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REVIEW

Role of Glyceraldehyde-3-phosphate Dehydrogenase in Vesicular Transport from Golgi Apparatus to Endoplasmic Reticulum

A. V. Bryksin* and P. P. Laktionov

Institute of Chemical Biology and Fundamental Medicine, pr. Akademika Lavrent'eva 8, 630090 Novosibirsk, Russia; E-mail: anton.bryksin@gmail.com

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Abstract—Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a well-studied glycolytic protein with energy production as its implied occupation. It has established itself lately as a multifunctional protein. Recent studies have found GAPDH to be involved in a variety of nuclear and cytosolic pathways ranging from its role in apoptosis and regulation of gene expression to its involvement in regulation of Ca^{2+} influx from endoplasmic reticulum. Numerous studies also indicate that GAPDH interacts with microtubules and participates in cell membrane fusion. This review is focused on the cytosolic functions of the protein related to vesicular transport. Suggestions for future directions as well as the model of protein polymer structure and possible post-translational modifications as a basis for its multifunctional activities in the early secretory pathway are given.

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It is known that some proteins in cells may serve several functions [1]. Recently, it was found that some of the so-called house-keeping proteins are also polyfunctional. For example, cytoplasmic aconitase looses its glycolytic activity on iron depletion and is converted into an iron-regulating protein factor. This protein factor binds to the specific IRP-binding loop on mRNA; as a result, expression of transferrin receptor gene is enhanced and finally, the amount of cell-penetrating iron is increased [2, 3].

Another example is glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a key enzyme of glycolysis, which catalyzes D-glyceraldehyde-3-phosphate oxidation coupled with phosphorylation [4]. Similar to aconitase, GAPDH also has non-glycolytic functions, but the number of functions ascribed to GAPDH is more that that of aconitase [5]. Non-glycolytic functions of GAPDH include nuclear RNA export [6, 7], phosphotransferase activity [8], participation in DNA replication and repair [9, 10], regulation of histone genes expression [11], participation in nuclear membrane fusion [12] and assembly of microtubules [13-17]. Some of the above-mentioned GAPDH functions are speculative and will remain hypothetical without additional studies; that included the role of GAPDH in cell apoptosis until recently. Accumulation of glycolytic enzyme in the cell nuclei under certain cultivation conditions discovered more than 15 years ago was initially perceived as experimental error or artifact of the method used, and most of the time the data were neglected. Only after several years, thorough analysis and new experiments supported the view that GAPDH is very important for apoptosis. In fact, it was recently shown that GAPDH is transferred into the cell nucleus in significant amounts during apoptosis. Use of antisense oligonucleotides that can decrease GAPDH expression level prevented enzyme penetration into the nucleus and stopped apoptosis. Detailed study of this phenomenon showed that such enzyme behavior is caused by its role in a new signal pathway, where the nitrogen monoxide molecule acts as the initial signal for apoptosis [18].

The role of GAPDH in vesicular transport is also unclear. In spite of the fact that reliable data indicate that this enzyme participates in microtubule assembly and fusion of lipid membranes, GAPDH function in integral cell processes remains unclear. The goal of this review is to summarize data on the interaction of GAPDH with

Abbreviations: GAPDH) glyceraldehyde-3-phosphate dehydrogenase; VTC) vesicular tubular cluster.

* To whom correspondence should be addressed.

components of the cell transport system, to analyze these data, and to develop a model of GAPDH functioning in membrane transport.

Membrane fusion is essential for existence of cells and whole organisms, because such vitally important processes as cell division, immune response, nervous impulse transduction, response to the external stimulus, water–salt balance, secretion, etc. [19] are based on this phenomenon.

Association of GAPDH with cell membranes during fractional separation of cells was discovered in the mid 1960s [20], and this stimulated studies on the nature of this interaction. Later association of radioactively labeled enzyme with synthetic monolayer and vesicles consisting of phosphatidyl choline/phosphatidic acid in various molar ratios was studied [21, 22]. The enzyme association constants with lipid monolayer (1 μM) [21] and vesicles (0.4 μM) were determined [22].

