Investigation of the dimerisation of the p3 and p4 proteins of the *European mountain ash ringspot-associated virus* (EMARaV) using the Yeast Two-Hybrid System

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Introduction

The *European mountain ash ringspot-associated virus* (EMARaV) leads to typical symptoms of mottling and chlorotic ringspots (Fig. 1) on leaves of the European mountain ash (*Sorbus aucuparia* L.) and is widely distributed in North and Central Europe (Büttner et al., 2013, Roßbach et al., 2015). EMARaV consists of four negative orientated single stranded RNA molecules (Fig. 2) each coding for a single protein (p1 – p4). The function of proteins p1 – p3 has been determined using sequence comparison methods (Mielke and Mühlbach, 2007). It has been proposed that p4 is a movement protein (MP). MP are essential viral proteins enabling viral spread in the host plant. MP facilitate cell to cell transport of virus particles by binding to viral nucleocapsid protein complexes in combination with tubule formation at plasmodesmata through MP multimerisation. A dimerisation of the EMARaV p4 protein and the interaction of p4 with the nucleocapsid protein (p3) was investigated using the yeast two-hybrid system (YTHS).

Method and materials

The EMARaV p3 and p4 coding regions (Fig. 2) were amplified by PCR using specific primers which were used to introduce restriction sites. The p3 and p4 coding regions were cloned into the yeast vectors pACT2 and pAS2; the introduced restriction sites allowing the inframe insertion with the GAL4-activation and the GAL4-binding domains respectively (Fig. 3). *Saccharomyces cerevisiae* (Y190) was subsequently transformed with both vectors and then analysed for protein-protein interaction by use of a LacZ assay and selection on synthetic dropout (SD) medium lacking histidine and supplemented with 30 mM 3-aminotriazole to supress non specific reporter activation. Single constructs were also tested for possible auto-activation.

Yeast Two-Hybrid System

The YTHS utilises the transcriptional activator GAL4 which has a separable activation (GAL4-AD) and binding domain (GAL4-BD). Through the inframe insertion of genome fragments (shown in Fig. 3) the two proteins of interest are expressed as C terminal fusions to the GAL4-AD and GAL4-BD. The interaction of the two proteins in a living yeast cell restores a fully functional GAL4 transcription factor and the reporter genes (*LacZ* or *His*3-gene) are expressed in the yeast cells (Fig. 4).

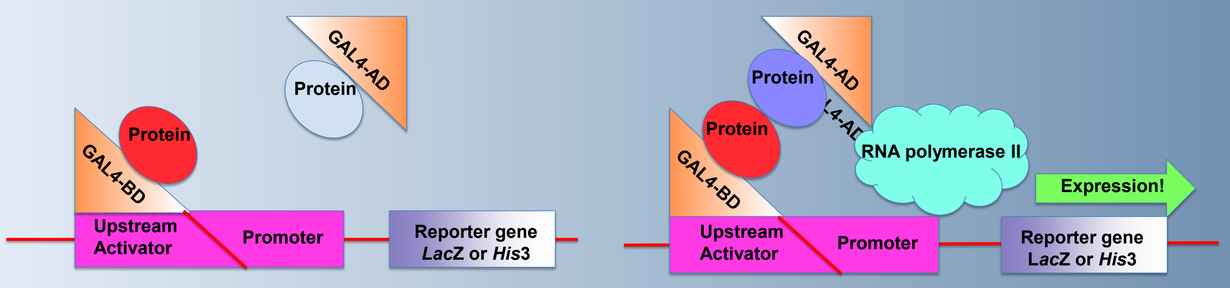


Fig. 4 Schematic representation of the yeast two-hybrid system. No protein interaction results in a non-functional transcription factor and the absence of reporter gene expression (left), interaction of „bait“ and „prey“ protein leads to a reconstructed transcription factor and to expression of the reporter gene (right)

Summary and conclusions

The p3 and p4 coding regions of EMARaV were successfully cloned into the yeast vectors pACT2 and pAS2 and confirmed by sequencing. The YTHS was used to test for protein-protein interaction.

- Dimerisation of the p4 proteins was observed on –His SD medium but was not confirmed by the LacZ assay.
- A p3-p3 interaction was seen, as expected of a nucleocapsid protein (table 1). However the single pAS2 - p3 construct produced an auto-activation of the reporter gene and should be excluded from further YTHS interaction studies (table 2).
- Additional plasmid constructs including p4 N and C terminal deletions, as wells as the pACT2-p3 showed no auto-activation of the reporter genes.
- **An interaction of the EMARaV p3 and p4 proteins was not observed and he results of this study do therefore not provide evidence for the role of the EMARaV p4 as a movement protein and its function remains elusive**.

