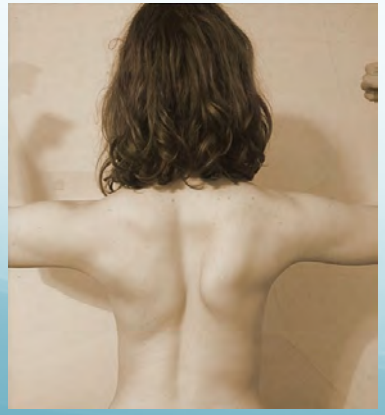
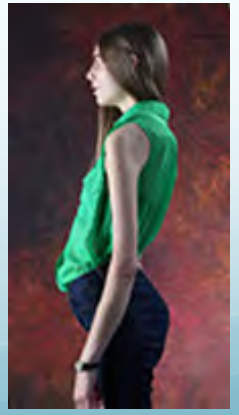
University of Nevada, Reno
School of Medicine
Department of Pharmacology

Emerging Treatment Strategies for FSHD

LA patient meeting Oct 21, 2017

Peter L. Jones, Ph.D. and Takako I. Jones, Ph.D.
Co-Principal Investigators



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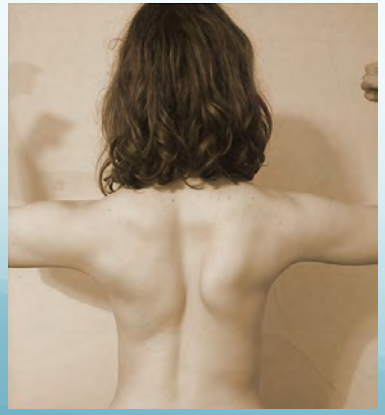
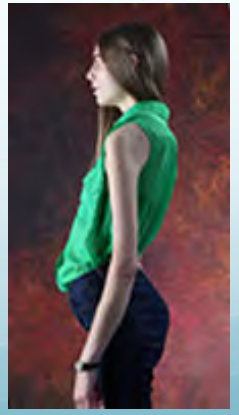
Disclosures:

Peter Jones, Takako Jones, and Charis Himeda are listed as inventors on US patent applications for epigenetic diagnosis of FSHD (PJ, TJ), epigenetic therapeutic targets for FSHD (PJ) and CRISPR therapy for FSHD (PJ, TJ, CH).

Peter Jones is on the SAB for Fulcrum Therapeutics






Rare Diseases as a group are not so rare
>90 Neuromuscular Diseases

~30 muscular dystrophies: progressive weakness and degeneration of the skeletal muscles that control movement.

Muscular dystrophy: 9 classes of disease

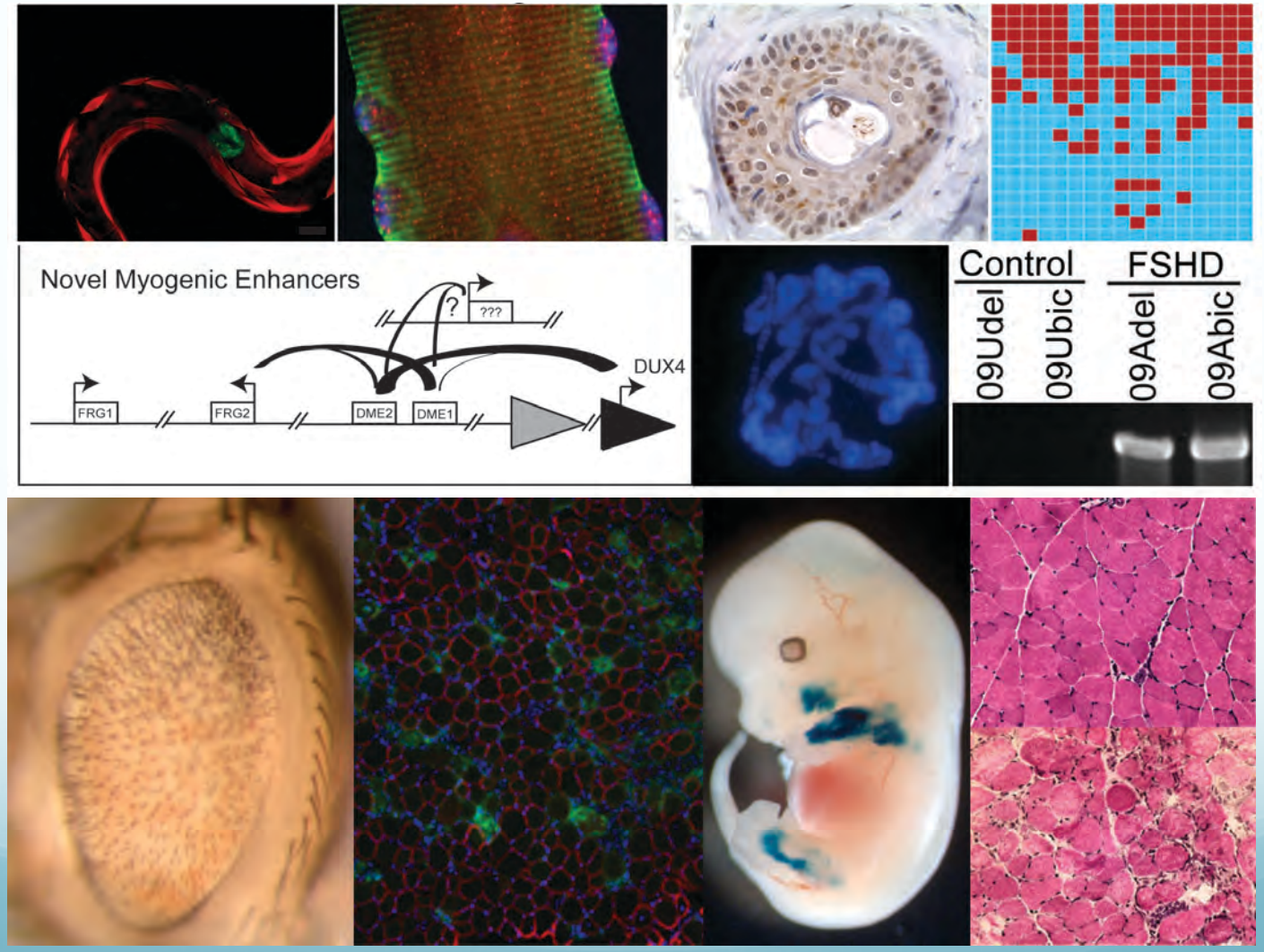
- **BMD (Becker)**
- **CMD (Congenital)**
- **DMD (Duchenne)**
- **DDM (Distal)**
- **EDMD (Emery-Dreifuss)**
- **FSHD (Facioscapulohumeral)**
- **LGMD (Limb-Girdle)**
- **MMD (Myotonic)**
- **OPMD (Oculopharyngeal)**

Aging can be considered a muscle disease

Big Picture Perspective

Research across the full spectrum of neuromuscular diseases leads to scientific and medical breakthroughs that accelerate treatments and cures. The power in this approach is that we can often apply learnings from one disease to progress in others to bring urgently-needed answers to affected patients and families.

Jones Lab expertise is epigenetics and developmental biology



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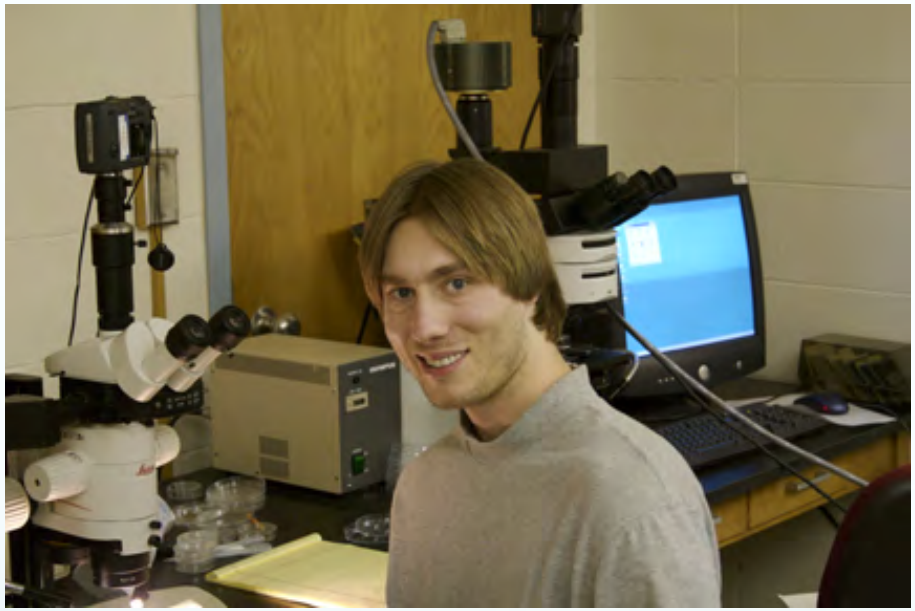


Ryan Wuebbles, PhD

2002 Patient meeting: FSHD is caused by a loss of epigenetic regulation

Introduced us to facioscapulohumeral muscular dystrophy

Epigenetics

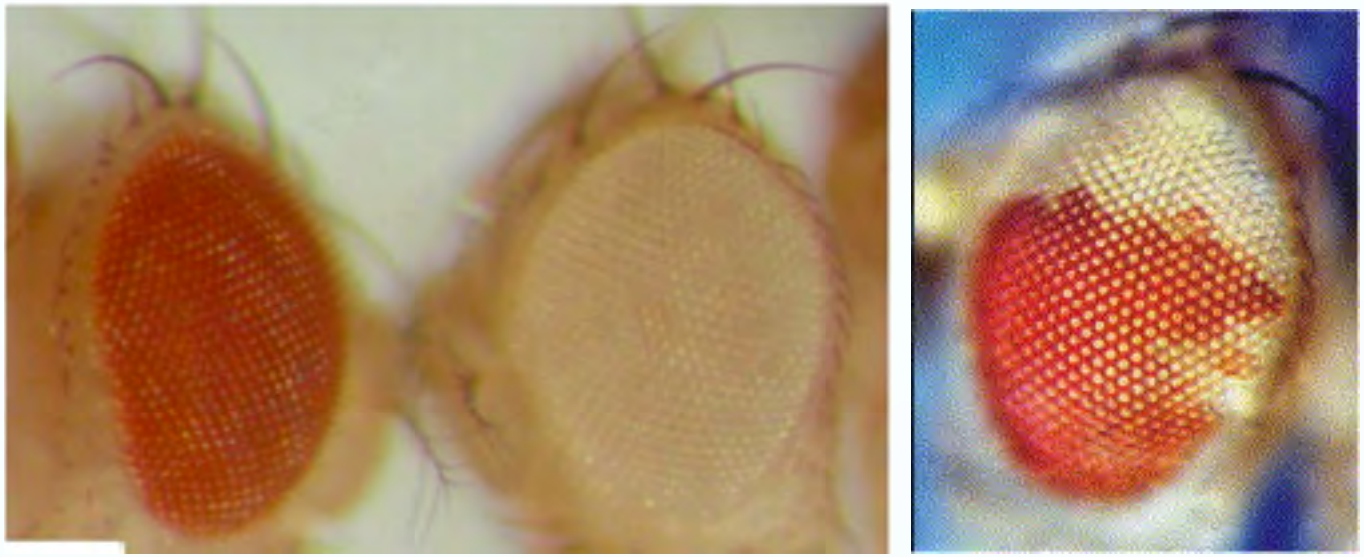
"Treasure your exceptions."

