The mutual interaction of glycolytic enzymes and RNA in post-transcriptional regulation

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ABSTRACT

About three decades ago, researchers suggested that metabolic enzymes participate in cellular processes that are unrelated to their catalytic activity, and the term "moonlighting functions" was proposed. Recently developed advanced technologies in the field of RNA interactome capture now unveil the unexpected RNA binding activity of many metabolic enzymes, as exemplified here for the enzymes of glycolysis. Although for most of these proteins a precise binding mechanism, binding conditions, and physiological relevance of the binding events still await in-depth clarification, several well explored examples demonstrate that metabolic enzymes hold crucial functions in post-transcriptional regulation of protein synthesis. This widely conserved RNA-binding function of glycolytic enzymes plays major roles in controlling cell activities. The best explored examples are glyceraldehyde 3-phosphate dehydrogenase, enolase, phosphoglycerate kinase, and pyruvate kinase. This review summarizes current knowledge about the RNA-binding activity of the ten core enzymes of glycolysis in plant, yeast, and animal cells, its regulation and physiological relevance. Apparently, a tight bidirectional regulation connects core metabolism and RNA biology, forcing us to rethink long established functional singularities.

Keywords: RNA-binding; moonlighting; enolase; glyceraldehyde-3-phosphate dehydrogenase; glycolytic enzymes; post-transcriptional regulation

INTRODUCTION

Metabolism is the central feature of living cells. Chemical reactions and pathways convert energy into usable forms, produce the molecular building blocks of the cell and eliminate degradation products and toxins. Thus, the metabolic network is central to cell maintenance, growth and reproduction as well as interaction with the environment. Anabolic and catabolic processes are catalyzed by enzymes that accelerate the reactions and are subject to regulation. Glycolysis is one of the most ancient metabolic pathways of biological systems and converts one molecule of glucose into two pyruvate molecules, thereby producing two ATP and two reduction equivalents in the form of NADH in a 10-step process. This central pathway occurs in all eukaryotes and most of archaea and bacteria.

Over time, it was found that these proteins not only function as classical highly specialized enzymes, as initially thought, but display additional features as scaffold proteins, binding partners and regulators. These so-called moonlighting functions are diverse and have physiological relevance for various cellular processes (Fig. 1). For some proteins, moonlighting functions are established for decades like the DNA-binding activity of *Escherichia coli* lactate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase (Grosse et al. 1986) or the thyroid hormone-binding activity of pyruvate kinase (Parkison et al. 1991). Possibly first considered as peculiarities of these enzymes at that time, moonlighting functions were discovered for all glycolytic enzymes in recent years.

Multifunctionality, not only of metabolic enzymes but of many or most proteins and other cellular molecular entities, might be essential to establish efficient regulatory circuitries adequately responding to the complex and variable environment. Multifunctionality enhances the functional capacity of the proteome, overcoming the limitation in protein-coding gene number.

EXPANDING THE RNA INTERACTOME: TECHNOLOGICAL ADVANCEMENT DRIVES NOVEL INSIGHT

Engineering progress in molecular methodology presently rejigs the field of RNA biology that so far was scarcely

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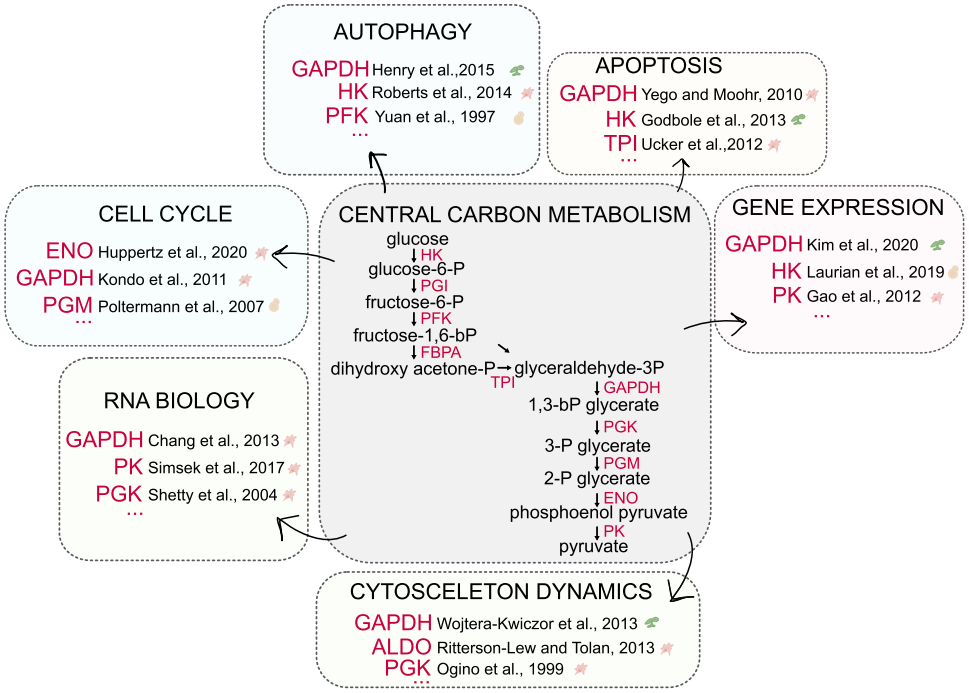

FIGURE 1. Moonlighting functions of core glycolytic enzymes. Enzymes involved in the conversion of glucose to pyruvate during glycolysis exhibit additional functions unrelated to their catalytic activity. These moonlighting functions regulate cellular processes like RNA biology, cytoskeleton dynamics, cell cycle control, apoptosis, autophagy, or gene expression. Due to space limitations, the number of given examples and references is limited to one.

linked to central metabolism. Different RNA types fulfill basal functions in cell biology mediating gene expression (mRNA), acting as cofactor or enzymes (rRNA, tRNA) or functioning as post-transcriptional regulators (regulatory noncoding RNAs) (Li and Liu 2019). In this context, RNA-binding proteins (RBP) present decisive factors regulating RNA processing, for example, by splicing, polyadenylation, other modifications, stability, localization, and translation efficiency. Their role in RNA metabolism and especially protein synthesis is indispensable as illustrated by the fact that malfunction of RBPs is cause of several human diseases (Brinegar and Cooper 2016; Gebauer et al. 2021).

Total resource and energy expenditure in protein synthesis is high. Therefore, protein synthesis is continuously adjusted post-transcriptionally according to environmental and developmental cues. In this regard, RBPs significantly contribute to a balanced energy and resource expenditure by dynamic and rapid regulation of protein synthesis. The mechanisms of post-transcriptional regulation may be particularly important under nonoptimal conditions such as disease, inflammation, abiotic or biotic stress. To realize RNA homeostasis, the RNA binding proteome dynamically responds to different types of external stimuli (Perez-Perri et al. 2018; Marondedze et al. 2019; Shchepachev et al. 2019; Backlund et al. 2020; Bresson et al. 2020; Matia-González et al. 2021). It determines the fate of RNAs and the rate of protein synthesis by targeting them to translation in polysomes, to degradation in processing bodies or storage in stress granules (Chantarachot and Bailey-Serres 2018).

The acknowledged relevance of RBPs and the growing interest in this field can be recognized by the increasing number of publications related to RNA binding proteins, as shown in Figure 2A. From this search, it is obvious that much more literature is available for the human system, indicating that the knowledge from a general point of view in yeast and plants is lagging behind.

Still, the enormous increase in the sensitivity of mass spectrometry (MS) in the last decade eased the improvement and development of new techniques to study the RNA binding interactome on a global scale. Implementation of RNA interactome capture (RIC) technologies gave novel insight into RNA–protein interactions (for review, see Ramanathan et al. 2019). Global identification of RBPs usually starts with cross-linking of the RNA–protein complexes in the cell by use of ultraviolet light or chemical cross-linking agents. This type of in vivo capture of RNA–protein interactions presented major progress compared to in vitro approaches like protein microarrays (Scherrer et al. 2010; Tsvetanova et al. 2010) or shift assays (Fillebeen et al. 2014) that do not allow the detection of binding events under native conditions. Depending on the research question, RNA–protein complexes are

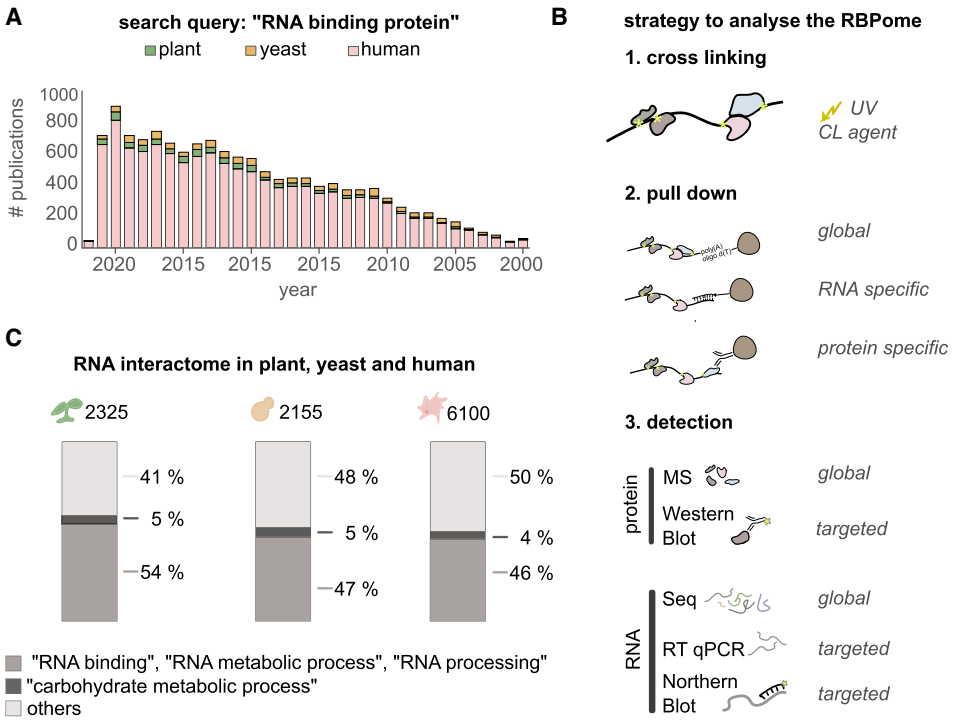

FIGURE 2. Strategies to identify the RBPome. (*A*) Increasing number of publications concerning RNA binding proteins. (*B*) General workflow of RNA-interactome capture with cross-linking, affinity purification and final identification of interaction molecules. (*C*) Current RNA binding proteome in *Arabidopsis thaliana*, *Saccharomyces cerevisiae*, and *Homo sapiens* according to RBP2GO database and its assignment to different gene ontology terms.

subsequently isolated in a global attempt (using, e.g., oligo[T]-oligonucleotides [Castello et al. 2013] or silica beads [Shchepachev et al. 2019]). In a more targeted analysis, different approaches focus either on a protein or on a mRNA of interest. For the first, researchers can use, for example, specific antibodies or nanobodies to purify a protein of interest with its associated RNAs (Marmisolle et al. 2018). For RNA-centric approaches, for example, sequence-specific oligonucleotides are exploited to select RBPs specific for the (m)RNA of interest (Rogell et al. 2017; Spiniello et al. 2018, 2019; Theil et al. 2019). Various RNA-centric strategies are at hand and reviewed elsewhere (Gerber 2021).

