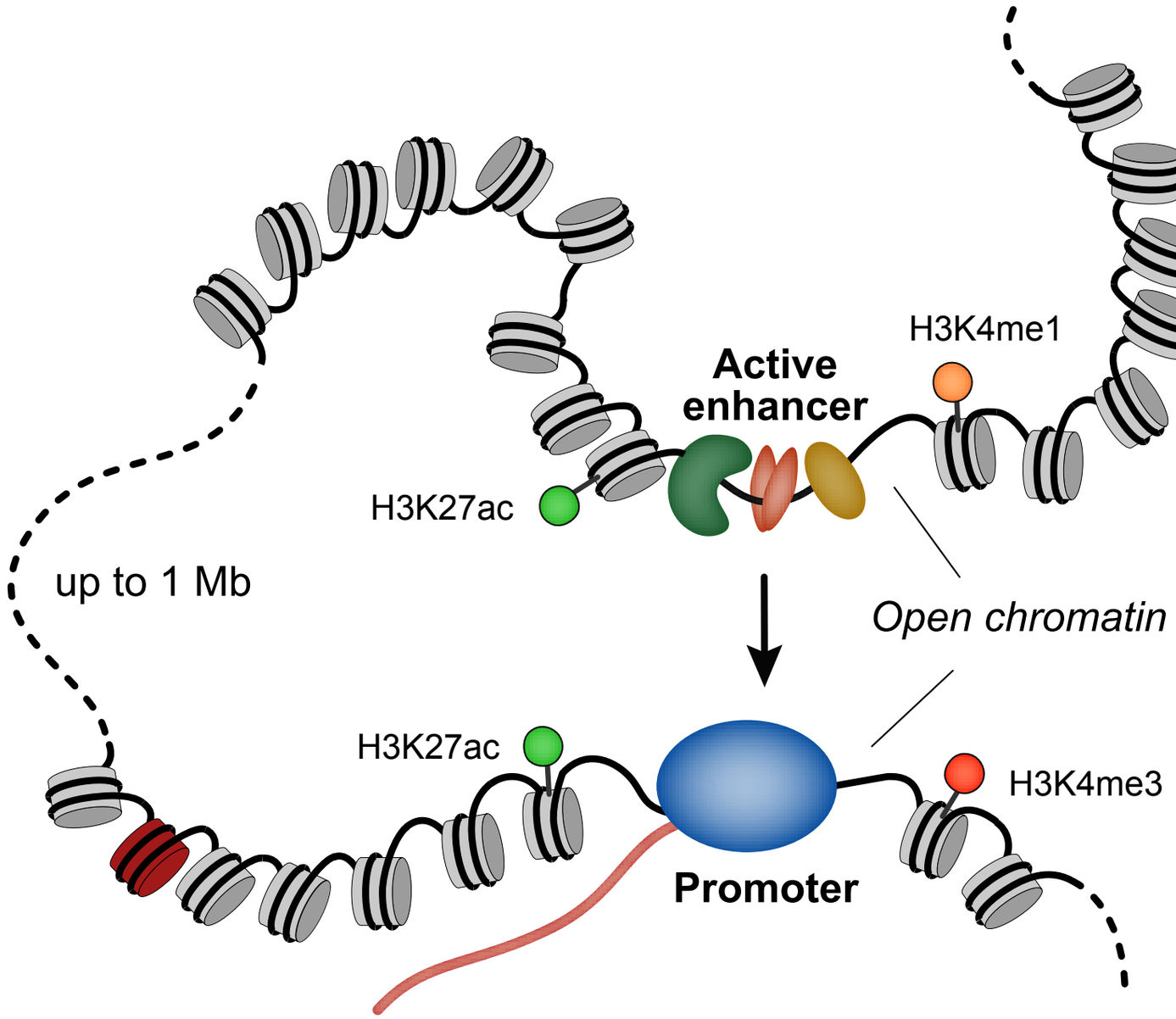
Transcription regulation in living human cells



Initiation of transcription in the bacterium *E. coli*

R (RNA Pol)
+
D (DNA)

k_{+1} k_{-1}

RD
(unspecific)

$K_B = k_{+1} / k_{-1}$

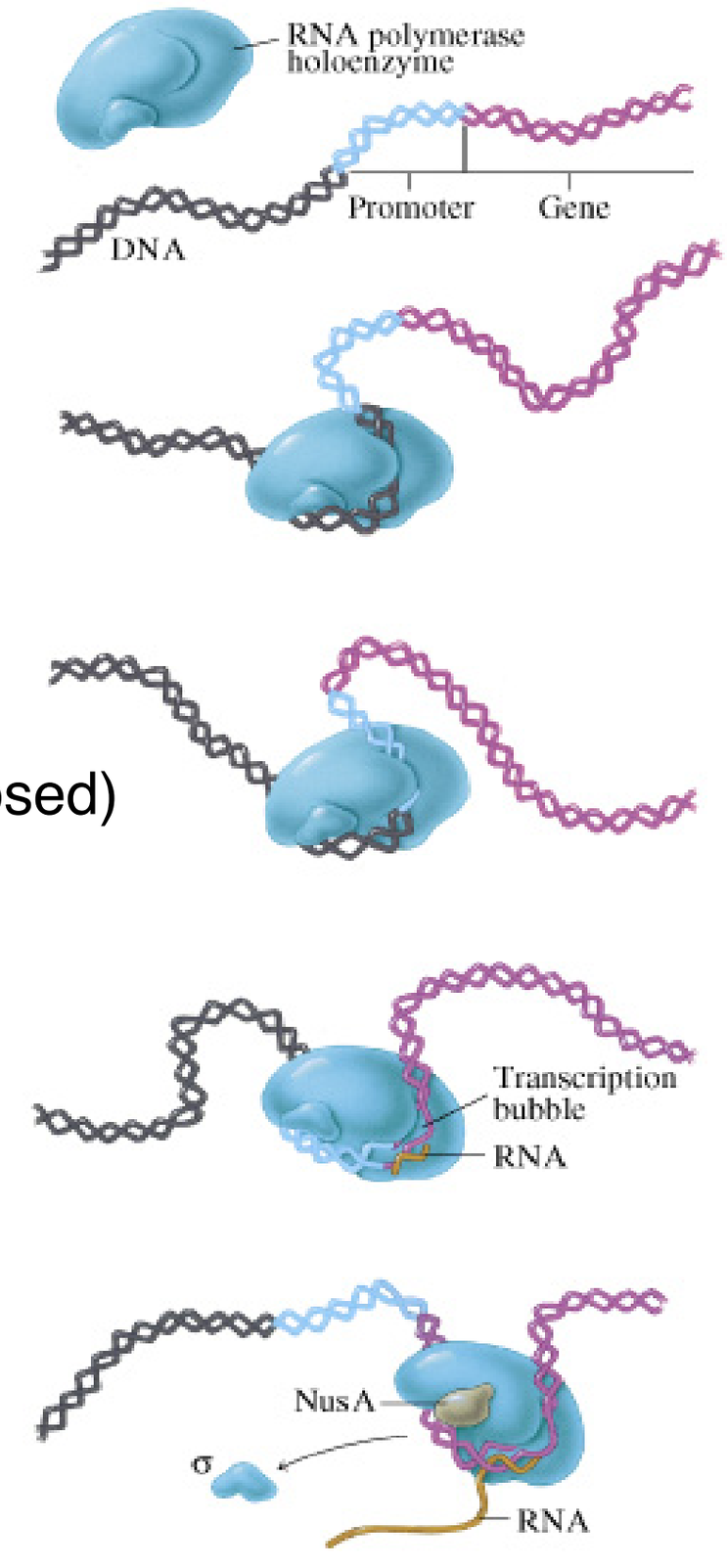
RPc
(Promoter closed)

k_{+2} k_{-2} ?

RPo
(Promoter
open)

k_{+3}

Re
(elongating)
+ RNA



(a) RNA polymerase holoenzyme binds nonspecifically to DNA.

(b) The holoenzyme conducts a one-dimensional search for a promoter.

(c) When a promoter is found, the holoenzyme and the promoter form a closed complex.

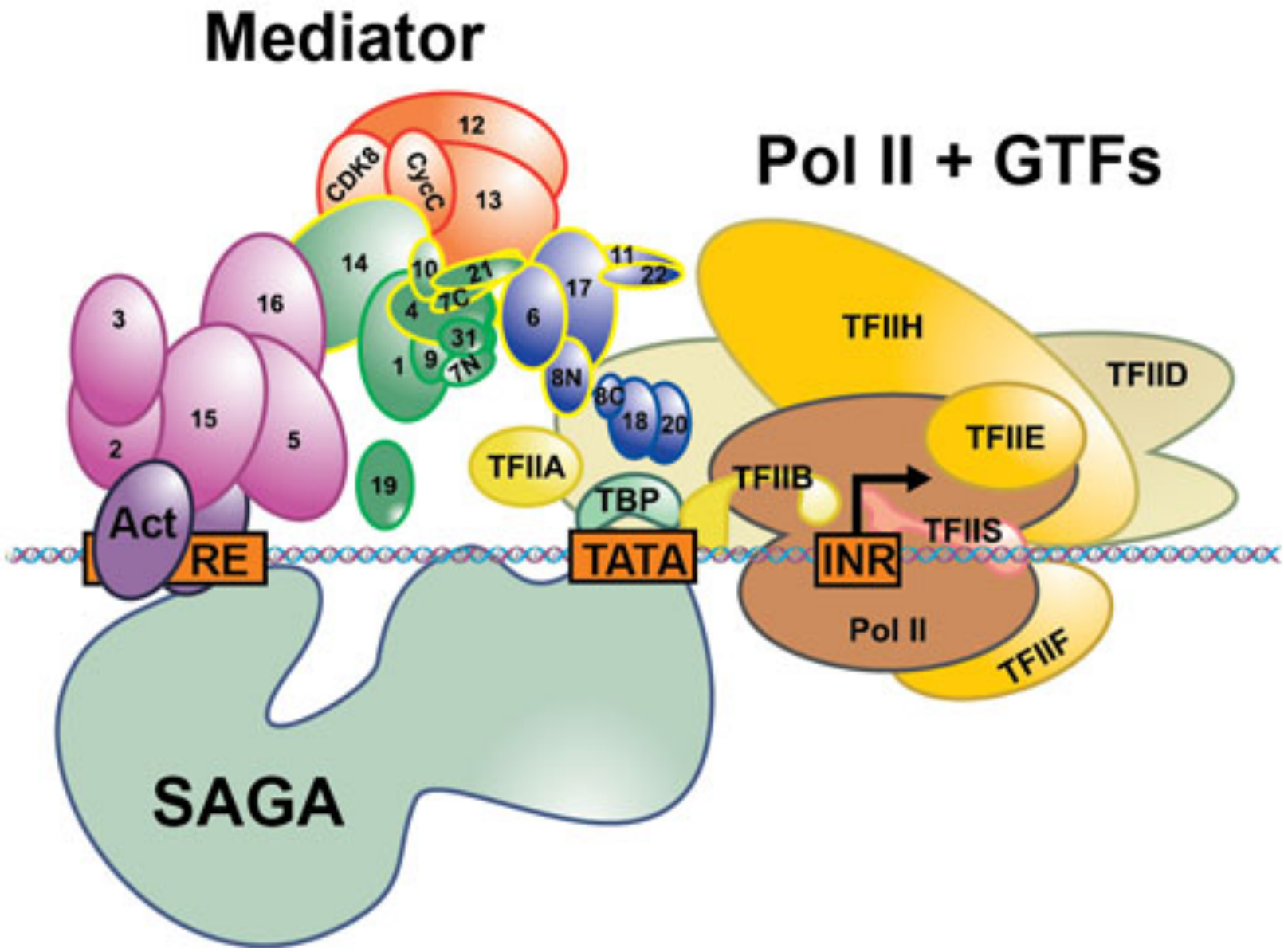
(d) A conformational change from the closed complex to an open complex produces a transcription bubble at the initiation site. A short stretch of RNA is then synthesized.

(e) The σ subunit dissociates from the core enzyme, and RNA polymerase clears the promoter. Accessory proteins, including NusA, bind to the polymerase.

Transcription initiation by RNA polymerase II

Cheung ACM, Cramer P: A movie of RNA polymerase II transcription. Cell 149, 1431-1437 (2009

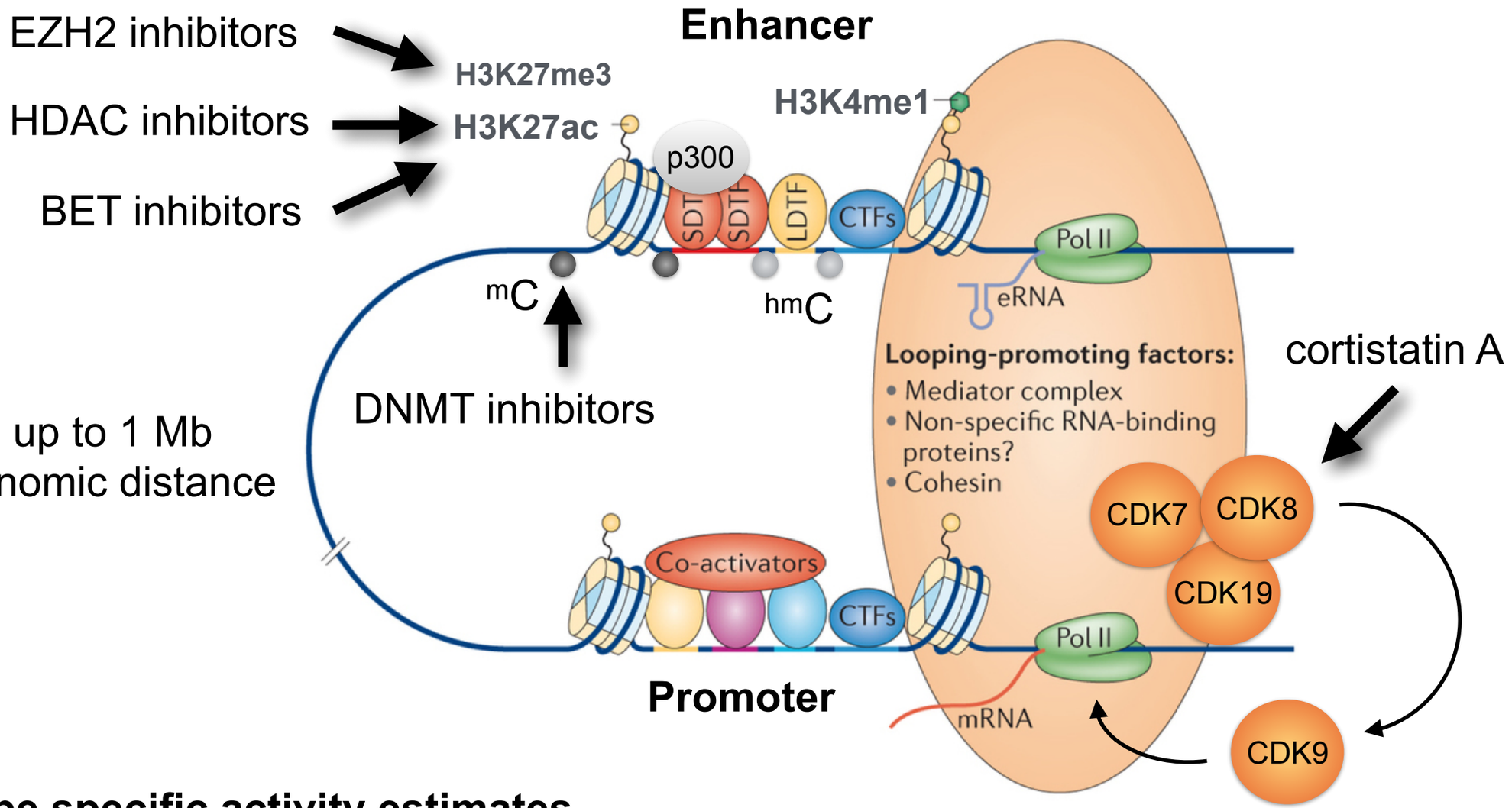
Yeast RNA polymerase II with activator complex



