, 707-723.

Beck, K. A., Buchanan, J. A. and Nelson, W. J. (1997). Golgi membrane skeleton: identification, localization and oligomerization of a 195 kDa ankyrin isoform associated with the Golgi complex. *J. Cell Sci.* **110**, 1239-1249

Bolton, S. J., Barry, S. T., Mosley, H., Patel, B., Jockush, B. M., Wilkinson, J. M. and Critchley, D. R. (1997). Monoclonal antibodies recognizing the N- and C-terminal regions of talin disrupt actin stress fibers when microinjected into human fibroblasts. *Cell Motil. Cytoskel.* **36**, 363-376.

Borowsky, M. L. and Hynes, R. O. (1998). Layilin, a novel talin-binding transmembrane protein homologous with C-type lectins, is localized in membrane ruffles. *J. Cell Biol.* **143**, 429-442.

Briesewitz, R., Kern, A. and Marcantonio, E. E. (1995). Assembly and function of integrin receptors is dependent on opposing α and β cytoplasmic domains. *Mol. Biol. Cell* **6**, 997-1010.

Briesewitz, R., Kern, A. and Marcantonio, E. E. (1993). Ligand-dependent

and independent integrin focal contact localization: the role of alpha chain cytoplasmic domain. *Mol. Biol. Cell* **4**, 593-604.

Burridge, K. and Connell, L. (1983). A new protein of adhesion plaques and ruffling membranes. *J. Cell Biol.* **2**, 359-367

Burridge, K. and Mangeat, P. (1984). An interaction between vinculin and talin. *Nature* **308**, 744-746.

Calderwood, D. A., Zent, R., Grant, R., Rees, D. J. G., Hynes, R. O. and Ginsberg, M. H. (1999). The talin head domain binds to integrin b subunit cytoplasmic tails and regulates integrin activation. *J. Biol. Chem.* **274**, 28071-28074.

Chen, H. C., Appeddu, P. A., Parsons, J. T., Hildebrand, J. D., Schaller,M. D. and Guan, J.-L. (1995). Interaction of focal adhesion kinase with cytoskeletal protein talin. *J. Biol. Chem.* **270**, 16995-16999.

Chen, Y. T., Stewart, D. B. and Nelson, W. J. (1999). Coupling Assembly of the E-Cadherin/beta-Catenin Complex to Efficient Endoplasmic Reticulum Exit and Basal-lateral Membrane Targeting of E-Cadherin in Polarized MDCK Cells. *J. Cell Biol.* **144**, 687-699.

Cosson, P. and Letourneur, F. (1994). Coatomer interaction with di-lysine endoplasmic reticulum retention motifs. *Science* **263**, 1629-31.

Dedhar, S. and Hannigan, G. E. (1996). Integrin cytoplasmic interactions and bidirectional transmembrane signalling. *Curr. Opin. Cell Biol.* **8**, 657-669.

De Melker, A. A. and Sonnenberg, A. (1996). The role of the cytoplasmic domain of alpha 6 integrin in the assembly and function of alpha 6 beta 1 and alpha 6 beta 4. *Eur. J. Biochem.* **214**, 254-264.

Dorner, C., Ciossek, T., Muller, S., Moller, P. H., Ullrich, A. and Lammers, R. (1998). Characterization of KIF1C, a new kinesin-like protein involved in vesicle transport from the Golgi apparatus to the endoplasmic reticulum. *J. Biol. Chem.* **273**, 20267-20275.

Engvall, E. and Ruoslahti, E. (1977). Binding of soluble form of fibroblast surface protein, fibronectin, to collagen. *Int. J. Cancer* **20**, 1-5.

Fath, K. R., Trimbur, G. M. and Burgess, D. R. (1994). Molecular motors are differentially distributed on Golgi membranes from polarized epithelial cells. *J. Cell Biol.* **126**, 661-675

Farquhar, M. G. and Palade, G. E. (1998). The Golgi apparatus: 100 years of progress and controversy. *Trends Cell Biol.* **8**, 2-10.

Fiedler, K., Veit, M., Stamnes, M. A. and Rothman, J. E. (1996). Bimodal interaction of coatomer with the p24 family of putative cargo receptors. *Science* **273**, 1396-1399.

Gilmore, A. P., Wood, C., Ohanian, V., Jackson, P., Patel, B., Rees, D. J. G., Hynes, R. O. and Critchley, D. R. (1993). The cytoskeletal protein talin contains at least two distinct vinculin binding domain. *J. Cell Biol.* **122**, 337-347.

Hauri, H. P. and Schweitzer, A. (1992). The endoplasmic-reticulum intermediate compartment. *Curr. Opin. Cell Biol.* **4**, 600-608.

