Proposition de stage

Parcours Master 2 « Microbiologie, Environnement, Santé »

1. Laboratoire / Entreprise d'accueil :

Intitulé : Natural variation in viral resistance of the nematode Caenorhabditis elegans

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2. Titre, description du sujet, approches utilisées, références (1 page maximum) :

Natural variation in viral resistance of the nematode *Caenorhabditis elegans*

The nematode *Caenorhabditis elegans* is widely used as a laboratory model organism. Among basic discoveries made using this organism is RNA interference (RNAi). RNAi corresponds to the silencing of the expression of a specific gene when double-stranded RNAs with the corresponding sequence are delivered. RNAi can potentially act against RNA viruses which all have a double-stranded stage during replication. The Felix team isolated the first (and still only) virus naturally infecting *C. elegans*, which was called the Orsay virus. This virus infects intestinal cells and is horizontally-transmitted. It is a single-stranded RNA virus, closely related to nodaviruses. The Orsay virus was thus used to test whether known RNAi pathway components acted in antiviral resistance: they do (Félix et al., 2011).

Natural pathogens such as the Orsay virus provide are relevant to study defense systems and their potentially rapid evolution. The team then performed a genome-wide association study (GWAS) of Orsay virus load after infection of a worldwide set of *C. elegans* isolates. This study pointed to a chromosome III locus segregating in the species. This major locus corresponds to a deletion inactivating the homolog of vertebrate RIG-I viral sensors, thus allowing viral replication. Variation at the *drh-1* locus explains a part of

the variation in Orsay virus resistance. However, some isolates, such as MY10 have a deleted *drh-1* allele yet are fully resistant (Ashe et al., 2013).

To find the cause of the viral resistance of the MY10 isolate, recombinant inbred lines were built between MY10 and the JU1580 sensitive strain (which initially contained the Orsay virus and also presents the *drh-1* deletion). The recombinant lines were tested for their level of virus resistance. Two pools of lines with either a resistant or a sensitive phenotype were then whole-genome sequenced. The difference between the two pools in the frequency of parental alleles points to a quantitative trait locus (QTL) in the middle of chromosome V (unpublished results).

The present project takes two approaches towards identifying the molecular locus underlying the high resistance of the MY10 strain.

First, in a genetic mapping approach, near isogenic lines will be built by successive backcrosses of the chromosome V locus in the other parent. This will on one hand confirm (or not) the QTL, and allow for further recombinant screening to narrow down the genetic interval. This will in turn narrow down the list of candidates to be tested.

Second, the analysis of the MY10 genome already pointed to possible candidate polymorphisms in the QTL region. A candidate will be tested by allele replacement using CRISPR/Cas9 genome editing.

The combination of these two approaches will help understanding the molecular variation underlying the high resistance of the MY10 strain to the Orsay virus.

References

Ashe, A., Bélicard, T., Le Pen, J., Sarkies, P., Frezal, L., Lehrbach, N.J., Félix, M.A., Miska, E.A., 2013. A deletion polymorphism in the *Caenorhabditis elegans* RIG-I homolog disables viral RNA dicing and antiviral immunity. eLife 2, e00994.

Félix, M.-A., Ashe, A., Piffaretti, J., Wu, G., Nuez, I., Bélicard, T., Jiang, Y., Zhao, G., Franz, C.J., Goldstein, L.D., Sanroman, M., Miska, E.A., Wang, D., 2011. Natural and experimental infection of *Caenorhabditis* nematodes by novel viruses related to nodaviruses. PLoS Biol. 9, e1000586.