Adaptation of Chlamydomonas reinhardtii High-CO₂- Requiring Mutants to Limiting CO₂

Kensaku Suzuki² and Martin H. Spalding*  
Department of Botany, Iowa State University, Ames, Iowa 50011

ABSTRACT

Photosynthetic characteristics of four high-CO₂-requiring mutants of Chlamydomonas reinhardtii were compared to those of wild type before and after a 24-hour exposure to limiting CO₂ concentrations. The four mutants represent two loci involved in the CO₂-concentrating system of this unicellular alga. All mutants had a lower photosynthetic affinity for inorganic carbon than did the wild type when grown at an elevated CO₂ concentration, indicating that the genetic lesion in each is expressed even at elevated CO₂ concentrations. Wild type and all four mutants exhibited adaptive responses to limiting CO₂, characteristic of the induction of the CO₂-concentrating system, resulting in an increased affinity for inorganic carbon only in wild type. Although other components of the CO₂-concentrating system were induced in these mutants, the defective component in each was sufficient to prevent any increase in the affinity for inorganic carbon. It was concluded that the genes corresponding to the ca-1 and pmp-1 loci exhibit at least partially constitutive expression and that all components of the CO₂-concentrating system may be required to significantly affect the photosynthetic affinity for inorganic carbon.

When grown under ordinary air (0.04% CO₂, 21% O₂), Chlamydomonas reinhardtii cells utilize inorganic carbon (C₃) very efficiently for photosynthesis. Even though the photosynthetic CO₂ fixation pathway and properties of rubisco, 1,5-bisphosphate carboxylase/oxygenase in this alga are the same as those in C₃ plants, Chlamydomonas cells grown at limiting CO₂ concentrations (air-adapted cells) have a much higher photosynthetic affinity for C₃ than that of terrestrial C₃ plants and exhibit no significant O₂ inhibition of photosynthesis (1, 3). Badger et al. (1) demonstrated that these characteristics most likely result from the operation of a CO₂-concentrating system. This system apparently is only fully functional when the cells adapt to low CO₂ conditions, since the cells exhibit much more “C₃-like” characteristics when grown under 5% CO₂ (CO₂-enriched cells). The CO₂-concentrating system has been suggested to involve at least two essential components: C₃ transport (1, 8, 9, 12, 18, 20) and intracellular carbonic anhydrase (CA) responsible for the supply of CO₂ for photosynthesis through dehydration of accumulated bicarbonate (1, 9, 11, 13, 19, 21).

Although intracellular CA is thought to be one of the essential components of the CO₂-concentrating system, most of the CA activity observed in air-adapted Chlamydomonas is periplasmic rather than intracellular (4). It has been shown that the periplasmic CA is not an essential component of the CO₂-concentrating system under all conditions, because it is not required for C₃ utilization at acidic pH (9, 25). It has been suggested that the periplasmic CA facilitates CO₂ uptake under alkaline, CO₂-limiting conditions by dehydration of bicarbonate (9, 10).

Exposure of CO₂-enriched Chlamydomonas cells to limiting CO₂ concentrations results in an apparent induction of the CO₂-concentrating system as exemplified by an increased photosynthetic affinity for C₃ and an apparent increase in the C₃ transport activity (1, 14, 16, 19). Induction of specific polypeptides during adaptation of Chlamydomonas cells to limiting CO₂ concentrations has also been reported recently (2, 7, 16). However, the periplasmic CA is the only identified enzyme that has been shown to be induced by limiting CO₂ concentrations in parallel with the increase of photosynthetic affinity for C₃. Although thought to be an essential component of the CO₂-concentrating system, the internal CA has recently been reported to be present in both CO₂-enriched and air-adapted cells (13).

