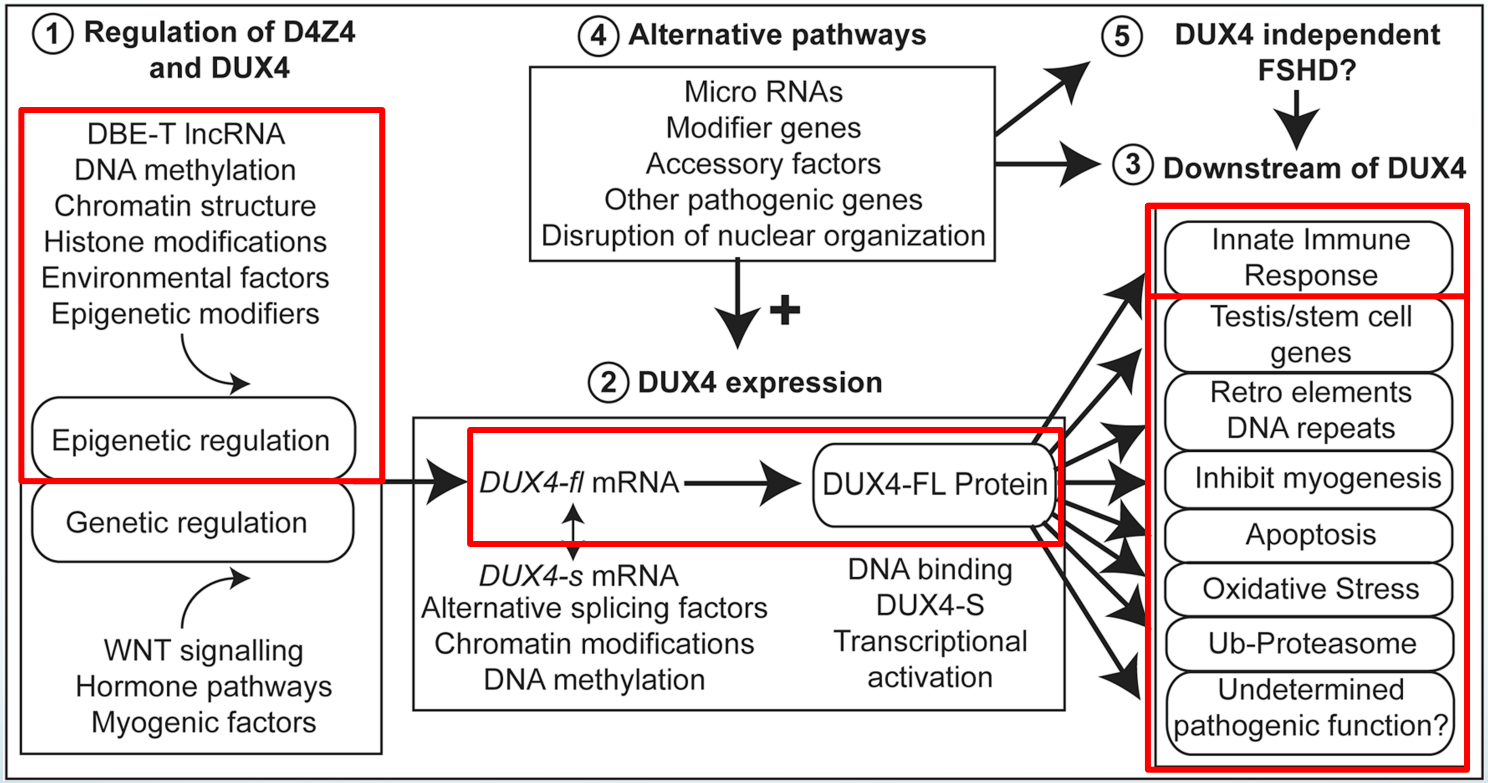
Numerous therapeutic avenues for FSHD



Himeda *et al*. (2015) *Antiox Redox Signaling*

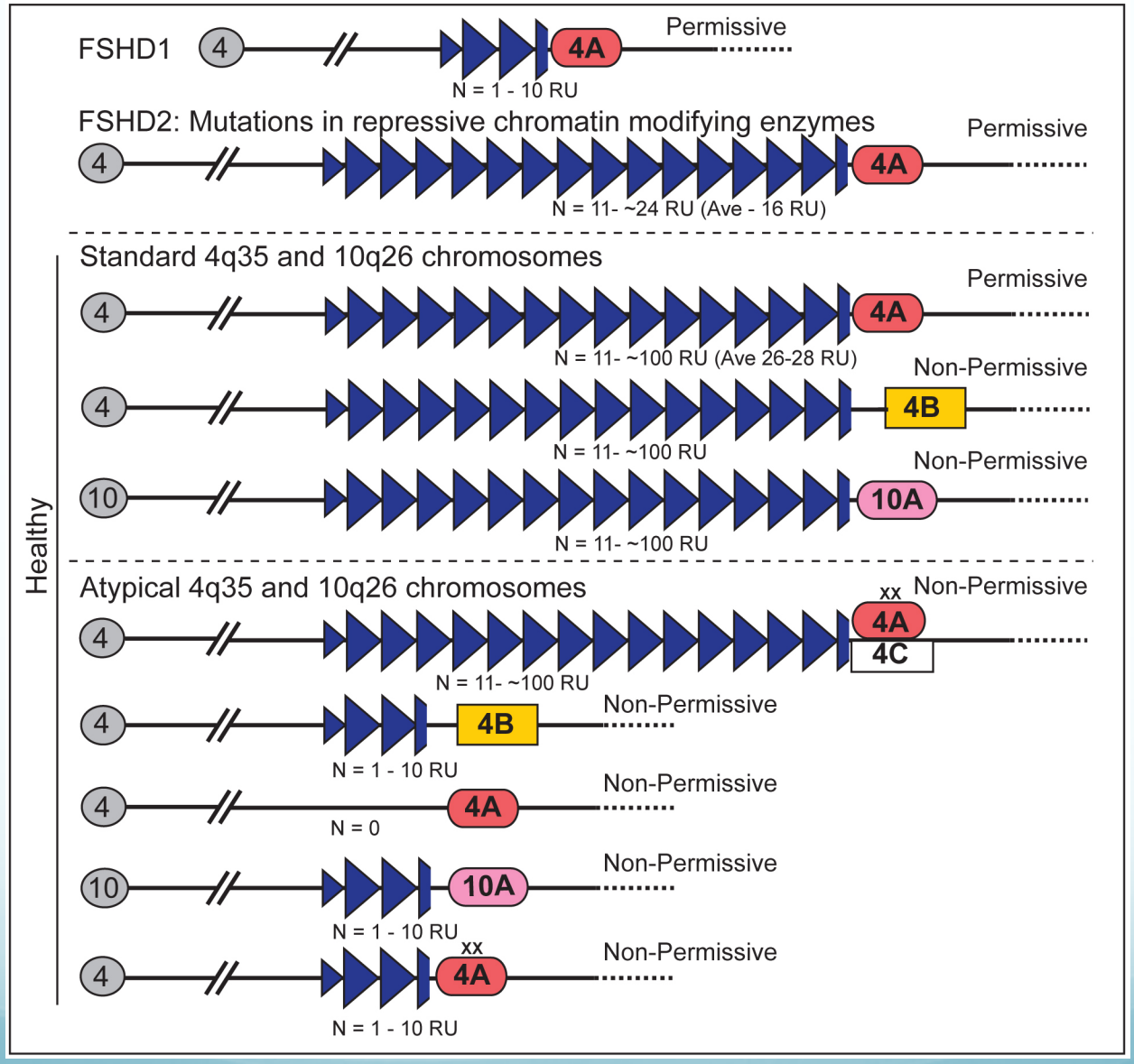
Epigenetics

"Treasure your exceptions."

Thomas Hunt Morgan

- **Context-dependent sequence independent gene expression**
- **Stable yet dynamic → Cellular memory**
