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Viewpoint

# A cytosolic oxidation–reduction cycle in plant leaves

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**Leaf cytosol contains non-phosphorylating and phosphorylating glyceraldehyde-3-phosphate dehydrogenase (np-GAPDH and p-GAPDH, respectively). From the viewpoint of carbon metabolism, np-GAPDH is redundant. However, mutants lacking np-GAPDH show significant metabolic adjustments and decreased growth, suggesting that np-GAPDH has central functions in plant metabolism. Here, I propose a cytosolic oxidation–reduction cycle. In its forward direction, np-GAPDH supplies NADPH. In the reverse direction, phosphoglycerate kinase and p-GAPDH consume ATP and NADH. Thus, the cytosolic oxidation–reduction cycle may constitute a central hub in energy metabolism.**

In the light, NADPH is primarily synthesized in chloroplasts. However, NADPH is highly compartmentalized (Heber and Santarius, 1965). Chloroplastic $NAD^+$ carrier proteins in reconstituted systems show low affinities for this reductant (Palmieri *et al.*, 2009). Thus, NADPH export to the cytosol is not straightforward.

Currently, it is thought that glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and $NADP^+$-dependent isocitrate dehydrogenase supply cytosolic NADPH (Geigenberger and Fernie, 2014). Additionally, carbon cycling around non-phosphorylating (np)-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was suggested to provide cytosolic NADPH (Fig. 1; Kelly and Gibbs, 1973*a*, *b*; Scagliarini *et al.*, 1990). In this cycle, (i) dihydroxyacetone phosphate is exported from chloroplasts to the cytosol by the triose phosphate translocator and converted to glyceraldehyde 3-phosphate (GAP) by triose phosphate isomerase, (ii) GAP is oxidized to 3-phosphoglyceric acid (3PGA) and $NADP^+$ is reduced to NADPH by np-GAPDH, and (iii) 3PGA is reimported into chloroplasts by the triose phosphate translocator and reduced to dihydroxyacetone phosphate by chloroplastic phosphoglycerate kinase (PGK) and phosphorylating (p)-GAPDH. A similar cycle involving p-GAPDH and PGK in the forward direction was suggested to provide cytosolic NADH and ATP (Fig. 1; Stocking and Larson, 1969). However, Heber and Santarius (1970) questioned its occurrence *in vivo*.

Here, another route supplying cytosolic NADPH is proposed: the cytosolic oxidation–reduction (COR) cycle (Fig. 1). In its forward direction, cytosolic np-GAPDH oxidizes GAP to 3PGA and reduces $NADP^+$ to NADPH. In the reverse direction, cytosolic PGK and p-GAPDH reduce 3PGA to GAP, dephosphorylate ATP to ADP and $P_i$, and oxidize NADH to $NAD^+$. COR cycling was shown to be operational in reconstituted enzyme systems (Serrano *et al.*, 1993; Arutyunov and Muronetz, 2003). Here, the COR cycle is discussed as an actual metabolic route with benefits for plant functioning. Specifically, I address (i) how differences in biochemical properties of np-GAPDH and p-GAPDH may promote COR cycling, (ii) how COR cycle requirements may be met, and (iii) physiological benefits of the COR cycle. My aim is to raise awareness that COR cycling may occur *in vivo* and encourage assessments of its metabolic feasibility, e.g. by flux network modelling (cf. Shameer *et al.*, 2019) and the development of methodology enabling flux quantification such as stable isotope techniques.

## Biochemical properties of GAPDH enzymes promote COR cycling

The cytosol of plant leaves contains two distinct GAPDHs. While np-GAPDH catalyses the irreversible conversion of

Abbreviations: 3PGA, 3-phosphoglyceric acid; COR, cytosolic oxidation–reduction; GAP, glyceraldehyde 3-phosphate; np-GAPDH, non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase; p-GAPDH, phosphorylating glyceraldehyde-3-phosphate dehydrogenase; PGK, phosphoglycerate kinase; ROS, reactive oxygen species.

