Labeling and perturbation of endogenous gene loci using CRISPR/Cas9

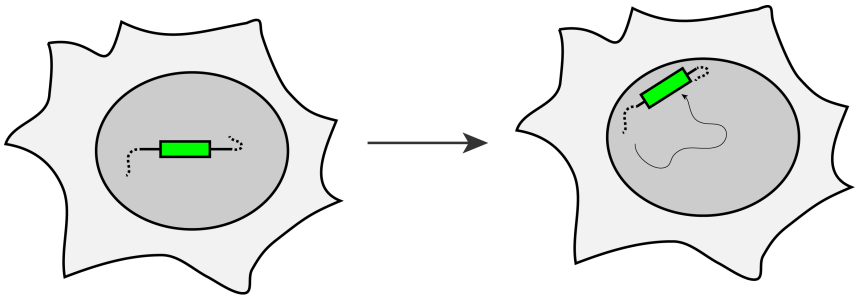
Outline

1. Live cell CRISPR imaging
   - state-of-the-art strategies
   - advantages & limitations

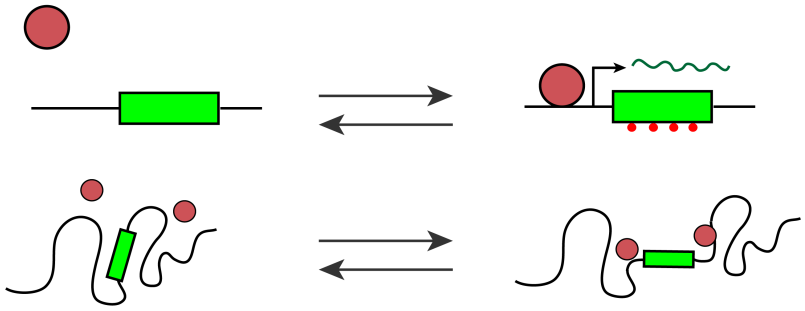
2. CRISPR-based chromatin editing
   - concept & examples
   - outlook: optogenetic chromatin editing

Why target effectors or fluorphores to gene loci?

observe localization & dynamics of genomic regions under different conditions

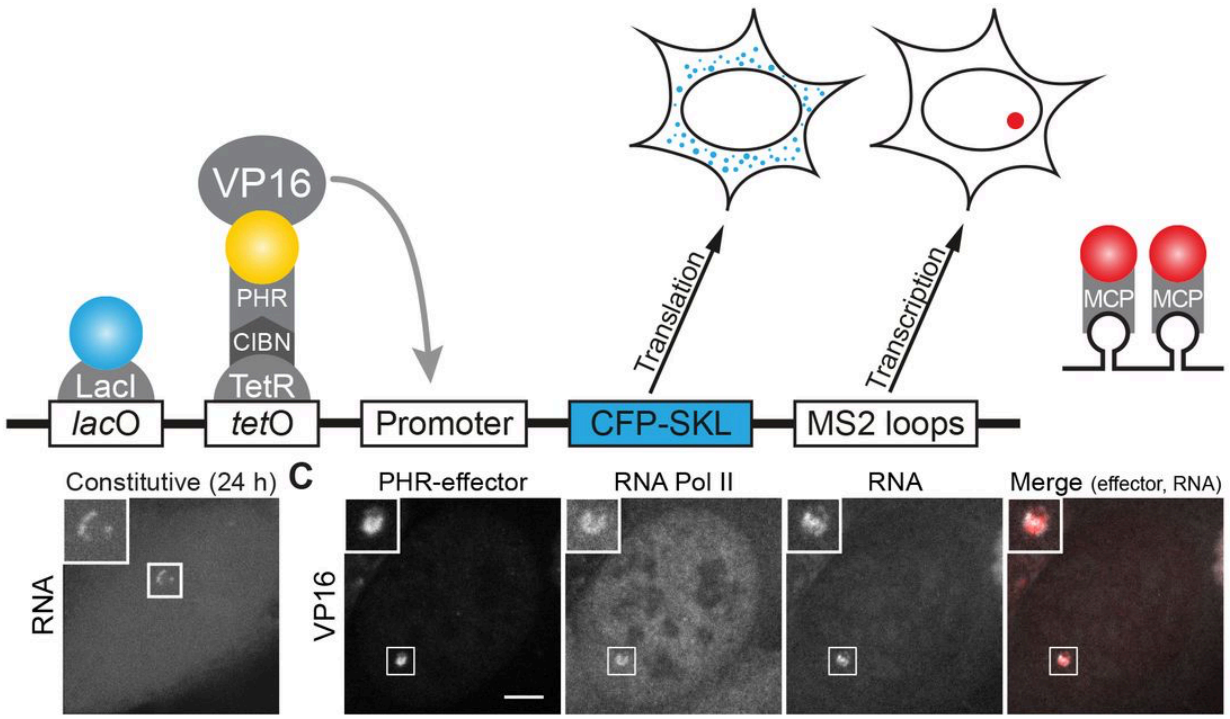


correlate the presence of an effector (e.g. TF) and its effects (e.g histone modifications) to a phenotype



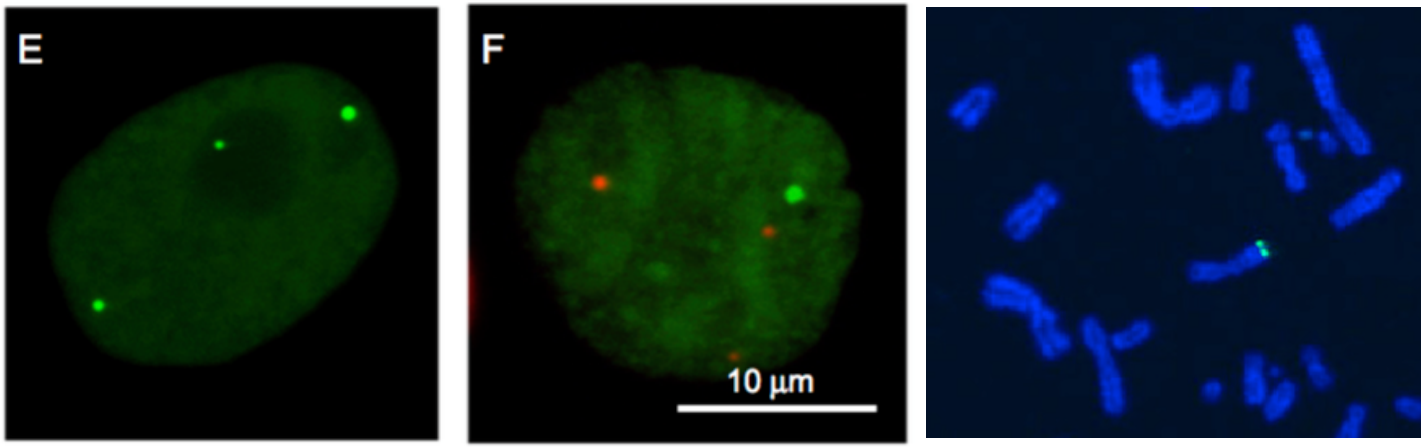
modulate gene transcription at the endogenous promoter level

