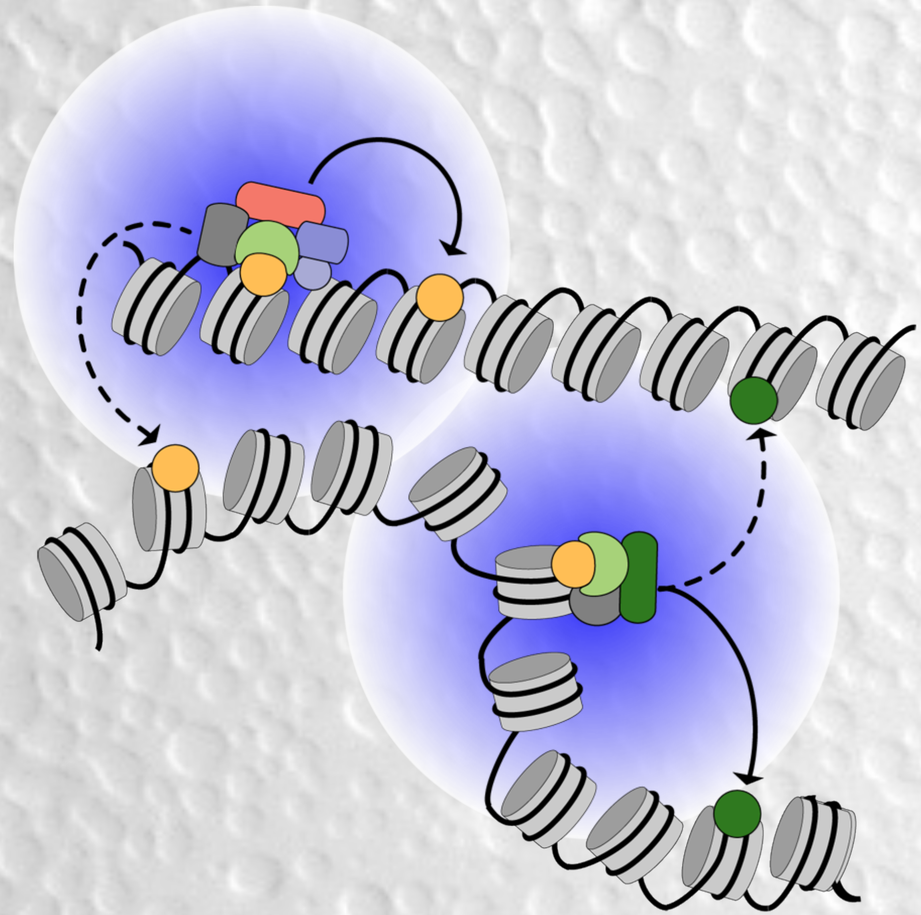
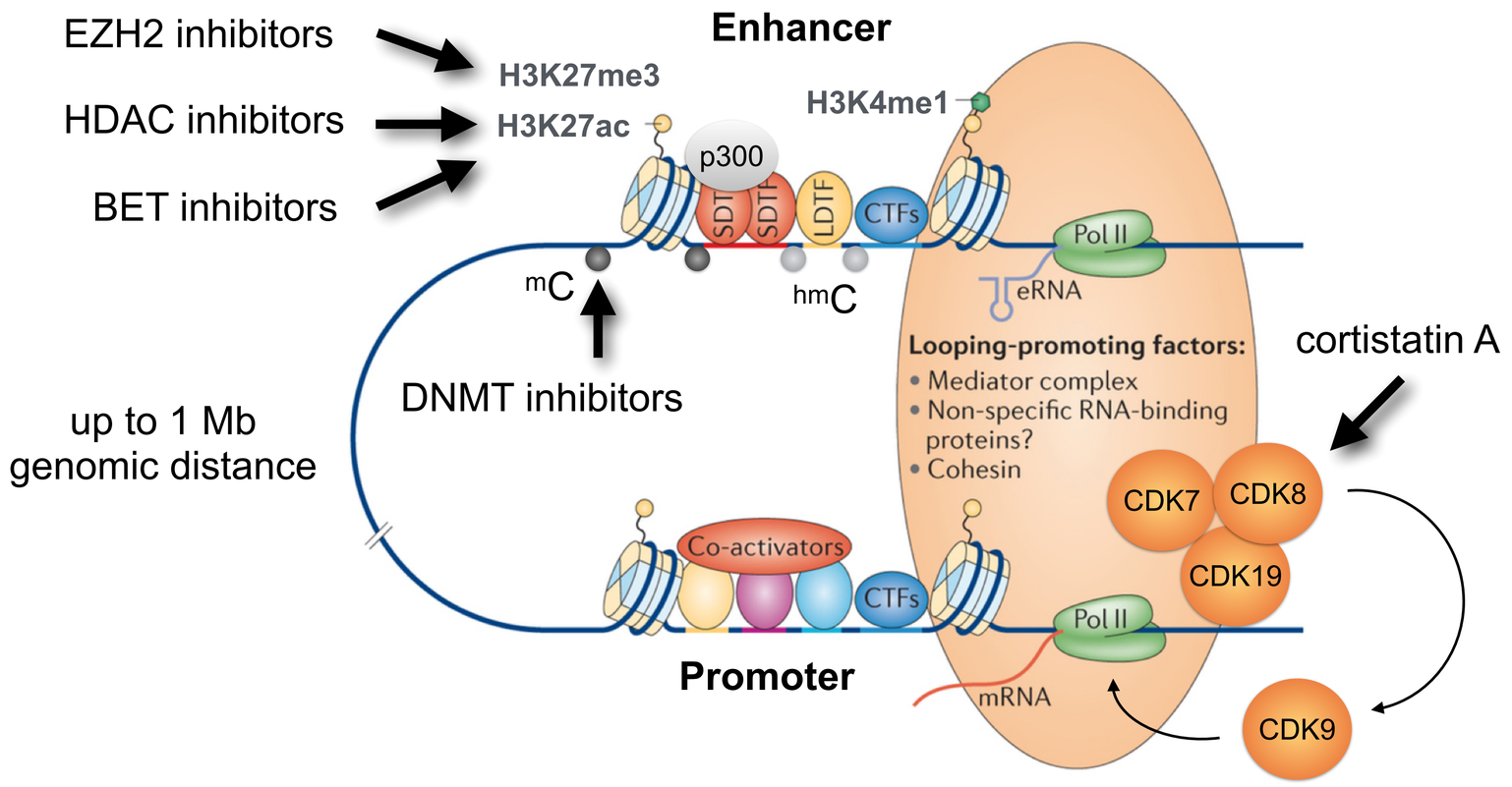
Interaction of DNA-bound proteins at a distance



More than **300 000** putative enhancers have been annotated in the human genome



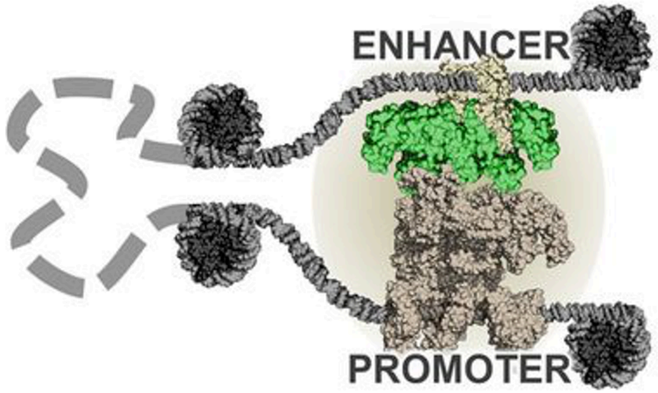
Cell type specific activity estimates

- ~20 000 coding genes (~54 000 including lncRNAs etc)
- 80 000 - 240 000 active enhancers
- typical: 1-2 target promoters per enhancer (~10 for some enhancers
- multiple enhancers for single promoter
- 300-500 “super-enhancers” > 10 kb

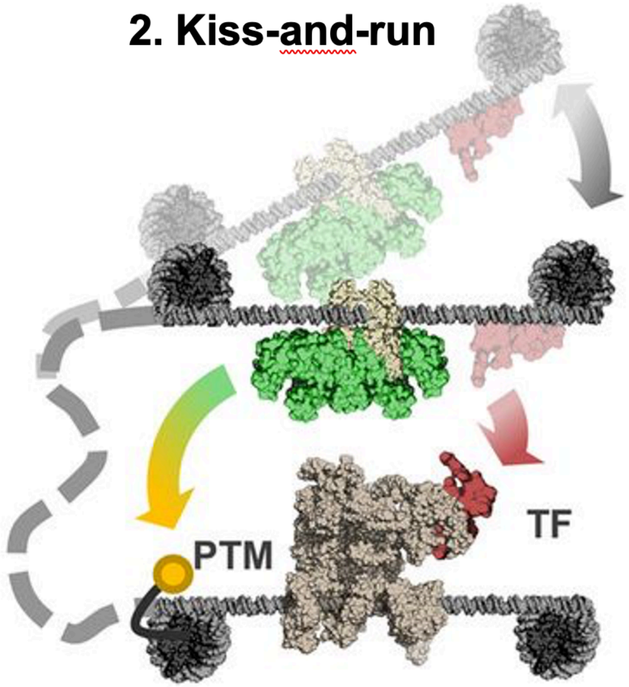
Heinz 2015, Nat Rev Mol Cell Biol
Roadmap Epigenomics Consortium 2015, Nature
FANTOM Consortium 2015, Nature

How do enhancers activate transcription?

