Am. J. Hum. Genet. 57:817–823, 1995

The Oculocerebrorenal Syndrome Gene Product Is a 105-kD Protein Localized to the Golgi Complex

Isabelle M. Olivos-Glander,[1,2] Pasi A. Jänne,[1] and Robert L. Nussbaum[2]

[1]Department of Genetics, University of Pennsylvania, Philadelphia; and [2]National Center for Human Genome Research, National Institutes of Health, Bethesda

Summary

The oculocerebrorenal syndrome of Lowe (OCRL) is a multisystem disorder affecting the lens, kidney, and CNS. The predicted amino acid sequence of the OCRL gene, OCRL-1, was used to develop antibodies against the OCRL-1 protein. Western blot analysis using affinity-purified serum against the amino terminus of the OCRL-1 gene product (ocrl-1) demonstrates a single protein of 105 kD in fibroblasts of a normal individual that is absent in fibroblasts of an OCRL patient who lacks OCRL-1 transcript. A single protein with the same electrophoretic mobility is found by western analysis in various human cultured cell lines, and approximately the same size protein is also found in all mouse tissues tested. Northern analysis of various human and mouse tissues demonstrate that OCRL-1 transcript is expressed in nearly all tissues examined. By immunofluorescence, the ocrl-1 antibody stains a juxtanuclear region in normal fibroblast cells, while no specific staining is evident in the OCRL patient who produces no transcript. Colocalization of the ocrl-1 protein to the Golgi complex was demonstrated using a known monoclonal antibody against a Golgi-specific coat protein, β-COP (beta coatomer protein).

Introduction

Lowe syndrome is a rare X-linked disorder characterized by congenital cataracts, Fanconi syndrome, and mental impairments (Lowe et al. 1952). Cataracts in male fetuses are evident as early as 20 weeks. Proximal tubular dysfunction usually develops within the first year but may appear much later. Mental retardation is common, but not universal, in this syndrome. Behavioral disturbances, including stubbornness and tantrums, are also frequently reported (Kenworthy et. al. 1993).

The oculocerebrorenal syndrome of Lowe (OCRL) gene, OCRL-1, was isolated by positional cloning with the aid of two female OCRL patients with X; autosome translocations. Northern blot analysis demonstrates that the OCRL-1 transcript is ~5.8 kb and contains an open reading frame of 2,910 nt (Attree et al. 1992). The majority of OCRL patients studied to date either lack OCRL-1 transcript or show an abnormal OCRL-1 transcript size (Attree et al. 1992). Specific mutations have been identified in a number of OCRL patients, confirming that the OCRL-1 gene is responsible for Lowe syndrome (Leahey et al. 1993).

Although no known functional domains were found in the predicted OCRL-1 protein sequence, a Genbank search revealed that it had a >50% identity to an inositol polyphosphate 5-phosphatase, INPP5B (Attree et al. 1992). This 75-kd type II enzyme has both 1,4,5-inositol trisphosphate 5-phosphatase activity and phosphatidyl inositol (4,5) bisphosphate phosphatase activity (Ross et al. 1991; Matzaris et al. 1994). The OCRL-1 protein was recently found to have activities similar to the INPP5B gene product, and, therefore, it represents the first human disease identified to date that results from abnormal phospholipid signal transduction and/or inositol metabolism (Zhang et al. 1995; S. F. Suchy and R. L. Nussbaum, unpublished data).

To identify and elucidate the function of the OCRL-1 protein, polyclonal antibodies were made to the amino portion of the protein. In this report, we used this antiserum to identify ocrl-1, to examine its expression in mouse tissues, and to localize the protein by immunocytochemistry. In addition, the expression pattern of OCRL-1 mRNA was studied by northern blot analysis using mRNA derived from human and mouse tissues. These studies are a first step in characterizing the OCRL-1 gene product.

Material and Methods

Antibody Production

A 764-bp fragment from the 5′ region of the OCRL-1 cDNA (Attree et al. 1992) was amplified using PCR in order to incorporate an *Nco*I restriction site on the 5′ end and a *Bam*HI site on the 3′ end of the segment. The two 25-mer primers consist of the following sequences: 5′-ACT GTC GAG GCC ATG GAG ATG

Received May 10, 1995; accepted for publication June 28, 1995.

