



FORUM REVIEW ARTICLE

Central Metabolism in Mammals and Plants as a Hub for Controlling Cell Fate

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Abstract

Significance: The importance of oxidoreductases in energy metabolism together with the occurrence of enzymes of central metabolism in the nucleus gave rise to the active research field aiming to understand moonlighting enzymes that undergo post-translational modifications (PTMs) before carrying out new tasks.

Recent Advances: Cytosolic enzymes were shown to induce gene transcription after PTM and concomitant translocation to the nucleus. Changed properties of the oxidized forms of cytosolic glyceraldehyde 3-phosphate dehydrogenase, and also malate dehydrogenases and others, are the basis for a hypothesis suggesting moonlighting functions that directly link energy metabolism to adaptive responses required for maintenance of redox-homeostasis in all eukaryotes.

Critical Issues: Small molecules, such as metabolic intermediates, coenzymes, or reduced glutathione, were shown to fine-tune the redox switches, interlinking redox state, metabolism, and induction of new functions *via* nuclear gene expression. The cytosol with its metabolic enzymes connecting energy fluxes between the various cell compartments can be seen as a hub for redox signaling, integrating the different signals for graded and directed responses in stressful situations.

Future Directions: Enzymes of central metabolism were shown to interact with p53 or the assumed plant homologue suppressor of gamma response 1 (SOG1), an NAM, ATAF, and CUC transcription factor involved in the stress response upon ultraviolet exposure. Metabolic enzymes serve as sensors for imbalances, their inhibition leading to changed energy metabolism, and the adoption of transcriptional coactivator activities. Depending on the intensity of the impact, rerouting of energy metabolism, proliferation, DNA repair, cell cycle arrest, immune responses, or cell death will be induced. *Antioxid. Redox Signal.* 34, 1025–1047.

Keywords: GAPDH, moonlighting, thiol switches, redox sensing, redox signaling, energy metabolism

Introduction

THE AVAILABILITY OF ENERGY in the form of ATP and reductant is a basic requirement for the growth and performance of each living organism. Central metabolism in heterotrophic conditions relies on the oxidation of organic molecules usually provided as carbohydrates. However, lipids and amino acids can also be metabolized to provide the required energy or building blocks for the synthesis of cellular components. In addition to oxidative metabolism for the provision of energy, plants possess the photosynthetic ma-

chinery to convert sunlight into energy, namely ATP during photophosphorylation by the thylakoid-bound ATP synthase in chloroplasts and the reductant NADPH, to produce biomass photoautotrophically (Fig. 1).

Plant cells, in general, are characterized by the presence of plastids. Besides the mitochondria, this is another compartment providing energy equivalents, not only in illuminated green tissues but also in nongreen tissues, and in green tissues in darkness (compare Figs. 1–3). Plant plastids are equipped with an additional set of enzymes for glycolysis and the oxidative pentose phosphate (OPP) pathway, thus allowing for energy

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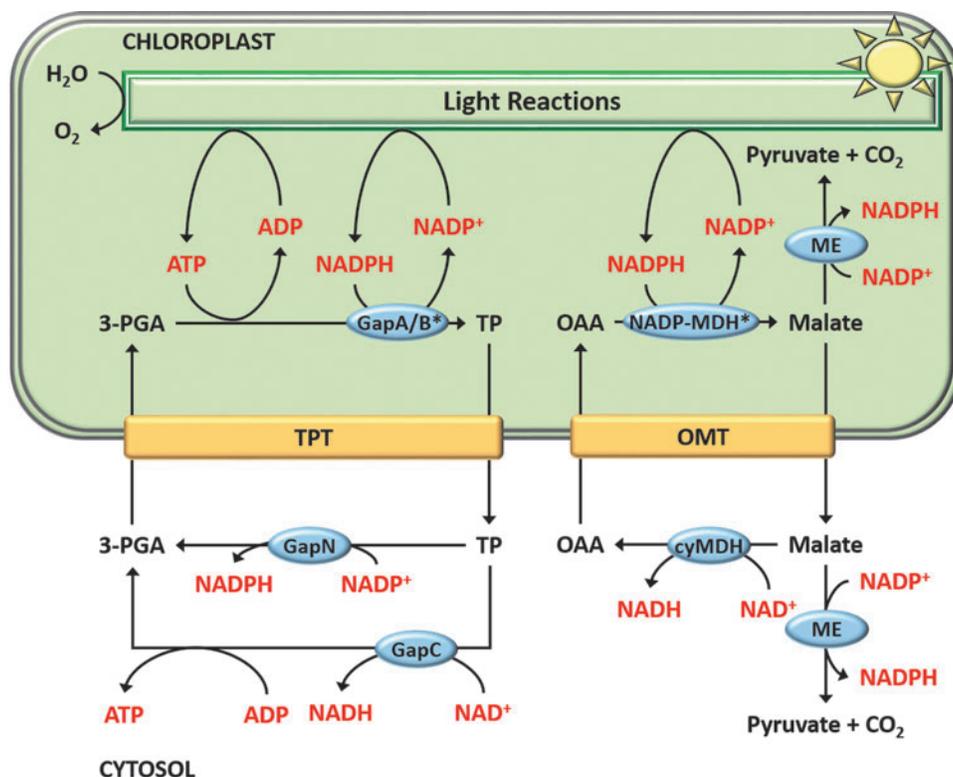


FIG. 1. Network of dehydrogenases for the exchange of energy between chloroplasts and the cytosol in the light.

The chloroplast enzymes (NADP-MDH and GapA/B) are subject to post-translational redox regulation and are only active in the light (*light/dark modulation). Besides, the NADP-MDH is only active when the NADP⁺/NADP(H) ratio is high (93). Only under these conditions, excess NADPH is exported enabling continued photosynthetic electron flow. TP export can serve for NADH and ATP generation or, alternatively, for NADPH production in the cytosol. 3-PGA, 3-phosphoglycerate; cyMDH, cytosolic NAD-dependent malate dehydrogenase; GapA/B, bispecific (NAD⁺/NADP⁺-dependent) glyceraldehyde 3-phosphate dehydrogenase; GapN, nonphosphorylating irreversible glyceraldehyde 3-phosphate dehydrogenase; ME, malic enzyme; NADP-MDH, NADP-dependent malate dehydrogenase; OAA, oxaloacetate; OMT, 2-oxoglutarate/malate transporter; TP, triose phosphate; TPT, triose phosphate/phosphate translocator.

provision from carbohydrates, not only by cytosolic but also by plastid isoforms of the respective enzymes (Table 1) (161, 162).

The membrane boundaries of the cellular compartments are largely impermeable for reducing equivalents and to some extent for ATP. Therefore, various translocators for the exchange of metabolites together with oxidoreductases on both sides of the membranes mediate indirect transport of reducing equivalents and in some cases ATP. Plastids and mitochondria are equipped with malate/oxaloacetate exchangers, namely the 2-oxoglutarate/malate transporter that functions as an oxaloacetate transporter in the malate/oxaloacetate shuttle across plastid membranes and the mitochondrial dicarboxylate carrier. These exchangers, in conjunction with the numerous isoforms of malate dehydrogenases (MDHs), act as efficient malate valves and function for transport of NAD(P)H from the site of (over-)production to the site of consumption (156, 162). The decarboxylating MDH NADP-malic enzyme (NADP-ME) can alternatively convert malate to pyruvate and CO₂ leading to NADPH release in the cytosol (Figs. 1 and 2) (60).

Triose phosphate (TP)/3-phosphoglyceric acid translocators (TP/phosphate translocator) acting together with the various isoforms of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) enable the indirect transport of NAD(P)H and ATP (Figs. 1 and 2) (52, 71). Specific to plants is the presence

of a nonphosphorylating aldehyde dehydrogenase (GapN) that generates NADPH in an irreversible reaction and no ATP in the cytosol (19) (Fig. 1). Under anaerobic conditions, NAD⁺ can be regenerated *via* the formation of lactate by lactate dehydrogenase (LDH) in the cytosol to avoid reductive stress (not shown in the figure for the sake of clarity).

Since the pool sizes of the energy carriers, ATP/ADP, and the reductants NAD(P)(H), are very small and need to function as acceptors as well as donors in the various exergonic and endergonic reactions, respectively, they need to be finely balanced at any time and in each compartment (157). Such flexible regulation of energy fluxes is achieved by continuous adjustment of the indirect exchange by the relevant energy carriers with the help of the so-called valve systems that are tightly controlled by the energy-converting enzymes on both sides. The cytosolic enzymes NAD-GAPDH and glucose 6-phosphate dehydrogenase (G6PDH) are present in all organisms.

However, plants possess an additional set of these enzymes in their plastids for the generation of energy in the absence of light or in nongreen plastids (161, 162). When compared to animals and yeast, plants are characterized by a number of unique enzymes and frequently more than a single isoform in one cell compartment. It is interesting to note that there are valve systems for translocation of C3- as well as for C4- compounds that carry both, energy and carbon skeletons for

