Deadline for application: 15 May 2018.

Title :
Cell fate and blade patterning during the early growth of *Saccharina latissima* sporophytes
(en français: "Cartographie des destins cellulaires dans les jeunes sporophytes en croissance de l'algue brune Saccharina latissima")

Supervision :
- Team 1 : Bénédicte Charrier, team « Morphogenesis of MacroAlgae » (MMA), SBR, CNRS-SU, France
- Team 2 (co-funding) : Hilde-Gunn Opsahl Sorteberg, Institute for Plant Sciences, Norwegian University of Life Science (NMBU), Norway

Starting/ End date : October 2018 / September 2021
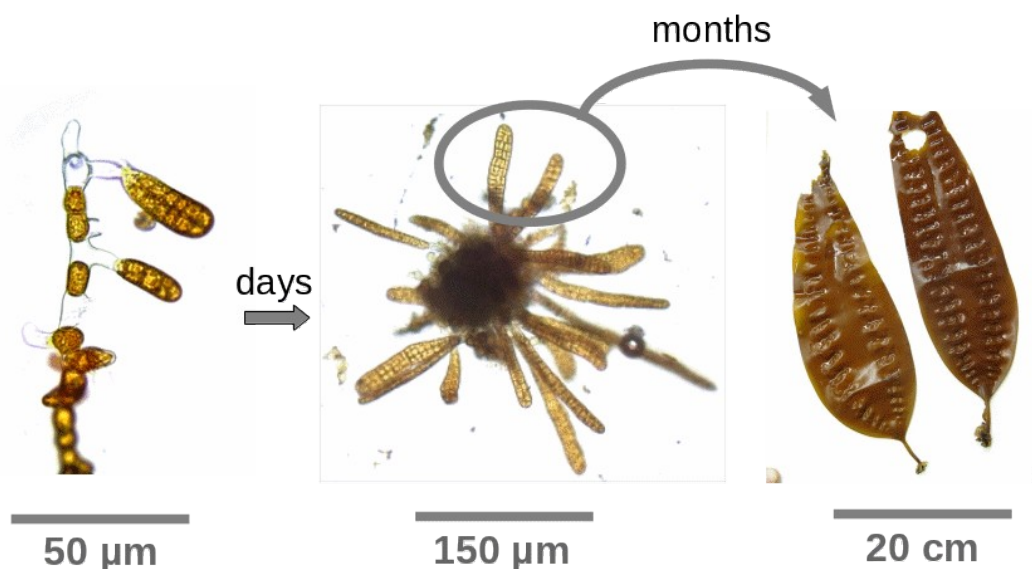**Salary** : 1422€ net per month.

HOW TO APPLY ? Please send CV (with marks or ranking) + letter of motivation (1 page max) + 2 contact names for references to Bénédicte Charrier (benedicte.charrier@sb-roscoff.fr) AND Hilde-Gunn Sorteberg (hilde-gunn.sorteberg@nmbu.no).

Project
Introduction
Saccharina latissima is a large brown alga growing along the European Atlantic coast. It is currently of high interest as a source of food, fuel and of extracted and processed products used in the pharmacology and cosmetic industries. As a result, in addition to mechanical harvest of wild populations, cultivation of *Saccharina* is currently deployed mainly in Norway and to a lesser extent in France.

The object of this PhD project is to acquire more knowledge on the early life stages of *S. latissima*. In addition to gaining insight in the underlying biological mechanisms controlling the growth of this alga, the project will allow progress in monitoring its cultivation in hatcheries by pinpointing the key developmental stages of juveniles, and will contribute to a better management of their growth and development before cultivation in open sea.

Development of the sporophytes of *Saccharina latissima* .

From the formation of the embryo (left, with 4-cell and ~ 16-cell sporophytes) to the mature sporophyte (right). The project will focus on the stages linking the first 2 photos (left and middle).

Scientific objectives

The PhD project will focus on the early stages of *S. latissima* sporophytes.