Address for correspondence and reprints: Dr. Robert L. Nussbaum, LGDR/NCHGR/NIH, Building 49, Room 4A72, 49 Convent Drive, Bethesda, MD 20892-4470. E-mail: rlnuss@nchgr.nih.gov

0002-9297/95/5704-0011$02.00

AAG G and 5′-CAG TTC TTG GGA TCC AAT TCA GTA G. The conditions for amplification were: 0.125 mM dNTPs (Cetus), 2.5 units of Taq polymerase (Cetus), 1 × buffer with 1.5 mM $MgCl_2$ (Cetus), 50 pmol of each primer, and 50 ng of cDNA template in a 50 μl volume. Cycling conditions were as follows: 5 min 95°C denaturation and 30 cycles of 94°C for 1 min, 64°C for 2 min, and 72°C for 2 min, with a final extension at 72°C for 7 min. The PCR product was digested with *Nco*1 and *Bam*HI (New England Biolabs) and cloned into pET vector 11d, (Novagen), that had been modified to include a 6-histidine tag in-frame and immediately prior to the *Nco*I cloning site (courtesy of F. Rauscher, Wistar Institute). Sequencing (Sequenase version 2.0, USB) of the clone at ligation junctures confirmed in-frame ligation. Plasmid DNA was isolated from this clone and transformed into BL21 DE3 (Novagen). A 500-ml log-phase culture was induced with 1 mM IPTG for 6 h. The bacterial cell pellet was lysed, and the supernatant was purified for the histidine-tagged protein by nickel-column purification according to manufacturer's protocol (Qiagen). Rabbits were inoculated (Hazelton Research Products) by standard protocols for polyclonal antibody production (Harlow and Lane 1988).

Antisera Affinity Purification

A membrane purification protocol (Tang 1993) was followed with a few modifications. Approximately 500 μg of OCRL-1 antigen from a nickel-column purification was run on a preparative 12% SDS polyacrylamide gel. This was transferred onto polyvinyl difluoride (PVDF) membrane (United States Biochemical) and stained with Coommassie brilliant blue to mark the antigen position on the membrane (Pluskal et al. 1986). One milliliter of polyclonal serum plus 0.05% sodium azide in a total of 50 ml of PBS plus 0.1% Tween-20 (PBS-T) was absorbed to the membrane overnight at room temperature. The blot was washed three times for 10 min each with PBS-T. The membrane strip containing the antigen was cut away from the blot and placed in a small tray on ice. Serum was eluted from the strip according to protocol. The purified serum was concentrated with an Amicon Centriprep-10 concentrator.

Description of OCRL Patient

MD is currently a 31-year-old male, born to a 19-year-old G1P0 mother after an uneventful pregnancy. Cataracts were seen at birth. At age 2 mo, glaucoma and hypotonia were noted, as was metabolic acidosis and generalized aminoaciduria. Cultured fibroblasts revealed no OCRL-1 transcript, by northern blot analysis (Attree et al. 1992).

Tissue Culture

All fibroblast lines were grown in Dulbecco's modified Eagle's medium with 10% fetal bovine sera (FBS) and 2 mM glutamine. Normal fibroblast (CRL 1509, ATCC), HEK 293 (CRL 1573, ATCC), and glioblastoma (CRL 1620, ATCC) cells were also grown in this media. Human lens epithelial cells (Andley et al. 1994) were grown in EMEM plus 20% FBS and 2 mM glutamine. Cells were grown in 150-cm^2 flasks at 37°C with 5% CO_2.

To prepare lysates for western analyses, cells were grown to confluency and trypsinized. Cell pellets were washed 2 times with PBS and resuspended in ~400 μl of water and stored at −70°C. Bio-Rad protein assays (Bradford 1976) were used to determine the protein concentration. A 5 × SDS loading buffer (Harlow and Lane 1988) was added, and the lysates were boiled for 5 min. Lysates were cleared by centrifugation and supernatants containing 150 μg were loaded onto the gel.

Mouse Tissue Homogenates

Mouse organs from 129SV/J mice (Jackson Laboratories) were dissected and immediately frozen in liquid nitrogen. Tissues were homogenized on ice in incomplete 2 × SDS loading buffer (20% glycerol, 4% SDS, 135 mM Tris pH 6.8). The homogenates were boiled and vortexed thoroughly. Bio-Rad protein assays (Lowry et al. 1951; Bradford 1976) were used to determine protein concentration. Samples in incomplete loading buffer were supplemented with 1 mM DTT and 0.02% (w/v) bromophenol blue prior to loading. Boiled lysates were cleared by centrifugation, and supernatants containing 150 μg were loaded onto the gel.

