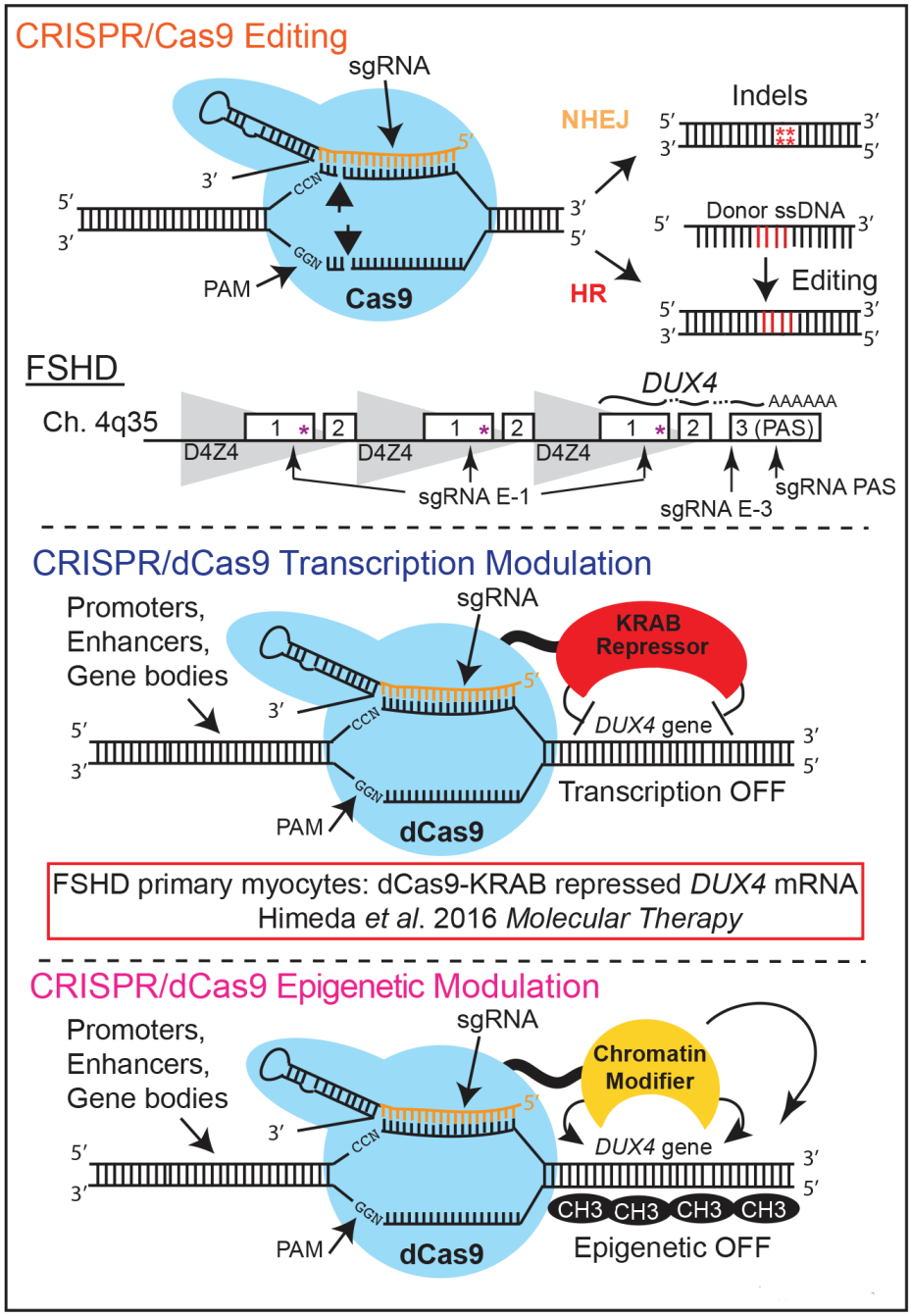
FSHD Therapeutic Approaches: CRISPR (Pt 1)

Therapeutic CRISPR/Cas9 approaches are essentially based on the very specific genome-targeting capabilities of the technology.



Annual Reviews of Genomics and Human Genetics (2019) 20:265-91

CRISPR editing: Targeted gene DNA sequence is cut and then repaired. This can result in Indels (insertions and deletions of DNA) or a template can be provided to specifically alter the sequence.

