Crassulacean Acid Metabolism. A Plastic Photosynthetic Adaptation to Arid Environments[1]

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Crassulacean acid metabolism (CAM) is an important elaboration of photosynthetic carbon fixation that allows chloroplast-containing cells to fix CO_2 initially at night using phospho*enol*pyruvate carboxylase (PEPC) in the cytosol. This leads to the formation of C_4 organic acids (usually malate), which are stored in the vacuole. Subsequent daytime decarboxylation of these organic acids behind closed stomata creates an internal CO_2 source that is reassimilated by Rubisco in the chloroplast. The refixation of this internal CO_2 generates carbohydrates via the conventional photosynthetic carbon reduction cycle. Thus, CAM involves a temporal separation of carbon fixation modes in contrast to the spatial separation found in C_4 plants. The first recognition of the nocturnal acidification process can be traced to the Romans, who noted that certain succulent plants taste more bitter in the morning than in the evening (Rowley, 1978). However, formal descriptions of the ability of succulent plants to conduct nocturnal CO_2 fixation or to acidify photosynthetic tissues at night and deacidify them during the day did not appear until the early 19th century (de Saussure, 1804; Heyne, 1815). The term CAM was coined to give credit to Heyne's observations that were made using *Bryophyllum calycinum*, a succulent member of the Crassulaceae.

Since these early descriptions, a detailed account of the sequence of biochemical reactions of the CAM cycle (Ranson and Thomas, 1960), the complexity of the biochemical variations in the pathway among different CAM species, and its regulation by the environment have been achieved (Osmond, 1978; Ting, 1985). Initial nocturnal CO_2 fixation by PEPC occurs when stomata are open and transpirational water losses are low. CO_2 release during the day promotes stomatal closure and concentrates CO_2 around Rubisco, suppressing its oxygenase activity, thereby minimizing photorespiration. The net effect of this CO_2-concentrating strategy is that CAM plants exhibit water use efficiency (WUE) rates severalfold higher than C_3 and C_4 plants under comparable conditions (Drennan and Nobel, 2000). Thus, CAM is typically, although not exclusively, associated with plants that inhabit extremely arid environments (e.g. deserts), semi-arid regions with seasonal water availability (e.g. Mediterranean climates), or habitats with intermittent water supply (e.g. tropical epiphytic habitats). Most notable among these are commercially or horticulturally important plants such as pineapple (*Ananas comosus*), agave (*Agave* subsp.), cacti (Cactaceae), and orchids (Orchidaceae). CAM is also correlated with various anatomical or morphological features that minimize water loss, including thick cuticles, low surface-to-volume ratios, large cells and vacuoles with enhanced water storage capacity (i.e. succulence), and reduced stomatal size and/or frequency.

The selective advantage of high WUE likely accounts for the extensive diversification and speciation among CAM plants principally in water-limited environments. Intensive ecophysiological studies over the last 20 years have documented that CAM is present in approximately 7% of vascular plant species, a much larger percentage than the percentage of C_4 species (Winter and Smith, 1996a). The widespread distribution of CAM among 33 taxonomically diverse families (Smith and Winter, 1996) suggests that CAM most likely evolved independently on numerous occasions in different families and even within individual families (Griffiths, 1989; Ehleringer and Monson, 1993; Pilon-Smits et al., 1996). More recent phylogenetic reconstructions using PEPC sequence information have provided more convincing support for the polyphyletic origins of CAM (Gehrig et al., 1998b, 2001). It is curious that CAM is also found in aquatic vascular plants where it presumably enhances inorganic carbon acquisition in certain aquatic environments where CO_2 availability can become rate limiting for photosynthesis (Keeley, 1996, 1998). Thus, the daytime limitation of CO_2 availability, brought about by water-conserving stomatal closure in arid terrestrial habitats or by competition from other species and the high diffusional resistances limiting access to CO_2 in aquatic habitats, appears to be the common factor responsible for the evolution of CAM.

