

SULSA-PECRE Report for Dr Abdellah Barakate's visit to SciLifeLab in October 2018.

Background

In Professor Robbie Waugh's group, Dundee we are studying meiosis in barley. Meiosis is a fundamental eukaryotic process that generates haploid gametes from the parental diploid meiocytes. During this specialised cell division, meiotic recombination creates new allelic combinations that drive evolution and underpin crop breeding programs. However, meiotic crossovers (CO) occur mainly at the sub-telomeric regions while genes in the centromeric areas rarely recombine. Understanding the mechanisms that regulate the distribution and frequency of COs would help improve the efficiency of trait mapping and crop breeding.

Several candidate genes are currently being manipulated by gene silencing (RNAi), chemical (TILLING) and CRISPR/Cas9 based mutagenesis to study their role in meiotic recombination. To determine the dynamics of gene regulation during male meiosis, barley anthers and meiocytes were manually isolated, staged by immunocytology and their transcriptome and proteome determined. However, meiocytes are embedded inside complex tissues of anthers and difficult to isolate in good numbers for all stages. The current bulk tissue-based methods will not allow the determination of meiosis specific chromatin epigenetics and post-translational modifications necessary to fully understand CO formation. INTACT technology (Isolation of Nuclei Tagged in Specific Cell Types) would allow quicker harvesting of large numbers of meiotic nuclei directly from barley inflorescences but relies on using a meiotic promoter that is not leaky in other tissues. While waiting to find such suitable promoter, the newly developed Spatial Resolved Transcriptome (SRT) technique will help improve the spatiotemporal transcriptome of meiosis.

The technique learned

Genome-wide SRT was developed by the group of Prof Joakim Lundeberg, SciLifeLab, Stockholm that I visited during October 2018 (Giacomello et al. (2017), doi:10.1038/nplants.2017.61). The technique is a tissue print that combines high resolution microscopy with massively parallel sequencing. The method involves placing 10 µm cryo-sections on oligo-dT array to capture mRNA and convert it to cDNA. This step is followed by tissue removal and cDNA library collection for massively parallel sequencing.

Before my visit, barley plants were grown for 5 – 7 weeks and meiotic spikes and isolated anthers were collected and embedded in Tissue-Tek compound and frozen. During my visit, the first three weeks were spent optimising the cryo-sectioning, pre-permeabilization and permeabilization steps to increase the proportion of target tissue (meiocytes) and reduced RNA leakage to preserve tissue-specificity. This quality control was performed by staining plant tissue on the slide with toluidine blue and labelling cDNA synthesis with fluorescent nucleotide Cy3-dCTP (see Figure). Toluidine blue staining reveals the preservation of the plant material anatomy while the fluorescence shows the capture of mRNA on the array.

Data analysis and dissemination

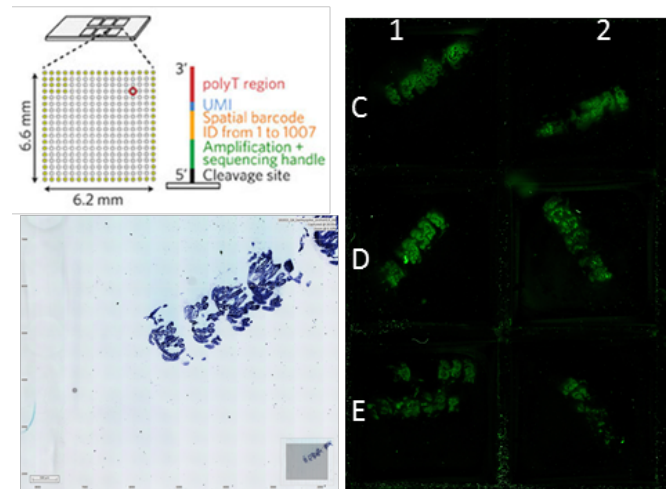
During the last week of my visit, I managed to perform a full experiment with optimised conditions and generated transcriptomes of spike sections in four sub-arrays (two other sub-arrays were not used as they did not pass the quality control). The technician who trained me checked the quality of obtained data and started its analysis using their Bioinformatic pipeline. The data represents the 3'UTR indicating the polyadenylation sites and is strand-specific, which will help improve our barley genome and transcriptome annotation and understand the meiotic gene regulation. I now have access to this data to continue the analysis with the help of our Bioinformatician. Once the analysis is finished, we will be able to decide whether the data can be published alone, or further experiments will be needed. Meanwhile, I am already

scheduled to present the learned technique and results in the Plant Sciences Division and The James Hutton Institute seminars, Dundee.

Figure: Spatially Resolved Transcriptome meiotic spikes of barley.

a) Each array contains 6 sub-arrays of 6.2 x 6.6 mm for replicates and/or different treatments. The array contains spots (100 μ m) of 2 million oligonucleotides made of polyT region for capturing mRNA, unique molecular index (UMI), spatial barcode, amplification and sequencing handle and a cleavage site for cDNA collection. The captured mRNA molecules are reverse-transcribed and their cDNAs collected for massively parallel sequencing. **b)** 10 μ m longitudinal section of barley spike at meiosis stage (anther size is 0.8 mm) stained with toluidine blue. **c)** Six sub-arrays showing

fluorescently labelled cDNA of longitudinally sectioned barley spikes.



Conclusions and Future prospects

In addition to learning SRT technique, I have now established a collaboration with the laboratory of Prof Joakim Lundeberg and Dr Stefania Giacomello, SciLifeLab, Stockholm to continue my analysis of meiotic transcriptome of barley spikes in different genetic backgrounds and address the following points:

- SRT data is currently analysed using genome sequence as a reference. We will expand the use of SRT by developing a transcriptome-based pipeline.
- As a complementary method, we will explore the feasibility of single-cell RNA-seq using barley meiotic nuclei. Such RNA-seq has been shown to improve further the resolution of SRT as the size of oligo-spots (100 μ m) on the current arrays are still too large and capture mRNA of multiple cells complicating the analysis.
- We are also interested in applying their expansion microscopy method to study the chromosomal dynamics during meiosis.
- To do this work, we are planning to apply for a Royal Society International Exchange grant this spring. My preliminary data will be used to apply for Marie Skłodowska-Curie or BBSRC fellowships.
- My aim is to establish SRT technique here in Scotland for both plant and biomedical sciences, but funding will be needed to purchase the necessary equipment.