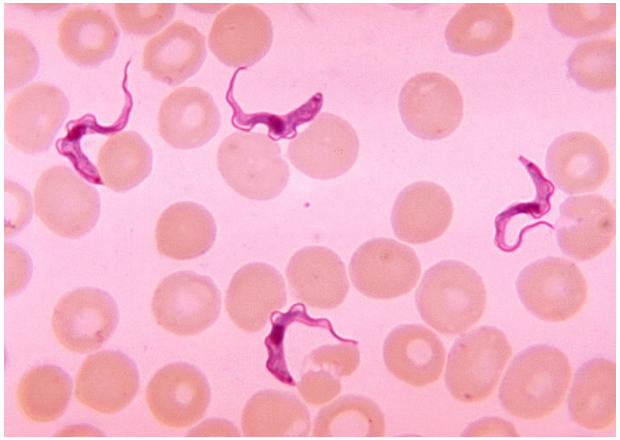
HAT Complex Protein **EAF6:** Role in Lifecycle Differentiation of *Trypanosoma brucei*

Fumi Tanizawa

BIO111 Fall2023 Final Presentation

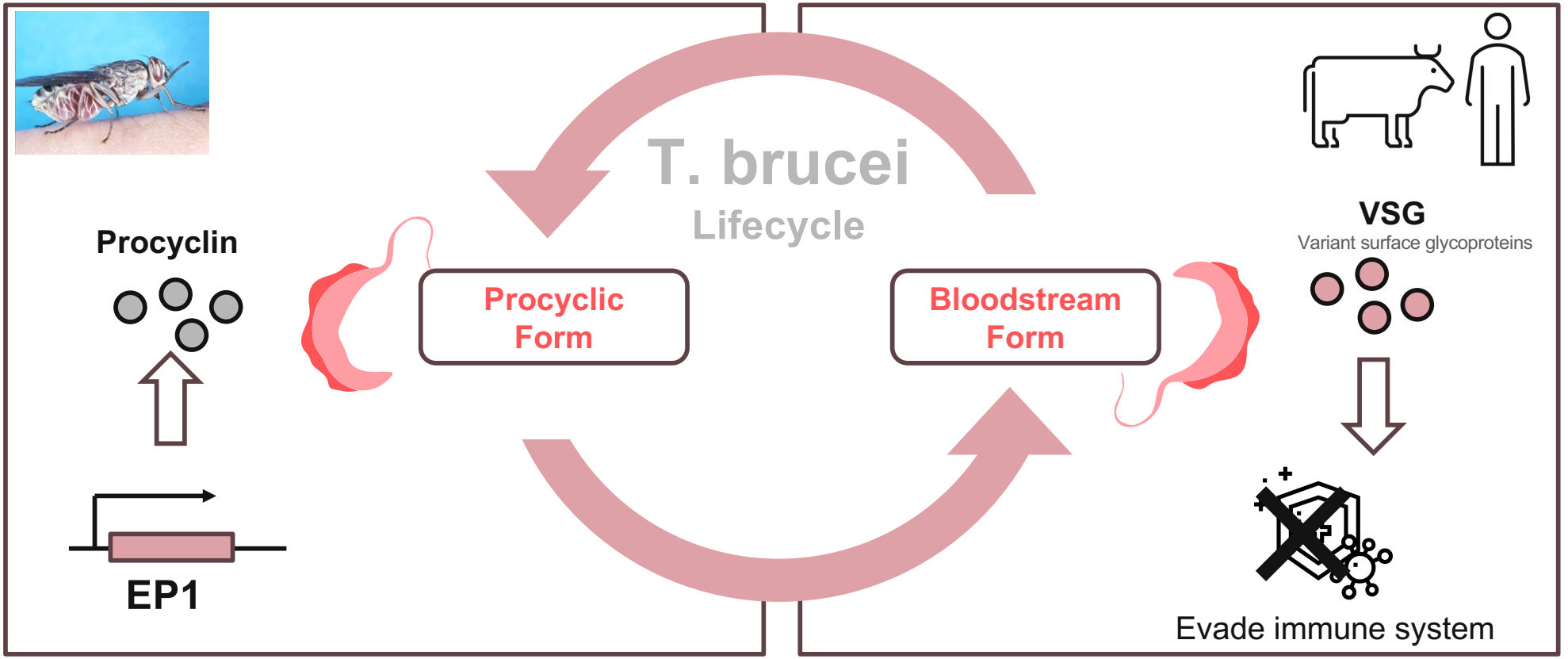
African trypanosomiasis

"Sleeping sickness" by a vector-borne parasitic disease

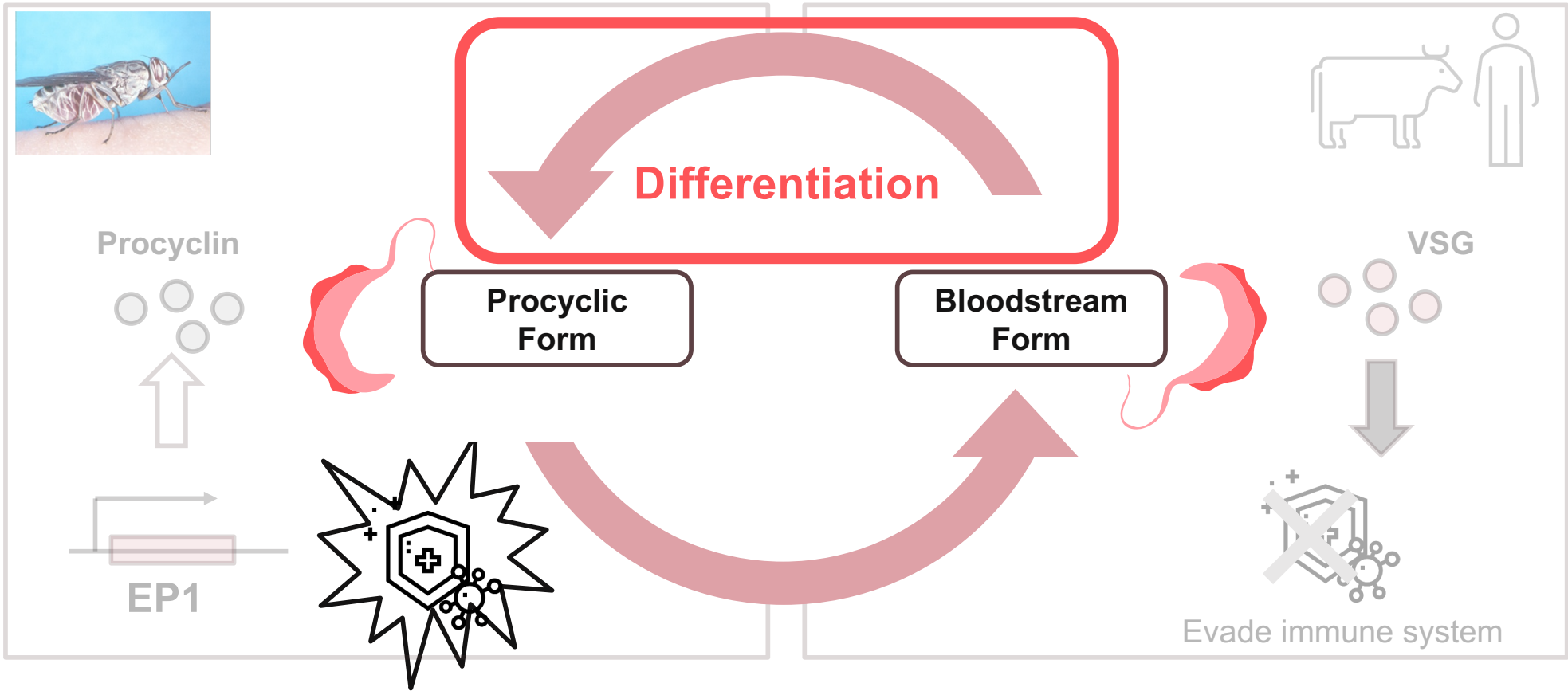


(PC from left: Wikipedia, CDC, Africa Health Organization)

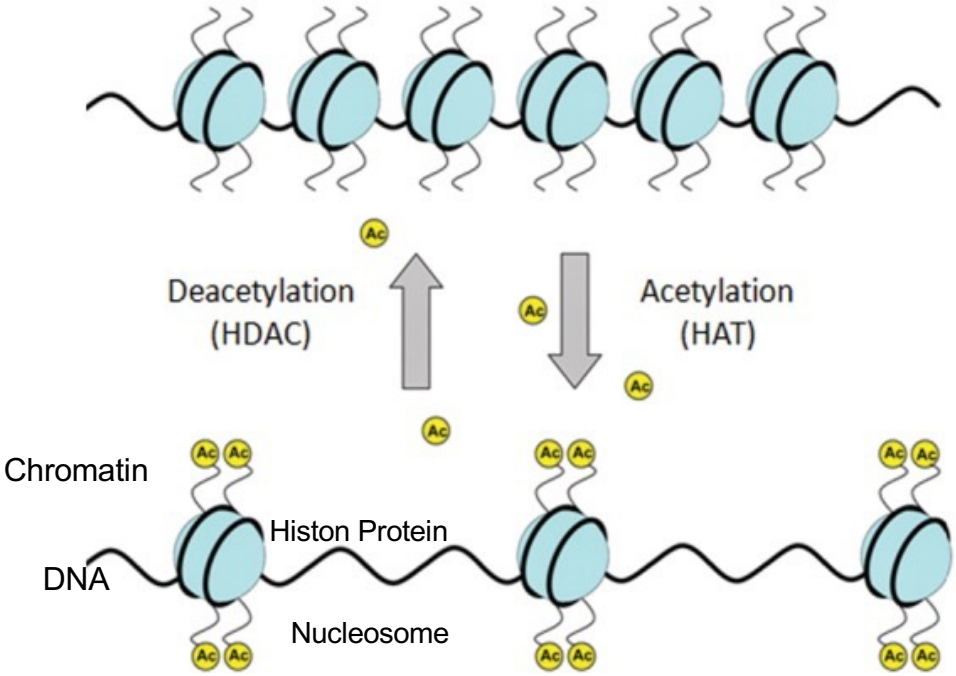
