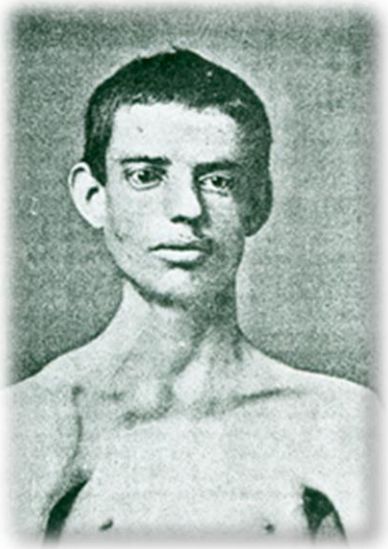
Genetics and Biology of FSHD

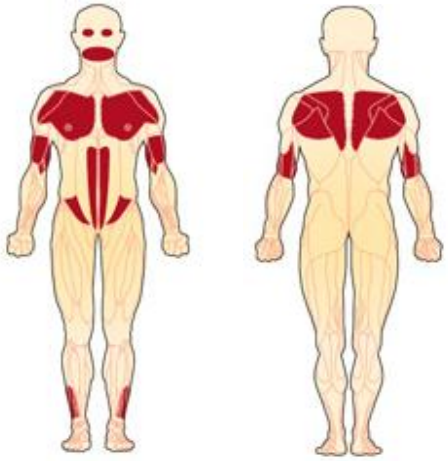
Stephen J Tapscott
Fred Hutchinson Cancer Research Center
Seattle, WA

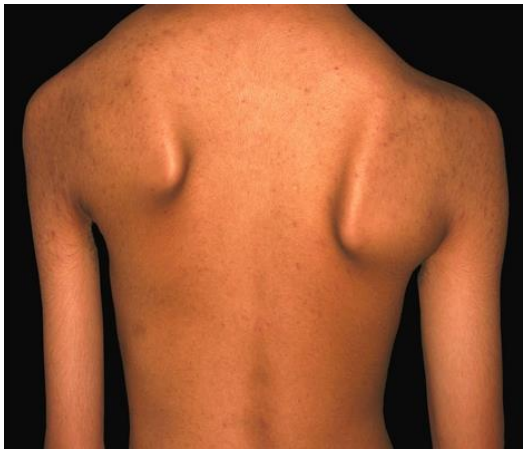
1884 and 1886
Landouzy and Dejerine describe a progressive muscular atrophy with involvement of facial (facio), shoulder (scapula), and upper arm (humeral) weakness



Landouzy and Dejerine

FSHD: Facioscapulohumeral muscular dystrophy





- Autosomal dominant
- Slowly progressive muscle weakness
- Onset in early adulthood
- ~1/10,000 prevalence

Mapping of facioscapulohumeral muscular dystrophy gene to chromosome 4q35-qter by multipoint linkage analysis and in situ hybridization.

Wijmenga C, Padberg GW, Moerer P, Wiegant , Liem L, Brouwer OF, Milner EC, Weber JL, van Ommen B, Sandkuyl LA, et al.

Genomics 1991; 9:570-5.

Determining the location of a disease mutation usually identifies the disrupted gene

- Duchenne muscular dystrophy
  - Mutation in the dystrophin gene
- Huntington's Disease
  - Mutation in the Huntingtin gene
- Myotonic Dystrophy
  - Mutation in the DMPK gene
- Breast Cancer
  - Mutation in BRCA1 gene

What was different with FSHD?

- Standard model of genetic disease
  - Mutation in a gene = disease
- FSHD
  - Reduced size of an array of gene copies

Each DNA sequence is like an instructive sentence

- Go to the zoo and let all of the monkeys out of their cages

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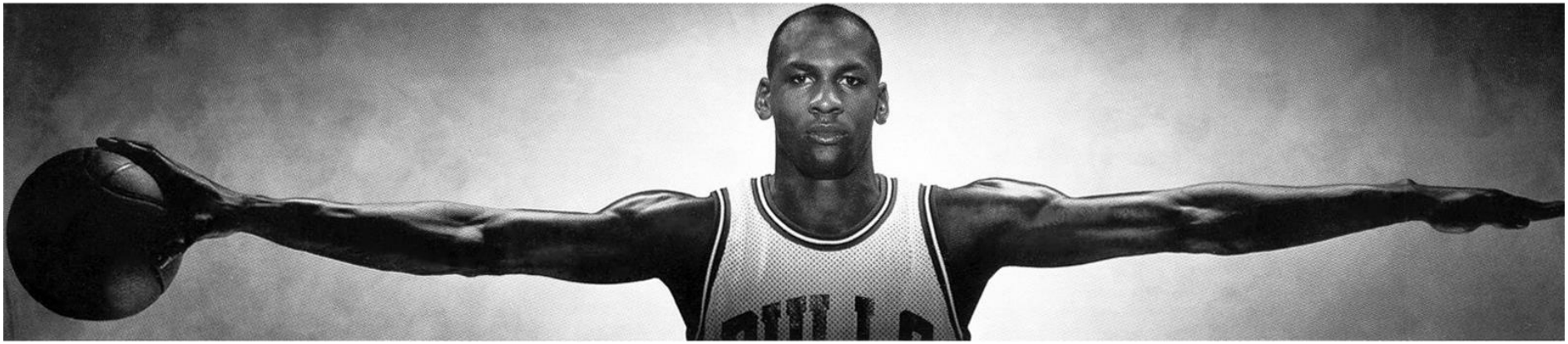
The FSHD mutation changed the packaging of the DNA and DUX4 gene

