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COMMENTARY

Glycogen: A must have storage to survive stressful emergencies

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ABSTRACT

Mechanisms of adaptation to acute changes in osmolarity are fundamental for life. When exposed to hyperosmotic stress, cells and organisms utilize conserved strategies to prevent water loss and maintain cellular integrity and viability. The production of glycerol is a common strategy utilized by the nematode *Caenorhabditis elegans (C. elegans)* and many other organisms to survive hyperosmotic stress. Specifically, the transcriptional upregulation of glycerol-3-phosphate dehydrogenase, a rate-limiting enzyme in the production of glycerol, has been previously implicated in many model organisms. However, what fuels this massive and rapid production of glycerol upon hyperosmotic stress has not been clearly elucidated. We have recently discovered an AMPK-dependent pathway that mediates hyperosmotic stress resistance in *C. elegans*. Specifically, we demonstrated that the chronic activation of AMPK leads to glycogen accumulation, which under hyperosmotic stress exposure, is rapidly degraded to mediate glycerol production. Importantly, we demonstrate that this strategy is utilized by *flcn-1* mutant *C. elegans* nematodes in an AMPK-dependent manner. FLCN-1 is the worm homolog of the human renal tumor suppressor Folliculin (FLCN) responsible for the Birt-Hogg-Dubé neoplastic syndrome. Here, we comment on the dual role for glycogen in stress resistance: it serves as an energy store and a fuel for osmolyte production. We further discuss the potential utilization of this mechanism by organisms in general and by human cancer cells in order to survive harsh environmental conditions and notably hyperosmotic stress.

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Introduction

When the extracellular osmolarity is higher than the intracellular osmolarity, cells experience hyperosmotic stress, which promotes water flux out of the cell by osmosis, causing cellular shrinkage, severe macromolecular damage, cell cycle arrest, and cell death.[1] Most organisms are exposed chronically or accidentally to high salinity environments and the ability to adapt to the availability of water is essential for life. In humans, many organs are exposed to water stress, due to water evaporation such as the skin, or through water osmosis into more concentrated aqueous environments due to physiological processes such as in kidneys, colon, and bladder.[1] Cells/tissues/organisms have developed strategies to adapt to threatening hyperosmotic environments. These strategies include cytoskeletal rearrangements to offset the mechanical pressure, the upregulation of antioxidant enzymes to neutralize the sudden increase in reactive oxygen species, the induction of transporters to regulate water transport, and the upregulation of heat shock proteins to ensure protein homeostasis.[1-3] In addition to the above-mentioned strategies, the synthesis of compatible organic solutes, also called osmolytes, is a widely-used strategy by all organisms which keeps cellular osmotic pressure equal to that of the external environment.[4]

The most common organic osmolytes include amino acids and derivatives (glycine, proline, taurine, etc.), carbohydrates, polyols and derivatives (trehalose, glycerol, inositol, myo-inositol, sorbitol, etc.), methylamines such as glycine betaine, and urea.[4]

In yeast and in the nematode *Caenorhabditis elegans* (*C. elegans)*, the exposure to hyperosmotic stress causes the rapid accumulation of glycerol via the transcriptional upregulation of glycerol-3-phosphate dehydrogenase, a rate limiting enzyme in glycerol

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synthesis.[5-7] Importantly, several hyperosmotic stress-resistant *C. elegans* mutants display heightened glycerol levels due to constitutive activation of *gpdh-1* and subsequent glycerol accumulation.[8-12] Although the use of glycerol in invertebrates to survive hyperosmotic stress is widely accepted, what fuels the rapid glycerol production upon hyperosmotic stress exposure has not been clearly elucidated.

