FEBS Letters 347 (1994) 273–278

FEBS 14176

Myelin membrane biogenesis by oligodendrocytes

Developmental regulation of low molecular weight GTP-binding proteins

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Received 20 April 1994; revised version received 25 May 1994

Abstract

Oligodendrocytes synthesize dramatic amounts of myelin membrane. We hypothesized that this requires unique aspects of vesicular trafficking. Specific stages of the oligodendrocyte lineage were assayed for low molecular weight GTP-binding proteins implicated in the regulation of vesicular transport pathway (two dimensional gel electrophoresis, [α-^{32}P]GTP overlay). Consistent with the hypothesis, as oligodendrocytes differentiate from early progenitors to mature myelin-producing cells, $\geq$ 12 small GTP-binding proteins become up-regulated. Myelin membrane also has a complex pattern of GTP-binding proteins. Several of these proteins may be specific to oligodendrocytes, suggesting that oligodendrocytes may utilize cell-type specific GTP-binding proteins for biogenesis and maintenance of the myelin membrane.

Key words: GTP-binding protein; Rab protein; Oligodendrocyte; Myelin biogenesis; Vesicular trafficking

1. Introduction

The oligodendrocyte (OL) ensheathes axons in the central nervous system with myelin, a cell-specific, multilamellar membrane continuous with the cell's plasma membrane [1]. During development oligodendrocytes undergo dramatic morphological and biochemical changes during a highly regulated course of differentiation from progenitors to mature oligodendrocytes which are capable of myelinating axons. This pathway is experimentally defined both in vitro and in vivo by stage specific antigens [2] (Fig. 1), and proceeds accurately in culture up to actual myelination even in the absence of neurons [3,4]. Oligodendrocytes grown in defined culture conditions can be expanded and developmentally synchronized through the application of specific growth factors (Fig. 1) [1,5–7], allowing for the analysis of developmental-stage specific cellular events. The manner in which an oligodendrocyte prepares for and executes the biogenesis of dramatic amounts of myelin membrane in a developmentally regulated manner is of specific interest.

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Abbreviations: PDGF-BB, B-subunit homodimer of platelet derived growth factor; bFGF, basic fibroblast growth factor; GD_3^+, cells expressing the ganglioside GD_3; Pro-OL, pro-oligodendroblasts; GalC, galactosylcerebroside; OL, oligodendrocytes; MBP, myelin basic protein.

Membrane biogenesis requires a number of molecular components for directing vesicular traffic between particular intracellular compartments [8–10]. Conserved constituents are required to accomplish steps in vectorial transport that occur similarly in all cells (e.g. transport of vesicles from the endoplasmic reticulum to the *cis*-Golgi); cell type specific constituents allow transport and sorting of proteins to specialized membrane domains (e.g. transport of proteins and lipids from the *trans*-Golgi network to the basolateral and apical domains of polarized epithelial cells [11]).

The pathways by which cells target newly synthesized proteins from donor to acceptor membranes utilize a number of small GTP-binding proteins [12]. The *rab* family is one class of these small GTP-binding proteins [13–15], and are the mammalian homologues of the yeast membrane transport proteins yptlp and Sec4p [16]. A number of rab gene products have been identified [17], primarily through differential cDNA library screening and/or RACE-PCR. These 21–25 kDa proteins are ~30% homologous to the *ras* gene product, and maintain four highly conserved regions in the open reading frame corresponding to either the GTP-binding region or the intrinsic GTPase activity [12,18].

Cell type specific rab proteins have been described. For example, rab3a is postulated to regulate Ca^{2+}-dependent secretion in the synaptic terminal of neurons [19], neuroendocrine [20], and also some exocrine and endocrine cells [21]; rab3d appears to be localized to glucose transporter-containing vesicles in adipocytes where it may control insulin-dependant exocytosis [22]; rab8 plays a role in membrane traffic from the *trans*-

SSDI 0014-5793(94)00562-A

Golgi network to the basolateral plasma membrane in Maden–Darby Canine Kidney (MDCK) cells [23] and dendritic processes in hippocampal neurons [24]; and rab17 is expressed by epithelial cells localized to the basolateral plasma membrane and apical tubules [25]. These data indicate that specific membrane trafficking programs exist that help achieve terminal differentiation by directing the placement of cell-type specific products.

