 doi:10.20944/preprints202208.0142.v1

Glycophagy – the physiological perspective on a newly characterized glycogen-selective autophagy

Delbridge LMD[1,2], Koutsifeli P[1], Fong SPT[1], Annandale M[1],
Weeks KL[2,5,6], Bell JR[2,4], Mellor KM[1,2,3]

[1] Department of Physiology, University of Auckland, New Zealand
[2] Department of Anatomy & Physiology, University of Melbourne, Australia
[3] Auckland Bioengineering Institute, University of Auckland, New Zealand
[4] Department of Microbiology, Anatomy, Physiology & Pharmacology, La Trobe University, Australia
[5]Baker Department of Cardiometabolic Health, University of Melbourne, Australia
[6]Department of Diabetes, Monash University, Australia

Running title: Selective autophagy: glycophagy

Corresponding author:
Prof Lea MD Delbridge
Cardiac Phenomics Laboratory
Department of Physiology,
University of Melbourne, Australia.
lmd@unimelb.edu.au

 doi:10.20944/preprints202208.0142.v1

Abstract

Degradation of intracellular components through autophagy is a fundamental process to maintain cellular integrity and homeostasis. Recently a glycogen-selective autophagy pathway has been described, termed ‘glycophagy’. Glycogen is a primary storage depot and regulator of glucose availability, and glycophagy is emerging as a critical physiological process involved in energy metabolism. Glycophagy-mediated degradation of glycogen appears to operate in parallel with the well-described canonical pathway of glycogenolysis involving glycogen phosphorylase. Evidence suggests that starch-binding domain protein-1 (Stbd1) is a key glycogen-binding protein involved in tagging glycogen for glycophagy, and that Gabarapl1 is primarily involved as the Atg8 family protein recruiting the Stbd1-glycogen complex into the forming glycophagosome. The nuances of glycophagy protein machinery, regulation and lysosomal glucose release are yet to be fully elucidated. In this mini-review, we critically analyze the current evidence base for glycophagy as a selective-autophagy process of physiological importance and highlight areas where further investigation is warranted.

Keywords (max. 6): glycogen, autophagy, lysosome, Stbd1, Gabarapl1, acid α-glucosidase (Gaa)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2022 doi:10.20944/preprints202208.0142.v1

Introduction

Macro-autophagy is a conserved cellular process resulting in lysosomal degradation of intracellular components [1,2]. Recently, an autophagy sub-type selective for glycogen has been described, termed 'glycophagy' [3,4]. Glycogen is a polysaccharide which represents the primary source of intracellular glucose storage [5]. Glycophagy-mediated degradation of glycogen appears to operate in parallel with the well-described canonical pathway of glycogenolysis involving glycogen phosphorylase and glycogen debranching enzyme. Emerging evidence suggests that glycophagy involves specific protein intermediaries to recruit glycogen to form the autophagosome ('glycophagosome'). Starch-binding domain protein 1 (Stbd1) has been identified as a key protein involved in tagging glycogen for autophagic degradation via its carbohydrate-binding domain (CBM20) [6]. The autophagosome membrane-bound protein from the Atg8 family, Gabarapl1, has been demonstrated to bind to Stbd1, recruiting glycogen into the glycophagosome structure prior to phagosome-lysosome fusion [4] (Figure 1).

Although these candidates for specific glycophagy machinery have only recently been described, the concept of lysosomal glycogenolysis has been known since the 1960s [7,8]. Acid α-glucosidase (Gaa) cleaves glycosidic bonds to degrade glycogen to free glucose in the lysosome [9,10] (Figure 1). Gaa mutations are the underlying cause of Pompe disease, an inherited glycogen storage disease (GSD) causing severe morbidity and premature death [11]. Several GSDs are characterized by suppressed autophagy activity. In GSD type 1a (glucose-6-phosphatase mutation), interventions to activate autophagy have been shown to restore glycogen (and lipid) stores to normal levels [12]. As a type of selective autophagy process, glycophagy may be an important metabolic route of glycogen degradation and glucose supply. Positioning of glycophagosomes in strategic subcellular locations may have physiological importance for bulk delivery of glucose fuel for glycolytic metabolism. There is some evidence that glycolysis is dependent on lysosomal glycogen degradation and glycophagy may represent a key process for cellular energy homeostasis. Several major protein players have been identified, and recent studies have explored the regulation of Gaa activity and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2022 doi:10.20944/preprints202208.0142.v1

transcription of glycophagy-related genes. Further knowledge of the glycophagy protein machinery is required, and an understanding of the regulation of lysosomal glucose release and glycophagy involvement in pathophysiology is lacking. In this mini-review, we critically analyze the current evidence base for glycophagy as a selective-autophagy process and highlight areas where further investigation is warranted.

