

Session 9:

***Epigenetics, Small RNAs & Chromatine
Structure***

Changes in nuclear architecture and DNA methylation pattern accompany the developmental programmed cell death of the tapetum.

P 09.1

The architecture of the cell nucleus is highly dynamic and organized in distinct functional domains which modify their organization in response to changes in the nuclear function and gene expression. DNA methylation of cytosine residues constitutes a prominent epigenetic modification of the chromatin fiber which is locked in a transcriptionally inactive conformation leading to gene silencing. However, the relationship between DNA methylation and large-scale organization of nuclear architecture is poorly understood. The tapetum, nursing tissue inside the anther, has a key role during specific stages of pollen development and when finishing, it follows programmed cell death (PCD) at late stages. In this work we analyze the dynamics of nuclear architecture and DNA methylation patterns, as defined epigenetic mark of the chromatin functional state, during development and PCD of tapetal cells in *Brassica napus* L and *Nicotiana tabacum* L. PCD is characterized by a series of cellular events which have been described in great detail in animal cells, but in plants the different ways of death, as well as their corresponding features, are not well established. Ultrastructural, cytochemical and immunocytochemical analysis showed structural features similar to animal cell apoptosis (cytochrome C releasing from the mitochondria, caspase-like expression, DNA fragmentation, chromatin condensation, cytoplasmic vacuolation and shrinkage). Sequential changes occurring from the early to the final death stages have been characterized in the nuclear architecture of tapetal cells by using different nuclear markers. Results showed that chromatin, interchromatin and nucleolar components became segregated, and interchromatin RNPs rearranged in HERDs-like bodies, new ectopic structures in plants which can also be considered markers of transcriptional arrest, as proposed in mammals. Immunofluorescence of 5-methyl-cytidine (5mC) and confocal analysis showed a specific distribution pattern in discrete foci at the periphery and the interior of tapetal nuclei at early developmental stages. With the progression of PCD at later stages, the 5mC distribution appeared in larger nuclear areas, over highly condensed chromatin masses. Genomic DNA methylation analysis by HPCE (high-performance capillary electrophoresis) at different developmental stages revealed that DNA methylation increased during the process. Results showed new evidences of changes in DNA methylation that accompany the reorganization of the nuclear architecture during plant PCD, giving new insights in the knowledge of the epigenetic control of nuclear architecture in plant development. Supported by Spanish MEC projects BFU2005-01094 and AGL2005-05104, and Spanish-Czech joint project 2006CZ0006 granted by CSIC and Czech Academy of Sciences.

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Epigenetic Mechanisms for Gene Expression Changes and Phenotypic Variation in Plant Polyploids

P 09.2

Polyploidy has occurred in the evolution of many plants and some animals. The merger of divergent genomes in allopolyploids creates genome-wide gene expression changes. We have shown transcriptome dominance of *Arabidopsis arenosa* over *A. thaliana* in the allotetraploids that contain these two divergent genomes. The underlying mechanisms are largely unknown. Here we report that the genes that are highly expressed in *A. thaliana* are associated with histone H3-Lys9 acetylation and H3-Lys4 methylation. The majority of genes that are highly expressed in *A. thaliana* are repressed in the allotetraploids and are associated with H3-Lys9 methylation, suggesting that chromatin modifications play a role in genome-specific expression changes in the allopolyploids. Consistent with the repression of *A. thaliana* genes, *A. thaliana* heterochromatic regions and centromeres are hypermethylated, which are associated with transposon activation and siRNA accumulation in the *met1-RNAi A. suecica* lines that contain *A. thaliana* and *A. arenosa* extant genomes. In contrast, microRNA loci are organized in chromosomal domains that are different from many differentially expressed loci in the allotetraploids. *A. thaliana* miRNA locus and its neighboring genes are hypomethylated and highly expressed, whereas the corresponding *A. arenosa*-derived homoeologous domain is epigenetically repressed in the allotetraploids. In the allotetraploids, accumulation of miRNAs correlates inversely with differential expression of ~50% of miRNA target mRNAs in the allotetraploids. Furthermore, genetic and epigenetic regulation of FLC loci is associated with the late flowering phenotype in resynthesized and natural allotetraploids. We suggest that interspecific hybridization induces progenitor-dependent chromatin modifications and locus-specific regulation of homoeologous chromosomal domains in polyploid genomes, which contributes to phenotypic variation in the new allopolyploid species. (Alternative session: Natural Variation and Ecosystem Genomics session)

