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The diverse functions of GAPDH: views from different subcellular compartments

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Abstract

Multiple roles for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) have been recently appreciated. In addition to the cytoplasm where majority of GAPDH is located under the basal condition, GAPDH is also found in the particulate fractions, such as the nucleus, the mitochondria, and the small vesicular fractions. When cells are exposed to various stressors, dynamic subcellular re-distribution of GAPDH occurs. Here we review these multifunctional properties of GAPDH, especially linking them to its oligomerization, posttranslational modification, and subcellular localization. This includes mechanistic descriptions of how *S*-nitrosylation of GAPDH under oxidative stress may lead to cell death/dysfunction via nuclear translocation of GAPDH, which is counteracted by a cytosolic GOSPEL. GAPDH is also involved in various diseases, especially neurodegenerative disorders and cancers. Therapeutic strategies to these conditions based on molecular understanding of GAPDH are discussed.

Keywords

GAPDH; Glyceraldehyde 3-Phosphosphate dehydrogenase; Siah; GOSPEL; oxidative stress; stress signaling; S-nitrosylation; cytoplasm; microtubules; vesicular trafficking; cytoskeleton; mitochondria; nucleus

1. Introduction

In the past two decades, the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) that was once considered a simple “housekeeping” protein has been shown to be involved in many cellular processes in addition to glycolysis. These include: DNA repair [1], tRNA export [2], membrane fusion and transport [3,4], cytoskeletal dynamics [5], and cell death [6–12]. The multifunctional properties of GAPDH are likely to be regulated, at least in part, by its oligomerization, posttranslational modification, and subcellular localization. Posttranslational modifications are divided into reversible and irreversible ones. Here we review this multifunctional nature of GAPDH exhibited in distinct subcellular domains of cytoplasm, vesicles, mitochondria, and nucleus. We propose a novel concept of

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how these functions may have a common biological significance in the role of stress response.

2. GAPDH in the cytoplasm (Fig. 1)

In the cytoplasm GAPDH exists primarily as a tetrameric isoform composed of four identical 37 kDa subunits, each with a single catalytic thiol group. GAPDH coverts glyceraldehyde-3-phosphate to D-glycerate 1,3-bisphospate, in the presence of nicotinamide adenine dinucleotide (NAD^+) and inorganic phosphate, and mediates formation of NADH and adenosine triphosphate (ATP). While GAPDH continues to retain its fundamental role as a glycolytic “housekeeping” protein of the cytoplasm, accumulating evidence indicates that posttranslational modifications of cytosolic GAPDH push this molecule into functional avenues that deviate from glycolysis.

In human monocytes GAPDH facilitates a metabolic shift from anaerobic respiration to the pentose phosphate pathway [13]. Oxidative stress following the respiratory burst during phagocytosis and monocyte activation induces *S*-thiolation of the reactive sulfhydryl groups on GAPDH. The corresponding cysteine residue of GAPDH in other organisms is also modified under oxidative stress, which was proposed to be a mechanism to protect the glycolytic enzyme from irreversible oxidative inactivation [14,15]. Since inactivation of GAPDH by *S*-thiolation is readily reversible, this posttranslational modification may allow GAPDH to function as a switch that enables cells to shift between metabolic functions and maintenance of oxidation/reduction balance. Indeed, Krobitsch and colleagues [16] provided the first direct evidence that oxidative inhibition of glycolytic enzymes, including GAPDH, is a controlled response that enables cells to redirect their carbohydrate flux from glycolysis to the pentose phosphate pathway, generating NADPH, the reducing power within cells to protect them from oxidative stress (Fig. 1).

Other studies have shown that with this redox-sensitive cysteine residue, GAPDH can modulate cellular signaling pathways in response to oxidative stress [17,18]. For example, GAPDH was shown to physiologically bind to inositol 1,4,5-trisphosphate receptor, delivering NADH in close proximity to the channel and thus regulating intracellular Ca^{2+} signaling (Fig. 1) [19].