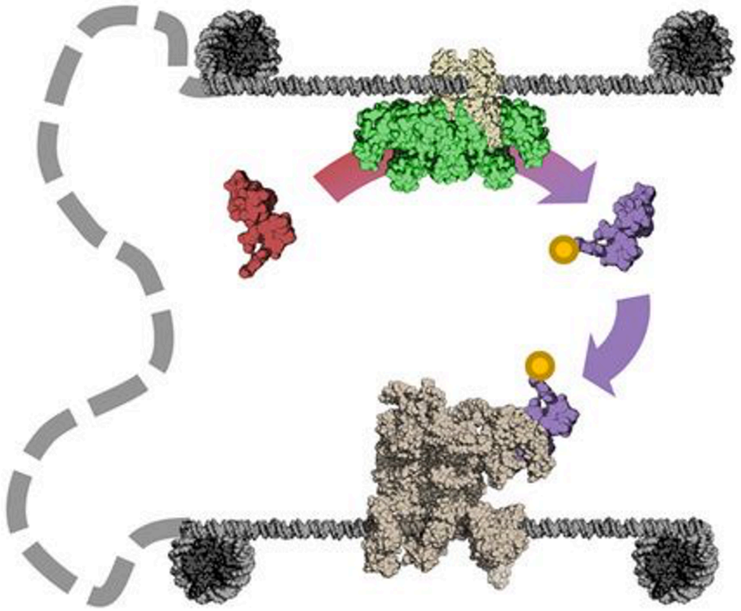
1. Stable contact



2. Kiss-and-run

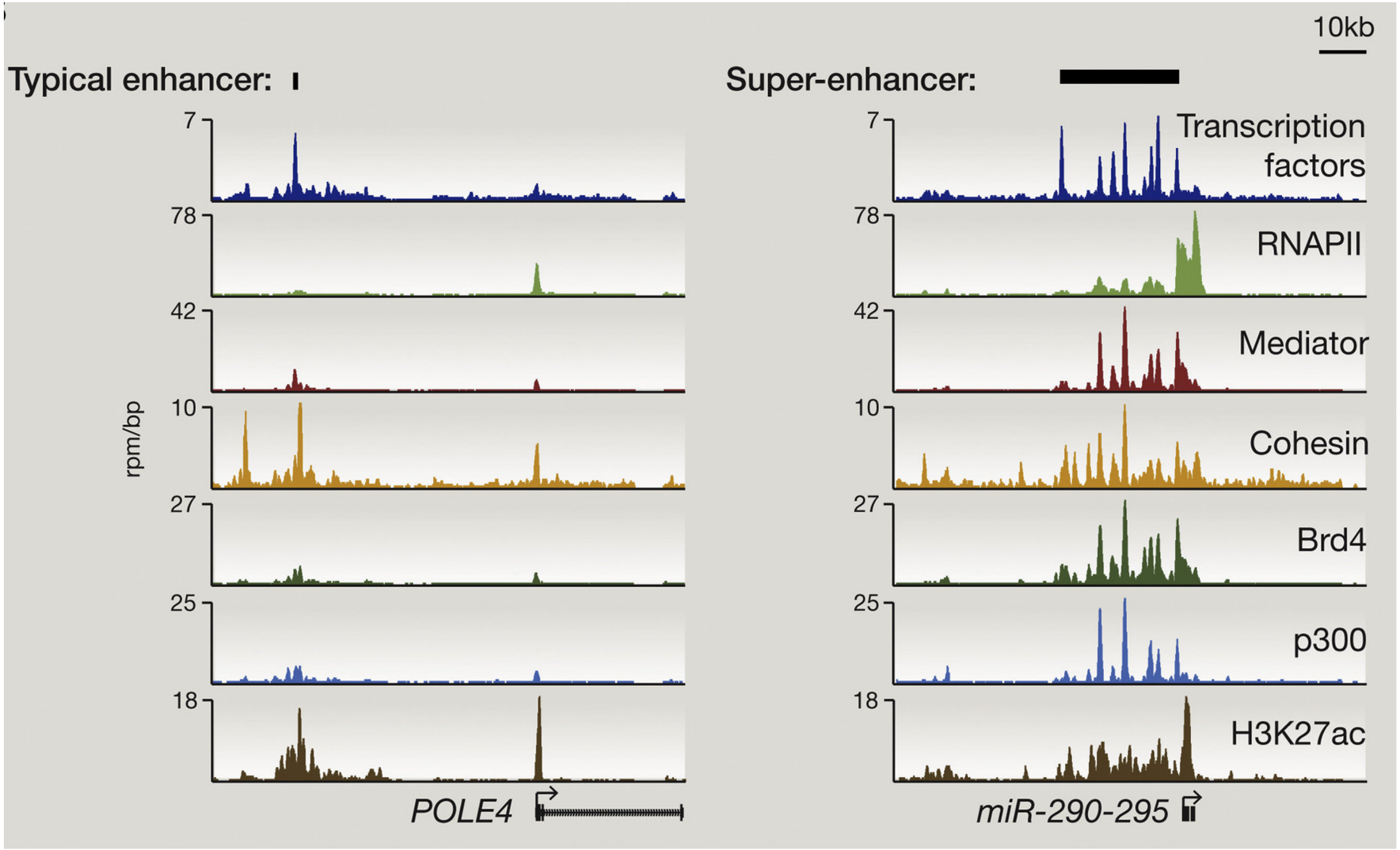


3. Transcription factor activity gradient



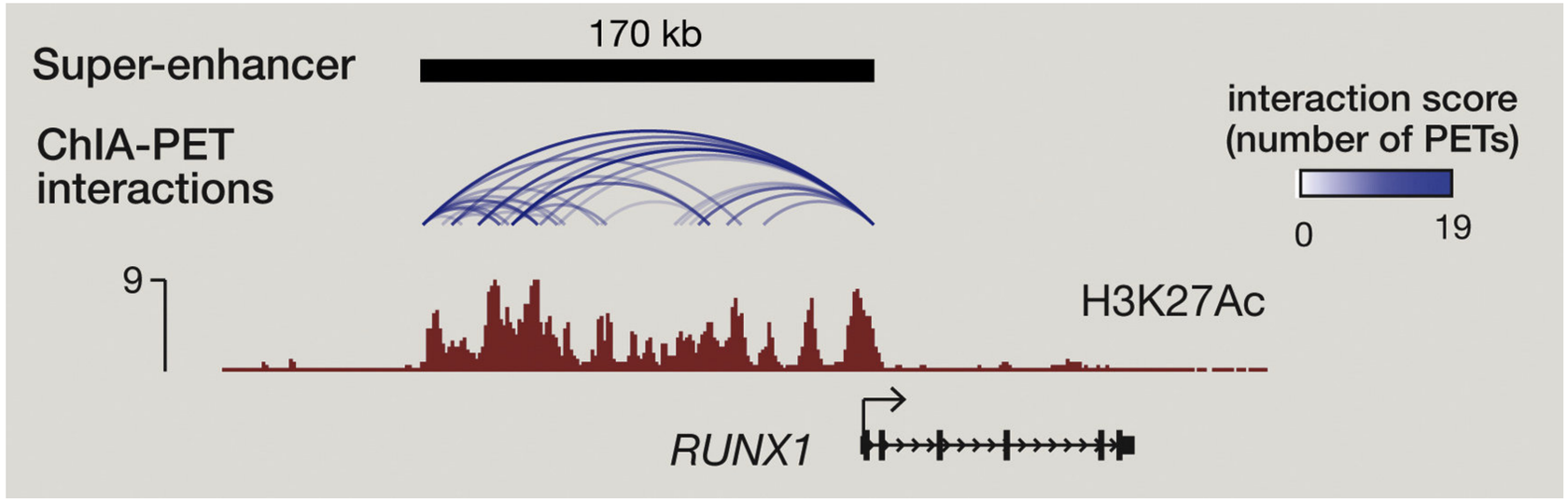
Karr et al. 2022, Genes & Dev 36, 7-16, https://doi.org/10.1101/gad.349160.121

