Cell. Mol. Life Sci. 64 (2007) 3261 – 3265
1420-682X/07/243261-5
DOI 10.1007/s00018-007-7381-7

Cellular and Molecular Life Sciences

Visions & Reflections (Minireview)

Phosphoinositides and Charcot-Marie-Tooth disease: New keys to old questions

U. Suter

Institute of Cell Biology, Department of Biology, ETH Zürich, ETH-Hönggerberg, 8093 Zürich (Switzerland), e-mail: usuter@cell.biol.ethz.ch

Received 19 August 2007; received after revision 3 October 2007; accepted 9 October 2007
Online First 29 October 2007

Abstract. Recent research into the genetic basis and the molecular disease mechanisms of Charcot-Marie-Tooth disease (CMT), also called hereditary motor and sensory neuropathies, has highlighted phosphoinositides, membrane-tethered phosphorylated metabolites of phosphatidylinositol, as key regulatory molecules in peripheral nerves in health and disease. Enzymes that dephosphorylate the endosomal phosphoinositides phosphatidylinositol-3-phosphate and/or phosphatidylinositol-3,5-biphosphate, and proteins with binding domains for these phosphoinositides, are mutated in subtypes of CMT. A hypothetical picture emerges suggesting that the precise regulation of phosphoinositide levels within neural cells, a process in turn critical for the correct dynamics of proteins binding to phosphoinositides, is a crucial bottleneck for the accurate function of myelinated peripheral nerves in both neurons and Schwann cells. The underlying molecular and cellular mechanisms are largely unknown. Some hypotheses are discussed in this essay.

Keywords. Charcot-Marie-Tooth disease, phosphoinositides, genetic basis, hypotheses, mechanisms.

Charcot-Marie Tooth disease (CMT) denotes a heterogeneous group of genetic diseases that affect peripheral nerves, leading to pronounced muscular atrophy and weakness of distal limbs. Based on electrophysiological, neuropathological and genetic data, it is thought that the initial causative damage due to the mutation involves only the myelinating Schwann cell in some disease subtypes. In others, only motor and sensory neurons might be affected, and in some cases, both cell types may be involved (for an updated list of the genes and mutations involved in CMT, see http://www.molgen.ua.ac.be/CMTMutations/default.cfm; for detailed information on the different disease subtypes, consult http://www.neuro.wustl.edu/neuromuscular/time/hmsn.html; for summaries of cellular and molecular disease mechanisms, see reviews [1–4]).

Delineating the initial trigger for the disease and the precise consequences at the cellular level are of utmost importance for understanding the underlying disease mechanisms and ultimately for development of promising treatment strategies. In parallel, such studies also provide considerable insights into the biology of myelinated peripheral nerves. The approximately two dozen genes identified today that lead to different forms of CMT present a rich source of proteins that are of eminent importance for the proper function of peripheral nerves. Elucidating the precise roles of these proteins and identifying potentially crucial networks in which these proteins may act in concert is a major challenge for the future. The main topic of this article, membrane-bound phosphorylated phosphatidylinositols (PI, or phosphoinositides) and their regulators and binding partners, appear to be

positioned right at the heart of such a critical node of a regulatory signaling network [5].

