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Subcellular localization of human glyceraldehyde-3-phosphate dehydrogenase is independent of its glycolytic function

Jennifer L. Mazzola[1], Michael A. Sirover*

Department of Pharmacology, Temple University School of Medicine, 3420 N. Broad Street, Philadelphia, PA 19140, USA

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Abstract

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was considered a classical glycolytic protein involved exclusively in cytosolic energy production. However, recent evidence suggests that it is a multifunctional protein displaying diverse activities distinct from its conventional metabolic role. These new roles for GAPDH may be dependent on its subcellular localization, oligomeric state or on the proliferative state of the cell. GAPDH is encoded by a single gene without alternate splicing. The regulatory mechanisms are unknown through which an individual GAPDH molecule fulfills its non-glycolytic functions or is targeted to a specific intracellular localization. Accordingly, as a first step to elucidate these subcellular regulatory mechanisms, we examined the interrelationship between the intracellular expression of the GAPDH protein and its glycolytic function in normal human fetal and senior cells. GAPDH localization was determined by immunoblot analysis. Enzyme activity was quantitated by in vitro biochemical assay. We now report that the subcellular expression of GAPDH was independent of its classical glycolytic function. In particular, in both fetal and senior cells, considerable GADPH protein was present in intracellular domains characterized by significantly reduced catalysis. Gradient analysis indicated that this lower activity was not due to the dissociation of tetrameric GAPDH. These results suggest that human cells contain significant intracellular levels of enzymatically inactive GAPDH which is age-independent. The possibility is considered that the functional diversity of GAPDH may be mediated either by posttranslational alteration or by subcellular protein:protein and/or protein:nucleic acid interactions.

Keywords: Glyceraldehyde-3-phosphate dehydrogenase; Subcellular localization; Protein structure/function

1. Introduction

Recent investigations reveal an emerging class of proteins characterized by new and independent activities separate from their presumed functions (reviewed in Ref. [1]). Although these proteins were originally identified and characterized specifically with respect to a unique activity, independent investigations from diverse laboratories revealed their participation in cellular pathways apart from their prior, well-identified and recognized function. Thus, glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC 1.2.1.12) was well studied for its role in glycolysis as a traditional protein with a significant role in energy production. In addition, it provided a useful experimental paradigm for the analysis of enzyme or gene structure and function. Nevertheless, recent investigations revealed that both eucaryotic and procaryotic GAPDH were, in reality, multidimensional proteins exhibiting a series of discrete activities independent of its classical glycolytic function (reviewed in Refs. [2–4]). In mammalian cells, these new activities included catalysis of membrane fusion and transport [5–7], microtubule bundling [8,9], phosphate group transfer [10,11], nuclear RNA export [12,13] and DNA repair [14–17]. It has also been identified as an RNA binding protein [18–24] especially involved in molecular mechanisms of viral infection [13,20–24] and as an A_p4_a binding protein [15]. Most recently, GAPDH has been implicated in vesicular transport and secretory pathways [25,26]. GAPDH can exist in vivo as a tetramer of identical 37-kDa subunits, as a dimer or as the 37-kDa monomer. Its glycolytic function is restricted to the tetramer. Its multiple activities may have distinct subcellular localizations which may be dependent on cell proliferation [27,28].

* Corresponding author. Tel.: +1-215-707-4351; fax: +1-215-707-7068.
E-mail address: msirover@temple.edu (M.A. Sirover).
[1] Present address: Scientific Connexions, 105 Terry Drive, Suite 118, Newtown, PA 18940, USA.

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As individual proteins have been shown to have multiple and independent functions, control mechanisms may exist which regulate their various subcellular localizations and possibly change their structure, thereby affecting their multidimensional activities. For these reasons, we have begun to examine the interrelationship between the subcellular distribution, structure and function of GAPDH. Initially, we examined the interdependence between the subcellular distribution of the GAPDH protein and its glycolytic function in non-cycling normal human cells (fetal and senior, $n=4$). Following subfractionation, the intracellular localization of GAPDH was determined by immunoblot analysis. In vitro biochemical assay of the identical samples was used to quantitate its classical enzyme activity. Gradient analysis was utilized to determine its oligomeric state. We now report that the subcellular distribution of the GAPDH protein was independent of its intracellular glycolytic function in both fetal and senior human cells. In particular, although the perinuclear and nuclear regions contained substantial quantities of the GAPDH protein, its dehydrogenase activity was severely reduced in each cellular fraction. Gradient sedimentation analysis revealed that most of the GADPH proteins appeared to be in the tetrameric form although the 37-kDa monomer was also observed. These results suggest that non-cycling fetal and senior human cells contain significant levels of GAPDH that is glycolytically inactive. The possibility is considered that human cells may regulate subcellular GAPDH activities by posttranslational modification. In addition, specific intracellular protein:protein or protein:nucleic acid interactions may affect GAPDH structure, thereby modulating its function.