Recruitment of effectors to transgene arrays to understand chromatin regulation



adapted from Rademacher et al., 2017 *JCS*

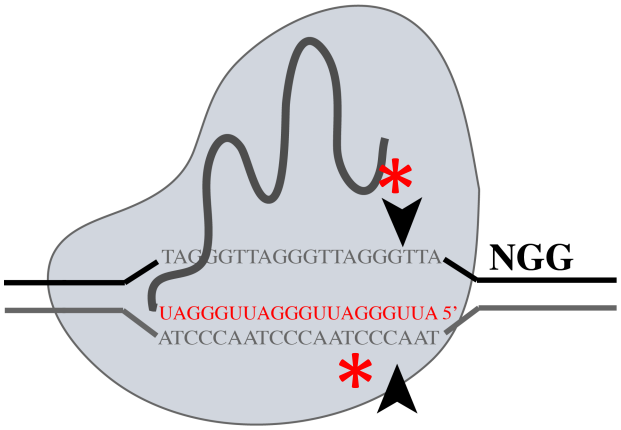
Limitations of transgene arrays to study properties and regulation of chromatin



Pankert et al., 2017

- introduction of foreign sequences → risk of artifacts
  - position & copy number effects
  - recombination/instability
  - heterochromatization
- laborious generation & screening
- multiplexed targeting possible (lacO, tetO) but limited

CRISPR/Cas9 enables targeting of endogenous loci without modification of the target genome



S. pyogenes Cas9 protein

single-guide RNA
(18-22 nt targeting region)

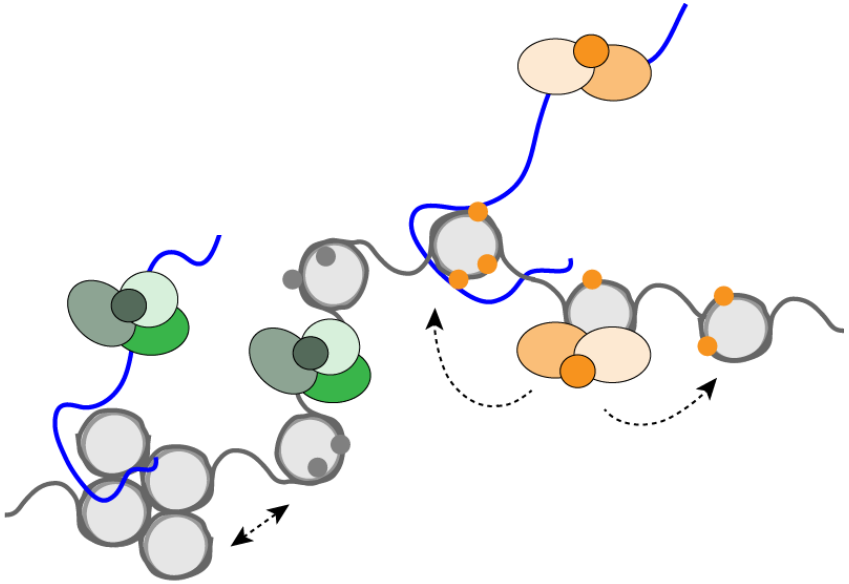
protospacer adjacent motif
(PAM)

catalytically-dead Cas9 (dCas9)

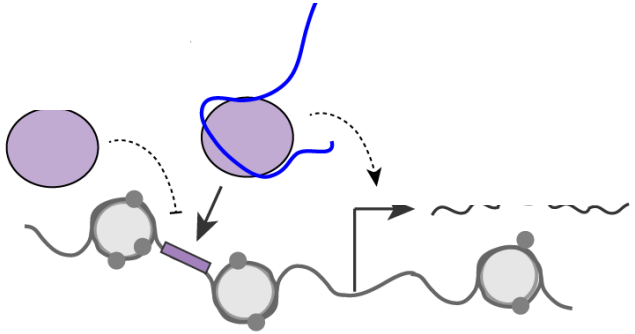
high affinity (K_D ~1 nM)

→ similar or higher than lacI/lacO

Chromatin function involves functional interactions between DNA, proteins and RNA

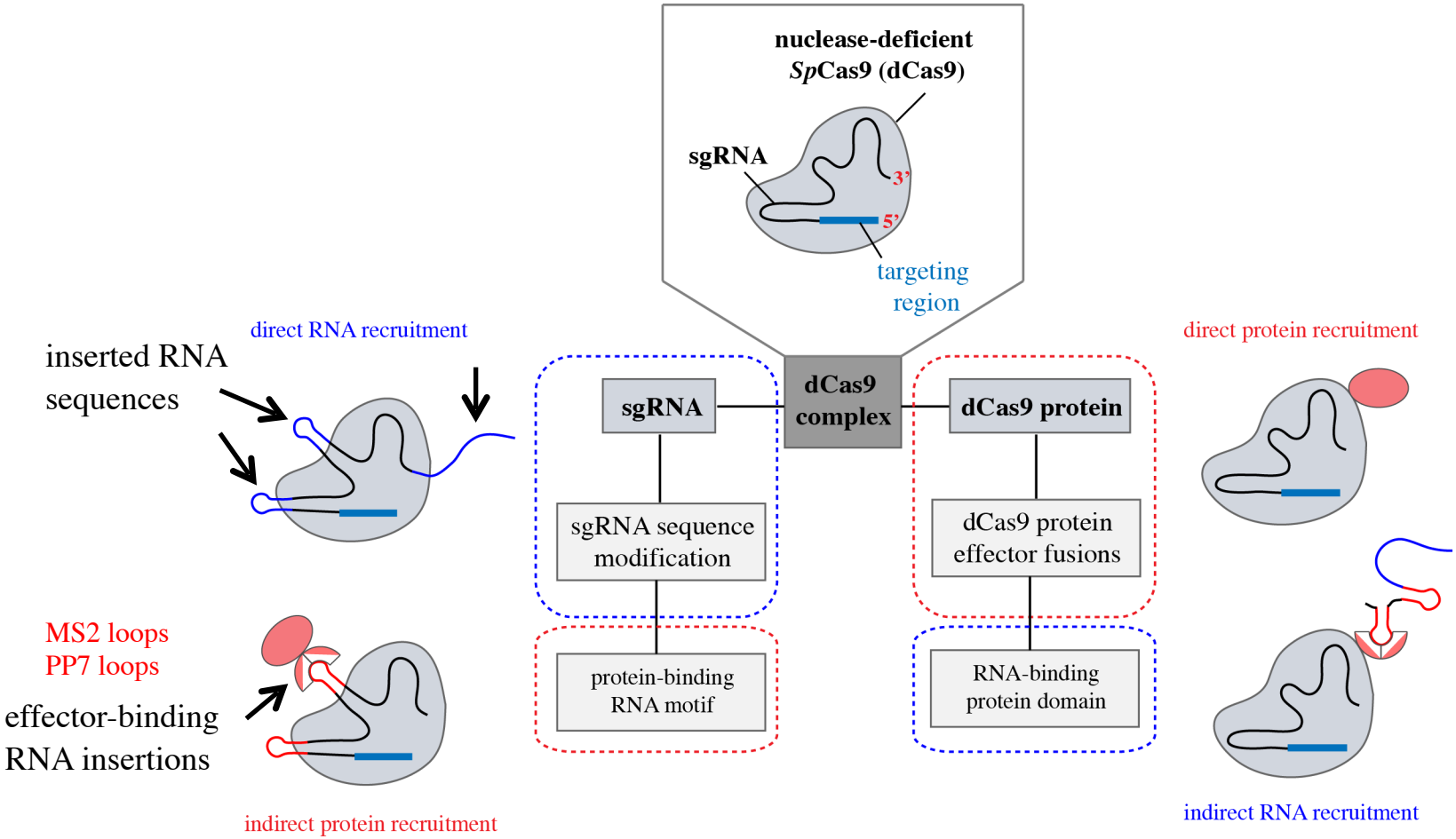


- histone proteins
- histone modifiers & remodelers
- transcription factors
- nascent & maturing RNA