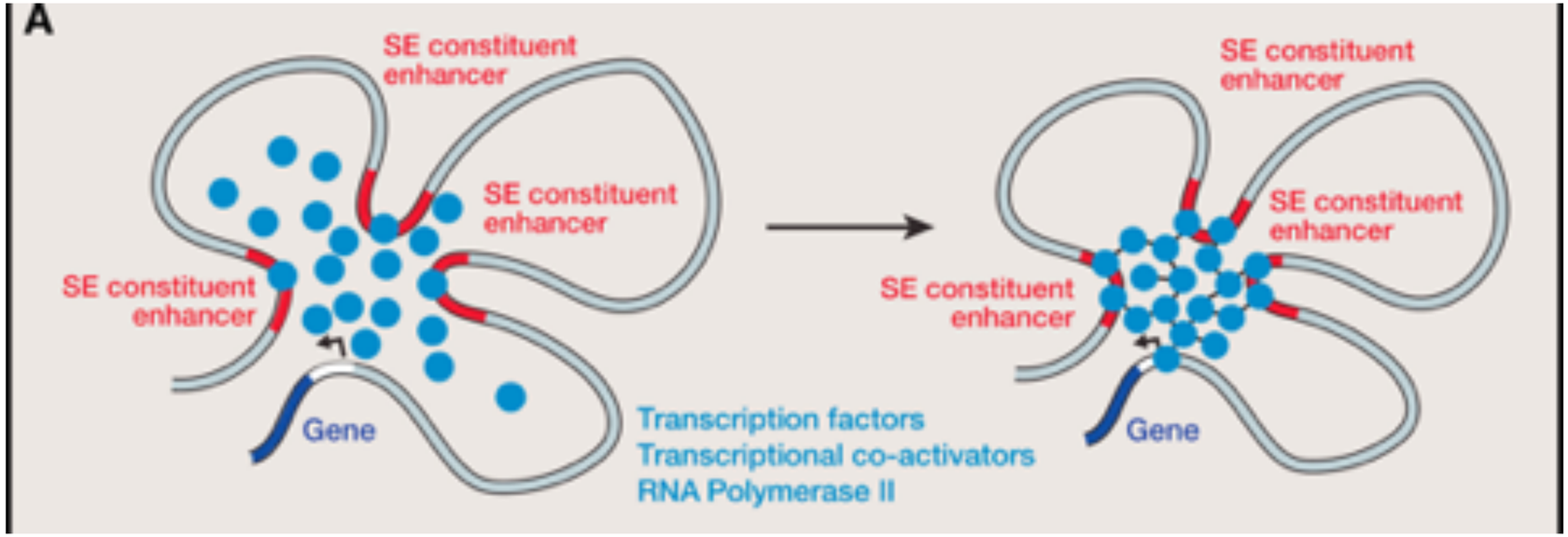
Enhancers vs “super-enhancers” (≥ 10 kb)



Hnisz D,… ,Young RA (2013) Super-enhancers in the control of cell identity and disease. *Cell* **155:** 934-947

Do super-enhancers activate via a phase separation mechanism?



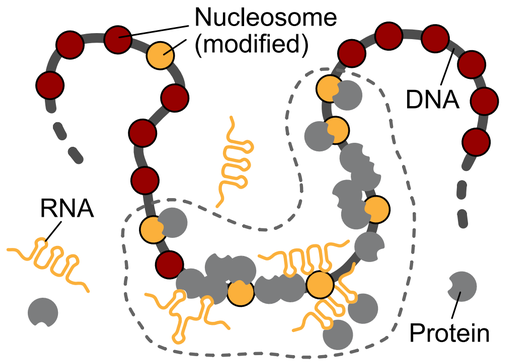


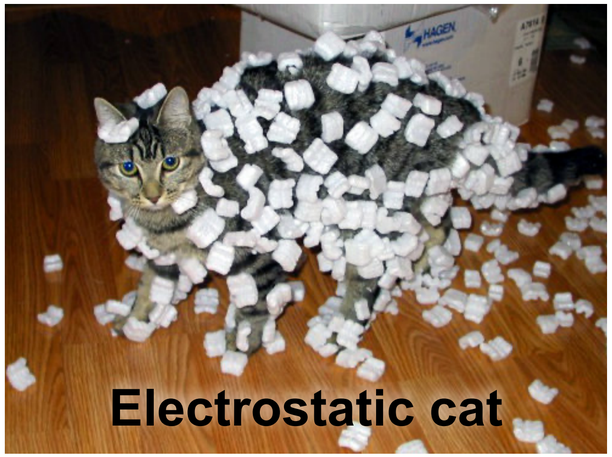
Hnisz D, Shrinivas K, Young RA, Chakraborty AK, Sharp PA (2017) A Phase Separation Model for Transcriptional Control. *Cell* **169:** 13-23

Mechanisms to form chromatin subcompartments

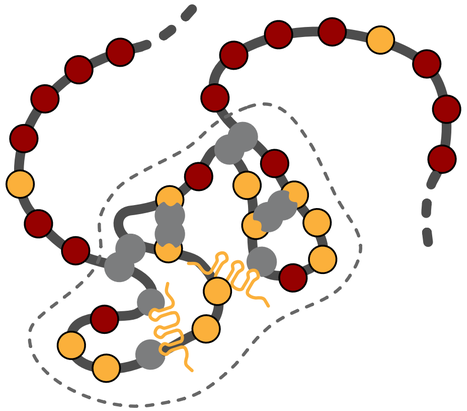
The “null hypothesis”

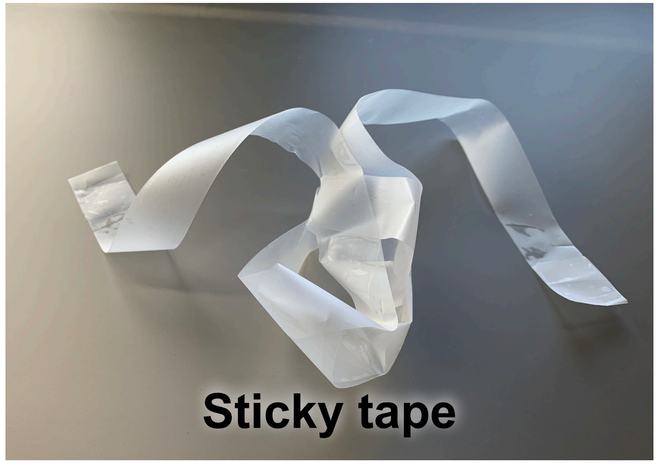
Binding site cluster



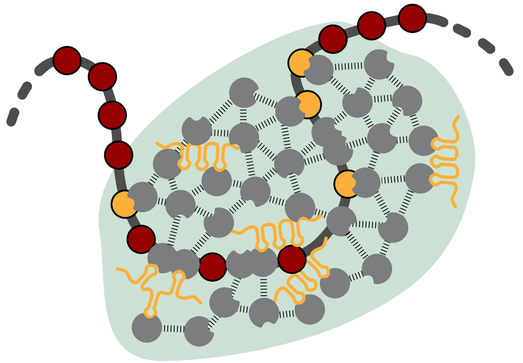


Chromatin bridging interactions



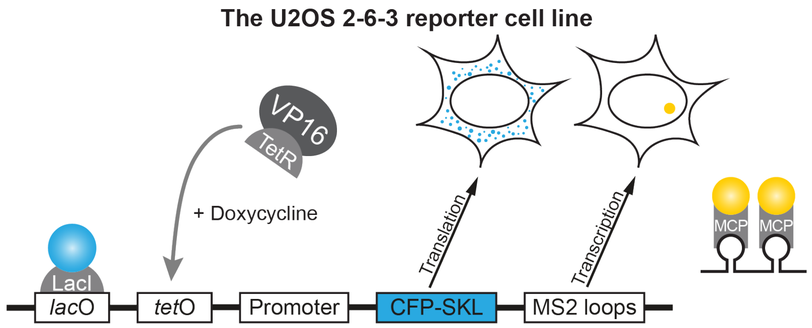


Liquid-liquid phase separation



Erdel & Rippe 2018 *Biophys J;* Frank & Rippe 2020 *J Mol Biol;* Rippe 2022 *Cold Spring Harb Perspect Biol*