- **Can be influenced by the environment (diet, aging, etc…)**

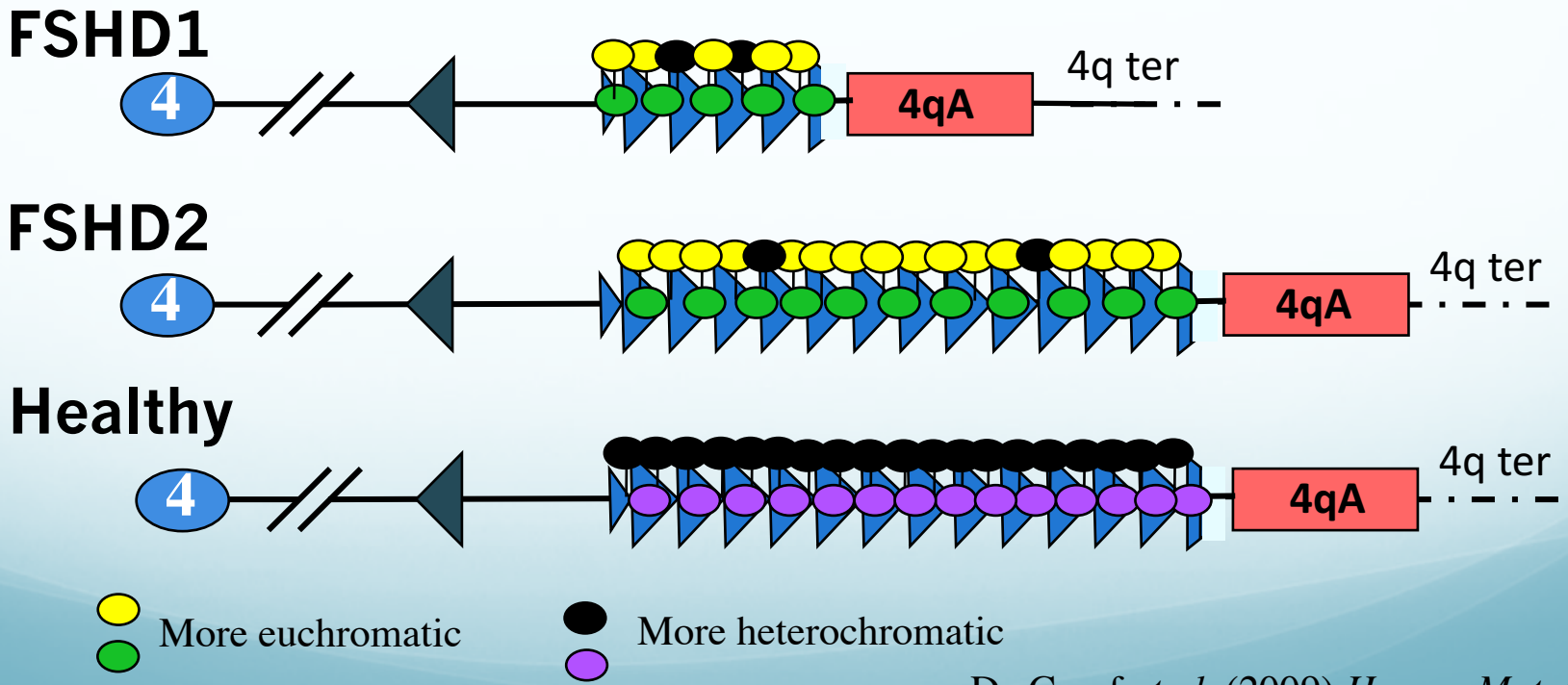
FSHD genetics are complex



Himeda *et al*. (2014) *Antiox Redox Signaling*

FSHD is primarily an epigenetic disease

High variability within the clinical and genetic population
→ Presentation, progression, severity, asymmetry, extramuscular, and age of onset



