The dynamic life of the glycogen granule

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Glycogen, the primary storage form of glucose, is a rapid and accessible form of energy that can be supplied to tissues on demand. Each glycogen granule, or "glycosome," is considered an independent metabolic unit composed of a highly branched polysaccharide and various proteins involved in its metabolism. In this Minireview, we review the literature to follow the dynamic life of a glycogen granule in a multicompartmentalized system, *i.e.* the cell, and how and where glycogen granules appear and the factors governing its degradation. A better understanding of the importance of cellular compartmentalization as a regulator of glycogen metabolism is needed to unravel its role in brain energetics.

Glycogen granules and their metabolic regulation differ widely between tissues, cell types, and even between intracellular compartments. Although the biochemical pathways of glycogen synthesis and degradation are similar between tissues, the enzymes involved and their regulation are uniquely adapted to the metabolic demands of each cell type (Table 1).

Each glycogen granule contains carbohydrate with a diverse complement of associated regulatory proteins, considered together as an organelle-like structure or "glycosome" (1). This Minireview is designed to summarize and integrate the existing physiology, cell biology and biochemical literature to propose a generalized model for the dynamic "life" of a glycogen granule or glycosome. Although this series of Minireviews is focused on the brain, a great deal of the understanding of the granule comes from investigations of muscle glycogen. Unless specified otherwise, the information in this Minireview originates from skeletal muscle; however, the overriding principles should apply to the brain as well.

The glycosome

Glycogen granules are composed of protein-glycogen (2). Three types of glycogen structures have been identified by EM and termed α- and β-granules and γ-particles (1, 3) (Fig. 1*A*). The α-granules are mainly found in liver and are formed by several β-granules arranged in a broccoli-like fashion. The β-granules are individual glycogen granules, which include several γ-particles, 3 nm protein-rich subunits that are highly electrodense after staining with lead and uranyl acetate (4) (Fig. 1, *B* and *C*). The β-granules are considered a rapid energy source and are ~20–30 nm in diameter and ~10^6–10^7 in molecular weight, whereas the liver α-granules are considered a slower energy source (5) and can be as large as 300 nm in diameter and of >10^8 in molecular weight (1, 3). The molecular structure of a β-granule includes a central priming protein, glycogenin (GN)[3] (6), covalently bound to the glucose polymer, which is formed by chains of ~13 glucose residues bound through α-1,4-glycosidic linkages and interconnected by α-1,6-glycosidic linkages at branching points (7).

According to the tiered model (8) for glycogen organization, β-granules are organized as concentric layers of glucose chains (tiers). The theoretical maximum size for an independent β-granule has been set to 42 nm, *i.e.* to consist of 12 tiers (9). Because the amount of glucose residues stored doubles in each increasing tier, a hypothetical 13th tier has been estimated to be physically impossible due to spatial constraints (7, 8, 10). In liver, several β-granules can form an α-granule; however, the process by which β-granules aggregate and the nature of the linkage between them remain elusive (11, 12). Based on the observation that GN accounts for 0.0025% of liver glycogen mass, 200-fold lower GN content than muscle glycogen (13), it was initially suggested that one GN molecule could prime the synthesis of several β-granules, forming an α-granule (6). However, the majority of the literature indicates that the β-granules in an α-granule are formed independently. EM studies of mouse liver glycogen during cycles of fasting/feeding show that when liver glycogen reaches a maximum concentration, it consists almost entirely of β-granules, which later form α-granules by binding via a protein backbone (5, 12, 14) capable of forming disulfide bonds (15). Further studies are needed to unravel the sequential processes involved in the initiation and formation of α-granules.

Glycogen fractions according to chemical properties

In addition to GN, many different proteins have been identified as part of the glycogen granule proteome (Table 2). The protein composition and/or the ratio of protein to carbohy-

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[3] The abbreviations used are: GN, glycogenin; GS, glycogen synthase; GBE, glycogen-branching enzyme; GDE, glycogen-debranching enzyme; GP, glycogen phosphorylase; SR, sarcoplasmic reticulum; PTG, protein targeting to glycogen; AMPK, 5′-AMP-activated protein kinase.

Table 1
Overview of differences in glycogen levels and metabolism in brain, skeletal muscle, and liver
Specialization of different tissues and cells types has led to the diversification of glycogen metabolism regulation. An overview of the main differences in glycogen content, inter- and intracellular localization, and glycogen-related regulatory enzyme expression in brain, muscle, and liver are presented.

Attributes	Brain	Skeletal muscle	Liver
Average particle size (inner diameter, nm)	10–30	10–40	110–290
Glycogen concentration (human, μmol/g wet weight)	3–10	30–100	100–500
Estimated % of tissue weight	0.1	1–2	6–8
Estimated whole organ content (human, fed state, g)	0.5–1.5	100	400
Tissue localization	Regional variability. Gray matter > white matter	Muscle type-dependent. Type II > type I	Uniform
Cellular/subcellular localization	Cell-dependent, highest in astrocytes. Greater in areas with high synaptic density, primary branches and fine processes	Subsarcolemmal > myofibrillar	Hepatocytes. subcellular location modulated by metabolic conditions
Glycogenin isoform	GN1	GN1	GN2
Glycogen phosphorylase isoform	GPB	GPM	PGPL
	GPM		
Glycogen synthase isoform	GS1	GS1	GS2