FSHD: Destroy the *DUX4* gene, or make the PAS nonpermissive.

CRISPR inhibition (CRISPRi) via transcription modulation: Targeted gene is turned off. The DNA sequence is not cut or altered. Repression is active.

FSHD: Turn off pathogenic expression of *DUX4*.

CRISPR inhibition (CRISPRi) via epigenetic modulation: Targeted gene is permanently turned off. The DNA sequence is not cut or altered. Repression is epigenetically inherited.

FSHD: Turn off pathogenic expression of *DUX4*.

FSHD Therapeutic Approaches: CRISPR (Pt 2)

CRISPR/Cas form a bacterial genome defense system evolved to help bacteria defend against invasive bacteriophages (viruses).

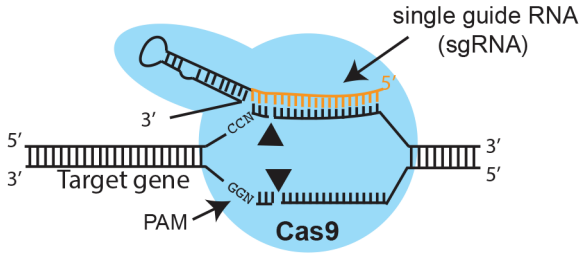
CRISPR (clustered regularly interspaced short palindromic repeats): DNA sequences in bacterial genomes derived from previous invasive entities (viruses/bacteriophages).

Cas (CRISPR-associated systems): These are the proteins that perform the defense activities, e.g., unwinding target DNA, cutting DNA targets, identifying sequences.

Cas9: Typically from the bacteria *Streptococcus pyogenes* (also called SpCas9). Originally contained four components.

Cas9 was engineered into a simpler two component system consisting of a DNA endonuclease (cuts DNA) and a single-guide RNA (sgRNA - targets the DNA sequence that is to be cut) for use in biotechnology (Jennifer Doudna and Emmanuelle Charpentier).

2020 Nobel Prize in Chemistry to Jennifer Doudna and Emmanuelle Charpentier

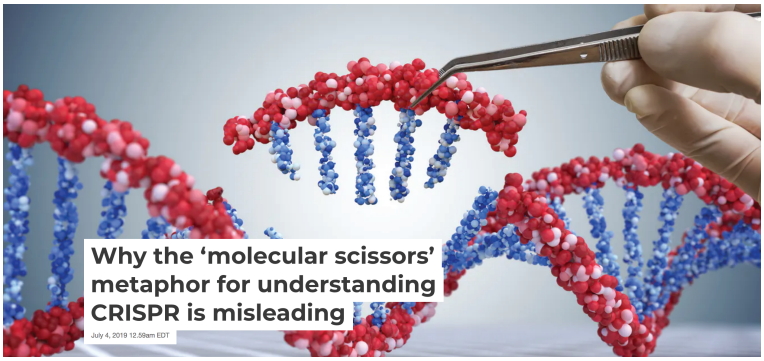


First genome editing in human cells (2013), Feng Zheng and George Church labs. *Science* 339:819-23 and *Science* 339:823-6.

CRISPR/Cas has been covered extensively in literature and online. We recommend the following resources if you would like to learn more:

What is CRISPR gene editing, and how does it work?
https://theconversation.com/what-is-crispr-gene-editing-and-how-does-it-work-84591

Why the “molecular scissors” metaphor for understanding CRISPR is misleading.
https://theconversation.com/why-the-molecular-scissors-metaphor-for-understanding-crispr-is-misleading-119812



Questions and answers about CRISPR -- Feng Zheng, the Broad Institute
https://www.broadinstitute.org/what-broad/areas-focus/project-spotlight/questions-and-answers-about-crispr

The heroes of CRISPR
https://www.broadinstitute.org/files/news/pdfs/PIIS0092867415017055.pdf

