Development and growth of the muscle tissue

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SCIENCE & IMPACT

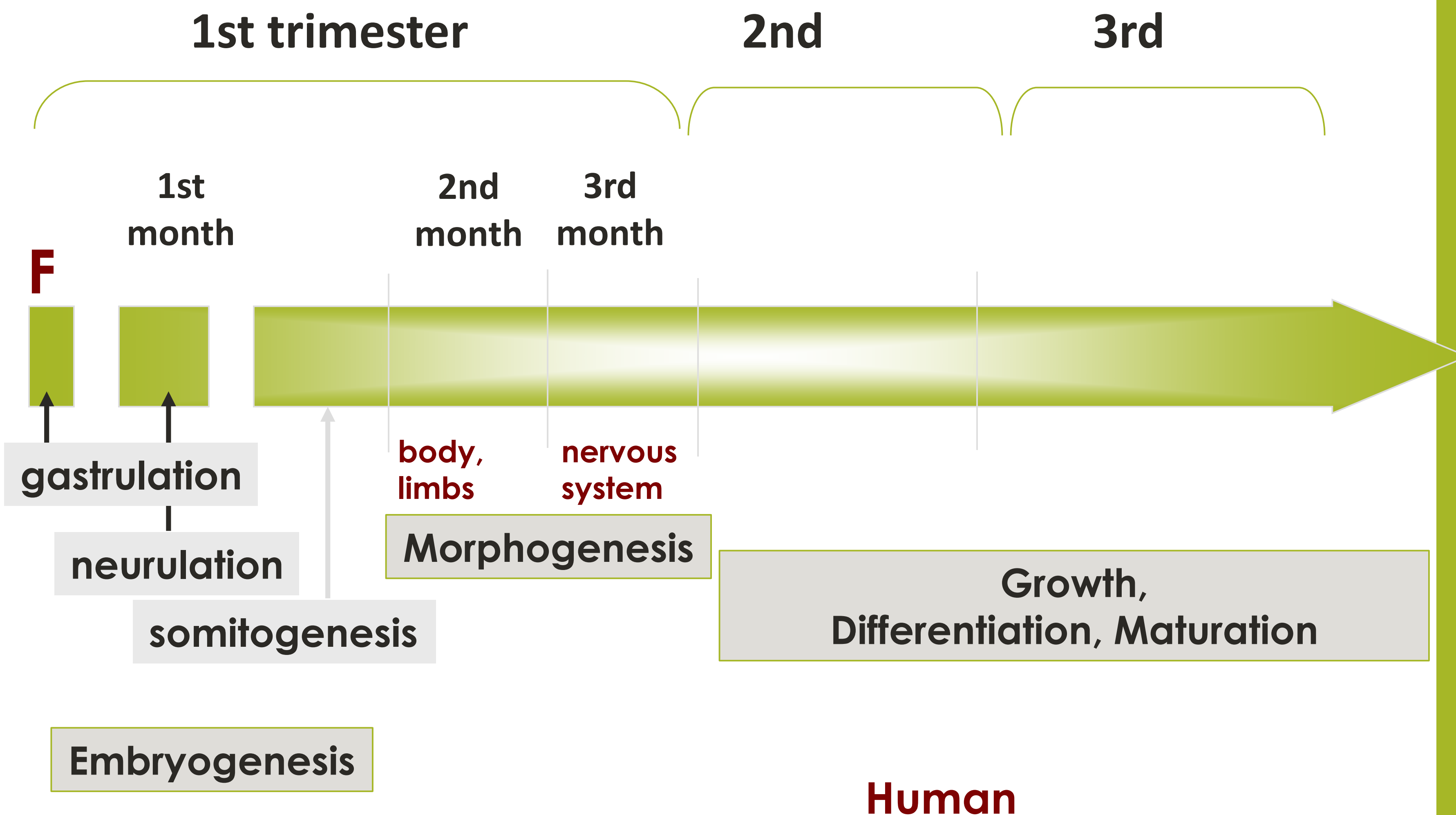
Myogenesis


Muscle development
Myogenesis
Fetal growth
Postnatal growth

1-ORGANOGENESIS

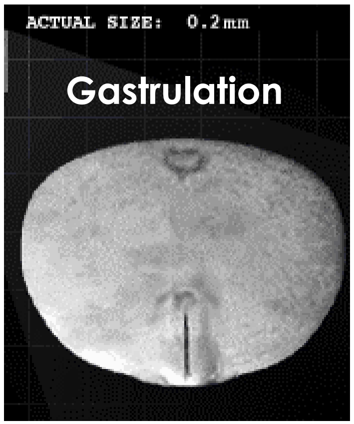
Skeletal muscles of higher vertebrates arise from the <u>embryonic mesoderm</u>.

Fetal development

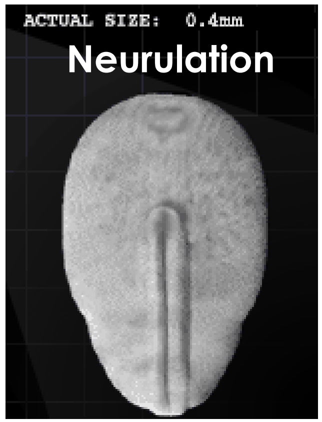


Embryogenesis

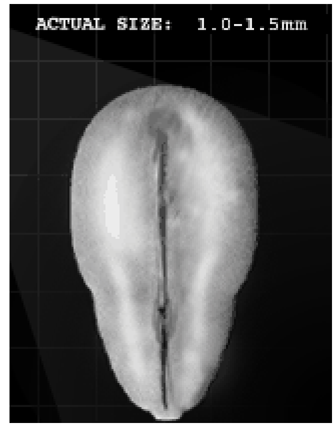
Stage 6 (~13 d p. ovulation)



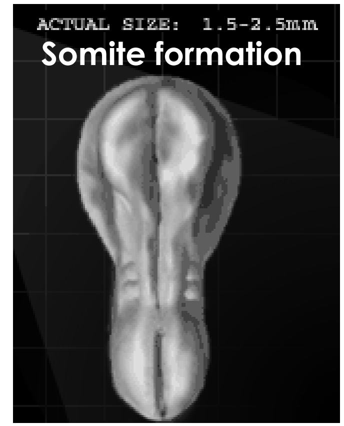
Stage 7 (~ 16 d p. ovulation)



Stage 8 (~ 17-19 d p. ovulation)



Stage 9 (~ 19-21 d p. ovulation)

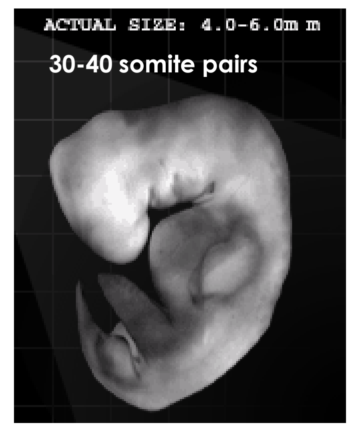


Stage 10 (~ 21-23 d p. ovulation)

1st month Human

Stage 11 (~ 23-25 d p. ovulation)

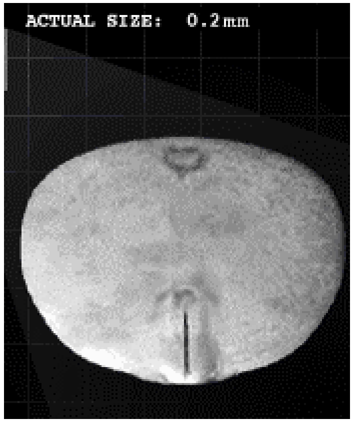
Stage 12 (~ 25-27 dp. ovulation)

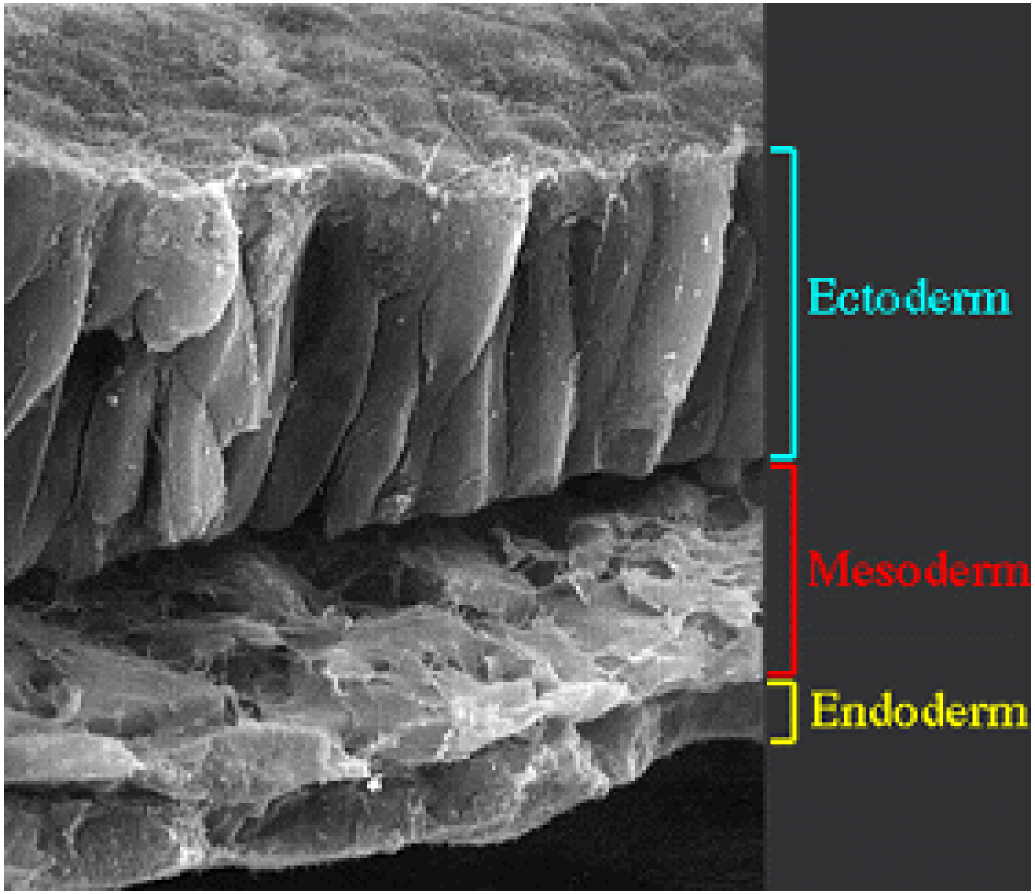


Stage 13 (~ 27-29 dp. ovulation)

http://www.visembryo.com/baby

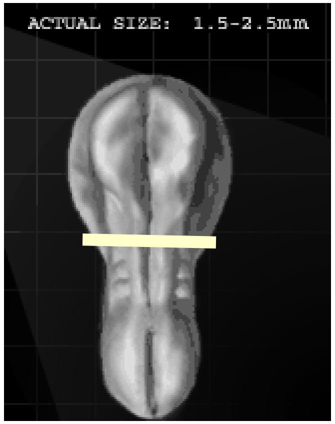
3 primary germ layers

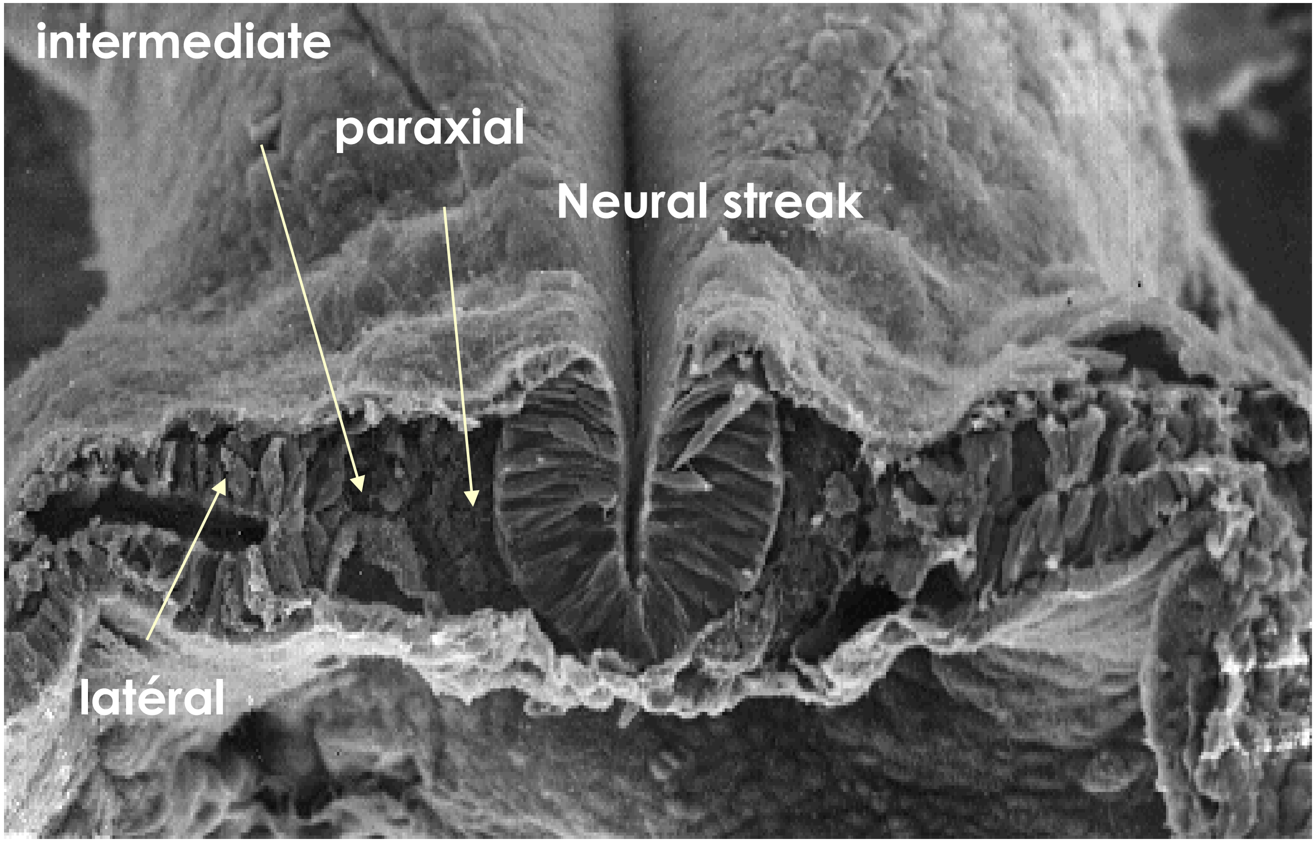




established during gastrulation

Differentiation of the mesoderm layer

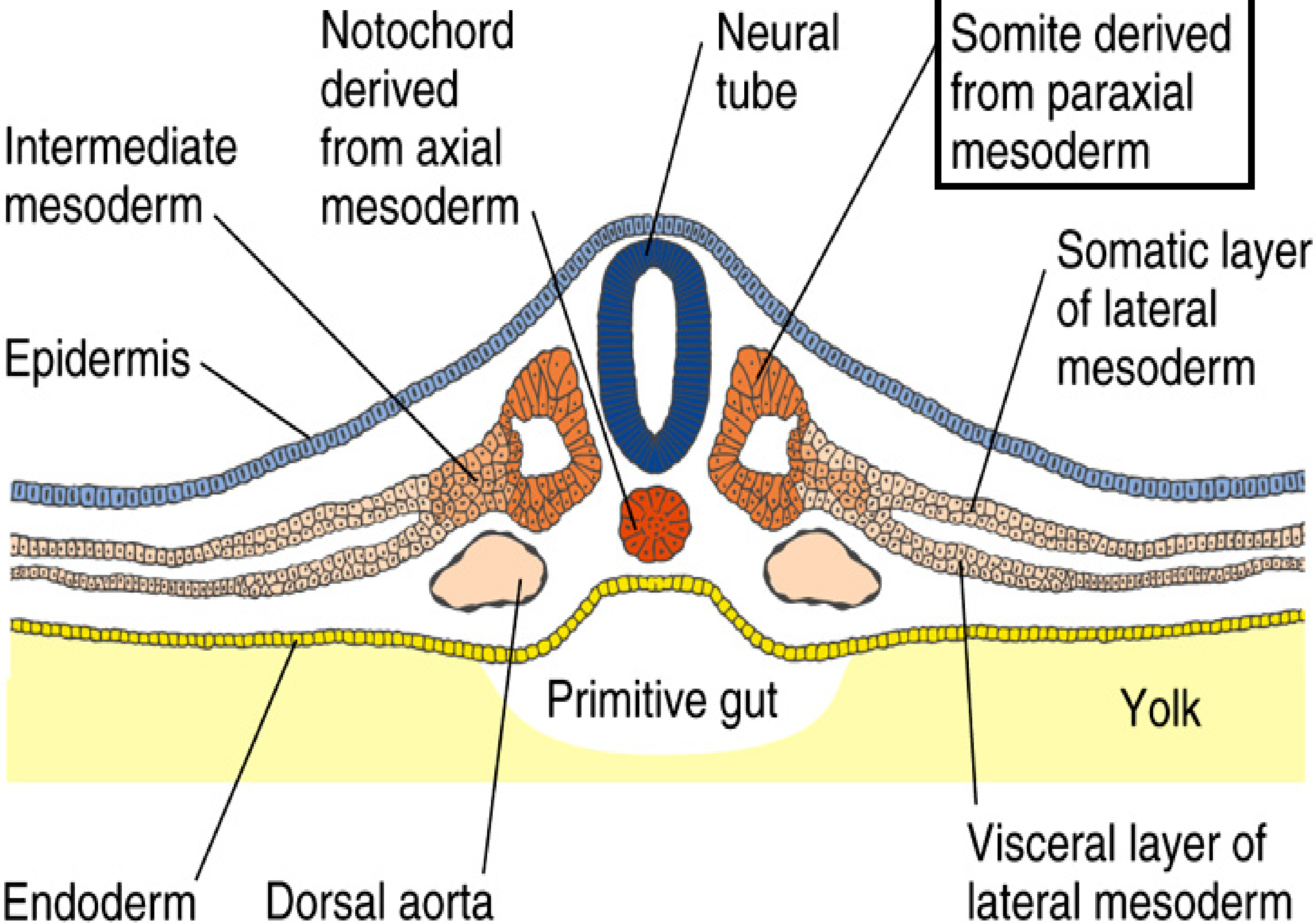




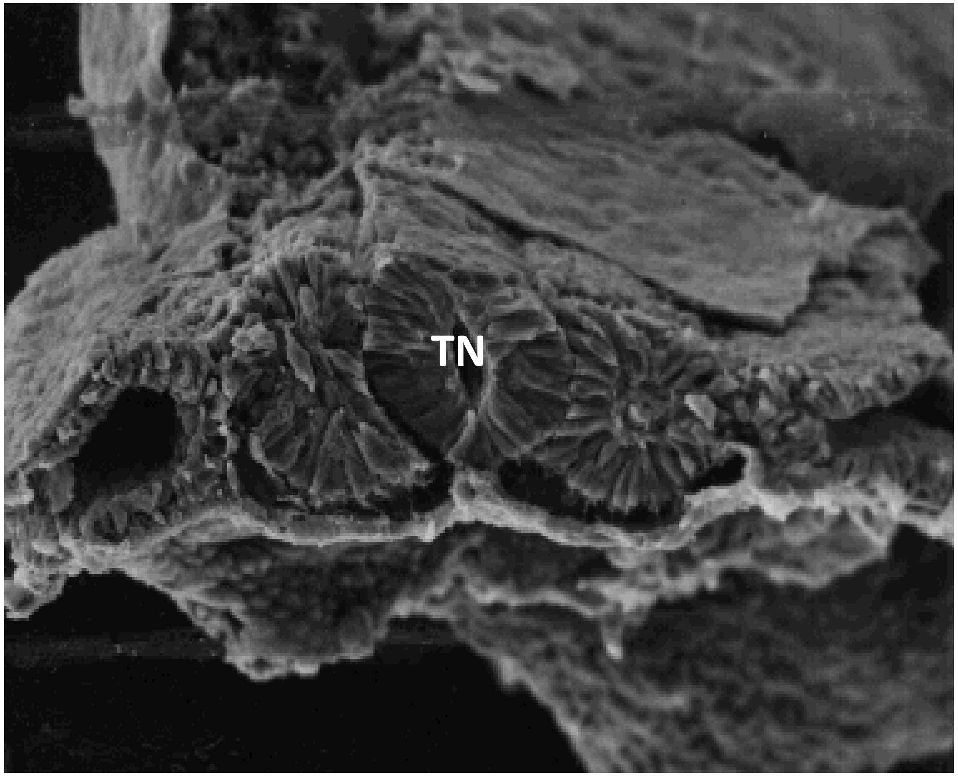
Human: 22 days **Mouse: 8 days**

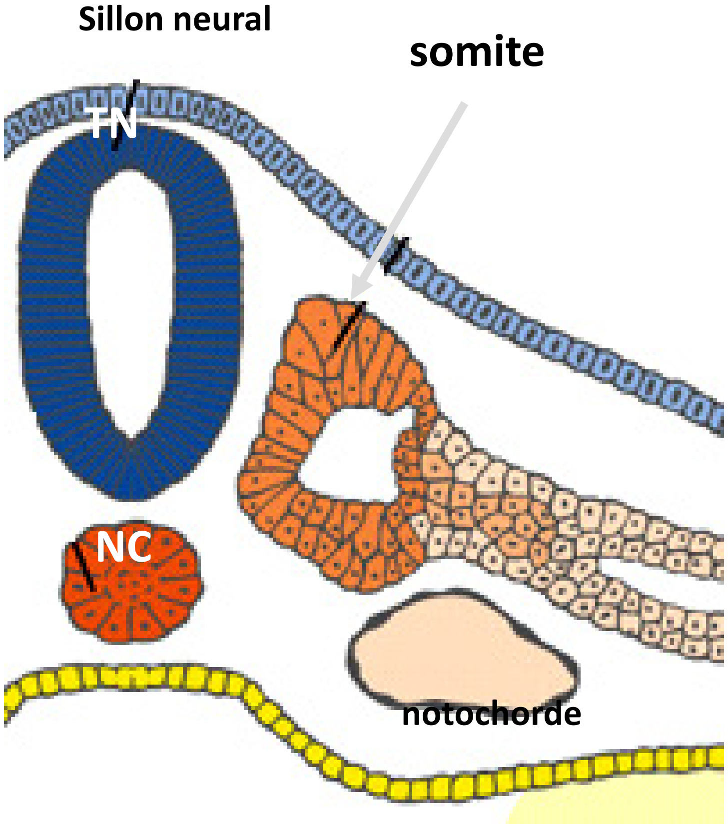
http://www.med.unc.edu/embryo_images/unit-mslimb/mslimb_htms/mslimb001a.htm