Western Blot Analysis

Next, 1.5 mm 6% SDS polyacrylamide gels were run with 1 × Tris-glycine-SDS buffer (Harlow and Lane 1988). Gels to be transferred onto PVDF membranes were equilibrated in transfer buffer (25 mM Tris, 0.2 M glycine, 15% methanol) for 30 min. Gels were transferred onto PVDF membrane (USB and Millipore) in a tank buffer system with the above transfer buffer for a total of 320 Vh at 4°C (Harlow and Lane 1988). The membrane was stained with Coomassie brilliant blue in methanol and acetic acid and destained to confirm protein transfer (Pluskal et al. 1986). Filters were blocked overnight in 2% casein in a 0.1-M NaCl and 0.1-M Tris pH 9.5 buffer plus 0.1% Tween-20. Washes were performed in PBS-T. Incubations were performed with purified primary antibody at a dilution of 1:50 and secondary antibody, anti-rabbit Ig HRP-linked, at a dilution of 1:10,000 according to the manufacturer's protocol (ECL western kit; Amersham).

Northern Analyses

Human and mouse multiple tissue northern blots were purchased from Clontech. Human blots (MTNI and MTNII) were hybridized according to the manufacturer's recommendations with the entire OCRL-1 cDNA

(Attree et al. 1992). The mouse MTN was hybridized with a partial murine *Ocrl-1* cDNA (gift of L. Charnas). Hybridization conditions and washes were performed according to the manufacturer's protocol. Blots were stripped and reprobed with a β-actin probe.

Immunofluorescence

Cells were grown on four-chambered glass slides (Nunc) in appropriate media for 24–48 h, until they reached 60%–90% confluency. Chambers were washed with PBS 2 × 5 min. Cells were fixed with 1% paraformaldehyde plus 0.1% Triton X-100 in PBS for 4 min. Cells were then immediately washed with PBS, 2 × 5 min. The purified ocrl-1 antiserum was diluted 1:30 with the monoclonal β-COP antibody, M3A5 (donated by J. Donaldson; characterized by Allan and Kreis 1986) diluted 1:1, and incubated on slides at room temperature for 1.5 h. Chambers were washed 2 × 5 min with PBS. Biotinylated anti-rabbit IgG (Vector Laboratories) was diluted 1:200 in PBS and incubated on slides for 30 min at room temperature. Chambers were washed again with PBS, 2 × 5 min. FITC-avidin D (Vector Laboratories) was diluted 1:400 with sheep anti-mouse Ig linked to Texas Red (Amersham) 1:200 in PBS and incubated on slides for 30 min at room temperature in the dark. The chambers were washed 3 × 5 min with PBS. Filter 4879-09 on a Zeiss fluorescent microscope was used to visualize FITC with exciter filter BP450-490, dichroic mirror ST510, and barrier filter LT520. Texas Red was visualized with filter 4879-15 containing exciter filter H546, dichroic mirror FT580, and barrier filter LP590.

Results

A construct containing part of the OCRL-1 sequence was prepared to overexpress a portion of this protein in bacteria. A peptide of 253 amino acids from the amino portion of the predicted OCRL-1 protein was expressed and used as antigen to produce polyclonal antibodies. This portion of the OCRL-1 gene was chosen because similarity between OCRL-1 and INPP5B proteins is <10% in this region (Attree et al. 1992), and we therefore hypothesized that the antibodies produced would not cross-react with the latter protein. Western blot analysis was performed with purified ocrl-1 antiserum against fibroblast lysates from a normal individual and an OCRL patient, MD, who produces no OCRL-1 transcript (Attree et al. 1992). A single protein of 105 kD is evident in the normal lysate but is absent, with no other proteins detected, in the fibroblasts from the OCRL patient, MD (fig. 1).

Various human cultured cell lines were assayed by western analysis to confirm the identification and size of the OCRL-1 protein. The two fibroblast lines mentioned above, one of a normal individual and one of an OCRL patient, were used as positive and negative controls, respectively. A human lens epithelial cell line, a human embryonic kidney cell line, and a human glioblastoma cell line were analyzed. These cell lines were chosen to represent the expression pattern in their normal human organ derivatives. A single protein of 105 kD was seen in all lanes except in that of the OCRL patient (fig. 2).