It is still not known how many components are involved in the CO₂-concentrating system or what essential components are induced during adaptation of the cells to limiting CO₂ concentrations. We have observed the changes in photosynthetic characteristics that occur when CO₂-enriched cells of wild type and four mutants of Chlamydomonas adapt to limiting CO₂ concentrations. Mutants representing the two loci included in this study have previously been characterized after induction of the CO₂-concentrating system (19, 20), but have not been characterized prior to the induction of the system. It was of interest to determine whether the mutations resulted in any significant physiological differences from wild-type Chlamydomonas in CO₂-enriched cells, in other words, whether the defective genes were expressed when the CO₂-concentrating system was not induced. Since the defective genes apparently were expressed prior to induction of the CO₂-concentrating system, it was also of interest to evaluate whether induction of the nondefective components of the system resulted in any increase in the affinity of the mutant cells for C₃ in photosynthesis. Although apparent induction of periplasmic CA and other characteristic components of the

1 This work was supported by grant CR8R-1-1591 from the Competitive Research Grants Office of the U.S. Department of Agriculture.
2 Present address: Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, Japan.
3 Abbreviations: C₃ inorganic carbon; CA, carbonic anhydrase; CO₂R, high-CO₂-requiring.
CO₂-concentrating system occurred, only in the wild-type cells was any substantial change in the photosynthetic affinity for C₄ observed.

**MATERIALS AND METHODS**

**Algal Strains and Culture Conditions**

Chlamydomonas reinhardtii strains CC124 mt−, CC125 mt+, CC198 mt−, CC801 mt−, CC1615 mt−, CC1861 mt−, and CC1930 mt− were supplied by the Chlamydomonas culture collection at Duke University (Durham, NC). Strains 2137 mt+ (22), CC124, CC125, CC801, and CC1615 were maintained on solid acetate medium (1.5% agar) in the light (26). Arginine-requiring mutants CC1861, CC1930, argcsp4 mt− (a gift from Dr. B. Sears), and y6arg7 mt− (a gift from Dr. C. Ford) were maintained on YA medium (solid acetate medium + 4 g/L yeast extract) in the light. High-CO₂-requiring (CO₂R) mutant strains ca-1-12-1C mt+ (19), pmp-1-16-5K (20), 18-6A mt+ (21, 23), and 18-7C mt+ (21, 23), and progeny from crosses involving these mutant strains were maintained on acetate medium in the dark, except that the progeny from crosses involving arginine-requiring mutants were maintained on YA medium in the dark (26).

**Physiological Analyses**

Cells were grown photoautotrophically in a liquid minimal medium (26) on a gyratory shaker (150 rpm) and aerated with 5% CO₂ in air (CO₂-enriched cells). "Air-adapted cells" were aerated with air for about 24 h prior to use, after being grown initially under 5% CO₂.

Photosynthetic O₂ exchange was measured at 25°C using a Clark-type O₂ electrode (Rank Brothers, Bottisham, United Kingdom) with the cells suspended in 1 mL of CO₂-free Mops-Tris (20 mm Mops, pH 7.4) after washing twice with the same buffer. The reaction was initiated by adding an appropriate concentration of NaHCO₃ after confirming the cessation of O₂ evolution under illumination (500 μmol photons m⁻² s⁻¹).

Accumulation of C₄ was determined using silicone oil filtering centrifugation (1, 5). The cells were suspended with 1 mL of CO₂-free buffer. The suspension (500 μL) was placed in the O₂ electrode chamber and incubated with 0.1 μCi of ¹³C's in the dark under illumination until O₂ evolution ceased. After adding NaH¹³CO₃, 200-μL aliquots of the cell suspension were layered over 100 μL of silicone oil (Wacker AR20:AR200, 5:1) in 400-μL centrifuge tubes in the light. The reaction was terminated 30 s after adding NaH¹³CO₃ by centrifugation through the silicone oil into 50 μL of 5 M KOH. Fixation of C₄ was measured at the same time by determining acid-stable ¹³C in the pellet. The intracellular volume was calculated using [¹⁴C]sorbitol and ¹³C's (5).

Carboxylase enzyme assays were performed by monitoring pH change at 2°C in 25 mM barbital-buffered solution (17). Enzyme units were calculated from the equation: U = tᵣ/tᵢ − 1, where tᵣ and tᵢ represent the time(s) measured for the pH change (8.3 to 7.3) with buffer alone (tᵢ) and with sample (tᵣ).

Chlorophyll was determined after extraction into 96% (v/v) ethanol (27).

