REVIEW / SYNTHÈSE

Glycogen: an overview of possible regulatory roles of the proteins associated with the granule[1]

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Abstract: While scientists have routinely measured muscle glycogen in many metabolic situations for over 4 decades, there is surprisingly little known regarding its regulation. In the past decade, considerable evidence has illustrated that the carbohydrate stores in muscle are not homogeneous, and it is very likely that metabolic pools exist or that each granule has independent regulation. The fundamental aspects appear to be associated with a complex set of proteins that associate with both the granule and each other in a dynamic fashion. Some of the proteins are enzymes and others play scaffolding roles. A number of the proteins can translocate, depending on the metabolic stimulus. These various processes appear to be the mechanisms that give the glycogen granule precise yet dynamic regulation. This may also allow the stores to serve as an important metabolic regulator of other metabolic events.

Key words: carbohydrate, glycogenin, larforin, malin, glycogen synthase.

Résumé : Depuis plus de quatre décennies, les scientifiques ont mesuré systématiquement le glycogène dans de nombreuses conditions métaboliques et curieusement, peu se sont penchés sur sa régulation. Au cours de la dernière décennie, beaucoup d'observations probantes révèlent que les réserves de sucres dans le muscle ne sont pas homogènes et qu'il est fort probable que des pools métaboliques existent et que chaque granule dispose de sa propre régulation. Ces fondements sont issus de la présence d'un ensemble complexe de protéines qui s'associent avec le granule et entre elles de façon dynamique. Certaines des protéines sont des enzymes et d'autres ont une fonction d'échafaud. Un certain nombre de protéines peuvent faire de la translocation selon le stimulus métabolique. Ces divers phénomènes semblent être à la base des mécanismes qui donnent au granule une régulation dynamique spécifique. De cette façon, les réserves peuvent jouer un important rôle de régulation des autres événements métaboliques.

Mots-clés : sucres, glycogénine, larforine, maline, glycogène synthase.

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Introduction

In this overview, I will be addressing the role of regulatory proteins (especially laforin and malin) and the glycogen granule. This may seem to be a very specific focus, but, in fact, it merits at least a full review, rather than this brief prospectus. The understanding of the organization and regulation of the granule is in its infancy, but it is already clear that the complement of proteins associated with the granule and the protein–protein interactions are extremely important. This supports the concept that the granule is indeed an organelle, and that regulation occurs at this level. In other words, all granules are not equal. The implication is that metabolic pools can and likely do exist to serve different roles in the cell. This complicates the science and restricts the ability to interpret investigations in which only total glycogen is measured.

I have been involved with 2 previous reviews (Shearer and Graham 2002, 2004), and in this report I will attempt to focus on the advances in this decade. Insightful work by Bergstrom et al. (1967), Hermansen et al. (1967), Hultman (1967), and Gollnick et al. (1974) in the 1960s and 1970s demonstrated what is now accepted as a fundamental understanding of the responses of muscle glycogen to exercise and diet. Text books list the basic enzymes that are involved: the synthases, phosphatases, and kinases. It is understood that the rate of depletion is exponential during prolonged exercise, that strenuous exercise reduces the glycogen concentration by approximately 80%, and that the subsequent ingestion of carbohydrates can increase it to 50%–80% in excess of the normal resting level. But it is not understood how this regulation is taking place in such a highly regulated manner.

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A number of scientists have shown that the concentration of glycogen can serve as a regulator: higher resting concentrations result in more rapid degradation during exercise, and low concentrations have been associated with an increased expression of a number of metabolic genes. In fact, the concentration of glycogen may be more important as a glucose uptake regulator than insulin itself (Nielsen and Richter 2003; Richter et al. 2001; Hargreaves 2004; Pilegaard et al. 2002). But how is this regulation occurring? Certainly, in this modest manuscript I cannot offer the explanations, but I will endeavour to present information that should add to the understanding of these metabolic events. It is startling to realize that we do not understand the basics of how a granule is formed, and we do not know where they are formed. Are they formed where they ultimately are found as mature granules, or are they synthetized in association with GLUT4 transporters and then moved to their functional locations? Granules are found in specific subcellular locations, suggesting that there are various metabolic pools of glycogen, but we do not understand what these are or how some granules are regulated differently from others.