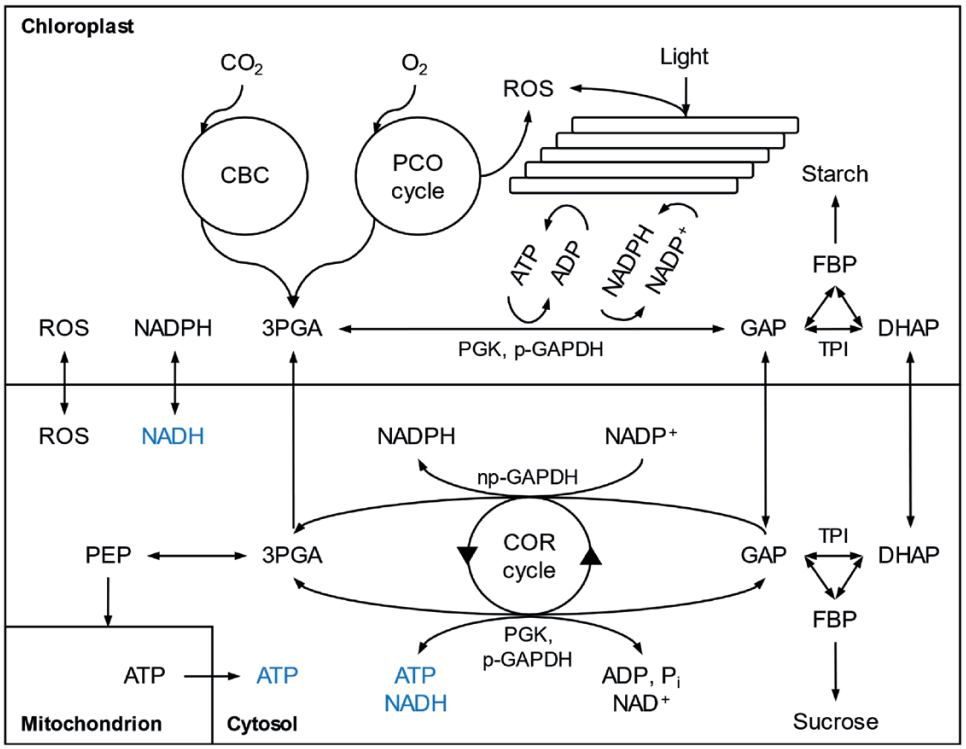


**Fig. 1.** Carbon and energy metabolism in plant leaves. Parts of the PCO cycle reside outside chloroplasts, in peroxisomes and mitochondria; notably, ROS synthesizing glycolate oxidase is peroxisomal. Abbreviations: 3PGA, 3-phosphoglyceric acid; ADP, adenosine diphosphate; ATP, adenosine triphosphate; CBC, Calvin–Benson cycle; COR cycle, cytosolic oxidation–reduction cycle; DHAP, dihydroxyacetone phosphate; FBP, fructose 1,6-bisphosphate; GAP, glyceraldehyde 3-phosphate; np-GAPDH, non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase; PCO, photosynthetic carbon oxidation; PEP, phosphoenolpyruvate; p-GAPDH, phosphorylating glyceraldehyde-3-phosphate dehydrogenase; PGK, phosphoglycerate kinase; $P_i$, inorganic phosphate; ROS, reactive oxygen species; TPI, triose phosphate isomerase.

GAP to 3PGA, p-GAPDH together with PGK catalyses the same reaction in a reversible manner (Fig. 1). Thus, from the viewpoint of carbon metabolism, np-GAPDH is redundant. However, np-GAPDH null mutants of Arabidopsis showed delayed growth compared with wild type (Rius *et al.*, 2006). Additionally, these mutants exhibited 3.5-fold higher transcript levels, 5.8-fold higher mRNA levels, and a 2.5-fold increased activity of p-GAPDH, indicating a compensation effect for the lack of np-GAPDH. Thus, np-GAPDH seems to be required for optimal plant functioning and growth (Rius *et al.*, 2006).