- DUX4 was normally not expressed in skeletal muscle
- The repeats moved the DUX4 gene to a silent region of the cell nucleus
- In FSHD the silencing of DUX4 was not complete in skeletal muscle

The human diploid genome
(6 x 10^9 bp/cell)

0.34 nm/base x 6 x 10^9 bases = 2 x 10^9 nm or

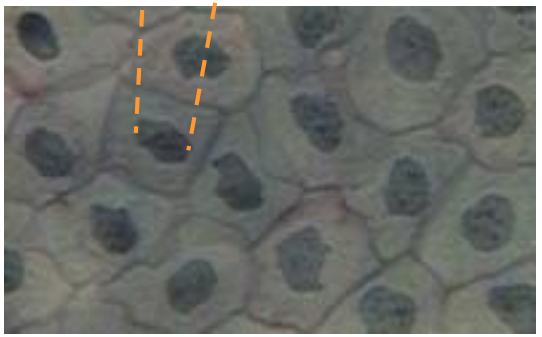
~ 2 meters (6.5 feet)



The average human chromosome is ~4.3 centimeters long.

Human nuclei are ~10 *um* in diameter: 10,000 X compaction

If the nucleus was the size of a tennis ball …



… it would have over 13 miles of DNA

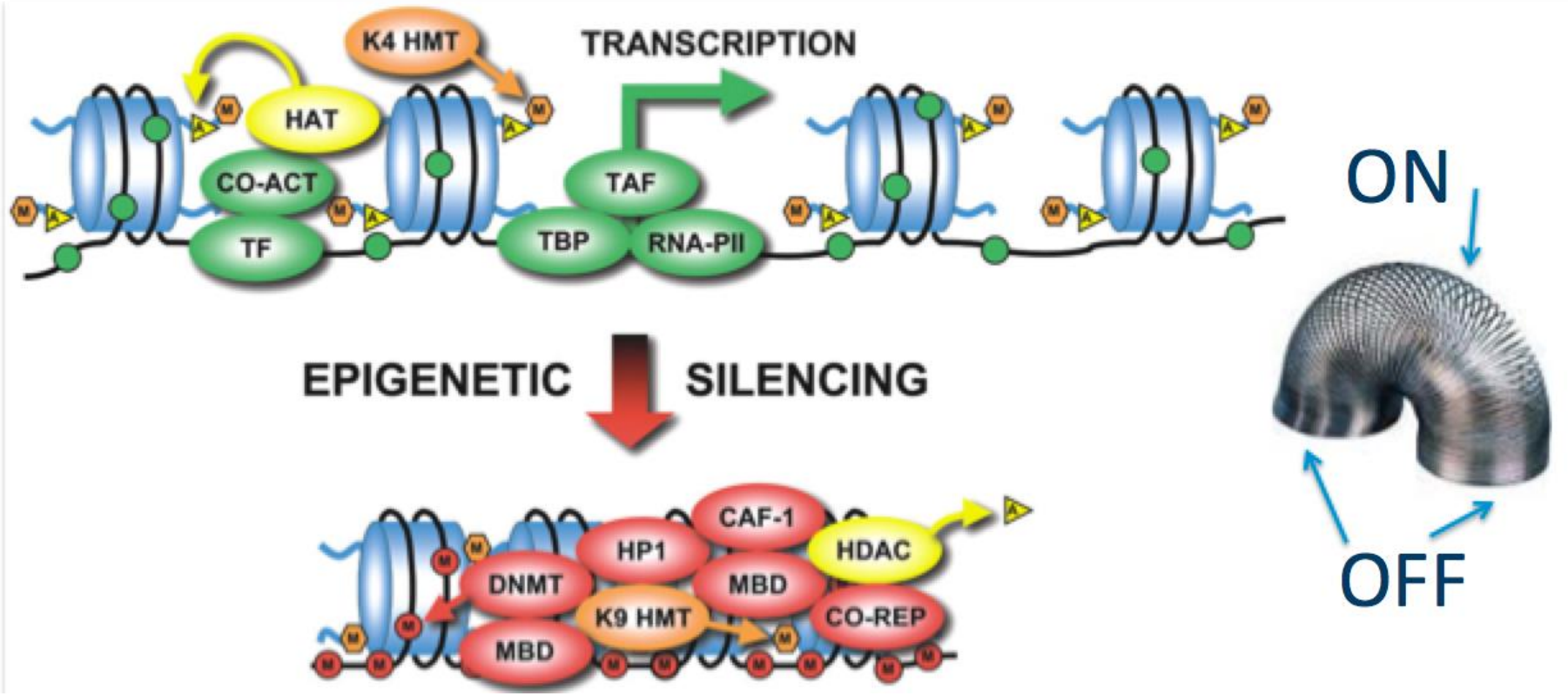
Regions that are open and can be used

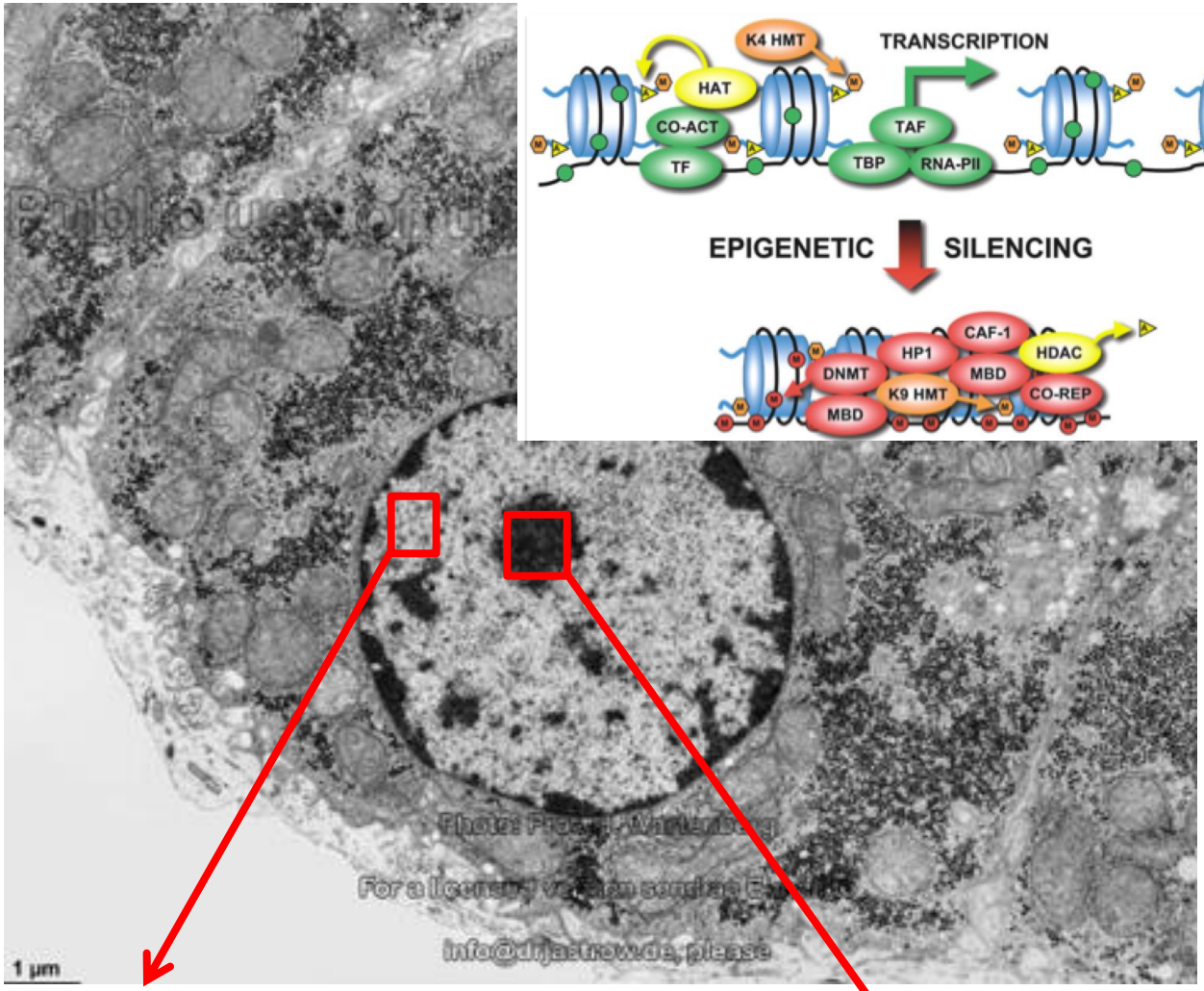
The book is open and the sentence can be read

