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Respiratory metabolism: glycolysis, the TCA cycle and mitochondrial electron transport

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The respiratory pathways of glycolysis, the tricarboxylic acid (TCA) cycle and the mitochondrial electron transport chain are ubiquitous throughout nature. They are essential for both energy provision in heterotrophic cells and a wide range of other physiological functions. Although the series of enzymes and proteins that participate in these pathways have long been known, their regulation and control are much less well understood. Further complexity arises due to the extensive interaction among these pathways in particular, and also between cytosolic and mitochondrial metabolism in general. These interactions include those between mitochondrial function in the photosynthetic and photorespiratory processes, amino-acid biosynthesis and the regulation of cellular redox. Recently, a wide range of molecular and biochemical strategies have been adopted to elucidate the functional significance of these interactions.

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Abbreviations

***Aco*1**	*Aconitase*1
AOX	alternative oxidase
NDA	NADH dehydrogenase
ROS	reactive oxygen species
TCA	tricarboxylic acid
UCP	uncoupling protein

Introduction

Despite the fact that the major respiratory pathways were elucidated decades ago, relatively little is known about their regulation and control. Respiration can be divided into three main pathways: glycolysis, the mitochondrial tricarboxylic acid (TCA) cycle and mitochondrial electron transport. Plants exhibit several unique features of respiratory metabolism, including multiple entry points from sucrose and starch, the duplication of pyrophosphate and ATP-dependent phosphorylation of fructose 6-phosphate, complementation between the cytosol, plastid and mitochondria, the loss of regulation of glycolysis by kinetic effects of ATP on phosphofructokinase and pyruvate kinase reactions, and the presence of non-phosphorylating transport systems. This review focuses on these three pathways separately but, as metabolism is an integrated network, discussing the pathways as discrete entities is liable to underestimate the importance of interactions between them. We therefore also discuss the interactions between the pathways, and the roles that these interactions play in various metabolic processes. Understanding of these pathways is further complicated by the large size of the annotated gene families that encode their constituent proteins. We conclude by highlighting both technical and biological questions that need to be addressed to further advance understanding in this research field.

Glycolysis

The glycolytic pathway, the oxidization of glucose to pyruvate, is arguably the least studied of those reviewed here. As for the other pathways, this is most probably due to the prevailing opinion that the glycolytic pathway is already well characterized. Beyond our understanding of the structural organization of the pathway, however, there are large gaps in our understanding of subjects as fundamental as the regulation of the pathway and even its precise cellular location. Here, we review both targeted and genomic strategies aimed at addressing this problem that have been published since the last major review of plant glycolysis [1]. We do not cover studies on the sensing role of hexokinase, which have recently been expertly reviewed elsewhere [2].

In the past few years, a wide number of reverse genetic strategies have been used to analyse the importance and control of the glycolytic pathway [3•,4–7]. These studies have demonstrated an important role for cytosolic phosphoglucomutase (but not for hexokinase- or pyrophosphate-dependent phosphofructokinase) in the control of glycolysis in heterotrophic tissues. Further, they revealed that the modification of the expression of hexokinase and of the cytosolic isoforms of phosphoglucomutase, phosphoglycerate mutase and pyruvate kinase had dramatic effects on photosynthetic metabolism. Surprisingly, these studies were unable to pinpoint where the majority of metabolic control lies within this pathway. It is crucial to note, however, that the reverse genetic studies reported to date do not represent pathway saturation. Indeed, despite the genome sequencing projects for *Arabidopsis* and rice, there is still no gene annotation

for an ATP-dependent phosphofructokinase in plants. In contrast to the poor understanding of the transcriptional control of plant glycolysis, several important observations have been made recently regarding the regulation of this pathway at the posttranslational level [8,9•]. Studies on transgenic potato plants exhibiting enhanced sucrose cycling revealed a general upregulation of the glycolytic pathway that is most probably mediated at the level of transcription [8]. The fact that this sucrose cycling places a large ATP demand on the cell makes it tempting to suggest that glycolysis in plants is demand-driven in a manner analogous to that in *Escherichia coli* [10]. This is surprising at first, given the lack of kinetic regulation of plant phosphofructokinase and pyruvate kinase by ATP. Correlative evidence of transcriptional regulation of glycolysis has also been provided by several transcriptomic studies [11,12]. The kinetic properties of most of the enzymes of glycolysis have long been established and are now readily accessible through the BRENDA database [13••]. Nevertheless, the recent discovery of two post-translational modifications of the cytosolic pyruvate kinase, which alter the regulatory properties of the enzyme and facilitate its degradation by the proteasome [9•], suggest that our understanding of such regulation is far from complete.