At the sequence level, np-GAPDH and p-GAPDH are entirely unrelated (Habenicht *et al.*, 1994; Michels *et al.*, 1994). I argue that a comparison of differences in enzyme properties may point to the physiological necessity for np-GAPDH.

### *GAPDH energetics, substrate affinity, and activity*

Conversion of GAP to 3PGA by np-GAPDH is energetically favourable over conversion by p-GAPDH and PGK ($\Delta G^{0\prime}$=−22.1 versus −13.3 kcal $mol^{-1}$, respectively). Additionally, np-GAPDH from various sources has an ~10-fold higher affinity for GAP than p-GAPDH ($K_m$ of np-GAPDH between 17 and 40 μM, $K_m$ of p-GAPDH between 239 and 400 μM; Rosenberg and Arnon, 1955; Kelly and Gibbs, 1973*b*; Duggleby and Dennis, 1974; Speranza and Gozzer, 1978; Iglesias and Losada, 1988; Scagliarini *et al.*, 1990). Furthermore, np-GAPDH reportedly exceeded the activity of p-GAPDH in a cell-free extract from *Pisum sativum* shoots at *in vivo* levels of GAP and reductants (Kelly and Gibbs, 1973*b*). Since np-GAPDH exhibits a higher affinity for GAP, Flügge and Heldt (1984) hypothesized cytosolic NADPH synthesis by np-GAPDH may be prioritized over NADH and ATP synthesis by p-GAPDH and PGK. I argue that growth delays and substantial biochemical compensation effects in np-GAPDH null mutants (Rius *et al.*, 2006) suggest a significant contribution of np-GAPDH to catalysing GAP to 3PGA conversions.

Reported concentrations of GAP and 3PGA in suspension-cultured cells of *Catharanthus roseus* correspond to equilibrium conditions around p-GAPDH and PGK (Kubota and Ashihara, 1990) even though np-GAPDH works against this equilibrium. Thus, p-GAPDH and PGK may readjust their disturbed equilibrium by more frequently catalysing the reverse reaction (3PGA to GAP) than the forward reaction (GAP to 3PGA), which results in COR cycling.

### *Activation of np-GAPDH by reactive oxygen species*

In natural settings, the cellular redox balance is regularly disturbed, e.g. by low chloroplastic $CO_2$ concentrations

($C_c$) due to drought. Low $C_c$ promotes an energy excess in chloroplasts due to decreased consumption of ATP and NADPH by the Calvin–Benson cycle but continued electron input by light-harvesting complexes (Wilhelm and Selmar, 2011). The resulting lack of electron acceptors promotes the generation of reactive oxygen species (ROS) especially superoxide and $H_2O_2$. Additionally, low $C_c$ promotes photorespiration and the generation of the photorespiratory side product $H_2O_2$. Under low $C_c$, photorespiration was estimated to generate >70% of all $H_2O_2$ (Noctor *et al.*, 2002). Thus, low $C_c$ promotes oxidative stress leading to increased oxidation of cytosolic NADPH by antioxidant systems. *In vivo*, the NADPH concentration proposedly exerts primary control over np-GAPDH activity with decreasing concentrations activating np-GAPDH (Kelly and Gibbs, 1973*b*; Iglesias and Losada, 1988; Scagliarini *et al.*, 1990). Thus, decreasing NADPH due to oxidative stress at low $C_c$ causes np-GAPDH activation and promotes the GAP to 3PGA forward reaction of COR cycling.

### *Inhibition of p-GAPDH by reactive oxygen species*

np-GAPDH is 63 times less susceptible to inhibition by ROS than p-GAPDH, with $H_2O_2$ being a particularly potent inhibitor of p-GAPDH (Piattoni *et al.*, 2013). Rising $H_2O_2$ levels are believed to progressively inhibit the reversible p-GAPDH (Hancock *et al.*, 2005; Bedhomme *et al.*, 2012; Piattoni *et al.*, 2013). This is corroborated by reported increases of glycolytic downstream metabolites including 3PGA under oxidative conditions (Baxter *et al.*, 2007; Lehmann et al., 2009, 2012; Rabara *et al.*, 2017). At PGK and p-GAPDH, increased 3PGA levels would promote the reverse reaction (3PGA to GAP). Thus, the COR cycling flux mode may be promoted under oxidative conditions.

