Reelin Is a Serine Protease of the Extracellular Matrix*

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Carlo C. Quattrocchi,a,b,c Francesca Wannenes,d,e Antonio M. Persico,a Silvia Anna Ciafre´,d Gabriella D’Arcangelo,f,g,h,i Maria G. Farace,j and Flavio Keller*j

From the “Laboratory of Neuroscience, Department of Physiology and Neuroscience, Universitá “Campus Bio-Medico,” Via Longoni 83, 00155 Roma, Italy, the gProgram in Neuroscience, Faculty of Medicine, University of Brescia, Via Valsabbina 19, 25123 Brescia, Italy, the hDepartment of Experimental Medicine and Biochemical Sciences, Università di Tor Vergata, Viale di Tor Vergata 135, 00133 Roma, Italy, the iDepartment of Internal Medicine, Università di Tor Vergata, Viale di Tor Vergata 135, 00133 Roma, Italy, and jThe Cain Foundation Laboratories, gDepartment of Pediatrics, hProgram in Developmental Biology and iDivision of Neuroscience, Baylor College of Medicine, Houston, Texas 77030

Reelin is an extracellular matrix protein that plays a pivotal role in development of the central nervous system. Reelin is also expressed in the adult brain, notably in the cerebral cortex, hippocampus, cerebellum, and several brainstem nuclei, as shown by spontaneous Reelin null mutations (i.e. the reeler mouse) (3, 4). In the developing cerebral cortex, Reelin is secreted by Cajal-Retzius cells, located in the marginal zone. Reelin must be secreted into the extracellular matrix to exert its biological effect (5).

In the reeler mouse, migrating neurons fail to pass through earlier-generated neurons, possibly because they are unable to penetrate the subplate, or because they maintain extensive contacts with the radial glial fibers (6). Several hypotheses have been suggested regarding the function of Reelin: (i) Reelin may act as an attractant molecule for migrating neurons; (ii) it may act as a repulsive molecule; or (iii) Reelin may interrupt the association between migrating neurons and radial glia (7, 8), thus allowing migrating neurons to switch from a “gliophilic” to a “neurophilic” state (9). Furthermore, Reelin has been recently shown to be expressed in several adult neuronal cells, including glutamatergic cerebellar granule neurons and specific GABAergic interneurons of the cerebral cortex and hippocampus (10), and in the adult mammalian blood, liver, pituitary pars intermedia, and adrenal chromaffin cells (11, 12). The cellular function of Reelin in the adult organism is unknown. Evidence is accumulating for involvement of Reelin in human diseases such as autosomal recessive lissencephaly (13), schizophrenia (14), and autistic disorder (15).

The mouse Reelin sequence (1) encompasses 3461 amino acids and possesses a signal peptide followed by a domain with 28% sequence identity with F-spondin (as assessed by Blast software), a protein secreted by floor plate cells and promoting cell adhesion and neurite growth (16). This region is followed by a unique region with no sequence homology, and then by eight internal repeats of 350–390 amino acids, each repeat containing two related subdomains flanking a cystein-rich sequence similar to the epidermal growth factor-like motif. The carboxyl terminus region contains many positively charged amino acids required for secretion (5). Human Reelin (2) is 94.8% identical to the mouse protein at the amino acid level, indicating strong functional conservation. Recent findings suggest that the Reelin signal transduction involves binding to the very low density lipoprotein receptor and to apoE receptor 2 followed by intracellular activation of the adapter protein disabled-1 (17–19). Other possible Reelin signal transduction pathways may involve interaction with the αβ integrin receptor (8, 9) and with cadherin-related neuronal receptors (20).

