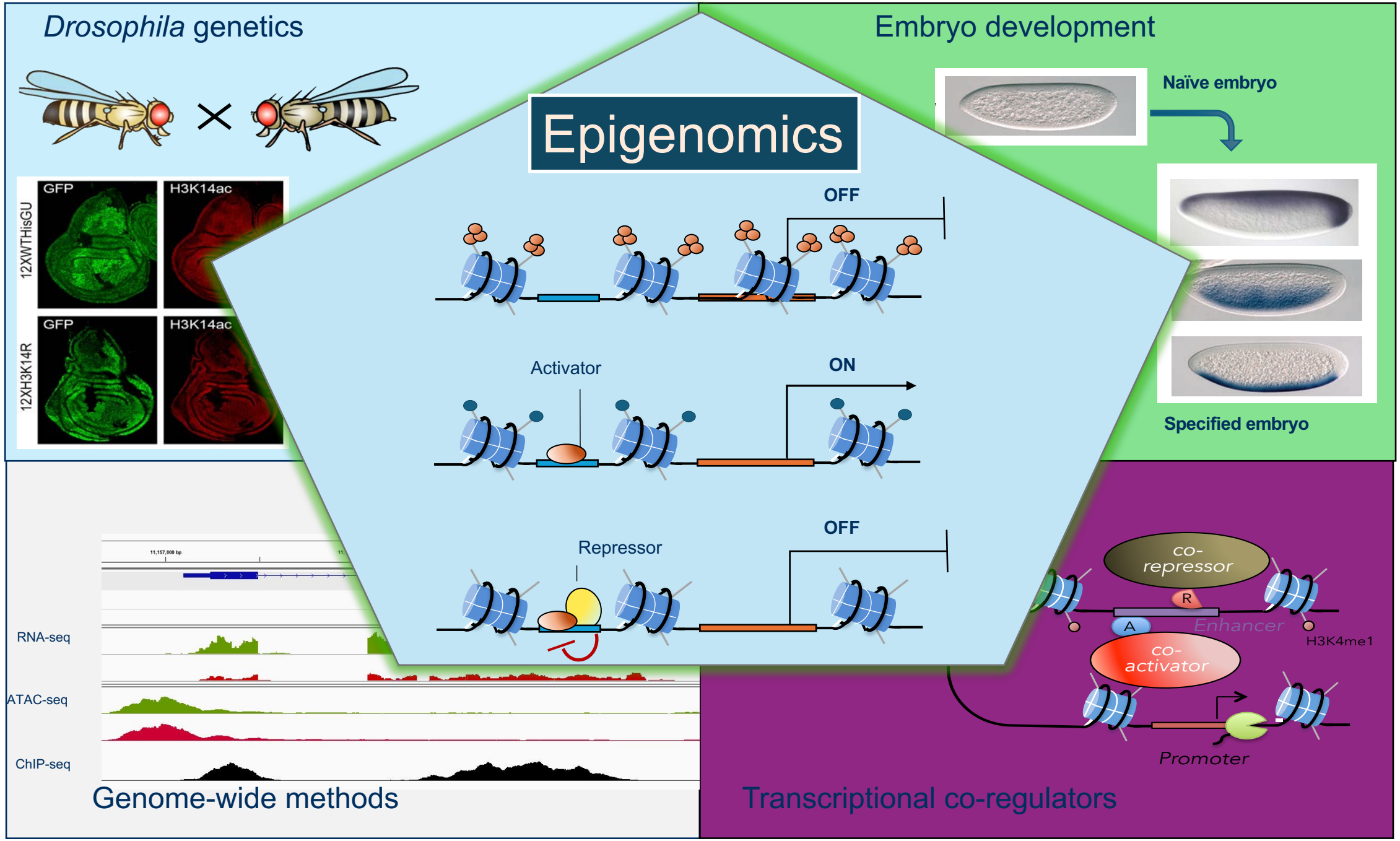
Stockholm University

SCENTINEL kick-off meeting:

The link between gene expression and chromatin in Drosophila development

Mattias Mannervik lab
Stockholm University
Sergei Pirogov, Ph.D. student

Crete 2024

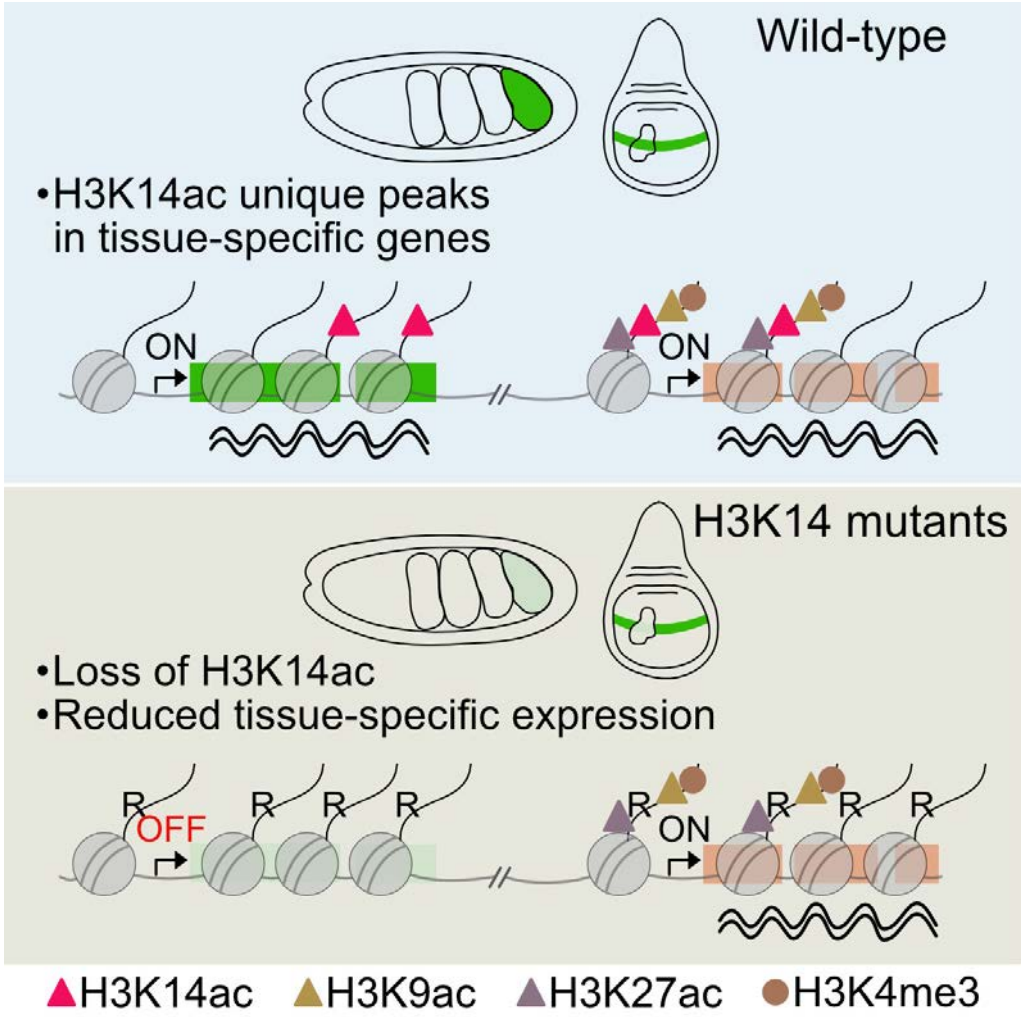

Drosophila genetics
GFP
H3K14ac
12XWTHisGU
GFP
H3K14ac
12XH3K14R
Epigenomics
OFF
Activator
ON
Repressor
OFF
Embryo development
Naïve embryo
Specified embryo
RNA-seq
ATAC-seq
ChIP-seq
Genome-wide methods
co-repressor
R
A
Enhancer
H3K4me1
co-activator
Promoter
Transcriptional co-regulators

Histone replacement projects

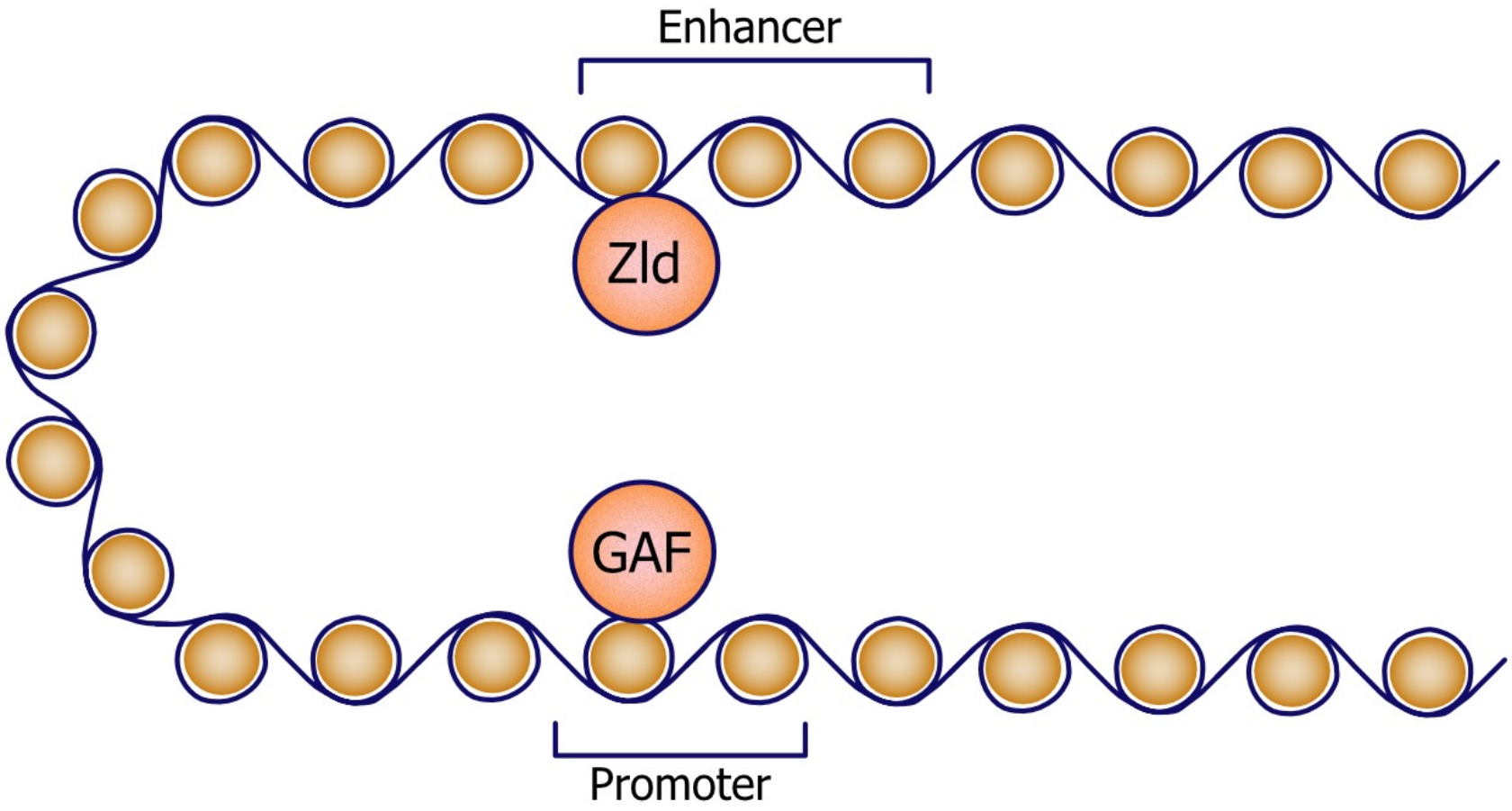
Our objects of study: embryos, wing discs, and S2 cell line.

1. H3K14ac replacement project (*Isabela Regadas et al, Mol Cell, 2021*)

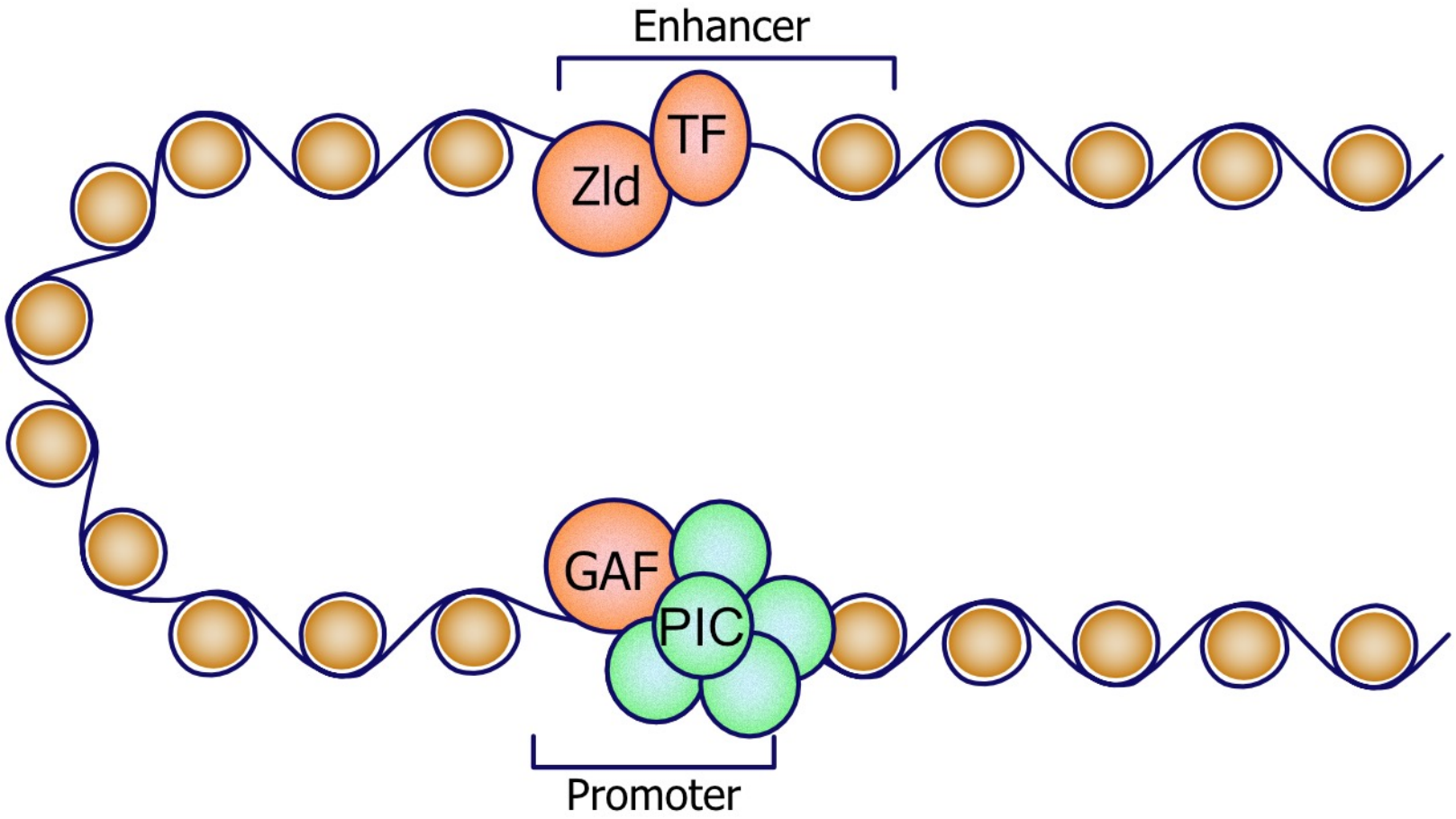
2. H3K79me project (Alexander Pfab, Hicham Houhou, *unpublished*)