Among pathways that lead to glycerol production, the degradation of glycogen leads to glucose-1-phosphate, which is rapidly converted to glucose-6-phosphate, a major metabolic intermediate that may enter the glycolysis pathway or produce glycerol-3-phosphate, a crucial metabolite for glycerol synthesis (Fig. 1).[13] Importantly, our recent work demonstrates that this strategy is utilized to survive hyperosmotic stress by *C. elegans* wild-type nematodes and is enhanced upon loss of *flcn-1*, the worm homolog of the renal tumor suppressor protein Folliculin (FLCN), responsible for the Birt-Hogg-Dubé cancer syndrome in humans.[14] Specifically, we also highlighted an important role for glycogen reserves in the rapid production of glycerol upon hyperosmotic stress exposure thereby enhancing organismal survival.[14]

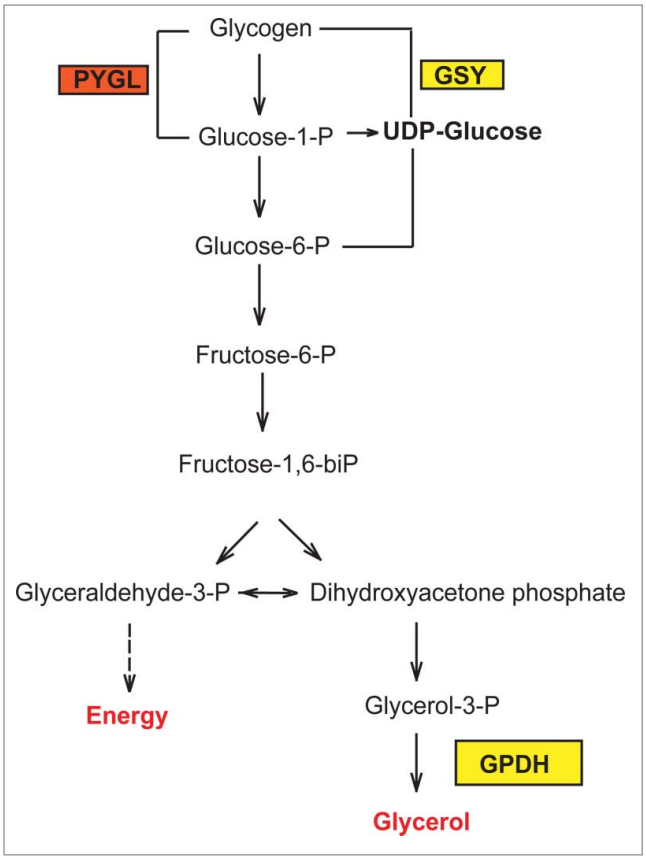

Figure 1. Representative scheme of glycogen metabolism and osmolyte production in *C. elegans*. PYGL: Glycogen phosphorylase, GSY: Glycogen Synthase.

FLCN-1/AMPK regulates hyperosmotic stress resistance in C. elegans

We have previously shown that FLCN-1 regulates resistance to energy stresses in *C. elegans* and mammalian cells including oxidative stress, anoxia, heat, and serum starvation.[15-17] We also showed that the increased resistance to energy stresses is evolutionarily conserved and requires the 5'AMP-activated protein kinase (AMPK), a major regulator of cellular energy homeostasis and stress response.[15,17] In our recent work, we demonstrate an important role for FLCN-1/AMPK in the regulation of resistance to hyperosmotic stress in *C. elegans*.[14] Specifically, we showed that loss of *flcn-1* enhanced the resistance of *C. elegans* nematodes to high NaCl conditions (400mM and 500mM NaCl) and improved their recovery from acute salinity attacks. Using the *flcn-1(ok975); aak-1(tm1944); aak-2 (ok524)* triple mutant animals that we generated, we showed that this FLCN-1-dependent hyperosmotic stress resistant phenotype strictly requires both AMPKα catalytic subunits AAK-1 and AAK-2.[14]

FLCN-1/AMPK regulates glycogen metabolism in C. elegans

Using electron microscopy and iodine staining, we observed a prominent accumulation of glycogen in different tissues of *C. elegans* nematodes upon loss of FLCN-1, especially in the hypodermis.[14] Glycogen is a polymer of glucose molecules widely used as an energy storage in animals. Glycogen is synthesized from UDP-glucose by glycogen synthase and is degraded into glucose-1-phosphate using glycogen phosphorylase, and both enzymes are highly evolutionarily conserved (Fig. 1).[13] Importantly, we observed that the inhibition of glycogen synthesis and degradation by RNAi against glycogen synthase and glycogen phosphorylase, respectively, abrogated the resistance of wild-type animals to hyperosmotic stress and strongly suppressed the advantageous resistance mediated by loss of *flcn-1*.[14]

