Deletion of CpSec66, a non-essential Translocase Gene, Reduces Virulence of Chestnut Blight Fungus

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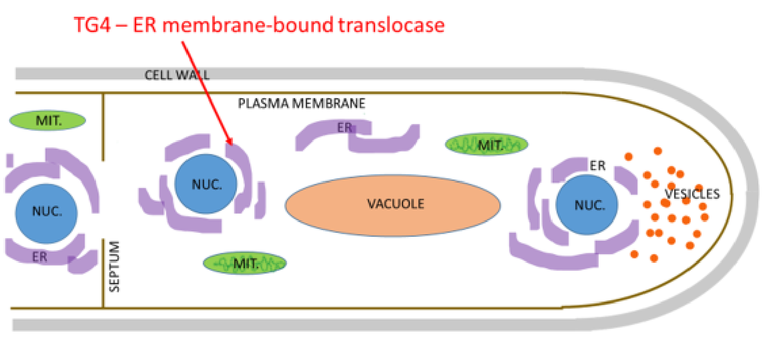
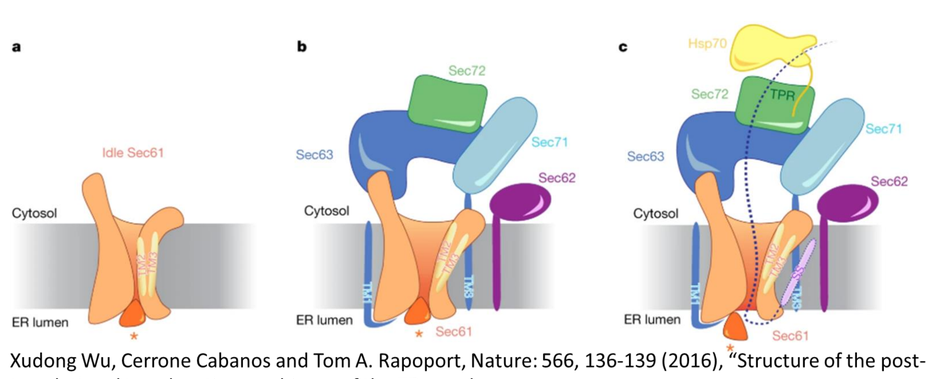
Summary:

An examination of suspected pathogenicity genes with close homologues in two ascomcete plant pathogenic fungi, the chestnut blight fungus, *Cryphonectria parasitica* (*Cp*) and the powdery mildew fungus *Golovinomyces cichoracearum* (*Gc*), revealed several auxiliary regulatory genes that appear to help the fungi adapt to life in their hosts. Deletion of one of these genes, the ER membrane-bound *CpSec66,* affected a number of cellular processed and reduced fungal virulence. This gene may present a good target for novel forms of resistance to Cp, and may lead to the discovery of other Cp genes directly involved in pathogenicity.

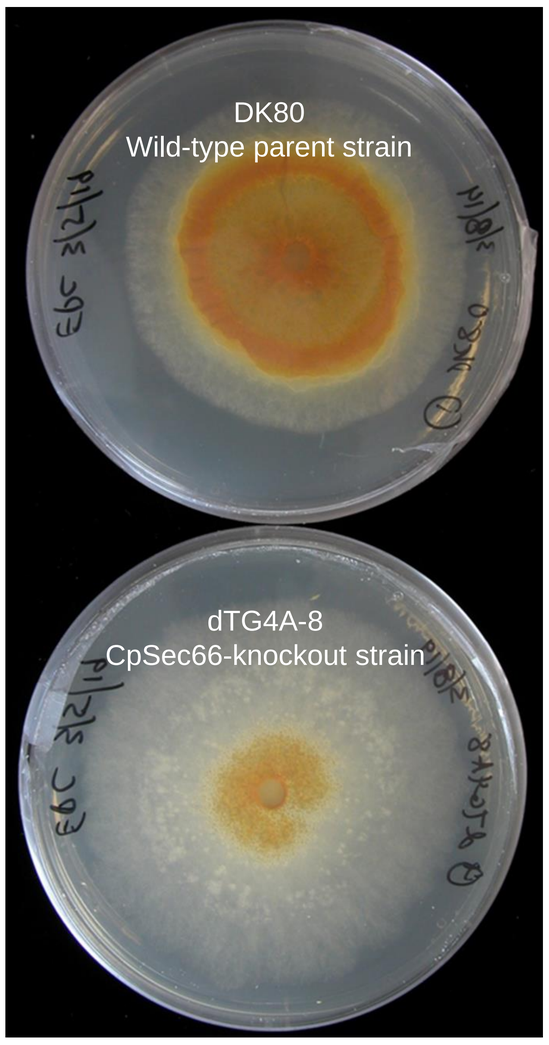


CpSec66 is a homologue of the Sec66 gene in *Saccharomyces cervisiae* where it serves as an auxiliary ER membrane-bound translocase that assists with the passage of selected proteins through the Sec61 pore complex, to be further processed in the ER and delivered to their final destinations. Without Sec66, certain proteins do not get fully synthesized or delivered to where they need to be.



Xudong Wu, Cerrone Cabanos and Tom A. Rapoport, Nature: 566, 136-139 (2016), "Structure of the post-translational translocation machinery of the ER membrane

Small stem assays using pure American seedlings from a single mother tree. By 49 days post-inoculation, all 14 seedlings inoculated with the wild-type DK80 strain, and 13 out of 14 inoculated with SG2,3, had died distal to the inoculation site. There was no mortality among seedlings inoculated with dTG4A-8, the CpSec66-knockout strain.



The wild-type DK80 strain (above) compared to the CpSec66-knockout (below), shown here growing on cellophane over PDA medium

Methods

- Selection of genes that have high aa sequence homology between Cp and the powdery mildew fungus Golovinomyces cichoracearum (Gc), which contain signal peptides, and whose homologues in Sc are not essential genes.
- Knock the genes out of Cp using homologous gene replacement
- Observe the effect on fungal phenotype in vitro, and on virulence in planta (detached stem and small stem assays)

Results

The *CpSec66*-knockout strain *in vitro*:

- Rapid radial growth with less accumulation of biomass than the WT parent, suggesting impairment of ability to sense or take up nutrients
- Lack of zonal growth, possibly due to impairment of ability to sense light

In planta:

- Detached stem cankers similar (α=0.05) to SG2,3 cankers
- Small stem assays resulted in small, superficial cankers, and no stem mortality over 49 days, during which all stems inoculated with the WT parent strain died.

Next steps

1. Test the feasibility of host-induced gene silencing (HIGS) targeting *GcSec66* with HIGS transgenic Arabidopsis plants

2. Observe protein-protein interactions involving CpSec66 to identify potential pathogenicity-related proteins that it may carry across the ER membrane.