The main cell types involved in peripheral nerves that are primarily affected by the various mutant proteins in CMT are very special cells in terms of their cell biology. This notion relates particularly to the peculiar structure of the cells and the tasks that they fulfill. Many peripheral neurons have long axonal projections that may extend up to a meter to their targets, so that these neurons face special cellular challenges with regard to building up and functionally maintaining these elaborate structures. Besides some critical basic issues of membrane and protein maintenance over long distances, general axonal transport of organelles and loaded vesicles must function efficiently in both directions during development and in homeostasis. Not surprisingly, based on the finding that distal muscles are mainly affected in CMT and supportive cell biological data, it has been suggested that deficient axonal transport may be a key issue in many forms of CMT [1]. Conversely, myelinating Schwann cells have the formidable task of producing and maintaining enormous amounts of proteins and lipids in the multi-lamellar myelin sheath. In CMT, it may still be a matter of debate how much of the disease mechanism in the individual subtypes is related to developmental alterations and how much is due to pure neural degeneration after initially normal development. Regardless of this uncertainty, which will hopefully be resolved by careful analysis of suitable animal models combined with patient data, the special challenges of Schwann cells and neurons remain. Throughout development, they have to generate bulk amounts of membranes in a highly coordinated manner. Once the cells are fully differentiated in the adult with little or no cellular turnover, both neurons and myelinating Schwann cells still have to metabolically maintain their proper structures and functions. From a cell biological point of view, another key issue in both nerve development and maintenance is ensuring accurate communication between the different cell types that critically regulates the physiology of neurons and Schwann cells in a bi-directional fashion [6, 7]. On the molecular level, the regulatory mechanisms involved include gene transcription, classical signaling pathways, membrane production, membrane and protein trafficking and function, and cytoskeletal rearrangements. Phosphoinositides have been implicated in many if not all of these crucial cellular processes [5]. Thus, it comes as little surprise given the complex cell biology of the cells involved that disturbance of the phosphoinositide metabolism is deleterious to the proper function of myelinated nerves. On the other hand, one might have expected that global alterations would not be compatible with life, given the broad role of phosphoinositides in different cell types. Is it only the complex requirements of myelinated peripheral nerves that make them particularly vulnerable, or are there also other possible explanations? To find potential answers to this question, it is instructive to have a closer look at the specific CMT-related proteins involved in phosphoinositide metabolism. One such group comprises the enzymes that dephosphorylate phosphatidylinositol-3-phosphate (PI3P) and phosphatidylinositol-3,5-biphosphate [PI(3,5)P2] [5, 8, 9]. These specific phosphoinositides are found on the cytosolic side of different categories of endosomes, and play major functional roles in the complex membrane system that regulates intracellular trafficking of membranes and proteins. PI3P is predominant on early endosomes and serves as a specific address to target proteins with PI3P-binding domains to these vesicles. There these proteins, usually in the form of larger complexes, exert their functions in various processes including membrane fusions and signaling events. PI(3,5)P2 is present in low levels in the late endosome/lysosome compartments. Its function is not well understood in mammalian cells, but might involve the control of membrane recycling between lysosomes and late endosomes.

The levels of different phosphoinositides are regulated by a set of phosphoinositide kinases and phosphatases. Myotubularin-related protein (MTMR)-2 dephosphorylates both PI3P and PI(3,5)P2 specifically at the D3 position and is mutated in recessive CMT4B1 [3, 10]. MTMR2 belongs to a large family of rather ubiquitously expressed proteins. The eight family members with catalytic activity appear to share the same substrate specificity with MTMR2, and many if not all of the disease-associated MTMR2 mutations lead to loss of enzymatic activity when assayed *in vitro*. The six inactive members of the MTMR family tend to form complexes with active MTMRs. The most relevant interaction with regard to CMT is the formation of a tetrameric complex between MTMR2 and MTMR13, since mutations in MTMR13 are associated with recessive CMT4B2 [3, 10]. This form of the disease is pathologically indistinguishable from CMT4B1 and can be modeled in the mouse [11]. Within the MTMR2/MTMR13 complex the phosphatase activity of MTMR2 is enhanced by an order of magnitude [12], consistent with the hypothesis that this enzymatic activity is crucial for the function of MTMR2 in myelinated peripheral nerves [13]. The regulatory situation might be more complex, however, since both MTMR2 and MTMR13 also contain protein domains that bind to phosphoinositides. In general, MTMR2 and MTMR13 are mainly cytoplasmic proteins, also in neurons and Schwann cells, but