Since the chronic AMPK activation has been shown to lead to glycogen accumulation in multiple model systems, and because we have previously demonstrated that loss of *flcn-1* chronically activates AMPK

in *C. elegans* and in mammalian cells,[15] we hypothesized that the increased accumulation of glycogen in *flcn-1* animals depends on AMPK. Indeed, we demonstrated using iodine staining, that AMPK is required for glycogen accumulation in both wild-type and *flcn-1* animals.[14] This result explains why loss of AMPK or inhibition of glycogen metabolism lead to the same phenotypic outcome in regards to hyperosmotic stress resistance in *C. elegans*.

Glycogen breakdown fuels glycerol production and enhances hyperosmotic stress survival

While glycogen has been also shown to mediate a parental-associated effect of stress resistance in *C. elegans* embryos,[18] two recent reports that were published while our manuscript was under review, have also linked glycogen to hypoosmotic-anoxic stress resistance in *C. elegans*.[19,20] However, how glycogen is leading to hyperosmotic stress resistance specifically has not been clearly elucidated. In our recent work, we showed that following hyperosmotic stress exposure, glycerol is rapidly produced in both wild-type and *flcn-1* animals, but more prominently in *flcn-1* nematodes, which is consistent with the massive glycogen breakdown in these animals.[14] We also showed that the enzymes responsible for glycogen synthesis, glycerol-3-phosphate dehydrogenases *(gpdh-1 and gpdh-2)* are strongly transcriptionally induced in both wild-type and *flcn-1* animals, but more prominently upon loss of *flcn-1*. Supporting the important role of glycerol in the resistance to hyperosmotic stress, we generated the *flcn-1; gpdh-1; gpdh2* triple mutant and determined its resistance to the *gpdh-1; gpdh-2* double mutant animals. Indeed, we found that the loss of glycerol-3-phosphate dehydrogenases, strongly suppressed the increased resistance to hyperosmotic stress conferred by loss of *flcn-1*.[14]

The glycogen accumulation conferred by loss of FLCN-1 is evolutionarily conserved

In this work, we also highlighted an evolutionary conserved role of FLCN/AMPK in the regulation of glycogen metabolism. Specifically, we showed that glycogen accumulates in the tumors of BHD patients and in renal tissues of kidney-specific *Flcn* KO mice. This result implies that glycogen could play an important role in BHD tumorigenesis. In accordance, heightened glycogen levels were also reported in the muscle tissues of muscle-specific *Flcn* KO mice as compared to the controls.[21,22]

A dual role for glycogen

The role of glycogen as an energy source has been widely demonstrated in multiple organisms. However, its role as a reservoir for the production of osmolytes upon acute exposure to hypertonic stress has not been clearly reported. In *Corynebacterium glutamicum*, the exposure to hyperosmotic shock was shown to result in glycogen degradation and the synthesis of the osmoprotectant trehalose.[23] In *C. elegans*, recent reports demonstrate an important role for glycogen in mediating survival to hypoosmotic-anoxic stress.[19,20] Our data suggest that glycogen degradation leads to different outcomes depending on the type of stress. It is possible that the glycogen degraded by energy stresses generates ATP while the glycogen degraded by hyperosmotic stress produces glycerol. In support to this, we observed that the pretreatment of wild-type and *flcn-1* mutant animals with Paraquat (PQ; oxidative and energy stressor) suppressed the increased resistance of *flcn-1* nematodes to NaCl, while the pretreatment of wild-type and *flcn-1* mutant worms with 200 mM NaCl increased their resistance upon PQ exposure.[14] This could imply that the pretreatment of the animals with PQ depletes them from glycogen, generating ATP, and abrogating their ability to produce glycerol later on upon NaCl exposure. However, the pretreatment of the worms with NaCl depletes the glycogen stores and produces glycerol, a carbon source that could be used to produce ATP upon exposure to PQ.