Thomas Hunt Morgan

Randy Jirtle/Duke University

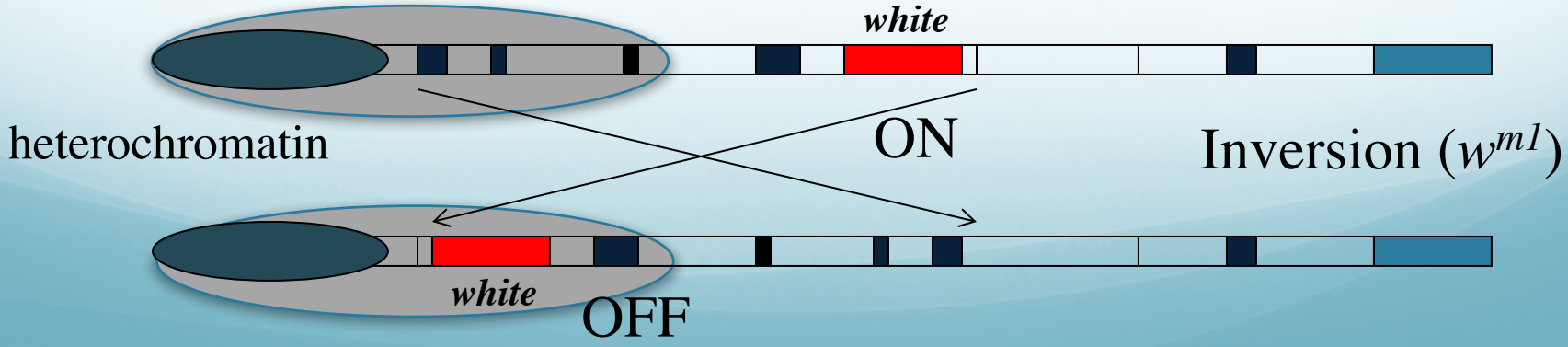
- **Non-Mendelian pattern of heritability**
- **Context-dependent sequence independent gene expression**
- **Can be influenced by the environment (diet, aging, etc…)**

The gene "environment" affects gene expression



Normal Genetic mutant Epigenetic mutant

Translocation of a gene from a euchromatic region to a heterochromatic region resulting in inactivation of nearby euchromatic genes



Epigenetic differences can have profound long-term health consequences

Epialleles

A^{IAP} allele, methylated

Brown, normal

A^{IAP} allele, unmethylated

Yellow, obese, spontaneous tumors

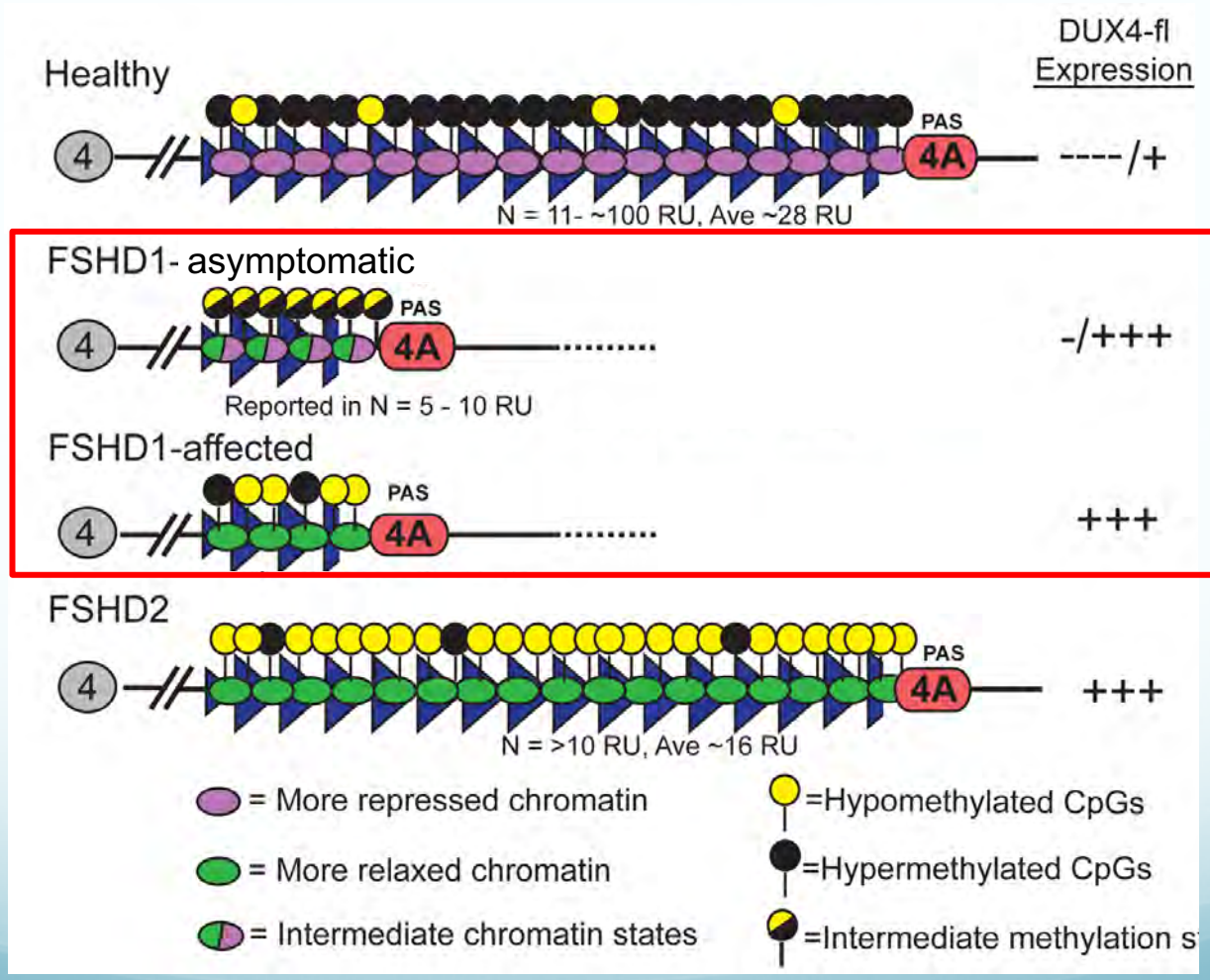
Genetically identical
Epigenetically different

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

Waterland and Jirtle, *Mol Cell Biol*, 2003

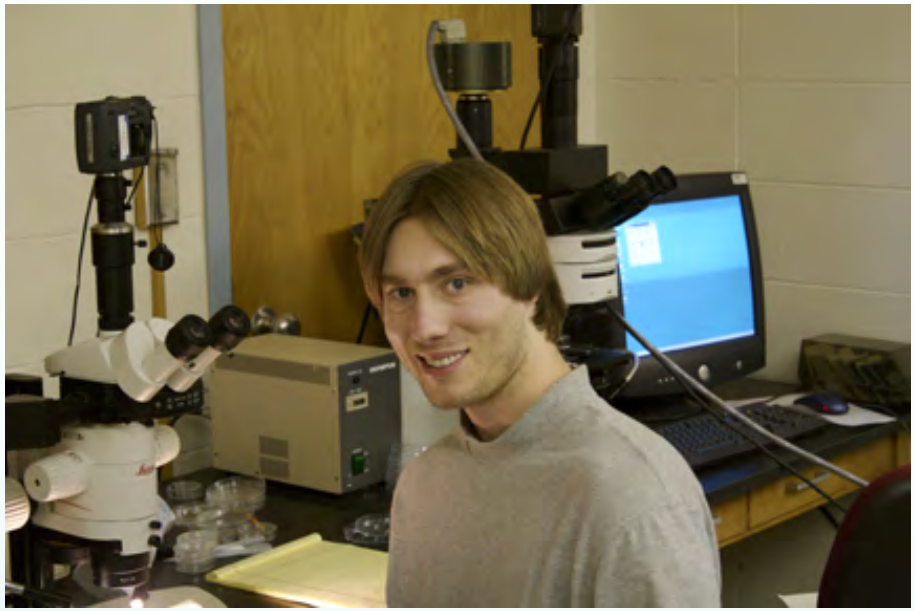
FSHD is an epigenetic disease

The FSHD gene, *DUX4*, is under epigenetic regulation
The "genetic environment" is changed in FSHD



T Jones *et al*. 2015 *Clinical Epigenetics*

Jones Lab expertise is epigenetics and developmental biology of FSHD



Ryan Wuebbles, PhD

FSHD is caused by a loss of epigenetic regulation

Since 2003, our focus has been on FSHD pathogenic mechanisms, therapeutic targets, and animal models

FSHD Therapeutic Development in 2003

FSHD gene?	**Unknown**
Pathogenic mechanism?	**Unknown**
Cellular models?	**Not significant**
Animal models?	**Non existent**