Subdivision of the mesoderm



Somitogenesis



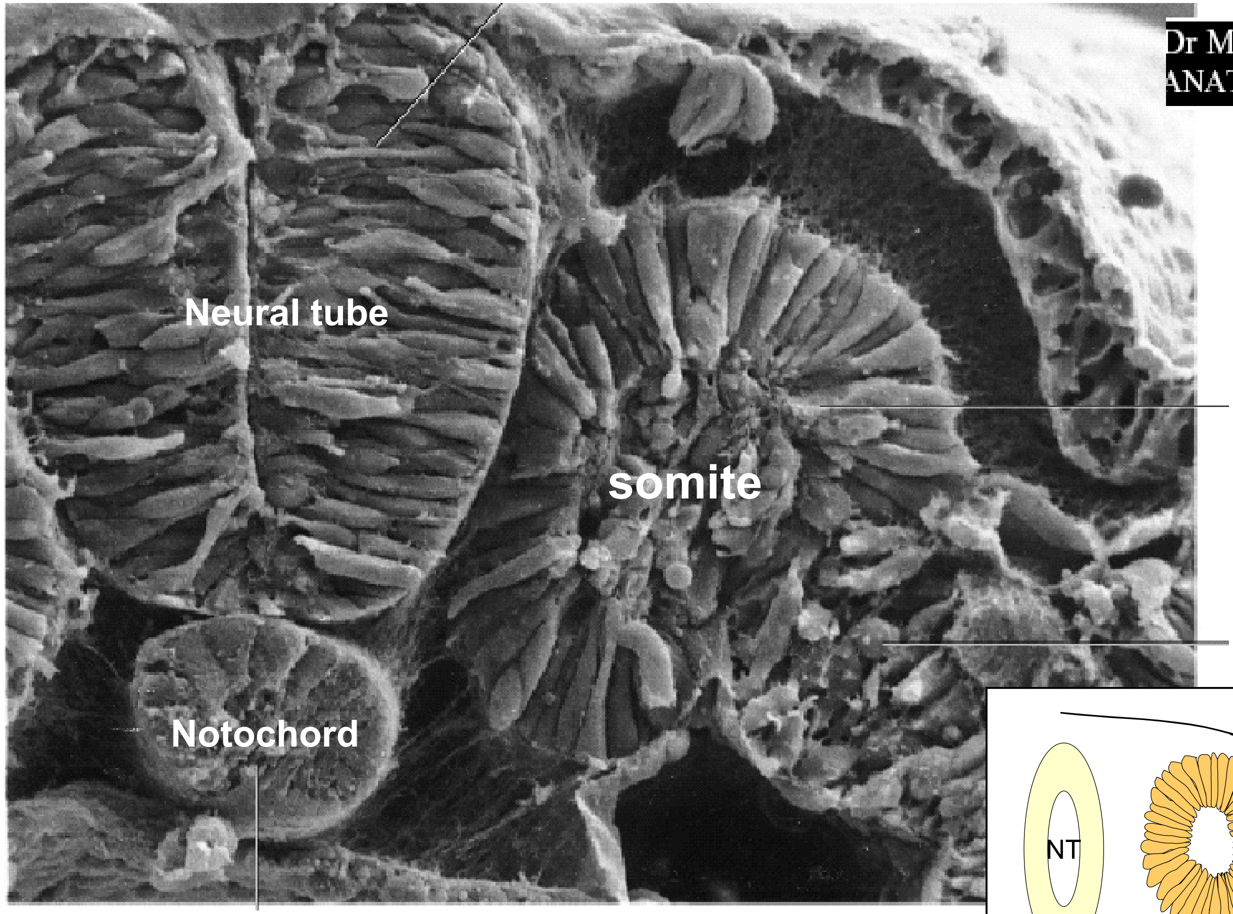


Human: 23 days   Mouse: 9 days

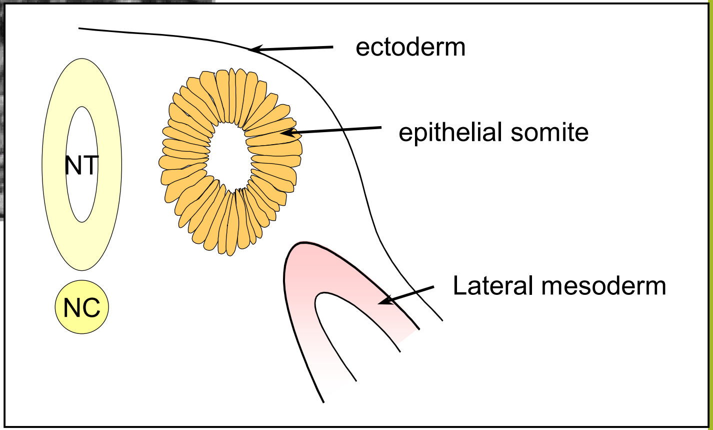
The paraxial mesoderm cells is segmented into cylindrical structures (somitomeres) early in the third week.

http://www.med.unc.edu/embryo_images/unit-mslimb

Somites



Dr Mark Hill, CBL, Anatomy, UNSW
ANAT 2300- Mesoderm Development



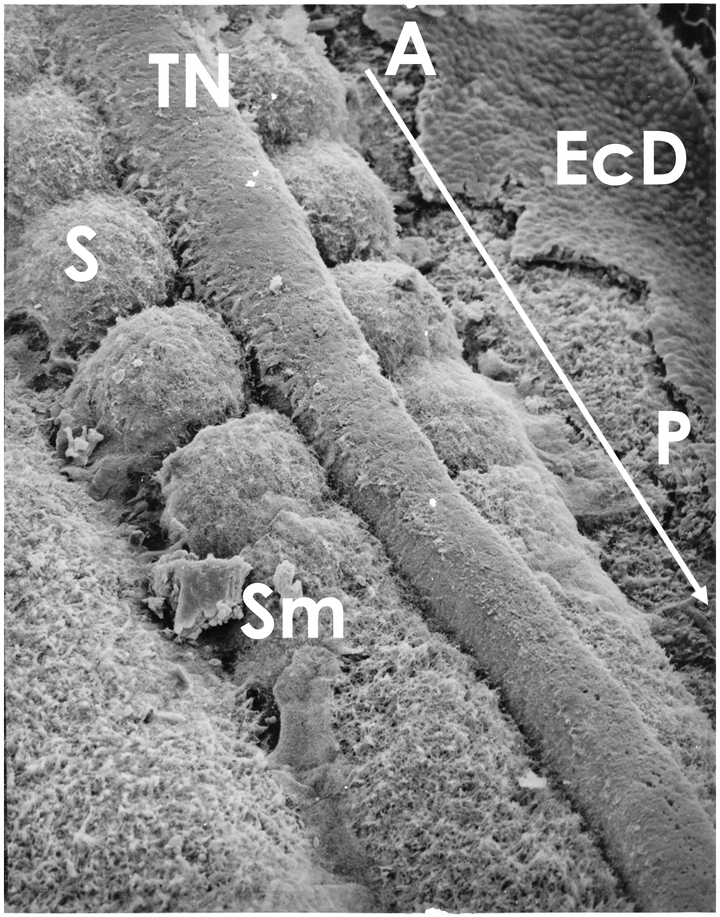
= pairs of cylindrical, epithelially-organized mesenchymal segments

Somitogenesis

Somitomeres → somites

Excepted Sm 1 to 7 **Final number =31**

Human embryo (28 days)



Metamerisation

Take-home message

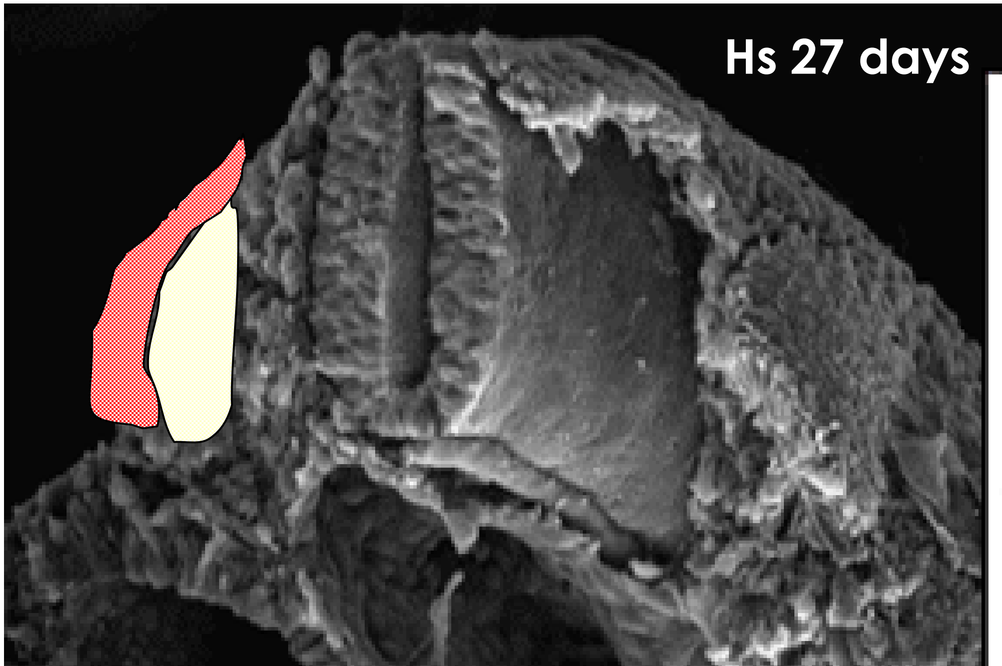
The somites are <u>transient</u> structures that form progressively along the anteroposterior axis by <u>segmentation</u> of the paraxial mesoderm and reorganize <u>without cell differentiation</u> (primary organs).

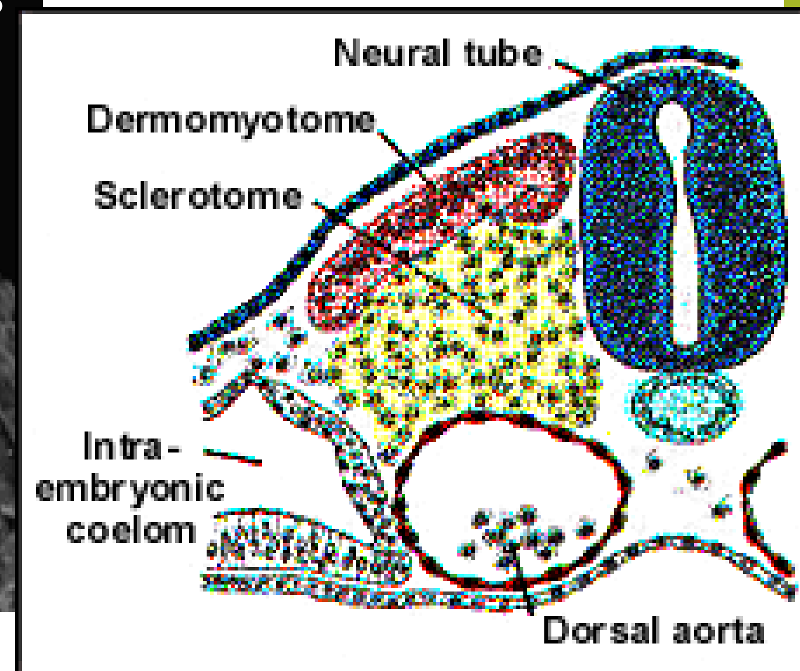
Take-home message

The somites are responsible for the segmental organization of the body.

They govern the metamerism of somite-derived tissues and spinal ganglia. Metameric division of the spine, the neural tube, the abdominal wall and thorax (ribs) depends on the organization of somites.

Subdivisions of somites





The somite subdivides into :

- **the sclerotome** (ventral medial part)
- **the dermomyotome** (dorsal part)

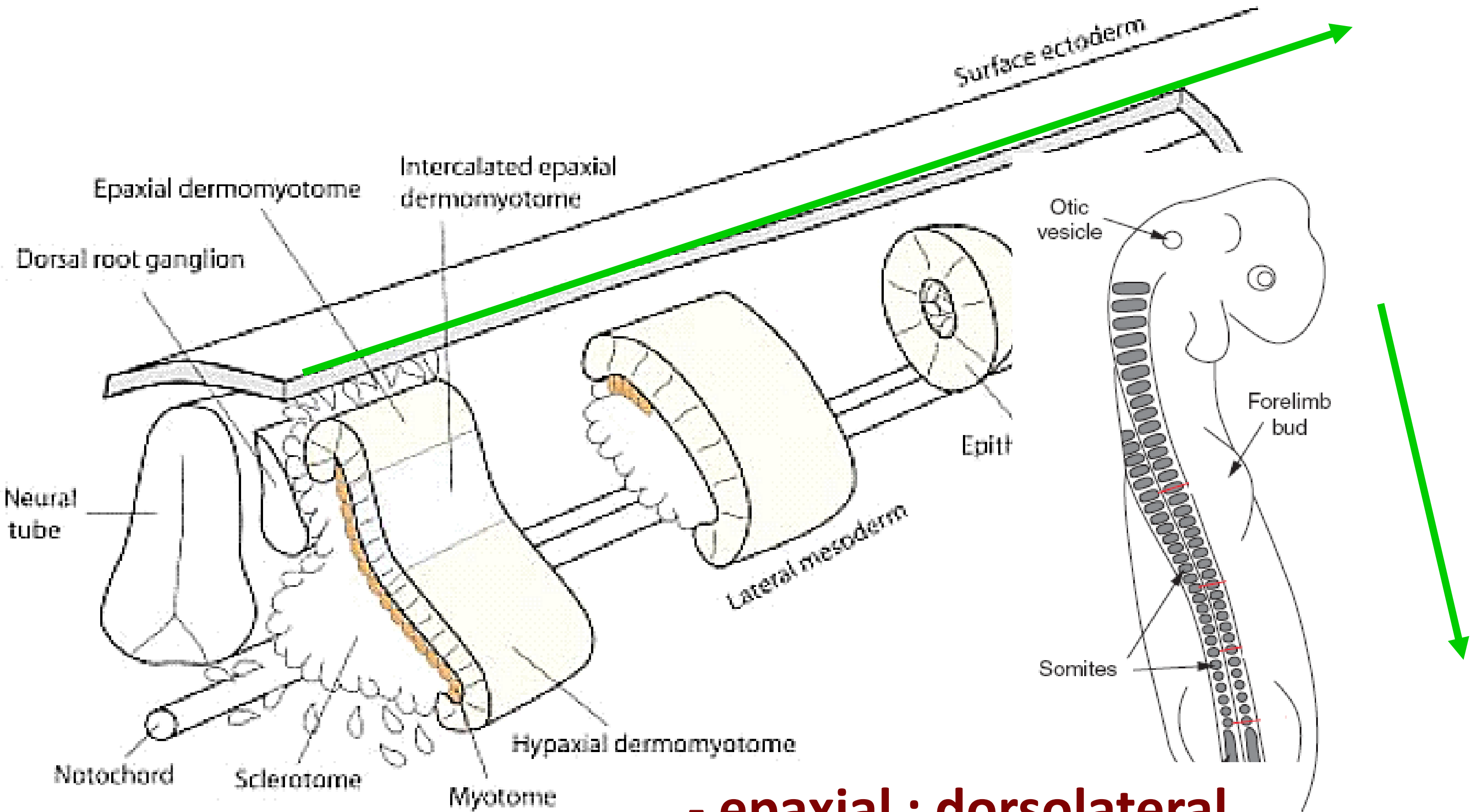
Derivatives of somites

- the sclerotome that gives rise to cartilage, vertebrae and most of the ribs.

- the dermomyotome, a double-layered structure composed of myotome in the lower layer and dermatome in the upper layer :

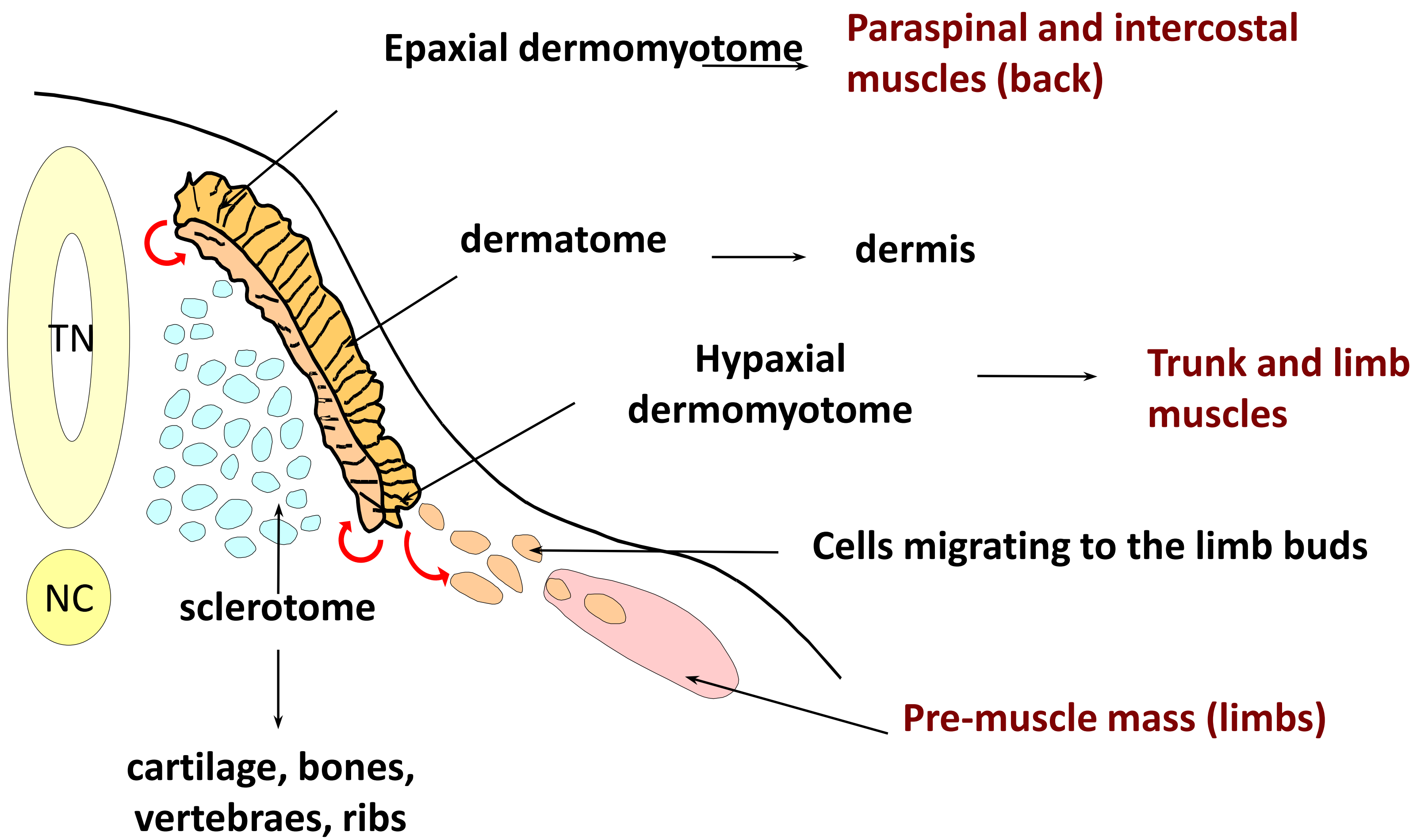
  ⇨ myotome : skeletal muscles (body, limbs)
  ⇨ dermatome, that generates dermis (skin)

Formation of the dermomyotome



- epaxial : dorsolateral
- hypaxial : ventrolateral

Derivatives of the dermomyotome



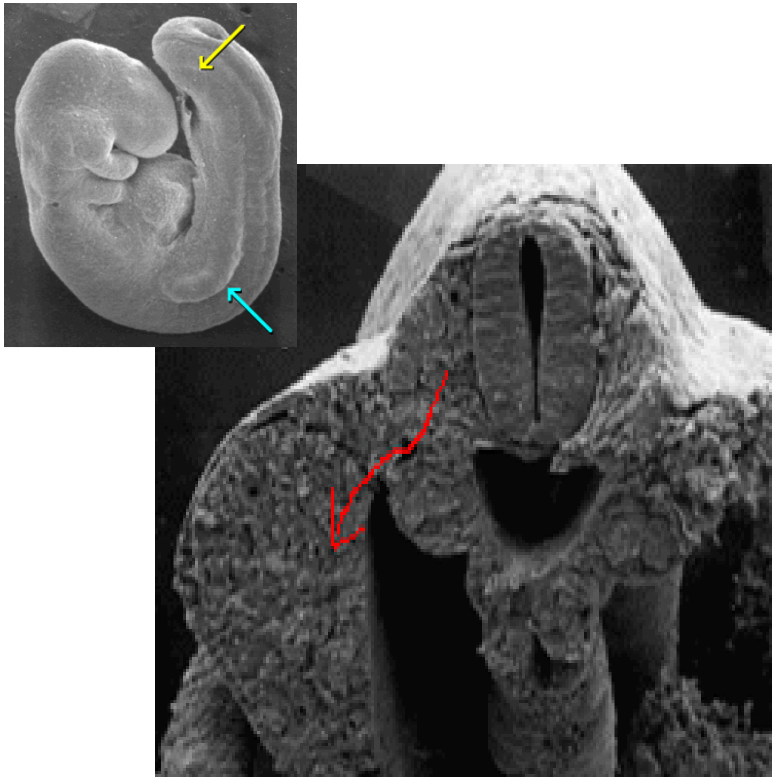
Adapted from Relaix et Buckingham, 1999

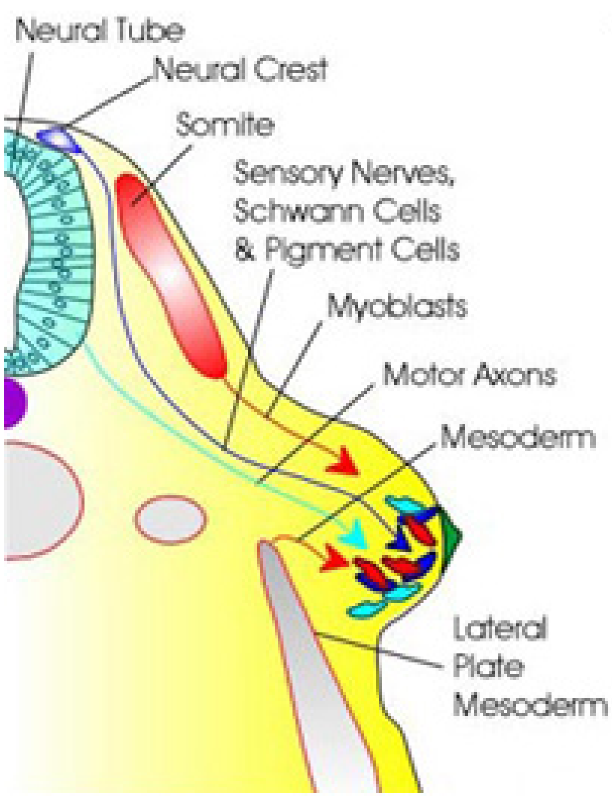
Epaxial vs. hypaxial muscles

	Epaxial muscles	Hypaxial muscles	
Position in the adult	Dorsal	Superficial, lateral, ventral	
Innervation	Dorsal ramus of spinal nerves	Ventral ramus of spinal nerves	
Embryonic origin	Medial somite	Lateral somite	
Precursors			
Induction	By synergistic signals from neural tube/ectoderm and from notochord/floor plate	By synergistic signals from lateral mesoderm and ectoderm	
Migratory profile	Non-migratory	Non-migratory (flank level)	Migratory (occipital, cervical, limb levels)