Molecular identification of glycogen for glycophagy

The early phase of autophagy involves the recruitment of cellular cargo into a forming autophagosome, mediated by 'receptor' proteins, which bind to both the cargo and Atg8 family proteins in the autophagosome membrane [1] (Figure 1). Molecular screening studies have identified potential glycophagy receptors using *in silico* and *in vitro* approaches. Stbd1 has been proposed as a selective glycophagy receptor as it contains both a glycogen binding domain (CBM20) and several binding sites for Atg8s, known as Atg8-interacting motifs (AIMs) [4,13]. Two Stbd1 AIMs in particular meet the 2D sequence and 3D conformational criteria [14] to be considered as functional AIMs (located at aa203-206, and aa212-215 in the Stbd1 sequence) [4]. There is some evidence from a chemical genetic screening study that Stbd1 may be phosphorylated by AMP-activated protein kinase (AMPK) at Ser 175 [15], but the regulatory outcomes of this phosphorylation site are unknown.

Consistent with a role for Stbd1 in glycophagy, Stbd1 has been shown to interact with Atg8 proteins using *in vitro* GST-pulldown assays [16] and to co-localize with lysosomal-associated membrane protein 1 (Lamp1) [6]. Furthermore, inhibition of autophagosome-lysosome fusion using bafilomycin induced accumulation of FLAG-labelled Stbd1 in HeLa cells [17], confirming that Stbd1 is at least partially processed by the autophagosome-lysosome pathway. Mutation of the Stbd1 aa203-206 AIM site in a cancer cell line resulted in increased glycogen content [18], suggesting that this Stbd1 AIM is involved in mediating glycogen recruitment into the glycophagosome for lysosomal glycogen degradation. Stbd1 may be the rate-limiting step in

glycophagy as demonstrated by *in vivo* Stbd1 knockout, preventing hepatic glycogen accumulation caused by Gaa deficiency [19]. Interestingly, this effect was not seen in skeletal muscle and heart tissues [19]. Collectively, these findings suggest that Stbd1 may be required for glycophagy processing of glycogen in some settings, but further work is required to fully elucidate the mechanisms involved and systematically evaluate tissue specificity.

In silico sequence analysis of common glycogen-related proteins revealed several putative glycophagy receptors by screening for AIMs [20]. In addition to Stbd1, seven other proteins were identified to contain AIMs in their amino acid sequence: glycogen synthase, glycogen branching enzyme, glycogen phosphorylase, glycogenin, glycogen debranching enzyme, laforin and malin. Whether these AIMs constitute functional binding partners for Atg8 proteins is yet to be determined. Conflicting evidence is found in the literature relating to glycogen synthase serving as a potential Atg8 binding partner. In *Drosophila*, glycogen synthase was shown to co-localize with Atg8 proteins [21]. Large-scale human proteomic analysis revealed that glycogen synthase interacted with several Atg8 family proteins, but subsequent *in vitro* experiments could not validate this result [16]. Thus, further work is required to map the molecular machinery of glycophagy fully. The current evidence base favors Stbd1 as the primary glycophagy receptor, but alternative/additional receptors may be identified with future investigation. It could be speculated that distinct glycophagy receptors have important functional implications for targeting glycogen in different physiological (or pathophysiological) settings. Understanding the nuances of how glycogen is tagged for glycophagy is an important priority.

Glycophagosome enclosure of glycogen

The processes of phagophore nucleation and autophagosome formation are likely to be similar for autophagy sub-types (see detailed reviews [22,23]). Interestingly, the proposed origin sites of autophagosome biogenesis, the endoplasmic reticulum (ER) and its associated compartments, are closely aligned with some subcellular pools of glycogen clusters [24–26]. The concept of an ER-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2022 doi:10.20944/preprints202208.0142.v1

glycogenolytic complex has been advanced [25], but to date, the focus has mainly been on cytosolic glycogen breakdown processes, and the involvement of glycophagy at this site remains unknown. Glycophagosome localization at the ER could be important for functional delivery of glucose fuel to sites of energy use. For example, in muscle tissues, the sarcoplasmic reticulum (specialized muscle form of ER) contains Ca^{2+} ATPases involved in excitation-contraction coupling, which have been shown to be dependent on glycolytic ATP supply.