2. Materials and methods

2.1. Cell culture

Fibroblast cell strains were obtained from the Coriell Institute for Medical Research (Normal human fetal: GM-6112, AG-4392, 16 fetal weeks, respectively; normal human senior: AG-4560, AG-8044, 59 and 55 years, respectively). Cells were grown as described [29]. All cultures were maintained at 37° in a humidified atmosphere of 5% CO_2 in air. Cells were grown in DMEM supplemented with 4 mM L-glutamine, 100 μg/ml streptomycin, 100 units/ml penicillin and 10% fetal calf serum. Medium was replaced every 3 days and the cells were trypsinized for passaging when confluent. Cell analysis was performed when cultures were visibly confluent.

2.2. Cell fractionation

Confluent cells were harvested by scraping with a rubber policeman then washed twice in cold 1× PBS. Cells were swollen for 10 min in hypotonic buffer (20 mM Tris–HCl, pH 7.1, 5 mM KCl, 1 mM $MgCl_2$ and 1% aprotinin) then disrupted with 20–30 strokes using a pestle in a tight-fitting Dounce homogeneizer. As defined by phase microscopy, this resulted in disruption of 90–95% of the cell population. The suspension was centrifuged at $375 \times g$ for 10 min at 4°. The supernatant was recovered and used as the postnuclear fraction. The pellet was resuspended in hypotonic buffer and repelleted at $375 \times g$ at 4°. The supernatant was removed and termed the perinuclear fraction. The pellet was resuspended in hypotonic buffer and comprised the nuclear preparation. Each fraction was sonicated for 30 s at 60 W at 4° using a needle probe and stored at −20°.

To radiolabel cellular DNA, cells were incubated with [^{3}H]thymidine (100 μCi/dish, specific activity 40–60 Ci/mmol) for 48 h prior to collection. Incorporation of [^{3}H]thymidine into DNA was determined by quantitation of acid-insoluble radioactivity in each fraction. LDH activity was determined by the disappearance of NADH as described by the manufacturer (Sigma).

2.3. Glycerol gradient density analysis

Size separation of proteins was examined by centrifugation through a 10–50% glycerol gradient in 10 mM Tris–HCl, pH 7.8, 100 mM NaCl, 1 mM k_2EDTA. Each sample was sedimented for 16 h at $150{,}000 \times g$ at 4°. Each gradient was fractionated by collection from the bottom of the tube (250 μl per aliquot). Through this procedure, early fractions would contain molecules with higher molecular weights while later fractions would include proteins with lower molecular weights. Quantitation of GAPDH activity that is restricted to its tetrameric form was used to define the gradient position of proteins with an $M_r=150$ kDa. The identical fractions were utilized to detect uracil DNA glycosylase activity to distinguish proteins with an $M_r=37$ kDa [14]. In parallel, sedimentation of BSA was examined to identify the position of proteins with an $M_r=76$ kDa.

2.4. Determination of GAPDH glycolytic function

GAPDH activity was measured in a reaction mixture (total volume, 1 ml) that contained: 100 mM sodium pyrophosphate (pH 8.5), 20 mM sodium phosphate, 0.25 mM NAD^+ and 3 μM dithiothreitol. The mixture was allowed to equilibrate to 25° for 5 min. The reaction was initiated by the addition of glyceraldehyde-3-phosphate (final concentration 1.6 mM). GAPDH activity was determined by quantitating the amount of NADH formed as defined by A_{340} for 5 min. The millimolar extinction coefficient of 6.3 was used to calculate nmol GAPDH. Data is expressed as μmol NADH/mg protein. One milliunit is the amount of protein needed for the production of 1 nmol NADH/min. Specific activity is expressed as the mean ± S.E.

2.5. Immunoblot analysis

Each sample (3 μg) was separated on SDS-PAGE then electrophoretically transferred to Hybond nitrocellulose membranes. To saturate nonspecific binding sites, each blot was incubated for 1 h at 25° with 5% nonfat dried milk in TBS-T (20 mM Tris–HCl, pH 7.6, 137 mM NaCl and 0.1% Tween 20). Each membrane was washed 3 × TBS-T (30 ml total; 10 min each wash). Each blot was then incubated for 2 h at 25° with first antibody (anti-human GAPDH monoclonal antibody 40.10.09, 1:50 dilution in TBS-T plus 5% milk). Following washing as described above, each membrane was incubated for 1 h at 25° with secondary antibody (horseradish peroxide-linked anti-mouse IgG, Amersham). Electrochemical detection was performed as described using Hyperfilm ECL film (Amersham).

3. Results

3.1. Subcellular expression of GAPDH in non-cycling human cells

Immunofluorescence analysis was used to initially examine the intracellular localization of GAPDH in confluent normal human cells [2,3]. A typical result is shown in Fig. 1. As illustrated, GADPH displays a diffuse, apparently non-nuclear distribution. Significant GAPDH protein is detected in the cytoplasmic or membrane regions with some, but apparently minor amounts, detected in the nucleus.