Another aspect of the regulation of the glycolytic pathway that is rarely considered is the spatial organization of the pathway within the plant cell. Several observations from studies of mammalian cells suggest that glycolytic enzymes may physically concentrate at sites of demand for ATP or other glycolytic intermediates. A combination of proteomic analyses of a highly purified mitochondrial fraction and enzyme activity assays recently established that the enzymes of glycolysis are functionally associated with the mitochondria in *Arabidopsis* [14••]. The sensitivity of these activities to protease treatments indicated that the glycolytic enzymes are present on the outside of the mitochondrion. This association was confirmed *in vivo* by the expression of enolase- and aldolase-yellow fluorescent protein fusions in *Arabidopsis* protoplasts. Furthermore, when supplied with appropriate cofactors, isolated intact mitochondria are capable of the metabolism of C^{13}-glucose to C^{13}-labeled intermediates of the TCA cycle, suggesting that the complete glycolytic sequence is present and active in this subcellular fraction.

TCA cycle

The respiratory process continues with the mitochondrial reactions of the TCA cycle, which converts phosphoenolpyruvate (PEP) to malate and/or pyruvate in the cytosol. These organic acids are then taken up into the mitochondria, through specific members of the mitochondrial carrier family (MCF; Table 1, Figure 1). There, they are subsequently interconverted, producing energy and reducing power, and ultimately yielding 15 ATP equivalents per pyruvate molecule. Additional technical hurdles are presented by plant cells in comparison with animal cells [15], and this partially explains why much of our knowledge of the operation of the TCA cycle has been gleaned from studies of mammalian cells. In the past few years, however, genetic (and to a lesser extent environmental) approaches have begun to uncover features of the cycle that are unique to plant cells [15,16•,17,18,19•,20]. That said, not all of the genes that encode enzymes that participate in the TCA cycle have been functionally characterised. For example, despite strong evidence for the activity of a succinyl CoA ligase [21] and the presence of such an enzyme in the mitochondrial proteome of *Arabidopsis* [22], the genes that encode this enzyme are as yet predicted only on the basis of homology in the *Arabidopsis* genome.

The first enzyme of the TCA cycle, the mitochondrial pyruvate dehydrogenase, is a multienzyme complex that catalyses the irreversible reaction that converts pyruvate