Studying transcription factor droplets in living cell at a reporter gene array

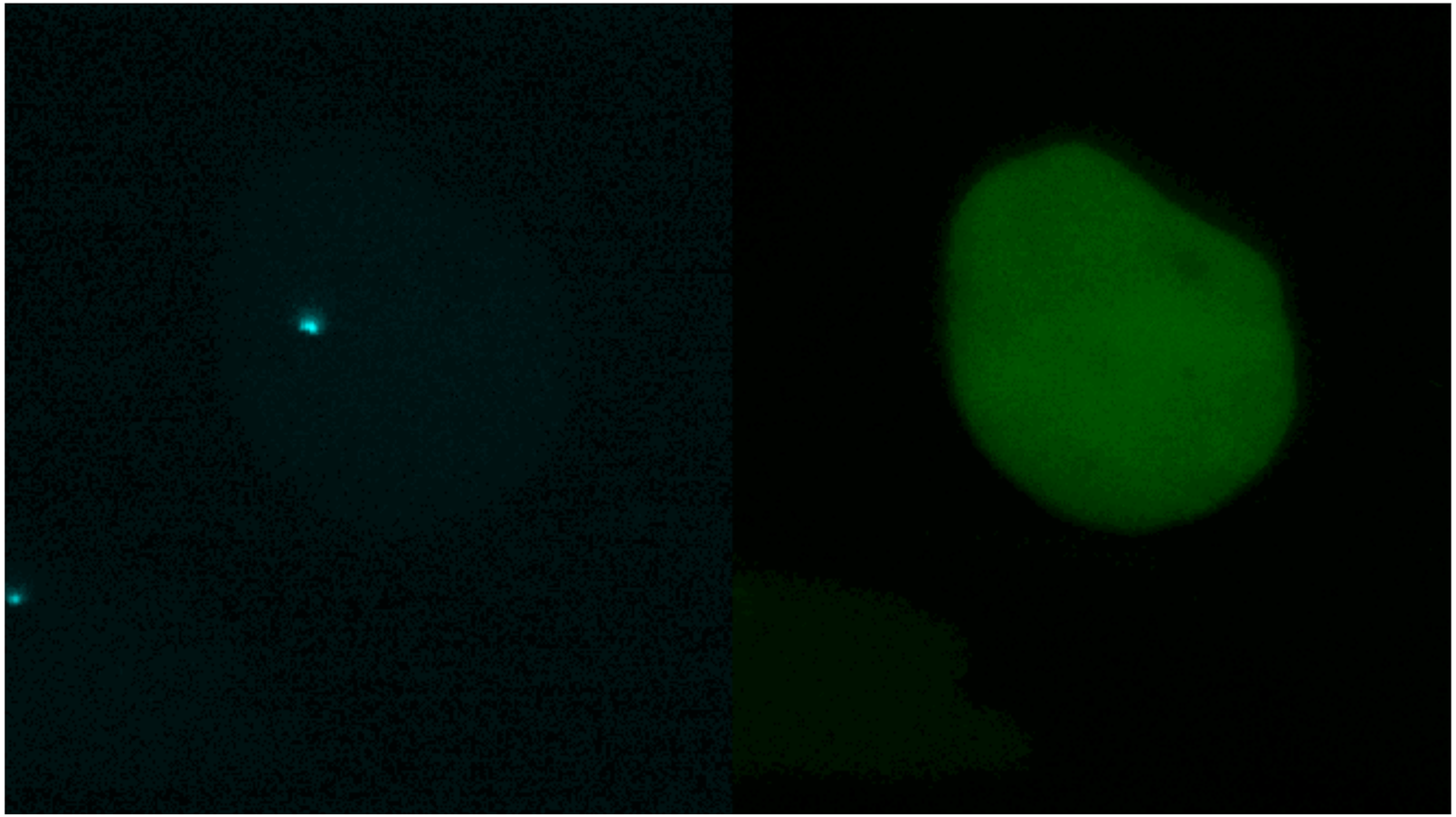


Heterochromatic locus:
- array with ~200 repeats
- compact chromatin state
- H3K9me3
- HP1 enriched
- Activated by VP16/VPR

Janicki *et al.* (2004) *Cell* 116:683-698.

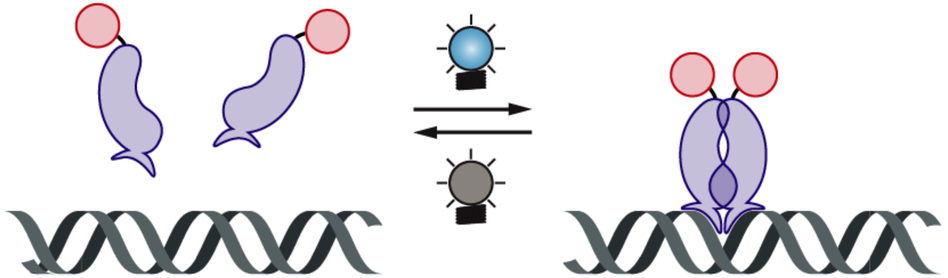
CFP-LacI (chromatin)
CFP (translated protein)

MS2-YFP (RNA)

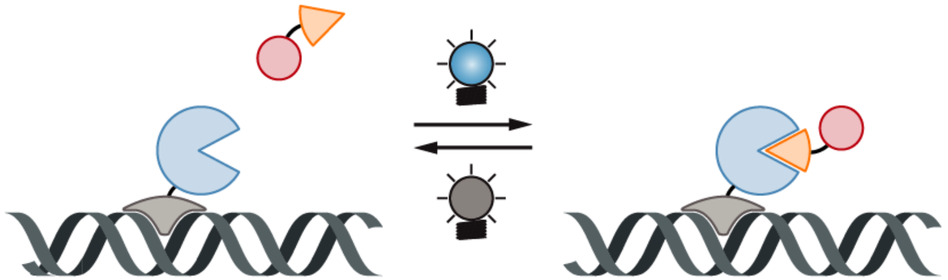


Optogenetics to control proteins in living cells with light

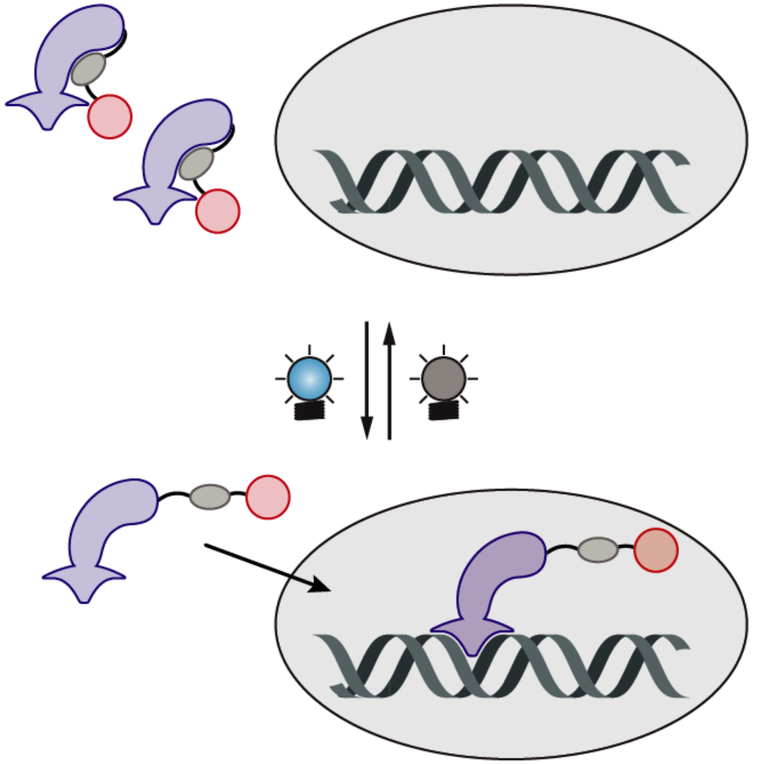
Homodimerization



Heterodimerization



Translocation to the nucleus



LOV domain-containing protein

PHR/CIBN

effector

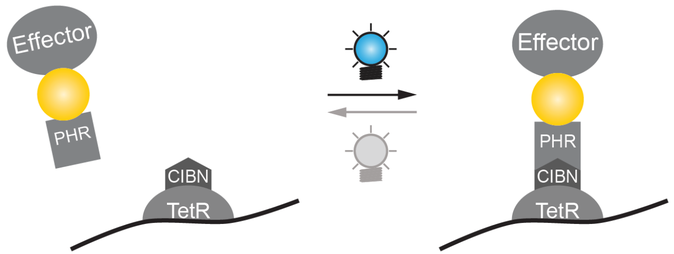
DBD

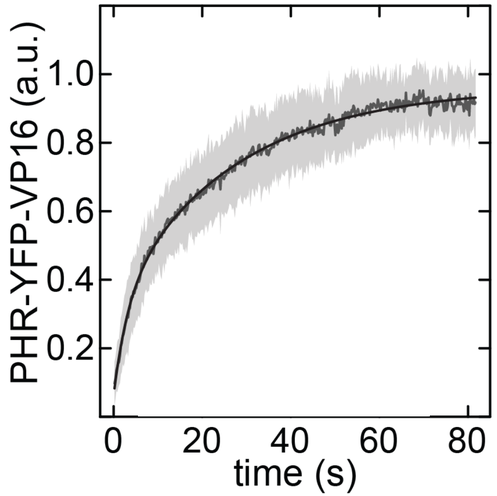
NLS

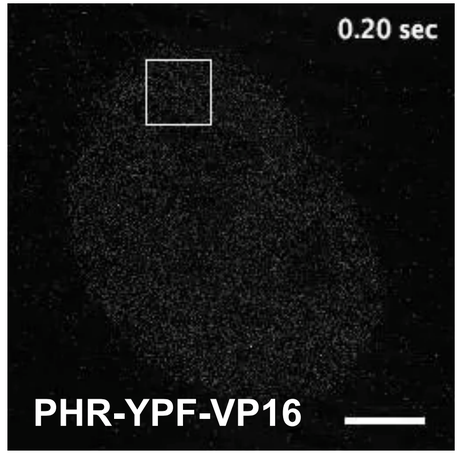
Tischer & Weiner (2014) *Nature Rev Mol Cell Biol* 15, 551-558.

Blue light induced recruitment of transcription activators within seconds

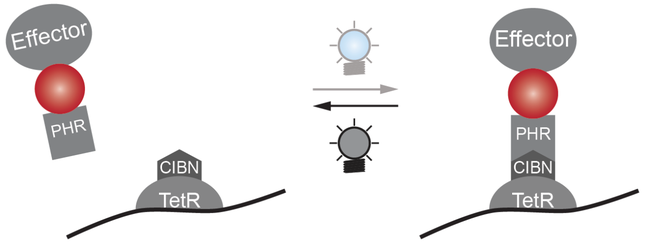
Binding within seconds

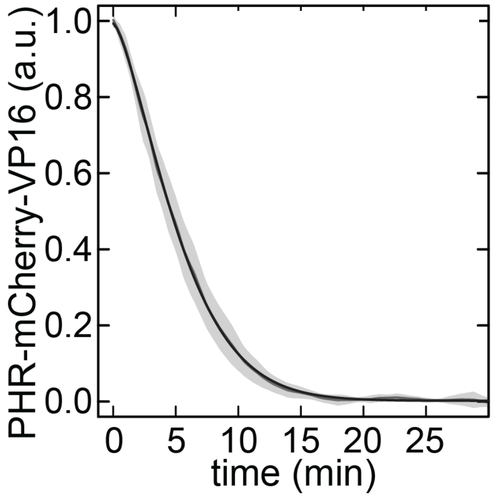






Dissociation within minutes





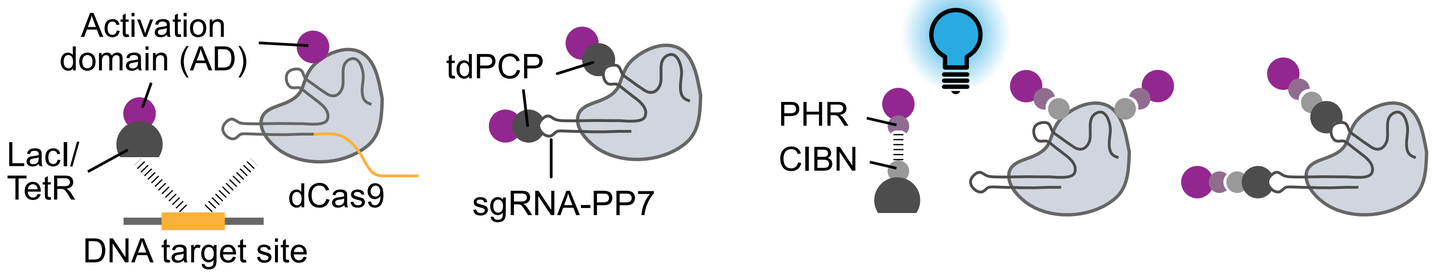
CIBN TetR iRFP713 localizer
+ PHR-mCherry effector