S-Nitrosylation, a covalent addition of a nitric oxide (NO) group to the thiol side chain of cysteine, has emerged as an important mechanism for reversible posttranslational regulation of many proteins including GAPDH [20]. However, its effect in mediating a metabolic flux is limited due to subsequent posttranslational modifications that inactivate GAPDH irreversibly. These include NAD^+ or NADH attachment, both of which are capable of more strongly inhibiting the catalytic activity of GAPDH [21,22]. When the cell is exposed to massive stressors beyond its stress tolerance level, the inactivation of GAPDH may have catastrophic “loss of function” effects by reducing the ability of the cell to meet the increased energy demands required to maintain homeostasis under extreme stress [23,24]. However, the posttranslational or conformational modifications to a small pool of the total cellular GAPDH are potentially unlikely to induce dramatic changes in the cellular glycolytic pathways. In this case, a small pool of *S*-nitrosylated GAPDH has further and irreversible modification (sulphonation) (Fig. 1), which with this modification, translocates to subcellular domains where it does not normally occurs, as seen with GAPDH-Siah association, may stimulate a “gain of function” that could provoke apoptosis or cellular dysfunction (see in subsection 5 below) [6].

The posttranslational modifications from *S*-nitrosylation to sulphonation commit GAPDH to an irreversible signaling cascade that begin in the cytosol and traverse to other cellular compartments. Thus, regulatory mechanisms for this cascade are important for cellular

homeostasis. We have recently reported a novel protein, GOSPEL (GAPDH's competitor Of Siah Protein Enhances Life), as a key regulator for GAPDH [25]. This cytosolic protein is highly expressed in organs with high levels of energy requirement and high expression levels of GAPDH, such as muscle, heart, and brain. In the presence of nitrosative stress, GOSPEL is quickly S-nitrosylated and retains GAPDH in the cytoplasm, promoting GAPDH-GOSPEL association while competitively preventing the cytotoxic interaction of GAPDH with Siah (Fig. 1) [25]. The competition between GOSPEL and Siah for GAPDH binding is likely to maintain cellular homeostasis when cells are exposed to stressors, by favoring the cytoprotective GOSPEL *S*-nitrosylation over the cytotoxic mechanisms mediated by GAPDH *S*-nitrosylation. However, once the level of nitrosative stress exceeds a threshold, GAPDH-Siah binding predominates over GAPDH-GOSPEL interaction and then leads to cell death/dysfunction [25]. This is analogous to *S*-nitrosylation of the NMDA-type glutamate receptor [26–28]: activation of NMDA receptor at a modest level contains a protective mechanism by *S*-nitrosylation (a type of negative feedback), inhibiting the overactivation of this receptor that might result in massive activation of nNOS (nitrosative stress) and cell death/dysfunction. Likewise, we reported that overexpression of GOSPEL is neuroprotective, whereas mutant GOSPEL lacking the *S*-nitrosylation site and its binding of GAPDH fails to block cell death in primary neuron cultures [25]. This neuroprotective action of GOSPEL was further validated in a model of NMDA excitotoxicity *in vivo* [25].

Furthermore, increased levels of oxidative stresses can promote GAPDH aggregation in the cytoplasm, which seems to be associated with cell death (Fig. 1) [29,30]. Oxidative stress *in vitro* elicits the formation of disulfide-bonded GAPDH aggregates, which in turn results in the production of amyloid-like fibrils [29]. Similarly, oxidative stress caused *in vivo* by methamphetamine, which produces massive oxidative stress, induces the formation GAPDH aggregates in mouse brain. In GAPDH transgenic mice, methamphetamine accelerated GAPDH aggregation and neuronal cell death [30].

3. GAPDH in association with microtubules, vesicular trafficking, and the cytoskeleton (membrane fusion) (Fig. 2)

GAPDH was one of the first glycolytic enzyme known to interact with tubulin and actin, facilitating microtubule bundling and actin polymerization, respectively (Fig. 2) [5,31]. Serum deprivation, likely to be associated with oxidative stress, promotes the association of GAPDH with the stress fibers (microfilament bundles) in NIH 3T3 cells [32]. GAPDH-microtubule associations directly modulate the glycolytic activity and quaternary structure of GAPDH by promoting the reversible dissociation of its tetrameric isoform into glycolytically inactive monomeric molecules of GAPDH [33]. Furthermore, catalytically active GAPDH may be transported within the cell via microtubule treadmilling during a process that may allow it to couple signal-stimulated glycolysis with the reorganization of the cytoskeleton (Fig. 2) [5,31].

