



Article

# Spectroscopic Investigation of the Kinetic Mechanism Involved in the Association of Potyviral VPg with the Host Plant Translation Initiation Factor eIF4E

Jocelyne Walter <sup>1</sup>, Amandine Barra <sup>1</sup>, Justine Charon <sup>2</sup>, Geneviève Tavert-Roudet <sup>1</sup> and Thierry Michon <sup>1,\*</sup>

<sup>1</sup> University of Bordeaux, INRAE, Biologie du Fruit et Pathologie, UMR 1332, F-33140 Villenave d'Ornon, France

<sup>2</sup> University of Sydney, Faculty of Sciences, Charles Perkins Center D17, Camperdown campus, 2026 NSW, Australia

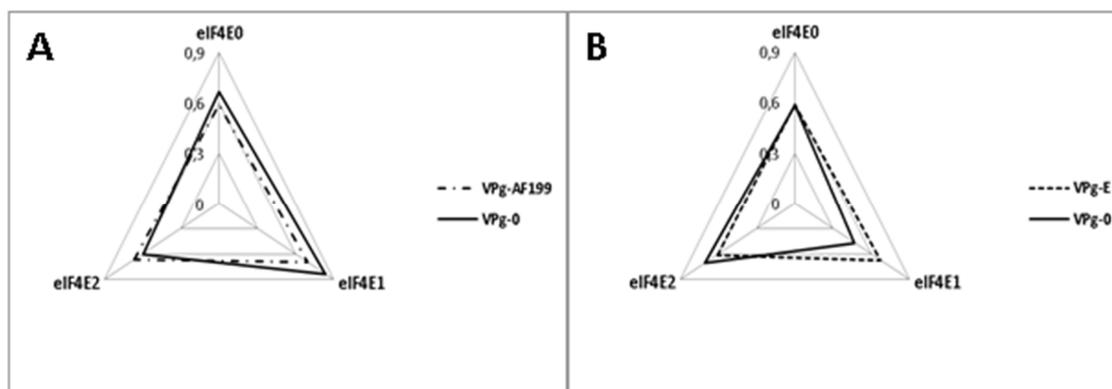
## Supporting Information

Figure S1: Comparison of all interaction combinations between eIF4E from resistant and susceptible lettuces and VPgs from resistance or non-resistance breaking LMVs.

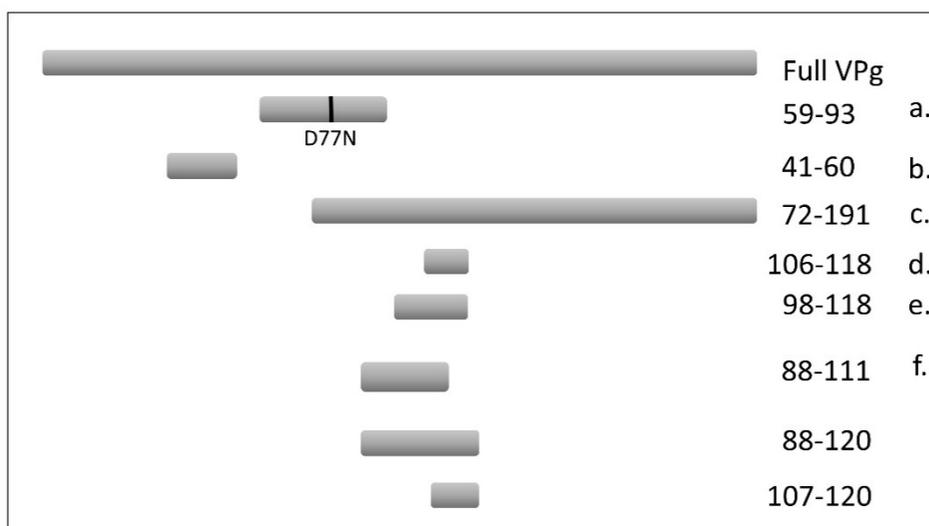
Figure S2: Mapping of eIF4E region involved in the interaction with VPg.

Figure S3: Transient kinetics of binary complex formation between eIF4E (0.3  $\mu$ M) and VPg88–111 or VPg88-120.