We have analyzed the primary amino acid sequence of human Reelin, and found several hints that Reelin might be a serine protease, since: (i) Reelin contains the sequence GKSDDG (amino acids 1280–1284 of human Reelin) (2), corresponding to the serine hydrolase consensus sequence GXXG; this sequence is 100% conserved among mouse, chicken, and human Reelin; (ii) Reelin shows significant structural similarities with serine hydrolases, such as the extracellular serine protease precursor (EC 3.4.21) of Serratia marcescens, and the probable ubiquitin carboxyl-terminal hydrolases FAM and FAF-Y (EC 3.1.2.15); (iii) Reelin contains eight epidermal growth factor-like repeats; epidermal growth factor-like repeats are observed in serine proteases, for example, coagulation factors VII, IX, and X, and protein C, Z (21), calcium-dependent serine protein-

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† Present address: Dept. of Pediatrics-Neurology, Baylor College of Medicine, 1102 Bates St., MC 3–6365, Houston, TX 77030.

‡ To whom correspondence should be addressed: Laboratory of Neuroscience, Università “Campus Bio-Medico,” Via Longoni 83, 00155 Roma, Italy. Tel.: 39-06-2254-1335; Fax: 39-06-22-54-14-56; E-mail: f.keller@unicampus.it.

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Ase (22) which degrades extracellular matrix proteins, and complement C1s and C1r components (23); (iv) several serine hydrolases, such as lipoprotein lipase and the urokinase-type plasminogen activator, bind very low density lipoprotein receptor and apoE receptor 2 (24, 25). In this study we present converging evidence that purified Reelin acts as a serine protease, and that this enzymatic activity may be relevant for its cellular function.

EXPERIMENTAL PROCEDURES

Chemicals—Cell culture media, antibiotics, and media supplements were purchased from Invitrogen (Gaithersburg, MD). All other chemicals were from Sigma, unless otherwise specified.

Amino acid Sequence Analysis of Reelin—All sequence analyses are based on human Reelin (accession number: NP_035391) (2). Homologies were evaluated by using V-Blat 2 sequences software (www.ncbi.nlm.gov/blast). Also, PropSearch software (EMBL, Heidelberg) was used to find homologies with other serine hydrolases. PattinProt software (PBIL, NPSA, Lyon) was used to identify putative conserved sequences for serine proteases.

Cell Culture, Transfection, and Expression of Recombinant Reelin—Human embryonic kidney (HEK) 293T cells (ATCC, CRL-11032) were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-Inactivated fetal bovine serum, penicillin/streptomycin (100 international units/ml and 100 μg/ml, respectively), and 10 mM L-glutamine. All cells were cultured at 37 °C, 5% CO₂, and 97% relative humidity. All cells were cultured at 37 °C, 5% CO₂, and 97% relative humidity. Cells were transfected with pCDNA3 empty vector or with 2 μg of pCDNA3 plasmid containing cDNA encoding human Reelin (27). The reaction was stopped with PBS containing 2 μg MLT. Alternatively, Reelin bands were revealed using a chemiluminescence method with anti-reelin antibody 142 (1:100,000 in TBST) for 1 h. Then, after three washes, 1:5000 peroxidase-conjugated streptavidin (CHEMICON Int., Temecula, CA) was added for 30 min. Filters or films were scanned with a XA4 crystal for digital scanning with XA4 software (Hewlett-Packard). The specificity of staining was checked by preincubating mAb 142 with protein SP, the Reelin fragment recognized by mAb 142 (27).

Labeling of Reelin with FP-Peg-biotin—Fluorescein phosphate biotin (FP-Peg-biotin) (a gift of Dr. Benjamin Cravatt) (30), stored as a 100 μM solution in water, was added directly to protein samples to a final concentration of 2 μg/ml. The reaction mixture was incubated at room temperature for 30 min, and stopped by adding an equal volume of 2× reducing sample buffer. As a control for the specificity of FP-Peg-biotin labeling, replica samples were incubated for 1 h with 11.4 μM disopropyl fluorophosphatase (DFP), 1 a potent and specific serine-hydrolase inhibitor, before incubation with FP-Peg-biotin. Samples were separated by SDS-PAGE and transferred by electroblotting to nitrocellulose (22). The membranes were blocked in TBST with 3% bovine serum albumin for 1 h at 25 °C or overnight at 4 °C, and then incubated for 15 min with an avidin-horseradish peroxidase conjugate (Pierce) diluted 1:3000 in blocking solution. The labeled filters were revealed with 1:2000 mAb 142, overnight, followed by 1:5000 alkaline phosphatase-labeled anti-mouse IgG (Promega Italia, Milan, Italy). The specificity of staining was checked by preincubating mAb 142 with protein SP, the Reelin fragment recognized by mAb 142 (27).