**Genetic Analyses**

Gametogenesis, mating, zygote maturation, and germination were performed as described by Sears et al. (15). Each CO₂R mutant was crossed with the mt− strains, CC198, CC801, CC1615, CC1930 (arg⁻2), argwsp4 (arg⁻2), CC1861 (arg⁻7), or y6arg7 (arg⁻7) for tetrad analysis to obtain mt−CO₂R mutants and/or to combine the CO₂R mutants with the arg⁻2 or arg⁻7 markers. Strain CC801 is a spectinomycin-resistant mutant (spr-1), but in this work it was used as a wild type with respect to CO₂ requirement. Mating type was determined by mating with CC124 (mt−) and CC125 (mt+). Complementation analyses were performed using arg⁻2 and arg⁻7 to select diploids (6, 11). Phenotypes of tetrads and diploids were determined using spot tests on agar plates (26).

**RESULTS**

**Genetic Analysis of 18-6A and 18-7C**

None of the crosses among the CO₂R mutants, ca-1-12-1C × 18-6A, ca-1-12-1C × 18-7C, and 18-6A × 18-7C, produced wild-type haploid progeny, with about 3000 germinated zygotes observed from each cross (data not shown). The crosses between each CO₂R mutant and wild type resulted in 2:2 segregation in all the tetrads tested (25 for 18-6A × wild type, 23 for 18-7C × wild type) with respect to the CO₂R phenotype (no data shown). Mutations in ca-1-12-1C, 18-6A, and 18-7C were not able to complement each other (Table I). Diploids from any cross among ca-1-12-1C, 18-6A, and 18-7C required elevated CO₂ for photoautotrophic growth, and the Kₙs(C₄) of each diploid was similar to those of the mutant haploids (data not shown). However, these three mutants were each able to complement pmp-1-16-5K (Table I). The above observations indicate that the CO₂R phenotypes of 18-6A and 18-7C result from single-gene, nuclear mutations, and, since neither recombination nor complementation occurred among ca-1-12-1C, 18-6A, and 18-7C, indicate that the mutations in 18-6A and 18-7C are probably in the same locus as that of ca-1-12-1C, the ca-1 locus (19).

**Adaptation to Limiting CO₂ Concentrations**

The photosynthetic response to CO₂ concentration for both air-adapted and CO₂-enriched cells of wild-type *Chlamydomonas* and two CO₂R mutants is compared in Figure 1 at two O₂ concentrations. As previously reported (20, 21), the apparent affinity for CO₂ of air-adapted *Chlamydomonas* was 25 μM (5).

**Table I. Phenotypes of Diploids Created by Crosses between arg-2 and arg-7 Haploid Strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>12-1C</th>
<th>18-6A</th>
<th>18-7C</th>
<th>18-5K</th>
<th>wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-1C</td>
<td>CO₂Ra</td>
<td>CO₂R</td>
<td>CO₂R</td>
<td>wt</td>
<td>ND</td>
</tr>
<tr>
<td>18-6A</td>
<td>CO₂R</td>
<td>CO₂R</td>
<td>CO₂R</td>
<td>wt</td>
<td>ND</td>
</tr>
<tr>
<td>18-7C</td>
<td>CO₂R</td>
<td>CO₂R</td>
<td>CO₂R</td>
<td>wt</td>
<td>ND</td>
</tr>
<tr>
<td>16-5K</td>
<td></td>
<td>CO₂R</td>
<td></td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>wt</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*CO₂Ra, elevated CO₂ required for photoautotrophic growth. **wt, wild type. †ND, not determined.
A variety of characteristics associated with the CO₂-concentrating system were compared for air-adapted and CO₂-enriched cells of wild type and CO₂R mutants ca-1-12-1C, pmp-1-16-5K, 18-6A, and 18-7C (Table II). In wild type, $K_{\text{C}}(\text{C})$ of photosynthesis under 21% O₂ was 200 μM for CO₂-enriched cells and decreased to 25 μM after 24 h of exposure to limiting CO₂ concentrations. However, as would be expected from the Ci response curves of Figures 2B and 2C, the $K_{\text{C}}(\text{C})$ of photosynthesis was not significantly changed in any of the CO₂R mutants following adaptation to limiting CO₂ concentrations and was much higher even than that of CO₂-enriched cells of wild type (Table II).

