Tissue specific gene expression in Drosophila - dissecting promoter architecture

Supervisory team:
Main supervisor: Prof Helen White-Cooper (Cardiff University)
Second supervisor: Dr Nathan Harmston (Cardiff University)

Collaborators: Prof James Wakefield (University of Exeter), Dr Fabrizio Costa (University of Exeter), Prof Luke Alphey (University of York)

Host institution: Cardiff University

Project description:
DNA sequence contains all the information required to ensure accurate transcriptional regulation, however for expression of many genes, we have limited understanding of how this information directs the appropriate programme in vivo. The Drosophila testis is an excellent model system for studying fundamental gene regulatory mechanisms. Testis-specific transcription is primarily activated in pre-meiotic spermatocytes by Testis Meiotic Arrest Complex (TMAC), a multi-subunit complex containing four DNA binding proteins and associated proteins. TMAC activates expression of over 1000 genes, and testis expression is driven by short proximal promoter sequences rather than distant enhancer. Despite the apparent simplicity of this system there is very limited primary sequence similarity between testis-specific promoters, and no "testis gene" DNA motifs have been identified.

We propose that testis-specific transcription depends on TMAC binding to the promoter as a complex, via any one (or any combination) of its constituent DNA binding subunits. Thus the "TMAC target site" could be a broad range of alternative sequences. The lack of a clear simple bioinformatics signature would be an emergent property of the mechanism by which TMAC identifies its binding sites, and would establish TMAC as a scaffolded complex, a new paradigm in cis regulatory module architecture. We have determined the DNA sequence binding preferences of TMAC subunits, used CRISPR to endogenously tag the proteins, and are performing ChIP to identify binding sites in vivo. We are generating fly lines that ablate the DNA binding activity, while allowing complex formation to occur. This combined wet-lab and computational PhD project will build on the extensive background work and tools to investigate cis regulatory module architecture in testis-specific promoters. You will use a computational approach to examine presence and spacing of TMAC subunit binding sites in target sequences, and predict for each gene which regions are implicated in TMAC association. You will test these predictions with a series of reporter constructs, examining the promoter behaviour in wild type flies and in flies with specific TMAC subunits mutations. You will complement these experiments with in vitro DNA binding assays. You will interrogate scRNAseq data to determine whether promoter sequence correlates with timing of onset of transcription in male germline cells.

This project will reveal fundamental insights into a) how tissue specific gene expression can be achieved with very short promoter regions, b) how a single transcriptional regulatory complex can interact with a very diverse range of DNA sequences.

Our aim as the SWBio DTP is to support students from a range of backgrounds and circumstances. Where needed, we will work with you to take into consideration reasonable project adaptations (for example to support caring responsibilities, disabilities, other significant personal circumstances) as well as flexible working and part-time study requests, to enable greater access to a PhD. All our supervisors support us with this aim, so please feel comfortable in discussing further with the listed PhD project supervisor to see what is feasible.