Investigating the spatiotemporal dynamics of meiotic crossover control

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BIOCHEMICAL SOCIETY

Introduction

Essential to meiotic cell division is the formation of crossovers – sites of reciprocal exchange of genetic material between homologous chromosomes that occurs during prophase I. The distribution of crossover sites along chromosomes is non-random: the occurrence of one crossover reduces the likelihood of occurrence of another one in its vicinity. This is known as crossover interference. Several models for the mechanism of this process have been proposed. The so-called coarsening model developed my supervisor, Dr. Chris Morgan [1], postulates that formation of crossover sites is mediated by HEI10, a conserved E3 ligase. In early prophase I, HEI10 is loaded onto, and allowed to diffuse along, the synaptonemal complex (SC), which is a proteinaceous scaffold that forms between paired chromosomes. Molecules of HEI10 then progressively accumulate, or coarsen, at distinct foci, which later become the designated crossover sites [1-2]. Current evidence that supports this model is mostly correlative and based on fixed cell imaging data. Morgan et al. now aim to test the model by obtaining live imaging data and comparing this to predictions from mathematical modelling. Apart from being a central process to eukaryotic life, crossover formation presents wider applications in crop breeding,

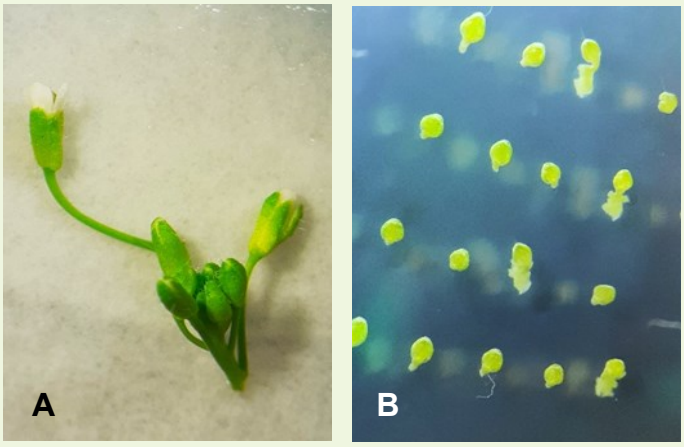

Figure 1. A: An *Ath.Tg* (HEI10:mEGFP) inflorescence as dissected from a six-week old plant using tweezers. **B**: An array of buds extracted from the inflorescence in (A) and placed onto MS culture medium in preparation for imaging.

for example. In wheat, crossover formation is restricted to the sub-telomeric regions of chromosomes, yet central regions are still gene-rich [3]. Harnessing the mechanism of crossover site designation could enable the generation of custom recombination sites and thereby the improvement of crop varieties by giving rise to new genetic combinations in regions that are crossover-suppressed.

Project summary

The project was divided into two major parts: generation of a transgenic SCEP1:mEGFP *A. thaliana* lineage using molecular cloning techniques; and performing live imaging on transgenic *A. thaliana* male meiocytes of HEI10:mEGFP lines, which had been prepared by my supervisor beforehand.

Obtaining data from a transgenic plant that I personally constructed would not have been feasible during a six-week timeframe, so the project was organised in this way to simulate a real workflow.

The pollen-bearing structure of the stamen, where meiocytes are found, is the anther. Anthers were prepared for microscopy by dissecting young inflorescences and transferring buds of the appropriate size to culture medium (Figure 1A-B). Buds were imaged live under a water-immersion objective of the Zeiss LSM980 confocal microscope, which includes the Airyscan 2 post-processing function. This is a detector module that improves the signal-to-noise ratio in the output images. Cells were screened for the appropriate stage of meiosis – late prophase I, when discrete HEI10 foci are visible – and suitable nuclei were subject to targeted illumination with high-energy laser light to induce immediate photobleaching. Since HEI10 is mobile along the axis of the chromosomes, fluorescence in the illuminated regions gradually recovers as the molecules redistribute. This technique, fluorescence recovery after photobleaching (FRAP), enables the dynamics of HEI10 turnover at recombination foci to be studied. Movies were recorded (such as Supplementary Video S1) and analysed using a tracking algorithm in the image analysis software FIJI [4]. This involves 3D segmentation methods that create a trajectory for every detectable HEI10 focus in the movie (Figure 2A). The fluorescence intensity can then be plotted as a function of time to generate a FRAP curve as presented in Figure 2B. Intensity values are normalised against a group of control foci from