- chromatin-associated RNAs with specific functions

A platform for site-directed delivery of proteins and RNA to chromatin



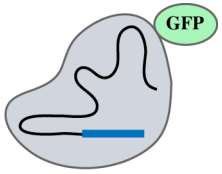
CRISPR imaging

→ strategies to target a detectable amount of fluorophores to one or more genomic loci of interest (in living cells)

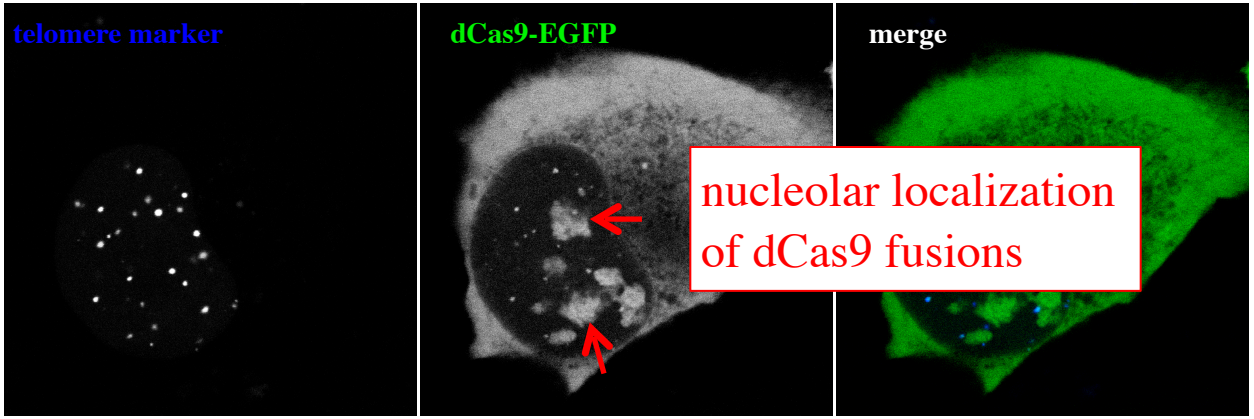
considerations

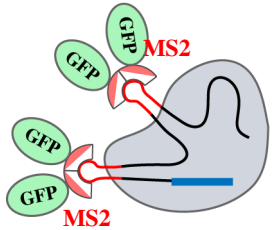
- number and density of binding sites (theoretical)
- number of fluorophores per binding site (recruitment strategy)
- expression levels, brightness & background of fluorophores
- sensitivity and S/N ratio of the microscopy setup

CRISPR imaging of highly repetitive loci is achievable with low fluorophore-to-binding site ratios

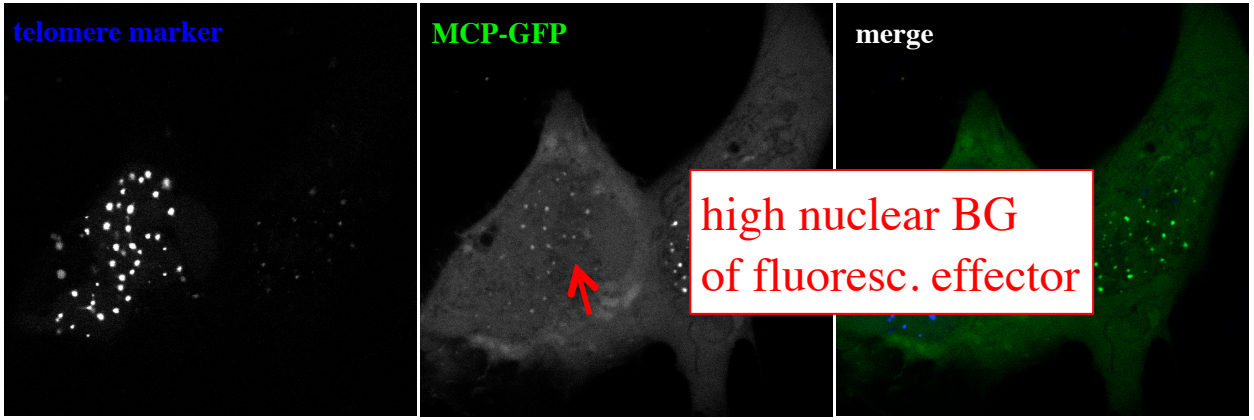


dCas9-EGFP





dCas9-HA



Example: telomere vs. single gene

10 kb of $(TTAGGG)_n$ repeats; footprint of Cas9: ~75 bp:

~130 dCas9-EGFP molecules per telomere assuming 100% occupation

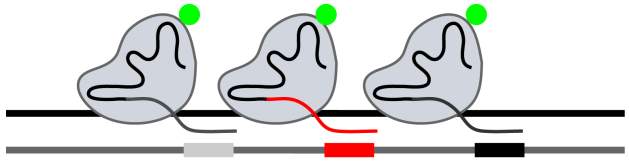
→ assuming 50% occupation: 65 fluorophores/telomere

in our microscopy/transfection setup

→ 50-60 fluorophores per gene locus in order to visualize it

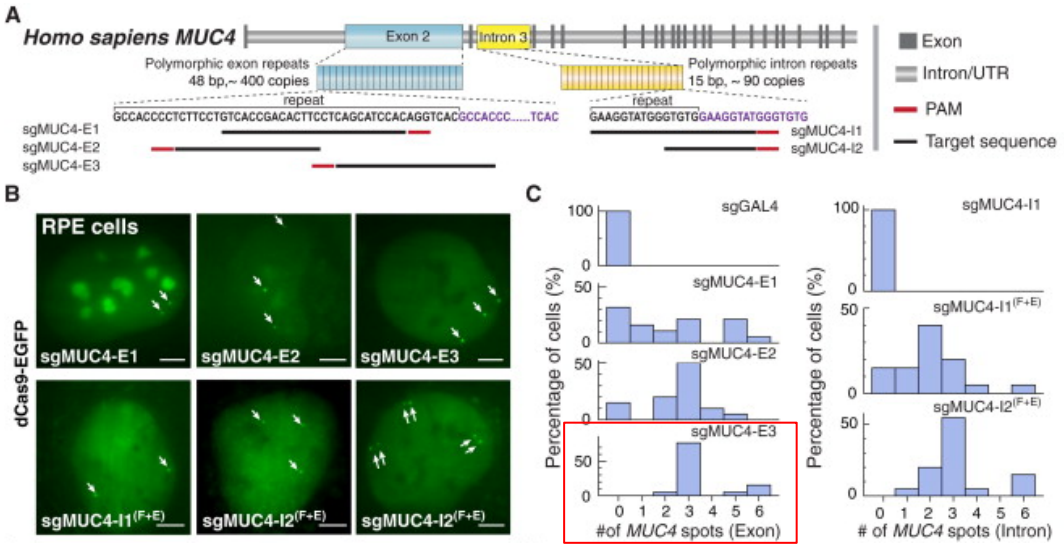
Strategies to target many fluorophores to one locus?