Seizl 2011

Packaging of the eukaryotic genome in chromatin/nucleosomes suppresses transcription
and additional activator complexes are needed to assemble active Pol II at the promoter

More than **300 000** putative enhancers regulate **~54 000** annotated human genes (including lncRNAs)

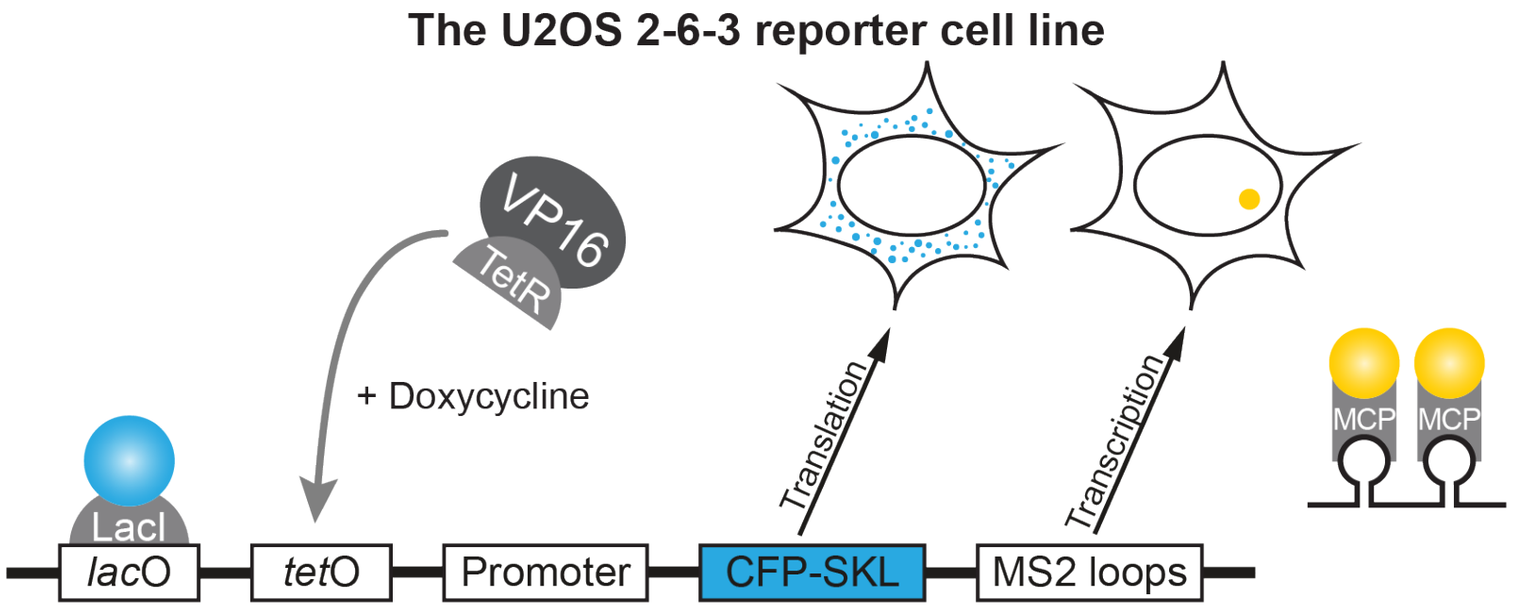


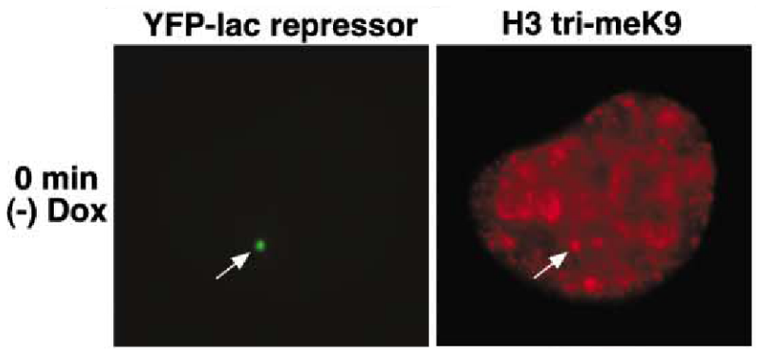
Cell type specific activity estimates

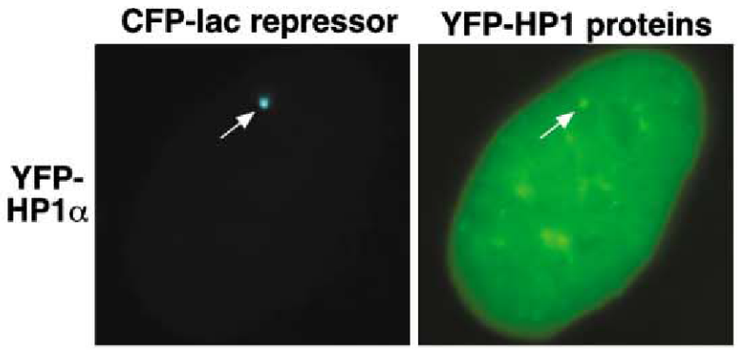
- ~20 000 active genes
- 80 000 - 240 000 active enhancers
- typical: 1-2 target promoter per enhancer
- ~10 different targets for some enhancers
- multiple enhancers for single promoter
- 300-500 super enhancers > 10 kb

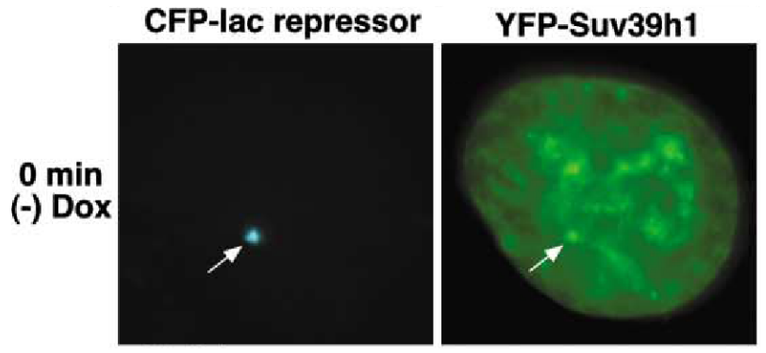
Heinz 2015, Nat Rev Mol Cell Biol
Roadmap Epigenomics Consortium 2015, Nature
FANTOM Consortium 2015, Nature

Following gene expression in living cells







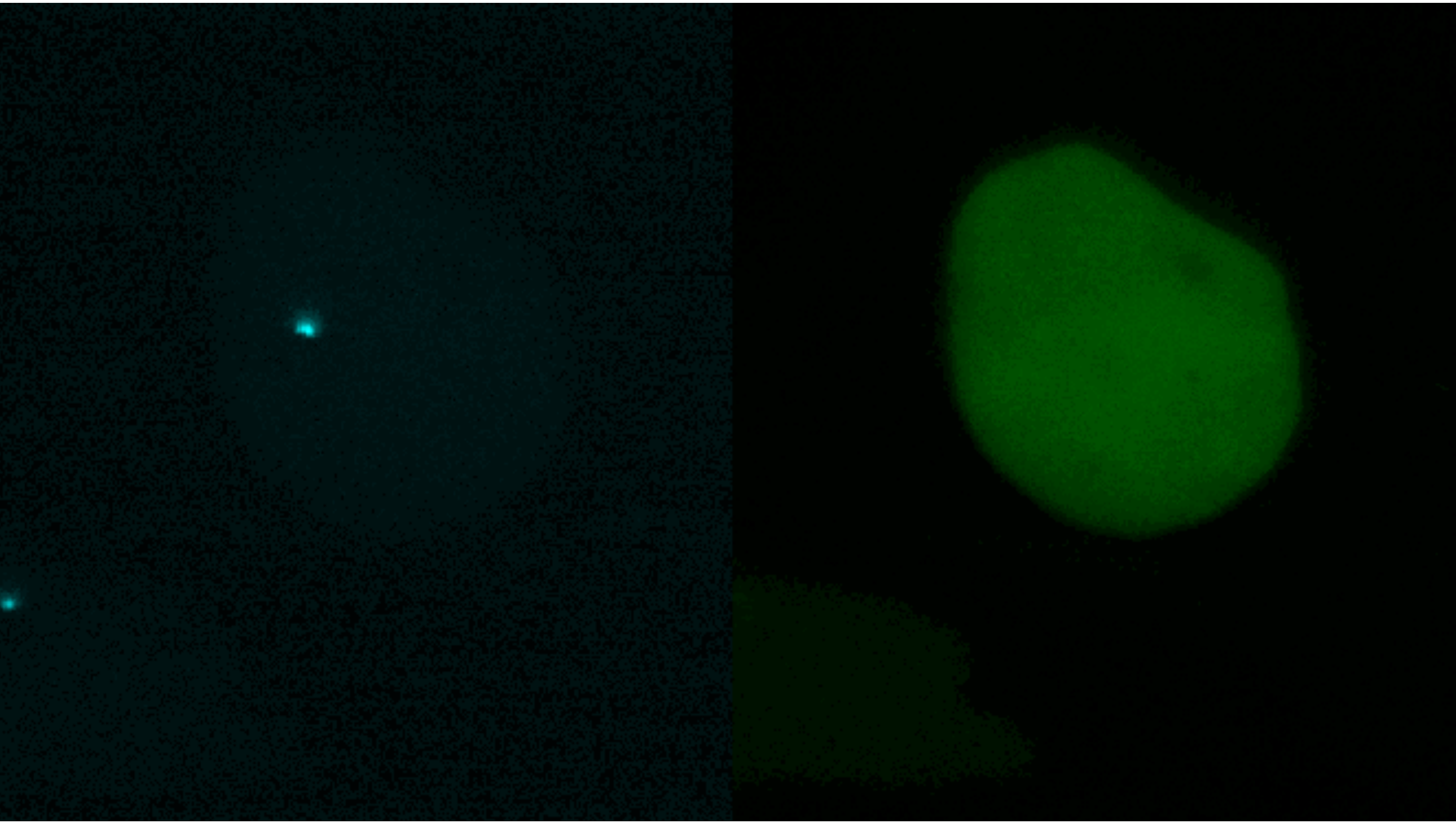


Heterochromatic locus:
- array with ~200 repeats of reported
- compact chromatin state
- H3K9me3
- HP1 enriched

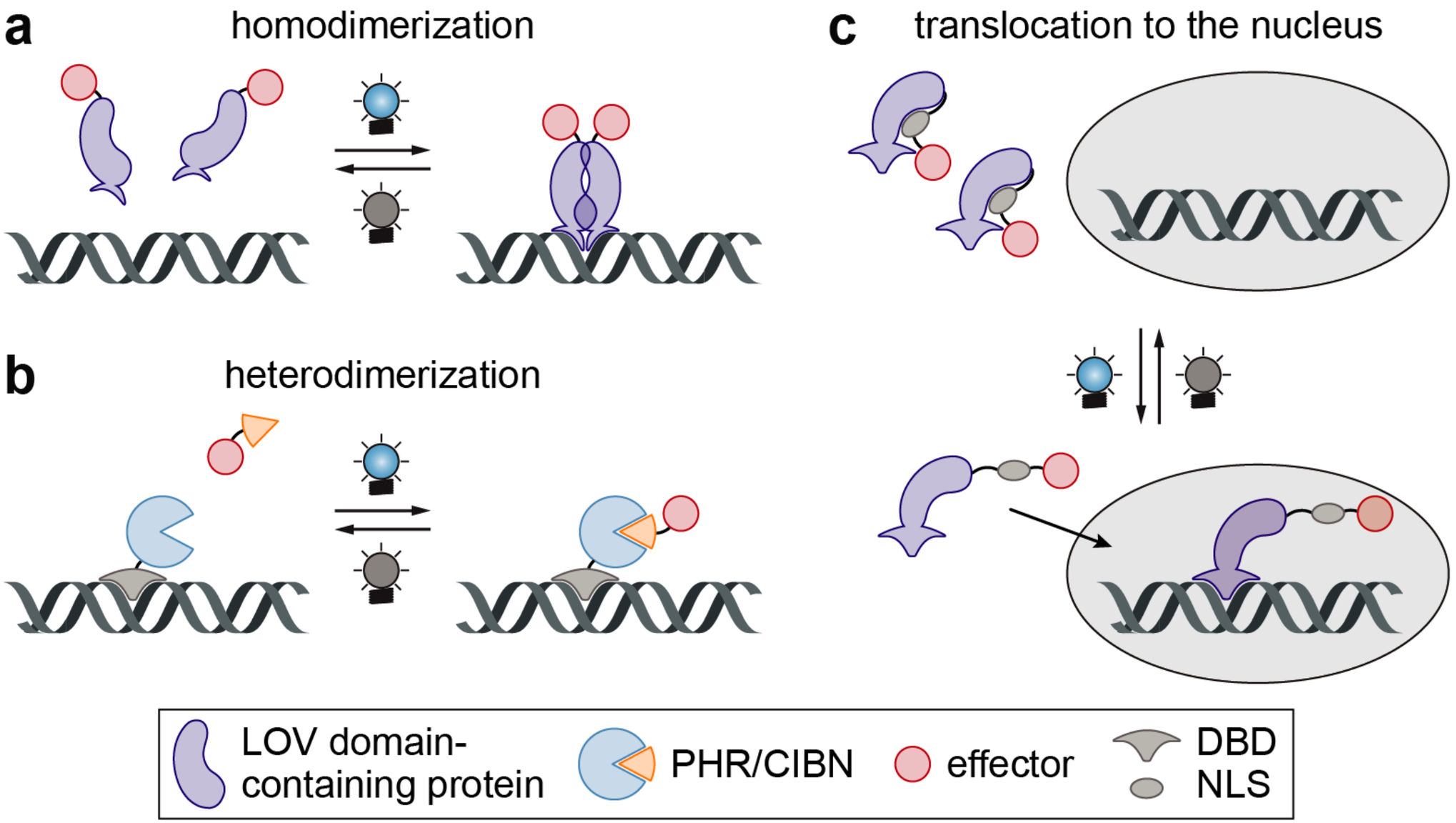
Janicki *et al.* (2004) From silencing to gene expression: real-time analysis in living cells. *Cell* 116:683-698.

CFP-LacI (chromatin)
CFP (translated protein)

MS2-YFP
(RNA)



Triggering protein relocalization in living cells



Tischer, D. & Weiner, O. D. (2014) Illuminating cell signalling with optogenetic tools. *Nature Reviews Molecular Cell Biology* 15(8): 551-558.

