Direct demonstration that histone modification impacts gene expression in trypanosomes

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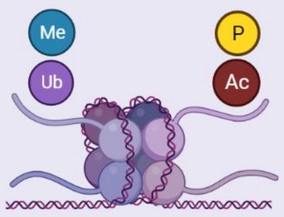
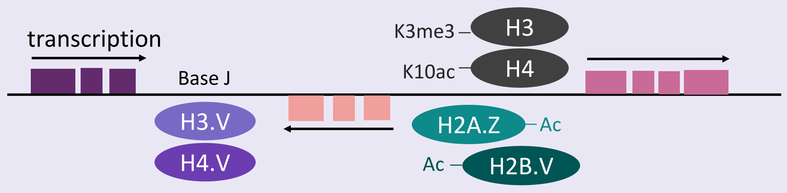
Background

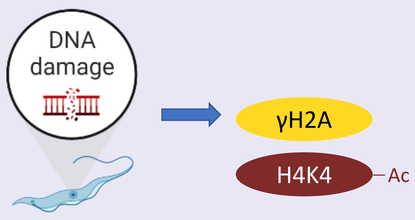
- It is unclear what mechanisms control transcription, DNA replication and DNA repair in trypanosomatids
- Epigenetic marks such as modification of core histone tails may play an important role in these processes
- To study this, we generated a cell line with a single H4 histone gene, which enables editing of a core histone gene in *T. brucei* for the first time
- The new strain has been used for site saturation mutagenesis of N-tail residues of H4 histone
- Fitness profiling of resulting mutants by amplicon-seq suggests that H4K10 is essential, while H4K4 and K14 can be replaced by other amino acids
- H4K4 acetylation mimic has shown increased expression of originally silent VSGs and DNA damage sensitivity

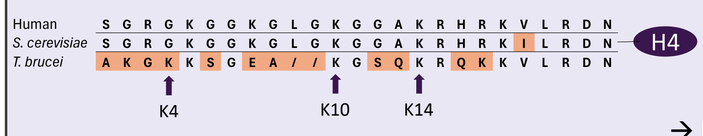
Introduction



Control of transcription initiation/termination, replication and DNA damage response







Organism	No. of H4 histone gene copies
Human	15
S. cerevisiae	2
T. brucei	~ 43

→ Mutagenesis of all gene copies is challenging

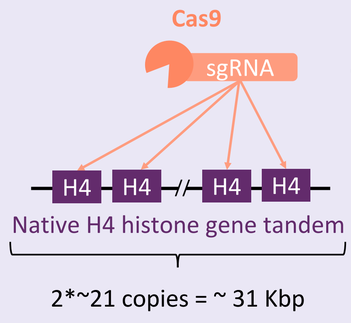
Histone modification enzymes → Resulting phenotype difficult to interpret

Aim: Study the roles of different histone residues using mutagenesis

→ Establish a cell line with a single core histone gene

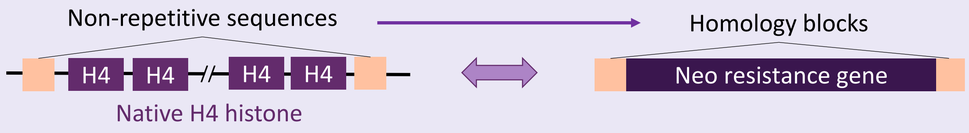
→ Use this cell line for saturation mutagenesis of different residues on the histone tail to gain insight into the function of the residues and their modifications