In spite of the apparent lack of change in the photosynthetic affinity of the mutants for Ci, CA activity in all four mutants, as well as in the wild type, increased dramatically following adaptation to limiting CO₂ concentrations (Table II). Although CA activity measured in intact cells (periplasmic CA) was less than half of the total activity, this probably results from some limit to the accessibility of the periplasmic CA. It has been shown previously that most of the CA activity is actually outside the plasma membrane in Chlamydomonas (4). The total CA activity (sonicated cells) of each of the four mutants was lower than that of wild type, but, contrary to previous reports (19, 21), that of ca-1-12-1C was not markedly lower (Table II). The reason for this contradiction with earlier work is not readily apparent, although the difference may be related to the loss of the light sensitivity initially observed in this mutant (22).

The Ci accumulation ratio (internal/external) in air-adapted cells of wild type was 16.5 (about 1.3 mm internal Ci) after 30 s photosynthesis in 0.08 mM NaH¹⁴CO₃, whereas the ratio was only 2.6 in CO₂-enriched cells (Table II). The Ci accumulation ratio also increased upon adaptation to limiting CO₂ in the three ca-1 mutants (Table II), although overaccumulation of Ci occurred in both air-adapted and CO₂-enriched cells of these mutants, to about 5.5 to 7.0 mm Ci, and 2.9 to 3.9 mm Ci in air-adapted cells and CO₂-enriched cells, respectively. In pmp-1-16-5K, on the other hand, Ci apparently was not accumulated in either air-adapted or CO₂-enriched cells after 30 s of photosynthesis in 0.08 mM NaH¹⁴CO₃ (Table II).

Both air-adapted and CO₂-enriched cells of wild type and pmp-1-16-5K fixed about 83% and 96% of the $^{14}$C taken up into cells during 30 s of photosynthesis (Table II). However, both air-adapted and CO₂-enriched cells of the three ca-1 mutants fixed less than 20% of $^{14}$C taken up into the cells. These results indicate that the wild-type and pmp-1 cells were able to use intracellular Ci efficiently for photosynthesis, but that the ca-1 mutants could not utilize most of the accumulated intracellular Ci under either growth condition.

**DISCUSSION**

So far, two requisite components of the microalgal CO₂-concentrating system have been defined genetically by CO₂R mutants of Chlamydomonas reinhardtii: internal CA (11, 13, 19) and Ci transport (20). Previously, only characteristics of such mutants following adaptation to limiting CO₂ concentrations have been investigated. Comparisons of the photosynthetic characteristics of four mutants, representing these two genetic loci, and of wild-type Chlamydomonas prior to
induction, as well as changes in the characteristics following induction of the CO₂-concentrating system by limiting CO₂ concentrations have been reported here.

Mutants representing the two loci investigated here have been extensively characterized previously, but only as air-adapted cells. Because of increased interest in components of the CO₂-concentrating system induced by limiting CO₂ concentrations (2, 7, 13, 14, 16), it was of interest to determine whether the components corresponding to the genetic lesions of ca-1 and pmp-1 are induced or constitutive components of the system. Comparisons of the photosynthetic characteristics of CO₂-enriched cells of the mutants and wild-type Chlamydomonas indicated that a lesion in either locus resulted in a greatly reduced affinity for Ci even in CO₂-enriched cells (Table II). If the genes corresponding to the loci ca-1 and pmp-1 were expressed only in air-adapted cells, then the photosynthetic characteristics of the mutants and wild type should have been nearly the same in CO₂-enriched cells. Our conclusion is that the genes representing both loci must be expressed at least in part constitutively.

One of the distinctive characteristics of ca-1-12-1C is an overaccumulation of intracellular Ci, which is thought to represent bicarbonate accumulation resulting from Ci transport in the absence of intracellular CA activity (9, 13, 19, 21, 24). This type of overaccumulation of Ci was observed in both CO₂-enriched and air-adapted cells of the three ca-1 mutants investigated here, although it was less extreme in CO₂-enriched cells (Table II). In addition to indicating that the defect in the ca-1 mutants impacts on CO₂-enriched as well as air-adapted cells, these results suggest that active Ci transport across the chloroplast envelope and/or cytoplasmic membrane occurs in both air-adapted and CO₂-enriched cells of these mutants, although at a lower rate in CO₂-enriched cells. This probably is the situation in wild-type cells as well, even though the lack of significant Ci accumulation in CO₂-enriched wild-type cells (Table II) seems to argue against this suggestion, since it has been observed previously that exposure of wild-type Chlamydomonas to the permeable CA inhibitor ethoxyzolamide results in overaccumulation of intracellular Ci in CO₂-enriched as well as air-adapted cells (1, 13). The ability of both air-adapted and CO₂-enriched cells to transport Ci would be consistent with the affinity for Ci of CO₂-enriched pmp-1 cells being lower than wild type, since this mutation is thought to affect Ci transport.

