

Several ways to go DUX hunting

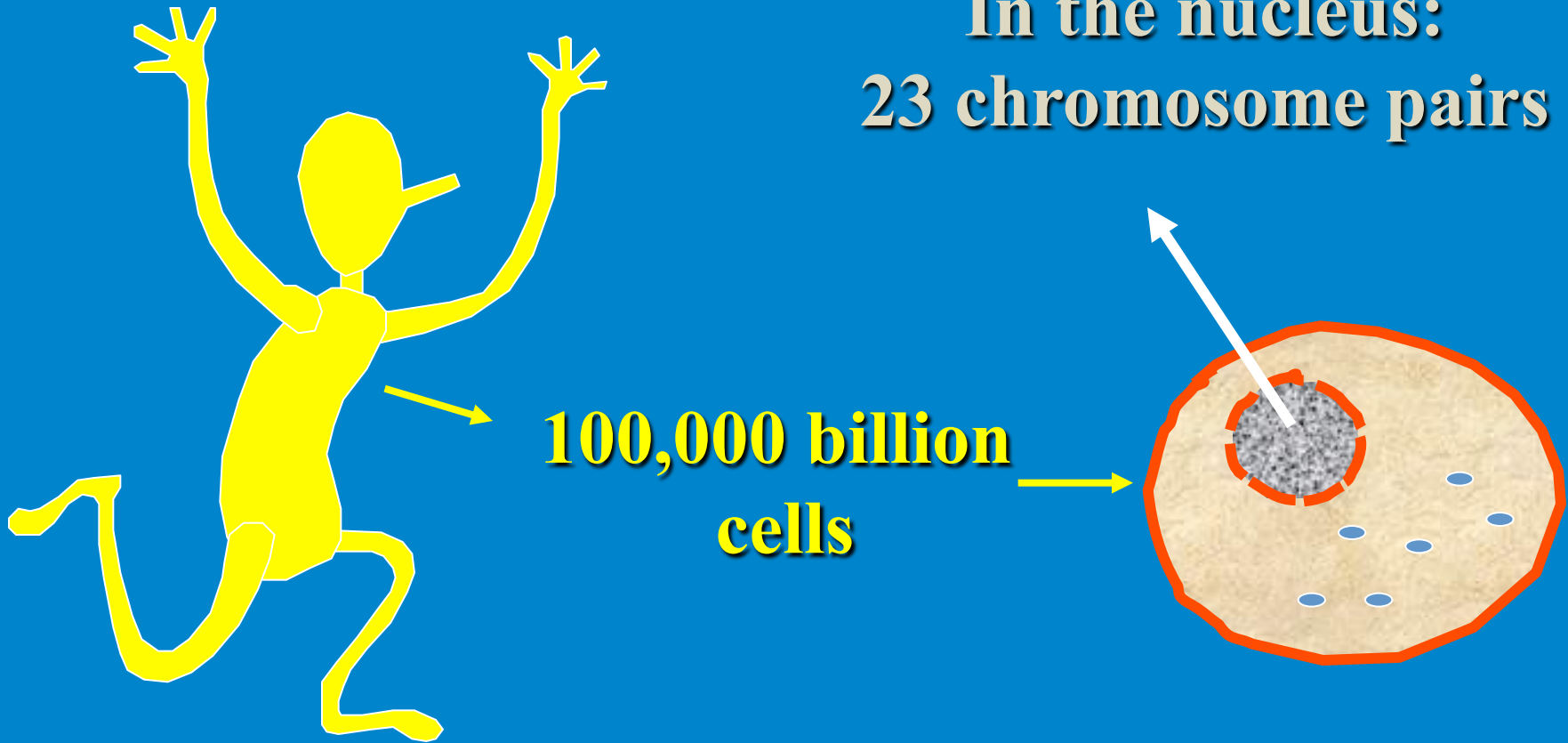
Alexandra Belayew

Frédérique Coppée

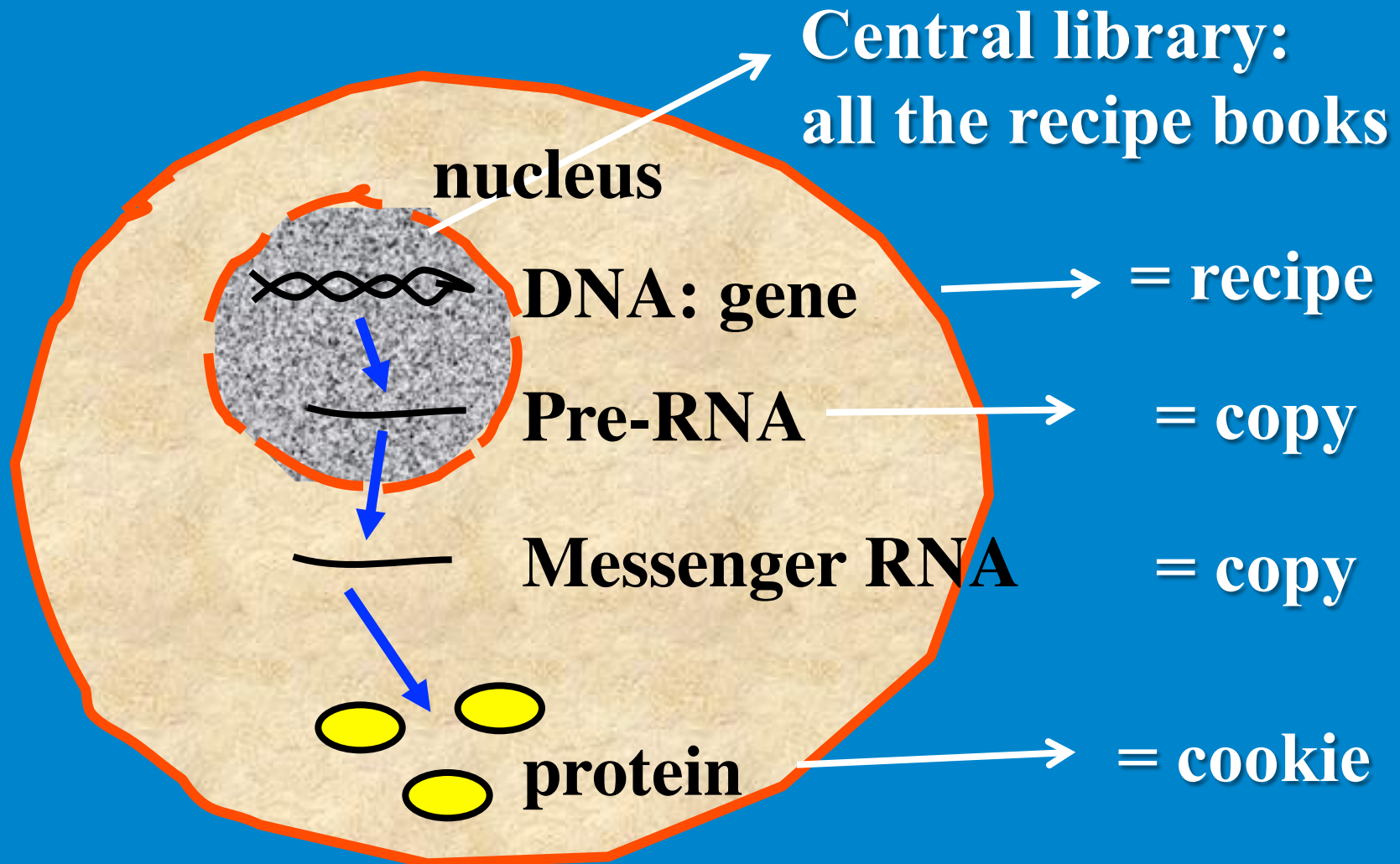
University of Mons, Belgium

Every cell of an individual has the same genetic program

In the nucleus:
23 chromosome pairs



Expressing a gene = baking a cookie!



Staffing the bakery

A cookie (= a protein)



Staffing the bakery

A cookie Chef!



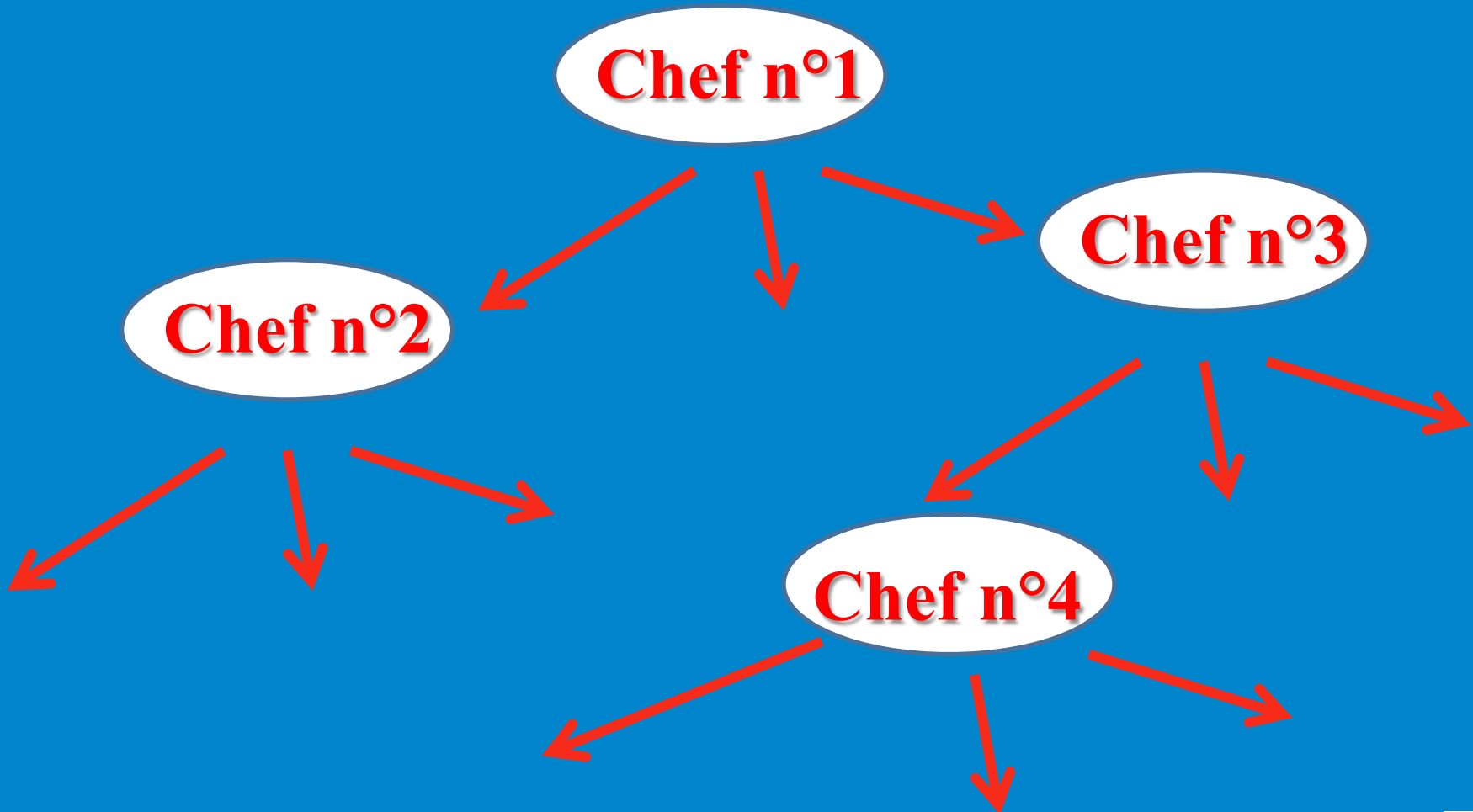
Staffing the bakery

A cookie Chef!