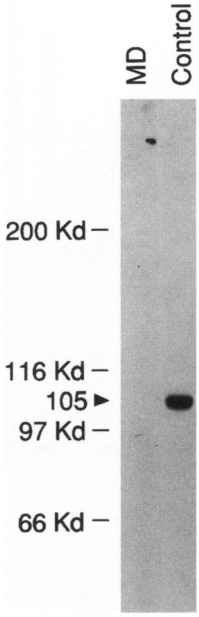

Figure 1 Western blot analysis of human fibroblast cells with purified ocrl-1 antiserum. Lane 1, OCRL patient, MD (produces no OCRL-1 transcript). Lane 2, Control (normal individual).

Northern blot analysis of various human tissues demonstrate that OCRL-1 is expressed in all tissues studied with the single exception of peripheral blood lymphocytes (fig. 3*A* and 3*B*). High levels of expression are seen in the testis and ovaries, and low levels are found in the liver, spleen, and thymus, when compared to the β-actin control. Northern analysis using mRNA derived from mouse tissues demonstrates the presence of the *Ocrl-1* transcript in all tissues examined (fig. 4). The mouse mRNA expression is highest in the brain, while the spleen and testis show low expression levels.

Expression of the mouse orthologue of the OCRL-1 protein in various tissues was also analyzed by western blotting with the purified antibody (fig. 5). Nine mouse tissues were examined along with the human fibroblast controls described previously. A protein of 106–109 kD, slightly larger than the protein identified in the normal human fibroblast lysate, was evident in most organs studied. Since the protein found in the mouse homogenates is similar in size to the human OCRL-1 protein and is the only protein evident in most tissues, this protein likely represents the mouse orthologue of ocrl-1. Lower molecular-weight proteins in some of the lanes

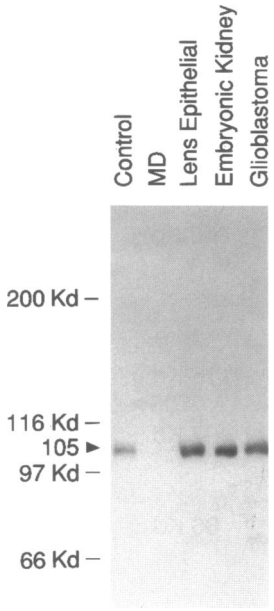

Figure 2 Western blot analysis of human cultured cell lines with purified ocrl-1 antiserum. Control lane, Fibroblast cells from normal individual. MD lane, Fibroblast cells from an OCRL patient.

may represent ocrl-1 degradation products or other proteins with similar epitopes. Repeated western analyses consistently demonstrate high levels of protein in the brain, eye, and testis and low levels in the liver. Both northern and western analyses reveal ubiquitous expression in mouse tissues. Differences between the expression levels of the OCRL-1 transcript and protein are observed only in the mouse liver and testis. The mouse liver appears to have much higher levels of OCRL-1 transcript than the mouse testis, while the opposite is true of their respective protein levels.

Indirect immunofluorescence with the ocrl-1 antibody was performed on fibroblast cells from a control individual and an OCRL patient, MD. The ocrl-1 antibody revealed a clear juxtanuclear stain and a faint cytoplasmic stain in all the normal cells, while no specific signal was evident in the OCRL patient's cells (data not shown). To determine subcellular localization of the OCRL-1 protein, a double-labeling immunofluorescence experiment was performed with β-COP, a coatomer protein residing in the Golgi (Allan and Kreis 1986; Duden et al. 1991). The two proteins colocalized to the same region of the cell, the Golgi apparatus (fig. 6).

Discussion

OCRL is an X-linked disorder with clinical manifestations in the eye, brain, and kidney. The organ pathology does not suggest an obvious common biochemical and/or cellular defect. In order to characterize the OCRL-1 gene product and elucidate its function, we initiated immunological studies of the protein.

Polyclonal antibodies were generated against the amino portion of the OCRL-1 protein and were shown to be specific by western blot analysis. This purified antiserum detects a single protein of 105 kD by western blot analysis in control fibroblast cells, which was absent in fibroblast cells from an OCRL patient who does not produce OCRL-1 transcript. Other human cultured cell lines, from brain, kidney, and lens, were of particular interest because they are derived from organs that are affected in OCRL patients. A single protein of 105 kD is recognized by the ocrl-1 antiserum in all three of these human cell lines as well, confirming their expression of the OCRL-1 gene product.