Internal CA, which apparently supplies CO₂ to ribulose-1,5-bisphosphate carboxylase/oxygenase from accumulated bicarbonate, has been reported to be present in both air-adapted and CO₂-enriched cells of C. reinhardtii (13), although the relative amount of CA activity in the two cell types was not investigated. Although Ci accumulation into the cells was not observed, CO₂-enriched cells of wild type and both air-adapted and CO₂-enriched cells of pmp-1-16-5K utilized intracellular Ci quite efficiently for photosynthesis (Table II), suggesting the presence of internal CA in both CO₂-enriched and air-adapted cells. Further support for this suggestion comes from contrasting this efficient use of intracellular Ci with the apparently inefficient use of intracellular Ci by the ca-1 mutants (Table II), which are thought to be deficient in intracellular CA. The overaccumulation of Ci and the inefficient use of the accumulated internal Ci in both CO₂-enriched and air-adapted cells of the putative internal CA mutant, ca-1, are entirely consistent with the presence in wild type of internal CA activity in both cell types. Since the defects in both the pmp-1 and ca-1 mutants apparently were expressed in CO₂-enriched as well as air-adapted Chlamydomonas cells, the effect of induction of

### Table II. Photosynthetic Characteristics of Wild Type (2137 mt+) and Four High-CO₂-Requiring Mutants of C. reinhardtii

| Strain | Cell Type   | K₅₅(Ci) (μmol O₂·mg Chl⁻¹·h⁻¹) | PSN (%) | Ci Ratio of Ci (in/out) | C Fixed/C Total C | CA Activity
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2137</td>
<td>Air-adapted</td>
<td>25</td>
<td>123</td>
<td>16.5</td>
<td>0.85</td>
<td>172.2</td>
</tr>
<tr>
<td></td>
<td>CO₂-enriched</td>
<td>200</td>
<td>171</td>
<td>2.6</td>
<td>0.83</td>
<td>14.0</td>
</tr>
<tr>
<td>16-5K</td>
<td>Air-adapted</td>
<td>600</td>
<td>88</td>
<td>1.9</td>
<td>0.96</td>
<td>106.9</td>
</tr>
<tr>
<td></td>
<td>CO₂-enriched</td>
<td>550</td>
<td>178</td>
<td>1.6</td>
<td>0.92</td>
<td>4.7</td>
</tr>
<tr>
<td>12-1C</td>
<td>Air-adapted</td>
<td>635</td>
<td>197</td>
<td>88.2</td>
<td>0.14</td>
<td>136.9</td>
</tr>
<tr>
<td></td>
<td>CO₂-enriched</td>
<td>640</td>
<td>210</td>
<td>49.3</td>
<td>0.13</td>
<td>3.4</td>
</tr>
<tr>
<td>18-6A</td>
<td>Air-adapted</td>
<td>620</td>
<td>108</td>
<td>75.4</td>
<td>0.15</td>
<td>112.8</td>
</tr>
<tr>
<td></td>
<td>CO₂-enriched</td>
<td>740</td>
<td>188</td>
<td>35.8</td>
<td>0.29</td>
<td>5.6</td>
</tr>
<tr>
<td>18-7C</td>
<td>Air-adapted</td>
<td>900</td>
<td>139</td>
<td>69.4</td>
<td>0.17</td>
<td>127.0</td>
</tr>
<tr>
<td></td>
<td>CO₂-enriched</td>
<td>810</td>
<td>176</td>
<td>48.8</td>
<td>0.48</td>
<td>5.9</td>
</tr>
</tbody>
</table>