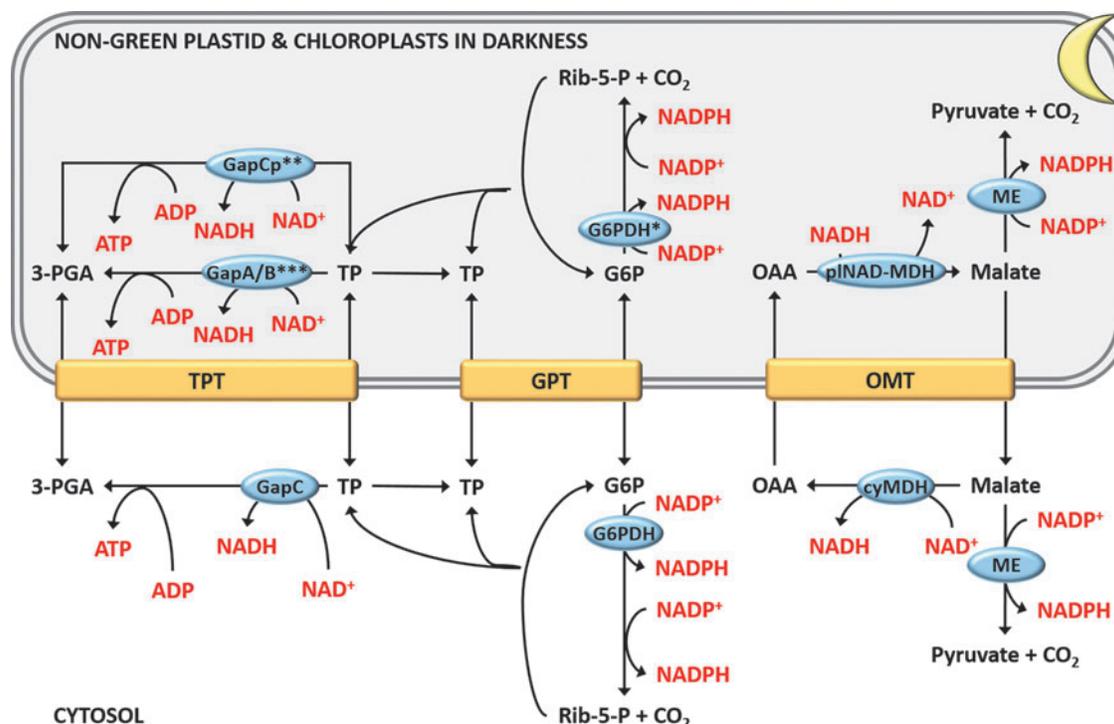


FIG. 2. Network of dehydrogenases and translocators for the exchange of energy between nongreen plastids or chloroplasts and the cytosol in darkness. The OPP-pathway enzyme G6PDH in chloroplasts is only active in the dark (*light/dark modulation). GapCp is only present in nongreen plastids (**). While GapA/B is active with NADP^+ only in the light (Fig. 1), its activity with NAD^+ is light-independent (***) (5, 6). Plastidial glycolysis can only generate ATP when NADH is continuously removed by the nonredox-regulated malate valve enzyme pINAD-MDH. Since NADPH is needed for biosyntheses, plastidial OPP-pathway activity is required. G6P, glucose 6-phosphate; G6PDH, glucose 6-phosphate dehydrogenase; GapC, cytosolic glyceraldehyde 3-phosphate dehydrogenase; GapCp, plastidial NAD-dependent glyceraldehyde 3-phosphate dehydrogenase; GPT, glucose-6-phosphate/phosphate translocator; OPP, oxidative pentose phosphate; pINAD-MDH, plastidial NAD-dependent malate dehydrogenase; Rib-5-P, ribose 5-phosphate.

biosyntheses across membranes. Besides, transport of C5/C6 compounds (2-oxoglutarate, isocitrate) and C2 compounds (acetate, glycolate) is possible due to less well-known shuttle systems together with the corresponding dehydrogenases on both sides (Table 1).

The focus of this review is on the multiple engagements of cytosolic oxidoreductases such as GAPDH as part of the strong interplay between redox metabolism (anabolic and catabolic reactions of photosynthesis, assimilation, glycolysis, respiration, and OPP pathway), redox sensing (redox regulation, rerouting of metabolism), and redox signaling (nuclear transcription). These multilevel controls are the basis for a stable, but also dynamic, and most sensitive and specific regulatory network. Such properties to sense and to signal multiple combinations of events lead to the maintenance of homeostasis and an appropriate outcome in each case. Examples of further enzymes of central metabolism such as enolase (ENO), MDH, isocitrate dehydrogenase (IDH), fumarase, and others that are, however, less studied will be mentioned as potential candidates for an even more complex and finely responding system.

Redox Imbalances and Redox Homeostasis Determine the Lifetime of Cells

Any imbalance between energy generation and consumption would lead to the production of excess reactive oxygen,

nitrogen, or sulfur species (ROS, RNS, RSS), and the subsequent modulation of enzyme activities. Furthermore, cellular components such as membranes, DNA, RNA, or proteins will be irreversibly damaged and necrotic cell death will be the result. Such a situation is often described as “oxidative stress” resulting from any detrimental impact caused by both, abiotic and biotic factors (168).

For the sake of the analysis of an organism’s stress resistance or tolerance, the development of necrosis, membrane leakage, and oxidation products of proteins and lipids as markers of oxidative stress damage is monitored. In most cases, such stress treatment is experimentally applied and a sudden increase in light, salt, or any other negative impact is given at a defined time point. Most likely, however, the damaged or necrotic tissue that appears after the applied stress treatment is the result of an impact the plant is not prepared for because such unphysiological shock rarely occurs in nature. In such a system, the redox buffers are rapidly exhausted, and the induction of the next level of defense is too slow resulting in irreversible damage and necrosis.

In contrast, under more physiological conditions, stepwise challenges will be managed, thus inducing priming and the increase of antioxidant activities after a first impact. To tolerate naturally occurring variations of the abiotic parameters, plants are well equipped with an ample inventory of antioxidants, and poisoning mechanisms such as the malate valve, and

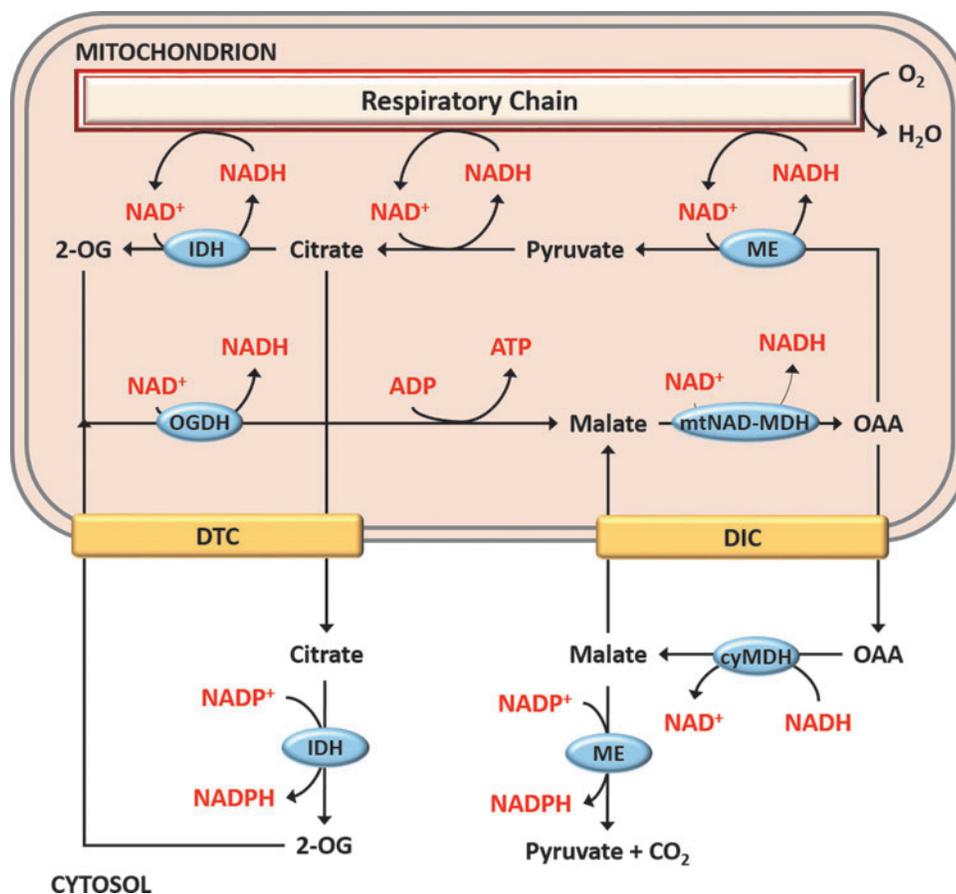


FIG. 3. Network of dehydrogenases and translocators for the exchange of energy between mitochondria and the cytosol. The exchange of energy is mediated by the malate and citrate valves. For the sake of clarity, further mitochondrial dehydrogenases were omitted in the scheme. For a detailed overview of plant mitochondria, see Schertl and Braun (158). 2-OG, 2-oxoglutarate; cyMDH, cytosolic NAD-dependent malate dehydrogenase; DIC, dicarboxylate carrier; DTC, mitochondrial dicarboxylate/tricarboxylate carrier; IDH, isocitrate dehydrogenase; mtNAD-MDH, mitochondrial NAD-dependent malate dehydrogenase; OGDH, 2-oxoglutarate dehydrogenase.

oxidative stress will rarely occur. Excessive ROS are immediately removed by the scavenging systems consisting of glutathione (reduced glutathione [GSH]) and ascorbate that are continuously regenerated when oxidized and serve as efficient redox buffers (54). In certain cases, however, even cell death is induced as a controlled response such as programmed cell death (PCD) in mammals that is mechanistically different in plants and poorly characterized (149). Such a response can be beneficial for the whole organism and serves to avoid continuing ROS formation. In other cases, developmental steps such as early flowering in plants are induced by a changed redox balance. Reducing conditions are a prerequisite for cell cycle progression and growth, while aging and senescence including PCD, as in the hypersensitive response, are governed by an increasingly more oxidizing cellular environment (37, 138).

Although ROS can be detrimental, adaptation to stress relies on a certain ROS level, for example, in striated muscle (1). In fact, a certain basal level of ROS appears to be required to allow for signaling and the appropriate responses resulting from changed gene expression (122). Whenever the actual enzymatic equipment is not sufficient to cope with the imposed changes, for instance upon sustained stress, ROS initiate a signal transduction chain ending in a defined positive or negative effect on transcriptional activities in the nucleus (110). Increased antioxidant capacities, and protection by rerouting energy metabolism, are then induced. Oxidative stress can also induce senescence (94, 95). In plants under stressful conditions, stress-induced early senescence is fre-

quently leading to an early seedset, allowing the next generation to possibly encounter more favorable conditions and to be more successful.

Reductive stress can also be detrimental, since it either leads to ROS formation or, under low oxygen, will be inhibitory for glycolysis and facilitating the induction of fermentation with products that are toxic in multicellular organisms (166, 200, 201). In mammals, increased glycolysis and/or OPP pathway activity, known as the Warburg effect, are associated with cell proliferation and cancer (204). For successful decisions under each given situation, the redox environment needs to be integrated with all other incoming information in a complex network, as is suggested in systems biology approaches in the new field of Quantitative Redox Biology (17). As a chance for future medical applications, the Reactive Species Interactome emerges now as a biological concept that places basic redox processes into the central role as an integrator of metabolism and signaling that is most important for human health (29, 153). Originating from the work with plants that are obviously exposed to a large extent of redox stress due to their sessile way of life and photoautotrophy, such a unifying concept emerges now as an essential basis also for understanding the physiology of health and disease of mammals.