drate within a glycogen granule and its association with cellular compartments affect its solubility in acid. As early as 1934, two fractions of glycogen were described according to their solubility in boiling water or cold TCA; the extractable fraction was named lyoglycogen, and the nonextractable fraction was named desmoglycogen (16). Desmoglycogen was reported to include glycogen granules associated with filaments and/or sarcoplasmic reticulum, and lyoglycogen included free unbound granules (17). Cells with a high content of glycogen contained mainly lyoglycogen, whereas the proportion of desmoglycogen increased as cell glycogen was depleted (18). The solubility of glycogen to acid treatment was also used to propose the existence of two distinct populations of glycogen granules, termed pro- and macro-glycogen (19–21). The acid-insoluble form, pro-glycogen, was thought to consist of smaller granules (1–8 tiers), which were the substrate for subsequent addition of carbohydrates, eventually becoming acid-soluble macro-glycogen (granule size >400,000 Da) (21, 22). Subsequently, James *et al.* (23) reported that alterations in the extraction conditions influenced the proportions of acid-soluble/insoluble glycogen and that these fractions did not correspond to specific granule sizes. Nevertheless, there is clear evidence in the literature of the existence in the cell of acid-labile (desmoglycogen/pro-glycogen) and acid-soluble (lyoglycogen/macro-glycogen) glycosomes, which within this Minireview will be termed acid-soluble and acid-insoluble fractions.

The birth of a new glycogen granule

Biosynthesis of a new glycogen granule is initiated by GN dimerization and autoglycosylation (Fig. 2*A*). GN autoglycosylation occurs in two sequential steps: initial intermolecular glycosylation and subsequent intramolecular glucose chain lengthening (Fig. 2*A*) (24, 25). During intermolecular glycosylation, GN catalyzes the transfer of glucose from UDP-glucose to Tyr-194 of a separate GN molecule, forming a 1-*O*-tyrosyl linkage (Fig. 2*B*) (24, 25). GN then catalyzes the addition of 6–17 additional glucose residues onto the *O*-linked glucose, forming α-1,4-glucosidic linkages (25). Dimerization of the proteins is weak, potentially allowing the units to separate after the initial intermolecular glycosylation reaction (26). GN binds to a conserved amino acid sequence in the C-terminal domain of glycogen synthase (GS) (27, 28). In skeletal muscle, GS and GN are expressed in equimolar amounts, suggesting an average of one GS molecule associated with each glycogen granule *in vivo* (28). As the glycogen granule grows, GS unbinds from GN and binds the polysaccharide through a glycogen-binding site at its C terminus (6), adding new glucosyl residues to the outer polysaccharide chains. Once the initial chains are formed, the glycogen-branching enzyme (GBE) cuts the distal end of the newly-formed chain and attaches it to a glucose residue from the older existing chain through an α-1,6 linkage (Fig. 2*C*) (29). The coordinated action of GS and GBE create the spherical and branched structure of the granule, which ensures solubility and creates a hydrophilic surface necessary to reduce the osmotic pressure exerted by each granule (30, 31).

The question arises as to where and when new glycogen granules are initiated and how this process is regulated. Accumulating evidence in the literature indicates that the start of