*K₅₅ of photosynthesis for Ci at 21% O₂ (μmol O₂·mg Chl⁻¹·h⁻¹) at 5 mM NaHCO₃ and pH 7.4.
*Photosynthetic rate (μmol O₂·mg Chl⁻¹·h⁻¹) at 5 mM NaHCO₃ and pH 7.4.
*Ratio of Ci internal/external. Ci accumulation was determined 30 s after incubating with 0.08 mM NaHCO₃ (pH 7.4).
*Ratio of fixed Ci to total Ci taken up during Ci accumulation experiments.
nondefective components of the CO$_2$-concentrating system on the photosynthetic affinity of the mutant cells for C$_i$ was evaluated. Although other characteristic components of the CO$_2$-concentrating system were induced in all four mutants upon exposure to limiting CO$_2$ for 24 h, the apparent $K_{S,Ci}(C_i)$ was not significantly decreased. In the three ca-1 mutants and in the pmp-1 mutant, the periplasmic CA was induced normally following exposure of the cells to limiting CO$_2$ concentrations (Table II). Although it is not clear whether the periplasmic CA is an essential component of the CO$_2$-concentrating system, induction of this enzyme indicates that the mutant cells were undergoing adaptive changes in response to the limiting CO$_2$ concentration.

All four mutants also exhibited an induction of specific polypeptides (ref. 7; M. Spalding, in preparation) as observed with wild-type cells exposed to limiting CO$_2$ concentrations (2, 7, 16). The induction of these polypeptides supports the contention that induction of the CO$_2$-concentrating system occurs in these mutants when exposed to limiting CO$_2$.

During adaptation to limiting CO$_2$, wild-type _Chlamydomonas_ cells typically exhibit an increase in C$_i$ transport activity, as indicated by an increase in the C$_i$ accumulation ratio (Table II). The C$_i$ accumulation ratio of the three ca-1 mutants also increased in response to exposure to limiting CO$_2$ (Table II), again indicating adaptive changes normally associated with the induction to the CO$_2$-concentrating system. On the other hand, the C$_i$ accumulation ratio of the pmp-1 mutant did not increase following exposure to limiting CO$_2$. However, this mutant is thought to be deficient in C$_i$ transport, so it would not be expected to exhibit an increase in C$_i$ transport activity.

Based on the observations reported in this paper, it was concluded that the genes corresponding to the mutant loci ca-1 and pmp-1 are expressed in and impact on the photosynthetic characteristics of CO$_2$-enriched as well as air-adapted cells. The data also indicate that C$_i$ transport, although active in both CO$_2$-enriched and air-adapted cells, probably increases in activity during induction of the CO$_2$-concentrating system. Thus, the pmp-1 locus, thought to represent an essential component of C$_i$ transport, might be up-regulated under limiting CO$_2$ concentrations. The data presented here and the conclusion that the putative mutations in internal CA (ca-1 mutants) are expressed in both cell types also are in agreement with the report that internal CA activity is present in both CO$_2$-enriched and air-adapted cells, but they do not indicate whether there is any up-regulation of this activity under limiting CO$_2$ concentrations.

Since the genes corresponding to the defects in the four mutants appear to be expressed in both CO$_2$-enriched and air-adapted cells, it was expected that induction of the nondefective components of the CO$_2$-concentrating system would result in some increase in the photosynthetic affinity for C$_i$ in each mutant. Based on the observations presented here, it is clear that all four of these mutants investigated exhibit adaptive responses to limiting CO$_2$ that are characteristic of the induction of the CO$_2$-concentrating system. However, photosynthetic affinity for C$_i$ did not change significantly in any of the four mutants after adaptation to limiting CO$_2$. Although other components of the CO$_2$-concentrating system were induced in these mutants upon adaptation to limiting CO$_2$, the defective component in each was apparently sufficient to prevent any significant increase in the affinity for C$_i$. Therefore, this evidence suggests that all components of the CO$_2$-concentrating system may be required to significantly affect the photosynthetic affinity for C$_i$ when _Chlamydomonas_ cells adapt to limiting CO$_2$, and all components of the CO$_2$-concentrating system may be required for the system to significantly reduce the $K_{S,Ci}(C_i)$ of the cells.

## LITERATURE CITED


Copyright © 1989 American Society of Plant Biologists. All rights reserved.