Thiol Switches Regulate Enzyme Activities, Localizations, and Moonlighting Functions

The cellular redox state is of central importance for functional metabolism and efficient regulation during normal

TABLE 1. OXIDOREDUCTASES IN PLANTS, HUMANS, AND YEAST INVOLVED IN THE INDIRECT ENERGY SHUTTLING SYSTEMS BETWEEN ORGANELLES AND THE CYTOSOL

Compartment	Enzyme	Isoform	Arabidopsis thaliana	Homo sapiens	Saccharomyces cerevisiae	
Chloroplast/ Plastid	Glucose-6-phosphate dehydrogenase	G6PDH1	AT5G35790 (EC 1.1.1.49)	n.e.	n.e.	
		G6PDH2	AT5G13110 (EC 1.1.1.49)			
G6PDH3		AT1G24280 (EC 1.1.1.49)				
G6PDH4		AT1G09420 (EC 1.1.1.49)				
Cytosol	Glyceraldehyde 3-phosphate dehydrogenase	GapA1	AT3G26650 (EC 1.2.1.13)	n.e.	n.e.	
		GapA2	AT1G12900 (EC 1.2.1.13)			
		GapB	AT1G42970 (EC 1.2.1.13)			
		GapCp1	AT1G79530 (EC 1.2.1.12)			
		GapCp2	AT1G16300 (EC 1.2.1.12)			
		Malate dehydrogenase	NADP-MDH	AT5G58330 (EC 1.1.1.82)	n.e.	n.e.
Cytosol	Malic enzyme	p1NAD-MDH	AT3G47520 (EC 1.1.1.37)			
		NADP-ME4	AT1G79750 (EC 1.1.1.40)	n.e.	n.e.	
	Glucose-6-phosphate dehydrogenase	G6PDH5	AT3G27300 (EC 1.1.1.49)	HGNC:4057 (EC 1.1.1.49)	YNL241C (EC 1.1.1.49)	
		G6PDH6	AT5G40760 (EC 1.1.1.49)			
	Glyceraldehyde 3-phosphate dehydrogenase	GapC1	AT3G04120 (EC 1.2.1.12)	HGNC:4141 (EC 1.2.1.12)	YJL052W (EC 1.2.1.12)	
		GapC2	AT1G13440 (EC 1.2.1.12)	HGNC:24864 (EC 1.2.1.12)	YJR009C (EC 1.2.1.12) YGR192C (EC 1.2.1.12)	
	Isocitrate dehydrogenase	GapN	AT2G24270 (EC 1.2.1.9)	n.e.	n.e.	
		NADP-IDH	AT1G65930 (EC 1.1.1.42)	HGNC:5382 (EC 1.1.1.42) HGNC:5383 (EC 1.1.1.42)	YLR174W (EC 1.1.1.42)	
	Malate dehydrogenase	cyNAD-MDH1	AT1G04410 (EC 1.1.1.37)	HGNC:6970 (EC 1.1.1.37)	YOL126C (EC 1.1.1.37)	
		cyNAD-MDH2	AT5G43330 (EC 1.1.1.37)			
		cyNAD-MDH3	AT5G56720 (EC 1.1.1.37)	HGNC:17836 (EC 1.1.1.37)		
	Malic enzyme	NADP-ME1	AT2G19900 (EC 1.1.1.40)	HGNC:6983 (EC 1.1.1.40)	n.e.	
NADP-ME2		AT5G11670 (EC 1.1.1.40)				
NADP-ME3		AT5G25880 (EC 1.1.1.40)				
Lactate dehydrogenase	LDH1	AT4G17260 (EC 1.1.1.27)	HGNC:6535 (EC 1.1.1.27) HGNC:6541 (EC 1.1.1.27) HGNC:6544 (EC 1.1.1.27)	n.e.		
Mitochondrion	Isocitrate dehydrogenase	NAD-IDH1	AT4G35260 (EC 1.1.1.41)	HGNC:5384 (EC 1.1.1.41)	YNL037C (EC 1.1.1.41)	
		NAD-IDH2	AT2G17130 (EC 1.1.1.41)			
		NAD-IDH3	AT4G35650 (EC 1.1.1.41)	HGNC:5385 (EC 1.1.1.41)	YOR136W (EC 1.1.1.41)	
		NAD-IDH4	AT1G32480 (EC 1.1.1.41)			
		NAD-IDH5	AT5G03290 (EC 1.1.1.41)	HGNC:5386 (EC 1.1.1.41)	YDL066W (EC 1.1.1.42)	
		NAD-IDH6	AT3G09810 (EC 1.1.1.41)			
	Malate dehydrogenase	mtNAD-MDH1	AT1G53240 (EC 1.1.1.37)	HGNC:6971 (EC 1.1.1.37)	YKL085W (EC 1.1.1.37)	
		mtNAD-MDH2	AT3G15020 (EC 1.1.1.37)			
	Malic enzyme	NAD-ME1	AT2G13560 (EC 1.1.1.39)	HGNC:6984 (EC 1.1.1.39)	YKL029C (EC 1.1.1.38)	
		NAD-ME2	AT4G00570 (EC 1.1.1.39)	HGNC:6985 (EC 1.1.1.40)		
	Lactate dehydrogenase	D-LDH	AT5G06580 (EC 1.1.2.4)	HGNC:19708 (EC 1.1.2.4)	YDL174C (EC 1.1.2.4)	
	2-Oxoglutarate dehydrogenase complex	OGDH-E1	OGDH-E1	AT3G55410 (EC 1.2.4.2)	HGNC:8124 (EC 1.2.4.2)	YIL125W (EC 1.2.4.2)
			OGDH-E2	AT4G26910 (EC 1.2.4.2)		YDR148C (EC 1.2.4.2)
			OGDH-E2	AT5G55070 (EC 1.2.4.2)		
			OGDH-E3	AT3G17240 (EC 1.2.4.2)		YFR049W (EC 1.2.4.2)
			OGDH-E3	AT1G48030 (EC 1.2.4.2)		
			OGDH-E3	AT3G13930 (EC 1.2.4.2)		

The numbers of isoforms and the used cofactors vary between organisms. Also, mammalian mitochondria possess two different malic enzymes that differ in their coenzyme usage [either NAD-(HGNC:6984) or NADP-dependent (HGNC:6985)]. Note that plants are unique in containing plastids (green and nongreen, see Figs. 1 and 2) that do not exist in human and yeast.

G6PDH, glucose 6-phosphate dehydrogenase; GapA/B, bispecific (NAD⁺/NADP⁺-dependent) glyceraldehyde 3-phosphate dehydrogenase; GapC, cytosolic glyceraldehyde 3-phosphate dehydrogenase; GapCp, plastidial NAD-dependent glyceraldehyde 3-phosphate dehydrogenase; IDH, isocitrate dehydrogenase; LDH, lactate dehydrogenase; ME, malic enzyme; mtNAD-MDH, mitochondrial NAD-dependent malate dehydrogenase; NADP-MDH, NADP-dependent malate dehydrogenase; n.e., not existent; OGDH, 2-oxoglutarate dehydrogenase; p1NAD-MDH, plastidial NAD-dependent malate dehydrogenase.

growth and development, as well as for protection from abiotic and biotic stresses (47, 168). Cysteine residues can undergo various redox-dependent modifications, thus altering the properties of the target proteins as their activity and binding properties are concerned (30). As a well-analyzed example, various chloroplast enzymes undergo redox modifications, primarily by reversibly forming disulfide bridges upon oxidation, thereby changing their structure locally and also allosterically (65). Oxidation leads to the inactivation of Calvin–Benson cycle enzymes and activation of G6PDH of the plastidial OPP pathway thus avoiding futile cycling. The coordination of metabolism during daily light and dark phases is controlled by this way in all green tissues (16, 41, 72, 121).

However, there is increasing evidence that cytosolic enzymes in plants and all other organisms are also subject to many different reversible and irreversible redox modifications at cysteine residues. Cysteines are readily oxidized to form disulfide bridges with other protein thiols, but also with the small thiol GSH or nitrosoglutathione (GSNO) leading to S-glutathionylation, with nitric oxide (NO) leading to S-nitrosylation, with H₂S to S-sulfhydration, or with ROS leading to sulfenic, sulfinic, or sulfonic acid residues. The local environment of a cysteine residue determines its reactivity by various mechanisms, that is, by thermodynamic and by kinetic aspects governing formation and accessibility of the thiolate anion for the nucleophilic attack (84, 140, 210). Thioredoxins (Trxs) with the low pK_a values of their regulatory cysteine residues can mediate redox changes by dithiol/disulfide exchange with reduced ferredoxin plus ferredoxin-thioredoxin reductase or NADPH plus NADP-dependent thioredoxin reductase (NTR) and oxidants such as ROS or O₂. In contrast to Trx, glutaredoxins possess a less negative redox potential and are involved in many thiol modifications as are the peroxiredoxins. Plants are particularly rich in their cysteine redoxome, that is, the sum of all redox active, small thiol-containing proteins, a high number of them belonging to the ubiquitously occurring main groups of small monothiol or dithiol redoxins (107, 119, 178, 203, 212).

Glycolysis, and in particular GAPDH, is central to energy metabolism and is, at the same time, involved in stress responses due to its properties as a redox sensor and its redox-dependent translocation into the nucleus (44). Under conditions of oxidative/nitrosative stress, GAPDH is among the first cytosolic proteins to be modified at its catalytic cysteines by reversible S-nitrosylation and S-sulfenation (Fig. 4), as other oxidative modifications, S-glutathionylation, S-sulfhydration, and oxidation of the sulfur to sulfenic, sulfinic, and sulfonic acid, were found to occur at the catalytic cysteines (3, 12, 76, 135, 213). However, these data have not yet been integrated into the database. The increase of nuclear localization of GAPDH in isolated *Arabidopsis* protoplasts when exposed to oxidants was demonstrated (3, 160). Furthermore, cadmium-treated *Arabidopsis* roots develop oxidative stress leading to oxidation and nuclear translocation of GAPDH (188). Other glycolytic enzymes, such as triosephosphate isomerase and aldolase, are also subject to reversible S-glutathionylation leading to their inactivation (43, 186).

Small Molecules Fine-Tune the Redox Switches

Both assimilation and dissimilation of carbon compounds coupled to redox reactions are processes comprising electron

transport chains. Upon changes at the input side or the acceptor/consumption end, rapid adjustment of the fluxes must prevent over-reduction of the redox carriers and subsequent formation of excess ROS that would affect cellular integrity. At various regulatory points of the metabolic network, post-translational redox modifications are responding by changing actual enzyme activities instantaneously as exemplified by light/dark-modulated chloroplast enzymes that possess thiol switches operated as dithiol/disulfide exchange reactions between Trx and a large number of target enzymes.

Continuous cycling between oxidized and reduced forms allows for adjustment of activities fine-tuned by metabolites since products and substrates of the respective reactions act also as effectors of reduction and reoxidation, respectively (93). The cost for fine-tuning is covered by some additional flow of photosynthetically energized electrons in a “futile cycle,” but in chloroplasts in the light, electrons for the all reductive processes are rarely limiting. Plant cytosolic GAPDH isoforms GapC1 and 2 are reversibly oxidized and inactivated by treatment with GSNO or with GSH in the presence of H₂O₂, and the substrate glyceraldehyde 3-phosphate protects GAPDH from inactivation (76).

Small molecules such as metabolites, coenzymes, GSH, and also phytohormones and Ca²⁺, can be seen as indicators for the actual situation inside the cell and in its environment and, in some cases, are key players. They are, therefore, the ideal molecules to reflect the current cellular activities, any deviation from, or the need for developmental changes and adaptive responses. These small molecules serve as (redox and metabolic) sensors when noncovalently binding to specific targets or modifying these targets covalently. Such an association or modification event can initiate a signal transduction chain that leads to an adjustment by changed gene expression. Binding of a phytohormone to its membrane-bound or soluble receptor is a well-known example (132) and redox effects in these interactions are widely distributed (193).