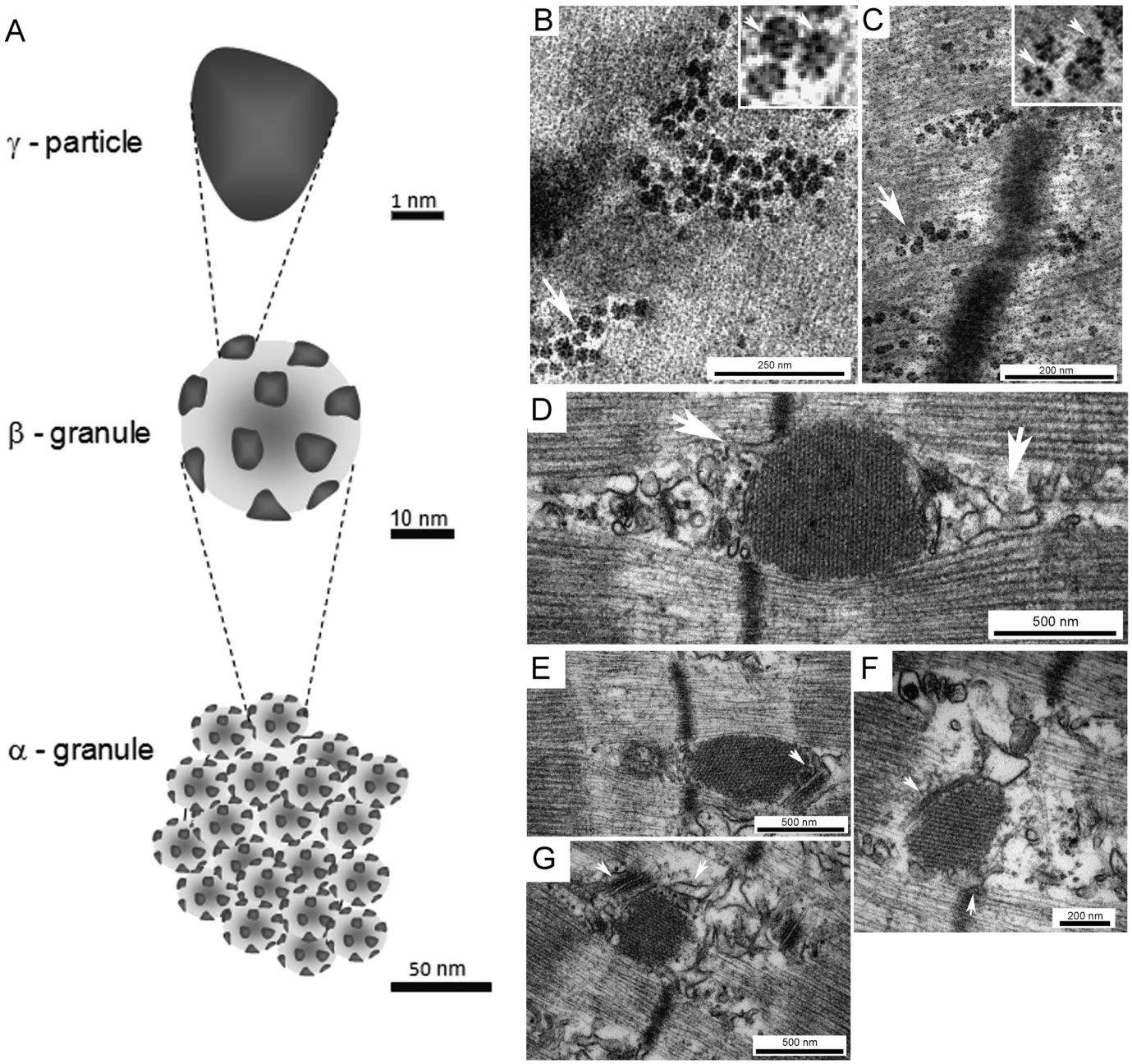

Figure 1. Glycogen granule and the actin-rich spherical structures: EM observations. Analysis of glycogen granules by transmission EM has led to the identification of three structural entities: the γ-particle, the β- granule, and the α-granule (*A*). The γ-particle is highly electrodense after lead and uranyl acetate staining visualized as black clusters in the magnified arrow-marked glycogen granules (*top-right corner*) (*B* and *C*). The β- granule includes the carbohydrate polymer and the bound γ-particles, and the α-granule is composed of several β-granules bound via a protein backbone rich in disulfide bonds. At the start of glycogen re-synthesis after severe storage depletion, actin-rich spherical structures form (37), which in skeletal muscle are visualized by transmission EM as electrodense structures at the I-band of the sarcomeres in close proximity to the sarcoplasmic reticulum and transverse tubuli (*D–G, white arrows*). *Scale bars, B*, 250 nm; *C* and *F*, 200 nm; and *D, E,* and *G*, 500 nm.

each glycogen granule is associated with the actin cytoskeleton, with actin binding a conserved DNIKKKL C-terminal domain of GN. Disruption of the actin cytoskeleton by cytochalasin D leads to granule dispersion (32), explaining previous observations of alignments of glycogen granules associated with cytosolic filaments (1). Interestingly, several independent studies have reported that the initial stages of glycogen re-synthesis after episodes of low glycogen levels are linked to cytosolic actin-rich spherical structures. Cid *et al.* (33) reported GS intranuclear localization in cultured human muscle and in 3T3-L1 cells under low glucose conditions and GS translocation to cytoplasmic spherical structures upon glucose administration. In response to glucose administration after glycogen depletion, similar structures have been reported in different cell types, among them *Saccharomyces cerevisiae* (34), 3T3-L1 adipocytes (35), hepatocytes (36), and rabbit and human skeletal muscle (37, 38). A combination of light and EM was used to characterize the spherical structures resulting from dynamic actin cytoskeleton rearrangement in low glycogen conditions, a process that required 1.5 h and was a prerequisite for glycogen

re-synthesis to start (37). These observations are in agreement with previous studies reporting that glycogenolysis results in the release of dissociated GN and GS to the cytoplasmic fraction; *in vitro*, 50% re-association of these two proteins took hours (28). Altogether, the start of new glycogen granules is associated with and regulated by the actin cytoskeleton; however, further studies are needed to understand the regulation and dynamics of the molecular pathways involved.

Glycogen storage (granule size *versus* number)

Changes in cell glycogen content may respond to changes in the size and/or number of granules. Given that the amount of carbohydrate storage increases exponentially with each tier, one might assume that to maximize storage efficiency granules would grow to their maximal size (~42 nm). Remarkably, most investigations of granule size in brain, liver, skeletal, and cardiac muscle have consistently reported a continuum of sizes, ranging from 10 to 44 nm in diameter, with an average granule diameter of roughly 25 nm (5, 39). Thus, most of the glycogen granules contain only about 20% of the theoretical maximum amount of carbohydrate that they could store. In agreement, Elsner *et al.* (40) determined granule size in cultured myotubes before and after insulin stimulation, and they showed that only 33% of the insulin-stimulated glucose uptake was used to increase average granule size (from 24.9 to 28.1 nm in diameter). Similarly, labeled glucose experiments in liver showed that granules that were synthesized early after glucose administration stopped growing, and other granules were then formed, rather than enlarging the previously formed granules to their maximal capacity (41). In the brains of mice, Oe *et al.* (42) have shown that glycogen granules are mainly localized in the primary branches and fine processes of cortical and hippocampal astrocytes (Table 1) and that the presence of large glycogen granules (~15 MDa (43)) is unique to glycogen-rich areas. Altogether, the growth of glycogen granules seems to be coordinated and limited in some manner, so that glycogen granules grow only modestly in size, rarely reaching their theoretical maximal capacity.

