Published in final edited form as:

Int J Biochem Cell Biol. 2014 December ; 0: 20–26. doi:10.1016/j.biocel.2014.09.026.

Structural Analysis of Glyceraldehyde-3-Phosphate Dehydrogenase Functional Diversity

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Abstract

Multifunctional proteins provide a new mechanism to expand exponentially cell information and capability beyond that indicated by conventional gene analyses. As such, examination of their structure-function relationships provides a means to define the mechanisms through which cells accomplish critical yet disparate activities required for cell viability and survival.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) may be considered the quintessential multidimensional protein which exhibits a variety of functions unrelated to its classical role in energy production. This review discusses new insights into the structure-function mechanisms through which defined GAPDH amino acid domains are utilized for its diverse activities, the importance of its post-translational modification, and, intriguingly, the logic inherent in the presence or the absence of specific signaling domains.

Keywords

glyceraldehyde-3-phosphate dehydrogenase; multifunctional protein; amino acid domains; mRNA stability; post-translational modification

“Necessity is the Mother of Invention”

An English Proverb

1. Introduction

Multifunctional proteins represent a new, recently identified class of cellular molecules. Initially isolated and characterized based on a single, well analyzed activity, subsequent studies demonstrated their unexpected role in a wide variety of cellular pathways distinct from their classical functions (Jeffery, 1999; Kim and Dang, 2005; Huberts and van der Kiel, 2010). Given these new activities, it's reasonable to suggest that analysis of multifunctional protein structure may provide the basis not only for understanding their

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diverse activities but also for determining specific mechanisms through which those functions are controlled.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC 1.2.1.12) may be considered as the quintessential example of such proteins (Sirover, 1995, 1999, 2011; Tristan et. al., 2011). Initially well characterized for its role in glycolysis, it is now recognized as a novel multifunctional protein exhibiting new functions including the regulation of mRNA stability (Bonafe et. al, 2005; Rodriguez-Pascual et. al., 2008; Zhou et. al., 2008; Backlund et. al., 2009, Kondo et. al., 2011; Ikeda et. al., 2012), intracellular membrane trafficking (Tisdale, 2001), iron uptake and transport (Raje et. al., 2007), heme metabolism (Chakravarti et. al., 2010), the maintenance of genomic integrity (Meyer- Sieglar et. al., 1991; Azam et. al., 2008; Demarse et. al., 2009), the regulation of gene expression (Harada et. al., 2007; Zheng et. al., 2003) as well as nuclear tRNA export (Singh and Green, 1993). Intriguingly, despite its multiplicity of functions, GAPDH is encoded by a single structural gene in somatic cells (Bruns and Gerald, 1976; Bruns et. al., 1979), no alternate transcripts have been identified (Mezquita et. al., 1998) and the protein itself is highly conserved across the phylogenetic scale. However, although it is translated as a single 37 kDa protein normally observed in its tetrameric form, past studies indicate specific GAPDH isozymes which differ both in pI and in catalytic activity (rev. in Sirover, 1999).

Accordingly, the purpose of this review is to define three distinct GAPDH structure-function relationships: the use of unique GAPDH domains for its new activities, the role of multiple post-translational GAPDH modifications that govern several of its new activities, and, intriguingly, the use (or lack of use) of specific signaling domains to regulate GAPDH activity. En toto, this provides a means to understand not only the basis for GAPDH functional diversity but also its utility as a model for the role of this new class of cellular proteins in normal cell function and in cell pathology.

2. Analysis of GAPDH structure and functional domains

Glyceraldehyde-3-phosphate dehydrogenase is a 335 amino acid long polypeptide which is highly conserved across the phylogenetic scale. It contains two major domains (Fig. 1), the NAD⁺ binding domain (amino acids 1→150) and the catalytic or glyceraldehyde-3-phosphate domain (amino acids 151→335) which contains an active site cysteine.