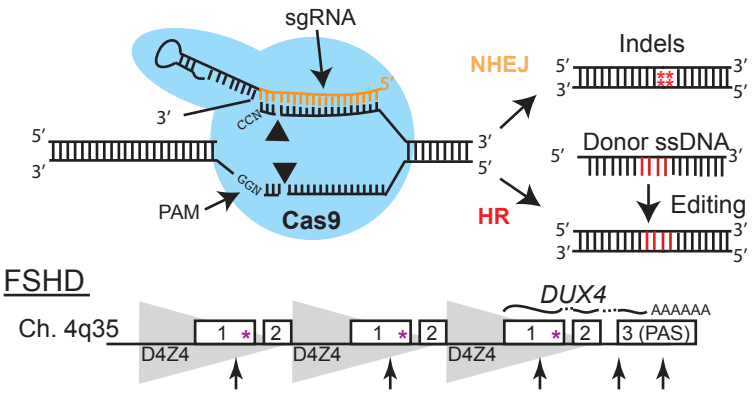
FSHD Therapeutic Approaches: CRISPR (Pt 3)

CRISPR/Cas technology potentially applicable to FSHD

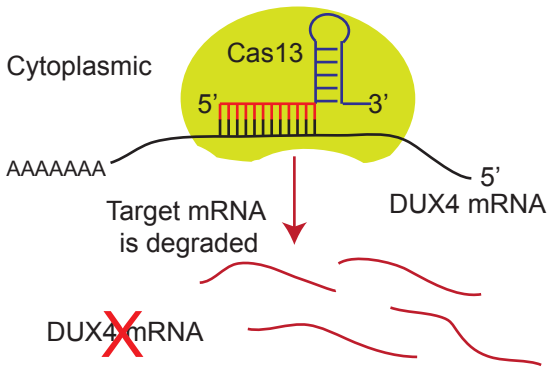
There are many types of Cas9 from different bacteria. The *Staphylococcus aureus* Cas9 (SaCas9) is relevant for FSHD because it is much smaller than the SpCas9 and this is important for gene therapy applications and delivering the CRISPR/Cas to your body's cells.

CRISPR/Cas Cutters

Cas9 cuts DNA and can be used, in theory, to destroy the *DUX4* PAS or the *DUX4* open reading frame. Concerns with high copy number targets.



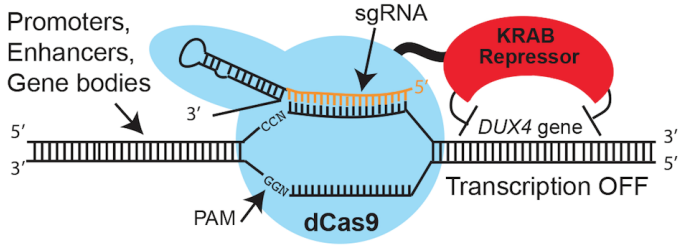
Cas13 cuts RNA instead of DNA and can be used to knock down the pathogenic DUX4 mRNA.



CRISPR/dCas Modifiers

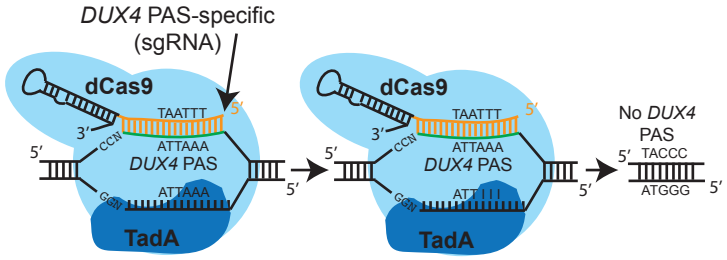
One does not need to use the active form of Cas. Dead Cas (dCas) is useful for genome targeting without cutting the DNA. One can fuse another protein to dCas9 and the sgRNA will direct that protein (and its activity) to a specific sequence. In FSHD, this is being used to turn OFF the *DUX4* gene directly (left) or modify the PAS via base editing.

CRISPR/dCas9 Transcription Modulation



Himeda *et al.* (2016) *Molecular Therapy* 24:527-35.

