



P9–EPIGENETICS

Organized by K. Lindsey and M. Holdsworth for the Plant Gene Structure and Function Group

P9.1–Epigenetic mechanisms in post-transcriptional gene silencing

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Post-transcriptional gene silencing (PTGS) is a sequence-specific RNA degradation mechanism that follows the recognition of certain RNAs. The products of degradation are small 21–25nt RNAs that subsequently associate with an RNase to allow targeting of other homologous RNAs. Double-stranded RNAs, such as replicating plant RNA viruses and transgenes transcribed as inverted repeats, are potent activators of PTGS. In these examples, PTGS is maintained because of the constitutive production of dsRNA. However, single-stranded RNAs can also activate PTGS, and in these examples, an RNA-dependent RNA polymerase (RdRP) is thought to produce the complementary strand. Thus, RdRP activity can provide a mechanism to maintain PTGS in examples where double-strandedness is not an inherent feature. These observations have raised several interesting questions that will be addressed: what features of RNA mark it as a template for RdRP, and what other proteins are involved in this mechanism?

PTGS can also be associated with DNA methylation of homologous nuclear sequences. For example, infection of a 35S-GFP transgenic plant with a cytoplasmically replicating RNA virus engineered to carry GFP sequences results in PTGS directed against the GFP RNA and DNA methylation of the GFP transgene. These observations suggest that there is communication between cytoplasm and nucleus and that RNA can direct DNA methylation. If the virus carries 35S promoter sequences, then the RNA-directed DNA methylation results in transcriptional gene silencing (TGS). The implication of these results for TGS, paramutation and transposon silencing will be discussed.

P9.2–Gene silencing in *Arabidopsis*

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Transgenes introduced into higher plants can result in the silencing of homologous transgenes or endogenous genes (homology-dependent gene silencing or HDG

silencing). The introduction of extra copies of the chalcone synthase gene (*CHS*) into *Arabidopsis* can result in silencing of the endogenous copy of the gene at the *TT4* locus. Under inducing conditions, wild-type plants are purple due to the accumulation of anthocyanin, and silenced plants are green. Using this simple system, we have investigated the genetics of HDG silencing of *CHS*. The results suggest that the *CHS* copy at *TT4* is modified by an interaction in trans with the transgene copies of *CHS*. This epigenetic change is correlated with increased DNA methylation of *TT4* and the rapid silencing of the gene in the F₁ generation of crosses to plants containing silencing transgenes. Mutants that show reduced DNA methylation (*ddm1*, *ddm2* and *cmt3*) relieve HDG silencing of *TT4* and some revertants of HDG silencing show genome-wide demethylation (*hog1*). Recent progress on the molecular characterization and genetics of the *hog1* mutation will be presented.

P9.3–A tale of gene silencing in tomato: inverted repeats and long and short RNAs

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We investigated the properties of small sense and antisense RNAs and larger aberrant RNAs in transgenic tomato plants exhibiting post-transcriptional gene silencing of both a fruit ripening-specific polygalacturonase (PG) gene and a truncated homologous sense transgene. Two lines of evidence indicate that small RNAs (23nt) arise from the transgene and not the endogenous gene. Firstly, small RNAs are formed in leaves and immature green fruits, where the transgene is expressed from its 35S promoter, but the endogenous PG gene is not expressed. Secondly, these small RNAs hybridise to the truncated transgene, but not the 3' half of the endogenous gene, which was excluded from the transgene. These small RNAs were preferentially generated from the 3' region of the transgene and correspond to a region where the endogenous PG mRNAs were cleaved when expressed in ripening fruit to produce two aberrant RNAs corresponding to the 3' and 5' halves of the endogenous mRNA. We also studied the accumulation of small RNAs in transgenic tomato plants showing gene silencing of ACC oxidase 1 (ACO1). As in the PG-

silenced line, small RNAs (21, 23 and 28nt) were preferentially produced from the 3' region of the ACO1 sense transgene. The introduction of inverted repeats (IRs) to the 5' end of the sense transgene, however, enhanced silencing and switched the preferential production of small RNAs to the 5' region. In addition, the strong silencing associated with IRs was correlated with the more abundant accumulation of 28nt RNAs.

P9.4—Regulation of genomic imprinting in flowering plants

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Genomic imprinting (parent-of-origin specific gene expression) has been shown to be of vital importance for endosperm development in flowering plants. In general, paternally expressed genes promote endosperm development, whereas maternally expressed genes inhibit endosperm development. An imbalance between these paternally and maternally expressed genes leads to aberrant endosperm development, and in severe cases, results in seed abortion.

What regulates genomic imprinting in plants? Like in mammals, methylation plays a role in the uniparental silencing of imprinted genes in flowering plants. We have previously shown that the removal of DNA-methylation erases genomic imprints in the *Arabidopsis* genome. For example, demethylation of the pollen parent leads to re-activation of normally imprinted, endosperm inhibiting genes, resulting in maternalisation of the endosperm: because of the extra set of active endosperm inhibiting genes, a small endosperm is formed. In addition, we propose that the FIS-complex is involved in the regulation of imprinting. FIS mutant ovules give rise to seeds, which abort with a strong paternalised phenotype. We explain this by assuming that the FIS complex downregulates the expression of maternally imprinted, endosperm-promoting genes.

Many questions still remain to be answered. For instance, what is the relationship between methylation and the FIS complex on the maternal side? And what is the link between uniparental methylation and gene silencing? In this presentation, we aim to give an overview of what is known about the regulation of imprinting in flowering plants. In addition, we will describe a screen designed to identify components of the uniparental gene-silencing complex.