The ability of GAPDH to influence the membrane fusion process (fusogenic activity) was first mentioned by Morero et al. [23]. Studying the effect of Ca^{2+} and various proteins (ribonuclease, concavalin A, BSA, protease, glucagon, lysozyme, L-lactate dehydrogenase, rabbit muscle GAPDH, and yeast GAPDH) on fusion of synthetic lipids consisting of phospholipids (phosphatidylcholine/phosphatidic acid were used in molar ratio 9 : 1), they found that GAPDH markedly catalyzed this process. Thus, vesicles incubation in the presence of GAPDH at concentration 0.15 μg/ml, pH 7.5, resulted in complete membrane fusion within 120 sec without addition of Ca^{2+} (the effect was evaluated by resonance energy transfer), whereas the control was stable for more than 60 h [23]. Such GAPDH concentration corresponds to about three enzyme molecules per vesicle.

It is interesting to note that BSA, ribonuclease, concavalin A, protease, and glucagon used in parallel experiments did not exhibit any noticeable activity even when added at extremely high concentrations (BSA, 500 mg/ml) to the reaction mixture, whereas lysozyme and L-lactate dehydrogenase along with GAPDH demonstrated fusogenic activity at concentration 40 μg/ml, but were less efficient than GAPDH.

Since these data raised doubts, the authors checked their results by five different detection methods: resonance energy transfer, fluorescence spectroscopy, gel filtration, electron microscopy, and phase mixture with water solutions, and the GAPDH preparation was checked for the presence of the residual amounts of Ca^{2+}; high Ca^{2+} concentrations can cause fusion of synthetic membranes in the absence of any additional factors [23].

Further study of vesicle fusion in the presence of GAPDH showed that the maximal fusion rate is observed at pH 4.5-5.0. GAPDH fusogenic activity significantly increased with increasing temperature and depended on vesicle charge (negatively charged vesicles fused more efficiently) [24].

Using Morero's methodology [23] described above, Hessler et al. studied various neutrophil factors able to influence cell membrane fusion [25]. According to their calculations, the contribution of GAPDH to cell membrane fusion is about 25%.

Although the data are convicting, it should be mentioned that test system represented by vesicles consisting only of phosphatidylcholine/phosphatidic acid in molar ratio 9 : 1 (Hessler et al., 3 : 1) is somewhat artificial, because phosphatidylcholine is a minor fraction of most mammal cell membranes, excluding endoplasmic reticulum (54%) and Golgi apparatus (45%) membranes [26]. Data by Glaser and Gross [27] indirectly confirm that this model is inadequate; they studied fusion of synthetic vesicles consisting of lipids combined so that lipid composition of mammalian cell membrane was matched as precisely as possible: phosphatidylcholine – 27%, phosphatidylethanolamine – 27%, phosphatidylserine – 6%, cholesterol – 40% (this composition coincides maximally with the composition of myelin membranes of mammalian neurons [26]). The authors studied the effect of plasmenylethanolamine in membrane composition on the rate of vesicle fusion [28]. Plasmenylethanolamine is a component of membranes undergoing fast fusion under physiological conditions, e.g. synaptic membranes. The developed model implied the presence of a protein catalyst accelerating fusion of such membranes [28]. The search for the catalyst among rat brain cytosol extract proteins by lysate chromatographic fractioning and fraction analysis for the presence of potential factor influencing membrane fusion revealed one protein later identified as a GAPDH isoform [27].

It is interesting to note that addition of protein at concentration 1 μg/ml to plasmenylethanolamine-free vesicles (but containing other lipids, including 27% phosphatidylcholine) did not cause their fusion. However, addition of plasmenylethanolamine to the vesicle composition revealed fusogenic activity of GAPDH isoform [27]; this supports the assumption that GAPDH-mediated fusion of physiological membranes strictly depends on their composition. The study of the fusion rate of membranes with high plasmenylethanolamine content demonstrated that one "activated" GAPDH molecule can cause fusion of more than one synthetic vesicle per msec, and that it was a Ca^{2+}-independent reaction. Another important conclusion was made—the necessity of activation of GAPDH for it to exhibit fusogenic activity. The data indicate that such activation most probably occurs through two steps—release from inhibitor and modification. Initial cytosol extract (GAPDH was then isolated from it) did not possess fusogenic activity, and this suggested that an inhibitor of GAPDH fusogenic activity is present in cytosol. Identity of a protein catalyzing membrane fusion to GAPDH was demonstrated by several methods: immunologically (using monoclonal antibodies against GAPDH) and chemically (sequenc-