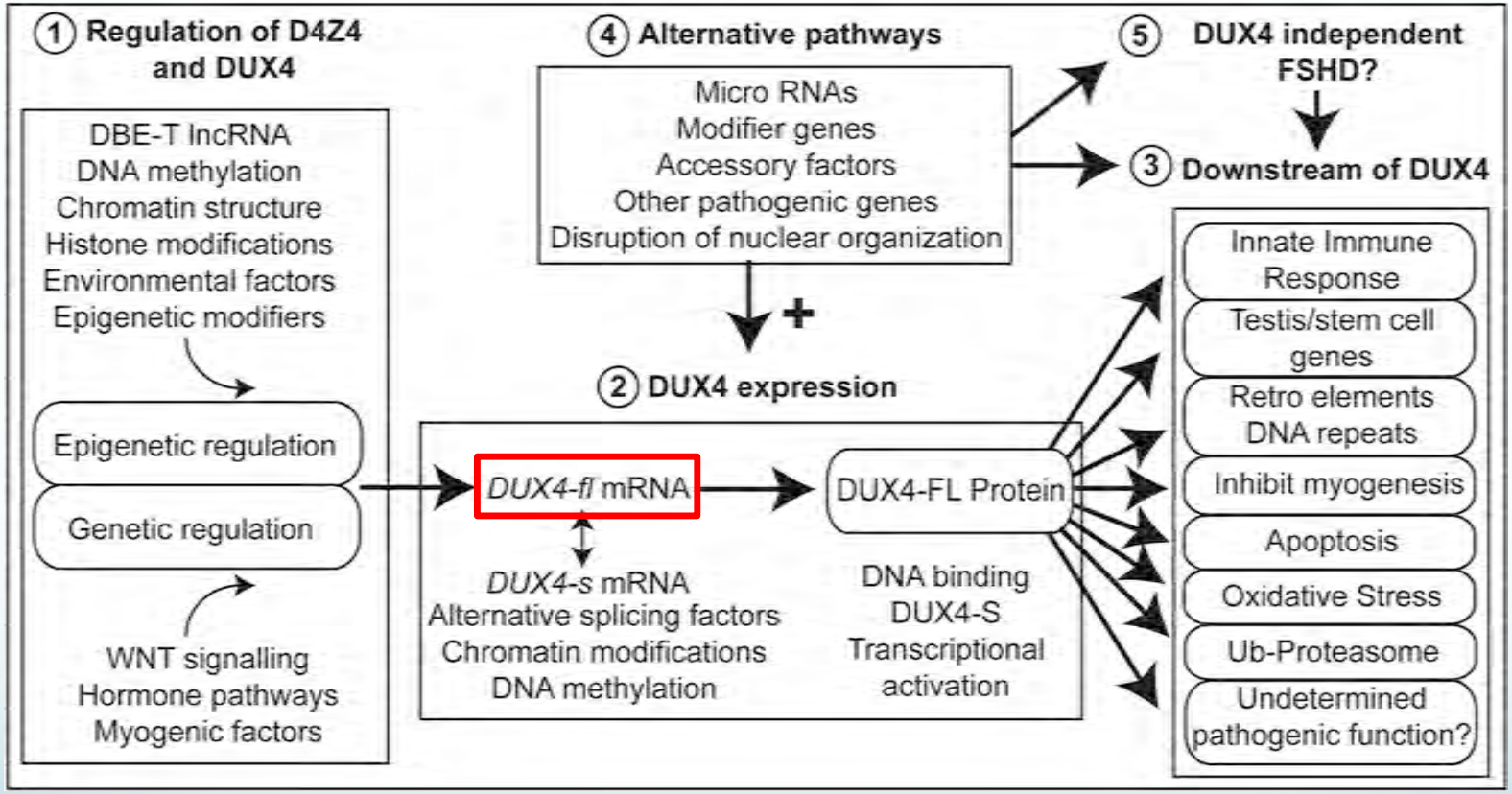
Treatments: Steroids, myostatin inhibition
Rationale: ~work for DMD, so why not

FSHD Therapeutic Development in 2017

FSHD gene?	**DUX4**
Pathogenic mechanism?	**Epigenetic dysregulation** **Still many possibilities**
Cellular models?	**Many**
Animal models?	**Mice, Fly, Zebrafish**

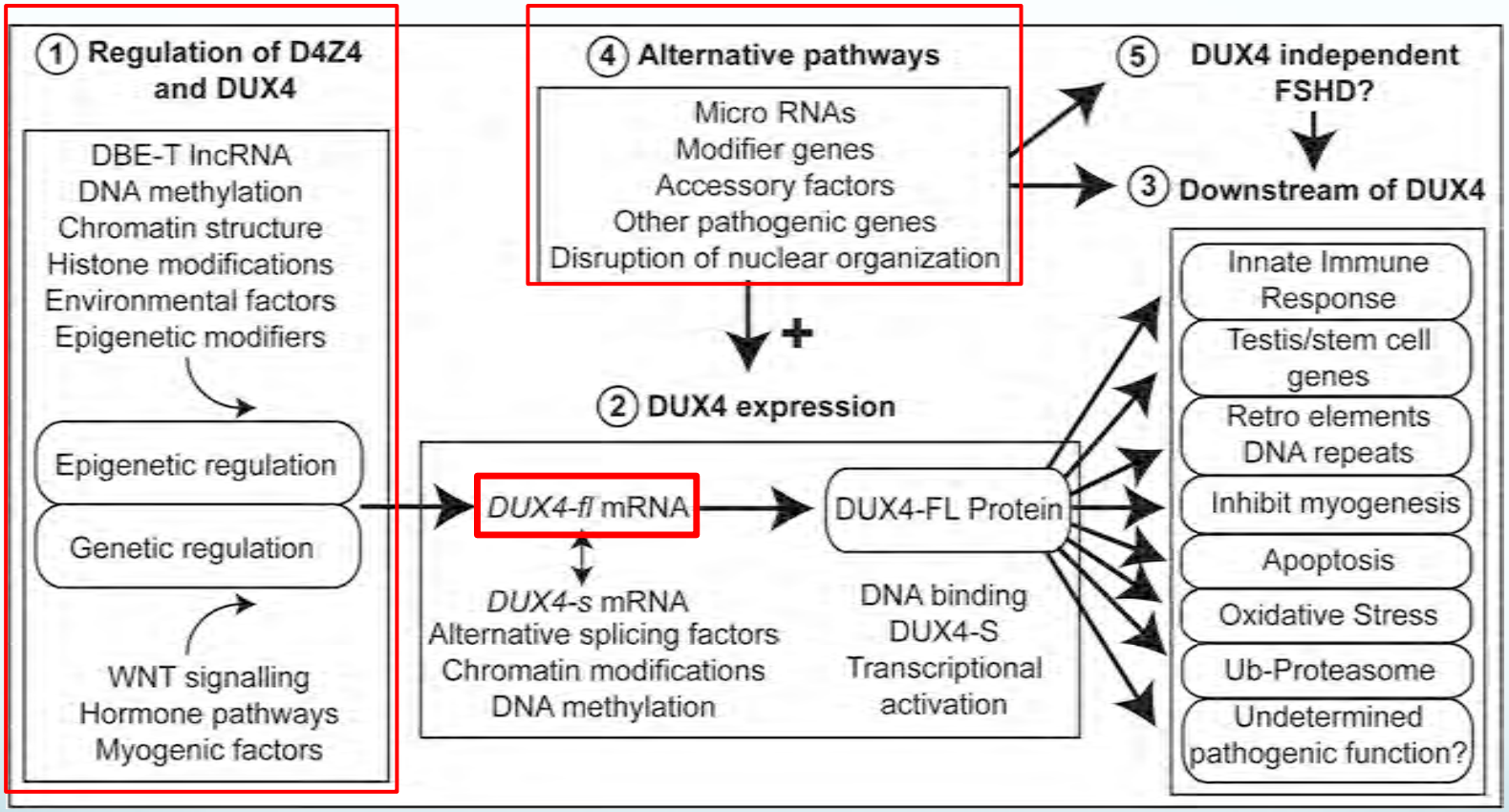
Treatments: **Myostatin inhibition, immune suppression**
Rationale: **FDA approved, biology-based**

FSHD in 2017
Many viable therapeutic approaches!



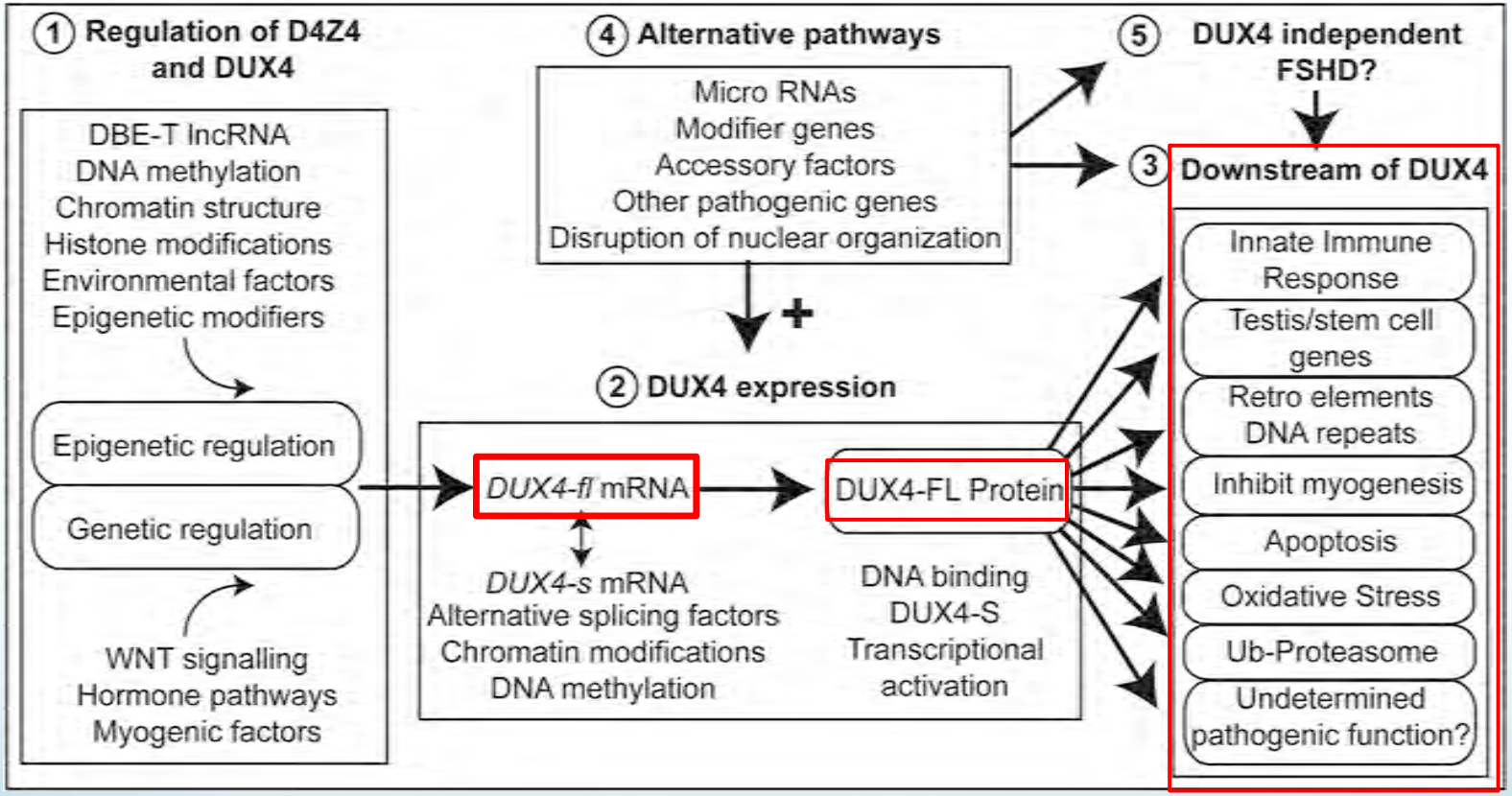
Anti-sense, morpholinos, PMOs, microRNAs
→ inactivate or destroy the DUX4-fl mRNA

FSHD in 2017
Many viable therapeutic approaches!