Although the 5′ UTR of the OCRL-1 gene has not

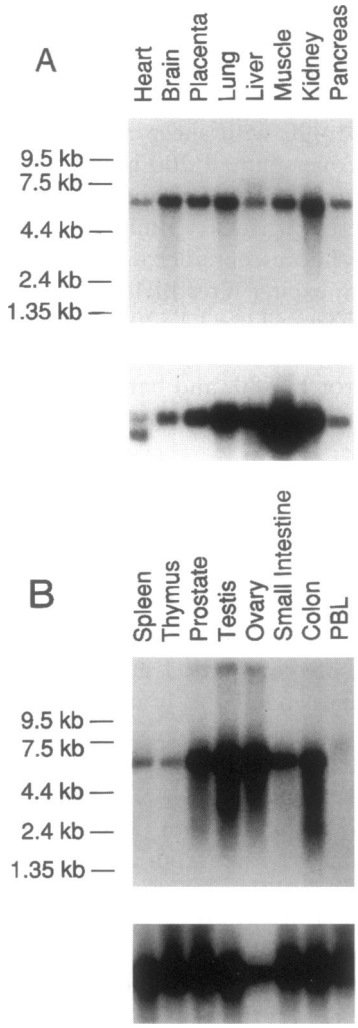

Figure 3 Northern blot analysis of human tissue homogenates. In both *A*, and *B*, the northern blots were probed with OCRL-1 (*top panel*) and with a β-actin probe (*bottom panel*). PBL = peripheral blood lymphocytes.

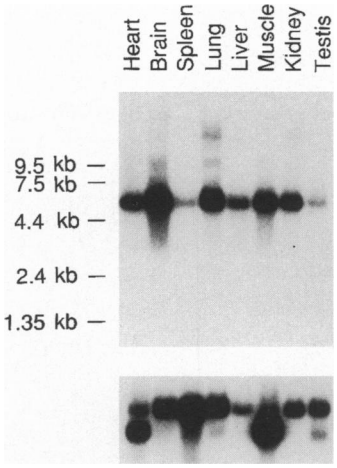

Figure 4 Northern blot analysis of mouse tissue homogenates. The northern blot was probed with murine *Ocrl-1* cDNA (*top panel*) and with a β-actin probe (*bottom panel*).

been precisely determined and the transcriptional start site has not been confirmed, a candidate methionine codon has been identified located at amino acid 98 (M88162). This methionine codon, although not the furthest 5′ after an in-frame stop codon, presents the best consensus Kozak sequence compared to other candidates (Kozak 1991). The predicted size of the ocrl protein using this AUG as the probable start site, is 101 kd, which is similar to the estimated size determined by western blot analysis. Therefore, it is likely this methionine codon represents the translational start site.

To examine the pattern of expression in tissues, we performed northern blot analysis of human and mouse tissues as well as western analysis of mouse tissues. Northern analysis of human tissues reveals expression in all tissues studied except for peripheral blood lymphocytes. Northern analysis in mouse tissues demonstrates that the mouse *Ocrl-1* gene is ubiquitously expressed with the highest level of expression found in the brain. Furthermore, western blot analysis on various mouse tissues shows that a protein of 106–109 kD is produced in all tissues examined. The highest level is found in the mouse brain, and the lowest is found in the liver.

Although the 106–109-kD protein seen in mouse tissues is consistently larger than the OCRL-1 protein found in the human cultured cell lines, it is recognized by the human ocrl-1 antiserum, and it is the most prominent protein detected in all mouse tissues. It is also absent in embryonal stem cells and mice in which the *Ocrl-1* gene has been knocked out by homologous recombination (P. A. Jänne and R. L. Nussbaum, unpublished data). Therefore, this 106–109-kD protein likely represents the mouse *Ocrl-1* protein. Although the limited *Ocrl-1* mouse cDNA sequence available shows >95% nucleotide identity to the human gene (R. L. Nussbaum, unpublished data) the entire mouse cDNA has not been sequenced, and it is possible that differences in the protein sequence between the mouse and human OCRL-1 gene accounts for the size difference. Furthermore, it is possible that the mouse protein undergoes different posttranslational modifications compared to its human counterpart.

