Crassulacean acid metabolism (CAM) can be defined as the ability to perform significant CO$_2$ assimilation into C$_4$ acids (mainly malic acid) in the dark. Reutilization of the stored C$_4$ acids results in a characteristic diel (24 h) pattern of organic-acid fluctuation along with a reciprocal pattern of fluctuating levels of storage glucan. CAM is found primarily, although not exclusively, in succulent plants, particularly those having large chloroplast-containing parenchyma cells with the ability to store large amounts of malic acid and water. CAM plants are mainly tropical or subtropical in origin and typically inhabit arid environments with periodic water deficits such as semi-deserts, or regions with Mediterranean climates, or epiphytic habitats in tropical forests (Kluge and Ting 1978). Their ecological distribution and the observation that CAM plants open their stomata at night and close them during the day to avoid excessive evaporative water loss have led to the general consensus that CAM is a functional adaptation to dry environments and a means of water conservation. However, not all CAM plants occur in arid environments. *Isoetes* and related species which grow in aquatic environments display CAM (Keeley and Busch 1984). In these instances, CAM provides the plants with a source of carbon when levels of CO$_2$ in the aquatic environment become limiting during the day.

Crassulacean acid metabolism exhibits great plasticity in its manifestations and variations on the central theme of CAM, such as CAM-idling (Ting 1985), a response to severe drought conditions. Many plants display CAM on a strictly constitutive basis, whereas others, such as *Peperomia*, clearly exhibit CAM on a developmental basis (Sipes and Ting 1985; Holthe et al. 1987; see also Chap. 13). Various other environmental stimuli can cause CAM to become elicited in a facultative manner. In certain plants, CAM is regulated by daylength (Brulfert et al. 1982) or season (Guralnick et al. 1984). CAM can also be brought about by exposure to water stress in various species such as *Portulacaria afra* (Ting and
Hanscom 1977), Sedum telephium (Groenhof et al. 1986; see also Chap. 11), and Mesembryanthemum crystallinum (Winter and von Willert 1972).

Mesembryanthemum crystallinum is the best-studied example of CAM inducibility at both the biochemical and physiological levels (for a review, see Bohnert et al. 1992). A number of other attributes, such as an interesting developmental profile, amenability to tissue culture regeneration (Meiners et al. 1991), a short life-span, and small genome size (Meyer et al. 1990; DeRocher et al. 1990) make this model even more attractive and should aid future research. Additional avenues of investigation have been initiated by the characterization of other adaptive mechanisms to salinity and drought stress, such as osmoprotective solute biosynthesis (Vernon and Bohnert 1992a) in transgenic plants (Tarczynski et al. 1993; Vernon et al. 1993b).

9.2 CAM Evolution

CAM is currently recognized in 33 plant families (Chap. 26), its widespread taxonomic distribution arguing strongly for multiple, independent origins (Moore 1982). One hypothesis suggests that CAM biochemistry arose from the multiplication and alteration of genes encoding enzymes for malate formation and decarboxylation in guard cells (Teeri 1982; Cockburn 1983). However, many other cell types exhibit the ability to produce malate. All enzymes required for a functional CAM pathway are derived from existing genes essential for photosynthetic carbon metabolism (Cockburn 1983). These enzymes perform indispensable reactions in glycolysis, gluconeogenesis, mobilization of carbon compounds into the TCA cycle and photosynthetic carbon flow (Fig. 9.1). The genetic alterations needed to transform guard-cell-specific gene expression to other cell types and bring about functional CAM are likely to be few. The necessary changes were to include adjustments in the spatial and/or temporal patterns of gene expression and evolutionarily selected alterations in regulatory properties. A discussion of the origins of CAM has been presented (Monson 1989), and is discussed further in Chapters 24 and 25.

Aside from alterations in expression patterns, a second important adaptation might have involved changes in the enzymes themselves. Very little work has been conducted to address the question of enzyme adaptation to a specific mode of photosynthetic carbon fixation. One of the best-studied enzymes in CAM is phosphoenolpyruvate carboxylase (PEPC), the enzyme responsible for the primary fixation of CO₂ (as HCO₃⁻) into oxaloacetate (Fig. 9.1). Tracing the evolution of this enzyme and the evolution of the genes that encode PEPC might give us clues about the rate of CAM evolution and potential molecular changes which the enzyme underwent in its different forms in CAM, as well as in C₃ and C₄ metabolism. Analyses of this type have been conducted for C₃ and C₄ species of Flaveria (Hermans and Westhoff 1992) and for the C₄ species Sorghum (Lepiniec et al. 1993). As more genes for this enzyme have been characterized, a larger number of sequences, including the CAM-specific form of PEPC from