Here we report the developmentally regulated expression of GTP-binding proteins in oligodendrocytes and myelin. We present data showing that as oligodendrocytes undergo differentiation from early progenitor cells to mature myelin-producing cells, the pattern of expression of small GTP-binding proteins, several of which may be unique to oligodendrocytes and myelin, becomes substantially more complex. These data are interpreted in terms of the predicted alterations in molecular cytoarchitecture as oligodendrocytes differentiate into cells capable of synthesizing and assembling the vast amounts of myelin membrane needed for normal brain development.

2. Materials and methods

2.1. Cell culture

Oligodendrocyte progenitors were collected by overnight shaking [26] of mixed primary cultures derived from perinatal rat telencephalon [4] grown for 11 days. Purified oligodendrocyte populations at specific stages of the lineage were prepared and maintained in a modified defined media [4] on 100 mm tissue culture dishes (Falcon) pre-coated with poly-ornithine. Progenitor expansion and synchronization of early progenitors (positive for ganglioside GD_3 (GD_3^+)) was achieved by the simultaneous addition of 10 ng/ml of both the platelet derived growth factor (PDGF-BB) (a generous gift of Upstate Biotechnology Institute) and basic fibroblast growth factor (bFGF) (Upstate Biotechnology Institute) each day for three days [5]. Later progenitor stage cells were obtained by releasing GD_3^+ populations into medium supplemented with bFGF in the absence of PDGF for two more days [6,7]. Populations of cells in later stages of the lineage (Pre-Galactocerebroside stage (Pre-GalC), Immature OL, Mature OL) were obtained by releasing the cells after five total days in growth factor into defined medium [4] without supplementation with PDGF or bFGF. Pre-GalC stage cells were also prepared by antibody perturbation with RmAb [27]. Each of these blocks is reversible, and upon release the cells resume normal lineage progression and enter terminal differentiation. Lineage stages were defined by the appearance of specific antigens determined by immunofluorescence microscopy ([1]; see below).

Astrocytes were isolated from the shaken mixed primary cultures used to generate oligodendrocytes as previously described [28], with the addition of complement lysis using both monoclonal antibody A2B5 [29] and RmAb [30,31]. Astrocyte populations were > 99% pure as judged by immunoflourescence microscopy with anti-glial fibrillary acidic protein and monoclonal antibody A2B5.

2.2. Immunofluorescence microscopy and antibodies

Detection of stage specific antigens has been described previously [1,27,31,32] (Fig. 1). The following definitions were used to delineate particular stages, using the monoclonal antibodies A2B5, O4, RmAb, O1, and anti-GD_3, and polyclonal anti-myelin basic protein (MBP): $A2B5^+O4^-$, GD_3^+(O-2A) Early Progenitors; $A2B5^+O4^+$, Late Progenitors (Pro-oligodendroblasts); $O4^+RmAb^+O1^-$, cells at the progenitor–oligodendrocyte interface (Pre-GalC); $O4^+RmAb^+O1^+$(GalC$^+$), Immature Oligodendrocytes (Immature OL); $O1^+MBP^+$, Mature Oligodendrocytes (Mature OL). In this particular study, the phenotypes of the analysed populations were as follows: Early Progenitors (97% $A2B5^+$, 3% $O4^+$, < 1% $O1^+$); Late Progenitors (> 85% $O4^+$, 4% R-mAb$^+$, < 1% $O1^+$); Immature OL (97% $O4^+$, > 85% R-mAb$^+$, 40% $O1^+$); More Mature OL (95% $O4^+$, > 85% $O1^+$, 40% MBP$^+$).

