Biochimica et Biophysica Acta 1857 (2016) 1715–1725

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Review

Metabolite transport and associated sugar signalling systems underpinning source/sink interactions

Cara A. Griffiths [a], Matthew J. Paul [a], Christine H. Foyer [b,*]

[a] Plant Biology and Crop Science, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK
[b] Centre for Plant Sciences, School of Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK

ARTICLE INFO

Article history:
Received 11 January 2016
Received in revised form 6 June 2016
Accepted 23 July 2016
Available online 31 July 2016

Keywords:
Phloem loading
Redox regulation
Source-sink interactions
Sucrose transporters
Sugar signalling
Trehalose

ABSTRACT

Metabolite transport between organelles, cells and source and sink tissues not only enables pathway co-ordination but it also facilitates whole plant communication, particularly in the transmission of information concerning resource availability. Carbon assimilation is co-ordinated with nitrogen assimilation to ensure that the building blocks of biomass production, amino acids and carbon skeletons, are available at the required amounts and stoichiometry, with associated transport processes making certain that these essential resources are transported from their sites of synthesis to those of utilisation. Of the many possible posttranslational mechanisms that might participate in efficient co-ordination of metabolism and transport only reversible thiol-disulphide exchange mechanisms have been described in detail. Sucrose and trehalose metabolism are intertwined in the signalling hub that ensures appropriate resource allocation to drive growth and development under optimal and stress conditions, with trehalose-6-phosphate acting as an important signal for sucrose availability. The formidable suite of plant metabolite transporters provides enormous flexibility and adaptability in inter-pathway coordination and source-sink interactions. Focussing on the carbon metabolism network, we highlight the functions of different transporter families, and the important of thioredoxins in the metabolic dialogue between source and sink tissues. In addition, we address how these systems can be tailored for crop improvement.

1. Introduction

Plant metabolism is driven by the energy-transducing reactions of the chloroplasts and mitochondria, which use ATP, reducing power {NAD(P)H)} and associated metabolites as the major currency of energy exchange. Plant cells synthesize all of the metabolic building blocks for growth, biomass production and defence such as sugars and carbohydrate polymers, lipids, amino acids and secondary metabolites such as alkaloids and terpenoids. Metabolite fluxes through parallel pathways often occur simultaneously in differing cellular compartments [1]. Moreover, the metabolic requirements of different developmental and defence processes change dynamically with time and according to prevailing environmental conditions, requiring overlapping layers of short and long-term regulation. Dynamic regulation of photosynthetic and respiratory metabolism involving extensive metabolite exchange provides tight but flexible delivery of the correct building blocks in appropriate amounts at the right time and place. While our understanding of the co-ordination of the pathways of primary carbon and nitrogen assimilation has greatly increased in recent decades, relatively little is known about regulation of the essential transport systems between compartments, cells and different plant organs [2–4]. Metabolites such as sugars and amino acids occupy central positions in the coordination of processes in different cellular compartments, facilitating multiple points of reciprocal control between pathways [5–7]. In addition, metabolites, such as sucrose and nitrate act as signals regulating gene expression to optimise pathway fluxes according to prevailing environmental conditions. In this review we discuss the importance of transporters in the metabolic coordination network within cells, between cells and between organs, with a particular focus on carbon metabolism and sugar transport and signalling, and the main pathways that interact with carbon metabolism to ensure appropriate provision of resources between source and sink organs.