Imaging of lowly/non-repetitive loci requires signal amplification strategies



multiple sgRNAs (“sgRNA tiling”)

“Titrating” the number of binding sites required to visualize a single gene with dCas9-EGFP



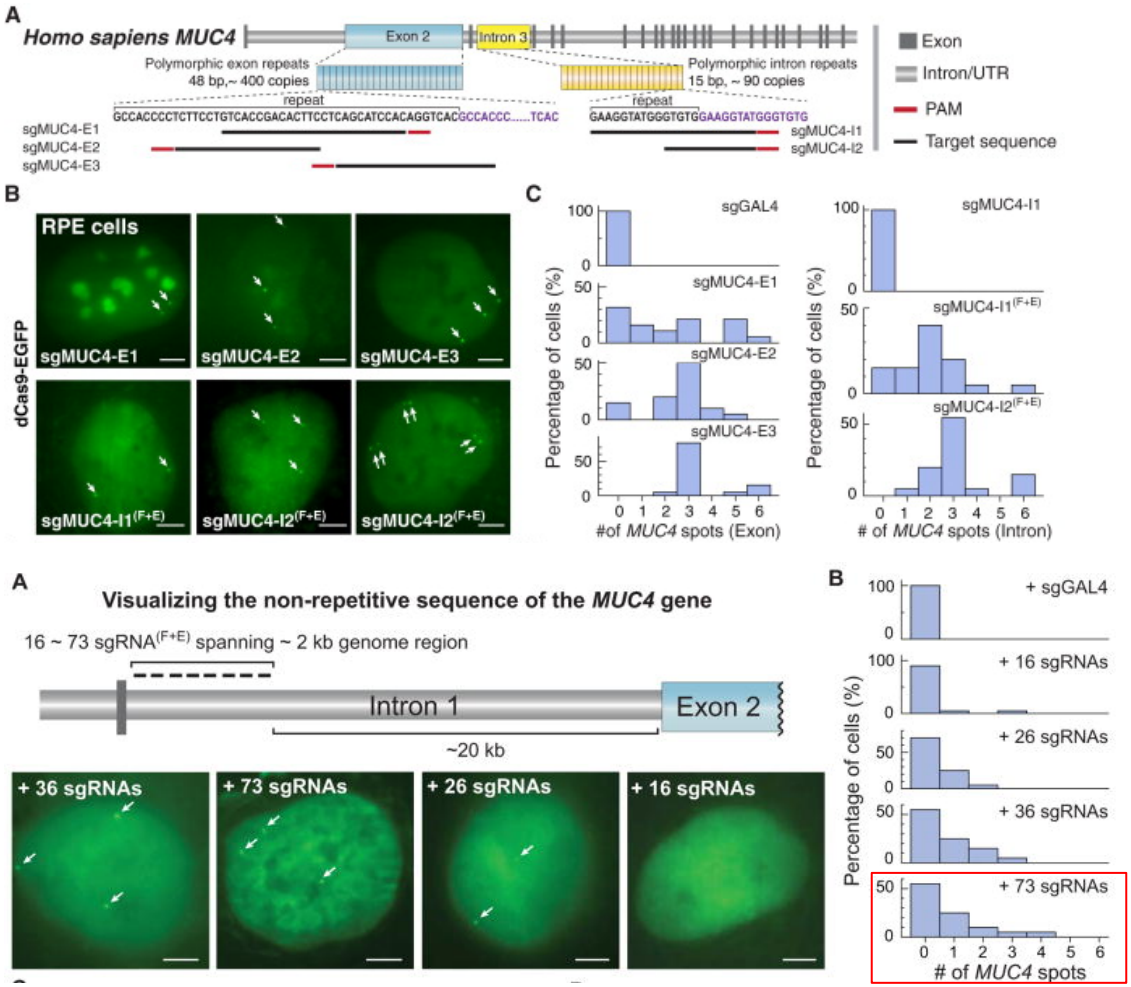
sgMUC4-E3
max 200x dCas9-EGFP

50%: ~100 dCas9-EGFP

3 spots
(HeLa, triploid for MUC4)

Chen et al., 2013 *Cell*

“Titrating” the number of binding sites required to visualize a single gene with dCas9-EGFP

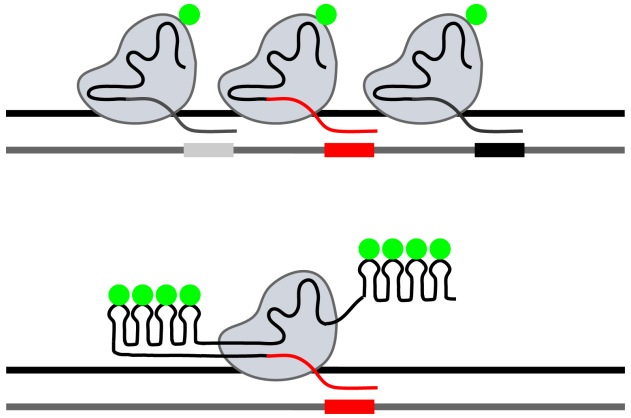


73 sgRNAs binding
in intron 1 of MUC4
max. 73 x dCas9-EGFP

50%: ~35 dCas9-EGFP

Chen et al., 2013 *Cell*

Imaging of lowly/non-repetitive loci requires signal amplification strategies

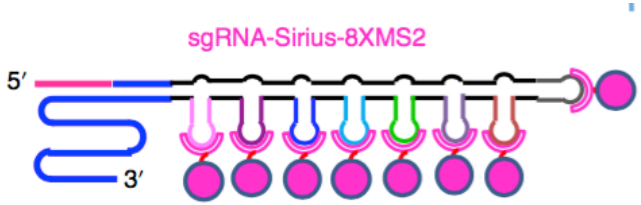


multiple sgRNAs ("sgRNA tiling")

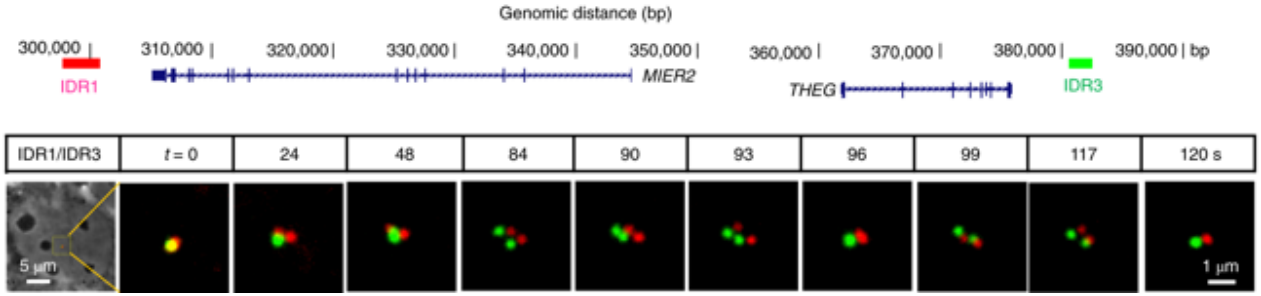
disadvantages ? many constructs, stronger perturbation (DNA replication)

multiple sgRNA-bound fluorophores

CRISPR-Sirius allows multiplexed imaging with fewer sgRNAs per target locus

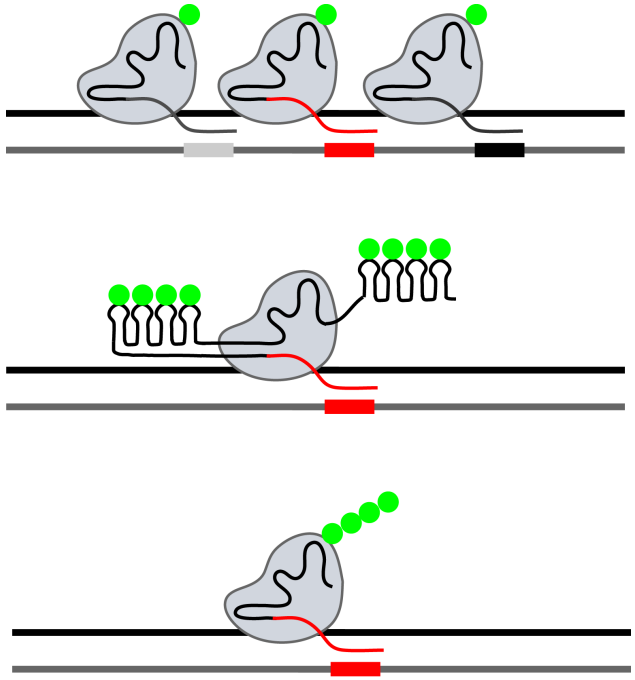


MS2 loops / PCP protein
PP7 loops / MCP protein
→ multiplexing



Ma et al., 2018 *Nat Comm*

Imaging of lowly/non-repetitive loci requires signal amplification strategies



multiple sgRNAs ("sgRNA tiling")