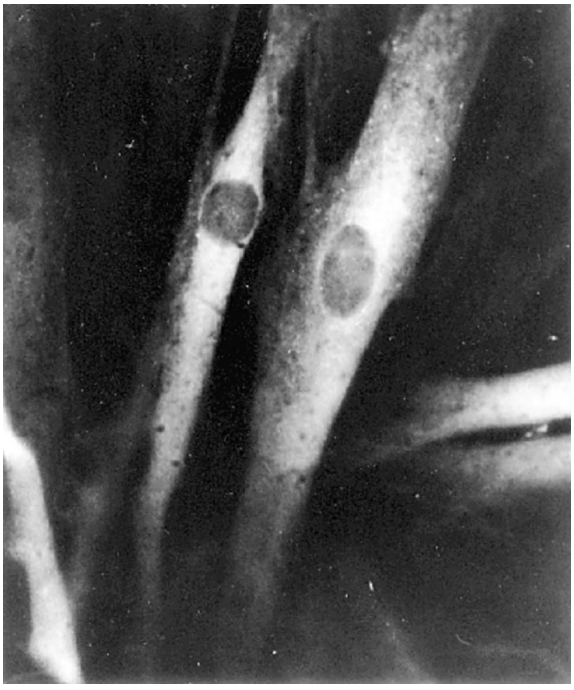

Fig. 1. Immunocytochemical analysis of GAPDH in non-cycling human cells. Immunofluorescence analysis was performed as previously described using anti-GAPDH monoclonal antibody 40.10.09 [27].

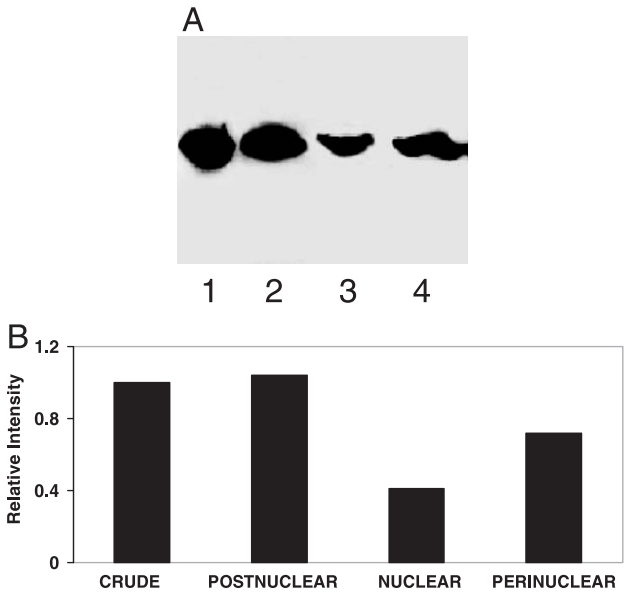

Fig. 2. Intracellular localization of GAPDH in non-cycling human cells. Subcellular fractionation and immunoblot analysis were performed as indicated in Materials and methods. Intensity was normalized to GAPDH expression in crude cell extracts.

Subcellular biochemical analysis was used to rigorously quantitate the subcellular distribution of the GAPDH protein in non-cycling cells. To confirm the validity of the fractionation protocols, the intracellular localization of lactate dehydrogenase (LDH) activity and the subcellular distribution of [^{3}H]thymidine labeled DNA were examined. As expected for a cytosolic enzyme, LDH was located predominantly in the postnuclear supernatant. Little or no activity was seen in the nucleus nor in the perinuclear region (results not shown). Similarly, virtually all of the [^{3}H]thymidine-labeled DNA was detected in the nuclear fraction.

The subcellular expression of the GAPDH protein was determined by immunoblot analysis. A representative experiment in fetal cells is shown in Fig. 2. A single 37-kDa band was detected in each fraction corresponding to the GAPDH monomer (Fig. 2A). As expected, significant GAPDH protein was detected in the postnuclear fraction in accord with its known glycolytic function. Further, reduced levels of the 37-kDa GAPDH protein were present in the nucleus. However, unexpectedly, as defined by densitometric analysis, the latter contained 39% of that detected in the postnuclear fraction. Similarly, surprisingly significant levels of GAPDH were detected in the perinuclear fraction. Densitometric analysis indicated that the latter contained 67% of that observed in the postnuclear region. Identical results were observed in senior cells (results not shown).

3.2. Intracellular distribution of GAPDH is independent of its glycolytic function

The subcellular distribution of GAPDH activity was then determined. As shown in Fig. 3A, catalysis was easily