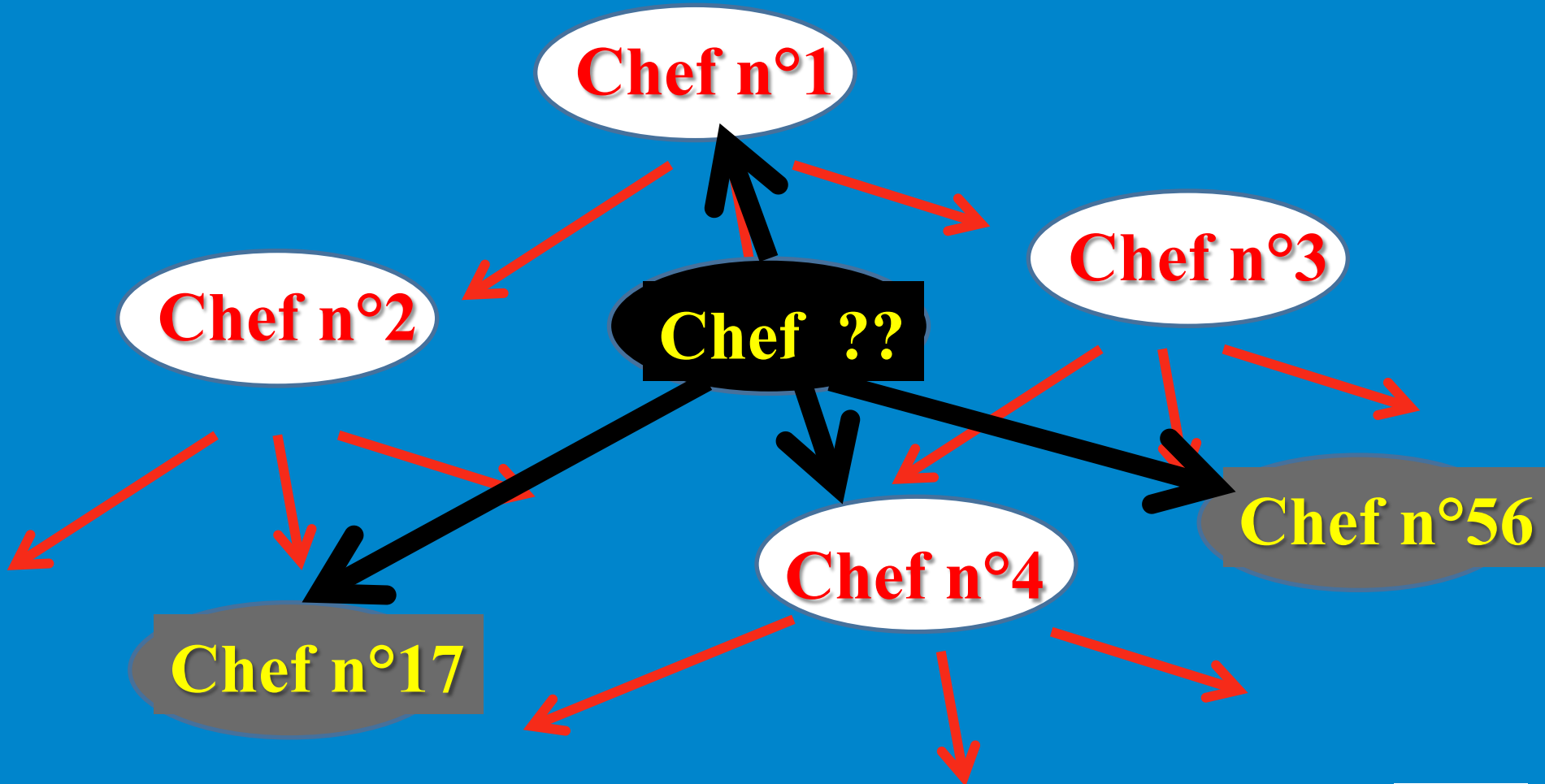
Decides
which cookies
get to be baked
in the cell
i.e.
which genes
are switched
on or off



The bakery staff: cookie Chefs and cookie workers need to be organized



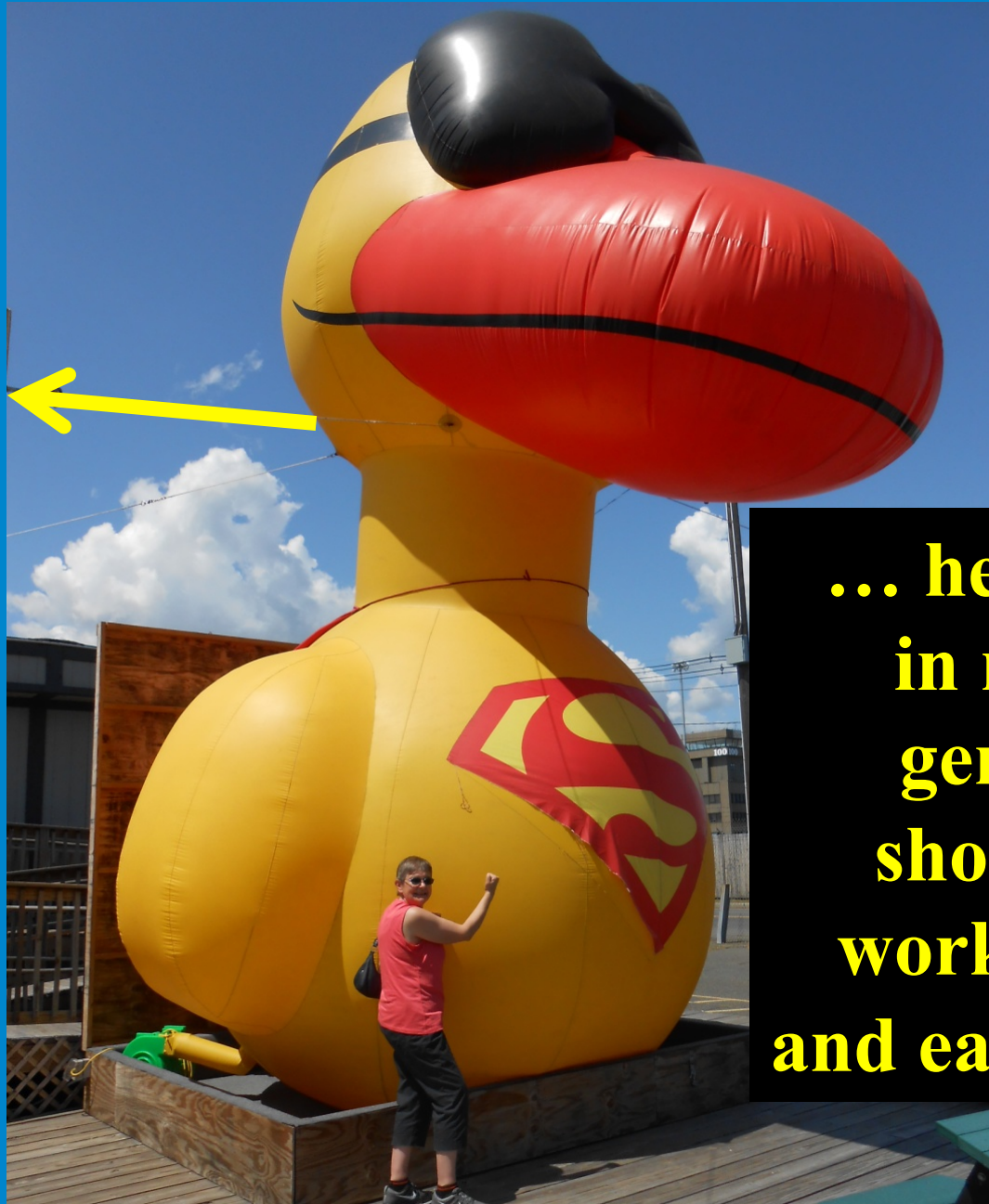
A crazy Chef



DUX4
is a
crazy
Chef
...



DUX4
is a
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Chef
...