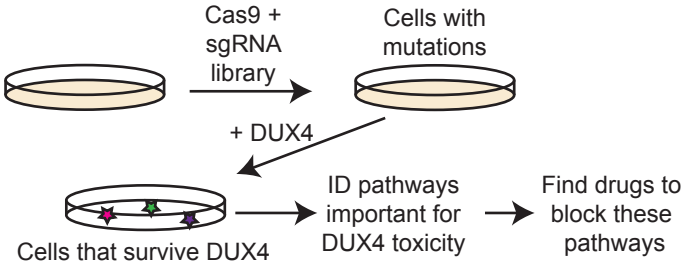
CRISPR/dCAS base editing the PAS



TadA was engineered to convert A to I.
Nature (2017) 551:464-71

CRISPR/Cas Discovery

CRISPR/Cas can be used in drug discovery as well. It has been used to identify potential druggable FSHD therapeutic pathways.



SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

MUSCULAR DYSTROPHY

Applying genome-wide CRISPR-Cas9 screens for therapeutic discovery in facioscapulohumeral muscular dystrophy

Lek *et al. Science Translational Medicine* (2020) 12:eaay0271

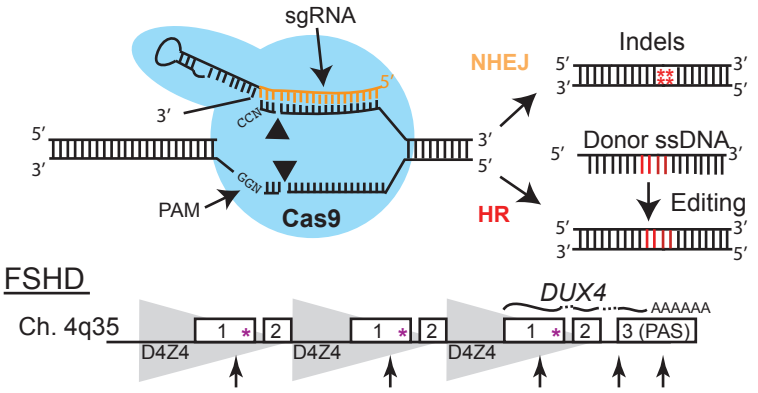
FSHD Therapeutic Approaches: CRISPR (Pt 4)

CRISPR/Cas cutting/editing may be detrimental in FSHD.