2.3. Purification of rat brain myelin

Myelin purification was carried out using a combination of methods [33–35] with some additional steps. Rat brain was homogenized in 0.85 M sucrose (5% w/v), overlayed with 0.32 M sucrose and centrifuged at 28,000 rpm for 60 min in an SW-28 rotor. The interfaces were homogenized in 100 ml 0.85M sucrose, overlaid with 0.32 M sucrose and centrifuged as above. The interfaces were resuspended in 200 ml ddH_2O and centrifuged at 32,000 rpm in a Ti-60 rotor. The pellets were twice resuspended in 10 mM EGTA (pH 7.5), homogenized, brought to 200 ml, stirred for 30 min at 4°C and the suspension was centrifuged at 10,000 rpm for 15 min. The pellets were homogenized in 0.85 M sucrose/2 mM EGTA (pH 7.5), overlayed with 0.32 M sucrose, and centrifuged in a SW-28 rotor at 28,000 rpm for 90 min. The interfaces were collected, and the sucrose washout repeated. The pellets were resuspended in 10 mM EGTA (pH 7.5), homogenized, stirred for 15 min at 4°C and the suspension was pelleted at 10,000 rpm for 15 min. The pellets were resuspended in a minimal volume of 50 mM Tris-HCl (pH 7.5) plus protease inhibitors (1 mM phenylmethyl-sulfonylfluoride, 1 μg/ml pepstatin A, 1 μg/ml antipain, 10 μg/ml aprotinin), snap frozen in liquid N_2 and stored at −80°C. In some experiments, the myelin pellets were homogenized in 1.2 M sucrose, overlaid with steps of 0.75 M, 0.55 M and 0.32 M sucrose in 50 mM Tris (pH 7.5) with 2 mM EGTA, and centrifuged at 28,000 rpm for 18 h. The floated myelin at the 0.75 M/0.55 M interface was collected, centrifuged and stored as above.

2.4. Assay for GTP-binding proteins

Dishes of cells were placed on ice and washed once in 3 ml of cold PBS (pH 7.4) and twice in 3 ml SIEP buffer (250 mM sucrose, 3.0 mM imidazole, 0.5 mM EDTA, 1 mM phenylmethylsulfonylfluoride, 1 μg/ml pepstatin A and antipain (Sigma), 10 μg/ml aprotinin (Calbiochem), pH 7.4). Cells were harvested by scraping them into the small remaining volume of buffer left on the dish plus a scrape of residual material with another 50 μl SIEP. Cells were homogenized by 10 passes through a 25 6/8 gauge needle affixed to a syringe, and centrifuged at 3,000 rpm for 10 min at 4°C in order to pellet the nuclei and cellular debris. The supernatant fraction was centrifuged (Beckman TL-100) at 60,000 rpm for 30 min at 4°C. The resulting membrane pellet was resuspended in a small volume of SIEP and stored at −80°C. Protein content was determined using a Lowry assay kit (Bio-Rad).

Resolution and detection of low molecular weight GTP-binding proteins was carried out by two dimensional gel electrophoresis and [α-^{32}P]GTP overlay blotting as previously described [23] using 50–100 μg of cellular microsomal membrane fraction or myelin membrane total protein per gel. Immunoblotting of specific regions of the resulting nitrocellulose membranes was performed with the enhanced chemiluminesence (ECL) Western blot kit (Amersham). Antisera to rab 3a and 3a/b were obtained from R. Jahn (Yale University), rab 1 and rab 6 from B. Goud (Pasteur Institute, Paris), rap 2 from J. de Gunzburg (INSERM U-248, Paris), ral A from R. Scheller (Stanford University, Stanford), rho A from A. Hall (Univ. College, London).

3. Results

3.1. Expression of small GTP-binding proteins as a function of oligodendrocyte development in culture

Oligodendrocyte cultures at several stages of the developmental lineage were harvested and analyzed for their expression of small GTP-binding proteins by two dimensional electrophoresis followed by [α-^{32}P]GTP-binding and autoradiography. The GTP-binding pattern (Fig. 2) changed from a relatively simple one in the early progenitors ('GD_3^+' progenitors, Fig. 1) to one significantly more complex in mature oligodendrocytes expressing

