A viral effector suppresses cell-to-cell spread of silencing by targeting two plasmodesmal receptor-like kinases

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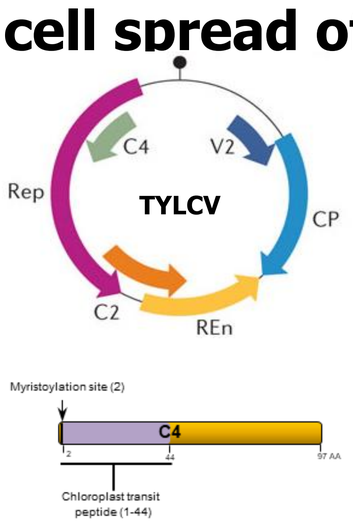
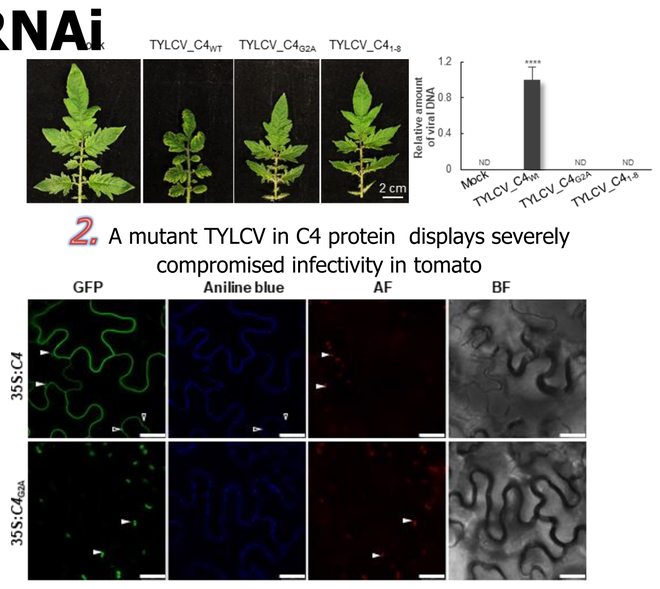
Abstract

RNA interference (RNAi) in plants can move from cell to cell, allowing for systemic spread of an anti-viral immune response. How this cell-to-cell spread of silencing is regulated is currently unknown. Here, we describe that the C4 protein from *Tomato yellow leaf curl virus* can inhibit the intercellular spread of RNAi. Using this viral protein as a probe, we have identified the receptor-like kinase (RLK) BARELY ANY MERISTEM 1 (BAM1) as a positive regulator of the cell-to-cell movement of RNAi, and determined that BAM1 and its closest homologue, BAM2, play a redundant role in this process. C4 interacts with the intracellular domain of BAM1 and BAM2 at the plasma membrane and plasmodesmata, the cytoplasmic connections between plant cells, interfering with the function of these RLKs in the cell-to-cell spread of RNAi. Our results identify BAM1 as an element required for the cell-to-cell spread of RNAi and highlight that signalling components have been co-opted to play multiple functions in plants.

C4 from TYLCV is a plasma membrane/plasmodesmal protein and suppresses cell-to-cell spread of RNAi