In the final step, interacting molecules are identified via MS (protein) or RNA-sequencing (RNA), or detected using targeted approaches like immunodetection (protein), northern blotting or quantitative real time PCR (RNA) (in Fig. 2B). Besides this general strategy to discover the RBPome, similar workflows were developed and optimized for specific organisms or specialized research questions that outreach the sole identification of interacting molecules (König et al. 2010; Perez-Perri et al. 2018; Bach-Pages et al. 2020), as reviewed in Hafner et al. (2021).

These new techniques helped to define the complexity and dynamics of the RNA-binding interactome in various organisms over recent years. In this course, the experimental approaches uncovered an unexpected high number of noncanonical RBPs (Fig. 2C), showing that in silico predictions of RBPs based on common RNA binding domains like the KH domain, RNA-recognition motif (RRM), zinc finger domain and others fall short in identifying the complete RNA-binding interactome. Several databases are available that summarize results from published RIC approaches and enable access to comprehensive data (EuRBPDB [Liao et al. 2020], RBP2GO [Caudron-Herger et al. 2020], RBPbase [Hentze group EMBL]). For RBP2GO, Caudron-Herger et al. (2020) collected results from 105 RIC experiments in 13 different organisms as well as information about protein–protein interactions to generate a database for RBPs and their reported functions.

The database allows us to query whether a protein of interest was found in any of the global RIC approaches, and it assigns so-called RBP2GO scores reflecting the likelihood for the protein to bind RNA based both on the occurrence in RIC approaches (listing score) and the listing score of the top ten interaction partners (Caudron-Herger et al. 2020). One protein class that is commonly detected in RIC studies across different species is the group of metabolic enzymes. The identification of metabolic enzymes as RNA binding proteins was often neglected and considered as unspecific binding due to their often high abundance. But with time it turned out that the interaction

between metabolic enzymes and RNA is indeed meaningful and fulfills physiological functions. Reliable RNA-binding activity was proven for several enzymes involved in different pathways like the tricarbonic acid (TCA) cycle, fatty acid metabolism, thymidylate synthesis, glycolysis and oxidative pentose cycle and more (for an extensive review, see Cieśla 2006). Figure 2C exemplarily depicts the portions of RBPs that are related to carbohydrate metabolic processes. In *Arabidopsis thaliana*, *Saccharomyces cerevisiae* and *Homo sapiens*, representatives of three different kingdoms of life, a significant fraction of 5% of the so far identified RBPome are involved in carbohydrate metabolism, whereas the dominant part is annotated with RNA binding/processing or metabolic processes, demonstrating that RNA binding of carbohydrate metabolic enzymes is a ubiquitous phenomenon rather than the sole exception.

Since their appearance as RBPs, several publications addressed the increasing diversity of the RBPome throughout different organisms and thoroughly discussed the potential molecular and physiological implications of different metabolic enzymes as noncanonical RNA binding proteins (Beckmann et al. 2015; Castello et al. 2015; Albihlal and Gerber 2018; Hentze et al. 2018; Marondedze 2020). An overview of the current state of identification as RNA binding proteins of the ten core enzymes is summarized in Figure 3. According to information from the RBP2GO platform, all core enzymes of the glycolytic pathway with at least one isoform were identified as candidate RBP in yeast and humans, whereas in plants, hexokinase, phosphoglycerate mutase and pyruvate kinase isoforms are the only enzymes that are not listed as noncanonical RBP. However, amino acid sequence comparison may provide the first hints to a common binding mechanism across the different species. The sequence similarities between yeast (*Saccharomyces cerevisiae*), plant (*Arabidopsis thaliana*), and human (*Homo sapiens sapiens*) differ from 15% for phosphoglycerate mutase and fructose bisphosphate aldolase to 50% for enolase (Fig. 3). The in part high similarities of the glycolytic enzymes indicate the existence of common and conserved protein domains beyond the structure needed for enzymatic activity. Therefore, one can hypothesize that the plant orthologs of human and yeast hexokinase, phosphoglycerate mutase and pyruvate kinase may also be able to bind RNA, if RNA recognition is realized with the conserved protein domains. The assumption is supported by the fact that prokaryotic glycolytic enzymes, as in the case of enolase, also show RNA binding activity and are involved RNA metabolism (Kühnel and Luisi 2001; Morita et al. 2004; Chandran and Luisi 2006, discussed below). However, the specific protein domains engaged in RNA recognition and binding still await elucidation in most cases.

Moreover, plant hexokinase, phosphoglycerate mutase and pyruvate kinase isoforms were recently identified as drought-responsive RNA-binding proteins in a study that is not included in the RBP2GO database (Marondedze et al. 2019). Besides HXK1 (plant hexokinase), PGAM1 (plant phosphoglycerate kinase), and PKP1 (plant pyruvate kinase), they identified several other drought-responsive glycolytic enzymes, with GAPC2 (plant glyceraldehyde-3-phosphate dehydrogenase) and PFK7 (plant phosphofructokinase) showing significantly increased association with RNA upon polyethylene glycol (PEG)-induced dehydration stress in cultured *Arabidopsis thaliana* cells (Marondedze et al. 2019).

However, Figure 3 illustrates that for most of these enzymes in eukaryotes, detailed analysis beyond identification as RBP awaits further experimentation (light gray boxes). Open questions concern the conditions of RNA binding, the nature of RNA targets, the RNA binding sites and the necessary protein domains involved in the interaction. Especially in plants, knowledge about the significance of these RNA-binding events is rare and deserves validation and investigation.

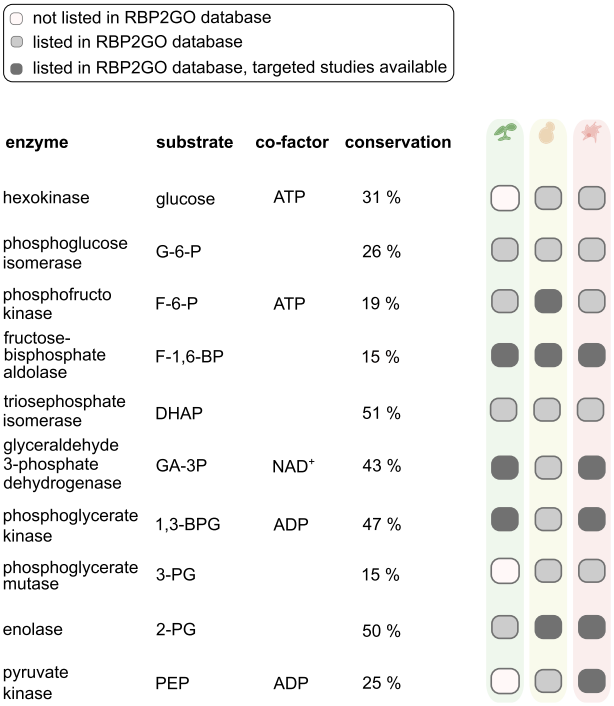

FIGURE 3. Overview of glycolytic enzymes and their identification as RNA-binding proteins. According to the RBP2GO database, glycolytic enzymes are highlighted with a light gray box when one isoform of the enzyme was identified in global RIC approaches, a dark gray box when additionally RNA binding was validated and characterized beyond global approaches, and with white boxes when a protein is not listed as an RNA-binding protein in the RBP2GO database. Multiple amino acid alignments were conducted using the UniProt alignment tool. For every glycolytic enzyme, all isoforms were compared. Shown are always the lowest values for protein sequence similarity. For a detailed percentage identity matrix, see Supplemental Figure 1.

The aim of this review is to compile the current knowledge about the ten core glycolytic enzymes in the three major kingdoms of life whose members are of utmost interest in basic research to identify and emphasize common molecular mechanisms. Moreover, it seems reasonable that moonlighting functions of these conserved enzymes are similar among these kingdoms. This allows knowledge transfer and opens new perspectives, especially for organisms that experienced less attention in this regard.

HEXOKINASE, PHOSPHOGLUCOISOMERASE, TRIOSEPHOSPHATE ISOMERASE AND PHOSPHOGLUCOMUTASE

Neither in animals, yeast nor plants was RNA-binding activity of hexokinases, phosphoglucoisomerases, triosephosphate isomerases, and phosphoglucomutases further analyzed for validation of global RIC results. Therefore, reliable information is unavailable on whether and how these interactions affect RNA function and metabolism. Nonetheless, hexokinases are still associated with post-transcriptional control of mRNAs in plant and human cells based on other evidence.

In rice (*Oryza sativa*), OsHXK6 contributes to the regulation of pollen fertility via physical interaction with restorer of fertility 6 (RF6) and promoting the cleavage of aberrant *atp6-orfH79*, a transcript associated with cytoplasmic male sterility (CMS) (Huang et al. 2015).

In human cells, HK2 (human hexokinase) regulates cellular stress response by controlling the subcellular localization of the heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1), an RNA-binding protein involved in viral infection (Courteau et al. 2015).

In both cases, hexokinase does not directly interact with the respective target RNAs, and post-transcriptional regulation occurs indirectly via additional protein–protein interaction(s). Nonetheless, this underlines the significance of hexokinases beyond their basal function in metabolism.

PHOSPHOFRUCTOKINASE

Phosphofructokinases catalyze the phosphorylation of fructose-6-phosphate forming fructose-1,6-bisphosphate. The ability of yeast phosphofructokinases to bind nucleic acids first became apparent in 2004 when Hall and colleagues conducted a screening with a yeast proteome microarray and DNA probes and found PFK26, a fructose 2,6-bisphosphate generating enzyme (Hall et al. 2004). Identification of the classical fructose 1,6-bisphosphate generating enzyme as RBP succeeded in 2010 using a high-density microarray with fluorescently labeled RNA (Scherrer et al. 2010). The study validated the RNA-binding activity of the yeast isoform PFK2 via RIP-CHIP and observed that PFK2 binds its own message. This process allows for efficient autoregulation of PFK2 including positive or negative feedback loops.