Small molecule inhibitors; CRISPR technology
→ prevent expression of *DUX4*

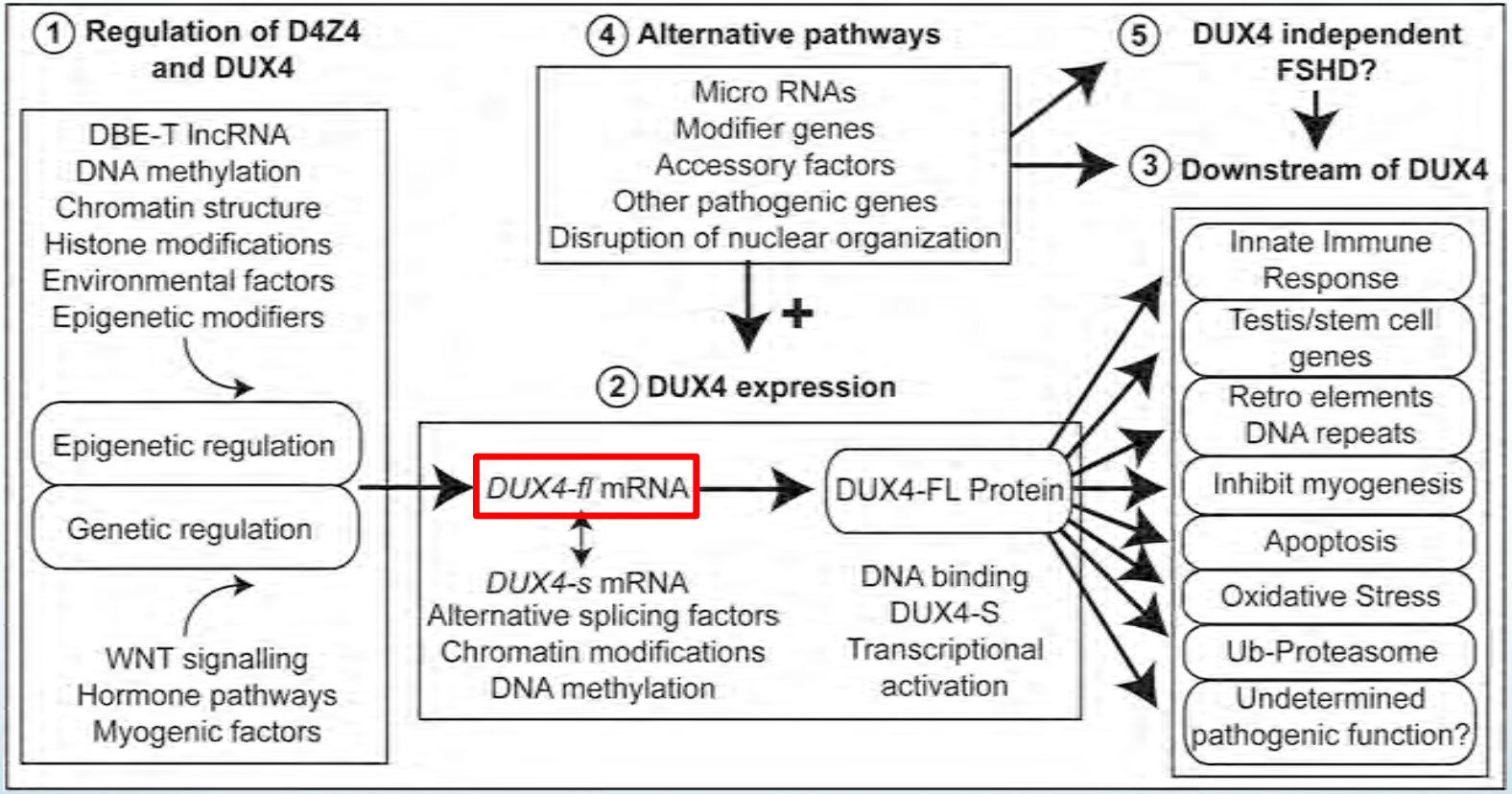
FSHD in 2017
Many viable therapeutic approaches!



Small molecule inhibitors

→ block downstream pathogenic effects of DUX4-FL protein

FSHD in 2017
Many viable therapeutic approaches!

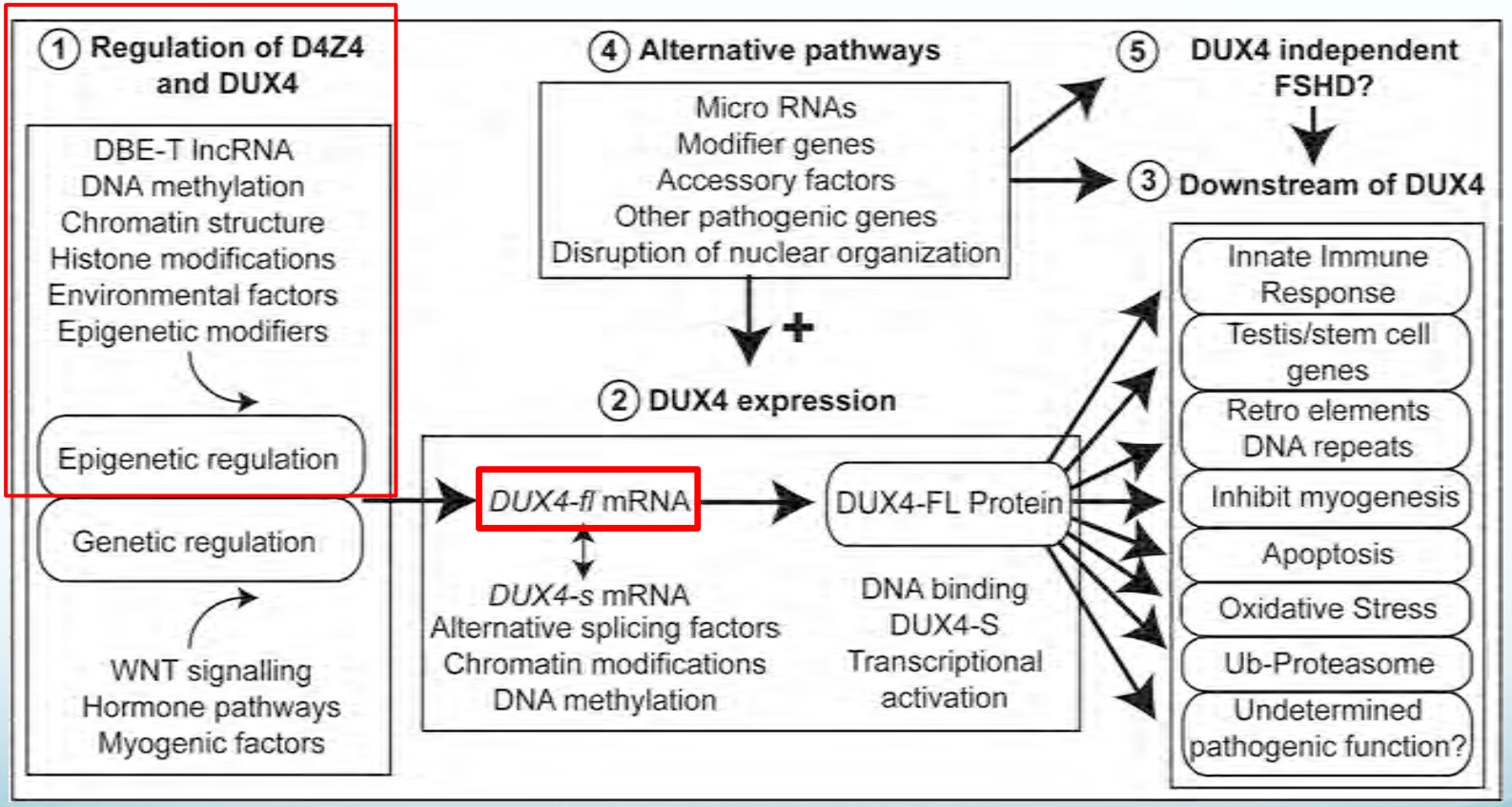


DUX4-independent approaches

→ Myostatin inhibition (Acceleron ACE-083 trial)

FSHD in 2017
Many viable therapeutic approaches!

