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Oral Communication Abstract – 4.01

PUTTING "SYSTEMS" BACK INTO SYSTEMS BIOLOGY

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Metabolic modelling, control analysis, supply-demand analysis, regulatory design, biotechnological manipulation

A central tenet of systems biology is that organisms, cells, genes and proteins are complex structures whose relationships and properties are largely determined by their functional organisation. Systems biology, therefore, should therefore go beyond the properties and amounts of individual biomolecules, and take seriously their organisation into a living whole. Sadly, much of what currently passes for systems biology has degenerated into what can be called the"system-wide" biology of all the -omics, which seems to be based on the belief that if we measure everything that can be measured in the cell we will understand cellular physiology. In this talk I shall argue for the necessity of a "systems view" for gaining the required understanding.

In terms of functional organisation metabolism can be regarded as a chain of coupled factories: a catabolic factory transforms nutrients into carbon skeletons and captures chemical energy and reducing power. These catabolic products serve as input to an anabolic factory that synthesizes the building blocks for macromolecular syntheses (amino acids, nucleotides, simple lipids, etc.). The factories for protein, polynucleotide, complex carbohydrate and lipid synthesis form the end of the chain and lead to growth. I show how this view of the functional organisation of the cell underlies a quantitative formalism and a general theory for understanding the cell as a integrated molecular economy of coupled supply and demand systems that have evolved regulatory mechanisms that enable them to fulfill specific functions such as control of flux or homeostatic maintenance of metabolite concentrations. In “classical” accounts of metabolic regulation the rates at which metabolic products are made are purported to be controlled by so-called rate-limiting steps within the supply pathways. Our supply-demand analysis[1] allows the control and regulation of metabolism as a whole to be understood quantitatively in terms of the elasticities of supply and demand, which are experimentally measurable properties of the individual pathways or processes. The kinetic and thermodynamic aspects of regulation[2] can be clearly distinguished, and a major consequence of enzyme regulation is that fluxes can respond to changes in demand or supply, depending on the type of function that the system fulfils, while the system remains both far from equilibrium and homeostatic. Supply-demand analysis shows that flux and concentration control are inextricably linked: the more control either supply or demand block has over flux, the less it determines the degree of homeostasis of the concentration of the linking intermediate, which becomes the function of the other block.

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Oral Communication Abstract – 4.02

PATHOGEN EVOLUTION WITHIN HUMAN HOST

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Bacterial pathogens evolve during the infection of human hosts, yet teasing apart adaptive and neutral mutations remains elusive. Here, we identify genes under adaptive evolution by tracking recurrent patterns of mutations in the same pathogenic strain during the infection of multiple patients. We sequenced the genomes of 112 Burkholderia dolosa isolates recovered from 14 people with cystic fibrosis over years. We found that 17 genes underwent convergent adaptive evolution, receiving non-synonymous mutations repeatedly in multiple patients. These mutations illuminate the genetic basis of important pathogenic phenotypes, including antibiotic resistance and bacterial membrane composition. Six genes under adaptive evolution - including three in an oxygen-related regulation pathway - have not been previously implicated in pathogenesis, suggesting novel therapeutic targets. Such convergent molecular evolution reveals the key selection forces acting on pathogens within humans and can help predict and prepare for their future evolutionary course.

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Oral Communication Abstract – 4.03

LOCAL SYSTEMS BIOLOGY AND ANALYSIS OF TRANSCRIPTIONAL-METABOLIC NETWORKS IN FRUITS OF TOMATO (*SOLANUM LYCOPERSICON* L.) CAROTENOID MUTANTS REVEAL NOVEL FINDINGS IN THE REGULATION OF FRUIT CAROTENOGENESIS

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Carotenoid, tomato, ripening, metabolomics, network analysis