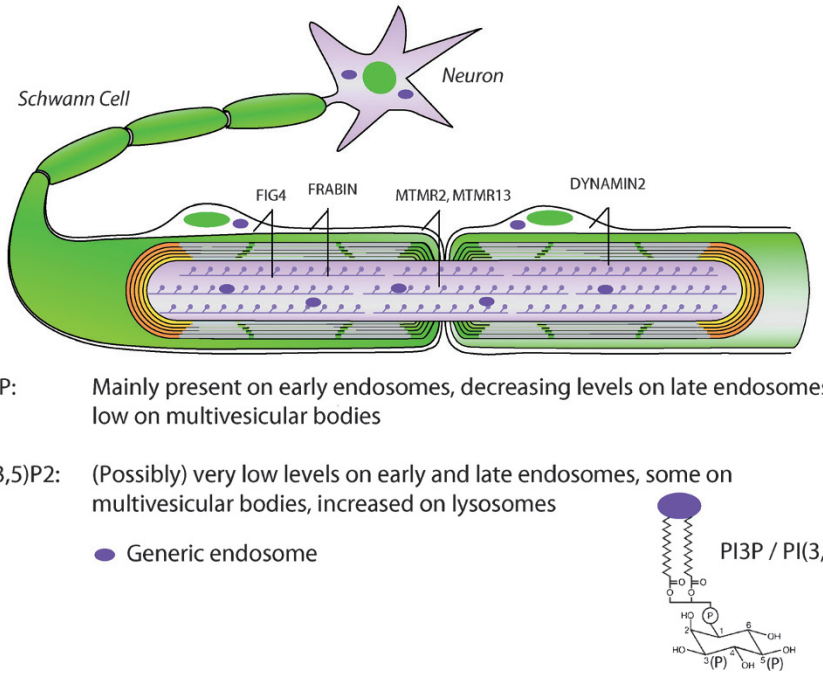

Figure 1. The upper part of the figure shows a schematic cutaway view of a myelinated peripheral nerve fiber. Proteins known to cause CMT that are directly connected with the phosphoinositide metabolism are indicated. They are shown as being expressed in both, neurons and myelinating Schwann cells. This is an assumption based on circumstantial evidence, with the exception of MTMR2 and MTMR13, and needs to be rigorously assessed in future experiments. The exact distribution of endosomes containing PI3P and/or PI(3,5)P2 in the myelinated neuron/axon and in the myelinating Schwann cells is also not worked out. Thus, only generic endosomes are indicated in the schema. In the lower part of the figure, the general structure of the phosphoinositide backbone is shown together with the phosphorylation sites.

binding of MTMR2 and MTMR13 to intracellular membranes has been shown in a heterologous cell system [12]. Based on those data, a model has been proposed in which MTMR2 and MTMR13 might be present both as dimers and as heterotetramers within a cell, but with different affinities for membranes likely mediated by the phosphoinositide-binding domains present in both proteins. It remains an open question how such presumably fine-tuned bindings may lead to changes in phosphoinositide composition on endosomes and, as a potential consequence, to altered localization of the MTMR complexes and other binding proteins. What is clear, however, based on elegant mouse genetic experiments is that MTMR2 deficiency exclusively in Schwann cells is sufficient to generate myelin outfoldings as seen in CMT4B1 and CMT4B2 [14–16]. Such aberrant structures contain redundant myelin membranes that originate during late development of myelinating Schwann cells mainly at the paranodes and Schmidt-Lanterman incisures, regions that contain Schwann cell cytoplasm with endosomes and lysosomes. Paranodes and Schmidt-Lanterman incisures are also thought to be the main regions of the myelin sheath where addition and turnover of myelin membranes and its protein components occur. These findings, together with the known functions of the phosphoinositide substrates of MTMR2, suggest that MTMR2 is involved in the regulation of membrane addition and/or remodeling. Altered membrane trafficking, recycling, or impaired degradation may lead to the observed phenotype. The finding that MTMR2 binds to Dlg1/SAP97, a scaffolding protein involved in membrane biology in other cell types, lends indirect support to this hypothesis [14]. Alternatively or concomitantly, classical signaling pathways might also be altered by the changes in phosphoinositide levels and contribute to the phenotype [17]. Taken together, the available data are consistent with the hypothesis that phosphoinositides and their regulators may control myelin growth in a critical manner providing a potential key to the largely unknown molecular processes that govern how myelin membranes are added and degraded.