ing). However, fusogenic protein fraction was chromatographically (by chromatography on MonoQ Sorbent and GTP affinity chromatography) separated from oxidoreductase active fraction and appeared to be a modified GAPDH form. Unlike oxidoreductase active GAPDH form with p*I* 7-8, this isoform has p*I* 8.5, and its fusogenic activity was not inhibited with koningin acid (an inhibitor of GAPDH glycolytic activity), but was inhibited with G3P [27].

In later work by the same authors, the search for the inhibitor of GAPDH fusogenic activity provided an unexpected result [29]. At the first, it was found that the inhibitor was a protein, because it could not be sorted out by dialysis, was susceptible to inactivation by elevated temperature, and was sensitive to treatment with trypsin. Sequential fractionating of the inhibitor on DE-52 sorbent, MonoQ, and hydroxyapatite allowed isolation of an individual protein inhibitor with molecular mass 55 kD. Protein sequencing identified inhibitor as tubulin [29].

It is interesting to note that the interaction of GAPDH with tubulin was also reported in an earlier study [13]. Tubulin is a cytoskeletal protein of eukaryotic cells and directs movement of vesicles and vacuoles in the cell. The tubulin molecule is a heterodimer formed by two globular subunits—α- and β-tubulin—and is a constituent of microtubules. The latter are dynamic structures, and it is obvious that their assembly may be catalyzed by other factors, including proteins. Microtubule assembly can be monitored either via optical absorption change at 350 nm occurring during the assembly, or by electron microscopy. Using the approaches described above, Kumangai and Sakai in 1983 discovered the interaction of GAPDH with microtubules during a quest for protein catalyst of microtubule assembly [13]. They isolated a protein with molecular mass 35 kD by affinity chromatography on a column with tubulin-Sepharose 4B. It was shown that this protein did not bind with sorbent in the presence of 2 mM ATP. The effect of protein on microtubule assembly was studied after additional purification. It appeared that the assembly rate increased after addition of the protein. This protein was identified as GAPDH by peptide mapping. To further prove the point on the participation of GAPDH in microtubule assembly authors presented the data on acceleration of tubulin strand assembly by commercial GAPDH purified from rabbit muscle [13].

Later these data were independently supported by Walsh et al. [14]: they showed that two proteins were coprecipitating during ultracentrifugation. Studying GAPDH interaction with tubulin, Volker et al. [15, 16, 30] showed that the enzyme interacted with the site of α-tubulin C-terminal fragment [15], and such interaction can result in microtubule assembly *in vitro* [15, 16] and perhaps in formation of a microtubule network *in vivo* [16]. Using synthetic peptides, it was later found that GAPDH binding site was localized between amino acid residues 408-451 on the α-tubulin subunit [30].

It should be mentioned that the interaction of GAPDH with tubulin inhibits not only fusogenic activity of the enzyme [29], but also its glycolytic activity [15] and thus tubulin can be considered as a universal regulator of GAPDH activity.

The abovementioned data indicate that GAPDH may participate in key stages of vesicular transport, namely, microtubule mediated vesicle movement (by providing for example enhanced affinity to the latter) and vesicle fusion with target membranes. It is unclear however under what conditions GAPDH (that is considered to be a cytosolic protein) would be found associated with the vesicle membranes. Also, the *in vitro* model used in the experiments mentioned above seems to be irrelevant to the real biological processes taking place inside the cell due to the very limited number of chemical components used. It is obvious that only *in vivo* experiments can prove the participation of GAPDH in vesicular transport. Tisdale et al. performed such experiments, studying vesicular transport via the early secretory pathway, which includes all vesicular traffic in cells between endoplasmic reticulum and the Golgi apparatus [17, 31, 32]. An intermediate stage of this transport is vesicular tubular cluster (VTC), where transported proteins are initially sorted (Fig. 1a, see color insert).

