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Transcriptome analysis of four chloroplast developmental barley mutants

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SUMMARY – We investigated the transcriptome of four *albina* (*alb-e*^{16} and *alb-f*^{17}) and *xantha* (*xan-s*^{46} and *xan-b*^{12}) barley mutants to provide an overall transcriptional picture of genes whose expression is interconnected with chloroplast activities and to search for candidate genes associated to the mutations. About 20% of the genes expressed in leaf cells showed a modified expression level in the mutants. Beside those encoding plastid localized proteins, more than 3000 genes involved in non-chloroplast localized metabolism were up-/down-regulated in the mutants revealing the network of chloroplast dependent metabolic pathways. Some keys metabolic pathways (e.g. chlorophyll or carotenoids biosynthesis) have been studied verifying the induction or repression of subsequent steps and the intermediates and pigment content. This permitted us to identify steps of pigment biosynthesis not functional in mutants and different subregulons of genes regulated as pigment synthesis proceeded.

Introduction

Chloroplasts are unique organelles of photosynthetic organisms and play a vital role in the cell. According to the endosymbiotic theory, plastids arose by endocytobiosis of a photosynthetic unicellular prokaryote into a eukaryotic host. Progressively the endosymbiont lost the capacity to function independently and part of the plastid genes were transferred to the host nucleus. This gene transfer required a continuous dialogue between nucleus and chloroplast in order to maintain a coordinated expression of protein function within the plastid. Chloroplasts still contain multiple copies of its own circular genome and encodes about 50-150 of the estimated 2000-5000 proteins that make up the plastidial proteome (Leister, 2003). To synthesize these proteins, chloroplasts retained the competence for DNA replication, transcription and translation. The introduction of oxygenic photosynthesis into the eukaryotic kingdom was not the only feature of the cyanobacterium that was retained during chloroplast evolution. Many of the metabolic capacities of the plant cell rely, at least in part, on processes taking place inside the chloroplast (i.e. fatty acid biosynthesis, nitrite and sulfate reduction and amino acid biosynthesis).

During the past several years many reports described chloroplast-related mutants affected in leaf coloration, chlorophyll fluorescence, plastid-to-nucleus signaling and plastid division (for review see Leister, 2003). The corresponding genes encode proteins located both in the chloroplast and in the cytoplasm and are related to protein translocation, proteolysis, enzyme activity, protein complex assembly, metal ion homeostasis, different subunits of the photosynthetic apparatus, xanthophyll cycle and porphyrin metabolism and transport.

Barley genetic stocks offer a unique collection of chloroplast deficient mutants, most characterized at the genetic and biochemical levels (Henningsen *et al.*, 1993). Although these mutations are generally lethal, the large endosperm of barley seeds supports plant growth for several weeks, allowing analysis of the mutants at the seedling stage. Previously, we had shown that barley plants carrying mutations preventing chloroplast development, in addition to the expected *albina* or *xantha* phenotype, are completely frost susceptible as well as impaired in the expression of several cold-regulated genes (Dal Bosco *et al.*, 2003). Interestingly they correctly induce many well-known CBFs

and their regulon(s), indicating that factors deriving from the chloroplast in addition to *Cbf* are required to promote the full suite of molecular changes associated with cold acclimation (Svennson *et al.*, 2006).

Aim of the work and preliminary results

The recent availability of a barley microarray (Close *et al.*, 2004) provided a new tool to describe the effect of a mutation at a whole genome level. In the present work we investigated four chloroplast barley mutants (*albina-e*[16], *albina-f*[17], *xantha-s*[46] and *xantha-b*[12], Fig. 1), representing successive steps in chloroplast biogenesis, and the corresponding wild type, with the Affymetrix Barley1 GeneChip® (*ca*: 22,000 probe sets) to assess the variations of gene expression associated with chloroplast development.

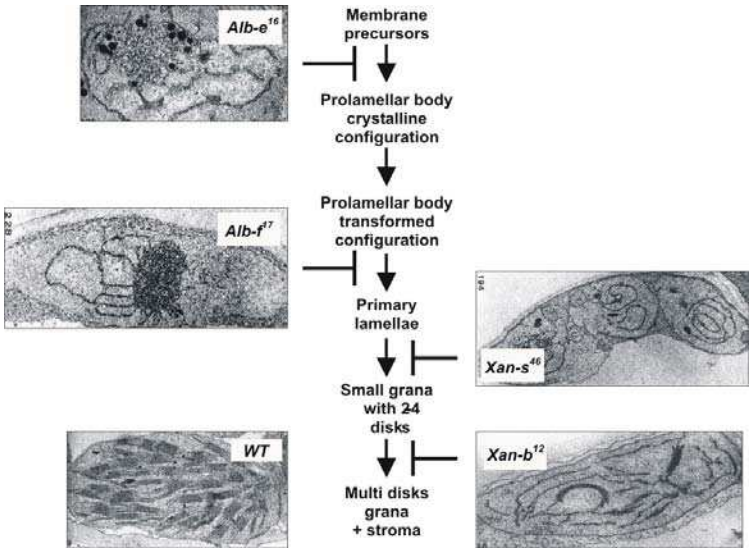

Fig. 1. Plastid development pathway. Simplified plastid development pathway showing the steps affected by *xantha* and *albina* mutants *(alb-e*[16], *alb-f*[17], *xan-s*[46] and *xan-b*[12]) and microscope pictures of plastids. Modified from Henningsen *et al.* (1993).

When mRNA isolated from leaves of mutant plants grown at 20℃ we re compared with mRNA extracted from green leaves of WT plants grown under the same conditions a number of probe sets were up-or down-regulated by more than 2 fold. The number of up- or down-regulated genes during growth at 20℃ was similar (19% of transcriptome) in the first three mutants (*alb-e*[16], *alb-f*[17] and *xan-s*[46]), while for *xan-b*[12] the number of genes modified were reduced to 9% showing a clear normalization of the transcriptome as chloroplast development proceed. Lists of up- and down-regulated genes in mutants with respect to WT were subdivided into classes in order to identify groups of genes differentially expressed in subsequent stages of chloroplast development. The up- and down- regulated gene lists were converted into the homologous sequences of *Arabidopsis* (cut off E-value = e^{-10}) and loaded onto the mapman software in order to gain information about the metabolic changes associated with a block in the chloroplast development. Some keys metabolic pathways (e.g. chlorophyll or carotenoids biosynthesis) have been studied thoroughly verifying the induction or repression of subsequent steps and the intermediates and pigment content. This permitted us to identify steps of pigment biosynthesis not functional in mutants and different subregulons of genes regulated as pigment synthesis proceeded. Collectively, our data provide a better understanding of the number and role of nuclear genes associated with chloroplast development.

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