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Tian L.
Liu J.
Ha M.
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DNA sequences involved in the tight DNA-protein complexes on different stages of the barley shoot development

P 09.3

Sjakste T.
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The tightly bound to DNA proteins (TBP) are a protein group that remains attached to DNA with covalent or non-covalent bonds after its deproteinisation. Enrichment of the TBPs in specific DNA sequences can determine potential function of such sequences in higher order structures of the genome of different organisms. In order to characterize TBP-associated sequences in barley DNA fragments associated with tightly bound proteins from shoot leaves, coleoptiles and roots were cloned, and about 600 inserts were sequenced. CT-motif sequences presented predominantly by the 16-bp CC(TCTCCC)₂TC fragment were found in many clones. 16-bp fragment was detected in the specimens of DNA associated to the tightly bound proteins purified out of all tissues, however it was absent from the free DNA. Enrichment in CT-motif was much more prevalent in the TBP-enriched DNA fractions from coleoptile and first leaf. In contrast to other specimens fraction of TBP-enriched DNA from root was often represented by GC-sequences presumably in the 49-GC fragment. Computational analysis of the motifs revealed their presence in barley ESTs as well their occurrence in other genomes and similarity with transposable elements. 6-bp repeated element -TCTCCC- of CT-motif is highly similar to the 5-bp -TCTCC- element previously described as highly enriched in the retained fraction of chicken DNA.

Loading time of the centromeric histone H3 variant makes maintenance of kinetochores different between plant and animal centromeres

Kinetochores are protein complexes established at eukaryotic centromeres and responsible for the correct chromosome segregation during nuclear divisions. Kinetochores formation is initiated by substitution of histone H3 by CENH3 within (some but not all) nucleosomes of active centromeres. Correct timing and targeting of this process are essential for centromere function, but are not well understood. Here we point out a general difference between animal and plant centromeres: For Arabidopsis and barley, CENH3 loading in plants occurs before mitotic sister centromere separation. Recent data for the holocentric chromosomes of the plant *Luzula nivea* and for a phylogenetically old red alga also indicate premitotic CENH3 loading, while for animals it was recently shown that CENH3 deposition occurs postmitotically, i.e. after sister centromere separation. Additionally, monocentric chromosomes of higher plants display distinct sister kinetochores immediately after loading of CENH3 during late G2, while the flanking centromere regions are cohesed until the onset of anaphase. Although the reason for the different timing of CENH3 deposition is not yet clear, it indicates that the initial step(s) of kinetochores maintenance, i.e. the mechanism(s) regulating CENH3 loading, differ between metazoans and plants.

P 09.4

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Fuchs J.
Schubert V.
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Epigenetic inheritance of CG methylation imposed on a coding region of a tobacco transgene by small RNAs.

Heritability of epigenetic marks is still unclear. We have studied the inheritance of the epigenetic state of tobacco transgenes whose expression and DNA methylation status were modified by the exposure to a silencer locus. The nptII reporter genes residing the target loci were posttranscriptionally silenced in hybrids containing a homologous trigger locus organized as an inverted repeat. Using locus-specific bisulfite genomic sequencing we show that the coding region of the target nptII genes were almost exclusively methylated in CG context with the exception of a few non-symmetric sites located close to the 3' end. On the other hand homologous sequences in the trigger were heavily methylated at both CG and non-CG motives. After segregation of the trigger only the CG methylation but not the non-CG methylation was transmitted to the next generations (F2-F4). In these plants we observed a redistribution and an increase of overall CG methylation. This pattern was inherited with some variation for at least over three meiotic cycles following segregation of the trigger. The epiallelic variants of the target locus re-expressed the reporter gene immediately after segregation of the trigger suggesting that PTGS was entirely dependent on the presence of the silencing trigger and that relatively dense CG methylation (60-80%) of the coding region did not block expression. We propose that body gene CG methylation seen in many actively transcribed regions may originate from ancient PTGS events and/or occasional production of methylation-inducing RNA molecules.