Sugar sensing reflects the nutritional status and metabolic pathways, as well as development, and sugar signaling is performed accordingly by integrating multiple additional signals for the execution of the correct response in each case. Redox status of the cell and the availability of nutrients are of prime importance to determine the actual cell fate. The impressive diversity of sugar signaling events and its connections with other cellular indicators have been reviewed recently for plants (105), and also for mammalian cells (191). In general, common principles are playing crucial roles in all organisms. For example, hexokinase, the target of rapamycin, and also glycolytic enzymes as well as redox active components are ubiquitously distributed and are involved in signaling and adaptation.

As light is of particular importance in plants, specific hubs, linking light and the sugar status of the cell, involve the plastidial NADP-dependent Trx reductase system that can transfer reducing power to redox-modified targets such as ADP-glucose pyrophosphorylase to activate starch synthesis by using NADPH resulting from the OPP pathway activity in nongreen plastids (120). Regulation and signal integration are achieved here by trehalose 6-phosphate indicating the sugar status and fine-tuning the redox-dependent activation state of ADP-glucose pyrophosphorylase at the post-translational level. Trehalose 6-phosphate is also acting as a

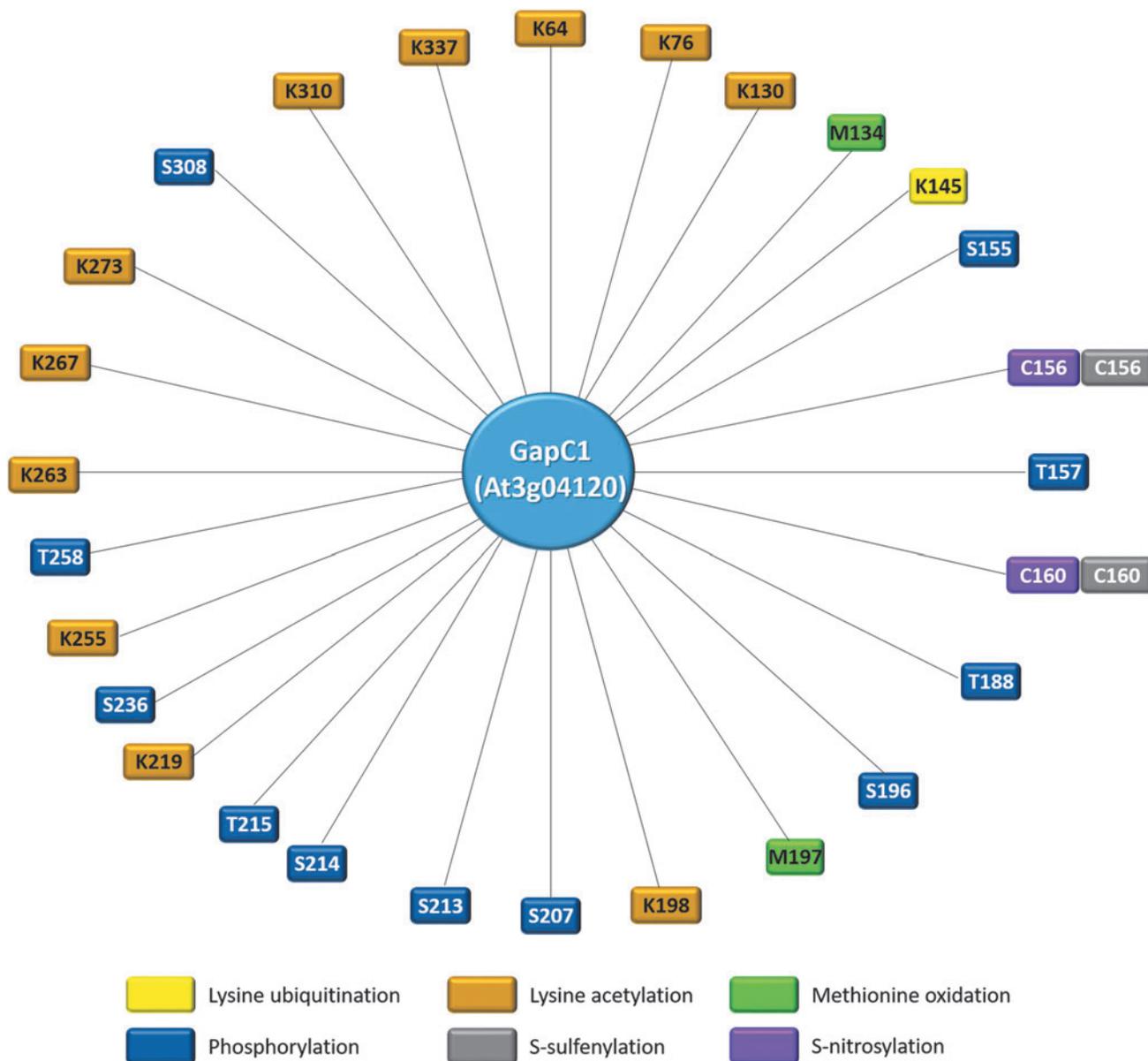


FIG. 4. Overview of identified PTMs of cytosolic GAPDH (GapC1) from *Arabidopsis thaliana*. PTMs that have been identified for GapC1 from *Arabidopsis* have been mapped using the bioinformatic tool PTMViewer (196). Eight different PTM types occur at 43 PTM sites (amino acids) as indicated. The high number of possible modification types and sites allows for the generation of GapC proteins with a myriad of possible shapes, properties, and localizations depending on the specific PTM code for each situation that requires specific functions of GapC. C156 and C160 are the cysteine residues present in the active site. Not all possible oxidative modifications of cysteines have been taken into the present database yet. PTM, post-translational modification.

central regulator to coordinate phytohormone responses with development, nutrient availability, and stress conditions at the transcriptional level (48, 134, 151, 198).

Inactivation of Cytosolic GAPDH Causes Rerouting of Metabolism

Upon rapid changes, for example, when oxidative stress is posed upon a system, a fast switch is required to accomplish protection from excess ROS and to provide NADPH as suitable energy equivalents. Carbohydrate oxidation in aerobic glycolysis as the common source for energy in the form

of ATP is then switched to an increased OPP pathway activity for the generation of NADPH. Since any transcriptional mechanism would be too slow, a rapidly responding switch that can alter metabolism and carbon fluxes to generate energy is required (148). A simple solution has been proposed recently by Dick and Ralser (38), namely the rapid shutdown of glycolysis due to the high sensitivity of cytosolic GAPDH for H₂O₂ inactivation (135). The subsequent increase of the metabolite pools before this step, that is, of glucose-6 phosphate (G6P), provides a high substrate concentration for G6PDH and the subsequent OPP activity (99). Such a mechanism is likely to also occur in yeast where a sudden

response toward oxidative stress has been described originally (148). When, finally, NADPH is not required anymore, NADPH as a competitive inhibitor of G6PDH acts directly in a short feedback loop to prevent exhaustion of the carbohydrate pools and to maintain redox homeostasis.

In mammalian cells, during normal metabolism and growth, cytosolic G6PDH provides NADPH as reducing equivalents for ROS defense and precursors for nucleic acid biosynthesis. For phases of rapid cell growth during development and also during tumor growth, glucose is not metabolized *via* glycolysis, the tricarboxylic acid (TCA) cycle, and oxidative phosphorylation for maximal energy conversion and ATP production. Instead, the upper glycolysis (“aerobic glycolysis”) from G6P is diverted to the OPP pathway to provide building blocks for the synthesis of new organic material (increased biomass) and the reductant NADPH for fatty-acid biosynthesis (Fig. 2).

Central for tumor growth is the provision of energy that requires an increase of glycolytic activity or even a switch to fermentation because oxygen is less available in rapidly proliferating cancer cells. Additional ATP required for high growth rates is provided by increased rates of aerobic glycolysis (the Warburg effect, as previously highlighted) with increased GAPDH levels (192). The Warburg effect is achieved at the transcriptional level by p53 and TP53-induced glycolysis and apoptosis regulator (TIGAR), which directly and indirectly induce and suppress the GAPDH and G6PDH activity, respectively (177). The Warburg effect consists of the direction of glucose consumption to aerobic glycolysis by increasing GAPDH activity in cancer cells in the presence of oxygen (106, 142). At the same time, p53 suppresses the OPP pathway activity by inhibiting G6PDH by binding of cytosolic p53 (86).

The products of aerobic glycolysis in cancer cells, namely lactate as the product of LDH, and pyruvate, were shown to

regulate gene expression by inducing the synthesis of hypoxia-inducible factor 1 α (HIF-1 α), which then binds to DNA leading to the production of a series of proteins that promote cell proliferation in tumor tissue (111). Due to the involvement of the lactate monocarboxylate transporter (MCT1) (34), a likely candidate for cancer therapy is available. Also, the HIF-1 α pathway in general is suggested as a promising target for the development of cancer medication (116).

Interlinkage of optimized energy supply (glucose) and ROS defense (need for NADPH from the OPP pathway to fuel Trx- and GSH-dependent antioxidant systems) is particularly important in the retinal cells during visual perception (104). To prevent oxidative damage and macular degeneration, 100% rerouting of the glucose flux through the OPP pathway and the complete loss of glucose as CO₂ might be the best solution in this instance. Rerouting of energy-producing pathways is also required under low oxygen when cells initiate fermentative glucose conversion. Here, increased glycolysis serves for ATP production in submerged plants leading to activation of sucrose-nonfermenting-related protein kinase-1 (SnRK1) and the stress response to cope with hypoxia (26). Central for signal transmission and coordination of the specific set of responses is GAPDH, highlighted in many studies (75). However, there are multiple modifications and structural changes known to cause the relocation of the protein, and the list of moonlighting functions seems to be never-ending (Fig. 5). In response to sustained pressure when enzyme activities reach their limit, ROS, RNS, or RSS can serve as signals indicating pending danger. Besides, central energy metabolism is also connected to nutrient availability (light, glucose, N-, S-, and P-availability). Very sensitive cytosolic proteins, for example, of glycolysis such as GAPDH, also also other metabolic enzymes, are redox