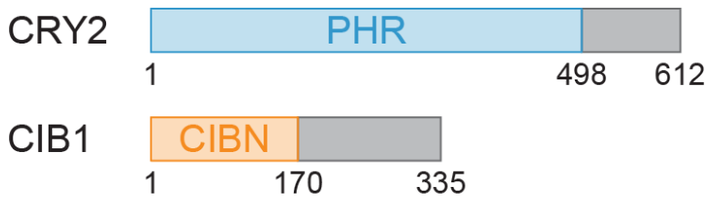
Light-inducible interactions are rapid and reversible

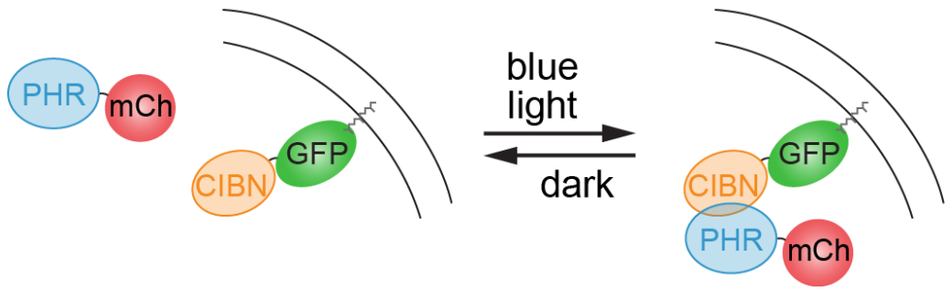
Photosensitive protein	Turn-on speed	Turn-off speed ($t_{1/2}$)	Chromophore requirement	Compatible imaging wavelengths (nm)	λ_{on} (nm)	λ_{off} (nm)	Effector affinity	Refs
PHYB	Seconds	• Seconds (illuminated at 750 nm) • Hours (dark reversion)	PCB; exogenous or synthesized *in situ*	≤514	650	750	• <100 nM (post 650 nm) • >100 µM (post 750 nm)	16–18
CRY2	Seconds	5 minutes	Flavin; endogenous	≥561	405–488	NA	Not determined	9–11
LOV	Seconds	Tens of seconds to minutes	Flavin; endogenous	≥514	440–473	NA	• 1 µM (dark) • 100 µM (light)	12–15, 67
Dronpa	Seconds	• Tens of seconds (illuminated at 390 nm) • Tens of minutes (dark reversion)	None	≥600	390	490	• 10 µM (post 490 nm) • >100 µM (post 390 nm)	19

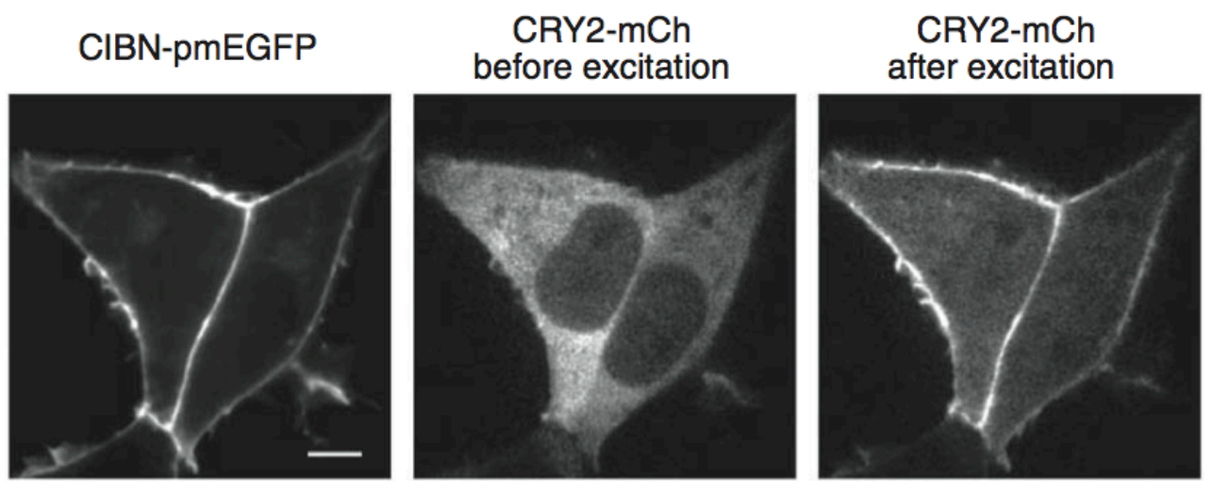
CRY2, CRYPTOCHROME 2; NA, not applicable; PHYB, PHYTOCHROME B; PIF, PHYTOCHROME INTERACTING FACTOR.

Tischer, D. & Weiner, O. D. (2014) Illuminating cell signalling with optogenetic tools. *Nature Reviews Molecular Cell Biology* 15(8): 551-558.

Triggering protein relocalization in living cells

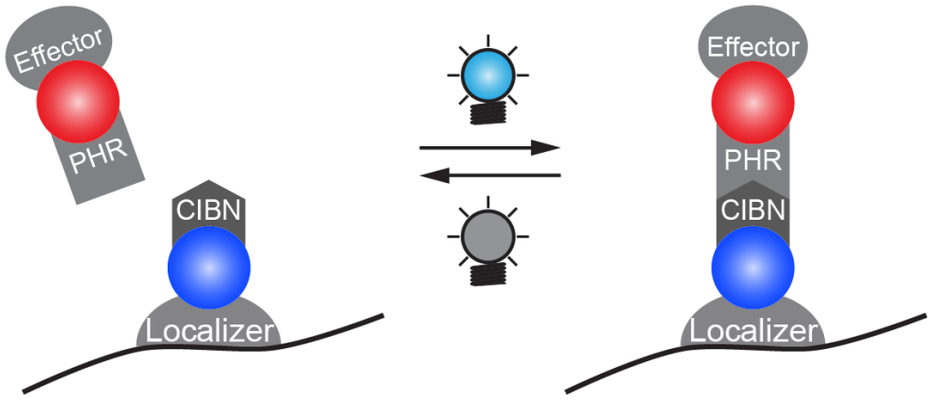


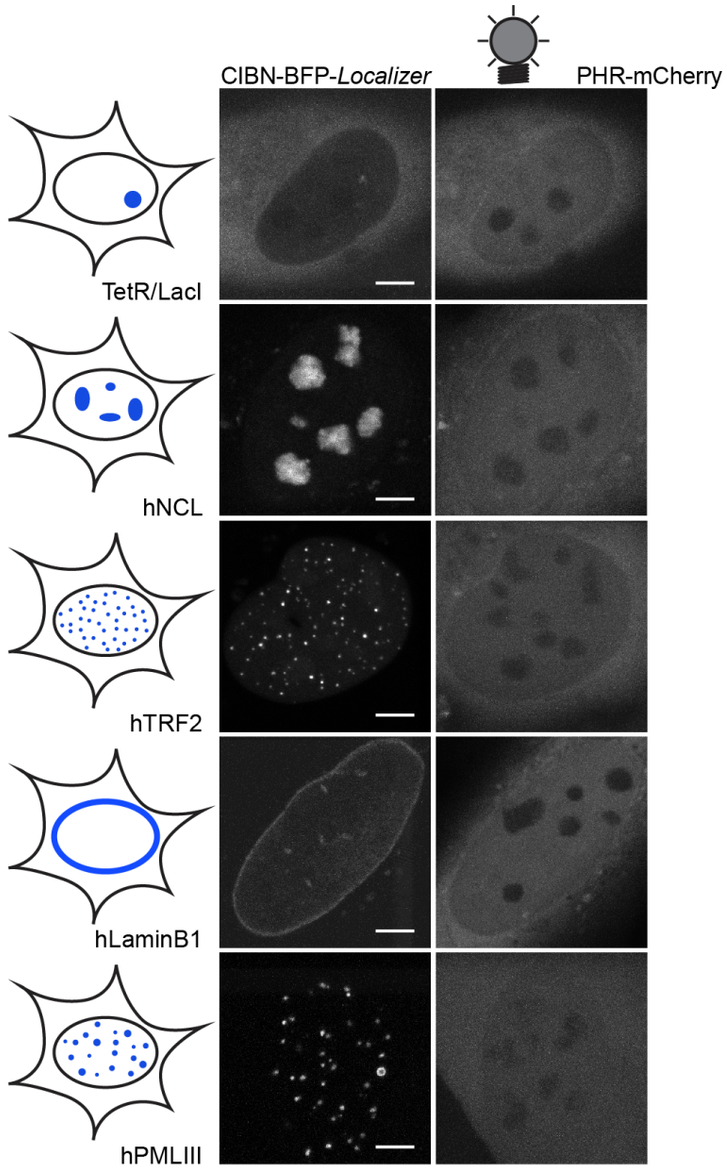




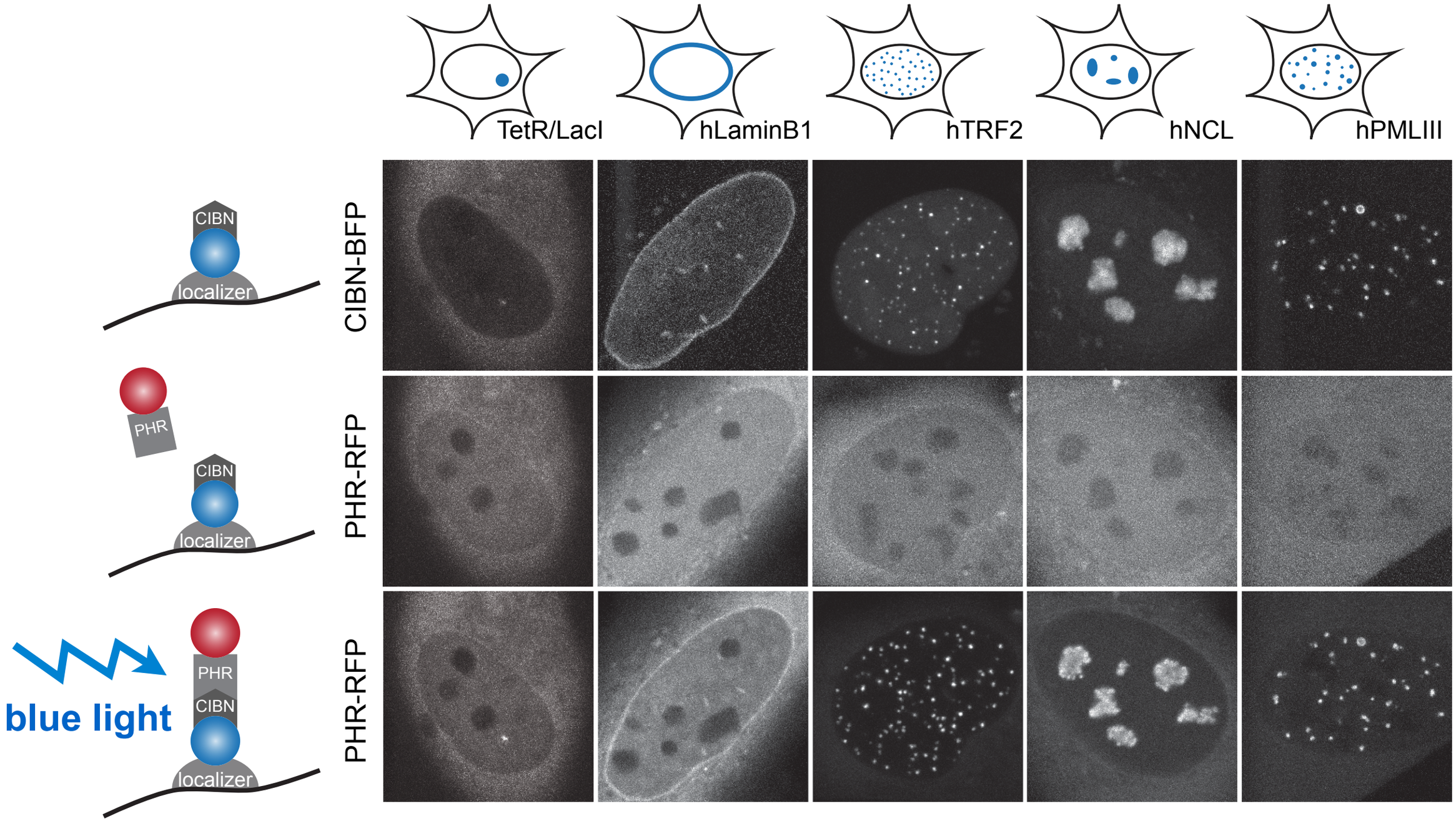
Kennedy, M. J., Hughes, R. M., Peteya, L. A., Schwartz, J. W., Ehlers, M. D. & Tucker, C. L. (2010) Rapid blue-light-mediated induction of protein interactions in living cells. *Nature Methods* 7(12): 973-975.

Blue **L**ight-**In**duced **C**hromatin **R**ecruitment **BLInCR**





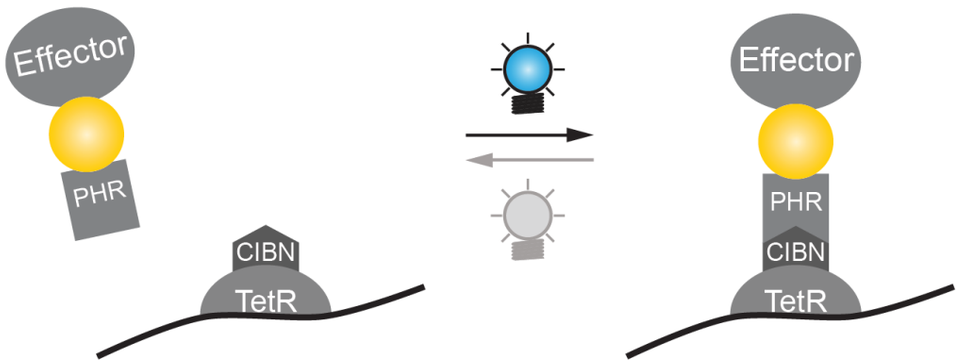
Blue **L**ight-**In**duced **C**hromatin **R**ecruitment **(BLInCR)**

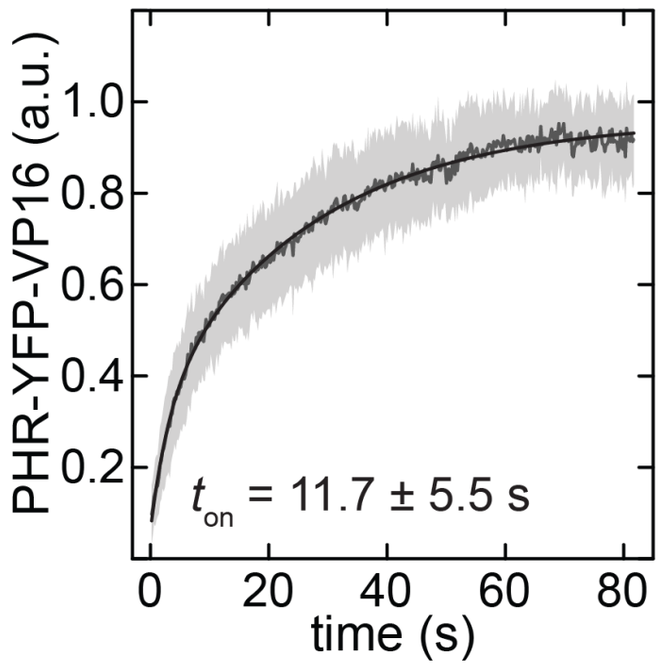


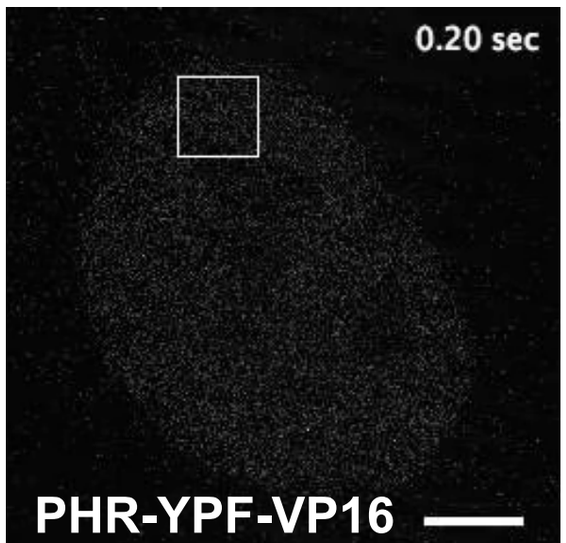
Rademacher, Erdel, Trojanowski, Schumacher & Rippe, Journal of Cell Science, 2017