Purification of Reelin—For gel filtration chromatography purification, 50 μl of supernatant from Reelin-transfected CER cells were concentrated first by osmotic dialysis with AQUACIDE I (Calbiochem, La Jolla, CA). A 5-ml concentrated sample was loaded on a FPLC system AKTAprime and passed through a HiLoad Superdex 200 26/60 column (Amersham Bioscience, Inc., Uppsala, Sweden). The run was performed at a constant flow rate of 3 ml/min and maximal pressure limit of 0.5 Pa. The eluate was collected in 1-ml fractions. After dot immunoblot screening and Western blot, positive fractions for Reelin were pooled and concentrated as described above.

For purification by SDS-PAGE, Reelin-containing supernatant from transfected 293T cells was concentrated as above and separated on a 5% gel. The gel was run for 3 h 30 min at 125 V to achieve a good separation in the >250 kDa range. Thereafter a thin vertical slice of gel was cut and stained with silver to reveal the 400-kDa Reelin band. A 5-μm wide horizontal side was cut from the remaining gel, using the stained slice as a reference, and 400-kDa Reelin was electroeluted at 60 V in 25 μl Tris, 250 mM glycine, 0.1% SDS buffer. Electroelution was carried out overnight at 4 °C. Purified Reelin was then, transferred to PBS, pH 7.2, by overnight dialysis in a Slide-A-Lyzer cassette (Pierce). Finally the protein was concentrated. The final protein concentration was estimated by 0.2 μg/ml Bradford reagent (Sigma). Purified Reelin was re-electrophoresed on a SDS gel to check purity of the protein.

1 The abbreviations used are: DFP, diisopropyl phosphorofluoridate; HEK, human embryonic kidney; mAb, monoclonal antibody; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; tPA, tissue plasminogen activator.
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Fig. 1. Consensus sequence analysis of Reelin around hypothetically catalytic amino acids (serine, histidine, and aspartic acid) of serine proteases. Identical or homologous residues are shaded.

Degradation of Extracellular Matrix Proteins—Reelin aliquots (10 ng) were incubated with 1 μg of fibronectin from human plasma (Sigma), or with laminin or collagen type IV from basement membrane of Engelbreth-Holm-Swarm mouse sarcoma (Sigma) for 0, 10, 30, or 120 min at 37 °C in PBS, pH 7.9. The reaction was stopped by adding sample buffer and heating the samples at 100 °C for 2 min. Samples were separated in a 8% SDS gel. After electrophoresis, the gel was fixed, silver-stained. The supernatant of mock-transfected cells showed a completely different labeling pattern with FP-Peg-biotin: the most evidently labeled band was a 150-kDa band, while no labeled bands were visible at 400 and 140 kDa (Fig. 4 B, lane 1, B). The 400- and 300-kDa band showed strong labeling with FP-Peg-biotin (Fig. 4 B, lane 2), while the 140-kDa band faint labeling with FP-Peg-biotin, while the 140-kDa band showed faint labeling with FP-Peg-biotin, while the 140-kDa band showed strong labeling with FP-Peg-biotin (Fig. 4 B, lane 1). A major Reelin band at approximately 400 kDa, and minor bands at 350 and 140 kDa (Fig. 2B, lane 2). The supernatant of mock-transfected cells did not show any stained bands (lane 1).