Adapted from Dietrich et al., 1999

Limb muscles





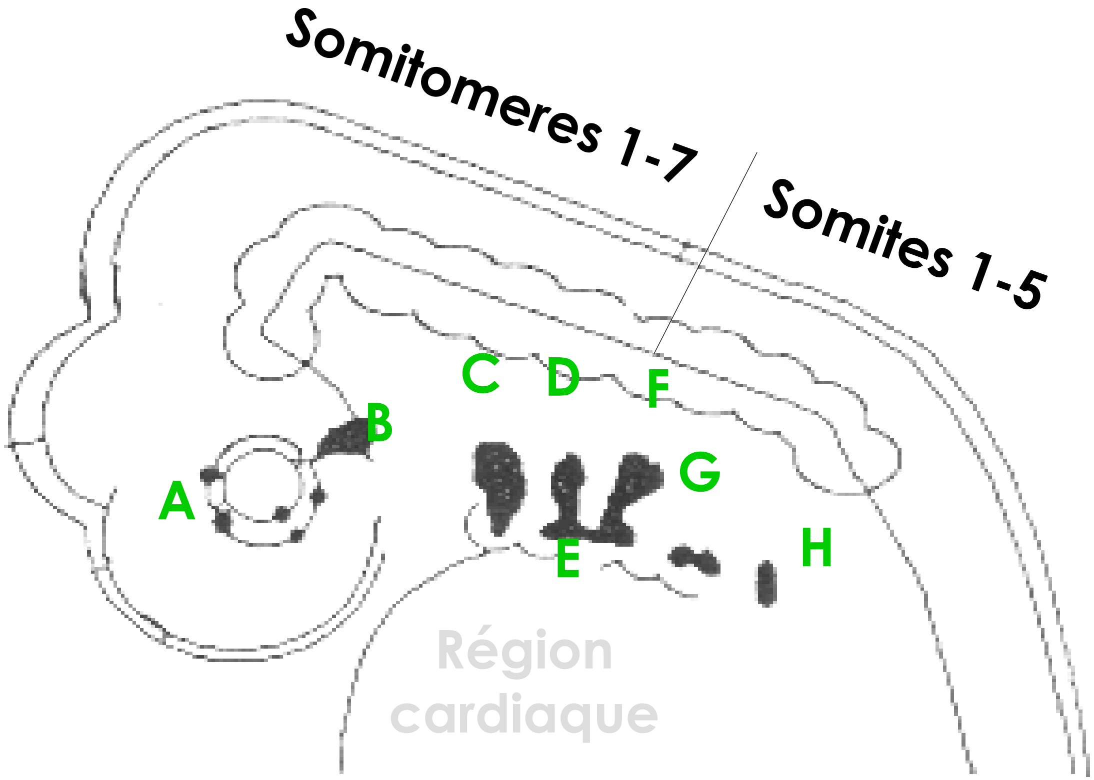
Cell migration

Limb colonisation

In the limb buds, the mesenchyme arises from

- **the lateral plate mesoderm (➔ skeleton)**
- **the ventrolateral part of the myotome (➔ muscles)**

Craniofacial muscles



A : ocular muscle
B : lateral rectus
C : masseter
D : facial muscles
E : tongue
F : pharyngeal muscles
G : laryngeal muscles
H : neck muscle

They arise from cephalic somitomeres
and from rostro-occipital somites.

Take-home message

- ectoderm
- endodem
- mesoderm:
  - lateral
  - intermediate
  - paraxial : ➔ somitomeres ➔ somites

    s1-s7 : m. cranio-facial

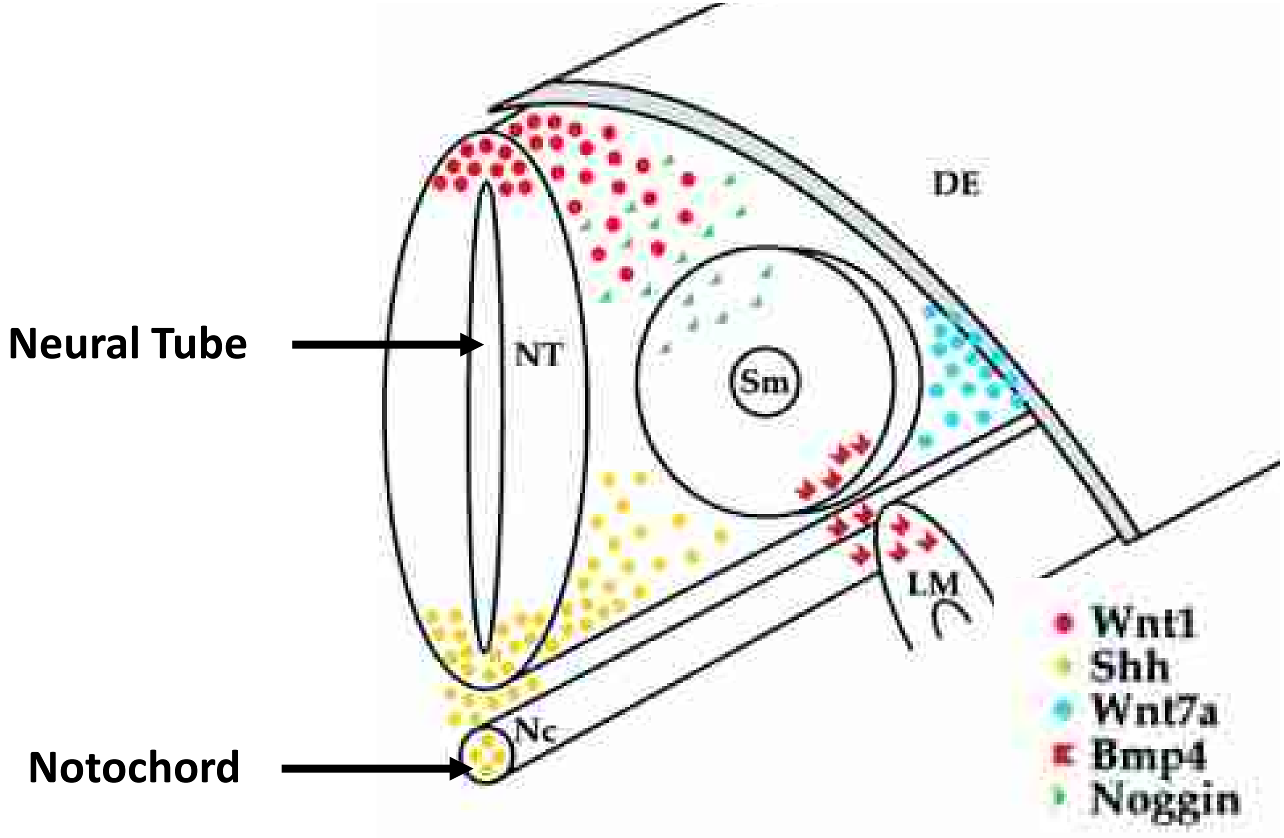
    - sclerotome (cartilage, vertebrae)
    - dermomyotome :
      - dermis
      - myotome :
        - epaxial (back muscles)
        - hypaxial (limb and trunk muscle)

REGULATION

Morphogenetic signals

Morphogenic signals

Signals secreted by the surrounding tissue **(paracrine)** **orchestrating muscle development**

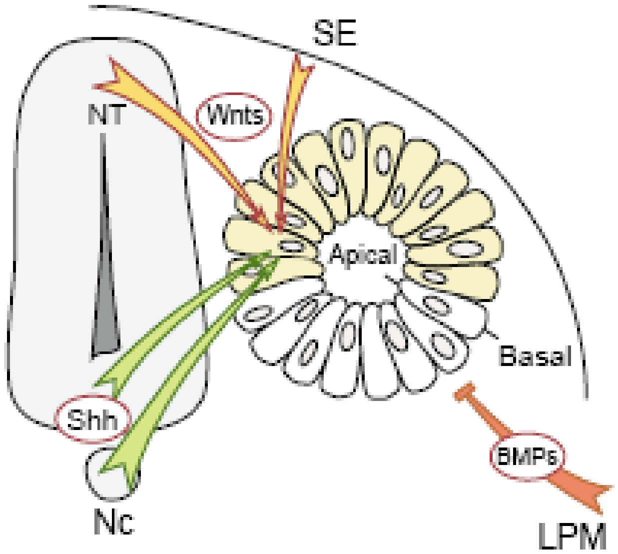


Morphogenic signals

Wingless

Wnt1 Wnt7

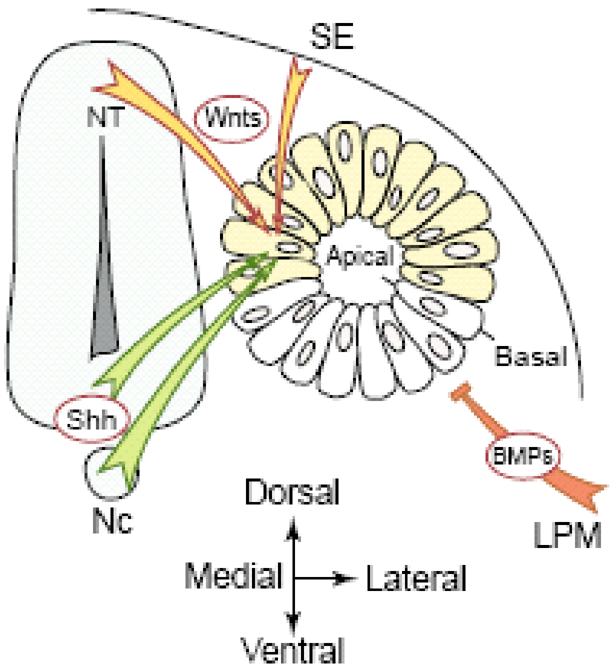
Sonic hedgehog



- **Wnt signals control the induction of muscle tissue morphogenesis**
- **Shh is necessary for the maintenance and proliferation of cells in the epaxial region**

Morphogenic signals

A gradient of BMP4 protein (lateral mesoderm) delays muscle differentiation in the ventrolateral area vs. the dorsomedial part.

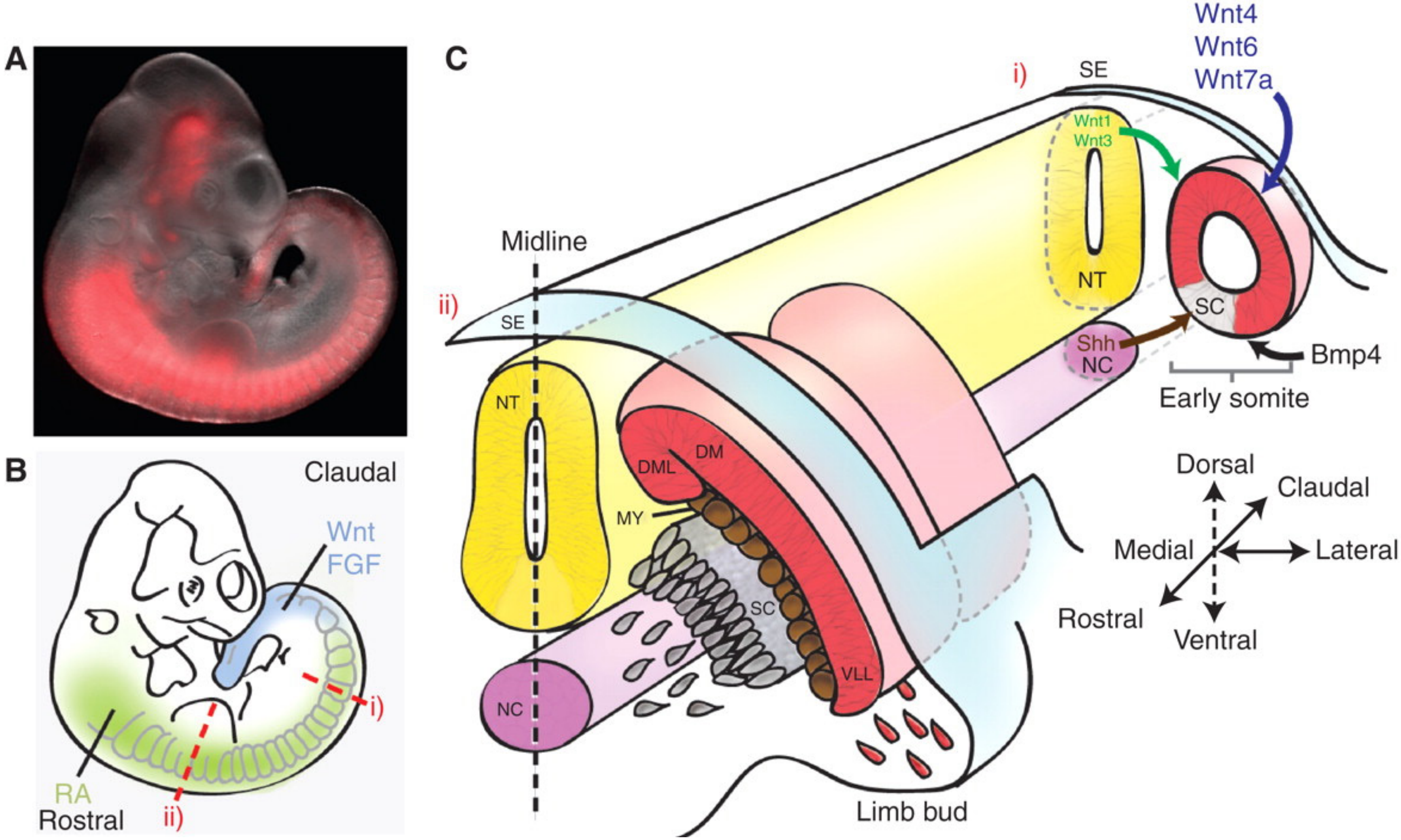


BMP4 induces lateralization of the somite.

Morphogenic signals

Growth factors secreted by the lateral mesoderm play an essential role in inducing the migration of precursor cells of hypaxial muscle.

- TGFs beta
- FGFs
- HGF (c-met receptor)

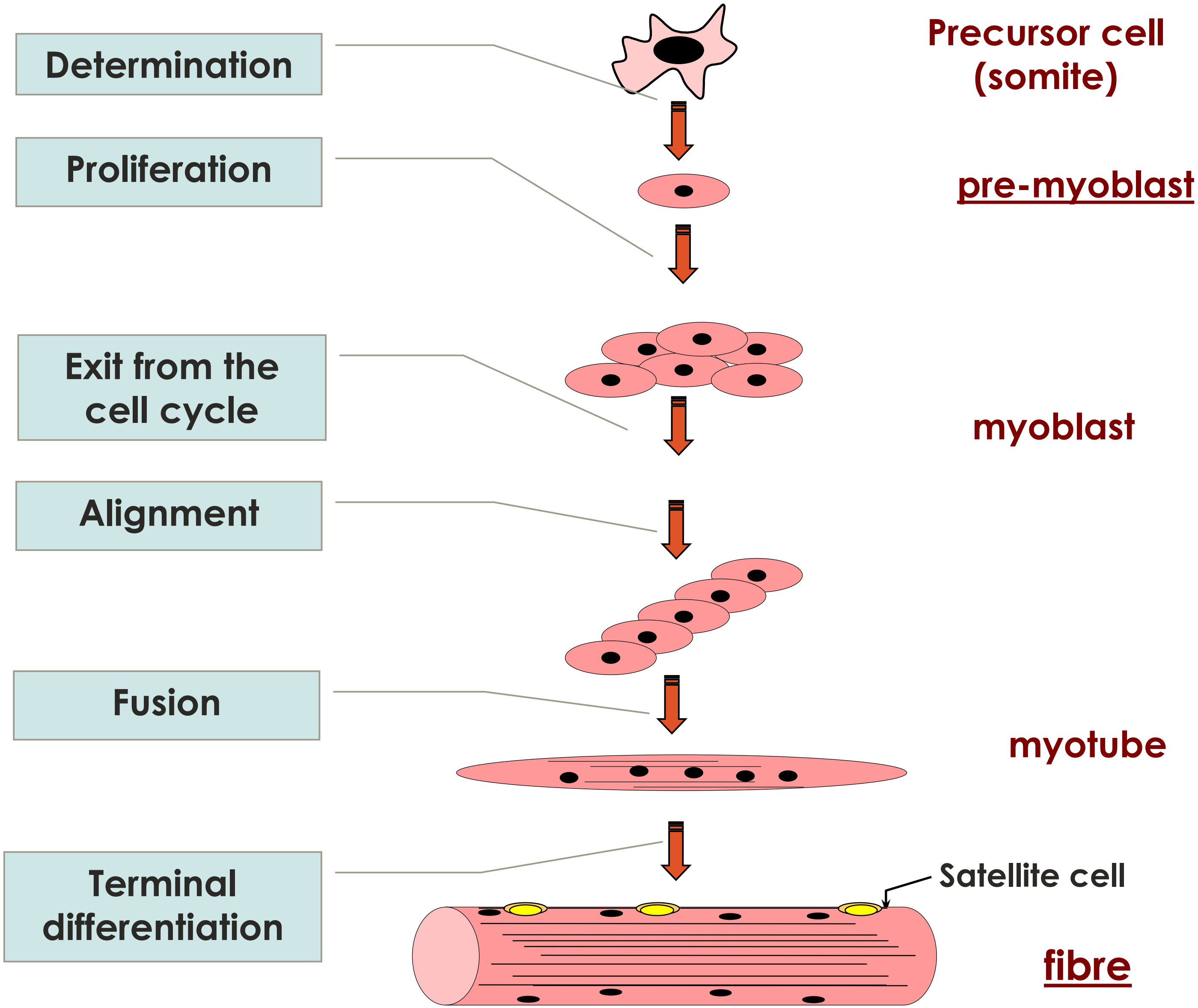


C. Florian Bentzinger et al. Cold Spring Harb Perspect Biol 2012;4:a008342

REGULATION

Genes controlling the commitment of cells into the muscle lineage and myogenic differentiation

Myogenesis (at the cell level)



Definitions

<u>Cell fate specification</u>: Labile state where a cell has reversibly acquired fate.

<u>Determination</u>: state where a cell has irreversibly acquired fate (ligneage commitment regardless of the environment ; changes in gene expression transmitted to offspring cell).

<u>Differentiation</u>: changes involved in the diversification of the structure and function of cells. Acquisition of the characteristics that allow different cell types to perform their functions.

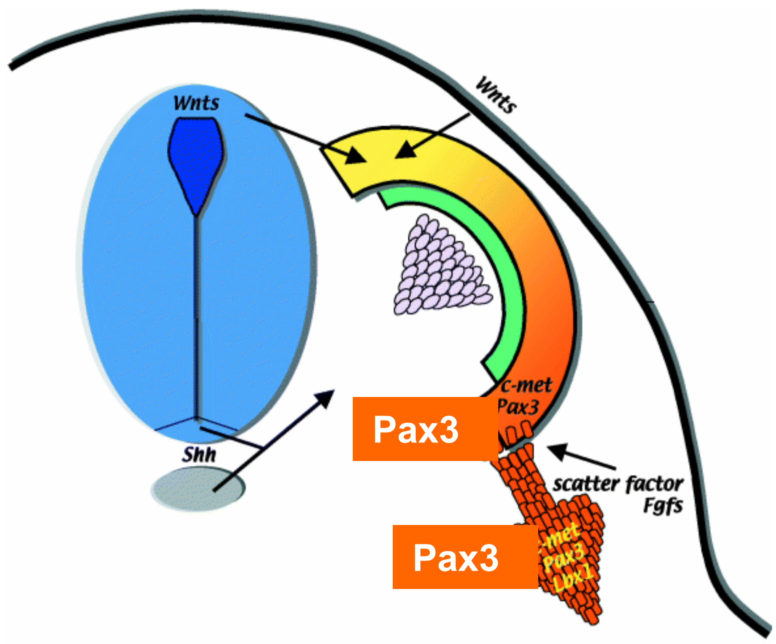
TRANSCRIPTION FACTORS

Key genes (transcription factors) control the expression of target genes.

examples covered:
1-Pax3 and cell specification
2-myogenic regulatory factors (MRFs)

1-Paired-box homeotic factor 3

Pax3



Expression

- paraxial mesoderm
- dermomyotome
- cells colonizing limb buds

- neurogenic precursors

Pax3/epaxial m. *vs* hypaxial m.

	Epaxial muscles	Hypaxial muscles	
Migratory profile	Non-migratory	Non-migratory (flank level)	Migratory (occipital, cervical, limb levels)
Marker gene expression			
Sim1	No	Yes	Yes
Pax3	Low levels	High levels	High levels
cMet[a]	Yes	Yes	Yes
Lbx1	No	No	Yes
Adult muscles (example)	Deep muscles of the back	Body wall muscles	Limb muscles

Adapted from Dietrich et al., 1999

Pax3 function and action

- No hypaxial myotome and limb musculature in Pax3 -/- mice.

- Pax3 induces myogenesis in totipotent cells.
- Overexpression of a dominant negative Pax3 abolishes myogenesis in mice.
- Pax3 controls the expression of myogenic factors

Role in myogenic specification

2-Myogenic Factors (MRFs)

The muscle IDENTITY is conferred by the expression of "myogenic regulatory factors" .