The autophagosome capture of specific cargo for degradation appears to be distinctive in selective autophagy sub-types. For glycophagy, the Stbd1-glycogen complex is enclosed (or 'captured') into the forming glycophagosome as it is bound to an Atg8 protein 'partner' (Figure 1). The mammalian Atg8 protein family consists of the Lc3 (Lc3a, Lc3b, Lc3c) and Gabarap (Gabarap, Gabarapl1, Gabarapl2) subfamilies [27]. Atg8 proteins are anchored into the autophagosome membrane via lipidation of a C-terminal glycine. Stbd1 has been shown to interact with all 6 Atg8 protein family members in a proteomic autophagy network analysis, with the highest binding affinity for Gabarap subfamily Atg8s [16]. Stbd1 interaction with Gabarap and Gabarapl1 was confirmed using co-immunoprecipitation pull-down approaches [6]. *In vitro* co-expression studies revealed that Stbd1 co-localizes with Gabarapl1, and to a lesser extent, with Gabarap [6]. Screening of Stbd1-Gabarapl1 co-localization using truncated mutant forms of human recombinant Stbd1 revealed that this interaction is dependent on the aa203-206 AIM in Stbd1 [4,18]. Thus the current evidence-base suggests that Gabarapl1 is the primary glycophagy Atg8 'partner' for Stbd1, but further work focusing on *in vivo* settings would be informative to fully understand the complex and distinctive roles of the six Atg8 family proteins in selective autophagy processes. Although some redundancy between Atg8s may be expected, the level of selectivity in the glycophagy protein intermediary partners has important implications for functional outcomes of upstream signaling regulation at a physiological level, and for future studies targeting glycophagy for intervention.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2022 doi:10.20944/preprints202208.0142.v1

Lysosomal glycogen degradation

The concept of lysosomal glycogenolysis has been documented for more than 50 years, particularly in the context of glycogen storage diseases. Inherited deficiency of the lysosomal glycogen degradation enzyme, Gaa, in Pompe disease is associated with marked accumulation of glycogen in several tissues, most prominent in the heart and skeletal muscle [11,28]. These early studies demonstrated that lysosomal degradation of glycogen is an essential physiological process and not merely a redundant parallel pathway of glycogen processing. The concept of glycophagic processing has only recently been recognized and knowledge of the implicated protein intermediaries is limited. Following glycophagosome-lysosome fusion, glycogen breakdown is mediated by the Gaa enzyme [5]. This process is distinct from cytosolic breakdown via glycogen phosphorylase, which exclusively cleaves α-1,4-glycosidic bonds. Gaa can hydrolyze both α-1,4-glycosidic strands and α-1,6-glycosidic branch points resulting in complete degradation of the glycogen granule [10,29]. Additionally, glycogen phosphorylase releases glucose-1-phosphate primed ready for glycolytic metabolism, whereas Gaa releases free glucose [9].

The implications of bulk release of free glucose from the lysosome have not been studied, and it could be speculated that this poses an osmotic and glycation challenge for the cell, and the physiology is therefore likely to be tightly regulated. It is not known how glucose is released from the phago-lysosome following glycogen degradation. Some reports show that the glucose transporter, Glut8, may exhibit lysosomal localization [30,31], but whether Glut8 is operational in transporting glucose out of the lysosome requires validation. The transmembrane protein, spinster 1 (Spns1), has also been advanced as a possible candidate for lysosomal glucose transport. Although little is known about Spns1, its sequence and structure aligns with proteins from the sugar transporter subfamily [32]. Co-localization of Spns1 with lysosomal markers, Lamp1 and the fluorescent lysosomal marker ‘lysotracker’ has been reported [33,34], and *in vitro* Spns1 knockdown induces glycogen accumulation [33]. The regulation of lysosomal glucose transport could be expected to have important consequences for cellular metabolism, but to date no

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2022 doi:10.20944/preprints202208.0142.v1

information is available. There is some evidence that glycophagy is upregulated in settings of high energy demand, thus activation/promotion of lysosomal glucose transport may occur via similar energy signaling pathways that positively regulate glucose metabolism. This is an emerging area of interest in the autophagy field where significant knowledge gaps warrant investigation to fully characterize the distal stages of the glycophagy pathway.

Regulation of glycophagy

Regulation of glycogen recruitment

Although the molecular pathways involved in regulating glycophagy are not well defined, certain structural characteristics of glycogen appear to be most commonly associated with glycophagy occurrence. Early studies using subcellular fractionation protocols reported that glycogen located in the lysosome has a higher molecular weight than glycogen in the cytosol [35,36]. When Gaa is inhibited by liposomal delivery of Gaa antibodies, hepatic cellular glycogen content shifts towards a low molecular weight profile [37]. High molecular weight glycogen is consistent with α-granules: rosette-like structures up to ~300nm in diameter and most prominent in the liver delivering slow glucose release [5]. Low molecular weight glycogen is more closely aligned with β-granules: less than ~50nm in diameter and most prominent in skeletal muscle where rapid glucose release is required in acute settings of high demand [5]. Thus the current evidence base suggests that glycophagy may favor the degradation of large α-granules (over small β-granules), but the functional implications are yet to be determined.

