Using genome-wide quantitative fitness profiling to identify the molecules responsible for detecting the oligopeptide differentiation signal in bloodstream T. brucei

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African trypanosomes are the causative agent of sleeping sickness in humans and nagana in livestock in Sub Saharan Africa. Within the infected mammalian host, *T. brucei* undergoes a density-dependent developmental transition from proliferative 'slender' cells, that establish infection, to cell-cell arrested 'stumpy' cells that are competent for transmission into the tsetse fly vector. Recent studies have established that this differentiation is triggered in response to an increasing concentration of extracellular oligopeptides, that are themselves generated by the release of parasite-derived peptidases into their environment. These oligopeptides are received by the parasite surface transporter *Tb*GPR89. Our previous screen to identify the components of the slender to stumpy quorum sensing (QS) signalling pathway used cell permeable molecules that bypassed the oligopeptide signal reception steps. Thus, the molecules that detect the oligopeptide differentiation signal and activate the previously characterised components of the signalling pathway are not known.

To identify these molecules we have recently used DRiF-Seq to perform a genome-wide quantitative fitness profiling in response to the physiological oligopeptide differentiation signal in a pleomorphic cell line capable of differentiation. We have identified a number of genes whose RNAi confers decreased sensitivity to BHI-derived oligopeptides and have validated these *in vitro* using independently generated inducible RNAi cell lines. We will next ask if the knock down of these novel regulators of oligopeptide sensitivity also creates defects in the QS signalling pathway. To do this, we will use cell cycle analysis and expression of the stumpy specific marker protein PAD1 to assess stumpy formation upon exposure to oligopeptides *in vitro* and during an infection *in vivo.*

I will present the outcomes of the DRiF-Seq screen and our current progress validating the identified molecules.