	Effector	Time $t_{on/off}$
Binding	VP16	8.0 ± 5.3 s
	NLS	9.2 ± 3.9 s
Disso-ciation	VP16	4.4 ± 0.8 min
	NLS	5.1 ± 0.5 min

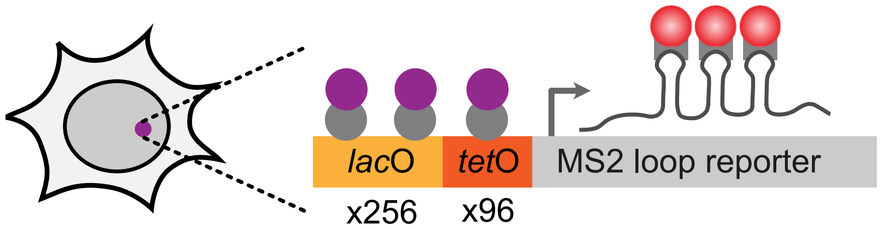
Rademacher 2017 *J Cell Sci*
Rademacher *Meth Mol Biol*

Controlling the recruitment of transcription activators to a reporter array

TF constructs with different ADs und DNA binding modules

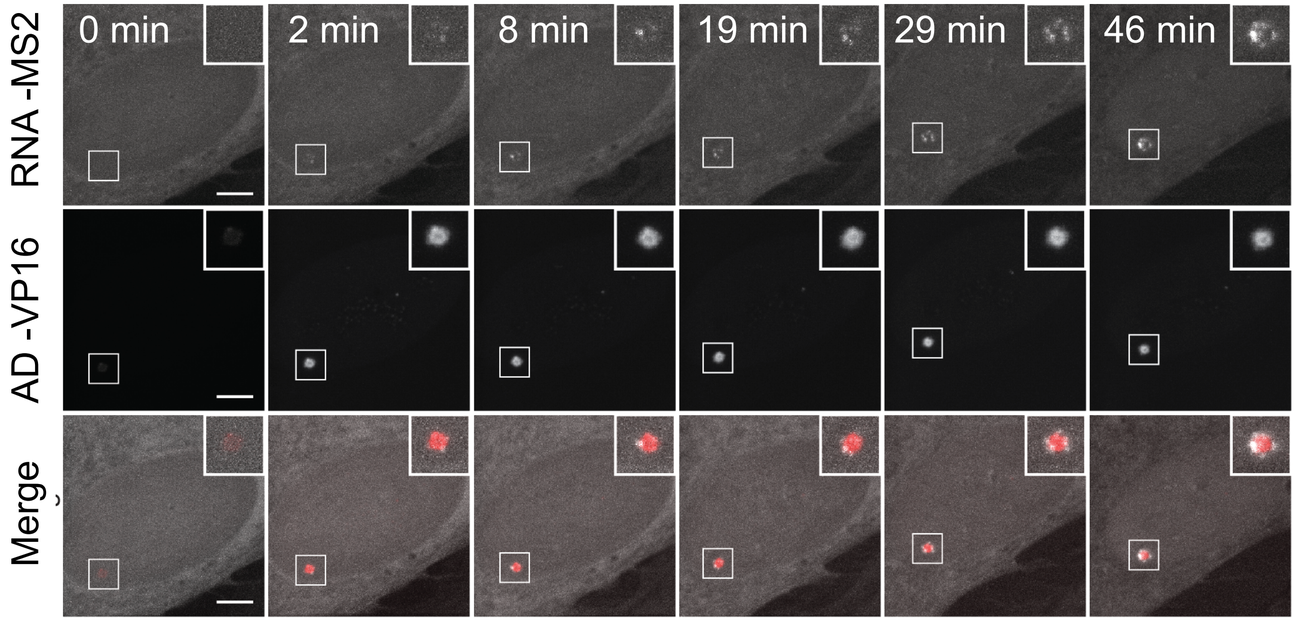


U2OS cell line with reporter array (~200 copies)



Janicki 2004 *Cell*

Transcription induction by light induced binding of the VP16 AD



Rademacher 2017 *J Cell Sci,* Trojanowski 2019 *Meth Mol Biol*

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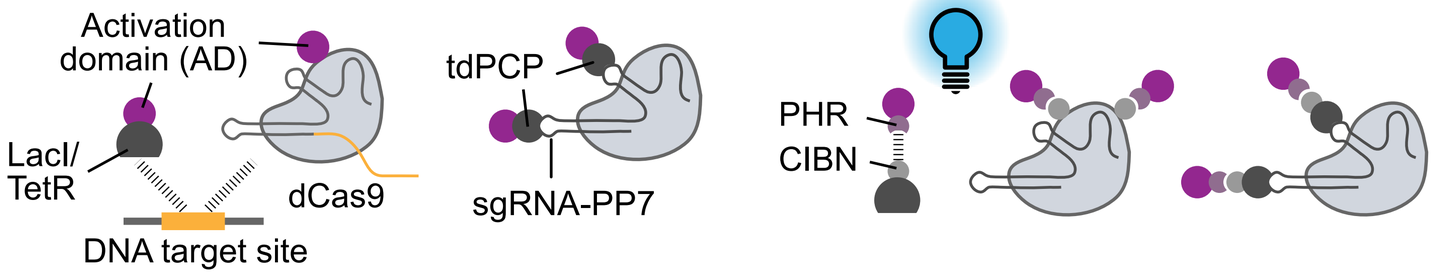

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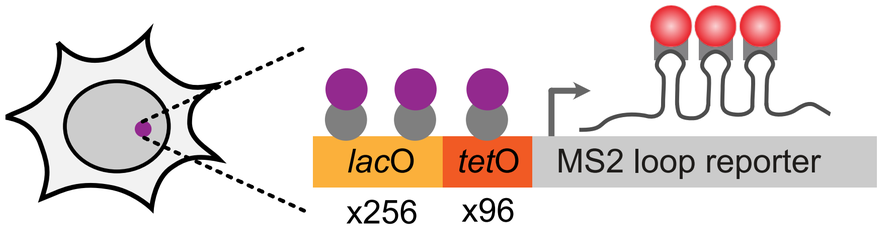

Janicki 2004 *Cell*

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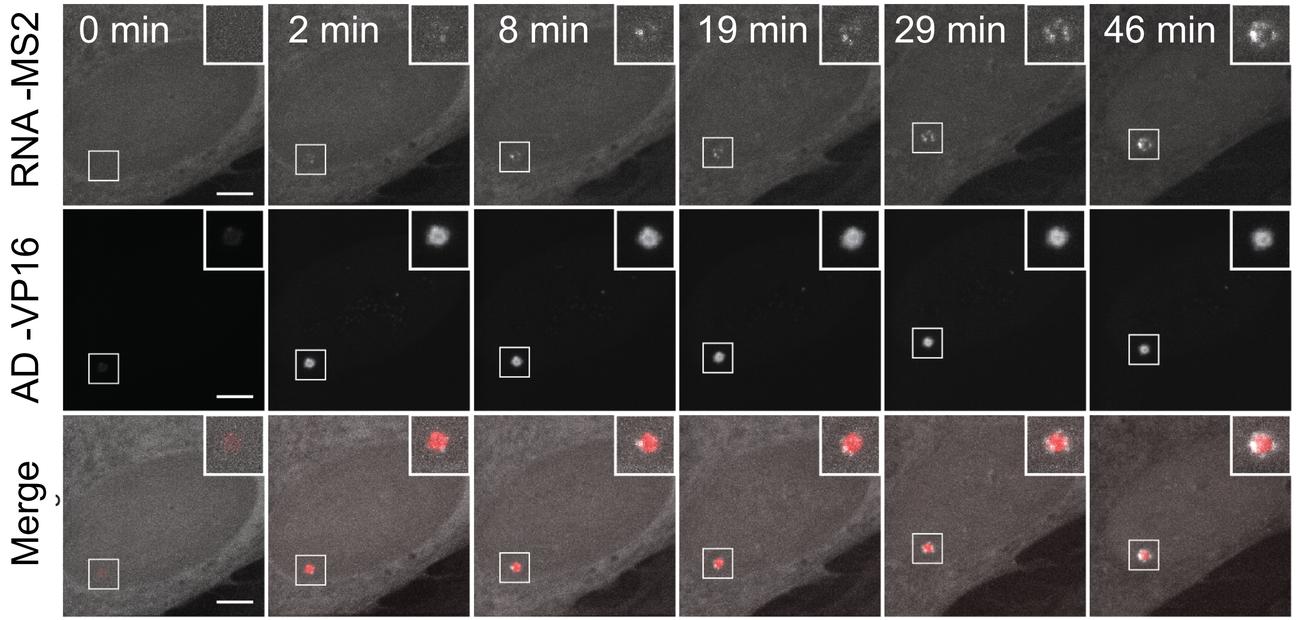
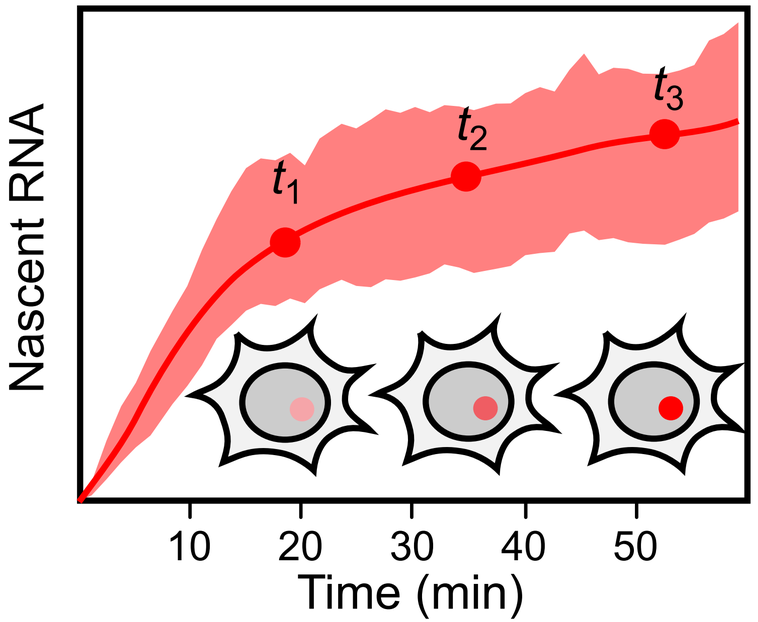