The granule

The theoretical structure of the granule has been well described (Melendez-Hevia et al. 1993; Melendez et al. 1997). Granules are formed on the protein glycogenin and grow to be as large as 42 nm in diameter in a highly organized manner, consisting of 12 tiers of carbohydrate. The carbohydrate density increases exponentially with each additional tier. Because of the progressive branching, it remains soluble, can contain approximately 55 000 glucosyl units, and the number of nonreducing ends is estimated to be 2100. This allows for fast mobilization by the abundant enzyme, glycogen phosphorylase. Because of the degree of branching with each tier, 34.6% of the total carbohydrate in a granule is in the outermost tier. For the maximal tier 12 granule, this is 19 000 residues. For a granule that has only 1 less tier, this decreases to 9500 residues. Thus, it is surprising that very few granules are found in this size range. Marchand et al. (2002) measured the diameter of approximately 55 000 granules in resting human muscle, and the average diameter was only 25 nm (i.e., approximately tier 8). Very few granules were found that were in the tier 10–12 range, even though increasing granule diameter from tier 9 (30.6 nm) to 12 (42 nm) would increase carbohydrate content from approximately 26 500 to 55 0000 glucosyl units per granule. One would expect that the goal for storage would be to take every granule to the 12 tier (42 nm) size; yet virtually all studies repeatedly report that granules average 20–30 nm diameter. Why would the cell elect to generate more granules instead of expanding the existing ones to their maximum size? What regulatory factors tend to stop synthesis at this mid-range size?

Subcellular pools

Lees et al. (2001) reported that the glycogen in the sarcoplasmic reticulum fraction from muscles fatigued by tetanic contractions decreased to merely 0.5% of the resting level, and the glycogen phosphorylase (GP) activity was reduced to only 4% of control. They speculated that this would compromise calcium homeostasis. Previously Shearer and Graham (2002, 2004) pointed out that various fractions or pools of glycogen granules appear to be degraded preferentially, depending on the exercise conditions. Many researchers (summarized in Shearer and Graham 2002) have employed the terms pro- and macroglycogen for the acid-insoluble and soluble fractions, respectively, and have interpreted these as differing in granule size. This has recently been challenged by James et al. (2009), who demonstrated that the 2 fractions had similar granule size in gel filtration chromatography, and suggested that a portion of the granules are likely trapped by undisrupted myofilaments. Nevertheless, their data are consistent with the notion that one fraction of granules is more responsive to a metabolic stimulus. This may well be based on subcellular location and (or) association with cytoskeletal elements rather than granule size.

Elsner et al. (2002) noted that tiers 9–12 (diameter 30.6 to 42 nm) would contain the majority of the glucosyl units that can be stored in granule. However, in their cell cultures, when glycogen storage was stimulated, the granules only increased from 24.9 nm to 28.1 nm and, similarly, during glycogenolysis, the average diameter decreased to 24.4 nm. The net change in carbohydrate stores led them to predict that the granule diameter should have changed by 11% rather than the observed 4.4%. They concluded that storage was associated with a simultaneous formation of granules, even though the average granule was only at tier 7 or 8, and that glycogen catabolism resulted in total destruction of some granules. This is a strong indication that regulation occurs at the level of the granule and that different metabolic pools exist.

A considerable amount of information from the past decade suggests that a large number of proteins is associated with the granule, and that many of these have regulatory properties. The physiology of these various enzymes and scaffolding proteins may very well be critical in metabolic regulation.

Glycogenin

Glycogenin is the protein that initiates the formation of a granule by catalyzing the addition of 7–11 glucosyl units to a specific tyrosine residue; it then acts as a substrate for glycogen synthase (GS) and the branching enzyme. It is essential for the synthesis of a granule. Thus, one would expect that the concentration of this protein would vital to glycogen stores. However, there have been 3 studies (Mamedova et al. 2003; Yang et al. 2002; Hansen et al. 2000) in which glycogenin was overexpressed in skeletal muscle, and the impact on glycogen stores was minimal. Unfortunately, the number of granules was not evaluated. It would appear that the amount of glycogenin is not a limiting factor in the regulation of glycogen, but the regulation of glycogenin itself is not well understood. For example, we do not know what happens to glycogenin as granules are degraded. Is it degraded or inactivated, or does it remain in an active form? Similarly, just as we do not know if granules can move, we do not know if glycogenin can translocate. This is vital to learning how and where new granules are synthesized.