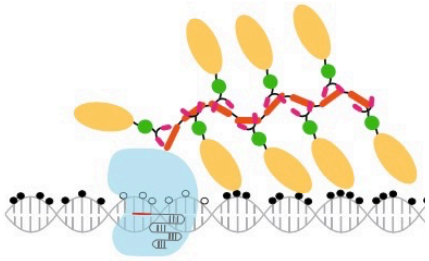
disadvantages ? many constructs, stronger perturbation (DNA replication)

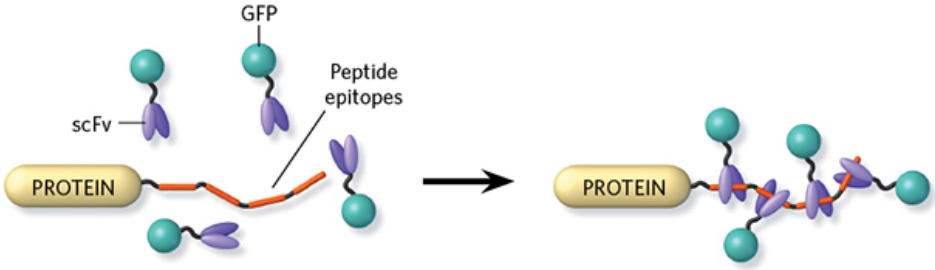
multiple sgRNA-bound fluorophores

disadvantages ? sgRNA stability & recognition

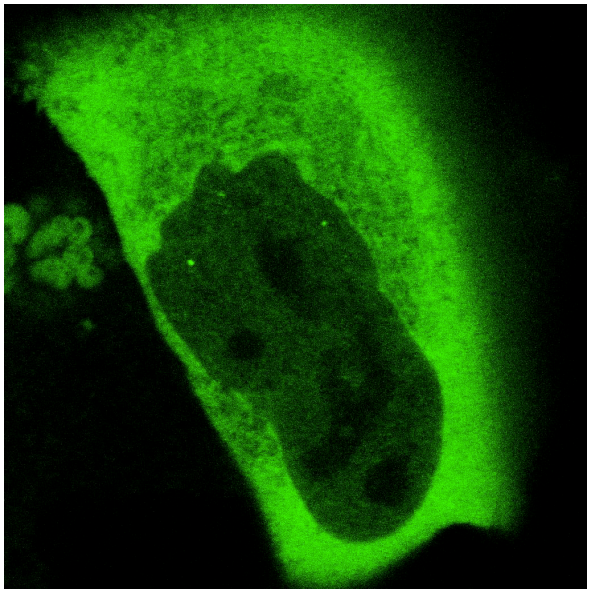
multiple dCas-bound/fused fluorophores

The SunTag system enables recruitment of up to 24 fluorescent proteins per dCas9 molecule





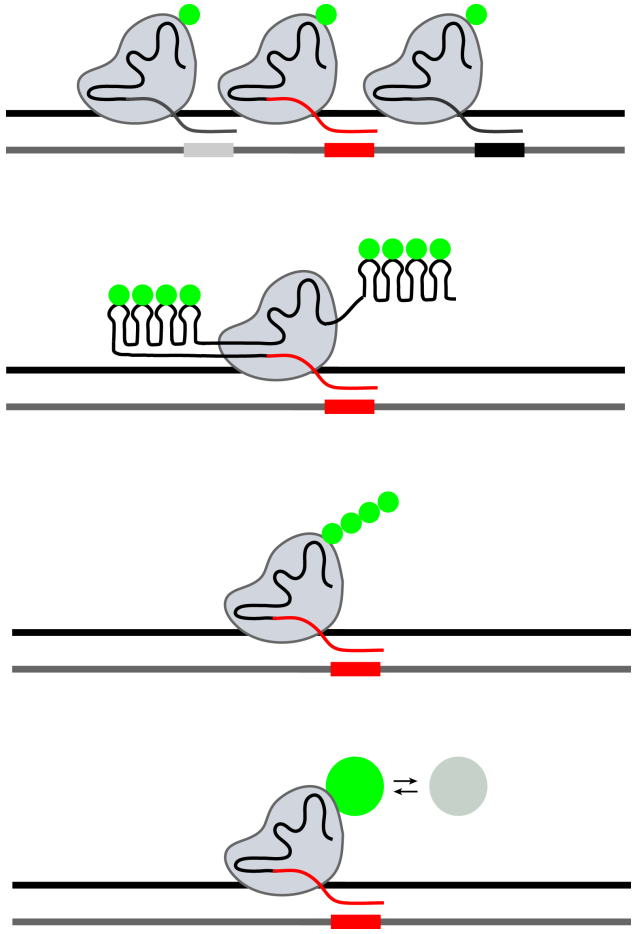
Tanenbaum et al., 2014, *Cell*



imaging of MUC4 locus (triploid)
in human osteosarcoma cells (U2OS)

dCas9-SunTag + sgRNA-MUC4-E3

Imaging of lowly/non-repetitive loci requires signal amplification strategies



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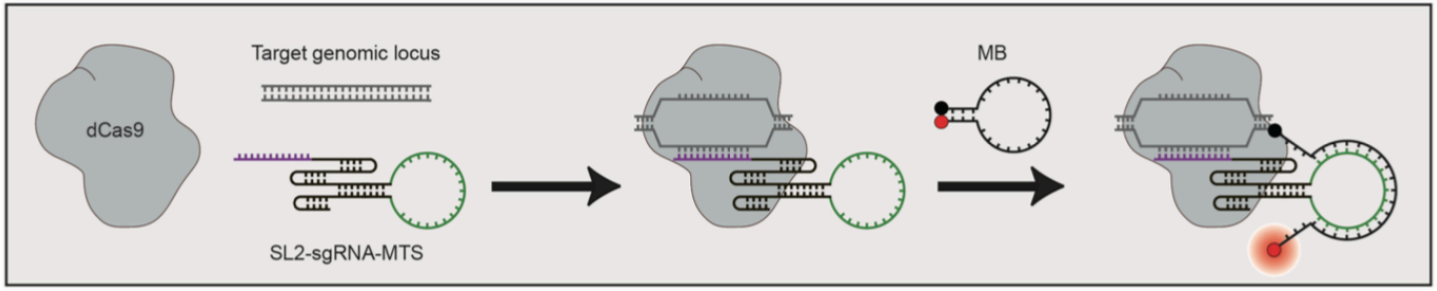
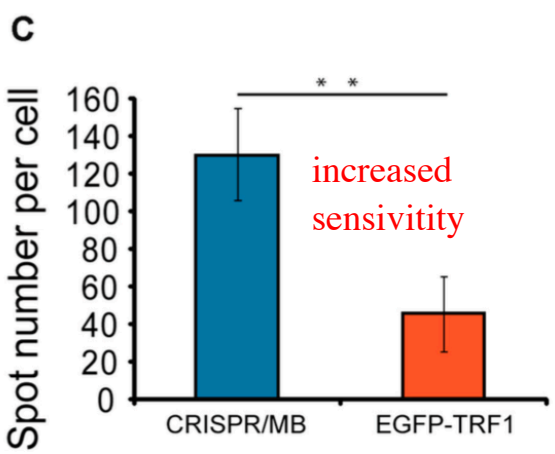
multiple sgRNA-bound fluorophores
disadvantages ? sgRNA stability & recognition