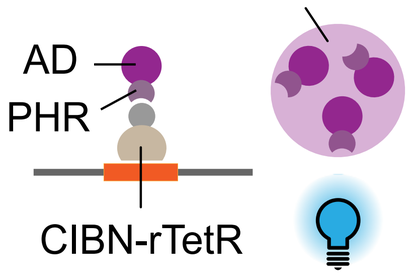
Rademacher 2017 *J Cell Sci,* Trojanowski 2019 *Meth Mol Biol*

Transcription activation kinetics

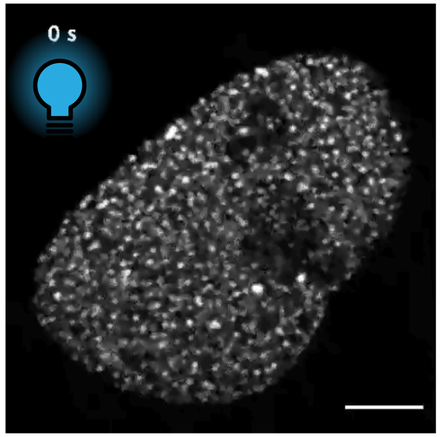


The activation domains (ADs) have different propensities to form optodroplets

PHR-AD optodroplets



FUSN droplets after blue light pulse



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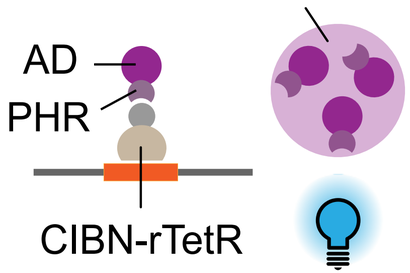
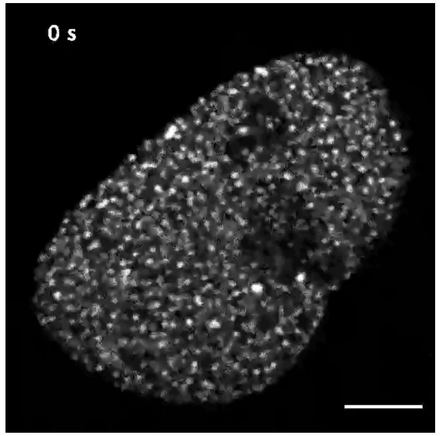
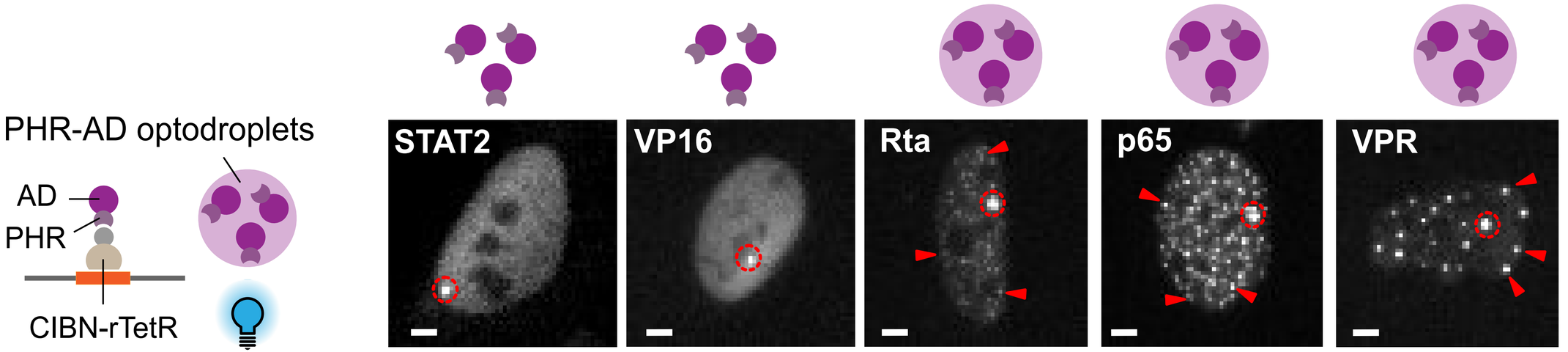
PHR-AD optodroplets

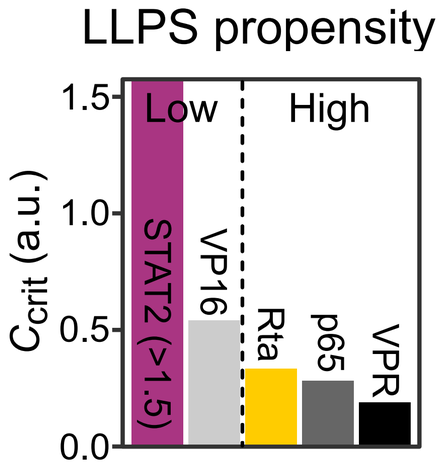


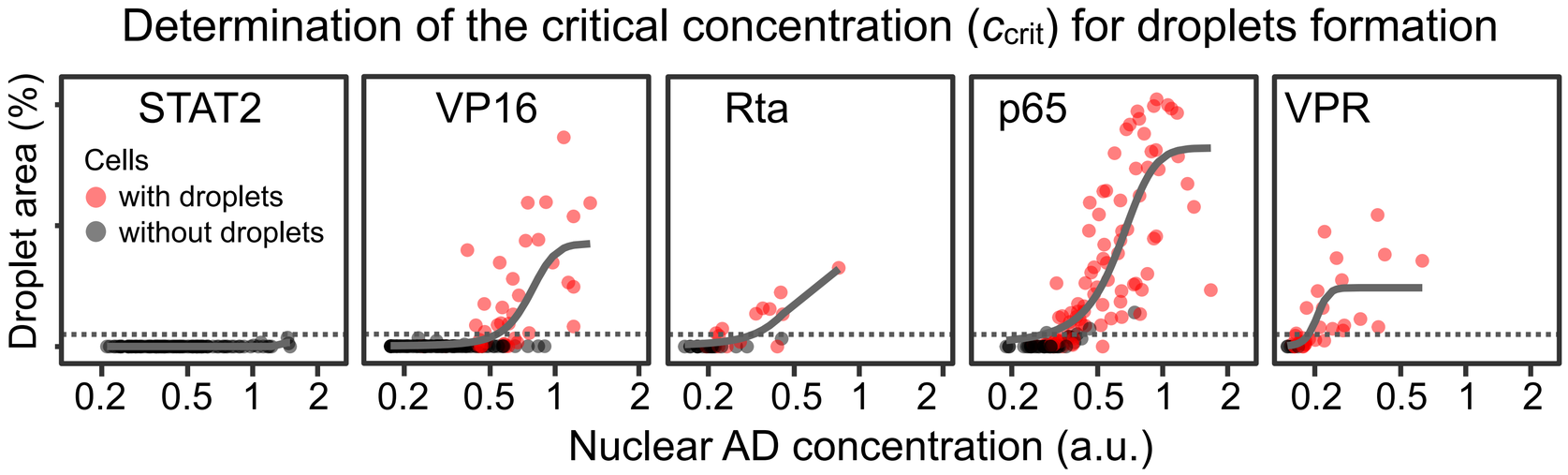
PHR-FUSN droplets
after blue light pulse