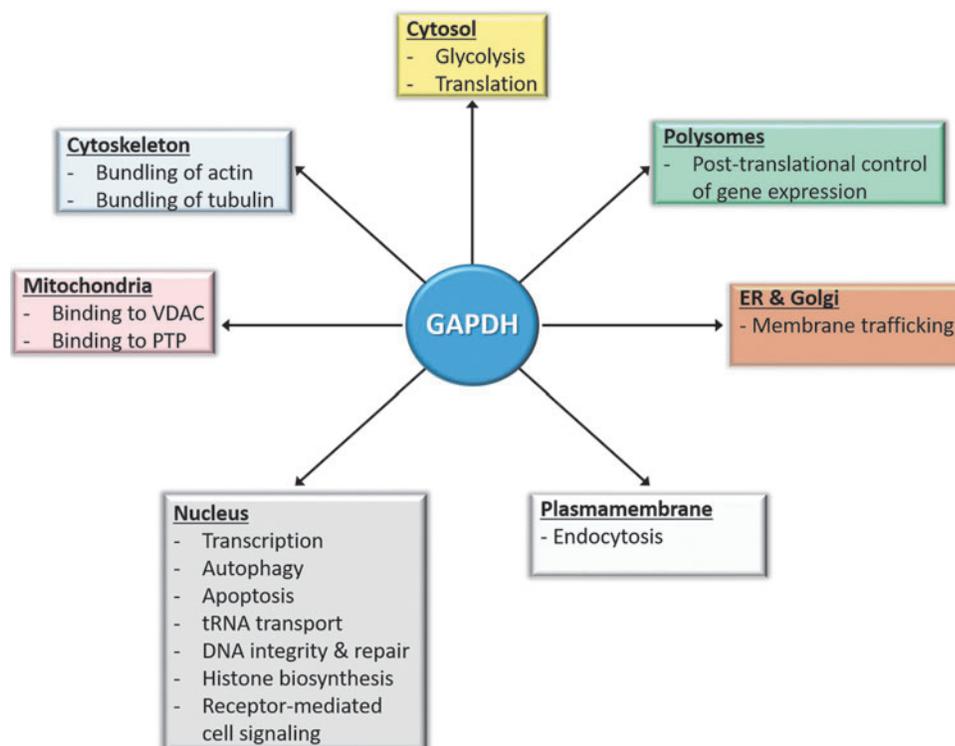


FIG. 5. Moonlighting functions of GAPDH in various cell compartments. GAPDH is a multifunctional protein with diverse activities and corresponding changes in subcellular localization. Besides its essential role in glycolysis, GAPDH has been shown to be directly involved in transcriptional and post-transcriptional gene regulation, translation, vesicular transport, receptor-mediated cell signaling, chromatin structure, bundling of actin and tubulin, the maintenance of DNA integrity, the cellular response to oxidative stress, apoptosis, autophagic gene regulation, and a variety of pathologies. GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PTP, permeability transition pore; VDAC, voltage-dependent anion channel.

modified and turned into the receptor for the signal transmitted by ROS, RNS, or RSS, that is, they sense the pending stress and transmit the signal by moving into the nucleus, and fulfill moonlighting functions (Fig. 5) (75).

Finally, due to its redox-sensitive cysteine residues in the active center, GAPDH tends to form abnormal intermolecular disulfide bonds leading to stress-induced aggregates that initiate cell death (125). Such protein aggregates were shown to be involved in the progression of Alzheimer's disease by binding to the amyloid precursor protein and amyloid β (32).

Taken together, inhibition of cytosolic GAPDH by oxidants and the subsequent rerouting of glucose to the OPP pathway for increased generation of NADPH are required for anabolism and antioxidant systems. However, at the same time, it induces a changed gene expression within a few minutes to rapidly respond to oxidative stress at the transcriptional level. This article focusses on cytosolic/nuclear effects and functions, often induced upon imbalances of the cellular redox state.

Cytosolic Enzymes Function As Redox Sensors and Take Over Moonlighting Functions

Moonlighting of metabolic enzymes after translocation into the nucleus or relocalization and binding to other cellular structures is a phenomenon described first for mammalian cells and yeast, but is known to occur in all eukaryotes (14, 15, 92, 167). In most studies, a single protein undergoing a specific modification was followed in its cellular location. However, depending on the experimental system, and the focus of the study, in each case, different modifications, as well as different cellular localizations (and likely functions), were described for the same protein. GAPDH, as a well-analyzed example, is involved in many regulatory processes (171) (Fig. 5). It is involved in cell death following oxidative stress and is recognized, therefore, as a central protein in many diseases (28, 185).

The occurrence of metabolic enzymes, such as GAPDH, in different cell compartments and their involvement in many different functions highlight them as sensors and integrators for many types and combinations of impact requiring dynamic adjustment of the cellular activities. This idea is presented in a recent overview summarizing cases of multiple protein localization and suggesting that the type of redox modification, whether one or more cysteine residues are S-nitrosylated, S-glutathionylated, or otherwise reversibly or irreversibly modified, determines the localization of the protein in the cell (53).

The complexity and variability of redox modifications are even more pronounced by the additional occurrence of other post-translational modifications (PTMs) such as lysine acetylation (49) and many others that have been identified so far and made available through the PTMViewer (Fig. 4) (196) or the functional analysis tool (FAT) for PTMs (31). Dependence of a certain PTM status, consisting of a certain combination of the different modifications, upon the type of stress and the specific developmental state in a spatiotemporal manner would allow for very specific responses that orchestrate cellular activity according to all incoming information (176).

Each modification of a specific amino acid influences the microenvironment and either facilitates or prevents further modifications of neighboring amino-acid residues. Such

cross talk between all potential sites for the different modifications gives rise to a unique PTM code by introduction or shielding of charged groups and by inducing conformational changes that influence accessibility or sensitivity (187). A cysteine redoxome, that is, all cysteine modifications qualitatively and quantitatively analyzed at multiple times in different cells and tissues, would allow for a better understanding of such complex relationships (73).

Binding of GAPDH to DNA, RNA, and Nucleotides Induces Epigenetic Effects

Epigenetic effects required for the adaptation of development and stress response to changing environmental conditions such as temperature, light, or other types of adverse growth conditions are induced either by DNA methylation/demethylation or by histone modifications, both epigenetic mechanisms changing the accessibility of nuclear genes for transcription (50, 174, 199). Lysine acetylation, as well as lysine succinylation, was found to occur side by side frequently on abundant proteins, but only in small portions of the total pools and in particular on metabolic enzymes (194). Apart from histone methylation and acetylation, also malonylation has been described to occur (202). As a product of aerobic glycolysis, lactate is the precursor for lactylation of histones at lysine residues, thereby providing an additional means for epigenetic regulation of metabolism by a metabolite acting on the chromatin structure (215). Since the activated sidechains for all these modifications must be provided as coenzyme A-bound building blocks, their availability is strictly linked to energy metabolism and subject to a complex regulatory network (173).

To comprehend the mechanisms involved in the complex regulatory network responsible for maintaining homeostasis while introducing new functions during development, first attempts have been made to identify lysine acetylation and succinylation in the proteome of *Brachypodium distachyon* leaves and many metabolic enzymes were found (219). Apart from histones, many other proteins, both in the cytosol and the nucleus, were identified to be subject to reversible lysine modifications, among these also components of the transcriptional complex (55). Sirtuins (SIRT in mammals, SIR in yeast, and SRT in plants) are NAD-dependent histone deacetylases (HDACs) occurring in all compartments where they regulate enzymes of energy metabolism by acting on their activity or stability (108). S-nitrosylated GAPDH is suggested to transnitrosylate nuclear proteins such as sirtuin-1, HDAC-2, and a DNA-activated protein kinase (96). By depending on NAD⁺, sirtuins are strictly coupled to the NAD⁺/NADH redox state and the energy status of the cell and can respond to any imbalances imposed upon the cell and requiring adjustment of energy metabolism (133, 181, 201). Sirtuins were also shown to regulate the antioxidant defense, with ROS affecting their activities (169). In another study, the relationship between cytosolic MDH providing NAD⁺ for sirtuin-1 activity and senescence in human fibroblasts was suggested (102).

Research concerning NAD⁺ metabolism and its role in the regulation of growth and development adds to the knowledge of the importance of central metabolism for gene expression (56). The role of NAD⁺ versus NADH binding in regulatory transcriptional complexes turns this small molecule into a sensor to adjust gene expression accordingly (51). Compartmentation of

NAD(P)(H) is of major importance, and their generation, their consumption, and their biosynthesis need to be strictly and individually controlled (100, 200). In each compartment, the pools of the energy carriers need to be balanced to function for energy transfer in metabolic processes and also for ROS defense. Any imbalance in these pools renders them to serve as versatile sensors for reductive or oxidative stress.

Since GAPDH binds NAD⁺, it can link the cytosolic redox state with the regulation of nuclear gene expression and serves as a sensor for the cellular redox state. GAPDH binds to Sir2 in yeast and regulates nuclear NAD⁺ levels and induces signaling (150). On the contrary, SIRT1 interaction with plant GAPDH was shown to prevent nuclear translocation of GAPDH under stressful conditions to regulate cell survival (90). Also, rice SRT1 prevents transcription of genes encoding glycolytic enzymes, since nuclear translocation and binding to their promoters are inhibited (216). GAPDH is also a component of the transcriptional activator complex OAC-S that is responsible for histone H2B transcription during the S-phase of the cell cycle (220). Here, the cellular redox state and DNA replication are coordinated by the transactivation potential of the p38 (GAPDH)-containing complex. Again, the NAD⁺/NADH ratio determines the binding of p38 to the H2B promoter.

Cytosolic Enzymes Take Over Multiple Roles in the Nucleus

Nuclear translocation and new moonlighting functions of GAPDH have been compiled in various reviews (Fig. 5). Its function as a DNA repair enzyme was found early when the human nuclear uracil DNA glycosylase gene was expressed and the sequence was identical to the 37-kDa subunit of GAPDH (118). Thereafter, GAPDH was identified as a DNA- and RNA-binding protein with many different functions in translation, transcription, and DNA repair (Fig. 5) (78). During oxidative stress, human GAPDH interacts with the endonuclease APE1, which is involved in DNA repair and reactivates the oxidized enzyme (4).

GAPDH is involved in multiple cellular reactions in yeast and mammals and, consequently, is localized not only in the cytosol and the nucleus but in various other positions in the cell depending on its many PTMs (Figs. 4 and 5) (75, 128, 170). In plants, post-translational redox modifications are similarly leading to multiple localizations and functions of cytosolic GAPDH (74, 211). Nuclear translocation of GAPDH under stress might be part of redox signaling and protection by its action in DNA repair, or induces cell death as in mammals and thereby prevents ongoing ROS formation in already damaged cells.

On the contrary, GAPDH acts as a proapoptotic protein supporting cell death (Fig. 5) (217). Apoptotic stimuli amplify NO formation that in turn initiates the so-called cell death cascade. NO formation leads to S-nitrosylation of GAPDH that abolishes its catalytic activity but confers the ability on GAPDH to bind to seven in absentia homologue 1 (Siah1), an E3-ubiquitin ligase that possesses a nuclear localization signal mediating the nuclear translocation of the GAPDH-Siah1 complex (68). In the nucleus, GAPDH stabilizes Siah1 facilitating its degradation activity of nuclear substrates, which leads to the induction of the cell death cascade. As in mammals, plant GAPDH interacts with an

Siah1-like E3-ubiquitin ligase, namely seven in absentia-like 7 (Sinal7), also responsible for its nuclear translocation (137). When Sinal7 is lacking in a knockout line, GAPDH does not appear in the nucleus, indicating that nuclear import of GAPDH is mediated indirectly by binding to Sinal7 as originally described for Siah1 in mammalian cells (69). Overexpression of Sinal7 appears to also improve drought resistance, prevents cell death, and delays senescence (130, 131, 136). Interestingly, in dividing *Arabidopsis* cells, the Sinal7 transcript level was found to be increased after ultraviolet-B irradiance (137).

