# Resolving the biological role of the Rhesus (Rh) proteins of red blood cells with the aid of a green alga

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The presence or absence of Rh antigens, abundant components of the red blood cell (RBC) plasma membrane, is a major cause of blood transfusion incompatibility and of hemolytic disease of the newborn. As deduced from studies on $Rh_{null}$ erythrocytes, the Rh complex of RBC includes the Rh-associated glycoprotein RhAG, the Rh polypeptides, CD47, LW proteins, and glycophorin B (1). RhAG is essential for the assembly of the Rh complex, and mutations in RhAG result in the $Rh_{null}$ syndrome (2). Despite their significance and intensive research, the biological role of the Rh protein complex remains controversial. This controversy may be accounted for by the fact that RhAG homologues are found in other mammalian tissues and in many lower organisms (3), including unicellular eukaryotes (4). Studies by Marini and colleagues (3) identified some homology (20–27% identity) between RhAG and Mep/Amt ammonium and methylammonium transporters in various organisms and suggested that the erythroid RhAG may function as an ammonium transporter (3). This finding was supported by the observation that RhAG (and its kidney-located homologue) could complement a yeast mutant impaired in ammonium uptake. Further, RhAG enhanced efflux of a preloaded methylammonium from yeast, suggesting that it might be involved in ammonium export as well (5). The notion that RhAG and its homologues are involved in $NH_3/NH_4^+$ transfer gained additional support by the observation that expression of RhCG, a mammalian nonerythroid homologue of RhAG, along the rat nephron, matched that expected from ammonium excretion activities of the respective nephron sections (6). An exciting contribution from the laboratory of S. Kustu (7) in this issue of PNAS provides supporting evidence for their earlier suggestion (4) that RH1 of the green alga *Chlamydomonas reinhardtii*, which is highly homologous to RhAG, may in fact function as a $CO_2$ channel. In their earlier study Soupene *et al.* (4) showed that the expression of *rh1* was strongly up-regulated after exposure of *Chlamydomonas* cells to high (3% vol/vol) levels of $CO_2$. In the present study (7) the authors used RNA interference technology to lower the abundance of RH1 mRNA. The three lines selected for further analysis did not express *rh1* but induced low-$CO_2$-dependent genes normally when grown in an air level of $CO_2$ and showed reduced growth under high-$CO_2$ conditions. Uptake of methylammonium hardly differed between the wild-type and the *rh1* RNA interference strains.

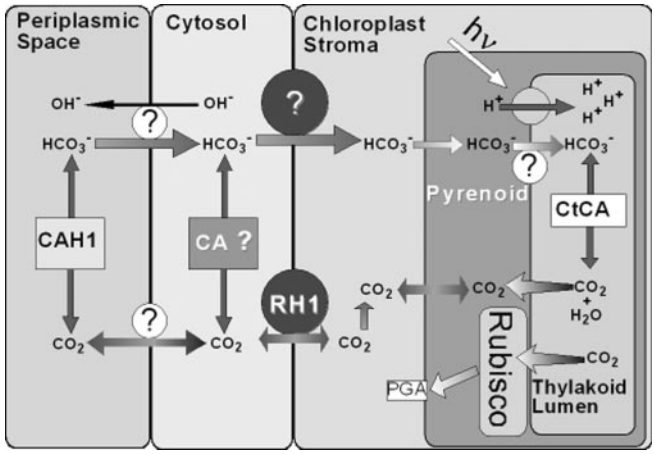


**Fig. 1.** A simplified scheme of inorganic carbon uptake and accumulation in *C. reinhardtii*. The putative function of RH1 as a chloroplast envelope-located $CO_2$ channel is emphasized. The means by which $HCO_3^-$ is taken up across the plasmalemma, the chloroplast envelope, and the thylakoid, if any, is not known. Possible involvement of other components, including the mitochondrial-located CA, LIP-36, and pmp-1 (8, 13), is not indicated. For the sake of simplicity, diffusion of $CO_2$ across the lipid bilayer of the various membranes is not shown. Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; PGA, 3-phosphoglyceric acid.