Targeting the *DUX4* open reading frame

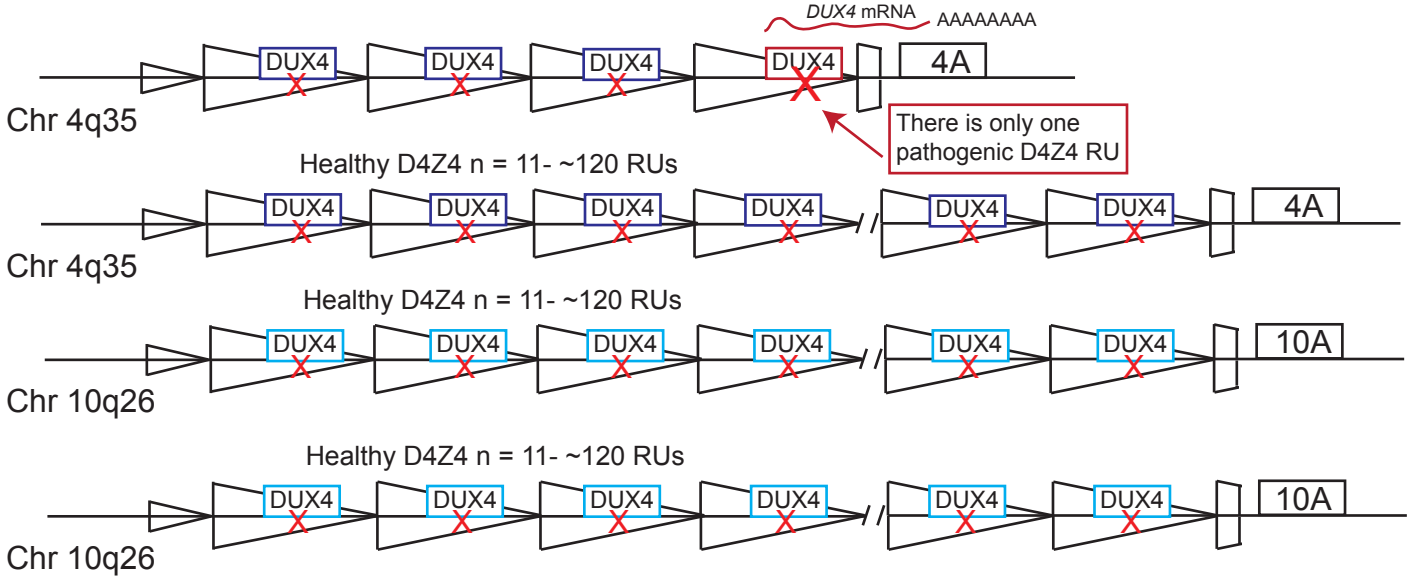
CRISPR/Cas Cutters

Cas9 cuts DNA and could be used, in theory, to destroy the *DUX4* PAS or the *DUX4* open reading frame. One would not even have to edit, just cut and make insertions or deletions (indels) to decrease expression of a functional DUX4 mRNA or protein.



However, unlike other diseases of single copy genes, there are concerns with the high copy number of D4Z4 repeats (targets) at chromosomes 4q35, 10q26, and elsewhere in the genome.

Genetically FSHD1: one chromosome 4q35 with D4Z4 n = 1-10 RUs.



DUX4 DUX4 DUX4 Have the same *DUX4* DNA sequence.

~DUX4 There are D4Z4s elsewhere in the genome with partial *DUX4* DNA sequence.

X Cas9 cutting in the D4Z4 leads to many double stranded DNA breaks.

Double stranded breaks in genomic DNA trigger programmed cell death (apoptosis) and are really bad!

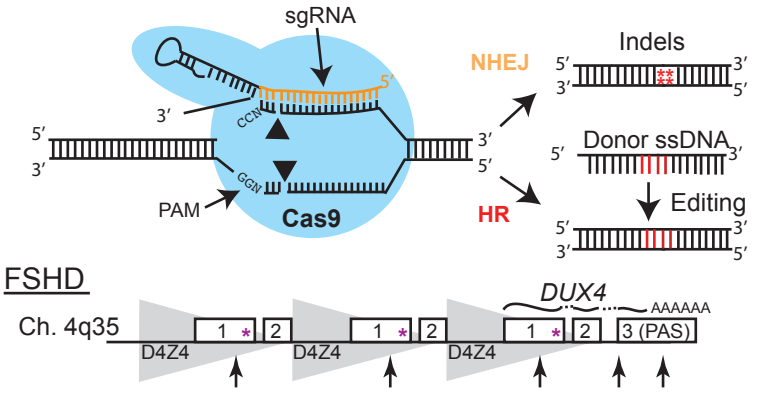
FSHD Therapeutic Approaches: CRISPR (Pt 5)

CRISPR/Cas cutting/editing may be detrimental in FSHD.

