Crucial Role of SmMBD2/3 and SmCBX during schistosome neoblast proliferation and oviposition

We have previously confirmed the presence of the epigenetic mark 5-methylcytosine (5mC) in the genome of the medically important blood fluke *Schistosoma mansoni*, as well as identified the core DNA methylation machinery components DNA methyltransferase 2 (SmDNMT2) and methyl-CpG binding domain protein (SmMBD2/3). While we demonstrated that SmDNMT2 is responsible for the establishment of this DNA modification, we did not further pursue the role of SmMBD2/3 during schistosome epigenetic processes. MBD proteins represent the readers of metazoan DNA methylation systems and specifically bind to methylated loci via a conserved N-terminal 5mC-binding domain. This event catalyses the recruitment of further co-repressors, thereby leading to a transcriptionally silent chromatin state. In *S. mansoni*, previous bioinformatics-led characterisation of the metazoan MBD family suggested that SmMBD2/3 is a functional member. Here we confirm that this schistosome protein is indeed a functional candidate by validating binding affinity of rSmMBD2/3 to a methylated substrate as well as demonstrating its primary nuclear localisation. A subsequent yeast-two-hybrid (Y-2-H) screen identifies the heterochromatin-associated SmCBX (*S. mansoni* chromobox protein) as a putative interaction partner of SmMBD2/3 and whole mount fluorescent *in situ* hybridisation (WISH) further confirms the co-expression of these proposed binding partners throughout mesenchymal-, germ- and proliferating somatic stem cells (PSCs or neoblasts). Interestingly, RNAi-mediated knockdown of *Smmbd2/3* and *Smcbx* results in PSC proliferation defects as well as reproductive deficiencies. Our data collectively suggest that SmMBD2/3 represents a functional epigenetic reader capable of binding to 5mC and its physical interaction with the repressor SmCBX further suggests a role for these partners in heterochromatin formation and regulation of gene expression. Additionally, the observed reductions in both PSC proliferation and egg laying, following *Smmbd2/3* and *Smcbx* knockdown, demonstrates a pivotal role for these gene products in schistosome development, transmission and immunopathology progression.