Recent studies indicate those GAPDH amino acid sequences that are utilized for its new functions (Fig. 1). In particular, its NAD⁺ binding site is the loci for its role in determining mRNA stability and translation (Bonafe et. al, 2005; Rodriguez-Pascual et. al., 2008; Zhou et. al., 2008; Backlund et. al., 2009, Koneda et. al., 2011; Ikeda et. al., 2012); its membrane functions based on a phosphatidyl serine binding site (Kaneda et. al., 1997) as well as its binding to glutathione (Puder and Soberman, 1997). Interactions with the active site cysteine are fundamental to its role in heme metabolism (Hannibal et. al., 2012), in the cellular response to oxidative stress (Hara et. al., 2006; Nakajima et. al., 2007) and in apoptosis (Hara and Snyder, 2006). Lastly, its unique CREM nuclear export signal is located in the distal portion of the molecule within the catalytic site (Brown et. al., 2004).

2.1. GAPDH structure and its functional modulation of mRNA stability

Protein RNA interactions represent a fundamental mechanism underlying post-transcriptional gene regulation. Recent studies identified GAPDH as an AU 3'-UTR binding protein mediated by its RNA binding domain within the GAPDH NAD⁺ binding site (Figure 1). The latter was defined by peptide mapping and by NAD⁺ competition (Nagy and Rigby, 1995). Furthermore, new studies indicate that a 3'-UTR mutation may convert an unrecognized mRNA to one which is now a GAPDH binding molecule (Zeng et. al., 2014). The latter may provide a molecular mechanism underlying the etiology of human disease.

Structure-function analyses indicated an intriguing complexity with respect to the effect of GAPDH on mRNA function. Three different effects were observed: diminution of translation due to mRNA degradation (Rodrogez-Pascal et. al., 2008; Ikeda et. al., 2012; Zeng et. al., 2014), stimulation of translation due to an increase in mRNA stability (Bonafe et. al. 2005; Kondo et. al., 2011), and, lastly, inhibition of translation due to GAPDH binding in the absence of mRNA degradation (Backlund et. al., 2009). These findings would appear to be in contrast to that normally observed as a consequence of protein: 3' UTR binding, i.e., a single effect on mRNA stability, facilitating or reducing the extent of translation.

To explain this apparent discrepancy, it's possible to suggest a two-step structure- function model as a mechanism through which GAPDH regulates mRNA function (Figure 2). First, the GAPDH: mRNA complex is formed mediated by the interaction of the GAPDH NAD⁺ binding site with the AU rich 3'UTR domain. Second, subsequently, this binding interaction orients the GAPDH catalytic site for subsequent activity based on mRNA: GAPDH amino acid active site interaction.

As presented, this structure-function model may be useful in explaining not only the documented disparate consequences of GAPDH: mRNA binding but also the significant secondary structure which characterizes AU rich 3'UTR sequence. The latter will have different tertiary conformations and will be located in different portions of the respective 3'-UTR sequence, i.e., GAPDH binding sites were mapped to residues 1–60, 1–100, and 924–1127 in the COX-2 (Ikeda et. al., 2012), AT1R (Backlund et. al., 2009), and ET-1 3'UTRs (Rodriguez-Pascual et. al., 2009), respectively. Further, it was mapped to the terminal 144 residues in the CSF-1 3'-UTR sequence (Zhou et. al., 2008) and to an 84 residue long element (CAESAR) at the junction of the 3'UTR and the coding region in *ccn2* mRNA (Kondo et. al., 2011).

Thus, it is reasonable to consider that each sequence will interact with the GAPDH catalytic site in a unique manner. For that reason, the model suggests that, although the RNA binding domain resides in its NAD⁺ site, the rate-limiting step in this new GAPDH function may be the mRNA: GAPDH catalytic site interaction.

2.2. GAPDH phosphatidylserine binding domain

Protein-protein interactions represent a fundamental mechanism underlying membrane structure, organization and function. GAPDH was identified not only as a peripheral membrane protein but also as a catalyst for membrane fusion (Nakagawa et. al., 2003).

Structure-function analysis identified that membrane bound GAPDH is a distinct isoenzyme with a unique pI and exhibits diminished catalytic activity (Glaser and Gross, 1995).