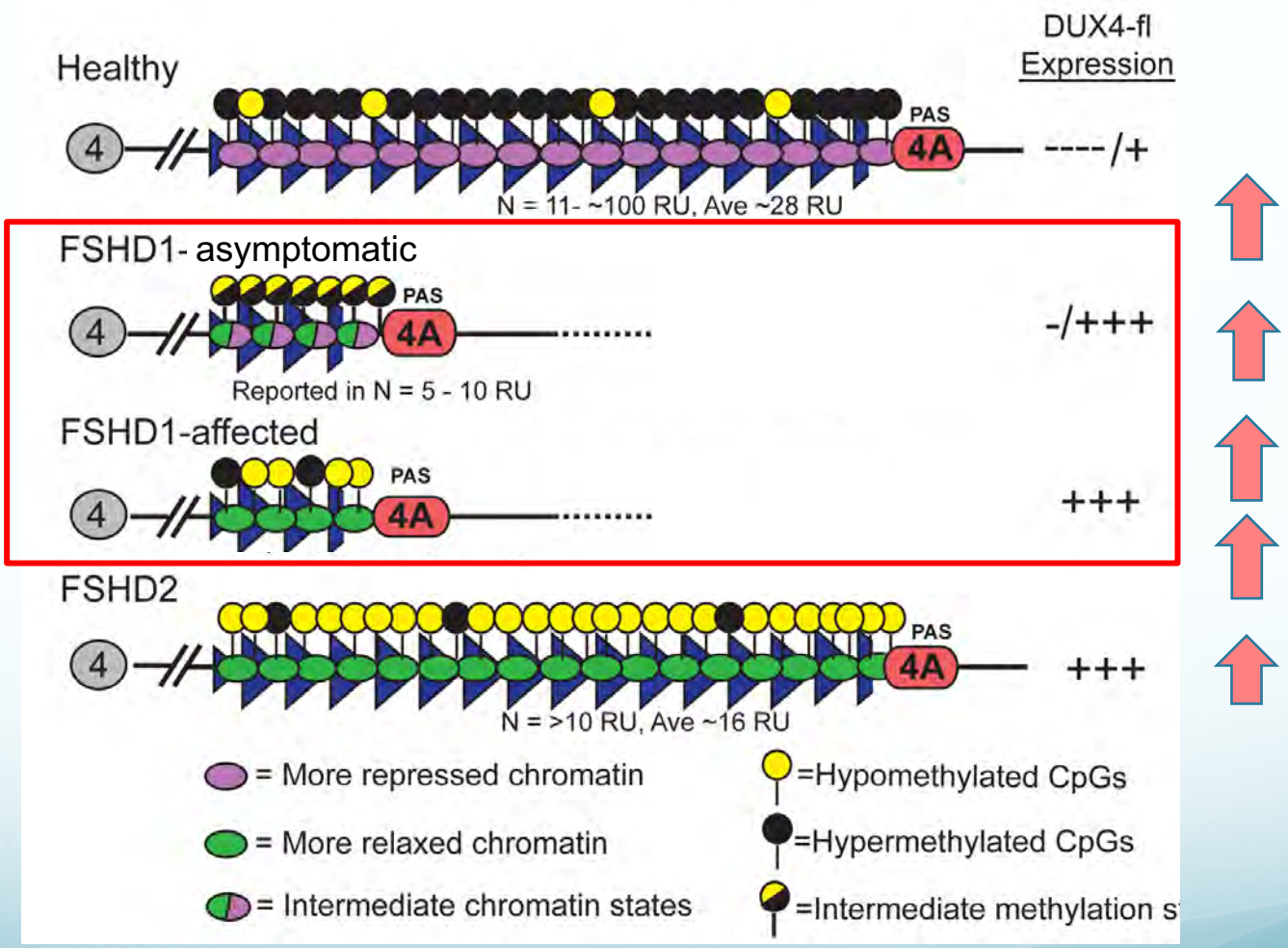
Jones Lab at UNRSOM



- **Small molecule epigenetic effectors**
- **CRISPR/dCas9 silencing**

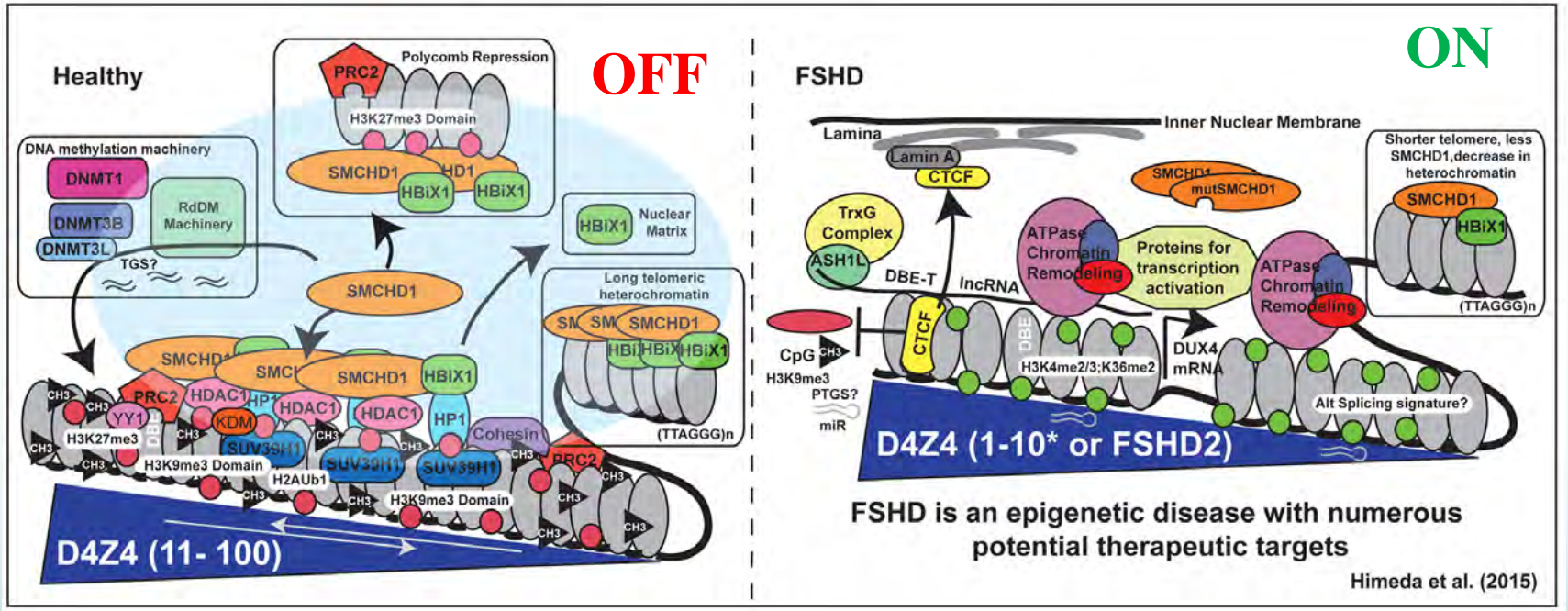
FSHD is an epigenetic disease

Can we therapeutically return to an FSHD non-affected epigenetic state?

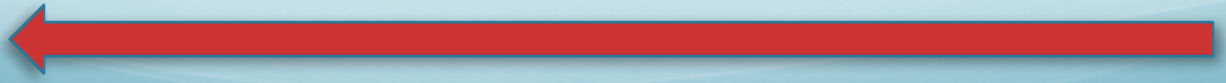


T Jones *et al*. 2015 *Clinical Epigenetics*

The FSHD genetic region normally is bound by negative regulators, in FSHD it is bound by positive regulators



Healthy and Asymptomatic **FSHD**



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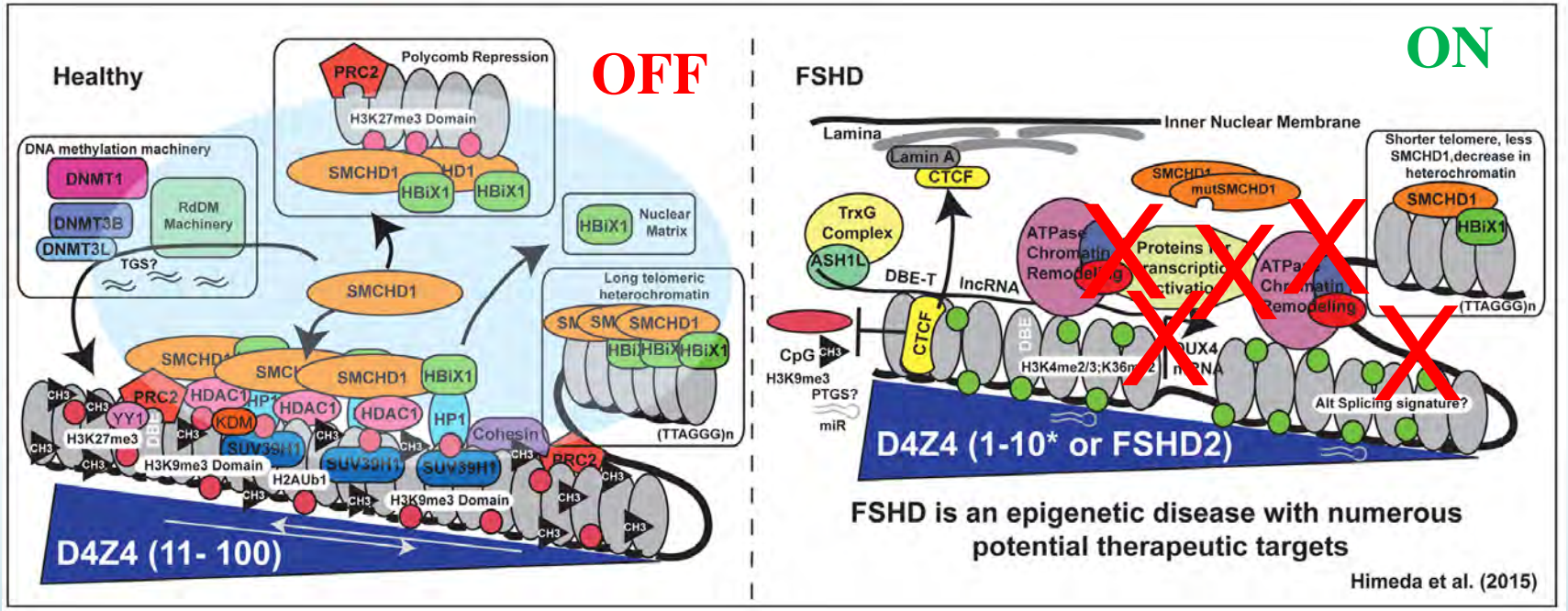
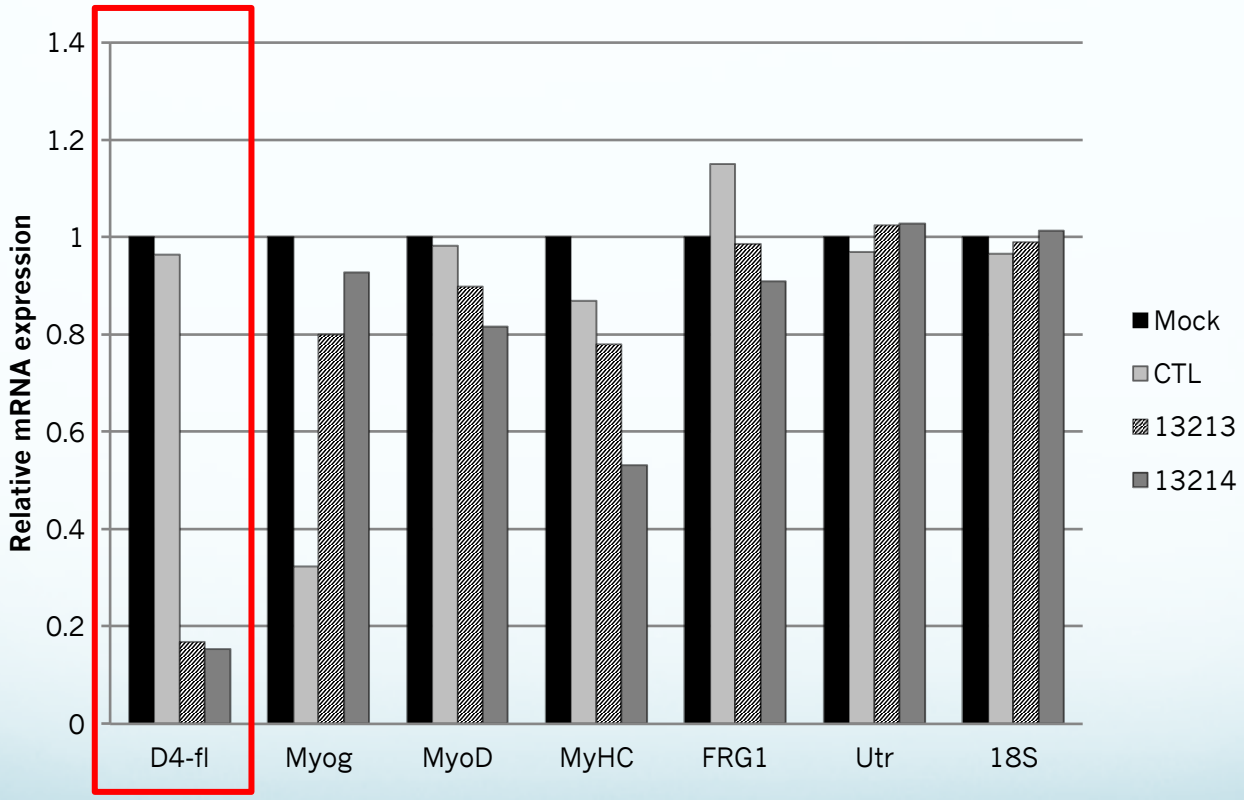