Many photosynthetic microorganisms possess inducible mechanisms that concentrate $CO_2$ at the carboxylation site, compensating for the relatively low affinity of ribulose-1,5-bisphosphate carboxylase/oxygenase for $CO_2$ and allowing acclimation to a wide range of $CO_2$ concentrations (see refs. 8–16 and references therein). The organization of the carboxysomes in prokaryotes and the pyrenoids in eukaryotes, and the presence of membrane mechanisms for inorganic carbon (Ci) uptake, are central to the activity of the $CO_2$-concentrating mechanism. The presence of multiple Ci-transporting systems in cyanobacteria has been indicated (17), but little is known about the mechanism of Ci uptake in eukaryotes, such as the green alga *Chlamydomonas* (13). As in most other photosynthetic organisms examined to date, *Chlamydomonas* can use either $CO_2$ or $HCO_3^-$ or both (13). Kinetic data based on initial rates of transport are available for Ci uptake in some organisms but difficult to obtain for *Chlamydomonas*, because the steady-state internal Ci concentration is attained rapidly. This finding is in agreement with the proposal that $CO_2$ can cross the membrane quickly by specific channels (7).

**RH1 of the green alga *Chlamydomonas reinhardtii* may function as a $CO_2$ channel.**

Facilitation of $CO_2$ formation from $HCO_3^-$ in the periplasmic space by carbonic anhydrase (CA) (18), encoded by *Cah1*; within the pyrenoid by thylakoid lumen-located CA (10, 19), encoded by *Cah3* (*ctCA*); and possibly the involvement of a mitochondrial-located CA,

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encoded by *mtCA* (20), enhances the supply of $CO_2$ to ribulose-1,5-bisphosphate carboxylase/oxygenase (Fig. 1). As opposed to $CO_2$ supply, there is far less information regarding the mechanism of $HCO_3^-$ uptake in *Chlamydomonas*. Nevertheless, isolated chloroplasts of *Chlamydomonas* can accumulate $HCO_3^-$ to levels higher than could be accounted for by passive diffusion (13). Furthermore, Ci uptake is higher in chloroplasts isolated from low- than from high-$CO_2$-grown cells (8). Genes involved in Ci uptake by *Chlamydomonas* are strongly induced when high-$CO_2$-grown cells are transferred to low-$CO_2$ conditions (12, 13, 19, 20). Mutants in which these genes were impaired required high $CO_2$ for growth. In contrast, expression of *rh1* is induced under high $CO_2$ and suppressed under low $CO_2$ (4, 7). The function of RH1 as a $CO_2$ channel, as suggested, would allow influx of $CO_2$ when all other (known) means of mediated Ci influx are suppressed (i.e., under high levels of ambient $CO_2$), whereas the absence of RH1 under low $CO_2$ would minimize wasteful leakage of $CO_2$ from the cells. Thus, assignment of RH1 as a $CO_2$ channel is novel to our understanding of the mechanisms and components involved in $CO_2$ acquisition by photosynthetic microorganisms.

Although not yet examined experimentally, the presence of a typical N-terminal transit peptide suggested that RH1 is located within chloroplast envelope (4) (Fig. 1) where the low-$CO_2$-induced LIP-36 also resides. Should RH1 be confined to the chloroplast envelope, transfer of $CO_2$ across the plasmalemma of *Chlamydomonas* may limit Ci uptake under high-$CO_2$ conditions.

**The function of RH1 as a $CO_2$ channel allows influx of $CO_2$ when other means are suppressed.**

The possibility that $CO_2$ does not merely diffuse through the bulk lipids within membranes was indicated in experiments where an aquaporin blocker severely inhibited $CO_2$ uptake by *Synechococcus* sp. strain PCC 7942 (21). Another means of passage of $CO_2$ across the membrane was suggested by Forster and colleagues (22) who provided 4,4′-diisothiocyanato-stilbene-2,2′-disulfonate to RBCs. This treatment resulted in reduced $CO_2$ permeability without affecting the CA activity. 4,4′-Diisothiocyanato-stilbene-2,2′-disulfonate is known to inhibit the $Cl^-/HCO_3^-$ exchanger (band 3) in the RBC membrane. Recent studies by Tanner (23) and Bruce *et al.* (24) showed that the two major complexes, Rh and the $Cl^-/HCO_3^-$ exchanger, are closely associated forming a single macrocomplex that may function as an integrated $CO_2/O_2$ gas exchange unit in the erythrocyte membrane.

Finally, both RBCs and the soil alga *Chlamydomonas* must cope with high $CO_2$ levels in their surroundings and have apparently adopted a similar channel mechanism to allow efficient exhaustion or uptake of $CO_2$. It is plausible that ability of the alga to acclimate to low $CO_2$ conditions was acquired simultaneously with development of the means to block the expression of *rh*.

This research was supported by grants from the Israel Science Foundation, the German Bundes Ministerium fur Bildung Wissenschaft, Forschung und Technologie, and the Avron-Evenari Minerva Center of Photosynthesis Research.

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