Proposition de stage M2

Année universitaire 2017-2018

1. Fiche « Structure d'accueil » / *Fact sheet on host structure*

LABORATOIRE ou ENTREPRISE / *Laboratory or company*			
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EQUIPE d'ACCUEIL ou SERVICE / *Host team or department*			
Nom / *Name*	**GENOPHY**		
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2. Titre du sujet : Investigating the molecular mechanisms of resistance to giant viruses in a marine algal model.

3. Description du sujet :

Marine unicellular Mamiellophyceae are abundant microalgae worldwide, and numerous complete genomes of them are available. They are attacked by prasinoviruses, whose ≈190 kb long genomes have been characterized in our lab, and in general viruses are known to regulate algal blooms. We will focus on the molecular responses of *Ostreococcus tauri*. which can be lysed by prasinoviruses, such as OtV5[1]. In cultures of *O. tauri*, OtV5 usually lyses susceptible host cells, but if the cleared lysate is left in culture, virus-resistant host cells usually arise spontaneously and can be re-grown as lines that show durable resistance to OtV5[2]. We have maintained 38 such OtV5-resistant cultures for 4 years and have shown that more than 20 genes clustered on chromosome 19 are over-expressed in these cultures[3]. However, we do not know the molecular basis of the specific change that leads to resistance.
The objective of this Master study is to further our knowledge about resistance/susceptibility using both experimental and bioinformatic procedures.

Procedure.
Bioinformatics. We have sequenced the complete transcriptomes of 20 virus-resistant and 4 virus-susceptible lines. We will align all of the reads in these transcriptomes, which cover almost all of the host genome, to the reference susceptible *Ostreococcus* RCC4221 genome, and search for point mutations and deletions. We will pay particular attention to any mutations for example in functions that might affect response to viral attack and to mutations that appear in the same gene in several of the independently resistant lines. Independently, by transcriptome analyses in virus-infected cells[4], we have identified a group of *viral* genes that appear to be expressed earlier than others. We will screen the viral genome for promoter motifs that govern viral gene expression.

Experimental. We are developing CRISPR technology in *O. tauri* in order to create mutations in specific genes, such as those which re up-regulated in chromosome 19, to investigate whether this affects resistance. Initially we plan to test new vectors that we are developing to knock out genes with screenable or selectable markers, such as fluorescent proteins or genes involved in nitrate assimilation. The student will help in the development of suitable markers and transformation techniques.

The student will be supported in the context of the ANR ALGALVIRUS, and might lead on to a doctoral thesis.

References:

1. Derelle, E. *et al.* Life-cycle and genome of OtV5, a large DNA virus of the pelagic marine unicellular green alga *Ostreococcus tauri*. *PLoS ONE* **3,** e2250, 1–13 (2008).
2. Thomas, R. *et al.* Acquisition and maintenance of resistance to viruses in eukaryotic phytoplankton populations. *Environ. Microbiol.* **13,** 1412–1420 (2011).
3. Yau, S. *et al.* A Viral Immunity Chromosome in the Marine Picoeukaryote, *Ostreococcus tauri*. *PLOS Pathog* **12,** e1005965 (2016).
4. Derelle, E., Yau, S., Moreau, H. & Grimsley, N.H. Prasinovirus attack of *Ostreococcus* is furtive by day but savage by night. *J. Virol.* in the press, (2017).