Targeting the *DUX4* 4qA PAS

CRISPR/Cas Cutters

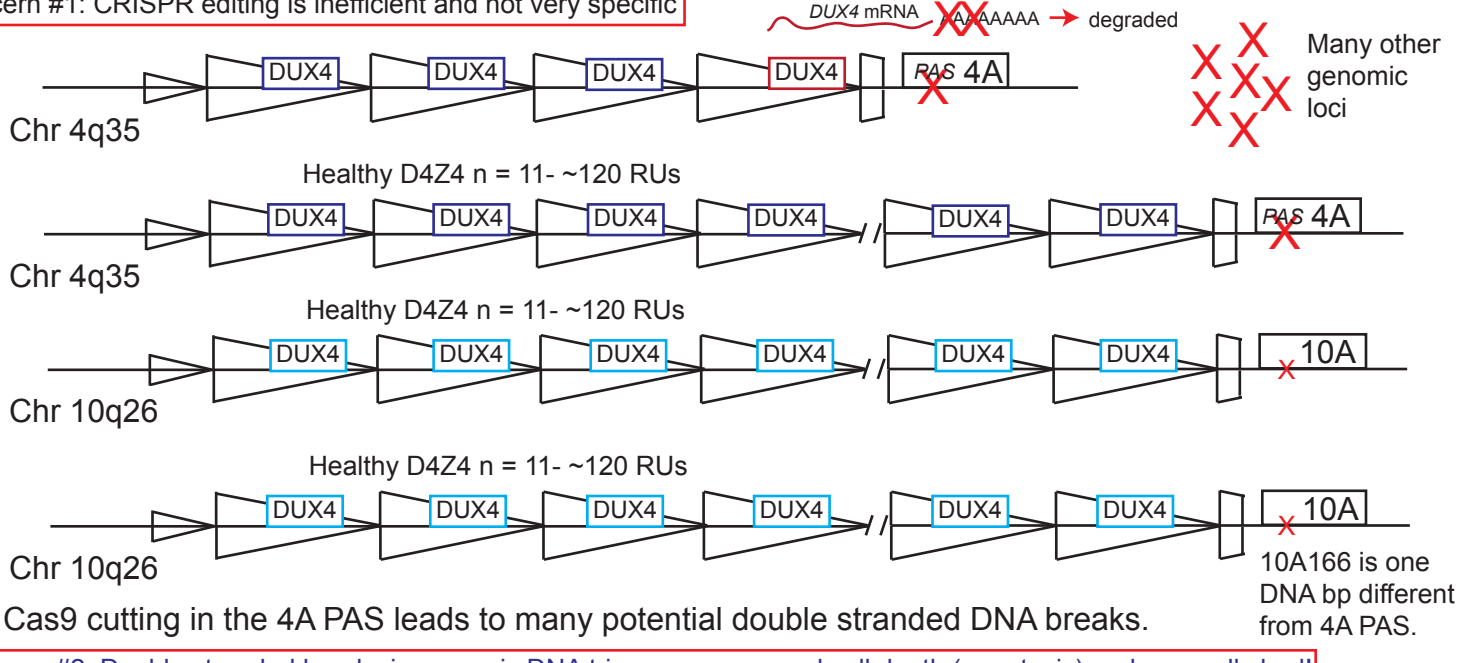
Cas9 cuts DNA and could be used, in theory, to destroy the *DUX4* PAS or the *DUX4* open reading frame. One would not even have to edit, just cut and make insertions or deletions (indels) to decrease expression of a functional DUX4 mRNA or protein.



However, unlike other diseases of single copy genes, there are concerns with the high copy number of D4Z4 repeats (targets) at chromosomes 4q35, 10q26, and elsewhere in the genome.

Genetically FSHD1: one chromosome 4q35 with D4Z4 n = 1-10 RUs.

Concern #1: CRISPR editing is inefficient and not very specific

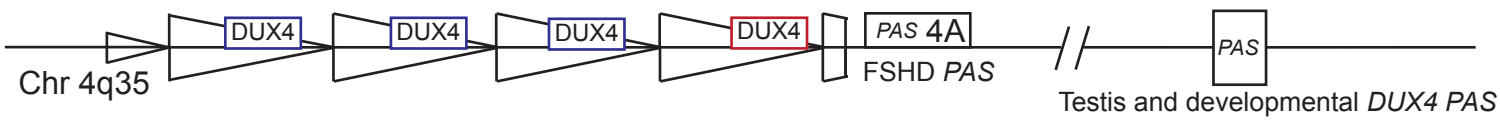


X Cas9 cutting in the 4A PAS leads to many potential double stranded DNA breaks.

Concern #2: Double stranded breaks in genomic DNA trigger programmed cell death (apoptosis) and are really bad!

Concern #3: Alternate polyadenylation site usage; even if successful, may not eliminate DUX4 polyadenylation.

There is another DUX4 PAS on chromosome 4 that is not normally used in FSHD, which could be activated.



FSHD Therapeutic Approaches: CRISPR (Pt 6)

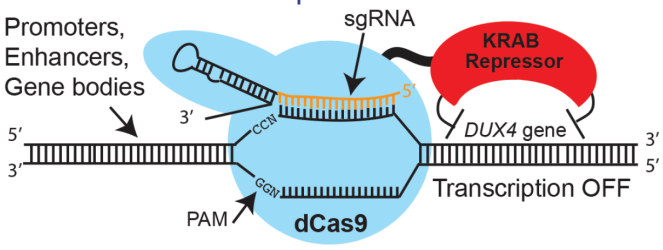
CRISPR/dCas repression is a viable approach for FSHD.

Targeting the *DUX4* gene for repression.