The activation domains (ADs) have different propensities to form optodroplets

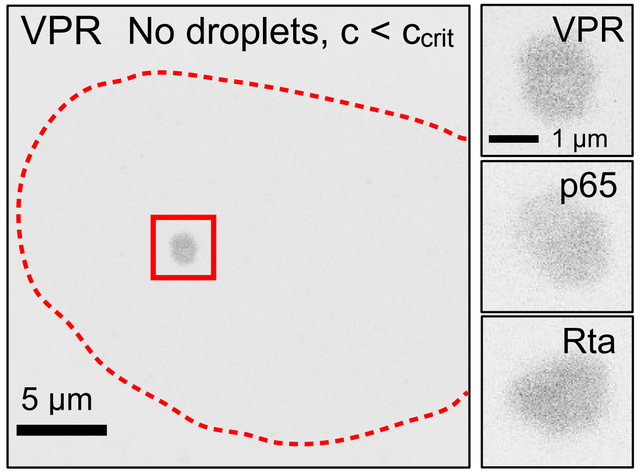


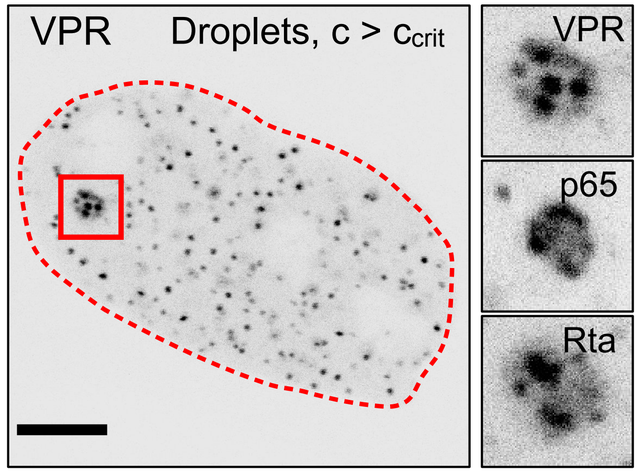




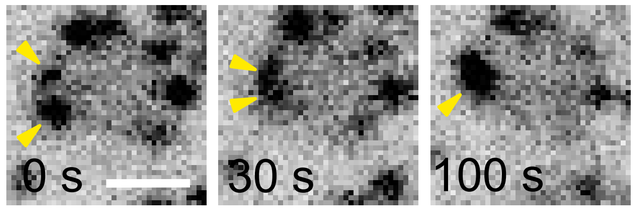
ADs accumulate into liquid like subdomains at the reporter above c_{crit}

Living cell confocal microscopy

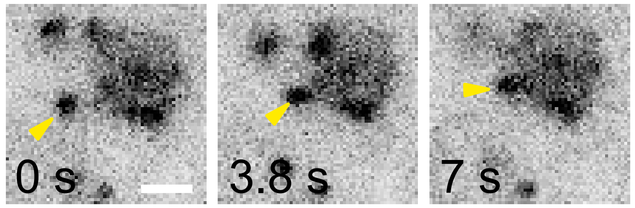




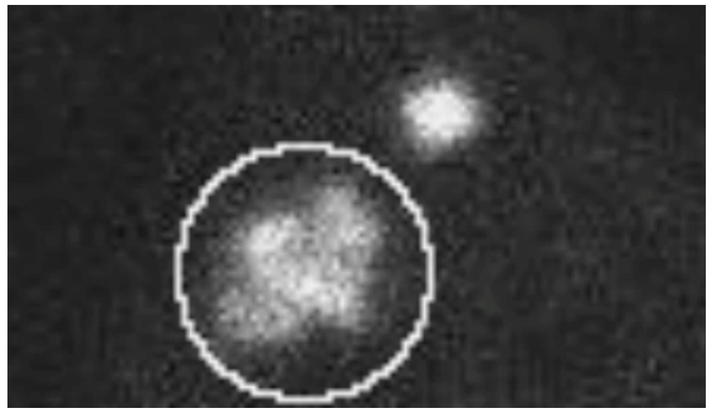
Rta: subdomain coalescence



Rta: fusion with reporter array

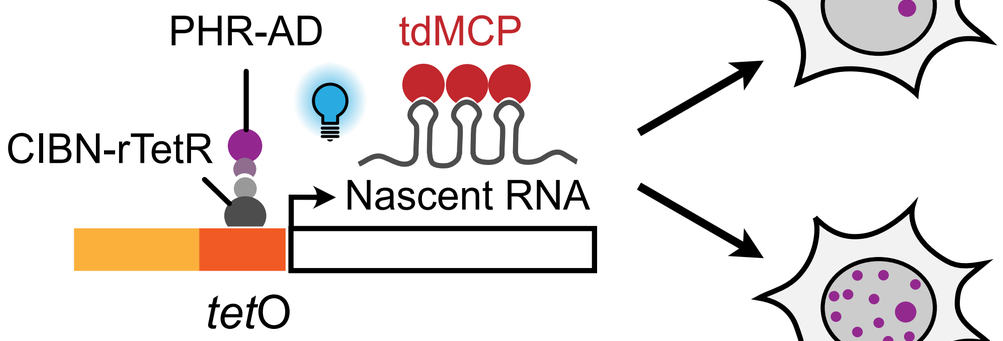


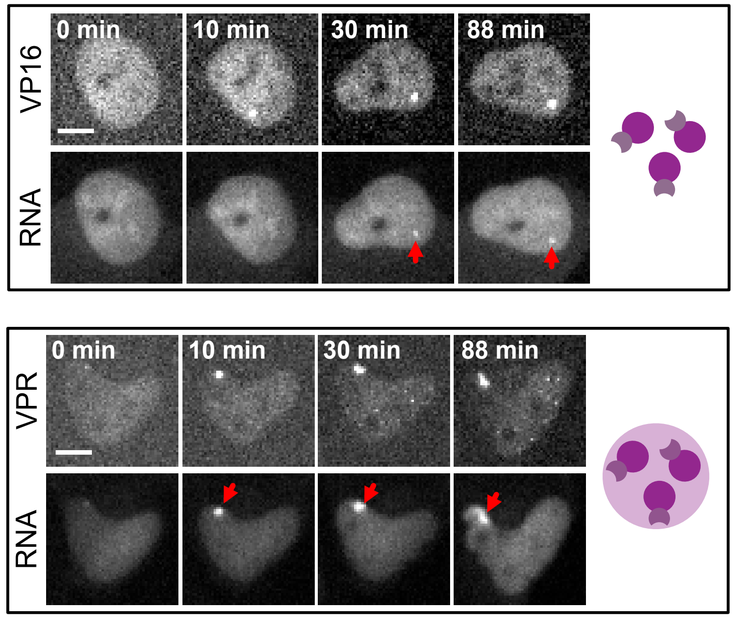
VPR: fusion with reporter array (21 s)



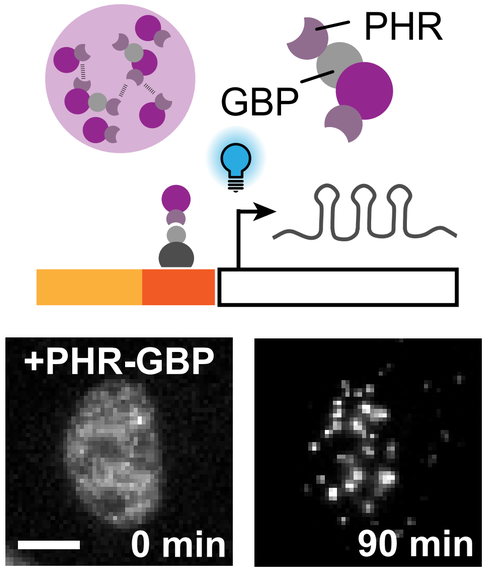
Droplet formation does not enhance transcription activation

Comparing transcription in cells with/without droplets

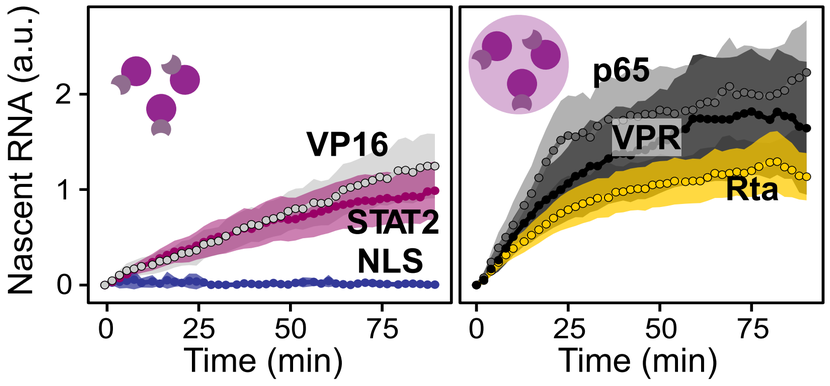




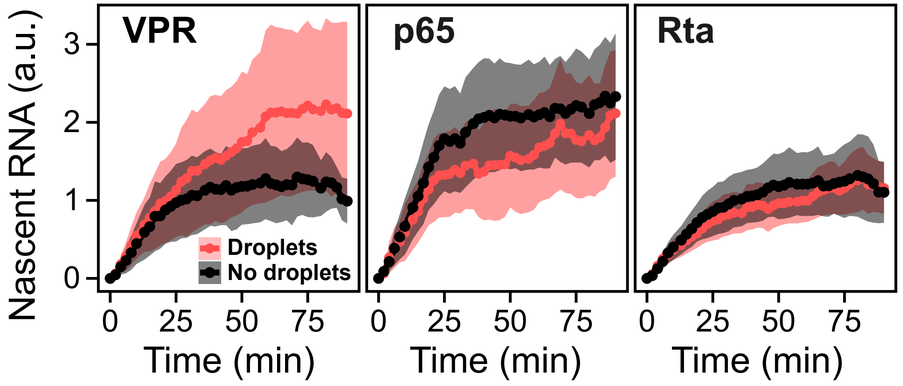
Repression of VP16 by enhanced droplet formation