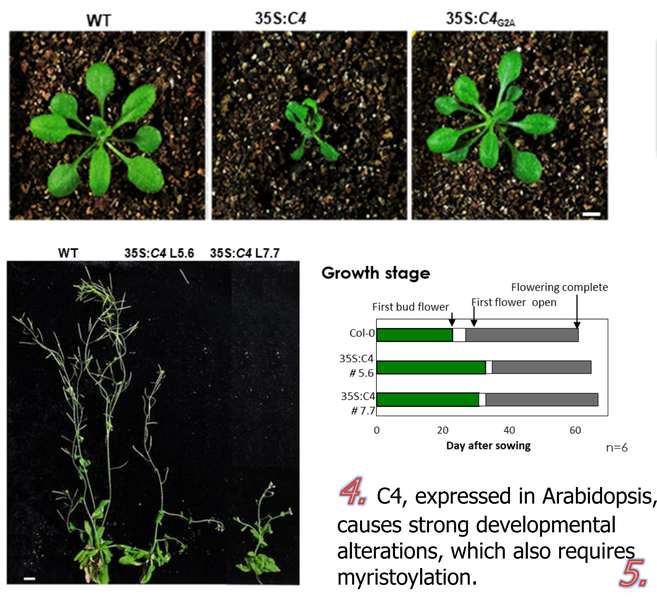


1. Genomic organization of *Tomato yellow leaf curl virus* (TYLCV) and localization motifs in its C4 protein.

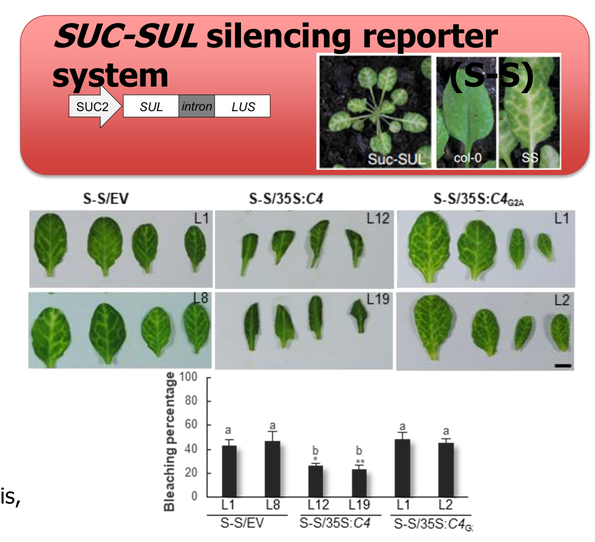


2. A mutant TYLCV in C4 protein displays severely compromised infectivity in tomato

3. C4 localizes to different compartments in the plant cell, mainly the plasma membrane, plasmodesmata and chloroplasts. Empty arrowheads indicate plasmodesmata; filled arrowheads indicate chloroplasts.