RESULTS

Reelin Contains Regions of Homology with Serine Proteases—Human Reelin contains the sequence GKSDG (amino acids 1280–1284), homologous to the consensus sequence GX-SXG of serine proteases (Fig. 1). Furthermore, searches of the Reelin sequence for consensus patterns (PROSITE www.ich/uel.ac.uk/cmgserpro.htm) around hypothetical amino acids of the catalytic triad (Ser, His, and Asp) using PattinProt (PBIL, ucl.ac.uk/cmgs/serpro.htm) around hypothetical amino acids of the catalytic triad (Ser, His, and Asp) using PattinProt (PBIL, ucl.ac.uk/cmgs/serpro.htm) have revealed several sequences sharing the catalytic triad (Ser, His, and Asp) using PattinProt (PBIL, ucl.ac.uk/cmgs/serpro.htm) around hypothetical amino acids of the catalytic triad (Ser, His, and Asp) using PattinProt (PBIL, ucl.ac.uk/cmgs/serpro.htm) around hypothetical amino acids of the catalytic triad (Ser, His, and Asp) using PattinProt (PBIL, ucl.ac.uk/cmgs/serpro.htm).

Reelin Inhibits Cell Attachment in Vitro—To obtain recombinant Reelin protein, HEK 293T cells were transfected with pCrl plasmid, and Reelin mRNA expression was assessed by RT-PCR, using primers complementary to exon 27 sequences. RT-PCR of pCrl-transfected cells revealed the expected 376-bp band (Fig. 2A, lane 3), while the band was absent in mock-transfected cells (lane 2). Reelin secretion into the supernatant was confirmed by Western blotting. The supernatant of pCrl-transfected cells showed a major Reelin band at approximately 400 kDa, and minor bands at 350 and 140 kDa (Fig. 2B, lane 2). The supernatant of mock-transfected cells did not show any significant adhesion as compared with mock-transfected HEK 293T cells. After a 2-h incubation, 44.7 ± 6.3 (mean ± S.E.) pCrl-transfected cells were attached to the substrate, as compared with 96.3 ± 10.8 mock-transfected cells (Fig. 3A, B). Furthermore, cell morphology was markedly different: Reelin-secreting cells appeared unable to spread on fibronectin and their processes were diminished in number and length, as compared with mock-transfected cells (Fig. 3B). To assess the biological significance of the enzymatic activity of Reelin, the effect of DFP, a potent and specific inhibitor of serine hydrolases, on cell adhesion was studied. Micromolar concentrations of DFP partially restored adhesion of Reelin-expressing cells on fibronectin, without affecting mock-transfected cells (Fig. 3A and 3B). The effect of DFP was dose-dependent, starting at concentrations ≥0.5 μM; maximal increase in adhesion of Reelin-expressing cells was seen at 5.4 μM DFP, while 5.4 μM was equally toxic for pCrl- and mock-transfected cells, inhibiting adhesion of >99% of the cells (Fig. 3C).

Reelin Binds a Serine Hydrolase Probe—FP-Peg-biotin is described to behave as a specific and irreversible probe for serine hydrolases, showing properties similar to those of common FP inhibitors, such as DFP (30). To explore Reelin labeling with FP-Peg-biotin, aliquots of transfected 293T cell culture supernatants incubated with 5 μM FP-Peg-biotin were separated on standard SDS-PAGE gels, blotted, and probed with avidin peroxidase; replica samples were stained with the monoclonal antibody 142. The supernatant of Reelin-expressing cells showed distinct Reelin bands at approximately 400, 300, and 140 kDa (Fig. 4A, lane 2). The 400- and 300-kDa bands showed faint labeling with FP-Peg-biotin, while the 140-kDa band showed strong labeling with FP-Peg-biotin (Fig. 4B, lane 1; arrows indicate corresponding bands in the two blots). Labeling of these three bands was inhibited by DFP (Fig. 4B, lane 2). The supernatant of mock-transfected cells showed a completely different labeling pattern with FP-Peg-biotin: the most evidently labeled band was a 150-kDa band, while no labeled bands were visible at 400 and 140 kDa (Fig. 4B, lane 3). Given the complex pattern of labeling with FP-Peg-biotin in supernatants, we decided to perform FP-Peg-biotin labeling on partially purified Reelin. The supernatant of the stable cell line CER, expressing high levels of Reelin, was concentrated and partially purified.
by gel filtration chromatography. The Reelin-positive eluate from the Superdex 200 gel filtration column was concentrated and then incubated in the absence or presence of the serine protease inhibitor phenylmethanesulfonyl fluoride (PMSF). The samples were separated by SDS-PAGE on a 4–12% gradient gel, blotted, and incubated with the monoclonal antibody E4 to reveal Reelin (Fig. 5A, lanes 1 and 2). The blot was then stripped and incubated with streptavidin to reveal the binding to FP-Peg-biotin (Fig. 5A, lanes 3 and 4). As with the crude supernatant (Figs. 2 and 4), the immunoblot showed two major Reelin-positive bands, one higher than the 250-kDa marker, corresponding to the 400- and 300-kDa isosforms, and a smaller band at about 140 kDa (Fig. 5A, lanes 1 and 2). FP-Peg-biotin binds to both major Reelin bands in the absence, but not in the presence of PMSF (Fig. 5A, lanes 3 and 4). The smaller band appeared to bind FP-Peg-biotin with a higher affinity than the