A REMARKABLE PLASTICITY

One of most striking themes to emerge in recent years is the extent to which the phylogenetic and ecological diversity of CAM plants is also reflected in

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a remarkable plasticity of the basic metabolic scheme described above. Genotypic, ontogenetic, and environmental factors such as light intensity, relative humidity, and water availability combine to govern the extent to which the biochemical and physiological attributes of CAM are expressed (Cushman and Borland, 2001). The photosynthetic plasticity of CAM occurs within a continuum of diel gas exchange patterns that fall into four phases as defined by Osmond (1978). The nocturnal uptake of atmospheric and respiratory CO_2 via PEPC to form C_4 acids (phase I) and daytime organic acid decarboxylation to generate elevated C_i and stomatal closure (phase III) are interspersed with transitional periods of net CO_2 uptake at the start (phase II) and end of the day (phase IV) when both PEPC- and Rubisco-mediated carboxylation can contribute to CO_2 assimilation. The proportion of CO_2 taken up via PEPC at night or directly during the day by Rubisco (net CO_2 assimilation) is dictated by the integration of stomatal behavior, fluctuations in organic acid and storage carbohydrate accumulation, and the abundance and activity of primary (PEPC) and secondary (Rubisco) carboxylating and decarboxylating enzymes (e.g. malic enzyme or PEP carboxykinase), as well as gluconeogenic/glycolytic enzymes responsible for the synthesis and breakdown of C_3 carbon skeletons.

Depending on developmental and/or environmental influences, a variety of CO_2 assimilation, acid flux, and stomatal behavior characteristics may be observed outside the conventional pattern of the four phases (Table I). "Nearly-C_3 " or "CAM cycling" species display daytime net CO_2 uptake with refixation of respiratory CO_2 at night accompanied by only small diel C_4 acid fluctuations. In plants growing in thin soils or rock outcrops, this nocturnal recapture of respiratory CO_2 is thought to help maintain a positive carbon balance during frequent episodes of drought (Martin, 1996). However, the potential conservation of water resulting from the induction of CAM cycling varies widely (5%–70%) in various species (Borland, 1996; Martin, 1996). In C_3-CAM intermediate species, such improvements in WUE are not always associated with CAM induction (Eller and Ferrari, 1997; Cushman and Borland, 2001). In "obligate" or "constitutive" CAM species, net CO_2 uptake occurs almost exclusively at night (phase I), with some net CO_2 assimilation occurring during phases II and IV, even under well-watered conditions, accompanied by large diel C_4 acid fluctuations. Under severe drought conditions, many CAM species will undergo "CAM-idling" wherein stomata remain closed day and night, preventing net CO_2 uptake, yet the plants will continue to conduct diel fluctuations in organic acids. Other modes of CAM such as latent CAM, indicated by organic acid concentrations elevated above those normally present in C_3 plants but without diel fluctuation, may represent a nascent C_3-to-CAM progression in some species (Schuber and Kluge, 1981). A hypothetical variation of CAM called "rapid-cycling CAM" has also been proposed in which the CO_2 acquisition and reduction

Table I. *Plasticity of CAM modes in relationship to environmental and developmental influences*

CAM Variation	Net CO_2 Uptake	Respiratory CO_2 Refixation?	Stomatal Behavior (Open)	C_4 Acid Flux	Developmental/ Environmental Impact	Proposed Functions
C_3	Day	No	Day	–[a]	–/–	–
CAM cycling	Day	Yes	Day	+	+/+	Maintain positive carbon balance; improved WUE?[b]; reduced photorespiration?
C_3-CAM (facultative CAM)	Day/night	Yes	Day/night	++	+/++	Maintain positive carbon balance; improved WUE?; reduced photorespiration
CAM (obligate CAM)	Phases I, II, and IV	Yes	Day/night	+++	+/+++	Improved WUE; reduced photorespiration
Phase II CAM	Phases I and II	Yes	Morning/night	++++	+/+++	Improved WUE; reduced photorespiration
Phase I CAM	Phase I only	Yes	Night only	+++	+/+++	Improved WUE; reduced photorespiration
CAM idling	None	Yes	Always closed	+	+/+++++	Protection of photosynthetic apparatus from photoinibition; maintain a positive carbon balance
Latent CAM	Day	Yes	Day	– (elevated)	?/?	C_3 to CAM progression?
Rapid-cycling CAM (theoretical)	All	?	?	– (rapid)	?/?	?

[a] Dashes indicate no substantial occurrence or effect. [b] Question marks indicate that no information is available.

phases of CAM may occur over time periods shorter than the normal diel cycle (Cockburn, 1998).