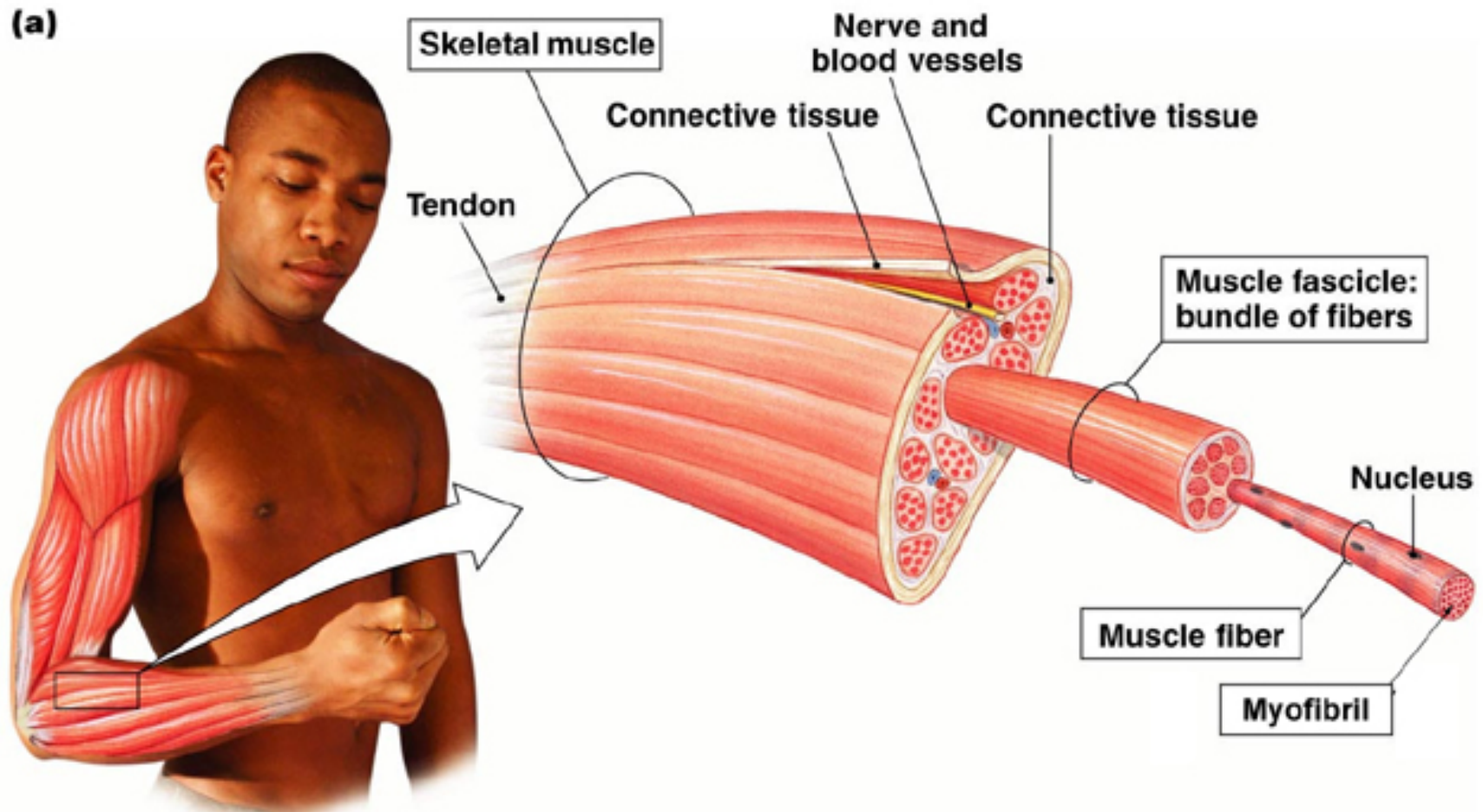
... he activates
in muscles
genes that
should only
work in testes
and early embryo

DUX4
is a
crazy
Chef.
Get
rid of
him!



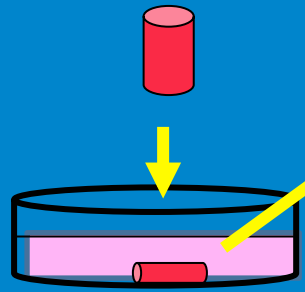
A needle!

A muscle = an assembly of fibers



Human primary myoblast cultures

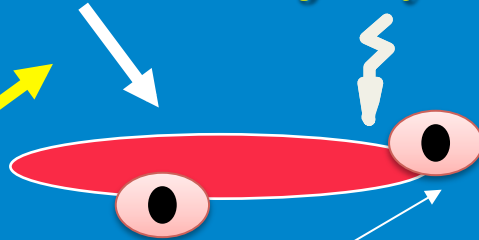
Muscle biopsy



Plastic dish

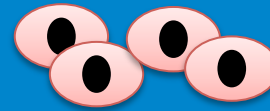
+ medium

Fiber Injury



Satellite cells

Activation, proliferation



Myoblasts

Differentiation: cells fuse

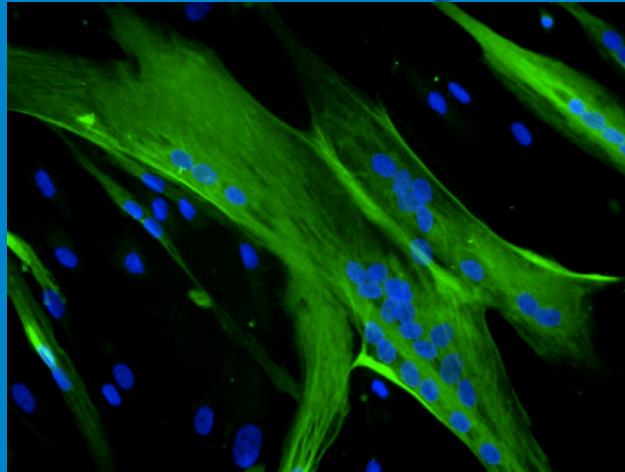


Myotubes

Collaboration Dr. D. Laoudj-Chenivresse (INSERM, Montpellier)