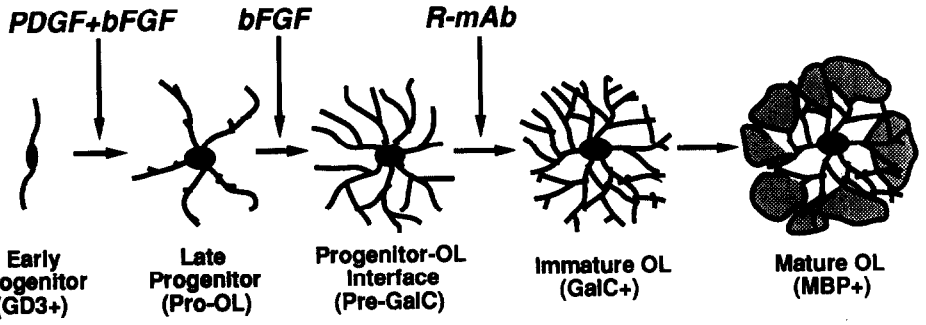

Fig. 1. Developmental lineage of oligodendrocytes. Developmental stages are identified by stage-specific panels of marker expression, some of which are shown in parentheses (see section 2; summarized more extensively in reference [1]). Three stages at which progenitor lineage progression can be reversibly blocked by growth factors (PDGF and bFGF together, or bFGF alone) or antibody perturbation (R-mAb) are indicated by vertical arrows.

later myelinogenic markers ('Mature OLs', Fig. 1). Proteins expressed by early progenitors were expressed throughout the lineage. The bottom panel of Fig. 2 shows a diagrammatic representation of the pattern exhibited by mature oligodendrocytes, with each spot assigned a name or letter designation (detailed below).

Two proteins (spots J and M) were markedly up-regulated as the cells progressed from Early Progenitors to Late Progenitors (Fig. 2). This pattern remained similar through the 'Progenitor-OL Interface' and 'Immature OL' stages, although over-exposure of the films demonstrated the appearance of some new spots in the Immature OL stage. As the cells matured further to the mature OL stage (Fig. 2, 'More Mature OLs') and began expressing late differentiation makers such as proteolipid protein and myelin basic protein, spots J and M were further up-regulated. In addition at this stage, three prominent spots (spots I, H and K) were markedly up-regulated, and ≥ 8 more weakly labeled spots appeared. Therefore, the overall pattern observed demonstrates a sequential up-regulation of additional small GTP-binding proteins during the course of oligodendrocyte lineage progression.

In contrast to oligodendrocytes, the pattern of GTP-binding proteins from extracts of cultures highly enriched for astrocytes (Fig. 2, astrocytes) was relatively simple, containing about 16 spots. One of these spots (asterisk) may be specific for astrocytes.

3.2. *Identification of small GTP-binding proteins expressed by oligodendrocytes*

Some of the small GTP-binding proteins expressed by oligodendrocytes could be identified with varying degrees of certainty by comparison to a GTP-binding protein mobility map generated from an analysis of other cell types, especially MDCK cells and Baby Hamster Kidney cells [23,36] based on over-expression of cloned cDNAs and immuno-blotting, and/or immuno-blotting of material generated in this study (Fig. 2, bottom panel). The two spots labeled A and B are reproducible positioning markers that are conserved among a number of different cell types [23]; the molecular identity of these spots is currently unknown.

Protein identifications presumed strictly on the basis of co-migration with previously identified proteins are indicated by parentheses in the bottom panel of Fig. 2. These include rab 5a,b,c, rab 7, rab 11, rab 22, rac, and rho D. Spots E and F had mobilities that were similar to rab 4 and rab 9, respectively, though due to the large number of spots in this area resolution and identification of these two proteins is difficult without immunostaining. Spots G, L, and N had mobilities previously observed in other cell types, but identification for these proteins is still lacking. A spot in the position expected for rab 8 was not observed.

Those proteins identified by both mobility and immunoblotting with available anti-sera, indicated in Fig. 2 (bottom panel) with an asterisk, are rab 3, rap 2, ral A and rho. Spots I (an early developmentally regulated protein), K (a late developmentally regulated protein), and M migrate at the positions of rab 1a/1b, rab 10, and rab 17, respectively, but failed to immunoblot with antisera to these proteins (see section 4). The Golgi-specific GTP-binding protein rab 6 was demonstrated by immunoblot to be present as a doublet, possessing a different mobility at a slightly higher pI than had previously been observed for this protein in other cell types ([23], Fig. 2).

A number of additional proteins (C, D, H, I, J, P, Q, R, S, T) did not correspond to previously observed examples, and are therefore candidates for oligodendrocyte/myelin-specific low molecular weight GTP-binding proteins.

