Regulation of root respiration and sugar-mediated gene expression in plants

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The respiration rate of plant tissues is related to both substrate supply and demand for respiratory energy. At the biochemical level, it is considered to be regulated by the supply of adenosine diphosphate. In the whole plants, respiration rate is correlated with carbohydrate content suggesting control by substrates. There is evidence to support both the hypotheses, and they can be reconciled if the capacity of tissues for respiration can alter within a few hours and sucrose can control that capacity. Supplying exogenous sugars to the starved tissue not only increases the rate of respiration of that tissue but also leads to induction of enzymes which are involved in reversing the carbohydrate starvation-induced effects in plants. Expression of proteins and metabolic systems may be under the direct or indirect control of sugar. Sucrose or some product of its metabolism can control gene expression of these proteins, probably by *increasing transcription.*

THE chief source of carbon from a growing root is sucrose produced by photosynthesis in the shoot. Sucrose provides carbon skeletons for use in biosynthesis, and acts as a respiratory substrate. The respiration rate of plant tissues is related to both substrate supply and demand for respiratory energy[1]. Plant roots respire about 50% of the carbon imported from photosynthetic tissues to provide adenosine triphosphate (ATP) to drive energy-consuming processes like ion uptake, maintenance and turnover of existing and biosynthesis of new tissues[1,2]. The advantage of using roots for respiratory measurements is that roots provide a good system for the study of respiration without complications from photosynthesis or photorespiration. There are two hypotheses proposed for the regulation of respiration: ATP demand- and substrate supply-hypotheses.

Adenylate control

The evidence that respiration is under the control of adenylates covers from experiments in which an increase in demand for ATP results in an increased rate of respiration[3,4]. Shortly after salts are added to roots, the rate of respiration increases as a result of the increased use of ATP to drive ion uptake[5,6]. The use of uncouplers such as dinitrophenol (DNP), carbonylcyanide chloromethoxyphenyl hydrazone (CCCP), and carbonylcyanide *p*-trifluoromethoxyphenyl hydrazone (FCCP) demonstrates that respiration is under the control of adenylates[7,8]. The respiration rate of roots increases in pruned barley plants after supplying FCCP, which suggests that turnover of ATP controls respiration[7]. During carbohydrate starvation of sycamore cultures, supply of respiratory substrates to the mitochondria cannot increase the respiration rate of starved cells, instead ATP-utilizing pathways are the main limiting factor for respiration[9]. The O_2 uptake of glucose-starved excised maize root tips increases by the addition of 10 mmol m^{-3} DNP which suggests that respiration is controlled by ATP-utilizing processes[10]. That respiration in whole plants is limited by ADP recycling is also supported by increase in respiration associated with the induction of energy-consuming processes such as ammonia assimilation[11] and translocation[12].

Addition of uncouplers produces an increase in oxygen uptake *in vivo* as a result of the recycling of protons through the mitochondrial inner membrane without ATP synthesis. Adenylates exert their regulation either on electron transport via a constraint on oxidative phosphorylation or on glycolysis where ATP is required and regulates certain key enzymes[13]. The supply of adenylates controls the rate of oxygen uptake in maize roots by limitation of the flux of electrons through the respiratory chain[3]. However, in roots of *Phaseolus vulgaris*, adenylates control respiration via substrate supply to the mitochondria, probably by their effect on glycolysis[3]. In barley roots, adenylates limit the rate of O_2 uptake by restricting the supply of reducing equivalents to the cytochrome and alternative pathways[7].

Substrate supply

The rate of respiration is linearly related to relative growth rate in many growing tissues[2,14]. Respiration rate can also be correlated with carbohydrate content[15,16]. Limitation of root respiration by the substrate is dependent on the state of the shoot. Therefore, treatments which lower the supply of carbohydrate to the root, for instance, reduced illumination (shading) or selective pruning of the shoot, lead to a reduction in the rate of

respiration, and supplying sugars exogenously increased the rate of respiration[7,8,17–19].

Supplying sucrose does not generally increase the rate of respiration immediately. However, in a few instances exogenous sugar does increase respiration rapidly[9,16,20], which suggests that sucrose may control respiration directly. Similarly, supplying sucrose exogenously stimulates the respiration of the apical 40 mm of roots of *Pisum sativum*[21]. Short-term exposure of plants to sugar does not usually increase the respiration rate, specially in situations where tissue carbohydrate content is not depleted prior to exposure (personal observation, unpublished). Under such a condition of excess sugar supply, if uncouplers are added, the adenylate control may not operate (personal observation, unpublished).