Another potential strategy for cells to up-regulate glycogen storage capacity could be to increase GN protein expression (6). 90% of GN has been reported to be isolated with the glycogen fraction in skeletal muscle (28), which suggests that the reservoir of un-primed GN available may be a limiting factor for cell glycogen storage capacity; however, that does not turn out to be the case, because overexpression of GN in COS-1 cells and rat fibroblasts with unlimited glucose supply had no effect on total cellular glycogen content. Not surprisingly, the overexpression did lead to an increased number of smaller glycogen granules (44, 45), but with no effect on the overall storage capacity. Consistent with the above observations, training-induced increases in glycogen storage in rodents showed no significant correlation with GN protein levels or activity (46, 47). Supporting the existence of coordinated mechanisms regulating the number and size of glycogen granules, Montori-Grau *et al.* (48) showed that the overexpression of different protein phosphatase 1 (PP1) glycogen targeting subunits in cultured myotubes resulted in distinct alterations in both granule numbers and size. Overexpression of any of the targeting subunits resulted in

Table 2
Primary proteins in the glycogen granule proteome and their interactions
List of main human glycosome-associated proteins as identified by name and UNIPROT identifier (ID) as well as primary interactions relevant to glycogen metabolism. Protein–protein interactions were derived from databases within UniProt (uniprot.org) (80), mainly The Biological General Repository for Interaction Datasets (BioGrid) (81) and the Protein Interaction Database and Analysis System (IntAct) (82). Abbreviations used are as follows: AMPK, 5′-AMP-activated protein kinase; EMP2A, laforin; GBE, branching enzyme; GDE, debranching enzyme; GN, glycogenin; GP, glycogen phosphorylase; GS, glycogen synthase; PhK, phosphorylase kinase; PP1, protein phosphatase; STDB1, starch-binding domain-containing protein 1; TRIM7, tripartite motif-containing protein.

Protein	Role	UniProt ID	Key glycogen-related interactions
Glycogenin (GN)	Initiation	P46976 (GN1, muscle) O15488 (GN2, liver)	GS, AMPK, GBE, GP, STBD1, PP1 (PPP1R3C, PPP1CA, PPP1R5), TRIM7
Tripartite motif-containing protein (TRIM7, GNIP)	Initiation, regulation	Q9C029 (TRIM7)	GN
Glycogen synthase (GS)	Synthesis	P13807 (GS1, muscle) P54840 (GS2, liver)	GN, AMPK, GBE, PP1 (PP1R3B, PPP1R3C, PPP1CA), STBD1, KAPCA, CSK21, MAPKAPK2, GSK3, PAST, laforin, MLP3B/3C, DYRK
Glycogen branching enzyme (GBE)	Synthesis	Q04446	GP, GS, GN, STBD1, GBE, VAPA
Glycogen phosphorylase (GP)	Degradation	P11217 (PYGM, muscle) P11216 (PYGB, brain) P06737 (PYGL, liver)	AMPK, PKC, GBE1, PP2, MAPKAPK2, PP1
Glycogen debranching enzyme (GDE, AGL)	Degradation	P35573	AMPK, PP1, malin, AMPK, STBD1
Malin (E3 ubiquitin-protein ligase NHLRC1)	Ubiquitin ligase	Q6VVB1	Laforin, GS, PP1, GDE, AMPK
5′-AMP-activated protein kinase (AMPK)	Kinase	P54646 (α2) Q9Y478 (β1) O43741 (β2)	Laforin, PP1 (PPP2CA, PPP2R1B), PHKG2, CAMK, GN
Laforin (EPM2A)	Carbohydrate phosphatase, ubiquitin ligase	O95278	AMPK, PP1 (PPP1R3C, PPP1R3D), GSK3B, STBD1, GS, malin,
Protein phosphatase I (PP1) and targeting subunits	Phosphatase, main regulatory and catalytic subunits	PP1	AMPK, laforin, GSK3B, GN, GS
		Q16821 (PPP1R3A, GM) Q86X16 (PPP1R3B, GL) Q0VCR4 (PPP1R3C, PTG) O95685 (PPP1R3D, PPP1R6) P67775 (PPP2CA) P62136 (PPP1CA) P30154 (PPP2R1B)	
Phosphorylase kinase (PhK)	Kinase	P15735 (γ, liver, testis) Q16816 (γ, muscle)	AMPK, PP1 (PPP1R3B), GP
Starch-binding domain- containing protein 1 (STBD1)	Cargo receptor for glycogen	O95210	GDE, GBE, PP1, malin, GN, GS, AMPK, GP, MLP3B/3C

