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PLANT PATHOGENS

Oomycete kinase blights potatoes

Phytopathogenic oomycetes such as *Phytophthora infestans* secrete hundreds of effector proteins into host plant cells, and many of these effectors lack similarity to known proteins and have uncharacterized biochemical activities. Writing in *PLoS Pathogens*, van Damme *et al.* now show that the effector crinkler 8 (CRN8) is an active kinase that is secreted into the plant by *P. infestans* to enhance virulence.

CRNs are an important family of oomycete effectors and consist of conserved amino termini that are required for translocation into the plant cell, as well as diverse carboxyl termini that are responsible for the effector activity. The C terminus of *P. infestans* CRN8 has previously been shown to induce plant cell death, and the D2 region of its C terminus bears similarity to the RD family of Ser/Thr kinases, but whether CRN8 is an active kinase was unknown.

> “CRN8 is the first plant pathogen effector shown to encode a functional kinase domain.”

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In this new study, van Damme *et al.* constructed FLAG-tagged versions of CRN8 (FLAG–CRN8) in which the Arg and Asp residues of the predicted RD kinase domain were either singly or doubly substituted (Asp470Asn, or Arg469Ala and Asp470Asn), and then expressed these tagged proteins in plants before purifying them and using them for *in vitro* kinase assays. The authors found that, although wild-type FLAG–CRN8 was autophosphorylated, both the singly and doubly substituted mutants lacked any bands indicative of autophosphorylation. Interestingly, when the Asp470Asn mutant was expressed in *Nicotina benthamiana*, cell death still occurred, indicating that kinase activity is not required for the cell death function of CRN8.

Using mass spectrometry, the authors identified five phosphorylated Ser residues in CRN8. Importantly, they observed that it was the phosphorylation of these residues that affected CRN8 activity, as a FLAG–CRN8 mutant carrying Ala substitutions for the five residues caused lower levels of cell death than the wild-type protein when expressed in plants. Finally, the authors found that, although overexpression of the wild-type CRN8 in *N. benthamiana* leaves enhanced growth of *P. infestans* lesions, overexpression of the doubly substituted kinase mutant actually decreased the lesion growth rate, suggesting that this mutant interfered with wild-type CRN8 (produced by *P. infestans*) in a dominant-negative manner.

Secreted kinases with important roles in pathogenicity have been identified for multiple animal parasites, such as *Plasmodium* spp. and *Toxoplasma gondii*, but CRN8 is the first plant pathogen effector shown to encode a functional kinase domain. Further work is now needed to determine the mechanism by which it acts and the processes that it hijacks in the plant host.

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ORIGINAL RESEARCH PAPER van Damme, M. *et al.* The Irish potato famine pathogen *Phytophthora infestans* translocates the CRN8 kinase into host plant cells. *PLoS Pathog.* **8**, e1002875 (2012)