MyoD	*Myogenic determination factor 1*	Davies et al., 1987
Myf-5	*Myogenic factor 5*	Braun et al., 1989
Myogenin	*Myogenin*	Wright et al., 1989
MRF-4	*Myogenic regulatory factor 4*	Rhodes et al.,1989

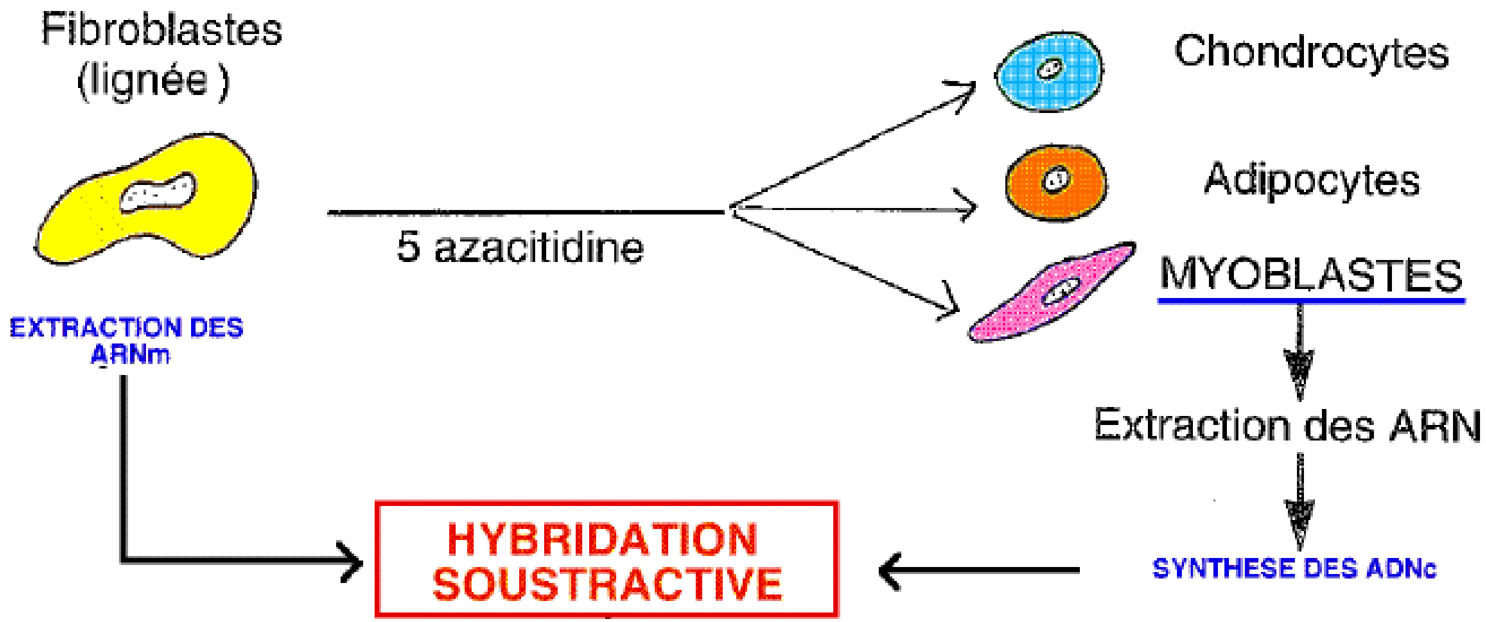
MyoD family

Experimental demonstration

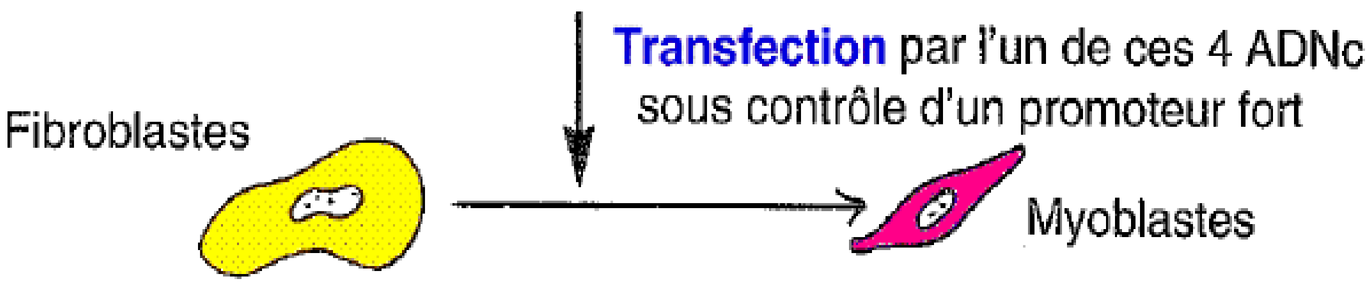
Fibroblasts from mouse embryos cultured in the presence of 5-azacytidine differentiate mainly in myoblasts.

MyoD family

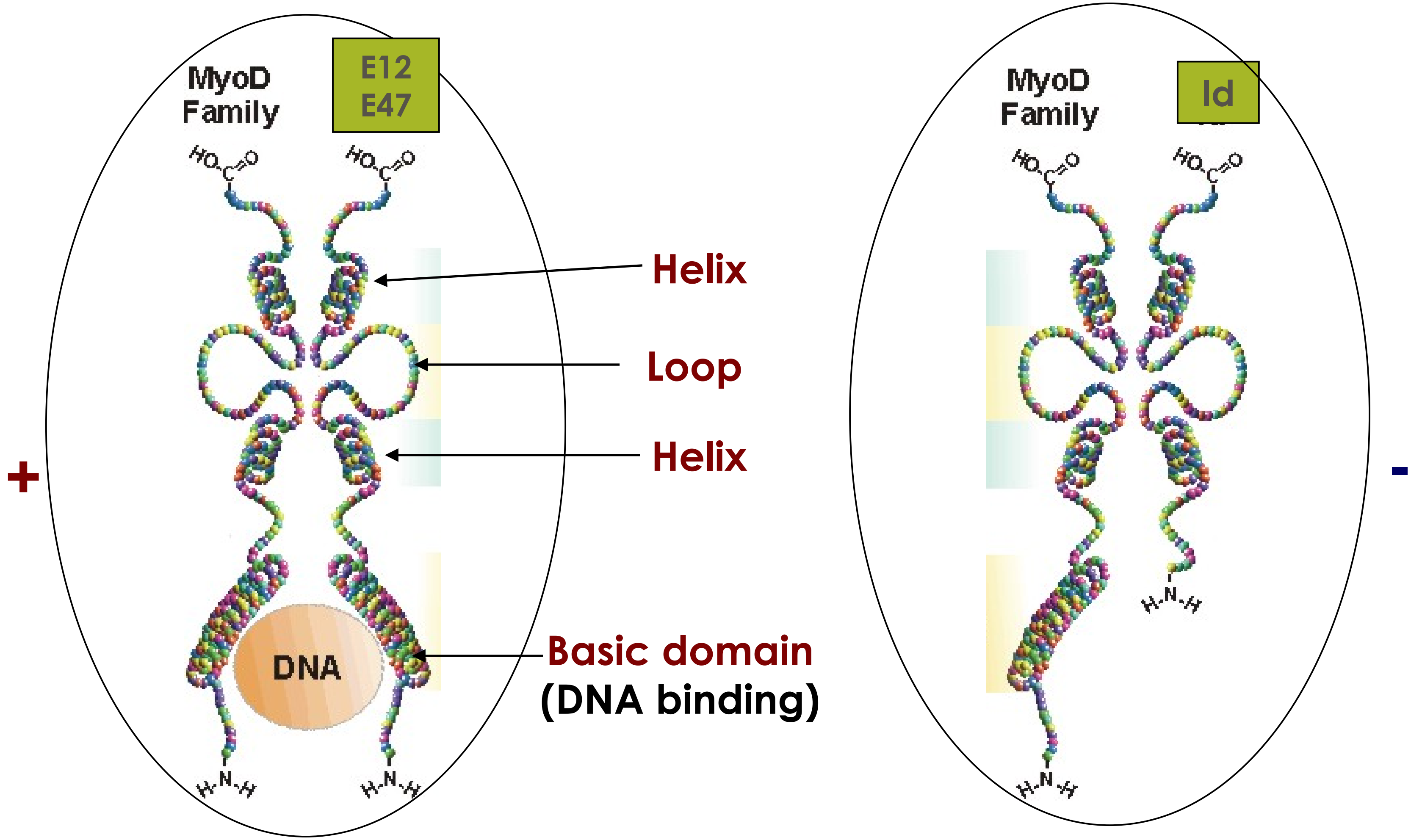
Identification



Identification of MyoD1, myogenin, Myf5, MRF4

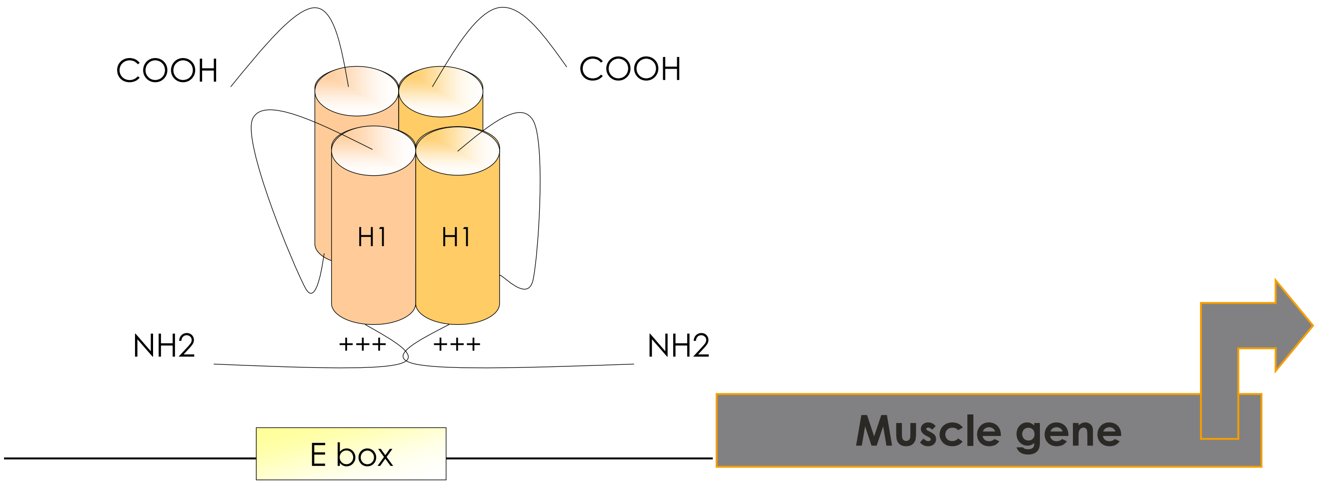


Structure



Basic Helix-Loop-Helix (bHLH) Transcription factors
Heterodimerization with bHLH proteins

Activity



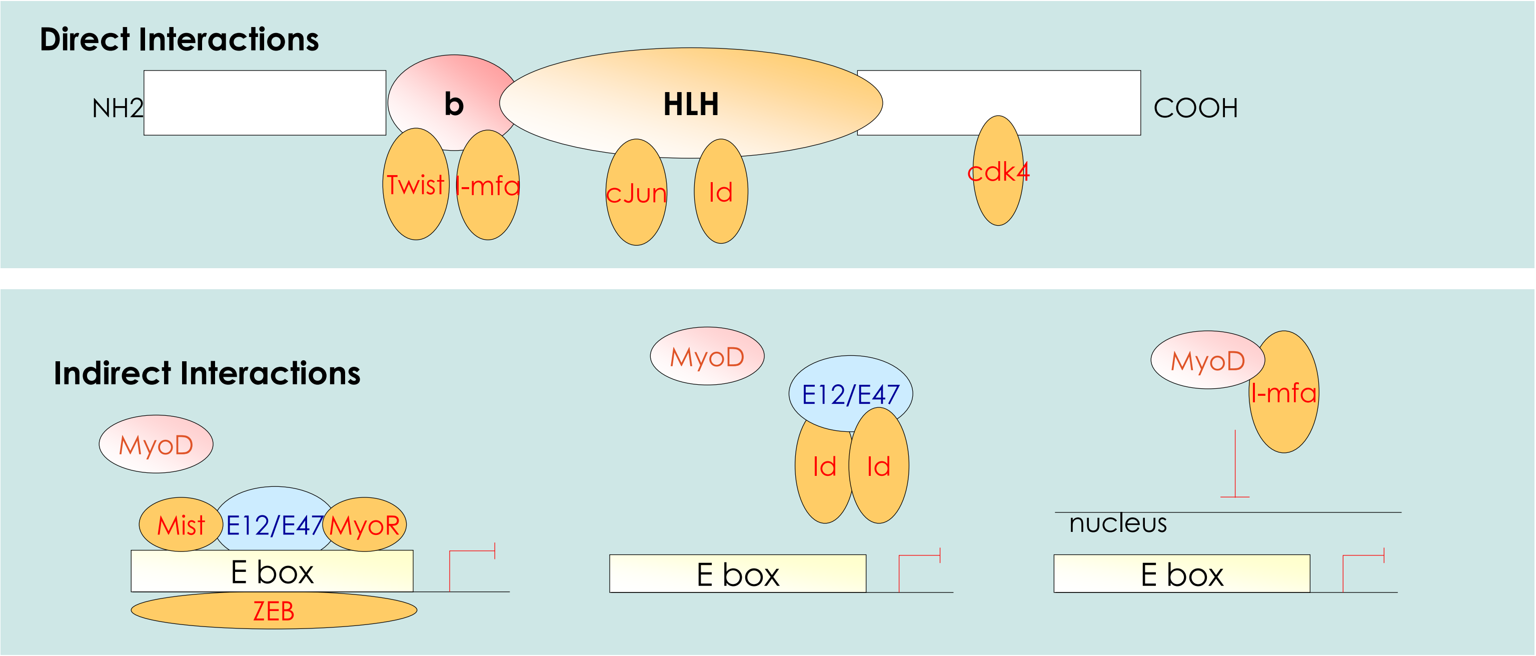
- **Transcriptional activity**
- **Bind to consensus E-box sequence (CANNTG): Found in promoters of many muscle-specific genes**
- **Autoregulatory loop**

Regulation

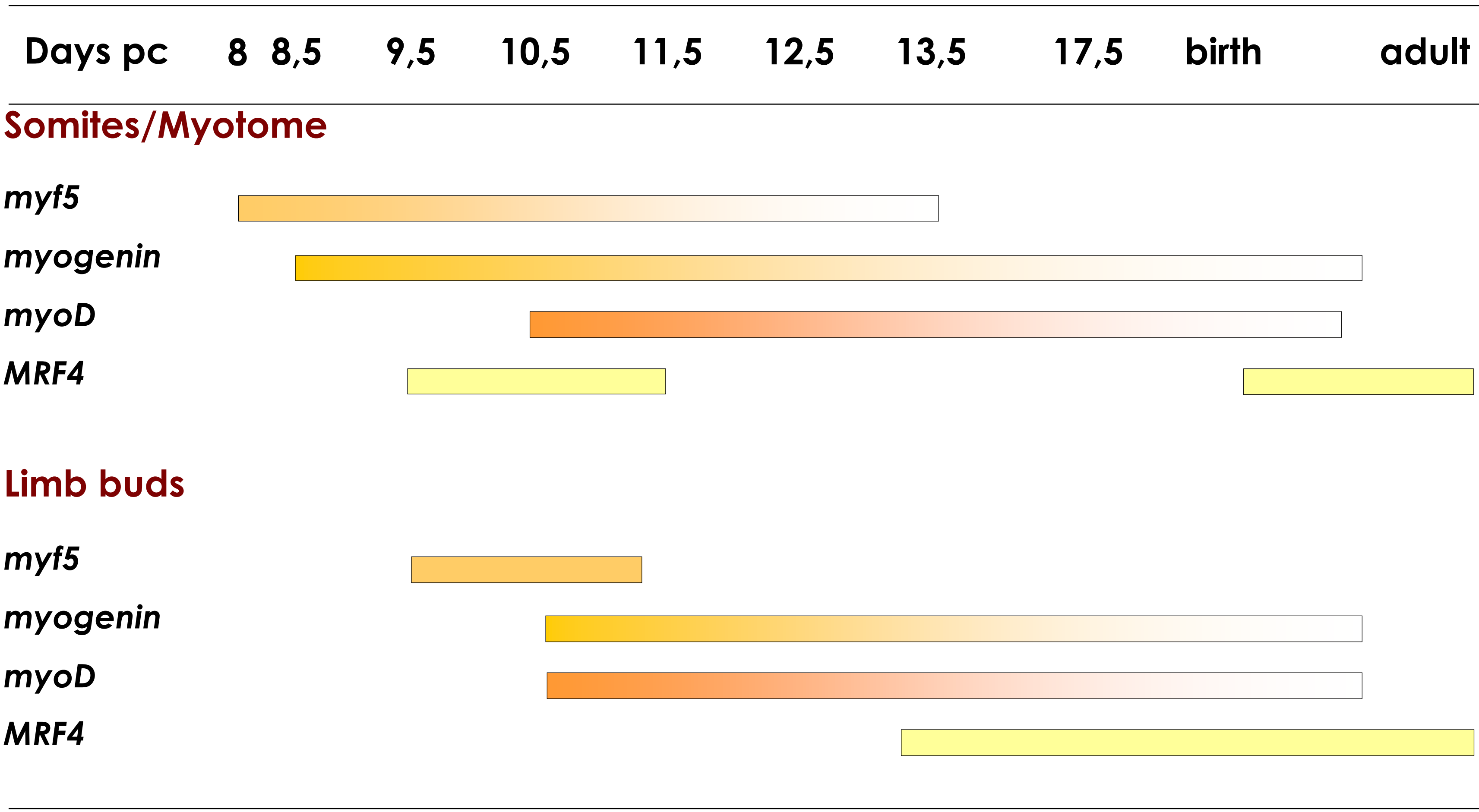
By positive partners

- E12/E47
- MEF2 family members (muscle enhancer factors: MEF2A, b, c, d)
- MLP (Muscle LIM Protein)

By negative partners



Expression

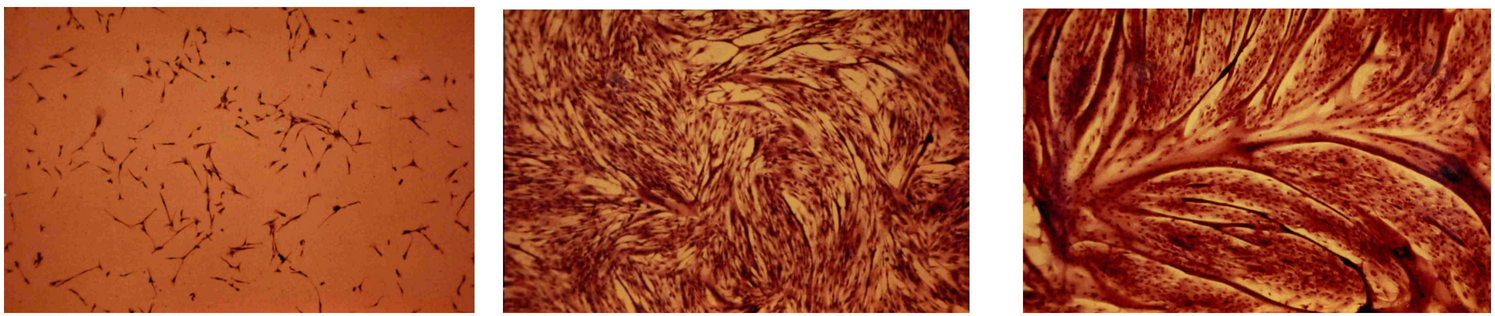


<u>Sequential</u> expression during mouse development (*in situ hybridization*).