Carotenoids represent one of the larger and more important class of natural compounds, playing essential roles in human nutrition on the basis, for many of them, of strong antioxidant activities. In plants, they have fundamental functions as photosynthetic pigments in leaves, hormone precursors, and secondary metabolite pigments in flowers and fruits. Tomato has been extensively investigated as model system for carotenoid pathway due to the extensive genetic variation affecting carotenoid composition during fruit ripening; anyhow, most of them have been, so far, characterized merely by through the identification of the source of the gained or lost activity. Here, we report an extensive investigation, both at the biochemical (LC-DAD-APCI-MS) and molecular (Real-Time PCR) levels, of a collection comprising the mutants "apricot" (*at*, loss-of-function of the chromoplast-specific isopentenyl diphosphate isomerase (*Ipi*)), "yellow flesh" (*r*, loss-of-function of the chromoplast-specific phytoene synthase gene (*Psy1*)), "tangerine" (*t*, loss-of-function of carotenoid isomerase gene (*CrtISO*)), "Delta" (*Del*, gain of function of the chromoplast-specific lycopene β-cyclase (*CrtL-e*)), "Beta" (*B*, gain of function of the chromoplast-specific lycopene β-cyclase (*CYC-b*)) and "white-flower" (*wf*, loss-of-function of the chromoplast-specific β-carotene hydroxylase gene (*CrtR-b2*)) throughout four different stages of fruit development.

A great extent of large alterations are observed, at late fruit stages, in the profiles of both carotenoids and other classes of isoprenoids and, more interestingly and unexpectedly, in the overall expression pattern of the endogenous genes, indipendently by the distance from the mutated enzymatic step. A powerful correlation networks approach has been used in order to reveal and predict regulatory relationships between metabolites, transcripts, and metabolite-transcripts so providing a better understanding of the mechanisms regulating fruit carotenogenesis in tomato. Furthermore, a powerful approach of untargeted non-polar metabolomics, has been, finally, carried out to identify a large set of differentially accumulated metabolites in each mutant so to deduce novel metabolic cross-talks within tomato fruit metabolome.

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Oral Communication Abstract – 4.04

DYNAMIC CHANGES IN ARABIDOPSIS TRANSCRIPTOME DURING SHADE AVOIDANCE RESPONSE

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R/FR, elongation, flowering, acclimation, phytochrome

The success of competitive interactions between plants determines the chance of survival of individuals and eventually of whole plant species. Shade-tolerant plants have adapted their photosynthesis to function optimally under low-light conditions. These plants are therefore capable of long-term survival under a canopy shade. In contrast, shade-avoiding plants adapt their growth to perceive maximum sunlight and therefore rapidly dominate gaps in a canopy. Daylight contains roughly equal proportions of red (R) and far-red (FR) light, but within vegetation that ratio is lowered as a result of the R absorption by photosynthetic pigments. This light quality change is perceived through the phytochrome system as an unambiguous signal of the proximity of neighbours resulting in the shade avoidance response. This adaptive reaction is achieved by a set of responses including enhanced internode and petiole extension growth, increased apical dominance, retarded leaf development, and an acceleration of flowering. However, if a plant succeeds in the attempt to overgrow its neighbours and the photosynthetic organs perceive daylight again, the shade avoidance response is rapidly switched off through phytochrome photoconversion. The adaptive responses result in changes in the distribution of assimilates between leaves, stems, and roots.

Genomic and genetic analyses by our and other laboratories have identified several low R/FR-regulated genes and key regulators involved in the shade avoidance response. However, very little is known about the cascade of events triggered by low R/FR that give rise to the full activation of the response and, later on, to the adaptation process when a plant does not succeed to overgrow its neighbours. Therefore, shade avoidance response was examined by genome wide expression profiling in wild type and genetically altered plants exposed to low R/FR light for different times. To identify gene networks, both computational and experimental approaches are being pursued. Informatic analyses provided insights into functional clusters and their dynamics, predictions of cis-regulatory elements for genes temporally regulated during shade avoidance response, inference of gene regulatory interactions. Together, these analyses uncovered novel aspects of shade avoidance, and generated testable hypotheses on gene regulatory circuitry underlying plant responses to light quality changes.