Yeast PFK2 also underwent detailed analysis in the context of glycolytic body (G-body) formation. Glycolytic enzymes aggregate in membrane-less cytoplasmic granules and form metabolic subcompartments in response to hypoxia (Jin et al. 2017) that rely on the participation of RNAs (Fuller et al. 2020). PFK2 directly interacts with RNA under normoxic conditions, as shown by PAR-CLIP autoradiography. Using photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP) and PAR-CLIP-seq, the authors identified 439 direct target transcripts of PFK2 with 559 discrete binding sites, most preferably located in the 3′ untranslated region of the mRNA. Binding sites contained AU-rich elements (ARE) that are also recognized by other metabolic enzymes (see glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate kinase). Gene annotation of the bound mRNA substrates displayed an enrichment in metabolic pathways, including glycolysis. Among these binding partners there were 13 out of 22 known mRNAs coding for glycolytic enzymes. This again is suggestive of some feedback autoregulation of glycolysis by its own enzymes like that shown in previous studies (Scherrer et al. 2010). Interestingly, the target transcripts of PFK2 largely overlap with mRNA substrates of yeast ENO1, ENO2, and FBA1 that were also analyzed by Fuller et al. (2020). Binding of common mRNA targets could promote G-body formation under hypoxia and thereby enhance glycolytic activity (Fig. 4A). This assumption is supported by the fact that RNAs bound to these glycolytic enzymes under normoxic conditions correlate with the RNAs associated with G-bodies under hypoxia.

Also in animal cells, phosphofructokinase is associated with G-body-like structures. Human PFK2 repartitioned to G-bodies in human hepatocarcinoma cells (Jin et al. 2017) and *Caenorhabditis elegans* PFK-1.1 was recruited to G-body-like structures in response to transient hypoxia-induced energy stress (Jang et al. 2021). So far, it remains elusive whether human PFK2 functions as a general scaffold through specific PFK2–RNA interactions and how this contributes to tumorigenesis. The same questions apply to the role of RNA-association of PFK-1.1 into metabolic subcompartments in nematodes.

FRUCTOSE-BISPHOSPHATE ALDOLASE

Fructose-bisphosphate aldolases convert fructose-1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate and represent one of the few enzymes of glycolysis for which RNA-binding activity was validated through additional approaches beyond global RIC studies in yeast, human and Arabidopsis.

RNA-binding of human ALDOA (human fructose-bisphosphate aldolase) was observed in 2002 by Kiri and

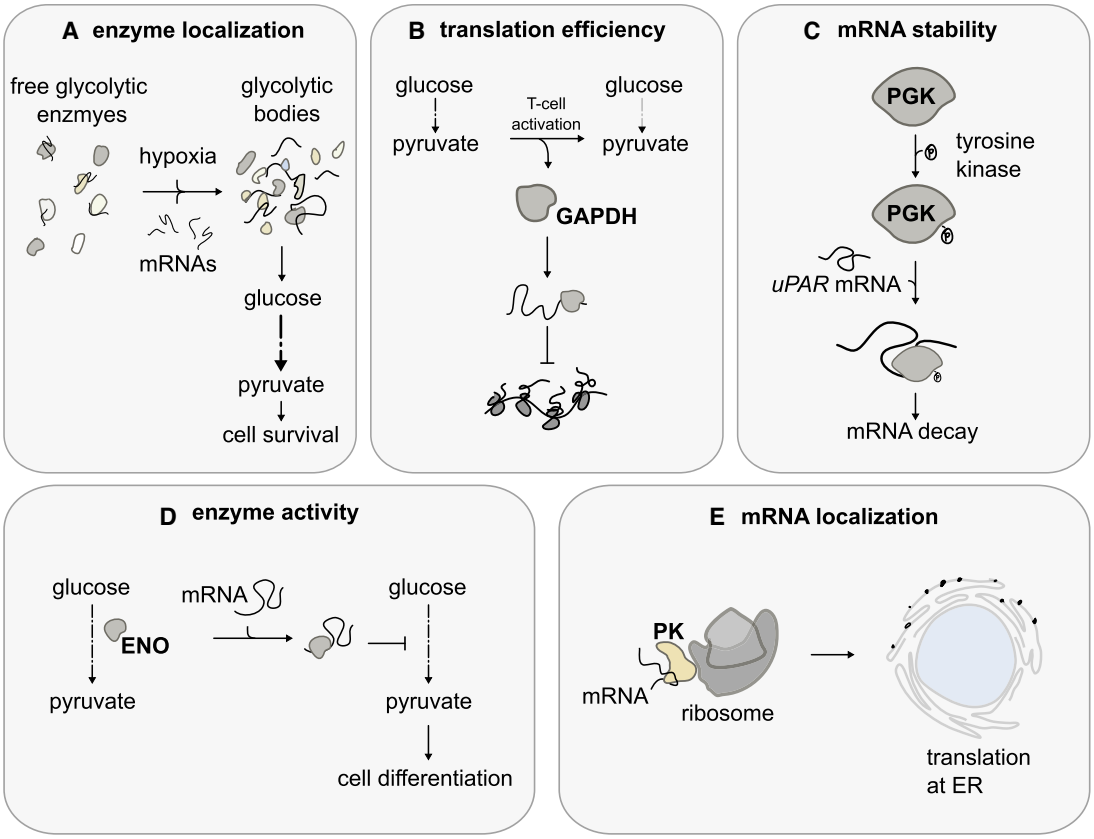

FIGURE 4. Examples for enzyme-mediated regulation of protein synthesis and riboregulation of metabolic activity through RNAs based on data from (*A*) Shetty et al. (2004), (*B*) Simsek et al. (2017), (*C*) Chang et al. (2013), (*D*) Huppertz et al. (2022), and (*E*) Fuller et al. (2020).

Goldspink (Kiri and Goldspink 2002). Using a gel retardation assay, they observed tissue-specific protein binding to the 3′-UTR of *myosin heavy chain* (*MyChC*) mRNA applying protein extracts from different human/mice organs. Via peptide sequencing, they identified ALDOA, a liver-specific isoform of the fructose-bisphosphate aldolase, and GADPH (human glyceraldehyde 3-phosphate dehydrogenase) as binding proteins. Competition assays revealed sequence specificity of the interaction.

In plants, chloroplast-localized FBA1 (plant fructose-bisphosphate aldolase) bound to the 3′-UTR of *PETD* that codes for subunit IV of the cytochrome b_6/f complex of the photosynthetic electron transport chain (Stenger et al. 2004). Because FBA1 was identified in several RNA–protein complexes, it was hypothesized that FBA1 is a major binding component in these ribonucleoprotein complexes. However, competition assays indicate a non-specific interaction with RNA. Presently, the circumstances and relevance of this binding event remain unknown for plants.

PAR-CLIP-seq with yeast FBA1 revealed 721 target RNAs with a total of 1024 binding sites that contained pyrimidine-rich motifs (Fuller et al. 2020). FBA1 preferably binds to noncoding RNAs and coding sequences. As for PFK2, several mRNAs coding for glycolytic enzymes were detected in the RNA interactome. The hypothesis that RNA substrates function as scaffold for efficient supramolecular assembly of glycolytic enzymes and stimulation of glycolysis needs further validation but seems likely based on these findings (Fuller et al. 2020).

GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE

Catalyzing the reversible conversion of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate, glyceraldehyde 3-phosphate dehydrogenase initializes the yield phase of glycolysis by reducing one NAD^+ to NADH and consuming an inorganic phosphate. It functions as a versatile multifunctional protein in animals, plants and yeast and is involved in different cellular processes, ranging from regulation of stomatal closure in plants to control of apoptosis, autophagy and cytoskeleton dynamics. Its role in regulating gene expression and protein synthesis is generally accepted and links to its nucleic acid binding activity. The glyceraldehyde 3-phosphate dehydrogenase participates in cell maintenance and stress responses by virtue of its DNA- and RNA-binding activities, of which the latter has

been comprehensively discussed (White and Garcin 2016; Garcin 2019).

Already in 1979, human GAPDH was isolated by poly(A)-sepharose chromatography, indicating the possibility for RNA binding (Milhaud et al. 1979). It was then Ryazanov and colleagues who found that human GAPDH interacts with *Escherichia coli* RNA or poly(U) (Ryazanov 1985) and detected its association with polysomes in rabbit reticulocytes (Ryazanov et al. 1988). It was hypothesized that the enzyme forms low affinity complexes with polyribosomes and promotes RNA-unwinding because a particular yeast isoform was shown to lower RNA melting temperatures (Karpel and Burchard 1981). This assumption still awaits confirmation.

In contrast to this scenario in which GAPDH acts as unspecific RBP, human GAPDH specifically recognizes sequences and structural features of tRNAs (Singh and Green 1993). Comparisons of wild type and mutant tRNA devoid of GAPDH binding ability suggest that the glycolytic enzyme facilitates nuclear export of tRNA.

Similarly, human GAPDH binds to distinct sequences or ternary structures in the UTRs of viral RNAs. EMSA verified animal GAPDH interaction with hepatitis B virus RNA (Zang et al. 1998), antigenomic hepatitis D RNA (Lin et al. 2000) and U-rich elements in the 3′-UTR of human papilloma virus RNA (De et al. 1996). Other studies unveiled its binding to certain AU-rich elements or internal ribosome entry sites (IRES) in 5′- or 3′-UTRs of hepatitis A and C viral RNA, partially destabilizing the RNA structure and suppressing translation (Petrik et al. 1999; Dollenmaier and Weitz 2003).