Healthy and Asymptomatic ← **FSHD**

Epigenetic drugs are a viable therapeutic approach to FSHD

We have identified strong candidates for targeted FSHD therapy

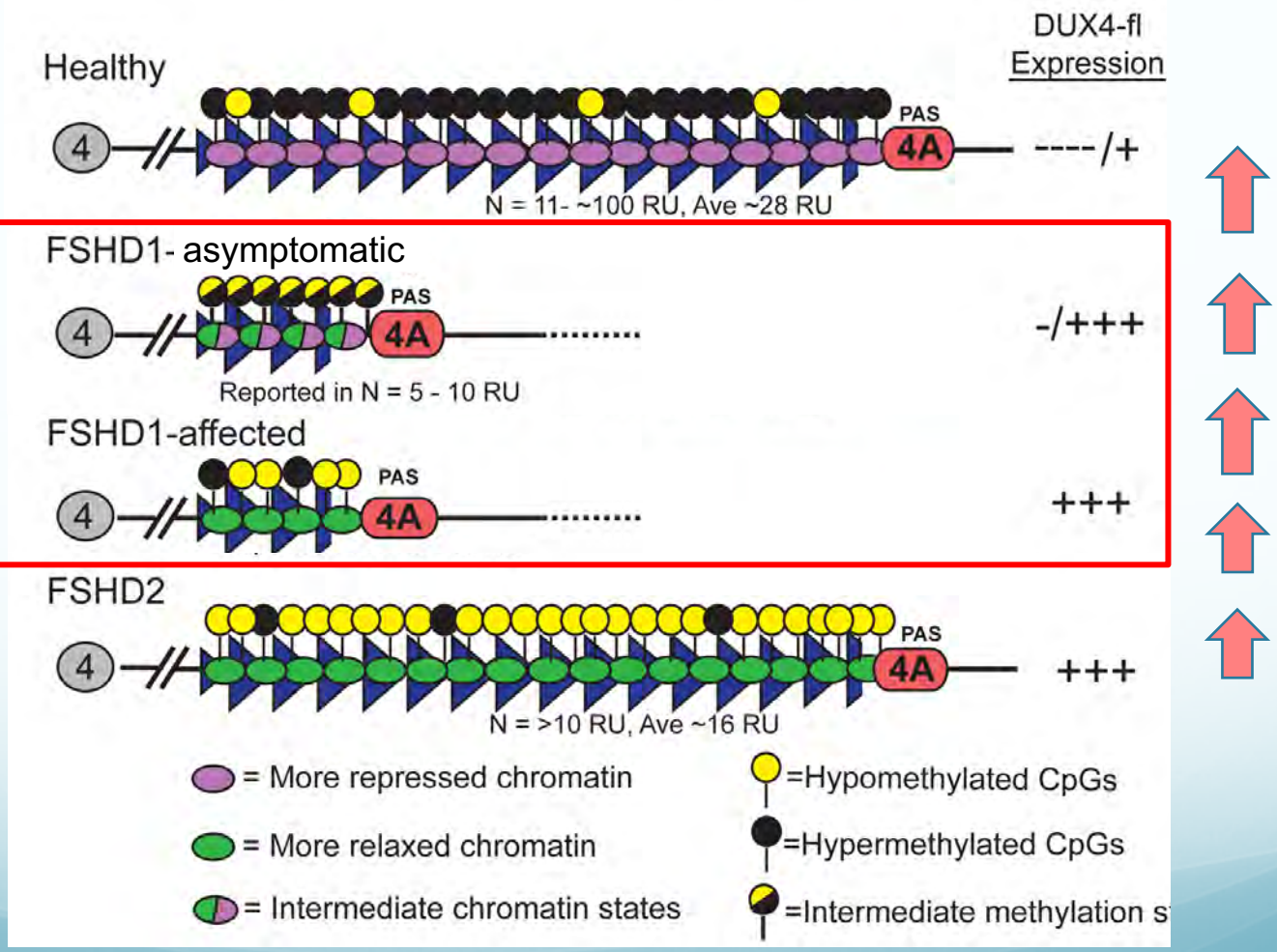
Knockdown this regulator returns the region to healthy state



Example: Epigenetic Regulator PT-2

Targeting FSHD epigenetics

Can we therapeutically return to a non-affected epigenetic state by recruiting OFF machinery?



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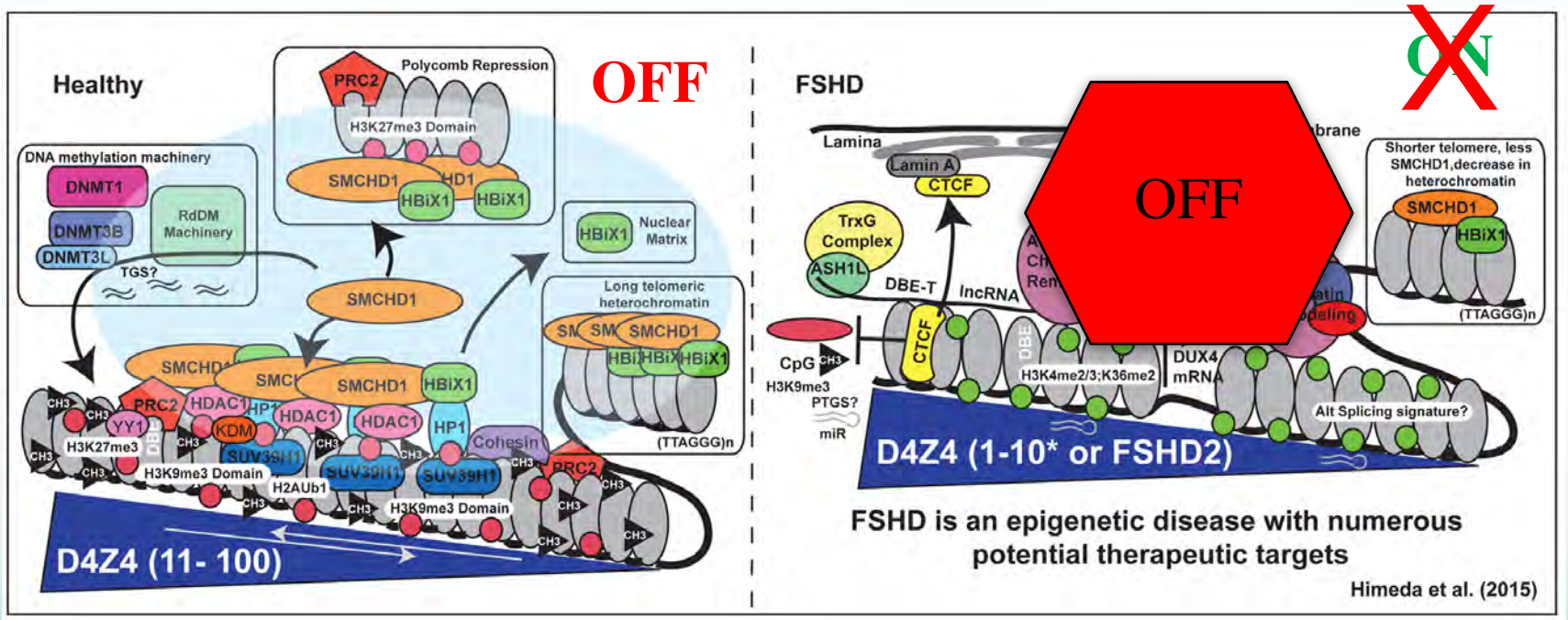


Healthy and Asymptomatic ← **FSHD**

CRISPR technology as a therapeutic approach to FSHD

CRISPR-mediated "genome editing"
Powerful, controversial, scary?

Not the whole story

CRISPR is much more than genome "editing"

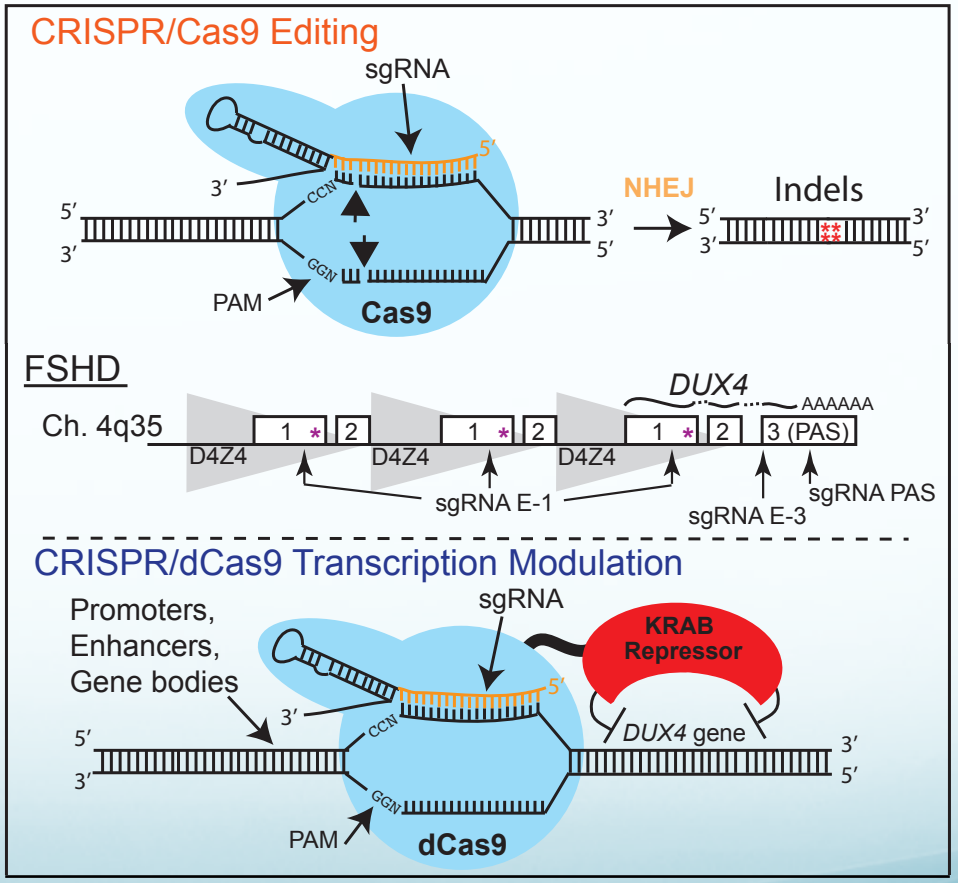
CRISPR/Cas technology is essentially a simple and more efficient way to specifically target ~any sequence of the genome of any organism

Sequence-specific genome targeting

- **Cut the DNA → editing**
- **Target an activator → turn a gene "ON"**
- **Target a repressor → turn a gene "OFF"**
- **Target a tag → "see" a gene, capture a gene**

Can we use CRISPR technology as a therapy for FSHD?