The question whether VTC belongs to the Golgi apparatus or should be considered a separate cell organelle is now actively discussed. Transport from endoplasmic reticulum to VTC mainly proceeds via the COPII pathway, which is different from the clathrin pathway.

At the first stage of this pathway, cytoplasmic soluble SarI-GTPase bound with GDP recognizes integral membrane Sec12 protein on the endoplasmic reticulum surface [33]. Sec12 catalyzes replacement of GDP for GTP of SarI–GTP complex, which in turn results in conformational change of SarI: extension of a hydrophobic part of the protein and its anchoring on endoplasmic reticulum membrane. The anchored SarI-GTPase then serves as a binding site for Sec23/Sec24 protein complex, which plays an important role in vesicle "protrusion" [34]. At this stage of "protrusion", Rab1-GTPase is bound via a mechanism that is still unclear; Rab1-GTPase marks VTC as a target for the vesicle delivery. Also binding of other factors such as Sec23 occur at this time [31, 34, 35]. Vesicle assembly is completed on subsequent addition of Sec13/Sec31 complex and fibrillar protein Sec16 interacting with it. After vesicle unbinding from membrane of endoplasmic reticulum, Sec23 interacts with SarI; this causes GTP hydrolysis, reverse change of SarI conformation, and release of protein coat factors bound with it [34, 35] (Fig. 1a). The vesicle is transported to VTC via microtubules from "+" to "–" pole, using dynein–dynactin complex as a motor [36]. On vesicles fusion so-called SNARE complex of GM130–p115-protein complex and transmembrane giantin—constituents of COPII vesicle membrane and VTC, respectively [37, 38]—is formed. It

is considered that formation of such a complex is the main factor fusing the vesicle with VTC.

The retrograde transport from VTC to endoplasmic reticulum mainly proceeds via the significantly less studied COPI retrograde pathway [39]. It is still unclear how vesicular COPI traffic is divided into anterograde and retrograde traffic and how the retrograde transport is also responsible for a part of transport from endoplasmic reticulum to the Golgi apparatus. Historically, the COPI mechanism was discovered as a mechanism of transport from endoplasmic reticulum to the Golgi apparatus, and later it appeared that only a negligible part of this transport proceeds via this mechanism. It is suggested that the mechanism of formation of COPI vesicles as a whole is similar to that of COPII vesicles, but is performed by other proteins. Arf1 plays a role of initiating GTPase, and coatomer proteins—α-COP, β-COP, γ1-COP, δ-COP, β'-COP, ε-COP, ζ1-COP, γ2-COP, and ζ2-COP—form vesicle coat [39]. Rab2-GTPase studied by Tisdale et al. was found to be associated with retrograde COPI vesicles and possibly defines direction of their transport from VTC to endoplasmic reticulum [40]. Studying Rab2-containing vesicles, it was found that they never contain anterograde (directing to the Golgi apparatus) components, but contain p53/gp58 recirculating protein complex, protein kinase Cι/λ (PKCι/λ) [31, 41], and GAPDH [31]. Study of the function of GAPDH as a component of such vesicles allows reevaluation of its role in vesicular transport.

First, it was shown that affinity-purified polyclonal antibodies against GAPDH block transport between endoplasmic reticulum and VTC in a cell system based on perforated NRK cells infected by ts405—a temperature-sensible VSV-G strain [31]. This virus synthesizes proteins, which are accumulated in endoplasmic reticulum at 39.5°C, but on decreasing the temperature to 32°C they are transported into the Golgi apparatus including Rab2-containing vesicles [42]. Perforation of these cells with subsequent incubation in cytosol solution containing ATP does not influence transport; however, cytosol preincubation with affinity-purified antibodies against GAPDH results in dose-dependent inhibition of transport of viral proteins [31]. This approach demonstrated that more than 60% of viral proteins are transported via the GAPDH-dependent pathway [31].