Heino, J., Ignotz, R. A., Hemler, M. E., Crouse, C. and Massagué, J. (1989). Regulation of cell adhesion receptors by transforming growth factor-β. *J. Biol. Chem.* **264**, 380-388.

Hemler, M. E., Ware, C. F. and Strominger, J. L. (1983). Characterization of a novel differentiation antigen complex recognize by a monoclonal antibody (A1A5): unique activation-specific molecular forms on stimulated T cells. *J. Immunol.* **131**, 334-340.

Hemmings, L., Rees, D. J. G., Ohanian, V., Bolton, S. J., Gilmore, A. P., Patel, B., Priddle, H., Trevithick, J. E., Hynes, R. O. and Critchley, D. R. (1996). Talin contains three actin binding sites each of which is adjacent to vinculin-binding sites. *J. Cell Sci.* **109**, 2715-2726.

Horwitz, A., Duggan, E., Buck, C., Beckerle, M. C. and Burridge, K. (1986). Interaction of plasma membrane fibronectin receptor with talin – a transmembrane linkage. *Nature* **320**, 531-533.

Hotchin, N. A., Gandarillas, A. and Watt, F. M. (1995). Regulation of cell surface β_1 integrin levels during keratinocyte terminal differentiation. *J. Cell Biol.* **128**, 1209-1219.28.

Hughes, P. E., Diaz-Gonzalez, F., Leong, L., Wu, C., McDonald, J. A., Shattil, S. J. and Ginsberg, M. H. (1996). Breaking the integrin hinge. A defined structural constraint regulates integrin signaling. *J. Biol. Chem.* **271**, 6571-6574.

Hynes, R. O. (1992). Integrins: versatility, modulation, and signalling in cell adhesion. *Cell* **69**, 11-25.

Jackson, M. R., Nilsson, T. and Peterson, P. A. (1990). Identification of a consensus motif for retention of transmembrane proteins in the endoplasmic reticulum. *EMBO J.* **10**, 3153-3162.

Jasmin, B. J., Cartaud, J., Bornens, M. and Changeux, J. P. (1989). Golgi apparatus in chick skeletal muscle: changes in its distribution during end plate development and after denervation. *Proc. Natl. Acad. Sci. USA* **86**, 7218-7222.

Knezevic, I., Leisner, T. M. and Lam, S. C.-T. (1996). Direct binding of the platelet integrin α_{IIb} β_{III} (GPIIb-IIa) to talin. *J. Biol. Chem.* **271**, 16416-16421.

LaFlamme, S. E., Ajiyama, S. E. and Yamada, K. M. (1992). Regulation of fibronectin receptor distribution. *J. Cell Biol.* **117**, 437-447.

Lenter, M. and Vestweber, D. (1994). The integrin chains β_1 and α_6 associate with the chaperone calnexin prior to integrin assembly. *J. Biol. Chem.* **269**, 12263-12268.

Mazaki, Y., Uchida, H., Hino, O., Hashimoto, S. and Sabe, H. (1998). Paxillin isoforms in mouse. Lack of the gamma isoform and developmentally specific beta isoform expression. *J. Biol Chem.* **273**, 22435-22441.

McCann, R. O. and Craig, S. W. (1998). Identification of a novel isoform of the focal adhesion protein talin. *Mol. Biol. Cell, 38th ASCB*, **9**, 138.

Mermall, V., Post, P. L. and Mooseker, M. S. (1998). Unconventional myosins in cell movement, membrane traffic, and signal transduction. *Science* **279**, 527-533

Miyamoto, S., Teramoto, H., Coso, O. A., Gutkind, J. S., Burbelo, P. D., Akiyama, S. K. and Yamada, K. M. (1995). Integrin functions: molecular hierarchies of cytoskeletal and signaling molecules. *J. Cell Biol.* **131**, 791-805.

Moulder, G. L., Huang, M. M., Waterston, R. H. and Barstead, R. J. (1996). Talin requires beta-integrin, but not vinculin, for its assembly into focal adhesion-like structures in the nematode *Caenorhabditis elegans*. *Mol. Biol. Cell* **7**, 1181-1193.

Muguruma, M., Matsumura, S. and Fukasawa, T. (1990). Direct interaction between talin and actin. *Biochem. Biophys. Res. Commun.* **171**, 1217-1223.

Munro, S. (1998). Localization of proteins to the Golgi apparatus (1998). *Trends Cell Biol.* **8**, 11-15.

Niggli, V., Kaufmann, S., Goldmann, W. H., Weber, T. and Isenberg, G. (1994). Identification of functional domains in the cytoskeletal protein talin. *Eur. J. Biochem.* **224**, 951-957.