The pattern of GTP-binding proteins observed for myelin isolated from postnatal day 15 rat brain was compared to that exhibited by mature oligodendrocytes in culture. A noticeable difference was the absence of rab 6 in myelin, and the greater relative amount of rab 3 (both confirmed by immunoblotting). Sequential immunoblotting with antisera to rab 3a and 3a/b suggested that rab 3 in myelin was the 3a subtype. Rab 3a is a known component of regulated exocytic vesicles in syn-

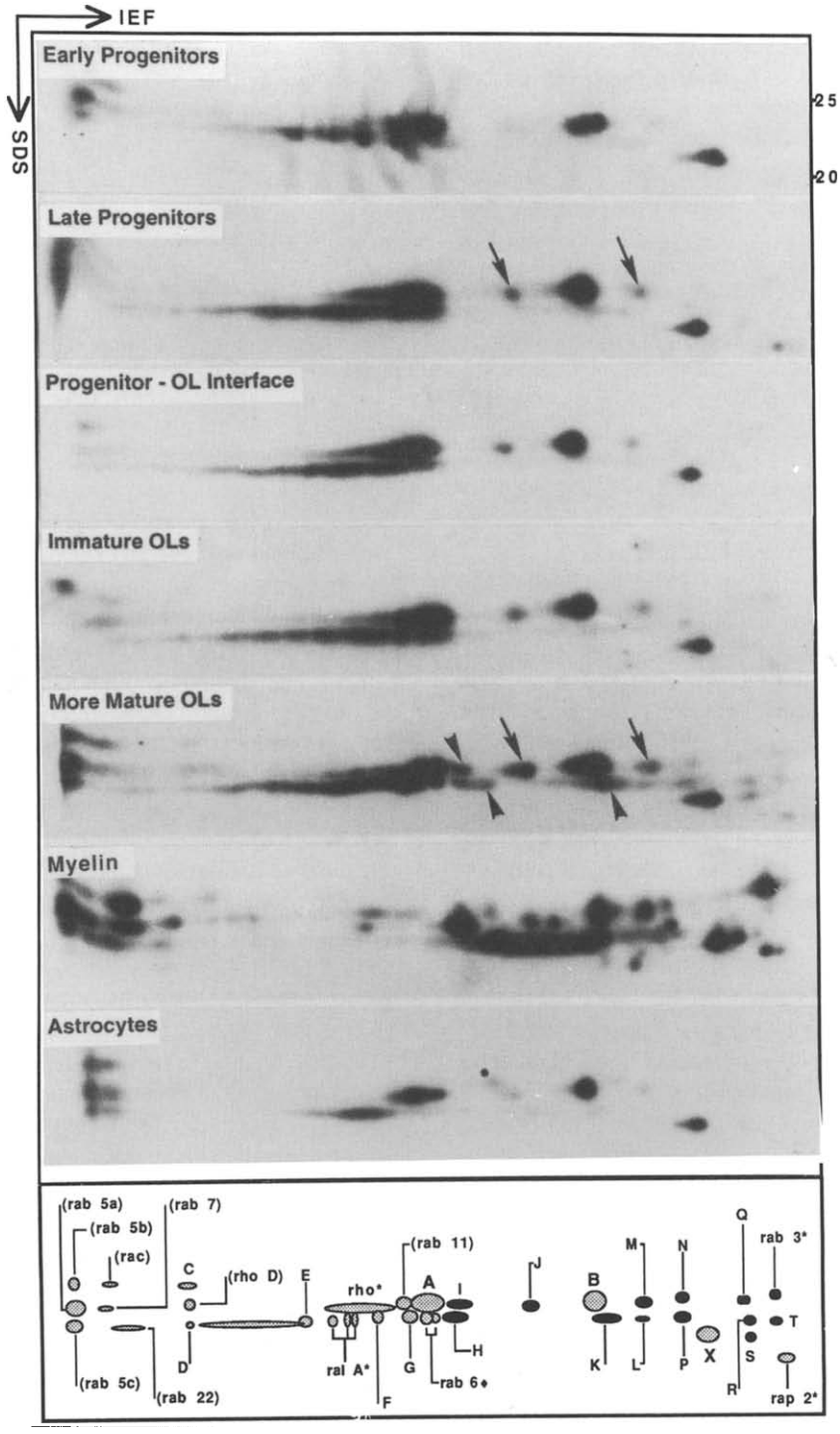

Fig. 2. Patterns of expression of small GTP-binding protein expression during development of oligodendrocytes, myelin and astrocytes. Two-dimensional gel electrophoresis (isoelectric focusing (IEF, pI ~4–9), followed by SDS-PAGE) was followed by [α-^{32}P]GTP overlay and autoradiography. The regions of the gels encompassing a molecular weight range of ~18–28 kDa are shown. Arrows indicate two prominent proteins (J and M) that are up-regulated at the Late Progenitor stage, and further at the More Mature OL stage. Arrowheads indicate three proteins (I, H, and K) in particular that are prominently up-regulated as cells enter the Mature OL stage. A small GTP-binding protein that may be novel to astrocytes is indicated with an asterisk. The continuous nature of the signal below postioning spots A and B in the myelin sample was uncharacteristic, and was resolved into discrete spots in other experiments. The bottom panel is a diagrammatic representation of the autoradiograph from an analysis of More Mature OLs in culture. The GTP-binding proteins have been labeled with specific names when warranted by identification by co-migration (parentheses) or immunoblotting (diamond), or both (asterisks). Other spots have been given arbitrary letter names pending further identification. The hatched symbols indicate GTP-binding proteins that were detected at all stages of oligodendrocyte differentiation; the solid symbols indicate GTP-binding proteins that were up-regulated during oligodendrocyte lineage progression.

aptic nerve terminals and certain exocrine and endocrine cells that is believed to function in the fidelity of the regulated exocytic process [37]. Recent analyses indicate that rab3a expression is regulated in OLs and may constitute an exocytic system in developing OLs and myelin synthesis [38]. Experiments with myelin isolated from adult rat brain produced results similar to post-natal day 15 myelin (data not shown).

4. Discussion

During development, OL progenitors mature from relatively simple cells into multi-processed cells producing and maintaining prodigious amounts of myelin membrane [1]. It is hypothesized that OLs must undergo substantial changes in their molecular cytoarchitecture as they enter terminal differentiation in preparation for myelin membrane biogenesis. Specific among these proposed changes are expected to be up-regulation of components (both general and cell type specific) of the machinery governing vesicular membrane trafficking, including the expression of small GTP-binding proteins involved in the formation and delivery of vesicles bearing proteins and lipids destined for myelin membrane. As expected, the overall result demonstrates substantially increased complexity in the expression of small GTP-binding proteins as the cells prepare for and execute myelin biogenesis.