Second, the effect of Rab2-GTPase concentration on GAPDH concentration related with the newly formed vesicles was evaluated. It appeared that incubation of 30 μg of microsomes isolated from NRK cell culture with 50 ng of Rab2-GTPase resulted in 50-fold concentration increase of GAPDH associated with microsomes [31]. Earlier the same authors demonstrated that incubation of microsomes containing Rab2 causes binding with membrane coatomer β-COP and protein kinase Cι/λ [43, 44].

Further study of Rab2-dependent GAPDH binding with microsomes demonstrated that GAPDH directly interacts with PKCι/λ regulatory domain (amino acid residues 1-247) and can serve as a specific substrate for the latter (it is interesting to note that phosphatidylserine is one of the factors of this interaction) [17]. As was found later, GAPDH directly interacts with Rab2: proteins were detected in coprecipitate of HeLa cell lysate after incubation with affinity-purified polyclonal antibodies against Rab2. Interaction of the proteins was confirmed by the two-hybrid system approach and GAPDH binding site with Rab2 was localized; it appeared to be between amino acids 20-50 [32].

Based on these data, Tisdale et al. proposed a hypothesis that a triple complex Rab2–PKCι/λ–GAPDH is formed on the surface of COPI retrograde vesicles. GAPDH as a component of this complex is subject to phosphorylation with PKCι/λ [32]. It was shown that phosphorylated GAPDH form (pGAPDH) is a factor of β-tubulin binding with microsomes, because dephosphorylation of pGAPDH associated with microsomes causes significant dissociation of β-tubulin from the microsome surface [17]. The authors found that GAPDH associated with the Golgi apparatus exists in tetrameric form and suggested that the glycolytic activity of the protein is important for its participation in vesicular transport. To prove this hypothesis, the wild type GAPDH and its mutant glycolytically inactive form with one amino acid replacement (C149G) were expressed in *E. coli*, purified by metal-affinity chromatography, and used for quantitative comparison of inclusion into microsomes prepared from HeLa cells. It appeared that the mutant form of GAPDH as well as the wild type GAPDH efficiently binds with microsomes in tetrameric form. Estimation of quantitative binding parameters of the mutant GAPDH with Rab2 and PKCι/λ and study of its phosphorylation did not reveal any distinction between the mutant GAPDH and the wild type enzyme [17, 32]. Also, binding with microsomes drastically inhibits the glycolytic activity of GAPDH. The data indicate that the role of GAPDH in vesicular transport from the Golgi apparatus to endoplasmic reticulum is completely independent of its glycolytic function, and that phosphorylated GAPDH form accelerates assembly of microtubules and increases vesicle affinity to them [32].

It is interesting to note that two years before Tisdale and his group published their work, Gross et al. [29] suggested a similar mechanism of GAPDH function while studying the role of the tubulin as an inhibitor of the GAPDH modulated fusogenic activity. According to this mechanism, GAPDH isoform is captured by the hypothetical GTP-activated Rab into vesicles associated with microtubules, phosphorylated with PKCι/λ, and then catalyzes membrane fusion.

As mentioned above, GAPDH possesses fusogenic activity, and synthetic vesicles fuse on incubation with GAPDH. The ability of GAPDH to catalyze fusion of physiological membranes was demonstrated by Han et al.

[45]: GAPDH isoform discovered earlier [27] and isolated from cytosol of rabbit brain extract efficiently caused fusion of transport vesicles with plasmatic membranes of β cells.

So, data obtained under conditions maximally similar to physiological ones demonstrate the participation of GAPDH in vesicular transport.

According to the suggested models, GAPDH provides interaction between vesicles and microtubules and membrane fusion when the vesicle reaches the target membrane. However, it should be mentioned that, first, experiments with cells infected with ts405 virus are now criticized because they study transport of a single protein, which is unnatural for a cell. Thus, transport mechanisms of viral proteins may differ from those of native cell proteins [46]. Examples when viruses use host cell proteins for functions usually not inherent for them are well known [47]. Second, GAPDH fusogenic activity was studied using transport vesicles, which are clathrinic in nature, whereas GAPDH association was detected only with COPI Rab2-containing vesicles [48].