Interestingly, inactive p-GAPDH functions as transcription factor triggering the induction of genes encoding antioxidant enzymes. For more information on GAPDH regulation including moonlighting functions see Scheibe *et al.* (2019) and references therein.

## Requirements of the COR cycle

### *Reverse reactions require ATP and NADH*

COR cycling reverse reactions (3PGA to GAP) catalysed by PGK and p-GAPDH require cytosolic ATP and NADH (Fig. 1). This requirement may increase with oxidative stress, e.g. due to low $C_c$ (see above). Much of the cytosolic ATP is believed to derive from the mitochondrial oxidation of photorespiratory glycine (Shameer *et al.*, 2019). Low $C_c$ promotes photorespiratory glycine oxidation. Moreover, low $C_c$ is often associated with excess amounts of NADPH in chloroplasts (see above). Excess NADPH in chloroplasts is shuttled out to the cytosol by the malate valve as NADH. Flux through the valve is regulated strictly by the activity of chloroplastic malate dehydrogenase, which increases with [NADPH]/[$NADP^+$] ratios (Fridlyand *et al.*, 1998). Under normal conditions, flux is low, yet kinetic modelling predicts much enhanced rates at high [NADPH]/[$NADP^+$] ratios, e.g. due to low $C_c$ under drought (Fridlyand *et al.*, 1998). Drought was shown to cause significantly increased activity of chloroplastic malate dehydrogenase in *Triticum aestivum* particularly under high light and moderate ambient $CO_2$ levels of 350 ppm (Biehler *et al.*, 1996). Thus, export of ATP and NADH to the cytosol is generally feasible. Mechanisms increasing ATP and NADH supply coincide with increased NADPH demands from COR cycling for ROS scavenging.

### *Retaining activity of p-GAPDH under oxidative conditions*

Reactive oxygen species inhibit p-GAPDH (see above). If all p-GAPDH were in its oxidized inactive state, COR cycling would halt. Hence, cycling requires retained p-GAPDH activity. Bedhomme *et al.* (2012) proposed cytosolic glutaredoxin and thioredoxin-based mechanisms reversing oxidative deactivation of p-GAPDH. Thus, part of the p-GAPDH population is likely always active.

## Benefits of COR cycling for plant functioning

COR cycling involves only three cytosolic enzymes and is carbon neutral. It requires no net carbon input, causes no net carbon loss, and does not produce carbon products that need to be consumed by other processes. Hence, COR cycling is independent of other parts of carbon metabolism as well as transmembrane transport of triose or pentose phosphates. This provides flexibility to its functions.

*In vivo*, NADPH concentration is believed to exert primary control over np-GAPDH activity (see above). Thus, COR cycling may help to maintain NADPH concentrations at high levels to steadily support all NADPH-consuming processes in the cytosol.

Optimal plant functioning requires a well-balanced energy supply versus consumption. Under most conditions, NADPH and ATP supply exceed metabolic demands, and several processes dissipating excess energy have been proposed (Wilhelm and Selmar, 2011). Export of reductant and ATP to the cytosol can remove excess energy from chloroplasts and mitochondria, respectively. COR cycling can dissipate this energy because each turn produces one molecule NADPH but consumes one molecule of ATP and NADH. Thus, COR cycling may counteract the generation of ROS. Additionally, NADPH from COR cycling may fuel cytosolic antioxidant systems and thus support ROS scavenging.

**Keywords:** Cytosolic oxidation–reduction cycle, energy metabolism, energy status, futile carbon cycling, glyceraldehyde-3-phosphate dehydrogenase, NADPH, oxidative stress, primary carbon metabolism, reactive oxygen species, redox status.

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