Direct demonstration that histone modification impacts DNA compaction and gene expression in trypanosomes

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Abstract

It remains unclear to what extent, and by which mechanisms, transcription, DNA replication and DNA repair rely upon chromatin-based controls in trypanosomatids. N-terminal histone tails, and tail modifications, such as lysine acetylation, play key roles in these processes in other eukaryotes. However, trypanosomatid histone N-terminal tails are highly divergent relative to the usual model eukaryotes, suggesting potential novel mechanisms. Notably, interpretation of 'writer', 'reader' and 'eraser'-defective phenotypes is complicated by potential perturbation of diverse or non-histone substrates. Genetic manipulation and subsequent study of histone functions have also proven particularly challenging because core histone genes are typically present in polycistronic transcription units of many identical copies of each gene (there are approx. 40 copies of H4 histone gene, for example).

We used an inducible CRISPR/Cas9 system in Trypanosoma brucei to delete all native copies of the histone H4 genes, as confirmed by genome sequencing, complementing the defect with a single, recoded and highly expressed ectopic copy. Further templated editing was then used for site saturation mutagenesis of lysine residues (K4, K10 and K14) in the N-terminal tail of the ectopic H4 gene in these 'histH4one' strains. Multiplex amplicon-seq profiling was used to monitor relative fitness, revealing those tolerated H4-K4 or H4-K14 mutations; H4-K10 mutations were not tolerated. Remarkably, viability was maintained even when H4-K4 or H4-K14 residues were removed. Using these outputs, a panel of strains exclusively expressing novel histone H4 mutants, including arginine (R; non-acetylated mimic) or glutamine (Q; constitutively acetylated mimic) substitutions, was phenotypically profiled; using proteomic, microscopy, growth, protein blotting, flow cytometry and DNA-damage sensitivity assays. The results suggest H4K4 facilitates DNA compaction. We also observed a specific defect in Variant Surface Glycoprotein gene silencing and the DNA damage response in H4-K4Q mutants – the first direct evidence that histone tails and their modification impact these processes in trypanosomes.