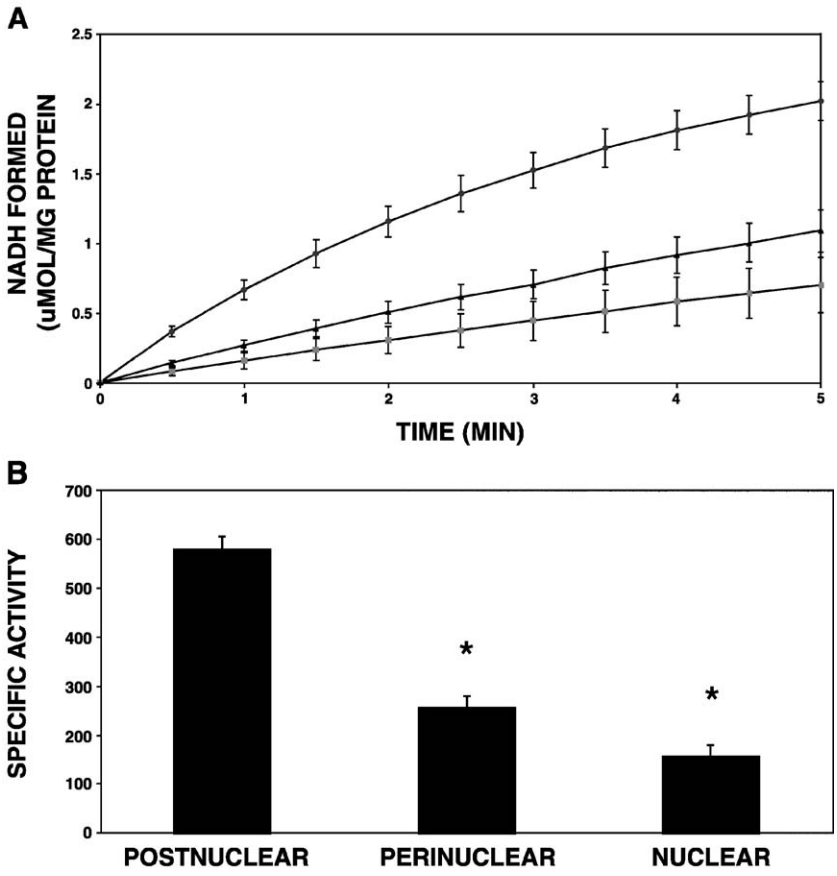

Fig. 3. Determination of intracellular GAPDH glycolytic function. GAPDH activity was measured as described in Materials and methods. (A) Determination of enzyme activity; (B) quantitation of specific activity.

detected in each of the three cell fractions. As expected, the postnuclear fraction displayed the highest activity. In contrast, both nuclear and perinuclear activities were reduced. The latter was reflected in quantitation of specific activity (Fig. 3B). As expected, the postnuclear and nuclear fractions displayed characteristically high and low levels of catalysis (579 ± 48, 156 ± 26 mU/mg protein, respectively, $P < 0.05$). Further, while demonstrable levels of the GAPDH protein were detected in the perinuclear region (Fig. 2, lane 4), GAPDH activity was modest, statistically different from that

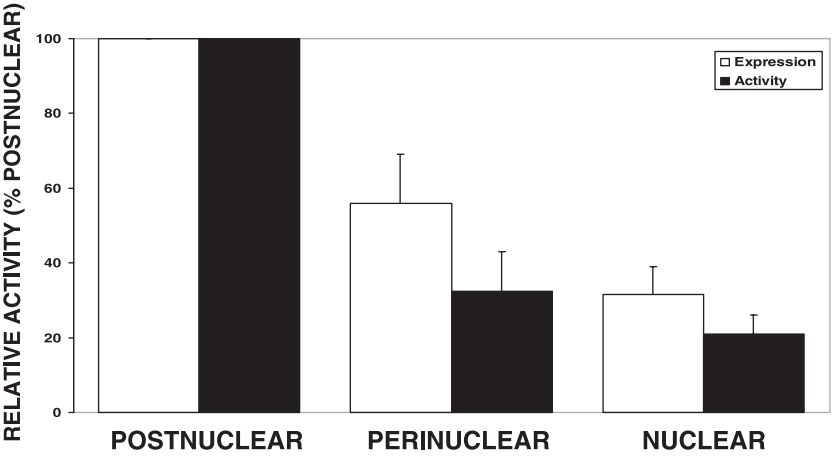

Fig. 4. Interrelationship between intracellular GAPDH localization and glycolytic function in human cells. The extent of the subcellular distribution of the GAPDH protein and quantitation of activity were normalized to that observed in the postnuclear fraction.

observed in the postnuclear fraction (254 ± 34 mU/mg protein, $P < 0.05$). An identical subcellular distribution of GAPDH activity was observed in senior cells (results not shown) thereby representing indistinguishable quadruplicate observations.

The interrelationship between the levels of GAPDH protein expression and its glycolytic function were then compared, normalizing each to that observed in the postnuclear region. As shown in Fig. 4, in human cells, GAPDH protein expression was significant both in the perinuclear fraction and in the nuclear region (open rectangles). However, in both fractions, enzyme activity was not coordinate with the level of protein expression. In both subcellular regions, catalysis was significantly reduced (closed rectangles). Thus, in both normal human fetal and senior cells, intracellular GAPDH activity was not proportional to the distribution of the 37-kDa monomer.

