Virus-induced gene silencing: mechanisms and applications

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Origin of siRNAs in response to virus infection in plants: plant virus-derived small interfering RNAs originate predominantly from highly structured single-stranded viral RNAs. RNA silencing is conserved in a broad range of eukaryotes and includes the phenomena of RNA interference in animals and posttranscriptional gene silencing (PTGS) in plants. In plants, PTGS acts as an antiviral system; a successful virus infection requires suppression or evasion of the induced silencing response. Small interfering RNAs (siRNAs) accumulate in plants infected with positive-strand RNA viruses and provide specificity to this RNA-mediated defence.

It has long been assumed that siRNAs are derived from double-stranded RNA intermediates occurring during the replication cycle of positive strand RNA viruses. This assumption is viewed with some scepticism by researchers involved in virus replication since it is not known if most positive-strand RNA viruses replicate through a completely double stranded RNA, partly double-stranded or predominantly single stranded replicative-intermediate. Moreover, the accessibility by Dicer of double-stranded RNA structure of a replicative-intermediate *in vivo* is questionable. In collaboration with Joszef Burgyán's team we published evidence that siRNAs are unevenly distributed across the viral genome, are mainly derived from the positive-strand, and are imperfect duplexes as would be expected if the siRNAs derived from highly base-paired RNA structures[1].

Several lines of evidence support this model. We previously demonstrated that inverted-repeats folding as hairpin upon transcription enhance the silencing response of positive-stranded RNA viruses, suggesting that formation of dsRNA is a limiting step in the silencing response[2]. Further analysis of siRNA accumulation in plants infected with viruses expressing inverted-repeats indicated that more siRNAs are accumulating than the corresponding antisense sequence. The polarity of the siRNAs was predominantly for the positive strand. A survey of virus specific siRNAs characterized by a sequence analysis of siRNAs from plants infected with *Cymbidium ringspot tombusvirus* (*CymRSV*) indicates that siRNA sequences originate for a large majority from the positive strand and have a non-random distribution along the length of the viral genome, suggesting that there are hot spots for virus-derived siRNA generation. Finally, an analysis of siRNAs derived from two other non-related positive-strand viruses (*Tobacco mosaic virus* and *Potato virus X*) showed that they both display the same over-representation for the positive strand as for *CymRSV* siRNAs. Taken together, these results suggest that virus-derived siRNAs originate predominantly by direct Dicer cleavage of imperfect duplexes in the most folded regions of the positive strand of the viral RNA. By this mean, the siRNA-based antiviral surveillance system would target preferentially the replicative intermediate negative strand for degradation, making this mechanism more effective as such intermediate is much less abundant for replication (See Fig. 1).

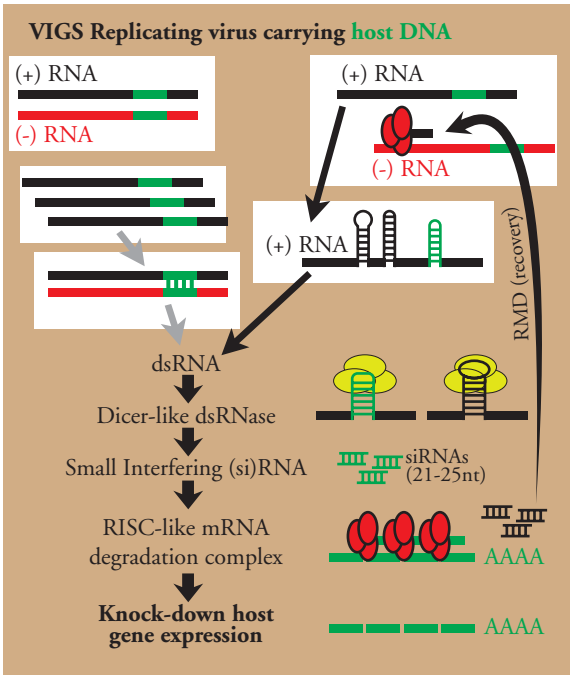

Figure 1 Virus-derived siRNAs originate predominantly from the positive strand as opposed from pairing of negative and positive viral RNA during replication. This in turn makes the surveyance RNA-mediated defence (RMD) mechanism against positive-strand viruses more efficient by targeting the negative strand replicative intermediate in a RISC complex guided by the siRNA, leading ultimately to a recovery phenotype.