Task 1. Cell patterning in the early sporophytic thallus

Preliminary observations of *S. latissima* early development (unpublished) supported by previous reports from other kelps (Fritsch 1954) indicate that building of kelp (Laminariales) body is subjected to an ordered succession of cell division modes that exhibit specific features. These features are i) the orientation of the division plane (anticlinal/periclinal divisions, and further parenchymatous growth), ii) the localisation of growth within the tissue (apical/intercalary growth, and further establishment of an intercalary meristematic tissue between the blade and the stipe), and iii) the thallus thickening process implying an additional dimension in cell division orientation (3-D). Characterisation of the spatio-temporal pattern of these cell division features will be undertaken using time-lapse optical microscopy, and a special focus on the transition between each division mode will be given. Imaging softwares previously developed for land plants (i.e. MorphoGraphX, Barbier de Reuille et al. 2015) will be used to tile the cell surface of the blade and to follow the fate of individual cells. Synchronous observations of several individuals will allow to assess the level of plasticity (statistical analyses of quantitative parameters like cell dimensions, connectivity, dynamics and other to be defined). In addition, the impact of environmental (mainly salt concentration and temperature, particularly relevant in a global warming climate context) and chemical (among which morphogenic molecules but also heavy metals) cues will be investigated. Altogether, these data will allow to advance knowledge on **how the *S. latissima* body is built up step by step and what can deviate or impair this process.**

Task 2. Cell-specific and position-specific transcriptomics

Kelp growth is characterised by the formation of an intercalary meristem, allowing the production of a new blade every year. Its functioning is therefore at the basis of the renewal of natural populations year after year. In addition, vascular, cortical, epidermis and meristoderm (peripheric meristem) tissues differentiate within the thallus as it grows. The cell division pattern deciphered in 1. will provide the classifying factors (e.g. sectors with the same cell division plane or with the same cell size) in an mRNA differential expression analysis. From the 10-cell stage up to the differentiation of the first tissues (~ 500-cell stages), cells or group of cells at specific position within the thallus will be dissected and collected using the technique of Laser-Capture Microdissection established in the other brown alga *Ectocarpus sp.* (Saint-Marcoux et al., 2015). Subsequently, transcriptomic pattern of these cells will be compared as a function of their position within the thallus and cellular activity. This experimental procedure will be carried out both in normal culture conditions and in response to the treatments shown to modify the growth pattern, identified in Task 1. Key genes will be identified by correlating the change of growth pattern in response to variation of the growth condition and the gene

expression pattern. Altogether, this part will allow to **potentially link the cell growth pattern to gene expression.**

Task 3. Blade patterning models and computer growth simulations

Planar growth provides a suitable tissue to perform morphogenetic modeling, because each cell can be observed and followed during growth. Previous results on brown algal growth (Billoud et al., 2008; Linardic et al. 2017) suggest that growth and differentiation are not deterministic and are mainly based on positional information, so far independently from chemical signaling (at least by the morphogene auxin). Dynamic models based on long-range or short-range positional information, potentially involving genes identified in Task 2., will be generated computationally, and further tested by simulation. These models will be used to simulate the impact of changing parameter(s) displayed in Task 1, and pinpoint the potential factors involved in the corresponding signaling pathway. Such predictions will be tested experimentally by knocking down/out the expression of these genes by RNAi expression in the egg (shown to work in *Fucus*, Farnham et al, 2013) or Genome-editing in the gametophytes (to develop). The recent development of a cryopreservation protocol for *S. latissima* gametophytes (team MMA, SBR, unpublished) will allow production of clones of sporophytes (identical at the DNA level), which will precludes variability in the results due to genetic variations of the algal material. Altogether, this part will build **an integrated multi-level model of planar morphogenesis in *S. latissima* sporophyte involving identified key genes.**

References

Barbier de Reuille P, Routier-Kierzkowska AL, Kierzkowski D, Bassel GW, Schüpbach T, et al.. MorphoGraphX: A platform for quantifying morphogenesis in 4D. Elife. **2015**

Billoud B, Le Bail A and Charrier B. A stochastic 1D nearest-neighbour automaton models the early development of the brown alga *Ectocarpus siliculosus*. Functional Plant Biology, 35: 1014-1024, **2008**

Farnham G, Strittmatter M, Coelho S, Cock JM, Brownlee C. Gene silencing in Fucus embryos: developmental consequences of RNAi-mediated cytoskeletal disruption. J Phycol. 49(5):819-29, **2013**

Linardić M, Braybrook SA. Towards an understanding of spiral patterning in the Sargassum muticum shoot apex. Sci Rep. 7(1):13887. **2017**

Saint-Marcoux D, Billoud B, Langdale JA and Charrier B. Laser capture microdissection in *Ectocarpus siliculosus*: the pathway to cell-specific transcriptomics in brown algae. Front. Plant Sci*. 6:54. **2015**