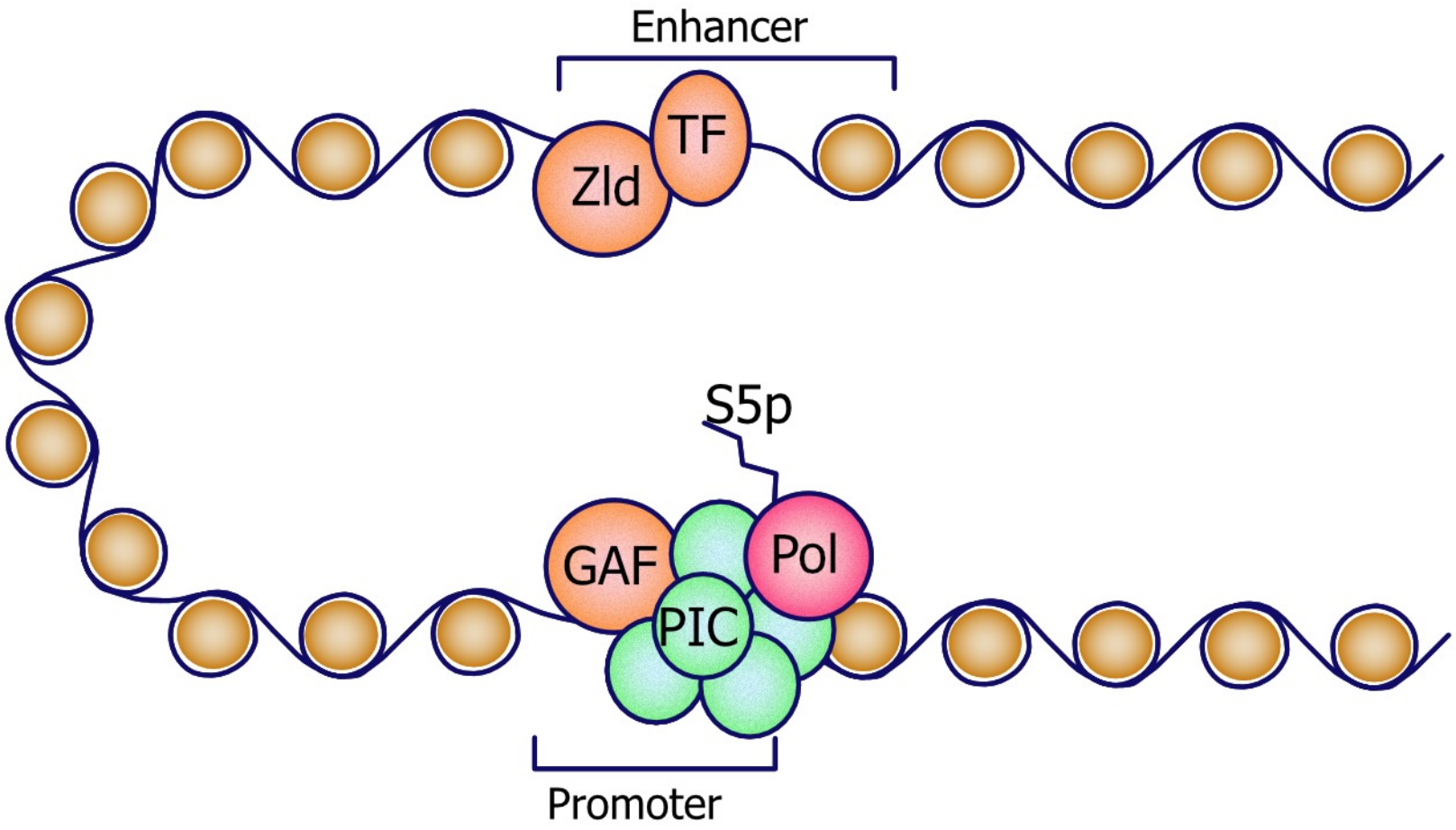
Gene activation model



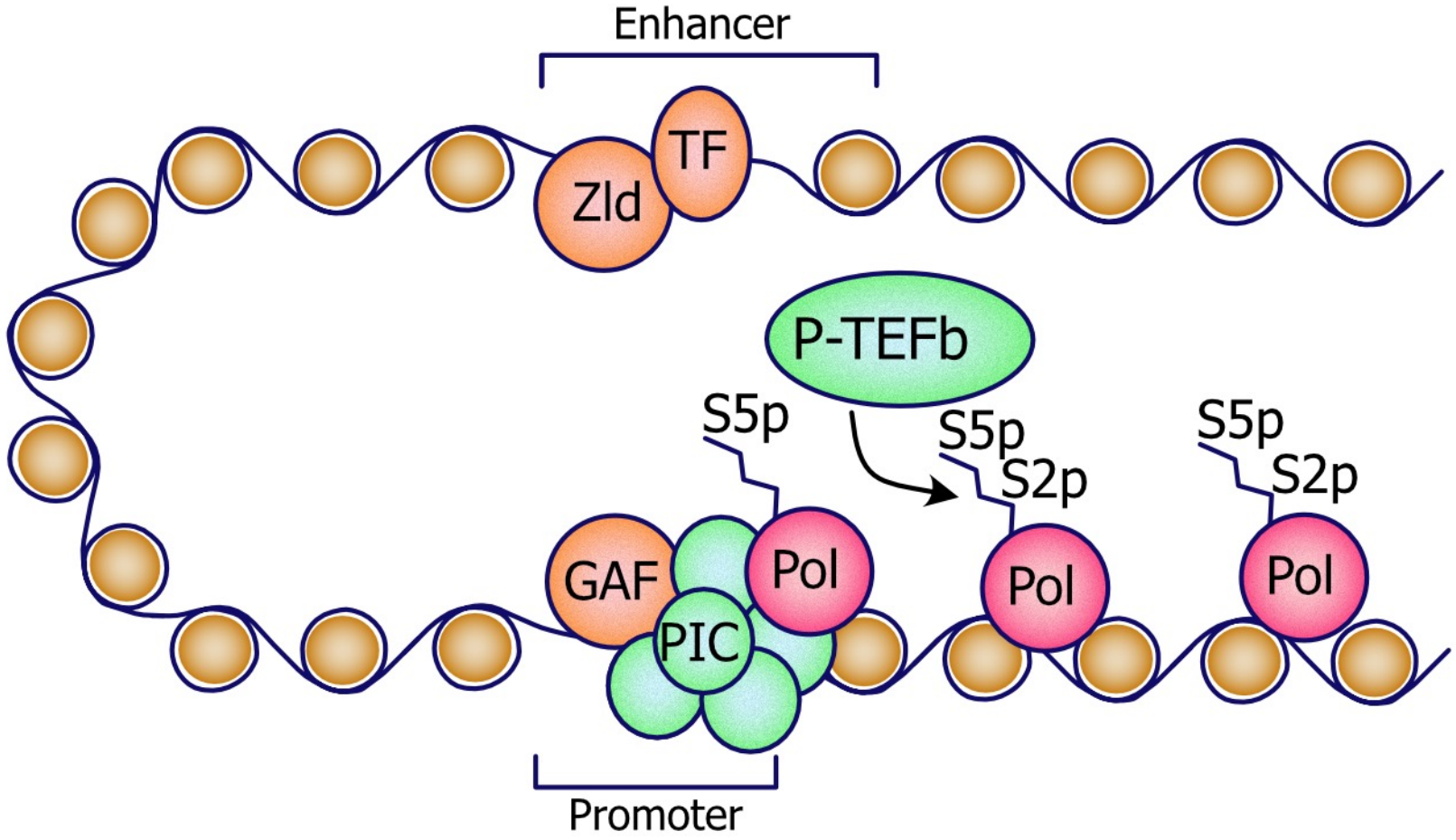
Gene activation model



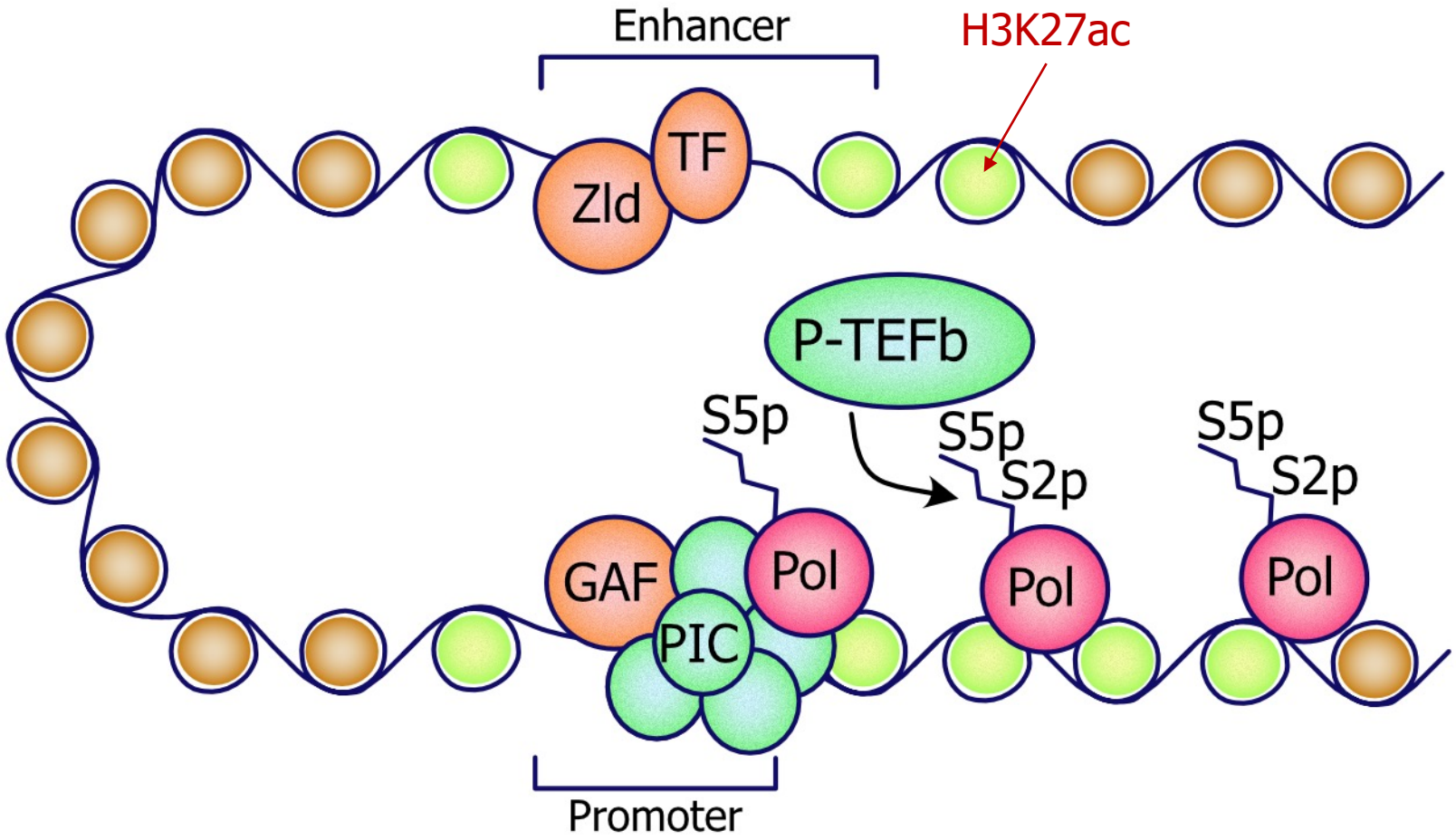
Gene activation model



Gene activation model



Gene activation model



Transcription factors
(Chan et al, 2019; Cho and Parick O'Farrell, 2023)

Transcription initiation

On cell cultures Boija et al, 2017;
Hogg et al, 2021; Narita et al, 2021

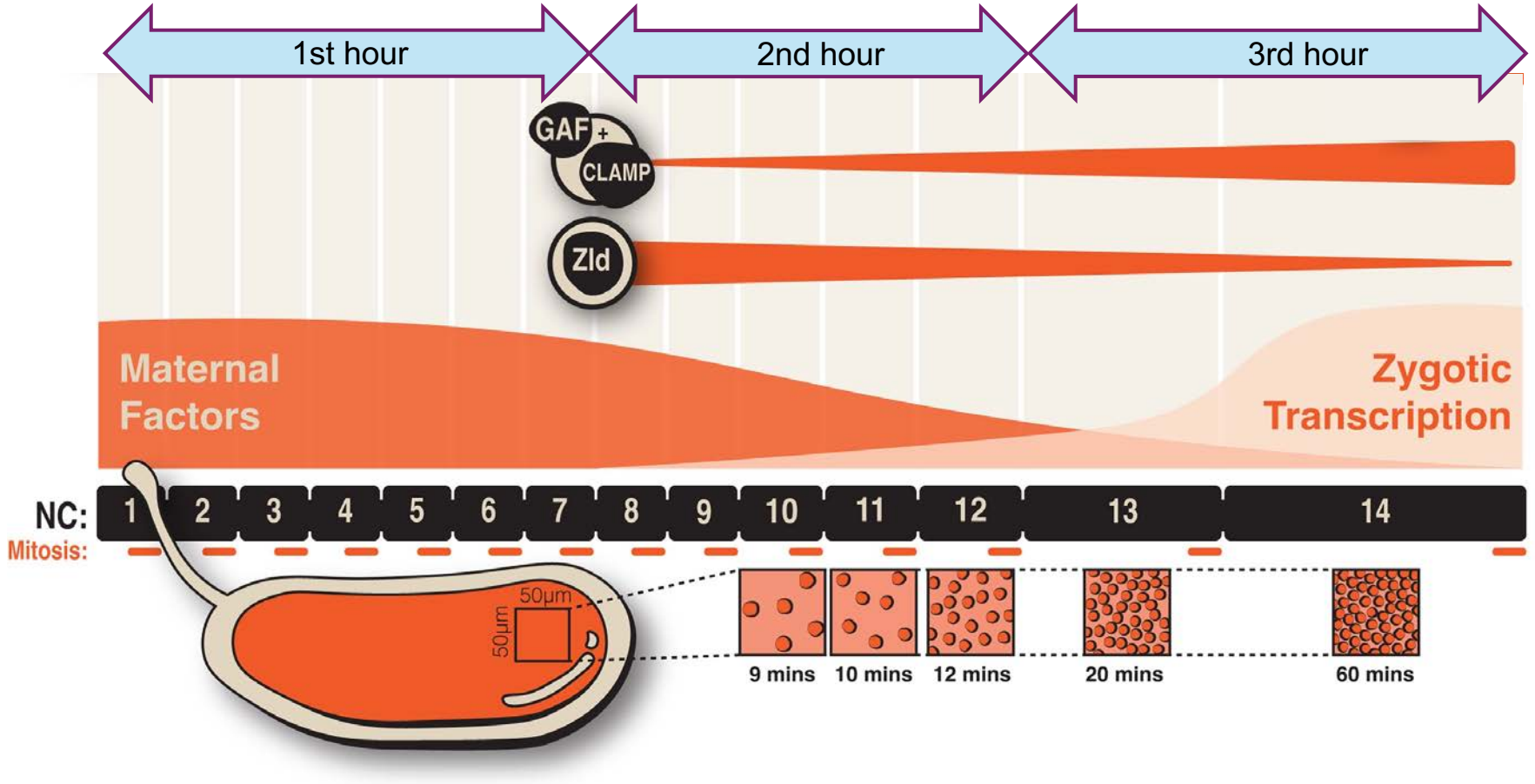
p300/CBP

Transcription pause release

H3K27ac
H3K18ac, H4K5ac,H2Bac
(Ogryzko et al, 1996)