The PHR-CIBN interaction is rapidly induced and reversible

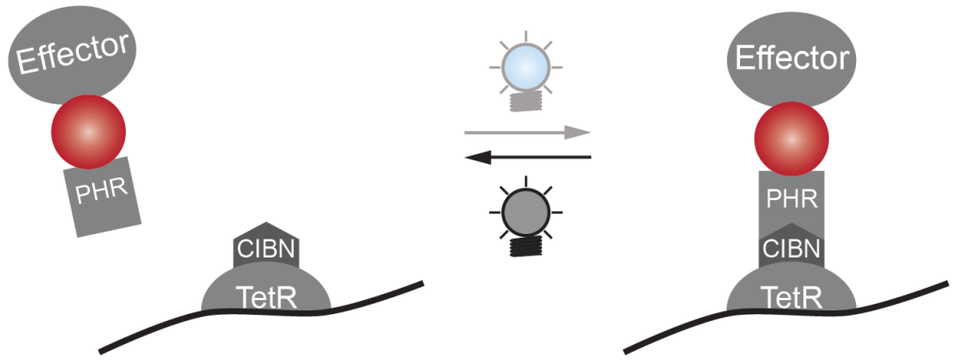
Binding within seconds

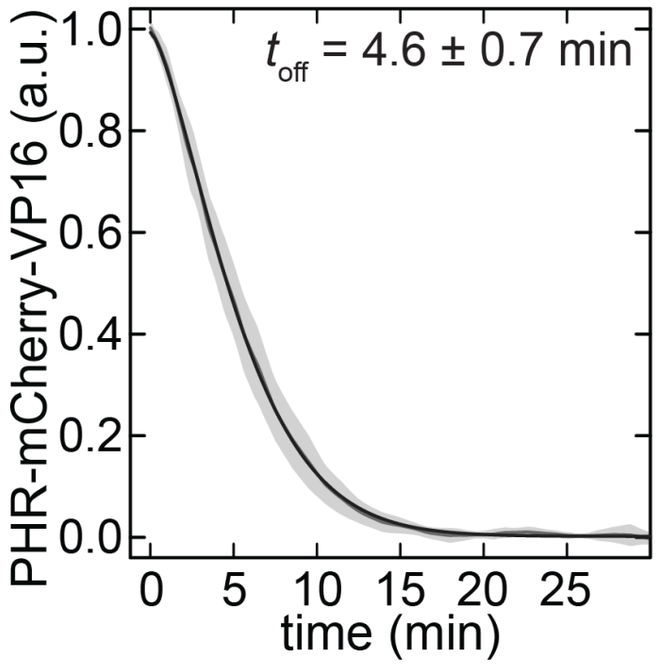




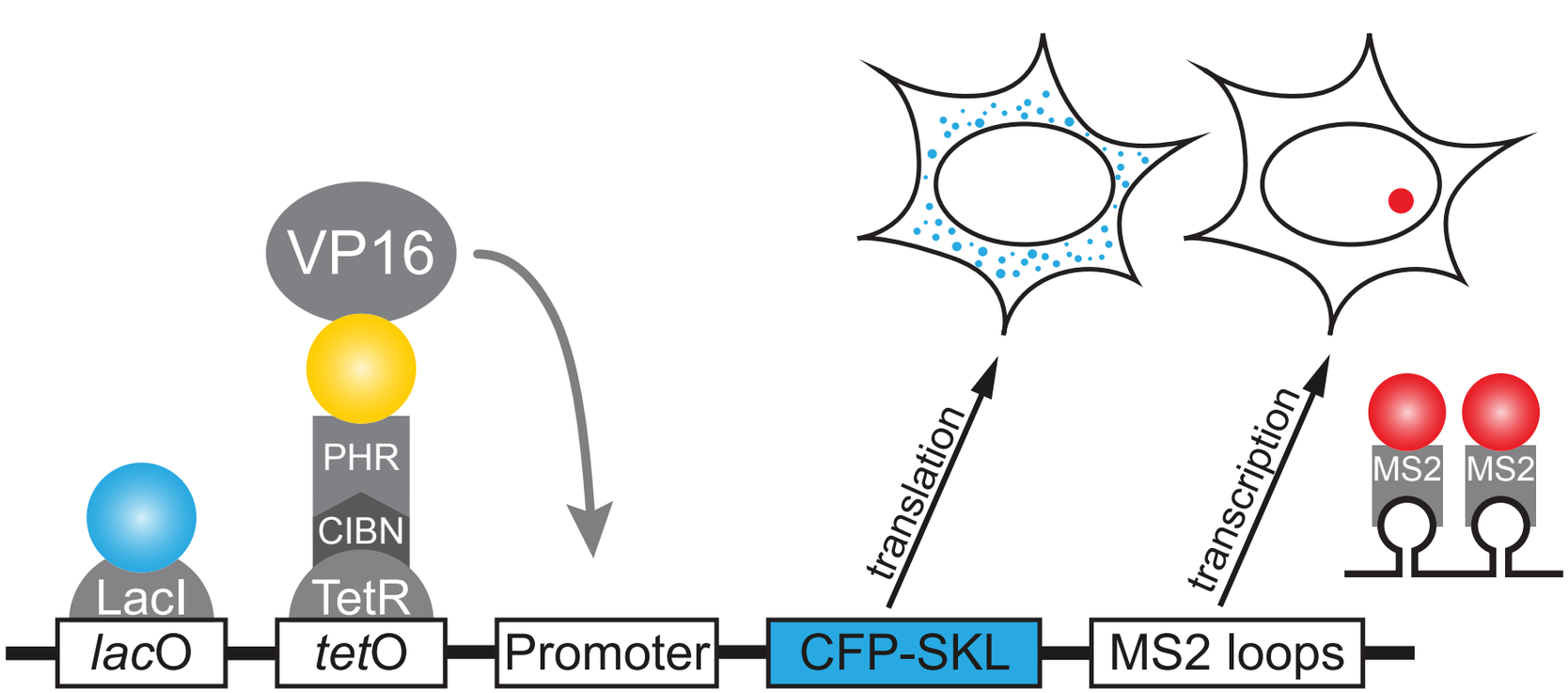


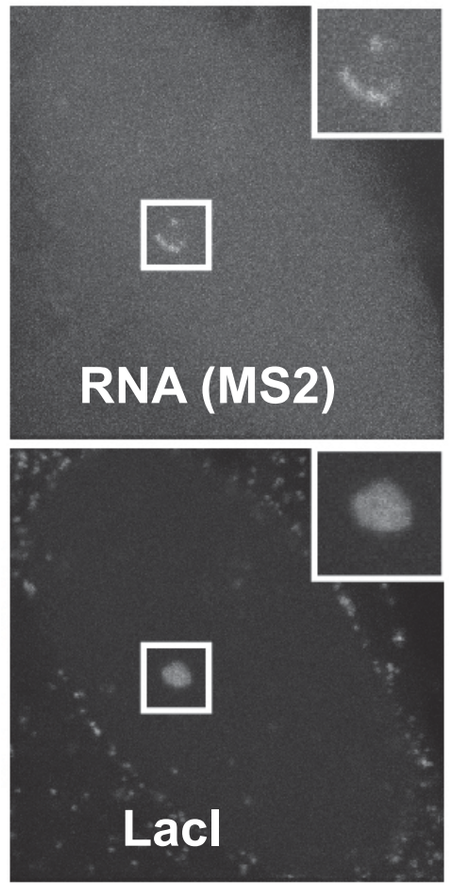
Dissociation within minutes





Tracing transcription activation with BLInCR in living cells

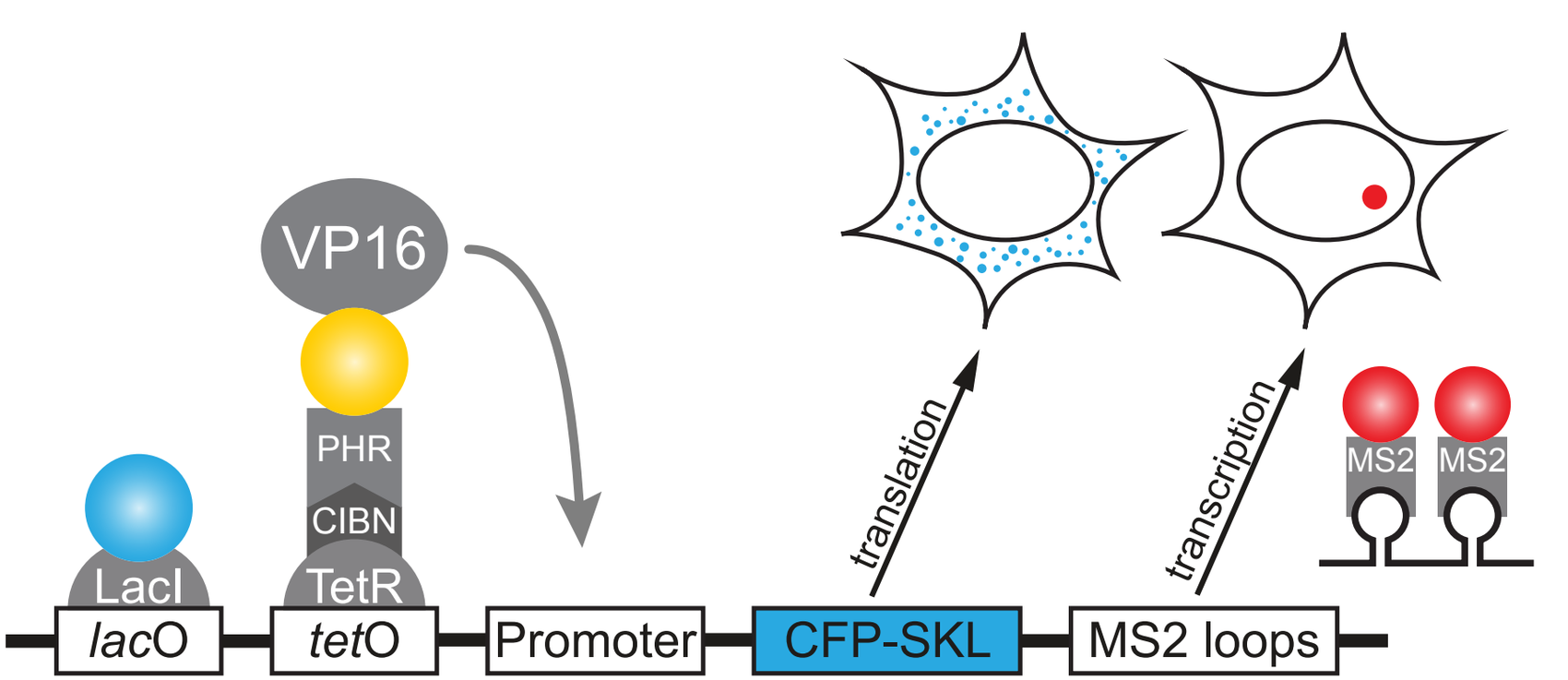


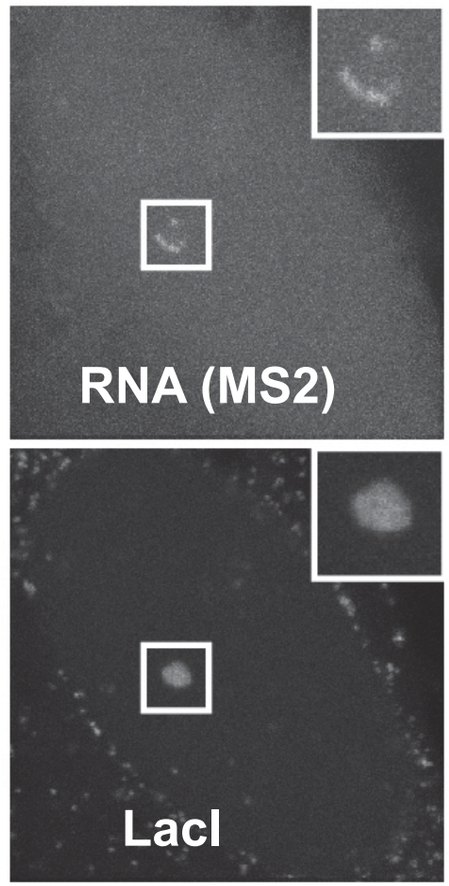


Reporter cell line U2OS 2-6-3 from Janicki 2004, *Cell*

Heterochromatic locus:
- array with ~200 repeats of reported
- compact chromatin state
- H3K9me3
- HP1 enriched

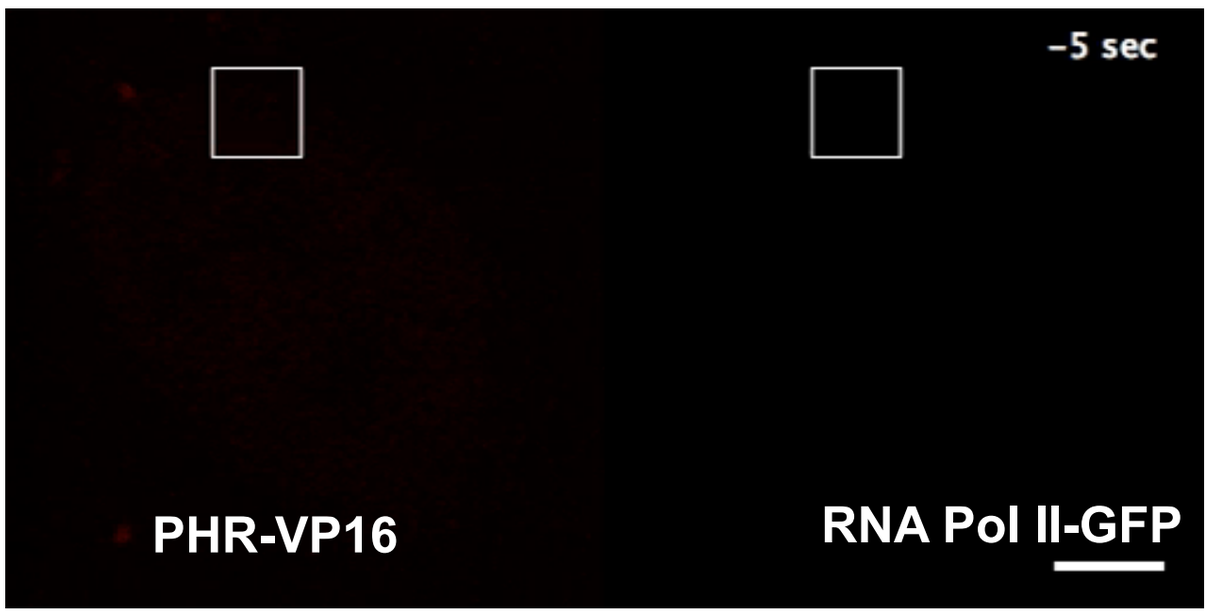
Tracing transcription activation with BLInCR in living cells