The best examples of CAM plasticity are the C_3-CAM intermediate species found predominantly among the Aizoaceae, Crassulaceae, Portulaceae, and Vitaceae (Smith and Winter, 1996). These facultative or inducible CAM species use the C_3 pathway to maximize growth when water is abundant, but then they undergo a gradual C_3-to-CAM transition often coincident with seasonal moisture availability (Winter et al., 1978). The C_3-to-CAM transition reduces water loss and maintains photosynthetic integrity under water-limited conditions that ultimately translates into reproductive success (Winter and Ziegler, 1992). Among facultative CAM species, the common ice plant, *Mesembryanthemum crystallinum*, has been most intensively studied (Adams et al., 1998; Bohnert and Cushman, 2001). This model species undergoes a gradual, largely irreversible, and partially developmentally regulated transition into CAM following water stress (Cushman et al., 1990; Herppich et al., 1992). In contrast, other inducible CAM species (e.g. Clusiaceae and Bromeliaceae) display more rapid and reversible shifts between C_3 photosynthesis and CAM in response to changes in water deficit, regardless of leaf or plant ontogeny (Schmitt et al., 1988; Zotz and Winter, 1993; Lüttge, 1996; Borland et al., 1998). The magnitude of CAM induction in facultative CAM plants tends not only to be influenced by water deficit, but also by associated environmental conditions such as temperature, light intensity, and humidity (Lüttge, 2000). For example, it is well established that high light intensity or light quality can enhance CAM induction in the ice plant in the presence or absence of salinity stress (McElwain et al., 1992; Cockburn et al., 1996; Miszalski et al., 2001).

MOLECULAR GENETICS OF CAM

Since the first molecular characterization of the common ice plant *Ppc1* gene encoding a CAM-specific isoform of PEPC more than a decade ago (Cushman et al., 1989), a large number of enzymes, transporters, and regulatory proteins required for CAM have been identified and characterized (for review, see Cushman and Bohnert, 1999, 2001; Cushman and Borland, 2001). Most studies have been restricted to inducible C_3-CAM models (e.g. common ice plant and *Kalanchoë* sp.) because the differential expression of genes induced in response to water deficit serves as a convenient and reliable indicator of their potential functional role(s) in CAM. Greater investments have been made in establishing molecular genetic resources for common ice plant than other CAM models because this species is also a halophyte and has been extensively investigated to understand salinity stress tolerance mechanisms (Bohnert and Cushman, 2001; Bohnert et al., 2001). CAM induction in response to salinity, water deficit, osmotic stress, or abscisic acid treatment is controlled primarily by transcriptional activation (Cushman et al., 1989, 2000b) initiated through a signaling cascade with apparent requirements for calcium and calcium-dependent protein kinase activities (Taybi and Cushman, 1999; Golldack and Dietz, 2001). In general, transcript and protein accumulation patterns are well correlated; however, discrepancies between transcript and protein abundance have suggested that changes in mRNA stability and utilization or translational efficiency are also likely to govern gene expression changes during the C_3-to-CAM transition (Cushman et al., 1990; DeRocher and Bohnert, 1993).

Detailed analysis of the PEPC gene families from facultative and obligate CAM species including pineapple (*Ananas comosus*), *K. blossfeldiana*, *K. daigremontiana*, common ice plant, and *Vanilla planifolia* has indicated that a single member of a four- to six-member PEPC gene family is typically recruited to fulfill the primary carboxylation and carbon flux requirements of CAM, as demonstrated by its enhanced expression in CAM-performing leaves (Cushman et al., 1989; Gehrig et al., 1995, 1998a). Remaining isoforms, which presumably fulfill anapleurotic "housekeeping" or tissue-specific functional roles, generally show lower transcript or protein abundance and remain unaffected in their expression following CAM induction. This "gene recruitment" paradigm likely pertains to other gene families as well. Enhanced expression of enzymes for C_4 acid metabolism is accompanied by corresponding increases in carbohydrate-forming and -degrading enzymes and transcripts (Holtum and Winter, 1982; Paul et al., 1993; Häusler et al., 2000). Elevated organellar PEP (Kore-eda et al., 1996) and triose and hexose phosphate transport activities (Neuhaus and Schulte, 1996; Kore-eda and Kanai, 1997) associated with CAM induction in common ice plant are matched by light-enhanced increases in transcript abundance and diurnal gene expression patterns of a PEP phosphate translocator and a Glc-6-P phosphate translocator (Häusler et al., 2000). However, the expression of a chloroplast Glc transporter and a triose phosphate transporter remain largely unchanged (Häusler et al., 2000; S. Kore-eda and J.C. Cushman, unpublished data). Tonoplast H^+-translocating ATPase transport activity and expression of corresponding tonoplast H^+-translocating ATPase subunit genes for energizing vacuolar malate storage is enhanced during the C_3-CAM transition in common ice plant (Rockel et al., 1998a, 1998b; Barkla et al., 1999; Golldack and Dietz, 2001). Molecular characterization of the vacuolar malate transporters, carriers, and channels for malate influx and efflux has remained a challenge (Lüttge et al., 2000). Recent measurements of vacuolar malate transport activities demonstrate an approximate 3-fold increase following CAM induction in common ice plant (Lüttge et al., 2000). A strategy to analyze differences in polypeptide expression patterns in C_3- versus CAM-performing leaves