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Oral Communication Abstract – 4.05

PHYLOGENOMICS APPLIED TO *SACCHAROMYCES CEREVISIAE* STRAINS REVEALS GENES WITH HIGH EVOLUTIONARY RESOLUTION

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Phylogenesis, yeast strains, genes, computational pipeline, evolution

The quest for genes representing genetic relationships of strains or individuals of a same population and their evolutionary history is acquiring a novel dimension of complexity and novel possibilities with the advancement of NGS technologies. In the past several authors have proposed candidate gene, or combinations of them, that were able to map the diversity of *Saccharomyces cerevisiae* strains, allowing to distinguish and cluster into separate branches closely related strains and therefore to recapitulate the natural diversity of *S. cerevisiae*, both in domesticated and in natural populations Nowadays, sequencing an entire genome is quite feasible and many genomes of different strains are available to the scientific community. This opens up the possibility to uncover genetic variation in coding and non-coding regions of a population of individuals of the same species. In fact, the knowledge of the sequence variation in a population of strains of the species *S. cerevisiae* offers the ideal model to search a gene or a set of genes representing the evolutionary relations among strains, that could be also inferred from the analysis of their entire genomes. In this work we propose an original strategy aimed at identifying a minimal set of genes able to characterize the population structure of natural yeast strains. By applying a combinatorial approach on gene selection to a pipeline for fast parallel phylogenetic analysis and to an efficient tree-screening strategy, we were able to isolate several genes with sufficient evolutionary resolution to be used in strain characterization and phylogenetic assessment, giving results comparable to those obtained with full genome sequencing.

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Oral Communication Abstract – 4.06

MASTER REGULATORS OF COLONY MORPHOLOGY SWITCH IN YEAST *S. CEREVISIAE*

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Morphotype, ecology, evolution, gene expression, SNPs

Colony morphology is a fascinating phenotype described in unicellular organisms as a possible step towards multicellularity.

We studied the environmental determinants of a specific type of morphology, called filigreed morphology. This phenotype first described by Cavalieri et al in 1998 and 2000 as present in heterozigosity in one *S. cerevisiae* strain from grapes of the Montalcino area, naturally reverts to normal colony morphology with a reversion rate that is dependent on the carbon source. We measured gene expression in cells grown in fermentable and non-fermentable carbon sources and used pathway analysis to evaluate the genetic determinants of filigreed phenotype. Our results support the hypothesis of an ecological function of filamentous phenotype in creating a community adaptable to the shifts of the environmental conditions. Ethanol is the main fermentation product and enables *S. cerevisiae* to inhibit the growth of other microorganism competing for space and nutrient. The increase in ethanol concentration is correlated to the decrease of fermentable sugars; in this perspective the stable and uniform morphotype, induced by ethanol, could reflect an adaptation to starvation and stress. The adaptive role of morphogenesis is further supported by the increased capability of this strain to invade agar, demonstrating a correlation between invasiveness, filamentous morphotype and pseudohyphal growth. Next-generation sequencing (NGS) of the sporal derivatives allowed to discover mutations in genes candidate to be the genetic determinants of the colony morphology phenotype. The combination of the results led to hypothesize that the colony morphology is dependent on "symmetric" processes resulting from synchronization of several genetic sub-networks involved in RAS signaling, bud site selection, cell cycle regulation and cell-cell adhesion.