Regions that are closed and cannot be used

The book is closed and the sentence cannot be read

Open regions can be read
Closed regions cannot be read





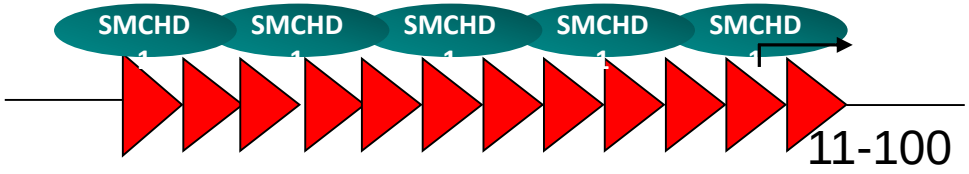
Light = Genes On

Open chromatin

Dark = Genes Off

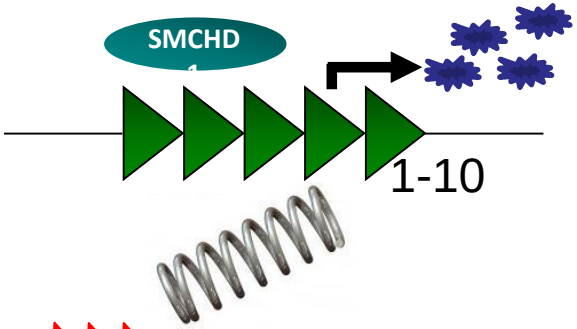
Closed chromatin

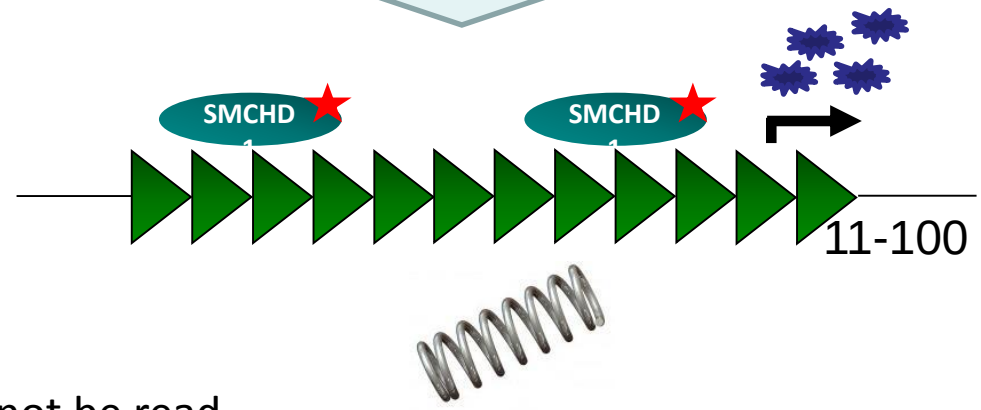
FSHD mutations move the repeats from closed to open regions in skeletal muscle



