Elucidation of regulatory networks of plant metabolism and their exploitation to improve plant growth and nutritional quality

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Growth and development of all organisms, and their ability to tolerate various stresses and diseases, are strongly regulated by dynamic changes in their metabolic networks. Concerted operation of metabolic networks is particularly important in plants because plants are sessile organisms that must continuously interact with their environment, adapting their metabolism accordingly. Our laboratory focuses on the aspartate-family metabolic network that regulates the production of the amino acids lysine, threonine and methionine (Figure 1). The importance of this metabolic network is dual: (i) the amino acids produced by this metabolic network are essential in the diets of human and farm animals because they cannot be synthesized by mammals; and (ii) one of the amino acids produced by this pathway, namely methionine, is also an important growth regulator, providing methyl groups for a large number of cellular processes, such as DNA replication. Hence, methionine metabolism in humans is an important regulator of growth of cancer cells, and in plants it is a major regulator of general growth and response to abiotic and biotic stresses. Our research aims to address several important objectives: (i) elevating the levels of the essential amino acids of the aspartate-family pathway in plants in order to improve their nutritional quality; (ii) using the aspartate-family pathway as a model for investigating the response of plants to metabolic perturbations; (iii) studying how do the metabolic networks of amino acids cross-interact with cellular processes of protein turn-over, particularly autophagy, under various developmental and environmental cues; and (iv) elucidating the functional role of the autophagy degradation processes in plant growth and response to the environment.

In order to elucidate regulatory networks associated with the aspartate-family pathway and also enhance the synthesis of lysine, we have expressed in transgenic plants a bacterial dihydrodipicolinate synthase (DHPS), a key regulatory enzyme of lysine biosynthesis that is insensitive to feedback inhibition by lysine. Plants expressing this bacterial enzyme over-produced lysine, showing that this bacterial gene can be exploited to alter plant metabolism. Since lysine is the most limiting essential amino acids in plant derived human foods and livestock feeds, our discovery led to a number of successful biotechnological programs, aiming to use bacterial DHPS enzymes to improve the lysine level in crop plants.

Our research using the bacterial DHPS enzyme has also led to the discovery that lysine accumulation in plants is regulated not only by the rate of lysine biosynthesis, but also by

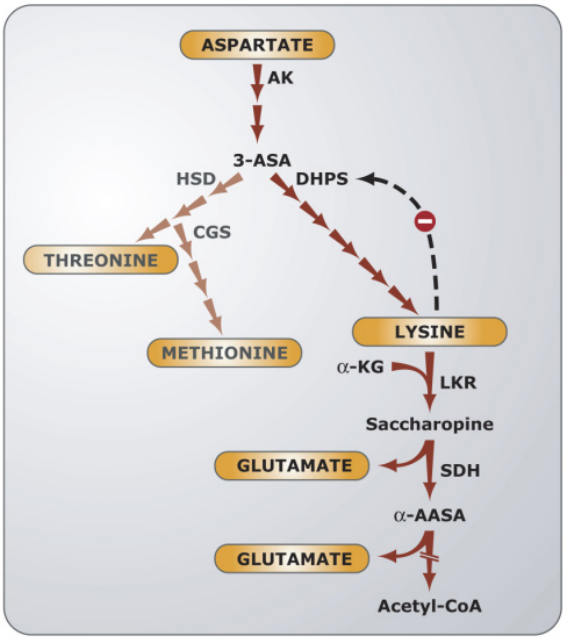

Fig. 1 Schematic diagram of aspartate-family pathway, leading to lysine, methionine and threonine synthesis as well as the pathway of lysine catabolism into glutamate and to acetyl CoA. *The dashed arrow with a "-" sign represents feedback inhibition of DHPS by lysine. Abbreviations: AK, aspartate kinase; DHPS, dihydrodipicolinate synthase; HSD, homoserine dehydrogenase; CGS; cystathionine g-synthase; LKR, lysine-ketoglutarate reductase; SDH, saccharopine dehydrogenase; 3-ASA, 3-aspartic semialdehyde; a-KG, a-ketoglutarate; a-AASA, a-amino adipic semialdehyde. Broken arrow at the bottom indicates several enzymatic steps leading to the production of acetyl-CoA.*