Recently, a second active phosphoinositide phosphatase called FIG4 (or Sac3) has been identified as the culprit protein mutated in CMT4J [18, 19]. The initial observations were made in a spontaneous mouse mutant called the “pale tremor mouse”, which shows a syndrome that includes widespread central (CNS) and peripheral (PNS) nervous system neurodegeneration. Of special relevance to CMT, a major loss of sensory neurons is evident during the neonatal period, while other sensory neurons contain large cytosolic vacuoles, suggesting that this stage precedes cell death. Mutant motor neurons develop morphologically similar vacuoles at the age of 6 weeks. The number of large myelinated axons is reduced, consistent with the observed neuronal alterations. Considerably slowed nerve conduction velocities indicate that alterations in myelinated Schwann cells may also be involved. The latter is supported by pathological findings in CMT4J showing, besides prominent axonal loss, thinly myelinated axons and signs of de- and remyelination. Clinically, CMT4J patients are heavily affected, also

by profound neuronal loss in the CNS, with complex genetics. The known patients are compound-heterozygous for FIG4, each carrying a null allele in combination with a particular mutation, I41T, on the other allele. This point mutation affects FIG4 function in yeast, where it causes a partial loss of function. On the molecular level, FIG4 dephosphorylates PI(3,5)P2 at the D5 position, consistent with the observed neuronal vacuoles that are most likely of late-endosomal-lysosomal nature in the FIG4 mutant [20].

One of the critical regulatory functions of phosphoinositides is the recruitment of proteins that contain phosphoinositide-binding modules, resulting in a highly dynamic signaling system. As mentioned earlier, the culprit genes in CMT4B1 (MTMR2) and CMT4B2 (MTMR13), besides being phosphoinositide phosphatases or regulators thereof, also contain phosphoinositide-binding motifs [9]. Recent work revealed that other CMT-causing disease proteins also contain such domains. Frabin/FGD4, an actin-binding guanine nucleotide exchange factor (GEF) for the RhoGTPase Cdc42 and mutated in CMT4H, contains a FYVE domain and two PH domains, motifs known to bind to phosphoinositides [21, 22]. Intriguingly, myelin outfoldings are the hallmark of CMT4H as in CMT4B1/2, hinting towards potentially connected disease mechanisms by a crucial interplay between phosphoinositide-binding proteins and phosphoinositide modulators. Furthermore, dynamin2, a large GTPase that plays a major role in the regulation of membrane dynamics and trafficking by mediating vesicle formation, is mutated in the dominant CMTDIB in which loss of large caliber axons and myelin deficiencies are the pathological features [23]. Most interestingly, the CMTDIB-causing mutations are clustered in a phosphoinositide-binding PH domain, indicating another connection to phosphoinositides [24].

In summary, the current knowledge strongly suggests that phosphoinositide metabolism plays a key role in a variety of CMT subtypes and in peripheral nerve biology. These phospholipids seem to represent critical converging regulatory points due to the unique cellular requirements of neurons and myelinating Schwann cells. Many fine points need to be work out, such as the exact contribution of phosphoinositide-mediated signaling in neurons and Schwann cells, the different forms and dynamics of the regulatory mechanisms, and the contribution and influence of other cellular mechanisms in the complex network of the physiology of the cells. This will require the elucidation of many different aspects of endosome biology with regard to endocytosis, membrane recycling, and the connected regulatory function of endosomes as critical platforms for controlling signal transduction [25]. Likely feedback mechanisms and quantitatively delicately balanced processes will have to be dissected. Novel techniques, in particular with regard to membrane dynamics of proteins and lipids, and especially phosphoinositides, will have to be developed in living cells [26].

One might argue that phosphoinositides have only been implicated in relatively rare subtypes of CMT, and that their general relevance for the more frequent causes of the disease is questionable. I would make a strong case for the theory that the proteins that are mutated in CMT play by definition a crucial role in the complex biology of peripheral nerves, and that the combined analysis of these proteins and their disease-causing forms will eventually converge to a rather coherent picture of the critical regulatory networks involved. Knowing these networks in detail will be beneficial for the understanding of each component and the system of myelinated peripheral nerves as a whole, and in this way contribute substantially to the rational development of potential therapies for diseases affecting the nerves as an integrated unit. To leave the reader with a highly speculative thought in this regard: Altered intracellular transport, biosynthesis and/or degradation have been put forward as potential disease mechanism in some of the most common forms of CMT [2, 3, 27], in particular PMP22 duplications, which account for the majority of cases [1, 28, 29]. Many of these processes are under stringent control of phosphoinositides and their regulators as they are essential players in regulating the endosomal-lysosomal system and autophagy. Maybe there are more connections than obvious at first glace, both related to myelinating cells as well as to neurons and their axon.