Usually sucrose exerts its stimulatory effect over a longer period. The rate of respiration of excised maize root tips decreases as carbohydrate content of the root tips falls, and adding 200 mol m^{-3} glucose stimulates respiration such that the control rate of respiration is restored after 8 or 24 h of addition[20]. The rate of respiration of barley roots can be manipulated by altering the sucrose supply to the root; shoot pruning leads to a decrease in the carbohydrate content 24 h after shoot pruning[8]. Shoot pruning also leads to a decline in the protein content and the rate of root respiration, and supplying roots with 20 mol m^{-3} sucrose for 24 h reverses the effects[22]. Addition of 200 mol m^{-3} glucose to glucose-starved maize roots increases the rate of respiration by 50% (ref. 10). In a separate study, it was noted that sucrose stimulates the rate of root respiration when supplied for 8 h, indicating that changes in carbohydrate availability may be integrated over a moderately extended period: a longer period of incubation in sucrose solution (4–8 h), either prevented the decline or increased the rate of O_2 uptake of roots of both *Arabidopsis* and barley during prolonged darkness (personal data, unpublished). All these observations where sugar stimulates the root respiration over a longer period of incubation indicate a role for sugar in inducing gene expression[14]; this effect may be due to the induction of respiratory genes[23] by sugars, including those encoding mitochondrial components[24].

Changes in the capacity of the cytochrome pathway can also be brought about by sucrose. The reduction in respiration rate of barley roots which follows partial shoot removal is prevented by supplying 25 mol m^{-3} sucrose either immediately or after 24 h. The addition of sucrose results in no reduction or a rise of rates, respectively. In each case the change in the rate of respiration is attributable to the capacity of the cytochrome pathway[7]; this implies that source-induced changes in sink respiratory capacity are regulated by sucrose.

The content of carbohydrate in the cytosol gives the best correlation with respiration rate[1]. It is suggested that the substrate controls both growth and respiration rate, not directly by mass-action on pre-existing enzyme systems, but rather by regulating the amount of enzymatic machinery (coarse control). Sucrose possesses the attributes necessary to act as both a sensor of the internal and external environment, and as a signal molecule which communicates messages between sources and sinks and vice versa[25].

Substrate supply vs adenylate control

The two hypotheses for the regulation of respiration, namely that based on the correlation between carbohydrate content and respiration in tissues, and the assertion that the requirement for ATP drives respiration, are apparently contradictory. The contradiction can be resolved when the time scale of the experiments which provide the evidence for these hypotheses is considered. The evidence that respiration is ADP-limited comes from short-term experiments, and it has been suggested that this reflects fine control of respiration operating in part via demand for ATP (ref. 8). The correlations between respiration and carbohydrate content have often been made several days or more after treatment of the plants. This long-term effect may involve coarse control[8]. Thus, the two hypotheses for the control of respiration can be reconciled if the capacity of the tissues for respiration can alter within a few hours and sucrose can control that capacity.

Although coarse control of respiratory metabolism is regulated by carbohydrate, fine control is exercised by adenylates[8]. The respiratory electron transport pathways are located in the mitochondria, but control of glycolysis in the cytoplasm has to be matched with the rate of use of its products[26]. The control of glycolysis may not be as tight as that of the respiratory chain[3], but it is possible that sucrose regulates the coarse control of sucrose cleavage[22] as well as the respiratory chain[7]. Apart from the hypothesis for 'coarse control' as a possible mechanism for substrate-regulated respiration, another study with plants shows a correlation between the amount of net photosynthesis and the rate of respiration[27]. This, together with observations that increased light intensity and raised CO_2 partial pressures increase plant growth, can be interpreted in terms of increased substrate supply leading to increased respiration.

Troubleshooters during measurement of rate of respiration

During some measurements, a rapid but short-term increase in the rate of O_2 uptake (respiration) is generally observed for 2–5 min but thereafter the rate becomes stable, and a linear trace is obtained for 25–30 min, the period within which almost all the measurements are made. It is because of this reason that a maximum time

(ca. 5 min) should be given to allow the recovery of the rate from a short-term increase in the rate induced by handling of the tissue, if at all, and this part of the trace must be excluded for the determination of the rate. The problems may be of greater concern when dealing with root systems that are more difficult to seal into the vessel[28]. It is here that the handling of roots and the time period for which a sudden increase in the respiration rate as a result of injury during handling become critical; a false measurement of the rate of respiration may be made. The nonlinearity of the traces of O_2 depletion against time, during measurement of the rate of O_2 uptake, is commonly encountered with detached roots or even roots of intact plants. Therefore, the O_2 dependence of root respiration must be checked: the rates are measured for more than 30 min, incubated in a buffer to record a non-limiting concentration of oxygen (personal observation).

