Localization of effectors from *Xanthomonas campestris* triggering plant reactions dependent on an N-myristoylation signal

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The Gram negative bacterium *Xanthomonas campestris* pv. *vesicatoria (Xcv)* is the causal agent of bacterial spot disease in pepper and tomato. Bacteria enter the plant via wounds and stomata and multiply in the intercellular spaces of the mesophyll, but they do not enter the cytoplasm of plant cells. Essential for pathogenicity is a specialized type III secretion system which delivers bacterial effector proteins directly into the plant cell. These effectors manipulate the plant cell metabolism to promote bacterial growth. So far, more than 20 effector proteins are known for *Xcv*. To study the localization of individual effector proteins we transiently expressed single effector genes via *Agrobacterium tumefaciens* in *Nicotiana benthamiana*.

We investigated the effectors XopE2 (*Xanthomonas* outer protein E2) and XopJ, both possessing a conserved putative N-myristoylation motif in the N-terminus. Myristoylation at residue G2 anchors proteins in the plasma membrane. Formation of the protein was determined by confocal laser scanning microscopy of the leaf epidermis. Leaf segments expressing the genes were chemically fixed, embedded in polyethylene glycol, sectioned and labelled for immunofluorescence. Immunogoldlabelling of chemically fixed samples failed. That's why we used high pressure frozen/freeze substituted material for the subcellular localization. The samples were subsequently embedded in HM20. Ultrathin sections if this material were appropriate for immunocytochemistry

Confocal laser scanning microscopy and immunocytochemistry revealed that GFP fusions of XopE2 and XopJ localized to the plant cell plasma membrane (Fig. 1). Targeting to the membrane is probably due to N-myristoylation, because a point mutation in the putative myristoylated glycine residues G2 in XopE2 and XopJ resulted in cytoplasmic localization of the mutant proteins.

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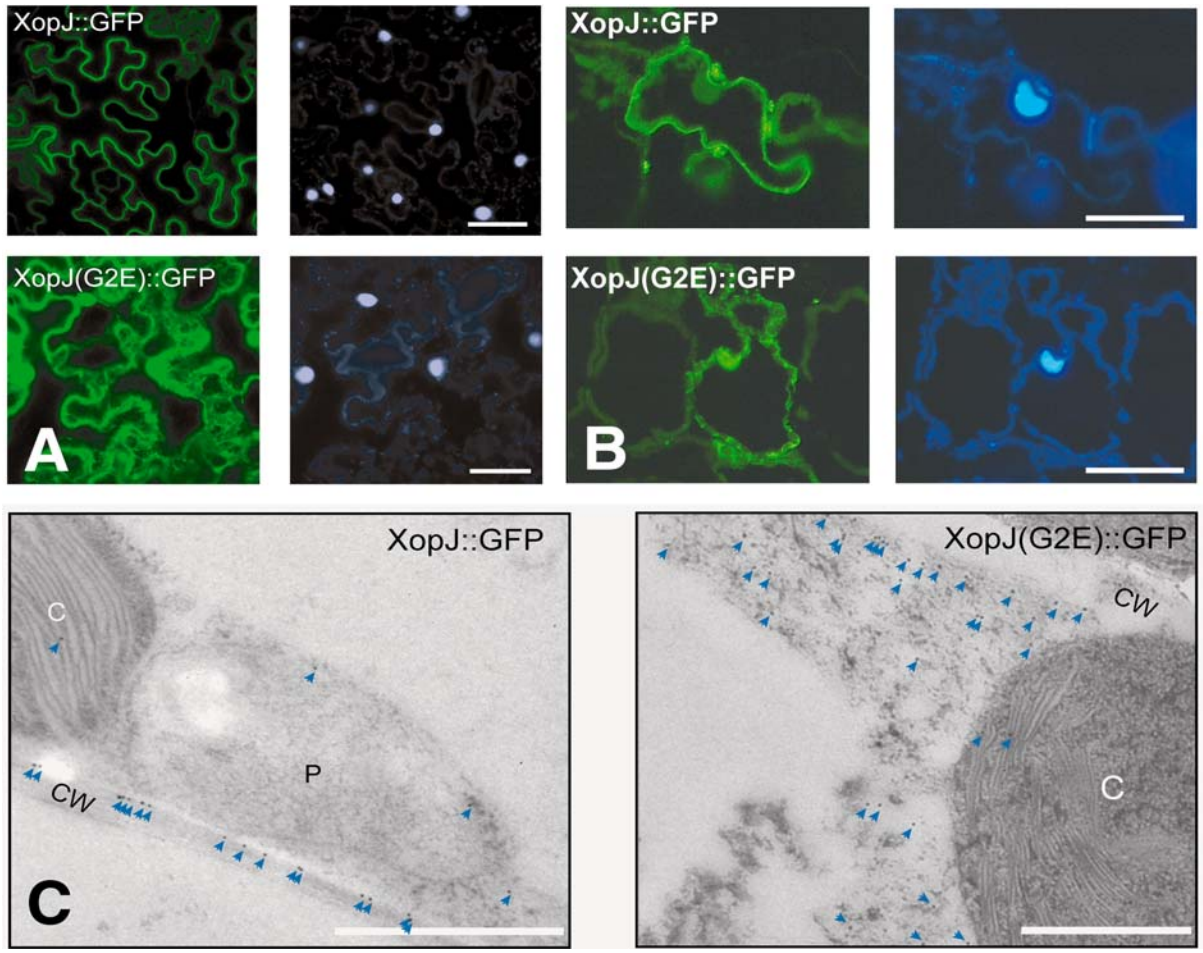

Figure 1: Localization of XopJ and of a mutated version of XopJ in cells of *Nicotiana benthamiana*. A: left panel: Localization of XopJ::GFP and XopJ(G2E)::GFP in epidermis cells by confocal laser scanning microscopy; right panel: DAPI-staining of corresponding cells; size bars correspond to 50 µm. B: left panel: Localization of XopJ::GFP and XopJ(G2E)::GFP in thin sections of mesophyll cells using an polyclonal antibody against GFP; right panel: DAPI staining of corresponding cells; size bars correspond to 50 µm. C: Ultrastructural localization of XopJ::GFP and XopJ(G2E)::GFP; size bars correspond to 0.5 µm. All three methods of localization reveal that XopJ is localized at the plasma membrane but mutation of the myristoylation site (XopJ(G2E)::GFP) causes the distribution of the protein in the whole cytoplasm.

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