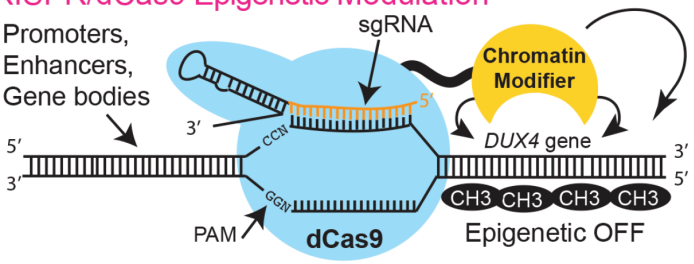
CRISPR/dCas genome regulation:

Dead Cas9 (dCas9) has no DNA cutting activity but retains its DNA targeting when paired with an appropriate sgRNA. Used to either transcriptionally shut off *DUX4* gene expression (using fusion with the KRAB transcriptional repressor) or to epigenetically silence *DUX4* gene expression (using fusion with repressive chromatin modifiers).

CRISPR/dCas9 Transcription Modulation

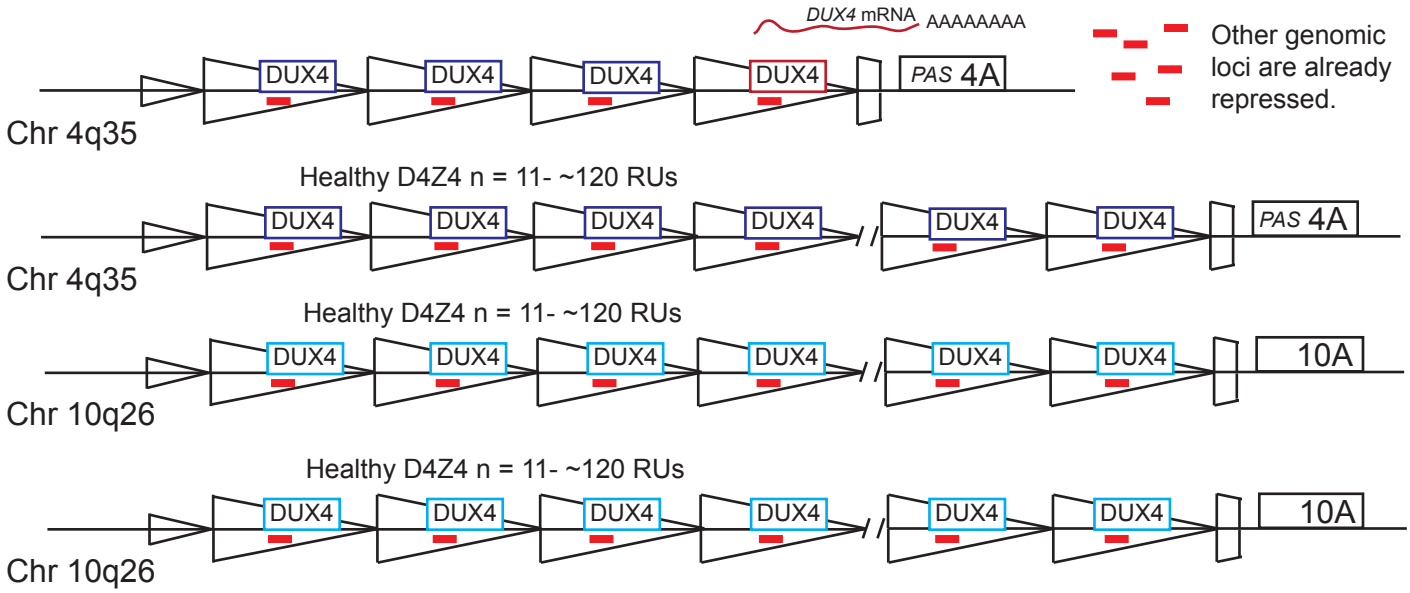


CRISPR/dCas9 Epigenetic Modulation



However, unlike with cutting or editing, the high copy number of D4Z4 repeats (targets) at chromosomes 4q35, 10q26, and elsewhere in the genome is not a problem and may actually be a benefit.

Works just as well for FSHD1 and FSHD2.



dCas9 targeting the D4Z4 gene shuts off the pathogenic *DUX4* gene, while other "target" D4Z4s are already OFF.

Targeted repression returns the pathogenic D4Z4 array to its healthy OFF state, maintains other D4Z4s in their natural OFF state, and does not damage the genomic DNA.