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Glycogen metabolism in tissues from a mouse model of Lafora disease

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Abstract

Laforin, encoded by the *EPM2A* gene, by sequence is a member of the dual specificity protein phosphatase family. Mutations in the *EPM2A* gene account for around half of the cases of Lafora disease, an autosomal recessive neurodegenerative disorder, characterized by progressive myoclonus epilepsy. The hallmark of the disease is the presence of Lafora bodies, which contain polyglucosan, a poorly branched form of glycogen, in neurons, muscle and other tissues. Glycogen metabolizing enzymes were analyzed in a transgenic mouse over-expressing a dominant negative form of laforin that accumulates Lafora bodies in several tissues. Skeletal muscle glycogen was increased 2-fold as was the total glycogen synthase protein. However, the −/+glucose-6-P activity of glycogen synthase was decreased from 0.29 to 0.16. Branching enzyme activity was increased by 30%. Glycogen phosphorylase activity was unchanged. In whole brain, no differences in glycogen synthase or branching enzyme activities were found. Although there were significant differences in enzyme activities in muscle, the results do not support the hypothesis that Lafora body formation is caused by a major change in the balance between glycogen elongation and branching activities.

Keywords: Lafora disease; Laforin; Glycogen; Dual specificity phosphatase; Glycogen synthase kinase-3; Branching enzyme; Glycogen synthase

Lafora disease is an autosomal recessive, progressive myoclonus epilepsy (OMIM #254780), with onset typically in teenagers followed by decline and death usually within 10 years [1–3]. The disease is characterized by the presence of Lafora bodies, periodic acid-Schiff positive structures containing an abnormal form of glycogen, the branched polymer of glucose that serves as a stored form of glucose in many tissues. Although Lafora body formation in neurons is believed to account for the symptoms of the disease, the bodies are present in other tissues including liver, muscle and skin [4–7].

Glycogen synthesis (Fig. 1) is mediated by glycogen synthase, which catalyzes formation of the predominant α-1,4-glycosidic linkage of the polymer and branching enzyme, which introduces the α-1,6-glycosidic branchpoints [8]. Degradation occurs in the cytosol through the action of glycogen phosphorylase and the debranching enzyme or alternatively in the lysosome via the activity of an α-glycosidase (acid maltase or GAA).[2] In Lafora disease, a less branched form of glycogen, also called polyglucosan, accumulates and is associated with the Lafora bodies.

Mutations in two genes, *EPM2A* and *EPM2B*, account for approximately 90% of Lafora cases [1,3]. *EPM2A*, encodes a protein, laforin, that belongs to the dual specificity

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[2] *Abbreviations used:* GAA, α-glycosidase; GS, glycogen synthase; BE, branching enzyme; GP, glycogen phosphorylase; DBE, debranching enzyme; GSK3α/β, glycogen synthase kinase-3α/β.

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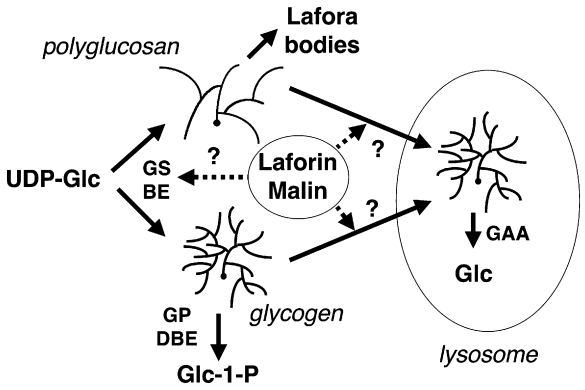

Fig. 1. Glycogen metabolism. Normal glycogen metabolism is shown in the lower part of the figure, with normal activities of glycogen synthase (GS) and branching enzyme (BE) leading to a regularly branched polysaccharide. Its degradation is mediated in the cytosol by glycogen phosphorylase (GP) and debranching enzyme (DBE) or in the lysosome by α-glucosidase (GAA). In Lafora disease, poorly branched glycogen or polyglucosan is formed. Possible sites of action of laforin and malin are indicated. One hypothesis is that they control the balance between branching and elongating activities. A second idea is that laforin in some way monitors the branching state of glycogen, enabling its effective removal, presumably via the lysosome.

protein phosphatase family [9]. Laforin also contains a functional polysaccharide binding domain [10–12] that binds preferentially to polyglucosan over glycogen [13]. Some forty mutations have been identified throughout all four exons of the *EPM2A* gene (http://projects.tcag.ca/lafora/) [9,12,14–17]. Most mutations cause a loss of phosphatase activity in recombinant laforin [11,18]. However, one disease mutation, W32G, in the polysaccharide binding domain blocks glycogen binding [10,11,19] without completely destroying phosphatase activity [10,19]. Therefore, binding to polysaccharide is required for normal laforin function *in vivo* and one might speculate that laforin is somehow involved in glycogen metabolism.