Branching content of the glycogen granule may also influence its degradation route. An *in vitro* study comparing glycogen with the less branched glucose polymer, amylopectin, provides some indication that Stbd1 has a higher affinity for less branched polysaccharides [6]. Similarly, Gaa has lower efficiency for cleaving α-1,6-glycosidic branch points than α-1,4-glycosidic strands [29]. There are also marked developmental differences in glycogen structure which appear to be associated with glycophagy activity. Fetal-type glycogen has less branching content than adult, and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2022 doi:10.20944/preprints202208.0142.v1

glycophagy activity (and more generally autophagy activity) is well documented to be highest in the neonatal setting [38,39]. To date, observations to support a glycophagy preference for large low-branched glycogen granules are mostly anecdotal but provide some suggestion that glycogen structure may influence glycophagy fate.

Regulation of Stbd1 & Gabarapl1

Stbd1 is phosphorylated by the master energy regulator, AMPK [15] (Figure 1). Although its regulatory action is still unclear, modulation of Stbd1 activity by AMPK would be consistent with the contention that glycophagy is responsive to cellular energy demand. Other phosphorylation sites on Stbd1 have been documented [15], but identification of the specific kinases involved has been elusive. The N-terminus of Stbd1 can be myristoylated, an irreversible post-translational modification involving addition of a 14-carbon saturated fatty acid group, and appears to play a role in localizing Stbd1 to the ER and mitochondria [40]. Further investigation into the enablers and barriers for Stbd1 tagging of glycogen for glycophagy is required to fully elucidate the mechanisms by which glycophagy may be initiated in various physiological settings.

The available literature documenting the regulation of the glycophagosome Atg8 protein, Gabarapl1, is sparse, but some evidence of transcriptional regulation has been reported. FoxO transcription factors, FoxO1 and FoxO3, have been demonstrated to target the transcription of Gabarapl1 [41] (Figure 1). Given that FoXO proteins are negatively regulated by the insulin signaling pathway, it is likely that Gabarapl1 expression levels are inhibited by insulin action, although this has not yet been experimentally demonstrated. Thus, glycophagy may be activated in settings of low insulin availability (e.g. fasting) via FoxO-induced transcription of Gabarapl1. We have previously shown that fasting-induced metabolic stress *in vivo* is associated with a prominent cardiac glycophagy response [42].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2022 doi:10.20944/preprints202208.0142.v1

Regulation of Gaa and lysosomal glycogenolysis

Accumulating evidence suggests that Gaa activity may be modulated by energy regulatory pathways, and has a role in liberating glucose fuel supply for energy metabolism. *In vitro* knockdown of Gaa decreased glycolytic metabolic rate and glucose oxidation, which was associated with a shift to fatty acid oxidation [43]. In Gaa deficient cells, the activity of the archetypal regulators of energy homeostasis, AMPK and PPARα, was increased [43]. Experimentally, *in vivo* modulation of the β-adrenergic pathway has been shown to regulate lysosomal Gaa. Specifically, adrenaline-induced β-adrenergic activation increased Gaa activity [38] and propranolol-induced β-adrenergic inhibition decreased Gaa activity [44]. Collectively, these findings provide further support to the contention that glycophagy is activated in physiologic settings of high energy demand.

Although conflicting reports exist [43,45], Gaa expression may be influenced by transcription factor EB (TFEB), potentially in a tissue-specific manner. TFEB is a known transcription factor of several autophagy and lysosomal genes, and nuclear localization of TFEB promotes autophagosome formation, autophagosome-lysosome fusion, and lysosomal biogenesis. TFEB has been identified as a regulator of macro-autophagy, mitophagy and lipophagy [46]. Its involvement in glycophagy is likely and further investigation is warranted.

Concluding remarks

Although the concept of lysosomal glycogen degradation has been known for some time, the term ‘glycophagy’ has only recently come into use with an enhanced understanding of selective-autophagy pathways. Identifying the specific molecular machinery involved in glycogen recruitment into the glycophagosome has advanced the field considerably. There is mounting evidence to support the contention that Stbd1 is the primary glycogen-binding protein involved in tagging glycogen for glycophagy, and that Gabarapl1 is the selective Atg8 family protein which recruits the Stbd1-glycogen complex into the forming glycophagosome. Significant knowledge gaps

 doi:10.20944/preprints202208.0142.v1

exist in understanding the nuanced processes of glycophagosome-lysosome fusion and the transport of glucose liberated from glycogen out of the lysosome. Some evidence suggests that key energy regulatory pathways play a crucial role in modulating glycophagy activity, and more work is required to map the precise mechanisms involved. It is clear from glycogen storage diseases that glycophagy is an essential cellular process and plays a vital role in the fundamental cell biology of glycogen metabolism. The glycophagy field is in an early discovery phase, and advancing the understanding of the physiology and pathophysiology of glycophagy is an important priority.