Phosphatidylserine (PS) is an acidic lipid located on the inner cytoplasmic side of the plasma membrane which functions in intracellular signaling and in protein translocation. Recent studies identified a unique PS binding domain localized to amino acid residues 70–94 within the GAPDH NAD⁺ binding site (Fig. 1). Structural analysis indicate several basic amino acids (lys⁷⁰, arg⁷⁸, lys⁸⁴) able to form stable bonds with PS PO₄⁻ groups. Of the former, lys⁷⁰ and lys⁸⁴ would appear most likely given the reactivity of other lysine residues involved in diverse GAPDH activities (Table 1).

Structural analyses indicated the relationship between GAPDH: PS binding and the functional diversity of GAPDH. Antibodies directed against the GAPDH PS binding domain suppressed the lipid bilayer fusion step of nuclear envelope assembly thereby inhibiting nuclear membrane formation. Similar results were observed using the 70–94 peptide itself. Surprisingly, although the PS domain is located in the GAPDH NAD⁺ binding site, NAD⁺ itself was unable to compete with antibody binding (Nakagawai et. al., 2003).

In contrast, although membrane bound GAPDH regulates Fe⁺⁺ uptake, transport and metabolism (Raje et. al., 2007), the dependence of that new function on its PS binding domain is unknown. With respect to the former, recent studies suggests a negative relationship between GAPDH membrane association and levels of the transferrin receptor proteins 1 and 2 (TR1 and TR2), i.e., cells actively increase GAPDH membrane concentration and simultaneously reduce TR1 and TR2 expression (Kumar et. al., 2012). That being said, at the present time, it is unknown whether the increase in GAPDH membrane concentration is due to new expression or a change in localization. Further, in times of Fe⁺⁺ scarcity, cells actively secrete membrane bound GAPDH as a means to facilitate Fe⁺⁺ absorption from the extracellular environment (Sheokand et. al., 2013). Accordingly, structural analysis of GAPDH-PS binding would establish the role of the GAPDH binding domain in this new GAPDH function.

3. Post-translational modification of GAPDH: Structural mechanisms of functional control

Post-translational modification is well-recognized as a means by which cells regulate protein function based on minute changes in protein structure, i.e, alteration of a single side chain in one amino acid can induce pleiotropic effects not only on activity in normal cells but also may change function related to cell pathology. As illustrated in Figure 1, GAPDH is subject to numerous post-translational modifications including acetylation, phosphorylation, nitrosylation, by modification with O-linked N-acetyl glucosamine or by oxidation. As indicated in Table 1, such changes in its protein structure may provide the basis for a number of its diverse functional activities.

3.1. Nitrosylation of GAPDH

Recent studies indicate the importance of nitric oxide (NO) post-translational modification of GAPDH at its active site cysteine including its function as a novel signal transduction

pathway regulating heme metabolism as well as its importance in apoptosis. The former is a reversible GAPDH S-nitrosylation that regulates inducible nitric oxide synthase (iNOS) function. This “on/off” mechanism and the rapidity with which SNO-GAPDH modification inhibits heme insertion and diminishes iNOS activity as well as the similar kinetics by which GAPDH denitrosylation restores each effect is reminiscent of the phosphorylation/dephosphorylation (or vice-versa) post-translational modifications which provide the basis for receptor mediated cell signaling.

With respect to the latter, GAPDH nitrosylation at its active site cysteine was not only an integral component of apoptotic induction but also this post-translational modification initiated a pleiotropic signal transduction cascade integral to programmed cell death. Recent studies suggest also that, not only does SNO-GAPDH initiate apoptosis, but also that it functions as a unique intracellular checkpoint to determine whether to proceed with programmed cell death. Intriguingly, there appears to be two such checkpoints, one of which is cytosolic (Hara et. al., 2005; Sen et. al., 2009) and one of which is nuclear (Lee et. al., 2012).