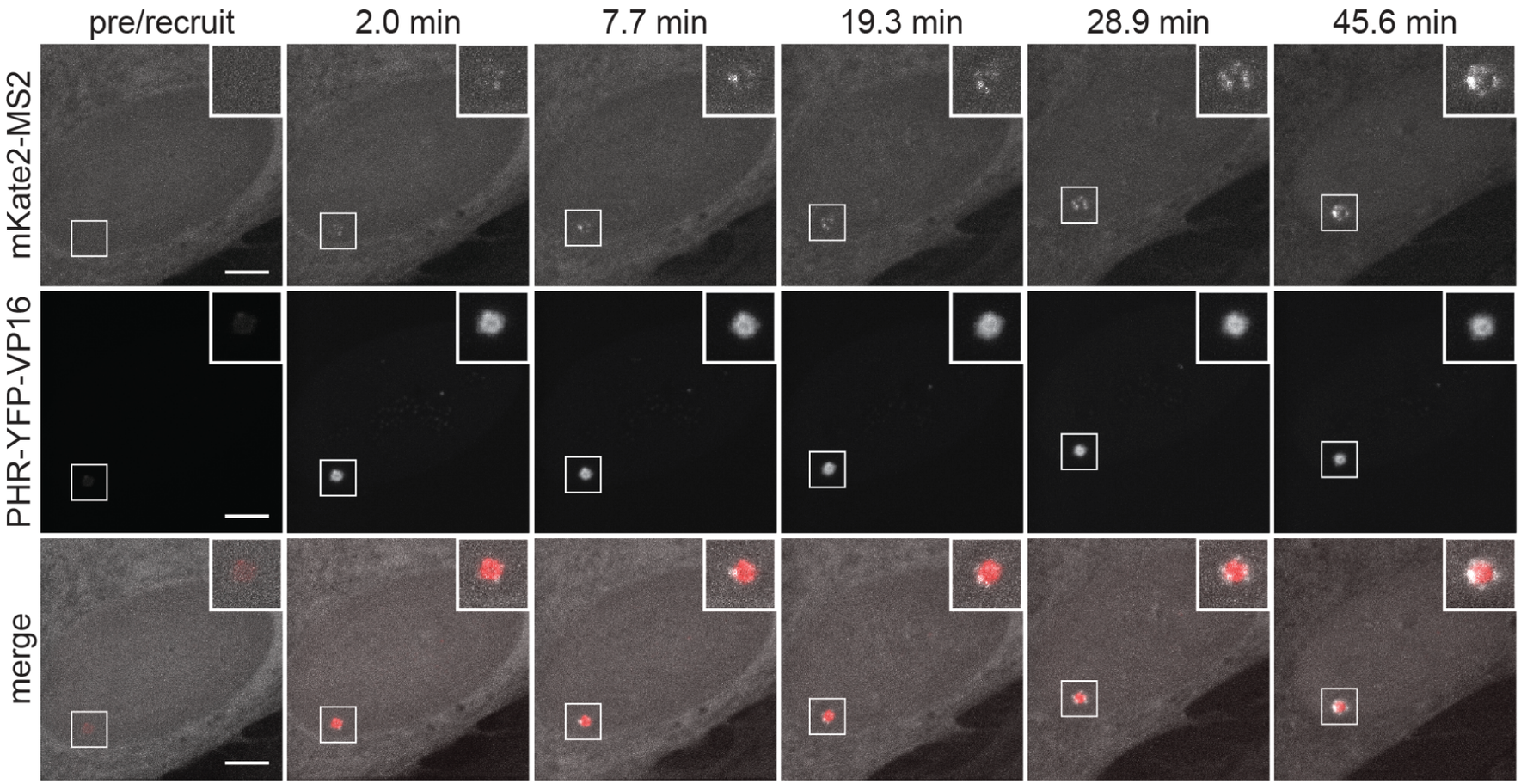


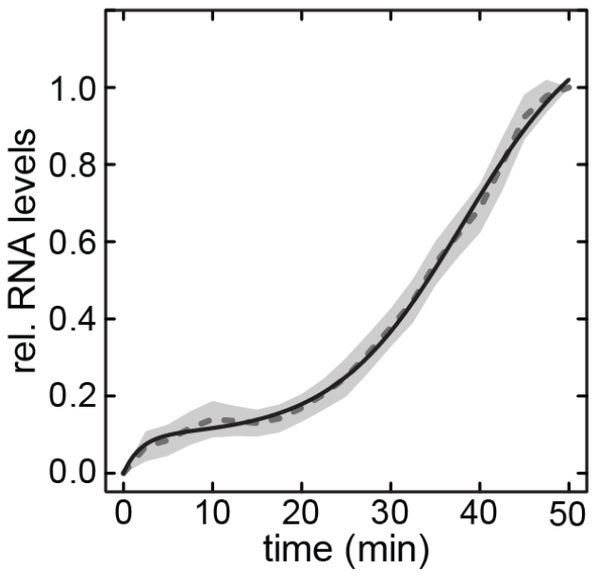
Reporter cell line U2OS 2-6-3 from Janicki 2004 *Cell*

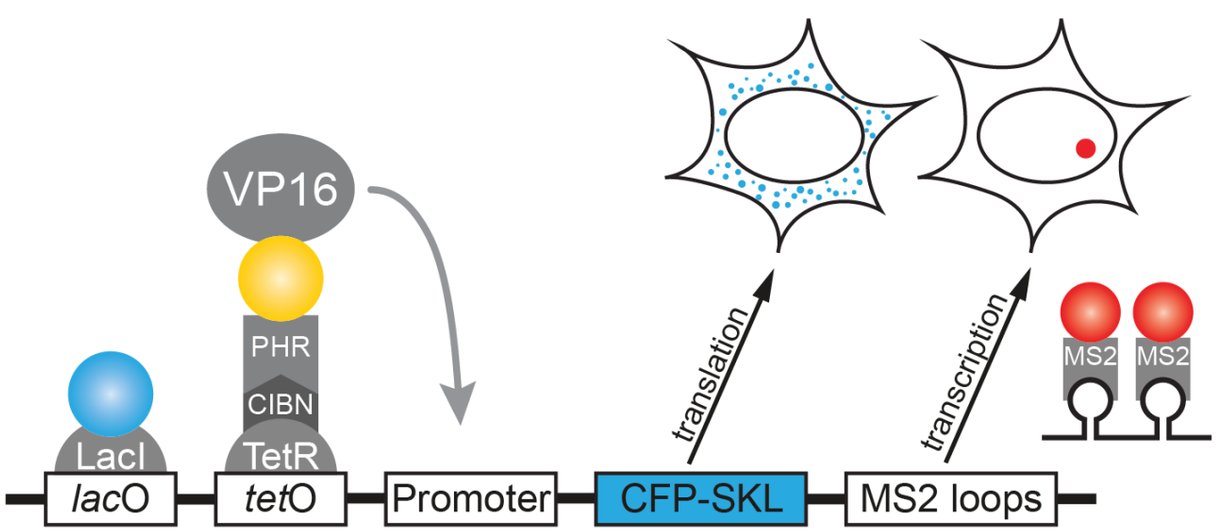
Heterochromatic locus:
- array with ~200 repeats of reported
- compact chromatin state
- H3K9me3
- HP1 enriched



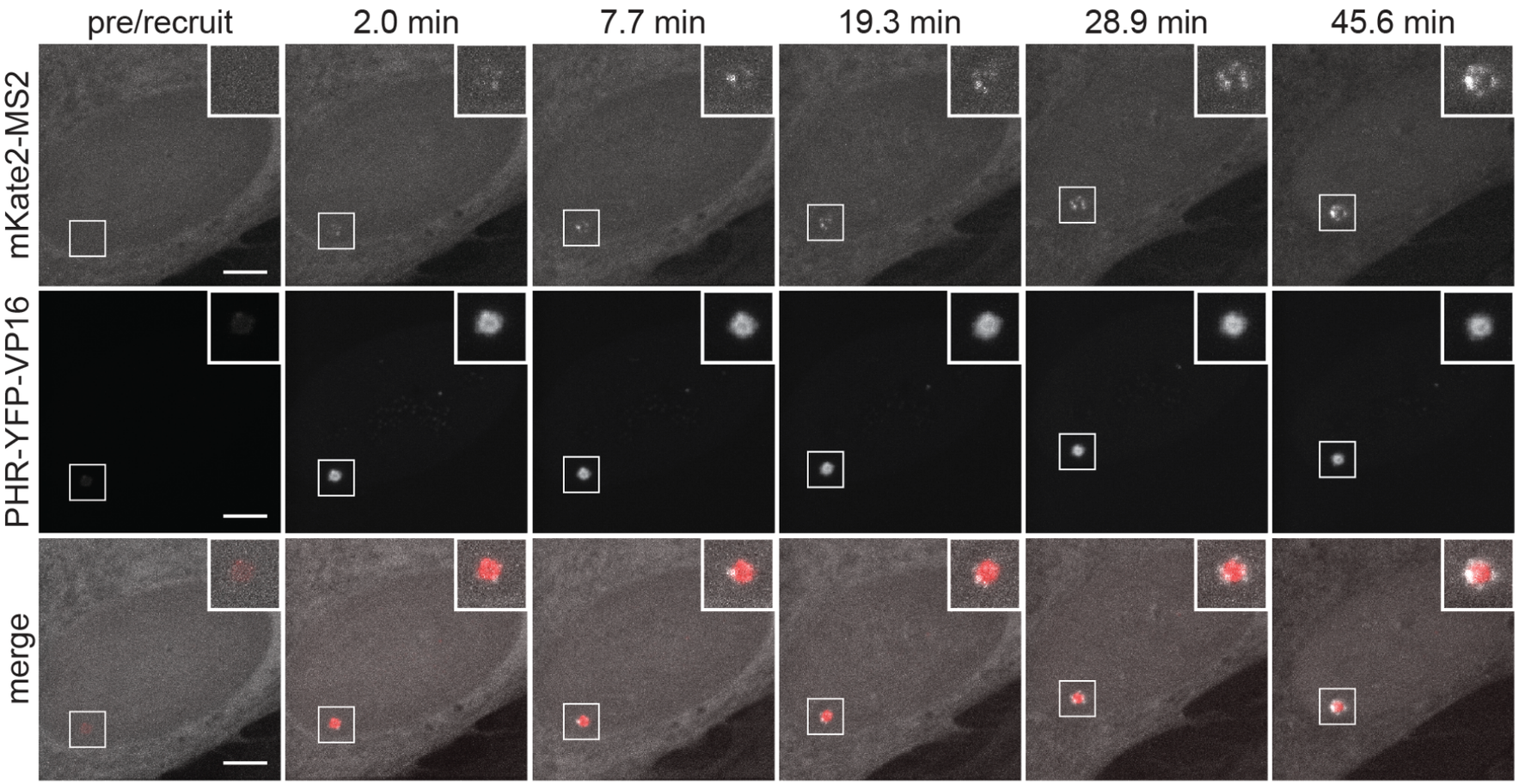
Transcription activation of the array proceeds in two phases