Multiple studies from different groups have demonstrated the participation of one or more GAPDH isoforms in membrane fusion and trafficking in biological systems. For example, Robbins and colleagues [34] demonstrated that a mutation of GAPDH altered membrane trafficking in Chinese hamster ovary cells. The small GTPase Rab2 localizes to vesicular tubular clusters (VTCs), which function as transport complexes carrying cargo between the endoplasmic reticulum (ER) and the Golgi complex. Tyrosine phosphorylation of GAPDH by atypical protein kinase Cι (aPKC) is facilitated by Rab 2, which increases phospho-GAPDH recruitment to VTCs. This process plays an important role for membrane trafficking between the ER and Golgi complex and for membrane trafficking form VTCs [4,35,36]. A tyrosine kinase Src-mediated phosphorylation of aPKC further facilitates protein associations of Rab2-Src-aPKC-GAPDH on VTCs [37], and the phospho-GAPDH

promotes the interaction of the microtubules and motor proteins with Rab2-generated vesicles (Fig. 2). Thus, GAPDH might act as an adaptor or scaffolding protein that mediates vesicular trafficking between cellular compartments (Fig. 2) [38]. In addition, this phosphorylation of GAPDH via aPKC counteracts tubulin-mediated inhibition of GAPDH-catalyzed membrane fusion [35,39]. Similarly, GAPDH increases the interaction of the microtubules with N-myrisotylated p22, an EF-hand Ca^{2+}-binding protein, which facilitates microtubule-membrane interactions [40].

4. GAPDH in the mitochondria (Fig. 3)

The levels of GAPDH in the mitochondria are low at the basal condition, but they are elevated under stressed conditions, such as serum deprivation and exposure to DNA-damaging agents [41].

When GAPDH is expressed exogenously, a pool of GAPDH is located to the mitochondria and induces pro-apoptotic mitochondrial membrane permeabilization (MMP) via an association with voltage-dependent anion channel 1 (VDAC1) [41]. Studies with isolated mitochondria have suggested that dimers and tetramers of GAPDH interact with VDAC1. Exogenous expression of GAPDH in the mitochondria also causes loss of the inner transmembrane potential, matrix swelling, permeabilization of the inner-mitochondrial membrane, and the release of two pro-apoptotic proteins, cytochrome c and apoptosis-inducing factor (Fig. 3) [41]. It is unclear whether specific posttranslational modifications may play a role in targeting GAPDH to the mitochondria and interaction of mitochondrial proteins.

In contrast, another study reports that GAPDH participates in the recovery from mitochondrial outer-membrane permeabilization (MOMP) [42]. In this scenario, GAPDH protects cells from death following MOMP, in the absence of caspase activation (Fig. 3). Here the association of GAPDH with cell survival may be by providing enough ATP to maintain the mitochondrial membrane potential via the F_0F_1 ATPase, helping counteract the effects of the energetic collapse by the loss of mitochondrial function.

Furthermore, it was recently demonstrated that rotenone, a common mitochondrial complex I inhibitor, induces GAPDH enrichment in particulate fractions, aggregate formation and reduces GAPDH glycolytic activity [43].

5. GAPDH in the nucleus (Fig. 4)

We reported that a small pool of GAPDH is translocated to the nucleus upon exposure to stressors and participates in cell death/dysfunction [12] with other groups also replicating this observation [10,44–51]. This indicates that GAPDH may act as a relay molecule between cellular compartments during cellular stress. The signal is conveyed by GAPDH that is S-nitrosylated by NO at active site Cys-150, allowing GAPDH to bind to the Siah (an E3 ubiquitin ligase), leading to nuclear translocation of GAPDH-Siah complex (Fig. 4) [6]. It seems that the nuclear localization signal in Siah can lead to the protein complex, probably maintaining tetrameric structure of GAPDH. Stabilized Siah together with *S*-nitrosylated GAPDH seems to facilitate ubiquitination and degradation of the nuclear co-repressor N-CoR [6,52]. Further studies have also shown that nuclear translocated GAPDH is further acetylated at Lys-160 by the histone acetyltransferase p300/CBP via direct protein interaction, which in turn stimulates the catalytic activity of p300/CBP. This nuclear event leads to the acetylation of downstream targets, including the tumor suppressor p53 (Fig. 4) [53]. By both of these mechanisms, the nuclear GAPDH-Siah complex may regulate gene expression via modulating histone modifications, which results in cellular dysfunction and death.