Abnormal shapes of FSHD myotubes

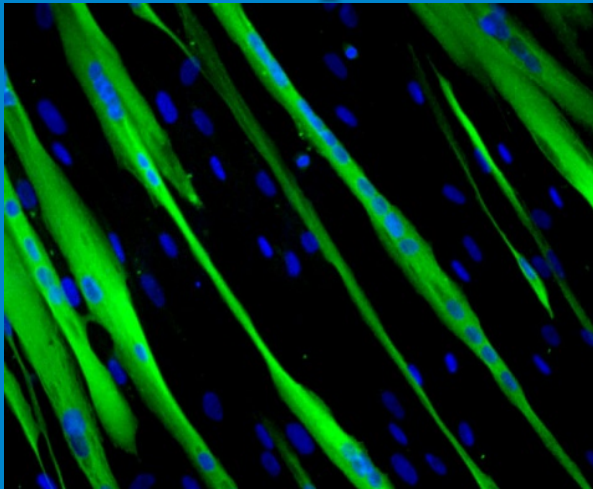
Healthy



Myosin

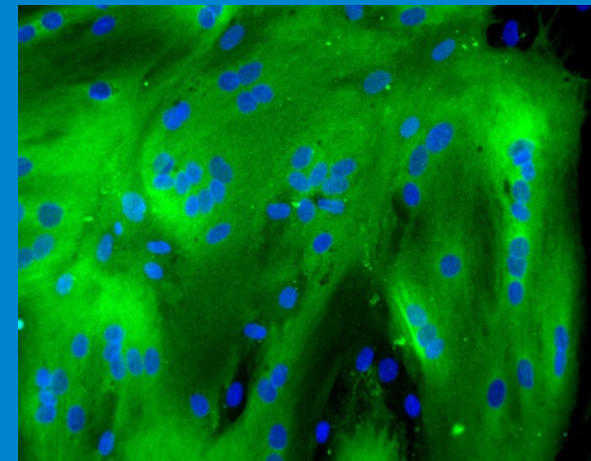
Nuclei

Atrophic



Very thin myotubes

Disorganised



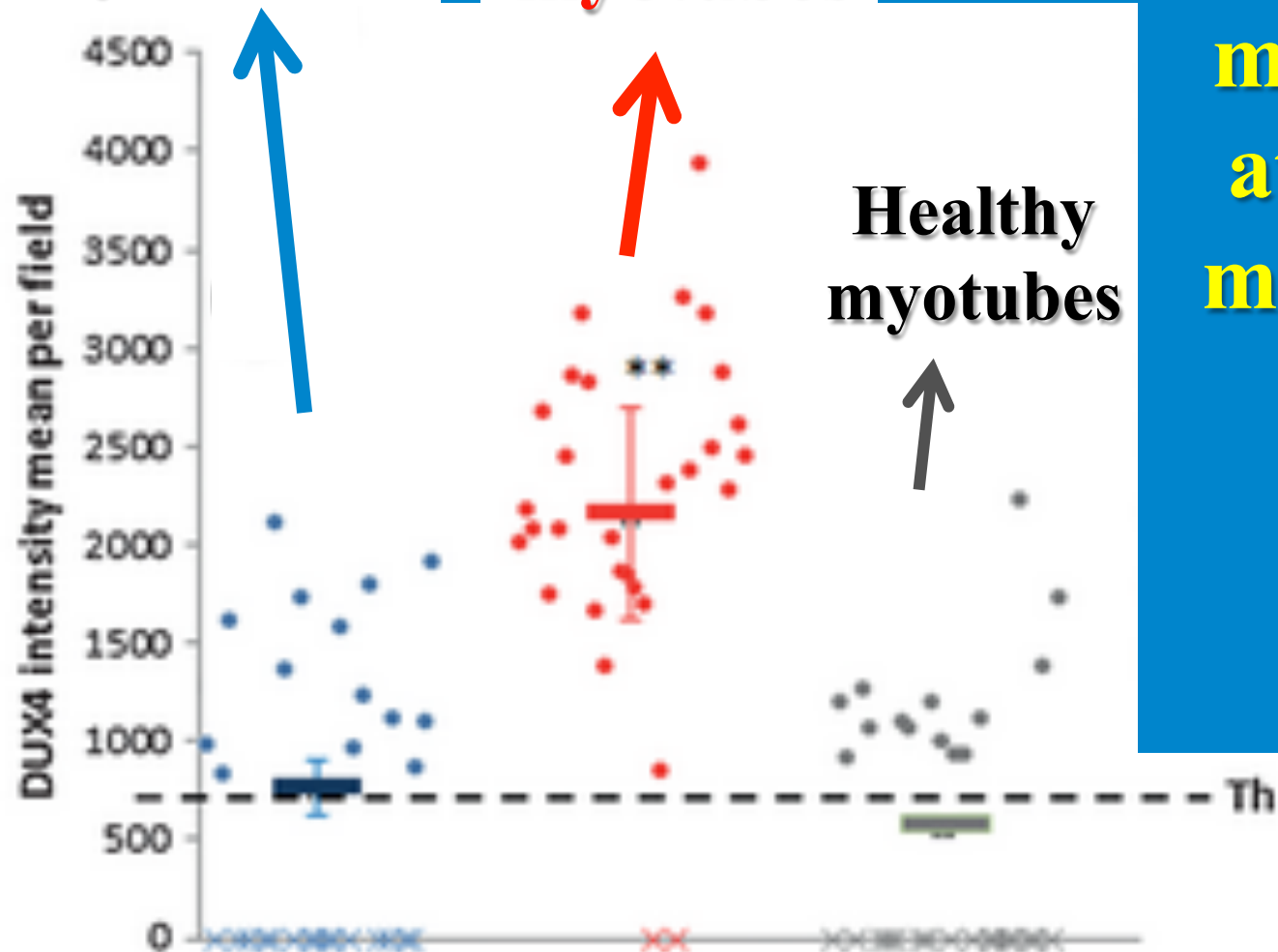
Large myotubes
+ clusters of nuclei

← FSHD →

**Disorganized
myotubes**

**Atrophic
myotubes**

**Increased
DUX4 levels
mostly in
atrophic
myotubes**

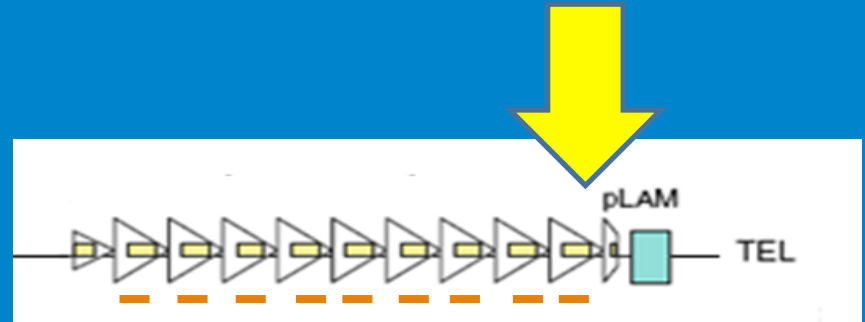


Tassin A. *et al*, 2013, J. Cell Mol. Med 17; 76-89

The human DUX genes

DoUble HomeoboX genes in repeated elements

- *D4Z4* locus at 4q35



➤ **DUX4 recipe + starts**

Gabriëls *et al.*, 1999

Kowaljow *et al* 2007

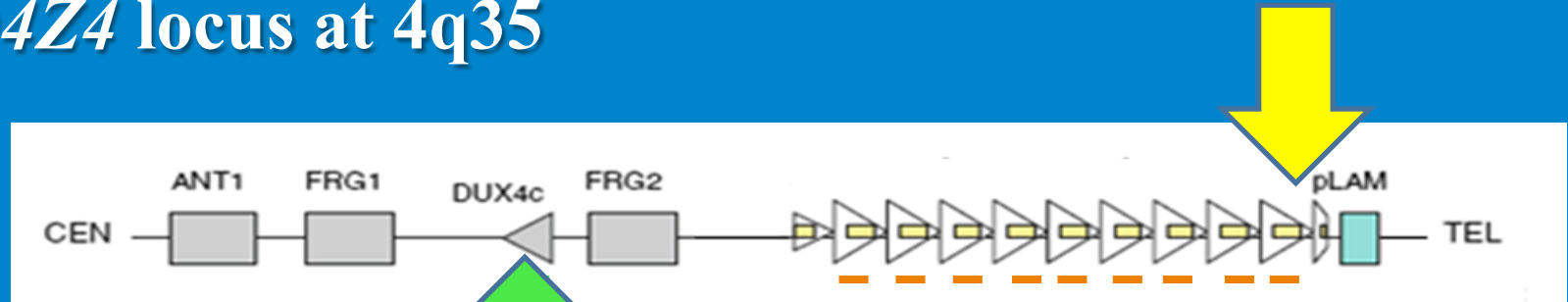
➤ **DUX4 messenger end**

Dixit *et al*, 2007

The human DUX genes

DoUble HomeoboX genes in repeated elements

- *D4Z4* locus at 4q35



➤ **DUX4c gene**
Ansseau et al, 2009

➤ **DUX4 recipe + start**
Gabriëls et al., 1999
Kowaljow et al 2007

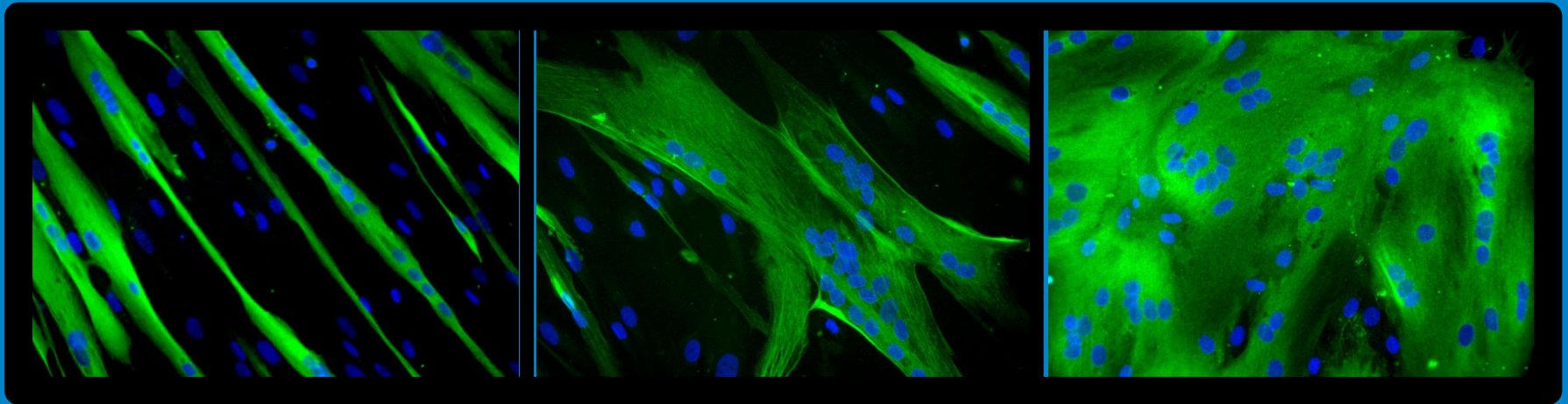
➤ **DUX4 gene end**
Dixit et al, 2007

Activation of DUX4 or DUX4c expression

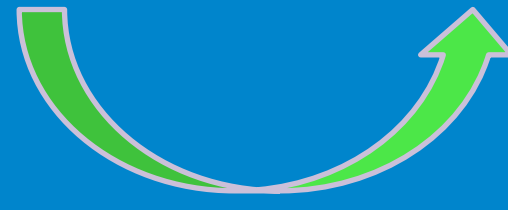
Atrophic

Healthy

Disorganized



DUX4



DUX4c

Vanderplanck et al., 2011 (*PLoS One*) and in preparation

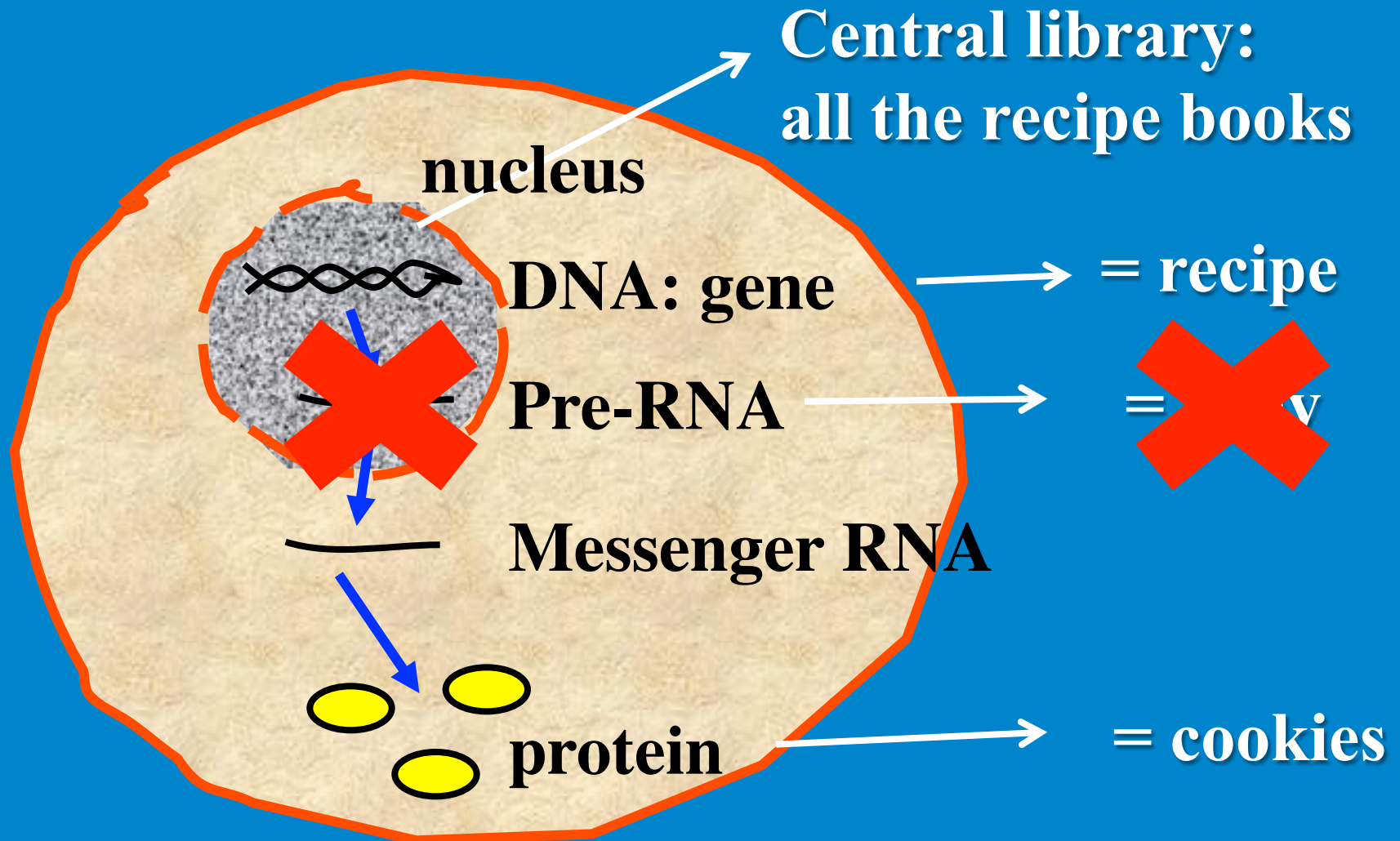
**DUX4
is a
crazy
Chef.
Get
rid of
him!**



