SHORT TERM SCIENTIFIC MISSION (STSM) – SCIENTIFIC REPORT

Action number: CA16212
STSM application number: 40234
STSM title: Deciphering how histone H1 variants drive light-controlled heterochromatin dynamics in Arabidopsis
Home institution: IBENS, Paris
Host institution: University of Zürich
STSM start and end date: 26/02/2018 to 09/03/2018
Grantee name: Gianluca Teano

PURPOSE OF THE STSM

The first perception of ambient light by naive embryonic leaves of the germinating seedling launches the establishment of a novel gene expression program underlying photosynthetic functionalities necessary to sustain plant growth. During this short developmental window, genome-wide reprogramming of the transcriptional landscape is accompanied by massive rearrangements of chromatin organization. More specifically, heterochromatin is mostly aggregated in 8-10 well-defined chromocenters in light-grown cotyledon nuclei, whilst in etiolated cotyledon heterochromatin is more relaxed in the nucleoplasm and chromocenters are hardly visible. Accordingly, the de-etiolation transition is associated with rapid condensation of heterochromatic domains with neo-formed chromocenters. Chromocenter formation also requires photoreceptor controls as well as the downstream DET1 and COP1 light signalling integrators, in a process that is independent from nuclear size variations and local DNA methylation changes. This phenomenology has recently led to the identification of histone H1.3 as a key molecular player in triggering light-induced chromatin dynamics.

In this context my PhD project aims at understanding how light perception regulates chromatin condensation and to identify the causative molecular determinants of these dynamics. The STSM purpose is to investigate the role of linker histones in the chromatin mobility changes during the de-etiolation transition in embryonic leaves (cotyledons) of *Arabidopsis thaliana*. The expert tutoring of Dr. C.Baroux (University of Zürich, Switzerland) in measuring chromatin mobility by Fluorescence recovery after Photobleaching (FRAP) approach allowed me investigate the impact of environmentally regulated changes in chromatin compaction on chromatin mobility. More over I have investigated the changes of chromatin mobility during de-etiolation in mutant plants impaired in heterochromatin dynamics or light signal transduction.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

During the scientific mission at the University of Zurich I have learned how to perform Fluorescence Recovery after Photobleaching (FRAP) using H2B mobility as a proxy of chromatin mobility in different light condition and mutant backgrounds. Following the first days of training, with the host supervisor Celia Baroux we have set-up a protocol for efficiently perform FRAP on dissected cotyledons from 5-day-old seedlings grown either under light or dark conditions. For FRAP experiments were used plants bearing the RFP-tagged H2B variant was introgressed either in a set of mutant lines impaired in chromatin condensation. Imaging was carried out using a Leica SP8 inverted confocal scanning microscope with a 63x water immersion objective. 5 days old seedlings were dissected and freshly mounted on liquid 0.5x MS. FRAP experiment were conducted at a controlled temperature of 22°C. Images were processed using the Fiji software (nojre-20151222). Intensity measurements were acquired and analysed using a double normalization according to Phair et al. (2004).

DESCRIPTION OF THE MAIN RESULTS OBTAINED

- Set-up of FRAP experiments on dissected dark grown cotyledons.
- 5-day-old WT cotyledon nuclei grown either under light or dark condition showed differences in chromatin mobility.
- Chromatin mobility is also affected in *det1-1* mutant plants, which not only lack cotyledon chromocenters decompaction but are more generally defective in etiolation and consequently display a constitutive photomorphogenic phenotype (*figure A*).
- Gained insight into the role of linker histone variants in defining this transition.

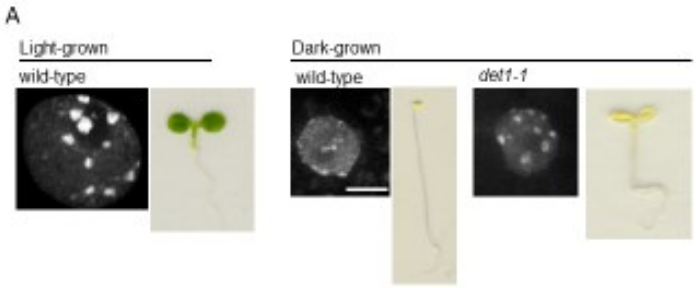

Figure A: Light grown WT plants have green and open cotyledons and short hypocotyl. At the nuclear level light grown WT cotyledons display 8-9 distinct DAPI-stained heterochromatin foci (chromocenters). Dark grown WT plants have a characteristic skoto-morphogenic phenotype with long hypocotyl and small pale closed cotyledons. Dark grown WT cotyledon nuclei are smaller with de-condensed chromocenters. *det1-1* mutant grows in dark displaying a constitutive photomorphogenic phenotype. At the nuclear level *det1-1* nuclei are small as in the dark-grown WT but we can identify 8-9 distinct chromocenters.

MUTUAL BENEFITS FOR THE HOME AND HOST INSTITUTIONS:

Benefits for the Home laboratory: gain of a novel expertise for analysing chromatin mobility in plant nuclei with state of the art protocol for FRAP experiments.

Benefits for the Host lab: knowledge transfer facilitating a future collaborative work that will result in joined projects and publications.

FUTURE COLLABORATION WITH THE HOST INSTITUTION (IF APPLICABLE):

This STSM provided preliminary data that will increase the chances of raising additional funds for this collaborative work.