Acknowledgements. I thank Ned Mantei for critically reading the manuscript. The work in the lab of the author is supported by the Swiss National Science Foundation and the NCCR "Neural Plasticity and Repair". I thank all my colleagues and past and current lab members who have contributed to this essay by stimulating discussions and publications. I would also like to apologize to all those authors whose work I have not been able to cite here due to space limitations.

1 Suter, U. and Scherer, S. S. (2003) Disease mechanisms in inherited neuropathies. Nat. Rev. Neurosci. 4, 714–726.
2 Niemann, A., Berger, P. and Suter, U. (2006) Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuromol. Med. 8, 217–242.
3 Berger, P., Niemann, A. and Suter, U. (2006) Schwann cells and the pathogenesis of inherited motor and sensory neuropathies (Charcot-Marie-Tooth disease). Glia 54, 243–257.
4 Nave, K. A., Sereda, M. W. and Ehrenreich, H. (2007) Mechanisms of disease: Inherited demyelinating neuropathies – From basic to clinical research. Nat. Clin. Pract. Neurol. 3, 453–464.
5 Di Paolo, G. and De Camilli, P. (2006) Phosphoinositides in cell regulation and membrane dynamics. Nature 443, 651–657.

6 Nave, K. A. and Salzer, J. L. (2006) Axonal regulation of myelination by neuregulin 1. Curr. Opin. Neurobiol. 16, 492–500.
7 Schafer, D. P. and Rasband, M. N. (2006) Glial regulation of the axonal membrane at nodes of Ranvier. Curr. Opin. Neurobiol. 16, 508–514.
8 Roth, M. G. (2004) Phosphoinositides in constitutive membrane traffic. Physiol. Rev. 84, 699–730.
9 Robinson, F. L. and Dixon, J. E. (2006) Myotubularin phosphatases: Policing 3-phosphoinositides. Trends Cell Biol. 16, 403–412.
10 Bolis, A., Zordan, P., Coviello, S. and Bolino, A. (2007) Myotubularin-related (MTMR) phospholipid phosphatase proteins in the peripheral nervous system. Mol. Neurobiol. 35, 308–316.
11 Tersar, K., Boentert, M., Berger, P., Bonneick, S., Wessig, C., Toyka, K. V., Young, P. and Suter, U. (2007) Mtmr13/Sbf2-deficient mice: An animal model for CMT4B2. Hum. Mol. Genet. (in press).
12 Berger, P., Berger, I., Schaffitzel, C., Tersar, K., Volkmer, B. and Suter, U. (2006) Multi-level regulation of myotubularin-related protein-2 phosphatase activity by myotubularin-related protein-13/set-binding factor-2. Hum. Mol. Genet. 15, 569–579.
13 Berger, P., Bonneick, S., Willi, S., Wymann, M. and Suter, U. (2002) Loss of phosphatase activity in myotubularin-related protein 2 is associated with Charcot-Marie-Tooth disease type 4B1. Hum. Mol. Genet. 11, 1569–1579.
14 Bolino, A., Bolis, A., Previtali, S. C., Dina, G., Bussini, S., Dati, G., Amadio, S., Del Carro, U., Mruk, D. D., Feltri, M. L., Cheng, C. Y., Quattrini, A. and Wrabetz, L. (2004) Disruption of Mtmr2 produces CMT4B1-like neuropathy with myelin outfolding and impaired spermatogenesis. J. Cell Biol. 167, 711–721.
15 Bonneick, S., Boentert, M., Berger, P., Atanasoski, S., Mantei, N., Wessig, C., Toyka, K. V., Young, P. and Suter, U. (2005) An animal model for Charcot-Marie-Tooth disease type 4B1. Hum. Mol. Genet. 14, 3685–3695.