3.2. Acetylation of GAPDH

Recent studies indicate the significance of GAPDH acetylation at lysine residues including its role not only in GAPDH nuclear translocation (Ventura et. al., 2010) but also its importance in apoptotic induced regulation of gene expression (Sen et. al., 2008). The former involves multiple GAPDH acetylations at lys^{117, 227 and 251} by the acetyltransferase p300/CBP-associated factor (PCAF). These results suggest the possibility that, instead of a straight-forward mechanism through which structure is altered to change function, there exists a relatively complex series of events which are required for acetylation-dependent GAPDH nuclear translocation. That being said, the temporal sequence with which each acetyl group is added is unclear as is the conformational changes that occur following each post-translational modification that permits the subsequent acetylation.

With respect to the latter, acetylation at lys¹⁶⁰ of SNO-modified GAPDH (again by PCAF) is required for GAPDH regulation of gene function in apoptosis. With this double modification, SNO-GAPDH^{lys160-Ac} initiates a signal transduction pathway involving not only the autoacetylation of PCAF but also the downstream regulation of a number of apoptotic genes (p53, PUMA, Bax and p21). Although GAPDH is the substrate and PCAF is again the acetylating enzyme, in the nucleus this enzyme-substrate interaction displays not only a different substrate specificity but also a dissimilar site of post-translational modification. By definition, this would require a distinctive GAPDH/PCAF active site interaction presumably mediated by the GAPDH^{cys152} nitrosylation.

3.3. Phosphorylation of GAPDH

Although past investigations described GAPDH phosphorylation (rev. in Sirover, 1999), recent studies indicate the significance of GAPDH phosphorylation as a mechanism for synaptic transmission, for membrane trafficking as well as a means for cancer cell survival. With respect to synaptic transmission, GAPDH was identified as a GABA_A receptor phosphorylating agent whose activity was necessary to maintain receptor function (Laschet

et. al., 2002). GAPDH mediated synaptic transmission requires a two-step, sequential phosphorylation. The first is a GAPDH autophosphorylation, historically a well reported GAPDH activity (Kawamoto and Caswell, 1986), whose significance is now only becoming evident. The second phosphorylation involves GAPDH as a phosphotransferase, utilizing its phosphate group to modify the GABA_A receptor. Although the site of GAPDH autophosphorylation is unknown, the GABA_A residues phosphorylated are thr³³⁷ and ser⁴¹⁶.

Structure-function analysis also revealed the role of GAPDH post-translational modification as a mandatory requirement for its role in rab2 GTPase mediated endoplasmic reticulum to Golgi membrane trafficking (Tisdale, 2001). The latter appears to require two sequential GAPDH post-translational phosphorylations each of which is catalyzed by a different kinase and each of which occurs at a different amino acid residue. The first phosphorylation is catalyzed by src which modifies GAPDH^{tyr41} (Tisdale and Artalejo, 2006); the second at an unknown GAPDH serine residue by PKC (Tisdale, 2002). The first appears to facilitate macromolecular complex formation while the second modulates tubulin bundling and cross-linking as well as dynein recruitment as a function of GAPDH^{ser-P}. The former facilitates protein complex formation, while the latter provides the “motor” for complex translocation. Of historical interest, although GAPDH mediated microtubule bundling was an early indication of its functional diversity, it is only recently that the significance of that activity has been elucidated.

Structure-function analysis also identified GAPDH thr²³⁷ not only as a new phosphorylation site for protein kinase B (Akt2) but also indicated that post-translational modification as a cancer cell survival mechanism (Huang et. al., 2011). In particular, once GAPDH was so phosphorylated, it was incapable of nuclear translocation, an a priori requirement for the initiation of programmed cell death. This translated into an increase in cell survival, a diminution in the rate of apoptosis as well as demonstrative resistance to apoptotic-inducing cancer chemotherapeutic drugs. Structural analysis coordinated with molecular modeling indicates that Akt2-induced phosphorylation of GAPDH^{thr237} may sterically hinder the formation of the apoptotic GAPDH-Siah1 complex that requires GAPDH^{lys225}.