In connection with this, mutation analysis performed by Robins et al. [49] is important evidence for the participation of GAPDH in membrane fusion and vesicular transport. To search for mutants with defective endocytotic transport, they used cells of hamster ovary (CHO) treated with ethyl methane sulfate. Change in endocytosis was monitored via cell absorption of FITC-labeled dextran. Selection resulted in FD 1.3.25 cell line, which differed from the initial parent line in accumulation of significant amounts of fluorescent label in non-endocytotic vesicles [49]. Moreover, these labeled vesicles were localized in the neighborhood of the Golgi apparatus and some of them were partly fused, this being typical of VTC in the contemporary view [50]. Since the labeled dye was never localized in the Golgi apparatus, Robbins et al. suggested that FD 1.3.25 cells are defective in one of proteins responsible for interaction between vesicles and microtubules [49]. Comparison of proteins associated with microtubules revealed a protein with molecular mass 36 kD in mutant cells. This protein possesses higher affinity to cytoplasmic microtubules polymerized in the presence of taxol as compared with the wild type cells (because this protein is not washed away with ATP and buffer with high ionic strength from microtubules obtained from mutant cells). The protein was further identified as GAPDH with one amino acid replacement, Ser234→Pro234.

It is interesting that in questioning the data, the authors transfected the initial cells with a construction bearing a mutant protein gene. According to evaluation of expression level of the mutant form, its content in some cells reaches 25% compared with the native gene, and this results in stable phenotype change for to the mutant one at mutant protein content from 15 to 25% [49]. Such distinct manifestation of phenotype in the presence of relatively small amount of mutant protein might be explained by the importance of GAPDH tetrameric form not containing mutant subunits for vesicular transport.

In conclusion, we would like to summarize all the abovementioned data and suggest a possible model for the functioning of GAPDH in vesicular transport.

Results obtained by now indicate that GAPDH participates in retrograde transport between VTC and endoplasmic reticulum (Fig. 1a). This transport is performed by COPI vesicles and mainly serves for transport of proteins delivered to VTC by mistake or being constituents of COPII vesicles and requiring reverse transport for subsequent recycling. Assembly of such vesicles most likely is initiated by accumulation of transmembrane recycling proteins, such as gp58/p53, which are recognized, and serve as a docking port for Arf1 and Rab2 cytosol GTPases (Fig. 1b). Arf1 and Rab2—GDP-associated GTPases—are then activated upon the interaction with transported cargo proteins (p23/p24 in the case of Arf1); this results in replacement of GDP by GTP and anchoring of protein fractions on VTC membrane. Then Arf1- and Rab2-mediated binding of coatomer proteins occurs, and the vesicle unbinds from the VTC membrane. Binding of ArfGAP, α-COP, γ1-COP, δ-COP, β-COP, β'-COP, ε-COP, ζ1-COP, γ2-COP, and ζ2-COP to a vesicle is Arf1-mediated [39]. Rab2 forms a triple complex with PKCι/λ and GAPDH tetramer (Fig. 1b) and mediates β-COP binding [48, 51].

After unbinding from the membrane, Arf1-mediated GTP hydrolysis occurs; this causes dissociation of Arf1 and coatomers associated with it from the surface of COPI retrograde vesicle. It is possible that at this stage, TyrK binds to PKCι/λ mediating subsequent phosphorylation of PKCι/λ. In turn, phosphorylated PKCι/λ phosphorylates some subunits of GAPDH tetramer, and this provides stable vesicle binding to microtubules, because dephosphorylated GAPDH form binds to α-tubulin as mentioned above, whereas pGAPDH binds to β-tubulin. Theoretically such interaction may function as a vesicular motor for vesicle transport from VTC to endoplasmic reticulum, because the Golgi apparatus as a rule (in animal cells) is positioned near the cell center of microtubule organization [50, 52], that is, in the same region where the "minus" end of microtubules is located. Microtubules consisting of α- and β-tubulin blocks always bear α-tubulin on their "minus" end which unmodified GAPDH form may efficiently bind to (Fig. 2, see color insert). After phosphorylation of GAPDH, this form should efficiently bind to tubulin accessible only as the second unit on the microtubule in the direction from VTC to endoplasmic reticulum (Fig. 2). For GAPDH functioning as a motor, either the presence of a hypothetical protein dephosphorylating pGAPDH, or replacement of the phosphorylated monomer by a dephosphorylated one from cytosol is necessary. In the latter case, phosphorylat-