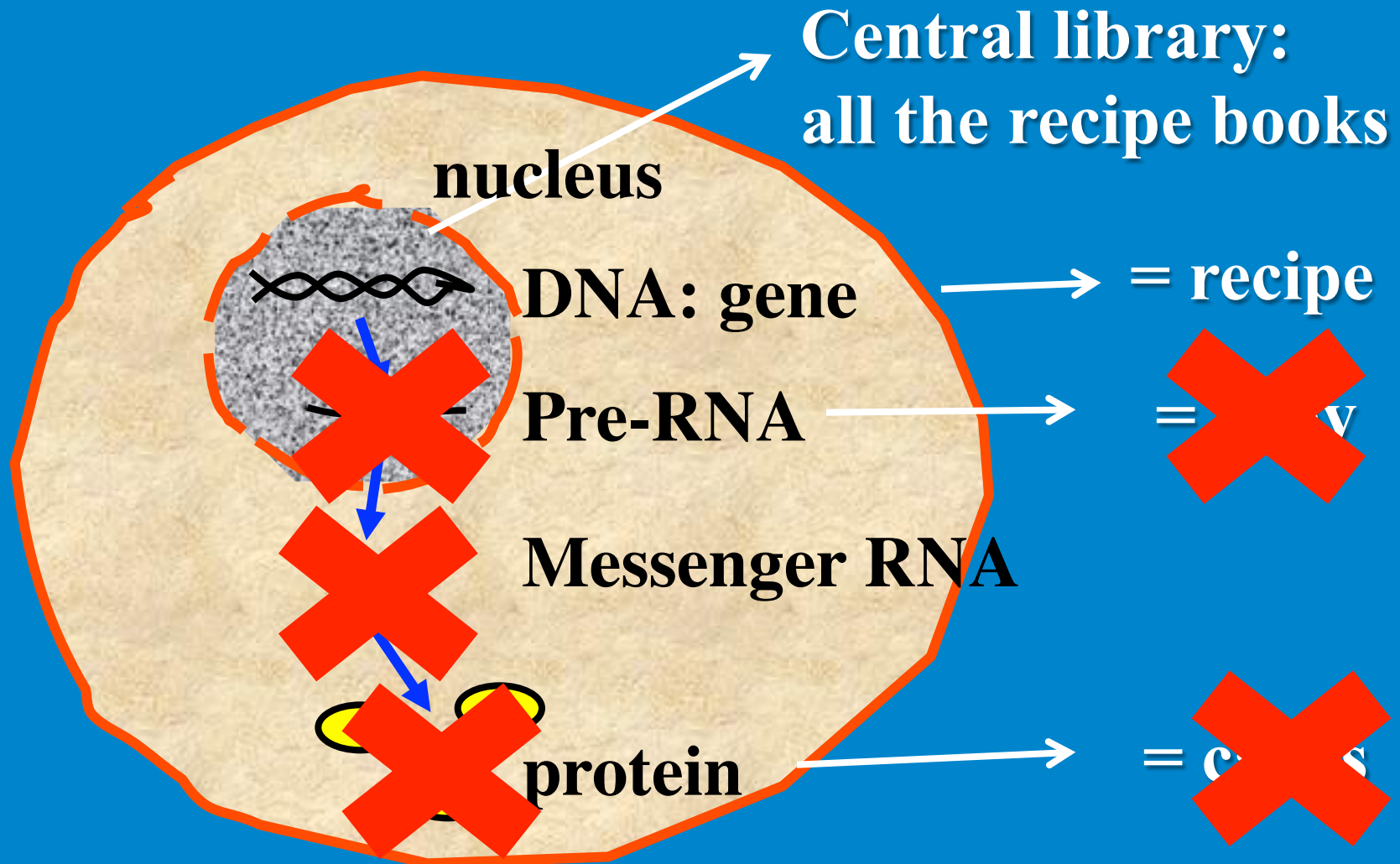
A needle!

**Antisense
agent**

Blocking DUX4 protein expression



Blocking DUX4 protein expression

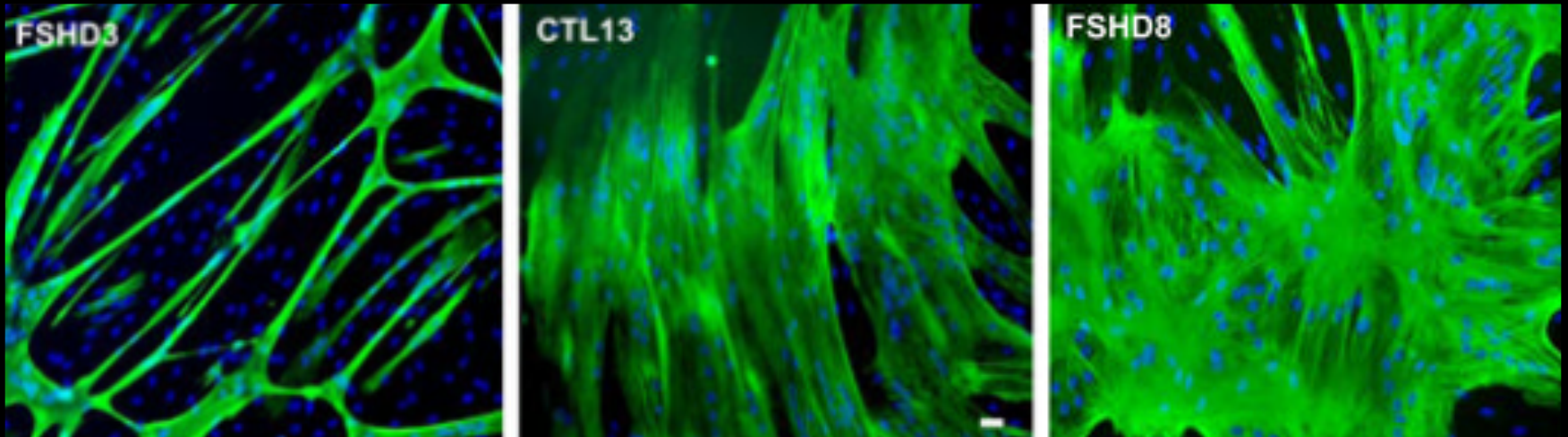


DUX4 inhibition

Atrophic

Healthy

Disorganized



~~DUX4~~

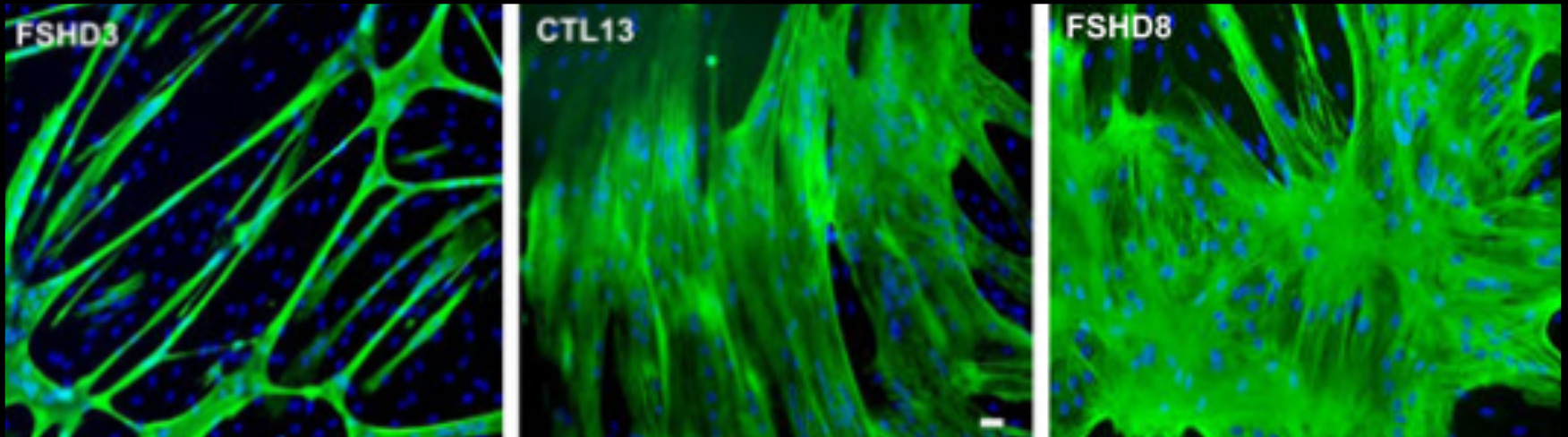
?