Translocation of glycogen synthase

In the past decade, there have been important advances in the understanding of the regulation of GS. While the basic

aspects of allosteric regulation have been well established, GS has recently been shown to translocate under different metabolic circumstances. Furthermore, physical association with the enzyme protein phosphatase 1 (PP1) has been demonstrated and, as discussed below, these and other protein–protein interactions appear to be critical in the regulation of the granule.

In 1999, Brady et al. (1999) showed that when cultured adipocytes were exposed to insulin, the increase in GS activity was associated with a marked decrease in cytosolic GS and a simultaneous increase and dephosphorylation (activation) of GS in the granule pool. They speculated that insulin stimulated the translocation and that the activation was facilitated by glucose 6-phosphate enhancing the action of PP1 and allosterically activating GS. Garcia-Rocha et al. (2001) also found that this translocation was dependent on the cytoskeleton in hepatocytes. Subsequently, 2 investigations (Nielsen et al. 2001; Prats et al. 2005) showed that exercise (and low glycogen) causes a similar shift in GS, from the glycogen granule fraction to the membrane and cytosolic fractions. Prats et al. (2005) also demonstrated that the GS translocation was dependent on phosphorylation (inactivation) of specific sites on the protein. Thus, this key enzyme appears to be able to change subcellular pools; this may be facilitated by the cytoskeleton, and its interaction with PP1 may be dependent on the location.

PP1

PP1 is the primary phosphatase acting on the key phosphorylation sites of GS, and its close association with GP allows for reciprocal control of these key enzymes. There are 5 glycogen targeting subunits of PP1, and the most notable are targeting protein glycogen (PTG or R5), R_{Gl} (or G_M), and the liver form, G_L. The investigation of the various targeting subunits is in its infancy, but they have been shown to have a number of key roles. PP1/R_{Gl} is a glycogen–sarcoplasmic-reticulum-associated phosphatase (Aschenbach et al. 2001), suggesting that it could be important in a specific glycogen metabolic pool. While insulin can stimulate GS without an active R_{Gl}, it is essential for the exercise-induced effects on GS and GP (Aschenbach et al. 2001; Suzuki et al. 2001). Similarly, G_L binds to GP more strongly than AMP, and this allows the PP1 to dephosphorylate and inactivate GP and GP kinase. PTG is primarily in insulin-sensitive tissues (Aschenbach et al. 2001), and binds to glycogen and forms complexes with GP kinase, GPa, and GS.

In their review of GS regulation, Nielsen and Richter (2003) addressed mechanisms for how exercise can stimulate GS independent of the insulin-signaling pathway. They pointed out that GS activity is closely coupled to the concentration of glycogen, and speculated that this could be partly due to subcellular location. They concluded that, in glycogen breakdown, the PP1/R_{Gl} complex, and possibly the translocation of GS, is important in the stimulatory regulation of GS during exercise, and that AMP-activated protein kinase (AMPK) and epinephrine (via protein kinase A) are essential for exercise-induced inhibition of GS.

These protein–protein interactions and translocations are important in the regulation of glycogen, and can add specificity to the responses of various metabolic pools. However, it has been difficult to visualize the way the various interactions and translocations occurred. Recent studies involving the proteins laforin and malin have added insight.