3.3. Oligomeric distribution of GAPDH in non-cycling human cells

To consider whether the striking reduction in nuclear and perinuclear GAPDH activity might be due to the dissociation of the GAPDH tetramer, gradient analysis was performed to quantitate tetrameric/monomeric GAPDH distribution. In particular, considering the extensive amount of the 37 kDa monomer present in those fractions, a comparative level of the monomer should be detected following sedimentation. In contrast, if there was no extensive dissociation, small amounts of the monomer should be observed consistent with the reported nuclear functions of the 37 kDa protein.

For that reason, glycerol gradient analysis was performed using both human fetal postnuclear and nuclear

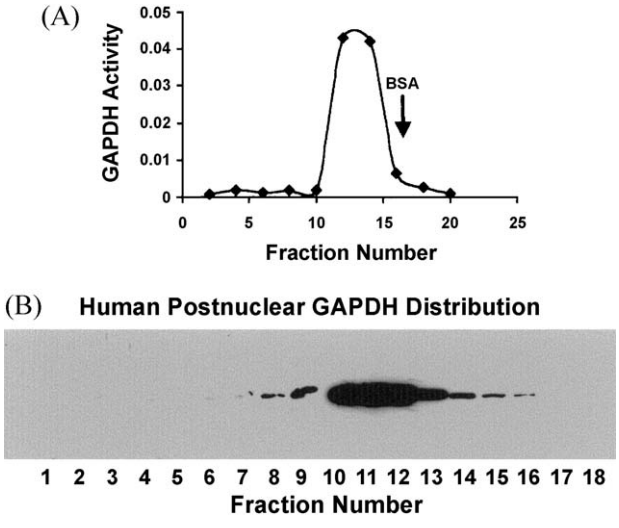

Fig. 5. Determination of fetal postnuclear GAPDH oligomeric association. Glycerol gradient sedimentation was performed as described in Materials and methods. (A) Enzymatic analysis of GAPDH glycolytic function; (B) determination of immunoreactive GAPDH.

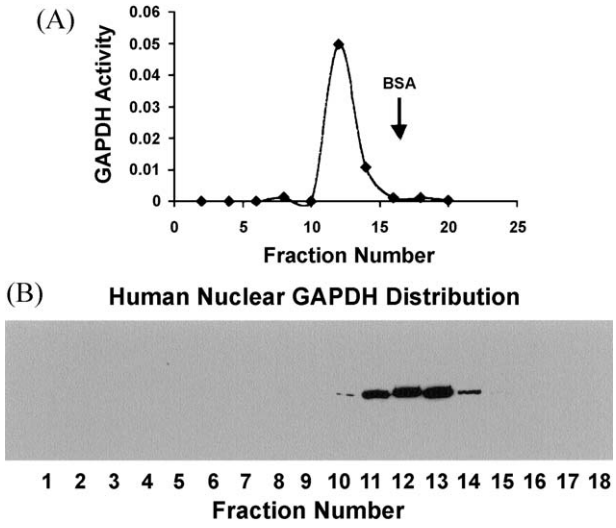

Fig. 6. Identification of fetal nuclear GAPDH oligomeric distribution. Glycerol gradient analysis was performed as described above [29,42]. (A) Quantitation of GAPDH activity; (B) immunoblot analysis of GAPDH distribution.

fractions. As shown in Fig. 5, postnuclear GAPDH activity was detected in fractions 10–13 (Fig. 5A). Immunoblot analysis was performed using the identical samples tested for GAPDH activity. As shown in Fig. 5B, the majority of GAPDH immunoreactivity was detected in those fractions characterized by GAPDH activity. A similar result was observed following sedimentation of the nuclear fractions. GAPDH enzyme activity was localized in fractions 11–14 (Fig. 6A). The distribution of the 37-kDa GAPDH protein paralleled the localization of GAPDH enzyme activity (Fig. 6B). In toto, these findings provide evidence to suggest that reduced subcellular GAPDH glycolytic function would not be due to GAPDH tetrameric dissociation. Instead, some other factor(s) would be responsible for the high level of subcellular GAPDH localization but low level of its glycolytic function.

4. Discussion

The results presented in this report indicate that the subcellular distribution of the GAPDH protein is unrelated to its intracellular glycolytic function. This was observed both in the perinuclear and nuclear regions in fetal and in senior human cells. As compared to the postnuclear fraction, these cellular domains were characterized both by substantial levels of the GAPDH protein and by significant reductions in catalysis. Perinuclear GAPDH may be involved in several non-glycolytic functions. These include its role in vesicular transport [25,26], cytoskeletal structure and translational control. The association of GAPDH with tubulin [8,30–32] and its ability to bundle microtubules [9,33,34] represented one of the first indications of its non-glycolytic functions. Its role as an mRNA binding protein was

reflected in its polysomal localization [19]. GAPDH has been implicated in several nuclear functions that may involve unique GAPDH: nucleic acid interactions (reviewed in Refs. [2,3]).