There are other possible links to glycogen metabolism. The *EPM2B* gene (also called NHLRC1) encodes malin, a 395 residue protein that contains an NH_2-terminal RING finger domain [20] characteristic of E3 ubiquitin ligases [21]. Gentry et al. [22] reported that malin interacts with laforin and catalyzes its polyubiquitination and in cultured cells leads to the degradation of laforin. Loss of function mutations in *EPM2B* should thus stabilize laforin making it hard to understand how recessive mutations in either *EPM2A* or *EPM2B* result in the disease. Lohi et al. [23] found that malin interacts also with glycogen synthase and propose that the laforin–malin–glycogen synthase complex is targeted for degradation. In the same study, it was also reported that laforin could dephosphorylate the NH_2-terminal inhibitory phosphorylation site of the protein kinase GSK-3 which should result in glycogen synthase inactivation. Another connection to glycogen came from the yeast two hybrid screen described by Fernandez-Sanchez et al. [18] which identified the type 1 protein serine/threonine phosphatase regulatory subunit, PTG. PTG, also called R5, binds glycogen and is implicated in the control of glycogen metabolism [24]. PTG has been proposed to act as a scaffold, binding also to glycogen synthase, phosphorylase and phosphorylase kinase [24]. Fernandez-Sanchez et al. [18] found that a disease-associated mutation of laforin, G240S, has no effect on either phosphatase activity or glycogen binding but impairs interaction with PTG.

Two mouse models of Lafora disease have been developed [13,25]. Disruption of the mouse Epm2a gene resulted in viable homozygous null mice that accumulated structures similar to Lafora bodies in neurons and other tissues [25]. Lafora bodies began to appear in the brain by two months at which time neurons began to degenerate and die but not by typical apoptotic mechanisms. Behavioral abnormalities were detected at 4 months and by 9 months the animals had myoclonic seizures, ataxia and epileptiform electroencephelogram activity. The second mouse model utilized transgenic over-expression of a dominant negative form of laforin generated by mutating the catalytic Cys266 to Ser [13]. The mice developed Lafora bodies in muscle, liver and neurons and, by immunogold electron microscopy, laforin was shown to be in proximity of the polyglucosan deposits.

Despite these significant advances, the cause of Lafora disease and the reason for the accumulation of Lafora bodies is not understood (Fig. 1). There are two prevailing hypotheses in the field. One idea is that the defect in laforin blocks the breakdown or removal of excess and/or aberrant glycogen molecules [11,13]. Some support for this hypothesis comes from the preferential binding of laforin to poorly branched glycogen [11,13,19], so that laforin may act in quality control of the synthetic process and initiate removal of defective molecules. The second idea is that there may be faulty glycogen synthesis, leading to the abnormal polysaccharide structure and reduced solubility. In this regard, it has been proposed that an imbalance between branching enzyme and glycogen synthase activities may cause the abnormal branching of the polyglucosan. In support of this idea, the GSL30 transgenic mouse that over-expresses constitutively active glycogen synthase in muscle hyper-accumulates glycogen that is less branched [26] and contains structures that resemble Lafora bodies [27]. One fundamental question yet to be answered is whether the activities of the glycogen metabolizing enzymes are affected in tissues that form Lafora bodies. To address this critical question, we analyzed several enzymes involved in glycogen metabolism in tissue from the transgenic mice over-expressing dominant negative laforin [13]. Although there were some statistically significant differences, there was no evidence for a gross imbalance between elongation and branching activities in the transgenic mice.

Experimental procedures

Genetically modified mouse models

Mice over-expressing mutant laforin, described in the Introduction, had gene expression driven by the β-actin promoter [13].