According to Buckingham, 1992

Expression in vitro

Proliferation **Fusion** **Differentiation**



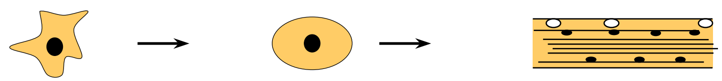
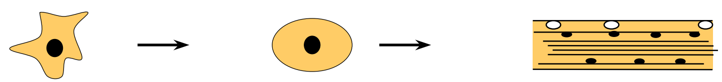
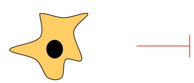
Myf5, MyoD ——————·····················→

Myogenin ——————————→

MRF4 ————→

Function

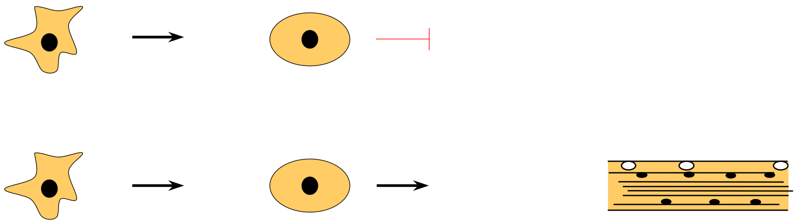
Invalidation experiments (knock-out)

Genotype	Somitic cell	Myoblasts	Myotubes	References
myf5-/-				Braun *et al.*, 1992
myoD-/-				Rudnicki *et al.*, 1992
myoD-/- et myf5-/-				Rudnicki *et al.*, 1993

- **Myf5 and MyoD: myogenic <u>induction</u> program (Determination)**
- **<u>Partially redundant</u> functions**
- **Myf5: epaxial muscle ; MyoD: hypaxial muscle**

Function

Gene-targeting experiments (knock-out)



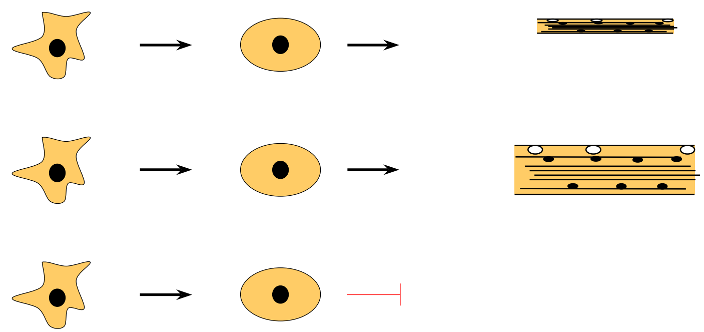
Genotype	Somitic cell	Myoblasts	Myotubes	References
myogenin-/-				Hasty *et al.*, 1993 Nabeshima *et al.*, 1993
MRF4-/-				Braun et Arnold, 1995 Patapoutian *et al.*, 1995 Zhang *et al.*, 1995

- **myogenin: crucial role in differentiation**
- **Compensation of MRF4 loss by myogenin ?**

Function

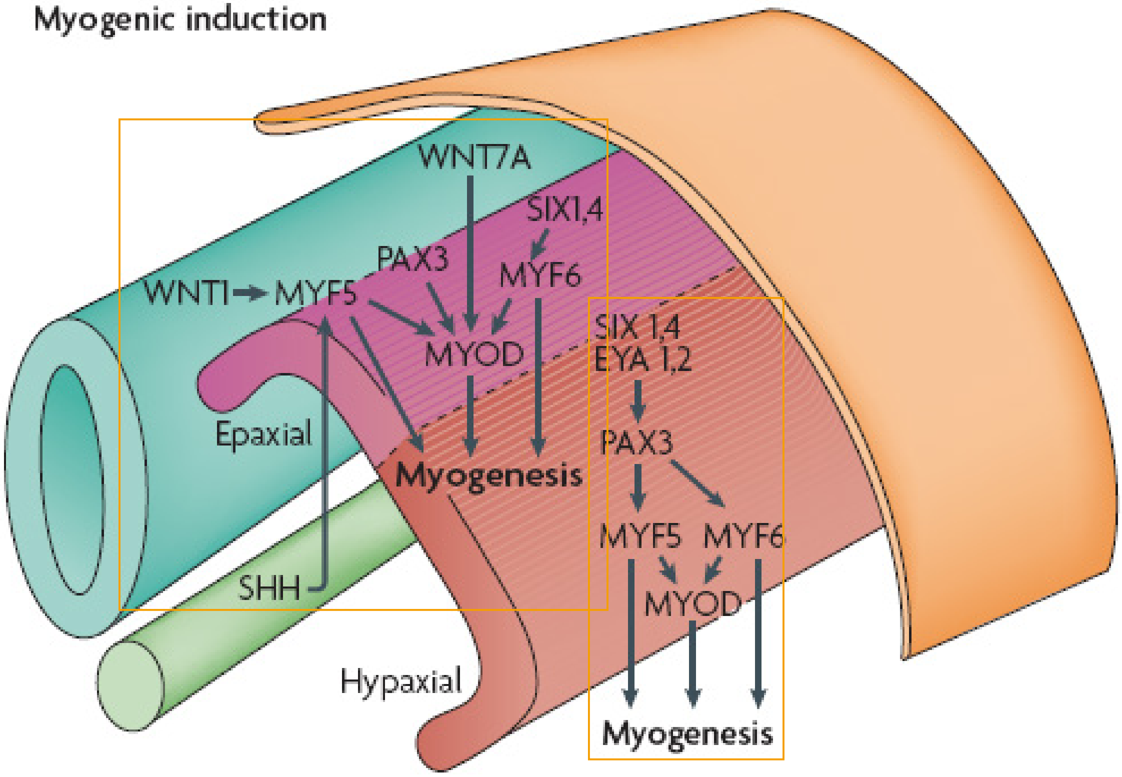
Knock-in experiments (gene x replaces gene y)

Genotype	Somitic cell	Myoblasts	Myotubes	References
myogenin KI myf5				Wang *et al*., 1996
MRF4 KI myogenin				Zhu et Miller, 1997
myogenin KI myf5 myogenin -/-				Wang et Jaenisch, 1997



- <u>myogenin:</u> role <u>downstream of Myf5 and MyoD</u>
- Mrf4 can <u>compensate</u> for the absence of myogenin
- importance of spatiotemporal expression

Regulation of myogenic induction



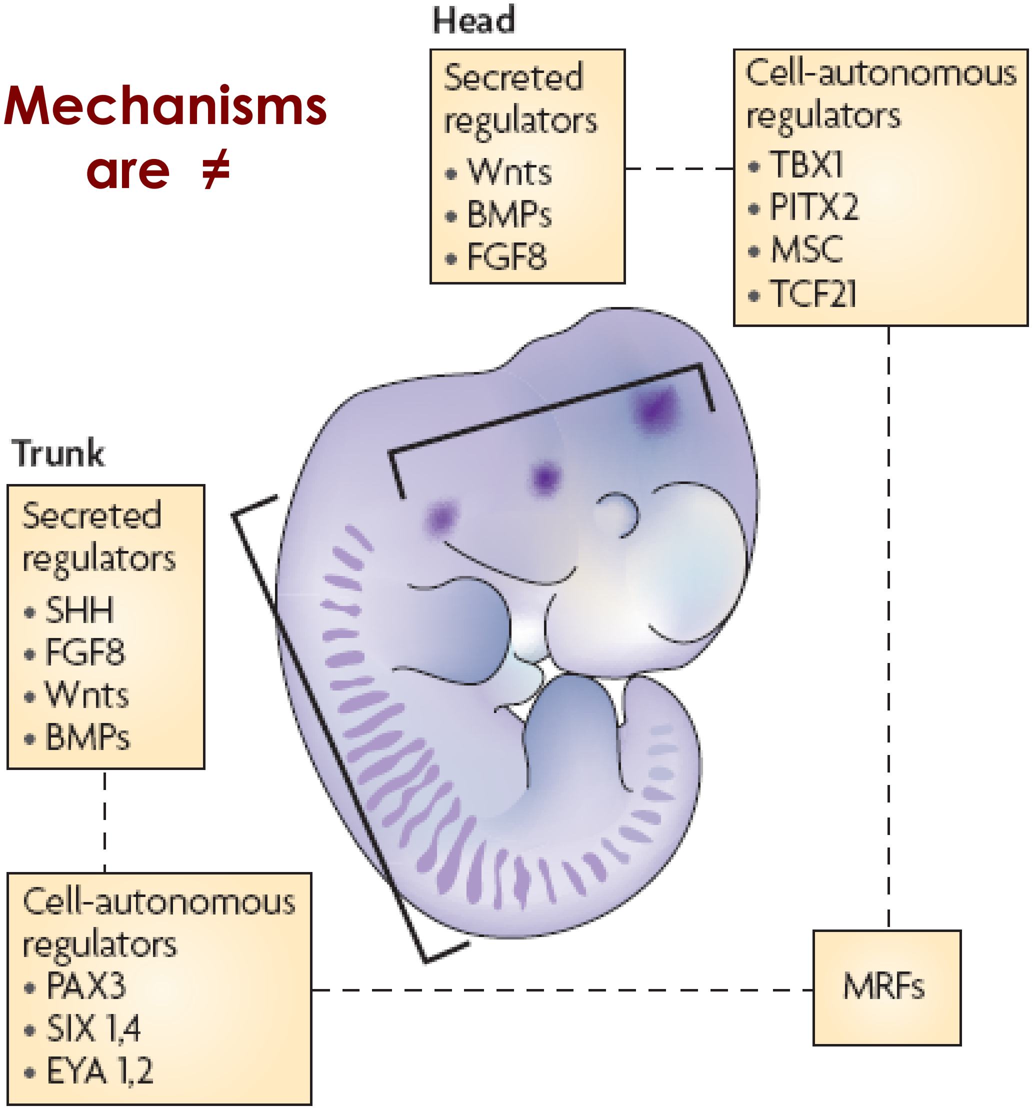
≠ muscle in epaxial and hypaxial muscle

Bryson-Richardson and Currie, 2008

Features head / trunk

Mechanisms are ≠

~~Pax3~~

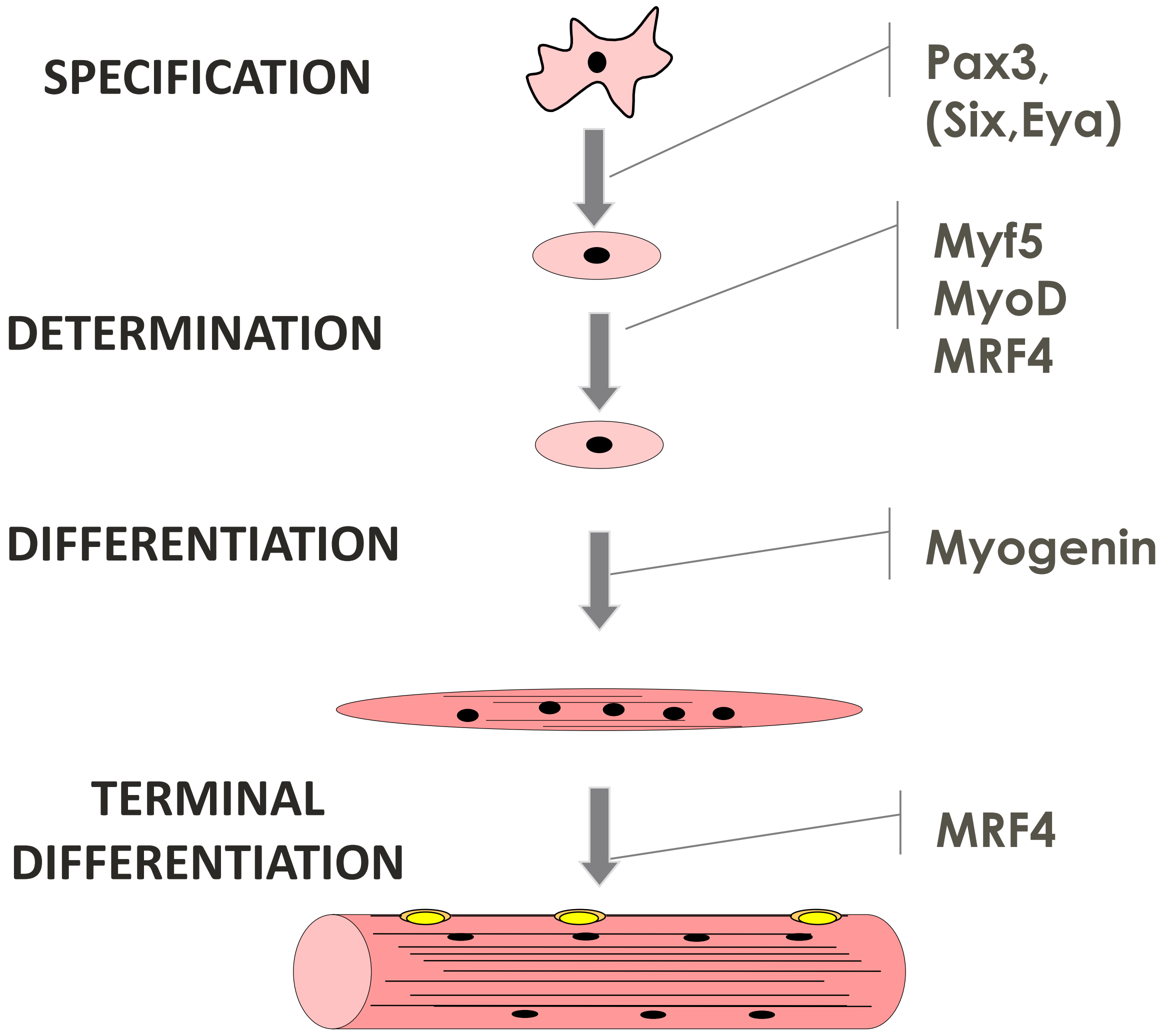


Bryson-Richardson and Currie, 2008; Tzahor, 2009

Myogenic lineages

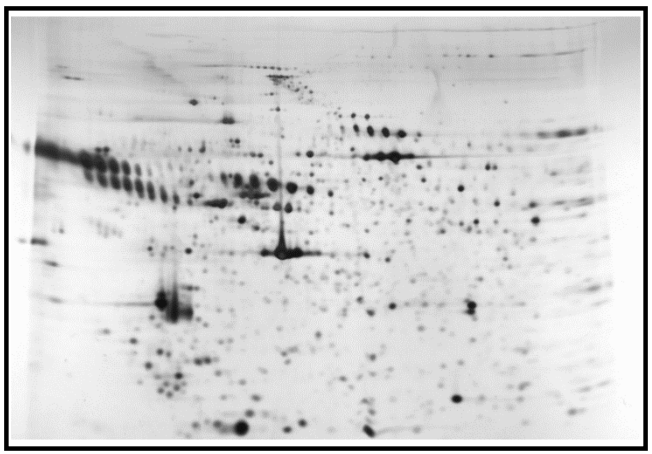
	Somitic dermomyotome	Progenitor cells (pax3/pax7)		
Muscle precursors	Myotomal cells	Embryonic myoblasts	Fetal myoblasts	Satellite cells
Muscle cells	Myoblasts (myotome)	Primary fibres	Secondary fibres	Satellite cells
		Myogenesis		*Fetal and postnatal growth, regeneration*

Take-home message

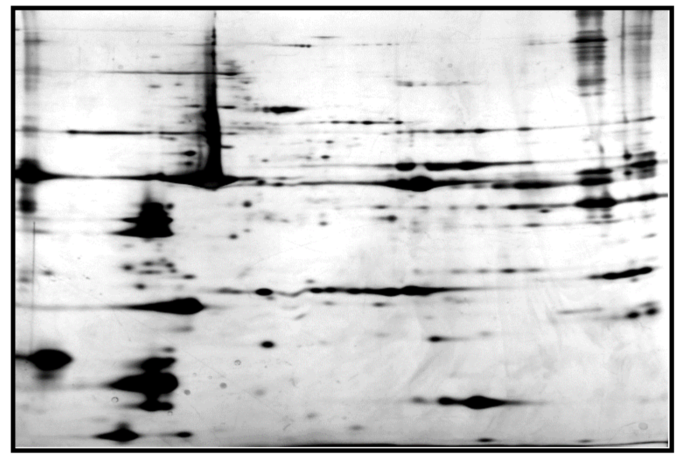


2-FETAL MUSCLE DEVELOPMENT

Expression profiles during myogenesis



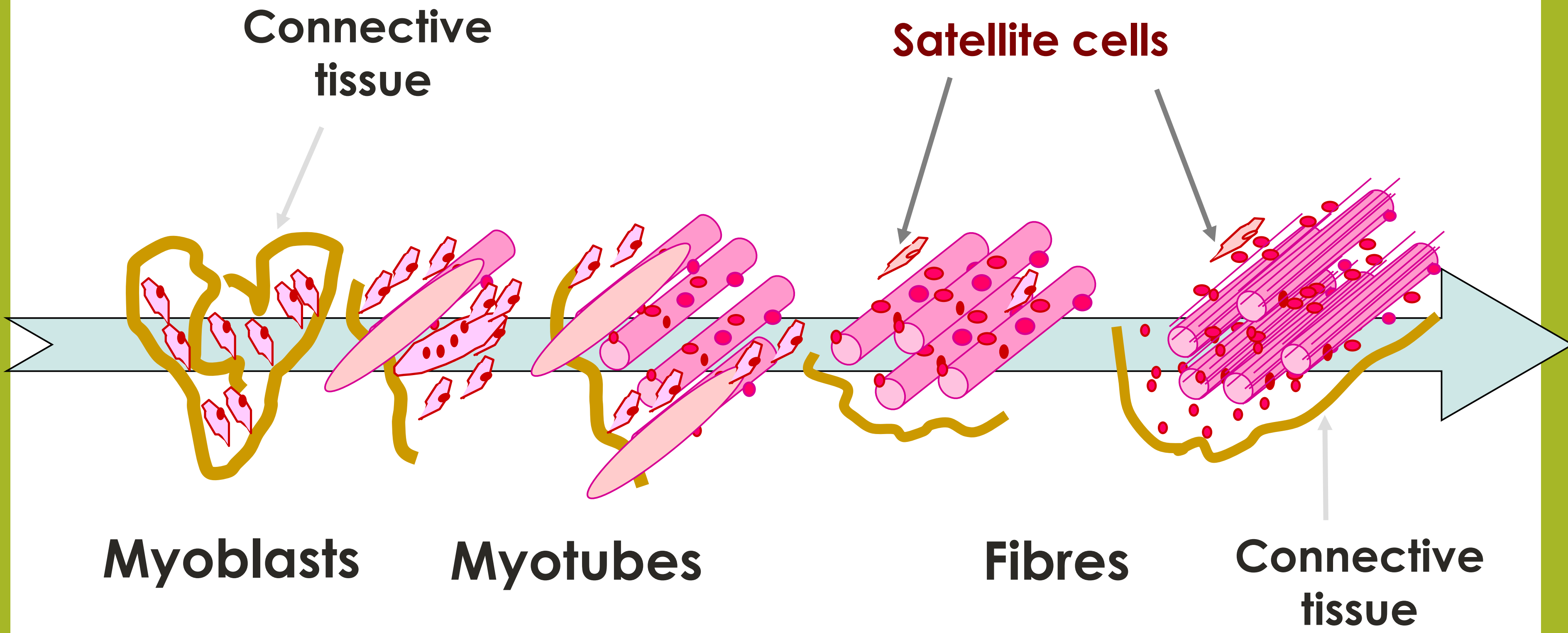
Semitendinosus muscle
(**D110 pc**)



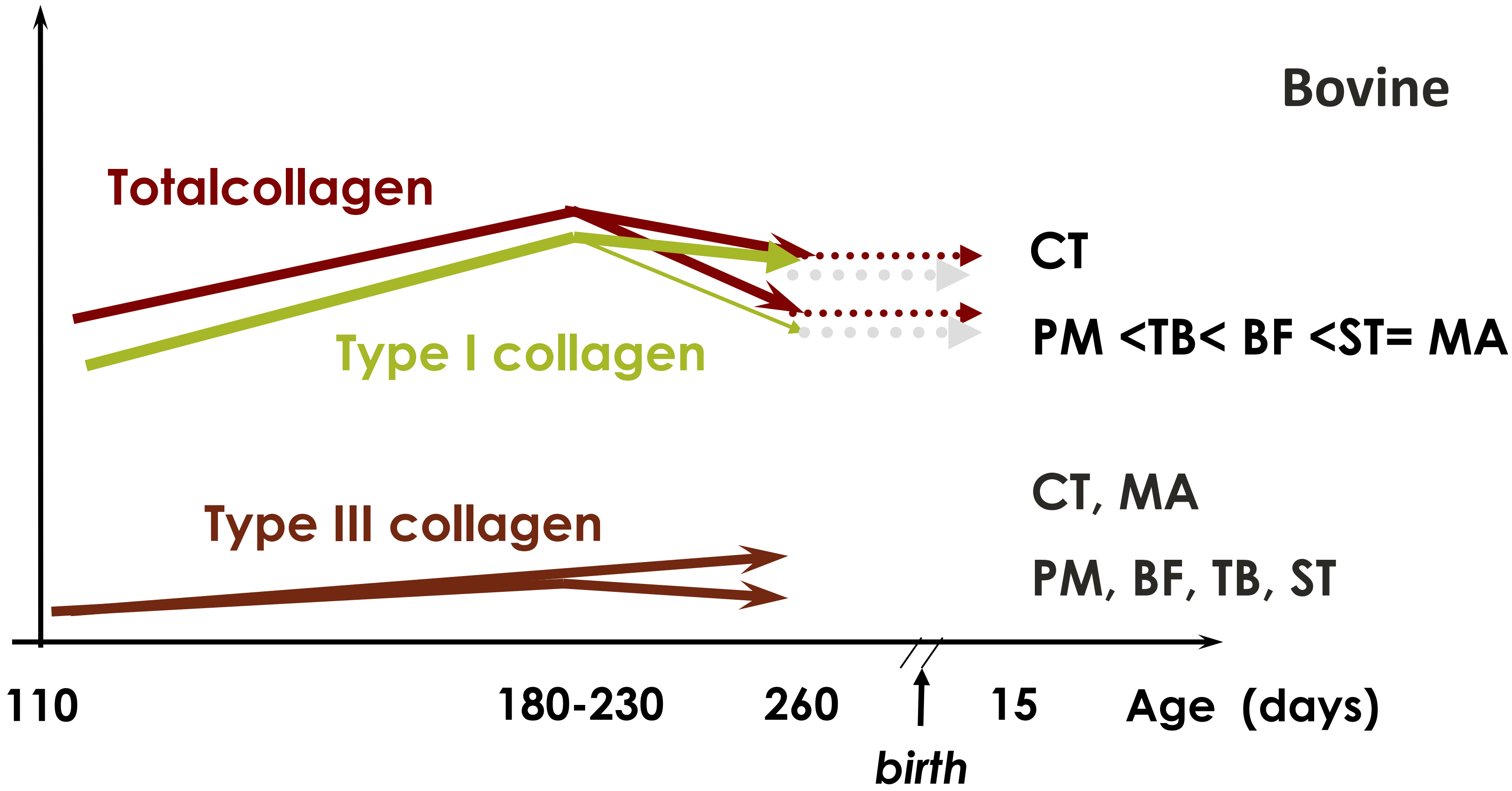
Semitendinosus muscle
(**Adult**)

The protein profile and transcript profile are specific to the developmental stage and the type of muscle.

Histogenesis of the muscle



Muscle connective tissue

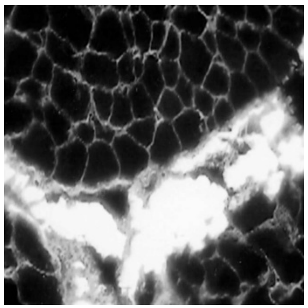


The collagen content increases during the development of fibres and decreases during their growth and differentiation.

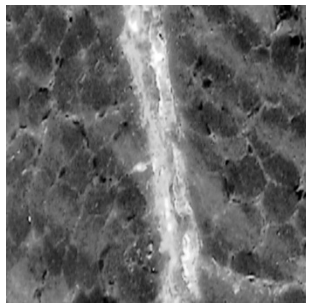
Muscle connective tissue

Collagen isoforms at the end of fetal life.