Although the cell death cascade is different in plants and does not involve the known set of proteins as this is the case in mammals (149), similar effects resulting from nuclear translocation of oxidatively modified GAPDH have been described. Biotic stress, leading to accumulation of long-chain bases such as sphinganine or dihydrosphingosine and induction of oxidative/nitrosative stress, in tobacco BY-2 cells, is similar to what is known in mammals, namely that nuclear translocation of the oxidized GAPDH induces cell death (Fig. 5) (184).

In humans, binding of a mutated form of huntingtin (mHtt) to the GAPDH-Siah1 complex causes Huntington's disease, a genetic disorder leading to neuronal cell death where nuclear translocation of the cytotoxic mHtt with its extended polyglutamine sequence is mediated by forming a ternary complex with GAPDH-Siah1 (8). The neuroprotective drug deprenyl, on the contrary, prevents GAPDH from binding to Siah1, thus avoiding nuclear translocation and the cell death-inducing activity of the complex (69). In retinal Müller cells, high glucose levels lead to increased expression of Siah1, enhanced nuclear translocation of the GAPDH-Siah1 complex, and accelerated cell death thus causing eye disease in diabetic rodents (206).

Salicylic acid (SA) is a phytohormone, and GAPDH was identified among others as an SA-binding protein. In subsequent screens, using human GAPDH, SA, a metabolite of Aspirin[®] (acetyl-SA), as well as some other compounds known to possess beneficial functions for health such as glycyrrhizin, was found to bind GAPDH and to prevent its nuclear translocation and its negative effect on the cellular lifetime (27).

GAPDH as a nucleotide-binding protein was found to also bind to polynucleotides such as DNA and RNA (Fig. 5). It specifically can bind to modified bases, for example, where adducts with alkylating drugs are formed (154). It has been shown that oxidized GAPDH is prone to bind to apurinic/apyrimidinic sites being the most frequently damaged regions of the DNA (98). GAPDH was found to bind to AU-rich RNA elements (ARE's), sequentially interacting with its dimer and tetramer interface (195). Several mRNAs and also tRNA recruit GAPDH and other ARE-binding transacting factors, resulting in structural changes and altered stability of the RNA, depending on the external impact. The association of GAPDH in its oxidized form to an RNA-splicing complex is one more such role in the nucleus (80). It also interacts with the telomerase-RNA component leading to inhibition of telomerase activity, and finally cell senescence. Under oxidizing conditions, this determines the progress of aging and the life span of the cells (128, 179). Nuclear GAPDH in brain cells, when oxidized in stroke patients, interacts with the acetyltransferase p300/CREB-binding protein (CBP) leading to transacetylation of GAPDH and subsequent activation of

p300/CBP and p53 thus causing cell death (163). The poly(ADP-ribose) polymerase-1 also interacts with nuclear GAPDH under oxidative/nitrosative stress conditions leading to a cell death cascade and finally brain damage (126).

The tumor suppressor p53 is central as a transcription factor determining the type of energy generated in cells. In general, ATP is provided by glycolysis and subsequent aerobic respiration, while NADPH for biosynthesis is generated by the OPP pathway activity. Both pathways are subject to transcriptional activities of p53 by controlling the expression of the next set of transcription factors that are essential for the expression of the required genes in each case. Problems with the ATP supply for growth in *Arabidopsis* can be sensed in a system of signal transduction and involves phosphorylation of suppressor of gamma response 1 (SOG1) by SnRK1, although details of this novel pathway and the actual sensor for low ATP are not yet known (66). In plants, SOG1 takes over a similar function as does p53 in mammals, although there is no sequence similarity (207), and there are also differences in the composition of the DNA damage response and the actual cell death cascade (208).

In view of the findings that metabolic enzymes act as transcriptional activators, it is of interest to find various cases where nuclear interactions of p53/SOG1 are not only with GAPDH (20, 21, 214) but also with other oxidoreductases of central metabolism such as G6PDH, and MDH (103, 204). These metabolic enzymes serve as direct sensors of the metabolic state and immediately can induce cellular responses at the transcriptional level after nuclear translocation as shown for various examples. These moonlighting functions in the nucleus depend on the conditions (oxidative stress, nutrient availability, hypoxia, *etc.*). Cell proliferation, cell cycle arrest, DNA damage repair, or cell death can be the result.

Direct interaction of GAPDH after translocation to the nucleus upon S-nitrosylation, binding to Siah1, and its association with p53/SOG1 would be a link to subsequent transcriptional changes. GAPDH binding to CBP and p53 activates the cell death pathway (163). GAPDH binding to p53 was suggested to be responsible for cell toxicity, while the disruption of this interaction with an interfering peptide prevents cell death in neuronal cells when exposed to glutamate (214).

Glycolytic Enzymes Take Over Many Cellular Functions

Various cellular functions depend on interactions of cytosolic GAPDH with proteins of the cytoskeleton such as F-actin (197), and tubulin as a microtubule-associated protein affecting its bundling properties even by the formation of intermolecular thiol/disulfide bonds (Fig. 5) (101). Binding of GAPDH and aldolase to the mitochondrial voltage-dependent anion channel (VDAC) appears to be redox dependent in plants (160) and mammals, leading to cell death in an alternative pathway involving the mitochondrial permeability transition pore (182). GAPDH was found to bind to membranes and mediate membrane fusion events and endocytosis (Fig. 5) [for reviews see refs. (20, 21)]. Binding of GAPDH to the phospholipase PLD δ contributes to H₂O₂ signal transduction in plants leading to stomatal closure upon induction by abscisic acid (63). GAPDH interacts with the receptor protein kinase Feronia to control plant cell expansion (205). In mammals, it can function as a macrophage receptor at the cell surface (24).

Protein kinases in signaling cascades are also targets of GAPDH binding thus linking different incoming signaling cascades for a coordinated output (124). Although GAPDH functions and interactions are involved in neurodegenerative diseases (Alzheimer, Parkinson, Huntington), and various other pathologies, it is often not sufficiently clear, how the moonlighting functions can be explained at the molecular level [see ref. (28)]. Frequently, the effects depend on post-translational protein modifications (Fig. 4), cell type, cellular context, and the external conditions, as they can be either proapoptotic or antiapoptotic.

GAPDH binding to GOSPEL (GAPDH's competitor of Siah protein enhances life) in the cytosol, in particular when S-nitrosylated, prevents nuclear translocation and cell death thereby protecting neuronal cells (13, 164). Phosphorylation of GAPDH by the protein kinase Akt2 prevents its nuclear translocation and promotes cell survival, for example, of ovarian cancer cells (79).

Other Enzymes of Central Metabolism Can Also Take Over Moonlighting Functions

Evidence is increasing that demonstrates the moonlighting roles of other metabolic enzymes from central energy pathways, thus adding to a yet rather complex network of perception of imbalances and transmission of diverse signals for appropriate responses. MDH isoforms in the various compartments are essential for the removal of NADH in glycolysis when aerobic respiration is not possible due to lack of oxygen, to regenerate NAD⁺ in glycolysis, as are LDHs or alcohol dehydrogenase in fermenting cells. MDH supports glycolysis in proliferating cells (67). During Cd²⁺-stress in the yeast *Candida tropicalis*, both MDH and GAPDH are relocated to the nucleus and induce cell cycle arrest (91).

In senescence and aging, p53 not only controls the expression of G6PDH to provide NADPH but also the expression of MEs (ME1 and ME2) (85), again demonstrating connections between energy metabolism and cell survival. Nucleocytoplasmic localization of MDH is another example of moonlighting functions of a metabolic enzyme controlling energy metabolism. Interaction of cytosolic MDH with p53 during glucose starvation modulates the transcriptional response (113). Energy responses after subjecting HEK cells to metabolic stress depend on MDH1 that interrupts the interaction between p53 and mouse double minute 2 homologue (MDM2) also known as the E3 ubiquitin-protein ligase Mdm2. However, even in cells lacking p53, MDH1 was translocated to the nucleus, most likely due to additional yet unknown pathways for the transfer of metabolic signals to change nuclear gene expression (103).

Furthermore, the glycolytic enzyme ENO was found to be localized in different compartments, and its nonglycolytic functions are multiple, being involved in many diseases (39). Both in plants and mammals, a shorter transcript of ENO represents the c-Myc-binding protein (MBP-1) and acts as a transcriptional repressor determining the fate of cancer cells, and of plant growth and stress responses (109). Finally, as another example, aldolase and fructose 1,6-phosphatase are involved in various moonlighting functions and could serve as a target for potential anticancer drug development (61, 62).

As further signaling hubs, IDHs, both as NAD- and NADP-dependent isoforms, can operate as "citrate/2-oxoglutarate valves" and link redox processes between organelles and the

cytosol (Fig. 3) (81). The cytosolic NADP-IDH from *Arabidopsis* and the recombinant human equivalents (HGNC:5382 and HGNC:5383; Table 1) were shown to be reversibly modified by the oxidant GSNO (127). Several cysteine residues of NADP-IDH are S-nitrosylated and Cys363 is S-glutathionylated thus opening multiple options for changed binding and localization properties as previously shown in many reports for GAPDH.

Cells with mitochondrial dysfunction make use of a pathway, including cytosolic IDH and cytosolic MDH activity, to generate ATP required for cell migration and other energy-requiring processes by increased glycolysis, where efficient recycling of NAD⁺ in the cytosol in proliferating cancer cells and activated lymphocytes takes place (58, 67). It has been suggested that MDH might be part of the glycolytic metabolon, although such variable quinary structures are dependent on the microenvironment *in situ* and are difficult to substantiate in biochemical terms under defined conditions. NAD-MDH isoforms in mitochondria and plastids (Figs. 1–3), allowing for malate transport *via* malate shuttles, are involved in the communication between organelles and prevent ROS accumulation and PCD in *Arabidopsis* (218). Malate applied to HeLa cells leads to ROS formation and cell death only when the mitochondrial MDH2 gene is functional, suggesting the mitochondrial origin of ROS (218).

The involvement of organic acids such as malate and oxaloacetate in redox balancing and redox signaling has been demonstrated in many cases in photosynthetic tissues (81, 82). Citrate can be seen as an important signaling molecule affecting transcriptional regulation of the major isoform of the alternative oxidase (AOX1A) in plants (64). As an upcoming candidate, fumarate might play a similar role, as the cytosolic isoform of fumarase, FUM2, was shown to be essential for cold acclimation of *Arabidopsis*, and again, a redox dependence of such sensing function is likely to be realized, both at the level of enzyme activity and of gene expression (45).