FSHD1: repeat contraction

FSHD2: SMCHD1 mutations



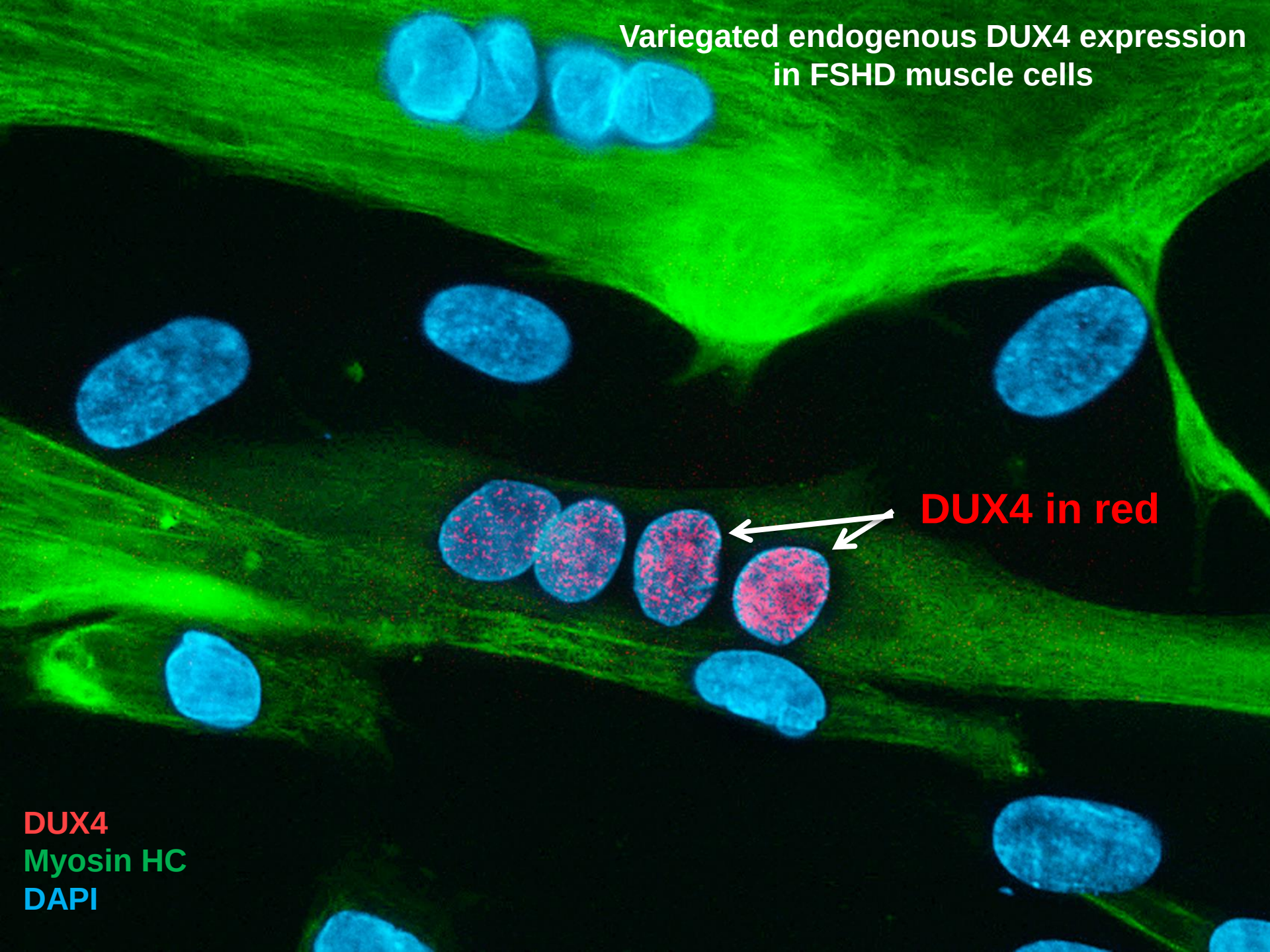


= closed chromatin = cannot be read

= open chromatin = can be read

= DUX4 protein

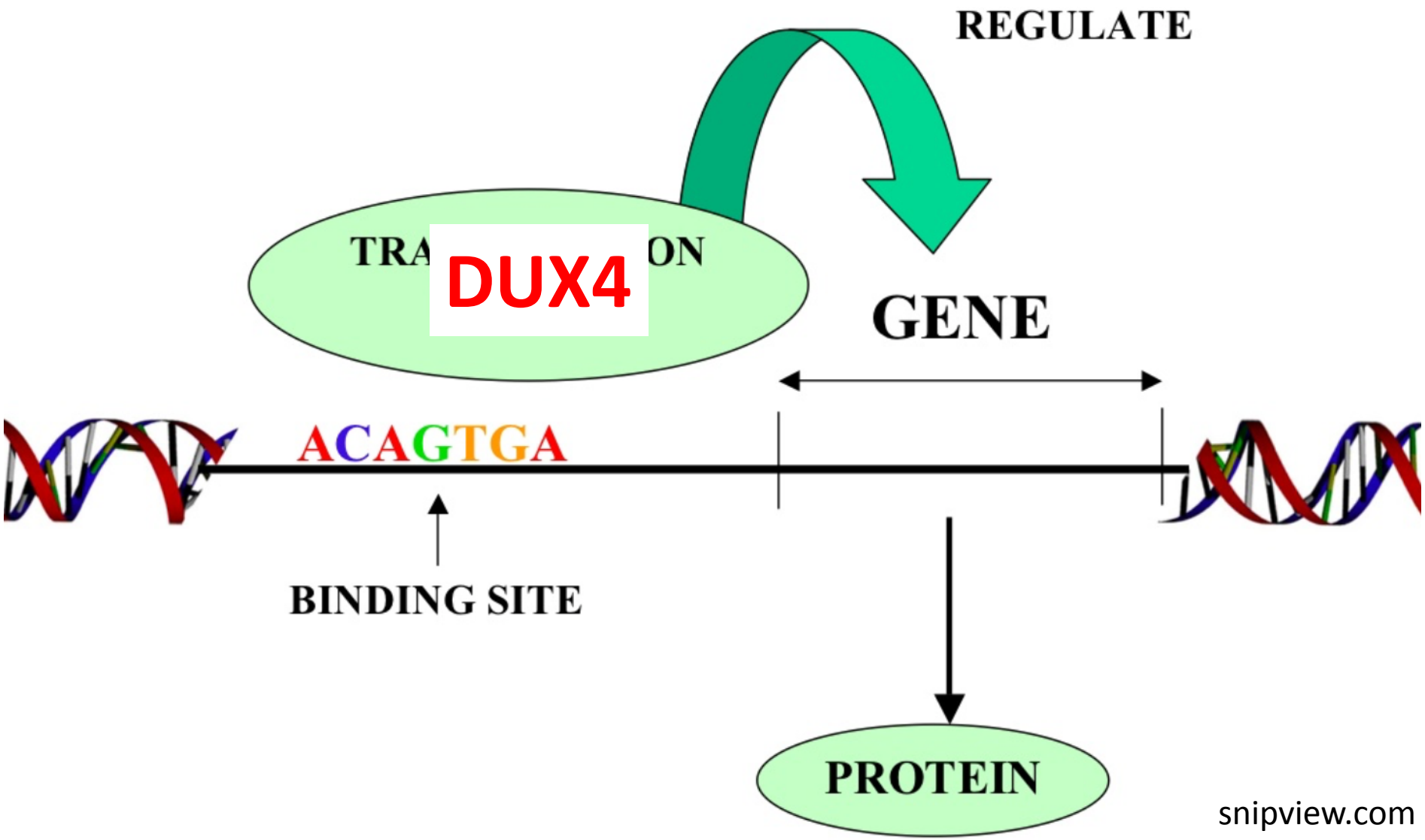
Balog et al, Epigenetics 2015


Variegated endogenous DUX4 expression
in FSHD muscle cells
DUX4 in red
DUX4
Myosin HC
DAPI

FSHD mutations move DUX4 into an open region

- The DUX4 gene can be made into RNA and protein
- DUX4 instructs the cell to turn on other genes
- What does DUX4 do?