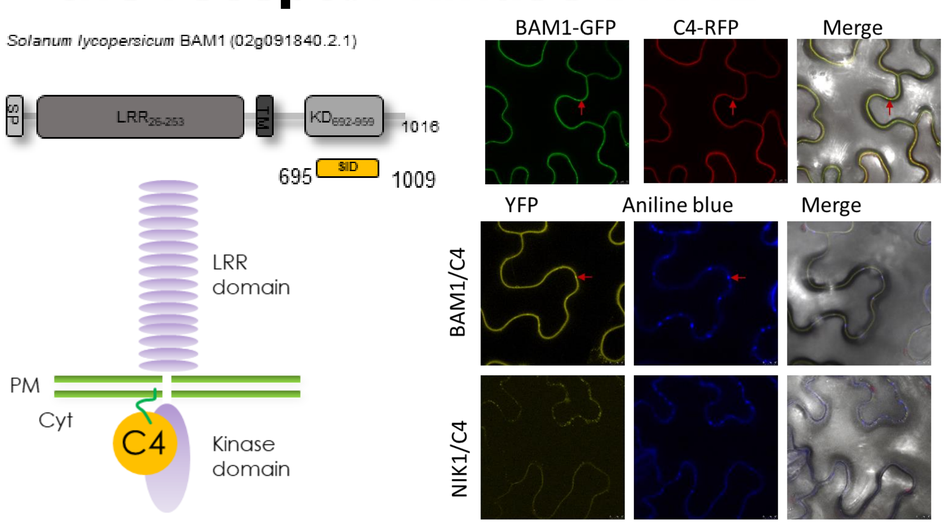


4. C4, expressed in Arabidopsis, causes strong developmental alterations, which also requires myristoylation.



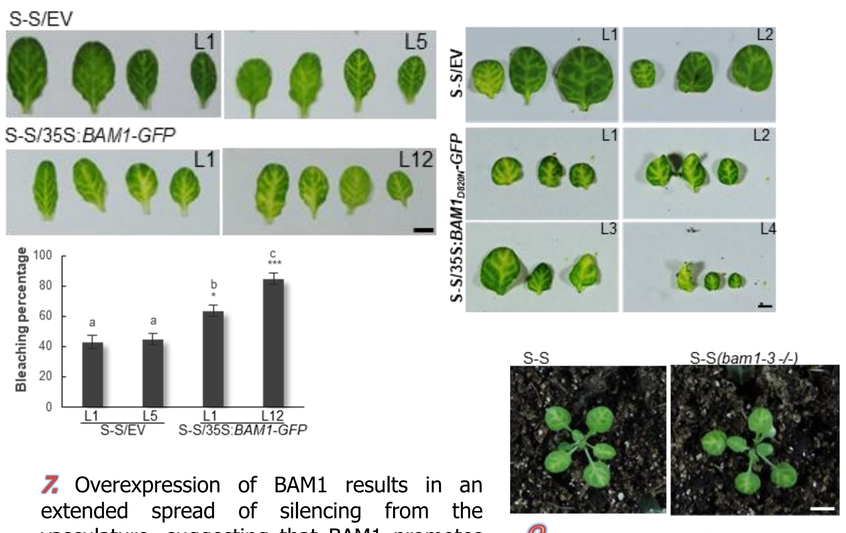
5. Expression of wild type C4, but not of the non-myristoylable mutant $C4_{G2A}$, greatly diminishes the spread of the silencing phenotype, suggesting that PM/PD-localized C4 may interfere with the cell-to-cell movement of silencing from the vasculature.

C4 from TYLCV interacts with the receptor kinase BAM1



6. The interaction between C4 and the receptor-like kinase (RLK) BARELY ANY MERISTEM 1 (BAM1) occurs through the kinase domain of BAM1, as shown by mapping of this interaction in yeast. This interaction was confirmed by BiFC, Co-IP, FRET-FLIM, and gel filtration. The signal peptide (SP), leucine-rich repeats (LRR), transmembrane domain (TM), and kinase domain (KD) are shown. Arrowheads indicate plasmodesmata.

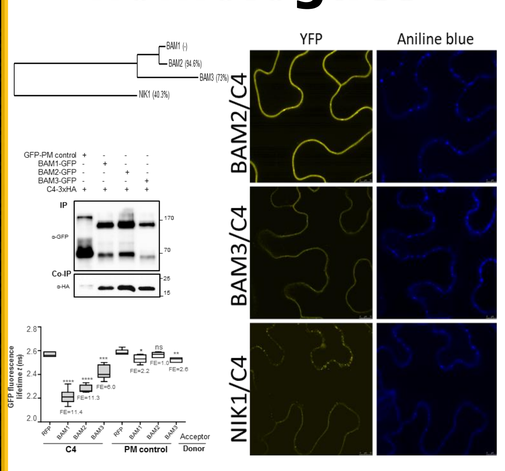
BAM1 promotes the cell-to-cell spread of RNAi



7. Overexpression of BAM1 results in an extended spread of silencing from the vasculature, suggesting that BAM1 promotes cell-to-cell movement of the silencing signal. BAM1 kinase, using a kinase-dead mutant, activity does not seem to be required for this function.