Charis Himeda, PhD



Himeda *et al*. (2015) *Mol. Therapy*
Himeda *et al*. (2016) *Trends Pharmacol*.

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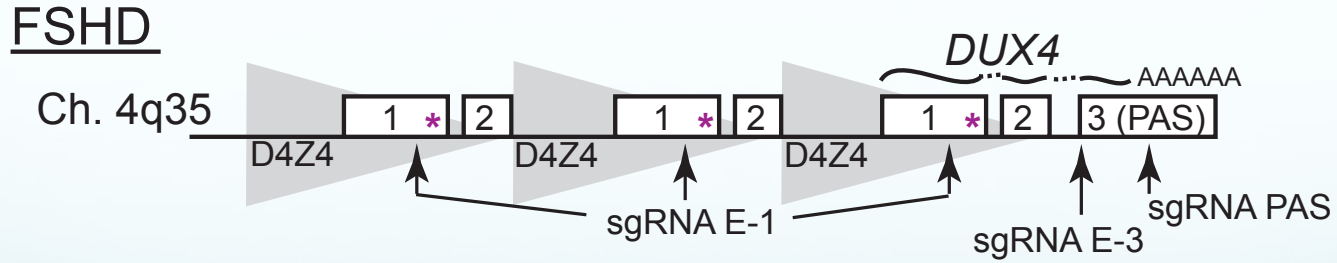
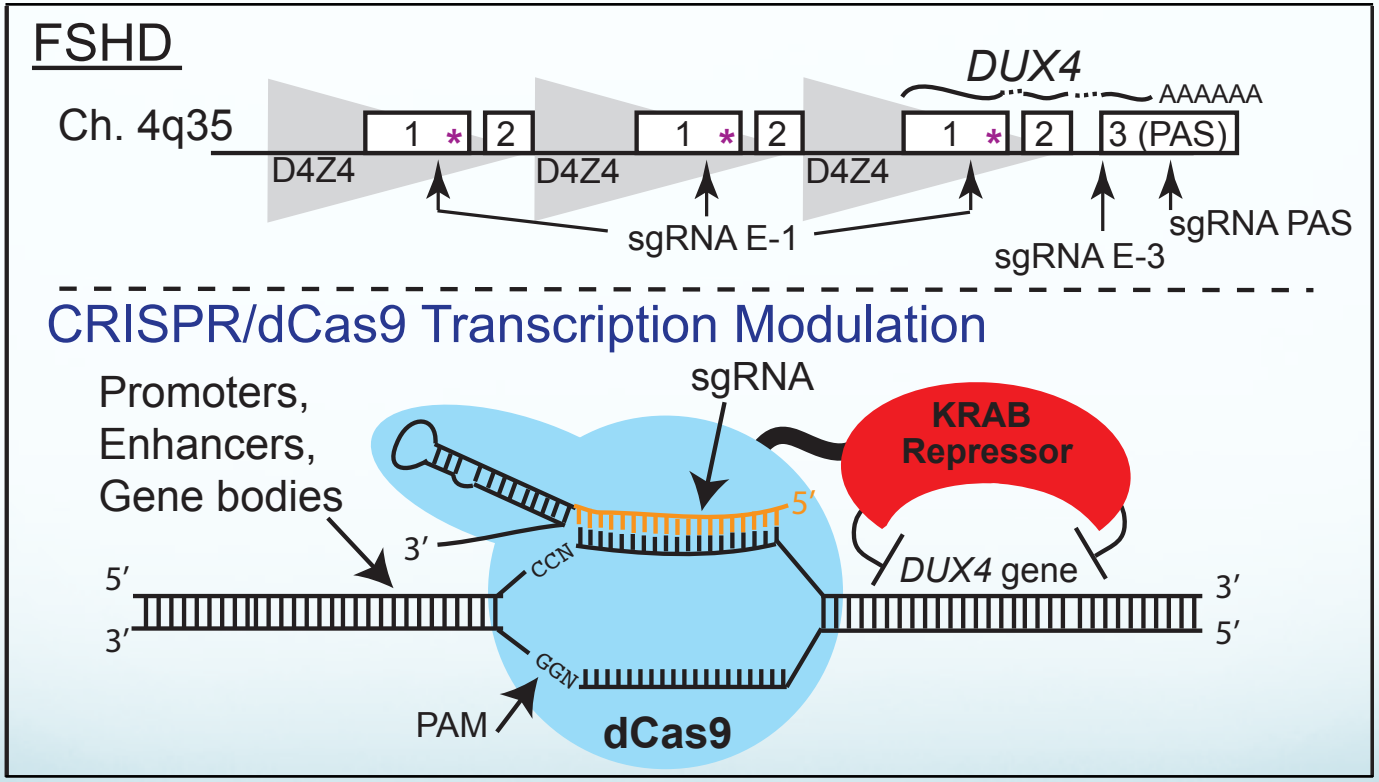


Sequence-specific genome targeting

CRISPR/dCas9 in FSHD therapeutic development

Efficient genome targeting of a transcriptional repressor



Himeda *et al*. (2015) *Mol. Therapy*
Himeda *et al*. (2016) *Trends Pharmacol*.

Proof-of-principle CRISPR "cure" for FSHD

original article

Mol Ther. 2016 Mar;24(3):527-35. doi: 10.1038/mt.2015.200. Epub 2015 Nov 3.

CRISPR/dCas9-mediated Transcriptional Inhibition Ameliorates the Epigenetic Dysregulation at D4Z4 and Represses *DUX4-fl* in FSH Muscular Dystrophy

Charis L Himeda[1], Takako I Jones[1] and Peter L Jones[1,2]

[1]*The Department of Cell and Developmental Biology, University of Massachusetts Medical School, Worcester, Massachusetts, USA;* [2]*The Department of Neurology, University of Massachusetts Medical School, Worcester, Massachusetts, USA*

The Washington Post

Innovations

How CRISPR could lead to a cure for muscular dystrophy

MOTHERBOARD Watch Machines Discoveries Space Futures Gaming Earth

CRISPR Technique Could 'Turn Off' Muscular Dystrophy Gene, Study Says

November 13, 2015 // 05:00 AM EST

Written by MELISSA CRONIN CONTRIBUTOR

BOSTON BUSINESS JOURNAL

BIOFLASH

UMass researchers achieve several 'firsts' in new use for CRISPR/Cas9

Nov 16, 2015, 11:31am EST

INDUSTRIES & TAGS Technology, Health Care, Biotech, Pharmaceuticals

How Controversial Gene Editing Could Lead To Groundbreaking Cures

This technology may change the way we think of some of the world's most challenging diseases.

Lila Shapiro
Senior Staff Reporter, The Huffington Post

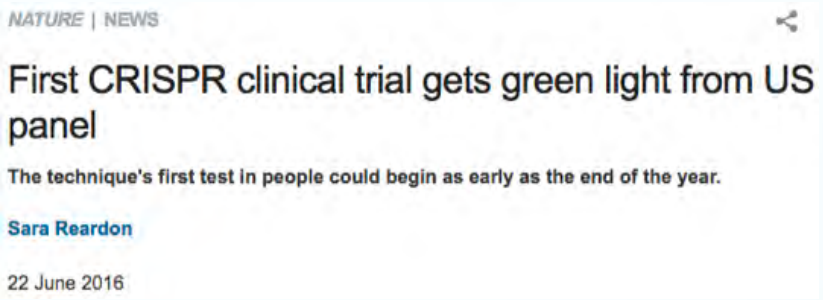
11/28/2015 07:31 am ET

Gregory Adams via Getty Images

Could CRISPR really become an FSHD therapeutic?

editas MEDICINE

CRISPR THERAPEUTICS


NATURE | NEWS

First CRISPR clinical trial gets green light from US panel

The technique's first test in people could begin as early as the end of the year.

