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Project Title:	Structure/Function of the Novel Proteins LCIB and LCIC in the Chlamydomonas CCM
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Specific Aims.

<u>Aim 1</u>. Determine the functional role of LCIB complexes in the microalgal CCM (Spalding).

1A. Verify role in Ci uptake and active CO_2 uptake (Spalding). LCIB complexes, specifically the LCIB/LCIC complex, are required in the microalgal CCM for active inorganic carbon (Ci) uptake in air levels of CO_2, and also contribute, along with Ci transporters (e.g., LCIA and HLA3), to active Ci uptake in CO_2 concentrations below air levels (very low CO_2). LCIB/LCIC is essential for an active CO_2 uptake pathway, whereas LCIA is essential for an active bicarbonate uptake pathway, and mutations in both eliminates the ability of Chlamydomonas to grow photoautotrophically in either very low CO_2 or in air levels of CO_2. Although LCIB mutants are unable to grow or assimilate CO_2 in air levels of CO_2, we have demonstrated that newly identified *lcic* mutants exhibit no identifiable growth or photosynthesis phenotype under any conditions. We have further demonstrated that *lcic* mutants still accumulate LCIB protein, that the LCIB protein forms an apparent LCIB homohexamer of the same apparent molecular size as the normal LCIB/LCIC heterohexamer, and that they fail to re-localize the LCIB complex to the peri-pyranoid space under very low CO_2 conditions, as is seen with the LCIB/LCIC complex in wild-type cells. Thus, LCIB can apparently assemble into a functional Ci uptake complex by itself, and the only role for LCIC may be to facilitate re-localization of the LCIB/LCIC complex in very low CO_2.

1B. Proteins functionally interacting with LCIB (Spalding). This sub-aim has focused on *su1*, an extragenic suppressor of the air-dier phenotype of *lcib* mutations. We have confirmed genetically that loss-of-function mutations in *LCI15* represent the *su1* locus and can suppress the air-dier phenotype of *lcib* mutants. Although we have not been able to identify proteins specifically interacting physically, it appears that *lci15* mutations result in an upregulation of LCIC and LCID, which may form a functional LCIC/LCID complex that functions in place of the LCIB/LCIC complex, thus suppressing the *lcib* mutant phenotype.

<u>Aim 2</u>. Investigate the formation and biochemical function of LCIB complexes (Spalding).

2A. LCIB Complex composition. (Spalding). As described above for Aim 1A, we have characterized *lcic* mutants and demonstrated a role for LCIC in LCIB/LCIC complex re-localization under very low CO_2 conditions. In experiments to determine whether LCIC interacts physically with proteins other than LCIB, we determined that both LCIC and LCIB can interact with the very similar, LCIB protein family member, LCID. In following up this observation, we have determined that LCID may function in a LCIC/LCID complex to replace LCIB/LCIC complex, but that this apparently does not happen except when LCI15 is mutated to become non-functional.

Publications: None, so far, although we are in the process of writing two manuscripts, one describing the characterization of *lcic* mutants and another describing the role of *LCI15* mutants in suppressing the *lcib* "air-dier" mutant phenotype.