2.4.2??The glycolytic pathway

Glycolysis originally described the sequence of reactions that convert glycogen to lactic acid in muscle and is usually considered to include the metabolism of hexose phosphates to pyruvate. In plant tissues, starch takes the place of glycogen in this scheme, and there is probably a second end-product, either oxaloacetate or malate (Figure 2.21).

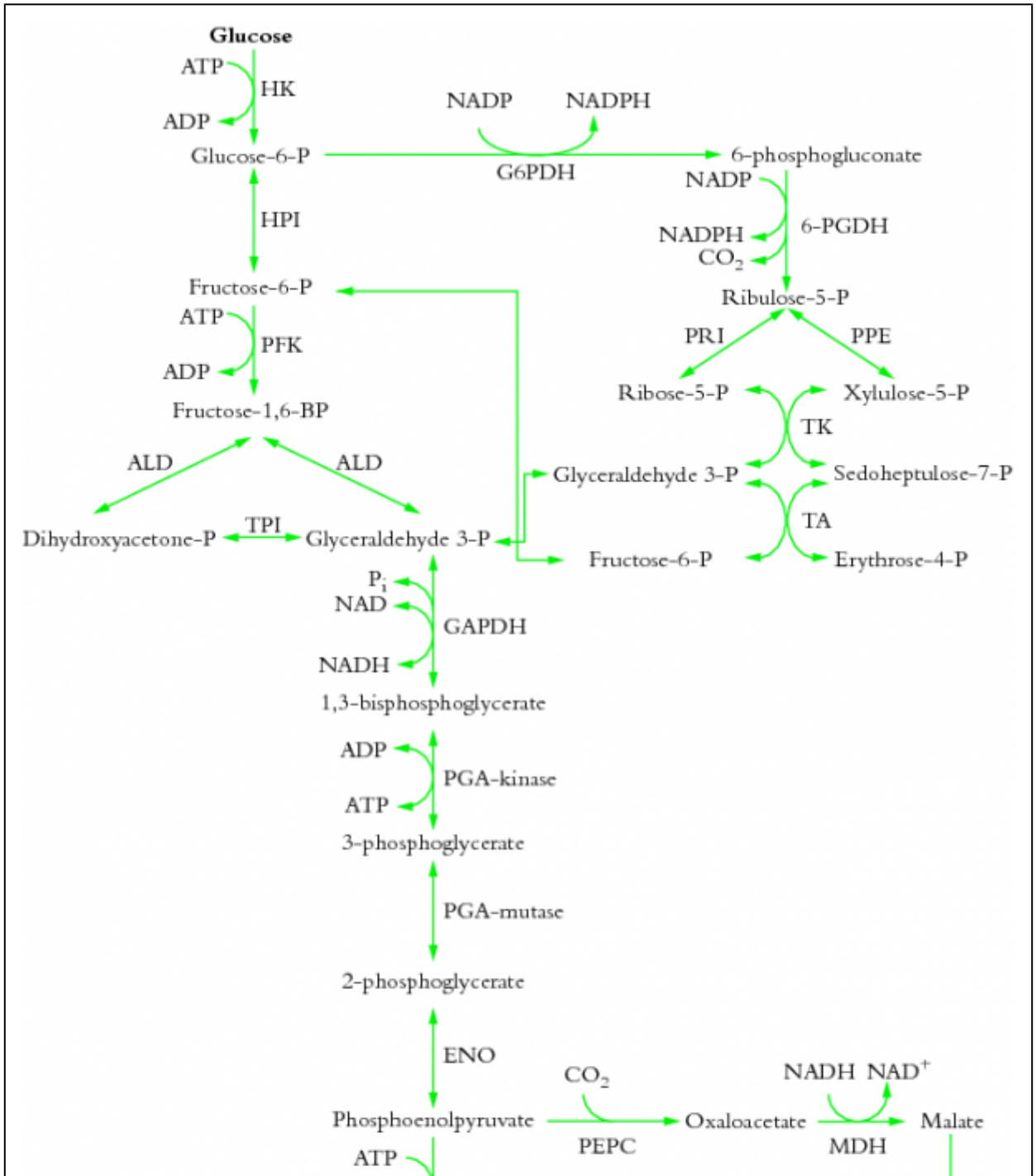

Figure 2.21 Interrelationships between glycolysis and the pentose phosphate pathway. Enzymes abbreviated are: HK, hexokinase; HPI, hexose phosphate isomerase; PFK, phosphofructokinase; ALD, aldolase; TPI, triose phosphate isomerase; GAPDI-I, glyceraladehyde 3-phosphate dehydrogenase; PGA, phosphoglycerate; ENO, enolase; PK, pyruvate kinase; PDC, pyruvate decarboxylase; ADH, alcohol dehydrogenase; LDH, lactate dehydrogenase; G6PDH, glucose-6-phosphate dehydrogenase; 6-PGDH, 6-phosphogluconate dehydrogenase; PRI,
phosphoriboisomerase; PPE, pentose phosphate epimerase; TK, transketolase; TA, transaldolase; PEPC, phosphoenolpyruvate carboxylase; MDH, malate dehydrogenase. Note that lactate and ethanol are formed only when mitochondrial function is inhibited, as under anaerobiosis. (Original drawing courtesy David Day)

Moreover, in plants glycolysis occurs in both cytosol and plastids, with reactions in the different compartments catalysed by separate isoenzymes. The ?rst step in the pathway is phosphorylation of fructose-6-P to fructose-1,6-P_2. Plant tissues contain two enzymes capable of catalysing this step: an ATP-dependent phosphofructokinase (PFK), which catalyses an essentially irreversible reaction and occurs in the cytosol and plastids, and phosphofructophosphotransferase (PFP) (now called PP_i-dependent phosphofructokinase, PP_i-PFK), which occurs only in the cytosol and utilises PP_i as the phosphoryl donor in a reaction that is readily reversible.