The OCRL-1 protein is expressed in many tissues that remain unaffected in patients with OCRL. There may be a number of reasons for this observation. It is possible that the function carried out by the OCRL-1 gene product may simply not be required in those tissues that escape significant pathology. It is also likely that the OCRL-1 protein function is required in a majority of these tissues, but different protein or proteins compensate for the defect. This raises the possibility that the OCRL-1 protein may be part of a family of proteins with partially overlapping functions and tissue distributions. The high amino acid similarity with the INPP5B gene product makes the enzyme encoded by this gene a reasonable candidate for providing compensating activity in some tissues. There is now evidence that these two proteins do have similar activities.

A partial, recombinant, ~90-kD OCRL-1 protein was recently described by Zhang et al. (1995) to have PIP_2 5-phosphatase activity. This correlates with findings in our laboratory, which show that fibroblasts from an OCRL patient, MD, which produce no OCRL-1 protein, are also deficient in PIP_2 5-phosphatase activity when compared to normal fibroblasts (S. F. Suchy and R. L. Nussbaum, unpublished data). Therefore, since OCRL-1 has PIP_2 phosphatase activity, our data suggest that the OCRL-1 protein is involved in phosphatidylino-

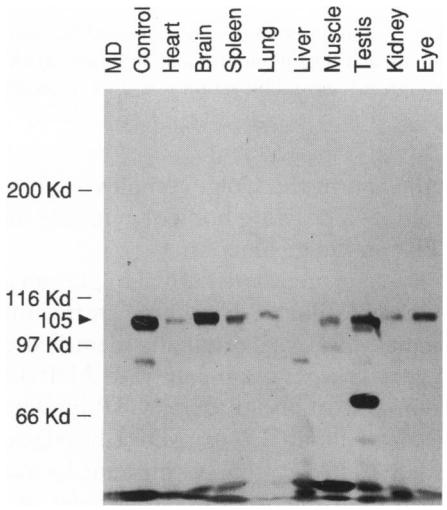

Figure 5 Western blot analysis of mouse tissue homogenates. PVDF membrane was probed with purified ocrl-1 antiserum. The lane labeled "MD" contains fibroblast cell lysate from an OCRL patient. The control lane contains fibroblast cell lysate from a normal individual.

