The Plant Metabolome in Action

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The term *METABOLOME* describes the complement of all metabolites expressed in a cell, a tissue or an organism during its lifetime. Already at the 19th century two classes of metabolites were defined; primary (or basic) and secondary. Secondary metabolism refers to compounds that are not necessary for the cell survival and propagation but are believed to play a role in the continued existence and adaptation to the ever changing environmental conditions. Secondary metabolites (SMs) are derived from primary metabolites (e.g. amino acids and carbohydrates), through modifications, such as methylation, hydroxylation, and glycosylation. Evolutionary processes directed towards enhancing plant fitness most probably stimulated formation of new structures. SMs are formed both as part of normal plant developmental pathways and upon diverse endogenous and environmental stimuli. Examples of SMs are fruit flavor and aroma compounds, flower and fruit pigments and "sun screen" metabolites. Up to date, a few hundred thousand different SMs structures have been identified in plants, most of which belong to the Phenylpropanoids, Isoprenoids and Alkaloids classes.

The main interest of our lab is in the regulation of plant metabolic pathways, in particularly those associated with secondary metabolism and its coordination with developmental and stress response programs.

Metabolomics: A Complementary tool in Systems Biology and Functional Genomics

The capacity to measure and identify metabolites is an essential component of our lab research activity. We are currently setting-up a Metabolomics platform that could generate a detailed metabolic profile for any given organism, tissue or cell type. Metabolomics holds great promise as an effective tool in numerous applied fields of research such as in metabolic engineering of crop plants, and identification of diagnostic biomarkers. Three interrelated components of Metabolomics technology development are currently addressed in our lab: (a), sample collection, extraction, recovery and validation for specific classes of metabolites; (b), analytical detection, identification, quantification, and structure elucidation; as well as (c), integrating metabolomics and transcriptomics to obtain a "bird view" of metabolic response. High-end analytical hyphenated instruments are employed (UPLC-qToF-MS-MS and GC-MS) and integrated into a computational infrastructure (Figure 1), which is currently being used to study plant and yeast biology.

On the Outer Surface of Plants

One of the fundamental changes in the adaptation of plants to the terrestrial environment was the formation of an outer surface, the cuticle. The plant cuticular layer plays multiple roles including the regulation of epidermal permeability, non-stomatal water loss and protection against insects, pathogens, UV light, and frost. It also functions in the prevention of post-genital organ fusion, pollen-pistil interactions and cell-to-cell communication. Cuticular components are generated in epidermal cells by four major independent biosynthetic pathways, that synthesize cutin monomers, aliphatic wax components, triterpenoids, and aromatic metabolites (e.g. flavonoids).

We are currently studying the regulation of the different pathways

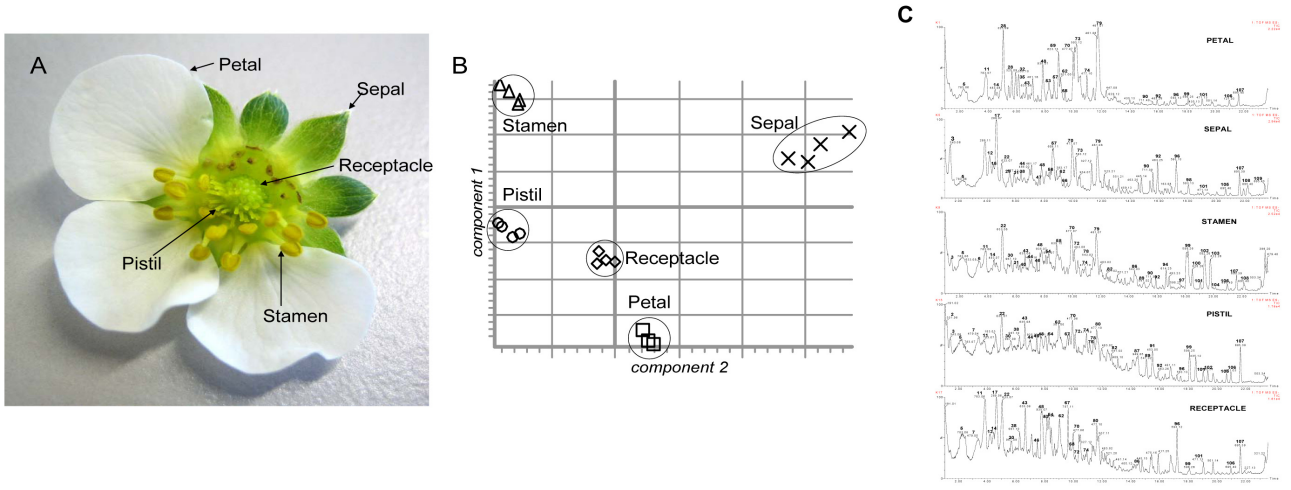

Fig. 1 *Example of Metabolomics analysis in strawberry flower organs using UPLC-qTOF-MS. A, Strawberry flower at the anthesis stage. B, Principal component analysis of the metabolite markers detected in extracts of the different flower organs. C, UPLC-qTOF-MS chromatograms of strawberry floral organs. The assigned peaks are marked by numbers.*