Background


T. brucei
Lifecycle
Procyclin
EP1
Procyclic Form
Bloodstream Form
VSG
Variant surface glycoproteins
Evade immune system

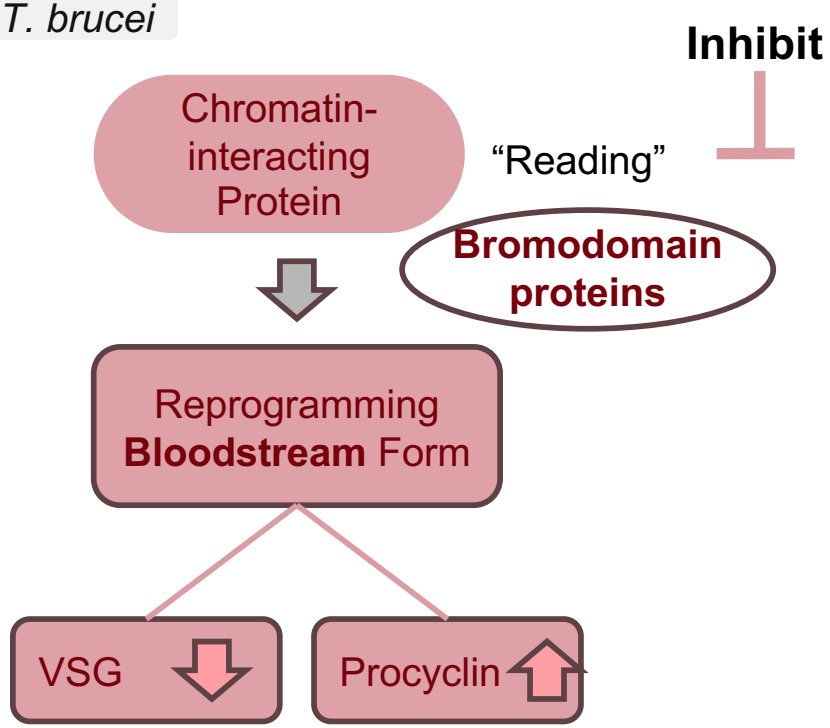
Motivation


Differentiation
Procyclic Form
Bloodstream Form
Procyclin
EP1
VSG
Evade immune system

Chromatin-Interacting Protein



T. brucei

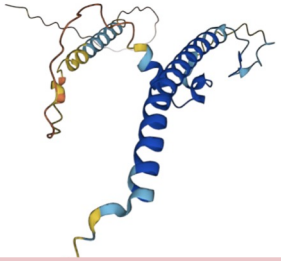


(Schulz et al. 2015)

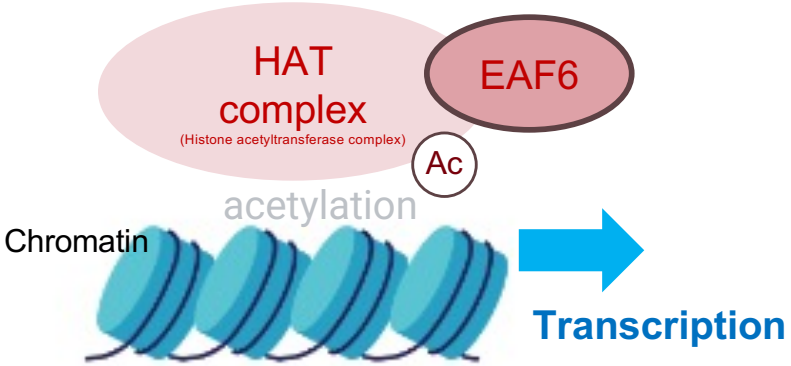
EAF6

(Tb927.9.2910)