Nishimura, N. and Balch, W. E. (1997). A di-acidic signal required for selective export from the endoplasmic reticulum. *Science* **277**, 556-558.

Nuckolls, G. H., Romer, L. H. and Burridge, K. (1992). Microinjection of antibodies against talin inhibits the spreading and migration of fibroblasts. *J. Cell Sci.* **102**, 753-762.

Otey, C. A., Pavalko, F. M. and Burridge, K. (1990). An interaction between α-actinin and the β_1 integrin subunit in vitro. *J. Cell Biol.* **111**, 721-729.

Pardi, R., Bossi, G., Inverardi, L., Rovida, E. and Bender, J. R. (1995). Conserved regions in the cytoplasmic domains of the leukocyte integrin alpha L beta 2 are involved in endoplasmic reticulum retention, dimerization, and cytoskeletal association. *J. Immunol.* **155**, 1252-1263.

Patil, S., Jedsadayanmata, A., Wencel-Dracke, J. D., Wang, W., Knezevic, I and Lam, S. C.-T (1999). Identification of a talin-binding site in the integrin β3 subunit distinct from the NPLY regulatory motif of post-ligand binding functions. *J. Biol. Chem.* **274**, 28575-28583.

Pavalko, F. M. and LaRoche, S. M. (1993). Activation of human neutrophils induces an interaction between the integrin beta 2-subunit (CD18) and the actin binding protein alpha-actinin. *J. Immunol.* **151**, 3795-3807

Peyruchaud, O., Nurden, A. T., Milet, S., Macchi, L., Pannochia, A., Bray, P. F., Kieffer, N. and Bourre, F. (1998). R to Q amino acid substitution in the GFFKR sequence of the cytoplasmic domain of the integrin IIb subunit in a patient with a Glanzmann's thrombasthenia-like syndrome. *Blood* **92**, 4178-4187.

Pfaff, M., Liu, S., Erle, D. J. and Ginsberg, M. H. (1998). Integrin beta cytoplasmic domains differentially bind to cytoskeletal proteins. *J. Biol. Chem.* **273**, 6104-6109.

Presley, J. F., Cole, N. B., Schroer, T. A., Hirschberg, K., Zaal, K. J. and Lippincott-Schwartz, J. (1997). ER-to-Golgi transport visualized in living cells. *Nature* **389**, 81-85

Ray, T. K. (1970). A modified method for the isolation of the plasma membrane from a rat liver. *Biochem. Biophys. Acta* **196**, 1-9.

Rees, D. J., Ades, S. E., Singer, S. J. and Hynes, R. O. (1990). Sequence and domain structure of talin. *Nature* **347**, 685-689

Retta, S. F., Balzac, F., Ferraris, P., Belkin, A. M., Fassler, R., Humphries, M. J., De Leo, G., Silengo, L. and Tarone, G. (1998). beta1-integrin cytoplasmic subdomains involved in dominant negative function. *Mol. Biol. Cell* **9**, 715-731.

Sampath, R., Gallagher, P. J. and Pavalko, F. M. (1998). Cytoskeletal interactions with the leukocyte integrin beta2 cytoplasmic tail. Activation-dependent regulation of associations with talin and alpha-actinin. *J. Biol. Chem.* **273**, 33588-33594.

Scales, S. J., Pepperkok, R. and Kreis, T. E. (1997). Visualization of ER-to-

Golgi transport in living cells reveals a sequential mode of action for COPII and COPI. *Cell* **90**, 1137-1148.

Stow, J. L., Fath, K. R. and Burgess, D. R. (1998). Budding roles for myosin II on the Golgi. *Trends Cell Biol.* **8**, 138-141.

Titus, M. A. (1997). Unconventional myosins: new frontiers in actin-based motors. *Trends Cell Biol.* **7**, 119-123.

Turner, C. E. and Burridge, K. (1989). Detection of metavinculin in human platelets using a modified talin overlay assay. *Eur. J. Cell. Biol.* **49**, 202-206.

Vignoud, L., Albigès-Rizo, C., Frachet, P. and Block, M. R. (1997). NPXY motifs control the recruitment of the $\alpha_5\beta_1$ integrin in focal adhesions independently of the association of talin with the β_1 chain. *J. Cell Sci.* **110**, 1421-1430.

Ylänne, J., Chen, Y. L., O'Toole, T. E., Loftus, J. C., Takada, Y. and Ginsberg, M. H. (1993). Distinct functions of integrin α and β subunit cytoplasmic domains in cell spreading and formation of focal adhesions. *J. Cell Biol.* **122**, 223-234.