16 Bolis, A., Coviello, S., Bussini, S., Dina, G., Pardini, C., Previtali, S. C., Malaguti, M., Morana, P., Del Carro, U., Feltri, M. L., Quattrini, A., Wrabetz, L. and Bolino, A. (2005) Loss of Mtmr2 phosphatase in Schwann cells but not in motor neurons causes Charcot-Marie-Tooth type 4B1 neuropathy with myelin outfoldings. J. Neurosci. 25, 8567–8577.
17 Fili, N., Calleja, V., Woscholski, R., Parker, P. J. and Larijani, B. (2006) Compartmental signal modulation: Endosomal phosphatidylinositol 3-phosphate controls endosome morphology and selective cargo sorting. Proc. Natl. Acad. Sci. USA 103, 15473–15478.
18 Chow, C. Y., Zhang, Y., Dowling, J. J., Jin, N., Adamska, M., Shiga, K., Szigeti, K., Shy, M. E., Li, J., Zhang, X., Lupski, J. R., Weisman, L. S. and Meisler, M. H. (2007) Mutation of FIG4 causes neurodegeneration in the pale tremor mouse and patients with CMT4J. Nature 448, 68–72.
19 Volpicelli-Daley, L. and De Camilli, P. (2007) Phosphoinositides' link to neurodegeneration. Nat. Med. 13, 784–786.
20 Sbrissa, D., Ikonomov, O. C., Fu, Z., Ijuin, T., Gruenberg, J., Takenawa, T. and Shisheva, A. (2007) Core protein machinery for mammalian phosphatidylinositol 3,5-bisphosphate synthesis and turnover that regulates the progression of endosomal transport: Novel Sac phosphatase joins the ArPIKfyve-PIKfyve complex. J. Biol. Chem. 282, 23878–23891.
21 Stendel, C., Roos, A., Deconinck, T., Pereira, J., Castagner, F., Niemann, A., Kirschner, J., Korinthenberg, R., Ketelsen, U. P., Battaloglu, E., Parman, Y., Nicholson, G. et al. (2007) Peripheral nerve demyelination caused by a mutant Rho GTPase guanine nucleotide exchange factor, frabin/FGD4. Am. J. Hum Genet. 81, 158–164.
22 Delague, V., Jacquier, A., Hamadouche, T., Poitelon, Y., Baudot, C., Boccaccio, I., Chouery, E., Chaouch, M., Kassouri, N., Jabbour, R., Grid, D., Megarbane, A. et al. (2007) Mutations in FGD4 encoding the Rho GDP/GTP exchange factor FRABIN cause autosomal recessive Charcot-Marie-Tooth type 4H. Am. J. Hum. Genet. 81, 1–16.
23 Zuchner, S., Noureddine, M., Kennerson, M., Verhoeven, K., Claeys, K., De Jonghe, P., Merory, J., Oliveira, S. A., Speer, M. C., Stenger, J. E., Walizada, G., Zhu, D. et al. (2005) Mutations in the pleckstrin homology domain of dynamin 2 cause dominant intermediate Charcot-Marie-Tooth disease. Nat. Genet. 37, 289–294.
24 McNiven, M. A. (2005) Dynamin in disease. Nat. Genet. 37, 215–216.
25 von Zastrow, M. and Sorkin, A. (2007) Signaling on the endocytic pathway. Curr. Opin. Cell Biol. 19, 436–445.
26 Balla, T. (2007) Imaging and manipulating phosphoinositides in living cells. J. Physiol. 582, 927–937.
27 Zuchner, S. and Vance, J. M. (2006) Mechanisms of disease: a molecular genetic update on hereditary axonal neuropathies. Nat. Clin. Pract. Neurol. 2, 45–53.
28 Fortun, J., Go, J. C., Li, J., Amici, S. A., Dunn, W. A. Jr. and Notterpek, L. (2006) Alterations in degradative pathways and protein aggregation in a neuropathy model based on PMP22 overexpression. Neurobiol. Dis. 22, 153–164.
29 Naef, R. and Suter, U. (1998) Many facets of the peripheral myelin protein PMP22 in myelination and disease. Microsc. Res. Tech 41, 359–371.