De Greef *et al*. (2009) *Human Mutation*

All types of FSHD are linked to epigenetic status of 4q35 D4Z4

FSHD1: Dominant deletions at 4q35 D4Z4 array
Apparently low penetrance
DNA Hypomethylation of shortened 4q35

FSHD2: Dominant inactivating mutations in *SMCHD1*
--ATPase chromatin remodeling protein
-- account for ~85% of FSHD2 → more mutations
DNA Hypomethylation of 4q35 and 10q26 arrays

IFSHD: Infantile form of FSHD1 or FSHD2, much more severe
DNA Hypomethylation of FSHD1 or 2

Epigenetic Gene and Genome Regulation

"The structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states" - Adrian Bird

Keys: DNA sequence independent
Context dependent
Stable/Heritable
Cellular Memory
Dynamic/reversible
Responsive

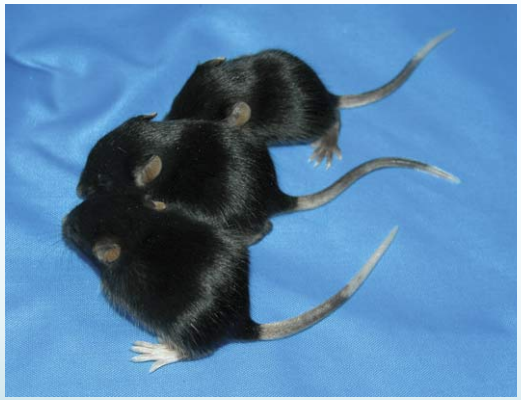
Chromatin: DNA, histones, non-histone proteins, RNA

Epigenetics: Nurture (Environment) vs. Nature

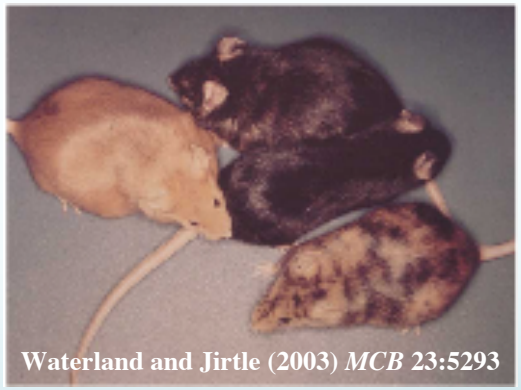
Heritable changes in gene activity that do not involve alterations to the genetic code

"Epialleles"

Nature: Genetics	*vs.*	Nurture: Epigenetics
Passed on by Mom and Dad		**Passed on by Mom and Dad**
Affected by: • **Mutagens** • **Mistakes**		**Affected by:** • **Stress** • **Sleep** • **Diet** • **Prenatal care** • **Environment**



Genetically identical

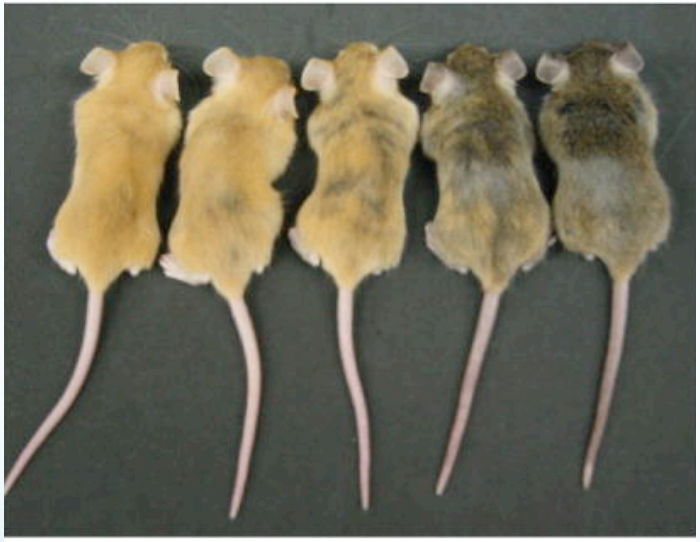


Genetically identical, epigenetically different

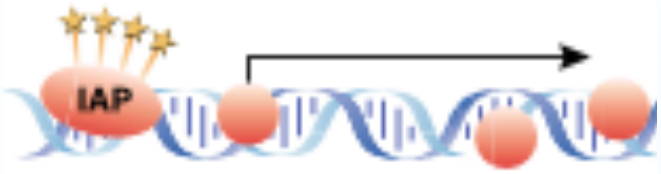
Epigenetic differences in genetically ~ equivalent people (i.e. identical twins) can have profound effects