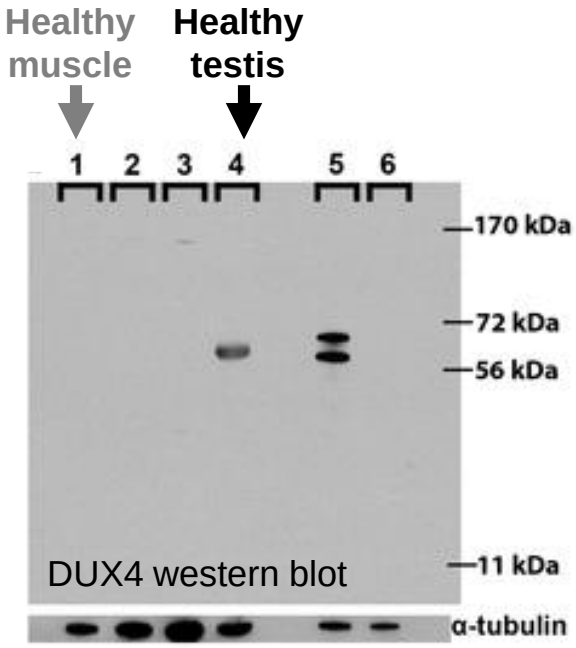
Legend: A transcription factor molecule binds to the DNA at its binding site, and thereby regulates the production of a protein from a gene.



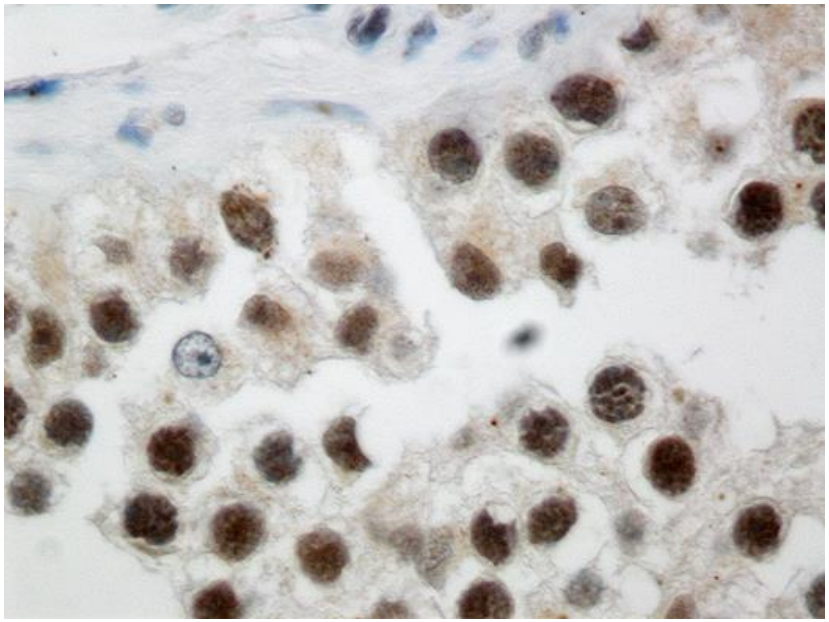
What is DUX4 instructing the cell to do?

- It gives the first instructions to become an early stem cell
- It is expressed in the germline that gives rise to sperm and eggs
- It turns on the first set of genes in the early embryo

DUX4 is expressed in testis but not in skeletal muscle



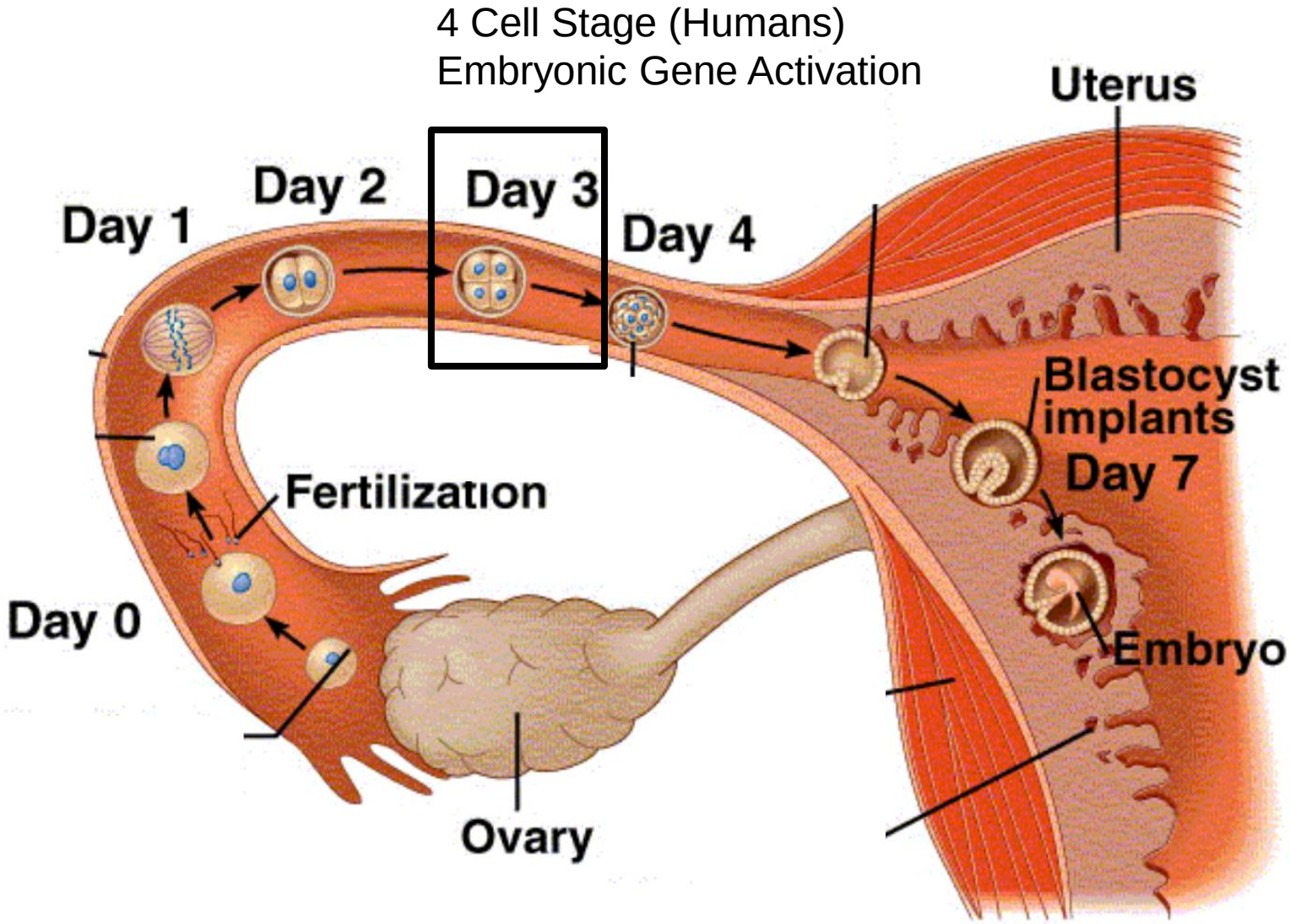
DUX4 expressed in stem cells in the testis