VIGS-based functional characterization of genes associated with powdery mildew resistance in barley The properties of this RNA-mediated defence mecha-

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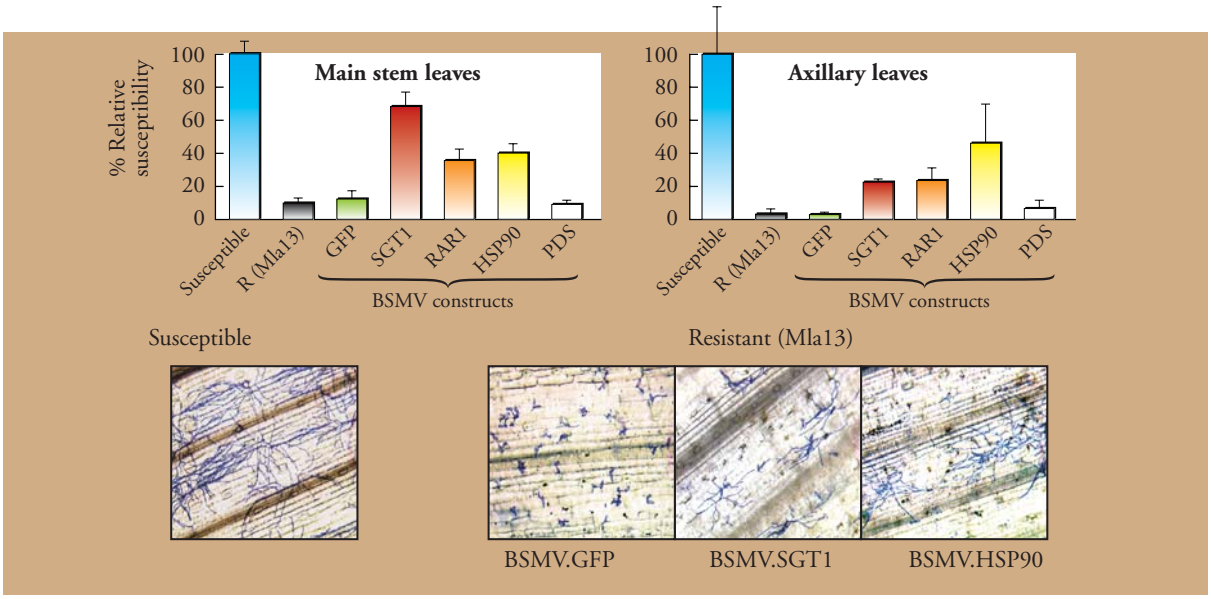

Figure 2 *Rar1*, *Sgt1* and *Hsp90* are required components for *Mla*-mediated resistance in barley to *Blumeria graminis* f.sp. *hordei* (*Bgh*). Percentage of relative susceptibility (to the Susceptible cultivar Golden promise) of BSMV-silenced Sgt1, Rar1 and Hsp90 on resistant cultivar harbouring the *Mla13* resistance gene to *Bgh* (indicative of a resistance-breaking phenotype) in comparison to either BSMV uninfected (R-Mla13) or challenged by a BSMV VIGS construct that does not trigger silencing of genes required for *Bgh* resistance (*GFP* and *PDS*). Observation of typical fungal structures (aniline blue staining) in silenced and control leaves from susceptible (non-infected by BSMV) and resistant barley cultivars infected by BSMV. GFP (no fungal growth) or by BSMV VIGS constructs BSMV.SGT1 and BSMV.HSP90 triggering silencing of respectively Sgt1 and Hsp90.

nism against virus infection offers the possibility to exploit plant viruses as a functional genomic platform based on gene silencing to elucidate plant gene function.

For this purpose, we successfully implemented virus induced gene silencing (VIGS) in barley (*Hordeum vulgare*) for the functional characterisation of genes required for resistance (*Mla*-mediated) towards the biotrophic barley pathogen *Blumeria graminis* f.sp. *hordei* (*Bgh*) (collaboration with K. Shirasu). Mutational analysis and map-based cloning in barley have identified *Rar1* (encoding a small zinc-binding protein with two highly similar domains CHORD-I and –II) and *Sgt1* (encoding a protein that associates with Skp1-Cullin-Fbox type E3 ubiquitin ligase complexes) as being required for *Mla*-resistance to *Bgh*. It has been shown that SGT1 interacts with RAR1 protein and as well with a cytosolic heat shock protein 90 (HSP90). So far the functional proof that *Hsp90* is a required component in disease resistance signalling pathways has been demonstrated only for dicotyledonous plants and is still lacking for *Mla*-mediated resistance in barley. Barley cultivar Clansman (a barley cultivar harbouring the *Mla13* resistance gene allowing *Barley stripe mosaic virus* [BSMV]-VIGS vector replication and systemic movement without causing excessive symptoms), was chosen as the most suitable host for BSMV-VIGS-based functional characterisation of *Rar1*, *Sgt1* and *Hsp90* in the *Mla*-mediated resistance towards powdery mildew. BSMV-induced gene silencing of these candidate genes, which are associated in many but not all race specific pathways, proved to be robust and could be detected at both mRNA and protein levels for up to 21 days post-inoculation. Systemic silencing was observed not only in the newly developed leaves from the main stem but also in axillary shoots. By examining fungal development from an incompatible mildew strain carrying the cognate *AvrMla13*-gene on BSMV-silenced plants for *Rar1*, *Sgt1* and *Hsp90*, a resistance breaking phenotype was observed, while plants infected with BSMV control constructs remained resistant[3].

These findings represent the first evidence in monocots that *Hsp90* is a required component for *Mla13*-mediated race specific resistance and demonstrates that BSMV-induced VIGS is a powerful tool for the rapid characterization of genes involved in pathogen resistance in barley (See Fig. 2).

References

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