Small GTP-binding proteins have been identified in brain [21,39,40] and in oligodendrocytes and myelin using one dimensional SDS-PAGE [41,42]. By adding a second dimension of characterization based on isoelectric point, and comparing the results with a substantial body of data obtained from other cell types, we have been able to extend such analyses. The overall GTP-binding pattern during oligodendrocyte development is a progression from a simple pattern to a more complex one as the cells enter terminal differentiation. GTP-binding proteins that are present throughout lineage progression may participate in aspects of vesicular transport common to most or all eucaryotic cells. In this regard, rab 4, 5, 7 and 9 (different compartments of the endocytic pathway), rab 6 (Golgi-associated), rab 11 (secretory pathway), and rap 2 (cytoskeletal-associated) are present throughout OL development. The endocytic proteins were strongly represented in OLs and in myelin extracts, therefore the possibility of endocytosis in myelin must be entertained. The presence of multi-vesicular bodies in myelin has been previously observed [43], which may be structural evidence for this possibility.

Most of the proteins that undergo developmental regulation in OLs are unidentified, and it is hypothesized that these proteins participate in specialized sorting functions associated with a differentiated phenotype. Specifically, two proteins (spots J and M) are up-regulated as the cells progress from early to later progenitors, and are further up-regulated as the cells enter terminal differentiation; three additional proteins (spots I, H and K) are strongly up-regulated only as the cells enter later stages of terminal differentiation; and a group of about 8 additional proteins are also up-regulated late in terminal differentiation, but are less strongly labeled in the overlay assay. This group of unknown developmentally-regulated GTP-binding proteins may participate in pathways specifically associated with trafficking of differentiated OL products into the myelin membrane. One of the known proteins that demonstrates developmental regulation is rab 3a (see results). Though spots K and M migrated similarly to rab 17 and rab 10, technical difficulties with the anti-sera precluded definitive identification of these two proteins. Rab 17 is localized to the basolateral plasma membrane and apical tubules [25]. Rab 8, involved in the specific transport of membrane proteins to the basolateral surface of kidney epithelial cells and to the dendritic membrane domains of hippocampal neurons [24], was notably undetectable.