Carbohydrate-mediated gene expression in plants

The role of sugar in modulating gene expression is a well-established fact in higher plants (Table 1)[29–57]. A number of studies described either the induction or repression of various plant genes, such as those encoding nitrate reductase[53], ADPG pyrophosphorylase[58], or storage proteins from potato tuber[59] and soybean[45]. Repression of gene expression by sugar has been observed for several other plant genes such as photosynthetic genes[60,61], sucrose synthase *Sh1* gene[62], α-amylase gene[63] and glyoxylate cycle gene[64].

Supplying exogenous sugar to the starved tissue not only increases the rate of respiration of that tissue but also leads to induction of enzymes which are involved in reversing the carbohydrate starvation-induced effects in plants (Table 1). It is clear from the Table 1 that carbohydrate status in the tissue can regulate the synthesis of individual proteins in both mitochondrial and non-mitochondrial systems. Respiratory genes are affected to a varying degree by sugar[30]. Both nuclear and plastid–encoded genes can exhibit positive responses, with the latter being upregulated through mRNA abundance[65]. Mitochondrial ubiquitin mRNA, and mitotic index of the meristematic cells and the amount of *cdk*-like protein are also strongly affected by sugar (Collis *et al.*, unpublished), as are levels of cytochrome oxidase and activity of fumarase[29]. Some respiratory genes encoding enzymes related to cytoplasmic portion of respiration are also regulated by sugar[23]. Recently, induction of a gene encoding a component of the mitochondrial respiratory pathway by sugar has been demonstrated[24]. This was achieved by an increase in cytochrome *c* mRNA levels by the sugar after detached sunflower leaves were incubated in a solution of 200 mol m^{-3} glucose for 6 h. Further, it was demonstrated that the phosphorylation of hexoses by hexokinase was sufficient to promote the observed change in cytochrome *c* mRNA levels. An important aspect of sugar-modulated gene expression may be that of adjustment in the respiratory rate in response to sugar availability; studies indicate that this may occur over time in root tips of both barley[14] and maize[10]. The suggestion that carbohydrate levels regulate the activity or the amount of enzymes of the respiratory machinery of the root is well supported by other studies; sugar regulates the expression of other mitochondrial components: some mitochondrial enzymes show increased activity in response to increased glucose levels[29]. Similarly, carbohydrate starvation produces a marked decrease in respiration rates and cytochrome aa_3 content[9,66]. It appears that the level of sugar may regulate not only the synthesis of mitochondrial components, but also their degradation under certain conditions[67]. Thus, it is now quite apparent that respiration typically increases in response to increasing levels of sugar[14,68], and it decreases with starvation[10,57] – as do expression levels of several genes related to respiratory processes. Simultaneously increase in the levels of mRNAs and of the associated respiratory activity have been observed as the sugar content rises in maturing leaves of transgenic plants overexpressing invertase[69].

Sucrose has a key role for the supply of both carbon skeletons and energy making it ideally suited to act as a messenger (signal) between the source and the sink. The notion that sucrose can control metabolic systems/processes in higher plants by acting as a messenger is not new; it has been shown to control plant cell differentiation[70], and cell division tends to cease in root meristems with low availability of sucrose[71]. Induction of some enzymes is under the control of carbohydrates; any treatment which leads to sugar depletion, such as darkening of plants or culturing cells without sucrose causes reduction in the activity of the enzymes pertaining to carbohydrate- and respiration-related metabolism, due either to differences in the activity of the enzyme, or changes in enzyme synthesis. When barley roots are starved for 24 h, their rate of respiration, activities of cytochrome *c* oxidase and acid invertase, and the amount of cytochrome *c* oxidase subunit II all decline. However, these changes can be reversed after supplying sucrose for a further period of 24 h; the amount of some specific proteins increases or decreases[8,55]. Similarly, mRNAs encoding for sucrose synthase are sensitive to sugar concentration in *Vicia faba* and potato[72,73]. Prolonged darkening of barley plants leads to reduction in the contents of Rubisco and cytochrome *c* oxidase subunit II (personal data, unpublished). It appears that the response of plant cells to carbohydrate starvation is characterized by changes in the patterns of normal gene expression. Such alterations of gene expression result in the induction of the synthesis of pre-existing or new