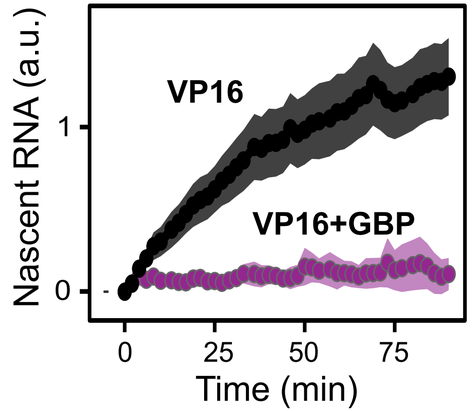


Nascent RNA kinetics



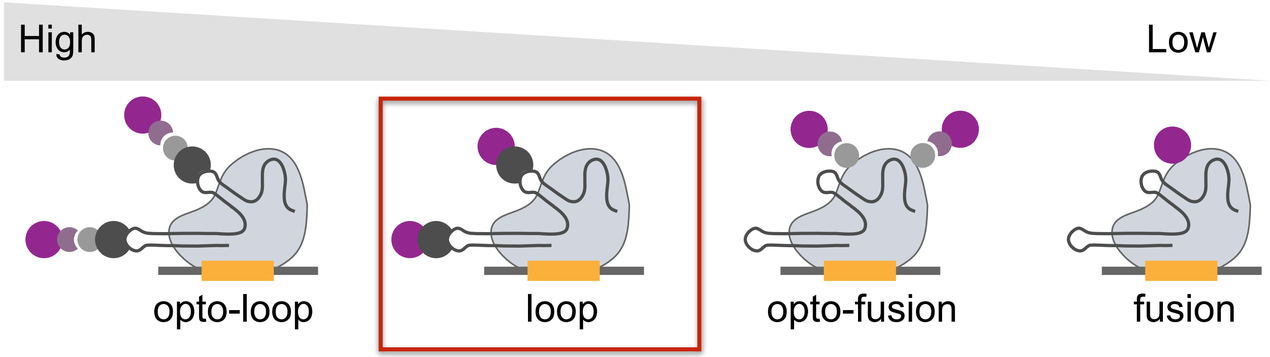
Nascent RNA kinetics above/below c_{crit}



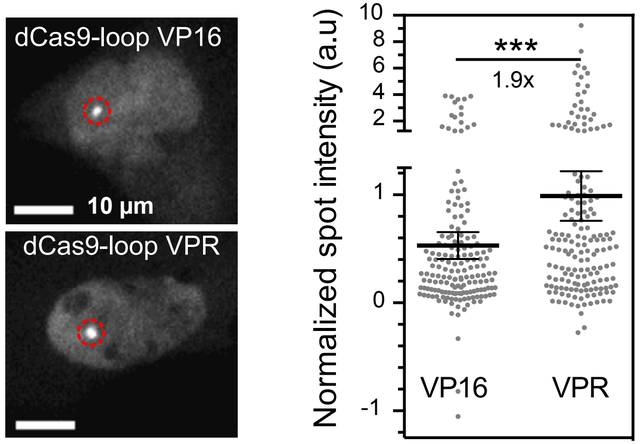


TF binding is stabilized by higher AD multivalency/LLPS propensity

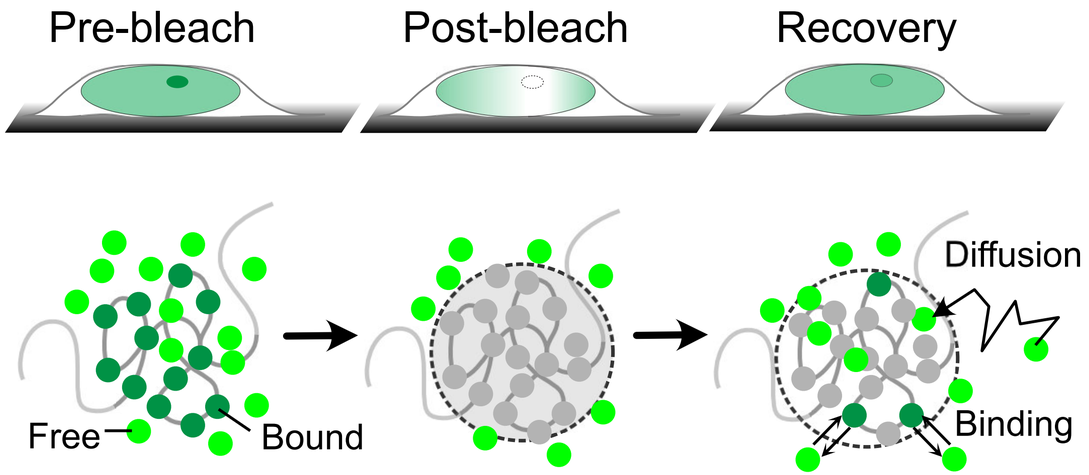
Activation domain turnover



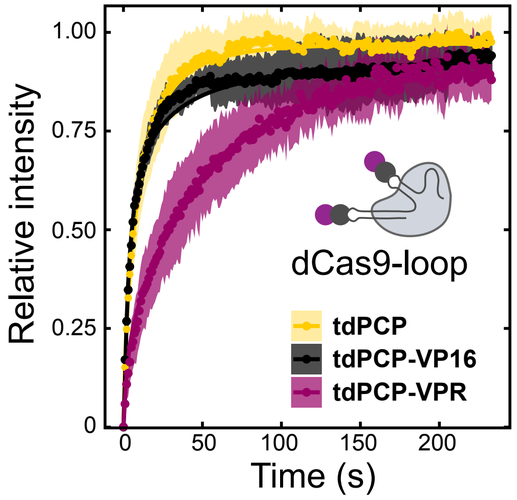
2-fold more VPR than VP16 at the array



Fluorescence recovery after photobleaching FRAP

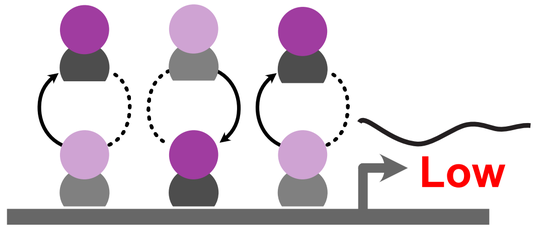


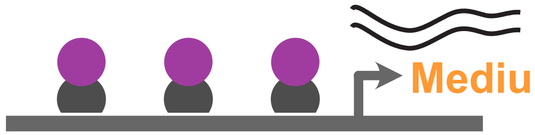
Longer residence time of VPR than VP16



Conclusions: transcription activation is enhanced by multivalent interactions independent of phase separation

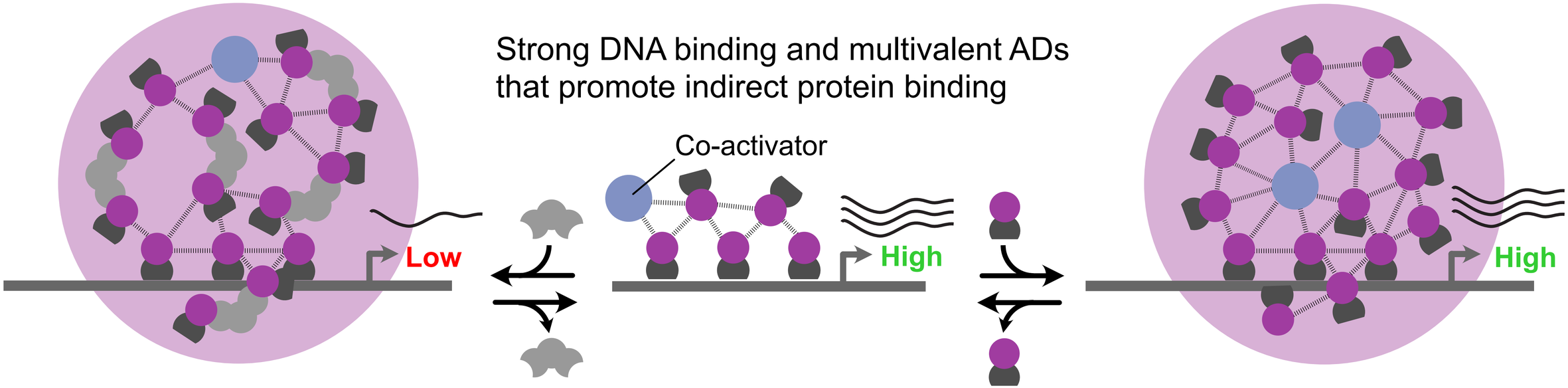
High turnover/
short residence time





Stable chromatin binding but
no multivalent AD interactions

Strong DNA binding and multivalent ADs
that promote indirect protein binding



Bridging factor enhanced droplets
=> repressed transcription

Liquid droplet formation above c_{crit}
=> no enhancement of transcription

Trojanowski, Frank et al. 2022 *Mol Cell*