"Writing"

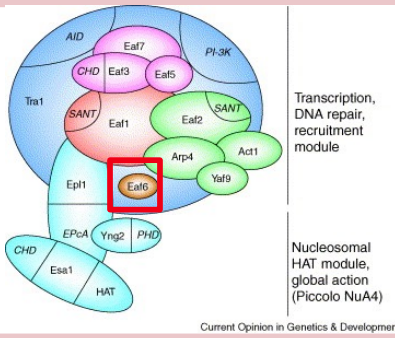


Non-catalytic component



Human, Yeast

NuA4 HAT complex



(Doyon & Cote 2004)

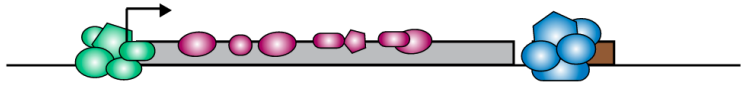
T. brucei

Class I TSR factors

CRD1
SET27

NuA4-related complex

BDF1
BDF4



BDF3 BDF5
HAT2

ZCW1
SET26

BDF2
HDAC3

HDAC1

BDF6
HAT1
EAF6

BDF7
ELP3b
PDH2
PDH4
DOT1A
TBP
TFIIS2-2
TRF

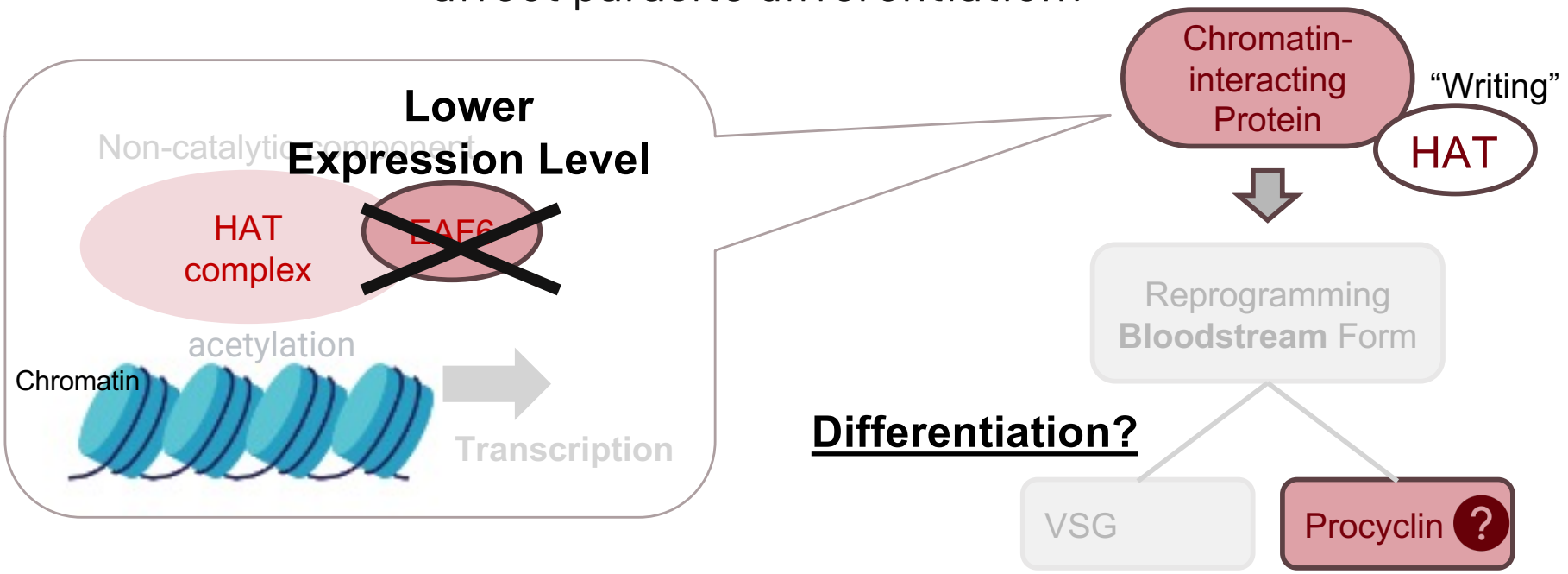
Class II TSR factors

TTR factors

(Staneva et al. 2021)

Question

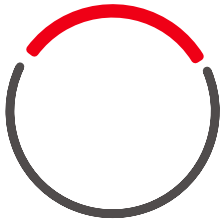
Does Knocking down a part of HAT complex (**EAF6**)
affect parasite differentiation?