Laforin

Recent work with the protein laforin (as well as malin) has provided preliminary information that may well lead to an integration of our understanding of the dynamic nature of many events that have been briefly outlined above. This protein was identified in studies of Lafora disease, a severe neurological condition characterized by Lafora bodies in the neurons and other tissues. These are insoluble, glycogen-like structures in which the carbohydrate chains are not as ordered as in glycogen. There are fewer branches (every 15–30 glucose monomers), and the insoluble structure has been described as starch-like. Laforin is the only known phosphatase to have a carbohydrate binding module (Worby et al. 2006), and its gene is 1 of 2 that are mutated in Lafora disease. Laforin can naturally create stable dimers, which are required for optimal activity (Liu et al. 2006). Worby et al. (2008) found that the enzyme will not dephosphorylate any of the proteins associated with glycogen, such as the targeting subunits of PP1, GS kinase, the branching enzyme, malin, or AMPK. However, it does dephosphorylate complex carbohydrate structures, and they proposed that this enzyme functions to remove phosphate groups from glycogen during synthesis to facilitate branching.

Tagliabracci et al. (2007, 2008) reported that mutation of the laforin gene in mice resulted in the mice developing Lafora bodies, and the muscle glycogen had a 4-fold increase in covalently linked phosphate per molecule. Furthermore, the phosphate groups are found, not merely on the surface, but throughout the granule. They propose that the excessive phosphorylation impaired normal branching of the granule, and that laforin normally functions to dephosphorylate the granule to allow for branching to take place. Fernandez-Sanchez et al. (2003) found that laforin interacts with itself and with PTG, and acts as a molecular scaffold, assembling PP1 with its substrate GS at the granule. Furthermore, laforin must be bound to glycogen to dephosphorylate the granule.

To get a more complete appreciation of the putative roles of laforin, one must also examine the protein malin; once again, much of our understanding comes from investigations of neurons and Lafora disease. Virtually all Lafora disease cases are associated with mutations of genes for laforin and (or) malin. Vilchez et al. (2007) stated that neurons normally do not synthesize glycogen because GS is phosphorylated (inactive) and the laforin–malin complex causes proteasome-dependent degradation of GS and PTG (PTG brings PP1 to GS for activation). Hence, protein–protein interactions and the binding to the granule appear to be even more vital to the normal regulation of glycogen.

Malin

Malin is a newly identified E3 ubiquitin ligase protein, which functions intimately with laforin and is yet another key regulatory protein that binds to PTG. Gentry et al. (2005) pointed out that malin ubiquitinates and targets a number of proteins that regulate glycogen synthesis for pro-

teasome-dependent degradation, and this contributes to the tight control of glycogen metabolism. The complex of laforin and malin downregulates PTG-induced GS activation (Gentry et al. 2005) by ubiqitinating and degrading PTG. Malin can also induce the ubiquitination of laforin itself and the debranching enzyme. These actions would obviously curtail the synthesis of glycogen and the growth of granules. However, to date, there are no reports regarding the responses of laforin or malin to exercise and recovery.

It is noteworthy that the interaction between laforin and malin is modulated by the energy sensor AMPK. AMPK increases the phosphorylation (inactivation) of GS while increasing glucose uptake. It also increases the formation of the laforin–malin complex and, ultimately, the degradation of PTG and the branching enzyme. This may be an important process in integrating the regulation of GS and the regulation of metabolic demand for glycogen.

Worby et al. 2008) reported that overexpression of any of several glycogen targeting proteins (PTG, R6, and G_L) resulted in increased glycogen storage. However, if this was associated with overexpression of either malin or laforin, the enhanced glycogen stores were impeded by 25% and, if coexpressed, the entire PTG effect was blocked. They also found that malin ubiquinates PTG in a laforin-dependent manner. They put forward an attractive hypothesis in which the phosphorylation of the glycogen granule is a byproduct of its synthesis. As the granule forms, PTG and the branching enzyme are targeted to the granule, as is laforin. Laforin then dephosphorylates the granule to facilitate branching. Malin is attracted and ubiquinates laforin, PTG, and the branching enzyme, releasing them from the granule and targeting them for degradation. If their hypothesis is correct, this could account for the reason it is relatively rare to find granules that are approaching the maximum size (tiers 11 and 12).