ed pGAPDH form may remain bound with the penultimate unit of β-tubulin for some time, thus blocking the reverse vesicle movement. However, since movement in microtubules is almost constant, interaction of the next vesicle with the origin of the microtubule and thus, blocking of the site for reverse movement of a vesicle going ahead, should be self-sufficient for one-way direction of the transport process. As shown by Tisdale et al. [17], GAPDH is phosphorylated via Ser, whereas Robbins et al. [49] showed that Ser234→Pro234 replacement resulted in malfunction of the vesicular transport. On the other hand, kinesin and dynein, two main motors defining movement along microtubules, were identified as VTC components [52, 53]. However, Lippincott-Schwartz et al. [52] note that kinesin as a VTC constituent is inactive and dedicated to further transport in cisterns of the Golgi apparatus, where it provides peripheral movement of secretory clathrin vesicles along microtubules after activation.

Whatever the mechanism of movement of COPI retrograde vesicles along microtubules, GAPDH glycolytic activity is not needed for its functioning [32].

However, GAPDH tetramers demonstrating glycolytic activity were associated with endoplasmic reticulum (Fig. 1a), which is the membrane target for vesicles [54]. Such GAPDH tetramers are bound in a covalent complex with IP3R—receptor regulating Ca^{2+} level in the cytoplasm (Fig. 1c) [54]. Since tetramer binds to IP3R receptor via a disulfide bond with Cys149 in the composition of two GAPDH monomers and Cys149 plays an important role in glycolytic enzyme activity, only dimer in the composition of tetramer possesses oxidoreductase activity [54]. Theoretically, such IP3 receptor specifically recognizing GAPDH may be an ideal site, which directs vesicles containing Rab1 in complex with GAPDH and forms a SNARE pair. The literature still lacks data on the SNARE pair participating in fusion of COPI-Rab2-associated retrograde vesicles. Burry et al. [55] as well as Dilcher et al. [56] suggest that Sec22/Sec20 and Use/Syn18 may be this pair, but no evidence for their participation is available in the literature. However, a model using IP3R–GAPDH complex as a SNARE pair or auxiliary docking mechanism looks attractive, considering that formation of such complex may result in displacement of the phosphorylated enzyme from vesicle surface, thus causing locally high pGAPDH concentration; the latter possesses high fusogenic activity and finalizes membrane fusion (Fig. 1d). The possible future of such complex is also of interest. Search for data indicating possible transport of IP3R–GAPDH complex from endoplasmic reticulum to the Golgi apparatus and back gave no result. However, the presence of sorting signals typical to transmembrane proteins in GAPDH primary sequence (Bryksin, unpublished data) and its covalent association with transmembrane protein indicates that such transport is possible.

In conclusion, it should be mentioned that the presented scheme is hypothetical and based on numerous assumptions, many of which do require experimental proof. For example, the position of Ser at which GAPDH is phosphorylated is still not determined. It is unclear whether phosphorylation is the factor activating fusogenic activity, because phosphorylation shifts protein p*I* to the acidic region, whereas modification under investigation is shown to shift protein p*I* to the basic region. The question whether all subunits of GAPDH tetramer in composition of COPI-Rab2-associated retrograde vesicle are phosphorylated and, if not, what is the difference in stability of such heterogenic polymer and monomeric polymer remains open. It is unclear how many GAPDH subunits participate in formation of Rab2–GAPDH–PKCι/λ triple complex and whether PKCι/λ and Rab2 interact with the same or different GAPDH subunits in the composition of this complex. Finally, the presence of kinesin and specific SNARE proteins in COPI-Rab2-containing retrograde vesicles should be supported or rejected. Answers to these questions will allow better understanding of the role of GAPDH in vesicular transport.

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