The molecular mechanism through which GAPDH is transformed into a multifunctional protein is unknown. Although a GAPDH gene family has been described [35,36], it is encoded by a single gene on chromosome 12 [37]. Alternate splicing has not been demonstrated in somatic cells [38]. The gradient analysis presented in this study indicates that most of the GAPDH protein is present intracellularly as the tetramer although the nuclear 37-kDa monomer is easily detectable in virtually all individual human cell strains that have been examined [29,42]. Thus, a change in oligomeric structure does not appear to be a viable control mechanism. As such, it is a reasonable hypothesis to suggest that inactivation of its glycolytic function may be necessary for tetrameric GAPDH to perform other intracellular activities.

Two posttranslational mechanisms may be considered initially. These include direct modification of the GAPDH protein itself or subcellular interactions that modulate GAPDH activity. With respect to the former, GAPDH isozymes have been identified which display individual levels of glycolytic function [39]. Alteration of isozymes were detected during apoptosis [40]. GAPDH phosphorylation may represent a rate-limiting step in vesicular transport [26]. With respect to the latter, normal protein:protein interactions [41] such as tubulin binding [8,9,30–32] or abnormal subcellular interactions such as those with age-related neurodegenerative proteins [29,42] may influence GADPH function. Of note, recent studies demonstrate that the binding of tubulin to GAPDH inhibits membrane fusion [43]. GAPDH:RNA interactions were reported both in the cytosol [18] and in the nucleus [12,13]. GAPDH:DNA interactions may be particularly significant both in cycling and non-cycling cells [44]. The high affinity of GAPDH for DNA was indicated as this interaction was not diminished by treatment with 5 M NaCl [45].

The results presented in this report demonstrate a specific GAPDH localization and function in non-cycling cells based on a defined level of GAPDH gene expression. This suggests the possibility that normal or abnormal alteration(s) of the latter may change the subcellular distribution of GAPDH and thereby affect its functional diversity. As indicated, recent studies demonstrate changes in GAPDH mRNA levels during cell proliferation that was accompanied by migration of the GAPDH protein into the nucleus [28]. Similarly, the intracellular localization of GAPDH and its protein:protein interactions were altered by serum depletion. [46,47]. Further, independent studies have revealed the alteration of GAPDH gene expression in prostate, renal and breast cancer [48–50]. These results suggest the possibility that alteration of intracellular GAPDH structure and function may occur during tumor initiation or progression. Thus, the results presented in this report may provide the basis to examine subcellular GAPDH structure in relation to its functional diversity both in the normal and pathological state.

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References

[1] C.J. Jeffery, Moonlighting proteins, Trends Biochem. Sci. 24 (1999) 8–10.
[2] M.A. Sirover, New insights into an old protein: the functional diversity of mammalian glyceraldehyde-3-phosphate dehydrogenase, Biochim. Biophys. Acta 1432 (1999) 159–184.
[3] M.A. Sirover, Role of the glycolytic protein, glyceraldehyde-3-phosphate dehydrogenase, in normal cell function and in cell pathology, J. Cell. Biochem. 66 (1997) 133–140.
[4] J.F. Alderete, K.W. Millsap, M.W. Lehker, M. Benchimol, Enzymes on microbial pathogens and *Trichomonas vaginalis*: molecular mimicry and functional diversity, Cell. Microbiol. 3 (2001) 359–370.
[5] P.E. Glaser, R.W. Gross, Rapid plasmenylethanolamine-selective fusion of membrane bilayers catalyzed by an isoform of glyceraldehyde-3 phosphate dehydrogenase: discrimination between glycolytic and fusogenic roles of individual isoforms, Biochemistry 34 (1995) 12194–12203.
[6] R.J. Hessler, R.A. Blackwood, T.G. Brock, J.W. Francis, D.M. Harsh, J.E. Smolen, Identification of glyceraldehyde-3-phosphate dehydrogenase as a Ca^{2+}-dependent fusogen in human neutrophil cytosol, J. Leukoc. Biol. 63 (1998) 31–336.
[7] A.R. Robbins, R.D. Ward, C. Oliver, A mutation in glyceraldehyde-3-phosphate dehydrogenase alters endocytosis in CHO cells, J. Cell Biol. 130 (1995) 1093–1104.
[8] H. Kumagai, A. Sakai, A porcine brain protein (35 K protein) which bundles microtubules and its identification as glyceraldehyde 3-phosphate dehydrogenase, J. Biochem. 93 (1983) 1259–1269.
[9] P. Huitorel, P.D. Pantaloni, Bundling of microtubules by glyceraldehyde-3-phosphate dehydrogenase and its modulation by ATP, Eur. J. Biochem. 150 (1985) 265–269.
[10] J.C. Duclos-Vallee, F. Capel, H. Mabit, M.-A. Petit, Phosphorylation of the hepatitis core protein by glyceraldehyde-3-phosphate dehydrogenase protein kinase activity, J. Gen. Virol. 79 (1998) 1665–1670.
[11] M. Engel, M. Seifert, B. Theisinger, U. Seyfert, C. Welter, Glyceraldehyde-3-phosphate dehydrogenase and Nm23-H1/nucleoside diphosphate kinase A: two old enzymes combine for the novel Nm23 protein phosphotransferase function, J. Biol. Chem. 273 (1998) 20058–20065.
[12] R. Singh, M.R. Green, Sequence-specificity binding of transfer RNA by glyceraldehyde-3-phosphate dehydrogenase, Science 259 (1993) 365–368.
[13] W.-Q. Zang, A.M. Fieno, R.A. Grant, T.S.B. Yen, Identification of glyceraldehyde-3-phosphate as a cellular protein that binds to the hepatitis B virus posttranscriptional regulatory element, Virology 28 (1998) 46–52.
[14] K. Meyer-Siegler, D.J. Mauro, G. Seal, J.C. Wurzer, J.K. de Riel, M.A. Sirover, A human nuclear uracil DNA glycosylase is the 37 kDa subunit of glyceraldehyde-phosphate dehydrogenase, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 8460–8464.
[15] M.D. Baxi, J.K. Vishwanatha, Uracil DNA glycosylase/glyceraldehyde-3-phosphate dehydrogenase is an $A_{p4}A$ binding protein, Biochemistry 34 (1995) 9700–9707.

