During the past years of this project we have made progress relative to the two major goals of the proposal: (1) to study the biochemistry and regulation of the reductive TCA cycle of CO_2 fixation and (2) to probe the physiological role of a RubisCO-like protein (RLP). Both studies primarily employ the green sulfur bacterium *Chlorobium tepidum* as well as other photosynthetic bacteria including *Rhodospirillum rubrum* and *Rhodopseudomonas palustris*.

1. Reductive TCA pathway of CO_2 assimilation

Many diverse microorganisms use the reductive TCA (RTCA) pathway for CO_2 assimilation. Included are photoautotrophic and chemoautotrophic organisms that occupy important niches in various ecosystems. Inasmuch as the biochemistry and regulation of the RTCA pathway has been virtually neglected, especially in comparison to the Calvin-Benson-Bassham (CBB) reductive pentose pathway of CO_2 fixation, we sought to develop a system that would allow for detailed biochemical analysis of the RTCA enzymes and associated proteins, along with the genes that encode these proteins. We have focused on the green sulfur photosynthetic bacterium *Chlorobium tepidum*, a fast growing moderate thermophile originally isolated by Professor Mike Madigan and colleagues. Because of its rapid growth and relative ease to produce massive cell amounts via high-density fermentator vessels, *C. tepidum* has become the organism of choice for investigators interested in studying all aspects of the physiology and biochemistry of green sulfur bacteria. Moreover, this organism possesses a very convenient natural transformation system that allows routine genetic transfer and the generation of knockout mutations via homologous recombination at specific genetic loci. The first such mutations were generated in our laboratory [Hanson & Tabita, PNAS USA, 98 (2001), 4397-4402] and the laboratory of Prof. Donald Bryant, such that these protocols have now become relatively routine. Moreover, the genome of *C. tepidum* was recently sequenced. Thus, all the tools are in place for productive analysis of key processes catalyzed by this organism, in particular for analysis of the RTCA pathway and the rather unique RubisCO-like protein (RLP) that we first discovered during the last grant period of this project [Hanson & Tabita, 2001].

We have concentrated on the enzymology of the key proteins of this pathway, in particular pyruvate synthase (PS), α-ketoglutarate synthase (KGS), and ATP-citrate lyase (ACL). In addition, we have also focused on key electron transfer proteins that must provide needed reducing equivalents to PS and KGS, including two separate ferredoxins that were shown to be abundantly produced by this organism.

2. Physiological/biochemical/genetic studies on the RubisCO-like Protein (RLP)

During the 2000-2003 grant period we identified what we believe is an evolutional precursor to bona fide RubisCO in *C. tepidum*, the RubisCO-like protein (RLP) [Hanson & Tabita, 2001]. Typical bioinformatics software incorrectly indicates that RLP is RubisCO, however our previous experience with RubisCO enabled us to establish that *C. tepidum* RLP has substitutions in 9 out of the 19 residues known to be important for RubisCO-catalyzed CO_2 fixation. After purifying recombinant RLP, we showed that the RLP is not a bona fide RubisCO that catalyzes RuBP-dependent CO_2 fixation, but appears to function in some aspect of the oxidation of reduced sulfur compounds by this organism. Indeed, this was first noted after we prepared an RLP knockout strain (Ω::RLP) and observed that Ω::RLP excreted copious quantities of elemental sulfur in the growth medium [Hanson & Tabita, 2001]. More recent studies [Hanson & Tabita, Photosynth. Res. 78 (2003) 231-248] during the past grant period have established that this effect is related to some aspect of thiosulfate oxidation in the reduced sulfur compound oxidation pathway, as sulfide oxidation was not affected. When we first discovered the RLP, we noted that RLP homologs were also found in other organisms, including heterotrophic bacteria

and at least one archaeon [Hanson & Tabita, 2001, 2003]. Finally, as long-time Rubiscologists we have always been intrigued with how the active site of RubisCO might have evolved for its key functional role in metabolizing CO_2 and O_2 [Tabita, Photosynth. Res. 60 (1999) 1-28; Tabita et al. Microbiol. Mol. Biol. Rev. **71** (2007) 576-599]. This was borne out by the recent elucidation of the structure of RLP via a collaborative with our group and the laboratory of Prof. David Eisenberg at UCLA [Li, Sawaya, Tabita & Eisenberg, Structure 13 (2005) 779-789]. Finally, in another collaborative effort with Prof. John Gerlt's group at the Univeristy of Illinois, we have shown that the RLP from *Rhodospirillum rubrum* catalyzes an unusual double isomerase reaction [Imker, Singh, Warlick, Tabita, and Gerlt, Biochemistry (2008) 11171-11173.] and is most likely involved in a completely novel pathway of sulfur salvage [Singh and Tabita, manuscript in preparation; Singh, J., Ph. D. Dissertation, The Ohio State University, 2008].