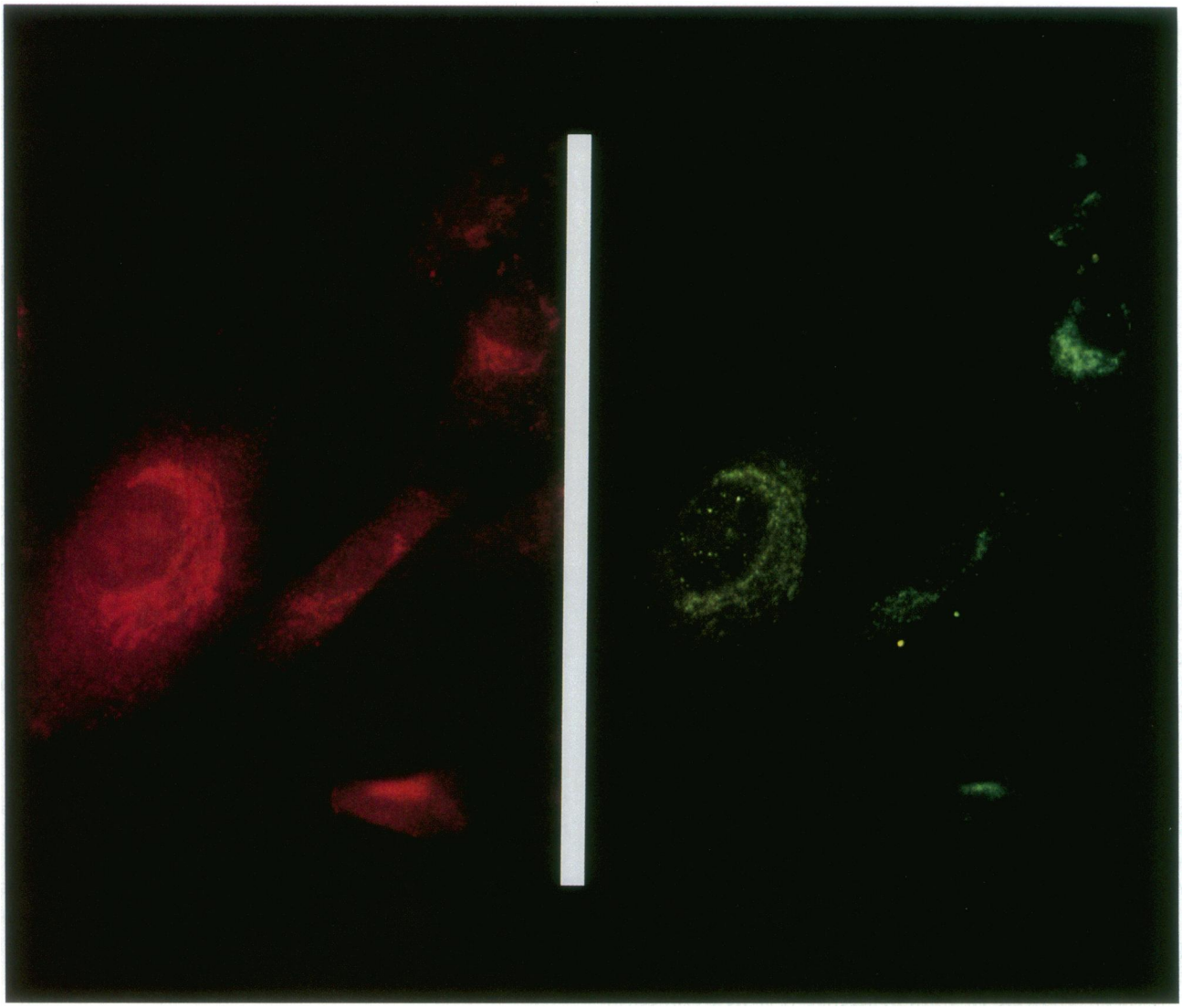

Figure 6 Immunocytochemistry of normal fibroblast cells with ocrl-1 and β-cop antisera. Cells were incubated with a mixture of purified ocrl-1 antiserum and β-COP monoclonal antibody (M3A5). A Texas Red (red) anti-mouse serum was used to recognize β-cop (*left panel*) and a fluorescein (green) anti-rabbit serum was used to recognize ocrl-1 (*right panel*). The magnification is 1,000×.

sitol metabolism in the Golgi complex. This suggestion is supported by a growing body of evidence for involvement of PIP_2 in Golgi function.

GTP-binding proteins are known to play an important role in the regulation of membrane trafficking. One of these proteins, ARF, was originally identified as a cofactor for in vitro cholera toxin-catalyzed ADP ribosylation of the α-subunit of the trimeric GTP-binding protein, G_s (Kahn and Gilman 1986). ADP ribosylation factors (ARFs) are a family of small G-proteins localized to the Golgi and responsible for the recruitment of coatomer proteins (Stearns 1990; Donaldson et al. 1991, 1992). These coat proteins are found on nonclathrin-coated vesicles and play an important role in vesicular traffic and organelle structure. The recruitment of these coat proteins by ARF is essential for the formation of budding vesicles from the Golgi. The GTPase activating protein for ARF (ARF-GAP) is stimulated directly by PIP_2. ARF-GAP is also stimulated by phosphatidic acid, which is produced when PIP_2 activates phospholipase D (Brown et al. 1993; Kahn et al. 1993; Liscovitch et al. 1994).

One might hypothesize that, if this pathway were disrupted through abnormal PIP_2 levels in Golgi membranes, protein trafficking out of the Golgi might be affected, leading to abnormalities in cell surface proteins required for differentiation or transport. These proteins required for cell-cell contact and differentiation in the lens may fail to reach the surface of developing lens cells and lead to cataract. Proteins or factors involved

in amino acid transport or in forming epithelial tight junctions might be affected and produce the Fanconi syndrome. Further studies will be required to test these hypotheses and to determine the pathogenesis of OCRL at the level of organelle and cellular function.

Acknowledgments

This work was begun in the Howard Hughes Medical Institute in Philadelphia and has been continued with support by the Division of Intramural Research of the National Center for Human Genome Research (to I.M.O.-G. and R.L.N.) and by grants R01HD23245 and T32GM07170 (to P.A.J.). We acknowledge Dr. L. Charnas for the for the kind gift of the partial cDNA of the mouse homolog of OCRL-1. We also thank Dr. S. Liebhaber for his support and encouragement of this work.