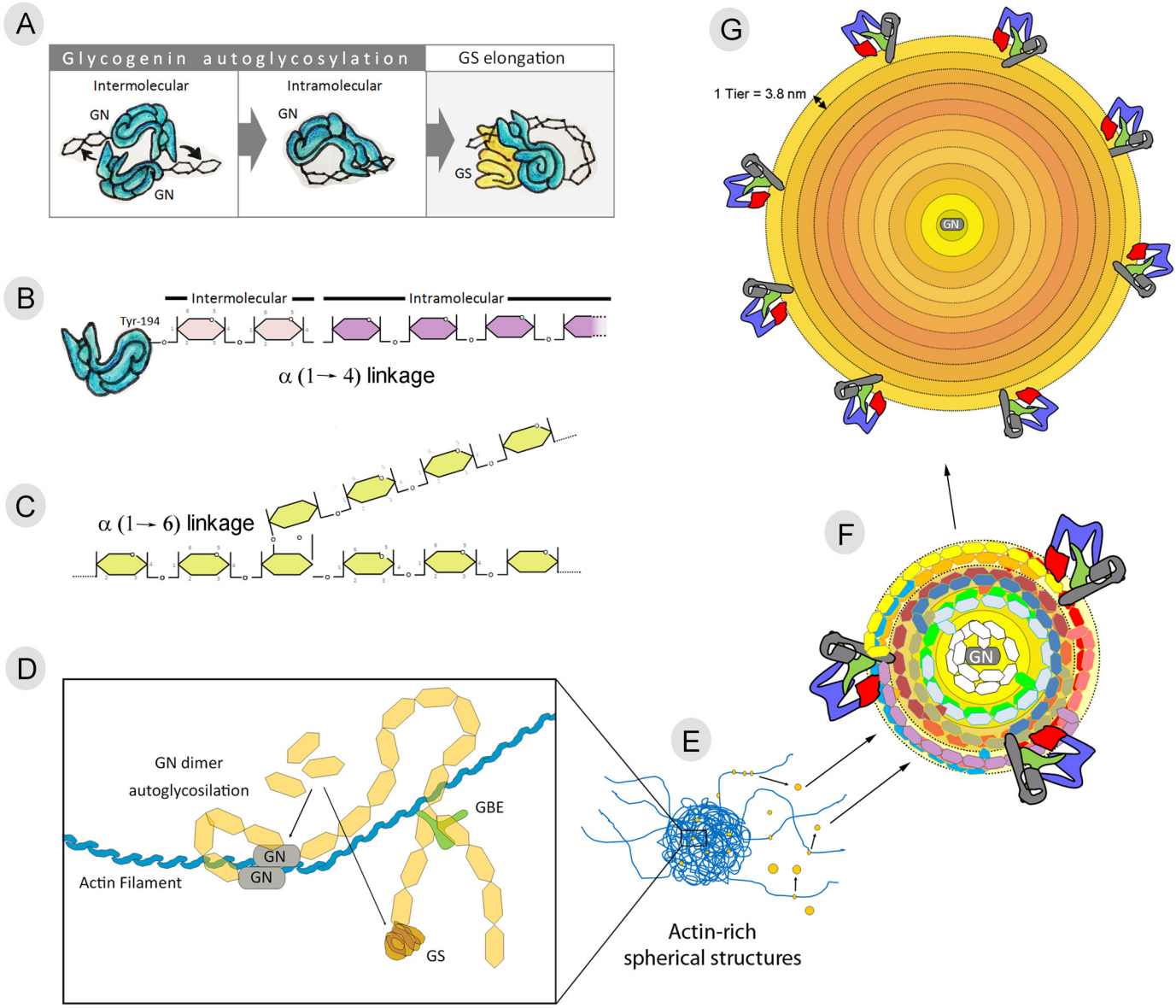

Figure 2. Life of a glycogen granule: birth to maturity. The start of a newly synthesized glycogen granule results from the coordinated dimerization and autoglycosylation of GN (*A*). GN glycosylation is believe to occur in the following two steps: intermolecular glycosylation as the transfer of 1–2 glucose units to tyrosine 194 (Tyr-194) from the other GN, and subsequent intramolecular glycosylation resulting in the elongation of the primer chain by the transfer of 7–16 glucose residues (*B*). Further elongation of the primer chain involves the coordinated action of glycogen synthase (*GS*) and glycogen-branching enzyme (*GBE*), adding glucose residues to the granule through α-1,4-linkage (*B*) and α-1,6-linkage (*C*), respectively. GN binds to actin filaments for the start of glycogen synthesis (*D*), and in situations of severe low glycogen levels, the formation of spherical actin-rich cellular structures (*E*) in which glycogen re-synthesis machinery gathers has been reported in several cell types. As glycogen granules mature, it may be released to the cytosol as an unbound acid-soluble glycogen granule (*F*), less metabolically active and ranging in size from 20 to 30 nm (*G*).

more granules: overexpression of PPP1R6 led to smaller average granule size (~14 nm), but overexpression of PPP1R3C/PTG increased the average granule size (to ~37 nm), resulting in 1.4- and 12-fold increases in glycogen content, respectively. Even though the molecular mechanisms regulating glycogen granule size and number remain elusive, a strong inverse correlation between GS activation and glycogen levels has repeatedly been reported, suggesting that glycogen regulates its own synthesis, perhaps through GS substrate inhibition.

Phosphorylation and granule growth

Although there have been reports of glycogen-containing phosphate groups (20), these were originally clouded with questions of contamination of the samples, etc. The identification of the glycogen phosphatase laforin and its mutation as a key factor in Lafora disease reaffirmed the relevance of glycogen phosphorylation in the regulation of glycogen metabolism. In skeletal muscle, glycogen granules contain approximately one phosphate per ~650 glucosyl units in rabbit and one phosphate per ~1500 glucosyl units in the mouse (49, 50), and these are found throughout the granule. Recently, Turnbull *et al.* (51) suggested that the hydrophilic phosphoryl groups could unfold the branch chains, exposing hydrophobic regions in a similar way as in starch. Laforin dephosphorylates glycogen, and it has been speculated that this dephosphorylation facilitates normal branching of glycogen during synthesis, allowing the granule to

