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UOS BARI

Programma Short Term Mobility 2014 –Relazione scientifica di attività svolta.

Period of activity: from 20 Nov. -14 Dec. 2014

Host Institution: Institute of Biochemistry and Biotechnology. Martin Luther University Halle-Wittenberg directed by Prof. S.E. Behrens

Title of the research: Studies on the functional relevance of deletions (indel) in the antiviral activity of *Nicotiana benthamiana* Argonaute 1 homeologs in a plant-based *in vitro* system.

Descripton of the scientific activity

Based on the transcript sequences, putative *N. benthamiana* AGO1-1H and L proteins were deduced and aligned using ClustalW (Thompson et al., 1994). A subsequent phylogenetic inspection of *A. thaliana* AGO proteins using MultiAlign (http://multalin.toulouse.inra.fr/multalin/) confirmed that the two putative proteins are strongly related and each belongs to the *A. thaliana* AGO1 clade (Vaucheret, 2008).

Considering that differences in the N-terminal sequence of the human AGO1 were earlier indicated to tune the catalytic activity of the protein (Hauptmann et al., 2013), this prompted us to determine whether the two AGO homeologs are functional, *i.e.* whether they display a comparable AGO/RISC mediated RNA cleavage ('slicer') activity. However, the evaluation of the biological relevance of the two AGO1 homeologs *in planta* is difficult mostly because a differential functional characterization of the H and L AGO1 proteins is impeded by their close similarity. For these reasons, we decided to perform this set of experiments in an *in vitro* system available at the Institute of Biochemistry and Biotechnology. This system, which is based on cytoplasmic extracts of *Nicotiana tabacum* BY-2 cells (BY-2 cell lysate, hereafter BYL), is capable of reconstituting active RISC with *in vitro* translated *Arabidopsis thaliana*, *N. benthamiana* or *Nicotiana tabacum* AGO1 proteins and effectively recapitulates sRNA-driven mRNA regulation (Iki et al., 2010) as well as vsiRNA-driven antiviral silencing (Schuck et al., 2013). In the performed experiments, *N. benthamiana NbAgo1-1H* and *L* cDNAs were *in vitro* transcribed and the corresponding proteins produced by *in vitro* translation in the BYL. Importantly, the translation reaction was carried out in the absence and presence of two types of siRNAs, siRNA 'gf698' and 'vsiRNA1'. These siRNAs were earlier demonstrated to program reconstituted AGO1/RISC such that a GFP-encoding target mRNA and a viral RNA (a Tomato bushy stunt virus defective interfering (DI) RNA) are efficiently targeted and cleaved,

respectively (Iki et al., 2010; Schuck et al., 2013). Both types of RISC reconstituted with either the NbAGO1-1H or with L protein, efficiently cleaved their respective target-RNA in dependency of the programming siRNA. Thus, in comparison with the control reaction, 5' and 3'-cleavage products were clearly detectable. Accordingly, we concluded that both NbAGO1-1 homeologs effectively incorporated siRNAs and formed RISC that was capable to slice mRNA and viral RNA targets, respectively.

References

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Schuck, J., Gursinsky, T., Pantaleo, V., Burgyan, J., and Behrens, S.E. 2013. AGO/RISC-mediated antiviral RNA silencing in a plant in vitro system. Nucleic acids research 41:5090-5103.

Thompson, J.D., Higgins, D.G., and Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic acids research 22:4673-4680.

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Comunication of results and preparation of the paper.

During my STM, beside the experimental activity above described, we have assembled the results previously separately in both Institutions (i.e. ISPP-CNR and UniHalle) in order to finalize a manuscript. The manuscript is ready to be submitted to one International Journal with high scientific impact in Plant Science field. In the aknownledgements section is highlighted the relevant support of CNR-STM 2014 program.

Others

The STM has given the possibility toi support and reinforce the collaboration between me and Prof. Behrens. We could also set up a common program of research that will be carried out in collaboration in the next future.

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