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Poster Communication Abstract – 4.07

"cAMP-SPONGE": A NEW GENETIC TOOL TO INVESTIGATE THE ROLE OF cAMP IN PLANTS

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cAMP-sponge, tobacco BY-2 cells, Agrobacterium tumefaciens-mediated transformation

Cyclic AMP is a well known second messenger which regulates a wide variety of cellular responses in living organisms such as bacteria, fungi, and animals. On the contrary, its presence and its role in plants have been debated for decades. The skepticism was finally overcome with the use of mass spectrometry that provided unequivocal evidence of its presence in higher plants. The information on the biological function of cAMP in plants remains very limited, mainly because its content in plant cells is significantly lower than in other organisms. To date, the cAMP involvement in several processes of higher plants, including cell cycle regulation, growth and reorientation of the pollen tube, seed germination and defense processes has been reported. However, in plants, the mechanisms involved in the cAMP-dependent signal transduction are yet unknown, especially for the failure to identify a kinase that responds specifically to cellular changes in cAMP concentration. Understanding both the biological events specifically attributable to cAMP, and the mechanisms by which these processes are regulated, through the combination of quantitative data with mathematical models, is a challenge for the study of plant signal transduction.

In order to obtain more information on the role of cAMP in plants we generated tobacco Bright yellow-2 (TBY-2) lines that constitutively express a non-invasive tool, the "cAMP-sponge", able to selectively perturb the cAMP concentration (Lefkimmiatis et al, 2009). The cAMP-sponge is composed of two high-affinity cAMP binding domains of the regulatory subunits I beta of human protein kinase A (PKARIbeta). These domains have been engineered to be unable to interact with the catalytic subunit of PKA itself or to homodimerize. This construct binds with high affinity cAMP but not cGMP. The cAMP-sponge in frame with the reporter gene mCherry has been inserted in the binary vector pGreenII (kan) under the control of the strong constitutive promoter CaMV 35S.The construct has been transferred into *Agrobacterium tumefaciens* GV3101 strain through electroporation and mobilized into TBY-2 cells via *A. tumefaciens*-mediated transformation. Transgenic TBY-2 lines have been selected in the presence of kanamycin, and several independent transgenic lines obtained.

Transformed lines have been analyzed through PCR, RT-PCR and immunoblotting to assess trans-gene integration and mRNA and protein expression. Finally the effect of the lower levels of cAMP on the growth of TBY-2 cells has been analyzed.

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Poster Communication Abstract – 4.08

ANALYSIS OF GENE REGULATORY NETWORKS IN PEACH: THE INTERPLAY BETWEEN TRANSCRIPTION FACTORS AND MICRORNAs

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MicroRNA, transcription factors, regulatory networks

MicroRNAs (miRNAs) are a class of non-coding small RNAs with fundamental roles in key plant biological processes such as development, signal transduction and environmental stress response. MiRNAs act on gene regulation at post-transcriptional level, a phenomenon known in plants as PTGS (Post Transcriptional Gene Silencing), through sequence-based interaction with target mRNAs. Transcription factor families comprise most of the highly conserved miRNA targets. At the same time microRNA genes expression is controlled by transcription factors and both the two types of *trans*-regulators are part of complex regulatory networks thus exerting a widespread impact on gene expression.

In this work the interplay between microRNAs and transcription factors has been studied in peach and complex regulatory networks have been identified. In particular feed-forward loops, involving a transcription factor regulating a target gene together with a microRNA, which is regulated in turn by the same transcription factor have been deeply analyzed.

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Poster Communication Abstract – 4.09

EFFECTS OF THE METALLOID OXYANION TELLURITE ON GROWTH OF THE YEAST *SACCHAROMYCES CEREVISIAE*

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Tellurite, Saccharomyces cerevisiae, mitochondria, transmission electronic microscopy