1 Strategy for establishing cell line with a single H4 gene



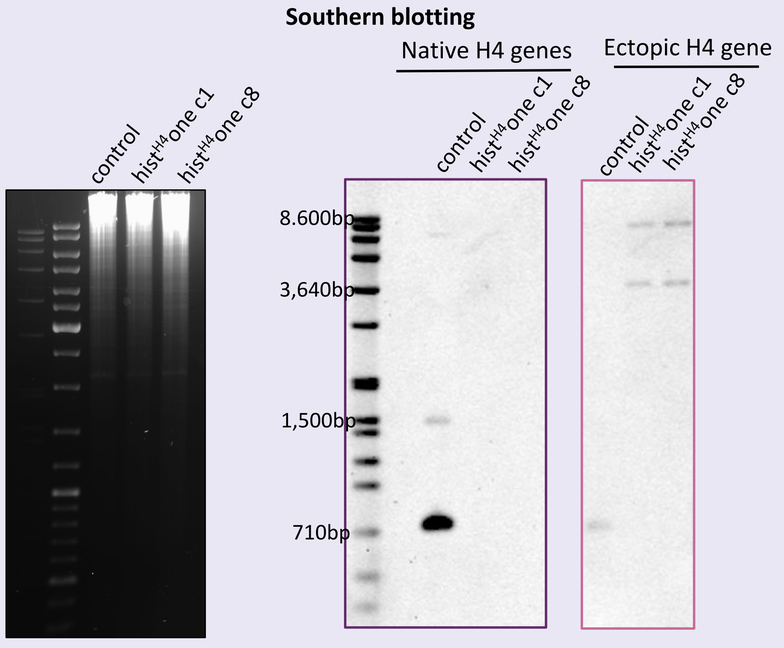
+ Ectopic H4 gene

→ Protected from sgRNA
→ Transcribed by T7 polymerase
→ Additional PAMs that will allow the use of Cas9 for precise mutagenesis

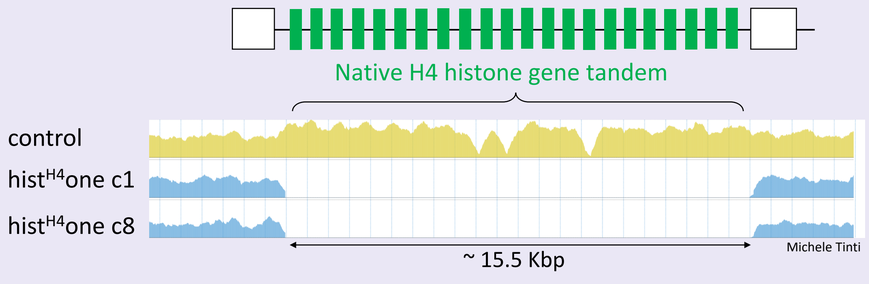


2 Cell line with a single H4 histone gene was successfully generated

Southern blotting



Whole genome sequencing



→ **All native H4 histone genes were replaced with an ectopic copy**

3 Site saturation mutagenesis of H4 gene: strategy

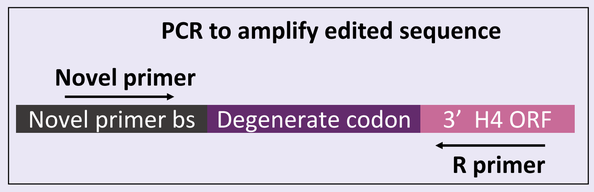
- Hist^{H4}one strain makes it possible to efficiently edit individual residues on H4 histone gene to investigate their function