P 09.5

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Fojtova M.
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Development of a gene targeting system based on in planta presentation of homologous donor DNA during meiosis.

P 09.6

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Currently, we are developing a system that allows efficient gene targeting in *Arabidopsis thaliana*. The outline of our approach is based on two main features: 1. the intrinsic characteristics – in regard to homologous recombination – of cells during meiosis 2. the in planta presentation of homologous donor DNA in the aforementioned cells. Our current assay is based on the restoration of a GUS:nptII gene, defective for kanamycin resistance. Target lines were created by introducing such a defective GUS:nptII reporter gene (containing a 3' nptII deletion) under control of a 35S promoter. Two separate target lines – both homozygous for a single copy of the defective GUS:nptII reporter gene – were selected. A targeting vector – carrying a lox cassette containing a promoterless gus:NPTII reporter gene (having a 5' gus deletion) and a downstream 5000 bp stretch of sequence homologous to the target sequence – was introduced in the two target lines. In the final step of our approach a Cre gene under control of a promoter active in the prophase I of the meiosis (promoter of the *A. thaliana* Solo Dancers (SDS) gene, AT1G14750, Azumi et al., 2002) was introduced into the target + targeting lines. Placing the expression of the Cre gene under control of the SDS promoter allows the induction of the Cre/lox recombination during the meiotic prophase I. The Cre/lox recombination will result in the formation of a circular donor DNA that can be presented for homologous recombination with the target sequence. Homologous recombination between both gus:NPTII reporter genes will lead to repair of the target GUS:nptII reporter gene (controlled by a 35S promoter) resulting in kanamycin resistance. First results show that in planta presentation of homologous donor DNA can lead to homologous recombination. Extensive analysis will identify the nature of the observed recombination events in order to discriminate between 'true' and ectopic gene targeting events. We recently extended the strategy to different promoters allowing activation of our gene targeting system in other specific cell types where homologous recombination is suspected to be the predominant recombination mechanism.

Cytological Study of Effectiveness of Plantlet Leaves Number in the Cold Perception during Vernalization using Epi-illumination Light Microscopy.

P 09.7

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M. Khosrowchahli^{1,*}, F. Valipour^{1,2}, J. Karapetian² and M.R. Dadpour³. 1- Department of Biology, University of Tabriz, Tabriz/Iran. 2- Department of Biology, University of Urmia, Urmia/Iran. 3- Department of Horticulture, University of Tabriz, Tabriz/Iran. Abstract Vernalization a process required for transition of the vegetative meristem into the reproductive one is promoted through exposure of plants to long period of cold temperature or winter. Different studies have shown that the apical meristem is the site of cold perception during vernalization. In this study, the transition of vegetative meristem into the flowering state through the different stages of plant development and different times of cold exposure was studied in rapeseed using Epi-illumination light microscopy. The results indicated that the developmental stage of plants measured by leaves number and the period of exposure time are crucial in vernalization effectiveness. The seedling at three leaves stage exposure to 4°C on period of 15, 30, 40, 50 and more days did not show any changes in vegetative meristem feature. In the case of seedling with 5 leaves at 4°C after 15 and 30 days transition was not observed, but after 40, 50 and 60 days cold exposure the transition into the flowering meristem have been occurred. Seedling at 7 leaves stage, transition occurred after 30 days cold exposure and remarkable changes in the developmental pattern of apical meristem, followed by the apparition of inflorescence and floral buds appeared after 30, 40, 50 and 60 days at 4°C. It seems that leaf number at seedling stage play a key role in cold perception during vernalization compared with cold period exposure. Key words: Development, Floral apex, Rapeseed, Vegetative apex, Vernalization. • - Corresponding author: mkhosrowchahli@yahoo.com • - Khosrow@tabrizu.ac.ir