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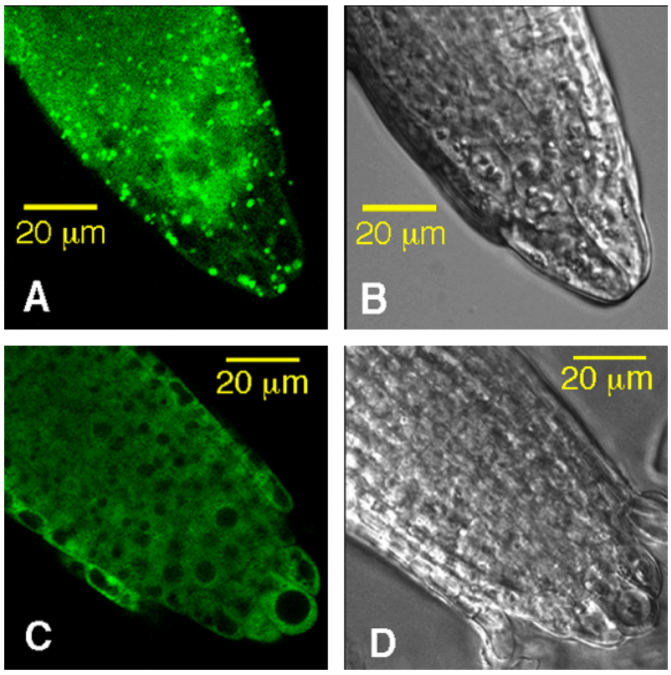

Fig. 2 GFP fluorescence in root tips of Arabidopsis plants expressing a GFP-Atg8 construct*. Ten days old Arabidopsis plants, transformed with GFP-AtAtg8 (A, B) or control GFP (C, D) constructs were observed in low magnification either in fluorescent microscope (A, C) or in a light microscope (B, D). Note that the GFP-Atg8 is localized in autophagosome-resembling spots, which are not seen in the control plants expressing GFP alone.*

the rate of its catabolism (degradation). Hence, we have cloned a novel *LKR/SDH* gene from plants, encoding a bifunctional enzyme that regulates the first two enzymatic steps of lysine catabolism. Eliminating lysine catabolism in transgenic plants, either by gene knockout or RNAi approaches, in combination with expression of a bacterial DHPS enzyme, synergistically improved lysine level in the plants. This new discovery is also being exploited biotechnologically in order to further improve lysine content in crop plants.

The *LKR/SDH* gene of plants appears also to be highly regulated by transcriptional and post-transcriptional controls, implying multiple functional roles for lysine catabolism in regulating plant growth and development. The potential functions of lysine catabolism are currently being studied in our laboratory, using genomics, metabolomics and bioinformatics approaches. Notably, lysine catabolism is not only important in plants, but also in human in which defective lysine catabolism causes a genetic disease, called familial hyperlysinemias. This genetic disease is associated in some patients with mental retardation. Based on our original discovery of the plant *LKR/SDH* gene, its homologue was also later cloned from human, and a mutation in this gene was shown to be associated with familial hyperlysinemias. Why is lysine catabolism so highly regulated in plants and what are the physiological functions of this pathway are two major questions in our present research activity.

Plant growth is regulated by both biosynthesis and degradation processes. We are also focusing on the functional significance of the autophagy degradation process in plant growth. Autophagy in plants has so far been mostly associated with metabolic starvation stresses, but we believe that this process also operates in plants grown under favorable conditions and are searching for such functions. Using a GFP fusion construct to one of the autophagy genes (*Atg8*), which regulates autophagosome development, we have shown, in collaboration with prof. Zvulun Elazar, that autophagosomes are formed in distinct tissues, such as root tips of plants grown under favorable conditions (Figure 2). In addition, by using autophagy knockout and RNAi mutants we plan to resolve the function of autophagy in these tissues.

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