Conflict of interest statement

No conflicts to declare.

Acknowledgements

K.M.M. is supported by funding from the Health Research Council of New Zealand (19/190) and the Marsden Fund of New Zealand (19-UOA-268).

References

*of special interest

1. Tanida I: **Autophagy basics**. *Microbiol Immunol* 2011, **55**:1–11.

*2. Delbridge LMD, Mellor KM, Taylor DJ, Gottlieb RA: **Myocardial stress and autophagy: mechanisms and potential therapies**. *Nat Rev Cardiol* 2017, **14**:412–425.

Delbridge et al provides a comprehensive critique of the literature related to autophagy machinery, selective autophagy subtypes (macrophagy, mitophagy & glycophagy) and autophagy regulation.

3. Delbridge LMD, Mellor KM, Taylor DJR, Gottlieb RA: **Myocardial autophagic energy stress responses-macroautophagy, mitophagy, and glycophagy**. *Am J Physiol Heart Circ Physiol* 2015, **308**:H1194-1204.

*4. Jiang S, Wells CD, Roach PJ: **Starch-binding domain-containing protein 1 (Stbd1) and glycogen metabolism: Identification of the Atg8 family interacting motif (AIM) in Stbd1 required for interaction with GABARAPL1**. *Biochem Biophys Res Commun* 2011, **413**:420–425.

This study identifies Stbd1 as a potential glycophagy 'receptor', recruiting glycogen into the glycophagosome by binding to the Atg8 family protein, Gabarapl1.

5. Prats C, Graham TE, Shearer J: **The dynamic life of the glycogen granule**. *J Biol Chem* 2018, **293**:7089–7098.

*6. Jiang S, Heller B, Tagliabracci VS, Zhai L, Irimia JM, DePaoli-Roach AA, Wells CD, Skurat AV, Roach PJ: **Starch Binding Domain-containing Protein 1/Genethonin 1 Is a Novel Participant in Glycogen Metabolism**. *J Biol Chem* 2010, **285**:34960–34971.

Jiang et al provides the first evidence that Stbd1 may be involved in glycogen-autophagy.

7. Nitowsky HM, Grunfeld A: **Lysosomal alpha-glucosidase in type II glycogenosis; activity in leukocytes and cell cultures in relation to genotype**. *J Lab Clin Med* 1967, **69**:472–484.

8. Phillips MJ, Unakar NJ, Doornewaard G, Steiner JW: **Glycogen depletion in the newborn rat liver: an electron microscopic and electron histochemical study**. *J Ultrastruct Res* 1967, **18**:142–165.

9. Adeva-Andany MM, González-Lucán M, Donapetry-García C, Fernández-Fernández C, Ameneiros-Rodríguez E: **Glycogen metabolism in humans**. *BBA Clin* 2016, **5**:85–100.

10. Palmer TN: **The substrate specificity of acid -glucosidase from rabbit muscle**. *Biochem J* 1971, **124**:701–711.

11. Chan J, Desai AK, Kazi ZB, Corey K, Austin S, Hobson-Webb LD, Case LE, Jones HN, Kishnani PS: **The emerging phenotype of late-onset Pompe disease: A systematic literature review**. *Mol Genet Metab* 2017, **120**:163–172.

12. Farah BL, Landau DJ, Sinha RA, Brooks ED, Wu Y, Fung SYS, Tanaka T, Hirayama M, Bay B-H, Koeberl DD, et al.: **Induction of autophagy improves hepatic lipid metabolism in glucose-6-phosphatase deficiency**. *J Hepatol* 2016, **64**:370–379.

13. Janecek S: **A motif of a microbial starch-binding domain found in human genethonin**. *Bioinformatics* 2002, **18**:1534–1537.

14. Popelka H, Klionsky DJ: **Analysis of the native conformation of the LIR/AIM motif in the Atg8/LC3/GABARAP-binding proteins**. *Autophagy* 2015, **11**:2153–2159.

15. Ducommun S, Deak M, Zeigerer A, Göransson O, Seitz S, Collodet C, Madsen AB, Jensen TE, Viollet B, Foretz M, et al.: **Chemical genetic screen identifies Gapex-5/GAPVD1 and STBD1 as novel AMPK substrates**. *Cell Signal* 2019, **57**:45–57.

*16. Behrends C, Sowa ME, Gygi SP, Harper JW: **Network organization of the human autophagy system**. *Nature* 2010, **466**:68–76.