remain soluble (50). The origin and role of the reversible phosphorylation of glycogen remain unclear, as reviewed in the accompanying Minireview by Gentry *et al.* (52). Even though the role of glycogen phosphorylation remains unclear, it should be noted that two starch kinases have been identified, raising the question of whether similar glycogen kinase(s) exist. A quality control role for glycogen phosphorylation has also been suggested (53), according to which a glycogen granule accumulates phosphate throughout its lifetime, becomes less soluble, and is eventually targeted for lysosomal disposal (Fig. 3*D*). This idea is supported by observations in laforin-KO mice, whose glycogen has ~4–6-fold higher phosphate content than the WT, reduced solubility, and forms characteristic Lafora bodies (49, 50). Independently of whether glycogen phosphorylation represents an enzymatic side-reaction error or is part of a regulated biochemical mechanism, there is no doubt that it has significant effects on glycogen metabolism.

Utilization of a glycogen granule and the fate of glycogen-bound proteins

Glycogen granules can be utilized by two pathways: 1) cytosolic degradation by the coordinated action of glycogen phosphorylase (GP) and glycogen-debranching enzyme (GDE) (Fig. 3*A*), and 2) lysosomal degradation by the action of α-glucosidase (Fig. 3*D*) (54).

Cytosolic degradation is mediated by the rate-limiting enzyme GP, which cleaves a terminal glucose residue bound to a glycogen branch by substitution of a phosphoryl group for the α-1,4 linkage. Four residues before a branching (α-1,6-linked glucosyl residue), the GDE catalyzes the transfer of three of the remaining four glucose units to the end of another glycogen chain, where they can be degraded by GP. The exposed α-1,6 branching point is then hydrolyzed by a second catalytic domain of GCE, releasing a molecule of glucose and eliminating a branch point. Thereafter, The exposed α-1,6-branching point is hydrolyzed by a second catalytic domain of GDE, releasing a molecule of glucose and eliminating the branch point. Thus, the degradation of a glycogen granule results in the release of Glc-1-P and glucose (Fig. 3*A*). Glc-1-P is converted to glucose 6-phosphate (Glc-6-P) by phosphoglucomutase, and glucose is phosphorylated to Glc-6-P by hexokinase. In most tissues, the resulting Glc-6-P is used internally to feed glycolysis flux; however, in gluconeogenic tissues, such as the liver, kidney, and intestine, endogenous Glc-6-P can be transported into the ER lumen, where it is dephosphorylated to glucose and secreted by the cell to the interstitial space (Fig. 3*B*).

In skeletal muscle, glycogen and glycogenolysis have been associated with the sarcoplasmic reticulum (SR). A high proportion of total GP and phosphorylase kinase (PhK) activities, 39 and 53% respectively, were recovered associated with purified SR vesicles (55), forming part of the ER–SR glycogenolytic complex, linking glycogenolysis to the SR calcium (Ca^{2+})-ATPase. Highlighting the compartmentalized nature of the complex, lowering extravesicular Ca^{2+} concentration (56) or inhibiting GDE (57) *in vitro* decreased the phosphorylation of only SR-bound GP, with no effect on the phosphorylation state of unbound GP. The coordination of GP activation with SR Ca^{2+}-flux in muscle allows for rapid GP activation at the onset of muscle contraction. A similar link between glycogenolysis regulation and the endoplasmic reticulum Ca^{2+}-ATPase has been reported in primary cultures of murine cerebellar and cortical astrocytes. By stimulating store-operated Ca^{2+} entry or blocking glycogenolysis, Müller *et al.* (58) showed that ER Ca^{2+}-uptake triggers astrocytic glycogenolysis in a cAMP-dependent manner. The existence of a compartmentalized cellular structure regulating GP activity, the glycogenolytic complex, can explain accumulating observations in the literature reporting that not all granules within a cell are regulated in an identical fashion; instead, specific intracellular pools of glycogen exist that are designated for cytosolic degradation (36). Furthermore, the brain expresses two different GP isoforms, the muscle and the brain isoforms (59), that are differently regulated by phosphorylation and AMP (60) and thus are expected to serve different physiological roles (60). Norepinephrine-induced up-regulation of glycogen degradation has been shown to be mainly mediated by the GP muscle isoform (60), whereas the glycolytic supercompensation seems to depend on brain GP activation (61). Whether the role of the two GP isoforms in the regulation of glycogen utilization can be explained by differential intracellular compartmentalizations in the astrocytes remains elusive.

Lysosomal glycogen is enriched in very large molecular weight granules, and its degradation affects 5% of total muscle glycogen and 10% of total liver glycogen (15, 62). The following questions arise. When and how are glycogen granules targeted for lysosomal degradation? What is the metabolic relevance of such a cellular process? In newborns, liver lysosomal glycogen is the product of glycogen autophagy, and it has been suggested to serve as an extra glucose source during and after birth (63). Several studies have reported evidence suggesting a role for laforin and malin in the regulation of glycogen and removal of glycogen-associated proteins via autophagy–lysosomal and ubiquitin–proteasome pathways, respectively (64). The autophagy–lysosomal pathway involves chaperone-mediated autophagy and unspecific invagination of a fraction of the cytoplasm into an autophagosome, which then fuses with a lysosome for content breakdown. Selective down-regulation of hepatic chaperone-mediated autophagy leads to glycogen depletion, potentially explained by the reduced degradation of glycolytic enzymes, leading to enhanced glycolysis rates (65). The ubiquitin–proteasome pathway, in which substrate proteins are targeted for 26S proteasome degradation by ubiquitination, is highly selective (66). Malin has been shown to ubiquitinate several glycogen-associated proteins *in vitro*, among them laforin, PTG, GDE, and GS (67–69); however, the substrates of malin *in vivo* remain unclear, as discussed by Gentry *et al.* (52). In contrast, starch binding domain 1 (Stbd-1) has been identified as a selective autophagy receptor for glycogen. Stbd-1 has a higher affinity for less branched polysaccharides (70), and it has an autophagy-related 8-family interacting motif (71).