Acetylates thousands of nuclear proteins
(Weinert et al, 2018)

Zygotic genome activation in Drosophila

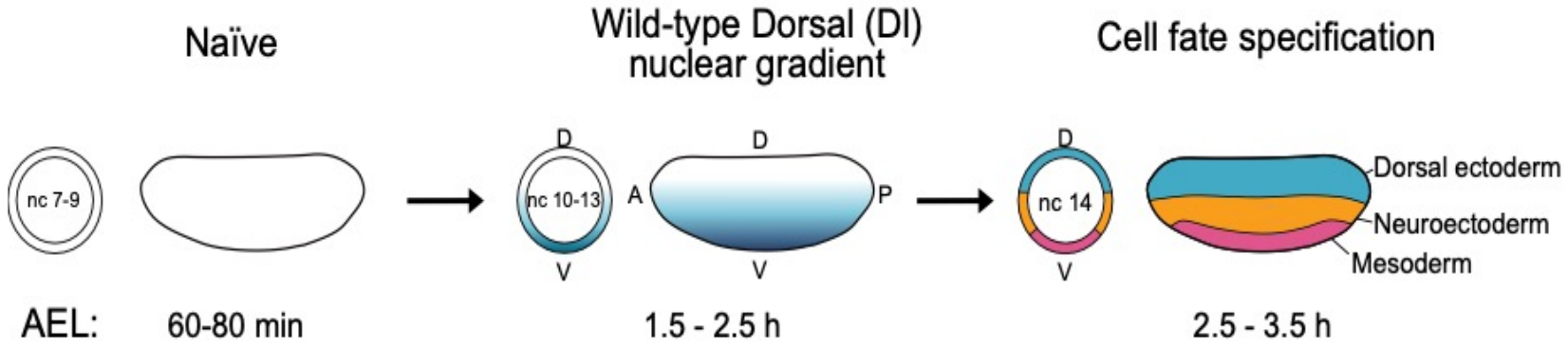


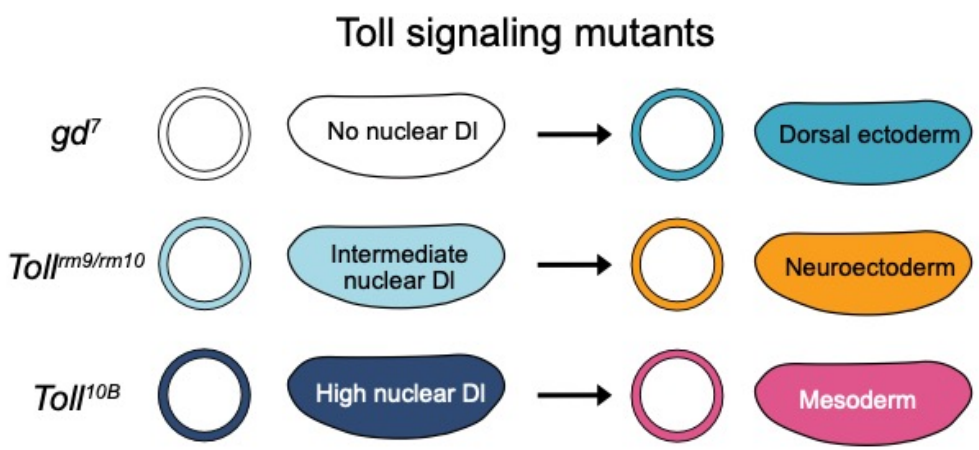
With modifications from Harrison et al, *Genetics*, 2023

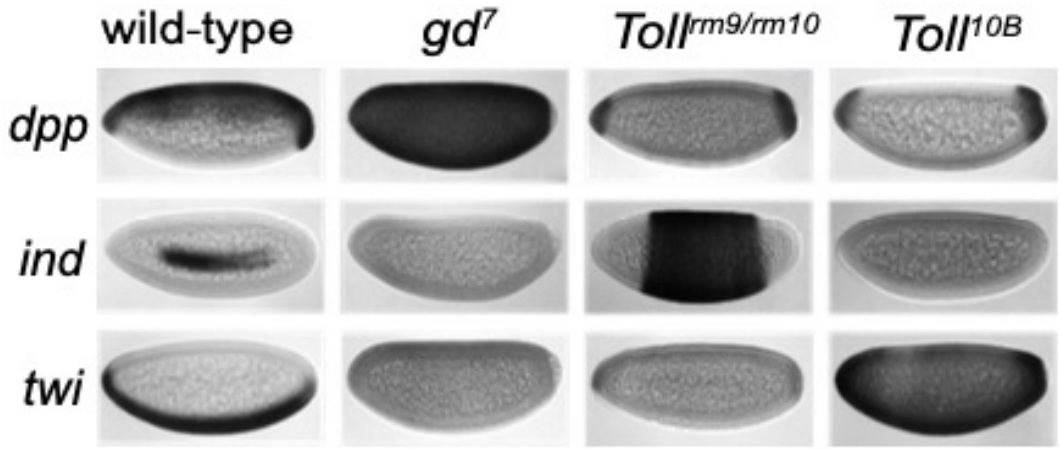
Tissue-specific RNA Polymerase II promoter-proximal pause release and burst kinetics in a Drosophila embryonic patterning network

(*George Hunt et al, Genome biology, 2024*)

Toll-signaling mutants provide cell type-specific resolution

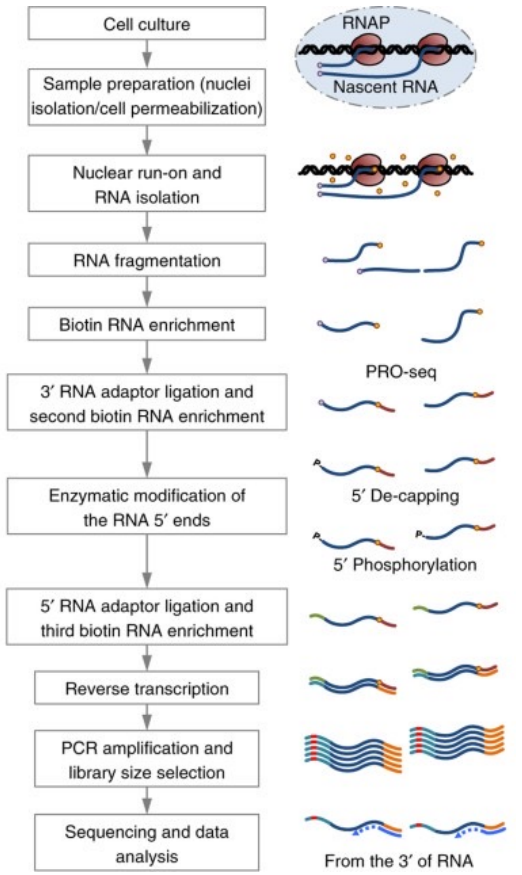


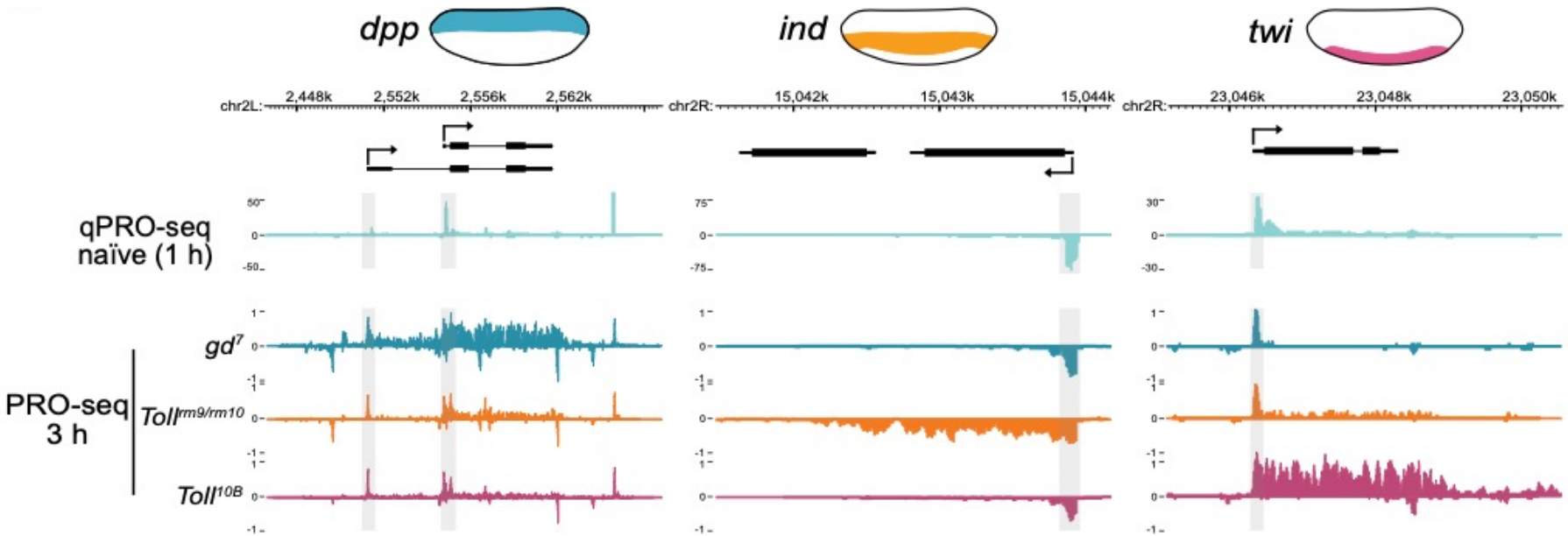




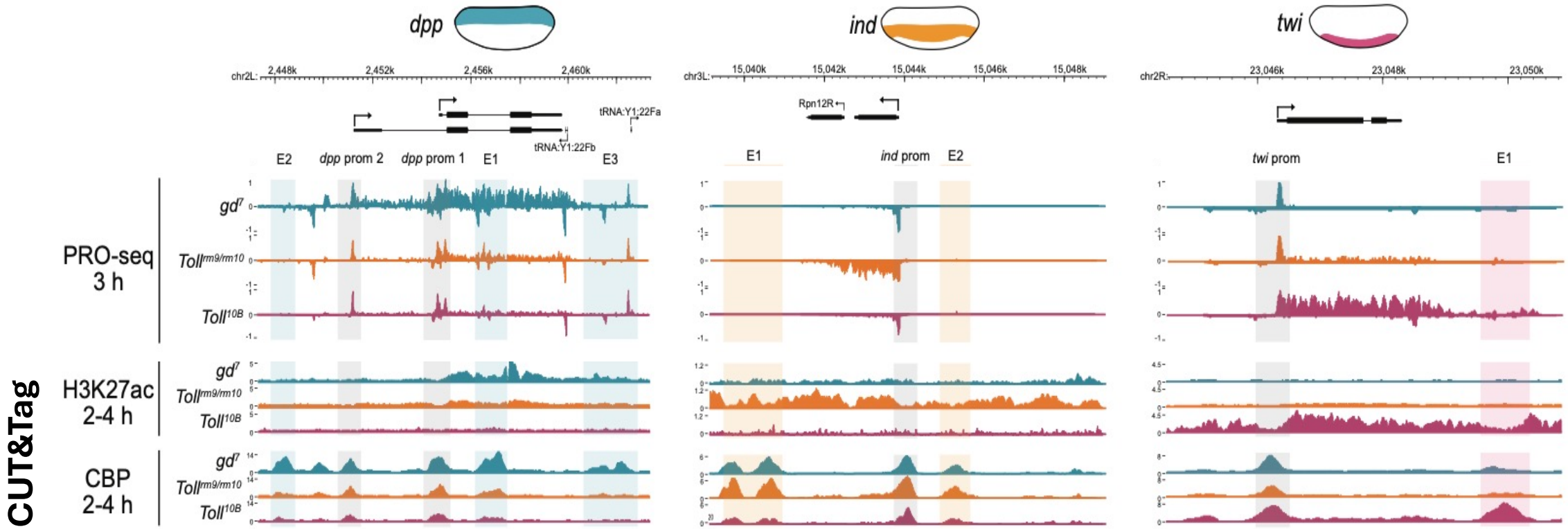
Paused Pol II is established at DV genes prior to ZGA but is released into elongation in a tissue-specific manner