Potential integration

The multiple protein–protein interactions appear to play major roles in the regulation of the synthesis and degradation of the granule. However, as noted earlier, it has frequently been observed that the magnitude of the glycogen stores is an important regulator and, of course, the stores must be sensitive to energy demands. AMPK is the key kinase for phosphorylation (inactivation) of GS. The beta subunit of AMPK has a glycogen-binding domain (McBride et al. 2009). High glycogen concentration inhibits AMPK activity, indicating that energy availability and energy sensing are inter-related. AMPK binds to nonreducing ends at the surface of the granule. Removal of 1 tier of carbohydrate from the granule releases 30% of available carbohydrate and halves the number of nonreducing ends. This could lead to release of AMPK to phosphorylate protein targets associated with insulin actions (McBride et al. 2009).

PTG has also been proposed as a key regulator of glycogen synthesis as PP1 activates GS and inhibits GP. Vernia et al. (2009) found that AMPK modifies the phosphorylation status of PTG, and they showed that this was specific to the PTG isoform of the glycogen-targeting protein. Thus, if this isoform is found in the granules in 1 subcellular compartment, it would allow those granules to be AMP-sensitive. Vernia et al. (2009) also resolved that the activity of PTG was downregulated by the laforin–malin complex. In addition, AMPK phosphorylation of PTG accelerates its own laforin–malin-dependent ubiquitination and degradation, resulting in a decrease in glycogenic activity.

While investigations of the many proteins and their associations with each other are in their infancy, the results to date shed considerable light on exciting possibilities for the way individual granules could be regulated independently, why granules rarely reach the maximum tier 12 structure, and how the magnitude of the glycogen store may operate to regulate cell metabolism.

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References

Aschenbach, W.G., Suzuki, Y., Breeden, K., Prats, C., Hirshman, M.F., Dufresne, S.D., et al. 2001. The muscle-specific protein phosphatase PP1G/R(GL)(G(M))is essential for activation of glycogen synthase by exercise. J. Biol. Chem. **276**: 39959–39967. doi:10.1074/jbc.M105518200. PMID:11522787.

Bergstrom, J., Hermansen, L., Hultman, E., and Saltin, B. 1967. Diet, muscle glycogen and physical performance. Acta Physiol. Scand. **71**: 140–150. doi:10.1111/j.1748-1716.1967.tb03720.x. PMID:5584523.

Brady, M.J., Kartha, P.M., Aysola, A.A., and Saltiel, A.R. 1999. The role of glucose metabolites in the activation and translocation of glycogen synthase by insulin in 3T3–L1 adipocytes. J. Biol. Chem. **274**: 27497–27504. doi:10.1074/jbc.274.39.27497. PMID:10488084.

Elsner, P., Quistorff, B., Hansen, G.H., and Grunnet, N. 2002. Partly ordered synthesis and degradation of glycogen in cultured rat myotubes. J. Biol. Chem. **277**: 4831–4838. doi:10.1074/jbc.M108226200. PMID:11724782.

Fernandez-Sanchez, M.E., Criado-Garcia, O., Heath, K.E., Garcia-Fojeda, B., Medrano-Fernandez, I., Gomez-Garre, P., et al. 2003. Laforin, the dual-phosphatase responsible for Lafora disease, interacts with R5 (PTG), a regulatory subunit of protein phosphatase-1 that enhances glycogen accumulation. Hum. Mol. Genet. **12**: 3161–3171. doi:10.1093/hmg/ddg340. PMID:14532330.

Garcia-Rocha, M., Roca, A., De La Iglesia, N., Baba, O., Fernandez-Novell, J.M., Ferrer, J.C., and Guinovart, J.J. 2001. Intracellular distribution of glycogen synthase and glycogen in primary cultured rat hepatocytes. Biochem. J. **357**: 17–24. doi:10.1042/0264-6021:3570017. PMID:11415431.

Gentry, M.S., Worby, C.A., and Dixon, J.E. 2005. Insights into Lafora disease: malin is an E3 ubiquitin ligase that ubiquitinates and promotes the degradation of laforin. Proc. Natl. Acad. Sci. U.S.A. **102**: 8501–8506. doi:10.1073/pnas.0503285102. PMID:15930137.

Gollnick, P.D., Piehl, K., and Saltin, B. 1974. Selective glycogen depletion pattern in human muscle fibres after exercise of varying intensity and at varying pedalling rates. J. Physiol. **241**: 45–57. PMID:4278539.