Intracellular compartmentalization and dynamics of a glycogen granule

An interesting quandary in glycogen metabolism involves the intracellular localization of granules. As introduced above, gly-

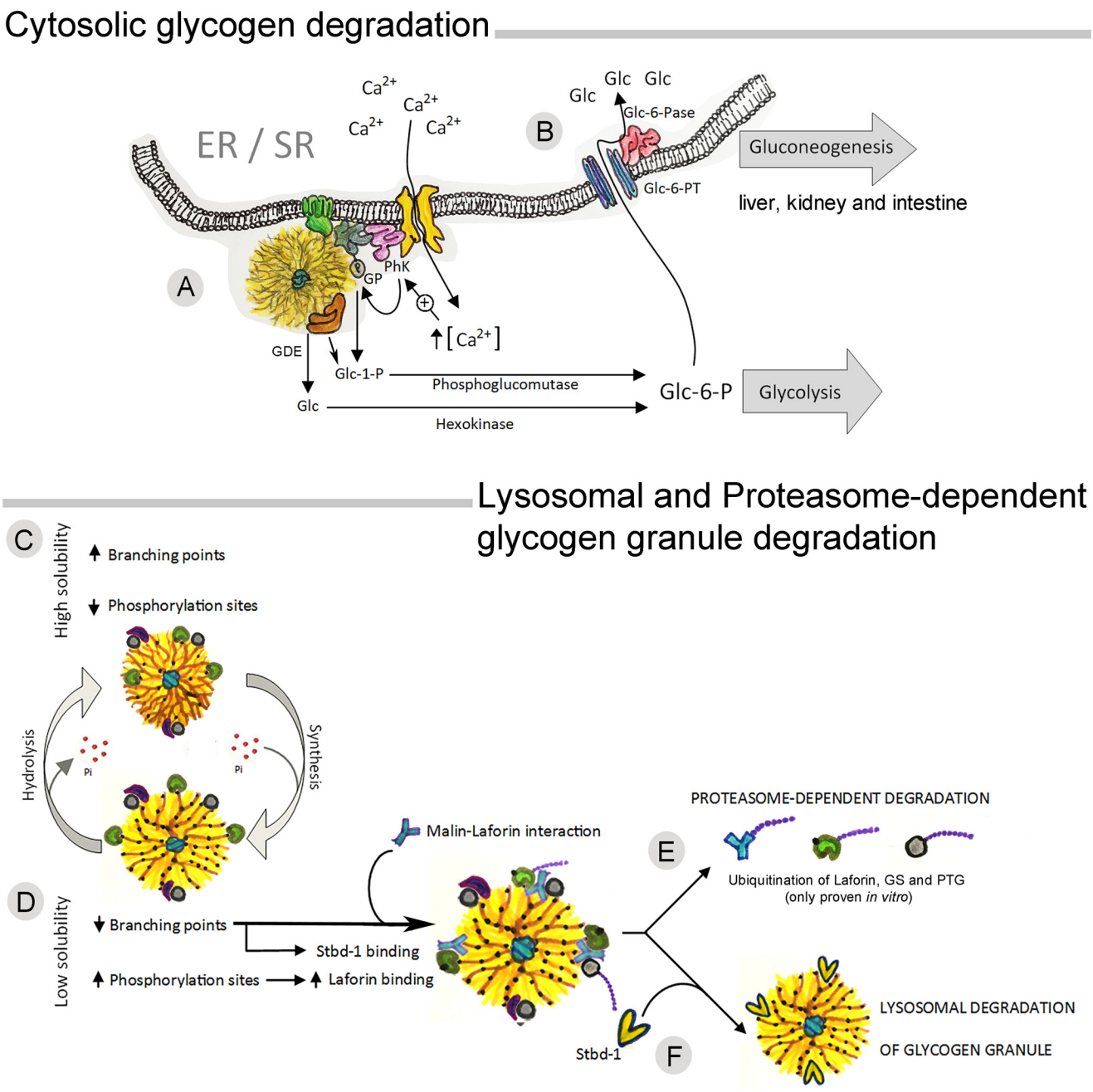

Figure 3. Life of a glycogen granule: partial and complete degradation. Glycogen granules can be utilized by cytosolic degradation or by lysosomal degradation. Cytosolic glycogenolysis has been associated with the endoplasmic and sarcoplasmic reticulum, where the glycogenolytic complex, formed by glycogen phosphorylase (*GP*) and phosphorylase kinase (*PhK*), links glycogen utilization with calcium-ATPase (*A*). The coordinated action of GP and glycogen-debranching enzyme (*GDE*) results in the release of glucose 1-phosphate (*Glc-1-P*) and glucose (*Glc*), which are converted to glucose 6-phosphate (*Glc-6-P*) by phosphoglucomutase and hexokinase, respectively. Glc-6-P will either be used as substrate for glycolysis or, in gluconeogenic tissues, will enter the ER/SR through a glucose 6-phosphate transporter (*Glc-6-PT*) and converted to Glc by glucose-6-phosphatase (*Glc-6-Pase*) (*B*). The mechanisms by which glycogen granules are tagged for lysosomal degradation remain elusive; however, evidence indicates that phosphorylation of glycogen granules may play a role. Increased glycogen phosphorylation has been associated with increased branching points and solubility (*C*), whereas increases in glycogen phosphorylation are associated with lower branching degree and solubility (*D*). Binding and phosphorylation of laforin leads to malin recruitment, which could result in ubiquitination of glycogen-bound proteins directing them toward proteasome degradation (*E*). However, the starch binding domain 1 (*Stbd-1*) will bind to less branched glycogen granules tagging them for lysosomal degradation (*F*).

cogenolysis is associated with the ER/SR, whereas glycogen synthesis seems to be associated with actin filaments. Thus, are glycogen granules formed in specific locations, and do they move during their lifetime?

As an attempt to integrate the reviewed literature, we take the opportunity to propose a generalized hypothetical model for the dynamic life of a glycogen granule (Figs. 1 and 2). Critically low glycogen levels trigger actin cytoskeleton rearrangement, forming actin-rich spherical-like structures, which could facilitate GN dimerization and interaction with GS for efficient glycogen re-synthesis (Fig. 2, *A* and *D*). In mouse astrocytes, the start of glycogen resynthesis after fasting has been reported in close association with the vasculature (72). Restored glycogen levels would lead to dissolution of the actin-rich structures and

explain the observation by Rybicka (1) of lines of cytoplasmic glycogen granules associated with filaments (Fig. 2*E*). As actin-associated glycogen granules grow, they may eventually dissociate from actin filaments, becoming free unbound granules (Fig. 2*F*) that could eventually associate with the ER/SR, becoming the glycogenolytic complex for cytosolic degradation (Fig. 3*A*). In skeletal muscle, ~75% of glycogen granules are found between myofibrils in the intermyofibrillar space (73) where the SR is located, and the rest are associated with contractile filaments inside the myofibrils at the actin I-band of the sarcomere or underneath the plasmalemma (39, 74). Investigation into the distinct roles for the skeletal muscle subcellular glycogen pools has suggested that intramyofibrillar glycogen is tightly associated with muscle resistance to fatigue, whereas intermyofibrillar glycogen appears to be linked with muscle relaxation time and the regulation of Ca^{2+} uptake by the SR (73). These observations support the proposed model in which actin-associated (intramyofibrillar) granules are key for glycogen re-synthesis after depletion, and intermyofibrillar glycogen granules associated with the SR–glycogenolytic complex link glycogenolysis with SR Ca^{2+} uptake.