qPRO-seq

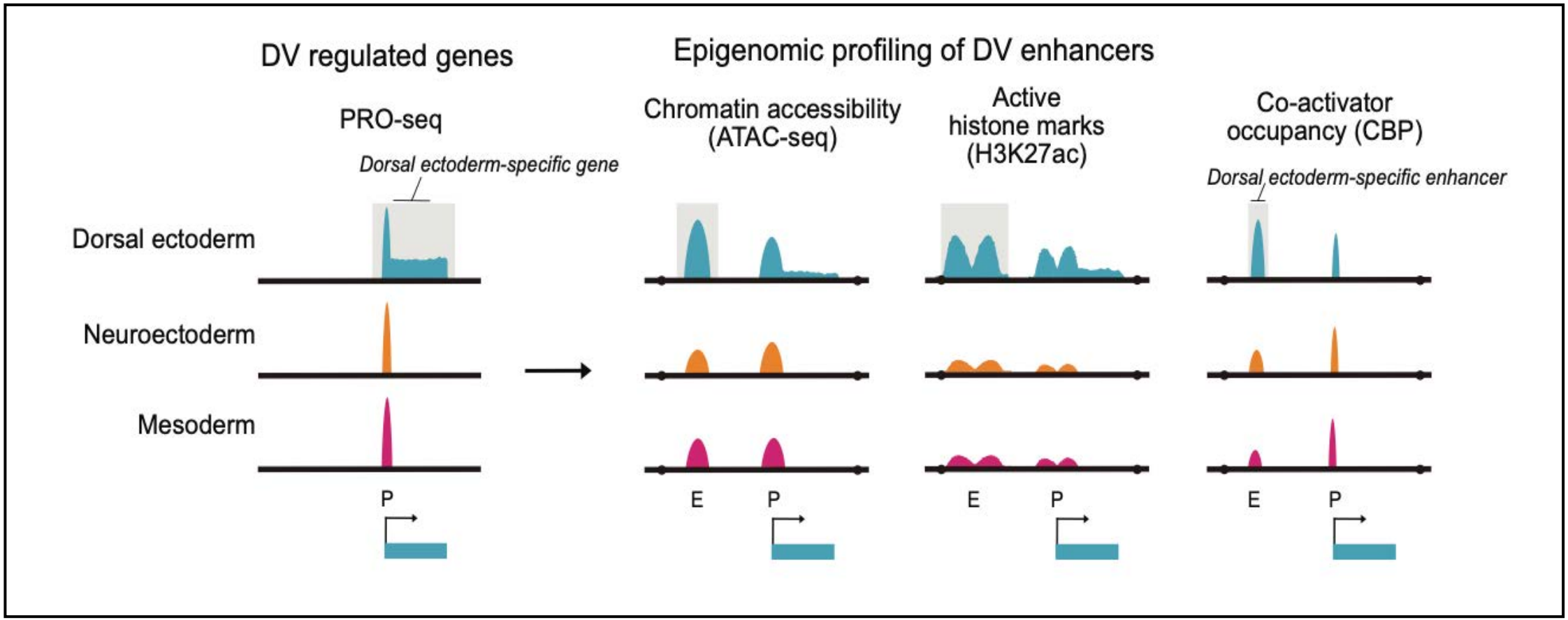




Enhancer chromatin state reflects tissue-specific DV gene transcription



Tissue-specific RNA Polymerase II promoter-proximal pause release



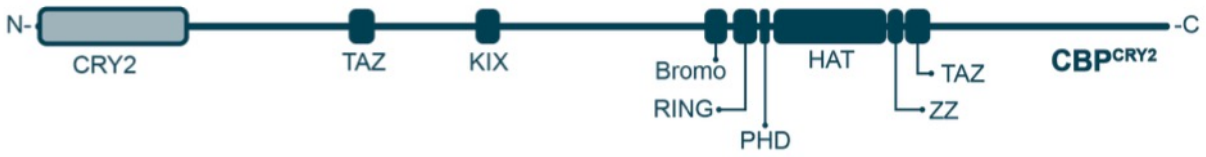
Catalytic and non-catalytic functions of CBP in zygotic genome activation

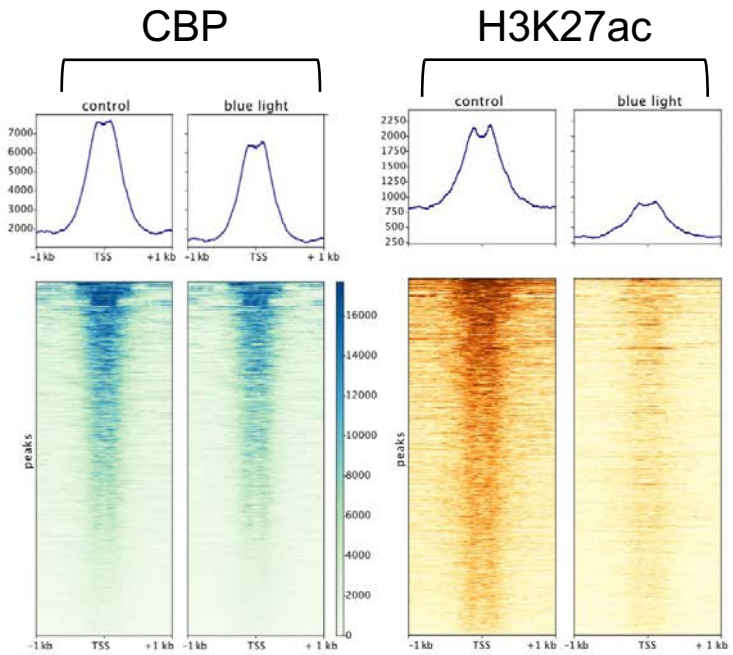
(Sergei Pirogov, *unpublished*)

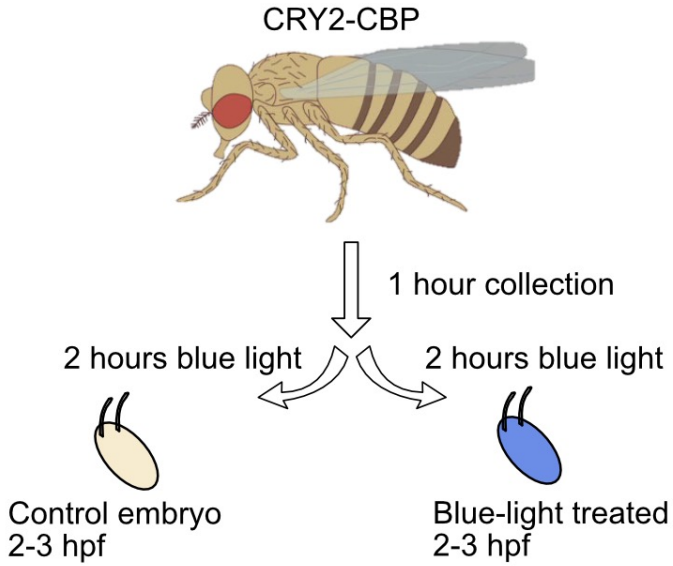
In collaboration with Audrey Marsh and Melissa Harrison, Wisconsin University, US

Catalytic function in ZGA

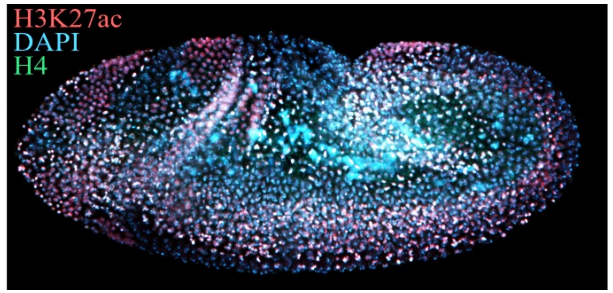
CRY2-CBP optogenetic (blue light) inactivation



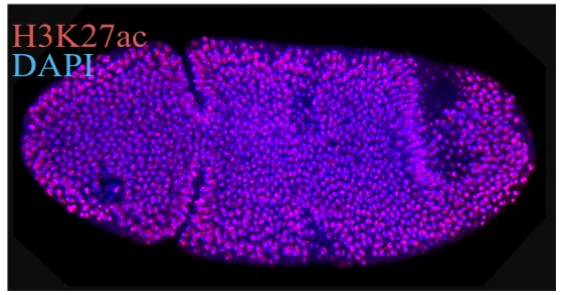




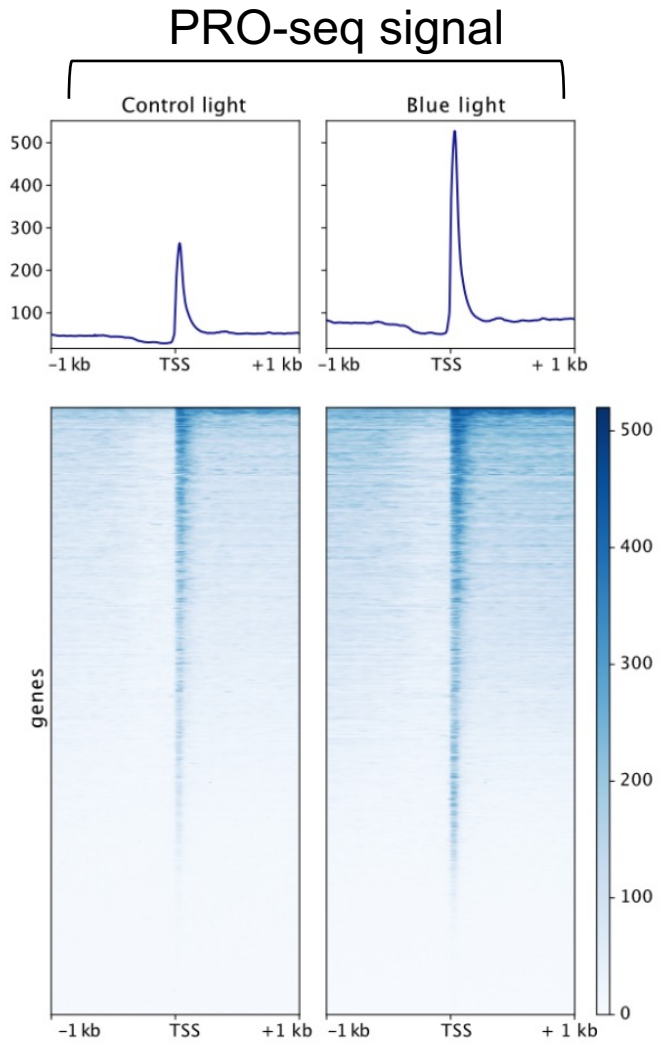
Normal gastrulation

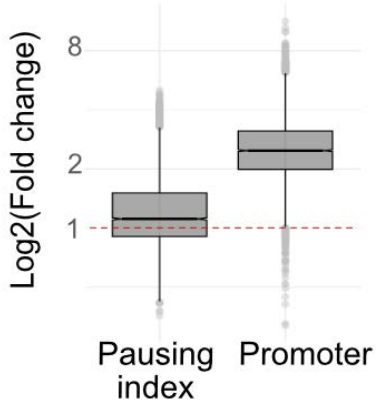


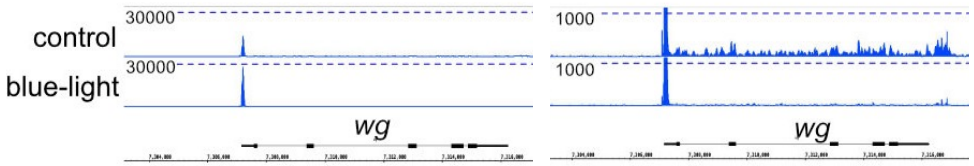
Incomplete gastrulation



Inactive CBP suppresses pause release

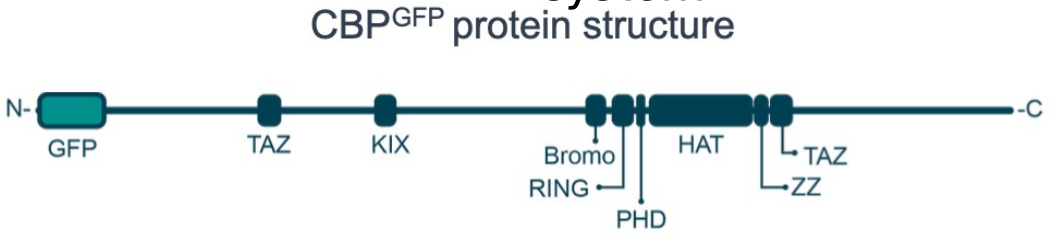


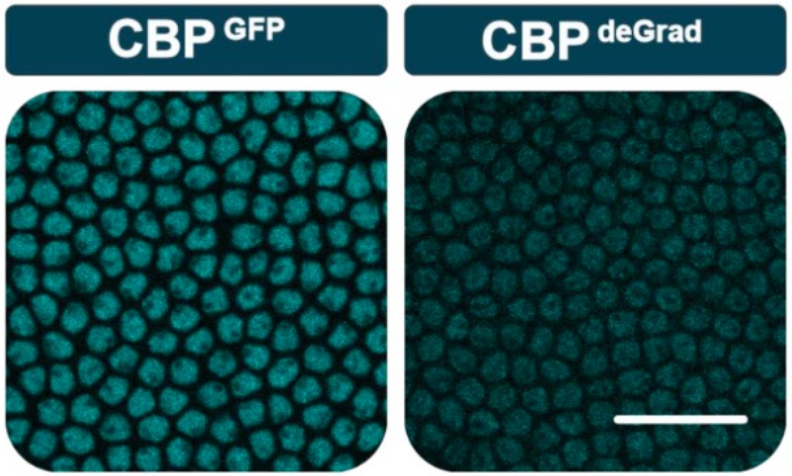


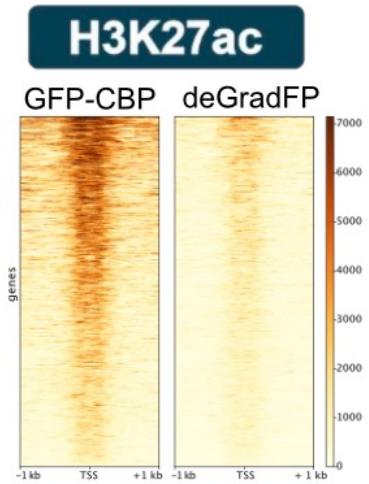


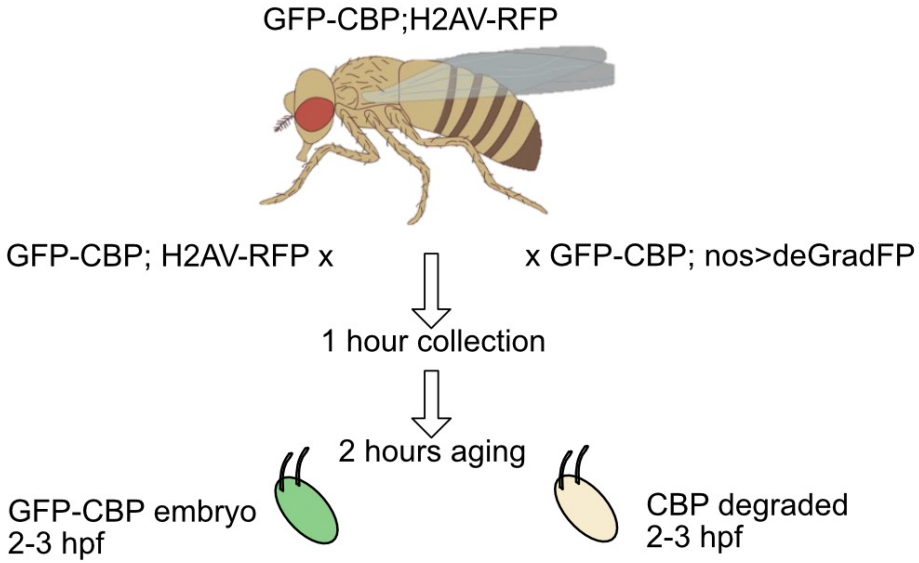
Non-catalytic function of CBP

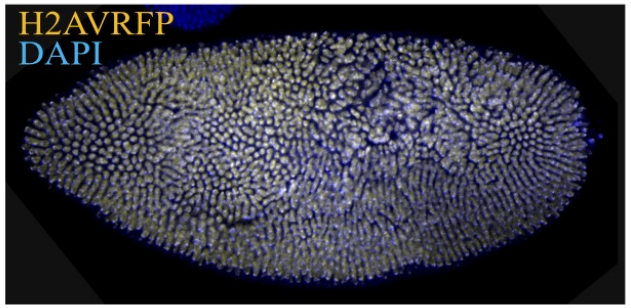
GFP-CBP removed from embryo by nos>deGradFP system