of common ice plant is being used to identify candidate vacuolar malate transporters. Antisera raised against affinity chromatography-purified tonoplast vesicle fractions from *K. daigremontiana* enriched for malate transport activity has been used to identify 32- and 33-kD common ice plant polypeptides that are induced or enhanced in the CAM state (Steiger et al., 1997; Lüttge et al., 2000). These low abundance polypeptides could be candidates for the vacuolar malate transporter. Amino acid sequence information from these polypeptides may facilitate the isolation of the corresponding genes.

CIRCADIAN CONTROL OF CAM

The circadian rhythm of CO_2 fixation, primarily studied in *K. fedtschenkoi*, is one of the earliest and best documented examples of circadian rhythms in higher eukaryotes (Wilkins, 1992). Diel oscillations in the activity of PEPC, controlled in part by circadian changes in its phosphorylation state, play a key role in directing carbon flux through the CAM pathway by changing the enzyme's sensitivity to allosteric inhibitors such as malate (Nimmo et al., 1987; Nimmo, 1998). PEPC phosphorylation state is controlled largely by changes in the activity of PEPC kinase (PPcK; Carter et al., 1991). In common ice plant, PPcK activity is induced concomitantly with a CAM-specific isoform of PEPC (Li and Chollet, 1994). Recent cloning of the gene for PPcK first in *K. fedtschenkoi* (Hartwell et al., 1999) and then in common ice plant (Taybi et al., 2000) demonstrated directly that this kinase is itself regulated at the level of transcript abundance by a circadian oscillator. A dissociable protein inhibitor of PPcK activity has also been described from *K. fedtschenkoi* that may function to suppress basal kinase activity during the light period and early stages of the dark period when carbon flux through PEPC is not needed (Nimmo et al., 2001a). In contrast to C_4 plants, elevations in cytosolic pH appear to have little (Bakrim et al., 2001) or no influence (Paterson and Nimmo, 2000) on PPcK activity in common ice plant or *K. fedtschenkoi*, respectively. However, circadian control of PPcK transcript abundance may be merely a secondary response to other factors such as the cytosolic malate concentration, which has been hypothesized to regulate the transcript abundance and activity of PPcK (Borland et al., 1999; Nimmo, 2000). Cytosolic malate concentrations are likely to be controlled by transport of malate across the tonoplast, a view that is well supported by temperature effects on tonoplast function and modeling studies (Rascher et al., 1998; Lüttge, 2000). Thus, response to environmental factors that alter organic acid content or malate partitioning between the vacuole and cytosol may be able to override circadian rhythms of PPcK activity, providing a possible mechanism for the rapid alterations in PEPC activity observed in some CAM species (Borland et al., 1999; Nimmo, 2000). In addition, feeding of detached *K. fedtschenkoi* and common ice plant leaves with various pharmacological reagents implicates the involvement of a phosphoinositide-dependent phospholipase C, inositol 1,4,5 P-gated tonoplast calcium channels, a putative Ca^{2+}-dependent/calmodulin protein kinase, and RNA and protein synthesis as possible components in the signaling cascade that regulates PPcK activity on a circadian basis (Hartwell et al., 1999; Bakrim et al., 2001; Nimmo et al., 2001b). However, these studies fail to address the influence of such inhibitors on the functioning of the underlying circadian oscillator, and so, observed changes in PEPC activity may not reflect alterations in the PEPC kinase-signaling cascade per se. One great challenge to understanding circadian regulation of CAM will be to dissect the mechanisms responsible for controlling the circadian oscillations in malate uptake and release across the tonoplast membrane. In particular, it will be important to understand how tonoplast malate transport is controlled by an underlying nuclear-controlled circadian clock. Rapid molecular identification of malate transport components in the tonoplast and circadian clock components from CAM species will be essential for this effort.