higher molecular weight isofrom (Fig. 5A, lane 3).

**Reelin Shows Protease Activity on Extracellular Matrix Proteins in Vitro**—To investigate the catalytic activity of Reelin, we first further purified the high molecular weight isofroms by SDS-PAGE and electroelution. As shown in Fig. 5B, separation

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**Fig. 3.** Expression of Reelin inhibits adhesion of HEK 293T cells to fibronectin, and DFP treatment reverses the effect of Reelin expression. A, quantification of the effect of various treatments. Bars show the numbers of cells attached to fibronectin-coated wells under different conditions. Each bar represents the mean and S.E. of five wells. Double-headed arrows indicate the statistical difference between groups (one-way ANOVA followed by LSD post-hoc test). B, phase-contrast images of cells grown in different conditions. DFP was applied at a concentration of 5.4 μM. C, dose-dependent effect of DFP on cell adhesion of mock- or pCrl-transfected HEK 293T cells. Each point represents the percent ratio between the number of attached cells after DFP treatment and the number of attached cells in the absence of DFP.

**Fig. 4.** Reelin can be labeled with FP-Peg-biotin, a serine trap probe, and labeling is inhibited by DFP. A, aliquots of supernatants were separated by SDS-PAGE, blotted, and stained with mAb 142. Lanes 1, Mock-transfected HEK 293T cells; 2, pCrl-transfected cells; arrows indicate Reelin bands. B, FP-Peg-biotin labels several bands in the supernatants. Lanes 1, supernatant of pCrl-transfected cells; arrows indicate bands corresponding to the 400-, 300-, and 140-kDa Reelin bands; 2, replica sample as in lane 1, but preincubated with 11 μM DFP; 3, supernatant of mock-transfected cells; 4, replica sample as in lane 3, but preincubated in the presence of 11 μM DFP. Two-hundred and fifty ng of total protein were applied to each lane.