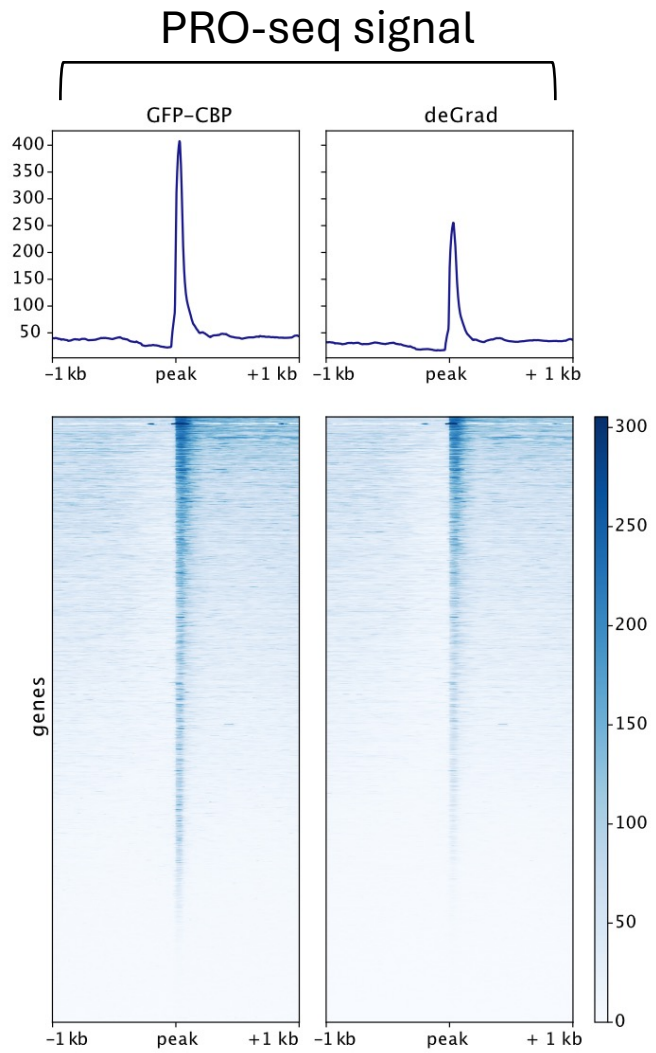


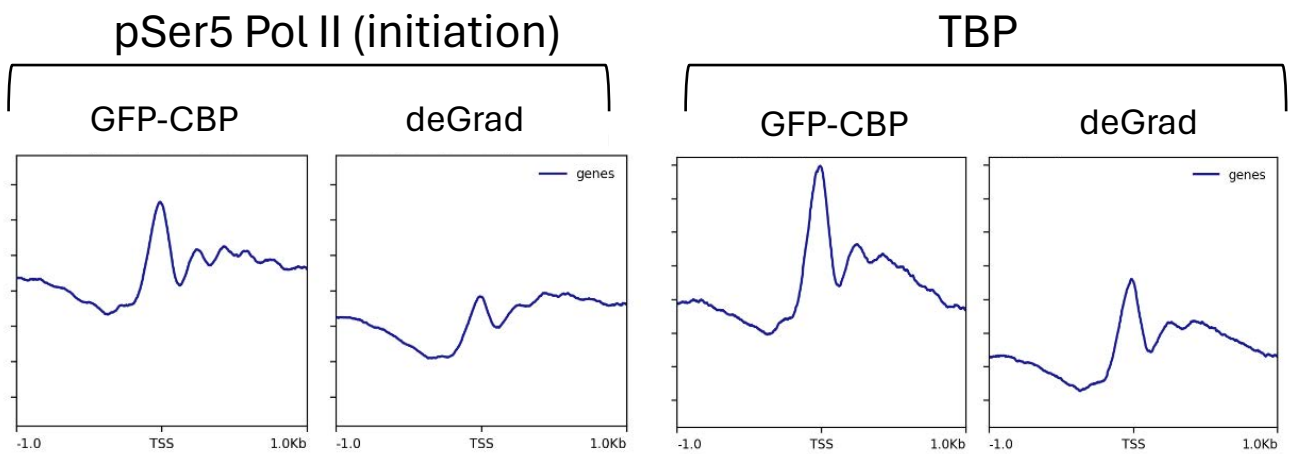




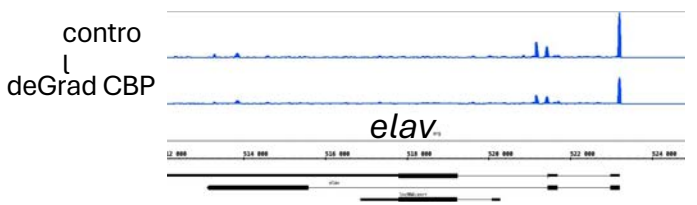


CBP depletion leads to decreased transcription initiation





CUT&Tag



Conclusions

- Catalytic activity of CBP mediates pause-release into productive elongation.

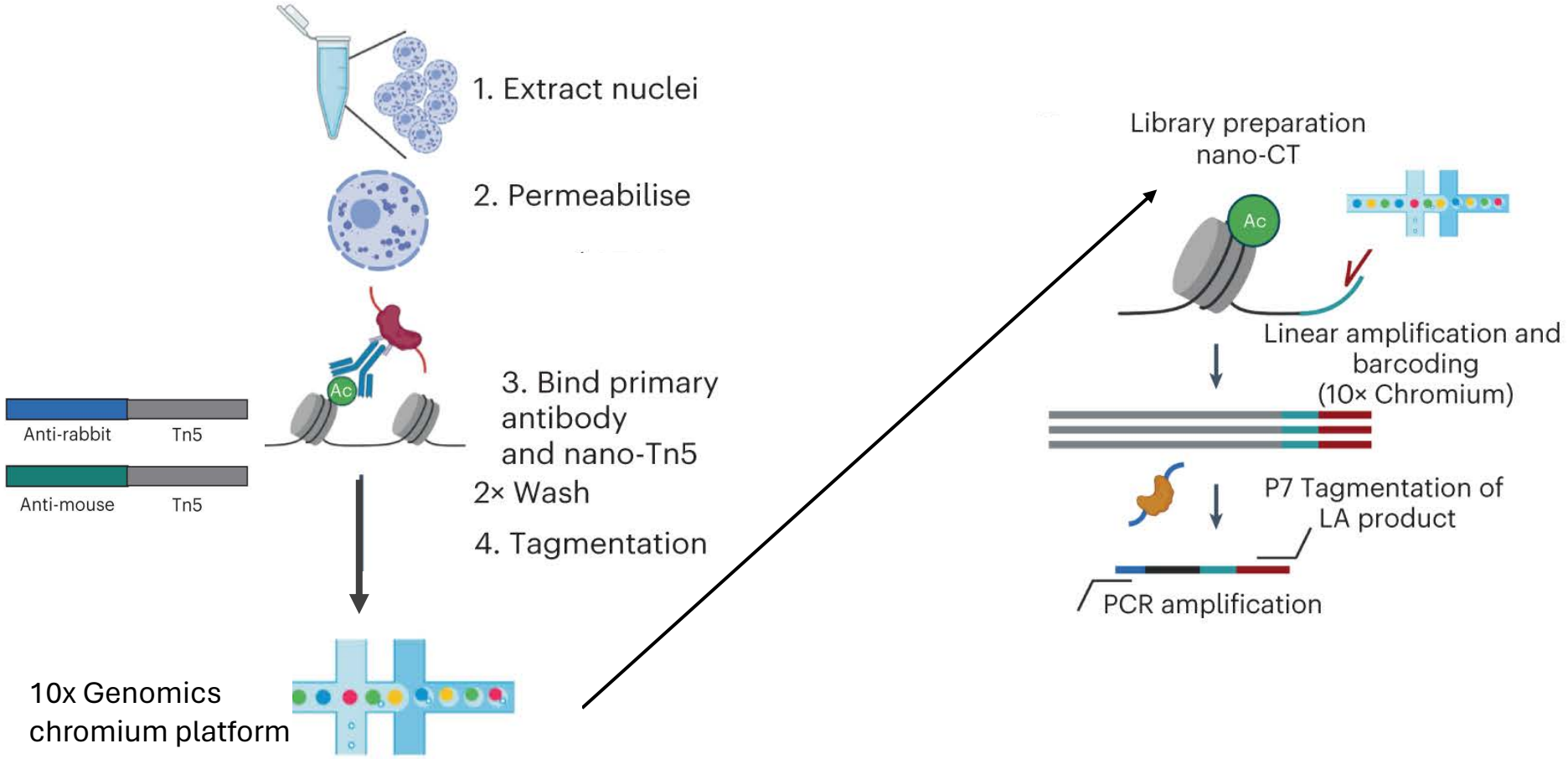
- CBP promotes transcription initiation in non-catalytic manner.

Bimodal profiling of epigenetic states through embryogenesis

(Sergei Pirogov, Aleksander Purik, *unpublished*)

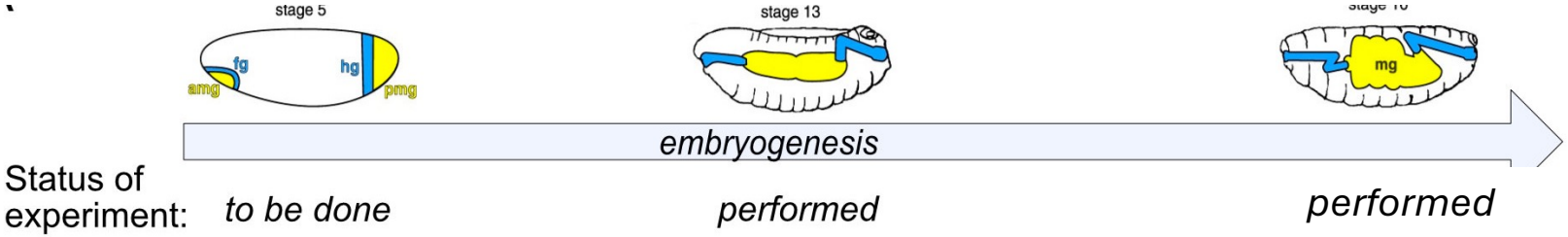
In collaboration with Marek Bartosovic, Stockholm University

Nano-CUT&Tag: the new approach for single-cell bimodal profiling of epigenome (Bartosovic and Castelo-Branco, 2023)