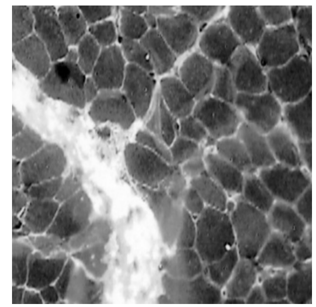
I



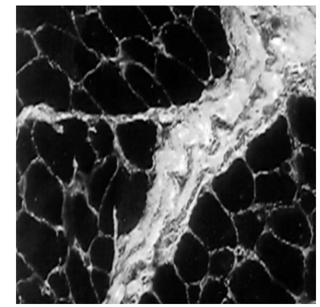
III



V

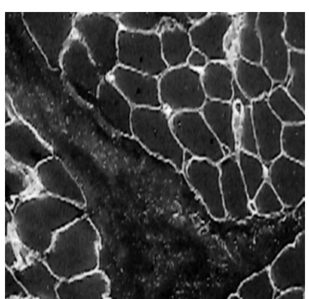


VI

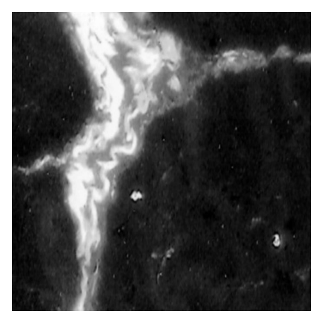


Semitendinosus Muscle (D260 pc) (250X)

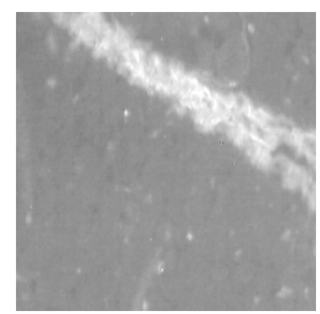
IV



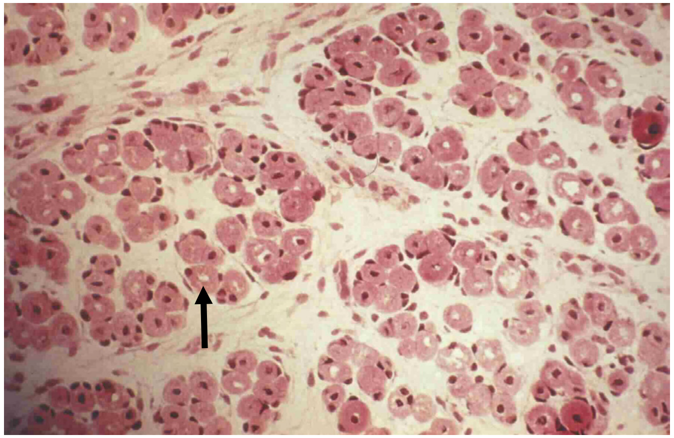
XII



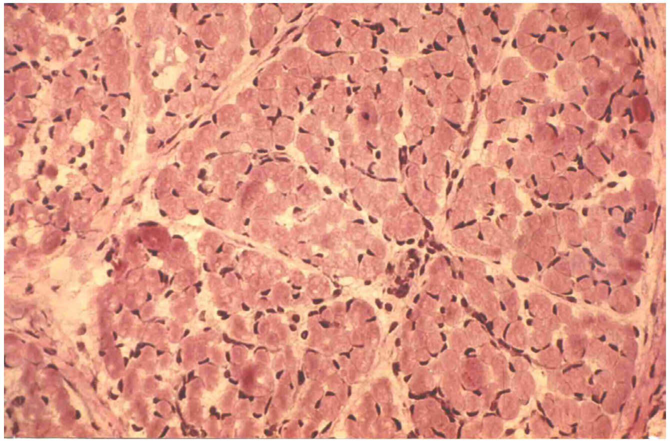
XIV



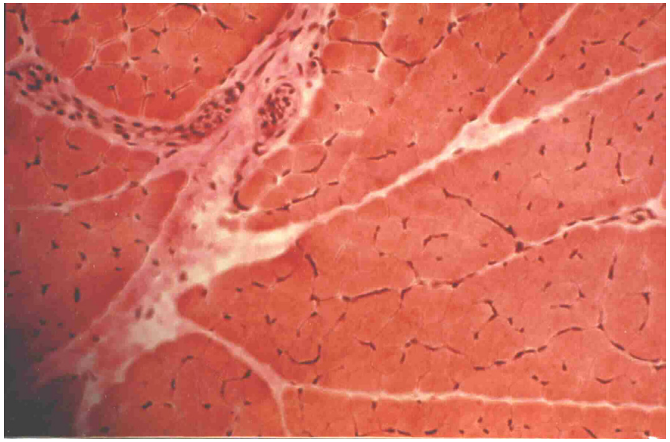
Myogenesis in cattle



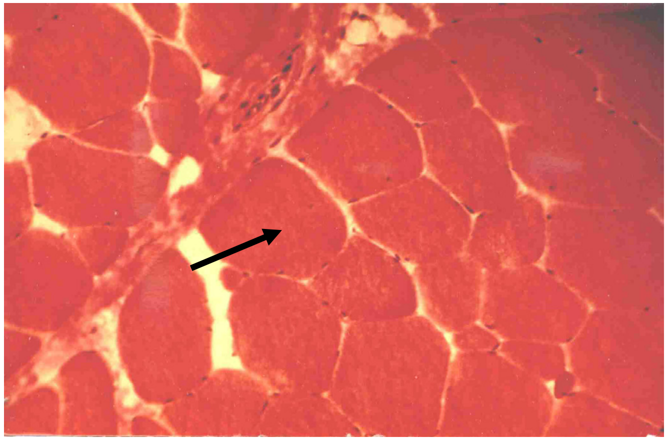
1st trimester



2nd trimester

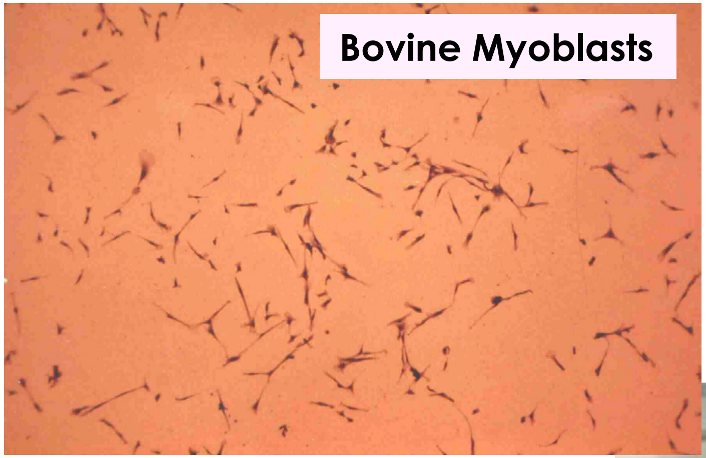


3rd trimester

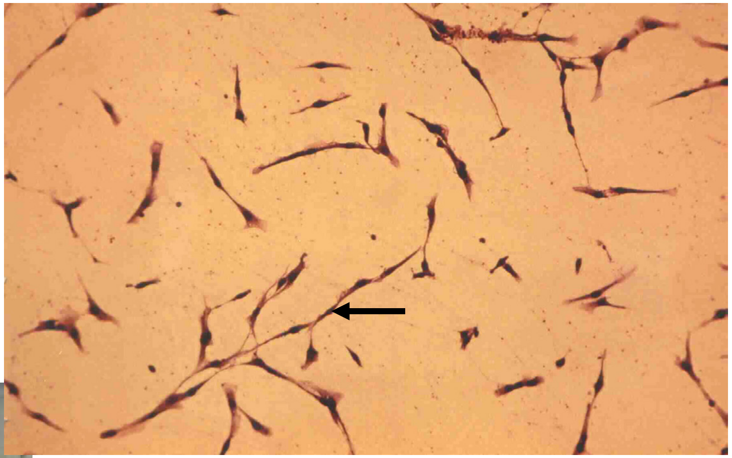


Adult

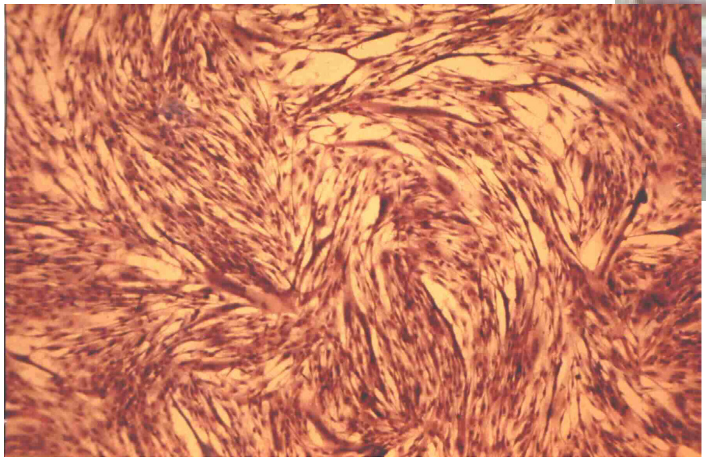
Myogenesis in vitro



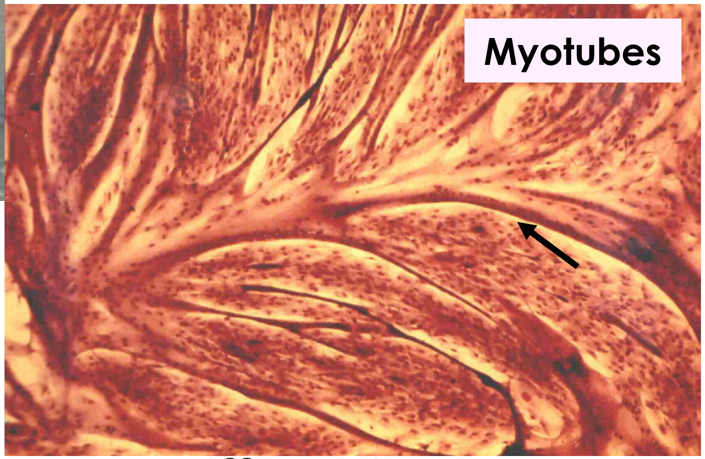
Proliferation



Alignment

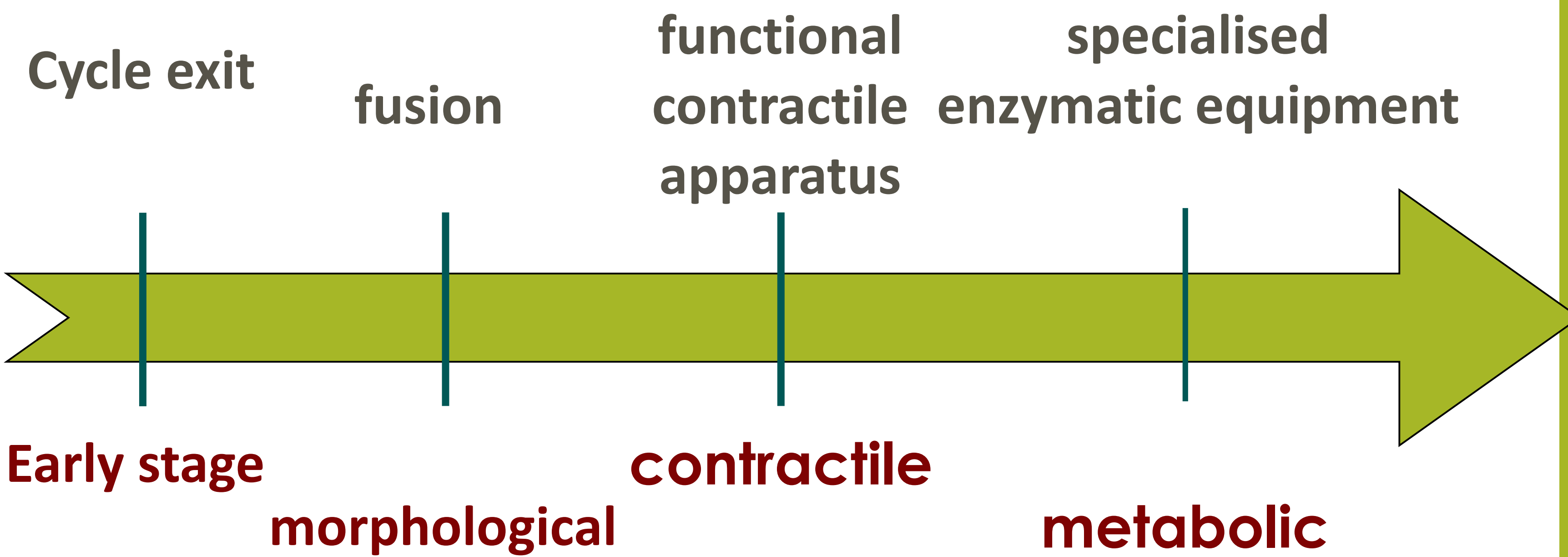


Fusion

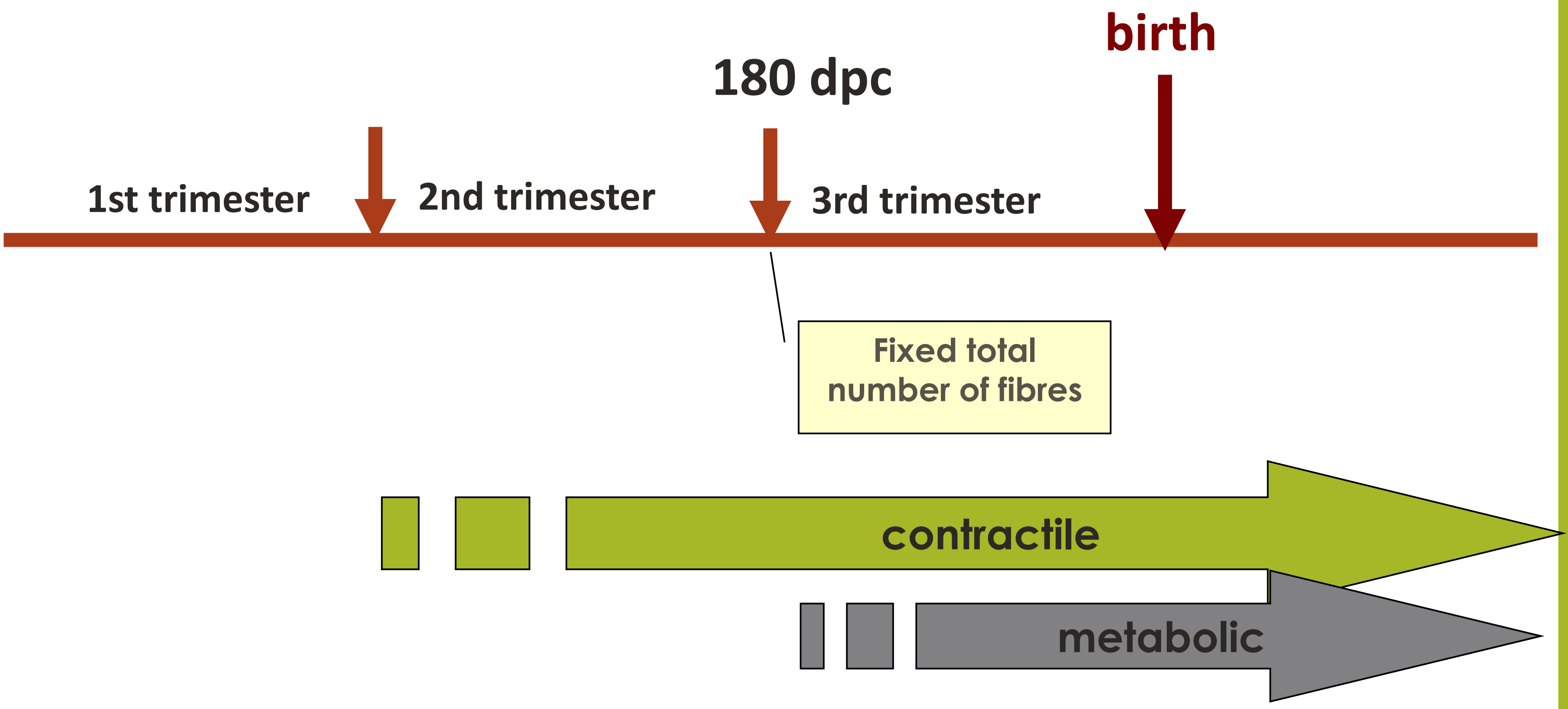


Differentiation

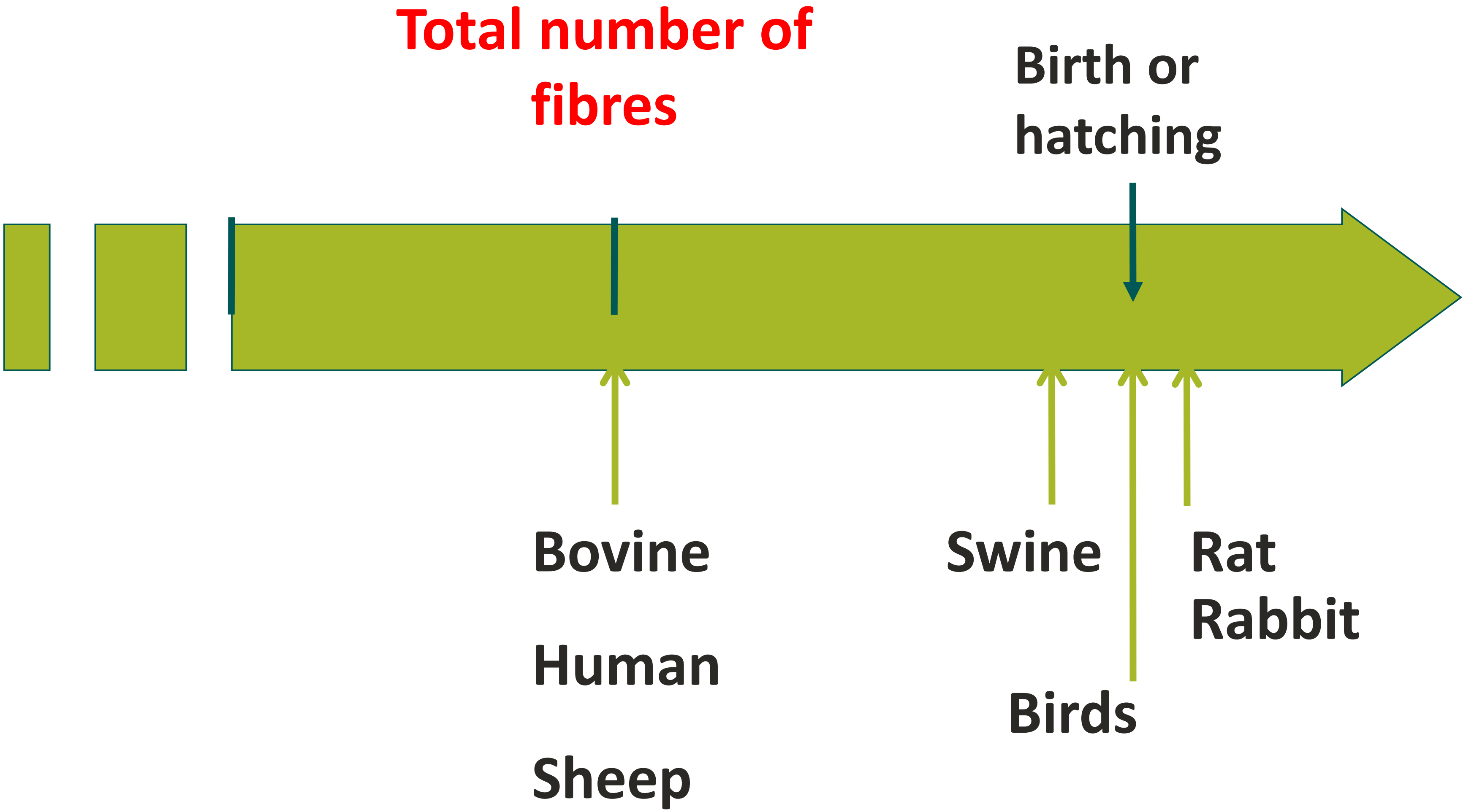
Stages of differentiation



Key stages in cattle



Precocity of myogenesis in cattle



Different generations of muscle fibres

40% 66% 78%

Total number of fibres

180 days

1st generation	30 dpc	I	I	
2nd generation	90 dpc		IIX	IIA X
3rd generation	110 dpc		IIA	IIC, I

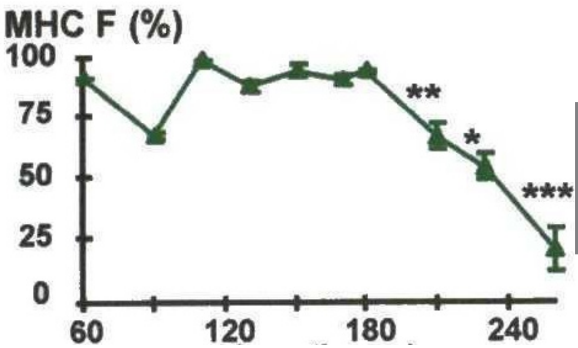
MyHC dev. 210 d

MyHC 2a 210 d

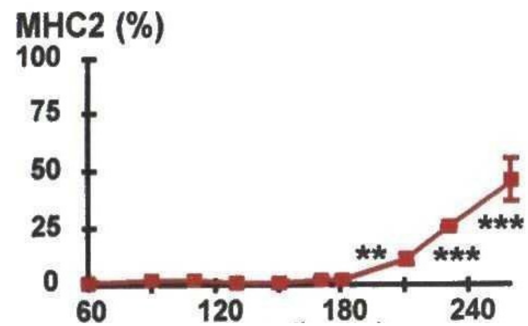
MyHC 2x 210 j

Gagnière et al, 1999; Duris, 1999

Precocity of muscle development in cattle

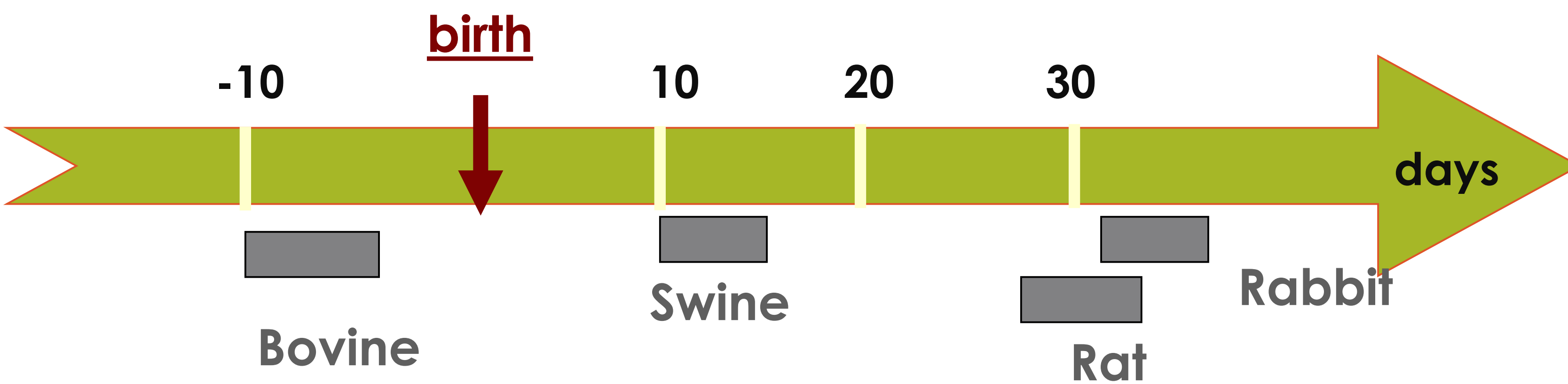


Developmental switch



Disappearance of the fetal MyHC

Appearance of adult fast MyHC



cattle: Picard et al, 1994
Rabbit: Gondret et al, 1996

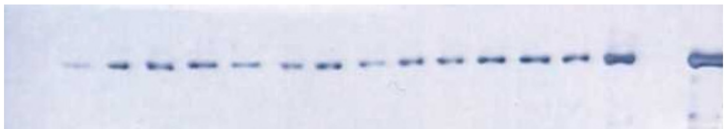
Swine: Lefaucheur et al, 1995
Rat: d 'Albis et al, 1989