**Fig. 5.** Purification of Reelin by fast protein liquid chromatography and SDS-PAGE electroelution, and labeling of purified Reelin with FP-Peg-biotin. A, the concentrated supernatant from CER cells was purified on a Sephadex-200 gel filtration column; the Reelin-positive eluate from the column was separated on a 4–12% gradient SDS-PAGE. Lanes 1 and 2, immunoblot (mAb E4) of the sample incubated with FP-Peg-biotin after pretreatment without and with PMSF, respectively. Lanes 3 and 4, the blot was stripped and developed with horseradish peroxidase-conjugated streptavidin to reveal bound FP-Peg-biotin. The 250-kDa calibration marker shown next to lane 1 is valid for all 4 lanes. B, supernatants of mock-transfected (lane 1) and pCrl-transfected cells (lane 2) were separated with SDS-PAGE (5% gel) for 3 h 30 min and the gel was stained with silver nitrate. The arrow next to lane 2 indicates the 400-kDa Reelin band. Lanes 3 and 4, blot corresponding to lanes 1 and 2, respectively, stained with anti-Reelin mAb 142. The arrow next to lane 4 points to 400-kDa Reelin. The 250-kDa calibration marker next to lane 1 is valid for lanes 2–4 as well. Lane 5, the 400-kDa Reelin band shown in lane 2 was electroeluted, electrophoresed in a second gel, blotted, and stained with mAb 142. The asterisks mark degradation products of Reelin at approximately 180 and 140 kDa. The 400-kDa band has practically disappeared.
of the concentrated cell culture supernatant using a 5% SDS gel resulted in a good separation of the Reelin isoforms. Silver nitrate staining indicated that only the supernatant of Reelin expressing cells contains a band at approximately 400 kDa corresponding to the Reelin isoforms of 400 (and 300) kDa (Fig. 5B, lanes 2 and 4). Therefore, the high molecular weight Reelin band was electroeluted from the gel to achieve a high degree of purification. The electroeluted sample was reanalyzed by SDS-PAGE and Western blotting (Fig. 5B, lane 5). However, we found that the purified high molecular weight Reelin protein quickly disappeared and smaller bands appeared around 180 and 140 kDa, probably corresponding to self-degradation products. The major proteolytic product of 140 kDa that we observed in this study may correspond to the 180-kDa degradation product that has been described by other investigators (8, 35).

To test for proteolytic activity on extracellular matrix proteins, purified Reelin was incubated with purified fibronectin, laminin, or collagen IV, and breakdown products were analyzed by SDS-PAGE and silver staining of gels. Fibronectin and laminin breakdown fragments were seen already after 10 min incubation (Fig. 6A and D), while collagen IV was degraded at a much slower rate (Fig. 6E). Fibronectin degradation was blocked by inhibitors of serine proteases (DFP, PMSF, and aprotinin), but not by inhibitors of other families of proteases (Fig. 6B). Fibronectin degradation was also partially inhibited by monoclonal antibody CR-50 (Fig. 6C), an antibody directed against the NH2-terminal portion of Reelin that has been demonstrated to inhibit Reelin function both in vitro and in vivo (see Ref. 31 and references quoted therein). Inhibition of fibronectin degradation was seen at a CR-50 concentration of 9.8 μg/ml, the highest concentration tested. Interestingly, this concentration is comparable with the concentrations that have been reported in the literature to inhibit Reelin function (20–200 μg/ml, Ref. 31).

**DISCUSSION**

In this paper we present converging biochemical and cellular evidence that Reelin is a serine protease of the extracellular matrix, and that its enzymatic activity is important for the modulation of cell adhesion. The fact that CR-50, a monoclonal antibody known to inhibit Reelin function both in vitro and in vivo, blocks the proteolytic activity of Reelin further supports the hypothesis that proteolytic activity is of fundamental importance for the function of Reelin. These findings appear interesting in view of the fact that serine proteases, such as tissue plasminogen activator (tPA), are already known to be important modulators of cell migration and axon growth (32).

HER 293T cells adhere quickly to fibronectin, due to endogenous expression of α5β1 integrin (28), which is a selective fibronectin receptor (33). In this paper we show that expression of Reelin leads to a marked decrease of adhesion of HEK 293T cells to fibronectin. These data, together with the demonstration that purified Reelin degrades fibronectin in vitro, are consistent with the hypothesis that Reelin, secreted by HEK 293T cells, inhibits cell adhesion by degrading the fibronectin substrate. Alternatively, Reelin might activate other targets, for example, cell membrane receptors or other proteases, which are in turn directly responsible for cell detachment. A third hypothesis is that Reelin induces the expression of another...
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serine protease, which is in turn responsible for cell detachment. This hypothesis appears remote, since we have demonstrated that Reelin binds FP-Peg-biotin, and degrades fibronectin in vitro.