From Bartosovic and Castelo-Branco, 2023 with modifications

Single-cell nano-CUT&Tag on Drosophila embryo

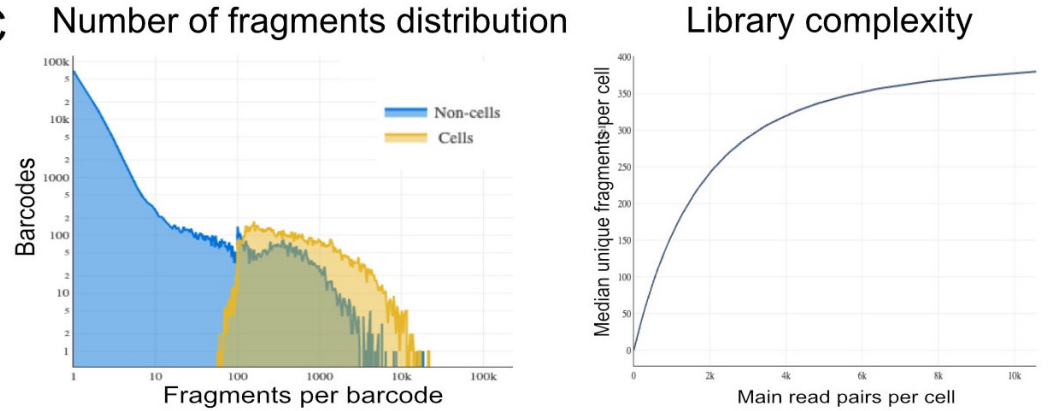


Status of experiment: *to be done* | *performed* | *performed*

9661
Estimated number of cells
380
Median high-quality fragments per cell

7680
Estimated number of cells
241
Median high-quality fragments per cell

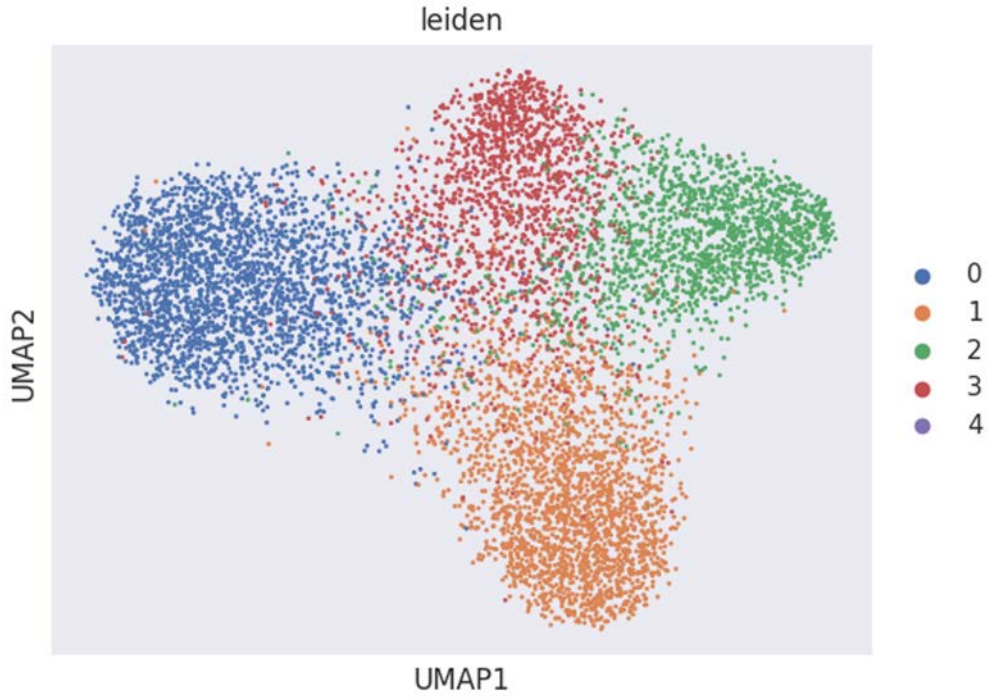
C



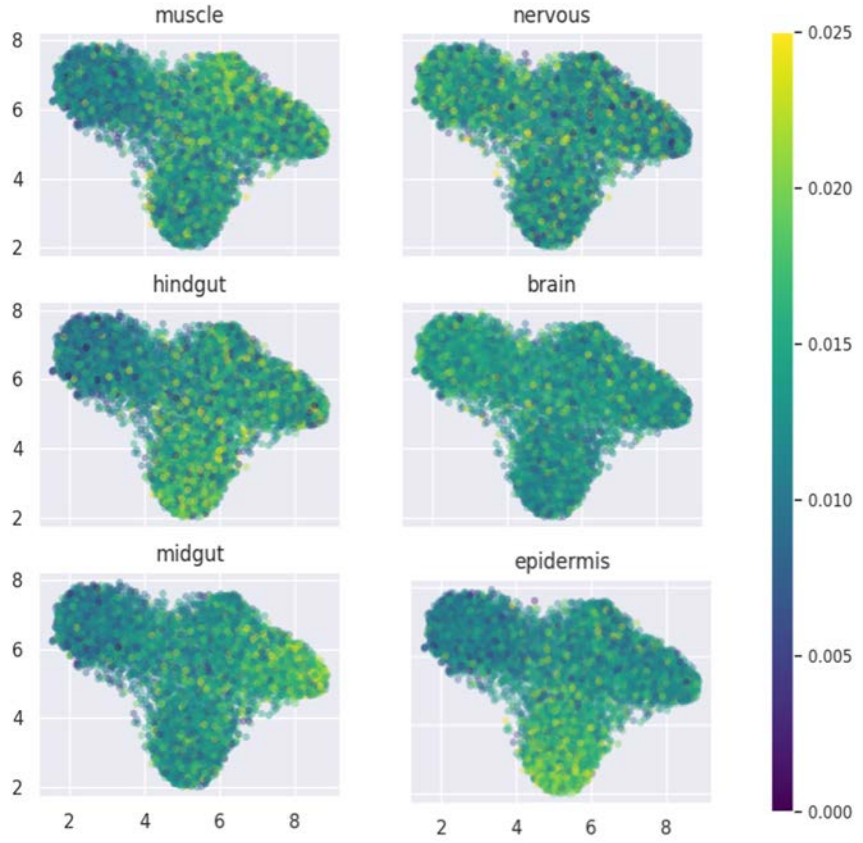
Dimensionality reduction by snapatac2 for acetylation

Dimensionality reduction on bin (5 kbp)

PCA (2:30), spectral algorithm, clusterization leiden

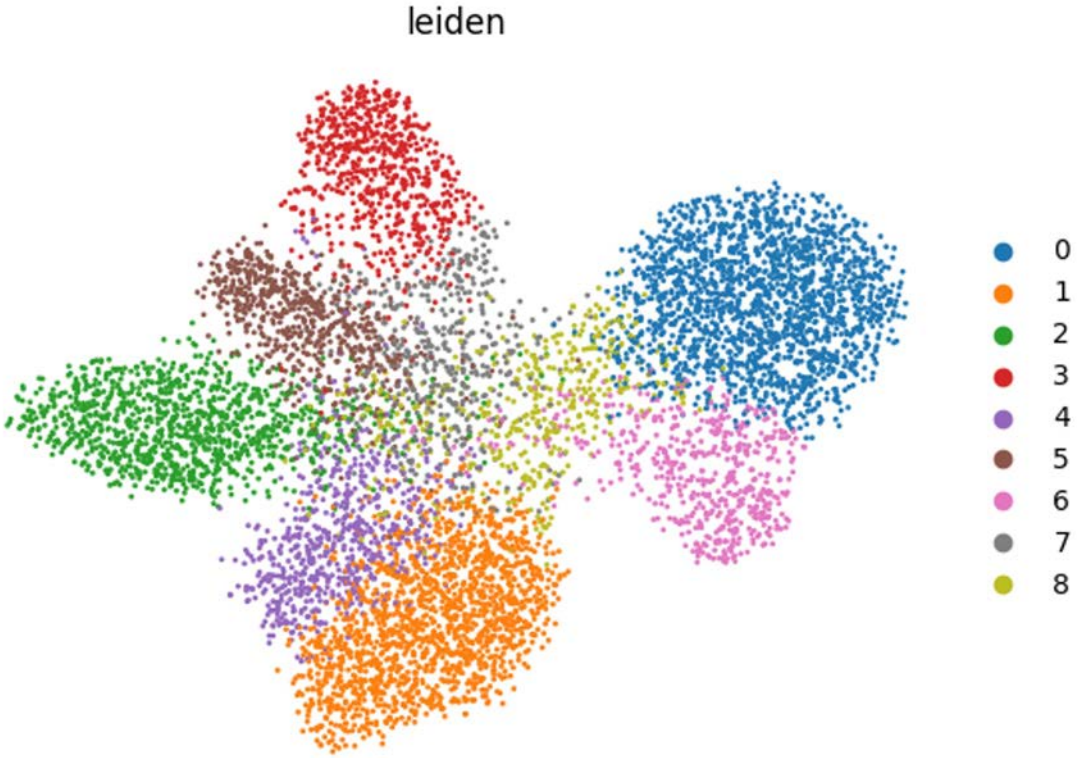


Patterns of gene expression based on *in situ* during embryogenesis (BDGP), 13-16 stages, pattern terms were parsed