GAPDH was shown to co-immunoprecipitate with promyelocytic leukemia protein (PML) and co-localize in a subset of nuclear bodies (Fig. 4) [54]. The localization of PML and GAPDH to the same nuclear bodies is reportedly dependent on the presence of RNA [54]. Since disruption of PML bodies reduces apoptosis in acute promyelocytic leukemia and GAPDH induces apoptotic neuronal death, the GAPDH-PML interaction may be involved in the regulation of cell death.

Nuclear GAPDH also has various functions unrelated to cell death. The correlation of an increased uracil DNA glycosylase (UDG) activity with an increase in cell cycle-regulated expression of monomeric GAPDH suggests that this multifunctional molecule also plays an important role in DNA repair (Fig. 4) [55,56]. Monomeric nuclear GAPDH also associates with DNA as a component of the multicomplex Oct-1 coactivator, OCA-S, that stimulates the expression of Histone 2B [57]. GAPDH acts as a redox sensor and binds directly to Oct-1 to regulate transcription during S phase (Fig. 4) [58]. GAPDH-NAD^+ association is required for Oct-1-mediated gene transcription. *O*-linked N-acetylglucosamine modifications of GAPDH (*O*-GlcNAcylation mainly on Thr227) are able to disrupt the tetrameric form of GAPDH, enabling its nuclear translocation [59]. Thus, it may be important to explore whether *O*-linked N-acetylglucosamine modifications may underlie roles of monomeric nuclear GAPDH.

GAPDH can also physically interact with apurinic / apyrimidinic endonuclease (APE1), an essential enzyme that functions in the base excision DNA repair pathway to process spontaneous and drug-induced abasic or apurinic/apyrimidinic sites as well as to regulate the redox state of a number of transcriptional factors (Fig. 4) [60]. GAPDH directly interacts with SET nuclear oncogene, a molecule that inhibits cyclinB-cdk1 activity, and reverses its inhibitory effects leading to an advanced cyclin B-cdk1 activity peak, increased mitosis, and accelerated the cell cycle (Fig. 4) [61].

Nuclear GAPDH also plays role in maintaining and protecting telomeric DNA from rapid degradation (Fig. 4) [62,63]. During oxidative stress, poly(ADP-ribose) polymerase -1 (PARP-1), a DNA-repair enzyme that is activated by severe DNA damage, may serve as an upstream regulator of GAPDH (Fig. 4) [64]. PARP-1 can inactivate GAPDH via ADP-ribosylation, which elicits greater cellular energy deficits and accelerates cell death. Through this process, GAPDH is believed to not only relay stress signals but also shuttle the generation of ATP to cells responsible for repairing and/or removing dead or dying cells, such as those surrounding ischemic tissue [65].

6. Significances of multifunctional roles for GAPDH

Several distinct pools of GAPDH appear to sense intra- and extracellular stresses via posttranslational and/or conformational changes and activate downstream pathways to maintain homeostasis or promote cell death (summarized in Table. 1). The prominent expression of GAPDH in the cytosol may allow it function efficiently as an intracellular sensor capable of directly relaying signals to various organelles, such as the nucleus. GAPDH functions as a double-edged sword capable of facilitating the completion of an apoptotic event or regulating the recovery from an insult, such as that seen following MOMP in the mitochondria. Of note, apoptotic cell death can contribute to the maintenance of homeostasis at the organism levels. Thus, overall functions of GAPDH may be to maintain homeostasis at multiple levels. Glycolytic enzymes function in a well-coordinated manner, and some of them, such as GAPDH and aldolase, form protein-protein complexes [66]. In analogy to GAPDH, other glycolytic enzymes may have multiple roles and function as intracellular sensors. Relationship of GAPDH with other glycolytic enzymes beyond glycolytic pathway may be an interesting subject to be studied in the future. GAPDH is

evolutionally well preserved. How come does GAPDH acquire such wide range of cellular roles during the evolution? This may be an interesting question in regard to homeostatic regulation in organisms.