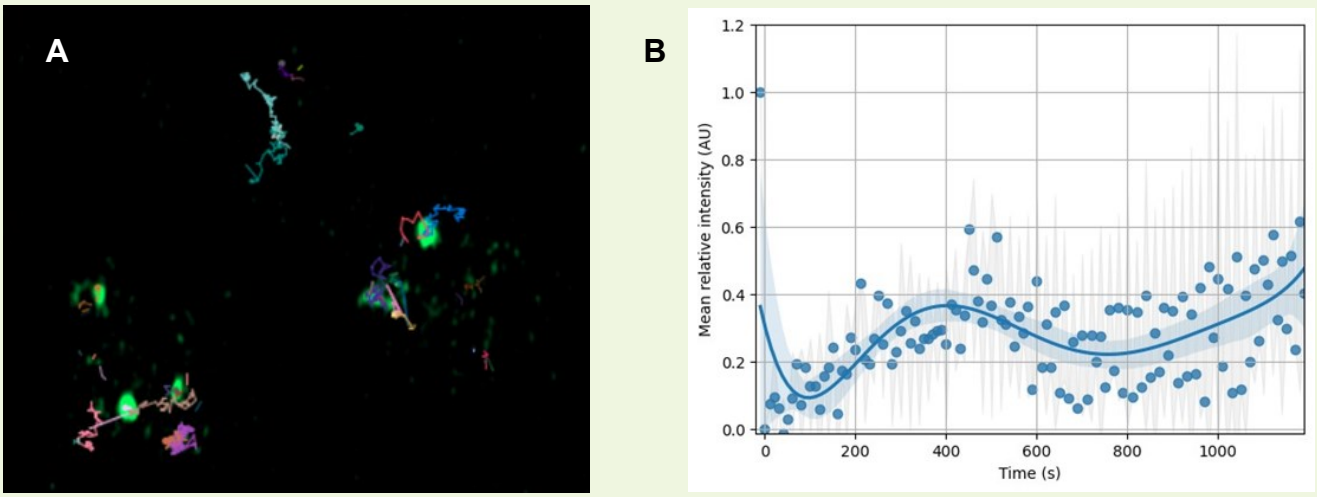

Figure 2. A: The output of the TrackMate algorithm in FIJI. The movement of HEI10 foci (green dots) is tracked in three dimensions over time to generate a motion path. The colour of the path is different for each focus. **B**: Average recovery of fluorescence intensity in photobleached nuclei (n = 9). Photobleaching occurs at time t = 0 seconds, after which fluorescence intensity is measured at regular 20 second intervals. The blue curve is a polynomial regression with 95% confidence interval represented by the lighter blue region around it. The grey shaded areas represent the error on each intensity measurement, given by average intensity ± one standard deviation.

adjacent nuclei to account for photo-bleaching that occurs as a matter of course due to light exposure during image. Custom Python scripts were then written to render the data analysis more streamlined and repeatable for future work to be carried out in Morgan's group. Focus intensity data from .csv spreadsheets was extracted, cleaned, ordered, normalised and averaged such that an average FRAP curve could be plotted for the nine imaging experiments that were performed. While an overall recovery in fluorescence can be seen, there are clear fluctuations in fluorescence intensity in the post-bleach stage of the experiment. This could simply be the result of noise in this small sample or indeed variable recycling dynamics due to different initial focus sizes and positions. Interchromosomal effects and exchange of HEI10 with its free pool in the nucleoplasm could also be at play. Further work could now focus on extracting biophysical parameters such as HEI10 diffusion coefficients to better characterise the role of HEI10 in crossover patterning.

In addition, HEI10 dynamics can be probed in the context of other nuclear components, such as the SC. To this end, a transgenic lineage containing a fluorescence reporter for SCEP1, a newly identified component of the SC, was constructed. SCEP1 is a long coiled-coil protein that forms the central element of the SC [5] and can be used as a marker for the putative conduit along which HEI10 diffuses in conjunction with the HEI10:mEGFP reporter to image double transgene lineages. This is a genetic engineering workflow that begins with amplification of *SCEP1* from *A. thaliana* genomic DNA. The gene is then cloned into a vector, in which site-directed mutagenesis is carried out to introduce restriction sites. These restriction sites are also attached to the mEGFP gene, such that subsequent digestion and ligation will generate a SCEP1:mEGFP construct (Figure 3) that was introduced into *A. thaliana* by means of an *A. tumefaciens*-mediated transformation. The final result of this work will be known in late autumn 2024, when any successful transformants will reach the right stage for imaging.

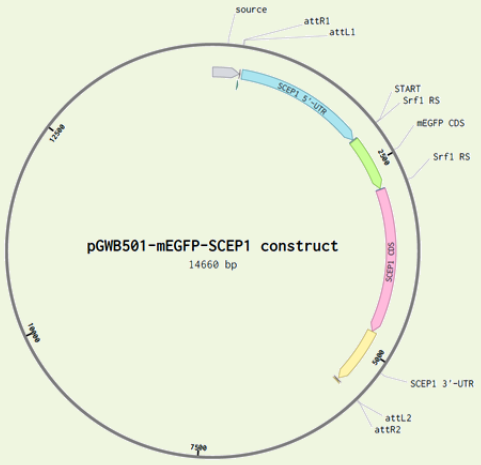

Figure 3. The destination vector containing the *SCEP1* transgene to be transformed into *A. thaliana*. The construct contains the native promoter of *SCEP1* in its 5'-UTR, an N-terminal mEGFP reporter, the *SCEP1* gene body, its 3'-UTR, and flanking recombination sites that were necessary for an upstream vector backbone exchange reaction.

Reflections

During my summer placement, I learned to work independently and efficiently in the laboratory while performing cloning experiments by managing my time effectively and planning ahead to minimise delays and long waiting times. I also became familiar with *A. thaliana* as a model organism, how it is prepared for live imaging and how to this end a confocal microscope is operated. At several stages of the cloning workflow, I simulated construct generation *in silico* using platforms such as Benchling, SerialCloner and SnapGene; and verified their correct formation by examining sequencing data using the online BLAST tool. I had also become more confident in using image processing software and data analysis libraries in Python such as pandas and seaborn.

I am certain that, having developed these experimental skills, I will be placed in a strong position to start my Master's project during my fourth year at university and beyond with further academic pursuits. During my time at the John Innes Centre, I also attended numerous talks, departmental seminars and delivered presentations at lab meetings myself. These opportunities enabled me to further develop my communication, presentation, and networking skills that will be essential for me as a researcher and science communicator in the future.

References

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