SUJET DE THESE : **Mechanisms of rhizoid growth in the green alga *Ulva mutabilis***

Spécialité : *Biologie cellulaire, Evolution*

Intitulé de l'équipe : Morphogenèse des Macro Algues, UMR8227

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Résumé du projet de thèse

Rhizoid growth in the green alga *Ulva*

Ulva is a marine green seaweed (responsible for the green tides). Phylogenetically, it belongs to the Chlorophyta lineage, which is a sister group of the Streptophyta comprising the land plants. *Ulva mutabilis* (Wichard, Front Plant Sci 2015) is currently developed as a model for the Ulvophyceae class. Its genome sequence will soon be published (first one for a multicellular chlorophyte) and genetic transformation has been developed (Oertel et al., J Phycol 2014).

U. mutabilis morphology is simple: the thallus is divided into the blade, made of 2 cell layers, stem cells at the basis of the blade, and rhizoids. The latter are thin row of cells. When the rhizoid is 4 cell long, the terminal cell of the rhizoid undergoes an asymmetrical division, while the other 3 ones stop dividing. This cell ensures the further growth of the overall rhizoid.

Recent data suggests that the rhizoid growth in this species relies on a different molecular toolkit than that described in the other organisms (not published). The present PhD project will characterize rhizoid growth in *U. mutabilis*. This will enlarge the range of organisms for evo-devo studies.

Objectives

1- Acquire knowledge about the mechanisms of rhizoid growth in the multicellular green alga *Ulva mutabilis*.

2- Compare with the other tip-growing organisms from the green lineage (filaments of the moss Physcomitrella, land plant pollen tubes and root hairs, the green filamentous alga Chara), and from other multicellular algae (among which the brown algae Ectocarpus filaments).

3- Develop supported scenarii about the evolutionary history of this cellular process.

(Facultatif)

Approaches

1) Modeling of Ulva rhizoid growth; *this part will rely on protocols recently developed by the host team. It includes first the acquisition of biological data and secondly a relevant model. The frame of the model needs to be defined beforehand by answering basic questions:*

- Is Ulva rhizoid growing by tip-growth? This will be carried out by pulse-chase experiments using e.g. calcofluor as a stain for cell wall component (cellulose and callose). Time-lapse observations will allow to define the growth area in the rhizoid and its growth rate in different conditions (mainly osmotic conditions).

- How does the cell wall expand during growth? Cell wall can reach different conformation during growth (expansion or compression, rotation). This pattern reflects the mechanical stretches of the cell wall during growth and is informative regarding the underlying mechanisms. This will be achieved by time-lapse experiments monitoring fluorescent markers oaded on the cell wall (Rabillé & Charrier, in prep).
- What turgor in rhizoid cells? In plant cells, turgor provides the basic force promoting cell growth. It will be measured either by the technique of Limit plasmolysis or by using a pressure probe.
- What is the detailed cell shape of rhizoid apical cell? Curvatures modulates the tensile stress undergone by the cell wall. Cell curvatures in apical cells will be inferred from the drawing of the contour of these cells. This work will be carried out with B. Billoud, bioinformatician in the team.
- What are the mechanical properties of the cell wall? Both physiological experiments (cell wall yielding by osmotic chocks) and biophysics measurements (atomic force microscopy; collaboration Plateform BiBS, INRA Nantes) will allow to infer the x,y elasticity and plasticity of the cell wall.
- Which specific features for rhizoid apical cells?: Transmission electronic microscopy performed on longitudinal section of *U. mutabilis* rhizoids will show the sub-cellular organisation and details about the cell components, with a particular focus on the cell wall thickness (collaboration S. Le Panse, Platform Merimage, SB Roscoff).

These biological parameters will be used to "feed" biophysical models.

Modeling rhizoid growth

- Ulva rhizoid growth will be modelled using elastic or viscoplastic models (depending on the results obtained above) previously used for land plants apical cells or other algal cells (e.g. Chara, Streptophyta). Simulations will allow to identify the master parameters in rhizoid growth in this species.

2) Identify genes involved in Ulva rhizoid growth; t*his part will be carried out in collaboration with Dr T. Wichard's lab, Univ Jena.*

Transgenic lines of Ulva expressing promoter-trap constructs fused to GFP will be produced by applying the protocol developed by the partner team (Oertel et al. J Phycol 2014). Lines expressing GFP in the rhizoids (which construct is inserted downstream from a start codon) will be screened for and isolated, and the genes into which the construct has been inserted will be identified e.g. by inverse PCR. The identity of the genes will be put in the context of the biophysical mechanism characterised in part 1).

Conclusion:

Thanks to the biophysical simulation approach, the PhD project will provide a mechanistic and dynamic view