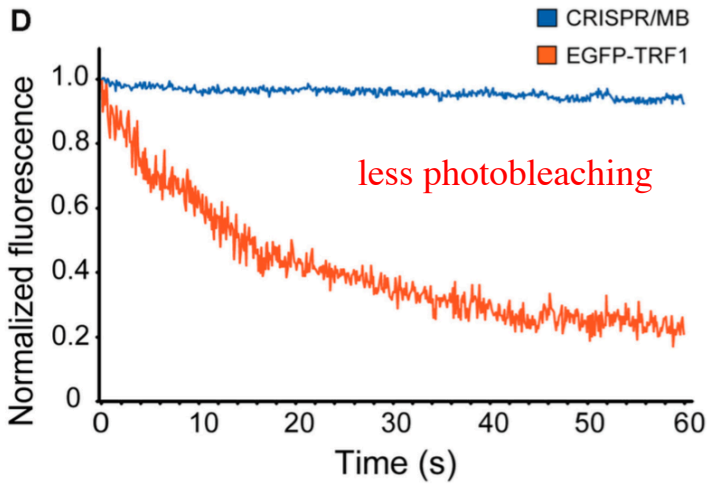
multiple dCas-bound/fused fluorophores
disadvantages ? dCas9 function & size

brigther & more stable fluorophore with reduced background fluorescence

Combining dCas9 with molecular beacons (MBs) for improved live cell CRISPR imaging

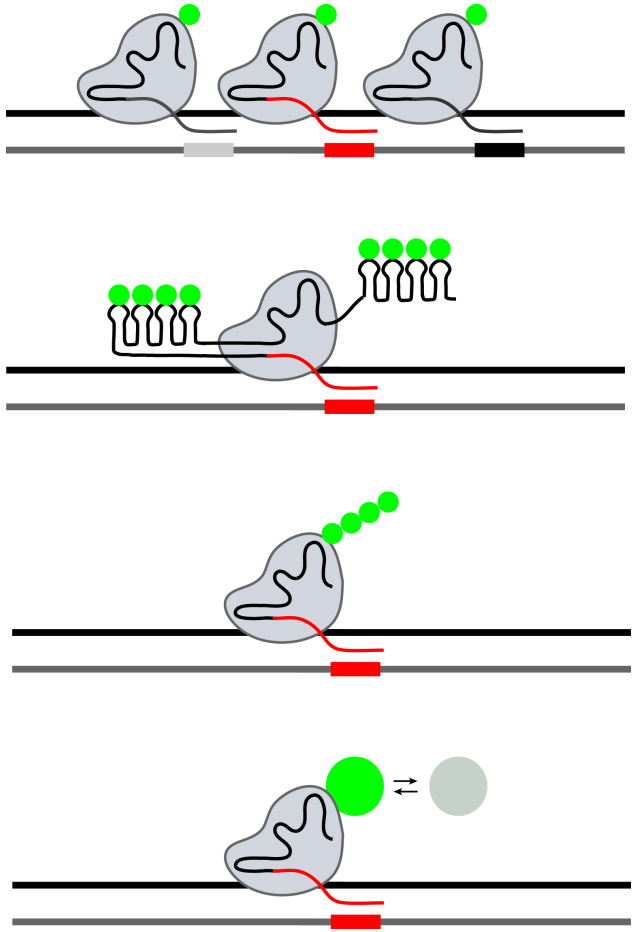






Wu et al., 2018 *Nucleic Acids Res*

Imaging of lowly/non-repetitive loci requires signal amplification strategies



multiple sgRNAs ("sgRNA tiling")

disadvantages ? many constructs, stronger perturbation (DNA replication)

multiple sgRNA-bound fluorophores

disadvantages ? sgRNA stability & recognition

multiple dCas-bound/fused fluorophores

disadvantages ? dCas9 function & size

brigther & more stable fluorophore with reduced background fluorescence

disadvantages ? costly, difficult delivery, toxicity (long-term imaging)

Summary (I)

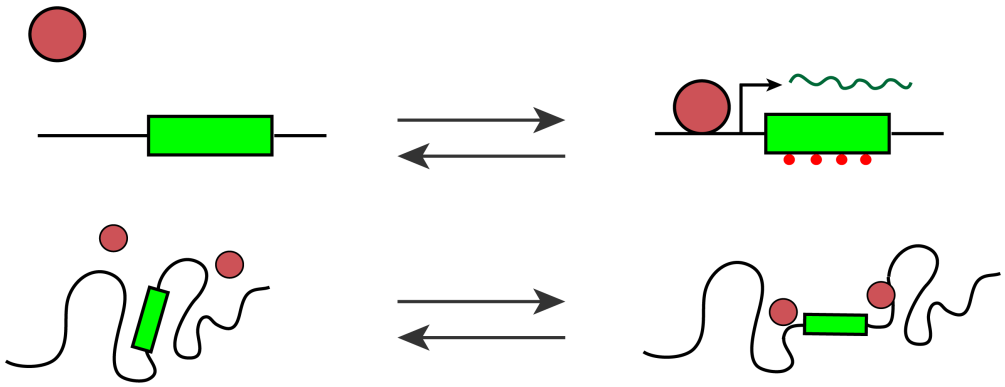
CRISPR imaging allows to study nuclear architecture in living cells without modifying the target genome

CRISPR imaging requires considerations about

- target copy number and binding site density
- biological question
- construct delivery
- signal amplification
- invasiveness of labeling strategy

CRISPR chromatin editing

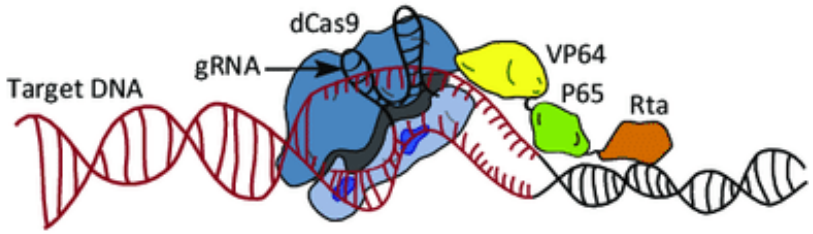
→ any type of modification or interaction induced by recruiting effectors via dCas9 complexes



e.g. correlate a deposited histone modification
with a phenotype (for example gene expression, accessibility)

CRISPRa and CRISPRi

- recruitment of transcriptional activators or repressors to gene loci in order to modulate their expression on the level of the endogenous promoter



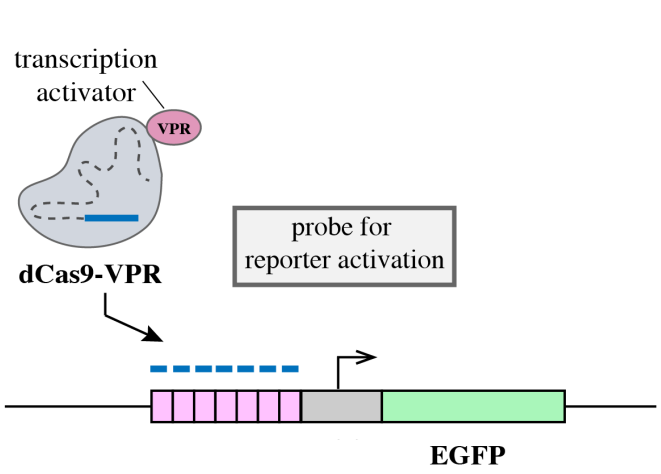
CRISPR-activation (CRISPRa) using dCas9-VPR

