Mutations in cucumber mosaic virus affecting interactions of the 2b silencing suppressor with AGO1 and CMV-induced effects on aphid performance

My 10-week research project was focused on how cucumber mosaic virus (CMV) induces disease symptoms in its hosts. CMV is a member of the *Bromoviridae* family and is the type species of the viral genus *Cucumovirus.* CMV has a very wide host range, around 1,200 species, which is thought to be the largest host range of any plant virus. Hosts include many important crops ranging from cucumbers and melons, to tomatoes and peppers, and across to peas, bananas, celery and spinach. The virus is generally found in temperate areas around the world, this is partly determined by the distribution of its main vector, aphids, although CMV can also be transmitted by sap, parasitic weeds and (in some hosts) through seeds. CMV-induced symptoms can differ between hosts but generally the whole plant suffers from stunted growth; hence the importance of the virus commercially, affecting crop yields. In some hosts there are severe developmental symptoms in which leaves suffer from narrowing of younger leaves ("shoestring" symptoms) and mosaic patterns, although the virus rarely kills its hosts.

CMV encodes five proteins. The smallest of these, the 12kDa 2b protein, interferes with antiviral silencing and also disrupts plant defence mechanisms controlled by jasmonic acid and salicylic acid as well as being an important virulence factor. The 2b protein also has effects on plant host resistance to the aphid vectors of CMV. Distinct domains within the 2b protein enable it to interfere with RNA silencing pathways by binding double-stranded short-interfering RNAs and microRNAs and by inhibiting activity of the Argonaute (AGO)-type endoribonucleases, AGO1 and 4. RNA binding activity is critical for suppression of antiviral silencing while in *Arabidopsis thaliana* the ability of the 2b protein to bind to AGO1 is important for its ability to interfere with microRNA-regulated plant gene expression and it is thought to be important for triggering a strong anti-aphid resistance mechanism. Not surprisingly, the induction of aphid resistance by 2b is normally inhibited by another viral gene production during infection. Previously published studies point to the central domain of 2b determining 2b-AGO1 interactions. However, more recent work suggests that this is not correct and that it is the C-terminal domain that controls 2b-AGO1 interactions. In *A. thaliana* plants the 2b protein of a severe CMV strain (Fny) interacts with AGO1 but the 2b of LS-CMV does not. There is one amino acid difference between the C-terminal domains of LS2b and Fny2b. Switching these residues affects the symptoms induced by the viruses so that the mild LS-CMV now causes severe symptoms.

The main aim of my project was to generate DNA constructs to investigate the interaction of the 2b protein with AGO1 by determining if replacement of the LS 2b protein's C-terminus with the C-terminus of the 2b protein of Fny-CMV allows it to interact with AGO1. I cloned the mutated LS 2b gene sequence, encoding a protein possessing a C-terminus identical to the Fny 2b protein, as well as the wild-type LS 2b gene sequence into plasmid vectors to generate chimeric genes encoding fusions with either the N- or C-terminal domains of the yellow fluorescent protein (YFP). These constructs were transformed into *Agrobacterium tumefaciens* and will be used with split YFP-AGO1 fusion constructs for bimolecular fluorescence complementation, a technique that allows protein-protein interactions to be monitored *in planta*. I also carried out patch assays in which *A. tumefaciens* cells carrying my constructs were co-infiltrated into *Nicotiana benthamiana* leaves together with cells carrying a green fluorescent protein reporter gene to assess the ability of wild-type and mutant 2b proteins to inhibit silencing of the *GFP* gene. Unfortunately, this experiment came at the end of my project and I was not able to complete that aspect of the work. Nevertheless, other members of the research group will use my constructs in future work.

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