Laboratoire Reproduction et Développement des Plantes

ENS DE LYON

Internship proposal (2024-2025):

Comparative proteomics to unravel the dynamics of cortical microtubules during early stages of cell division in *Arabidopsis*

Host: Laboratoire de Reproduction et de Développement des Plantes (RDP)
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Background:

Cell division, including the orientation of the cell division plane, is a key element for the proper development of a multicellular organisms. This is especially true in plants, where cells are embedded in a rigid extracellular matrix that prevent any cell migration. In plants, cell division relies on a peculiar cytoskeletal structure called the preprophase band (PPB). The PPB is mainly composed of cortical microtubules (CMT), and forms a ring-shaped structure at the cell cortex, which prescribes the future division plane. The first question is: how do cells determine the position of the PPB? This question is particularly intriguing since, in epidermal cells, interphasic CMT align with the direction of maximal tissue tensile stress, which does not necessarily correspond to the orientation of the future PPB. This suggests that the PPB doesn't result from the gradual narrowing of existing CMT, thus raising a second question: how do CMT change their orientation when cell enters division?

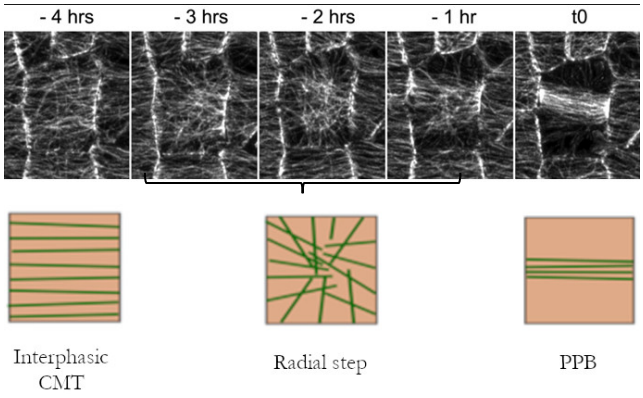

Figure.
A time course of CMT dynamics during the 4 hours preceding the PPB formation and the onset of mitosis. 4h before the PBB formation, CMT display a biased orientation, along the axis of maximum tensile stress. From 3h before the PPB formation, CMT lose this biased orientation to show a non-biased radial orientation. In this instance, PPB orientation aligns with interphasic CMT orientation, but this is not always the case.

We have recently characterized a new cell cycle stage occurring 2-3h before mitosis, and during which CMT go through a transient non-biased radial orientation (figure, Melogno et al., 2024, PNAS: 121 (29) e2320470121). We hypothesized that this radial step makes cells blind to external cues, allowing the cells to correctly position their division plane (orientation and symmetry). While we have characterized this step at the cellular level, the molecular mechanisms remain elusive, and the proteins involved are still unidentified. Identifying these proteins would provide a better mechanistic understanding of this transient cell cycle phase.

Research proposal:

We propose to conduct a deep comparative proteomic analysis of the radial step, using synchronized cell suspension and focusing on microtubule-interacting proteins.

The first step will be to establish and synchronize tobacco BY-2 cell suspension lines expressing various reporter lines (including MT reporter lines). In the second step, synchronized cells will be collected at different time points of the cell cycle, and proteins will be extracted (CoIP or TurboID using MT as a bait). Finally, we will perform a comparative proteomic analysis, comparing datasets to filter out specific from non-specific candidates (dataset from non-dividing/dividing cells, dividing cells at various cell cycle stages).

Technics:

- BY-2 cell culture, transformation and synchronization.
- Protein extraction, TurboID, CoIP.
- Comparative proteomics.