PhD project title: Investigating cross species plasticity of meiotic protein complexes to modulate recombination using synthetic biology

University of Registration: Leicester

Project outline

1. Project outline describing the scientific rationale of the project (max 4,000 characters incl. spaces and returns)

Genetic exchange is a key process for genome evolution and is dependent on crossover (CO) formation during meiosis. The frequency and distribution of COs is highly regulated to ensure that each pair of homologous chromosomes receives at least one CO, thus ensuring correct chromosome segregation and normal fertility. CO formation depends on the interaction between chromatin, recombination protein complexes, cell cycle progression and formation of the synaptonemal complex, a meiosis specific proteinaceous structure. CO formation is highly conserved in eukaryotes, and a considerable number of orthologues have been identified across species. However, the frequency and distribution of COs is highly variable between species, suggesting that small changes can have large effects and that genome structure is also important. In the majority of organisms with large genomes, the frequency and distribution of COs is often restricted. This is problematic for generating novel crop plants with advantageous alleles. Budding yeast on the other hand has proportionally high levels of COs, which could be useful if transferred to crop plants.

In this project we aim to transfer known meiotic protein complexes from budding yeast to Arabidopsis and vice versa. We will use a synthetic biology approach to generate ‘biobricks’ that can be easily transferred between organisms with CRISPR/Cas for precise gene targeting. We will initially investigate cross species function of recombination proteins HEI10, MSH4/5 and SGS1 from budding yeast and Arabidopsis. We will analyse the products using state-of-the-art fluorescence microscopy (including confocal and super-resolution) as well as molecular marker techniques involving next generation sequencing and bioinformatics analysis.

The specific questions for this project are: 1) How do HEI10, MSH4/5 and SGS1 function in a cross-species genetic background? 2) Can we modulate COs over cross-species barriers to improve crop plants?

Miniproject - Use a bioinformatics approach to identify recombination proteins from different strains of yeast and Arabidopsis to analyse co-evolution of particular amino acid substitutions and protein compatibility.

Months 0-1: Use a bioinformatics approach to identify appropriate recombination proteins in yeast and Arabidopsis for experimental analysis.

Months 1-12: From selected material generate ‘biobricks’ by cloning recombination genes into yeast and Agrobacterium vectors using standard methods and CISPR/Cas. Obtain mutant lines for recombination genes and cross to generate triple/quadruple mutants for complementation of the cross-species genes.
**Months 12-18**: Transform mutant lines for complementation with cross-species genes.

**Months 18-26**: Analyse material using cytological techniques in Arabidopsis, and NGS in yeast, to characterize the frequency and distribution of recombination events.

**Months 26-30**: Model data for effect of allelic variation on crossover interference and CO formation.

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**Relevant BBSRC Strategic Research Priority**: Food security

Techniques that will be undertaken during the project.

We will use CRISPR/Cas and cloning techniques to generate and transfer synthetic biobricks from Arabidopsis to yeast and vice versa. Cytological techniques including fluorescence microscopy, tetrad analysis, QTL analysis (using R), multivariate analysis (using Relative Information Gain and correlation statistics), molecular genetics, Next Generation Sequencing and analysis of the data generated, basic genetics of two different systems, bioinformatics and comparative genomics.

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