Epigenetic differences can have profound long-term health consequences

Epialleles



A^{IAP} allele, methylated



Brown, normal

A^{IAP} allele, unmethylated

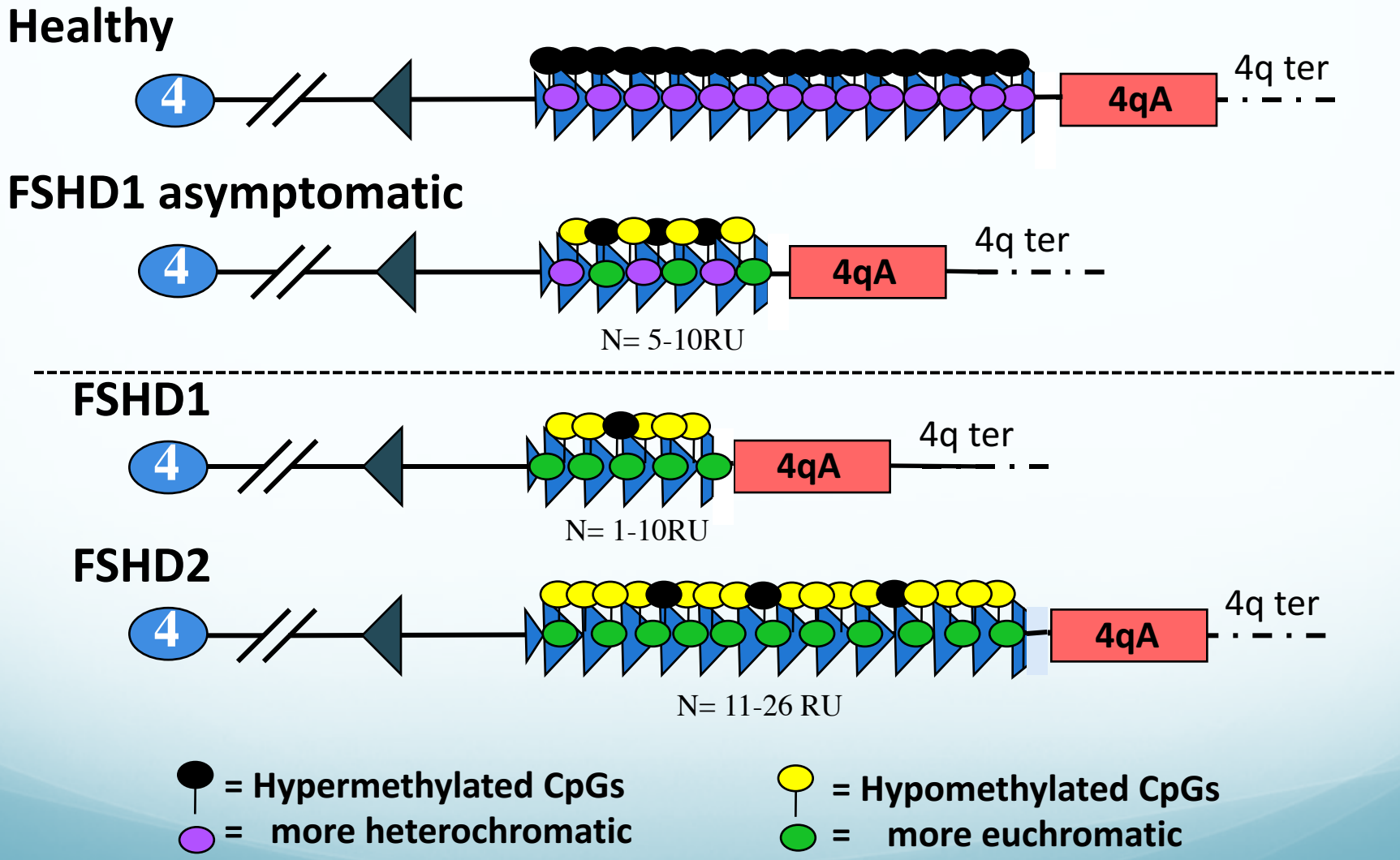
Yellow, obese, spontaneous tumors

Genetically identical
Epigenetically different

→ **Affects long-term health**
→ heritable?

Waterland and Jirtle, *Mol Cell Biol*, 2003

FSHD epigenetics dictate disease



Epigenetic Therapies

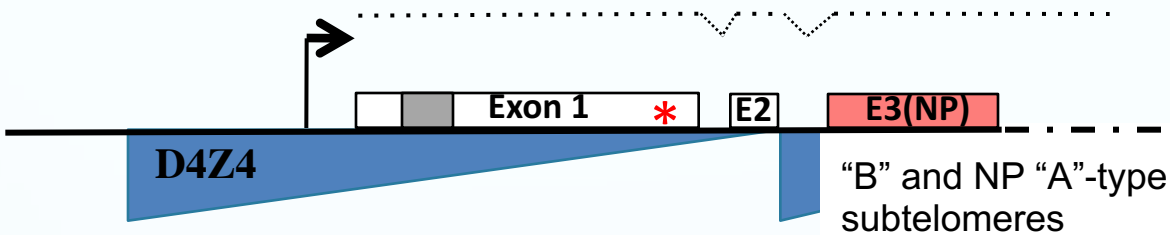
Disease	Epigenetic target	Approaches
FSHD	Euchromatic D4Z4 Chromatin structure	Small molecule inhibitors CRISPR/Cas9/dCas9
	Heterochromatic structure	
Rett Syndrome	Reactivation of silenced X chromosome	Small molecule inhibitors
Fragile X Syndrome	Reactivation of FMR1 gene	Small molecule inhibitors
Prader-Willi Syndrome Leukemia Cancer	Reactivation of tumor suppressors	Small molecule inhibitors
Duchenne MD Myotonic Dystrophy	Induction of compensatory genes	Small molecules
Emery-Driefuss MD	Nuclear architecture	???

Sciencexpress Report

A Unifying Genetic Model for Facioscapulohumeral Muscular Dystrophy

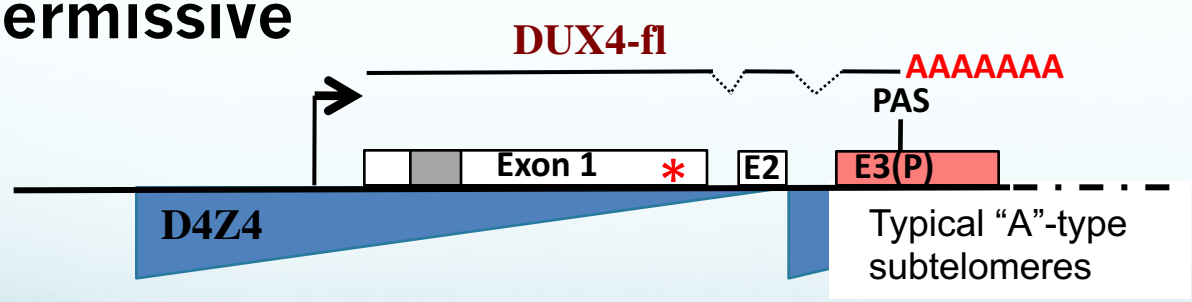
Richard J. L. F. Lemmers,[1] Patrick J. van der Vliet,[1] Rinse Klooster,[1] Sabrina Sacconi,[2] Pilar Camaño,[3,4] Johannes G. Dauwerse,[1] Lauren Snider,[5] Kirsten R. Straasheijm,[1] Gert Jan van Ommen,[1] George W. Padberg,[6] Daniel G. Miller,[7] Stephen J. Tapscott,[5] Rabi Tawil,[8] Rune R. Frants,[1] Silvère M. van der Maarel[1]*