[16] S.E. McNulty, W.A. Toscano Jr., Transcriptional regulation of glyceraldehyde-3-phosphate dehydrogenase by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, Biochem. Biophys. Res. Commun. 212 (1995) 165–171.
[17] E.Y. Krynetski, N.F. Krynetskaia, M.E. Bianchi, W.E. Evans, A nuclear protein complex containing high mobility group proteins B1 and B2, heat shock cognate protein 70, ERp60, and glyceraldehyde-3-phosphate dehydrogenase is involved in the cytotoxic response to DNA modified by incorporation of anticancer nucleoside analogues, Cancer Res. 63 (2003) 100–106.
[18] A.G. Ryazanov, Glyceraldehyde-3-phosphate dehydrogenase is one of the three major RNA-binding proteins of rabbit reticulocytes, FEBS Lett. 192 (1985) 131–134.
[19] E. Nagy, W.F.C. Rigby Jr., Glyceraldehyde-3-phosphate dehydrogenase selectively binds AU-rich RNA in the NAD^+-binding region (Rossman Fold), J. Biol. Chem. 270 (1995) 2755–2763.
[20] B.P. De, S. Gupta, H. Zhao, J.A. Drazba, A.K. Banerjee, Specific interaction in vitro and in vivo of glyceraldehyde-3-phosphate dehydrogenase and LA protein with *cis*-acting RNAs of human parainfluenza virus type 3, J. Biol. Chem. 271 (1996) 24728–24735.
[21] D.E. Schultz, C.C. Hardin, S.M. Lemon, Specific interaction of glyceraldehyde-3-phosphate dehydrogenase with the 5′-nontranslated RNA of hepatitis A virus, J. Biol. Chem. 271 (1996) 14134–14142.
[22] S.-S. Lin, S.C.-S. Chang, Y.H. Wang, C.-Y. Sun, M.-F. Chang, Specific interaction between the Hepatitis delta virus RNA and glyceraldehyde-3-phosphate dehydrogenase: an enhancement on ribozyme catalysis, Virology 271 (2000) 46–57.
[23] M. Yi, D.E. Schultz, S.M. Lemon, Functional significance of the interaction of hepatitis A virus RNA with glyceraldehyde-3-phosphate dehydrogenase (GAPDH): opposing effects of GAPDH and polypyrimidine tract binding protein on internal ribosome entry site function, J. Virol. 74 (2000) 6459–6468.
[24] G. Dollenmaier, M. Weitz, Interaction of glyceraldehyde-3-phosphate dehydrogenase with secondary and tertiary RNA structural elements of the hepatitis A virus 3′ translated and non-translated regions, J. Gen. Virol. 84 (2003) 403–414.
[25] E.J. Tisdale, Glyceraldehyde-3-phosphate dehydrogenase is required for vesicular transport in the early secretory pathway, J. Biol. Chem. 276 (2001) 2480–2486.
[26] E.J. Tisdale, Glyceraldehyde-3-phosphate dehydrogenase is phosphorylated by protein kinase C_L/λ and plays a role in microtubule dynamics in the early secretory pathway required for vesicular transport, J. Biol. Chem. 277 (2002) 3334–3341.
[27] B.L. Cool, M.A. Sirover, Immunocytochemical localization of the base excision repair enzyme uracil DNA glycosylase in quiescent and proliferating normal human cells, Cancer Res. 49 (1989) 3029–3036.
[28] I.R. Corbin, Y. Gong, M. Zhang, G.Y. Minuk, Proliferative and nutritional dependent regulation of glyceraldehyde-3-phosphate dehydrogenase expression in the rat liver, Cell Prolif. 38 (2002) 173–182.
[29] J.L. Mazzola, M.A. Sirover, Alteration of nuclear glyceraldehyde-3-phosphate dehydrogenase structure in Huntington's disease fibroblasts, Mol. Brain Res. 100 (2002) 95–101.
[30] A.H. Caswell, A.M. Corbett, Interaction of glyceraldehyde-3-phosphate dehydrogenase with isolated microsomal subfractions of skeletal muscle, J. Biol. Chem. 260 (1985) 6892–6898.
[31] J.L. Walsh, T.J. Keith, H.R. Knull, Glycolytic enzyme interactions with tubulin and microtubules, Biochim. Biophys. Acta 999 (1989) 64–70.
[32] J.F. Launay, A. Jellali, M.T. Vanier, Glyceraldehyde-3-phosphate dehydrogenase is a microtubule binding protein in a human colon tumor cell line, Biochim. Biophys. Acta 996 (1989) 103–109.