DUX4 or DUX4c inhibition

Atrophic

Healthy

Disorganized



~~DUX4~~



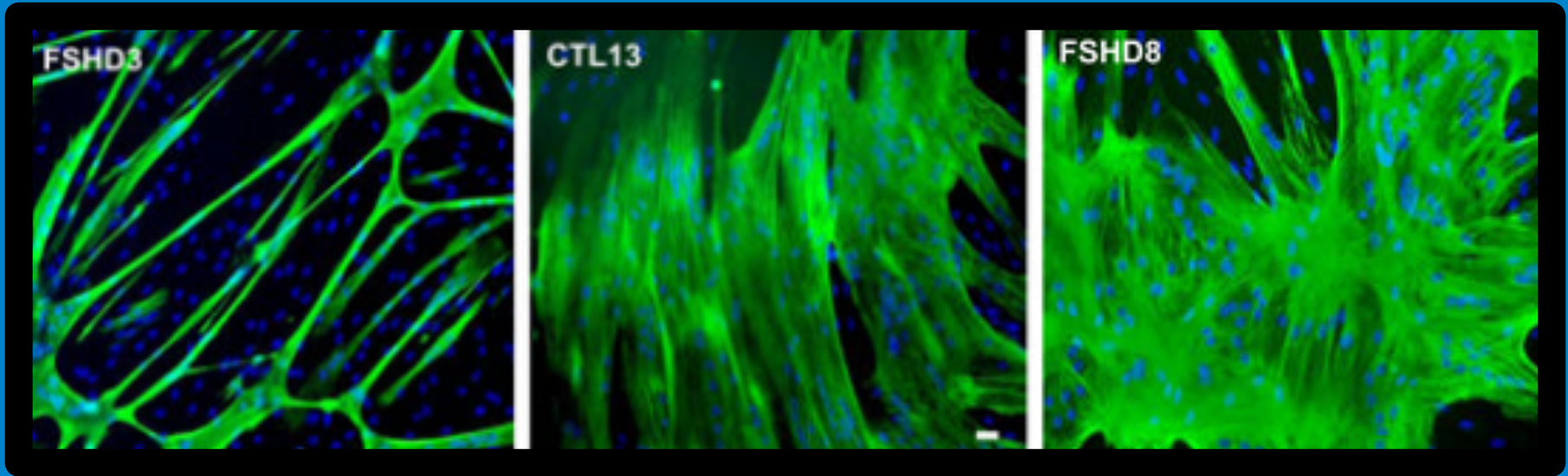
~~DUX4c~~

DUX4 inhibition shifts to a disorganized shape

Atrophic

Healthy

Disorganized



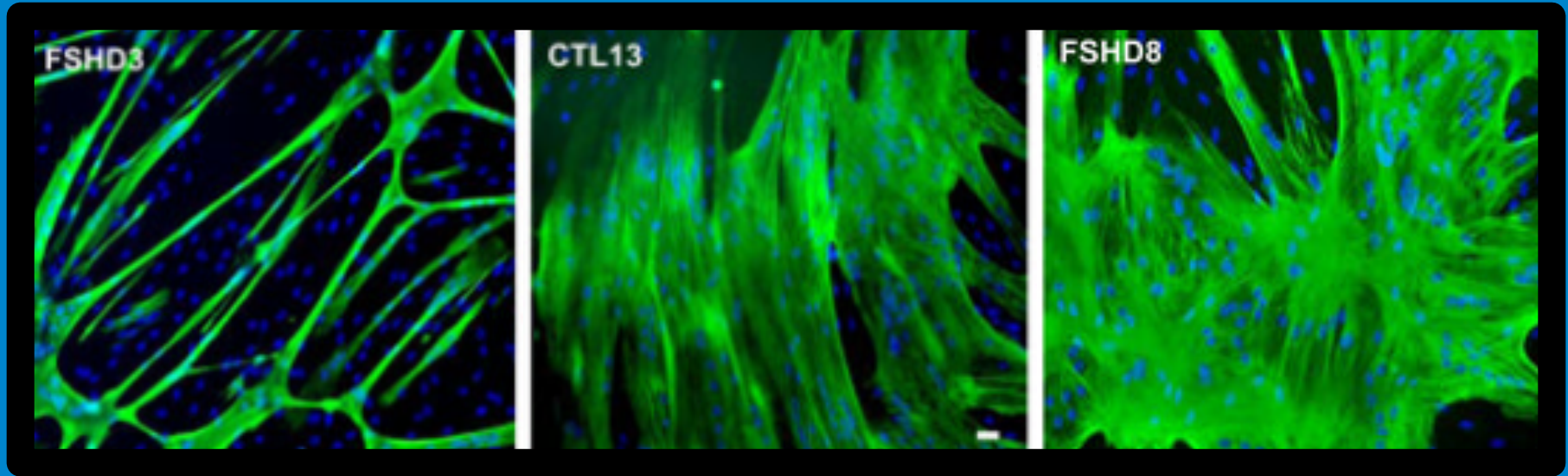
~~DUX4~~

DUX4 inhibition shifts to a disorganized shape

Atrophic

Healthy

Disorganized



~~DUX4~~

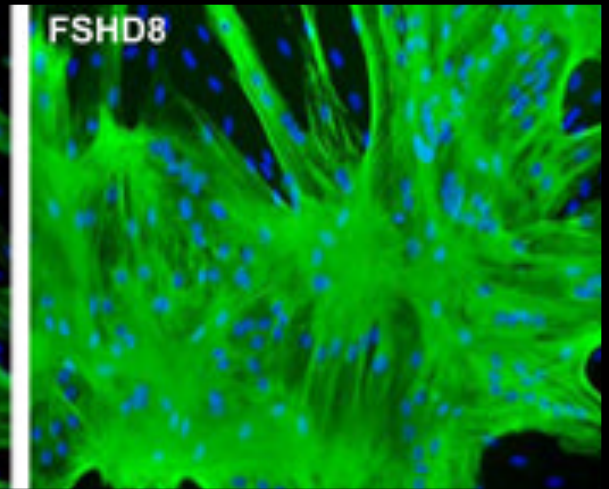
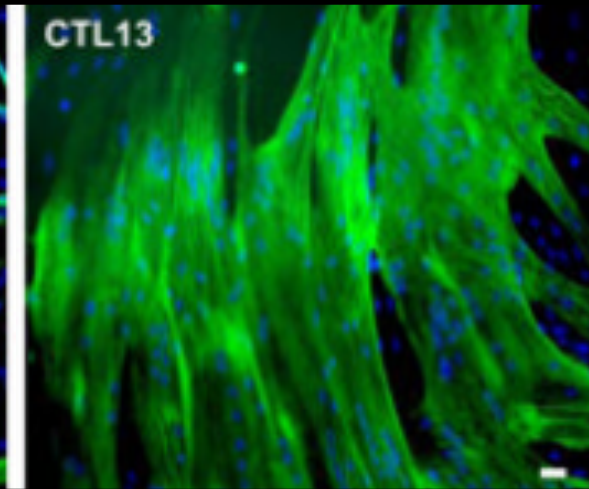
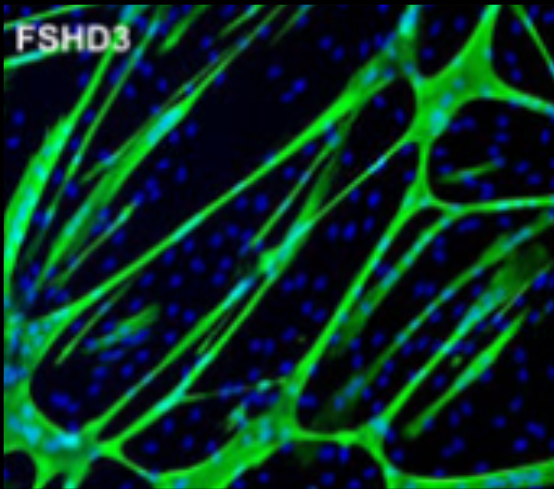


DUX4c inhibition shifts to an atrophic shape

Atrophic

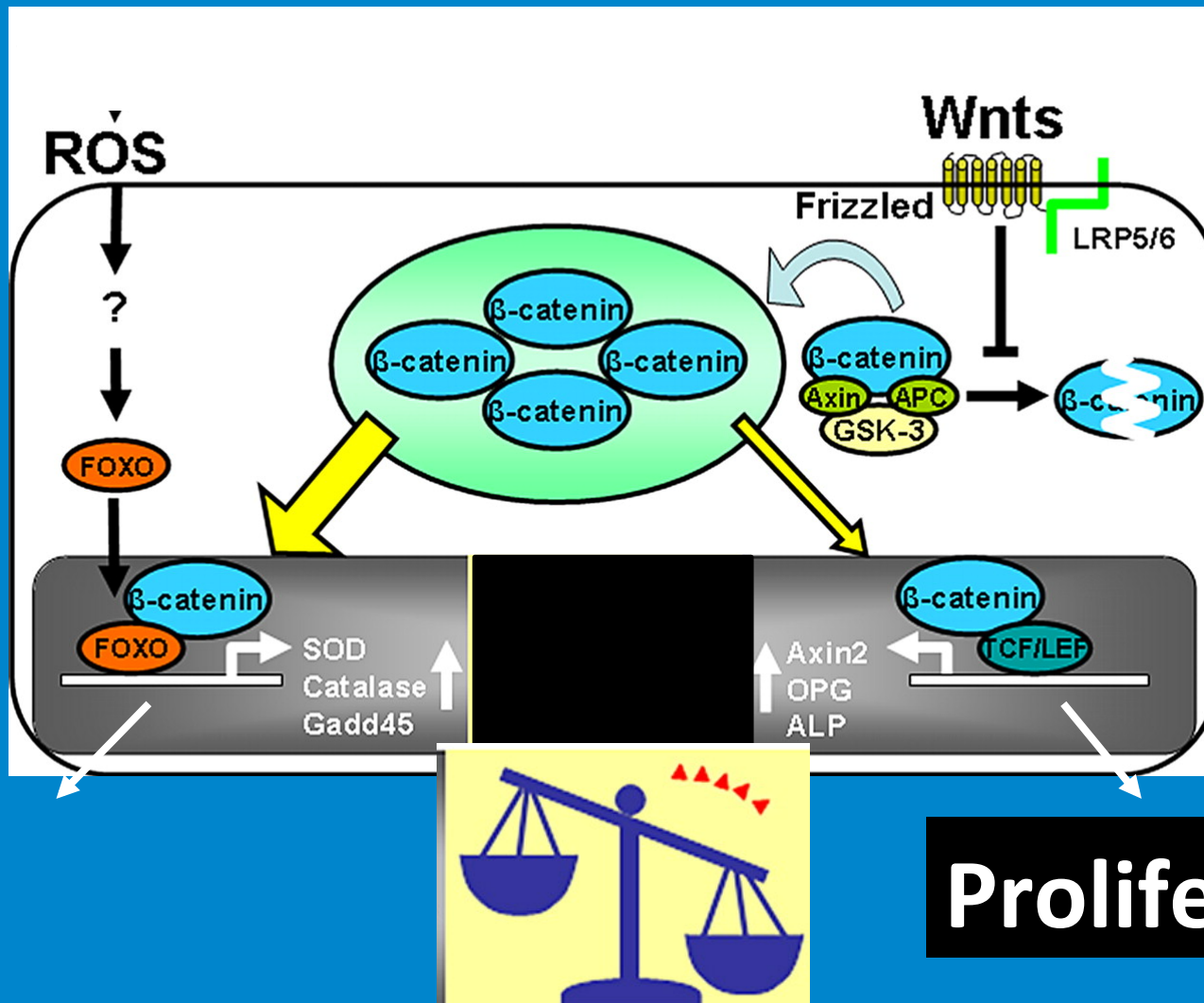
Healthy

Disorganized



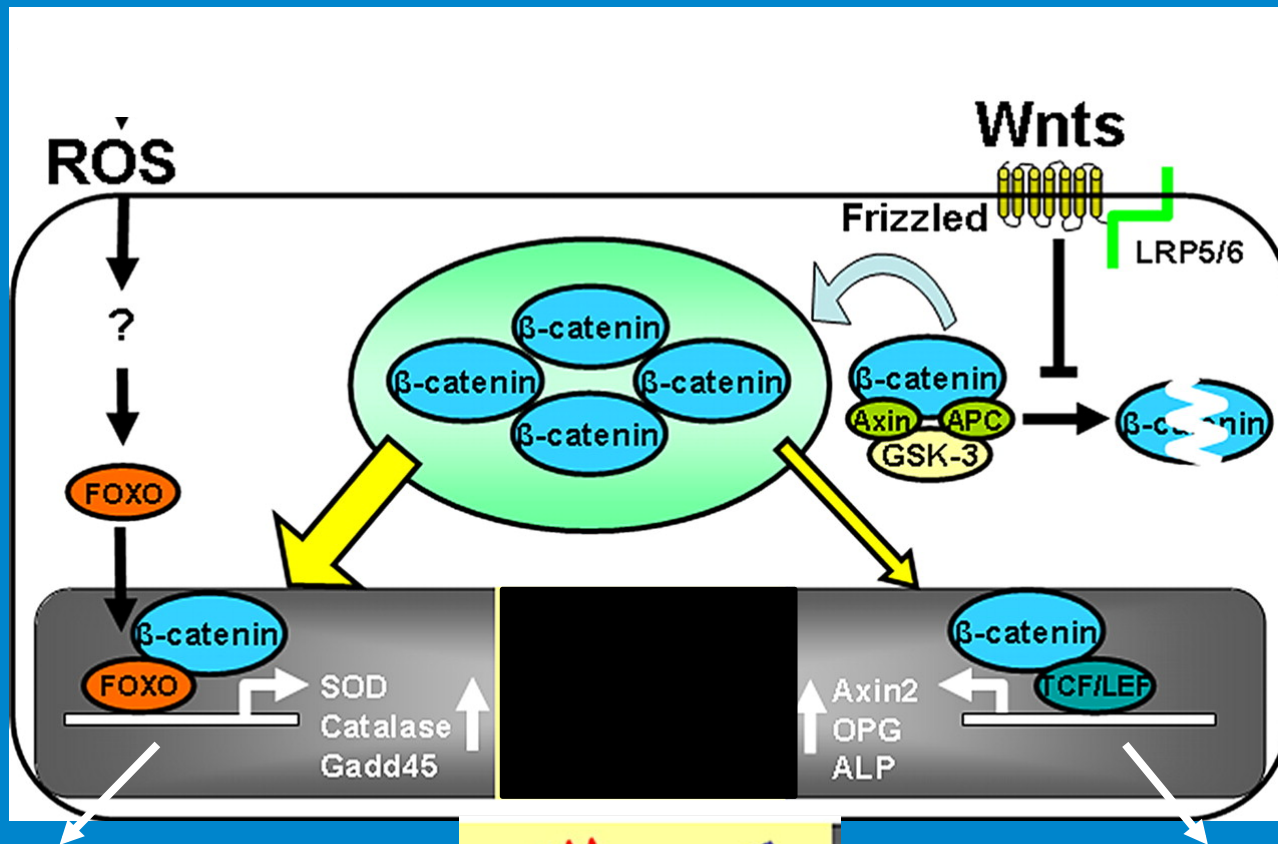
~~DUX4c~~

Muscles: signals to expand or to shrink?