Method

Cloning

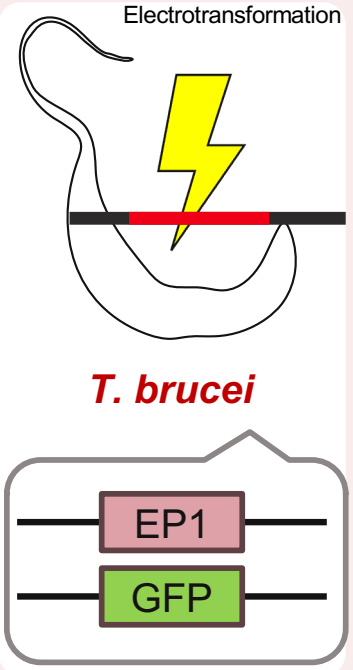
Part of EAF6



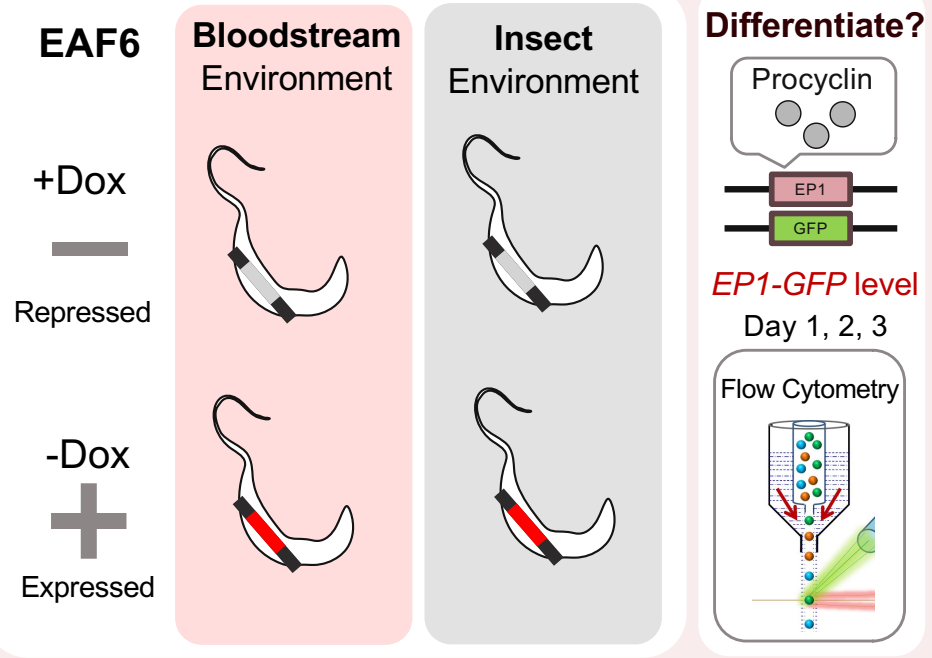
RNAi vector

- Bacteria Heatshock
- Screening & Apmlify
- PCR verification
- Midprep

Transformation



Differentiation Experiment

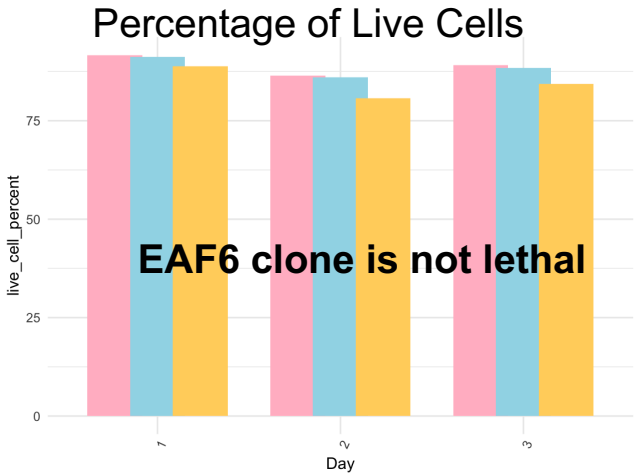


Results

The **following slides** show extracted data of **live cells** for

- EAF6 Clone with Dox
- EAF6 Clone no Dox
- Parent(WT) with Dox (≈ no Dox)

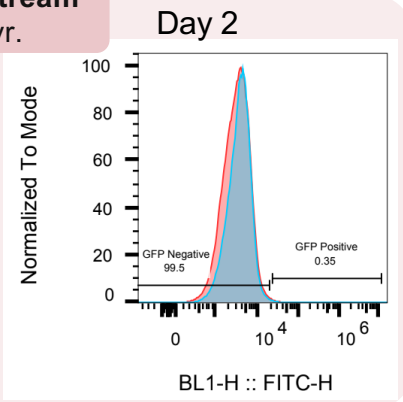
Measurement timing is different!
No quantitative comparison; qualitative only



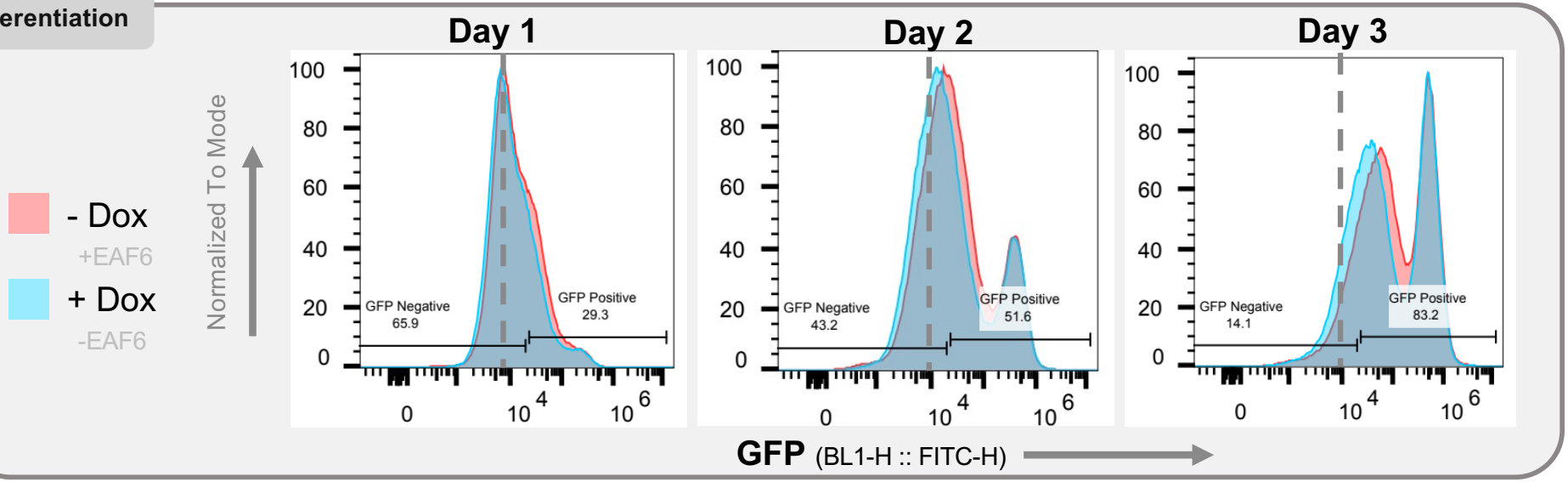
EAF6 Clone

No significant difference in differentiation between **+Dox** and **-Dox**

Bloodstream Envr.



Differentiation



EAF6 Clone

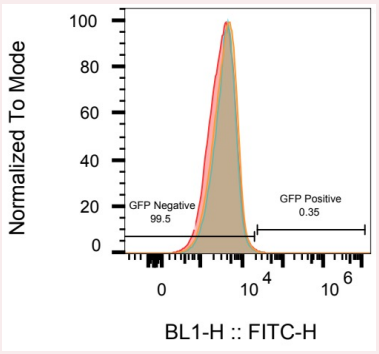
No significant difference in differentiation of **+Dox** and **-Dox**

Both +Dox and -Dox are **slower differentiation than parent**

Slight difference in differentiation of +Dox and -Dox

Bloodstream Envr.