Behrends et al provides an important data-rich resource of the autophagy protein network, comparing 'receptor'-Atg8 interactions.

17. Zhu Y, Zhang M, Kelly AR, Cheng A: **The carbohydrate-binding domain of overexpressed STBD1 is important for its stability and protein–protein interactions**. *Biosci Rep* 2014, **34**.

18. Han Z, Zhang W, Ning W, Wang C, Deng W, Li Z, Shang Z, Shen X, Liu X, Baba O, et al.: **Model-based analysis uncovers mutations altering autophagy selectivity in human cancer**. *Nat Commun* 2021, **12**:3258.

19. Sun T, Yi H, Yang C, Kishnani PS, Sun B: **Starch Binding Domain-containing Protein 1 Plays a Dominant Role in Glycogen Transport to Lysosomes in Liver**. *J Biol Chem* 2016, **291**:16479–16484.

*20. Koutsifeli P, Varma U, Daniels LJ, Annandale M, Li X, Neale JPH, Hayes S, Weeks KL, James S, Delbridge LMD, et al.: **Glycogen-autophagy: Molecular machinery and cellular mechanisms of glycophagy**. *J Biol Chem* 2022, **298**:102093.

This is a comprehensive review article critically analyzing the evidence for glycogen-autophagy.

21. Zirin J, Nieuwenhuis J, Perrimon N: **Role of autophagy in glycogen breakdown and its relevance to chloroquine myopathy**. *PLoS Biol* 2013, **11**:e1001708.

22. Chang C, Jensen LE, Hurley JH: **Autophagosome biogenesis comes out of the black box**. *Nat Cell Biol* 2021, **23**:450–456.

23. Hu Y, Reggiori F: **Molecular regulation of autophagosome formation**. *Biochem Soc Trans* 2022, **50**:55–69.

24. Wanson JC, Drochmans P: **Role of the sarcoplasmic reticulum in glycogen metabolism. Binding of phosphorylase, phosphorylase kinase, and primer complexes to the sarcovesicles of rabbit skeletal muscle**. *J Cell Biol* 1972, **54**:206–224.

25. Entman ML, Keslensky SS, Chu A, Van Winkle WB: **The sarcoplasmic reticulum-glycogenolytic complex in mammalian fast twitch skeletal muscle. Proposed in vitro**

 doi:10.20944/preprints202208.0142.v1

counterpart of the contraction-activated glycogenolytic pool. *J Biol Chem* 1980, **255**:6245–6252.

26. Nielsen J, Schrøder HD, Rix CG, Ortenblad N: **Distinct effects of subcellular glycogen localization on tetanic relaxation time and endurance in mechanically skinned rat skeletal muscle fibres**. *J Physiol* 2009, **587**:3679–3690.

27. Jatana N, Ascher DB, Pires DEV, Gokhale RS, Thukral L: **Human LC3 and GABARAP subfamily members achieve functional specificity via specific structural modulations**. *Autophagy* 2020, **16**:239–255.

28. Meena NK, Raben N: **Pompe Disease: New Developments in an Old Lysosomal Storage Disorder**. *Biomolecules* 2020, **10**:E1339.

29. Roig-Zamboni V, Cobucci-Ponzano B, Iacono R, Ferrara MC, Germany S, Bourne Y, Parenti G, Moracci M, Sulzenbacher G: **Structure of human lysosomal acid α-glucosidase-a guide for the treatment of Pompe disease**. *Nat Commun* 2017, **8**:1111.

30. Augustin R, Riley J, Moley KH: **GLUT8 contains a [DE]XXXL[LI] sorting motif and localizes to a late endosomal/lysosomal compartment**. *Traffic* 2005, **6**:1196–1212.

31. Alexander CM, Martin JA, Oxman E, Kasza I, Senn KA, Dvinge H: **Alternative Splicing and Cleavage of GLUT8**. *Mol Cell Biol* 2020, **41**.

32. Dermaut B, Norga KK, Kania A, Verstreken P, Pan H, Zhou Y, Callaerts P, Bellen HJ: **Aberrant lysosomal carbohydrate storage accompanies endocytic defects and neurodegeneration in Drosophila benchwarmer**. *J Cell Biol* 2005, **170**:127–139.

33. Rong Y, McPhee CK, McPhee C, Deng S, Huang L, Chen L, Liu M, Tracy K, Baehrecke EH, Baehreck EH, et al.: **Spinster is required for autophagic lysosome reformation and mTOR reactivation following starvation**. *Proc Natl Acad Sci U S A* 2011, **108**:7826–7831.