Proliferation

Muscles: signals to expand or to shrink?

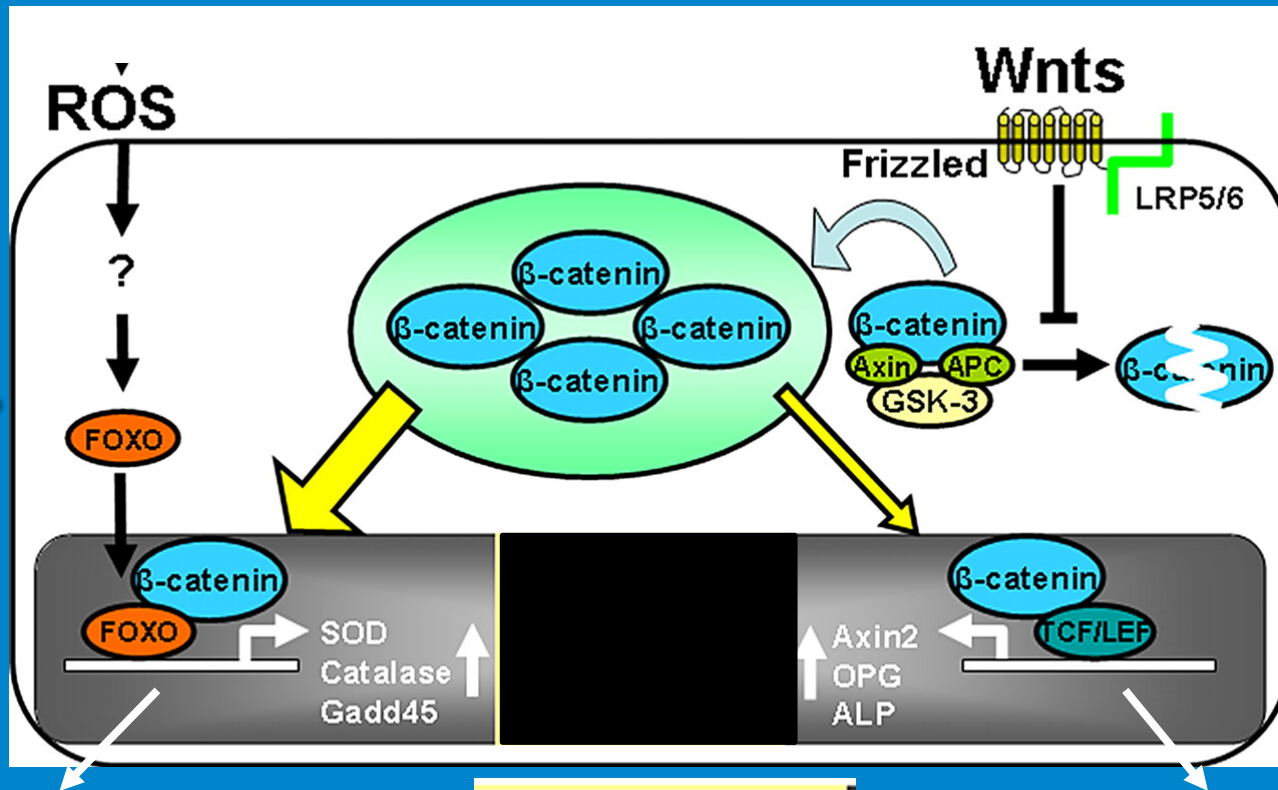


Atrophy



FSHD myotubes

Atrophic



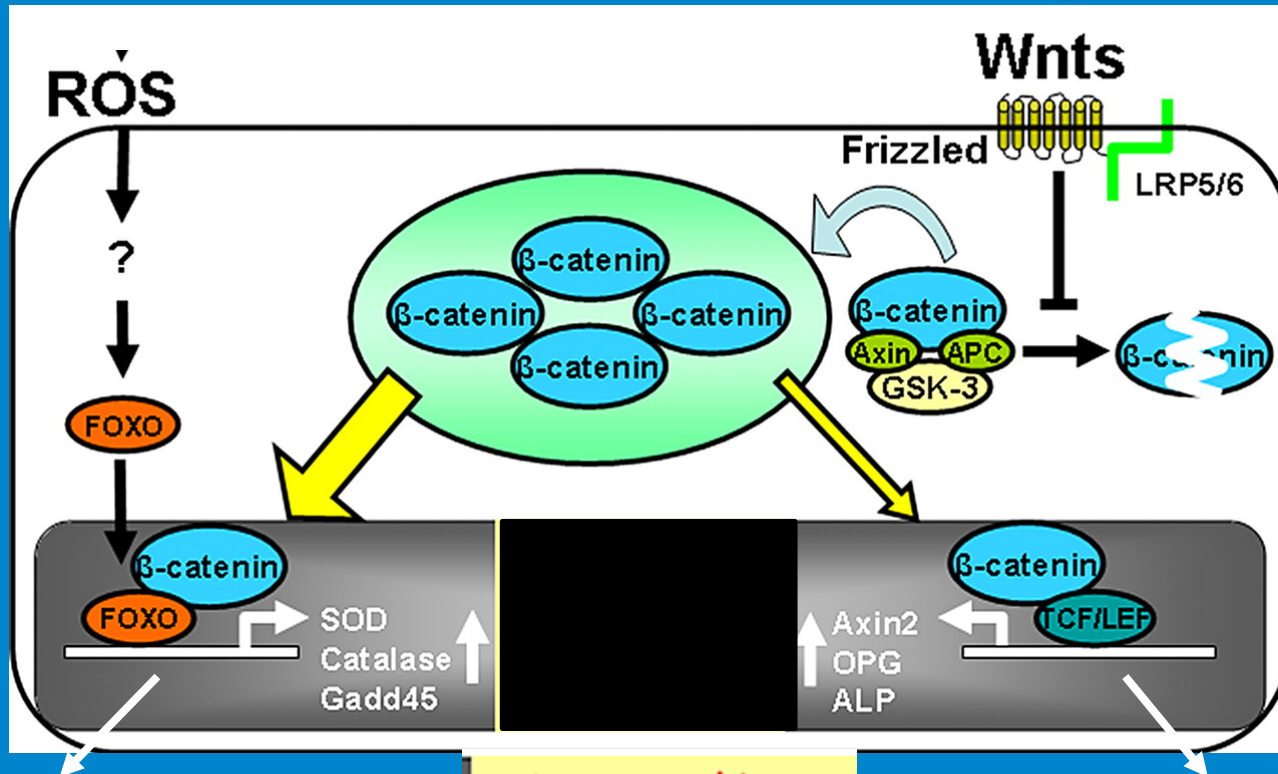
DUX4

Atrophy



FSHD myotubes

Disorganized



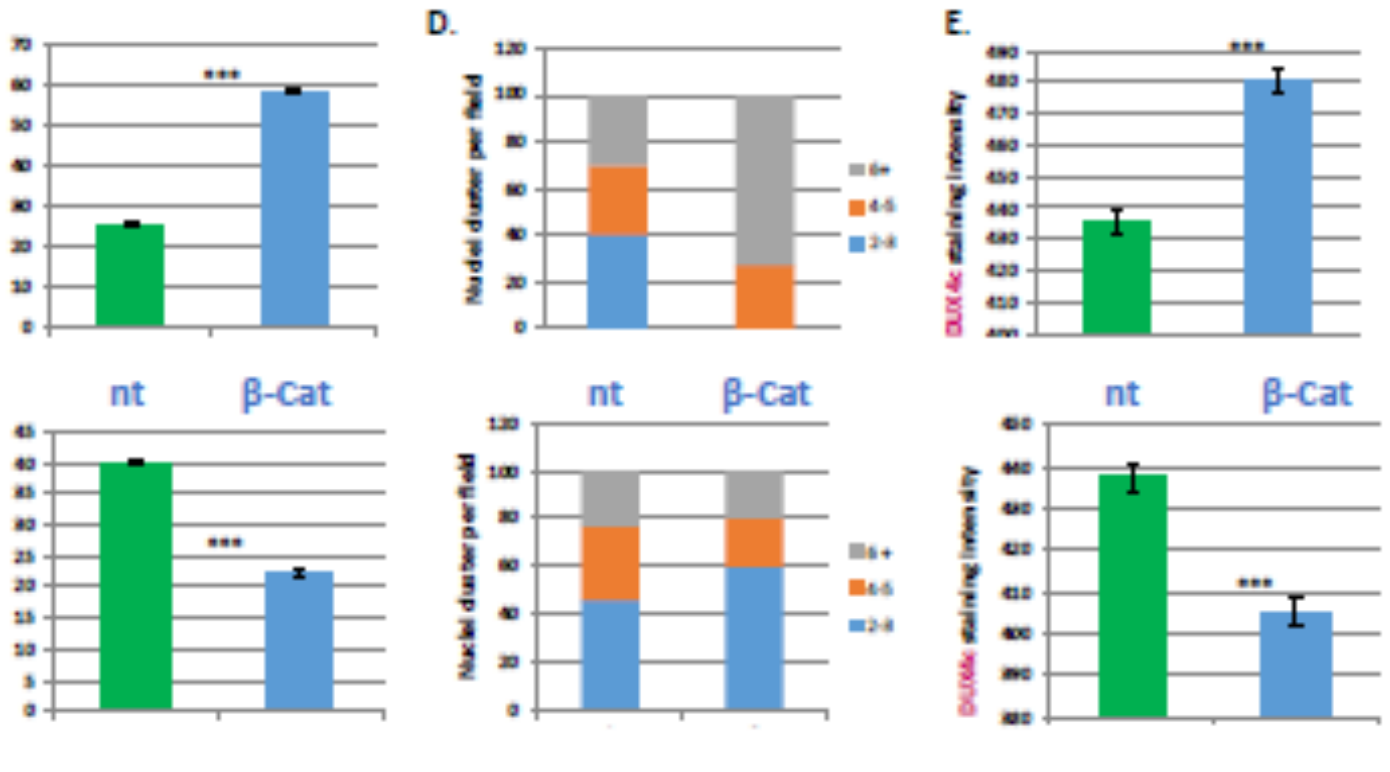
DUX4c

Proliferation

WNT pathway inhibition: tilting the balance

FSHD atrophic

=> disorganized

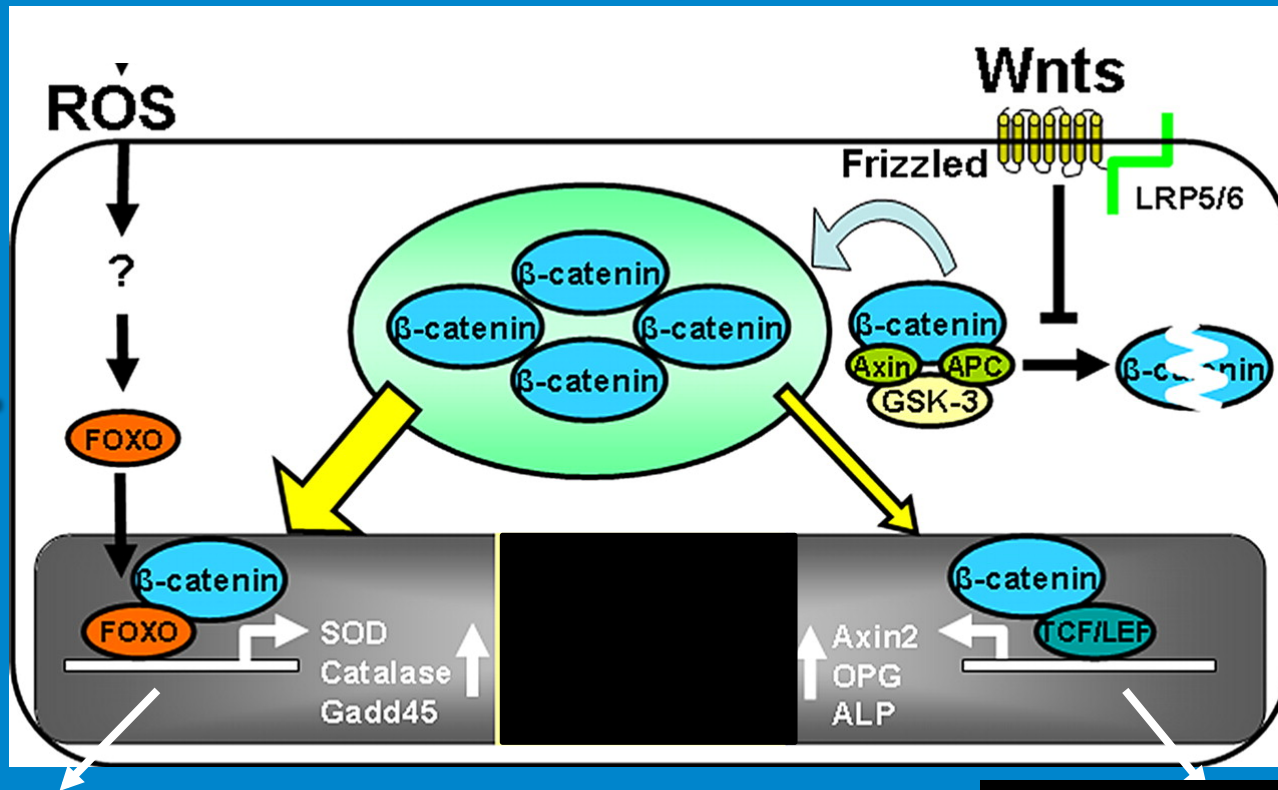


FSHD disorganized

=> atrophic

FSHD myotubes

Atrophic



DUX4

DUX4c

Atrophy

Proliferation

A woman with short grey hair and glasses, wearing a brown jacket and a blue patterned scarf, is sitting on a large, weathered bronze duck sculpture. She is smiling and has her hands on the duck's neck. In the background, a smaller, similar bronze duck is visible on a cobblestone path. The ground is covered with fallen leaves. The image is framed by a blue border.

DUX4c

DUX4

**A delicate balance must be kept
between DUX4 and DUX4c levels**

European Patent Application n° 10175125.3
filed on September 2nd 2010,
“Agents useful in treating
facioscapulohumeral muscular dystrophy”

Antisense agents specific against DUX4
and others against DUX4c

Soon to be granted...

Collaborations

- **Dr. Yi Wen Chen**