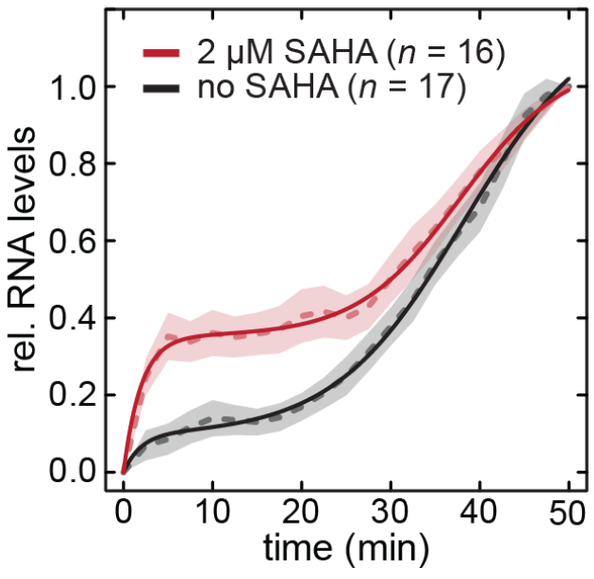


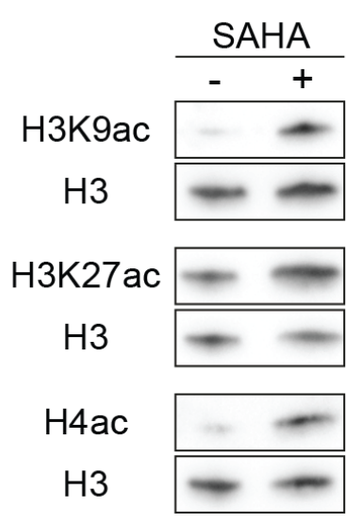


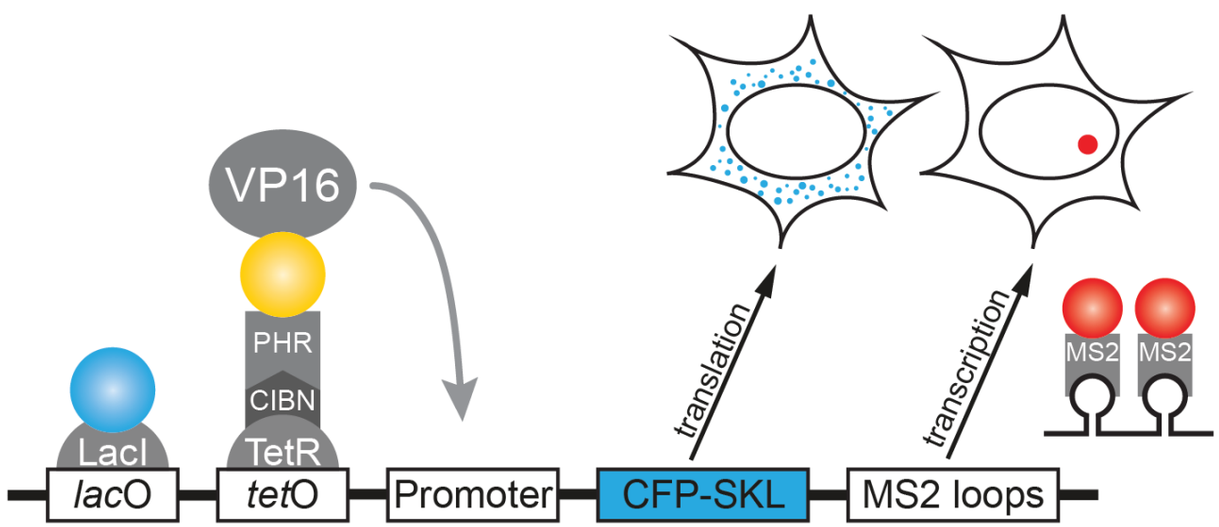


HDAC inhibition increases the fast activatable fraction

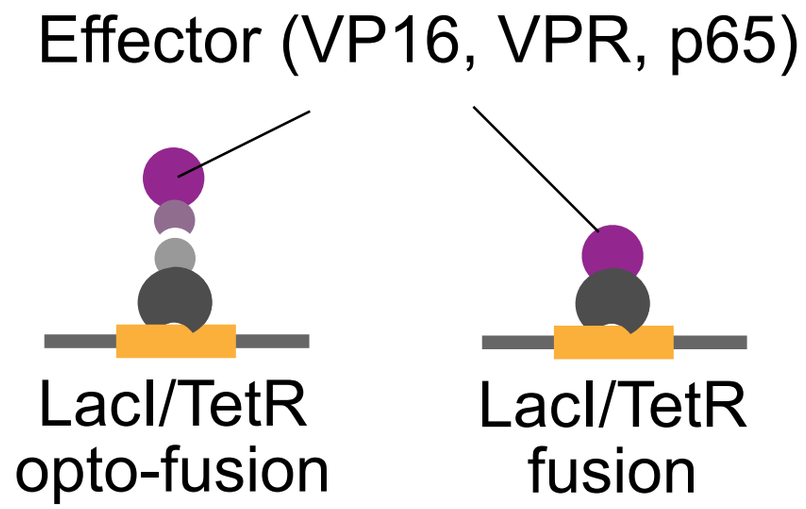


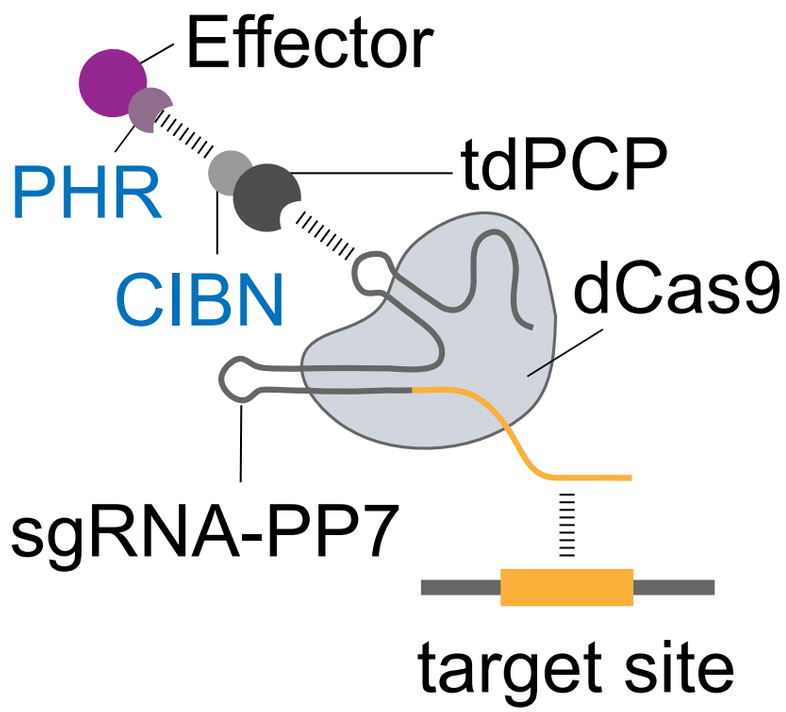


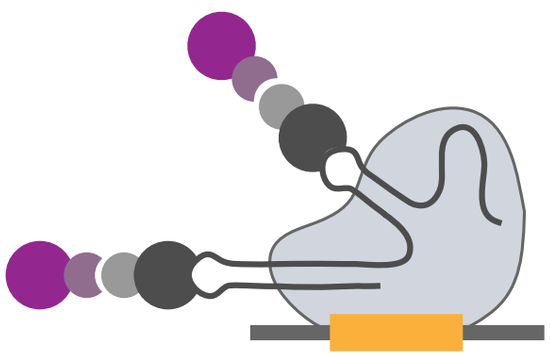


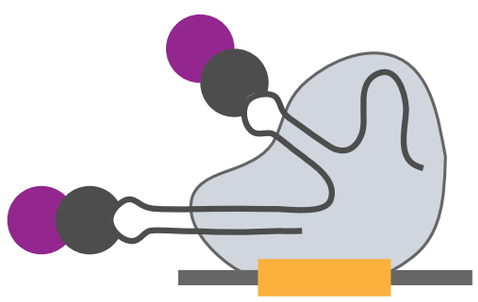


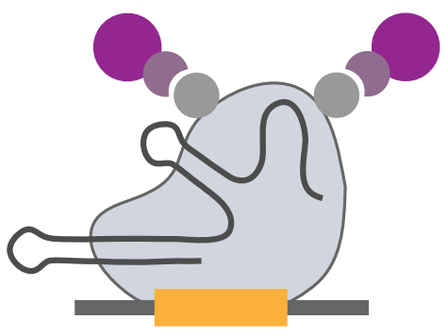
Assembling activation complexes around dCas9 for light-induced and reversible targeting of endogenous loci

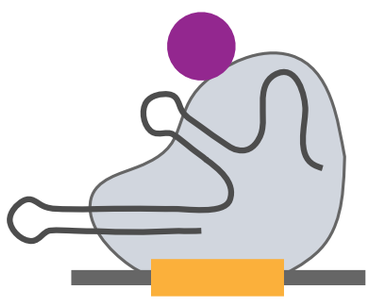




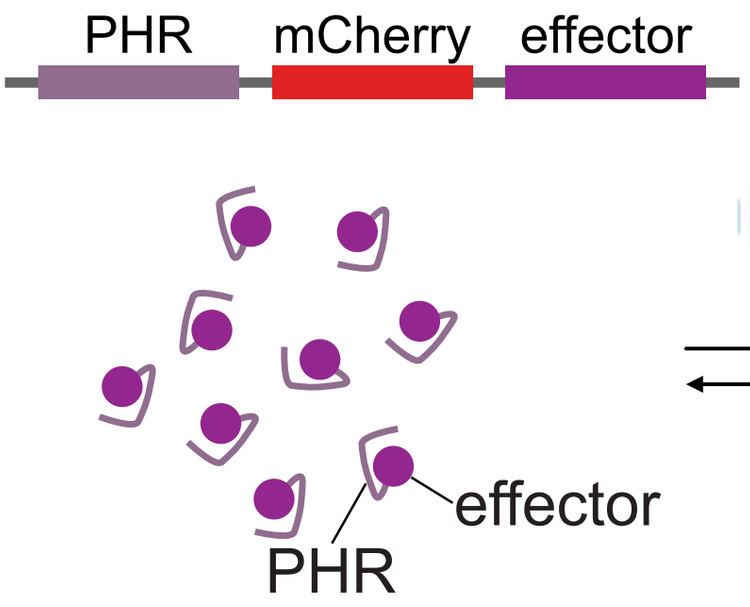

opto-loop

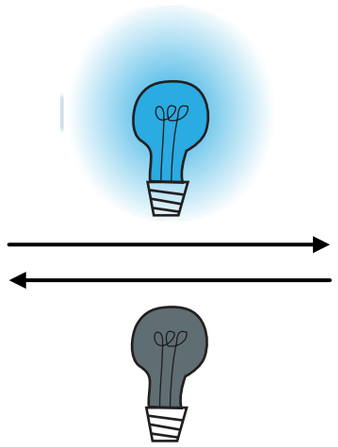

loop


opto-fusion

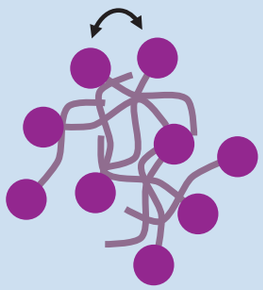

fusion

Modulating liquid-liquid phase separation propensity through optodroplet formation

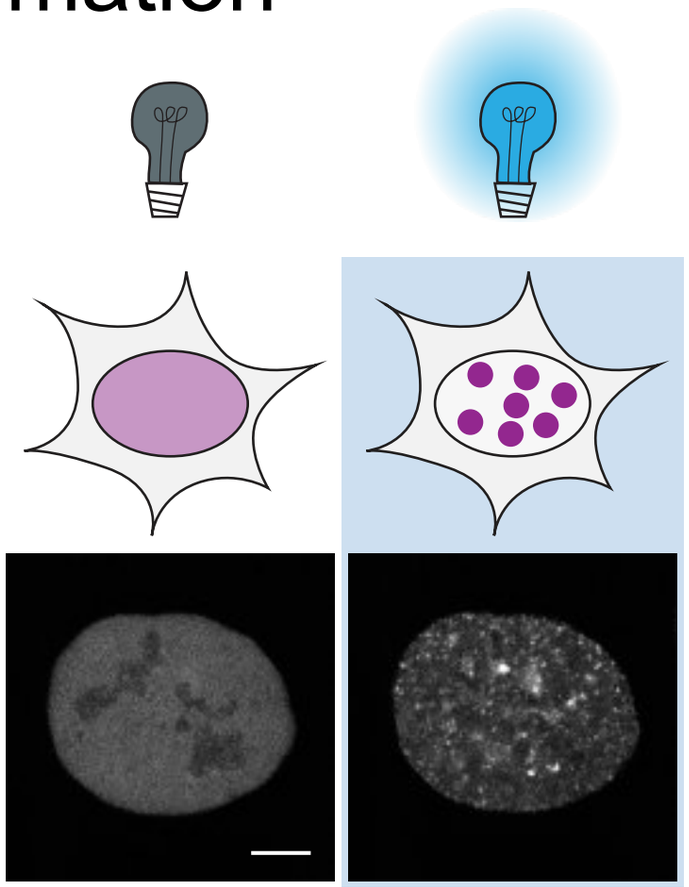




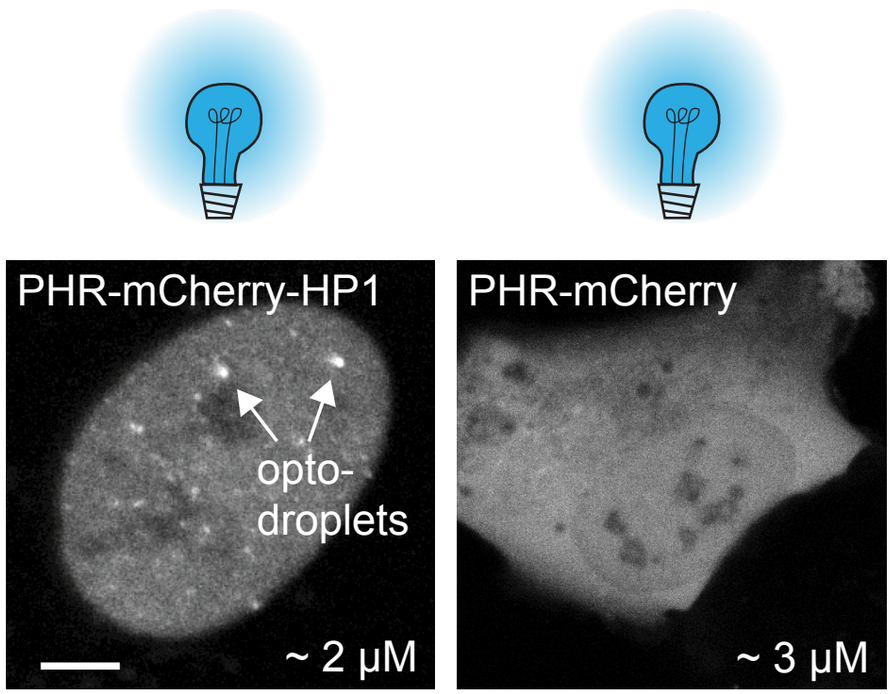
stabilizing interactions?



optodroplet



HP1α optodroplets

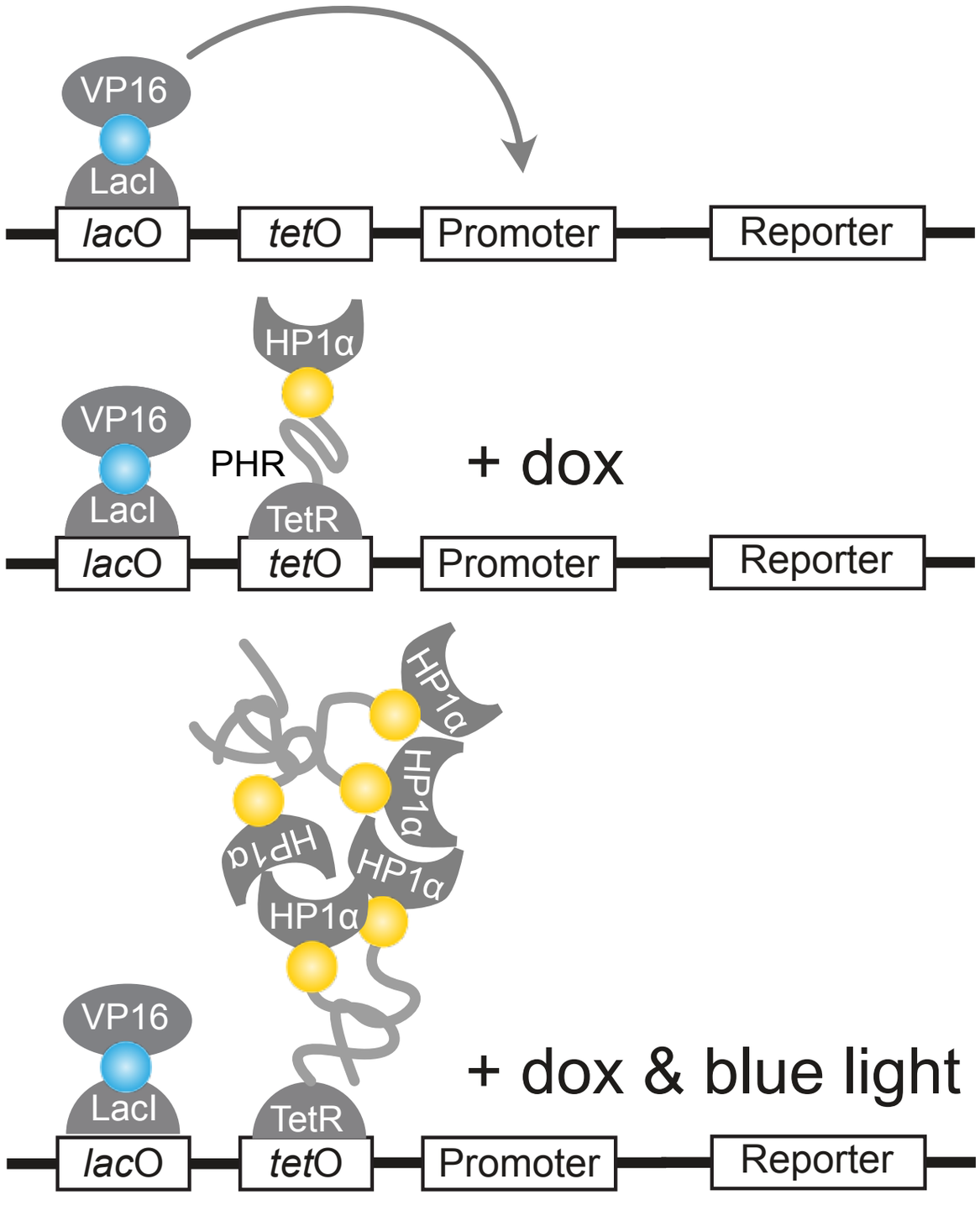


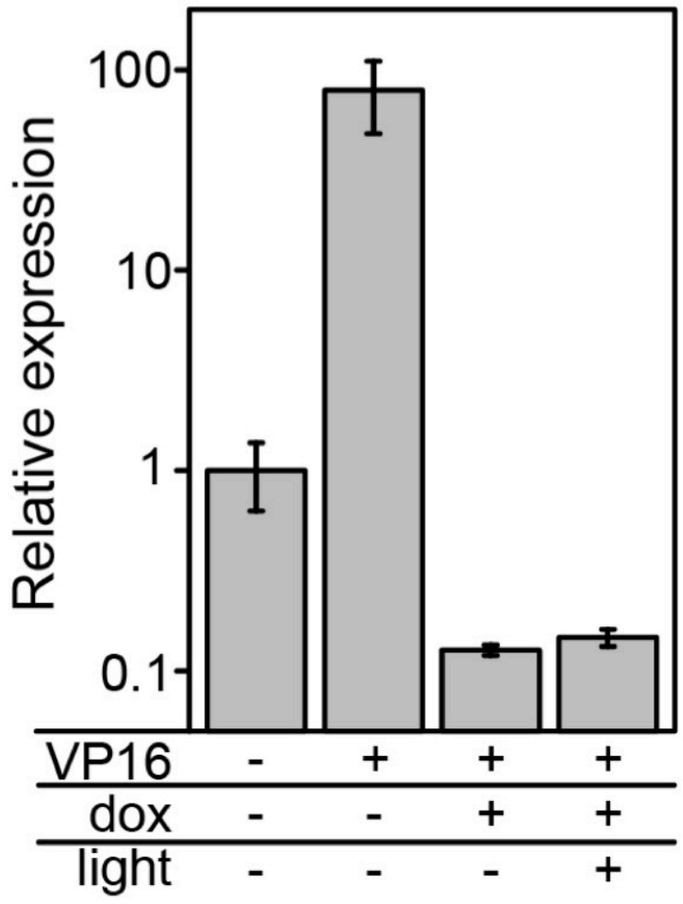
PHR fusion optodroplets are favored by

- fused multivalent effector
- high protein concentration
- high blue light intensity