A GENETIC MODEL FOR CAM?

To date, ecophysiological investigations have surveyed a wide variety of CAM species to determine which ones actually perform CAM. Alternatively, studies have focused on comparative analysis of specific aspects of CAM such as the degree of CAM induction by water limitation (Cushman and Borland, 2001), intercellular localization of carboxylation and decarboxylation processes (Borland et al., 1998), or the patterns of carbohydrate partitioning within a particular family (Christopher and Holtum, 1996, 1998). However, unlike C_3 and C_4 plants, which have the well-developed genetic models Arabidopsis and maize (*Zea mays*), respectively, there has been, until recently, no investment in the development of a genetic model for CAM. This deficiency has hindered our understanding of many of the molecular mechanisms that regulate CAM. In the past, CAM models were selected for their physiological characteristics. For example, certain obligate CAM species such as *K. daigremontiana* are often favored for gas exchange and biochemical studies due to their reproducible behavior. Other CAM models such as common ice plant can show hyperplastic stress responsiveness to slight changes in growth conditions, which can be a problem for reproducible physiological studies. *Kalanchoë* species, however, lack potential for development as a genetic system as well as any significant molecular genetic resources.

A comparison of the attributes of well-studied or commercially important CAM models from diverse families indicates that common ice plant has many

desirable features that make it an attractive genetic model (Table II). This fast-growing annual produces large quantities of small seeds (typically 10,000–15,000 plant^{-1}) under standard greenhouse or growth chamber conditions in 1-L pots. The plant is self-fertile, yet outcrossing is possible. In contrast, the perennial or semi-perennial pineapple, *Kalanchoë*, and *Clusia* species grow more slowly and are poor seed producers. Although the common ice plant grows more slowly than models such as Arabidopsis, compared with other CAM models, the common ice plant life cycle is quite rapid. Furthermore, it is possible to accelerate the normal life cycle of common ice plant from 4 to 5 mo under natural conditions (Winter et al., 1978) to approximately 7 weeks under growth chamber conditions under continuous light or extended photoperiods and limited rooting volumes (Cheng and Edwards, 1991). Acceleration of the life cycle is conveniently accompanied by a miniaturization of the plant. This is an important consideration when conducting genetic screening because growth chamber or greenhouse space is often a limiting factor. Alternatively, genetic screens could be conducted in a recently identified dwarf mutant background that displays CAM (see below). Finally, mutant collections have been established in common ice plant from fast neutron- or gamma-irradiated (Cushman et al., 2000b) or ethylmethane sulfonate-treated seeds (Adams et al., 1998). Expansion of existing fast neutron collections would create a useful resource for a fast neutron mutagenesis-based reverse genetic screening system in the common ice plant, similar to related resources recently developed in Arabidopsis and rice (*Oryza sativa*; Li et al., 2001). Facile screening procedures have been developed for the isolation of CAM-defective mutants (Cushman et al., 2000b). Identification of CAM-defective mutants is based on a simple pH assay that detects a failure in nocturnal C_4 acid accumulation. Mutant collections are not currently available in other CAM models. A useful by-product of such mutant screens is the identification of mutants with morphological (e.g. dwarfism and absence of epidermal bladder cells) or physiological defects (e.g. salt sensitivity; J.C. Cushman, unpublished data).