Day 2

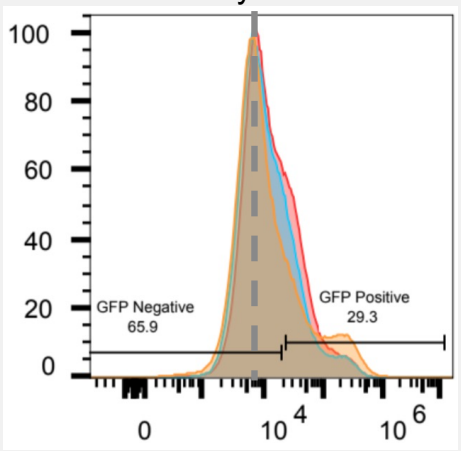


Differentiation

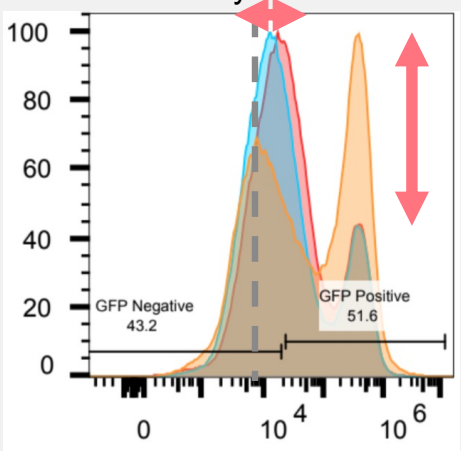
- Dox
+EAF6
+ Dox
-EAF6
WT + Dox

Normalized To Mode

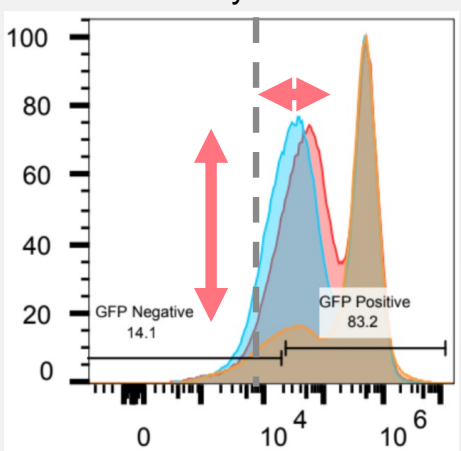
Day 1



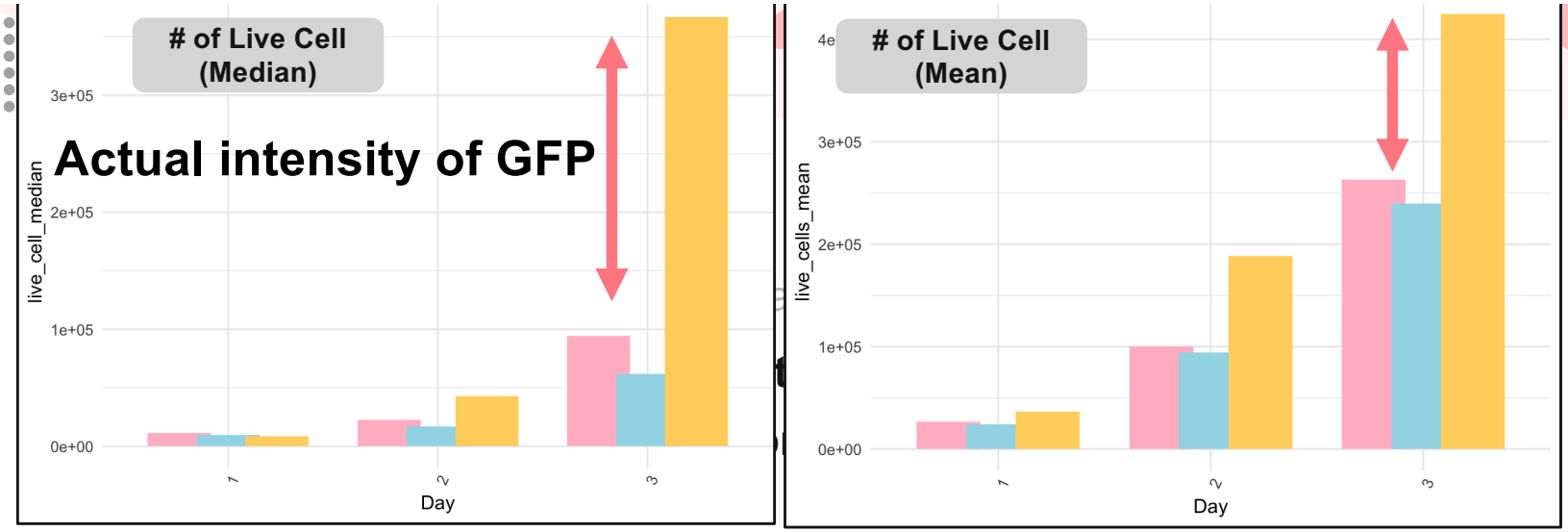
Day 2

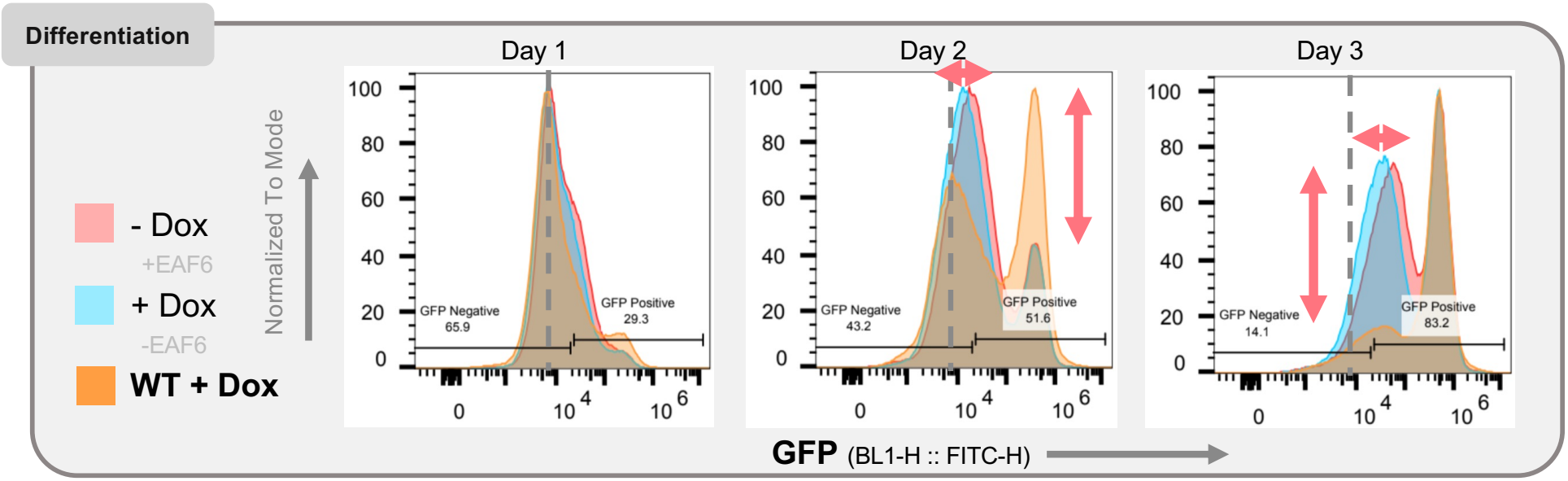


Day 3



GFP (BL1-H :: FITC-H)