Hansen, B.F., Derave, W., Jensen, P., and Richter, E.A. 2000. No limiting role for glycogenin in determining maximal attainable glycogen levels in rat skeletal muscle. Am. J. Physiol. Endocrinol. Metab. **278**: E398–E404. PMID:10710493.

Hargreaves, M. 2004. Muscle glycogen and metabolic regulation. Proc. Nutr. Soc. **63**(2): 217–220. PMID:15294033.

Hermansen, L., Hultman, E., and Saltin, B. 1967. Muscle glycogen during prolonged severe exercise. Acta Physiol. Scand. **71**: 129–139. doi:10.1111/j.1748-1716.1967.tb03719.x. PMID:5584522.

Hultman, E. 1967. Muscle glycogen in man determined in needle biopsy specimens. Scand. J. Clin. Lab. Invest. **19**(3): 209–217. doi:10.3109/00365516709090628. PMID:6057997.

James, A.P., Barnes, P.D., Palmer, T.N., and Fournier, P.A. 2008. Proglycogen and macroglycogen: Artifacts of glycogen extreaction? Metabolism, **57**: 535–543. PMID:18328357.

Lees, S.J., Franks, P.D., Spangenburg, E.E., and Williams, J.H. 2001. Glycogen and glycogen phosphorylase associated with sarcoplasmic reticulum: effects of fatiguing activity. J. Appl. Physiol. **91**: 1638–1644. PMID:11568145.

Liu, Y., Wang, Y., Wu, C., Liu, Y., and Zheng, P. 2006. Dimerization of laforin is required for its optimal phosphatase activity, regulation of GSK3beta phosphorylation, and Wnt signaling. J. Biol. Chem. **281**: 34768–34774. doi:10.1074/jbc.M607778200. PMID:16971387.

Mamedova, L.K., Shneyvays, V., Katz, A., and Shainberg, A. 2003. Mechanism of glycogen supercompensation in rat skeletal muscle cultures. Mol. Cell. Biochem. **250**: 11–19. doi:10.1023/A:1024980710799. PMID:12962138.

Marchand, I., Chorneyko, K., Tarnopolski, M.A., Hamilton, S., Shearer, J., Potvin, J.R., and Graham, T.E. 2002. Quantification of subcellular glycogen in resting human muscle: granule size, number and location. J. Appl. Physiol. **93**(5): 1598–1607. PMID:12381743.

McBride, A., Ghilagaber, S., Nikolaev, A., and Hardie, D.G. 2009. The glycogen-binding domain on the AMPK beta subunit allows the kinase to act as a glycogen sensor. Cell Metab. **9**: 23–34. PMID:19117544.

Melendez, R., Melendez-Hevia, E., and Cascante, M. 1997. How did glycogen structure evolve to satisfy the requirement for rapid mobilization of glucose? A problem of physical constraints in structure building. J. Mol. Evol. **45**: 446–455. doi:10.1007/PL00006249. PMID:9321423.

Melendez-Hevia, E., Waddell, T.G., and Shelton, E.D. 1993. Optimization of molecular design in evolution of metabolism: the glycogen molecule. Biochem. J. **295**: 477–483. PMID:8240246.

Nielsen, J.N., and Richter, E.A. 2003. Regulation of glycogen synthase in skeletal muscle during exercise. Acta Physiol. Scand. **178**: 309–319. doi:10.1046/j.1365-201X.2003.01165.x. PMID:12864735.

Nielsen, J.N., Derave, W., Kristiansen, S., Ralston, E., Ploug, T., and Richter, E.A. 2001. Glycogen synthase localization and activity in rat skeletal muscle is strongly dependent on glycogen content. J. Physiol. **531**: 757–769. doi:10.1111/j.1469-7793.2001.0757h.x. PMID:11251056.

Pilegaard, H., Keller, C., Steensberg, A., Helge, J.W., Pedersen, B.K., Saltin, B., and Neufer, P.D. 2002. Influence of pre-exercise muscle glycogen content on exercise-induced transcriptional regulation of metabolic genes. J. Physiol. **541**: 261–271. doi:10.1113/jphysiol.2002.016832. PMID:12015434.