Another desirable feature of the ideal CAM model is the availability of an efficient transformation system, preferably one that employs a non-tissue culture-based methodology such as vacuum infiltration or floral dipping in *Agrobacterium tumefaciens* suspensions (Bechtold et al., 1993; Clough and Bent, 1998). A transformation system with adequate efficiency would allow systematic functional genomic investigations to be performed involving reverse genetic screens for T-DNA insertion/activation-tagged gene knockouts, suppression or overexpression studies, and ultimately targeted gene replacement of regulatory or structural genes of interest with key roles in CAM. Of the possible candidate model CAM species, several are amenable to genetic manipulation using an *A. tumefaciens*-mediated transformation system (Truesdale et al., 1999). However, given the ice plant's susceptibility to *A. tumefaciens* transformation in tissue culture (Andolfatto et al., 1994; Ishimaru, 1999) and the availability of a high efficiency regeneration system (Cushman et al., 2000b), an experi-

Table II. *Comparison of desirable attributes of well-studied CAM models*

Attribute	Common Ice Plant (*M. crystallinum*)	Mother-of-Thousands (*K. daigremontiana, K. blossfeldiana, K. fedtschenchoi*)	Balsam Apple (*Clusia minor, C. major, C. rosea*)	Pineapple (*A. comosus*)
Family	Aizoaceae (dicot)	Crassulaceae (dicot)	Clusiaceae (dicot)	Bromeliaceae (monocot)
Growth habit	Annual	Perennial	Perennial	Semiperennial
Commercial/horticultural importance (relative value)	Ornamental, ground cover, and fire break ($)	Ornamental ($$)	Ornamental (−)	Edible crop ($$$$)
Mode of CAM	C_3-CAM and stress inducible	Obligate and developmentally regulated	C_3-CAM, rapid and reversible	Obligate and developmentally regulated
Propagation mode	Seed	Clonal	Clonal	Clonal
Seed production	10,000–15,000 plant^{-1}[a]	Poor to none	None	Poor to none
Growth rate (to adulthood)	Rapid (6 weeks)	Intermediate (2–3 mo)	Intermediate (2–3 mo)	Slow (18–24 mo)
Mutant collections?	Ethylmethane sulfonate, fast neutron, and gamma irradiated	No	No	No
Transgenic plants?	No (callus, yes)	Yes	No	Yes
Transformable?	Yes	Yes	?[b]	Yes
Genome size (Mb)	390	790–1,500	?	526
No. of expressed sequence tags (ESTs) available	>15,000	0	0	0
Microarray availability?	Yes	No	No	No

[a] Soil-grown plants under standard laboratory conditions in 1-L pots. [b] Question marks indicate that no information is available.

mental platform for future transgenic analysis in common ice plant appears highly feasible.

The other major limitation for CAM research has been the lack a genetic model with a wealth of available molecular genetic information, such as the complete nucleotide sequence of the genome or at the very least, sizeable collections of ESTs. The common ice plant genome is approximately 390 Mb, as estimated by flow cytometry (DeRocher et al., 1990) in nine chromosomes ($2n = 18$; Adams et al., 1998) or approximately 2.5 times larger than the Arabidopsis genome (approximately 145 Mb) and slightly smaller than the rice genome (approximately 420 Mb). The common ice plant genome is also smaller than all other CAM models for which such data are currently available. For example, the pineapple genome ($2n = 25$) is somewhat larger, with a DNA content approximately 3.7 times the size of the Arabidopsis genome (Arumuganathan and Earle, 1991; Williams and Fleisch, 1993), whereas *K. fedtschenkoi* and *K. blossfeldiana* are two (approximately 790 Mb) and four times (approximately 1,500 Mb) the size, respectively, of the common ice plant genome (DeRocher et al., 1990). Thus, the small size of the common ice plant genome makes it a most attractive target for genome sequencing.

In lieu of genomic sequence information, the availability of information-rich sequence data from EST collections would add strong incentives for investigators to invest in a particular CAM model. Although cDNA libraries are available for *K. daigremontiana* (Bartholomew et al., 1996) and *K. fedtschenkoi* (Hartwell et al., 1999), the most comprehensive collection of cDNA libraries for any CAM plant is available for the common ice plant. More than 30 cDNA libraries exist from tissues that span the entire life cycle, from seedling to adult and flowering stages, as well as different tissues such as meristems, roots, shoots, leaves, epidermal bladder cells, flowers and seed capsules, and different stress treatments (Bohnert and Cushman, 2001). Furthermore, more than 15,000 ESTs are now available (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html; Bohnert and Cushman, 2001). In addition, a gene index has been recently created that allows easy access to the EST sequence information in the form of nonredundant genes (singletons) and tentative consensus sequences derived from redundant cDNAs (http://www.tigr.org/tdb/mcgi/). However, similar investments in other intensively studied models such as *K. daigremontiana* and *Clusia spp.* in which cDNA libraries are also under development (T. Taybi and A.M. Borland, personal communication) will be needed for comparative analyses of the functional significance of genes encoding signaling and regulatory components, enzymes, and transporters and to extend cross-species comparison beyond current physiological or biochemical investigations.