7. Medical implications and future perspectives

Participation of GAPDH in multiple pathways of homeostatic regulation indicates that this molecule may also play a role, when it is disturbed, in the manifestation of certain diseases. Accumulating evidence suggests that nuclear GAPDH may be involved in several neurodegenerative disorders [67]. Nuclear GAPDH has been found in fibroblasts and in postmortem brains from patients with polyglutamine diseases (such as Huntington's disease or dentatorubral-pallidoluysian atrophy) [68,69], Parkinson's disease [51], and Alzheimer's disease [48,70]. Some studies suggest that GAPDH interacts with β-amyloid peptides, mutant huntingtin, androgen receptor, and atrophin-1 [71–75]. In an experimental model of brain ischemia, accumulation of nuclear GAPDH is observed [50]. Moreover, promising pharmacological evidence further supports a role for nuclear GAPDH in cell dysfunction and death: deprenyl used for symptomatic amelioration for patients with Parkinson's disease potentially may block GAPDH-Siah binding, in addition to its classic action as a monoamine oxidase B (MAO-B) inhibitor [76–79]. Some of the structural derivatives of deprenyl, even those lacking this inhibitory action on MAO-B, are still neuroprotective [76–82]. Among them, TCH346 shows neuroprotective action largely via blockade of GAPDH-Siah binding and nuclear translocation of the GAPDH-Siah protein complex [83] and rasagiline has shown neuroprotective effects in ethanol-induced cell death mediated by a novel GAPDH-MAO-B pathway [84,85]. To the contrary, saframycin, an antiproliferative agent for the treatment of leukemia- and tumor-derive cells is capable of forming a ternary complex with GAPDH and DNA to induce cytotoxic effects [86]. Further understanding of GAPDH may aid novel therapeutic strategies for many disorders.

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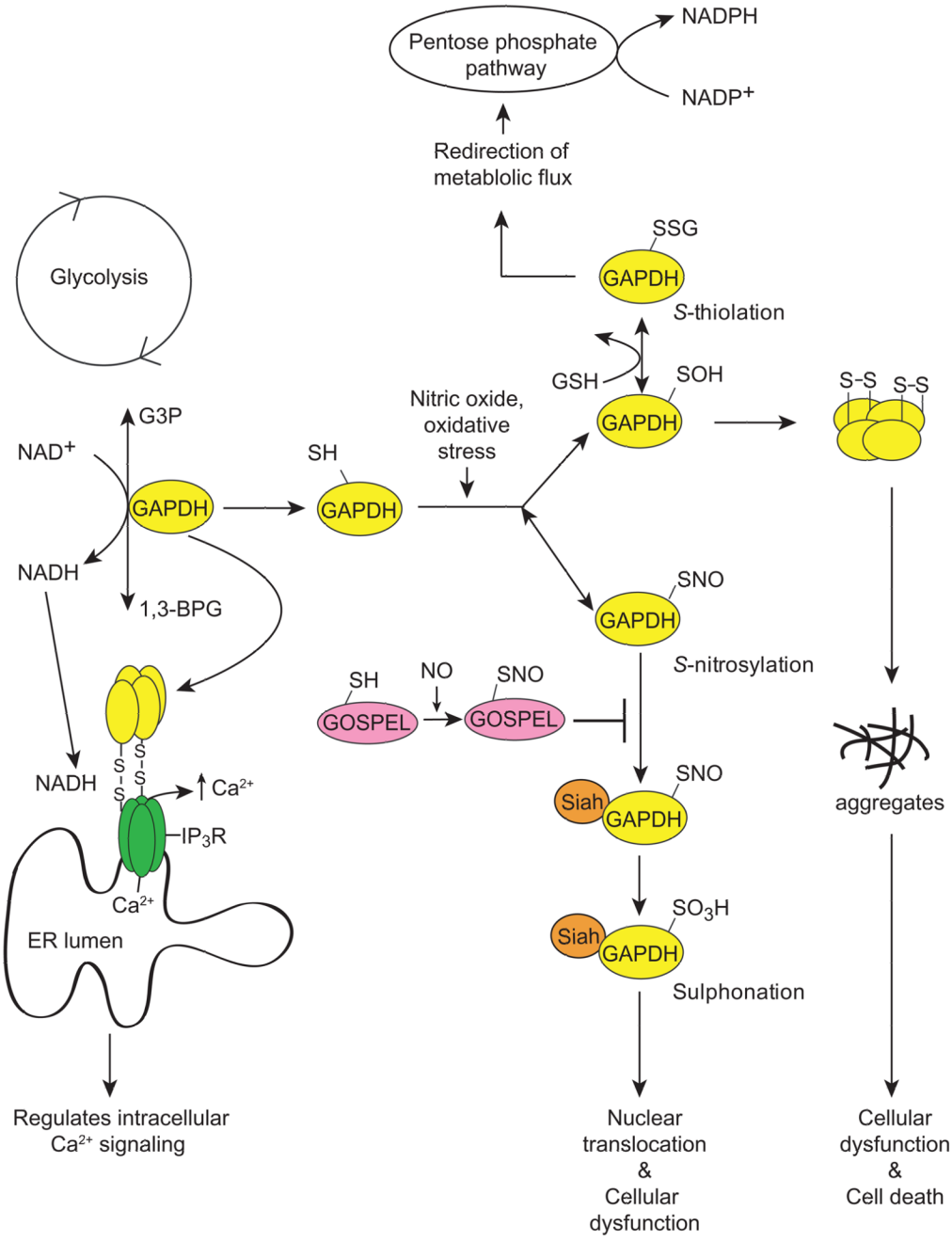