It is important to note that 'purified' myelin is a biochemical entity, based on a high lipid to protein ratio and thus on the ability to float myelin to a characteristic density on sucrose gradients. The possibility of contamination with other membranes, in particular axonal membranes, is always present. We have addressed this by including in our purification procedures a variety of techniques designed to minimize axonal contamination. These include, in addition to the traditional purification steps, homogenization and incubation in 10 mM EGTA [35] and floating the material to a 0.55 M sucrose step (1.06 g/cc). Even after extensive further purification, the pattern of GTP-binding proteins shown in Fig. 2 was retained, and we conclude that myelin has indeed a rich population of these proteins. This complexity of GTP-binding proteins in myelin, while perhaps surprising in its variety, is consistent with its recognized metabolic activity [44].

In summary, the present data demonstrate an extensive up-regulation in oligodendrocytes of a family of proteins intimately involved in vesicular transport. The developmentally regulated expression of these small GTP-binding proteins correlates well with changes in other parameters of myelinogenesis, including dramatic changes in morphology, and in the synthesis of myelin-specific lipids and proteins. The expression of several previously unrecognized proteins suggests that oligodendrocytes may utilize cell-type specific GTP-binding proteins to effect this unique developmental program that culminates in the biogenesis, and maintenance, of the myelin membrane.

Acknowledgements: We are pleased to acknowledge Kerren Murray for expert technical assistance, and Klaus Fiedler for reviewing the manuscript. DLM is supported by a Combined Degree (MD/PhD) fellowship from the University of Connecticut School of Medicine. This work was

supported supported by a Sonderforschungsbereich of the Deutsche Forschungsgemeinschaft (SFB 352, DFG) (K.S., L.A.H.), a RISE grant from the University of Connecticut Health Center (D.L.M.), and grants from the National Institutes of Health (NS10861) and the National Multiple Sclerosis Society (RG2182) (S.E.P.).