Abundant molecular genetic resources will facilitate integrative approaches to phenomena ranging from gene expression to gas exchange characteristics. Such integration is required to identify and distinguish the functional contribution and regulation of specific gene products, especially among circadianly regulated genes. Large EST collections and associated databases provide the foundation of nucleotide sequence information on which to build anticipated genome sequencing efforts (see below), as well as materials with which to print cDNA-based microarrays or to synthesize oligonucleotide-based GeneChips for large-scale gene expression-profiling experiments. Extensive or comprehensive expression data can often provide important clues about the function of specific isogenes in CAM or implicate roles in CAM for previously uncharacterized genes. Analysis of the existing common ice plant EST database compiled from salinity-stressed, CAM-induced plants indicates the presence of large numbers of genes, perhaps up to several thousand, that are not represented in other plant databases (Bohnert and Cushman, 2001). Such unknown or novel ESTs in the common ice plant database may arise, in part, from the evolutionary distance between common ice plant and the other plant models. We also expect that gene family expansion has occurred in the common ice plant, a native of the Namib Desert, to meet the additional requirements of CAM for long-term survival and reproductive success in arid environments. Evidence for this can be seen in, for example, the PEPC gene family. In Arabidopsis, this gene family is comprised of four members. In the common ice plant, however, at least six members make up this gene family, with only one specifically recruited to function in CAM (Cushman and Borland, 2001).

SEQUENCING A CAM PLANT GENOME?

Recent technological improvements in high-throughput, automated DNA sequencing systems and access to large capacity sequencing facilities make it reasonable to call for the sequencing of the complete genome of a CAM plant in the near future. The common ice plant is a logical choice for such an undertaking because it has the smallest genome among well-studied CAM models and the largest EST collection for gene identification (Table II). This effort will also provide important genomic information for comparative genomic studies of a species within the Caryophyllales. Most genome sequencing efforts target the major crop species in the Cruciferae, Poaceae, and Solanaceae. In contrast, very few Caryophyllales, which includes such plant families as the Aizoaceae, Amaranthaceae, Cactaceae, Chenopodiaceae, Caryophyllaceae, Phytolaccaceae, and Portulacaceae, are targets for genomic sequencing efforts because most are crop or ornamental species of relatively minor economic value. Yet, many spe-

cies in the order Caryophyllales have evolved to colonize environments characterized by water deficit, salinity, or extreme temperatures. As such, these species can be expected to be useful sources of novel genes involved in extending unusual biochemical pathways for plant secondary metabolites or abiotic stress tolerance. For example, many species of the Caryophyllales accumulate chromogenic betacyanins instead of anthocyanins and other complex substituted flavonoids. Thus, access to complete sequence information for the common ice plant would facilitate discovery of genes with CAM-specific functions or regulation (e.g. circadianly regulated genes), as well as of new gene products for abiotic stress adaptation and natural product biosynthesis and chemistry (Vogt et al., 1999a, 1999b).

PERSPECTIVES

The C_3 and C_4 photosynthetic pathways have been extensively investigated at the molecular genetic level. Much of this research has been greatly facilitated by the availability of excellent and well-studied genetic models and an abundance of cDNA and genomic sequence information. In contrast, our understanding of the complex regulation of the CAM photosynthetic pathway has lagged behind these other models. However, recent advances toward the creation of one or more viable genetic models for CAM, coupled with increasing availability of gene sequence and expression information, forecast a bright and productive future for CAM researchers. Future development and application of genomic, proteomic, and metabolic profiling technologies in selected CAM models such as the common ice plant is expected to rapidly improve our understanding of CAM induction by environmental and developmental influences and the circadian rhythms that dictate the diel patterns of CO_2 fixation characteristic of CAM plants. Thus, the greatest challenge facing CAM researchers in the future will be to develop teams of interdisciplinary researchers using genomic, biochemical, and physiological research approaches in selected CAM models. This approach will provide an integrated view of the complex regulatory dynamics that allow such remarkably plastic responses to the environment that has become one of the great hallmarks of CAM plants.

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