McGowan and Pekala (1996) demonstrated human GAPDH binding to AU-rich regions in the 3′-UTR of the mRNA coding for the basal glucose transporter GLUT1 (McGowan and Pekala 1996). GLUT1 expression is mainly regulated at the post-transcriptional level via alterations in mRNA stability (Cornelius et al. 1990; Stephens et al. 1992). Whether human GAPDH is the decisive factor in this context requires further validation. Many other examples are at hand that demonstrate regulatory functions of human GAPDH in post-transcriptional regulation, namely by affecting mRNA stability and translation efficiency. mRNA stability of *endothelial-derived vasoconstrictor endothelin-1* (*ET-1*) is controlled by GAPDH binding to ARE in the 3′-UTR, mediating RNA-unwinding in a redox-dependent manner in umbilical vein endothelial cells (Rodríguez-Pascual et al. 2008). With a similar mechanism, the enzyme negatively regulates the stability of *cyclooxygenase* (*cox2*) mRNA in mouse hepatoma cells (Ikeda et al. 2012). Also, in this scenario it binds to ARE in the 3′-UTR of the target transcript. Interestingly, the GAPDH-mediated control of mRNA stability was associated with the Dravet syndrome, a form of epilepsy (Zeng et al. 2014). A certain allele of the 3′-UTR of the voltage-gated sodium channel 1A (SCN1A) subunit forms a binding site for human GAPDH that negatively regulates mRNA stability and correlates with disease phenotype. GAPDH also influences mRNA stability of mouse *scn1a* and *scn3a* by binding to a conserved region in the 3′-UTR (Zeng et al. 2014). As an mRNA stabilizer, human GAPDH also interferes with the *colony stimulating factor-1* (*csf-1*) that is overexpressed in epithelial ovarian cancer cells (Bonafé et al. 2005; Zhou et al. 2008). Here, GAPDH acts as an AU-rich element binding protein (AUBP) that prevents mRNA decay and thereby enhances rates of CSF1 protein synthesis.

Besides stabilizing mRNA, experimental evidence proves the ability of GAPDH to directly regulate translation. Backlund et al. discovered in 2009 that AREs in the 3′-UTR of the *angiotensin II type receptor* (*AT1R*) mRNA bind human GAPDH. This mechanism represses translation of the mRNA. Moreover, GAPDH binding to ARE in the 3′-UTRs of γ-*interferon* (*inf-γ*) and *interleukin-2* (*il-2*) mRNA in vitro was reported by Nagy and Rigby (1995). Later, Chang et al. (2013) linked human GAPDH binding to cytokine mRNAs to the physiological switch from oxidative phosphorylation to aerobic glycolysis, which is crucial for T-cell activation. Analysis of polysomal fractions and results from other experiments indicate that the enzyme regulates INF-γ and IL-2 protein synthesis despite unaltered mRNA abundance (Fig. 4B).

In a very similar metabolism-dependent way, animal GAPDH regulates protein synthesis of the tumor necrosis factor (TNF) and hypoxia-inducible factor 1α (HIF1α) (White et al. 2015; Millet et al. 2016; Xu et al. 2016). It binds the 3′-UTR of *TNF* mRNA and represses translation and, thereby, regulates the inflammation response of human leukemia monocytes (White et al. 2015; Millet et al. 2016). Similarly, GAPDH binding to *TNFα* was observed in mice bone-marrow-derived macrophages (BMDM) and shown to suppress translation of the transcripts under resting conditions (Galván-Peña et al. 2019). Dissociation and translational activation of *TNFα* RNA upon macrophage activation by lipopolysaccharide (LPS) induction was linked to malonylation of GAPDH lysine 213. GAPDH-dependent control of HIF1α synthesis, on the other hand, modulates the T-cell response to hypoxia by repressing HIF1α translation upon binding to ARE in the 3′-UTR (Xu et al. 2016).

The given examples underline the multifaceted functions of human GAPDH in post-transcriptional control of protein synthesis in animal systems. Various studies demonstrate the diversity of RNA substrates and functional implications on RNA biology by GAPDH. With its strong preference for ARE in the UTRs of the RNA substrates, GAPDH can be considered as AUBP. Additional intermolecular interactions are likely required for effect specificity and need further investigation.

In contrast to mammalian GAPDH, RNA-binding activity of plant or yeast glyceraldehyde 3-phosphate dehydrogenases is less understood. In 2021 only, GAPC1 and GAPC2

(plant glyceraldehyde 3-phosphate dehydrogenase) were shown to interact in vitro with a sequence motif that was overrepresented in translationally regulated transcripts in response to high light treatment (Moore et al. 2021). The authors hypothesize a translation-repressing function of GAPC1/C2 induced by low energy conditions. Future investigations will have to address the sequence specificity and the post-transcriptional effect of GAPC1/C2 in planta.

PHOSPHOGLYCERATE KINASE

The phosphoglycerate kinase reversibly converts 1,3-bisphosphoglycerate into 3-phosphoglycerate, generating the first ATP molecule during the yield phase of glycolysis. More detailed knowledge about phosphoglycerate kinase function in RNA binding and post-transcriptional regulation is available for human and plant isoforms.

Human PGK1 binds mRNA in human bronchial epithelial cells (Shetty et al. 2004). uPAR is a receptor for an urokinase-type plasminogen activator whose expression is regulated by PGK1 post-transcriptionally. EMSA and northern blot approaches revealed direct binding of PGK1 to the coding sequence (CDS) of *upar* mRNA. Interestingly, binding to the ARE in the 3′-UTR as described for other metabolic enzymes above was excluded. RNA binding was validated in vivo via RIP-qPCR. The results indicate that destabilization of the *upar* mRNA through PGK1 contributes to post-transcriptional control of uPAR expression (Fig. 4C). uPAR plays a pivotal role in uPA-mediated plasminogen activation at the cell surface, and its expression level correlates with lung cancer prognosis (Pedersen et al. 1994), highlighting the high significance of PGK1 for the cell.

Besides its role in regulation of mRNA stability, human PGK1 is associated with viral RNA transcription. Ogino et al. (1999) proved a transcription-stimulating activity of human PGK1 for the Sendai virus (–)RNA, probably through interaction with tubulin and the transcription initiation complex. Later on, they identified ENO1/ENOA (human enolase) as another glycolytic enzyme associating with the host factor activator complex (Ogino et al. 2001). The transcriptional activation does not rely on the catalytic activity of PGK1. Unanswered is the question whether direct RNA-binding of PGK1 is involved in the mechanism.

Similar observations were made for plant PGK1. Direct binding of chloroplast localized PGK1 to viral RNA was experimentally demonstrated in 2007 (Lin et al. 2007). Gel shift assays and LC-MS analysis unveiled association with the poly(A)-tail of the Bamboo mosaic virus (BaMV) (–) RNA, which was required for efficient replication and reproduction of the virus. PGK1 facilitates the localization of viral RNA to the chloroplast, providing an environment for virus replication superior to cytosol and nucleus (Cheng et al. 2013).

ENOLASE

Enolases interact with both DNA and diverse RNA species in different organisms. In 2015, in vivo mRNA capture targeting poly(A) mRNAs isolated novel RNA-binding proteins in yeast (Matia-González et al. 2015). Among others, they found ENO1 and validated its RNA binding by RIP-qPCR. The set of identified RNA substrates of ENO1 comprised its own message as well as mRNAs of other glycolytic enzymes. This is in accordance with the results from Fuller et al., who analyzed the RNA interactome of ENO1 using PAR-CLIP-seq and also found metabolic enzymes to be enriched in the RNA substrates (Fuller et al. 2020). In total, they unveiled 1001 target transcripts with more than 1400 binding sites, often containing pyrimidine-rich elements. Whether the interaction of ENO1 with RNA mediates autoregulation of glycolytic enzymes through post-transcriptional control of their protein synthesis or represents a riboregulation by RNA-based association of glycolytic enzymes in G-bodies remains open and might be target-dependent. Another study from 2019, however, had different findings when they analyzed the yeast ENO1 RNA interactome via cross linking and cDNA analysis (CRAC) after they identified ENO1 as RNA binding protein via total RNA-associated protein purification (TRAPP) (Shchepachev et al. 2019). The authors could neither identify a specific binding site for ENO1 nor an enriched class or subset of target mRNAs besides from highly expressed genes. In contrast, a high fraction of recovered sequences comprised cytosolic tRNAs. Localizing the cross-linking site revealed that ENO1 binds specific structural aspects of the tRNA. These discrepancies between the studies need to be addressed.

tRNA interaction was also shown for the other yeast enolase isoform ENO2. This isoform was characterized as RNA chaperon by Entelis et al. (2006). Yeast ENO2, notably not yeast ENO1, interacts with the iso-acceptor $tRNA_{Lys}$ CUU (tRK1). ENO2-induced conformational changes in the tRNA structure enable the interaction with the cytosolic precursor of mitochondrial lysyl–tRNA synthetase (preMsk1p), a crucial factor for tRK1 transport to the mitochondria. Enhanced mitochondrial import of tRK1 is associated with temperature changes, indicating a role of ENO2 in cell acclimation (Entelis et al. 2006). Later it was shown that human ENO1, ENO2, and ENO3 also can be engaged in yeast tRK1 complex formation (Baleva et al. 2015).

Besides that, human ENO1/ENOA shows DNA-binding activity. In 2000, ENO1/ENOA was identified as binding protein of the *C-MYC* promotor down-regulating the transcription of the cell development-related protooncogene (Subramanian and Miller 2000). The authors showed that binding and regulation of *C-MYC* promotor region relies on the N-terminus of ENO1 hinting to the potential protein domain involved in the recognition and binding of nucleic acids.

Moreover, human ENO1/ENOA stimulates transcription of the Sendai virus (−)RNA as described for human PFK1 (Ogino et al. 2001), by associating with the transcription initiation complex together with tubulin and PGK1. Whether this effect relies on direct ENO1–RNA interaction remains open.

In other cases, distinct binding sites of mammalian enolase could be established. ENO1 from *Rattus norvegicus* acts as CUG triplet repeat-binding protein in gel shift assays (Hernández-Pérez et al. 2011). Moreover, using eCLIP, Huppertz et al. (2022) described about 2000 RNA binding sites of human ENO1 predominantly in the 5′-UTR of target transcripts in HeLa cells. They could show, that human ENO1 is target of complex riboregulation. They in vitro confirmed the interaction of ENO1 with mRNA of poly(A) binding protein 1 (PABPC1), protein tyrosine phosphatase 4A1 (PTP4A1), and ferritin heavy chain 1 (FTH1) via EMSA and showed that these targets inhibit enzymatic activity of ENO1 by noncompetitive inhibition (Fig. 4D). Regulation of ENO1 activity by RNAs plays a major role in embryonic stem cell differentiation. Moreover, the authors reported that RNA binding was enhanced upon ENO1 acetylation allowing for discussions on the regulation of ENO1 RNA binding activity.

Recently it was observed by Zhang et al. (2022) that human ENO1 is involved in post-transcriptional regulation. The glycolytic enzyme, after recruiting CNOT6, regulates expression of the iron responsive protein IPR1 by enhancing its mRNA decay rate. Thereby, ENO1 massively contributes to iron homeostasis and survival of hepatocellular carcinoma (HCC) cells.