Non-permissive



-Unstable mRNA
-DUX4 protein not made
-Nontoxic

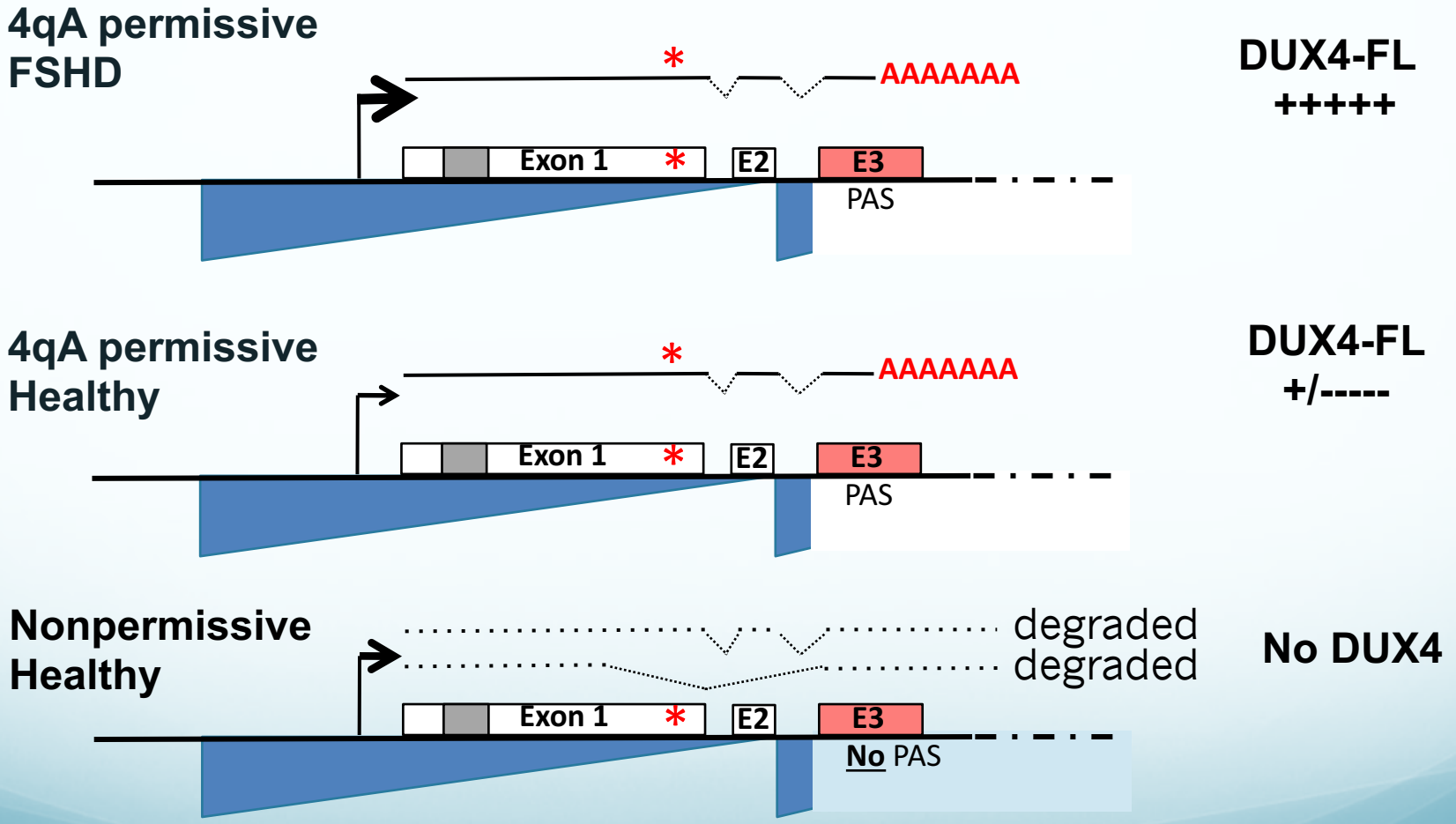
Permissive



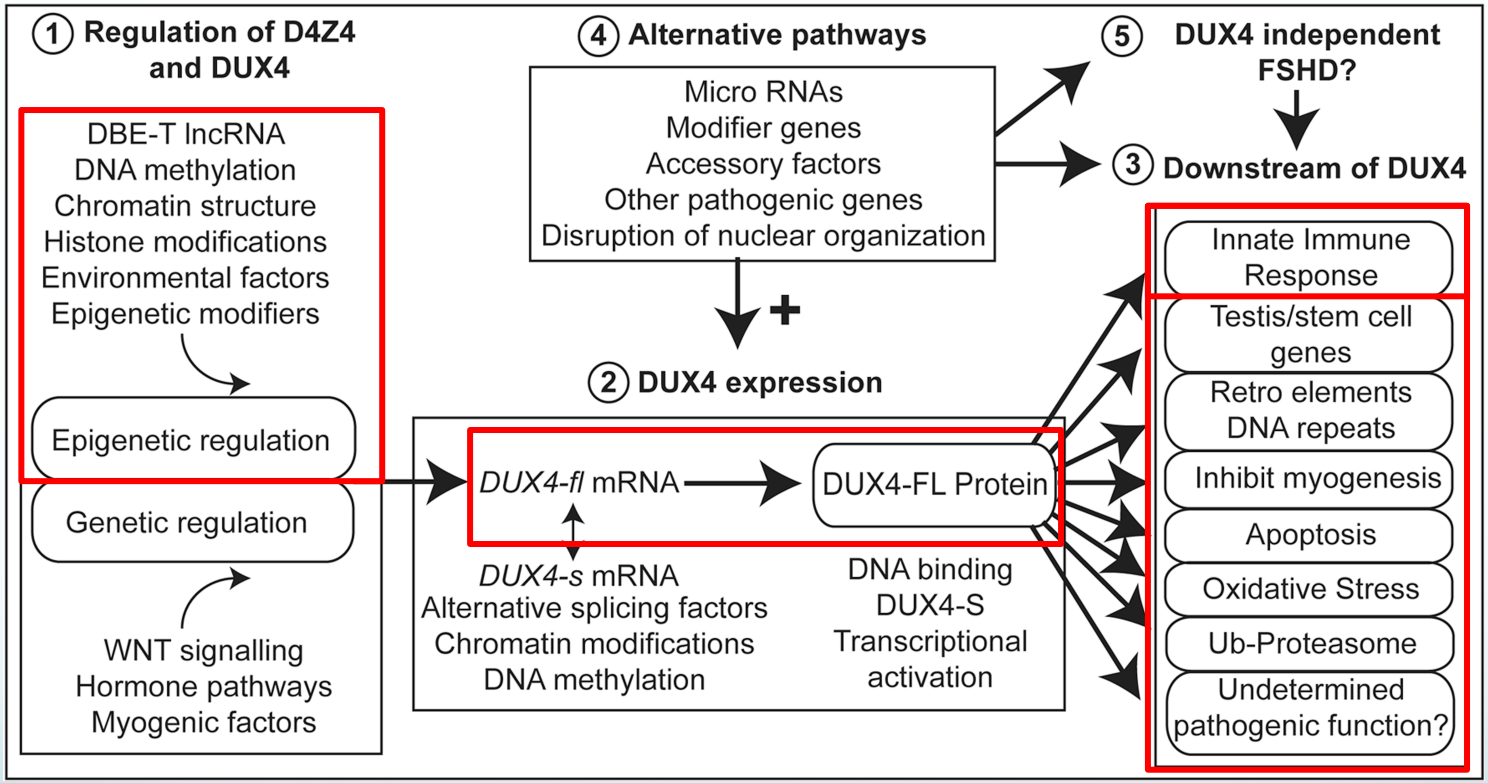
-Stable poly A mRNA
-DUX4 protein produced
-Cytotoxic

PAS = polyadenylation site
* = translation stop
NP = non-permissive
P = permissive

