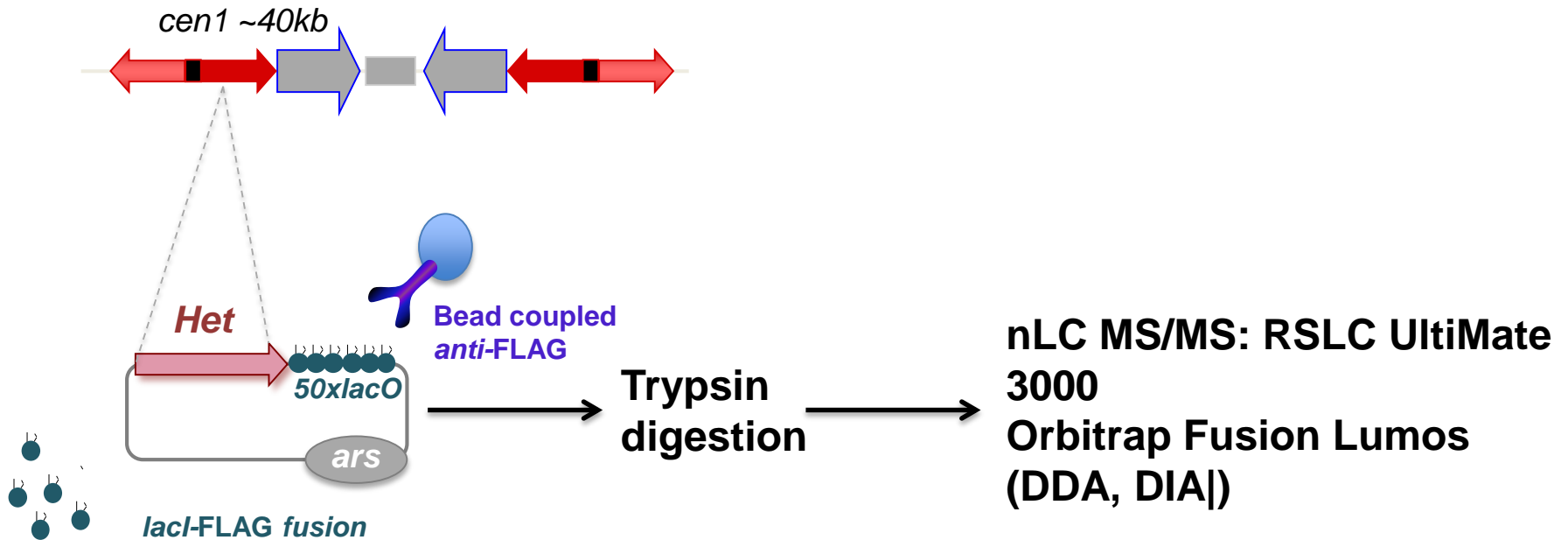


Deciphering pathways to heterochromatin establishment and maintenance using chromatin-region purification

Tania Auchynnika
Allshire lab

Proteomic analyses of purified chromatin regions allow answering important questions about epigenetic inheritance

Experimental methodology for isolation of chromatin regions:



Proteomic analyses of purified chromatin regions allow answering important questions about epigenetic inheritance

Heterochromatin nucleation and epigenetic memory screening:

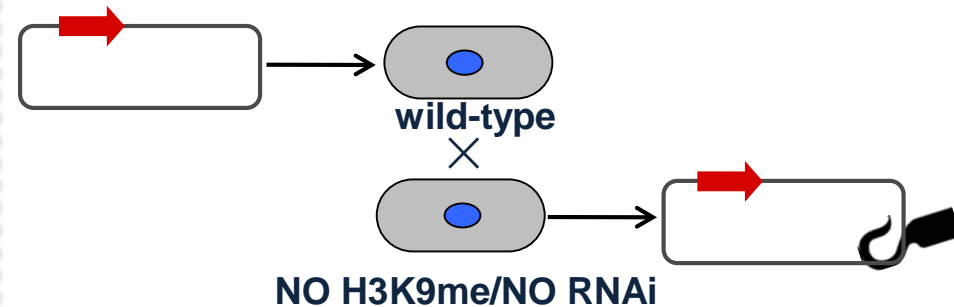
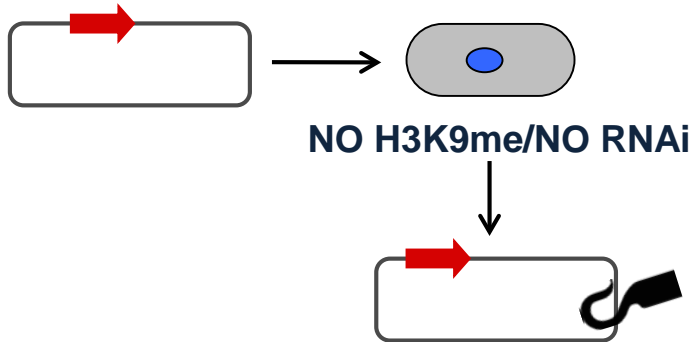
Same sequence poised for repression – different exposure to repressive marks

Naked DNA – no exposure

Chromatinised DNA which had assembled repressive domain – prior exposure

Heterochromatin establishment

Heterochromatin maintenance



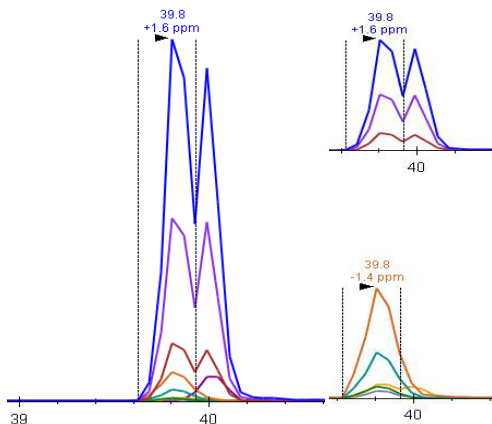
Despite identical sequence, are they different in their proteomic/histone PTMs content?