Fig. 1. GAPDH in the cytoplasm

Glycolytic enzyme, GAPDH, catalyzes the conversion of glyceraldehyde-3-phosphate (G3P) into 1,3-biphosphoglycerate (1,3-BPG). GAPDH can undergo different posttranslational modifications, which may determine some of its non-glycolytic functions. Under conditions of cellular oxidative stress, GAPDH can undergo reversible *S*-thiolation (-SSG), a mechanism to protect the glycolytic enzyme from irreversible oxidative inactivation and consequently redirecting the metabolic flux from glycolysis to the pentose phosphate pathway to maintain an optimal NADPH/$NADP^+$ ratio. GAPDH contributes to local $NADH^+$ and may regulate IP_3R-mediated Ca^{2+} signaling. Nitric oxide stress also leads to reversible *S*-nitrosylation (−SNO) of cystine-150 of GAPDH that facilitates its binding to

Siah, and results in translocation of the complex to the nucleus and irreversible sulphonation ($-SO_3H$) of GAPDH. This cascade mediates cell death/dysfunction in a gain-of-toxic manner. *S*- Nitrosylation of GOSPEL augments binding of GOSPEL with GAPDH, competing with binding of GAPDH with Siah, which is a cytoprotective mechanism against GAPDH-Siah cascade. Exposure to oxidants can induce an irreversible oxidation of cysteine residues that favor intermolecular disulfide bonds and the subsequent formation of cytosolic aggregates of GAPDH. This insoluble protein may ultimately promote cellular dysfunction and cell death.

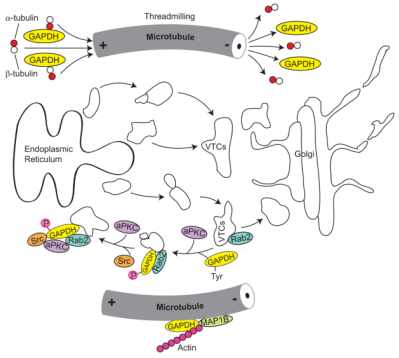

Fig. 2. GAPDH in association with cytoskeleton and vesicular transport

GAPDH is known to interact with tubulin and actin, facilitating microtubule bundling and actin polymerization, respectively. GAPDH may be transported within the cell via microtubule treadmilling. GAPDH also plays an important role in VTCs (vesicular tubular clusters) where phosphorylated GAPDH by atypical protein kinase Cι (aPKC) is recruited. This process plays an important role for membrane trafficking between the ER and Golgi complex without requirement of GAPDH glycolytic activity. A tyrosine kinase Src-mediated phosphorylation of aPKC further facilitates protein associations of Rab2-Src-aPKC-GAPDH on VTCs, and the phospho-GAPDH promotes the interaction of the microtubules and motor proteins with Rab2-generated vesicles.