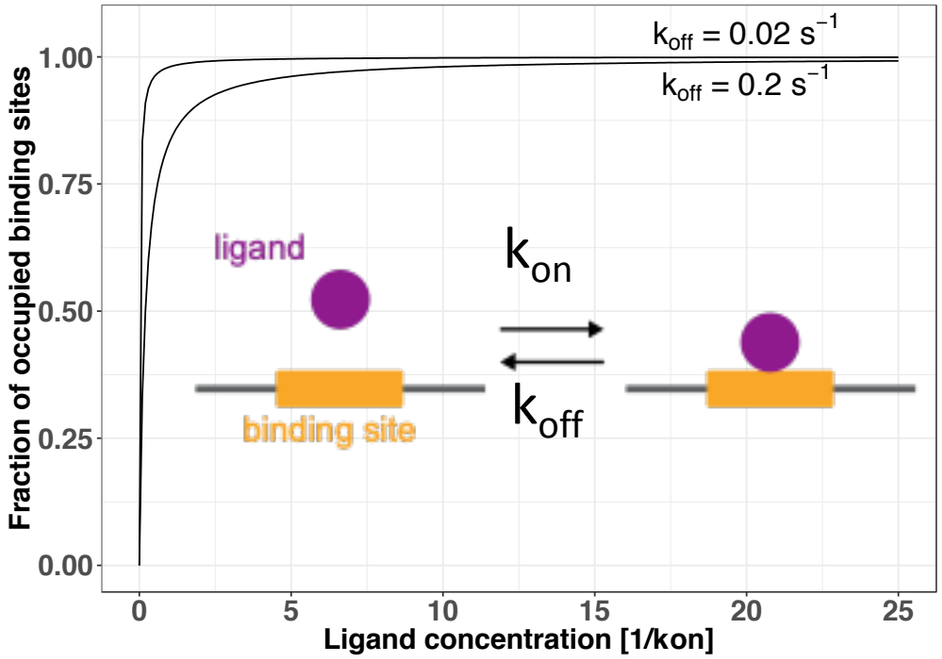
→ tunable system for droplet formation

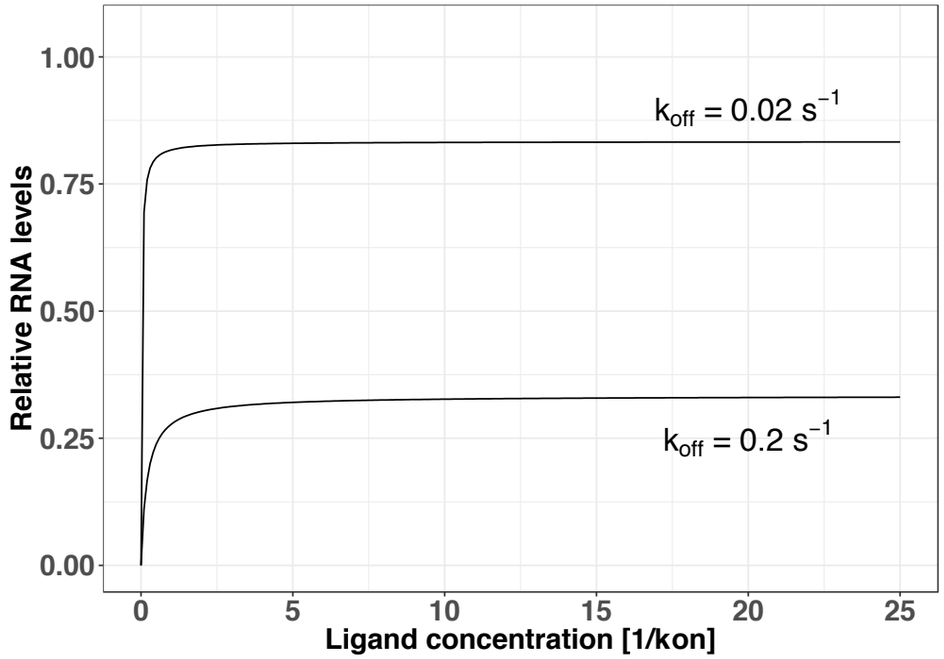
HP1α silences transcription independent of droplet formation

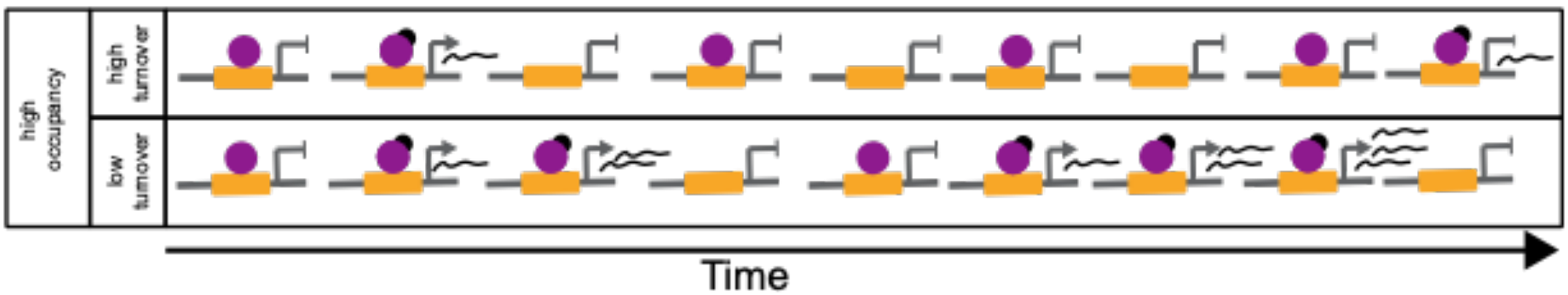




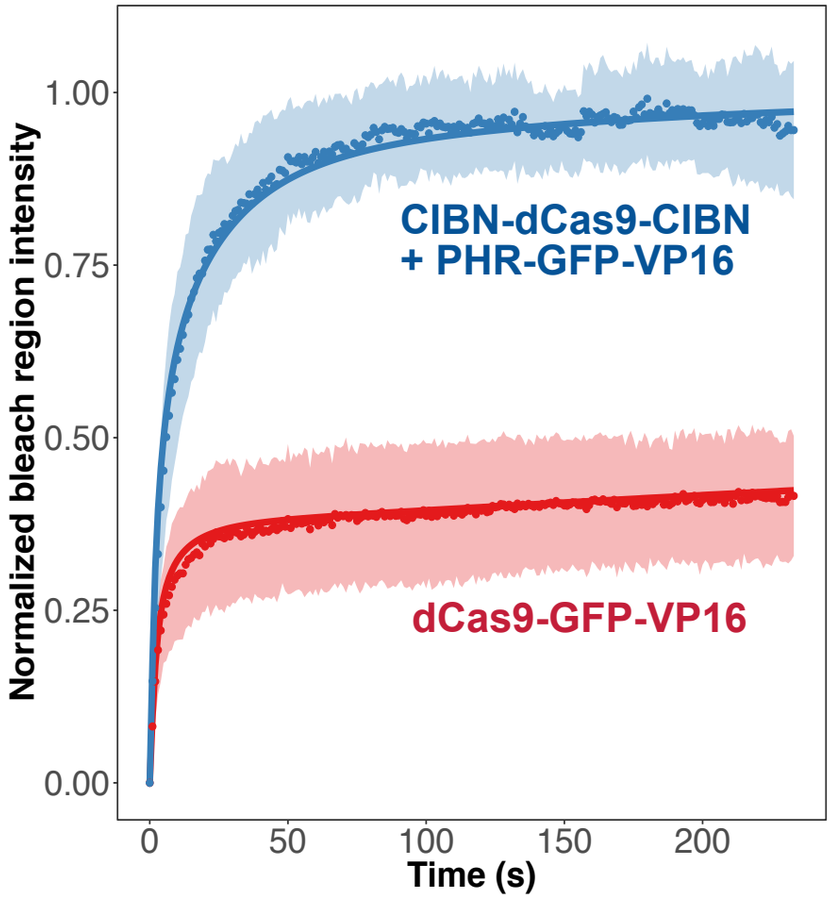
High activator turnover rate could lead to a reduced activation potential

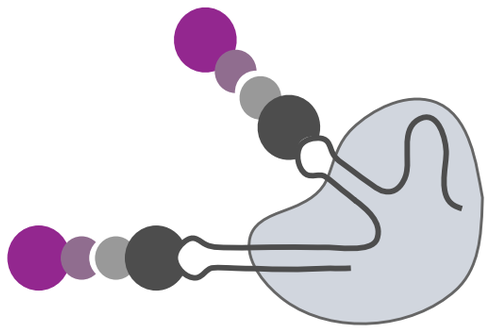




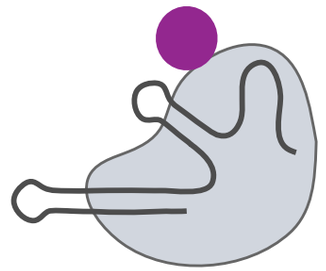


FRAP reveals large differences in turnover rates between dCas9 effector complexes