# of Live Cell (Median)
Actual intensity of GFP
live_cell_median
3e+05
2e+05
1e+05
0e+00
1
2
3
Day
# of Live Cell (Mean)
live_cells_mean
4e
3e+05
2e+05
1e+05
0e+00
1
2
3
Day


Differentiation
- Dox
+EAF6
+ Dox
-EAF6
WT + Dox
Normalized To Mode
Day 1
GFP Negative
65.9
GFP Positive
29.3
Day 2
GFP Negative
43.2
GFP Positive
51.6
Day 3
GFP Negative
14.1
GFP Positive
83.2
100
80
60
40
20
0
0
10 4
10 6
GFP (BL1-H :: FITC-H)

EAF6 Clone

No significant difference in differentiation of **+Dox** and **-Dox**

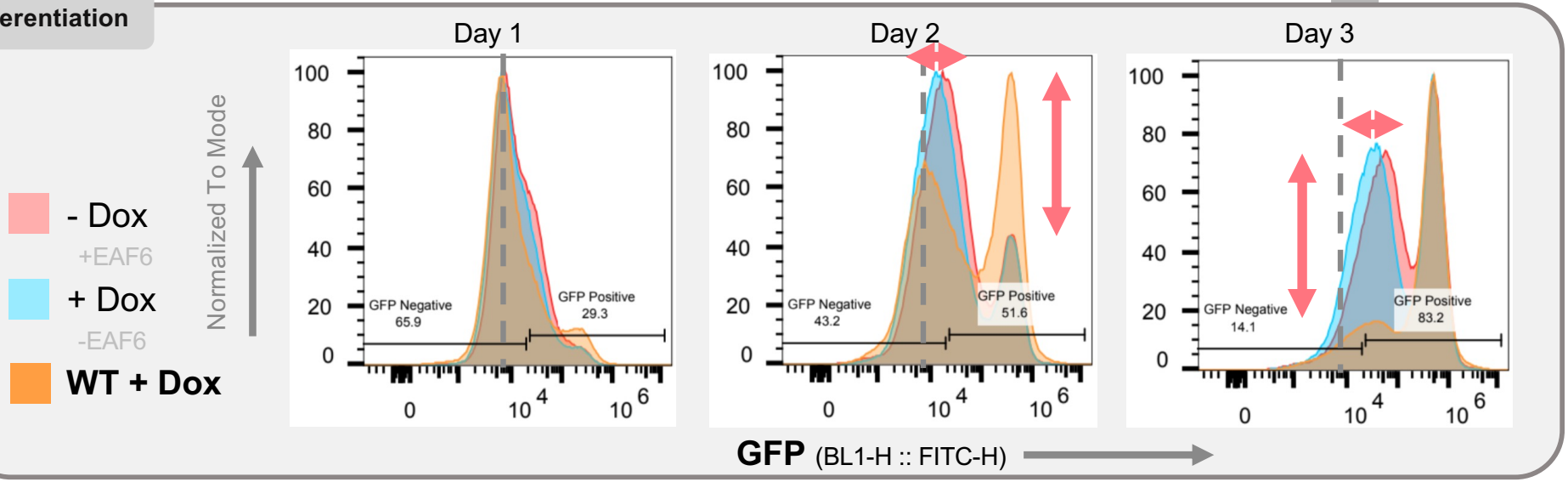
Both +Dox and -Dox are **slower than parent**

Slight difference in differentiation of +Dox and -Dox

Result

EAF6 has an **impact**, but our clone has an **error** in RNAi system

Differentiation



Discussion

① Experiment

② Role of EAF6

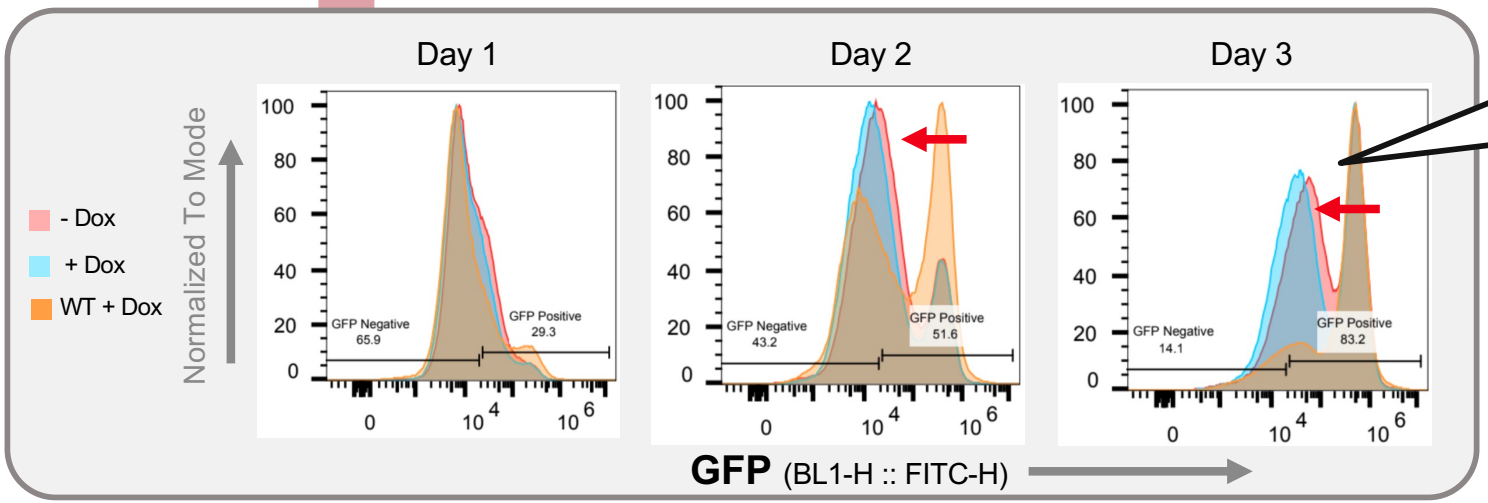
① Experiment

Results

EAF6 has an impact, but our clone has an error in RNAi system

EAF6 is **poorly expressed** and sensitive

Too much expression due to clone in chromosome



"Leak"
EAF6 is **repressed without** dox?