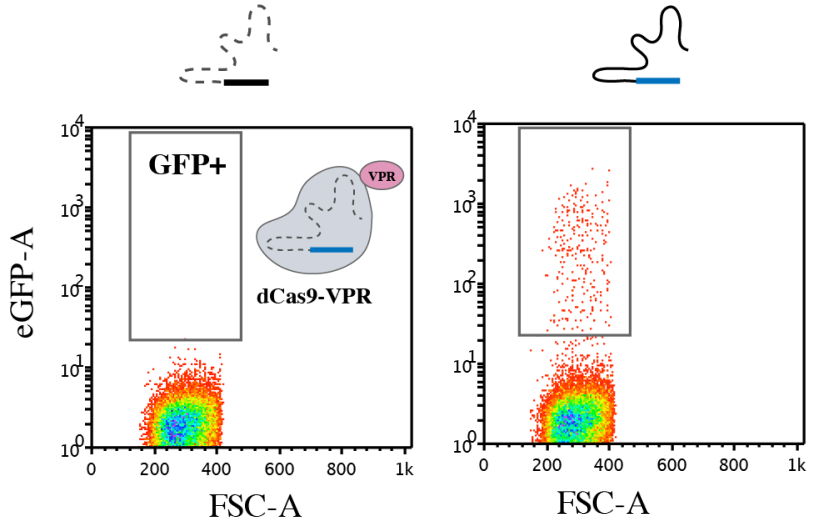
RNA polymerase II
histone acetylation
decondensation

adapted from Jusiak et al., 2016

CRISPRa and CRISPRi

- recruitment of transcriptional activators or repressors to gene loci in order to modulate their expression on the level of the endogenous promoter



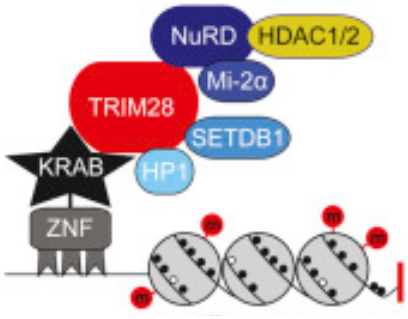


activating a silent reporter gene

CRISPRa and CRISPRi

- recruitment of transcriptional activators or repressors to gene loci in order to modulate their expression on the level of the endogenous promoter

dCas9-KRAB for efficient gene repression



KRAB domain: Krüppel associated box of some transcriptional repressors

- H3K9me3 deposition
- histone deacetylation
- ...

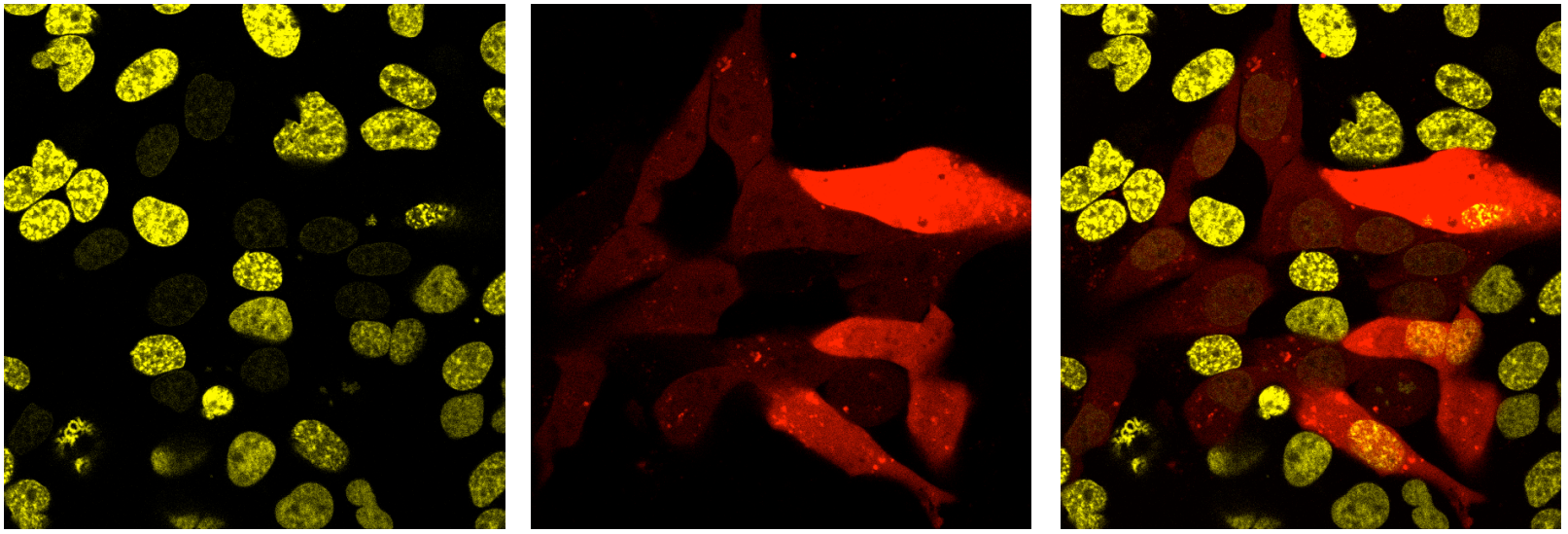
adapted from Oleksiewicz et al., 2017

CRISPRa and CRISPRi

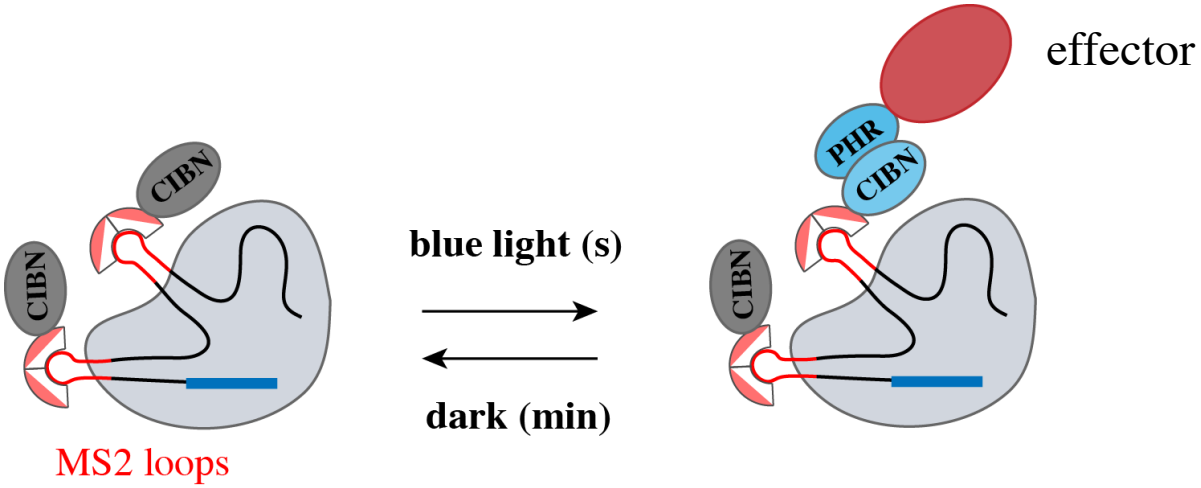
- recruitment of transcriptional activators or repressors to gene loci in order to modulate their expression on the level of the endogenous promoter