The tolerance of microorganisms to potentially toxic metals has received considerable attention. Microbial resistance to the metalloid tellurium (Te) as potassium tellurite, a higly toxic compound, is poorly understood but is thought to be associated with tellurite reduction and precipitation of metallic tellurium . Growth in the presence of tellurite is often associated with reduction of the oxyanion to Te°, which leads to blackening of the cells due to either cytoplasmic or periplasmic Te° crystalline precipitates (Zannoni et al., 2008). The capacity to reduce Te is not restricted to prokaryotes, as eukaryotes, including fungi, yeasts and plants, as well as animal tissues may carry out various reactions leading to black Te° precipitates (Zannoni et al., 2008). To gain insight about the nature of such biological mechanisms, the objective of the present work was to analyze the effects of potassium tellurite on growth, survival and micro-morphology of the model yeast, *Saccharomyces cerevisiae*. The yeast strains Sc57 rho$^+$ and its rho° derivative Sc57-R3 (Del Giudice et al., 2005) were used. Both rho$^+$ and rho° strains grew on a fermentable carbon source with up to 1.2 mM K_2TeO_3, while rho$^+$ yeast cells grown on a non-fermentable carbon source were inhibited at tellurite levels as low as 50μM suggesting that this metalloid specifically inhibited mitochondrial functions. Growth of rho$^+$ yeast cells in the presence of increasing amount of tellurite resulted in dose-dependent blackening of the culture, a phenomenon not observed with rho° cultures. The percentages of Sc57 and Sc57-R3 colony forming survivors were determined for cells growing in media containing glucose and different potassium tellurite concentrations. The number of viable cells of both strains decreased by increasing the potassium tellurite concentration in the media, while the addition of Bacto peptone in the media increased the percentage of viable cells in presence of tellurite suggesting an antagonistic metabolic reaction between Bacto peptone into the media and potassium tellurite. To analyze the tellurium uptake by yeast cells, Sc57 cultures growing in YED (yeast extract and glucose) media containing potassium tellurite were observed microscopically. Transmission electron microscopy (TEM) of *S. cerevisiae* rho$^+$ cells grown in the presence of tellurite showed that blackening was likely due to elemental tellurium (Te°) that formed large deposits along the cell wall and small precipitates in both the cytoplasm and mitochondria. The exact location of Te° grains in this organelles (i.e., the internal membrane of mitochondrial crests or the interspace between the internal and external mitochondrial membranes) remains to be

established. Nevertheless, the TEM data support the above-mentioned hypothesis that mitochondria may be involved in both tellurite reduction to Te° and, very likely, tellurite toxicity in *S. cerevisiae* (Massardo et al., 2009).

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Poster Communication Abstract – 4.10

CONTROLLING HEAVY METAL ACCUMULATION IN PLANTS

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Heavy metals, Pseudomonas putida, CzcCBA transporter, phytoremediation

The present study is aimed at understanding the interactions between plants, microorganisms and heavy metals. We have considered a natural environment, in Northern France, that shows high cadmium, zinc and lead pollution. Plants of the metal hyperaccumulator *Arabidopsis halleri* were collected together with their rhyzospheric soil. Several bacterial strains were then isolated from this soil and identified. Among these, a strain of *Pseudomonas putida* was considered for further analyses. Proteomic analysis was performed on this strain (called *P. putida-Cd001*) grown with and without Cd added to the nutrient medium. The differential patterns of protein expression were visualized by two dimensional electrophoresis technique and proteins were identified by MS analysis. Results showed that many different membrane proteins were up-regulated upon Cd treatment, in particular membrane transporters.

This study is focalized on the expression *in planta* of a membrane transporter CzcCBA, a member of the Czc family (cobalt/zinc/cadmium) efflux transporters. This system is a trans-envelope pump, typical of gram-negative bacteria, and acts as a chemiosmotic antiporter. The CzcCBA complex is constituted of three components: A, B and C. The first is localized at the inner membrane of the bacterial cell and allows the efflux of metal cations from the cytosol to the periplasmic space. The B subunit is located in the periplasmic space and directs ions towards the third component, C - located in the outer membrane, which opens a channel extruding metal ions outside the bacterium.