DUX4-fl expression is dependent upon the PAS and FSHD/healthy status

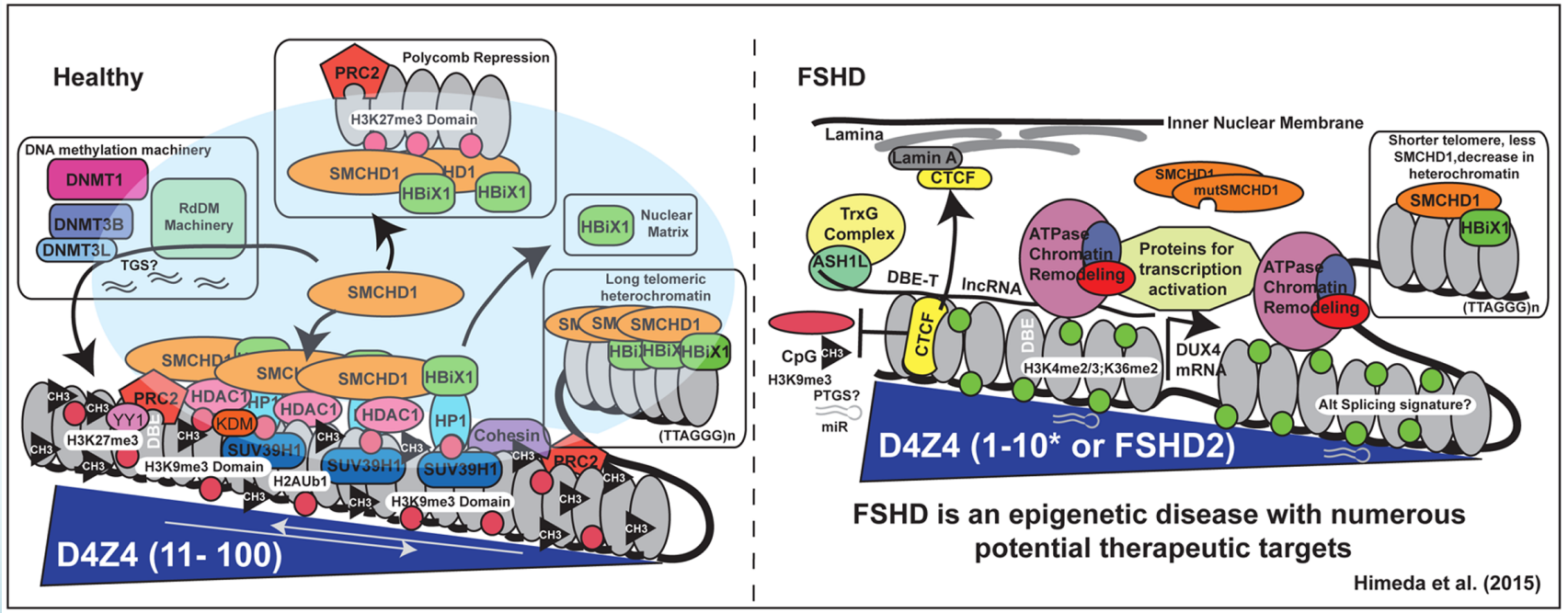


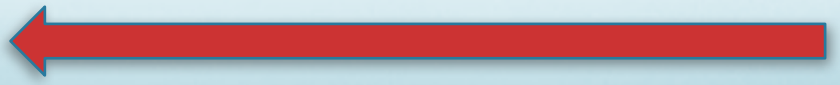
Therapeutic approaches to FSHD



Himeda *et al*. (2015) *Antiox Redox Signaling*

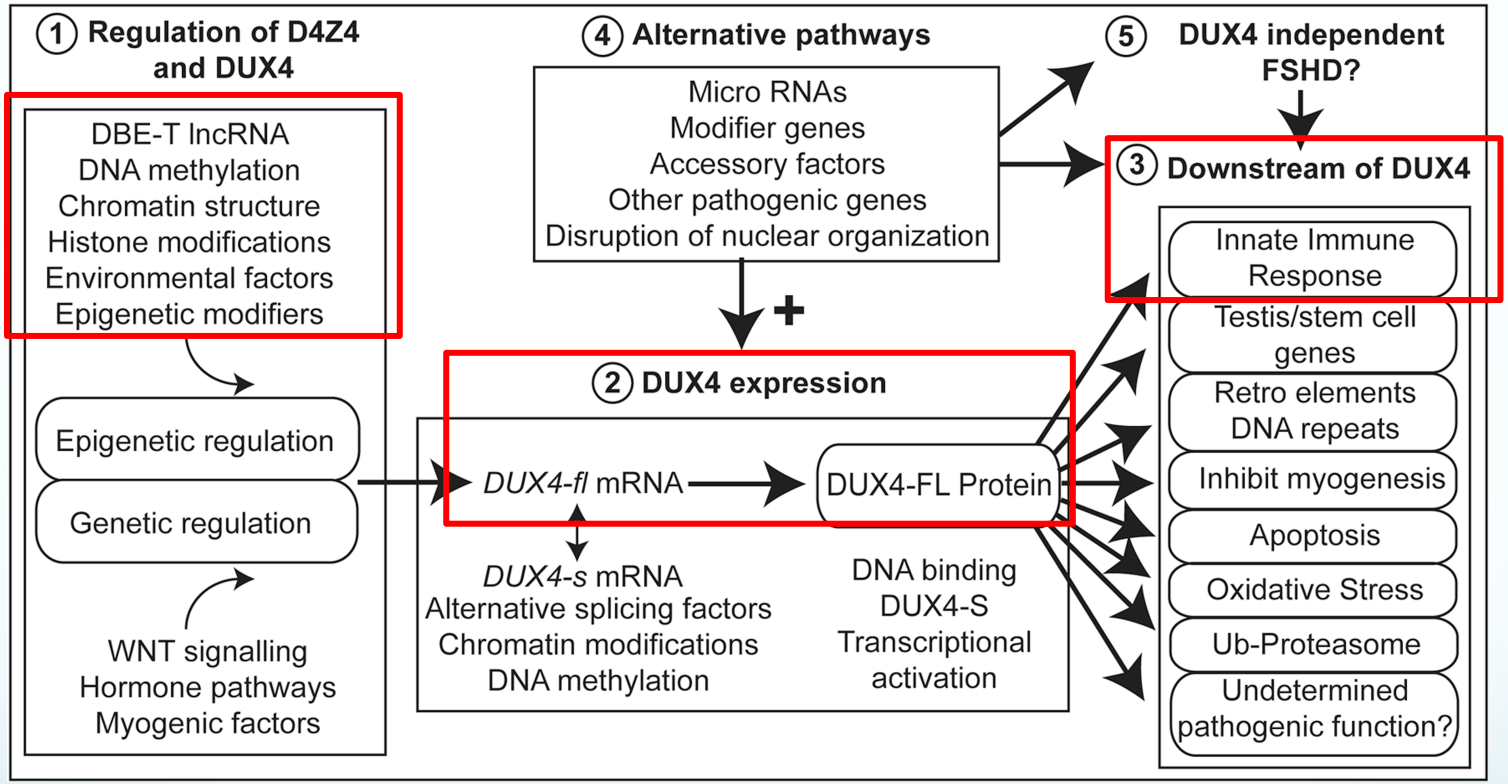
Epigenetic regulation is a therapeutic target for FSHD