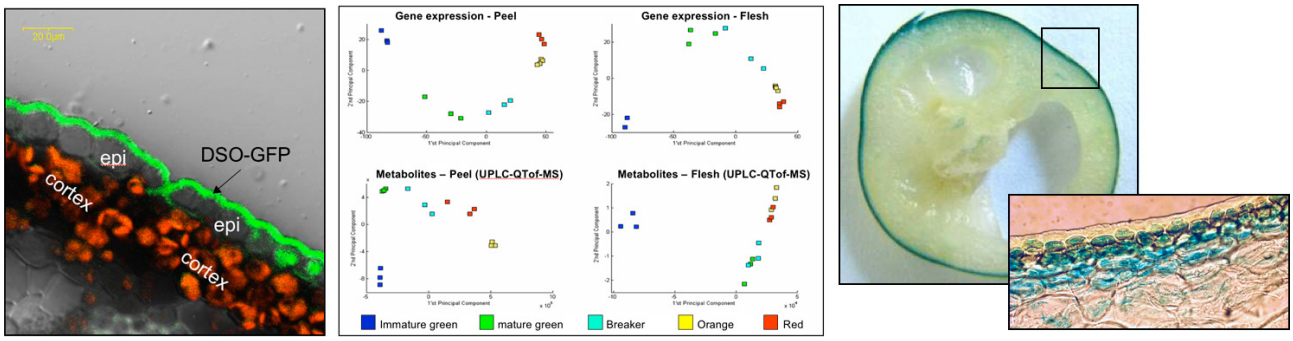

Fig. 2 *The outer surface of plants. Left, polar localization of the DSO protein, a wax and cutin transporter in the Arabidopsis stem epidermis (epi) cells. Middle, gene expression and metabolic profiles in tomato fruit. PCA of Transcriptome and Metabolome analyses demonstrate the profile differences between peel and flesh tissues during fruit development. Right, GUS expression driven by an upstream region of a tomato peel-associated gene. Intense staining is observed in the epidermis cell below the outer cuticular layer.*

constructing the cuticle and the secretion of the end products from the epidermal cells to the cuticle surface. Recently, we have identified an Arabidopsis ATP Binding Cassette (ABC) type transporter gene (*DSO*) that its protein product localizes in a polar manner in the plasma membrane of epidermal cells and is involved in the transport of cuticle components, (e.g. wax), from the epidermal cell layer to the cuticle (Figure 2). We are also conducting an in-depth characterization of the Arabidopsis SHINE/WIN clade of AP2/EREBP transcription factors and their putative interacting proteins that regulate cutin and wax biosynthesis in Arabidopsis. In a different project related to plant surfaces we study the transfer and deposition of Silica in the plant epidermis and address the open question on its role in plants, particularly in the cereals wheat and barley.

In parallel to the study of cuticular metabolism in vegetative tissues we investigate the cuticle in reproductive organs such as tomato fruit skin. Metabolic and expression profiling conducted in our lab revealed metabolites, enzymes, transporters and transcriptional factors related to cuticle metabolism in this tissue (Figure 2).

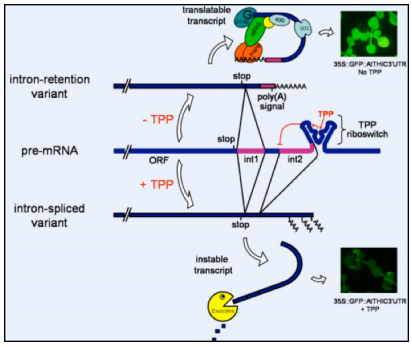

Fig. 3 *Riboswitch-mediated control of metabolic pathways in plants. The proposed mode of action of the plant TPP Riboswitch is illustrated in the scheme. The Riboswitch is employed to control a metabolic feedback loop through differential processing of precursor-RNA 3'-terminus.*

Riboswitches in Plants: Post Transcriptional Regulators of Metabolic Pathways

Riboswitches are natural RNA sensors that affect gene control via their capacity to bind small molecules. Their prevalence in higher eukaryotes is yet unclear. We discovered a post-transcriptional mechanism in plants that employs a riboswitch to control a metabolic feedback loop, through differential processing of precursor-RNA 3'-terminus (Figure 3). When cellular thiamin-pyrophosphate (TPP) levels rise, metabolite sensing by the riboswitch located in TPP biosynthesis genes directs the formation of an unstable splicing variant, which consequently leads to a decrease in TPP levels. When transformed in plants, engineered TPP riboswitches can act autonomously to modulate gene expression. In an evolutionary perspective, the presence of TPP riboswitch in ancient plant taxa suggests that this mechanism is active since the emergence of vascular plants 400 million years ago. We are currently deciphering the role of riboswitches in plant physiology and evaluating their potential use as metabolite sensors in whole plants.