P9.5—Imprinted genes in maize

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Endosperm is a product of the double fertilization process that occurs in most angiosperms. Development of the endosperm in most angiosperms is required for the

viability of the embryo and it has been demonstrated that the genomic ratio 2:1 (2 maternal/1 paternal) can be crucial for the successful development of this tissue. The molecular and cellular consequences for parental genomic interactions are, thus, highly important. A molecular mechanism must exist to sense the balance between the contribution of both parental genes, and when an incorrect balance is detected, to arrest development. There is accumulating evidence that this molecular mechanism involves a system of gametic imprinting.

In plants, current evidence suggests that the embryo is less susceptible to the effects of gametic imprinting than the endosperm. To date, only a few different genes have been found to be imprinted in the plant endosperm. In order to explore the role that gametic imprinting plays in endosperm development, and to reveal the molecular mechanisms involved, we have attempted to identify and characterise a range of imprinted genes in maize.

P9.6—Paternally inherited transgenes are down-regulated but retain low activity during early embryogenesis in *Arabidopsis*

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Early seed development seems largely subjected to parent-of-origin effects, especially of maternal origin (1). This can originate from: (i) the unequal maternal-to-paternal genome ratio in the triploid endosperm (dosage effects); (ii) stored gene products in the egg cell (storage effects); or (iii) differential parental gene expression (genomic imprinting). Recent reports support the hypothesis that, in *Arabidopsis*, many of the paternal alleles of genes expressed during embryogenesis, if not all, are not expressed following fertilisation, but are activated later during seed development (2). We present here evidence that paternal gene expression during early embryogenesis may not be controlled by an all-or-none mechanism, and that a basal expression level may be retained for some loci. Our work is based on transgene expression analysis during early seed development in *Arabidopsis*, using the *GUS* reporter gene and the cytotoxic *BARNASE* gene. Both were expressed under the control of two transgenic promoters (from the *AtCYCB1* and *AtLTP1* genes) and using either a trans-activation system or a direct transcriptional fusion. Our results first confirmed a lack of expression of the paternal components, as previously reported (1), but also showed the occurrence of paternal transgene expression, though at a low level, soon after fertilisation. Since multiple transgenic lines were used, our study is likely to reflect a situation affecting endogenous paternal loci.

1. Chaudhury and Berger (2001). *Semin. Cell Dev. Biol.* 5, 381–386.
2. Vielle-Calzada et al. (2000). *Nature* 404, 91–94.

P9.7—Genetic control of genomic imprinting during seed development: isolation and characterisation of genetic modifiers of *meadea*

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Genomic imprinting conveys a parent-of-origin epigenetic control on gene expression and has only been described in mammals and angiosperms so far. Despite its importance in development, human disease, plant breeding and apomixis in crops, the genetic regulation and the mechanisms of imprinting are very poorly understood. Grossniklaus and co-workers isolated the *meadea* (*mea*) mutant, which displays a gametophytic maternal effect embryo-lethal phenotype, and produces 50% aborted seeds (1). *MEDEA* is imprinted and shares several features with the mammalian imprinted genes at the structural, functional and regulatory levels; therefore, *mea* constitutes a unique entry point for studying the genetic hierarchy that controls genomic imprinting. We propose to identify novel trans-acting regulator loci of *MEDEA* imprinting through a genetic suppressor/enhancer screen in the *mea/MEA* mutant background. The phenotypic screen is based on a shift in the seed abortion. Among 1700 M1 plants, several candidate mutants (modifier lines) were isolated: 19 lines suppress seed abortion down to a range of 12–45% and five lines enhance abortion to a range of 55–68%. Four classes of mutations are expected: (i) mutations altering the establishment of the imprint; (ii) mutations altering the maintenance of the imprint after fertilisation; (iii) mutations enabling a functional substitution for *MEDEA*; and (iv) embryo-lethal mutations or other gametophytic mutations ('false-positive'). The rationale to isolate preferentially modifier loci involved in establishing the imprint, and the stage of progress for the genetic characterisation of the mutants, will be described.

1. Grossniklaus et al., 1998. *Science* 280, 446.

P9.8—Endogenous post-transcriptional gene silencing (PTGS) of *Arabidopsis* ion channel genes

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Post-transcriptional gene silencing (PTGS) in plants is an RNA degradation mechanism that shares a lot of similarities with RNA interference (RNAi) in animals. Recent studies of both processes revealed many common elements, such as the involvement of double stranded (dsRNA) and small interfering RNA (siRNA), a similar way of spreading within the organism, and dependence on several key proteins such as RdRP, RNA helicases, etc.

In this work, we present data supporting the idea that PTGS\RNAi can manifest itself endogenously, i.e. without introduction of transgenes or exogenous dsRNA. Our experiments carried out on two cyclic nucleotide gated channel (CNGC1, CNGC3) genes and one putative calcium channel (AtTPC1) gene revealed an accumulation of RNAs of discrete sizes, often more abundant as truncated versions of the expected fully spliced gene products. These products appeared as both sense and antisense, showing distinctive variations, depending on changes in the conditions of treatment.

Interestingly, antisense but not sense AtTPC1 RNA products of 20–30 bp were discovered in the roots and shoots of *Arabidopsis*. This siRNA-like material was found to increase significantly in the roots of plants treated with certain concentrations of NaCl.

Furthermore, analysis of the Marathon PCR sense and antisense products at the 3'-end of AtTPC1 implicates a hypothetical mechanism operating via specific RNA structures as substrates of a RdRP\RNA helicase\RNAseIII-like complex amplification of dsRNA trigger. Also, a possible link between PTGS\RNAi and dsRNA editing can be inferred from the high coincidence of exact A/U to G/C transitions in RNA's sharing identical sequence and length.