The paradoxical role of AMPK in glycogen metabolism

The AMPK-dependent regulation of glycogen metabolism has long been a paradox. The acute activation of AMPK has been shown to inhibit glycogen synthase leading to glycogen degradation.[24-27] However, the chronic activation of AMPK has been shown to lead to glycogen accumulation. Mechanistically, the chronic activation of AMPK has been shown to increase glucose uptake and result in the accumulation of glucose-6-phosphate, which allosterically activates glycogen synthase and leads to glycogen synthesis.[28-30] Accordingly, the constitutive activation of AMPK via mutations in the $\gamma 2$ and $\gamma 3$ subunits has been

associated with glycogen accumulation in the skeletal and cardiac muscles of pigs and mice.[30-36] In agreement, and similarly to what we have reported,[14] yeast snf1 mutants display decreased levels of glycogen as compared to the control.[37] Importantly, whether the osmotic stress-dependent acute activation of AMPK leads to the activation of glycogen phosphorylase is still not clear and needs further investigation.

Using the nematode to understand the Birt-Hogg-Dubé disease

Birt-Hogg-Dubé is an autosmal dominant neoplastic syndrome characterized by skin lesions named fibrofolliculomas, pulmonary cysts, pneumothorax, and an increased presdisposition to renal cysts and tumors.[38-51] BHD is caused by germline mutations in the BHD gene, which encodes FLCN, a 64KDa protein, expressed in most tissues.[52] Since the discovery of the FLCN gene, diverse FLCN-related cellular functions have been reported. However, it remains unclear whether these biological processes are directly regulated by FLCN or they are simply a result of indirect effects related to FLCN.

Although mammalian model organisms such as mice and rats are highly advantageous to study disease-related biological processes in humans due the close anatomical and physiological similarities between systems, they have disadvantages including space, cost, and time-consuming transgenic technologies. The nematode *C. elegans* has emerged as an excellent model organism to study conserved signaling pathways. In fact, many biological processes are highly evolutionary conserved such that findings in *C. elegans* are applicable to humans. Importantly, deregulation in pathways that regulate proliferation, cell death, and metabolism is associated with tumor formation and dissemination. Although *C. elegans* nematodes do not develop tumors *per se*, the molecular pathways that lead to cancer in humans are conserved across evolution and lead to other phenotypic outcomes in the worm, which have been successfully used by researchers, to genetically determine molecular interactions and to screen for anticancer drugs.[53-55]

In analogy with the important role of glycogen in survival to stress, glycogen could also be an important molecule that fuels tumorigenesis. Glycogen accumulates in many cancer types including ovarian cancer,[56] kidney cancer,[57] colorectal cancer,[58] bladder cancer,[59] and others.[60] In addition, higher glycogen levels were detected in breast, kidney, bladder, uterus, skin, ovary, and brain cancer cell lines[60], and recent studies have demonstrated a critical role for glycogen in the survival of cancer cells to hypoxic environments and glucose restriction.[57,61] Importantly, the inhibition of glycogen degradation induced apoptosis in pancreatic cancer cells and impaired the *in vivo* growth of tumor xenografts.[57]

The AMPK-dependent regulation of hyperosmotic stress that we observed in *C. elegans* is a very interesting aspect in regards to BHD disease, which is mostly manifested by enlarged renal cysts and tumors in all mammalian models including rats, mice, dogs, and men.[40-42,44,48] Since the kidney is an organ chronically exposed to hyperosmotic stress, it is possible that the BHD renal tumors and cysts are formed because of an increased resistance to hyperosmotic conditions, which could lead to DNA damage. In support of this, loss of *FLCN* and *VHL*, which are both renal tumor suppressor genes, predispose to renal clear cell carcinomas which are glycogen-rich tumors. Based on our recent findings in *C. elegans*, we speculate that glycogen plays a dual role in BHD neoplasm: it supplies cancer cells with energy and helps them resist the renal hyperosmotic environment.

Concluding remarks

Future experiments aiming to determine which osmolytes are produced following glycogen degradation in animals are necessary to understand the role of glycogen in hyperosmotic stress resistance. Although we show that glycerol is a major osmoprotectant, other osmolytes resulting from the hyperosmotic-stress dependent degradation of glycogen could also contribute to the survival of cells/organisms.

As a continuation of this work, it will be important to assess this pathway in cell culture systems and in BHD mouse models and to target it in cancer models to determine potential therapeutic benefits in the treatment of BHD disease in specific and kidney cancers in general.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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