The StructuraLEP project: structural and functional characterization of Leptosphaeria maculans effectors and of their interactants

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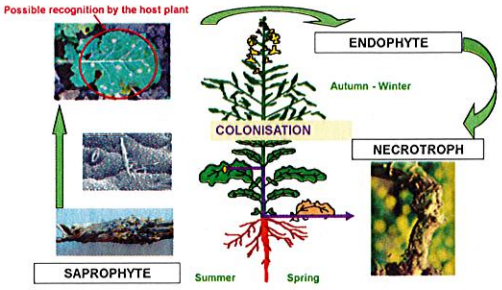
Context

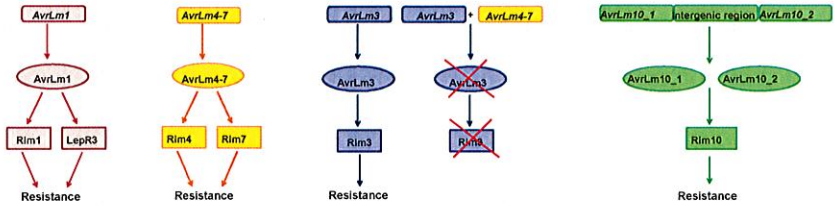
Fungal effector genes are very diverse and typically encode small proteins, predicted to be secreted, with no or low homology in databases, and absence of known motif. As such their function or role in pathogenesis is mostly unknown and structure information provides an elegant way to resolve functional traits. Recently, three-dimensional (3-D) structures of several fungal and oomycete avirulence effectors have been determined and have provided key advances in our understanding of plant-pathogen interactions including: the identification of structural similarities in effectors that were not visible in the sequence data, the identification of protein functions that were not apparent from sequences alone, and the visualization of molecular interfaces of relevance to pathogen virulence and plant immunity [1-2]. On these bases, the project StructuraLEP aims at elucidating the involvement of *L. maculans* effectors in pathogenicity through the structural and functional characterization of a few major effector proteins and the determination of their interactants.

Leptosphaeria maculans displays complex interactions with oilseed rape resistance genes

– 11 interactions *AvrLm* / *Rlm* genetically characterized
– 9 *AvrLm* genes cloned



The Dothideomycete *Leptosphaeria maculans* causes stem canker, one of the most devastating diseases of oilseed rape (*Brassica napus*) worldwide. Control of fungal crop diseases necessitates a global understanding of fungal pathogenicity determinants and of their evolution. Genetic studies demonstrated gene-for-gene relationships between *L. maculans* and *B. napus* and allowed us to genetically identify eleven avirulence (*AvrLm*) genes in the pathogen and eleven corresponding resistance (*Rlm*) genes in the host plant [3]. We are investigating five *L. maculans* effectors chosen for their biological significance (involvement in fungal fitness, cognate R gene identified) or because they may represent novel modes of interaction with their plant target (two AVR genes necessary to be recognized by a specific R gene) [4-5; Plissonneau, Petit, Degrave, Rouxel and Balesdent, pers. comm.].



I. Structural and functional characterization of *L. maculans* effectors

Structural and functional characterization of *L. maculans* effectors will include production of effectors in heterologous systems (*Escherichia coli and* / or *Pichia pastoris*) and determination of their 3-D structure, determination of *in planta* localisation and identification of the cellular processes targeted by effectors.

Heterologous production in *E. coli* or *P. pastoris* and determination of 3D structure

Biological function of effectors
Structural analogies ?

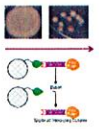
Localisation *in planta* (transient expression in tobacco leaves, immuno-cytolocalisation in oilseed rape during infection by *L. maculans*

Cellular processes targeted by effectors

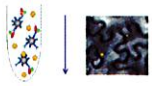
Stable expression in *Arabidopsis thaliana* (coll. M. Gourgues, Sophia-Antipolis)

II. Screening of plant proteins and molecules interacting with *L. maculans* effectors

Yeast two-hybrid
Pull-down



Plant proteins targeted by effectors



Validation (BiFC, FRET-FLIM, CO-IP)

The search for molecules interacting with effectors will be performed using a Fluorescent Thermal Shift assay with a ligand library (including metals and co-factors). The search for host proteins interacting with effectors will be performed by yeast two hybrid screens using a cDNA library of oilseed rape infected by *L. maculans*. In parallele, pull-down assays using effector proteins produced in heterologous system against a set of plant proteins will be initiated (or, in case heterologous proteins are not available, effectors will be transiently expressed with a tag in *Nicotiana bentamiana*). Interesting candidates will be validated through independent methods such as colocalisation in *N. benthamiana* coupled to FRET / FLIM, Bimolecular Fuorescence Complementation (BiFC) or Co-immunoprecipitation (CoIP)

III. Functional and physical understanding of the identified interactions

The most interesting interactions identified will be characterized through co-crystallisation of interactants coupled to directed mutagenesis and through manipulation of plant targets in *A. thaliana.*

- Co-crystalisation effector / plant target or molecule.
- Polymorphism of *effectors* in *L. maculans* populations / site directed mutagenesis.

Protein regions or amino-acids involved in interaction

Conclusions

Integration of knowledge on effector 3-D structure, localization, targeting of host proteins and host processes by *L. maculans* effectors studied in the StructuraLEP project will give insight into the fundamental processes governing the close association of a pathogenic fungus with its host plant. Progresses in these fields are necessary for sustainable crop production through the development of novel strategies to control fungal diseases.

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References

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