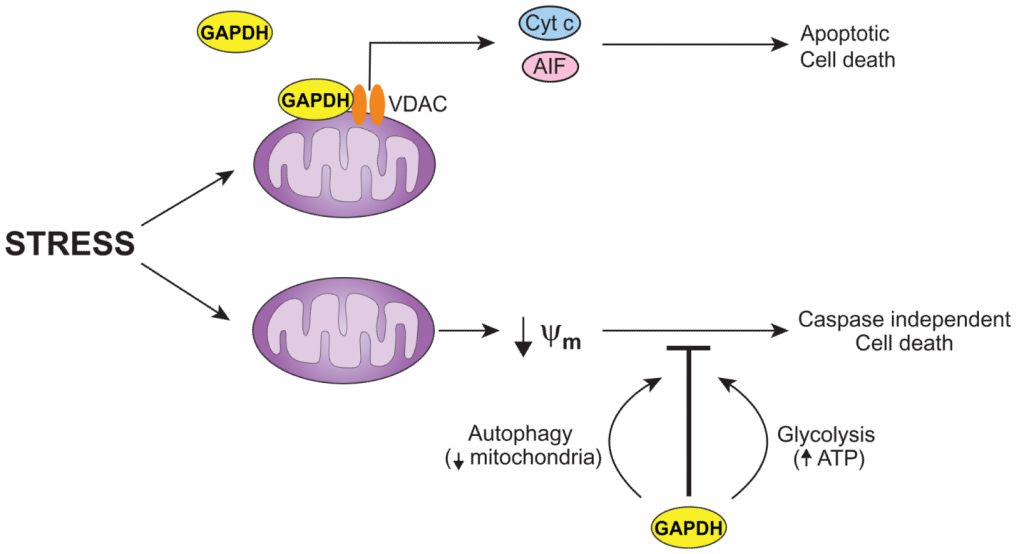

Fig. 3. GAPDH in mitochondria

GAPDH has been also localized in the mitochondria and have distinct functions in a context-dependent manner. GAPDH can bind with the voltage-dependent anion channel (VDAC), which may promote the release of cytochrome c (CytC) and apoptosis-inducing factor (AIF), leading to apoptotic cell death. In stressed conditions, a decrease in mitochondrial membrane potential (ψ_m) leads to caspase-independent cell death (CICD). In this context, GAPDH can inhibit cell death by simultaneously increasing ATP levels through glycolysis and stimulating autophagy-mediated clearance of permeabilized mitochondria.