① Experiment

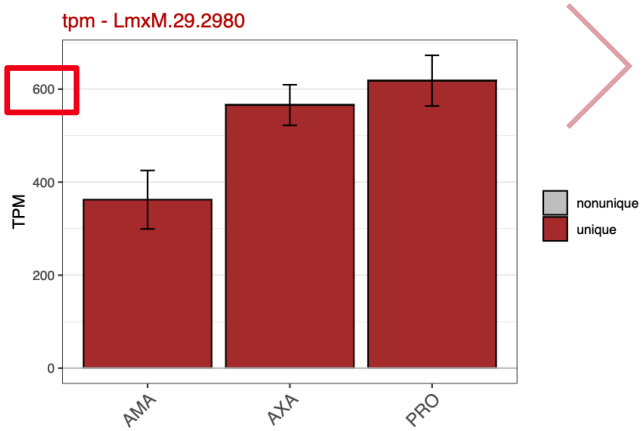
Results

EAF6 has an impact, but our clone has an error in RNAi system

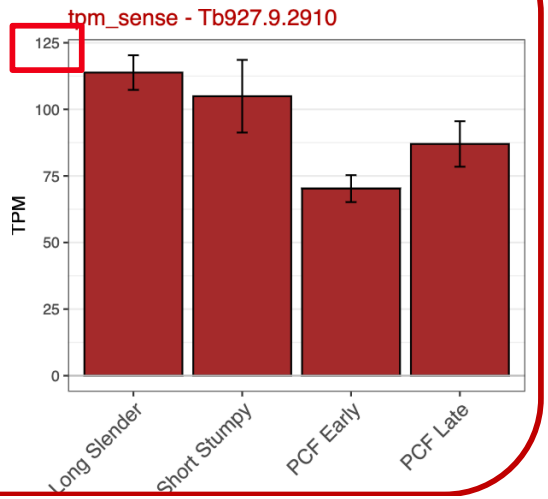
EAF6 is **poorly expressed** and sensitive?

Too much expression due to clone in chromos ne

GAPDH (“housekeeping” gene)



EAF6



To-do

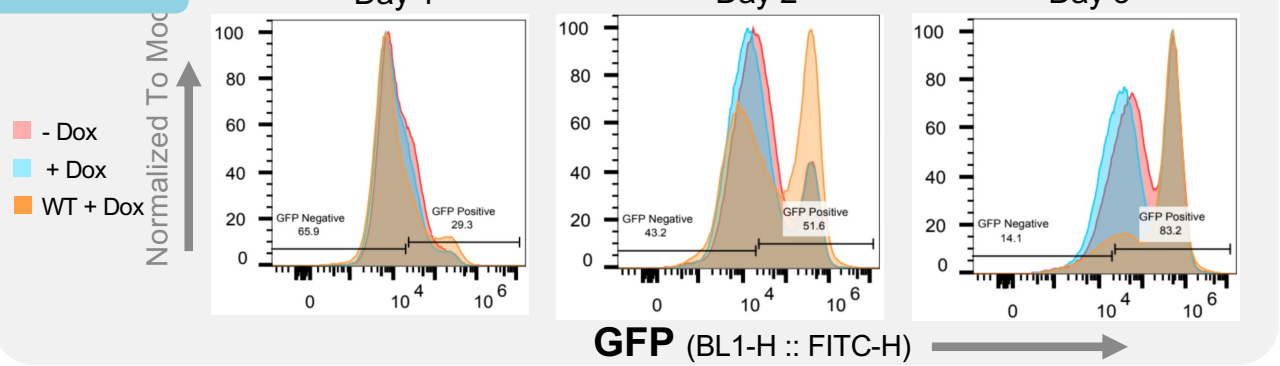
- Western Blot with EAF6 protein marker
- Re-do experiment with independent clone

RNA-seq analysis: Transcriptomes of T. brucei culture-derived slender/stumpy bloodstream and early/late procyclic forms

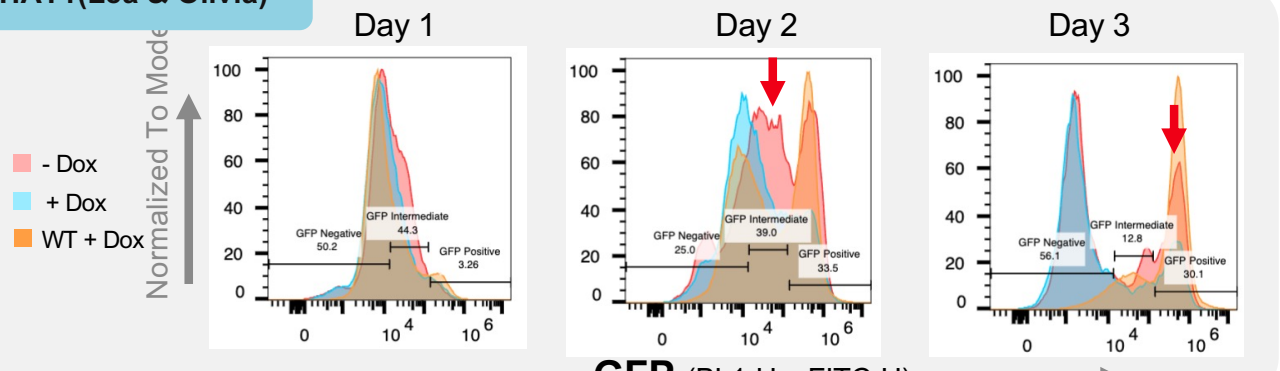
If there was an error in RNAi…

② Role of EAF6

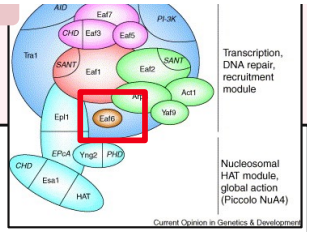
EAF6



HAT1(Lea & Olivia)



yeast

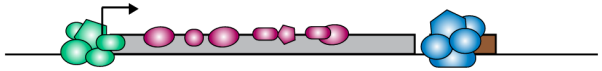


Tryp

Class I TSR factors

CRD1
SET27

BDF1
BDF4



BDF3 BDF5
HAT2

ZCW1
SET26

HDAC1

BDF2
HDAC3

BDF6
HAT1
EAF6

Class II TSR factors

BDF7
ELP3b
PDH2
PDH4
DOT1A
TBP
TFIIS2-2
TRF

TTR factors

Hypo

Perhaps **EAF6** and **HAT1** help **BDF6** act as a HAT complex?

Together, BDF6, EAF6, and HAT1 appear as part of a putative NuA4-related complex that functions at *T. brucei* TSRs. HAT1 has been shown to be required for H2A.Z and H2B.V acetylation and efficient RNAPII engagement and transcription (Kraus et al. 2020). BDF6, YEA2, or both may bind acetylated histones at TSRs to promote stable association of interacting chromatin modification and remodeling activities that enable the required histone dynamics to take place in these highly specialized regions and thereby facilitate efficient transcription.