Also in prokaryotes, enolases affect RNA metabolism. *E. coli* ENO is a component of the bacterial RNA degradosome significantly contributing to the rapid response to phospho-sugar stress (Kühnel and Luisi 2001; Morita et al. 2004; Chandran and Luisi 2006). Phospho-sugar stress occurs in cells if, following import of sugars and phosphorylation by hexokinase, the generated phosphosugars like Glc-6-P accumulate and are not consumed in central metabolism. The involved mechanism remains to be explored. In contrast, direct RNA-binding was shown for *Streptococcus pneumoniae* ENO that is localized on the cell surface of the bacteria, recognizes host RNA and facilitates infection (Zakrzewicz et al. 2016). These examples reveal important functions of enolases as evolutionary conserved RBPs and post-transcriptional regulators.

PYRUVATE KINASE

Pyruvate kinases catalyze the irreversible and final step of glycolysis and is the enzyme identified in most RIC studies similar to enolase (Table 1).

Human pyruvate kinase PKM was reported as poly (A)-RNA binding protein already in 2012 (Castello et al. 2012). Only recently, the circumstances of RNA binding and, more importantly, the relevance of RNA binding were clarified, first for mouse PKM (Simsek et al. 2017). Using ribosome affinity purification, the authors identified PKM as a ribosome-associated protein (RAP) in mouse embryonic stem cells (ESCs). FAST-iCLIP analysis confirmed RNA-binding activity of PKM. Ribosomal RNAs (18S and 28S mature rRNA) and mRNAs enriched in transcripts coding for endoplasmic reticulum (ER)-destined proteins dominated the set of main RNA substrates of PKM. Binding sites were mainly located in the CDS and the 3′-UTRs of the RNA targets. RNA binding is hypothesized to enhance protein synthesis of components of the ER and the cell membrane, most probably by tethering ribosome components and transcripts (Fig. 4E). It will be interesting to explore whether the interaction represents a constitutive mechanism or is subject to regulation by external stimuli and, therefore, contributes to conditional stress response and cell acclimation.

But also, human PKM was found to associate to ribosomes. Kejiou et al. (2019) discovered that PKM regulates polysome association of mRNAs related to cell cycle. It binds the coding sequence of transcripts with polyacidic stretches and stalls ribosome elongation in human osteosarcoma (U2OS) cells in response to pyruvate and glucose. PKM binding activity and translational regulation is cell cycle-dependent. Binding to ribosomal RNA is also critically discussed by the authors but lacks solid support by their data (Kejiou et al. 2019).

Interestingly, ribosome association of pyruvate kinase was also observed in prokaryotes. NMR spectroscopy revealed PK (*E. coli* pyruvate kinase) interaction with the A-site, the L1-stalk and the mRNA entry pore of the 70S ribosome (Yu et al. 2021). The authors found that the PK-ribosome interaction introduces quinary structures that regulate PK activity (k_{cat} +25%, K_M −65%) in a similar manner as it was found for other metabolic enzymes and they introduce the term of ribosome-amplified metabolomics (RAMBO) as a general mechanistic phenomenon that describes ribosome-dependent regulation of metabolic rates. Also in this case, PK-induced changes in ribosome performance seem reasonable, as all ribosome surfaces contributing to PK-interaction are involved in translation. These examples highlight the strong reciprocal regulation by pyruvate kinase association with the translation machinery.

The role of pyruvate kinases in post-transcriptional regulation of gene expression is not restricted to the cytosol at free or ER-bound ribosomes. A recent study uncovered that human PKM regulates RNA processing by enhancing splicing rates of precursor mRNAs in the nucleus (Anastasakis et al. 2021). It was found to bind folded RNA G quadruplexes (rG4) and most likely compete with splicing repressive rG4 binding proteins. Nuclear accumulation of PKM was often observed in cancer and it associated with its malignancy. Moreover, the identified targets of

TABLE 1. Compilation of glycolytic enzyme isoforms in *Arabidopsis thaliana*, their substrates, ID, presence in the RBP2GO database (Caudron-Herger et al. 2020) and information on reported functions

Enzyme (substrate, cofactor)	Isoform	Organism specific ID	RBP2GO (Score)	Drought responsive	RNA target, (effect)	Reference
Hexokinase (Glucose, ATP)	HXK1	AT4G29130	0/7 (3.6)	Yes		
	HXK2	AT2G19860	0/7 (2.9)	No		
	hexokinase-3	AT1G50460	0/7 (5.7)	No		
	hexokinase-4	AT3G20040	0/7 (4.3)	No		
	hexokinase-like 1	AT1G47840	0/7 (2.9)	No		
Phosphoglucose isomerase (Glucose-6-phosphate)	PGI1	AT4G24620	0/7 (5)	Yes		
	PGIC	AT5G42740	1/7 (11.4)	Yes		
Phosphofructokinase (Fructose-6-phosphate, ATP)	PFK1	AT4G29220	0/7 (2.1)	No		
	PFK2	AT5G47810	0/7 (3.6)	No		
	PFK3	AT4G26270	1/7 (10.7)	No		
	PFK4	AT5G61580	0/7 (3.6)	No		
	PFK5	AT3G22480	0/7 (4.3)	No		
	PFK6	AT4G32840	0/7 (2.1)	No		
	PFK7	AT5G56630	0/7 (3.6)	Yes		
Fructose-bisphosphate aldolase (Fructose-1,6-bisphosphate)	FBA1	AT2G21330	3/7 (35)	No	*petD* 3¢-UTR, unspecific	Stenger et al. 2004 (EMSA)
	FBA2	AT4G38970	3/7 (34.3)	No		
	FBA3	AT2G01140	0/7 (7.9)	No		
	FBA4	AT5G03690	0/7 (1.4)	No		
	FBA6	AT2G36460	1/7 (8.6)	No		
	FBA8	AT3G52930	1/7 (8.6)	No		
Glyceraldehyde-3-phosphate dehydrogenase	GAPC1	AT3G04120	0/7 (7.9)	Yes	*sap3* 5′UTR (translation repression?)	Moore et al. 2021 (EMSA)
	GAPC2	AT1G13440	0/7 (7.9)	Yes	*sap3* 5′ UTR (translation repression?)	Moore et al. 2021 (EMSA)
Glyceraldehyde-3-phosphate dehydrogenase (glyceraldehyde-3-phosphate, NAD^+, P_i)	GAPA	AT3G26650	2/7 (25.7)	No		
	GAPB	AT1G42970	2/7 (27.1)	No		
	GAPCP1	At1G79530	0/7 (7.9)	Yes		
	GAPCP2	AT1G16300	0/7 (7.9)	No		
Triosephosphate isomerase (dihydroxyacetonphosphate)	TIM	AT2G21170	1/7 (16.4)	Yes		
	CTIMC	AT3G55440	1/7 (14.3)	No		
Phosphoglycerate kinase (1,3-bisphosphoglycerate, ADP)	PGK1	AT3G12780	3/7 (31.4)	No	3′-UTR of BaMV RNA (+) (RNA localization)	Lin et al. 2007 (EMSA)
	PGK2	AT1G56190	1/7(14.3)	No		
	PGK3	AT1G79550	2/7 (22.1)	No		
Phosphoglycerate mutase (3-phosphoglycerate)	PGAM1	AT1G09780	0/7 (5.7)	Yes		
	PGAM2	AT3G08590	0/7 (6.4)	No		
	gpmA1	AT1G22170	0/7 (7.9)	No		
	gpmA2	AT1G78050	0/7 (8.6)	No		
Enolase (2-phosphoglycerate)	ENO1	AT1G74030	1/7 (13.6)	No		
	ENO2	AT2G36530	1/7 (12.9)	No		
	ENO3	AT2G29560	0/7 (6.4)	No		

Continued

TABLE 1. *Continued*

Enzyme (substrate, cofactor)	Isoform	Organism specific ID	RBP2GO (Score)	Drought responsive	RNA target, (effect)	Reference
Pyruvate kinase (phosphoenolpyruvate, ADP)	PKP1	AT3G22960	0/7 (3.6)	Yes		
	PKP2	AT5G52920	0/7 (3.6)	No		
	PKP3	AT1G32440	0/7 (3.6)	No		
	PKP4	AT3G49160	0/7 (3.6)	No		
	PK1	AT3G55650	0/7 (4.3)	No		
	PK2	AT2G36580	0/7 (4.3)	No		
	PK3	AT5G63680	1/7 (9.3)	No		
	PK4	AT5G08570	0/7 (1.4)	No		
	PK5	AT3G55810	0/7 (4.3)	No		
	PK6	AT5G56350	0/7 (3.6)	No		
	Putative pyruvate kinase	AT4G26390	0/7 (4.3)	No		

Isoforms written in green letters are localized in the chloroplast. Isoforms are stated as drought responsive according to Marondedze et al. (2019).

PKM mainly corelate with mRNAs that were found to be deregulated in cancer cells, making PKM a promising drug target for cancer therapies.

RNA BINDING OF GLYCOLYTIC ENZYMES OCCURS UBIQUITOUSLY

Tables 1–3 summarize the herein presented current knowledge about the RNA binding activity of the ten core glycolytic enzymes. While RNA binding activity can be found within the entire pathway across the different species the relevance of these RNA binding events remains elusive or speculative for many enzymes. Most case studies focused on animal systems and validated and mechanistically dissected the observed interactions whereas most studies in plants fail to address questions concerning the regulation and function of the RNA binding activity and the physiological significance *in planta*.

RNA binding of glycolytic enzymes occasionally is associated with aggregation of these enzymes in macromolecular assemblies as experimentally shown in yeast in response to hypoxic conditions (Fuller et al. 2020). Beyond that, the formation of metabolons is widely observed across diverse species and seems evolutionarily conserved (Jang et al. 2021; Zhang and Fernie 2021). Although the requirement of RNAs for formation of these complexes has not been proven for many organisms, observations in yeast allow the assumption that RNA binding activity of plant and animal glycolytic enzymes also massively contributes to the generation and integrity of glycolytic subcompartments.

Moreover findings in prokaryotes, like the involvement of *E. coli* enolase in RNA degradasomes, strongly suggest that not only the ability of highly conserved glycolytic enzymes to bind RNA is evolutionarily preserved but also the functions and mechanisms may be highly similar across even distant related enzymes (Kühnel and Luisi 2001; Morita et al. 2004; Chandran and Luisi 2006).

DECIPHERING THE RNA INTERACTOME OF GLYCOLYTIC ENZYMES: A FEASIBLE PROJECT?