Target mimicry: a novel mechanism for regulation of microRNA activity

MicroRNAs (miRNA) are non-RNA coding class containing 21-23 nucleotides that regulate key aspects of development and physiology in animals and plants. These regulatory RNAs act as guides of effector complexes to recognize specific mRNA sequences based on sequence complementarity, resulting in translational repression or site-specific cleavage. In plants, most miRNA targets are cleaved and show almost perfect complementarity with the miRNAs around the cleavage site. We examined the non-protein coding gene IPS1 (INDUCED BY PHOSPHATE STARVATION1) from *Arabidopsis thaliana* as a negative regulator of a miRNA during the phosphate starvation (Pi). IPS1 contains a motif with sequence complementarity to the phosphate starvation-induced miRNA miR-399, but the pairing is interrupted by a mismatched loop at the expected miRNA cleavage site. We show that IPS1 RNA is not cleaved but instead sequesters miR-399. Thus, IPS1 overexpression results in increased accumulation of the miR-399 target PHO2 mRNA and, concomitantly, in reduced shoot Pi content. Engineering of IPS1 to be cleavable abolishes its inhibitory activity on miR-399. We coin the term 'target mimicry' to define this mechanism of inhibition of miRNA activity. Target mimicry can be generalized beyond the control of Pi homeostasis, as demonstrated using artificial target mimics. Note: no comunicación oral

P 09.8

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Todesco M.
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Puga María I.
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Changes in nuclear architecture and DNA methylation pattern accompany the developmental programmed cell death of the tapetum

Changes in nuclear architecture and DNA methylation pattern accompany the developmental programmed cell death of the tapetum Testillano PS1, Corredor E1, Solís MT1, Chakrabarti N1, Raska I2, Risueño MC1 1Centro de Investigaciones Biológicas. CSIC. Ramiro de Maeztu 9, 28040 Madrid. Spain. 2 Dept. Cell Biology, Inst. Physiology, Acad. Sciences Czech Republic. Prague.Czech Republic. E-mail: testillano@cib.csic.es The architecture of the cell nucleus is highly dynamic and organized in distinct functional domains which modify their organization in response to changes in the nuclear function and gene expression. DNA methylation of cytosine residues constitutes a prominent epigenetic modification of the chromatin fiber which is locked in a transcriptionally inactive conformation leading to gene silencing. However, the relationship between DNA methylation and large-scale organization of nuclear architecture is poorly understood. The tapetum, nursing tissue inside the anther, has a key role during specific stages of pollen development and when finishing, it follows programmed cell death (PCD) at late stages. In this work we analyze the dynamics of nuclear architecture and DNA methylation patterns, as defined epigenetic mark of the chromatin functional state, during development and PCD of tapetal cells in *Brassica napus* L and *Nicotiana tabacum* L. PCD is characterized by a series of cellular events which have been described in great detail in animal cells, but in plants the different ways of death, as well as their corresponding features, are not well established. Ultrastructural, cytochemical and immunocytochemical analysis showed structural features similar to animal cell apoptosis (cytochrome C releasing from the mitochondria, caspase-like expression, DNA fragmentation, chromatin condensation, cytoplasmic vacuolation and shrinkage). Sequential changes occurring from the early to the final death stages have been characterized in the nuclear architecture of tapetal cells by using different nuclear markers. Results showed that chromatin, interchromatin and nucleolar components became segregated, and interchromatin RNPs rearranged in HERDs-like bodies, new ectopic structures in plants which can also be considered markers of transcriptional arrest, as proposed in mammals. Immunofluorescence of 5-methyl-cytidine (5mC) and confocal analysis showed a specific distribution pattern in discrete foci at the periphery and the interior of tapetal nuclei at early developmental stages. With the progression of PCD at later stages, the 5mC distribution appeared in larger nuclear areas, over highly condensed chromatin masses. Genomic DNA methylation analysis by HPCE (high-performance capillary electrophoresis) at different developmental stages revealed that DNA methylation increased during the process. Results showed new evidences of changes in DNA methylation that accompany the reorganization of the nuclear architecture during plant PCD, giving new insights in the knowledge of the epigenetic control of nuclear architecture in plant development. Supported by Spanish MEC projects BFU2005-01094 and AGL2005-05104, and Spanish-Czech joint project 2006CZ0006 granted by CSIC and Czech Academy of Sciences.