Contractile proteins

MyHC

Immunoblotting

MyHC 1

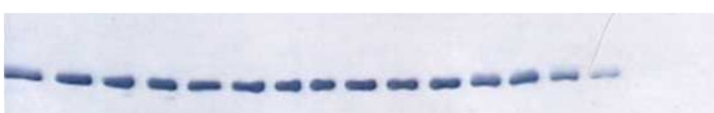


60 260 CT MA

MyHC 2

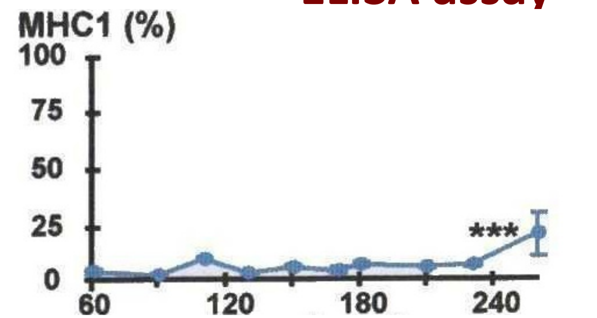
60 260 CT MA

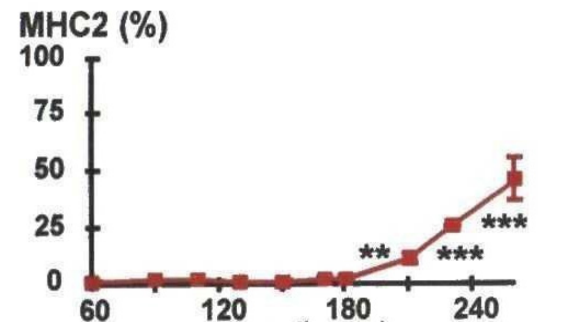
MyHC F

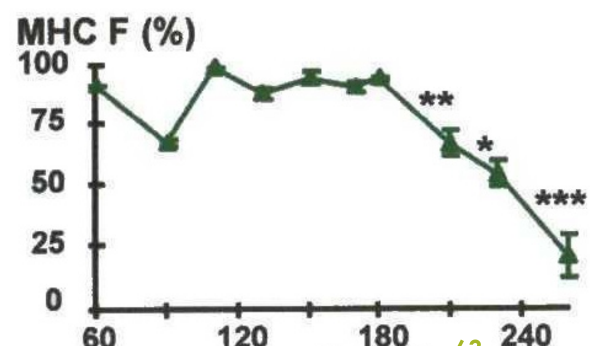


60 260 CT MA

ELISA assay

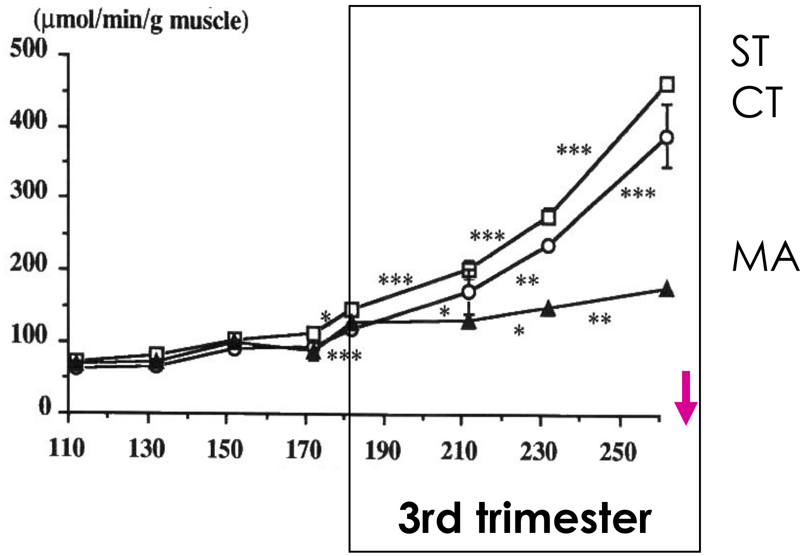






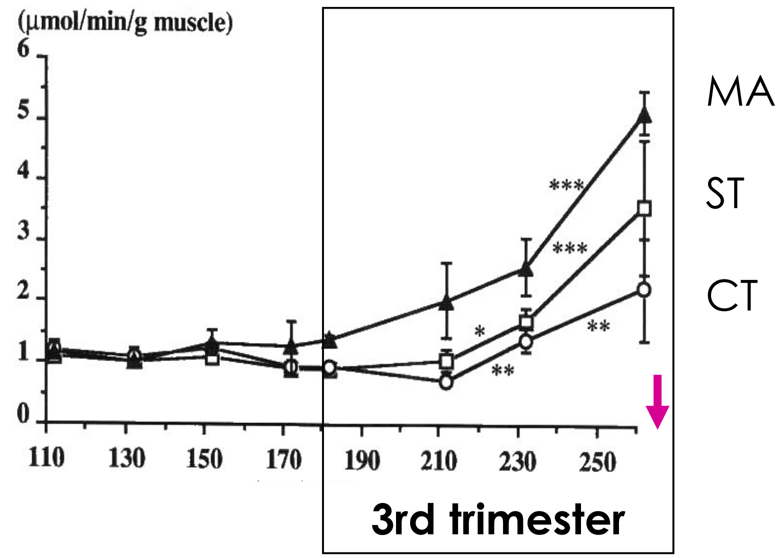
Muscle metabolism

GLYCOLYTIC



Lactate deshydrogenase

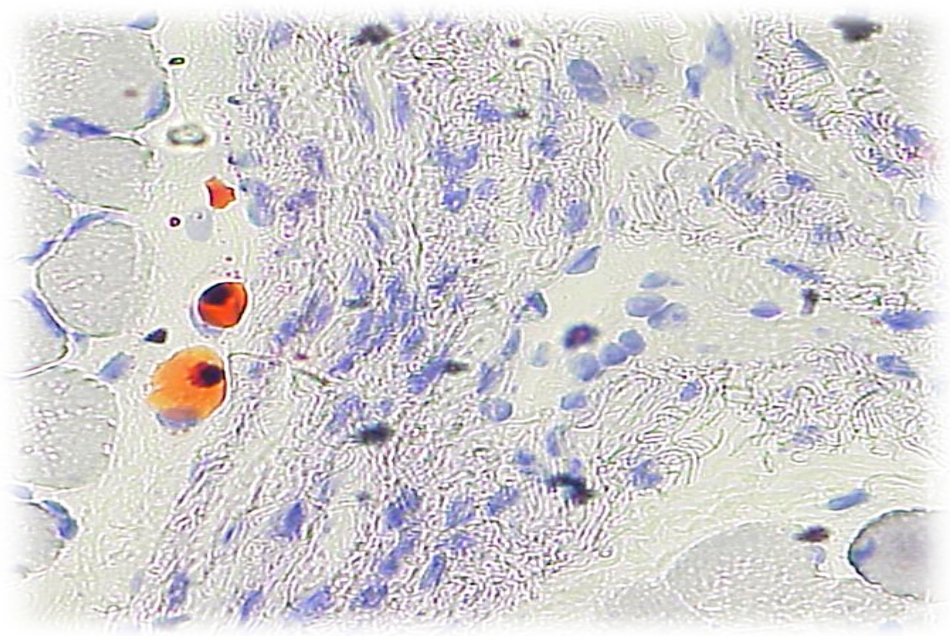
OXIDATIVE

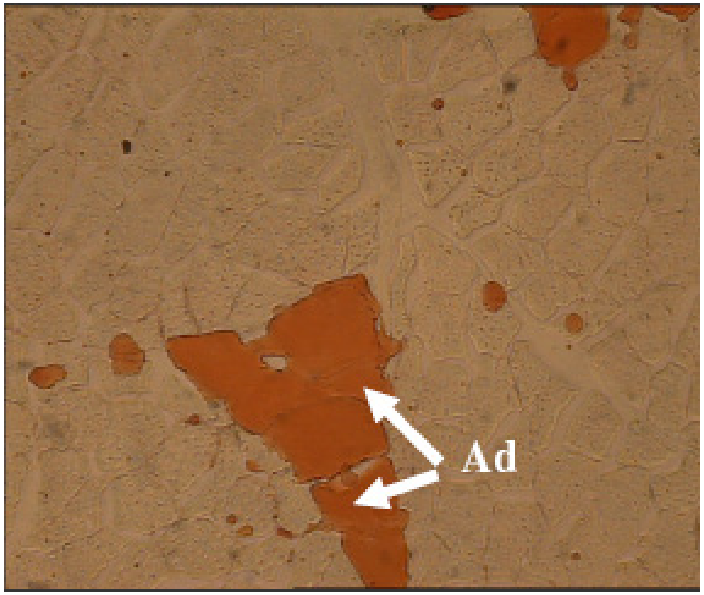


Isocitrate deshydrogenase

Cattle

Intramuscular adipose tissue





<u>Cattle</u>

ST muscle (fetus)

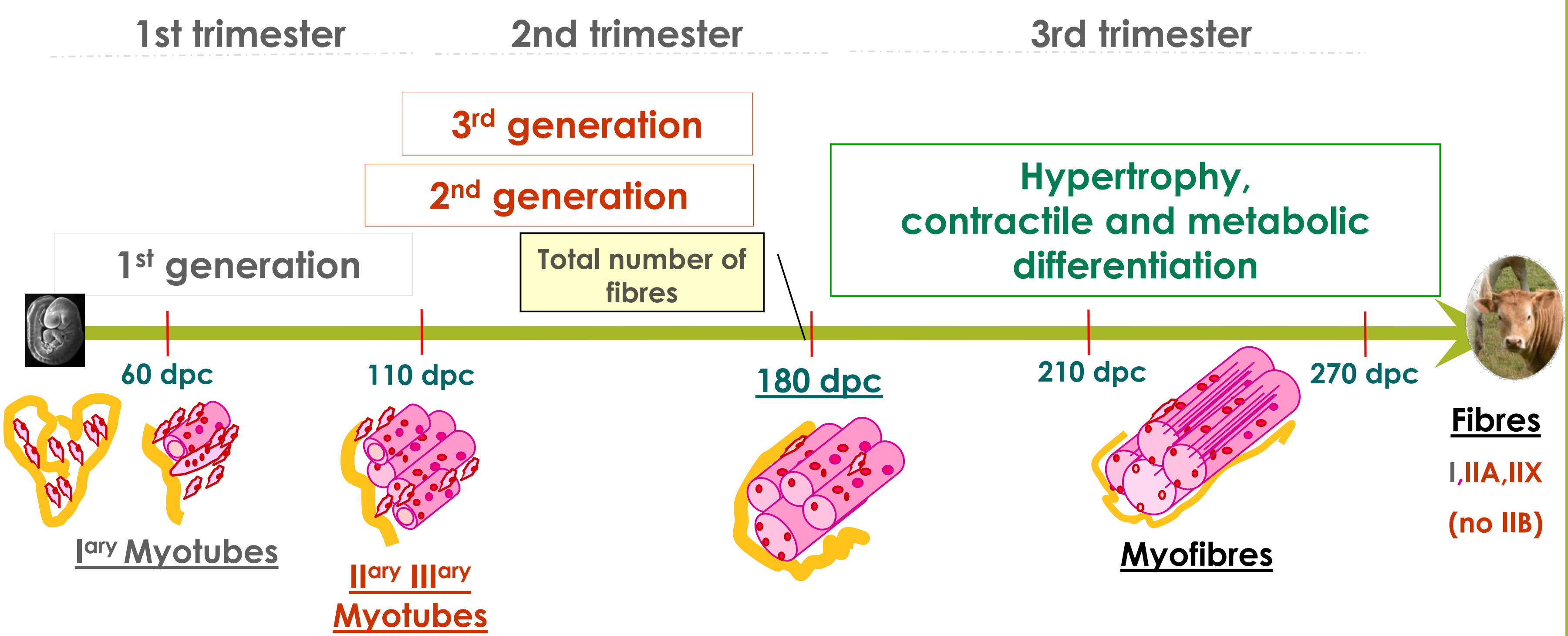
ST muscle (adult)

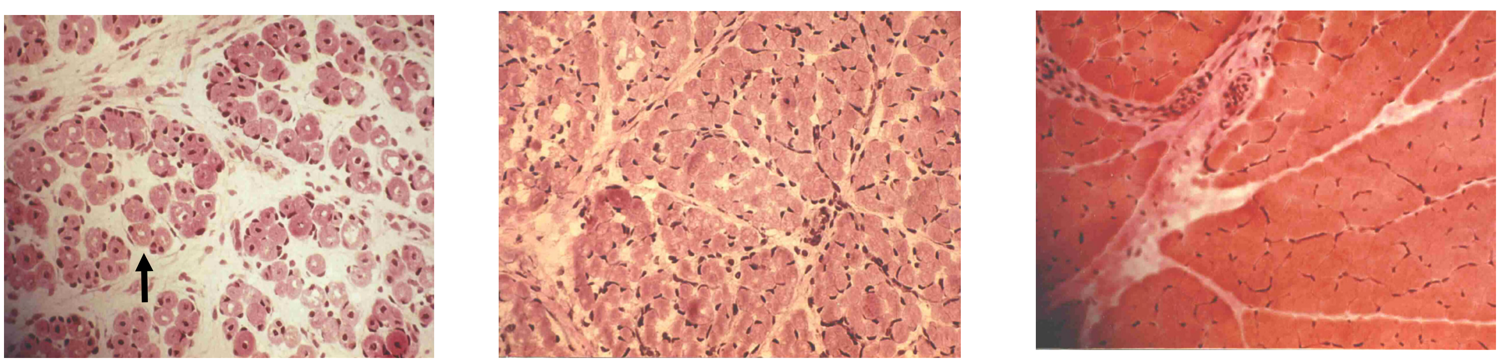
AT appears late (Late pregnancy, early postnatal life)
→ See M Bonnet lecture

Take-home message (1/2)

- Most of myogenesis occurs during fetal life (precocity)
- Three generations of fibres
- Fixed total number of fibres by the end of the second trimester of gestation
- The last third of gestation is marked by the acquisition of contractile and metabolic properties
- Postnatal myogenesis is characterized by the growth of the fibres and the plasticity of their properties

Bovine myogenesis : key stages (2/2)





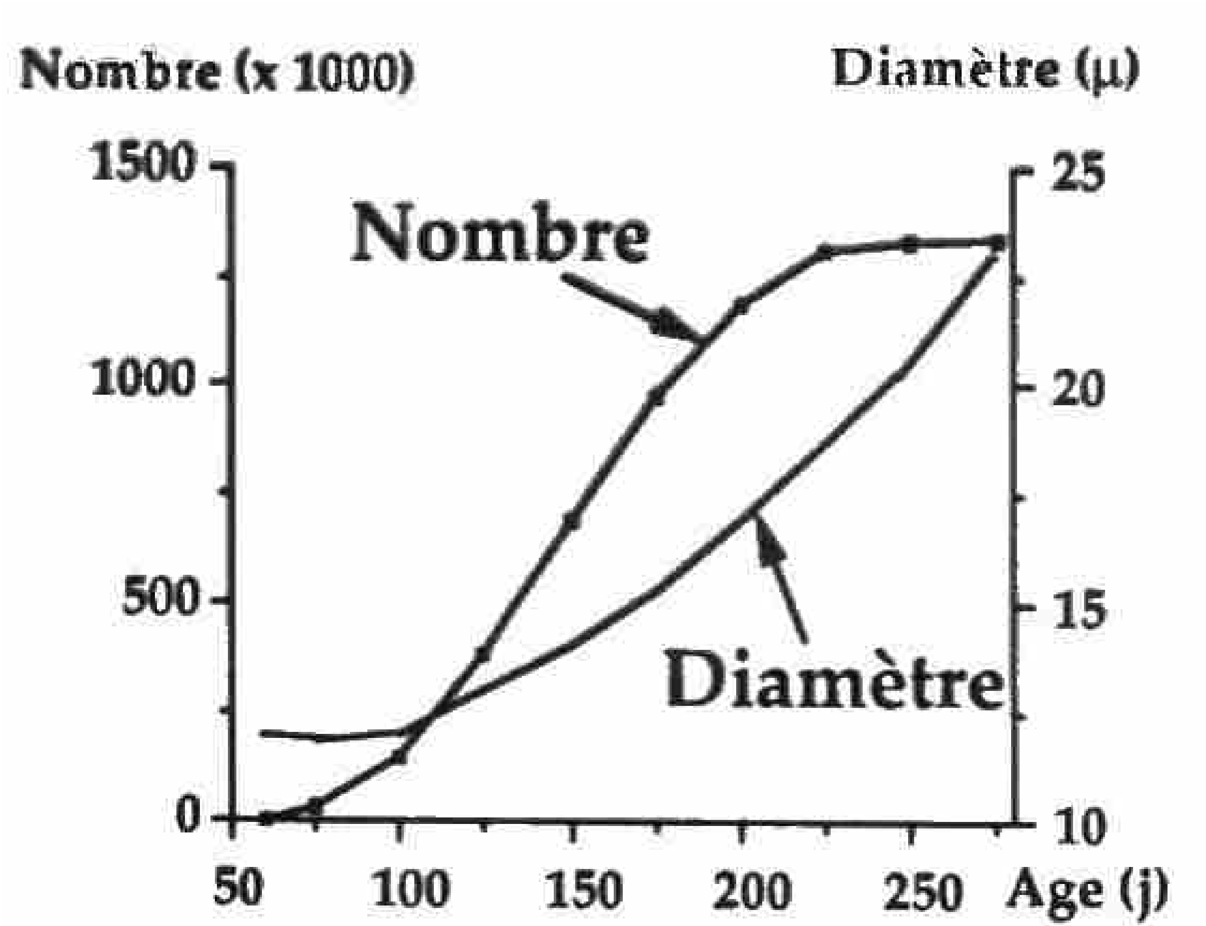
B. Picard et al, 2002

3-GROWTH

In the fetus

Muscle growth in the fetus

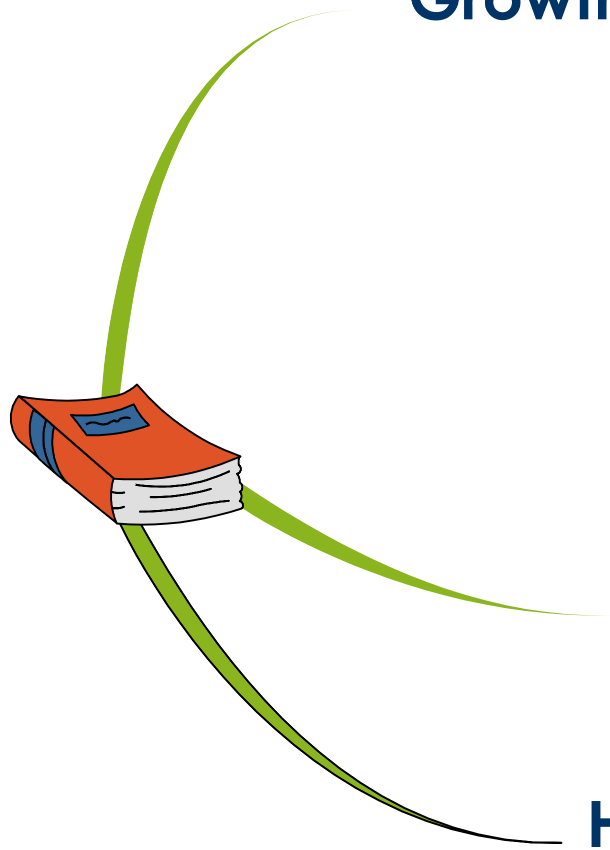
Bovine ST muscle : mass x500 between 80 and 260 dp.c