We found that, after purification, Reelin appears to undergo rapid self-degradation. Our data suggest that the major 140-kDa fragment is enzymatically active, since its binding to FP-Peg-biotin is even stronger than that of full-length Reelin. Interestingly, we also observed strong labeling with FP-Peg-biotin of the smaller fragment after immunoaffinity purification of Reelin from mouse brain.2 These data support the idea that the proteolytic processing of Reelin is functionally important, and that full activity of Reelin might require degradation of the 400-kDa full-length precursor to generate smaller, more active isoforms.

Reelin appears to behave as a specific serine protease, as collagen IV is degraded at a much slower rate than fibronectin or laminin. However, this hypothesis needs further confirmation, using model peptide substrates.

Reelin has been suggested to allow migrating neurons to grow past previously migrated cells and to promote detachment of neurons from radial glial fibers (6–8). The α3 integrin subunit, expressed in HEK 293T cells, shows high homology with the α5 subunit, which is expressed on migrating neurons, and appears to be involved in the inhibitory effect of Reelin on neuronal migration along radial glial processes. Reelin has been demonstrated to bind to α3β1 integrin (8). In situ hybridization experiments and double immunolabeling with antibodies against fibronectin and antibodies against radial glial cells demonstrate transient fibronectin expression on radial glia processes during early stages of cortical development, until completion of corticogenesis (34). On the basis of the available evidence, we propose that α3β1 integrin might immobilize extracellular Reelin on the surface of migrating neurons and thus focus its proteolytic activity on fibronectin expressed on radial glial cells. Alternatively, binding to α3β1 integrin might enhance the activity of Reelin by protecting it from degradation. In this respect it is interesting to notice that elevated levels of cleaved Reelin have been detected in the absence of α3β1 integrin. This finding has been interpreted as evidence that α3β1 integrin inhibits degradation of Reelin by modulating the activity of a zinc-dependent metalloproteinase (8, 35). Our data are consistent with the simpler hypothesis that the proteolytic processing of Reelin is functionally important, and that full activity of Reelin might require degradation of the proteolytic active isoforms.

Interestingly, we also observed strong labeling with FP-Peg-biotin, and degradation activity, and that full activity of Reelin might require degradation of the 400-kDa full-length precursor to generate smaller, more active isoforms.

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2 C. C. Quattrocchi and F. Keller, unpublished observations.
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Additions and Corrections

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Oxidative stress induces impairment of human erythrocyte energy metabolism through the oxygen radical-mediated direct activation of AMP-deaminase.

Barbara Tavazzi, Angela Maria Amorini, Giovanna Fazzina, Donato Di Pierro, Michele Tuttobene, Bruno Giardina, and Giuseppe Lazzarino

Page 48084, left column, line 16: The word “biphosphoglycerate” should be deleted from this sentence. The correct sentence is: “Results, corroborated by data of experiments effected on quinine-treated erythrocytes and hemolysates and obtained from patients suffering from glucose-6-phosphate dehydrogenase (G-6-PDH) deficiency, demonstrate that AMP-deaminase is activated by oxidative stress through the modification of its accessible –SH groups and is responsible for a profound and probably irreversible derangement of erythrocyte energy metabolism.”

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Reelin is a serine protease of the extracellular matrix.

Carlo C. Quattrocchi, Francesca Wannenes, Antonio M. Persico, Silvia Anna Ciafre, Gabriella D’Arcangelo, Maria G. Farace, and Flavio Keller

Page 303: The affiliation for Dr. Farace was incorrect. The correct affiliation is: “Department of Experimental Medicine and Biochemical Sciences, Universita di Tor Vergata, Via di Tor Vergata 135, 00133 Roma, Italia.”

Also, the grant footnote should read: “This work was supported by Consiglio Nazionale delle Ricerche, Programma “Biomolecole per la salute umana,” Grant 99.00555.PF33 (to F. K.) and by grants from the Consiglio Nazionale delle Ricerche and Ministero dell’Istruzione, dell’Universita e della Ricerca (to M. G. F. and S. A. C.)."