3.4. O-linked N-acetyl glucosamine (O-GlcNAc) modification of GAPDH

Structural analysis identified thr²²⁷ as the site of O-GlcNAc GAPDH modification (Park et. al., 2009). Subsequent functional investigation revealed two alterations in GAPDH properties: 1) a shift in oligomeric association; and, 2) an alteration in subcellular localization. With respect to the former, a shift in oligomeric association was observed. Substantial monomeric 37 kDa GAPDH was detected which is not in accord with its normal form as a tetramer of 4 identical 37 kDa subunits with an M_r = 148 kDa. Molecular modeling analysis suggested the critical role of thr²²⁷ in intersubunit interactions indicating steric interference by the hydrophilic, bulky moiety of O- GlcNAc.

With respect to the latter, subcellular analysis of GAPDH^{O-GlcNAc} indicated its preferential nuclear localization. This is in contrast to that observed for unmodified GAPDH which has a predominantly non-nuclear localization in unstressed cells. Mutational analysis of GAPDH^{thr227} mutant (T227A) indicated the absence of O-GlcNAc modification and a primarily cytoplasmic localization.

3.5. Oxidative modification of GAPDH

Structural analysis identified cys¹⁴⁹ as a site for oxidative GAPDH modification (Kim et. al., 2003). Functional investigation revealed alterations in GAPDH binding to nucleic acids as well as changes in GAPDH oligomeric association (Arutyunova et. al., 2003). GAPDH oxidation enhanced its binding to nucleic acids, most notably to tRNA and to DNA. Further, using GAPDH monoclonal antibodies which recognized non-native GAPDH, increased immunoreactivity was detected in monomeric and dimeric oxidized GAPDH.

Subsequently, immunofluorescent analysis using those antibodies demonstrated the proliferative and apoptotic dependent recognition of nuclear GAPDH species (Arutyunova et. al., 2013). En toto, these findings suggest that this post-translational GAPDH modification could affect its function as a nuclear tRNA export protein (Singh and Green, 1993) as well as its nuclear role in programmed cell death (Hara et. al., 2006).

4. Nuclear translocation signaling domains

Although structural analysis suggests a potential nuclear localization sequence (KKVVK) at positions 259–263 in the GAPDH G-3-P binding site (Sirover 1999), functional investigations have yet to be performed to confirm that suggestion. However, studies on the functional diversity of GAPDH identified nuclear translocation signaling domains within GAPDH interacting proteins involved actively in the latter's multiple activities. These include Siah1, whose GAPDH protein complex is required for apoptosis (Hara et. al., 2005), as well as the androgen receptor (Jenster et. al., 1993), whose GAPDH protein complex is a prerequisite for GAPDH mediated control of transcriptional expression (Harada et. al., 2007).

In contrast, although nuclear translocation of GAPDH containing OCA-S is a precursor for regulation of histone gene expression (Zheng et. al., 2003), no protein containing the requisite nuclear localization signal has been identified. Similarly, although basal levels of GAPDH are required for its “constitutive” nuclear functions (nuclear tRNA export, maintenance of genomic integrity), the structural basis for its nuclear transport is unknown. That being said, the structure-function analyses available to date suggest that the signaling domains which control GAPDH nuclear localization may reside not within the GAPDH protein itself but in the binding proteins associated with new GAPDH activities.

In contrast to the apparent lack of a nuclear localization signaling domain, as indicated in Figure 1, recent evidence determined the presence and utilization of a defined nuclear export signal (KKVVKQASEGPLK) at position 258–270, also in the catalytic site (Brown et. al., 2004). Molecular modeling indicated the presence of a free GAPDH helix within that signal region capable of CRM1 binding. Mutation of lys²⁵⁹ (K259N) precluded export. This structure-function analysis suggests there is a defined mechanism through which GAPDH is transported out of the nucleus. The presumption is that this signaling mechanism is utilized once GAPDH has performed that activity.

5. Discussion

Multifunctional proteins present both an advantage and a challenge with respect to their utilization in vivo. As to the former, they may provide a means to expand the use of cellular genetic information beyond that indicated by DNA analysis. As to the latter, there may need to be control mechanisms through which such proteins exhibit one of their many activities to the exclusion of their other functions (Sirover, 2012).