Further studies should test protein-protein interaction between deletion mutants of the p3 and p4 coding regions (examples shown in Fig 2.) to corroborate these first findings and identify possible functional domains.

Fig.1 *Sorbus aucuparia* L. (a & b)) and EMARaV infected leaves of *S. aucuparia* displaying chlorotic ringspots (c) and symptoms of mottling (d & e)

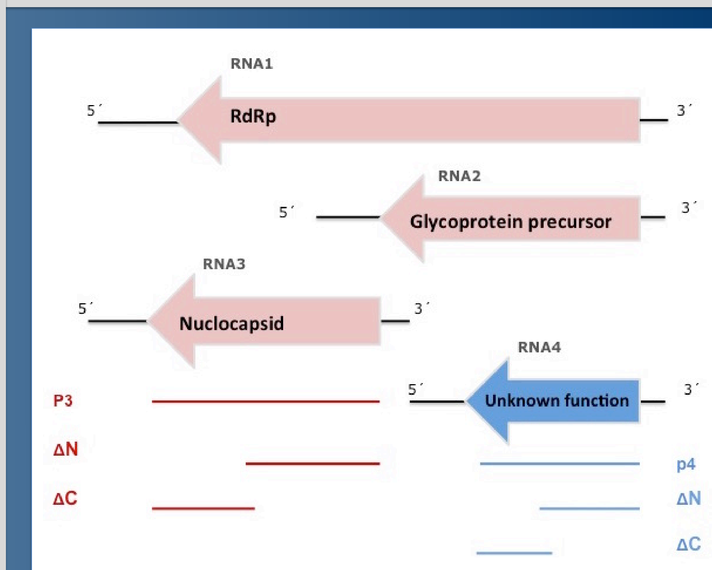


Fig.2 Genome organisation of EMARaV and schematic representation of p3 and p4 regions (complete and deletion mutants) cloned into YTHS vectors pACT2 and pAS2

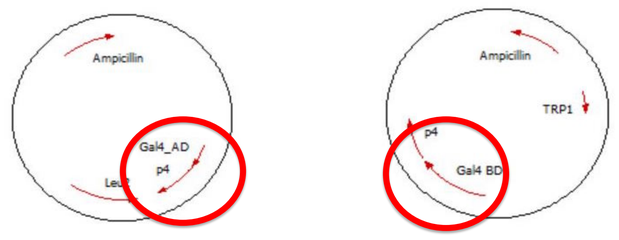


Fig.3 Schematic representation of pACT2 (left) and pAS2 (right) vectors with inframe insertion of p4 is illustrated as an example

Results

Table 1. Results of LacZ assay and selection on –His SD medium containing 30 mM 3 aminotriazole

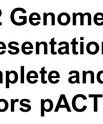
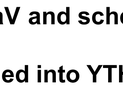
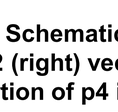
			GAL-BD: pAS2- p3	GAL-BD: pAS2-p4
GAL-AD	pACT2 - p3	LacZ		
		-His Test	+++	+
	pACT2 - P4	LacZ		
		-His Test	+++	+++

Table 2. Results of LacZ assay of single transformants testing for auto-activation

Plasmid construct	LacZ assay	Result
pAS2 - p3		positive
pAS2 - p4 ΔN1		positive

The following plasmid constructs tested negative for auto-activation, so can be used in further YTHS studies: pACT2, pAS2, pACT2- p4, pAS2 - p4, pACT2 - p4 ΔN1, pACT2 - p4 ΔC2, pACT2 - p3, pAS2 - p4 and pAS2 - p4 ΔC2

Legend: LacZ: Blue = activation of the LacZ reporter gene, +++ = strong growth on –His SD medium (100% of the clones), + = no or weak growth on –His SD medium (< 50% of the clones)

References

Büttner, C., V B, S., Bandte, M., Mühlbach, H-P.: Forest diseases caused by viruses. In*: Infectious forest diseases*. Gonthier, P. und G. Nicolotti, Oxfordshire, CABI, 50-75 S.
Mielke, N., H-P. Mühlbach, 2007: A novel, multipartite, negative-strand RNA virus is associated with the ringspot disease of European mountain ash (*Sorbus aucuparia* L.). J. Gen. Virol. **88**, 1337-1346.
Roßbach J., H-L. Dieckmann, T. Büttner, H-P. Mühlbach, S. von Bargen, C. Büttner, 2015: Genetic variability and phylogeny of European mountain ash ringspot-associated virus RNA3 and RNA4. Forests, **6**, 4072-408.

This research is funded by the DFG (BU 890 / 27-1)