Acclimation of photosynthetic microorganisms to changing ambient CO₂ concentration

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Photosynthetic microorganisms can acclimate to a wide range of CO₂ concentration, from as low as 0.001% to ≈10% CO₂ (vol/vol in the air in equilibrium with their environment). Some can even grow in the presence of 40% CO₂ (1). Acclimation to a limiting CO₂ level, well below the $K_m(\text{CO}_2)$ of their carboxylating enzyme, Rubisco (ribulose 1,5-bisphosphate carboxylase/oxygenase), is achieved by substantial physiological and structural changes at various cell levels (2–4). The most prominent of these is the induction of a CO₂ concentrating mechanism (CCM), which raises the [CO₂] in close proximity to Rubisco. The latter is for the most part located in pyrenoids or carboxysomes in eukaryotes and prokaryotes photosynthetic microorganisms, respectively (5). This CCM involves light energy-dependent inorganic carbon uptake and accumulation of HCO₃[−] within the cell. In cyanobacteria, the accumulated HCO₃[−] penetrates the carboxysomes where carbonic anhydrase (CA) catalyzes the formation of CO₂ in the close vicinity of Rubisco. The elevated CO₂ concentration is thus confined to the carboxysomes (3). This model is most likely also applicable to eukaryotic algae where pyrenoids, densely packed with Rubisco and also containing CA, may have the same function as carboxysomes (2–7). The elevated [CO₂] in these bodies compensates for the relatively low affinity of Rubisco for CO₂ and consequently also decreases photorespiration.

Exciting contributions appearing in this issue of PNAS, which have emerged simultaneously from the laboratories of Hideya Fukuzawa (8) and Donald Weeks (9), have now identified a key component of the transduction pathway between ambient [CO₂] and expression of the CO₂-responsive genes involved in the CCM. Several CO₂-responsive genes already have been identified, both in the green alga *Chlamydomonas reinhardtii*, which is the subject of Fukuzawa's and Weeks' research, and cyanobacteria. Various approaches have been used, including analyses of changes in the polypeptide patterns (2), detection of low-CO₂-induced cDNAs

(10), and characterization of mutants impaired in specific steps of the CCM (2, 11–15). Doubtless many more CO₂-responsive genes will be reported in the near future as a result of the application of technologies such as DNA microarray and proteomics. More than 40 low CO₂-induced genes recently have been detected in the cyanobacterium *Synechocystis* sp. PCC6803 (T. Ogawa, personal communication) and more than 170 in *Chlamydomonas* (H. Fukuzawa, personal communication). It should be noted that some of the apparently CO₂-sensitive genes may in fact respond to a general stress situation. For example, *ndhD3* (directly involved in CO₂ uptake in *Synechocystis*) is up-regulated both by high light (16) and low CO₂ (15), suggesting response to the reductive state of photosynthetic electron transport carriers. Further, some CO₂-responsive genes may not be involved in the CCM but in other CO₂-affected processes such as purine (17) or cyclophilin metabolism (18).

Information is lacking both with regard to the [CO₂] sensor, the DNA elements conferring CO₂ responsiveness on the relevant genes, and the components of the transduction pathway. On the basis of observed photosynthetic performance at various ambient CO₂ and pH levels, it has been postulated that [CO₂] is sensed at the plasmalemma by specific receptors (19). Modified acclimation behavior of *Chlamydomonas* mutants impaired in phosphoglycolate phosphatase activity (2) and of a *Synechococcus* sp. PCC7942 mutant overexpressing this enzyme (20) supported the notion that metabolites in the photorespiratory cycle may play an important part in the transduction pathway. The level of photorespiratory metabolites would be expected to increase with rise in the ratio [O₂]/[CO₂] and the consequent elevated oxygenase activity of Rubisco. These metabolites might be directly involved or might be sensed as starvation signals. Provision of organic nutrients down-regulates acclimation, indicating that ample supply of nutrients is sensed, and can counter acclimation even under low [CO₂]. Conversely, lack of certain nutrients, including

NO₃[−], may trigger acclimation even under sufficient CO₂ (21). Perception of the low CO₂ signal may alter during the course of the cell cycle as demonstrated by use of synchronously grown *Chlamydomonas* cells (22–24).