An interesting finding is the fact that mitochondrial-targeting sequences not only direct proteins to mitochondria but some are also found in the nucleus (123). In cancer cell metabolism, the pyruvate kinase PKM2 appears to play multiple moonlighting roles, and, among other subcellular localizations, it is also transferred to the nucleus when post-translationally modified (2). Metabolic kinases, such as hexokinases, pyruvate kinase, and 3-phosphoglycerate kinase, appear to also take over moonlighting functions and open the field for more therapeutic targets in cancer research (112). These examples and others might indicate that retrograde signaling originating from energy/redox imbalances in the mitochondria involves similar moonlighting functions of mitochondrial enzymes of energy metabolism (pyruvate dehydrogenase, 2-oxoglutarate dehydrogenase, phosphoglycerate mutase, and others) (18, 158). In summary, metabolic enzymes not only from the cytosol but also from mitochondria are thought to take over moonlighting functions, for example, inhibiting cell proliferation (22, 83).

Cellular Compartmentation Is Even More Complex Due to Metabolons

Due to the compartmentation of the eukaryotic cell, as described above, balancing and coordination of the various energy-requiring and energy-consuming pathways are es-

sential, and GAPDH appears to be a suitable sensor for any imbalance, concerning both ATP and reductant. At the same time, glucose/TP is serving as the C3/C6-carbohydrate fuel. It is feasible to assume the C4- and C5-compounds (2-oxocarboxylic acids originating from carbohydrate oxidation in the TCA cycle), and the oxidoreductases involved in their interconversions in mitochondria, plastids, and the cytosol play similar roles, that is, they might be targets of PTMs and the subsequently resulting moonlighting functions (Table 1). Isoforms of MDH and ME, as well as of IDH, LDH, and others, might turn out to take over similar tasks, however, in different energetic situations (Figs. 1–3). Only a few reports are available but they point to a more complex, finely tuned network that is capable to sense energy imbalances and to respond in multiple ways as required in each specific case.

Specific scenarios depend on the actual state of the cell (development, priming), its resources (nutrients), its genetic disposition (genotype), as well as on the type of impact or the set of factors to be considered by the plant for a most effective outcome. The response of the plant to multiple stress factors has been coined as “stress-induced morphogenic response” (144). It is assumed that these responses are evolutionary conserved and can result in similar outcomes after the integration of different sets of environmental factors (143). The phytohormones ethylene and auxin often mediate the observed growth effects, but ROS and energy supply are central to essentially all of these responses. The nutrient resources available for the response will determine the outcome. The metabolic network that is responsible for balancing the energy and redox state needs to satisfy the general requirements, namely sensitivity and robustness (129). Costs spent on maintenance, growth, or defense will depend on the activities and capacities of the respective systems of central metabolism and the available resources. The functional diversity of the moonlighting proteins such as GAPDH is the prerequisite for controlled responses (171, 172).

Protein–protein interactions in large complexes are not yet completely understood, in particular when they are influenced by the noncovalent association of small molecules and subject to PTMs that depend on the actual cellular activities. In plants, it appears that reduced GAPDH might be part of the glycolytic metabolon attached to the mitochondria *via* VDAC, and the oxidized form is found more frequently in the nucleus (160, 197).

In general, multiple locations of proteins appear to be redox dependent in plants due to the frequently occurring changes resulting from environmental challenges that need to be integrated with the lifestyle and the actual ontogenic state (53). It is interesting to note that the response of an annual weed such as *Arabidopsis* to suddenly increased light intensity depends on the previously applied daylength. Long-day conditions that are causing the early switch from vegetative growth to the reproductive phase to produce seeds result in the induction of only stress defense genes, while a reinforcement of the malate valve by inducing expression of NADP-MDH for the adjustment of energy distribution only takes place when the plants are grown under short-day conditions and the leaves remain in their actively growing state (11). Similarly, the expression of antioxidant genes strongly depends on H₂O₂ and CO₂ levels, but differently in long-day- and short-day-grown *Arabidopsis* (146). Also, the site of ROS formation, whether originating from the chloroplasts

under light stress or being generated extracellularly upon respiratory burst upon pathogen infection, is considered for an integrated, but very specific response (10).

Activation of NADPH oxidases (NOXs) facilitates localized ROS production at the plasma membrane following activation of receptors by associated ligands (*i.e.*, growth factors or hormones). In mammals, such an ROS-dependent signaling mechanism is involved in angiogenesis by controlling vascular physiology as summarized in a recent review (145). Similarly, NOX isoforms play an important role in plant immunity (190).

The nuclear localization of various proteins coincides with the presence of redox systems for the respective thiol/disulfide exchange reactions thought to occur within the nucleus (36). Trx, glutaredoxins, and nucleoredoxins together with the

NTRs, as well as the small thiol GSH, are localized in the cytosol and nucleus (35, 114, 115, 159). The redoxins were found to bind to a large number of proteins in nuclear extracts, many of them with moonlighting functions (*e.g.*, GAPDH) involved in signaling and redox-dependent gene expression (36).

Eukaryotic cells possess several compartments either defined by membranes as borders, with translocators and indirect shuttle systems for the exchange of energy equivalents, or by metabolons of variable composition depending on the metabolic state. Redox imbalances are often locally restricted by specific origins of ROS formation outside or inside the cell at the plasma membrane as well as in hotspots in the cytosol or mitochondria (87), or the photosynthesizing chloroplast (40). This scenario is well suited for sensing any kind of information, for signal transduction, and an outcome that

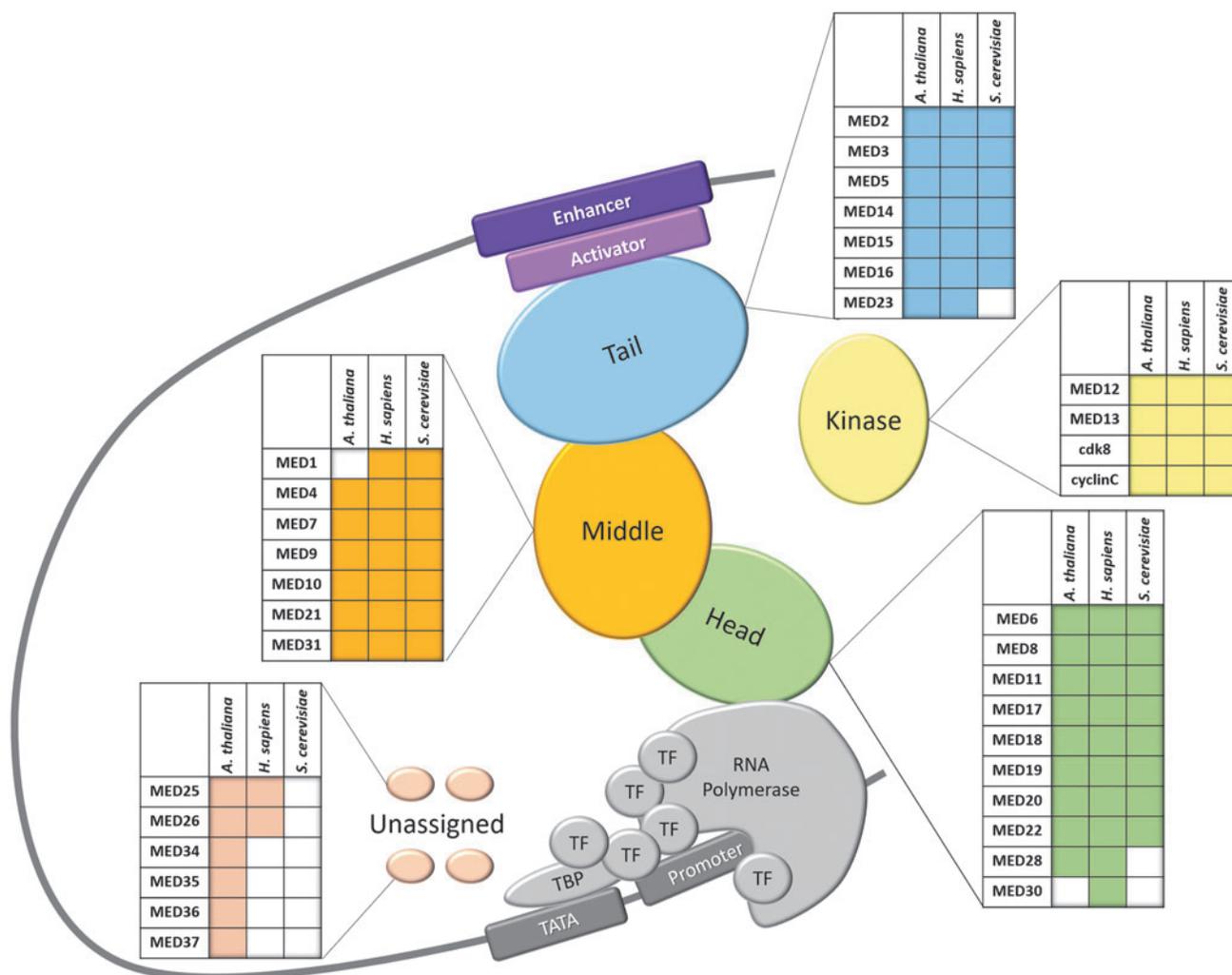


FIG. 6. Composition of the Mediator complex in plants, humans, and yeast. The Mediator complex stabilizes promoter/enhancer loops by physically bridging activators (transcription factors, TF) that are bound to the enhancer elements with the RNA polymerase transcription machinery and coordinates the initiation of transcription events. The Mediator core complex is composed of 26 subunits that are present in plants, humans, and yeast, respectively. The subunits are grouped according to their locations within the complex (head, middle, tail, kinase, and unassigned). Compared with yeast, the Mediator complex from *Arabidopsis* contains additional subunits (MED23, MED25, MED 26, MED28, MED34, MED35, MED36, and MED37), while the MED1 subunit is lacking. The Mediator complex in humans also comprises additional subunits (MED23, MED25, MED26, MED28, and MED30) when compared with the Mediator complex subunits of yeast. However, MED34, MED35, MED36, and MED37 represent plant-specific subunits that are absent in humans and yeast. TATA, TATA box; TBP, TATA-box binding protein.

allows for the best possible response to each set of impact factors (70, 139). GAPDH, aldolase, and other enzymes of glycolysis are often found as transiently forming dynamic microcompartments that depend on the cellular redox state, and induce various cellular responses (160, 197, 209). Mul-

iple localizations of proteins together with the small molecules and the different modifications are the basis for such dynamic and flexible networks at all levels of regulation, exhibiting also the required stability. Corresponding examples have been compiled in several reviews (59, 189).

