CHAPTER 18

METABOLIC ENGINEERING OF CHLOROPLASTS
FOR ABIOTIC STRESS TOLERANCE

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Abstract. Plants must be capable of coping with various environmental stresses. These stresses range from high and low temperatures to salinity, drought, oxidative, and radiation damage. Much of the biochemical machinery localized in the chloroplast makes it possible to regulate specific reactions associated with stress tolerance in this organelle. In the case of heat stress, it has been noted that there are chloroplast-localized heat shock proteins that may be involved in the acquisition of thermotolerance in plants. Following an initial heat episode and recovery period, HSP21 was localized in the soluble fraction of the chloroplast. Furthermore, heat stress resulted in a temperature-dependant redistribution of HSP21 from a soluble form to an insoluble chloroplast protein fraction. Other proteins, such as components of the PSII complex, are thought to be most sensitive to both heat and light. Thermotolerance and acclimation of PSII varies widely among species in general. Even in the case of PSII thermoduction, small heat shock proteins appear to offer thermo-protection.

Glycine betaine accumulates in plants that are drought tolerant and so it appears to act as an osmoprotectant. Protection may occur as a result of the stabilization of the quaternary structure of enzymes and enzyme complexes. Accumulation of trehalose in chloroplasts also has a positive effect on protein against drought. Thus, the insertion of genes into the chloroplast genome that resulted in an over-production of glycine betaine or trehalose, conferred high levels of salt and drought tolerance. Exposure of plants to light intensities higher than that required to saturate photosynthesis may cause light damage or photoinhibition of the thylakoid proteins of the chloroplast. To maintain normal functions under light stress, plants have developed several repair and protective mechanisms. The induction of a family of proteins, called early light-induced proteins (elips) consist of low molecular proteins localized in the thylakoid membranes of chloroplasts. These proteins may be genetically engineered to provide greater stability against photoinhibition. Therefore, there is a great potential for the genetic manipulation of key enzymes involved in stress metabolism in plants within plastids.

One set of metabolic activities involves the over-production of glutathione in transgenic plants. There are several enzymes that play an important role in the oxidation/reduction of compounds that are involved in oxidative stress. When transgenic maize plants were created by targeting iron superoxide dismutase (FeSOD) to chloroplasts, enhanced oxidative stress tolerance was observed. Similarly, MnSOD, localized in chloroplasts of bundle sheath cells conferred stress tolerance.

Transgenes that confer tolerance to abiotic stress should not outcross with weeds and permanently transfer these valuable traits to the weed nuclear genome. Therefore, maternal inheritance of transgenes that confer abiotic stress tolerance via the chloroplast genome may be ideal for this purpose. Because chloroplast genomes of major crops including cotton and soybean have been successfully transformed, this offers an exciting new approach to create transgenic plants with abiotic stress tolerance. While there

may be some limitations (including substrates or intermediates in complex pathways) to chloroplast genetic engineering for stress tolerance, there appears to be tremendous potential for increasing tolerance in plants to a number of stresses by expression of appropriate genes within chloroplasts.

1. INTRODUCTION

Chloroplasts are a unique class of intracellular organelles found in green plants. They contain their own DNA molecules organized into discrete protein-associated nucleoids, and chloroplast genome structure and overall gene order in higher plants are highly conserved. The chloroplast genome encodes genes for ribosomal and transfer RNA and some of the proteins involved in chloroplast functions, while nuclear DNA encodes the rest. The chloroplast genome generally varies from 120-200 KB and contains a large and a small single copy region separated by two large (~25 KB) inverted repeat regions. The genome has a capacity to code for 4 subunits of RNA polymerase, 4 ribosomal RNAs, 23 ribosomal proteins, 30 tRNAs, 30 thylakoid proteins for light reactions and the large subunit of Rubisco (Tyangi, 1999). In addition, there are over a dozen genes capable of coding for NADH dehydrogenase subunits and other products. There are also a few open reading frames that have the capacity to code for proteins of other as yet unidentified functions.

2. HEAT STRESS

In response to hyperthermia stress, most cells activate a small set of genes and preferentially synthesize heat shock proteins (HSP) encoded by those genes. The heat shock response was first described in *Drosophila* (Tissieres et al., 1974), and has since been reported in essentially all living eukaryotic and prokaryotic cells. In the past several years, an understanding of the mechanism of heat shock gene activation and expression has increased considerably, but the biological response to heat shock is still not well understood. While there have been numerous molecular and biochemical studies concerning the response of plant cells to induced heat stress, ultrastructural studies have indicated (a) the formation and distribution of heat shock granules, (b) morphological alterations of nucleoli with accumulation of pre-ribosomal ribonucleoprotein, and (c) the intracellular localization of plant HSP (Dylewski et al., 1991). Other experiments (Neumann et al., 1998) indicate that at the onset of heat shock there is a loss of ribosomes, dictyosomes, and endoplasmic reticulum (Belanger et al., 1986) accompanied by morphological alterations of chloroplasts and mitochondria.

Accumulation of small heat shock proteins (sHSPs) in response to high temperature stress is thought to contribute to the development of thermotolerance in eukaryotic organisms, but the mechanism of action is unknown. Osteryoung and Vierling (1994) have investigated the chloroplast-localized HSP21 to define how it contributes to the acquisition of thermotolerance in plants. Following an initial heat stress and recovery period, HSP21 was found to be localized in the soluble fraction of the chloroplast. Further heat stress resulted in a temperature-dependent redistribution of HSP21 from a soluble form to an insoluble fraction of the