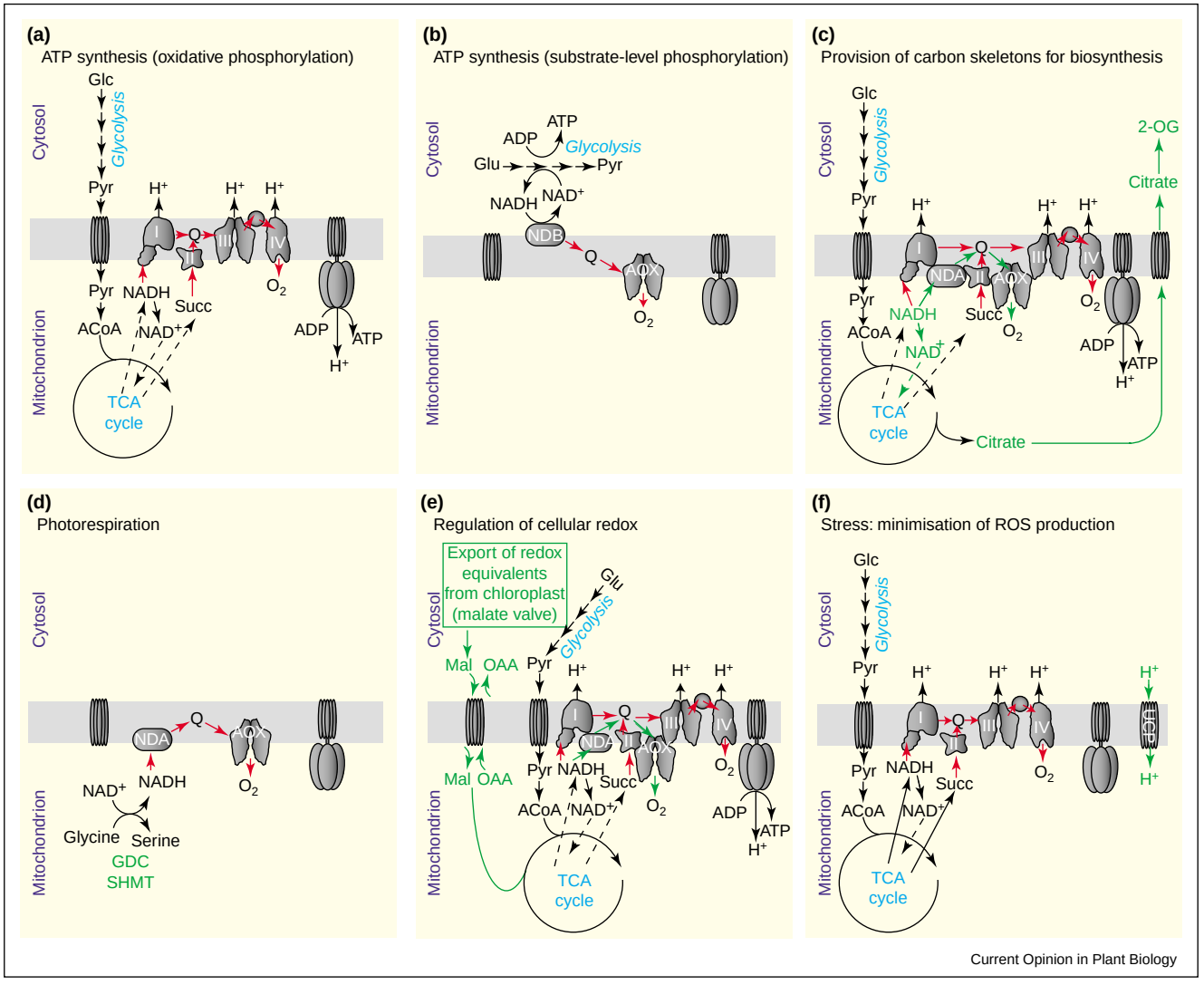
Table 1

Functionally characterised mitochondrial carriers from plants.

Carrier	Gene(s)	Function	Reference(s)
DTC	At5 g19760	Dicarboxylate-tricarboxylate carrier	[67•,68]
	D45073/4/5[a]	2-oxoglutarate/malate translocator	
SFC	At5 g01340	Succinate-fumarate carrier	[69]
CAC	At5 g46800	Carnitine carrier	[70]
BAC	At2 g33820/At1 g79900	Basic amino-acid carrier	[71,72]
PiC	At5 g14040	Phosphate carrier	[73]
AAC	At5 g13490	ADP/ATP carrier	[73]
UCP	At3 g54110/At5 g58970	Uncoupling protein	[45]
PYR	*YIL006w*[b]	*Pyruvate carrier*	[74•]

This table shows only those transporters that have been functionally characterized; these are only a subset of the 50 putative members of the mitochondrial carrier family (MCF) identified in the *Arabidopsis* genome by *in silico* and proteomic approaches [73,75]. Unless otherwise stated, the genes described are from *Arabidopsis*. Although no plant gene has yet been functionally identified as encoding a pyruvate carrier, the pyruvate transporter from *Saccharomyces cerevisiae* is included (in italics) because of its vital importance in linking glycolysis with the TCA cycle.
[a]Gene from *Panicum miliaceum*.
[b]Gene from *Saccharomyces cerevisiae*.

Figure 1



Possible interactions of mitochondrial electron transport with other pathways. **(a)** ATP synthesis (i.e. oxidative phosphorylation). Pyruvate (Pyr) supplied by glycolysis is oxidised by the mitochondrial TCA cycle, and electrons from the resulting reductant are transferred through the electron transport chain with a chemiosmotically coupled synthesis of ATP. Complexes I–IV of the electron transport chain are shown. **(b)** ATP synthesis (substrate-level phosphorylation). Glycolysis may also contribute to ATP production, particularly under conditions in which the oxidative phosphorylation pathways are impaired [66]. Glycolytic flux is dependent upon the recycling of cytosolic NAD^+, which can be achieved via the external NADH dehydrogenase (NDB). The activity of AOX could provide an entirely non-proton-pumping electron transport pathway, in which electron flux is not limited by mitochondrial ATP synthesis. **(c)** Provision of carbon skeletons for biosynthesis. Withdrawal of TCA-cycle intermediates (the export of citrate to support nitrogen assimilation is illustrated) may necessitate a higher flux of portions of the TCA cycle and a higher rate of entry of electrons into the electron transport chain. These extra electrons may be accommodated by a non-proton-pumping pathway that consists of the internal NDA and AOX, such that electron flow is not restricted by the rate of ATP synthesis. **(d)** Photorespiration. The oxidation of photorespiratory glycine in the mitochondrial matrix requires the recycling of NAD^+. This can be achieved by entry of electrons from NADH into the electron transport chain. The non-phosphorylating pathway explained above may operate to avoid electron flow being limited by the rate of ATP synthesis. **(e)** Regulation of cellular redox. Photosynthesis requires a precise balance between the generation of NADH and ATP. One way in which this may be achieved is to export excess NADH via metabolite shuttles. The 'malate valve' exports excess chloroplastic reductant as malate and imports it into the mitochondrion via oxaloacetate (OAA) exchange. Mitochondrial malate dehydrogenase releases the NADH. The extent to which this NADH supports ATP synthesis depends on the route of electrons through the electron transport chain (red arrows represent the phosphorylating pathway, green arrows represent the non-phosphorylating pathway). **(f)** Stress: minimisation of ROS production. A high mitochondrial membrane potential restricts electron flow and increases the leakage of electrons to form superoxide. This can be minimised by the activity of mitochondrial UCP, which dissipates the proton gradient. UCP is activated by superoxide, providing a regulatory loop for this pre-oxidant defence mechanism. AcoA, acetyl-CoA; GDC, glycine decarboxylase; Glu, glucose; 2-OG, 2-oxoglutarate; Mal, malate; SHMT, serine hydroxymethyl transferase; Succ, succinate.