(Staneva et al. 2021)

Todo

- BDF6

Conclusion

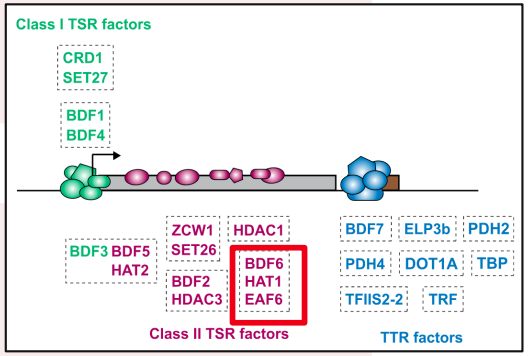
Q: Does Knocking down a part of HAT complex (EAF6) affect parasite differentiation?

- **Yes: EAF6** is **important** in lifecycle differentiation of *T. brucei*
- Perhaps EAF6 and HAT1 help BDF6 act as a HAT complex collectively?
- Our EAF6 clone had an error in RNAi regulation system

Future Direction

① Experiment

- **Repeat** the experiment with independent transformed clone
- Perform Western Blot with **marker protein** on EAF6

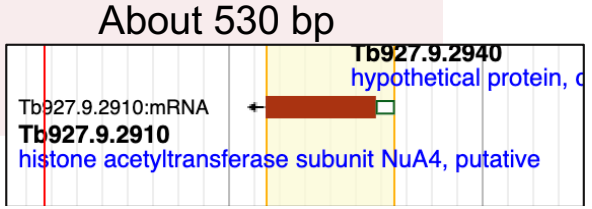


② Role of EAF6

- Do the same experiment with **BDF6**

  > structure and function

- Global Knock out by (inducible?) **CRISPR-Cas9**



Reference

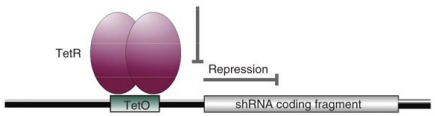
- Staneva DP, Carloni R, Auchynnikava T, Tong P, Rappsilber J, Jeyaprakash AA, Matthews KR, Allshire RC. A systematic analysis of Trypanosoma brucei chromatin factors identifies novel protein interaction networks associated with sites of transcription initiation and termination. Genome Res. 2021 Nov;31(11):2138-2154. doi: 10.1101/gr.275368.121. Epub 2021 Aug 18. PMID: 34407985; PMCID: PMC8559703.
- Schulz D, Mugnier MR, Paulsen E-M, Kim H-S, Chung C-wW, Tough DF et al. 2015. Bromodomain Proteins Contribute to Maintenance of Bloodstream Form Stage Identity in the African Trypanosome. PLoS Biol 13(12): e1002316.
- BIOL 111 Lab Manual (Fall 2023)
- R core
- https://tritrypdb.org/tritrypdb/app/

Thank You

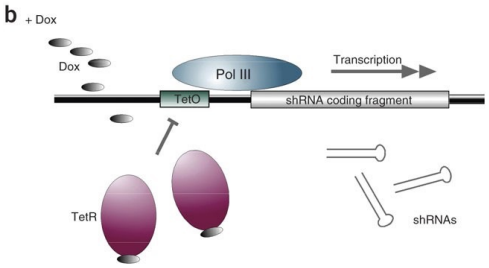
Any questions?

Acknowledgement

- Professor Danae Schulz
- Schulz Lab Members
- BIO111 Classmates
- Special thanks to Ethan Goroza for being an outstanding lab partner
- Harvey Mudd College Department of Biology

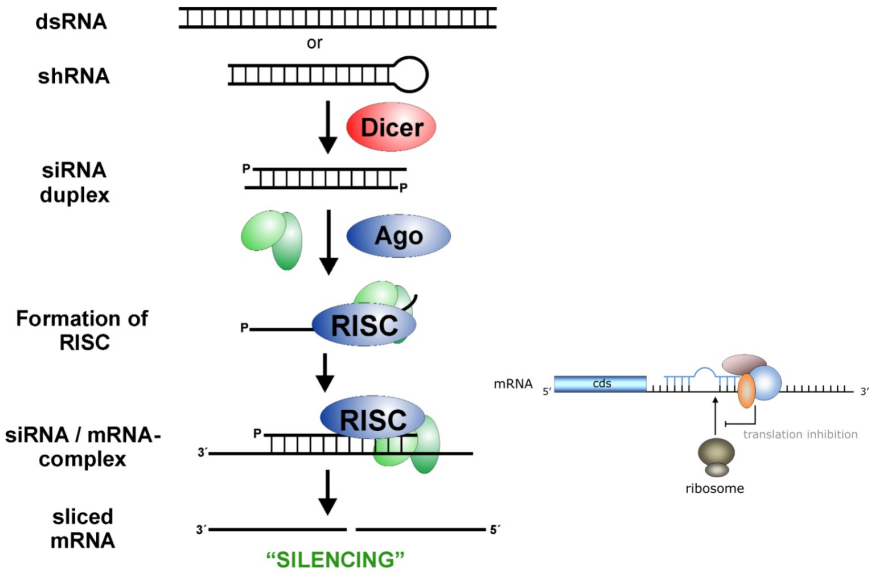


Supplement

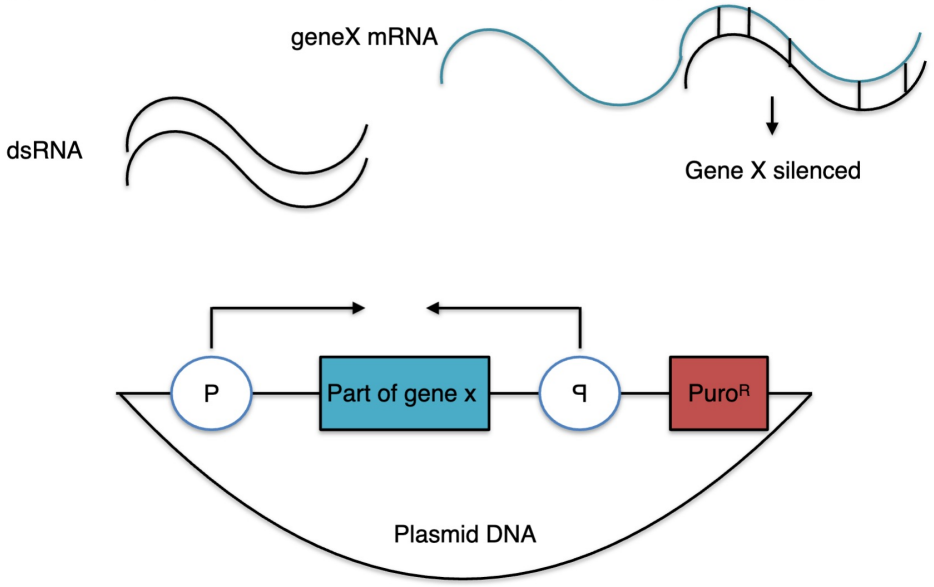


How RNAi works on gene of interest?

What is RNA interference (RNAi)?



How will we do RNAi in our parasite system?



Prof Schulz Lecture 3 slides

Supplement

Cloning Plasmid Design