8. A mutation in the *BAM1* gene alone did not affect the silencing phenotype of the SUC:*SUL* plants, which suggests functional redundanc

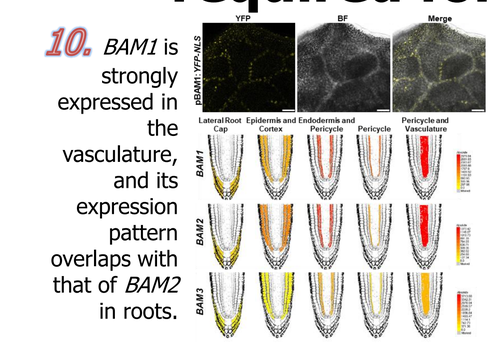
BAM1 has two homologues

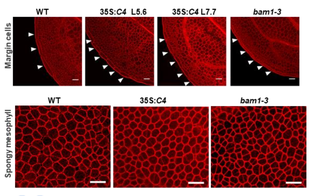


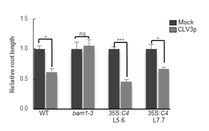
9. The kinase domains of BAM1 and BAM2 are 94% identical at the protein level, whereas those of BAM1 and BAM3 are 73% identical. Consistent with this, C4 can also interact with BAM2 and, more weakly, with BAM3.

BAM1 and its homologue BAM2 are required for the cell-to-cell spread of RNAi

10. *BAM1* is strongly expressed in the vasculature, and its expression pattern overlaps with that of *BAM2* in roots.

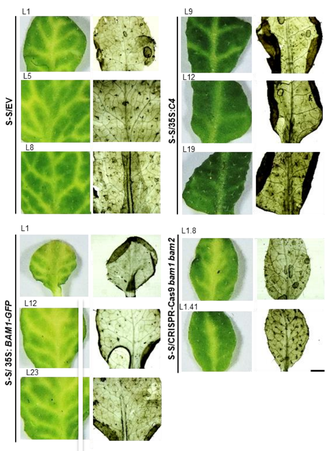




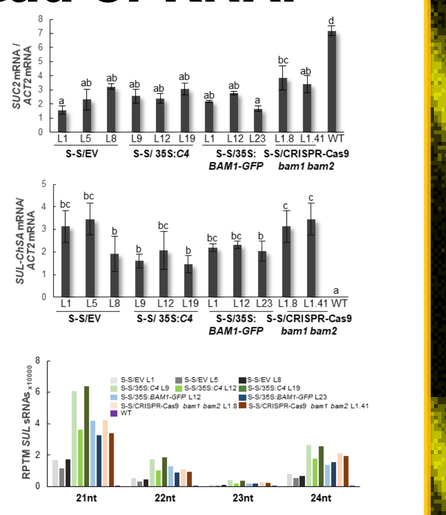


11. Similar developmental phenotypes in the transgenic Arabidopsis lines expressing *C4* and the *bam1-3* single or *bam1-3 bam2-3* double mutant.

12. C4 impairs a subset of all activities of BAM1. For example, the response to CLV3p is not affected.

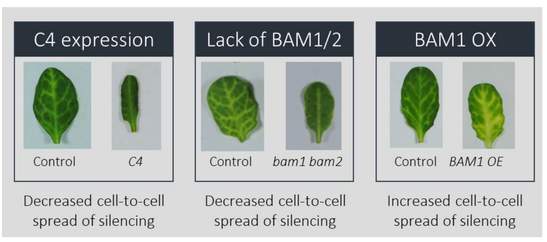


13. Spread of silencing and venation pattern in SUC:*SUL* lines expressing *C4*, overexpressing BAM1, or mutated in *BAM1* and *BAM2*.



14. Expression of the endogenous *SUC2* gene and the SUC:*SUL* transgene and accumulation of *SUL* sRNAs in SUC:*SUL* transgenic plants expressing *C4*, overexpressing *BAM1*, or mutated in *BAM1* and *BAM2*.

Conclusions



- BAM1 promotes the cell-to-cell spread of RNAi
- BAM1 and BAM2 act redundantly
- C4 targets BAM1/2 and suppresses their activity

This is the first description of an element (BAM1/BAM2) required for the cell-to-cell spread of RNAi.

Our work identifies a novel function of the RLKs BAM1/BAM2 in the cell-to-cell spread of RNAi; however, the underlying molecular mechanism is still elusive.