TABLE 2. SUBUNITS OF THE MEDIATOR COMPLEX IN PLANTS, HUMANS, AND YEAST

Location	Subunit	Arabidopsis thaliana	Homo sapiens	Saccharomyces cerevisiae
Head	MED6	AT3G21350	HGNC:19970	YHR058C
	MED8	AT2G03070	HGNC:19971	YBR193C
	MED11	AT3G01435	HGNC:32687	YMR112C
	MED17	AT5G20170	HGNC:2375	YER022W
	MED18	AT2G22370	HGNC:25944	YGR104C
	MED19	AT5G12230 (MED19a)	HGNC:29600	YBL093C
		AT5G19480 (MED19b)		
	MED20	AT2G28230 (MED20a)	HGNC:16840	YHR041C
		AT4G09070 (MED20b)		
	MED22	AT1G16430 (MED22a)	HGNC:11477	YBR253W
		AT1G07950 (MED22b)		
	MED28	AT3G52860	HGNC:24628	n.e.
	MED30	AT5G63480	HGNC:23032	n.e.
	Middle	MED1	n.e.	HGNC:9234
MED4		AT5G02850	HGNC:17903	YOR174W
MED7		AT5G03220 (MED7a)	HGNC:2378	YOL135C
		AT5G03500 (MED7b)		
MED9		AT1G55080	HGNC:25487	YNR010W
MED10		At5G41910 (MED10a)	HGNC:28760	YPR168W
		AT1G26665 (MED10b)		
MED21		AT4G04780	HGNC:11473	YDR308C
MED31		AT5G19910	HGNC:24260	YGL127C
Tail		MED2	AT1G11760	HGNC:23074
	MED3	AT3G09180	HGNC:2377	YGL025C
	MED5	AT3G23590 (MED5a)	HGNC:22963	YGL151W
		AT2G48110 (MED5b)		
	MED14	AT3G04740	HGNC:2370	YLR071C
MED15	AT1G15780 (MED15a)	HGNC:14248	YOL051W	
	AT1G15770 (MED15b)			
Kinase	MED16	AT4G04920	HGNC:17556	YNL236W
	MED23	AT1G23230	HGNC:2372	n.e.
	MED12	AT4G00450	HGNC:11957	YCR081W
	MED13	AT1G55325	HGNC:22474	YDR443C
	cdk8	AT5G63610	HGNC:1779	YPL042C
	CyclinC	AT5G48640 (CYCC1-1)	HGNC:1581	YNL025C
		AT5T48630 (CYCC1-2)		
Unassigned	MED25	AT1G25540	HGNC:28845	n.e.
	MED26	AT3G10820 (MED26a)	HGNC:2376	n.e.
		AT5G05140 (MED26b)		
		AT5G09850 (MED26c)		
	MED34	AT1G31360	n.e.	n.e.
	MED35	AT1G44910 (MED35a)	n.e.	n.e.
		AT3G19670 (MED35b)		
		AT3G19840 (MED35c)		
	MED36	AT4G25630 (MED36a)	n.e.	n.e.
		AT5G52470 (MED36b)		
	MED37	AT5G28540 (MED37a)	n.e.	n.e.
		AT1G09080 (MED37b)		
		AT3G12580 (MED37c)		
		AT5G02490 (MED37d)		
		AT5G02500 (MED37e)		
	AT5G42020 (MED37f)			

The number of subunits and genes encoding subunits of the Mediator complex varies between organisms. Note that plants are unique in containing additional subunits (MED34, MED35, MED36, and MED37) that do not exist in human and yeast.

n.e., not existent.

Multiple Signals Must Be Integrated for Specific Responses

Redox imbalances can originate in the different compartments of the cell, depending on the initial environmental stress, or the subsequent metabolic dysregulation that leads to the generation of ROS. Each of the different situations requires specific responses for maintenance of homeostasis or for the execution of diverging cellular fates being repair, proliferation, growth arrest, acclimation, senescence, or cell death. In each case, redox signaling involving thiol modifications of the proteins in signal perception and transduction needs to be specific and unique (33, 42, 57, 88, 180, 183). The integration of all incoming information requires coordination by cellular integrators such as the enzymes of central metabolism. The amalgamation of the redox state and energy metabolism is achieved by the redox sensitivity of several enzymes of central metabolism (147).

Mammalian cells are exposed to potential stress when they encounter problems with O₂ supply, glucose feeding, or isotonic conditions, mostly upon pathogen infection, or deviation from proper development or hormone homeostasis, for example, due to suboptimal blood vessel formation or function. Plant cells are challenged by additional stress situations from changes in temperature or turgor. This is due to the fact that plants are not homoiothermic and differences in osmotic pressure are connected to essential functions such as turgor-dependent movements. Because of their photoautotrophic way of life, they are exposed to dramatic imbalances from changing light intensities as the energy carriers are concerned. All this additional information needs to be integrated and controlled by very fast *in situ* reactions and also through regulation of gene expression. Therefore, it is not surprising to find a Mediator complex in all eukaryotes associated with the transcriptional machinery in the nucleus regulating gene expression (97, 141).

Multiple subunits of the Mediator complex show specificity in relaying information from signals and transcription factors to the RNA polymerase machinery enabling the control of expression of specific genes. Furthermore, this multisubunit complex contains additional subunits that are unique in plants (152). When comparing the Mediator-subunit composition of yeast and mammals with that of plants (Fig. 6; Table 2), the higher number of subunits as analyzed for *Arabidopsis* and rice (117) can be explained by the need for additional functions under challenging conditions of energy availability and stress acting on a sessile organism. Subunits MED34, MED35, MED36, and MED37 cannot yet be fitted into the existing structural model of the Mediator complex, and only some of the more ubiquitous ones among the subunits MED1–33 could be connected with specific functions in plants (Fig. 6; Table 2) (9). The additional subunits of the plant Mediator complex, MED34–37, provide the potential for additional docking domains to bind factors required for proper signaling and efficient responses during development and stress specifically in plants.

Signals from disturbed redox homeostasis due to external impact such as high light are likely to depend on novel PTMs or new combinations of those on the signal integrators. GAPDH as a universal hub to signal energy and redox imbalances has been found in preliminary pull-down experiments with nuclear extracts from *Arabidopsis* to bind to

Mediator subunit MED36 as well as some subunits of MED37, among others, At5g02500 described as MED37e or as HSC70 in the databases (personal communication, Minhee Kang, 2014). The expression level of nucleo-cytosolically localized HSC70-1 was found to be responsible for various growth and stress tolerance effects (23). This heat-shock factor was also found among the *in vivo* S-nitrosylated proteins (46). Therefore, it is tempting to speculate that PTMs of the Mediator complex subunits can lead to the adjustment of gene expression activities according to the demand of the cell. The presence of highly conserved cysteine residues and their redox-dependent conformational changes found for some of the subunits that bind to a cryptochrome 1 response element underlines this option (165).

It is not yet possible to predict any outcome for a given set of stress factors since experimental setups are not comparable in the many different studies that have already been performed. If, however, more knowledge will be available to analyze the relationship between each impact or set of factors acting and the PTM patterns of each signaling component as well as on the respective targets, then a future goal of synthetic biology or directed pretreatments for chemical priming might provide means to generate tailored plants that are acclimated to the actual stress factors (155). An updated database listing moonlighting proteins such as MoonProt 2.0 (25), and the various possible modifications of these proteins compiled in the PTMViewer or FAT-PTM database (31, 196) will support these attempts, but the correlation of each impact, applied for different times and intensities, alone or in combination, to well-defined model systems with defined pretreatments, requires the detailed analysis of modifications at each of the amino-acid residues of a protein since a dose-dependent pattern can be expected and will determine the specific outcome (175).

Conclusion

Enzymes from the central energy metabolism, namely GAPDH as the best-analyzed example, and also others, appear to function as versatile and dynamic hubs for signal integration due to their spatial distribution over many subcellular locations and their functions in multiple cellular processes. They are subject to a large number of PTMs that influence their biochemical properties as well as their localization. Their affinity to a variety of protein complexes as well as nucleic acids indicates the potential of these metabolic enzymes to influence a wide range of biological processes and to serve as targets for early diagnosis and clinical intervention in human diseases such as cancer (77). They also are of importance when tailoring crop plants for resistance when grown under changing or unfavorable conditions or for the production of high-value compounds in plants (89).

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Abbreviations Used

2-OG = 2-oxoglutarate
3-PGA = 3-phosphoglycerate
ARE = AU-rich RNA element
CBP = p300/CREB-binding protein
cyMDH = cytosolic NAD-dependent malate dehydrogenase
DIC = dicarboxylate carrier
DTC = mitochondrial dicarboxylate-tricarboxylate carrier
ENO = enolase
FAT = functional analysis tool
G6P = glucose 6-phosphate

Abbreviations Used (Cont.)

G6PDH = glucose 6-phosphate dehydrogenase
 GAP = glyceraldehyde 3-phosphate
 GapA/B = bispecific (NAD⁺/NADP⁺-dependent)
 glyceraldehyde 3-phosphate
 dehydrogenase
 GapC = cytosolic glyceraldehyde 3-phosphate
 dehydrogenase
 GapCp = plastidial NAD-dependent glyceraldehyde
 3-phosphate dehydrogenase
 GAPDH = glyceraldehyde 3-phosphate
 dehydrogenase
 GapN = nonphosphorylating irreversible
 glyceraldehyde 3-phosphate
 dehydrogenase
 GPT = glucose-6-phosphate/phosphate
 translocator
 GSH = reduced glutathione
 GSNO = nitrosoglutathione
 HDAC = histone deacetylase
 HIF-1 α = hypoxia-inducible factor 1 α
 IDH = isocitrate dehydrogenase
 LDH = lactate dehydrogenase
 MDH = malate dehydrogenase
 MDM2 = mouse double minute 2 homologue
 ME = malic enzyme
 mHtt = mutated form of huntingtin
 mtNAD-MDH = mitochondrial NAD-dependent malate
 dehydrogenase
 NADP-MDH = NADP-dependent malate dehydrogenase

NO = nitric oxide
 NOX = NADPH oxidase
 NTR = NADP-dependent thioredoxin reductase
 OAA = oxaloacetate
 OGDH = 2-oxoglutarate dehydrogenase.
 OMT = 2-oxoglutarate/malate transporter
 OPP = oxidative pentose phosphate
 PCD = programmed cell death
 pNAD-MDH = plastidial NAD-dependent malate
 dehydrogenase
 PTM = post-translational modification
 Rib-5-P = ribose 5-phosphate
 RNS = reactive nitrogen species
 ROS = reactive oxygen species
 RSS = reactive sulfur species
 SA = salicylic acid
 Siah1 = seven in absentia homologue 1
 Sinal = seven in absentia-like 7
 SnRK1 = sucrose-nonfermenting-related protein
 kinase-1
 SOG1 = suppressor of gamma response 1
 TATA = TATA box
 TBP = TATA-box binding protein
 TCA = tricarboxylic acid
 TIGAR = TP53-induced glycolysis and apoptosis
 regulator
 TP = triose phosphate
 TPT = triose phosphate/phosphate translocator
 Trx = thioredoxin
 VDAC = voltage-dependent anion channel