Since these membrane transporters contribute to the resistance to high heavy metals concentration in *P. putida*, we tried to utilize them to modulate heavy metal accumulation in transgenic plants. Both *Arabidopsis thaliana* and *Nicotiana tabacum* were selected as model species. Firstly, to understand the localization of the three genes in the plant cell, *CzcA*, *CzcB* and *CzcC* were cloned into the pUC-35S::NosT plasmid, in fusion with the eGFP reporter gene, under the control of the CaMV 35S promoter. Protoplasts transient transfection highlighted a probable plasma-membrane and nuclear localization of CzcC, while for CzcA and CzcB results need to be confirmed. The three genes were also cloned into appropriate plant-overexpression vectors, and transferred into the plant genome via *Agrobacterium* transformation. Preliminary data on the expression of *CzcA* and *CzcB* in tobacco, show that these genes alone are able to modulate cadmium amounts absorbed by transgenic plants, when compared to wild type control. In particular, transgenic plants overexpressing CzcA accumulate lower cadmium amounts in comparison to the wild-type.

Considering that the Czc system is constituted of three subunits, transgenic plants harboring the three single Czc genes are being crossed, to isolate individuals that carry the complete CzcCBA complex and to verify whether the entire transporter confer different cadmium transport ability to plants.

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Poster Communication Abstract – 4.11

IONOME CHARACTERISATION OF *SOLANUM LYCOPERSICUM* CV. M82 *X S. PENNELLII* INTROGRESSION LINES (ILs)

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Ionome, Solanum lycopersycum, Introgression Lines, metals accumulation, PCA

The ionome is the mineral nutrient and trace element composition of an organism and represents the inorganic component of cellular and organismal systems. It is a dynamic network of elements that are controlled by the physiology and biochemistry of the plant, which are ultimately controlled by the genome, in response to the environment. Ionomics is the study of elemental accumulation in living systems using high-throughput elemental profiling. A central theme of ionomics is the study of change in the ionome in response to physiological stimuli, environmental conditions and genetic modifications.

Several tomato (*Solanum lycopersicum*) cultivars and breeding lines are coming from interspecific hybridisation with *S. pennellii*, a wild tomato relative bearing useful commercial traits. To study the contribution of the introgression of *S. pennellii* genome on *S. lycopersicum* traits, Eshed et al. (Theor. Appl. Genet. 83:1027-1034, 1992) have developed 76 introgression lines (ILs), each characterised for the location of the introgressed *S. pennellii* chromosome sequences into the *S. lycopersicum* cv M82. Those lines are being extensively studied for several traits, thus giving a wealth of information about genetic and physiology of both species (Gur et al., Theor. Appl. Genet. 122:405-420, 2011). However, until now the contribution of the genome of *S. pennellii* in the tomato cultivated variety for ions biofortification and food safety has not yet been studied. In this regards, our research line is aimed to characterize the ionome of those ILs, in order to find modifications induced by the introgression of the wild tomato genome into the cultivated one.

Fifthy nine ILs, covering all 12-tomato chromosomes, were grown in a greenhouse. Shoot apices from 4 45 days-old plants were harvested and then analysed by inductively coupled plasma (ICP) spectroscopy for the amount of the following elements: Ca, Mg, Fe, Cu, Mn, Mo, Ni, Zn, Al, Na, Co, V, Cr, Sr, As, Cd, Pb, Sn and Ba. Statistic analysis (T-test Student's) carried out on the mean concentration of each element for each ILs compared with the parental line (cv M2) showed that the introgression of S. *pennellii* genome into *S. lycopersicum* cv. M82 modified the ionome of all the 59 ILs. In particular the Na+ concentration in all ILs was significantly lower than in cv M82 and Cd, Cu, Mg, Ca and Zn concentrations decreased in most of them, whereas Co and Mn concentrations increased. It is worthy to note that some ILs accumulated more toxic metals as As, Pb and Cr than the cultivated genotype. Results on PCA analysis will be also reported. Our results showed that the introgression of the wild genome into the cultivated one produced new phenotypes

in which the traits co-related to macro/micronutrients, trace elements and toxic elements accumulation in apical leaves were significantly modified in response to specific introgressions, thus opening the way to a genetic analysis of introgressed gene expression.