When a granule is not recruited for cytosolic degradation, accumulative glycogen phosphorylation would lead to alterations in the polysaccharide's structure (branching) (50), making it less soluble. Binding of 5′-AMP-activated protein kinase-phosphorylated laforin to the highly phosphorylated granule would increase malin binding (Fig. 3*D*), ubiquitination of glycogen-associated proteins, and subsequent proteasome-dependent degradation. In addition, because Stbd-1 has high affinity for less branched polysaccharides, it may tag aging granules toward lysosomal degradation (Fig. 3*F*) (71, 75). This idea is supported by the Lafora bodies that form and accumulate when laforin is absent, and these granules are water-insoluble, phosphate-rich, and ubiquitin-positive (76, 77).

In the proposed model, the acid-insoluble glycogen fraction would include the nascent glycogen granules associated with actin filaments and glycogen granules associated with the ER–SR-glycogenolytic complex, whereas the acid-soluble fraction may include cytosolic unbound glycogen granules. This idea is supported by studies reporting that the acid-insoluble glycogen fraction is more metabolically active (10) and has lower average external chain lengths (11). Interestingly, the amount of glycogen acid-soluble fraction has been reported to be more responsive to fasting/feeding and exercise/re-feeding cycles (23, 78). These results may seem contradictory; yet, they can be rationalized by the proposed model. In the model, the amount of metabolically active acid-insoluble glycogen granules (actin- and ER/SR-bound) could be stable, with the regained glycogen storage capacity predominantly occurring in the less metabolically active acid-soluble unbound glycogen granules. Whether glycogen synthesis and degradation co-exist in individual glycogen granules remains unsolved. It is interesting to note that newly formed granules in periportal hepatocytes have been reported to be closely associated with ER (79), and the actin-rich spherical structures in which glycogen re-synthesis localizes interact closely with SR–membrane systems and transverse tubules in skeletal muscle (Fig. 1, *D–G, white arrows*). A close physical proximity between the protein complexes regulating glycogen synthesis (actin-associated GN and GS and GLUT4 glucose uptake) and degradation (ER/SR-associated PhK, GP, and Ca^{2+}-ATPase) suggests that the regulation of the two events is likely coordinated.

Closing remarks and open ends

The dynamic life of a glycogen granule is tissue-specific. A large amount of the available literature originates from skeletal muscle and liver, and thus further studies investigating the regulation of glycogen metabolism in other tissues, especially brain, are needed. In addition, the importance of intracellular compartmentalization in the regulation of glycogen metabolism makes the integration of physiology, biochemistry, and structural biology studies essential.

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