opto-loop

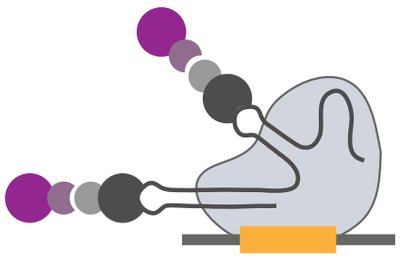


fusion

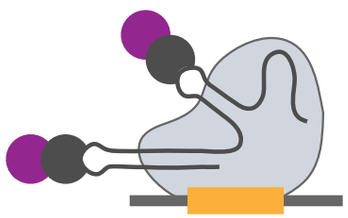
Effector turnover

High

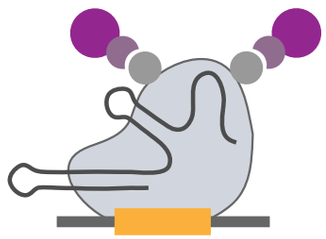
Low



opto-loop



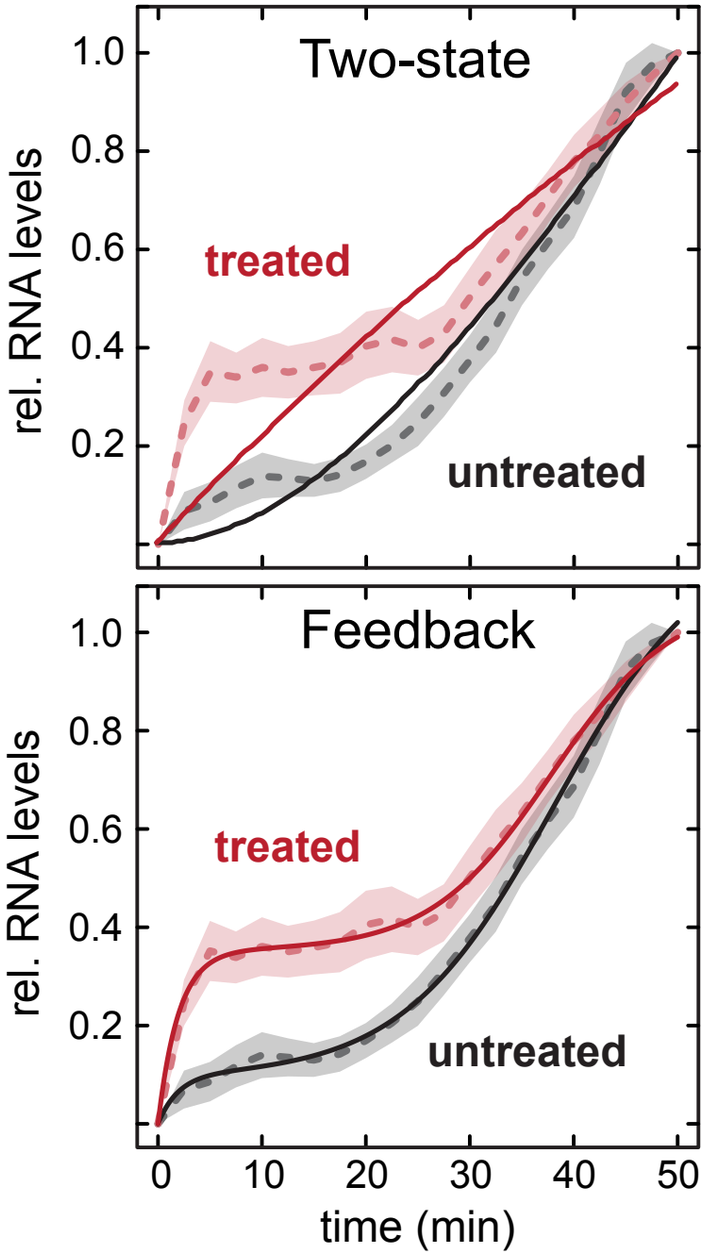
loop

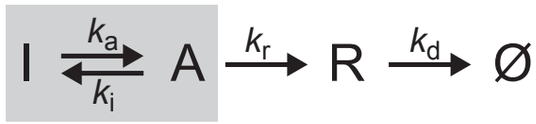


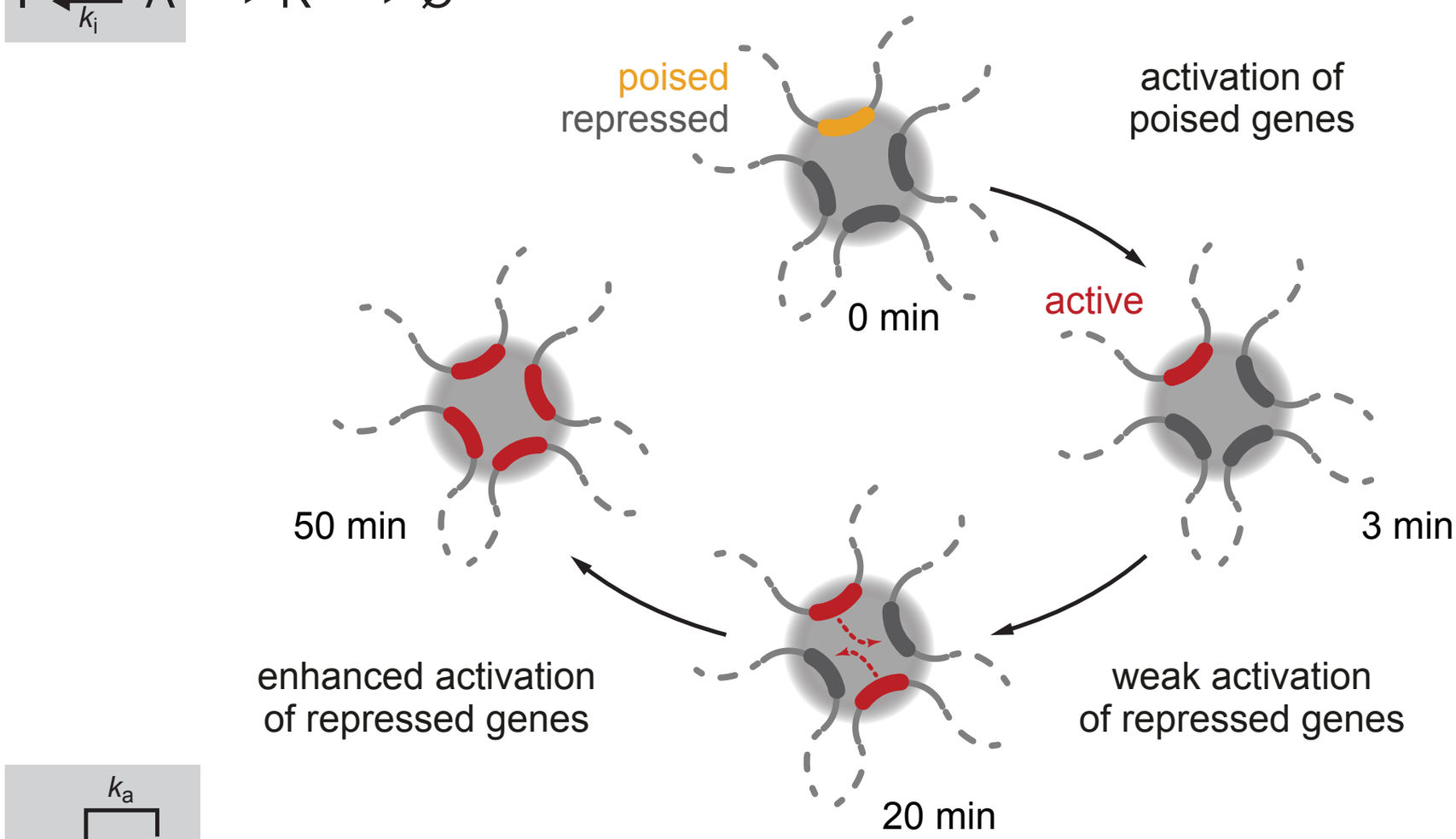
opto-fusion

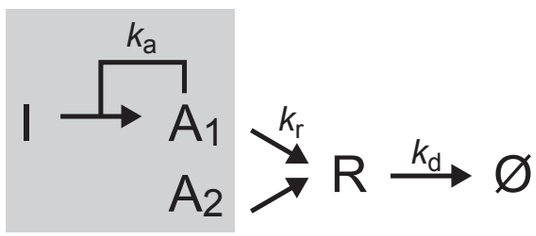
fusion

The activation kinetics indicate the presence of a feedback loop

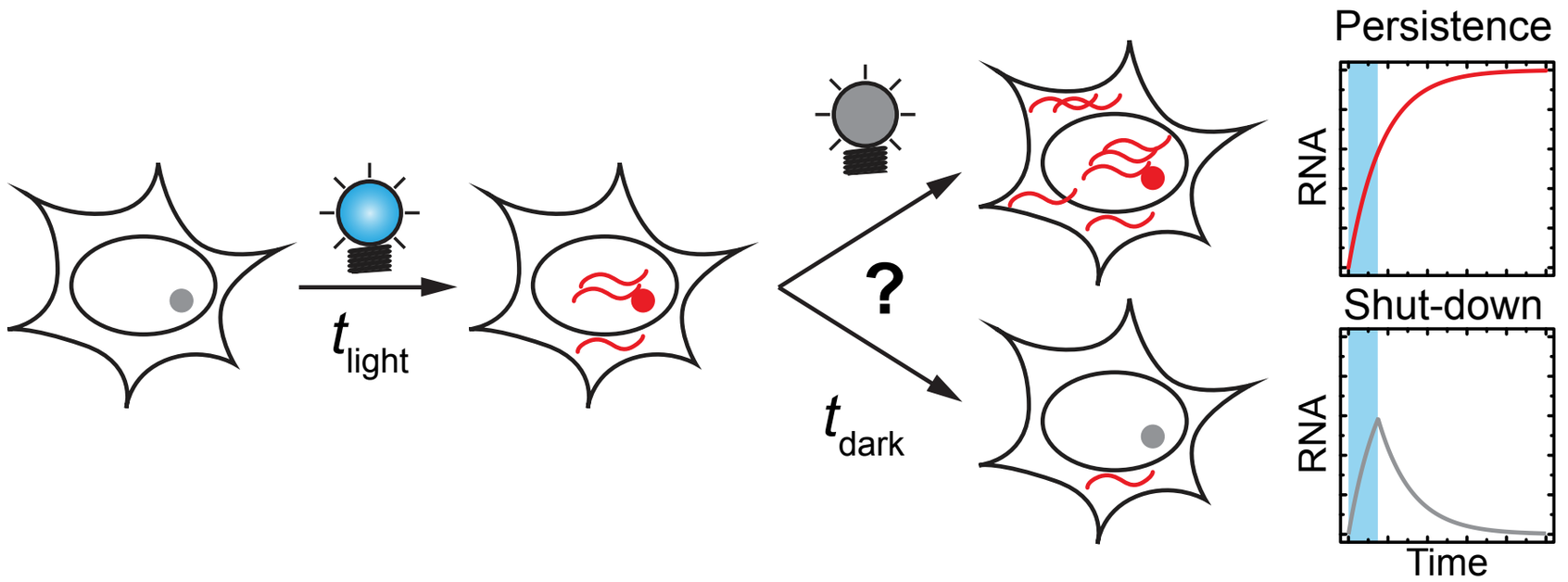


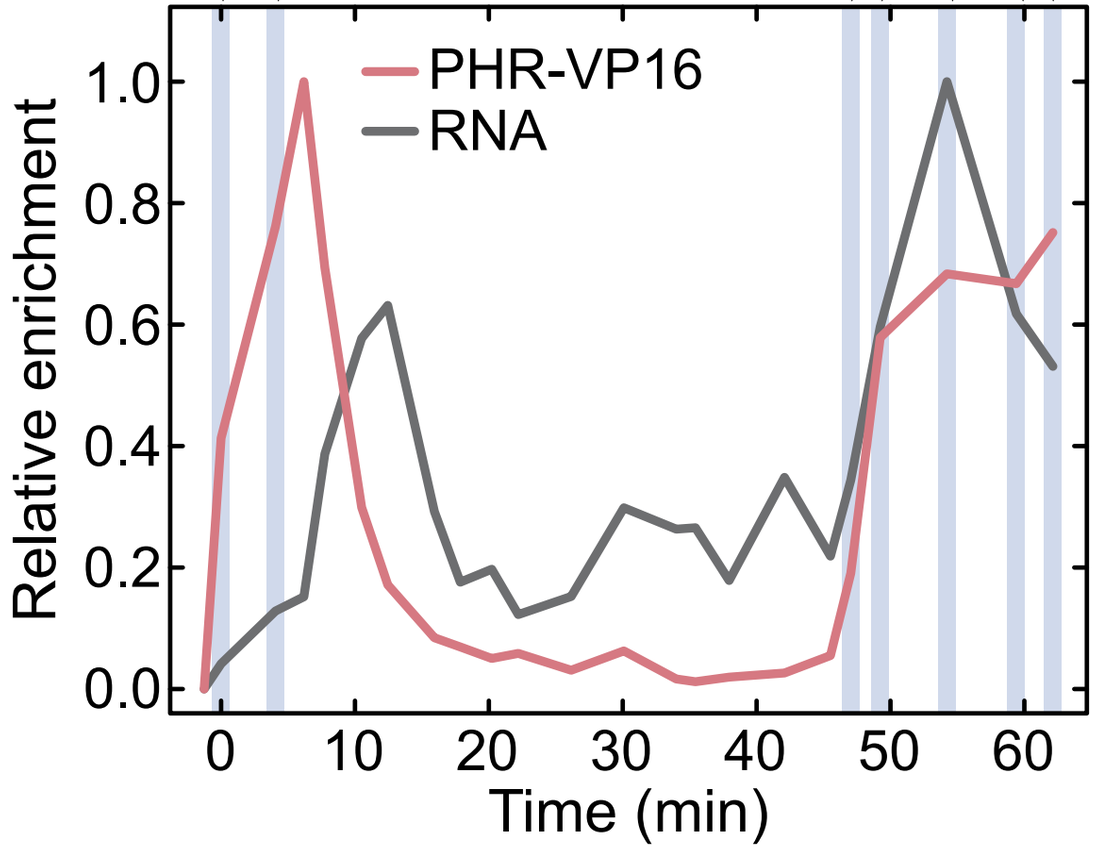






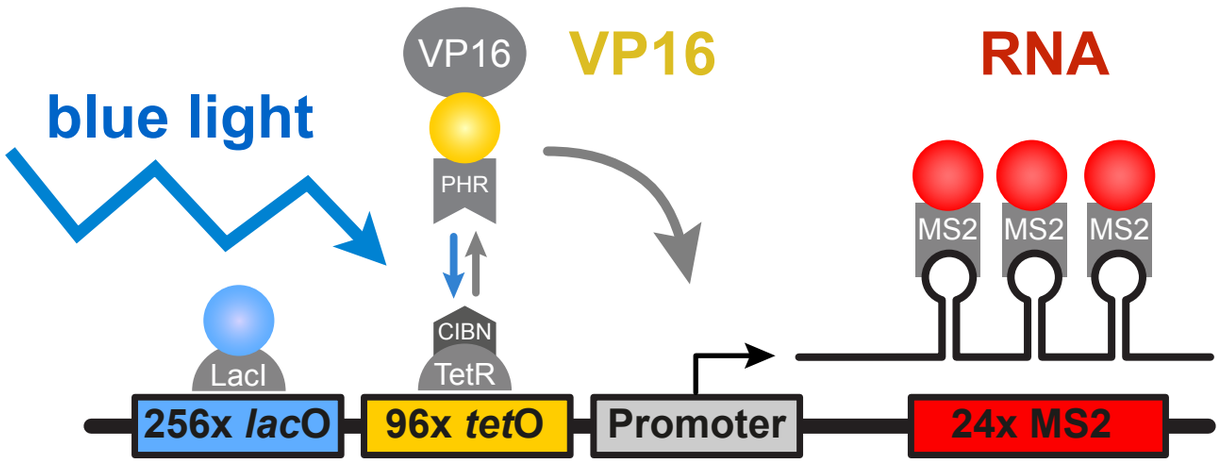
Transcription decays after an activating light pulse



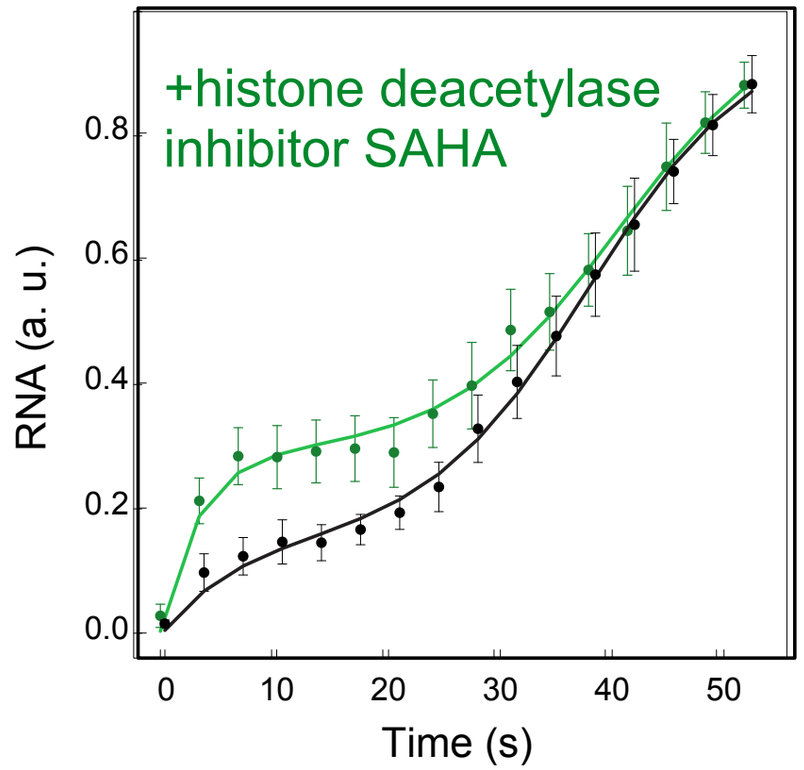


Light-induced measurements of transcription activation kinetics

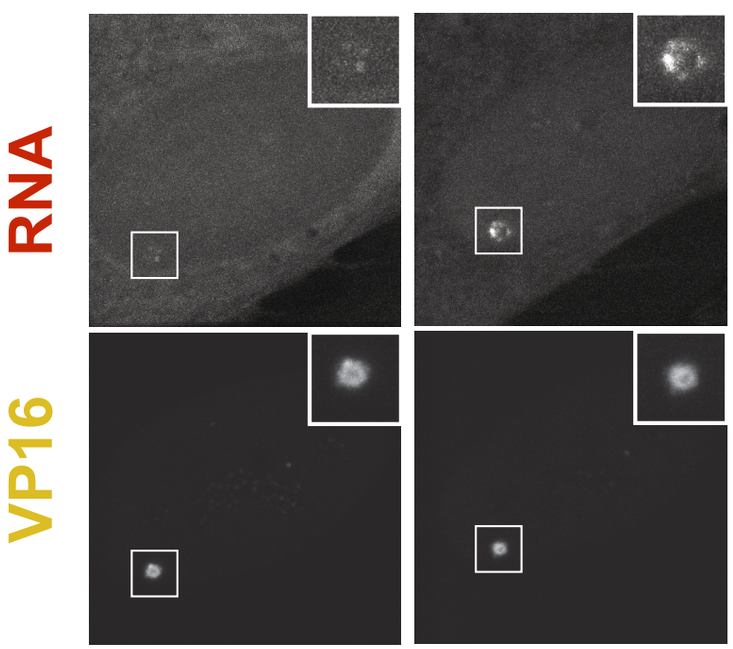
Reporter construct (200 copies) human U2OS cell line

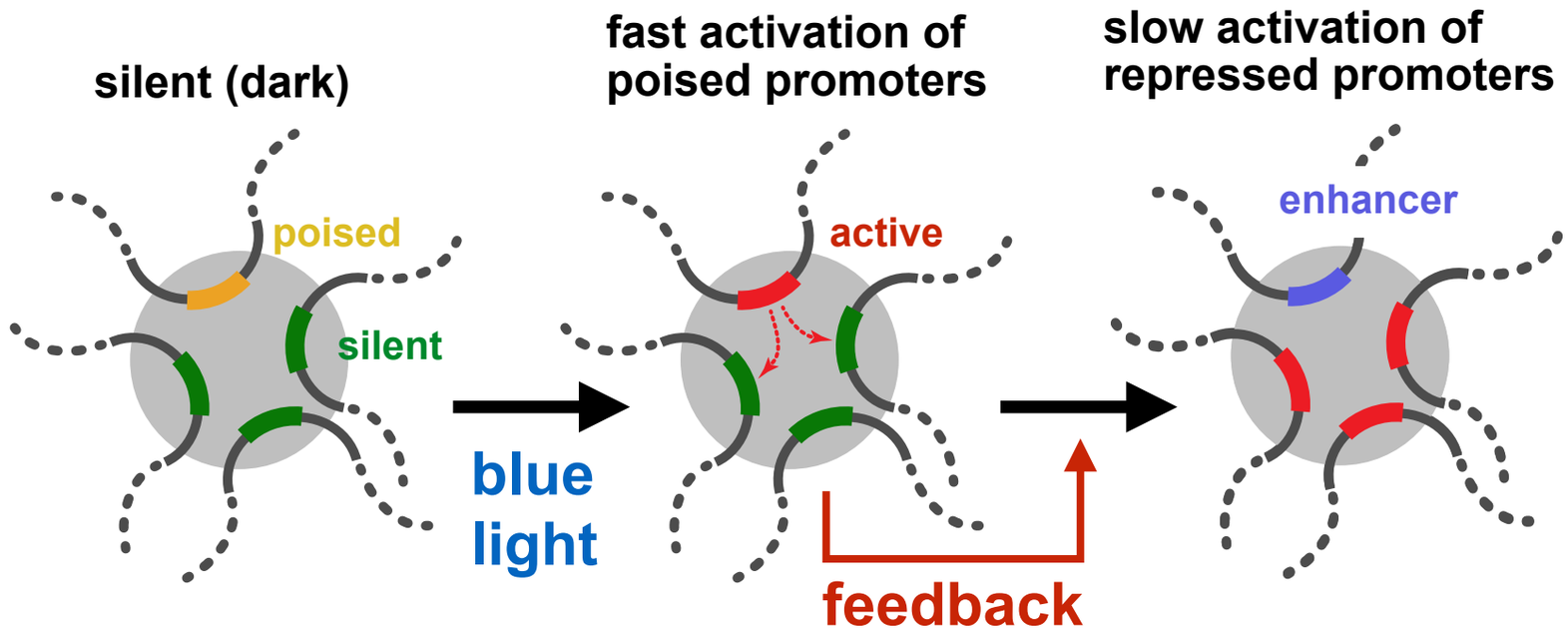


Transcription kinetics



Microscopy readout





Rademacher, Erdel, Trojanowski & Rippe, submitted