34. Sweeney ST, Davis GW: **Unrestricted synaptic growth in spinster-a late endosomal protein implicated in TGF-beta-mediated synaptic growth regulation**. *Neuron* 2002, **36**:403–416.

35. Geddes R, Stratton GC: **The influence of lysosomes on glycogen metabolism**. *Biochem J* 1977, **163**:193–200.

36. Geddes R, Taylor JA: **Factors affecting the metabolic control of cytosolic and lysosomal glycogen levels in the liver**. *Biosci Rep* 1985, **5**:315–320.

37. Geddes R, Otter DE, Scott GK, Taylor JA: **Disturbance of lysosomal glycogen metabolism by liposomal anti-alpha-glucosidase and some anti-inflammatory drugs**. *Biochem J* 1983, **212**:99–103.

38. Kondomerkos DJ, Kalamidas SA, Kotoulas OB, Hann AC: **Glycogen autophagy in the liver and heart of newborn rats. The effects of glucagon, adrenalin or rapamycin**. *Histol Histopathol* 2005, **20**:689–696.

39. Iwamasa T, Tsuru T, Hamada T, Takeuchi T: **Physicochemical and ultrastructural studies on glycogenosomes in newborn rat hepatocytes**. *Pathol Res Pract* 1980, **167**:363–373.

40. Demetriadou A, Morales-Sanfrutos J, Nearchou M, Baba O, Kyriacou K, Tate EW, Drousiotou A, Petrou PP: **Mouse Stbd1 is N-myristoylated and affects ER-mitochondria association and mitochondrial morphology**. *J Cell Sci* 2017, **130**:903–915.

41. Sengupta A, Molkentin JD, Yutzey KE: **FoxO transcription factors promote autophagy in cardiomyocytes**. *J Biol Chem* 2009, **284**:28319–28331.

*42. Reichelt ME, Mellor KM, Curl CL, Stapleton D, Delbridge LMD: **Myocardial glycophagy - a specific glycogen handling response to metabolic stress is accentuated in the female heart**. *J Mol Cell Cardiol* 2013, **65**:67–75.

This paper provides the first evidence that glycophagy is operational in the adult heart and responsive to fasting-induced cardiac metabolic stress.

43. Heden TD, Chow LS, Hughey CC, Mashek DG: **Regulation and role of glycophagy in skeletal muscle energy metabolism**. *Autophagy* 2021, doi:10.1080/15548627.2021.1969633.

44. Kalamidas SA, Kotoulas OB: **The degradation of glycogen in the lysosomes of newborn rat hepatocytes: glycogen-, maltose- and isomaltose-hydrolyzing acid alpha glucosidase activities in liver**. *Histol Histopathol* 1999, **14**:23–30.

45. Sardiello M, Palmieri M, di Ronza A, Medina DL, Valenza M, Gennarino VA, Di Malta C, Donaudy F, Embrione V, Polishchuk RS, et al.: **A gene network regulating lysosomal biogenesis and function**. *Science* 2009, **325**:473–477.

46. Settembre C, Di Malta C, Polito VA, Garcia Arencibia M, Vetrini F, Erdin S, Erdin SU, Huynh T, Medina D, Colella P, et al.: **TFEB links autophagy to lysosomal biogenesis**. *Science* 2011, **332**:1429–1433.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2022 doi:10.20944/preprints202208.0142.v1

Figure legend

Figure 1. Glycogen degradation by glycophagy. Starch-binding domain protein 1 (STBD1) is the likely cargo 'receptor' protein, tagging glycogen for glycophagy. STBD1 is phosphorylated by AMP-activated kinase (AMPK), although the functional consequences of phosphorylation are unknown. Evidence suggests that glycogen tagged for glycophagy is more likely to be high molecular weight α-granules with low branching content. GABA Type A Receptor Protein Like 1 (GABARAPL1) is an Atg8 family protein implicated in recruiting the glycogen-STBD1 complex into the glycophagosome. FoxO1 and FoxO3 are transcription factors for GABARAPL1. Following glycophagosome-lysosome fusion, acid α-glucosidase (GAA) degrades glycogen into free glucose. Transcription factor EB (TFEB) may promote transcription of Gaa and β-adrenergic signaling has been shown to increase Gaa activity. Transport of glucose out of the lysosome occurs via an unknown mechanism and may provide glucose fuel for local glycolytic energy metabolism. Created with Biorender.com.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 August 2022 doi:10.20944/preprints202208.0142.v1