References

[1] Pfeiffer, S.E., Warrington, A.E. and Bansal, R. (1993) Trends Cell Biol. 3, 191–197.
[2] Warrington, A.E. and Pfeiffer, S.E. (1992) J. Neurosci. Res. 33, 338–353.
[3] Raff, M.C. (1989) Science 243, 1509–1524.
[4] Gard, A.L. and Pfeiffer, S.E. (1989) Development 106, 119–132.
[5] Bögler, O., Wren, D., Barnett, S.C., Land, H. and Nobel, M. (1990) Proc. Natl. Acad. Sci. USA 87, 6368–6372.
[6] McKinnon R.D., Toshimitsu, M., Dubois-Dalcq, M. and Aaronson, S.A. (1990) Neuron 5, 603–614.
[7] Gard, A.L. and Pfeiffer, S.E. (1993) Dev. Biol. 159, 618–630.
[8] Rothman, J.E. and Orci, L. (1992) Nature 355, 409–415.
[9] Zerial, M. and Sternmark, H. (1993) Curr. Opin. Cell Biol. 5, 613–620.
[10] Novick, P. and Brennwald, P. (1993) Cell 75, 597–601.
[11] Simons, K. and Zerial, M. (1993) Neuron 11, 789–799.
[12] Pfeffer, S.R. (1992) Trends Cell Biol. 2, 41–46.
[13] Touchot, N., Chardin, P., and Tavitian, A. (1987) Proc. Natl. Acad. Sci. USA 84, 8210–8214.
[14] Zahraoui, A., Touchot, N., Chardin, P. and Tavitian, A. (1989) J. Biol. Chem. 264, 12394–12401.
[15] Chavrier, P., Parton, R.G., Hauri, H.P., Simons, K. and Zerial, M. (1990) Cell 62, 317–329.
[16] Salminen, A. and Novick, P.J. (1987) Cell 49, 527–538.
[17] Chavrier, P., Simons, K. and Zerial, M. (1992) Gene 112, 261–264.
[18] Takai, Y., Kaibuchi, K., Kikuchi, A. and Kawata, M. (1992) Int. Rev. Cytol. 133, 187–230.
[19] Fischer von Mollard, G., Sudhoff, T.C. and Jahn, R. (1991) Nature 349, 79–81.
[20] Darchen, F., Zahraoui, A., Hammel, F., Monteils, M.-P., Tavitian, A. and Scherman, D. (1990) Proc. Natl. Acad. Sci. USA 87, 5692–5696.
[21] Thomas-Reetz, A.C. and De Camilli, P. (1994) FASEB J. 8, 209–216.
[22] Baldini, G., Hohl, T., Lin, H.Y. and Lodish, H.F. (1992) Proc. Natl. Acad. Sci. USA 89, 5049–5052.
[23] Huber, L.A., Pimplikar, S., Parton, R.G., Virta, H., Zerial, M. and Simons, K. (1993a) J. Cell Biol. 123, 35–46.
[24] Huber, L.A., de Hoop, M.J., Dupree, P., Zerial, M., Simons, K. and Dotti, C. (1993b) J. Cell Biol. 123, 47–56.
[25] Lütcke, A., Jansson, S., Parton, R.G., Chavrier, P., Valencia, A., Huber, L., Lentonen, E. and Zerial, M. (1993) J. Cell Biol. 121, 553–564.
[26] McCarthy, K.D. and DeVellis, J. (1980) J. Cell Biol. 85, 890–902.
[27] Bansal, R. and Pfeiffer, S.E. (1989) Proc. Natl. Acad. Sci. USA 86, 6181–6185.
[28] Gard, A.L., Pfeiffer, S.E. and Williams W.C. II (1993) Neuroprotocols 2, 209–218.
[29] Dubois, C., Manuguerra, J., Hauttecouer, B. and Maze, J. (1990) J. Biol. Chem. 265, 2797–2803.
[30] Ranscht, B., Clapshaw, P.A., Noble, M. and Seifert, W. (1982) Proc. Natl. Acad. Sci. USA 79, 2709–2713.
[31] Bansal, R., Warrington, A.E. and Pfeiffer, S.E. (1989) J. Neurosci. Res. 24, 548–557.
[32] Bansal, R. and Pfeiffer, S.E. (1992) J. Neurosci. Res. 32, 309–316.
[33] Norton, W.T. and Poduslo, S.E. (1973) J. Neurochem. 21, 749–757.
[34] Fujimoto, K., Roots, B.I., Burton, R.M. and Agrawal, H. C. (1976) Biochim. Biophys. Acta 426, 659–668.
[35] Haley, J.E., Samuels, F.G. and Ledeen, R.W. (1981) J. Neurochem. 32, 175–187.
[36] Jahn, R. and Sudhoff, T. (1993) J. Neurochem. 61, 12–21.
[37] Huber, L.A., Ullrich, O., Takai, Y., Lütcke, A., Dupree, P., Olkkonen, V., Virta, H., deHoop, M.J., Alexandrov, K., Peter, M., Zerial, M. and Simons, K. (1994) Proc. Natl. Acad. Sci. USA, in press.
[38] Madison, D.L., and Pfeiffer, S.E. (1994) J. Neurosci. Res., in press.
[39] Yamashita, T., Yamamoto, K., Kikuchi, A., Kawata M., Kondo, J., Hishida, T., Teranishi, Y., Shiku, H. and Takai, Y. (1988) J. Biol. Chem. 263, 17181–17188.
[40] Elferink, L.A., Anzai, K. and Scheller, R.H. (1992) J. Biol. Chem. 267, 5768–5775.
[41] Larocca, J.N., Golly, F. and Ledeen, R.W. (1991) J. Neurochem. 57, 30–38.
[42] Braun, P.E., Horvath, E., Yong, V.W. and Bernier, L. (1990) J. Neurosci. Res. 26, 16–23.
[43] Trapp, B.D., Andrews, S.B., Cootauco, C. and Quarles, R. (1989) J. Cell Biol. 109, 2417–2426.
[44] Norton, W.T. and Cammer, W. (1984) in: Myelin (Morell, P., Ed.) pp. 147–195, Plenum Press, NY.