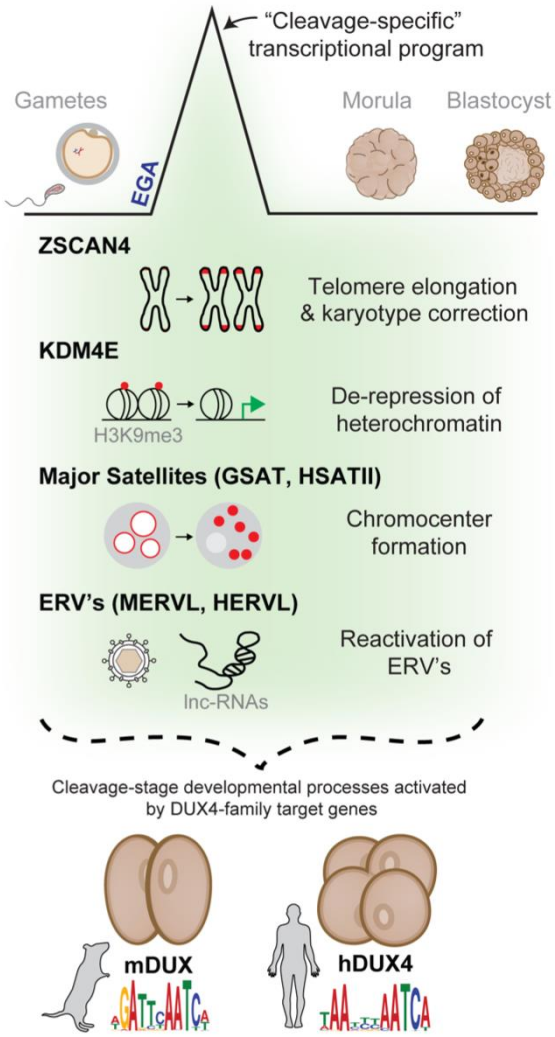
Brown = DUX4 immunodection

Snider, Geng et al. *PLoS Genet,* 2010

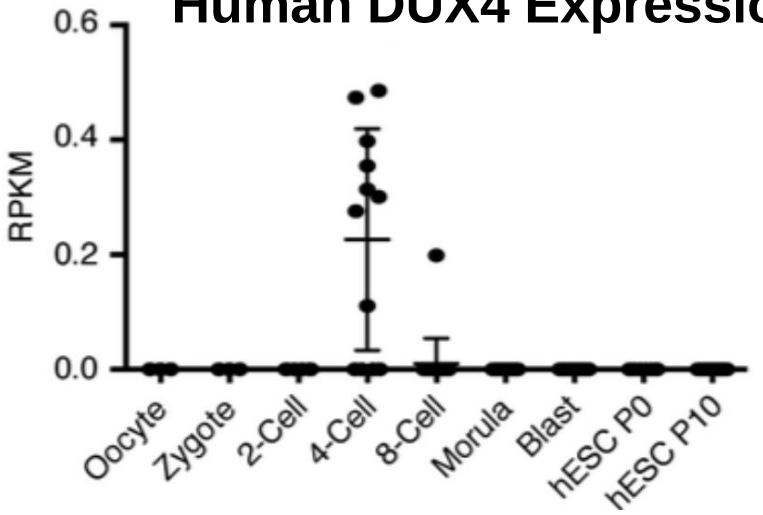
Embryo development from fertilization to implantation



