Genome-wide RNAi screen for innate immunity genes in *C. elegans*

FUNGENOMICS ANR - 07 - MIME

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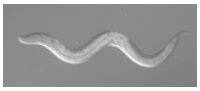
INTRODUCTION

We use *C. elegans* to investigate innate immune signaling. We focus mainly on the interaction of *C. elegans* with the fungus *Drechmeria coniospora*. This fungus infects the worm by attaching to its cuticle and sending hyphae across the epidermis, thus, invading the whole animal. One of the responses of the worm to this aggression is the up-regulation of a variety of antimicrobial peptide (AMP) genes in the epidermis. Transgenic worms carrying a GFP reporter under the control of an AMP promoter fluoresce green after infection by *D. coniospora*. The level of GFP in individual worms can be quantified. We are now screening for signaling molecules using a semi-automated RNAi approach. If a gene required for AMP gene expression is inactivated, the reporter strain will not turn green upon infection. We are screening 2 different RNAi libraries that together cover 95% of the genome. The screen is conducted on solid media and we can currently screen 3000 genes a week. The results of the screen will allow us to identify a great number of genes implicated in the immune response of *C. elegans*.

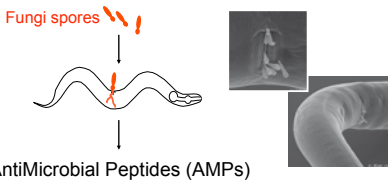
EXPERIMENTAL MODEL

1. *Caenorhabditis elegans* as model host

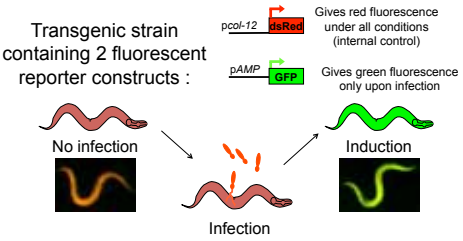


- transparent
- clonal
- easy transgenesis
- innate immune system

2. *Drechmeria coniospora* infects the worm



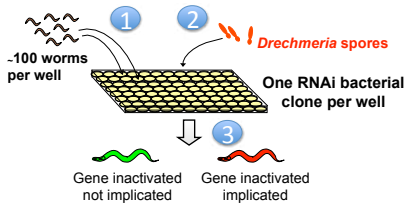
3. *in vivo* visualisation of AMP induction



HIGH-THROUGHPUT RNAi SCREENING

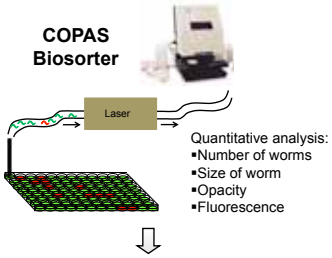
1. Genome-wide RNAi

RNAi bacterial clones stocked in libraries of 96-well plates : 18,000 clones = 95% genome



➡ **96 clones tested at once**

2. High-throughtput technologies

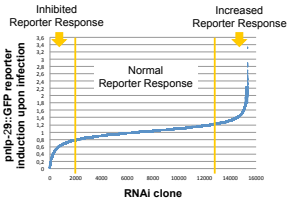


Data storage and treatment

Custom tools for handling very large data sets: **LIMS**

RESULTS

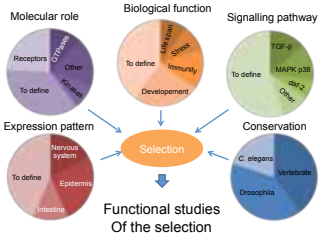
Candidate genes



Data analysis of the screen allows to establish 2 groups of interesting clones: positive regulators (their inhibition blocks the response) and negative regulators (inactivation leads to constitutive response)

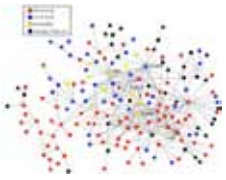
PERSPECTIVES

1.Gene group distribution



2.Gene interaction network

Microarrays, proteomics, tiling arrays…



CONCLUSIONS

The results that will be obtained from this genome-wide RNAi screen will lead to the discovery of new genes implicated in the innate immunity of the worm. This will allow a better characterization of the different pathways leading to an immune response against *Drechmeria* and possibly lead to the finding of conserved elements in mammals.

PUBLICATIONS

Pujol, N., Zugasti, O., Wong, D., Couillault, C., Kurz, C.L., Schulenburg, H., and Ewbank, J.J. (2008). Anti-fungal innate immunity in *C. elegans* is enhanced by evolutionary diversification of antimicrobial peptides. PLoS Pathog *4*, e1000105.

Engelmann, I., Griffon, A., Tichit, L., Montañana-Sanchis, F., Wang, G., Reinke, V., Waterston, R.H., Hillier, L., and Ewbank, J.J. A comprehensive analysis of gene expression changes provoked by bacterial and fungal infection in *C. elegans*. PloS one. 2011;6(5):e19055.

Ziegler, K., Kurz, C.L., Cypowyj, S., Couillault, C., Pophillat, M., Pujol, N., and Ewbank, J.J. (2009). Antifungal innate immunity in *C. elegans*: PKCdelta links G protein signaling and a conserved p38 MAPK cascade. Cell Host Microbe *5*, 341-352.

Pujol, N., Cypowyj, S., Ziegler, K., Millet, A., Astrain, A., Goncharov, A., Jin, Y., Chisholm, A.D., and Ewbank, J.J. (2008). Distinct innate immune responses to infection and wounding in the *C. elegans* epidermis. Curr Biol *18*, 481-489.

Zugasti, O., and Ewbank, J.J. (2009). Neuroimmune regulation of antimicrobial peptide expression by a noncanonical TGF-beta signaling pathway in *Caenorhabditis elegans* epidermis. Nat Immunol *10*, 249-256.

Dierking, K., Polanowska, J., Omi, S., Engelmann, I., Gut, M., Lembo, F., Ewbank, J.J., and Pujol, N. An unusual STAT-SLC6 protein complex in epidermal innate immunity in *C. elegans*. Cell Host Microbe.May 19;9(5):425-35.

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