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Poster Communication Abstract – 4.12

PROTEIN, AMINO ACID, AND TRANSCRIPTOME ANALYSES OF THE *ZEA MAYS* MUTANTS *OPAQUE-2* AND *OPAQUE-7*

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Zea mays L., endosperm mutants, Opaque-2, Opaque-7, microarray analysis

The changes in storage reserve accumulation during maize (*Zea mays* L.) grain maturation are well established. However, the key molecular determinants controlling carbon flux to the grain and the partitioning of carbon to starch and protein are more elusive. The *Opaque-2* (*O2*) gene, one of the best-characterized plant transcription factors, is a good example of the integration of carbohydrate, amino acid and storage protein metabolisms in maize endosperm development. Evidence also indicates that the *Opaque-7* (*O7*) gene plays a role in affecting endosperm metabolism. The focus of this study was to assess the changes induced by the *o2* and *o7* mutations on maize endosperm metabolism by evaluating protein and amino acid composition and by transcriptome profiling, in order to investigate the functional interplay between these two genes in single and double mutants.

We show that the overall amino acid composition of the mutants analyzed appeared similar. Each mutant had a high Lys and reduced Glx and Leu content with respect to wild type. Gene expression profiling, based on a unigene set composed of 7,250 ESTs, allowed us to identify a series of mutant-related down (17.1%) and up-regulated (3.2%) transcripts. Several differentially expressed ESTs homologous to genes encoding enzymes involved in amino acid synthesis, carbon metabolism (TCA cycle and glycolysis), in storage protein and starch metabolism, in gene transcription and translation processes, in signal transduction, and in protein, fatty acid, and lipid synthesis were identified. Our analyses demonstrate that the mutants investigated are pleiotropic and play a critical role in several endosperm-related metabolic processes. Pleiotropic effects were less evident in the *o7* mutant, but severe in the *o2* and *o2o7* backgrounds, with large changes in gene expression patterns, affecting a broad range of kernel-expressed genes.

Although, by necessity, this research is descriptive and more work is required to define gene functions and dissect the complex regulation of gene expression, the genes isolated and characterized to date give us an intriguing insight into the mechanisms underlying endosperm metabolism.

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Poster Communication Abstract – 4.13

FRIEND OR FOE: USING SYSTEMS BIOLOGY TO ELUCIDATE THE INTERACTION BETWEEN FUNGI AND THEIR HOSTS

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Systems biology, fungal disease, immune response, ecology, evolution

Modelling the networks subtending the fruitful coexistence between fungi and their mammalian hosts is becoming increasingly important in the effort to control emerging fungal pathogens. In a "Systems Biology" perspective the microbiota and the host should be seen as an ecosystem, and disease considered as an alteration of the equilibrium. The tasks of recognizing an invading pathogen and activating the host response are accomplished by pattern-recognition receptors, which recognize conserved microbial components called pathogen-associated molecular patterns. Recent evidence suggests that the use of distinct recognition receptors contributes to the disparate patterns of reactivity observed locally in response to challenge with pathogenic and harmless fungi. To contrast fungal infection, augmenting the ability of the immune system to eliminate a pathogen requires a sophisticated understanding of the molecular mechanisms that are involved in pathogen recognition and in the host immune response. The integration of a Systems Biology approach to functional data will offer new interpretive clues to the mechanisms of fungal virulence. This work addresses host-yeast interaction with special focus on the ecology of the yeasts, and the environmental determinants of the expression of potentially pathogenic traits. Knowledge of "friendly" organisms, generally recognized as safe, such as *Saccharomyces cerevisiae*, will significantly improve our ability to understand fungal ecological niches and the selective forces shaping their population structure.