The Primary-Secondary Metabolism Interface

Primary or central metabolism (i.e. carbohydrates, amino acids and lipids) is the source of backbone precursors for the production of SMs. The examination of two different transcription factors sets revealed coordinated activation of SMs biosynthesis and their corresponding primary metabolism pathways. In a first set, we investigate six factors that regulate the biosynthesis of Tryptophan- and Methionine-derived glucosinolates (insect deterrence substances). Transcriptome and Metabolome analyses of transgenic plants demonstrated that in parallel to the activation of the glucosinolate biosynthetic pathways these factors also induce several major sources of primary metabolites that generate precursors for glucosinolates formation. A second set of transcription factors studied in our lab regulate phenylalanine-derived SMs. Preliminary results shows that these factors retain targets of activation upstream in the Shikimate pathway that generates aromatic amino acids, including Phenylalanine. Currently, we focus on the identification of the metabolic pathways and genes that are direct targets of these transcription factors,

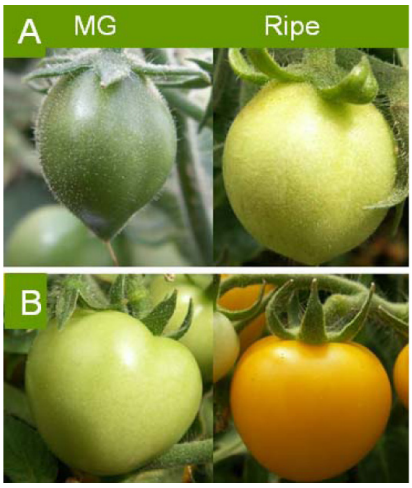

Fig. 4 *The olive mutant in tomato identified in the transposon tagging population generated by the lab. Mature green (MG) and Ripe stages of fruit development in the olive mutant (panel A) and control fruit (rin background; panel B). Note the change in shape and the intense green color of the olive mutant.*

and on elucidating the dynamics of the coordinated activation of primary and secondary metabolism.

Metabolic pathways of both primary and secondary metabolism are active in specific plant tissues and are often split into different sub-cellular locations. We have recently initiated experiments in which bioinformatics, metabolomics and cell sorting are used to unravel the distribution of active metabolic pathways in unique cell layers, and reconstruct the subcellular network of metabolic pathways. In this project we aim to resolve the extent and importance of metabolites flow between organelles in plant cells and the biological relevance of certain metabolic pathways activity in unique plant cell layers.

Regulatory Networks Coordinating Tomato Fruit Ripening and Secondary Metabolism

The process of ripening is a unique aspect of plant development. It makes fruit attractive for consumption by organisms that assist seed release and dispersal. The nutritional content of ripe fruit, such as minerals and vitamins makes the ripening process important in the diet of human and animals. We are interested in understanding the genetic control of ripening and the coordination of developmental and metabolic programs during this process. The primary aim of the project is to identify genes involved in the control of fruit ripening, specifically in the tomato fruit model. In addition, we study the regulation of the Isoprenoid pathway during tomato fruit ripening. This pathway generates both primary metabolites (e.g. sterols) and SMs including glycoalkaloids (triterpenoids) and carotenoids such as Lycopene that provides the fruit its typical red color. We employ various approaches including gene discovery through integrative expression-metabolic profiling, one-hybrid system and mutagenesis in order to identify regulators of this pathway. In the mutagenesis approach, a large population of mutants, consisting of several thousand individual plants is currently generated by introducing an heterologues transposon to tomato. The majority of the lines in this population will retain a gain of function mutations due to the incorporation of an enhancer in the transposon sequence. We intend to use Metabolomics for screening fruit that derive from this population for alterations in their metabolic profiles.

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Acknowledgements

A. Aharoni is an incumbent of the Adolfo and Evelyn Blum Career Development Chair. Research at the Aharoni laboratory is supported by the Minerva Foundation, The Lubin family foundation, Israel Ministry of Science (IMOS), Israel Ministry of Industry and trade (MAGNET program), The BARD fund, The EU FP6 program and The Israel Science Foundation (ISF).

INTERNAL support

Research at the Aharoni laboratory is supported by the The Sir Charles Clore Research Prize, William Z. & Eda Bess Novick New Scientists Fund, The Henry S. and Anne Reich Family Foundation, Sir Harry Djanogly, CBE, Mrs. Louise Gartner, Dallas, TX, Mr. and Mrs. Mordechai Segal, Israel and The Estate of Fannie Sherr, New York, NY.