[33] M. Somers, Y. Engelborghs, J. Baer, Analysis of the binding of glyceraldehyde-3-phosphate dehydrogenase to microtubules, the mechanism of bundle formation and the linkage effect, Eur. J. Biochem. 193 (1990) 437–444.
[34] C. Durrieu, F. Bernier-Valentin, B. Rousset, Microtubules bind glyceraldehyde 3-phosphate dehydrogenase and modulate its enzyme activity and quaternary structure, Arch. Biochem. Biophys. 252 (1987) 32–40.
[35] M. Piechaczyk, J.M. Blanchard, S. Riaad-el Sabouty, C. Dani, L. Marty, P. Jeanteur, Unusual abundance of glyceraldehyde 3-phosphate pseudogenes in vertebrate genomes, Nature 312 (1984) 469–471.
[36] A. Hanauer, J.T. Mandel, The glyceraldehyde 3 phosphate dehydrogenase gene family: structure of a human cDNA and of an X chromosome linked pseudogene; amazing complexity of the gene family in mouse, EMBO J. 3 (1984) 2627–2633.
[37] G.A.P. Bruns, P.S. Gerald, Human glyceraldehyde-3-phosphate dehydrogenase in man-rodent somatic cell hybrids, Science 192 (1976) 54–56.
[38] J. Mezquita, M. Pau, C. Mezquita, Several novel transcripts of glyceraldehyde-3-phosphate dehydrogenase expressed in adult chicken testis, J. Cell. Biochem. 71 (1998) 127–139.
[39] M. Ryzlak, R. Pietruszko, Heterogeneity of glyceraldehyde-3-phosphate dehydrogenase from human brain, Biochim. Biophys. Acta 954 (1988) 309–324.
[40] P.A. Saunders, R.-W. Chen, D.-W. Chuang, Nuclear translocation of glyceraldehyde-3-phosphate dehydrogenase isoforms during neuronal apoptosis, J. Neurochem. 72 (1999) 925–932.
[41] P.A. Srere, H.R. Knull, Location-location-location, Trends Biochem. Sci. 23 (1998) 319–320.
[42] J.L. Mazzola, M.A. Sirover, Subcellular alteration of glyceraldehyde-3-phosphate dehydrogenase in Alzheimer's disease fibroblasts, J. Neurosci. Res. 71 (2003) 279–285.
[43] P.E. Glazer, X. Han, R.W. Gross, Tubulin is the endogenous inhibitor of the glyceraldehyde 3-phosphate dehydrogenase isoform that catalyzes membrane fusion: implications for the coordinated regulation of glycolysis and membrane fusion, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 14104–14109.
[44] K.A. Lee, M.A. Sirover, Physical association of base excision repair enzymes with parental or replicating DNA in BHK-21 cells, Cancer Res. 49 (1989) 3037–3044.
[45] A. Sawa, A.A. Khan, L.D. Hester, S.H. Snyder, Glyceraldehyde-3-phosphate dehydrogenase: nuclear translocation participates in neuronal and nonneuronal cell death, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 11669–11674.
[46] H.-D. Schmitz, Reversible nuclear translocation of glyceraldehyde-3-phosphate dehydrogenase upon serum stimulation, Eur. J. Cell Biol. 80 (2001) 419–427.
[47] H.-D. Schmitz, J. Bereiter-Hahn, Glyceraldehyde-3-phsophate dehydrogenase associates with actin filaments in serum deprived NIH 3T3 cells only, Cell Biol. Int. 26 (2002) 155–164.
[48] D.E. Epner, A.W. Partin, J.A. Schalken, J.T. Issacs, D.S. Coffey, Association of glyceraldehyde-3-phosphate dehydrogenase expression with cell motility and metastatic potential of rat prostatic adenocarcinoma, Cancer Res. 53 (1993) 1995–1997.
[49] M.R. Vila, A. Nicolas, J. Morote, I. de Torres, A. Meseguer, Increased glyceraldehyde-3-phosphate dehydrogenase expression in renal cell carcinoma identified by RNA-based, arbitrarily primed polymerase chain reaction, Cancer 89 (2000) 152–164.
[50] F. Revillion, V. Pawlowski, L. Hornez, J.-P. Peyrat, Glyceraldehyde-3-phosphate dehydrogenase gene expression in human breast cancer, Eur. J. Cancer 36 (2000) 1038–1042.