2. Carbon/nitrogen interactions

The efficient operation of carbon metabolism leading to carbon gain intrinsically depends on the successful uptake of other nutrients particularly nitrogen and phosphorus. Increasing focus is being placed on this interdependence because of the uncertainties that persist regarding how plant yields will be influenced by climate change and the increasing availability of carbon dioxide in the atmosphere. On one hand, studies in FACE (Free-Air CO_2 Enrichment) systems have demonstrated that nitrogen use efficiency will increase as atmospheric carbon dioxide becomes more available [8]. Conversely, the concomitant inhibition of

* Corresponding author.
E-mail address: c.foyer@leeds.ac.uk (C.H. Foyer).

http://dx.doi.org/10.1016/j.bbabio.2016.07.007

photorespiration will have an adverse effect on primary nitrogen assimilation because of limitations on redox cycling [9]. In addition, for future agriculture to be sustainable it is crucial that further yield gains are achieved with current and preferably low levels of soil fertilization [10]. Nitrogen use efficiency (NUE) is a highly complex trait involving N uptake efficiency (NUpE) and N assimilation efficiency (NUtE). NUpE is influenced by root architecture and the activities of large families of NO_3^- and NH_4^+ transporters [10]. Several families of nitrate transporters (NRT1, NRT2 and CLC) mediate the uptake and transport of nitrate in plants [10]. In general, NRT2 transporters have a high affinity for nitrate, while most of the NRT1 family have a low affinity for nitrate. Perhaps the best characterised example is NRT1.1, which is a dual affinity transporter. It is switched from low- to high-affinity transport forms by phosphorylation of Thr101 [10]. The post-translational modification of NRT1.1 enhances the affinity of the protein for nitrate, while high nitrate also acts as a transcriptional suppressor of NRT1.1expression. NRT1.1 is an important nitrate-sensing component that regulates lateral root development [11] by facilitating auxin transport [12]. This transporter also participates in the control of the expression of genes such as the high-affinity nitrate transporter, NRT2.1, whose expression is also regulated by nitrate availability [13–15].

Nitrate reduction in the cytosol is catalysed by NADH-dependent nitrate reductases (NR). While the activity of this enzyme is regulated in response to environmental and metabolic triggers as well as protein phosphorylation, the rate of nitrate assimilation can be limited by the availability of NADH [2,3,16,17]. Metabolite transport between the chloroplasts and cytosol is important in boosting the cytosolic NADH pool, involving dicarboxylates transport and shuttle systems for malate and oxaloacetate. The 2-oxoglutarate/malate transporter, AtpOMT1 plays an important role in this process, functioning both as an oxaloacetate/malate transporter in the malate valve pathway and as a 2-oxoglutarate/malate transporter mediating the transfer of carbon skeletons [18,19].

Nitrite generated by the action of NR is transported into the chloroplasts where it is reduced by nitrite reductase (NiR) to ammonium (NH_4^+), which is assimilated into amino acids by the glutamine synthetase/glutamine-2-oxoglutarate aminotransferase (GS-GOGAT) pathway. Thereafter, a raft of aminotransferases and other enzymes catalyse transfer of the amino group to form other amino acids. Provision of the 2-oxoglutarate required for ammonia assimilation requires partial operation of the TCA cycle in the mitochondria [3].

3. Pathway co-ordination of by reversible thiol-disulphide exchange

The extensive cycling of reducing equivalents facilitated by metabolite transport [9,20,21] also facilitates pathway co-ordinates through influences on post-transcriptional protein modifications (PTM) that provide dynamic and reversible protein processing to modulate enzyme activity, binding properties and function. Many types of PTM (over 450) have been identified to date. Many proteins involved in carbon and nitrogen metabolism are subject to PTMs such as protein acetylation, succinylation, malonylation, butyrylation, and propionylation. For example, the large subunit of ribulose-1, 5-bisphosphate carboxylase (RuBisCO) is extensively succinylated and acetylated. Deacetylation of the RuBisCO protein has been shown to activate the enzyme [22]. However, in most cases the functional significance of these PTMs has yet to be resolved. In contrast, the functions of protein phosphorylation and thiol-disulphide exchange processing (Fig. 1) have been extensively characterised. For example, the light and thiol-dependent activation of photosynthetic CO_2 fixation pathway enzymes requires thioredoxins (TRX), which are small proteins with disulphide reductase activities [23]. TRX reductases in the stroma reduce TRXs using either reduced ferredoxin or NADPH produced by the photosynthetic electron transport chain [24]. Conversely, TCA cycle enzymes such as succinate dehydrogenase and fumarase are reductively inactivated by TRX [25–27]. In this way, the TRX systems in the plastids, mitochondria and cytosol link photosynthetic and respiratory electron transport activities to functional changes in enzyme activities [23,28].