Children's National Medical Center, Washington DC, USA.

- **Dr. Scott Harper**

Nationwide Children's Hospital, Columbus, Ohio, USA.

- **Dr. Dalila Laoudj-Chenivesse**

INSERM, University of Montpellier, France.

- **Drs. Rita Perlingeiro and Michael Kyba**

Lillehei Heart Institute, Dept Pediatrics, Univ Minnesota, USA.

- **Drs. V. Mouly and G. Butler-Browne**

Institut de Myologie, Paris, France.

- **Dr. Ruddy Wattiez**

- **University of Mons, Belgium**

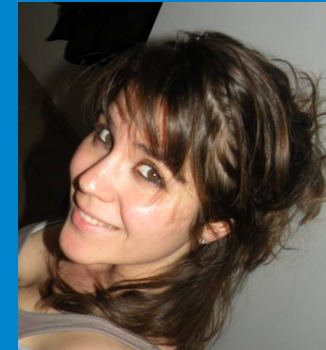
- **Dr. Steve D. Wilton**

Molecular therapy, Murdoch University, Western Australia,

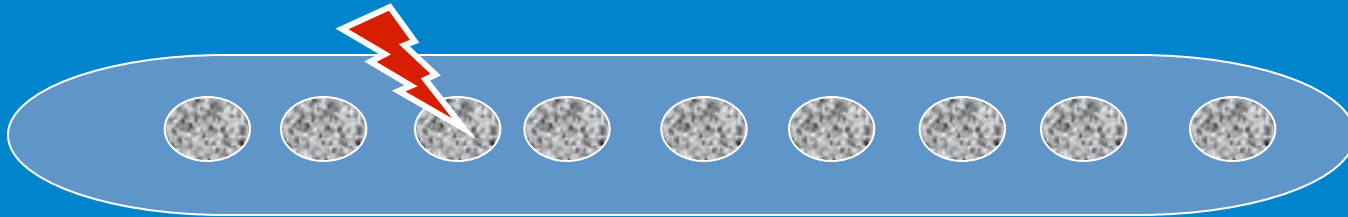
Laboratory of Molecular Biology

alexandra.belayew@umons.ac.be

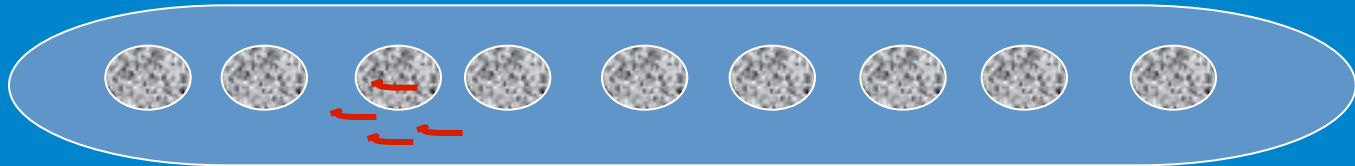
frederique.coppeee@umons.ac.be



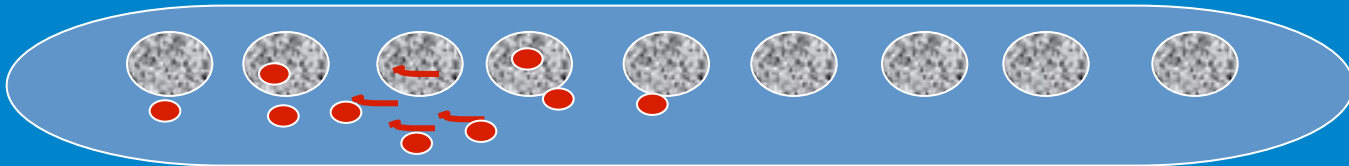
Myotube: *DUX4* gene activation in one nucleus.



DUX4 mRNA molecules

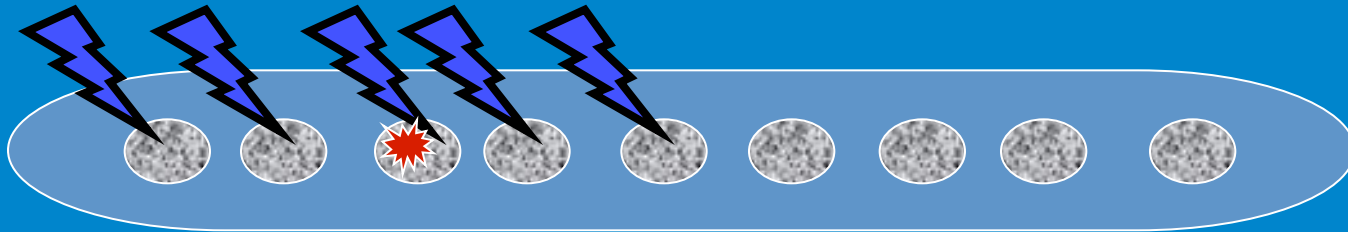


DUX4 protein molecules

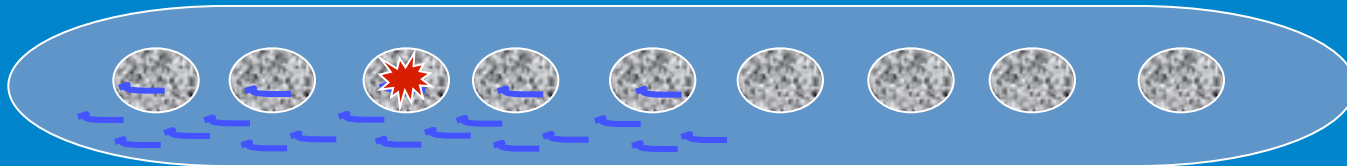


DUX4 proteins imported in several nuclei

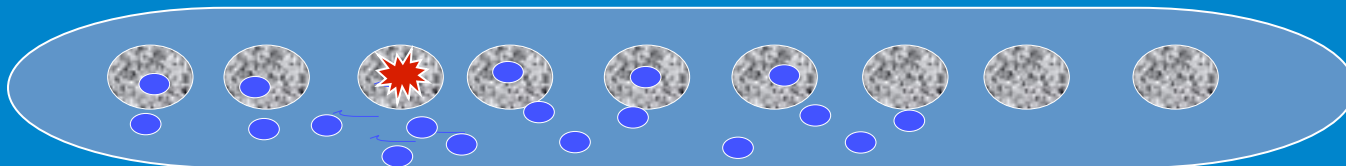
Activation of a DUX4 target gene expressing another *trans*-factor .



trans-factor mRNA molecules ↓



trans-factor protein molecules ↓



trans-factor proteins imported in several nuclei