Sara Reardon

22 June 2016

exonics THERAPEUTICS

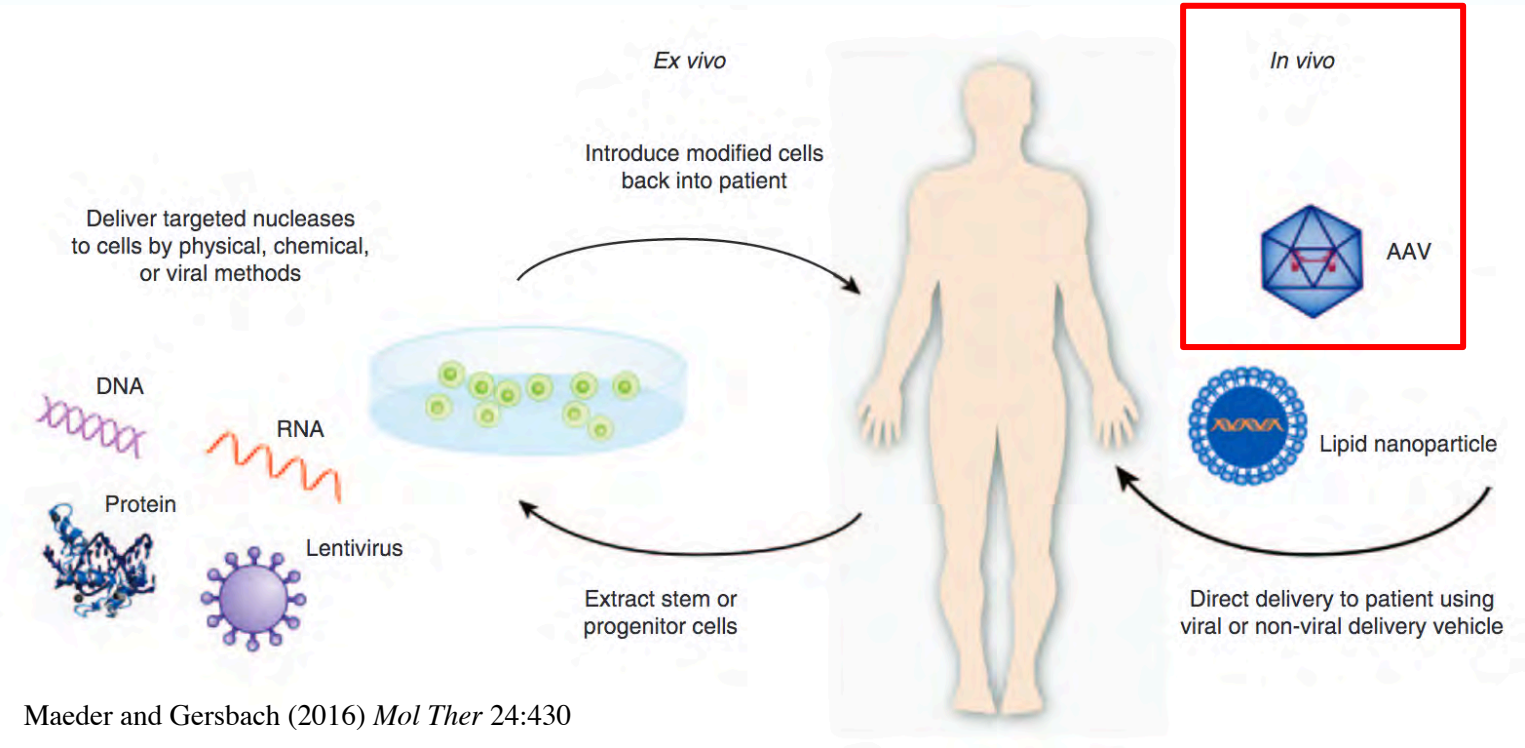
Intellia THERAPEUTICS

Projected market of $5.5 billion by 2021

New CRISPR and CRISPR-like systems being discovered

Therapeutic delivery of CRISPR/Cas *in vivo* is challenging

FSHD is a skeletal muscle disease



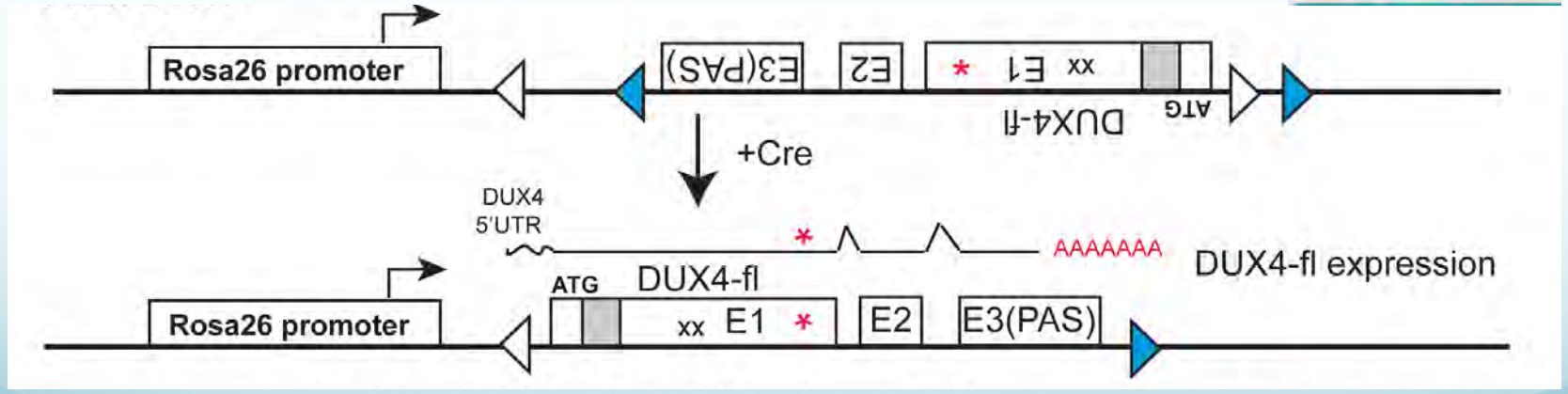
Maeder and Gersbach (2016) *Mol Ther* 24:430

Need an animal model → pathogenic gene is primate-specific

Generation of a viable, phenotypic FSHD-like mouse based on DUX4-fl expression

Takako Jones, PhD

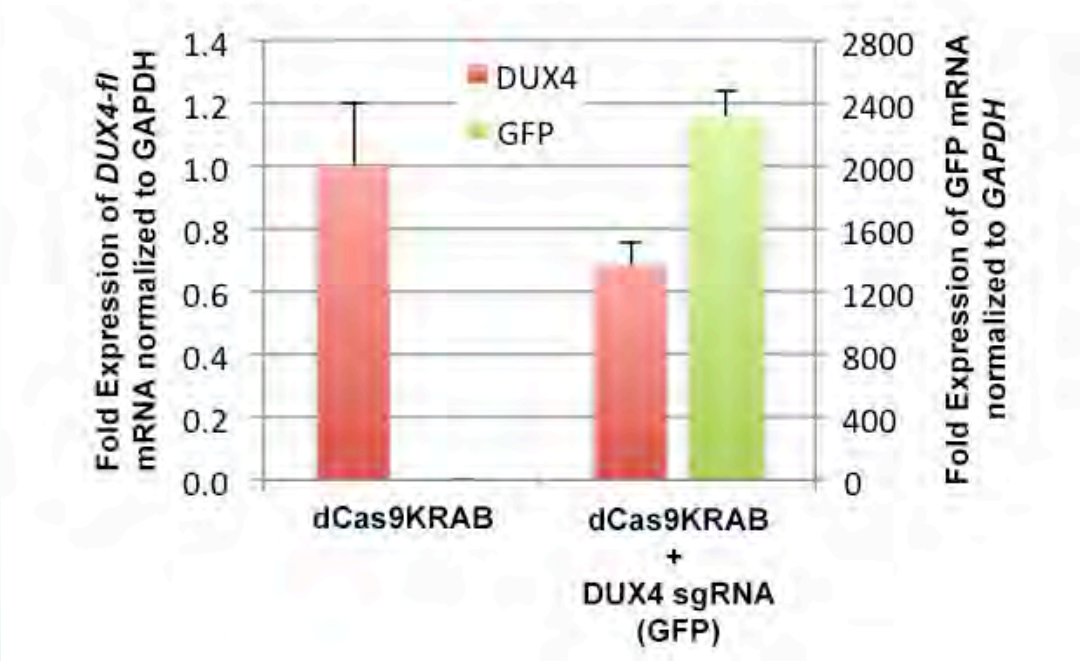
FLExDUX4



The *Rosa26* promoter ensures robust DUX4-fl expression in all cells that underwent cre-mediated inversion

FLExDUX4 model mice show rapid decline in mobility and a severe FSHD-like myopathy

In vivo delivery of AAV9-dCas9-KRAB + AAV9-sgRNA leads to significant DUX4 knockdown

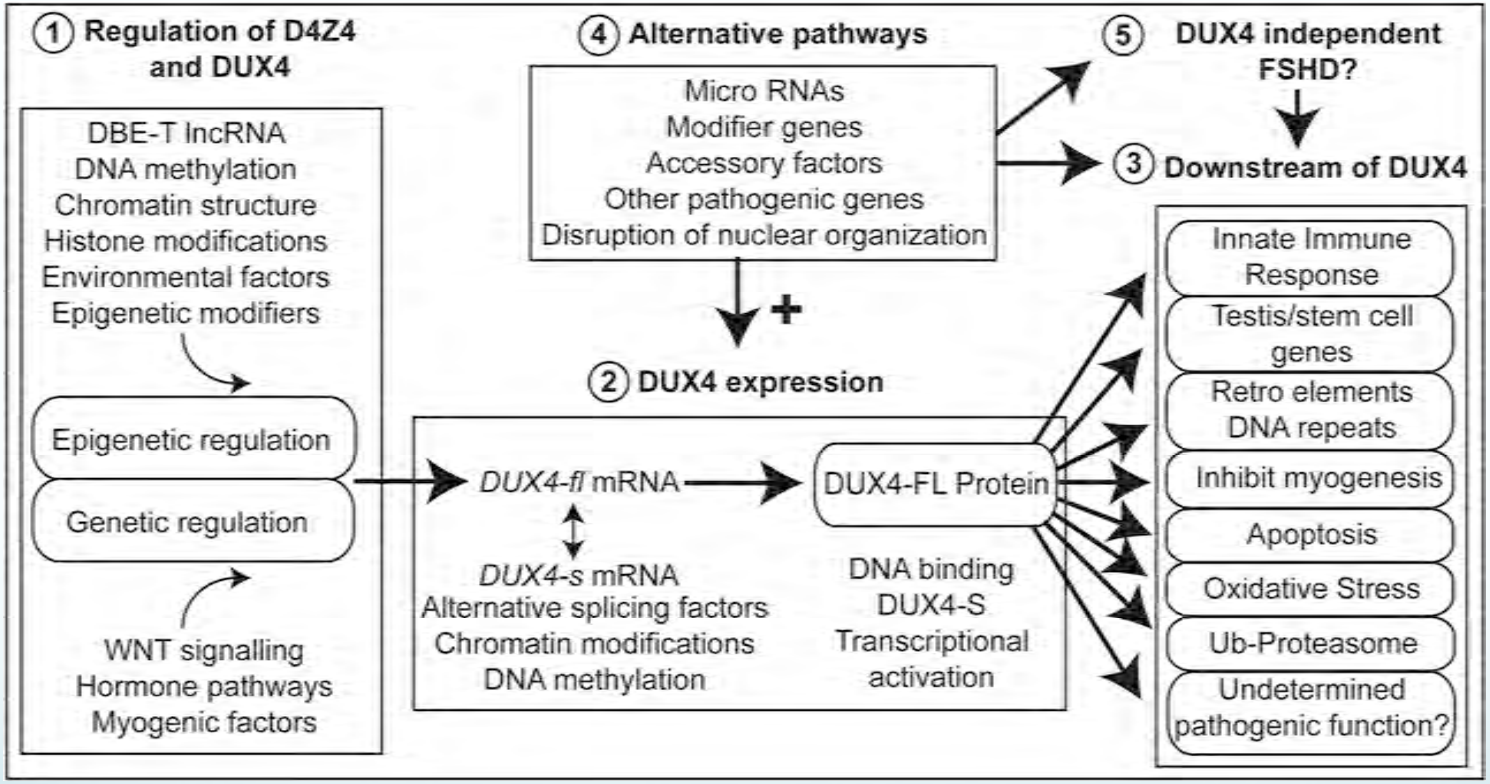


AAV9 delivery results in 30% decrease in TA muscle

Enough to be therapeutic?

Only need to dial back expression from affected to asymptomatic

Our recent increased understanding of FSHD pathogenic mechanisms has led to the development of numerous therapeutic approaches and tools



CRISPRi/dCas9-KRAB; CRISPR/Cas9; Myostatin inhibition
Morpholinos/PMOs/shRNAs; miRNAs; Anti-inflammatory
Small molecules targeting epigenetic regulators; more…

The FSHD field will be translating discoveries to the clinic and the future is bright

Steven Blier: Musician, Professor at Julliard, FSHD patient, and friend
Kelli O'Hara: Tony award winning actress and advocate for FSHD

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Acknowledgements

Mick Hitchcock, PhD, Endowed Chair in Medical Biochemistry

FSH SOCIETY
FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY

A Voice for FSHD
The Chris Carrino Foundation for FSHD

AFM
Association Française contre les Myopathies

NIAMS
National Institute of Arthritis and Musculoskeletal and Skin Diseases

MDA®

Eunice Kennedy Shriver
NICHD
National Institute of Child Health & Human Development