Small molecule inhibitors

Therapeutic approaches to FSHD



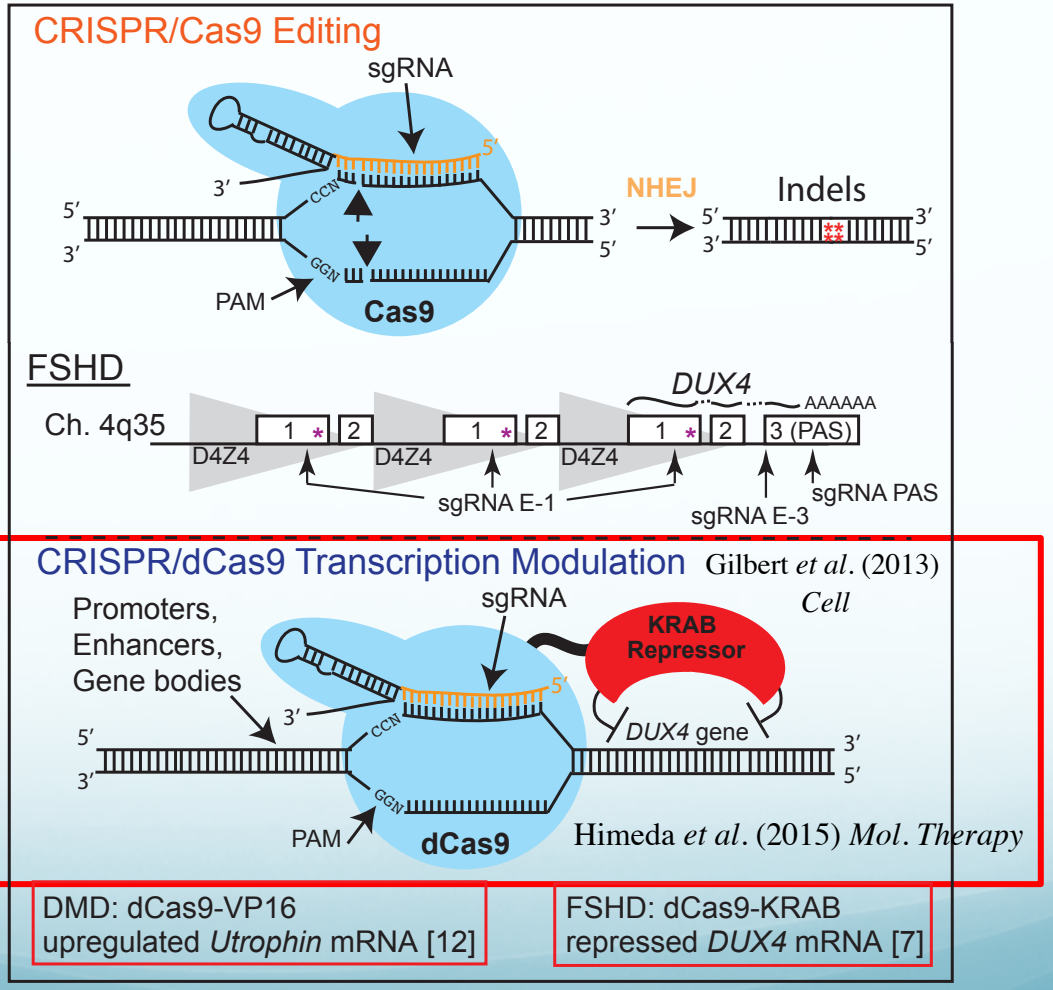
Morpholinos/PMOs/shRNAs/miRNAs

CRISPR/Cas9 **CRISPRi/dCas9-KRAB**

Anti-inflammatory **Myostatin inhibition**

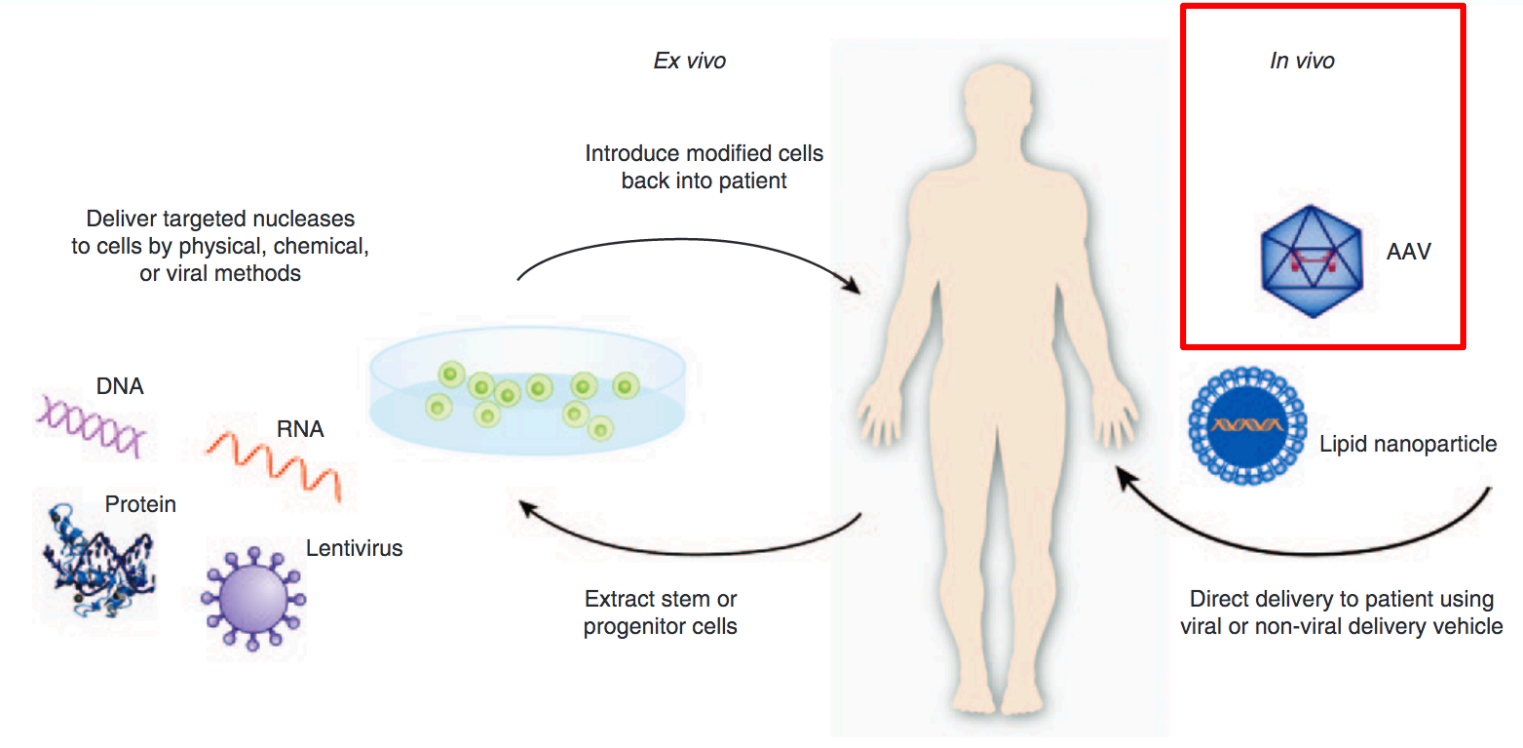
CRISPR-Cas9 and dCas9 in muscular dystrophy research and therapeutic development

Efficient genome targeting



Himeda *et al*. (2016) *Trends Pharmacol*.

Therapeutic delivery of CRISPR/Cas is challenging



Maeder and Gersbach (2016) *Mol Ther* 24:430