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Silencing suppressor activity of the Tobacco Rattle Virus-encoded 16-kDa protein and interference with endogenous small RNA-guided regulatory pathways

The final outcome of viral infections in plants is largely influenced by interactions between the host RNA silencing machinery and the plant virus. Higher plants use RNA silencing as an efficient mechanism against viral infections, but viruses may encode proteins with silencing suppressor activities to counteract these defenses. In addition, several virus-encoded suppressor proteins exert an inhibitory effect on endogenous small RNA regulatory pathways leading to dramatic changes in the host transcriptional profile and developmental defects. In this work, we identify the Tobacco rattle virus-encoded 16 kDa (TRV-16K) protein as a viral suppressor that blocks RNA silencing induced by ssRNA and dsRNA at a step downstream of small interfering RNA (siRNA) formation. We show that TRV-16K did not prevent formation of transgene-derived, aberrant transcripts which likely serve as a trigger for RNA silencing. Microarray hybridization revealed that infection of Arabidopsis plants by TRV was accompanied by virus-specific changes in host gene expression affecting a broad range of cellular processes. However, this effect was not due to virus-mediated interference with the activity of microRNAs or trans-acting siRNAs. Our data indicate that plant viruses may use a number of strategies to modulate host gene expression without disturbing endogenous small RNA-regulatory functions. We propose that plant viruses interact with the RNA silencing machinery likely to counteract its antiviral role but not to accommodate gene expression in order to benefit virus infection.

P 09.10

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Yu A.
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Donaire L.
Llave C.

Structural and Genetic Requirements for the Biogenesis of Tobacco Rattle Virus-derived Small Interfering RNAs

In plants, small RNA-guided processes referred to as RNA silencing control gene expression and serve as an efficient antiviral mechanism. Plant viruses are inducers and targets of RNA silencing as infection involves the production of functional virus-derived small interfering RNAs (siRNAs). We investigate the structural and genetic components influencing the formation of Tobacco Rattle Virus (TRV)-derived siRNAs. Non-exclusive mechanisms for virus-derived siRNA biogenesis include processing of replicative intermediates of RNA viruses, base-paired structures within the positive genomic RNA strand or virus-derived dsRNA formed through the activity of host-encoded RNA dependent-RNA polymerases (RDR). We found that most virus-derived siRNAs are generated by Dicer-like (DCL)-mediated processing of perfectly complementary dsRNA, as opposed to cleavage of local structures along the positive-strand viral RNA. We present evidence that biogenesis of a large portion of TRV-derived siRNAs is RDR-dependent, and identified RDR1, RDR2 and RDR6 as host factors required for production of siRNAs and antiviral silencing. We show that the entire TRV is targeted by DCL4, DCL3 and DCL2 to produce siRNAs of distinct size classes. Taken together, we conclude that the overall composition of siRNA derived from the RNA virus in systemically infected tissues reflects the combined action of several interconnected pathways involving different DCL and RDR activities.

P 09.11

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ARGONAUTE 4 is required for resistance to Pseudomonas syringae in Arabidopsis

