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## THIOREDOXIN AND EVOLUTION

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Thioredoxins are a widely distributed group of small proteins with a conserved dithiol active site. Chloroplasts contain two types of thioredoxin (**f** and **m**) for which functional differences coincide with differences in structure. These thioredoxins, which are both reduced photochemically by way of ferredoxin and ferredoxin-thioredoxin reductase, regulate biochemical activity in chloroplasts in response to light by reducing specific target enzymes and changing their activities. Thioredoxin **f** preferentially activates enzymes of the photosynthetic carbon cycle (fructose biphosphatase, sedohepulose biphosphatase, phosphoribulokinase, NADP-glyceraldehyde 3-P dehydrogenase). Thioredoxin **m** preferentially activates an associated enzyme, NADP-malate dehydrogenase, and deactivates, also apparently by enzyme reduction, an enzyme of carbonate breakdown (glucose 6-P dehydrogenase). Both thioredoxins interact with CF1-ATPase, the enzyme forming ATP. Recently the regulatory thiol site has been identified for most of these enzymes. In each case, a specific disulfide (S-S) group is reduced to the sulfhydryl (SH) level, leading to the activation of the enzyme. This redox modulation enables chloroplasts to use light for the regulation of these enzymes, thereby achieving "biochemical order"--i.e., futile cycling is minimized so that starch can be built up in the light and broken down in the dark.

Comparisons of primary structure have revealed significant homology between the **m** type thioredoxins of chloroplasts and the thioredoxins from a variety of bacteria. Chloroplast thioredoxin **f**, by contrast, remains an enigma: certain residues are invariant with those of other thioredoxins, but a phylogenetic relationship to bacterial or **m** thioredoxins seems distant. Knowledge of the evolutionary history of thioredoxin **f** is, nevertheless, of interest because of its role in photosynthesis. We have, therefore, attempted to gain information on the evolutionary history of chloroplast thioredoxin **f**, as well as **m**. Our goal was first to establish the utility of thioredoxin as a phylogenetic marker, and, if found suitable, to deduce the evolutionary histories of the chloroplast thioredoxins. To this end, we have constructed phylogenetic (minimal replacement) trees using computer analysis. The results show that the thioredoxins of bacteria and animals fall into distinct phylogenetic groups--the bacterial group resembling that derived from earlier 16s RNA analyses and the animal group showing a cluster consistent with known relationships. The chloroplast thioredoxins show a novel type of phylogenetic arrangement: one (**m** type) aligns with its counterpart of eukaryotic algae, cyanobacteria and other bacteria, whereas the second (**f** type) tracks with animal thioredoxin. The results give new insight into the evolution of photosynthesis.