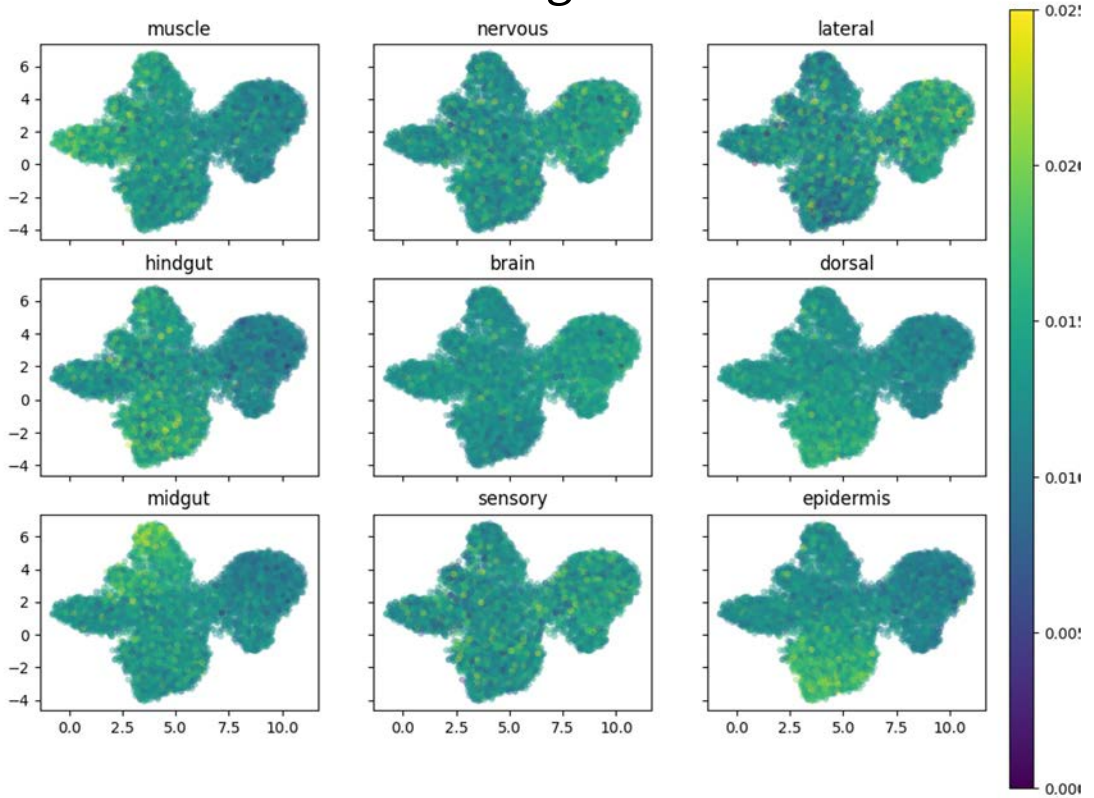


Dimensionality reduction of combined methylation and acetylation fragments

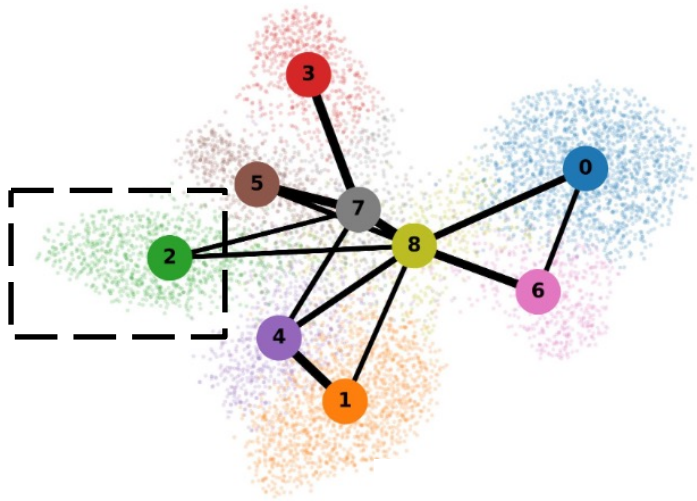
multispectral algorithm

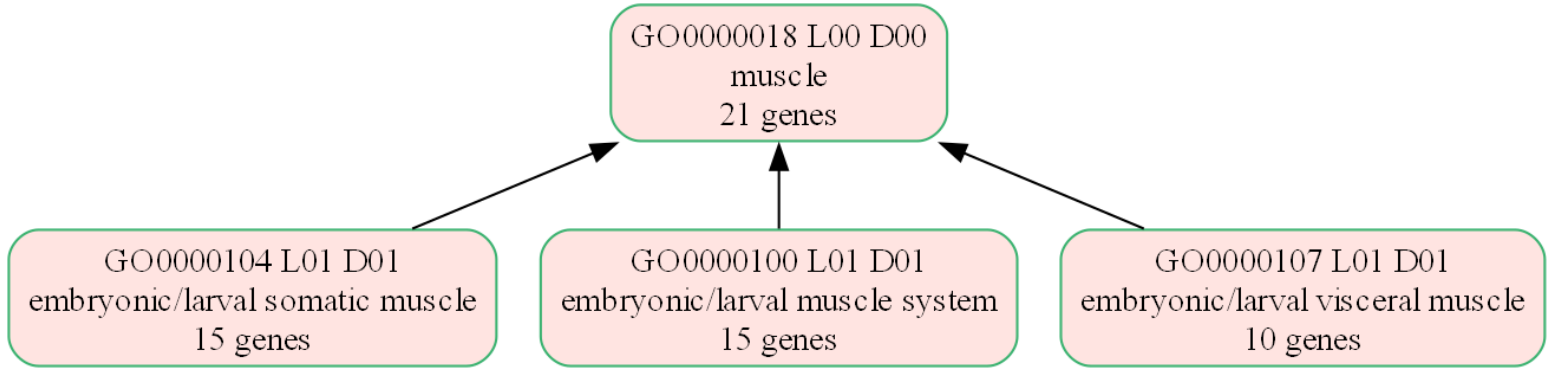


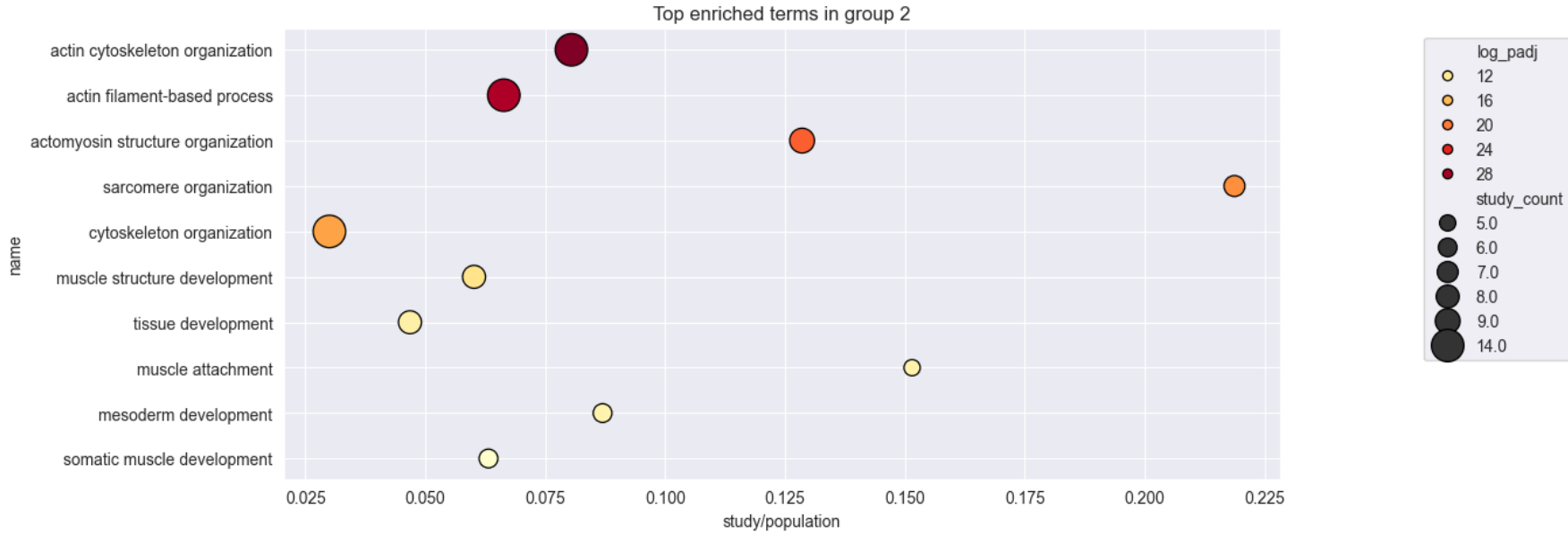
Scores based on in situ gene data



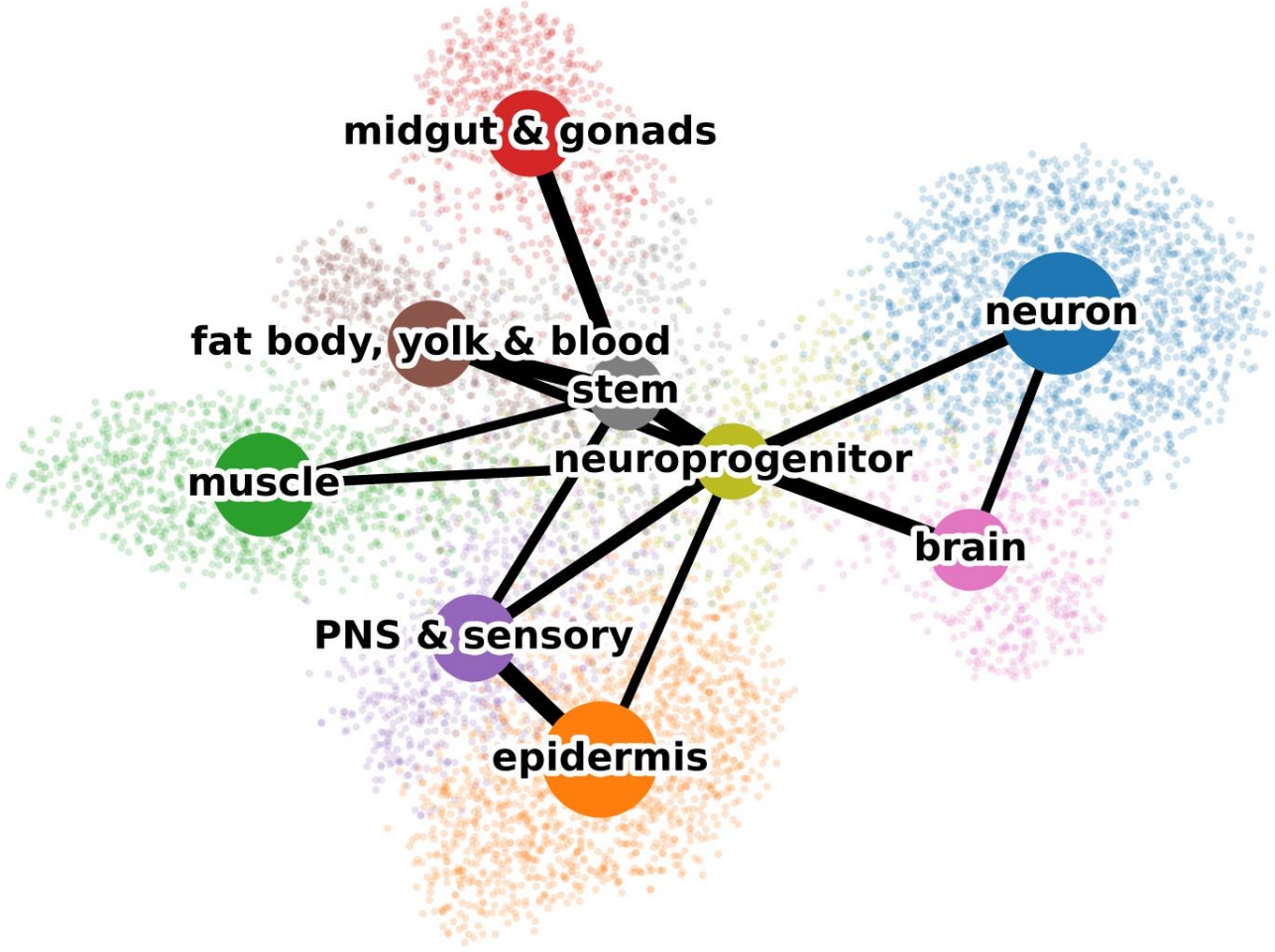
Cluster annotation based on GO-terms and *in situ* patterns





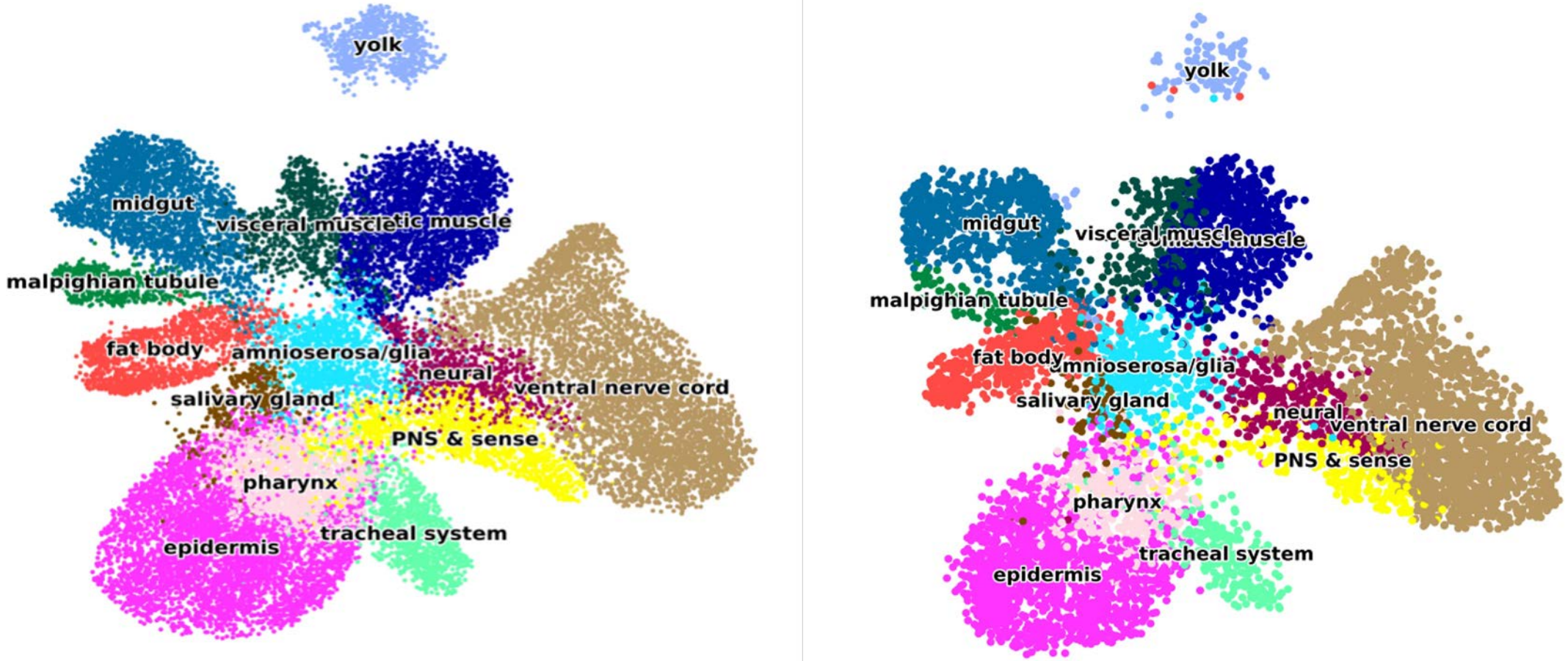


Cluster annotation based on GO-terms and *in situ* patterns



Integration of scATAC-seq with acetylation nano-CT

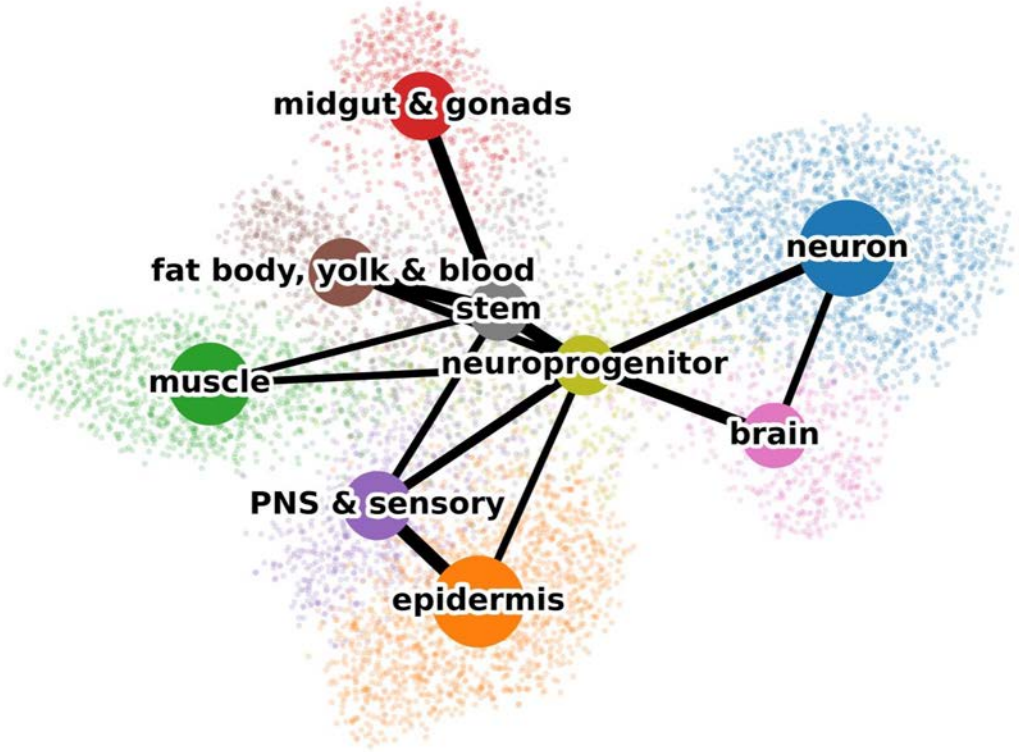
Embedding: scATAC (25000 cells) nanoCut&Tag (8000 cells)



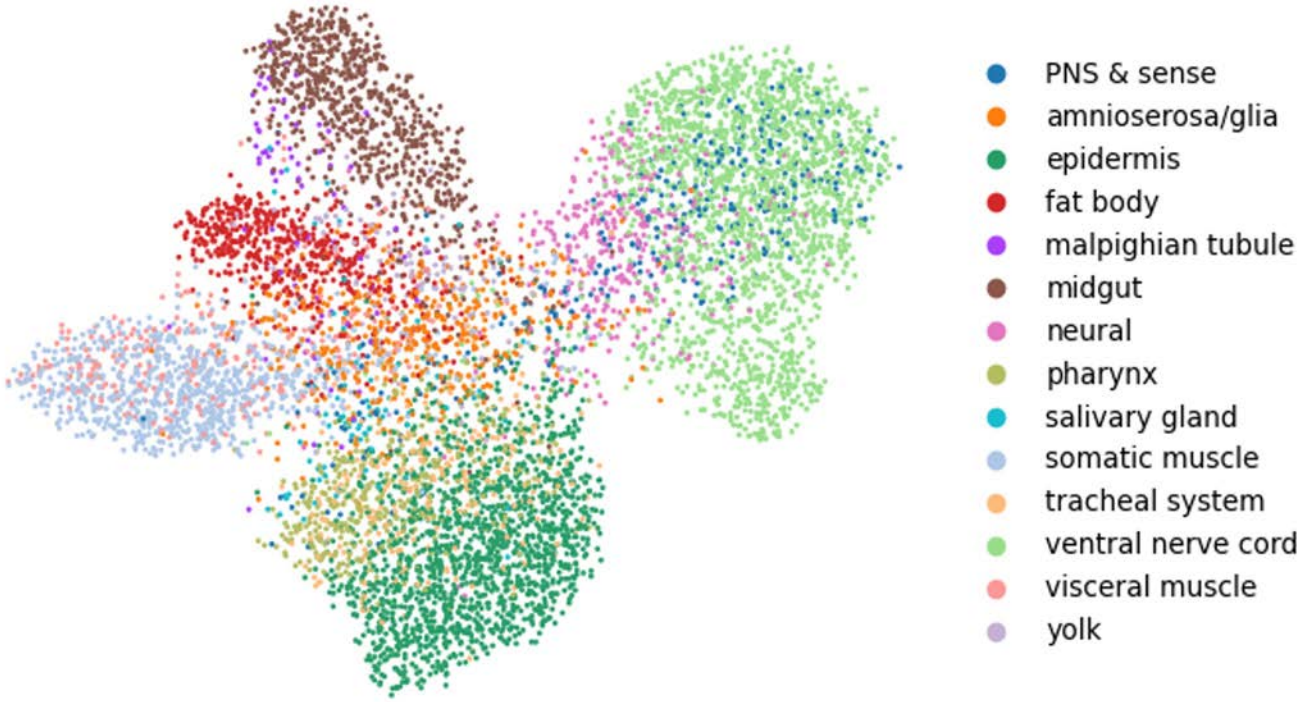
Calderon, Blecher-Gonen, Huang, Secchia,…, Furlong, Shendure, Science, 2022

Comparison of clusters annotations

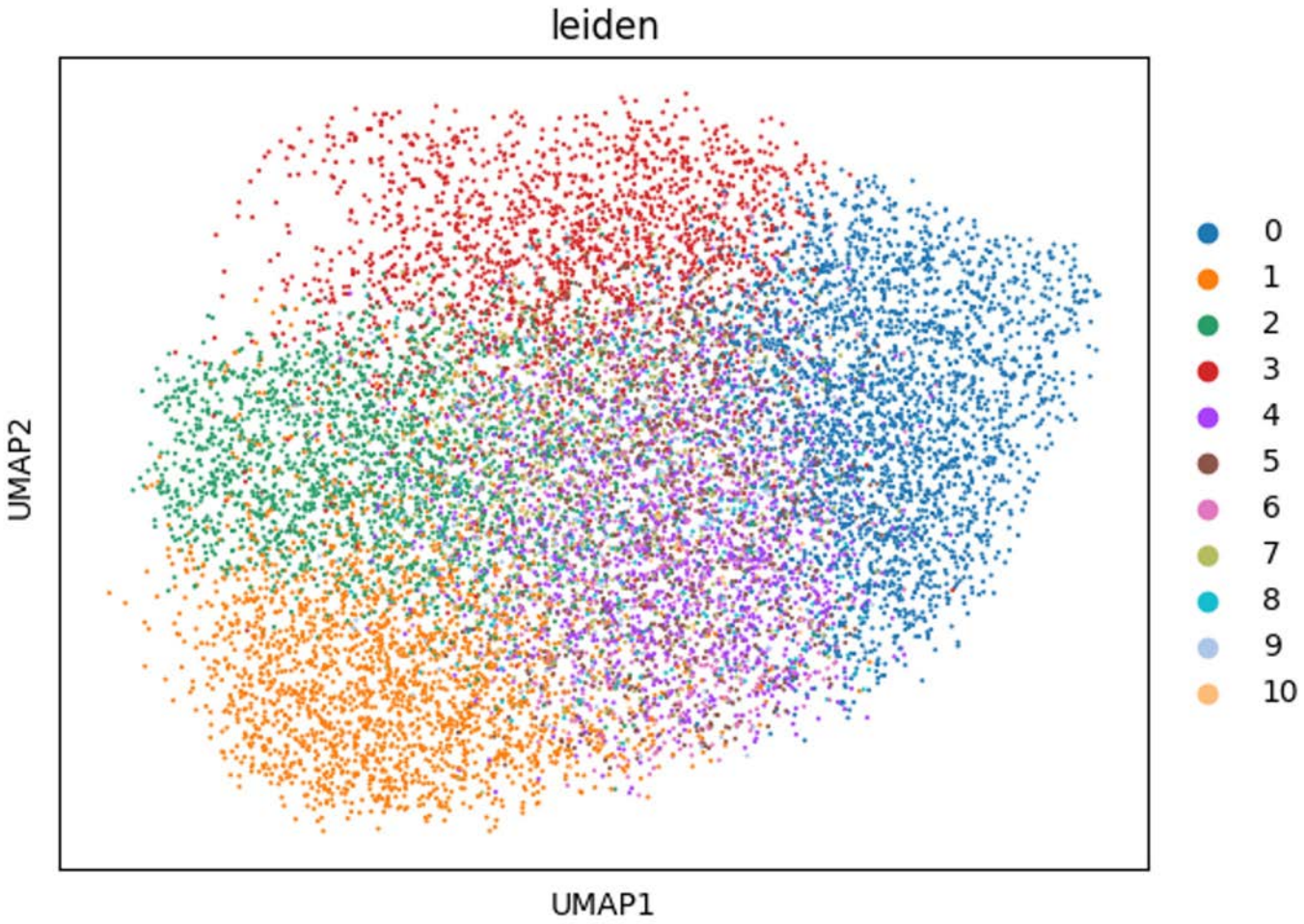
Bimodal clusterization with the combined GO-term and in situ annotation