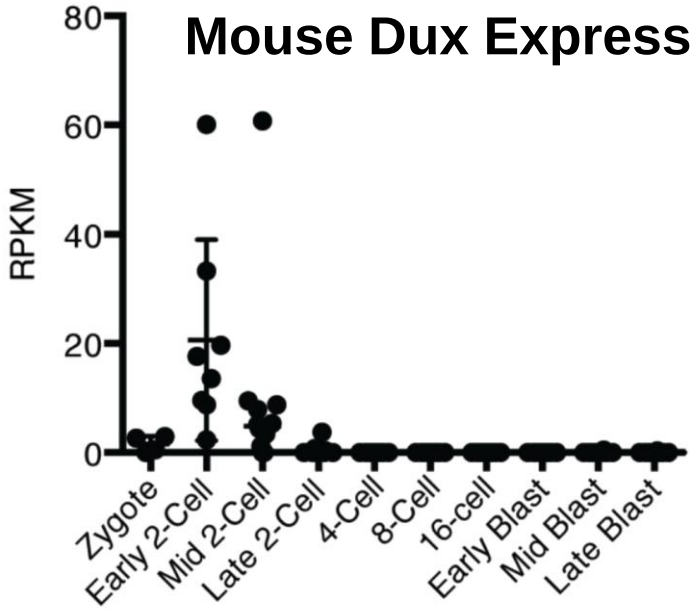
DUX4 activates the cleavage-stage transcriptional program



Human DUX4 Expression



Mouse Dux Expression



Hendrickson … Cairns Nat Gen 2017

Consequences of DUX4 expression in muscle cells

- Induces expression of stem cell genes
- Stem cell genes might induce an immune response
- Inhibits muscle gene expression
- Activates stress response pathways
- Leads to accumulation of toxic RNAs
- Leads to muscle cell death (apoptosis)

Expression of DUX4 in Skeletal Muscle

- DUX4 activates expression of stem cell genes
  - Incompatible with normal muscle function?
  - Might induce immune response similar to CTAs
- DUX4 in muscle causes cell death: apoptosis
  - Why does it not cause cell death in early stem cells?
- DUX4 alters RNA processing
  - Accumulation of abnormal RNAs and proteins
  - Expression of repetitive RNAs and retrotransposons
- Inhibition of muscle regeneration
  - Expression of beta-defensins and abnormal Wnt signaling
- Other …

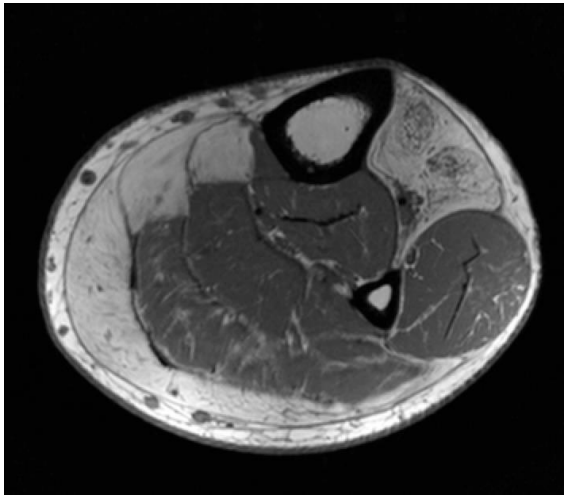
Therapeutic Opportunities

- **Suppress DUX4 expression**
  - DUX4 should be “off” in muscle cells
  - Increase the normal chromatin repression of DUX4
    - SMCHD1 pathway or other repressors
- **Decrease DUX4 mRNA stability/translation/splicing/pA**
  - sh-, si-, mi, or mo-RNA; small molecule inhibitors
- **Block DUX4 protein activity**
  - Dominant negative or target protein interactions
- **Interfere with pathological mechanism(s)**
  - Why does DUX4 kill the muscle cells and not the stem cells?
  - Does an immune response contribute to the disease?
  - In what other ways does DUX4 cause muscle damage?

Identifying Candidate Therapies

- Screen existing chemical compounds
  - FDA approved compounds
  - Clinical candidate compounds
  - Diverse libraries
- Rational development of new drugs
  - Targeting a specific protein/RNA
    - Small molecule drugs and siRNAs
- Immunomodulation?

Clinical Trial Milestones



- Demonstration of drug activity
  - DUX4 mRNA or regulated genes
  - Immune response or regeneration
- Biological response
  - MRI or serum markers of muscle damage
- Halt or reverse disease progression
  - Slowly progressive disease
    - Requires long-term study
    - Large numbers of participants
  - Natural history studies and FSHD registries

How long will it take?

- Within a few years if … ?
  - FDA approved drug
  - Repurposed drug
  - Class of drugs in development for other diseases
- Within a decade if … ?
  - New drug development
  - Progressively more effective drugs

When will we start?

- We have, thanks to you.
  - Consensus model of disease
  - Candidate biomarkers
  - Clinical history studies & infrastructure
  - Multiple efforts at drug development
    - Academic
    - Pharmaceutical Companies

THANK YOU!!!!!

- For coming today
- For supporting FSHD research
- For participating in this important research