Identifying Variant Surface Glycoprotein Gene Regulators in *Trypanosoma brucei*

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Trypanosoma brucei is a single-celled, flagellated, extracellular protozoan parasite and the causative agent of African trypanosomiasis in humans and animals. *T. brucei* evades the host antibody responses by periodically switching its Variant Surface Glycoprotein (VSGs) coat. VSGs are highly antigenic proteins expressed in a monogenic manner from a repertoire of 2,500 genes and pseudogenes, at subtelomeric expression sites (ES). However, the mechanisms that control VSG switching remain unclear. Previously, immunoprecipitation coupled with cross-linking and mass spectrometry revealed that, as part of the phosphatidylinositol signaling pathway, phosphatidylinositol 5-phosphatase (PIP5Pase) associates with the VSG silencer repressor-activator protein 1 (RAP1) to regulate VSG expression and switching in *T. brucei*. Additionally, PIP5Pase and RAP1 strongly interact with several additional proteins, including eight candidate regulators which we hypothesize are essential genes for parasite survival and VSG expression control. Our objective is to investigate the role of these candidate regulators in antigenic variation in *T. brucei*. We constructed tetracycline-inducible RNA-interference (RNAi) cell lines in bloodstream form *T. brucei* SM427 to induce gene-specific knockdowns, followed by gene expression analysis. Five genes were cloned into the MC177VSG221RNAi vector, and two constructs were successfully transfected to generate tetracycline-inducible RNAi cell lines. RNAi-mediated knockdown of the two candidate regulators did not significantly affect parasite growth or morphology. Therefore, these two genes are unlikely to be essential for parasite viability. Gene expression analysis and VSG switching assays are in progress to determine the consequence of the knockdowns on VSG expression and better understand the host evasion mechanisms used by *T. brucei*.