Clusterization of integrated scATAC with nano-CT with label transfer



Early embryogenesis time point has a bad cluster resolution in bimodal nano-CT embedding

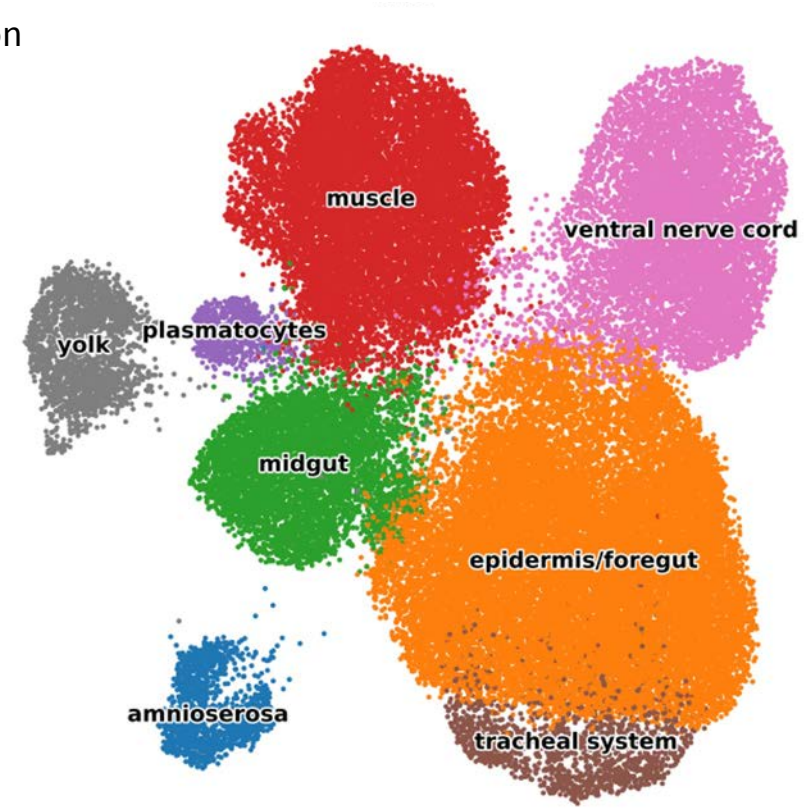


Integration with ATAC-seq significantly improves cluster resolution

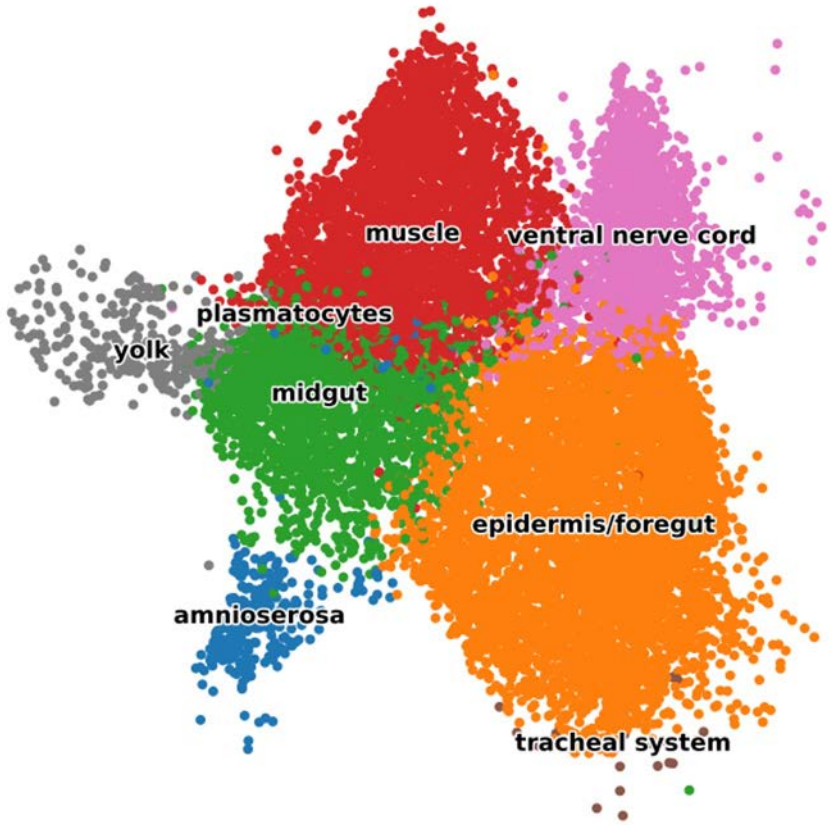
Embedding:

scATAC embedding before integration

scATAC (50’000 cells)



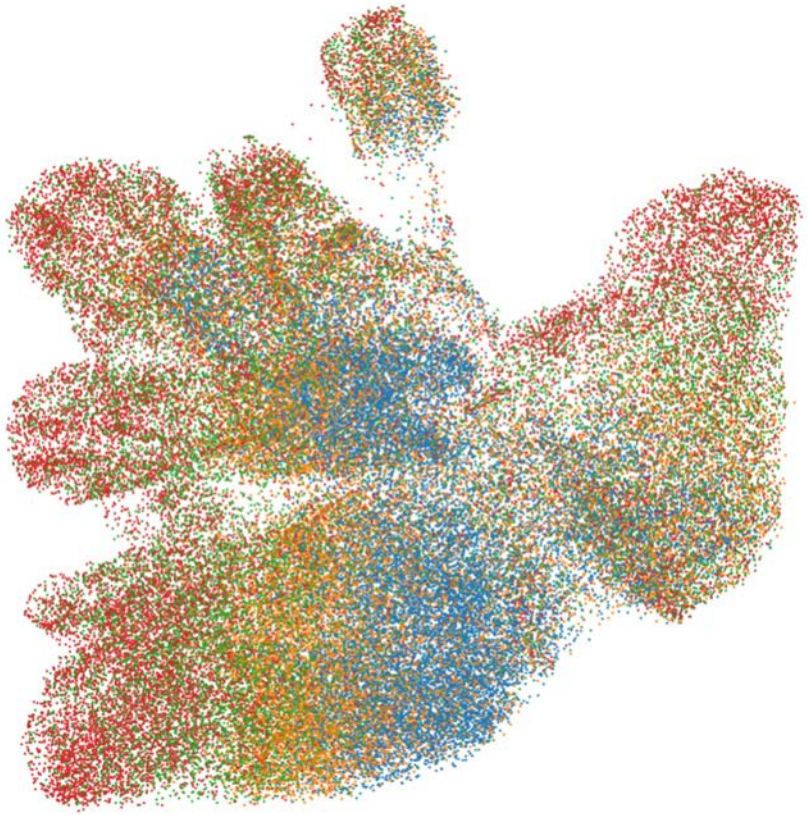
nanoCut&Tag (9000 cells)



Integration of nano-CT and scATAC of different time points

Embedding:

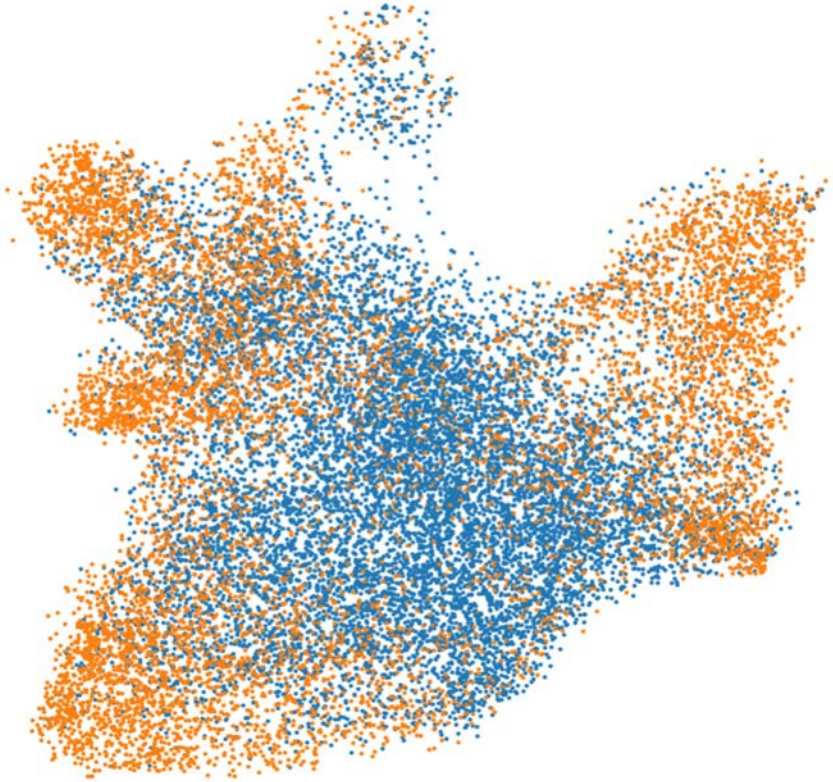
scATAC



Times:

8-10
10-12
12-14
14-16

nano CutTag

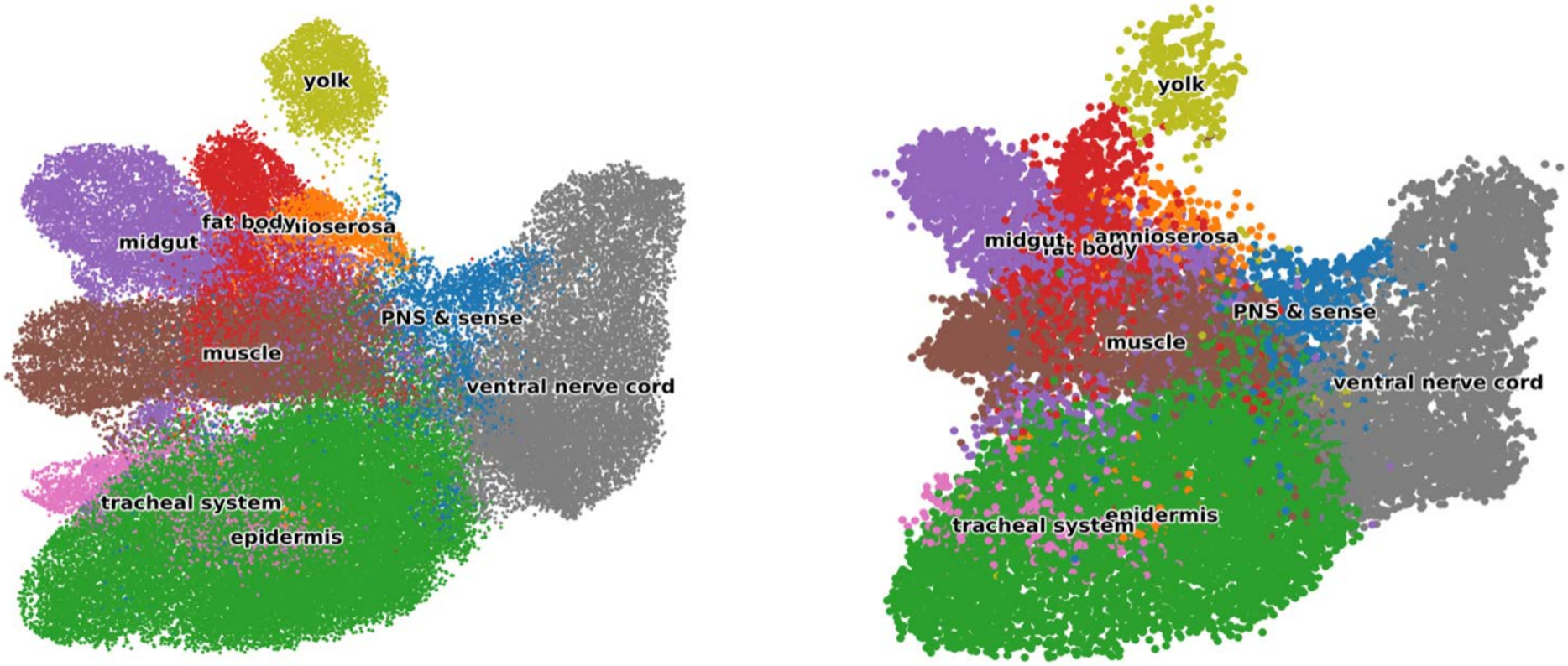


Times:

8-10
14-16

Label transfer from scATAC to nano-CUT&Tag

Embedding: scATAC nano CutTag



Aims of the study

- How repressive state is distributed in time-cell space
- Do cell move from more repressive state to more permissive in their trajectories
- Is chromatin accessibility precedes acetylation
- Is chromatin accessibility more permissive than acetylation
- What is the distribution of the “void” state
- How much chromatin accessibility and acetylation are predictive for gene expression

Acknowledgements

Mattias Mannervik

Sergei Pirogov, Ph.D. student
Aleksander Purik
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Vetenskapsrådet

CANCERFONDEN

Thank you for attention!

p300/CBP sustains Polycomb silencing by non-enzymatic functions

George Hunt, Ann Boija & Mattias Mannervik, Mol Cell, 2022