Modulation of glyceraldehyde-3-phosphate dehydrogenase functional diversity suggests that there is a “pool” of GAPDH in the cell from which otherwise identical GAPDH molecules can be selected to be utilized for a specific purpose. Such regulation uses defined GAPDH amino acid sequences, the formation of macromolecular complexes or post-translational modification as the basis for control of GAPDH function. In this manner, GAPDH may be targeted selectively not only to assume one of its many activities but also to define its subcellular localization (Tristan et. al., 2011, Sirover, 2012).

GAPDH targeting as a mechanism underlying its functional diversity may be illustrated by the apparent structural paradox which underlies its diverse nuclear activities (Sirover, 2012). The absence of a nuclear localization signal, coordinate with the presence of a nuclear export signal, suggests that “free” GAPDH does not undergo non-specific nuclear translocation. Instead, GAPDH is present only in the nucleus when it is recruited for a specific function. The latter may be facilitated by formation of a macromolecular complex in the case of the androgen receptor and Siah1 each of which contains its own nuclear localization sequence. Alternatively, post-translational modification (O-GlcNAc) may be necessary for GAPDH-containing OCA-S nuclear localization and subsequent regulation of histone gene transcription, as it is for a variety of transcription factors. In either instance, not only does this represent a mechanism for targeted GAPDH translocation but also obviates the need for its own nuclear localization sequence.

In contrast, the presence of a GAPDH nuclear export signal suggests that cytoplasmic translocation of GAPDH is non-specific. In this instance, once GAPDH has performed its specific nuclear function (and is thus no longer needed), it can be quickly exported by now classical nuclear export mechanisms. The supposition is that a mechanism exists for the transfer of expendable GAPDH to the latter complex for export out of the nucleus. En toto, given this mechanism of regulation and control, it is possible to understand the rational design of the GAPDH protein with respect to its nuclear functions.

Acknowledgments

Work in the author's laboratory was funded by the National Institutes of Health (CA 119285)

Abbreviations

GAPDH	glyceraldehyde-3-phosphate dehydrogenase
SNO	S-nitrosylated
AT1R	angiotensin II type 1 receptor

NLS	nuclear localization signal
ccn2	connective tissue growth factor

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Highlights

- GAPDH structure define its functional diversity
- GAPDH interactions are based on its NAD⁺ binding domain
- Post-translational modifications specify distinct GAPDH activities
- GAPDH domains determine its cell signaling functions

Figure 1. Structural Basis of GAPDH Functional Diversity: The position of GAPDH functional domains, post-translational modifications, and binding sequences are localized as indicated within the GAPDH protein.

Figure 2. Mechanisms of GAPDH mediated control of mRNA translation. A: GAPDH- mediated degradation of mRNA: ET-1 mRNA, COX-2?; B: GAPDH-mediated mRNA stabilization: Colony Stimulation Factor mRNA; CCN-2 mRNA; C: GAPDH-mediated inhibition of mRNA translation: AT1R mRNA.

Figure 3. Mechanisms of GAPDH Nuclear Translocation. The dependence of GAPDH functional diversity on nuclear translocation sequences and nuclear export sequences within GAPDH binding proteins and GAPDH, respectively, are indicated.

Table 1

Structural Analysis of GAPDH Functional Diversity

Amino Acid Modified	Location	Post-translational Modification	Functional Property
Lysine	117	Acetylation	Nuclear Localization
Lysine	227	Acetylation	Nuclear Localization
Lysine	251	Acetylation	Nuclear Localization
Threonine	227	O-linked N- acetyl Glucosamine	Nuclear Localization
Threonine	237	Phosphorylation	Nuclear Localization
Cysteine	149	Nitrosylation	Heme Metabolism
Cysteine	149	Nitrosylation	Apoptosis
Cysteine	149	Oxidation	Nuclear Localization
Lysine	160	Acetylation	Apoptosis
Tyrosine	41	Phosphorylation	Membrane Trafficking
Serine	N.D.	Phosphorylation	Membrane Trafficking
N.D.	N.D.	Phosphorylation	Synaptic Transmission