Table 1. Examples of enzymes and metabolic systems directly or indirectly controlled by sugar abundance

Genes/enzymes (function) or systems	Evidence: plant, tissue	Effectors tested	References
AGPase [Sh 2] (Starch)	*Chenopodium* cell cultures + spinach		29
	Transgenic potato leaves		30
	Potato/detached leaves in dark		31
Starch phosphorylase	Potato/detached leaves in dark	S	31
Starch synth [GBSS]	Potato/detached leaves in dark	S,G,F	31
Branching enzyme [BE]	Cassava stem and leaves	S,G,F	32
Acid invertase	Sugarcane stem		33
	Maize root tips [Ivr2]	S,G,F	31, 34
	Chenopodium rubrum	S,G,F,6dG	35
Neutral invertase	Barley roots	S	22
Sucrose synthase	Maize [Sus1] roots	S,G,F	36, 37
	Eggplant		38
	Rice embryos	S,G,F	39
	Chenopodium cell cultures	S,G,F	40
Sucrose phosphate synthase	Transgenic potato tubers	Sol sugs	41
	Sugar beet petioles	G	42
Sucrose–sucrose fructosyl transferase	Ryegrass leaves	S	43
Phleinase (fructan exohydrolase)	Orchard grass		44
Lipoxygenase (storage proteins)	Soybean V_{sps}	S,G,F, MeJA	45
'Carbohydrate responsive proteins'	Pearl millet		46
Patatin class I	Transgenic potato	S	47
	Transgenic potato leaves and tubers	S, starch	48
	Potato tuber/transgenic tobacco	S	49
	Transgenic potato tuber	Sol sugs	41
Proteinase inhibitor II [Pin 2]	Transgenic potato tubers	Sol sugs	41
	Transgenic tobacco	S,G,F	50
Chaperonin 60*B* (protein synth)	*Arabidopsis* leaves	S	51
PP-F-6-P phosphotransferase	*Chenopodium* cell cultures, tobacco and spinach leaves	G	30
Nitrate reductase	*Arabidopsis* leaves light/dark	S,G,F	52
	Arabidopsis plants light/dark	G	53
rolC gene of Ri plasmid	Transgenic tobacco/phloem	S	54
Cytochrome *c* oxidase	Barley roots	S	55
Mitochondrial cytochrome pathway	Barley roots	S	7
	Sycamore cell cultures	S	56
Endoproteases	Maize roots	G	57
	Barley roots	S	P. Dwivedi, unpublished

6dG, 6-deoxy-glucose; F, fructose; G, glucose; MeJA, methyl jasmonate; PP-F-6-P phosphotransferase, pyrophosphate fructose-6-phosphate-phototransferase; S, sucrose.

proteins, repression of normally expressed proteins, and as a result major biochemical modifications are seen in response to starvation. Sugar can also modulate the changes that take place in chlorophyll and carotenoid patterns during fruit ripening and senescence as these processes are governed by carbohydrate-sensitive genes[74]. Sugar is a positive regulator of genes encoding storage proteins in potato (patatin)[75] and sweet potato through the appearance of mRNA[59], and sucrose synthase (*Sus1*) in maize[62]. Putative regulatory elements involved in sugar response, in the case of root storage protein genes of sweet potato, have been identified in the 5′ upstream region of these genes[76]. Identical sequences have been shown to be present in the promoter region of the petunia chalcone synthase gene, which is induced by elevated sugar levels in transgenic *Arabidopsis*[77]. The induction of starvation-related endopeptidases is controlled by the sugar level[57]. In another study, endoproteases are also regulated by the sugar level in the tissue (personal data, unpublished) as evinced from sugar-starved systems.