Histone PTMs analysis benefits from DIA MSX

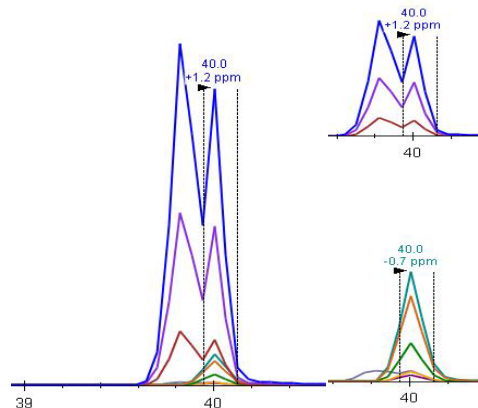
- Histones PTMs are the primary source of epigenetic inheritance in *Schizosaccharomyces pombe*
- *Notoriously difficult to analyse due to the presence of multiple isobaric peptides*

DIA for histone PTMs analysis: DIA MSX 6x4 (Sidoli et al., Proteomics 2016)

H3 (9-17)KSTGGKAPR
ac



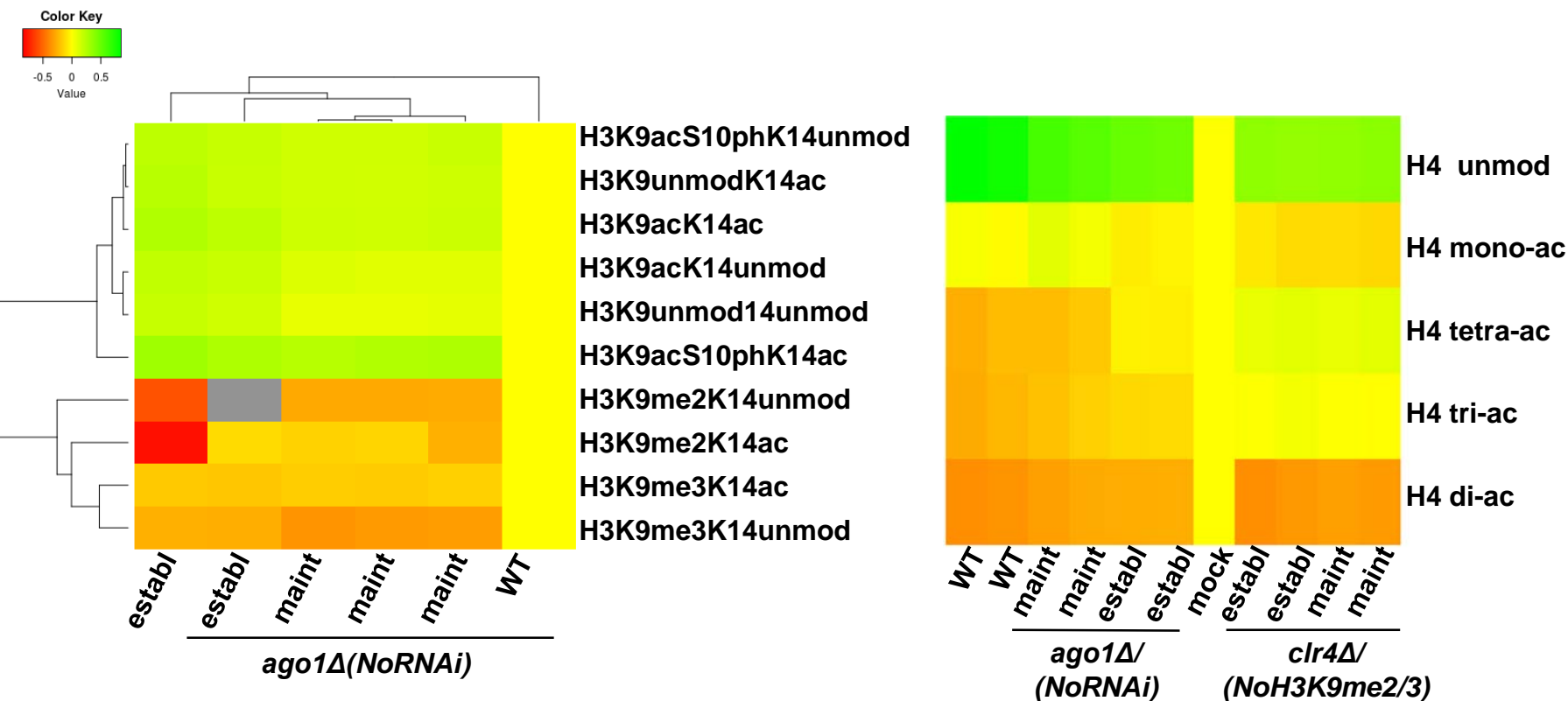
H3 (9-17)KSTGGKAPR
ac



LC set up: EasySpray
50cm 2 μ m beads – high
resolution, high back
pressure (550-600bar at
50°C), extremely sharp
peaks. Examples -
150min gradient.

“memories” of heterochromatin persist after de-repression

- pHet/maint is associated with higher levels of H3K9me2/3 and lower level of polyacetylation of N-termini of histones H3 and H4
- Proteomic data from pHet/maint indicate lower transcriptional potential for chromatin regions exposed to repressive marks



Acknowledgements

Allshire lab



Juri Rappsilber



THE UNIVERSITY *of* EDINBURGH

welcometrust

 **Epigenesys**