References

Allan VJ, Kreis TE (1986) A microtubule-binding protein associated with membranes of the Golgi apparatus. J Cell Biol 103:2229–2239

Andley UP, Rhim JS, Chylack Jr LT, Fleming TP (1994) Propagation and immortalization of human lens epithelial cells in culture. Invest Ophthalmol Vis Sci 35:3094–3102

Attree O, Olivos IM, Okabe I, Bailey LC, Nelson DL, Lewis RA, McInnes RR, et al (1992) The Lowe's oculocerebrorenal syndrome gene encodes a protein highly homologous to inositol polyphosphate-5-phosphatase. Nature 358:239–242

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Brown HA, Gutowski S, Moomaw CR, Slaughter C, Sternweis PC (1993) ADP-ribosylation factor, a small GTP-dependent regulatory protein, stimulates phospholipase D activity. Cell 75:1137–1144

Donaldson JG, Cassel D, Kahn RA, Klausner RD (1992) ADP ribosylation factor, a small GTP-binding protein, is required for binding of the coatomer protein β-COP to Golgi membranes. Proc Natl Acad Sci USA 89:6408–6412

Donaldson JG, Kahn RA, Lippincott-Schwartz J, Klausner RD (1991) Binding of ARF and β-COP to Golgi membranes: possible regulation by a trimeric G protein. Science 254:1197–1199

Duden R, Griffiths G, Frank R, Argos P, Kreis TE (1991) β-COP, a 110 kd protein associated with non-clathrin-coated vesicles and the Golgi complex, shows homology to β-adaptin. Cell 64;649–665

Harlow E, Lane D (1988) Immunoblotting. In: Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp 471–510

Kahn RA, Gilman AG (1986) The protein cofactor necessary for ADP-ribosylation of G_s by cholera toxin is itself a GTP binding protein. J Biol Chem 261:7906–7911

Kahn RA, Yucel JK, Malhotra V (1993) ARF signaling: a potential role for phospholipase D in membrane traffic. Cell 75:1045–1048

Kenworthy L, Park T, Charnas LR (1993) Cognitive and behavioral profile of the Oculocerebrorenal syndrome of Lowe. Am J Med Genet 46:297–303

Kozak M (1991) An analysis of vertebrate mRNA sequences: intimations of translational control. J Cell Biol 115:887–903

Leahey A-M, Charnas LR, Nussbaum RL (1993) Nonsense mutations in the OCRL-1 gene in patients with the oculocerebrorenal syndrome of Lowe. Hum Mol Genet 2:461–463

Liscovitch M, Chalifa V, Pertile P, Chen C-S, Cantley LC (1994) Novel function of phosphatidylinositol 4,5-bisphosphate as a cofactor for brain membrane phopholipase D. J Biol Chem 269:21403–21406

Lowe CU, Terrey M, MacLachlan EA (1952) Organic-aciduria, decreased renal ammonia production, hydropthalmos, and mental retardation. Am J Dis Child 83:164–184

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275

Matzaris M, Jackson SP, Laxminarayan KM, Speed CJ, Mitchell CA (1994) Identification and characterization of the phosphatidylinositol-(4,5)-bisphosphate 5-phosphatase in human platelets. J Biol Chem 269:3397–3402

Pluskal MG, Przekop MB, Kavonian MR, Vecoli C, Hicks DA (1986) Immobilon PVDF transfer membrane: a new membrane substrate for western blotting of proteins. Biotechniques 4:272–283

Ross TS, Jefferson AB, Mitchell CA, Majerus PW (1991) Cloning and expression of human 75-kDa inositol polyphosphate-5-phosphatase. J Biol Chem 266:20283–20289

Stearns T, Willingham MC, Botstein D, Kahn RA (1990) ADP-ribosylation factor is functionally and physically associated with the Golgi complex. Proc Natl Acad Sci USA 87:1238–1242

Tang W-J Y (1993) Blot affinity purification of antibodies. In: Asai DJ (ed) Antibodies in cell biology. Vol 37 in: Wilson L, Matsudaira P (eds) Methods in cell biology. Academic Press, San Diego, pp 99–100

Zhang X, Jefferson AB, Auethavekiat V, Majerus PW (1995) The protein deficient in Lowe syndrome is a phosphatidylinositol-4,5-bisphosphate 5-phosphatase. Proc Natl Acad Sci USA 92:4853–4856