Prats, C., Cadefau, J.A., Cusso, R., Qvortrup, K., Nielsen, J.N., Wojtaszewki, J.F.P., et al. 2005. Phosphorylation-dependent translocation of glycogen synthase to a novel structure during glycogen resynthesis. J. Biol. Chem. **280**: 23165–23172. doi:10.1074/jbc.M502713200. PMID:15840572.

Richter, E.A., Derave, W., and Wojtaszewski, J.F.P. 2001. Glucose, exercise and insulin: emerging concepts. J. Physiol. **535**: 313–322. doi:10.1111/j.1469-7793.2001.t01-2-00313.x. PMID:11533125.

Shearer, J., and Graham, T.E. 2002. New perspectives on the storage and organization of muscle glycogen. Can. J. Appl. Physiol. **27**(2): 179–203. PMID:12179957.

Shearer, J., and Graham, T.E. 2004. Novel aspects of skeletal muscle glycogen and its regulation during rest and exercise. Exerc. Sport Sci. Rev. **32**: 120–126. doi:10.1097/00003677-200407000-00008. PMID:15243208.

Suzuki, Y., Lanner, C., Kim, J.-H., Vilardo, P.G., Zhang, J., Cooper, L.D., et al. 2001. Insulin control of glycogen metabolism in knockout mice lacking the muscle-specific protein phosphatase PP1G/RGL. Mol. Cell. Biol. **21**(8): 2683–2694. doi:10.1128/MCB.21.8.2683-2694.2001. PMID:11283248.

Tagliabracci, V.S., Turnbull, J., Wang, W., Girard, J.M., Zhao, X., Skurat, A.V., et al. 2007. Laforin is a glycogen phosphatase, deficiency of which leads to elevated phosphorylation of glycogen in vivo. Proc. Natl. Acad. Sci. U.S.A. **104**: 19262–19266. doi:10.1073/pnas.0707952104. PMID:18040046.

Tagliabracci, V.S., Girard, J.M., Segvich, D., Meyer, C., Turnbull, J., Zhao, X., et al. 2008. Abnormal metabolism of glycogen phosphate as a cause for Lafora disease. J. Biol. Chem. **283**(49): 33816–33825. PMID:18852261.

Vernia, S., Solaz-Fuster, M.C., Gimeno-Alcaniz, J.V., Rubio, T., Garcia-Haro, L., Foretz, M., et al. 2009. AMP-activated protein kinase phosphorylates R5/PTG, the glycogen trageting subunit of R5/PTG-PP1 holoenzyme and accelerates its downregulation by the laforin-malin complex. J. Biol. Chem. **284**(13): 8247–8255. doi:10.1074/jbc.M808492200. PMID:19171932.

Vilchez, D., Ros, S., Cifuentes, D., Pujadas, L., Valles, J., Garcia-Fojeda, B., et al. 2007. Mechanism suppressing glycogen synthesis in neurons and its demise in progressive myoclonus epilepsy. Nat. Neurosci. **10**: 1407–1413. PMID:16901901.

Worby, C.A., Gentry, M.S., and Dixon, J.E. 2006. Laforin, a dual specificity phosphatase that dephosphorylates complex carbohydrates. J. Biol. Chem. **281**: 30412–30418. doi:10.1074/jbc.M606117200. PMID:16901901.

Worby, C.A., Gentry, M.S., and Dixon, J.E. 2008. Malin decreases glycogen accumulation by promoting the degradation of protein targeting to glycogen (PTG). J. Biol. Chem. **283**(7): 4069–4076. doi:10.1074/jbc.M708712200. PMID:18070875.

Yang, R., Cao, L., Gasa, R., Brady, M.J., Sherry, A.D., and Newgard, C.B. 2002. Glycogen-targeting subunits and glucokinase differentially affect pathways of glycogen metabolism and their regulation in hepatocytes. J. Biol. Chem. **277**: 1514–1523. doi:10.1074/jbc.M107001200. PMID:11600496.