The possibility of higher plant genes responding to sugar levels was initially evident from the studies in a variety of tissues. These studies support the notion that sugar regulates the activities of enzymes involved in its own metabolism: exogenous sucrose leads to increase in the activities of acid invertase in root sections of bean[78] and stem segments of Avena[79], sucrose synthase in detached leaves of eggplant[38], and sucrose-sucrosyltransferase in detached leaves of *Lolium temulentum* and barley[80,81]. Increase in enzyme activity may be due either to induced synthesis or activation only. A reduction in the activity of some of the enzymes of glycolysis and the citric acid cycle is associated with sucrose deple-

tion[9]. There are several examples available demonstrating that the sugar-specific changes in enzyme activity are due to alterations in *de novo* synthesis. For example, the accumulation of new protein (sucrose-sucrosyltransferase) in leaves of *Lolium temulentum*[43], induction of the patatin gene in potato tubers[75] and transgenic tobacco leaves[49] by sucrose, the inhibition of acid invertase synthesis in sugarcane internodes by glucose[82], the induction of phleinase in *Dactylis glomerata* by glucose[44], and the repression of several photosynthetic gene promotors by sucrose or glucose in maize[83]. It is hypothesized that sucrose regulates the capacity of sink metabolism via coarse control. In a complex multicellular organism, sucrose is proposed to act as a signal enabling the sink metabolism to adjust to the rate of supply from source leaves while minimizing cellular investments in expensive metabolic machinery[14]. There are instances, however, when it is not always the carbohydrate metabolism which responds to sugar level. For example, nitrate reductase is synthesized partly as a result of glucose supply[84]. Genes that encode enzymes involved in nitrogen metabolism, including nitrate reductase, nitrite reductase and glutamate dehydrogenase are regulated by light, and the regulation can be mimicked by sugars[85]. Pea root meristems change the patterns of protein synthesis when deprived of sugar supply – a starvation 'stress protein' is reported[86]. Evidences from bacteria, yeast, animals and higher plants suggest that induction of protein synthesis is regulated by carbohydrate[23,87–90].

Sugar regulates the transcript levels of genes, for instance, genes encoding glyoxysomal malate synthase (MS) and isocitrate lyase (ICL) in higher plants. After four days of starvation of the culture medium of heterotrophic cucumber cells, both the MS and ICL transcripts increased. Upon addition of 20 mol m^{-3} sucrose to the culture medium, both the MS and ICL transcripts fell to undetectable levels after the next 48 h (ref. 64); it has been shown that induction of MS and ICL genes is dependent on the intracellular concentration of sugar falling below a critical threshold concentration. In maize root tips starved of carbohydrate, the mRNA levels of ribosomal protein genes encoding protein S28 and ubiquitin-fused protein S27a decrease rapidly and become undetectable after 24 h of starvation[91]. This effect of carbohydrate starvation on Ubi-S27a and S28 mRNA expression is reversed by supplying the root tips with glucose, sucrose and fructose. Transcripts of the *cdc2a* gene coding a regulator of the cell cycle are under the influence of sucrose as their abundance declines in cell suspension upon reduction in sucrose supply by one-tenth[92].

Thus studies made so far apparently suggest that sugar affects the expression of genes involved in several processes, such as photosynthesis, respiration, lipid metabolism, nitrogen metabolism, sucrose and starch metabolism. Both glucose and sucrose are important signalling molecules in plants. Genes which are regulated by sucrose are often also regulated by glucose and fructose[25]. Sucrose is converted to its hexose components, or more probably a close metabolic derivative, before it becomes the signal and controls gene expression[93]. Hexokinase may function in the signal transduction pathway of a sugar-sensing mechanism. The phosphorylation of hexoses by hexokinase and the sugar-sensing mechanism have been thoroughly studied in yeast[94,95]. Hexokinase could also be responsible for sensing sugars to repress a number of plant genes[64,96]. Whereas the sugar-sensing complex in prokaryotes (e.g. *Escherichia coli*) is the phosphotransferase system, the sugar-sensing system in plants comprises a complex formed by hexokinase and a hexose transporter to serve as the active form of the sugar sensor[94] – this, however, is just hypothetical and has not been proved yet. There are some evidences available to support hexose-independent changes in gene expression[97] and in proton-Suc symporter activity[98]. In a recent study, *WPK4*, a gene encoding a protein kinase in wheat was shown to be upregulated by cytokinin. It was also shown that sucrose negatively regulates this upward regulation mediated by cytokinin[99].

Recent studies have revealed that an interaction between carbon and nitrogen metabolism may play a crucial role in influencing gene expression of several enzymes, specially those involved in nitrogen metabolism and respiration[86]. Nitrate has been shown to act as a signal to initiate coordinated changes in the carbon and nitrogen metabolism, and that several genes affected by sugar are also governed by nitrate[100]. Amidst recent findings on the role of factor(s) acting as signals influencing gene expression in plants, carbohydrates are the strongest candidates that serve as the signal molecules and modulate gene expression.

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