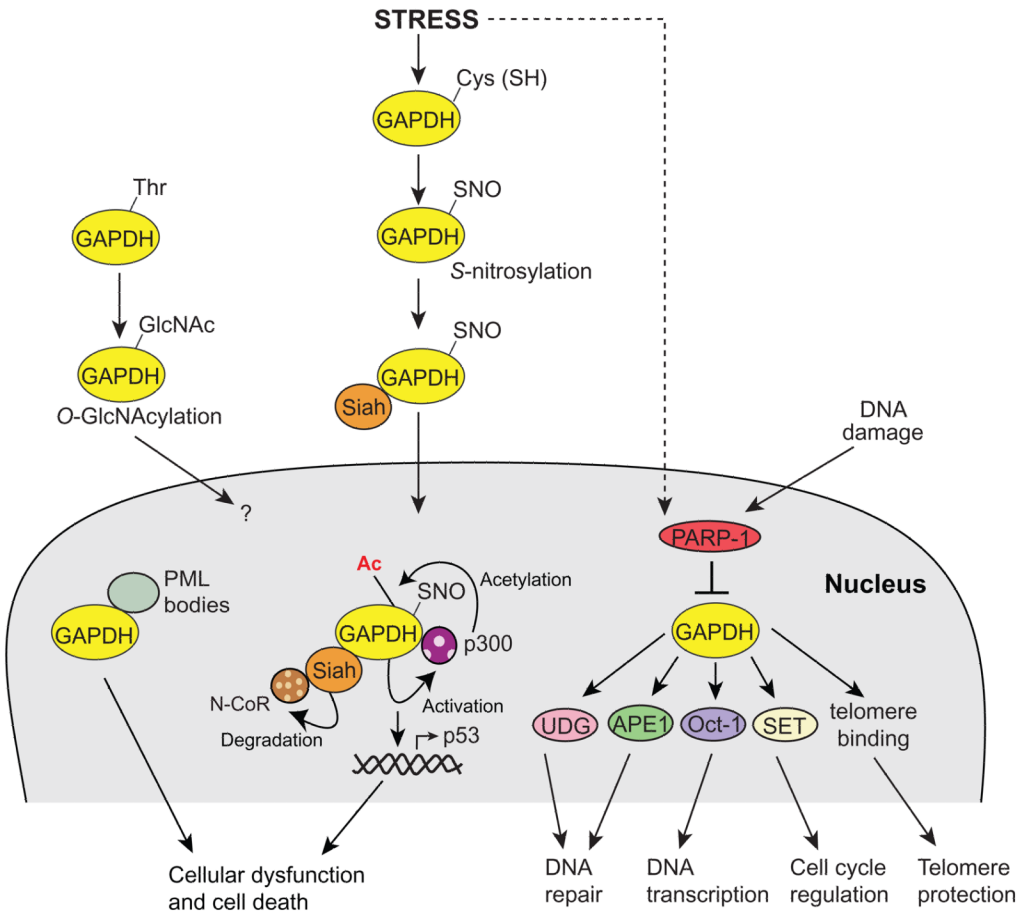

Fig. 4. GAPDH in nucleus

Oxidative modifications such as, *S*-nitrosylation, of GAPDH increases binding to Siah, which mediates its nuclear translocation. GAPDH stabilizes Siah, facilitating its degradation of nuclear proteins such as nuclear co-repressor (N-CoR). Nuclear GAPDH is acetylated by the p300/CREB-binding protein (CBP), which in turn stimulates the catalytic activity of p300/CBP. Consequently, downstream targets of p300/CBP, such as p53, can be activated and cause cellular dysfunction. In addition, GAPDH can undergo O-linked *β*-N-acetylglucosamine glycosylation (O-GlcNAcylation) at threonine residues, which also may mediate its nuclear translocation in a different mechanism. GAPDH also binds with PML, which may be involved in cell death. Nuclear GAPDH can participate in DNA repair, regulations of gene transcription and cell cycle, turnover of telomeric DNA. During oxidative stress, PARP-1 may serve as an upstream regulator of nuclear GAPDH.

Table 1

Modification of GAPDH	Amino Acid Site	Structural Form	Reversible	Catalytic Activity	Functional Relevance
Acetylation	K160	Tetramer	Yes	No	Stress Response Cytotoxicity Cellular Dysfunction
O-GlcNacylation	T227	Monomer	Yes	No	Stress Response Cytotoxicity Cellular Dysfunction Cell Proliferation
Phosphorylation	Y40 A81 S149 T182 T244	Unknown	Yes	Unknown	Stress Response Cytotoxicity Cellular Dysfunction Cellular Trafficking
S-nitrosylation	C150	Tetramer Monomer Dimer	Yes	No	Stress Response Cytotoxicity Cellular Dysfunction
S-thiolation	-SH groups	Tetramer Monomer Dimer	Yes	No	Stress Response Cytotoxicity Cellular Dysfunction
Sulphonation	C150	Tetramer Monomer Dimer	Yes	No	Stress Response Cytotoxicity Cellular Dysfunction
Aggregation	C150 and C282	Aggregate	No	No	Stress Response Cytotoxicity Cellular Dysfunction
Gospel attachment	P80 - S120	Monomer?	Yes?	No	Stress Response Cytotoxicity Cellular Dysfunction
NAD/NADH attachment	Rossmann-fold	Tetramer	No	No	Stress Response Cytotoxicity Cellular Dysfunction
Covalent NADH attachment	C150	Tetramer	No	No	Stress Response Cytotoxicity Cellular Dysfunction
Polyglutamine association	M1 – V169	Tetramer Monomer Dimer	No	Yes/No	Stress Response Cytotoxicity Cellular Dysfunction
Siah attachment	P220 - (K225*) - V238	Tetramer	No	No	Stress Response Cytotoxicity Cellular Dysfunction