P 09.12

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To identify novel plant components operating in pathogen-induced signalling cascades, a large-scale screen using Arabidopsis plants carrying the B-glucuronidase reporter gene under control of the H₂O₂-responsive Ep5C promoter was previously done and the ocp (for overexpressor of cationic peroxidase) mutants were identified (Coego et al., 2005; Plant Cell 17, 2123-2137). Here we report the characterization of ocp11 in which the reporter construct is constitutively expressed. Strikingly, the ocp11 mutant shows enhanced disease susceptibility to the virulent bacteria *Pseudomonas syringae* pv. tomato DC3000 (P.s.t. DC3000) and also to the avirulent P.s.t. DC3000 carrying the effector avrRpm1 gene. In addition, ocp11 plants are also compromised in the resistance to the non-host pathogen *P. syringae* pv. tabaci (P.s. tabaci). Moreover, genetic and molecular analyses reveal that ocp11 plants are not affected in SA perception and express normal levels of PR genes after pathogen attack. Our results thus points towards OCP11 as an important cellular component mediating disease resistance against *P. syringae*. We cloned OCP11 and show that it encodes ARGONAUTE 4, a component of the pathway that mediates the transcriptional gene silencing associated with siRNA that direct DNA methylation at specific loci, a phenomenon known as RNA directed DNA methylation (RdDM). Thus, we renamed our ocp11 mutant as ago4-2 as it represent a different allele to the previously characterized recessive ago4-1. We demonstrate that ago4-2 is a dominant negative mutant and show that ago4-1 plants, like ago4-2, are compromised in resistance. Both mutants decrease the extent of DNA cytosine methylation at CpNpG and CpHpH positions present at different DNA loci and show commonalities in all the molecular and phenotypic aspect that we have considered. Interestingly, we show that AGO4 works independently of other components of the RdDM pathway in mediating resistance to P.s.t. DC3000, and loss of function in other components of the pathway operating upstream of AGO4 such as in RDR2, DCL3, or operating downstream such as DRD1, CMT3, DRM1 or DRM2 do not compromise normal resistance to this pathogen. Finally, we show evidences indicating that transcriptional activation of the defence-related Ep5C gene correlates with a shift from methylation to demethylation at CpHpH and CpNpG positions in a promoter region of the Ep5C gene. Furthermore, this methylation program is modulated, and can be partially inhibited, by signals generated during the course of a plant-pathogen interaction.

A role for SHORT LIFE (SHL) in the regulation of the Floral transition in Arabidopsis

The SHL gene from Arabidopsis encodes a small nuclear protein that contains a BAH domain and a PHD finger (Müssig et al., 2000). Both domains are found in transcriptional regulators and chromatin remodelling factors. Proteins with the same modular architecture as SHL have been found in Arabidopsis and other plant species, but not in other organisms, suggesting that these proteins are part of a plant-specific family of transcriptional regulators. In Arabidopsis, another member of this family previously characterized in our laboratory and named EARLY BOLTING IN SHORT DAYS (EBS), is required to delay flowering under non-inductive photoperiodic conditions by repressing the expression of the floral integrator FT (Piñeiro et al, 2003). To understand the role of this family of proteins related to chromatin remodelling factors in the control of plant development, we have analysed loss-of-function alleles of SHL and we show that this locus also has a role in the repression of flowering. Moreover, we have performed double mutant analyses combining different shl and ebs alleles and the results obtained suggest that SHL has partially overlapping functions with EBS in the control of the floral transition in Arabidopsis. Progress in the genetic and molecular analysis currently underway in our laboratory to understand the role of SHL in the control of flowering time will be presented.

P 09.13

López L.
Martínez-Zapater J.
Jarillo Q.
Piñeiro G.

Silencing suppressor activity of the Tobacco Rattle Virus-encoded 16-kDa protein and interference with endogenous small RNA-guided regulatory pathways

The final outcome of viral infections in plants is largely influenced by interactions between the host RNA silencing machinery and the plant virus. Higher plants use RNA silencing as an efficient mechanism against viral infections, but viruses may encode proteins with silencing suppressor activities to counteract these defenses. In addition, several virus-encoded suppressor proteins exert an inhibitory effect on endogenous small RNA regulatory pathways leading to dramatic changes in the host transcriptional profile and developmental defects. In this work, we identify the Tobacco rattle virus-encoded 16 kDa (TRV-16K) protein as a viral suppressor that blocks RNA silencing induced by ssRNA and dsRNA at a step downstream of small interfering RNA (siRNA) formation. We show that TRV-16K did not prevent formation of transgene-derived, aberrant transcripts which likely serve as a trigger for RNA silencing. Microarray hybridization revealed that infection of Arabidopsis plants by TRV was accompanied by virus-specific changes in host gene expression affecting a broad range of cellular processes. However, this effect was not due to virus-mediated interference with the activity of microRNAs or trans-acting siRNAs. Our data indicate that plant viruses may use a number of strategies to modulate host gene expression without disturbing endogenous small RNA-regulatory functions. We propose that plant viruses interact with the RNA silencing machinery likely to counteract its antiviral role but not to accommodate gene expression in order to benefit virus infection.