Information on the DNA elements that may confer responsiveness of gene expression to [CO₂] is scarce. Analysis of the promoter region of the low CO₂-induced *cmpA*, encoding a component of an ABC-type HCO₃[−] transporter (25) in *Synechococcus* PCC7942, led to the identification of enhancing and suppressing regulatory elements (26). Deletion of the latter potentiates transcription under high CO₂ (20). In *Chlamydomonas*, CAH1, which encodes a periplasmic CA, is among the genes up-regulated by low CO₂ in the light and down-regulated by high CO₂ or dark conditions. Analysis of its promoter region identified silencer and enhancer cis-elements, which control the promoter under low CO₂ conditions in the light (27), but, before the publication of the two papers in this issue (8, 9), transcription factors involved were not recognized.

Isolation of high CO₂-requiring *Chlamydomonas* mutants, *cia5* and C16, which appear to lack all of the presently recognized responses to low CO₂, opened the way to dissection of the signal transduction path between ambient [CO₂] and gene expression. Mutant *cia5* was produced by random mutagenesis (12) and C16 by gene interruption (28). In the two seminal papers in this issue (8, 9), it is proposed that the newly discovered component of the signal transduction pathway is a transcription factor. A genomic region that complements the phenotype of both *cia5* and C16 has been identified (8, 9) and the relevant gene has been designated *Cia5* and *ccm1*, respectively. *Ccm1* (CIA5) contains a putative zinc-finger motif in its N-terminal region (where the H54Y mutation in *cia5* is located) and a Gln repeat typical of transcription factors. In transformed onion epidermal cells, the *Ccm1*

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apparently was located in the nucleus (9) but this may not be the case in *Chlamydomonas* (H. Fukuzawa, personal communication). *ccm1* apparently is expressed constitutively, and Ccm1 is present under both high and low CO₂ conditions. It is thus plausible that Ccm1-dependent transcription of low CO₂-induced genes requires posttranslational modification of Ccm1 under low CO₂. Strikingly, when the *cia5* mutant was complemented with a truncated *ccm1* missing the region coding for the last 54 C-terminal amino acids, the transformant expressed low CO₂ characteristics even under high CO₂ conditions. It remains to be seen whether the putative protein kinase C phosphorylation site in the C terminus of Ccm1 (missing in the truncated construct; ref. 9) participates in the control of CO₂-dependent gene expression via phosphorylation/dephosphorylation cycles, or possibly even via phospho-relay cascade (see the proposed scheme in Fig. 1). Use of an anti-CCM1 antibody (H. Fukuzawa, personal communication) indicated that Ccm1 is not the nuclear protein that binds to the promoter region of CAH1 and presumably activates its transcription under limiting CO₂. Because CAH1 is among the genes under the control of Ccm1 it follows that if the latter is a transcription factor it must function higher in the hierarchy.

In cyanobacteria, transcription of *cmpA* is under the control of CmpR, belonging to the LysR transcription factor family (29). CcmN, a putative DNA-binding protein, is required for expression of *cmpA* in low CO₂-grown *Synechococcus* cells. In *Synechocystis*, transcription of *ndhD3* is up-regulated by low CO₂ (15) and repressed by NdhR. The latter is itself up-regulated by low CO₂ (30).

Fig. 1. A possible scheme for the transduction path between ambient [CO₂] and gene expression in *Chlamydomonas*.

In the race to break open the “black box” that up to now has enveloped the transduction path between ambient CO₂ concentration and expression of the CCM, researchers into *Chlamydomonas* thus have gained a signal advantage over their colleagues investigating cyanobacteria. Identification of other components of this pathway is likely to follow clarification of

the manner in which Ccm1 is modified in response to changing CO₂ concentration.

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