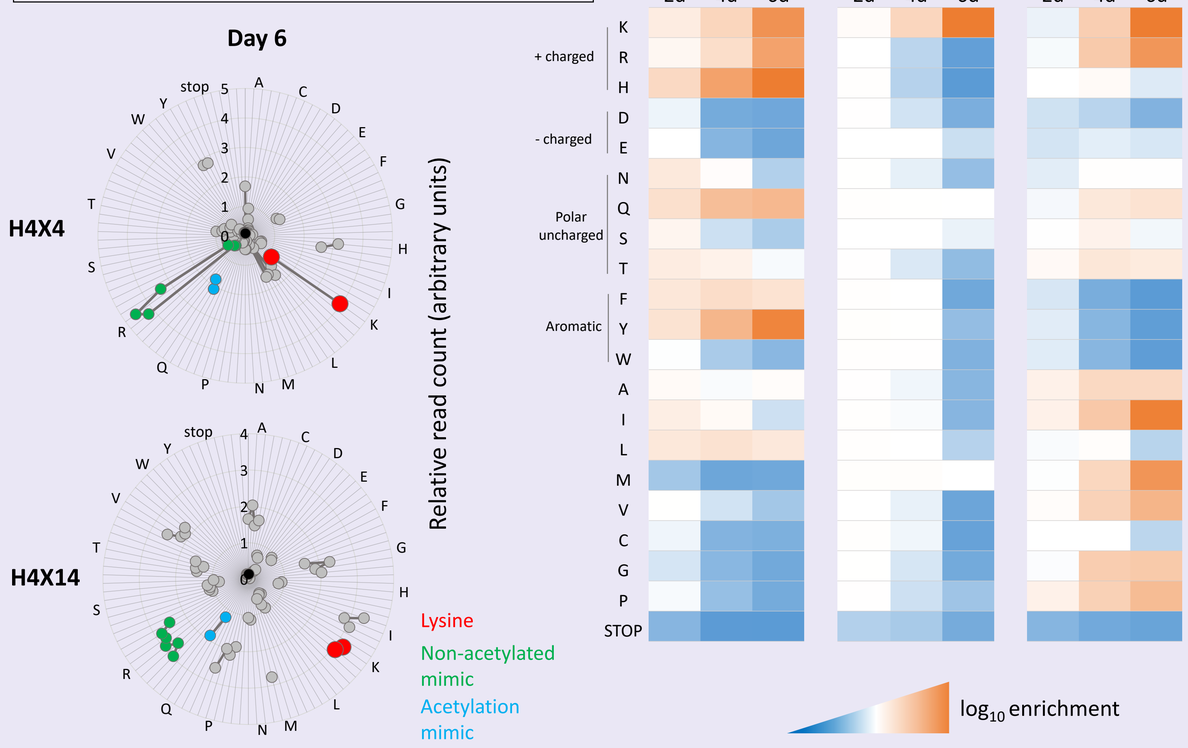
H4K4/10/14 codon → Any possible 3 nucleotide combination

- sgRNA targeting H4 N-tail in Hist^{H4}one strain inserted, Cas9 was induced, and cells were transfected with a template with degenerate codon on position 4, 10 or 14

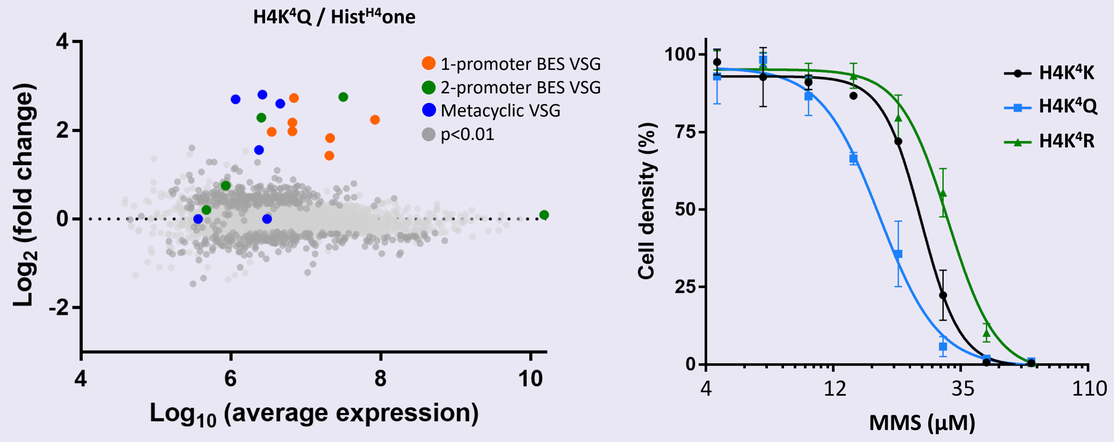
- Collection of cells for 6 days to profile fitness

4 Fitness profiling of H4 histone N-tail residue mutants





5 Variant surface glycoproteins expression and DNA damage sensitivity increased in H4K^{4}Q mutant



Summary

- Hist^{H4}one strain with only one H4 gene has been established and validated
- This strain has been successfully used for saturation mutagenesis of different residues
- Acetylation of H4K4 and K14 is likely not essential for viability
- H4K10 cannot be replaced with any other amino acid: its modifications and their regulation likely essential
- Mimicking acetylation of H4K4 causes increased expression of VSGs

Acknowledgements

- David Horn and Horn group
- Joana Faria (University of York): supervision in 1st year of PhD
- Michele Tinti (University of Dundee): sequencing data analysis