The RNA substrates of the 10 core glycolytic enzymes so far comprise diverse RNA species like viral RNA, precursor mRNA, mRNA, rRNA, or tRNA. However, the RNA interactome of these enzymes might be far from being complete. Like the RBPome itself, the RNA interactome of a protein also might be complex and highly dynamic. The cases of HXK1 (plant hexokinase), PGAM1 (plant phosphoglycerate kinase) and PKP1, which were identified as RNA binding proteins so far only under drought stress (Marondedze et al. 2019), demonstrate that RNA binding activity can be restricted to specific conditions. This strongly complicates the identification and further characterization of RBPs. This finds support by the comparative analysis by Köster et al. (2017) who inspected and discussed the Arabidopsis RBPome derived from studies with leaves, cell culture, etiolated seedlings or mesophyll protoplast. The rather small overlap of the identified RBPs suggests that different cell systems, different developmental stages or altering culturing conditions greatly affect the composition of the RNA binding proteome.

Similar conditional effects might be observable in studies aiming at identifying RNA targets. In addition, the use of different techniques might also contribute to discrepancies in the RNA interactome of a protein like seen in the case of ENO2 that was analysed by PAR-CLIP and CRAC, respectively (Shchepachev et al. 2019; Fuller et al. 2020). Whether the observed differences are due to the application of different techniques or differences in culturing conditions will have to be addressed in future work. The results of this process will either reveal potential physiological

TABLE 2. Compilation of glycolytic enzyme isoforms in *Saccharomyces cerevisiae*, their substrates, ID, presence in the RBP2GO (Caudron-Herger et al. 2020) database and information on reported functions

Enzyme (substrate, cofactor)	Isoform	Organism specific ID	RBP2GO (score)	Target, motif	Reference
Hexokinase (glucose, ATP)	HXK1	YFR053C	4/11 (27.3)		
	HXK2	YGL253W	4/11 (26.4)		
Phosphoglucose isomerase (glucose-6-phosphate)	PGI1	YBR196C	3/11 (30.5)		
Phosphofructokinase (fructose-6-phosphate, ATP)	PFK1	YGR240C	4/11 (24.5)		
	PFK2	YMR205C	5/11 (35)	Own transcript (autoregulation?)	Scherrer et al. 2010 (RIP-Chip)
				AU-rich elements, 3′ UTRs, coding sequence of, e.g., glycolysis-related transcripts, noncoding RNAs (mRNA localization to G-bodies)	Fuller et al. 2020 (PAR-CLIP-seq)
Fructose-bisphosphate aldolase (fructose-1,6-bisphosphate)	FBA1	YKL060C	6/11 (35.9)	Noncoding RNAs and coding sequences, pyrimidine-rich motifs (mRNA localization to G-bodies)	Fuller et al. 2020 (PAR-CLIP-seq)
Glyceraldehyde-3- phosphate dehydrogenase (glyceraldehyde-3-phosphate, NAD^+, P_i)	TDH1	YJL052W	7/11 (31.4)		
	TDH2	YJR009C	8/11 (31.8)		
	TDH3	YGR192C	9/11 (35.9)		
Triosephosphate isomerase (dihydroxyacetone phosphate)	TPI	YDR050C	3/11 (31.8)		
Phosphoglycerate kinase (1,3-bisphospho glycerate, ADP)	PGK1	YCR012W	6/11 (44.1)		
Phosphoglycerate mutase (3-phosphoglycerate)	GPM1	YKL152C	4/11 (34.5)		
Enolase (2-phosphoglycerate)	ENO1	YGR254W	5/11 (40)	Glycolytic enzymes, pyrimidine-rich (localization to G-bodies)	Fuller et al. 2020 (PAR-CLIP-seq)
	ENO2	YHR174W	5/11 (40)	Several glycolytic proteins	Matia-González et al. 2015 (fluorescence-based protein–RNA interaction assay [RIP], RIP-qPCR)
				tRK1 (import into mitochondria)	Entelis et al. 2006
Pyruvate kinase (phosphoenolpyruvate, ADP)	PYK1	YAL038W	4/11 (33.6)		
	PYK2	YOR347C	2/11 (20.9)		

relevance or point to methodological shortcomings or variations.

Another open question concerns the specificity of binding, because specific RNA binding substrates are unknown for several glycolytic enzymes like for most metabolic enzymes. They may function either as specific RBPs targeting selected RNAs or as nonspecific RBPs recognizing a broad spectrum of RNA species. These two different modes of action might come along with distinct implications of moonlighting functions of glycolytic enzymes in RNA binding. On the one hand, RNA-binding enzymes could serve as scaffolds for macromolecular structure formation through multiple intermolecular interactions, thereby, regulating either RNA state or protein localization and metabolic activity. This scenario might mainly involve nonspecific RNA-binding processes if we consider that most of the glycolytic enzymes are highly abundant and thus the molar ratio of enzyme to specific transcript is usually quite high. Unspecific binding to numerous RNAs would increase the number of RNA substrates and facilitate efficient regulation of the metabolic activity of the protein. On the other hand, binding to specific RNA could enable targeted regulation of RNA fate and activity.

Because naked RNA in the cell appears to be the rare exception rather than the common form, a competitive process between specific and general RNA-binding proteins has to be expected and might be determinant in the occupation of the accessible RNA surfaces with glycolytic enzymes.

TABLE 3. Compilation of human glycolytic enzyme isoforms, their substrates, ID, presence in the RBP2GO (Caudron-Herger et al. 2020) database and information on reported functions

Enzyme (substrate, cofactor)	Isoform	UniProt ID	RBP2GO (score)	RNA target (effect)	Reference (method)
Hexokinase (glucose, ATP)	HK1	P19367	4/43 (7.9)		
	HK2	P52789	5/43 (9)		
	HK3	P52790	0/43 (2.6)		
Phosphoglucose isomerase (glucose-6-phosphate)	GPI	P06744	6/43 (12.7)		
Phosphofructokinase (fructose-6-phosphate, ATP)	PFKL	P17858	3/43 (11.2)		
	PFKM	P08237	2/43 (13.1)		
	PFKP	Q01813	6/43 (15.7)		
Fructose-bisphosphate aldolase (fructose-1,6-bisphosphate)	ALDOA	P04075	13/43 (26.4)	*MyHC 3′UTR* (localization to cytoskeleton?)	Kiri and Goldspink 2002 (EMSA)
	ALDOB	P05062	0/43 (4.5)		
	ALDOC	P09972	7/43 (17.1)		
Glyceraldehyde-3-phosphate dehydrogenase (glyceraldehyde-3-phosphate, NAD^+, P_i)	GAPDH	P04406	16/43 (30.7)	tRNA (nuclear export)	Singh and Green 1993 (EMSA)
				GLUT1 mRNA 3′-UTR (ARE),	McGowan and Pekala 1996 (EMSA)
				Ribosomal RNA (RNA unwinding?)	Ryazanov 1985 (RNA affinity chromatography)
				HAV RNA 5′ UTR (IRES) (destabilization of RNA structure, suppression of translation)	Chang et al. 1993, Schultz et al. 1996, Yi et al. 2000 (EMSA, IP)
				HAV RNA 3′-UTR (ARE) and 3′ coding,	Dollenmaier and Weitz 2003 (RNA footprinting)
				HCV RNA 3′-UTR,	Petrik et al. 1999 (RNA affinity chromatography N terminal sequencing)
				HBV, EMSA	Zang et al. 1998
				HPIV RNA 3′-UTR (U-rich)	De et al. 1996 (EMSA, RIP)
				HDV antigenomic RNA	Lin et al. 2000 (EMSA, RNA footprinting)
				c-myc	Nagy and Rigby 1995 (EMSA)
				GM-CSF	Nagy and Rigby 1995 (EMSA)
				CCN-2, RNA-affinity chromatography-MS,	Kondo et al. 2011 (EMSA)
Glyceraldehyde-3-phosphate dehydrogenase (glyceraldehyde-3-phosphate, NAD^+, P_i)	GAPDH	P04406	16/43 (30.7)	*ET-1 3′-UTR* (ARE) (destabilization of mRNA/ translational regulation?)	Rodríguez-Pascual et al. 2008 (RNA-affinity chromatography-MS, EMSA)
				Cox2 3′-UTR (ARE) (destabilization of mRNA)	Ikeda et al. 2012 (EMSA)
				SCN1A (3′-UTR), (destabilization of mRNA)	Zeng et al. 2014 (EMSA)
				SCN3A (stabilization of mRNA)	Lin et al. 2017 (EMSA, RNA-affinity chromatography-MS)
				CSF-1 (stabilization of mRNA)	Bonafé et al. 2005, Zhou et al. 2008

Continued

TABLE 3. *Continued*

Enzyme (substrate, cofactor)	Isoform	UniProt ID	RBP2GO (score)	RNA target (effect)	Reference (method)
					(EMSA, RNA foot printing analysis)
				IFN-γ (translational repression)	Nagy and Rigby 1995, Chang et al. 2013 (EMSA, RIP-qPCR)
				IL-2 (translational repression)	Nagy and Rigby 1995, Chang et al. 2013 (EMSA, RIP-qPCR)
				AT1R (translational repression)	Backlund et al. 2009 (EMSA, RNA-affinity chromatography-MS)
				TNF-α (translational repression)	White et al. 2015, Millet et al. 2016 (EMSA, FRET, RIP-qPCR)
				HIF1α (translational repression)	Xu et al. 2016 (RIP-qPCR)
Triosephosphate isomerase (dihydroxyacetone phosphate)	TPI	P60174	7/43 (18.8)		
Phosphoglycerate kinase (1,3-bisphosphoglycerate, ADP)	PGK1	P00558	13/43 (22)	*uPAR* coding sequence (mRNA destabilization)	Shetty et al. 2004 (EMSA, RIP-qPCR)
				glycoLINC (metabolon formation)	Zhu et al. 2022 (affinity pull down, RIP)
	PGK2	P07205	0/43 (6.9)		
Phosphoglycerate mutase (3-phosphoglycerate)	PGAM1	P18669	8/43 (17.3)		
	PGAM2	P15259	0/43 (7.2)		
Enolase (2-phosphoglycerate)	ENO1	P06733	19/43 (30.1)	RNA CUG triplet,	Hernández-Pérez et al. 2011 (affinity assay, EMSA)
				5′ UTRs, *PABPC1*, *PTP4A1*, *FTH1*	Huppertz et al. 2020 (eCLIP, EMSA)
				tRK1 (import into mitochondria)	Baleva et al. 2015 (in vitro import test)
				IRP1 (accelerates mRNA decay)	Zhang et al. 2022
				glycoLINC (metabolon formation)	Zhu et al. 2022 (affinity pull down, RIP)
	ENO2	P09104	1/43 (9.5)	tRK1 (import into mitochondria)	Baleva et al. 2015 (in vitro import test)
	ENO3	P13929	1/43 (8)	tRK1 (import into mitochondria)	Baleva et al. 2015 (in vitro import test)
Pyruvate kinase (phosphoenolpyruvate, ADP)	PKLR	P30613	0/43 (3.8)		
	PKM	P14618	18/43 (29.5)	18S and 28S mature rRNAs and mRNAs (translational activator)	Simsek et al. 2017 (FAST-iCLIP)
				mRNAs (CDS, 3′UTR): ER component, cell membrane proteins (translational activator)	Simsek et al. 2017 (FAST-iCLIP)
Pyruvate kinase (phosphoenolpyruvate, ADP)				Cell cycle-related transcripts (stalling of translation elongation)	Kejiou et al. 2019 (eCLIP)
				rG4 of precursor mRNAs (promotes splicing)	Anastasakis et al. 2021 (PAR-CLiP)
				glycoLINC (metabolon formation)	Zhu et al. 2022 (affinity pull down, RIP)

DIFFERENT SITES FOR MOONLIGHTING OF GLYCOLYTIC ENZYMES IN THE CELL

The "REM-hypothesis" (RNA, enzyme, metabolite) proposed the concept of bidirectional regulation of the two interaction partners. Hentze and Preiss (2010) summarized the complex regulatory network of RNAs, enzymes and metabolites linking metabolic and RNA activity as illustrated in Figure 5. The hypothesized reciprocal relationship between metabolic enzymes and RNA has several mechanistic and spatial levels that are addressed in the following section.