dCas9-KRAB

H2B-Citrine | mCherry (co-transfection) | merge

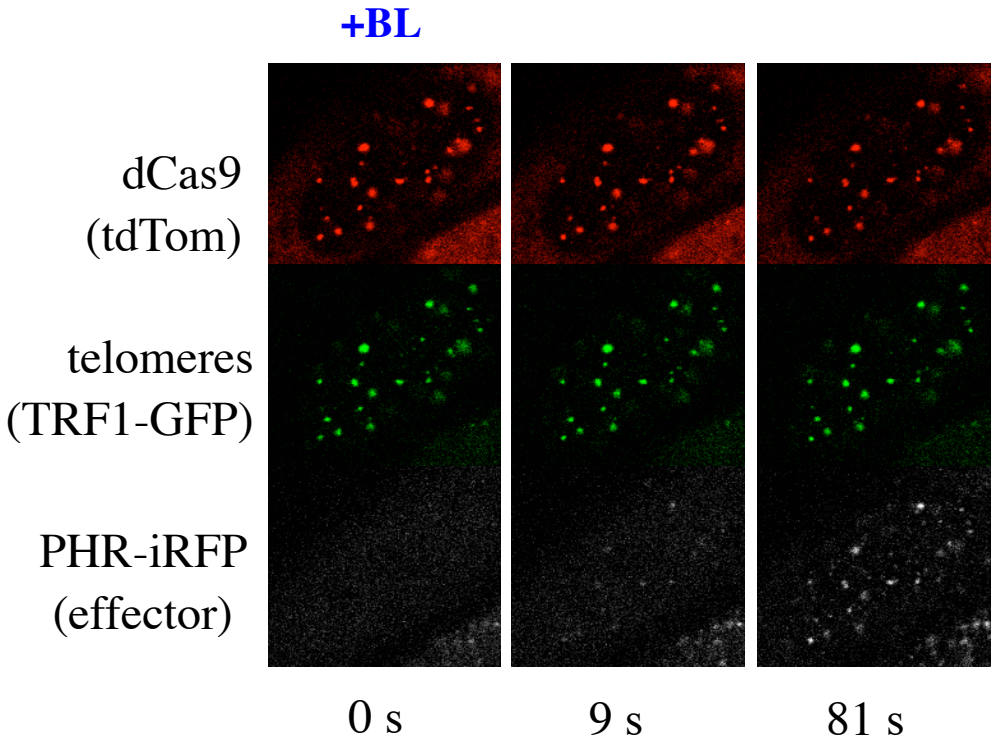


Reversible effector recruitment to dCas9 for dynamic chromatin editing

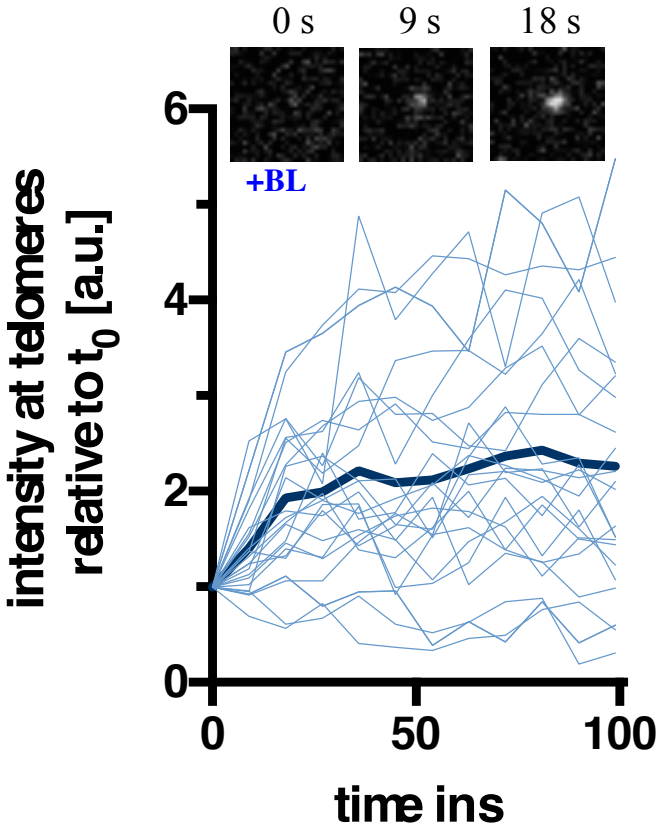


optogenetic control of effector recruitment
other ways: drug-induced (e.g Rapamycin, ABA)

Light-induced dCas9 based effector recruitment to telomeric chromatin occurs within seconds

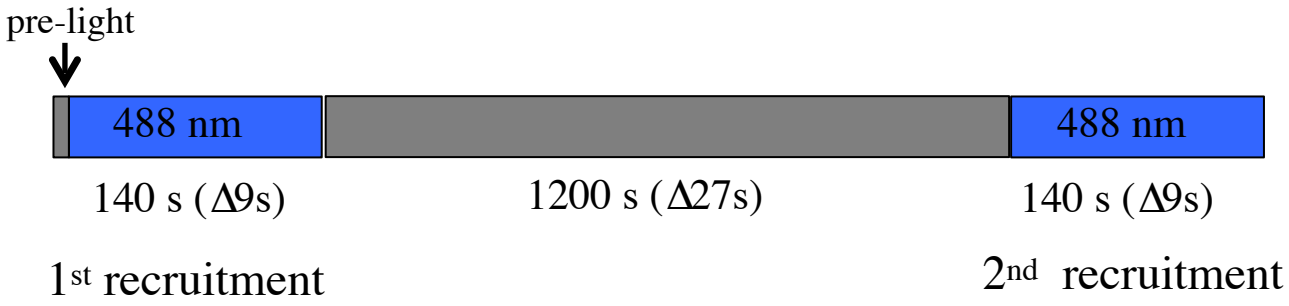


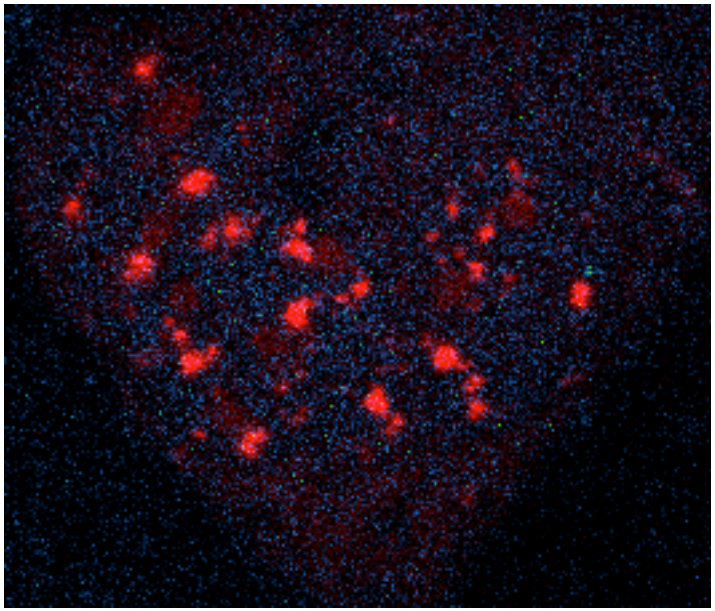
U2OS



25 telomeres/1 cell

Recruitment to dCas9 docked on telomeres is reversible and can be repeated





dCas9-tdTom
PHR-iRFP713

Summary (II)

- CRISPR chromatin editing allows to causally correlate chromatin features (for example histone PTMs) with a phenotype of interest

- CRISPR activation/repression is a great tool to modulate gene expression at the level of endogenous promoters

- reversible strategies for recruitment of effectors to dCas9 complexes on chromatin are particularly valuable

→ dissection of molecular mechanisms at the chromatin level