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Mutations in Arabidopsis homologs of proteins of the SWR1 complex accelerate flowering time

P 09.14

Our interests is focused on the analysis of the molecular mechanisms involved in the regulation of flowering time and, particularly, on those factors required for the repression of flowering until plants are in optimal environmental conditions or reach the appropriate developmental stage to flower. Initially, we characterized early in short days (*esd1*) mutations, which cause early flowering and several vegetative and reproductive developmental defects. *esd1* abolishes the FLC-mediated late flowering phenotype of plants carrying active alleles of *FRI* and of mutants of the autonomous pathway (Martin-Trillo et al., 2006). We found that *ESD1* is required for the expression of *FLC* and other members of the FLC-like/MAF gene family to levels that inhibit flowering. The *ESD1* locus encodes *ARP6*, a homolog of the actin-related protein family that is part of the SWR1 chromatin-remodeling complex in yeast, which catalyzes the exchange of histone H2A for the histone H2A.Z variant in nucleosome arrays that ensures full activation of underlying genes. Now, we have isolated *Arabidopsis* mutants in homologs of others SWR1C components. Mutations in *AtSWC6* caused similar developmental defects as *esd1*, including serrated leaves, weak apical dominance, flowers with altered number and size of organs and early flowering by reduction in expression of *FLC* and other MAF genes. *swc6* suppresses late flowering phenotype of *FRI* and of five autonomous pathway mutant plants. Protein interaction analyses suggest the formation of a complex between *ESD1* and *AtSWC6*. In addition, we will present evidence that *AtSWC6* is needed to achieve both the levels of H3 acetylation and H3K4 methylation required for high *FLC* expression, on the same manner as *ESD1* does. These observations are consistent with the presence of an SWR1C-like complex in *Arabidopsis* that may regulate plant development controlling gene expression, being required for modulation of chromatin structure. Bibliography Martin-Trillo et al. (2006). *Development*, 133, 1241-52

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López-González L.
Piñeiro M.
Jarillo J.

Evolutionary dynamics of two Arabidopsis BAH/PHD-containing proteins involved in the control of developmental transitions through chromatin modification

Narro- Diego L
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Piñeiro M.

Our laboratory is interested in understanding the role of chromatin remodelling processes in the control of plant development. In particular, we are focused in the analysis of a family of nuclear proteins characterised for containing a BAH and a PHD domain, motifs present in chromatin remodelling factors. Proteins with this modular architecture have been found in Arabidopsis and in other plant species but not in other organisms suggesting that they constitute a plant-specific family of transcriptional regulators involved in the modulation of chromatin organization. Previous studies indicate that the two members of this family in Arabidopsis, EARLY BOLTING IN SHORT DAYS (EBS) and SHORT LIFE (SHL), are involved in the control of different developmental programs. EBS is involved in the repression of flowering, preventing the expression of the floral integrator FT under noninductive photoperiods (Piñeiro et al, 2003), and SHL has partially overlapping functions with EBS in the control of the floral transition in Arabidopsis. In order to understand the evolutionary dynamics of the genes encoding these two partially redundant transcriptional regulators, we have analysed the patterns of nucleotide variation in the corresponding genomic regions in several Arabidopsis accessions. In contrast to the SHL locus that shows an excess of non-synonymous replacements relative to synonymous mutations, no non-synonymous polymorphisms have been found in the coding regions of EBS, suggesting that positive selection constrains the variation of this gene. Natural variation data with implications in the evolution of this family of plant specific proteins likely involved in chromatin remodelling processes will be discussed. On the other hand, beside their role in flowering time, these proteins participate in the control of other plant developmental transitions, and at least EBS has been shown to regulate seed dormancy (Gómez-Mena et al., 2001). Progress in understanding the role of these gene expression regulators in the repression of seed germination during the period of dormancy will be also presented. Piñeiro, M., et al. (2003) Plant Cell 15, 1552-1562. Gómez-Mena, C., et al. (2001) Plant Cell 13, 1011-1024.