EU Sixth Framework Programme (FP6), Integrated Project (IP)

Silencing RNAs: organisers and coordinators of complexity in eukaryotic organisms (SIROCCO)
http://www.sirocco-project.eu/

Project overview

SIROCCO is an integrated project funded by the EU sixth framework programme inside of the *life sciences, genomics and biotechnology for health research* thematic area (priority 1).
The project, coordinated by Prof. Dr. David Baulcombe of the The Sainsbury Laboratory (TSL, Norwich), involves 18 participants from whole Europe. The consortium "*constitutes a highly competent grouping that is capable of achieving the project objectives. It includes individuals who are pioneers of sRNA. These individuals have contributed to and continue to contribute to the rapid recent progress in this field*".
The goal of SIROCCO is to improve human health and to stimulate biotechnology through the enhanced understanding of regulatory small (s)RNAs in eukaryotes.
More in details SIROCCO will

- characterise the full complement of miRNAs and siRNAs in animals and plants. Using bioinformatics, genomics, biochemistry, cell biology and genetics, the consortium members will reveal how these RNAs are produced and processed, how they are transported and how they target specific genes and RNAs for silencing
- investigate the miRNA and siRNA profiles associated with development, with phenotypic divergence within populations, and with diseased states including cancer. The functional genomics of silencing RNAs will be addressed by up- or down-regulation of miRNA and siRNA species. There will also be an assessment of miRNA and siRNA regulatory networks and their interaction with other cellular control mechanisms
- generate databases of silencing RNA sequence and function in several organisms, new technologies for detection and manipulation of these RNAs, and information that will allow siRNA and miRNA profiles to be used as molecular markers and diagnostic methods for natural biological variation including the perturbations associated with disease.
- identify potential targets of disease therapy amongst the components of the small RNA silencing systems.
- improve the specificity with which small RNAs can be employed as therapeutic tools through the insights generated in the project.

AlPlanta contribution

SIROCCO is structured in 8 subprojects. The AlPlanta´s Epigenetics group will be involved in subproject 3 "*The mechanisms of the sRNA function*" more specifically in the study of "*mechanism of RNAi and role of sRNAs in transcriptional and post-trancriptional gene silencing"*. The research will be focused on the investigation of siRNA targeting mechanisms by exploring an unexplained difference between transgenes and endogenous genes as targets of siRNAs.

When silencing is initiated in a localized region of a transgene (i.e. upon introduction of primary dsRNA sharing homology with the region), maintenance of RNAi and transitive silencing is induced – transitive silencing describes a process in which secondary siRNAs are

produced that do not share homology with the initiating primary siRNAs. Silencing thus proceeds from the original siRNA/template binding sites into flanking sequences.
In contrast, if the primary trigger RNA is homologous to an endogene only RNA cleavage but neither maintenance of nor transitive silencing are detectable.

In this context we will investigate the differential efficiency of trans- and endogene silencing. Maintenance of and transitive silencing require RNA-directed RNA polymerase 6 (RDR6) -mediated production of secondary sRNAs. It is reasonable to speculate that only primary transcription of transgenes is associated with the production of so-called aberrant RNAs (abRNAs) that can serve as a template for RDR6.
Because endogenes behave like transgenes when re-introduced as transgenes, the sequence context can hardly be assigned to this phenomenon. Generally, transgene constructs consist of artificial regulatory elements and of coding regions lacking introns. These features may cause that only an incomplete transcription machinery is recruited which could lead to RDR6 template RNA production. Alternatively, the splicosome may sequester abRNA into RNA-degrading compartments preventing its availability for the RNAi machinery.

Based on genetic and biochemical approaches our group will study secondary sRNA production processes in plants.
A focus will be put on the features that render endogenes resistant against maintenance of RNAi and transitive silencing.
Reporter gene constructs lacking or containing functional introns or plant regulatory elements will be introduced into tobacco plants. Plant lines expressing the reporters will be used to study cell-to-cell movement of and transitive silencing by local induction of RNAi. A tagged *RDR6* will be then introduced into transgenic tobacco lines expressing the reporter gene producing double transformants for the investigation of protein complexes and RNA molecules that are associated with the RDR6.

Another focus will be put on the role of siRNAs as triggers of *de novo* DNA methylation in plants.
At least in plants, RNAi can initiate specific *de novo* methylation of DNA sequences that share homology with the trigger RNA. Our group has discovered this RNA-directed DNA methylation (RdDM) mechanism and established systems to induce RdDM in plants.

Initiation of RdDM requires nuclear dsRNA and involves scanning for complementarity of the entire genome by the RNA. However, little is known about the complex that directs the trigger RNA to its target. Moreover, the nature of the ‘scanning RNA’ is still elusive.

Among other essential proteins the domains rearranged methyltransfereases (DRMs) and the RNA-directed RNA polymerase 2 (RDR2) are required for RdDM.

Our group will use biochemical approaches based on the DRM and a host-encoded RDR that has been implicated in sRNA-directed transcriptional gene silencing (TGS). The objective is to determine whether the putative RdDM complex contains short or long, single or double stranded RNAs and whether this complex is associated with only DRM or also with RDR2 (one of the RDR family member polymerases).