Manipulating meiosis: crossovers from Arabidopsis to crops

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Over the past decade, studies in Arabidopsis have progressed our understanding of how plant meiosis and recombination are controlled at the molecular level, revealing two pathways to generating meiotic crossovers (COs). Transferring this knowledge to crops could enable the manipulation of recombination to improve plant breeding. This would be particularly useful for wheat, barley and other grasses where recombination and distribution of crossovers is restricted to the ends of chromosomes such that many genes rarely recombine, limiting the genetic diversity that can be exploited.

Our objectives are to take the knowledge and techniques that elucidated meiosis in Arabidopsis and use them to determine how meiotic recombination is controlled in barley in order to illuminate the basis for the skewed recombination pattern. We will then explore strategies for manipulating recombination to improve future crop breeding.

Confirming skewed crossover distribution in barley

The relationship between the physical and genetic maps of barley chromosome 3H and the physical map of syntenic rice chromosome 1 is shown in Fig 1A. Lack of recombination in the centre of 3H means that 70% of the physical map is represented by only 5% of the genetic map, although co-linearity with rice 1 indicates that this region may contain 50% of the genes on 3H. Cytological evaluation of crossover distribution by scoring chiasmata confirms this skewed CO distribution (Fig 1B; see chromosome 5 where pale blue arrows point to the centromeres and white arrows to the chiasmata at the ends of the chromosome).

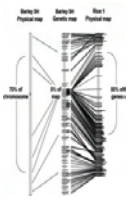


Fig 1A

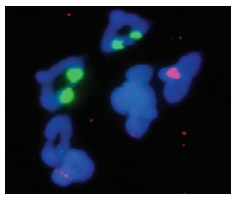


Fig 1B

Transferring molecular cytogenetic tools from Arabidopsis to barley

Understanding meiosis in Arabidopsis was enabled by the development of novel molecular cytogenetic techniques/reagents to underpin detailed characterisation of gene function. We are using the same approaches and antibodies in barley to determine the chronology of meiosis and to identify similarities and differences between Arabidopsis and barley. Many aspects of meiosis are similar in both species but some details differ between Arabidopsis and barley. Barley nuclei show a "classical" bouquet organization at zygotene (Fig. 2A). In early leptotene, Rad51 predominantly localises to the "mature" chromosome axes in sub-telomeric regions in barley (Fig. 2B), but this polarization is not so apparent in Arabidopsis (Fig. 2C).

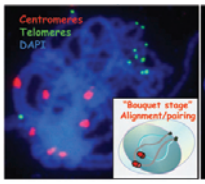


Fig 2A Barley

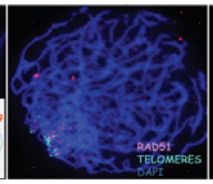


Fig 2B Barley

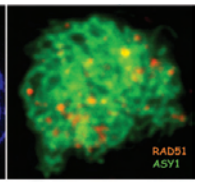


Fig 2C *Arabidopsis*

Developing a recombination assay

We are developing a new cytological assay for monitoring recombination in barley to underpin future work on CO manipulation. Pachytene nuclei are isolated, embedded in polyacrylamide, and probed with the Arabidopsis Asy1 antibody to detect synaptonemal complexes (SCs). Probed nuclei are optically sectioned and individual bivalents tracked with Imaris software. Fig. 3 shows traces of each of the 7 individual SCs holding each bivalent together. FISH of BACS markers will be used to generate a chromosome-specific barcode and location of MLH1/3 will mark COs, allowing changes in recombination to be monitored.

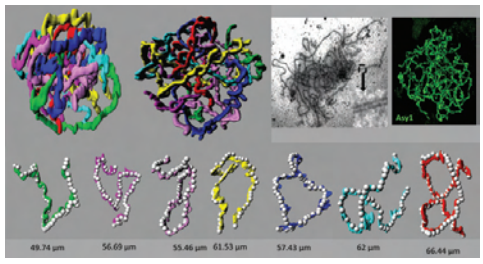


Fig 3

Identifying barley orthologues of Arabidopsis recombination genes

Sequences representing barley genes involved in meiotic recombination have been identified in EST databases by homology to Arabidopsis and rice genes, and full-length sequences are currently being cloned (Fig. 4A). Another route to cloning barley meiotic genes is to isolate the genes underlying desynapsis (*des*) mutations (Fig 4B). 14 non-allelic *des* mutants have been crossed into a common Bowman background (J.D. Francowiak). Putative map positions have been identified for the mutations (A. Druka) and some are being subjected to fine mapping to identify candidate genes.

Fig 4A Extending barley EST sequences (black lines) to generate complete cDNA sequences (green lines). Degree of completeness is illustrated against a generalized cDNA sequence (blue box).

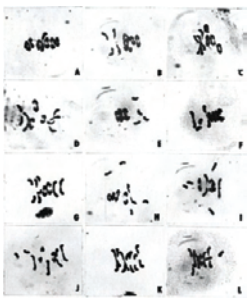
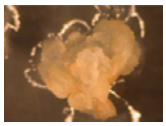
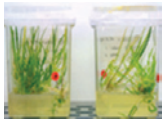


Fig 4B Metaphase 1 configurations of cells with desynapsis of chromosomes 1-7

Manipulating recombination in barley





The function of these genes in barley meiosis and their potential for recombination manipulation is being determined using RNAi and overexpression in transgenic barley (Fig. 5).

Fig 5

Acknowledgements
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