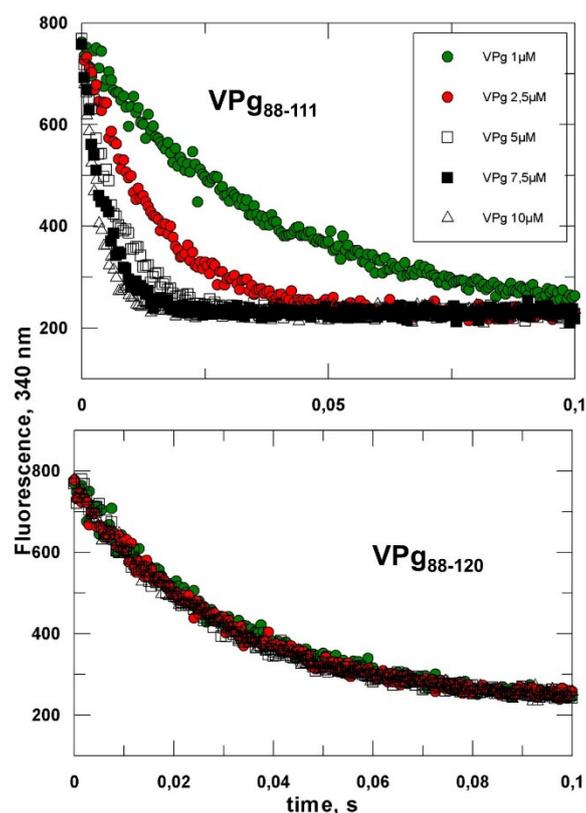
Figure S4: Steady state titration of lettuce eIF4E association with VPg from LMV.



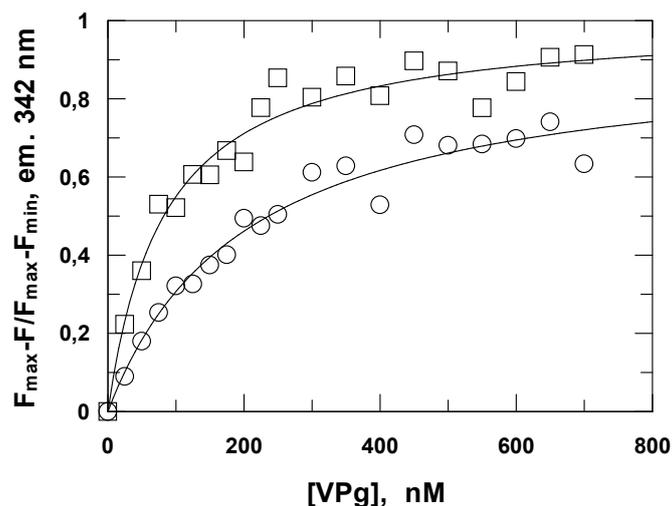
**Figure S1.** Comparison of the interactions between eIF4E from susceptible (eIF4E<sup>1</sup>, eIF4E<sup>2</sup>) lettuce cultivars and VPg from non-resistance-breaking (VPg<sup>0</sup>) or resistance-breaking LMV isolates (VPgAF199 and VPgE). Interactions were assessed by ELISA-based assays, as described previously [1]. (A) VPgAF199 versus VPg<sup>0</sup>. (B) VPgE versus VPg<sup>0</sup>. Purified eIF4Es bait (4  $\mu$ g ml<sup>-1</sup>) were immobilized in the wells, and VPgs prey (6  $\mu$ g ml<sup>-1</sup>) were added. Interactions were revealed using a combination of a monoclonal antibody directed against LMV-VPg (mAb 1H5) and anti-mouse alkaline phosphatase conjugates. Values in the graphs correspond to absorbance at 405 nm after background noise subtraction (no immobilized bait protein in the wells). All proteins were prepared following the protocol detailed in the material and method section, except that the His tag was not removed.



**Figure S2.** Scheme of the VPg regions proposed as being involved in the interaction with eIF4E. (a). TuMV, D77N mutation was reported to prevent TuMV infectivity [2]; (b). PVY [3]; (c). TuMV [4]; (d). TEV [5]. (e). PVY [6]. (f). Synthetic peptides derived from the LMV VPg central region, tested in this study for their ability to bind eIF4E from lettuce.



**Figure S3.** Transient kinetics of binary complex formation between eIF4E (0.3  $\mu\text{M}$ ) and VPg88-111 or VPg88-120. Peptide concentrations varied between 1 and 10  $\mu\text{M}$ .



**Figure S4.** Steady state titration of lettuce eIF4E association with VPg from LMV. Open circle, VPg from non-resistance-breaking virus strain LMV0. Open square, VPg from resistance-breaking virus strain LMV AF199. Measurements were performed as in [7].  $K_D$  was  $202 \pm 28$  nM and  $82 \pm 10$  nM for VPg of LMV0 and AF199 strains, respectively.

## References

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