RNA regulating metabolism

Riboregulation of glycolytic enzymes may depend on different mechanisms. The observation that RNA contributes to the formation, fusion and integrity of G-bodies in yeast in response to hypoxia (Fuller et al. 2020) leads to the assumption that glycolytic metabolons might rely on RNA in other organisms as well. In this scenario, RNA might act as scaffold for the assembly of supramolecular complexes of glycolytic enzymes that allow for efficient substrate channeling. In this scenario, the RNA–enzyme interactions indirectly regulate metabolic fluxes and glycolytic activity. Very recently, RNA-mediated metabolon formation was detected in human cell lines (Zhu et al. 2022). The long noncoding RNA (lncRNA) glycoLINC (gLINC) builds the scaffold for the four glycolytic payoff phase enzymes as well as lactate dehydrogenase A (LDHA) and this enhances glycolytic flux and ATP production. The c-Myc responsive RNA contributes to cell survival under serine deficiency. Association of glycolytic proteins in macromolecular assemblies was observed in plants in various studies (Zhang and Fernie 2021) but was not yet investigated in regard to the involvement of nucleic acids.

In this context, RNA–enzyme interactions might not only support metabolon formation of glycolytic enzymes but also tune their conditional optimal localization. It was repeatedly observed in *Arabidopsis thaliana* and in several other organisms (Taylor et al. 2003; Brandina et al. 2006), that the entire glycolytic pathway associates with the cytosolic side of the outer mitochondria membrane (Giegé et al. 2003; Graham et al. 2007). Moreover, it was shown that PGAM1/PGAM2 (plant phosphoglycerate mutase) and ENO2 significantly contribute to the colocalization of chloroplasts and mitochondria forming large metabolons containing, for example, PK4 and allowing for fast supply of pyruvate to the mitochondria (Zhang et al. 2020). It might be worthwhile to investigate whether the colocalization of organelles for efficient metabolism by glycolytic enzymes is mediated or supported by RNA.

Similarly, the recruitment of glycolytic enzymes to other organelles like the nucleus, as usually observed for human PKM in cancer cells, inevitably impacts the catabolic process in the cytosol. In cancer cells, reduced glycolytic flux channels the carbon source from glucose to biosynthetic processes and promotes cell growth. At the same time, repartitioning of metabolic enzymes will also affect enzymatic reactions in the target destination. On the one hand this enables alternative enzymatic reactions catalyzed by the relocated enzyme as recently shown for mice pyruvate kinase M2 (Gao et al. 2012). PKM2 promotes cell proliferation by activating transcription of cell cycle-related genes through phosphorylation of the transcription factor STAT3 in the nucleus. The authors could show that the protein kinase function is conducted as dimer whereas conversion of PEP is catalyzed as a tetramer.

On the other hand, relocated glycolytic enzymes might change the metabolite constitution in the nucleus with broad implications on gene expression. This was shown for human triosephosphate isomerase TPI1 (Zhang et al. 2021). Upon phosphorylation by the cyclin-dependent kinase 2 (CDK2), TPI1 translocates to the nucleus where it decreases the concentration of DHAP. This promotes nuclear acetate accumulation as less DHAP is available for scavenging acetate through 1-acetyl-DHAP formation. Consequently, this leads to histone hyperacetylation with impacts on chromatin condensation, gene transcription and DNA replication. This pathway is regulated by the cell cycle via the involvement of nutrition-regulated mammalian target of rapamycin complex 1 (mTORC1) kinase that phosphorylates and activates CDK2. In a similar scenario, RNA-mediated translocation could alter metabolism in other cellular compartments and impact gene expression.

More direct regulation of metabolic activity in situ might occur through competitive inhibition when RNA binding site is identical to the substrate or cofactor binding site as in the case of human ENO1 (Huppertz et al. 2022) or through allosteric inhibition as observed for prokaryotic pyruvate kinase (Yu et al. 2021). Dynamic binding

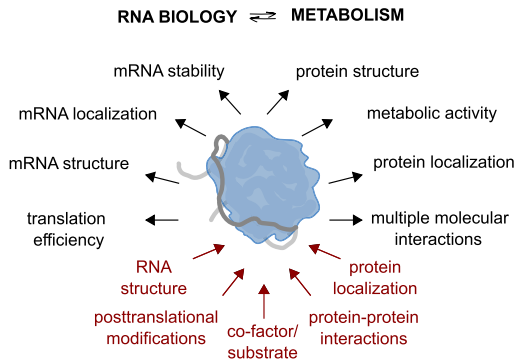

FIGURE 5. Summary of interconnection between RNA biology and metabolism. The schematics illustrate the diverse implications (in black letters) of RNA–enzyme interactions on mRNA features and enzyme activity and potential targets (in red letters) to regulate the binding event.

of metabolic enzymes to RNA would regulate the availability of active enzyme. In all scenarios, the implication on overall enzyme performance is difficult to estimate and should be addressed by metabolic flux analysis. The question how many fully active enzymes are needed to keep up overall glycolysis or one specific conversion within the pathway would depend on the chemical equilibrium of the involved enzymatic reactions, metabolite concentrations, the presence of other metabolic enzymes and the stoichiometric relationship between the moonlighting enzyme and the RNA. Glycolysis likely is the metabolic pathway with the highest number of mathematical models (e.g., Lambeth and Kushmerick 2002). Such in silico predictions need to be linked to metabolome, proteome and flux data in order to estimate the rate-controlling function of individual RNA-binding events on glycolysis.

Moreover, it needs to be considered that many of the 10 glycolytic enzymes exist in several functionally redundant forms, namely as isoforms or paralogs. RNA binding or moonlighting functions in post-transcriptional regulation may differ between paralogs (Tables 1–3). Moreover, paralog-specific stress response of metabolic enzymes has been observed and discussed as a strategy to maintain basic metabolic function but simultaneously fulfil additional roles (Matia-González et al. 2021). This functional specification may have evolved in response to the evolutionary pressure in complex environmental scenarios, as discussed elsewhere (Conant and Wolfe 2008; Matia-González et al. 2021).

Enzymes regulating protein synthesis

Many studies demonstrate the broad implications of the RNA binding activity of glycolytic enzymes on processes such as mRNA processing, stability and degradation, localization, and translational regulation. Transcripts that are subject of targeted regulation by glycolytic enzymes are diverse, ranging from mRNAs for different cytokines, membrane proteins, substrate transporters to sodium channels. Apparently, regulation by glycolytic enzymes can target distinct cellular processes or pathways. Regulation of RNA regulons by enzymes to coordinate expression of functionally related transcripts is also conceivable (Keene 2007; Imig et al. 2012). This assumption finds support by Matia-González et al. (2015) and Fuller et al. (2020) who reported that some glycolytic enzymes bind their own message. This allows for efficient feedback regulation of central carbon metabolism. RNA binding of glycolytic enzymes represents a perfect link between the central (carbon) metabolism and protein biosynthesis that is necessary to coordinate the highly ATP-demanding process in accordance with the cellular energy state.

In general, the moonlighting functions of glycolytic enzymes in post-transcriptional regulation of target transcripts is associated with distinct subcellular localizations. Control of precursor mRNA processing takes places in the nucleus, as in the case of human PKM2 (Anastasakis et al. 2021), while control of protein synthesis rates by metabolic enzymes is associated with the cytosol. Here moonlighting might appear in the cytoplasm on the RNA substrate, represented by human GAPDH regulating polysome association of certain cytokines (Chang et al. 2013), but also in association with free or ER-bound ribosomes. The later scenario is demonstrated again by human PKM2 promoting the translation of mRNAs coding for ER components or cell membrane proteins (Simsek et al. 2017).

The cytoskeleton is also a potential site of moonlighting function of glycolytic enzymes. Binding of *MyHC* transcript by human ALDOA and GAPDH, for example, is tentatively discussed as a mechanism to localize the mRNA to the cytoskeleton (Kiri and Goldspink 2002). This example of ALDOA and GAPDH might be one hint to a more general phenomenon of targeted RNA distribution mediated by glycolytic enzymes that acts as linker components. This is supported by the fact, that interaction of glycolytic enzymes with cytoskeleton components is observed in several organisms.

These examples indicate that the moonlighting function of glycolytic enzymes in RNA binding is not restricted to a specific subcellular compartment, but occurs at several, distinct sites in the cell. One and the same glycolytic enzyme can have different subcellular sites of activity as illustrated by human GAPDH or PKM2. On top, these multilocal RNA binding events can be controlled differentially as shown by a study addressing the stress response of nuclear and cytoplasmic RBPs (Backlund et al. 2020). The authors reported that for some RBPs the response to arsenite-induced oxidative stress was different in the nucleus than in the cytoplasm. This adds additional complexity to the regulatory REM network that should be considered when investigating the moonlighting functions of glycolytic enzymes and unveils the limitations of whole-cell analysis.

HOW TO COORDINATE RNA BINDING ACTIVITY OF GLYCOLYTIC ENZYMES

The moonlighting function of glycolytic enzymes as RBPs may occur constitutively or conditionally and might differ between enzymes and paralogous forms. For some glycolytic enzymes, specific stimuli might decide whether the protein at all binds to RNA or not (see plant HXK1, PGAM1, or PKP1) while for other enzymes the exact conditions might determine the RNA substrates. The proven responsiveness of the RNA binding activity to certain stress conditions implies that mechanisms to coordinate moonlighting functions of glycolytic enzymes are absolutely mandatory for the cell to maintain cell growth and function

and needs to be investigated when understanding the complex REM network.