The photosynthetic electron transport chain provides the reductive "push" that keeps the stromal TRXs reduced in the light, ensuring that the activation states of the thiol modulated enzymes involved in CO_2 fixation are matched to rate of production of reduced ferredoxin and NADPH [24,29–31]. When this push is removed in the dark, the TRXs revert to their oxidized forms, which in turn allow oxidative inactivation of the thiol-modulated enzymes. In addition, chloroplast TRXs also regulate malate and oxaloacetate transport [32] and starch metabolism through effects on adenosine diphosphate (ADP)-glucose pyrophosphorylase (AGPase). The small subunit of this key enzyme of starch biosynthesis is regulated by redox-dependent dimerization in response to sugar availability [33–35].

Appropriate resource allocation between different plant organs might also be achieved by post-translational modification of sucrose transporters. Sucrose export from leaves occurs both in the light and dark to ensure the continuous and stable provision of adequate carbon resources to drive plant growth and development. The transport of sucrose is mediated by membrane-localised sucrose transporters, whose properties will be discussed later in detail. The activities of these transporters are regulated by changes in cellular redox status and by protein-protein interactions [36]. For example, SUT1 interacts with a small cysteine-rich cell wall protein in potato called SN1 [37]. The SN1 protein belongs to the Snakin/gibberellic acid stimulated (GAS) family in *Arabidopsis* [38,39]. Silencing of SN1 leads to perturbations in cellular redox metabolism, particularly antioxidant activities [39]. SUT1 is able to form dimers with a protein disulphide isomerase in a redox-dependent interaction and is hence able to interact with other proteins that are involved in metabolism or secretion [40]. However, no increases in the transport activity of StSUT1 have been shown to be dependent on this dimerization [41].

4. Transport of sugars between the chloroplasts and cytosol

Triose phosphate/phosphate, glucose, maltose transporters are important mediators of carbon transfer between the plastids and cytosol [7]. Carbon assimilated during photosynthesis is either transported out of the chloroplast or used for starch biosynthesis in the stroma. Carbon is exported from the chloroplasts in the light as triose phosphate or 3-phosphoglyceric acid. This occurs across the triose-phosphate/phosphate translocator (TPT) in strict stoichiometric exchange for inorganic phosphate (Pi). TPT belongs to the plastidial phosphate translocator family, which has three other members: the pentose phosphate/phosphate translocator (XPT), phosphoenolpyruvate (PEP)/phosphate translocator (PPT) and the glucose 6-phosphate/phosphate translocator (GPT).

TPT is relatively abundant in the chloroplast inner membrane, constituting 10–12% of the protein content [42]. Triose-phosphates transported by TPT are utilised for sucrose synthesis in the cytosol or for the generation of organic acids through the anaplerotic pathway. Inorganic phosphate produced during sucrose synthesis is transported back into the chloroplast via the TPT to be used in ATP synthesis [43–45]. Therefore, the re-cycling of phosphate maintains photosynthetic electron transport and the pentose phosphate pathways [46]. While transgenic potato plants that have reduced TPT levels or where TPT is knocked out completely show no phenotype, the chloroplasts have a reduced capacity to import phosphate by up to 30%, together with a 40–60% reduction in maximal photosynthesis [47]. The TPT is therefore central to the regulation of carbon partitioning between starch and sucrose [48,49]. Transgenic tobacco plants that over-express TPT did not show a marked phenotype or any marked effects on amino acid production [50]. However, they incorporated more CO_2 into sucrose and had higher leaf starch/sucrose ratios than the wildtype [50]. Transgenic *Arabidopsis* lines over-expressing TPT and also fructose-1, 6-bisphosphatase had higher photosynthetic carbon assimilation