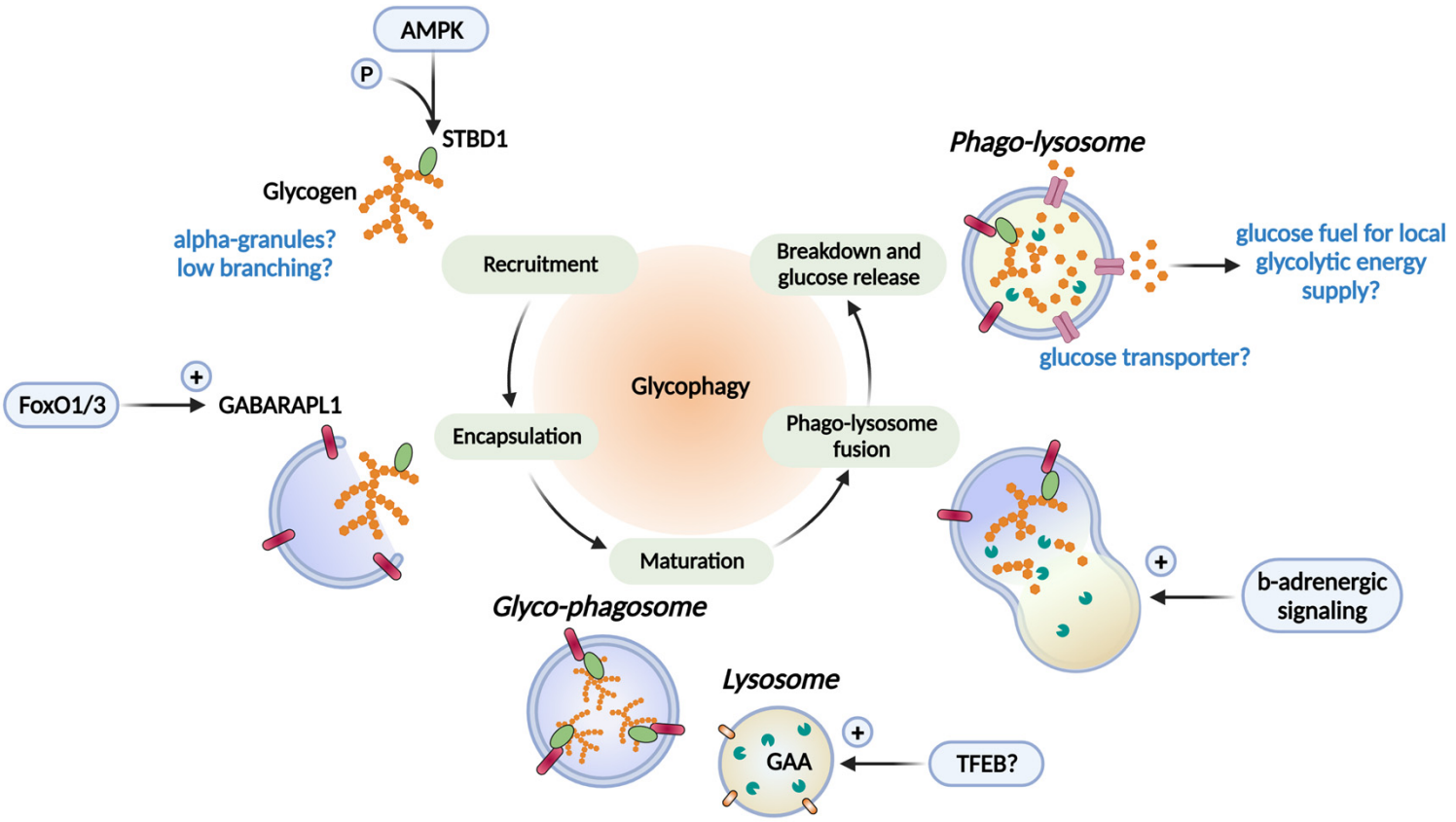

Figure 1. Glycogen degradation by glycophagy. Starch-binding domain protein 1 (STBD1) is the likely cargo 'receptor' protein, tagging glycogen for glycophagy. STBD1 is phosphorylated by AMP-activated kinase (AMPK), although the functional consequences of phosphorylation are unknown. Evidence suggests that glycogen tagged for glycophagy is more likely to be high molecular weight α-granules with low branching content. GABA Type A Receptor Protein Like 1 (GABARAPL1) is an Atg8 family protein implicated in recruiting the glycogen-STBD1 complex into the glycophagosome. FoxO1 and FoxO3 are transcription factors for GABARAPL1. Following glycophagosome-lysosome fusion, acid α-glucosidase (GAA) degrades glycogen into free glucose. Transcription factor EB (TFEB) may promote transcription of Gaa and β-adrenergic signaling has been shown to increase Gaa activity. Transport of glucose out of the lysosome occurs via an unknown mechanism and may provide glucose fuel for local glycolytic energy metabolism. Created with Biorender.com.