Deciphering the molecular mechanisms that control RNA-binding activity of the metabolic enzymes also involves the identification of protein domains that are involved in RNA binding. As glycolytic enzymes usually lack canonical RNA-binding domains, the protein interface involved in RNA recognition remains mainly unknown. Different protein regions are discussed as RNA binding site based on individual examples or global investigation of RNA binding peptides by, for example, RBPmap (Castello et al. 2016).

Several metabolic enzymes display a Rossmann-fold (Rao and Rossmann 1973), the binding site for the NAD^+/NADH cosubstrate. As NAD(H) is a dinucleotide, it seems reasonable to assume that the Rossmann-fold might also recognize RNA molecules. Competition experiments with NAD^+/NADH in vitro suggest the involvement of the Rossman-fold in RNA-binding of mammalian GAPDH (Nagy et al. 2000). These observations are complemented by findings that GAPDH binding to *INF-γ*, *TNFα*, and *HIF1α* depends on the metabolic state of the cell (Chang et al. 2013; White et al. 2015; Millet et al. 2016; Xu et al. 2016). However, involvement of the substrate binding site of the enzyme cannot be excluded at this point. This protein region contains a high number of positively charged amino acids and, therefore, has been proposed as RNA-binding site as well (Carmona et al. 1999). Also, Castello et al. (2016) observed that the binding sites of several metabolic enzymes is located in close proximity to their substrate binding pocket. On the other hand, White et al. (2015) demonstrated that mutations in the dimer-dimer interface of mammalian GAPDH interfere with RNA-binding. Similarly, the moonlighting of PKM2 in the nucleus is associated with the dimeric form. This finding hints to a strong contribution of the quaternary structure of the enzymes to their binding activity (Gao et al. 2012).

Another protein region that might mediate protein–RNA interaction are intrinsically disordered regions (IDR). IDRs are commonly present in proteins that undergo phase transition (Wiedner and Giudice 2021) and enable intermolecular interactions due to their flexible structure (Tompa et al. 2009; Lin et al. 2017). From the glycolytic enzymes, only phosphofructokinases show less structured regions with high flexibility. Indeed, it was shown that the IDR of yeast PFK2 is required for its recruitment to RNA-dependent G-bodies upon hypoxia (Jin et al. 2017). The specific role of the IDR in RNA-binding, for example, as flexible linker or binding interface, is unclear. Future experiments need to dissect the structural features required for RNA recognition and interaction.

Depending on the protein domain involved in RNA-binding and its proximity to sites for PTMs or metabolite binding, diverse regulatory mechanisms of RNA-binding are possible and allow for tight control of moonlighting functions of glycolytic enzymes (Fig. 5).

Regulation of RNA binding activity by metabolite and cofactors

In the case of animal GAPDH, the presence of the cosubstrate NADH interferes with RNA-binding. The addition of NAD^+ or NADH reduces the RNA-binding activity of GAPDH in gel shifts assays (Nagy et al. 2000). Similarly, RNA-binding of ENO1 is suppressed by the enzyme-specific substrates 2-phosphoglycerate and PEP in vitro (Huppertz et al. 2020). In these cases, RNA and the substrate may compete for the respective binding site. However, indirect (de)stabilizing effects of substrates, cosubstrates or cofactors on the oligomeric enzyme structure may also control RNA binding ability. In vivo evidence supports the view that metabolite concentrations regulate the RNA-binding activity of the enzyme because binding to different target transcripts depends on the metabolic state of the cell (Chang et al. 2013; White et al. 2015; Millet et al. 2016; Xu et al. 2016).

Regulation of RNA binding activity by post-translational modifications

Regulation of moonlighting functions of glycolytic enzymes by PTMs would allow for rapid and accurate responses to environmental changes. A clear correlation between RNA binding activity and certain PTMs of the glycolytic enzymes has so far only been reported for few of the enzymes. Phosphorylation of PGK1 inhibits its binding to *upar* mRNA (Shetty et al. 2004; Shetty and Idell 2004). On the other hand, redox-related modifications interfere with the RNA-binding activity of human GAPDH. S-glutathionylation, but not S-thiolation, of the catalytically active residue Cys 152 induced by oxidative stress blocks the binding *to et-1* (Rodríguez-Pascual et al. 2008). Similarly, a free sulfhydryl group is a requirement for binding of human GAPDH to *ccn-2* mRNA (Kondo et al. 2011). Also, malonylation was shown to regulate RNA binding activity of mammalian GAPDH (Galván-Peña et al. 2019). Riboregulation of human ENO1 is controlled by acetylation (Huppertz et al. 2022). This modification presents an additional link to metabolite concentrations as acetylation of proteins is based on sufficient supply with acetyl-CoA (Xing and Poirier 2012). Presumably, many other cases exist, where RNA binding is controlled by PTMs as indicated by the results of global identification of RNA binding sites in HeLa cells using RBPmap (Castello et al. 2016). The authors found that the identified RNA binding domains represent hot spots for PTMs suggesting that modification of these sites might interfere with RNA binding activity.

Regulation of RNA binding activity by protein–protein interactions

Several mechanisms involving glycolytic enzymes in post-transcriptional regulation of protein synthesis engage formation of supramolecular assemblies as discussed. A more specific example in this context is mammalian GAPDH that functions as individual RNA-binding protein but also as part of the γ-INF-activated inhibitor of translation (GAIT) complex that regulates translation of inflammation-response mRNAs in myeloid cells (Tristan et al. 2011). Also, the stimulation of transcription of the Sendai virus RNA by PGK1 and ENO1 relies on complex formation of both enzymes with tubulin (Ogino et al. 1999, 2001). It is reasonable to assume that conditional protein–protein interactions specifically induce or inhibit the RNA-binding activity of glycolytic enzymes in these cases. Association of glycolytic enzymes with regulatory proteins could modulate the RNA-binding activity and enhance target and effect specificity.

Regulation of RNA binding activity by subcellular partitioning

Binding only occurs if both partners get together in time and space. Consequently, the repartitioning of glycolytic enzymes to another cellular compartment will reduce its RNA binding activity in the previous compartment. Cellular redistribution of glycolytic enzymes has been widely observed in dependence on certain conditions or developmental state as discussed in this review. For example, plant GAPC1/C2 and human GAPDH partition to the nucleus in response to stress treatments (Vescovi et al. 2013; Schneider et al. 2018; Kim et al. 2020). Nuclear import separates the enzyme from cytosolic RNA and may indirectly regulate RNA binding activity. In some cases, the nuclear localization is correlated with distinct PTMs (Sen et al. 2008; Ventura et al. 2010; Aroca et al. 2017). But also the association with the cytoskeleton or the attachment to organelles like the chloroplast or the mitochondria, as it was shown for several glycolytic enzymes (Giegé et al. 2003; Taylor et al. 2003; Brandina et al. 2006; Graham et al. 2007), likely influence the RNA-binding activity of the proteins. As discussed above, the spatial distribution of the enzymes adds an additional dimension to the regulation not only of the RNA binding itself but also to the mode of actions. Thereby, translocation to other cellular sites might not increase or decrease RNA binding activity but rather alter the RNA interactome.

Regulation of RNA binding activity by protein abundance

In the beginning, researchers often regarded RNA binding activity of metabolic enzymes as artifacts due to their high abundances in the cell. It is target of discussion whether high abundant enzymes are more likely to face and bind RNA than others. Indeed, analysis of paralog-specific RNA binding activity revealed that in several cases the more abundant enzyme paralog shows RNA association while the lower abundant does not (Matia-González et al. 2021). However, there is a need to distinguish between non- or low-specific binding of abundant proteins and specific binding of low abundant proteins. The occupation of RNA binding sites depends on competition during the on-reaction and the life-time of the association before dissociation. Yet, features like protein structure, exposed domains and PTMs contribute to the binding capability more than protein abundance. This finding is supported by studies, investigating changes in the RBPome in response to stress (Marondedze et al. 2019; Bresson et al. 2020). In these cases, stress-responsive RNA binding activity of metabolic enzymes does not correlate with changes in the overall protein abundance. On the contrary, Backlund et al. (2020) reported a subgroup of proteins that show moonlighting in RNA binding in the compartment where they displayed lower protein abundance. This suggests that specific regulatory mechanism control the RNA–enzyme interactions. Nonetheless, the high abundance of glycolytic enzymes, likely facilitated the evolution of moonlighting functions.

Various mechanisms are at hand to regulate the diverse moonlighting functions of metabolic enzymes. Ultimately, various processes will determine RNA binding activity, RNA substrates and the modes of action of the glycolytic enzymes and decide between constitutive or conditional binding. Constitutive binding might contribute to general cell maintenance. Conditional binding would depend on particular stimuli that switch the function of the enzyme. Taken together, their role in central metabolism, their sensitivity to metabolic changes and abundance make glycolytic enzymes ideal linker modules between glycolysis and RNA biology.

CONCLUDING REMARKS

Technical advancements push molecular biology and likewise methodological innovation has driven RNA biology as an emerging field and moved RNA-binding proteins into the focus of research. Often enzymes of central metabolic pathways were considered as contaminants in RNA technologies due to their high abundance. This simplified view has fundamentally changed. A wealth of studies unveiled important functions of these enzymes in different cellular processes and proved their RNA-binding activity and moonlighting functions in post-transcriptional control of protein synthesis. In a reverse manner, RNA binding to these enzymes participates in the regulation of metabolic pathways. However, we felt that especially in plant research, recognition of glycolytic enzymes as RNA binding

proteins advances slowly and knowledge about RNA substrates and binding mechanisms is lagging behind. Comparison with the homologous enzymes in mammalian or yeast system provides blueprints to form new hypothesis and clarify the environmental and metabolic conditions that trigger the bidirectional responses, to dissect the molecular features involved in regulation and RNA binding and to quantitatively describe the dynamics of ribonucleoprotein assemblies with target RNAs in plants. In general, there is an urgent need for a more nuanced analysis of the moonlighting functions on several levels. It will be necessary to analyse the role of enzyme paralogs and how they contribute to moonlighting functions or maintain the basic functions of the enzymes in central metabolism. Moreover, it will be essential to address the potential interconnection between the moonlighting mechanisms in different subcellular compartments of one and the same enzyme to complete the widespread REM network and to fully understand the complex relationship between central metabolism and RNA biology.

SUPPLEMENTAL MATERIAL

Supplemental material is available for this article.

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