Regulation of PFK is achieved by a combination of mechanisms, including pH, the concentration of substrates and effector metabolites, changes in the state of aggregation, and covalent modi?cation by phosphorylation/dephos-phorylation of the protein. The relative importance of these mechanisms varies depending on the organism. In plants phosphoenolpyruvate (PEP) is probably the most potent regulator, inhibiting at μM concentrations, but 3-PGA and 2-PGA also strongly inhibit. P_i activates the cytoplasmic PFK, and to a lesser extent that from plastids, and overcomes the inhibition by PEP. The enzyme is also activated by Cl^- and other anions. The regulatory metabolite fructose-2,6-P_2, a powerful activator of PFK from animals, has no effect on the enzyme from plants.

PFP, discovered subsequently, is ubiquitous in plants and has a catalytic potential higher than that of PFK. Fructose-2,6-P_2 strongly activates PFP, but the physiological signi?cance of this activation and, indeed, the role of PFP, in plants have not yet been clearly established. Fructose-2,6-P_2 is a potent inhibitor of cytosolic fructose-1,6-bisphosphatase which is an important control point of sucrose biosynthesis regulating the partitioning of photosynthate between sucrose and starch in leaves. Whether fructose-2,6,-P_2 has a role in the control of glycolysis through its activation of PFP is not clear.

Since the reaction catalysed by PFP is reversible and the concentration of fructose-2,6-P_2 in the cytosol is usually high enough to maintain PFP in an activated form, the direction of this reaction *in vivo* is likely to depend on availability of substrates. In tissues where sucrose breakdown is occurring, PFP may function to generate PP_i to facilitate the conversion of UDP-glucose to glucose-1-P (Figure 2.20). Under these conditions, the simultaneous and opposing action of PFK and PFP in the cytosol could set up a potentially wasteful substrate cycle between fructose-6-P and fructose-1,6-P_2. The operation of such a cycle may be a cost of having a mechanism to generate PP_i and, ultimately, UDP for the breakdown of sucrose by sucrose synthase. PFP may also act as an inducible enzyme in some plant tissues, providing increased glycolytic capacity when required during certain stages of plant development or during adjustment to adverse environmental conditions.

Fructose-1,6-P_2 is cleaved by aldolase to form dihydroxyacetone-P and glyceraldehyde 3-P, and these triose phosphates are interconverted in a reaction catalysed by triose phosphate isomerase. Glyceraldehyde 3-P is oxidised to glycerate-1,3-P_2 by an NAD-dependent glyceraldehyde 3-P dehydrogenase in the cytosol and an NADP-linked enzyme in plastids. Glyceraldehyde 3-P dehydrogenase is sensitive to inhibition by the reduced pyridine nucleotide cofactor, which must be reoxidised to maintain the flux through the glycolytic pathway. In chloroplasts, the reactions catalysed

by fructose-1,6-P_2 aldolase, triose phosphate isomerase and NADP-dependent glyceraldehyde 3-P dehydrogenase also form part of the PCR cycle. The remaining steps for PEP formation are shown in Figure 2.21; all steps from fructose-1,6-P_2 to PEP are reversible.

The end-product of glycolytic reactions in the cytosol of plants is determined by the relative activities of the two enzymes that can utilise PEP as a substrate: pyruvate kinase, which forms pyruvate and a molecule of ATP, and PEP carboxylase, which forms oxaloacetate (Figure 2.21). Both of these reactions are essentially irreversible and there are ?ne controls that regulate the partitioning of PEP between these reactions. Pyruvate kinase requires monovalent cations and is inhibited by ATP (and therefore is sensitive to the energy status of the cell), whereas PEP carboxylase is inhibited by malate and is independent of cell energy status. The sensitivity of PEP carboxylase to malate is regulated by phosphoryl-ation of the enzyme by a protein kinase: the phosphorylated form is less sensitive to malate inhibition. This phosphorylation may form part of an important diurnal regulatory cycle in the leaves of crassulacean acid metabolism plants (see Section 2.1).

Oxaloacetate is reduced by malate dehydrogenase to malate which, along with pyruvate, can be taken up into mitochondria and metabolised further (see below). The reduction of oxaloacetate in the cytosol could provide a cytosolic mechanism for oxidising NADH formed by glyceraldehyde 3-P dehydrogenase (Figure 2.21).

In chloroplasts glycolysis is most active in conjunction with the breakdown of starch to form sucrose for export to non-photosynthetic tissues. There is some doubt about the occurrence of phosphoglycerate mutase in chloroplasts, and therefore the main products of the glycolytic reactions may be triose phosphates and 3-PGA. These could be exported through the P_i translocator in the chloroplast envelope to the cytosol, where sucrose synthesis takes place. In photosynthetic cells, the triose P exported to the cytosol for sucrose synthesis (Section 2.1.8) could also enter the glycolytic pathway directly to provide mitochondrial substrates.

Studies of changes in the content of glycolytic intermediates in plant tissues that undergo an altered rate of
respiration (e.g. during the climacteric in ripening fruits or in plant tissues placed under low-O_2 stress) indicate that the conversion of fructose-6-P to fructose-1,6-P_2, and PEP to pyruvate, are major regulatory steps. For example, a decrease in activity of PEP carboxylase and pyruvate kinase (the latter in response to a lower energy demand as indicated by an increase in the cytosolic ATP/ADP ratio) can lead to an increase in the concentration of inhibitory metabolites of PFK and, consequently, a decrease in the rate of glycolysis. The rate of oxidation of NAD(P)H is also likely to have a bearing on the glycolytic flux at the glyceraldehyde 3-P dehydrogenase step.