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Poster Communication Abstract – 4.14

DIFFERENTIAL GENE EXPRESSION OF POLYAMINE OXIDASES IN *ARABIDOPSIS THALIANA*

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Polyamine oxidase, GUS, guard cells, pollen, roots

Polyamine oxidases (PAOs) are FAD-dependent enzymes involved in polyamine catabolism. In *Arabidopsis thaliana*, five PAOs (AtPAO1-5) are present with cytosolic or peroxisomal localization. Here, we present a detailed study on the expression pattern of *AtPAO1*, *AtPAO2, AtPAO3* and *AtPAO5* during seedling growth and flower development through analysis of promoter activity in *AtPAO*::*GUS* transgenic *Arabidopsis* plants. Characterization of these plants showed distinct expression patterns for each one of the *AtPAOs* studied, such as in the transition region between the meristematic and the elongation zone of roots and anther tapetum for *AtPAO1*, in the quiescent center, columella initials and pollen for *AtPAO2*, in columella, guard cells and pollen for *AtPAO3* and in the vascular system of roots and hypocotyls for *AtPAO5*. Furthermore, treatment with the plant hormone abscisic acid increased *AtPAO1*-related GUS staining in the root tip and *AtPAO2*-related GUS staining in the guard cells. These data suggest distinct physiological roles for the various *AtPAOs*. Studies are in progress to determine *AtPAO* involvement in gravity response and stomata closure.

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Poster Communication Abstract – 4.15

MOLECULAR CHARACTERIZATION OF THE OPAQUE-6 MUTATION OF *ZEA MAYS* L.

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Zea mays L., endosperm mutant, Opaque-6, microarray analysis, bio-informatics

Maize endosperms accumulate during development a large amount of storage proteins (zeins). The rate of zein accumulation is under the control of several regulatory loci, many of which lower the zein level, thus improving the nutritional quality of maize meals. Among these regulatory loci is *Opaque-6* (*O6*), located on the long arm of chromosome 8, which when present in recessive form determines a general reduction of zein accumulation. Curiously, the extent of growth of the *opaque-6* locus, which has been demonstrated to be allelic to the *pro-1* locus, is limited by the availability of Proline. Without Proline the mutant plants show abnormal leaves, reduced growth and lethality at the second leaf stage. Moreover, at the ultrastructural level, primary leaves of *o6* mutants exhibit an abnormal chloroplast development. Various explanations for these phenomena were conceived: 1) the mutants cannot make Proline in sufficient amounts; 2) Proline degradation is enhanced in comparison to synthesis; 3) Proline transport across intercellular compartments is blocked; 4) Proline is having some effect related to its known role as a stress reliever. More recently, it was demonstrated, by means of an *in vitro* growth assay, that the *o6* mutant can be rescued not only through Proline addition, but also by supplying Arginine, Asparagine, Glycine, Leucine, Methionine, and Tryptophan during plant growth. These findings dismantle the previously proposed explanations of *O6* functionality.

In order to increase our knowledge of the *Opaque-6* locus, we performed microarray experiments on homozygous wild type (WT) and o6 plantlets. For this purpose, mature WT and mutant seeds were sterilized and germinated in Petri dishes for 48h at 27°C. Embryos were then dissected and placed in test tubes containing basal medium, or basal medium with Proline. On the basal growth medium, the WT seedlings showed a normal phenotype, whereas the mutant seedlings exhibited the described abnormalities of leaves, reduced growth and lethality at the second leaf stage. Mutant embryos cultivated on basal medium added with Proline showed a complete recovery of the normal phenotype. Plantlets were collected after 1- and 5 days of *in vitro* growth. Subsequently, total RNA was extracted from each of the eight sample types (WT and mutant genotype; basal and Proline supplied medium; 1 and 5 days of growth). Sample extractions were performed in threefold and used to prepare Cy-3 and Cy-5 labelled hybridization probes. A 55,000 oligonucleotide maize microarray was then hybridized with the molecular probes, using a dye-swapping strategy. The collected hybridization data were first normalized at the slide level, in order to remove dye-related signal differences, and subsequently across slides. All statistical procedures were performed with the use of the lemma package for the R software suite. The obtained results will be discussed in relation with the morphological abnormalities exhibited by the homozygous *o6* mutant plants.