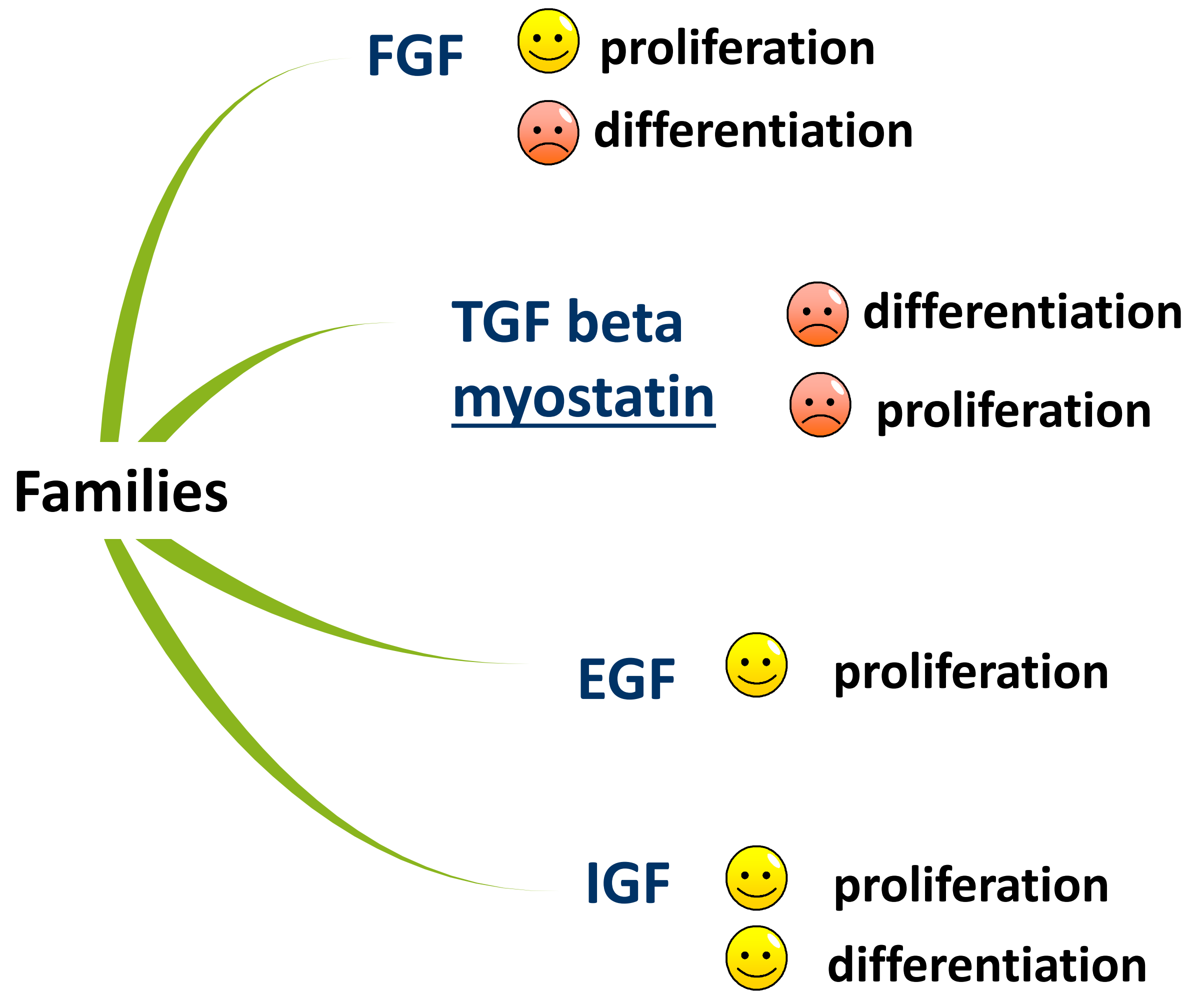
Hyperplasia
Hypertrophy

Robelin, 1990

Regulation



GROWTH FACTORS



The example of Myostatin

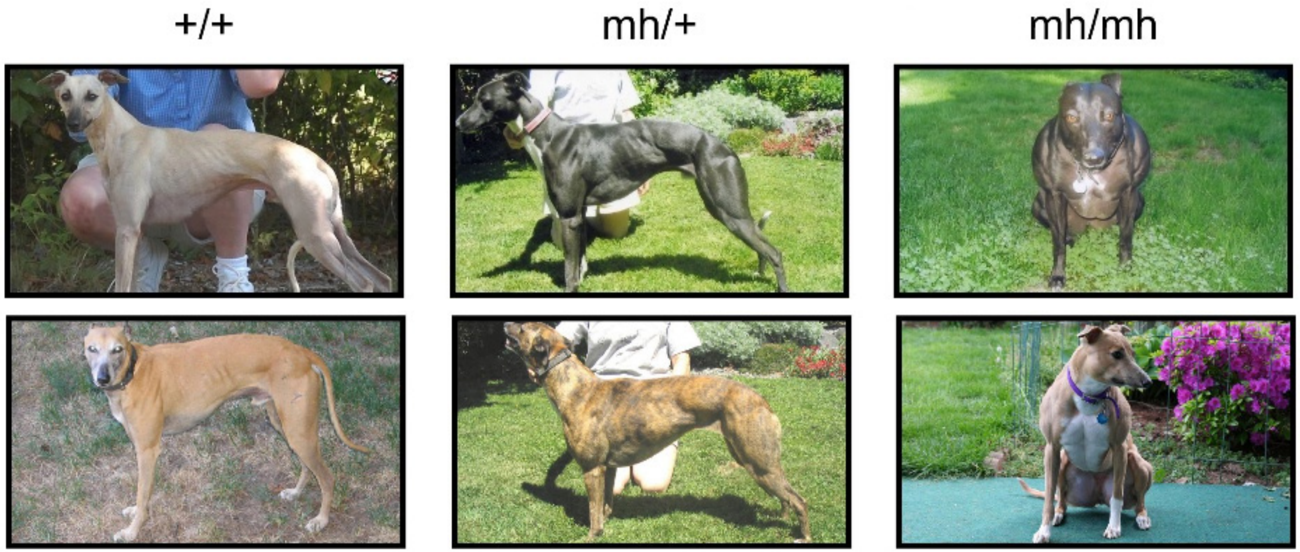
Mutation in the *myostatin* (gdf8) gene

Muscular Hypertrophy

Mice (knock-out)

Double-muscled charolais (selection for mh mutation)

Myostatin phenotypes



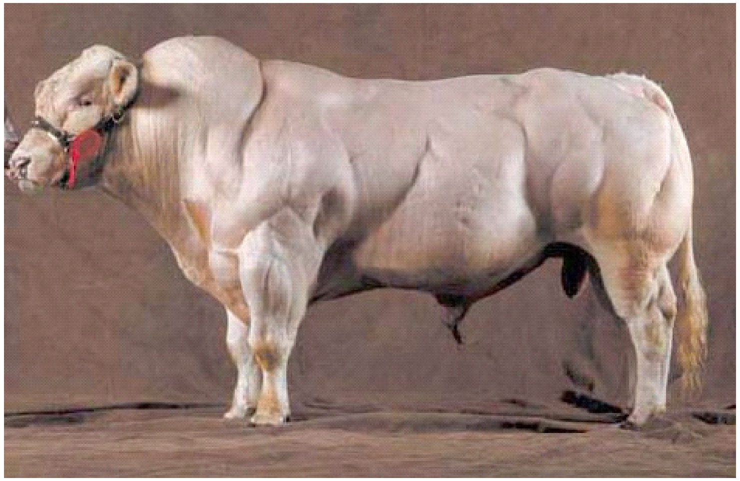
Whippet (race dog)

Human

Overgrowth of muscle tissues = hypertrophy

Double-muscled cattle

Hypermusculature (double-muscled)



- **hyperplasia**
- **fibre hypertrophy**

- **increased glycolytic fibres %**
- **decreased collagen content**
- **decreased intramuscular fat content**

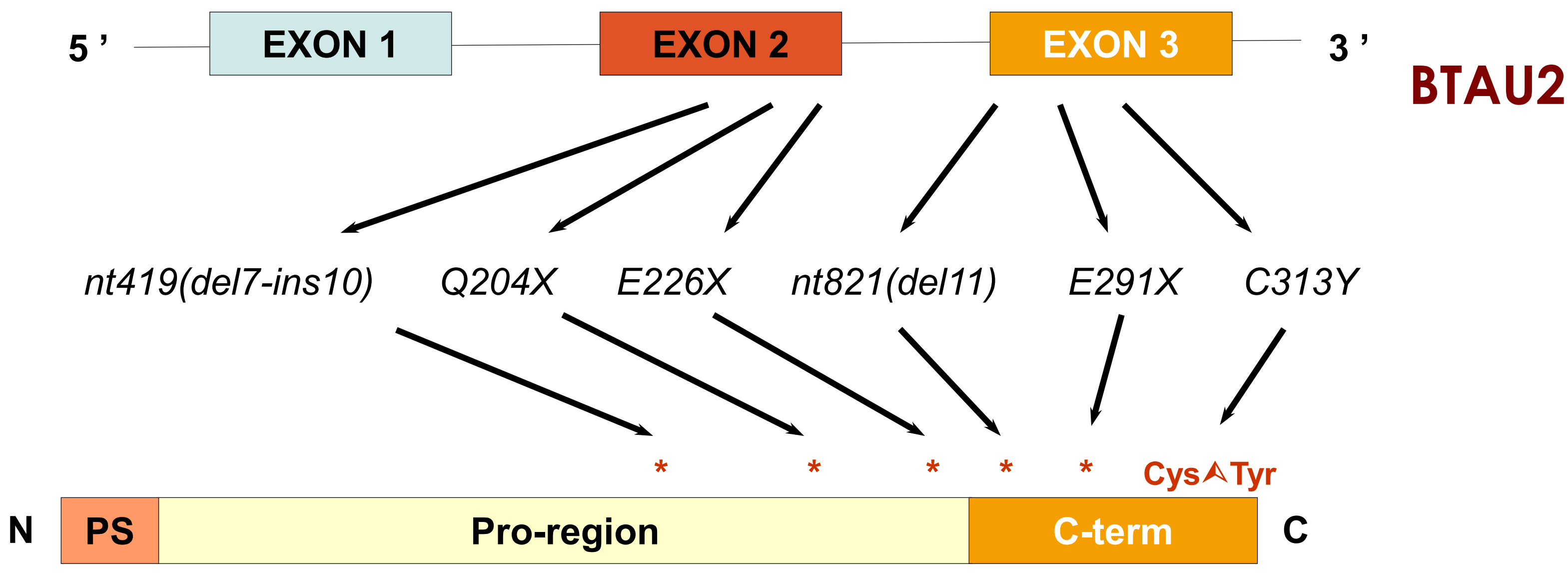
- **<u>originating in fetal life</u>**

A model to understand the mechanisms underlying muscle growth and hypertrophy

A monogenic, autosomal segregation pattern

- A gene initially suspected : *mh (BTAU2)*
- The gene encoding myostatin was found at the *mh* locus (mstn as a candidate gene)
- Loss-of-function mutations in mstn gene lead to double-muscling
- a wild type "+" allele and a recessive “-" allele, causing the double-muscled phenotype in the homozygous condition.

Mutations in cattle

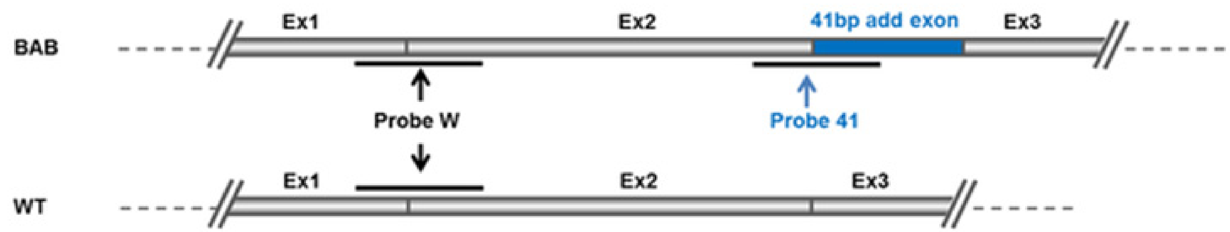


nt419(del7-ins10)	Q204X	E226X	nt821(del11)	C313Y
STOP codon	STOP codon	STOP codon	STOP codon	Cysteine loss

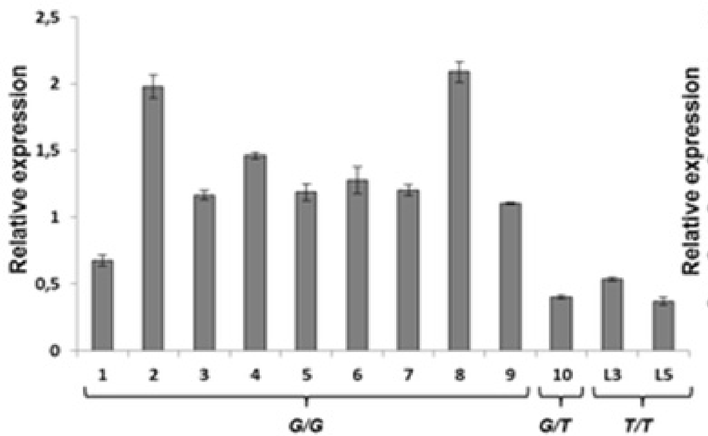
An intronic mutation was recenly identified in Blonde Aquitaine (fixed in the population):
→ aberrent transcript (Bouyer et al, 2014)

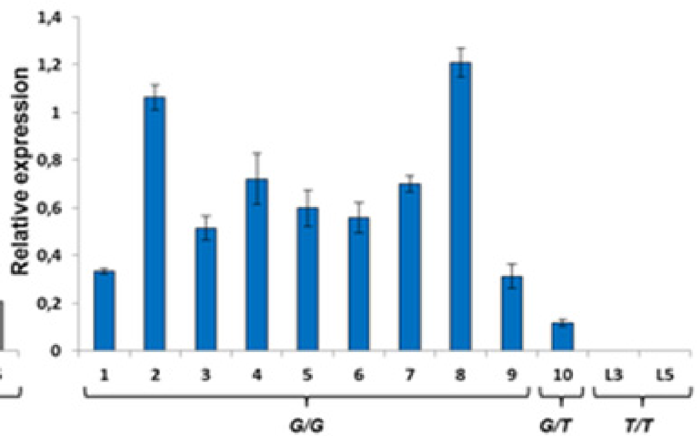
Une nouvelle mutation du gène myostatine

- Substitution **T** (3811)→ **G** (3811) dans l'intron 2 du gène de la myostatine en B Aq
- Création d'un site cryptique illégitime d'épissage → transcrit anormal



- **Allèle mutant fortement exprimé**





- **↘ myostatine fonctionnelle et phénotype hypertrophique modéré de la B Aq?**

Bouyer et al. 2014. PLoS ONE 9(5): e97399. doi:10.1371/journal.pone.0097399

Function

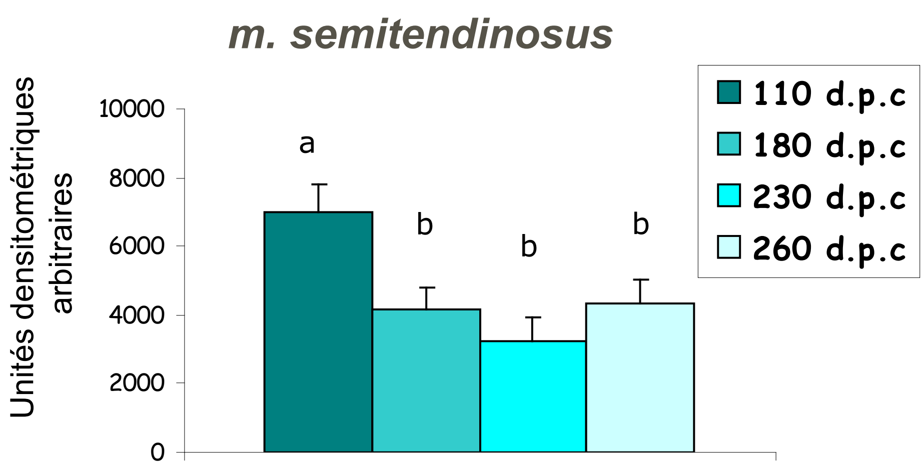
Mutations in the MSTN/gdf8 gene
⇨ reduce the production of functional myostatin
⇨ overgrowth of muscle tissue

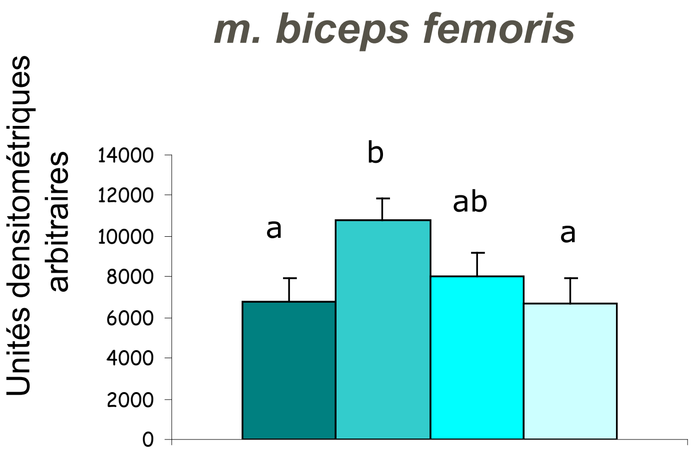
- The protein normally restrains muscle growth, ensuring that muscles do not grow too large (statin function),
- is involved in muscle mass homeostasis,
- is involved in regulation of adipogenesis

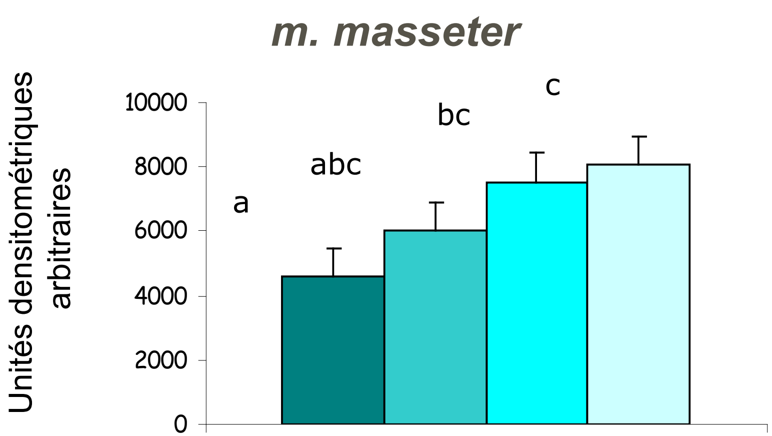
Myostatin

- Muscle-specific expression
- Regulation of expression during myogenesis
- Fibre-specific expression
- Muscle mass regulation

Expression







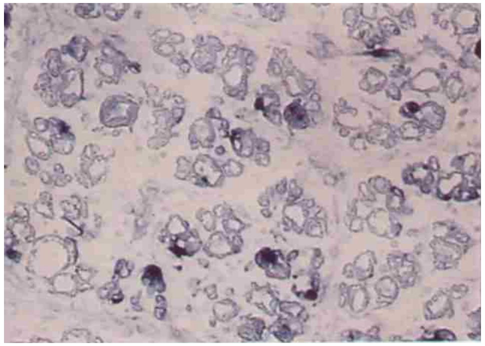
Bovine fetus

- **low expression (but > to postnatal)**
- **changes during development**
- **differentially according to muscle-type**

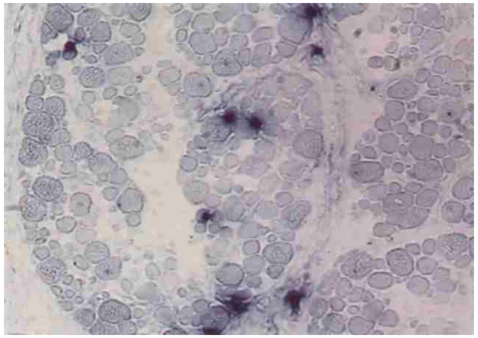
Location

Hybridization in situ

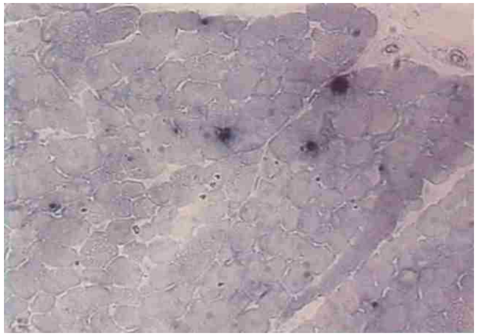
90 dpc



180 dpc



260 dpc

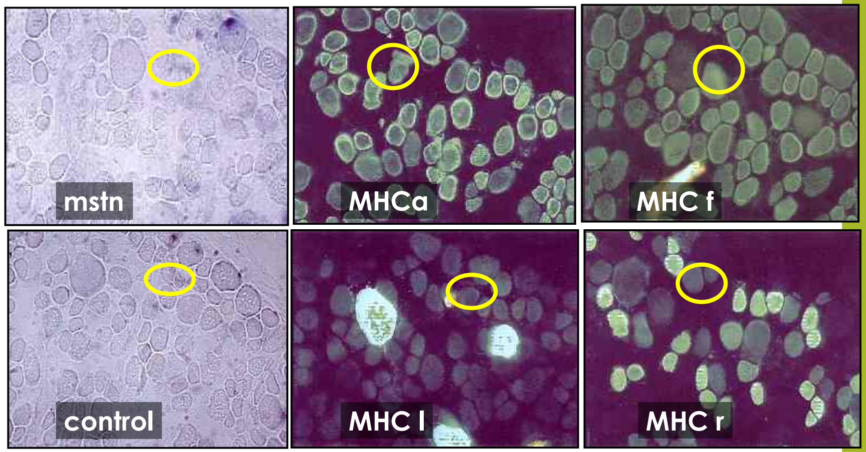


Bovine ST Muscle

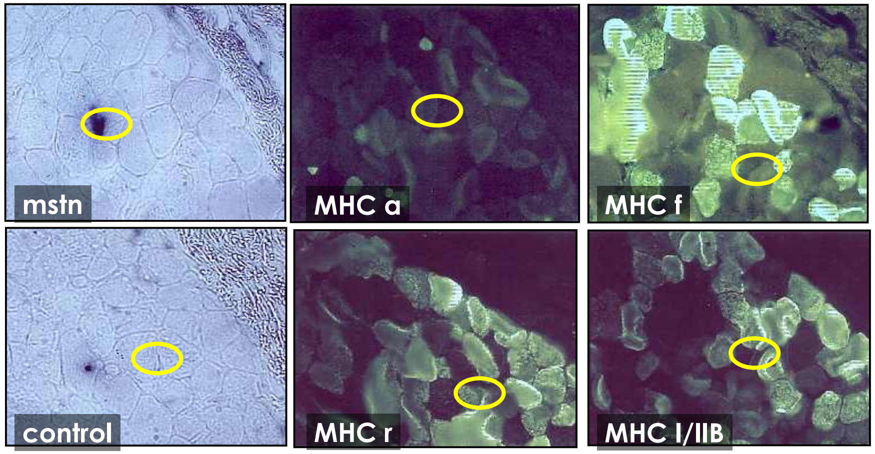
A low number of muscle cells express the *myostatin* gene.

« Fibre-specific » expression

- in 2nd et 3rd generations
- in the less differentiated cells



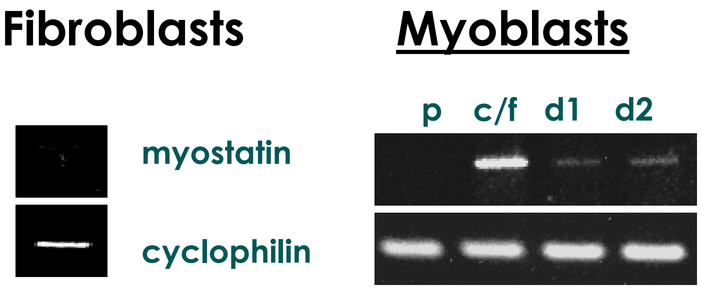
180 dpc

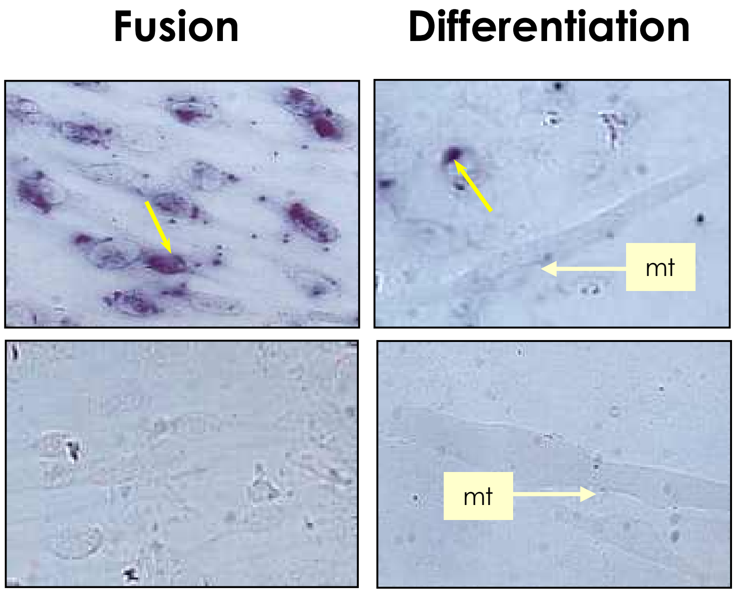


260 dpc

In IIA fast fibres at 260 dpc

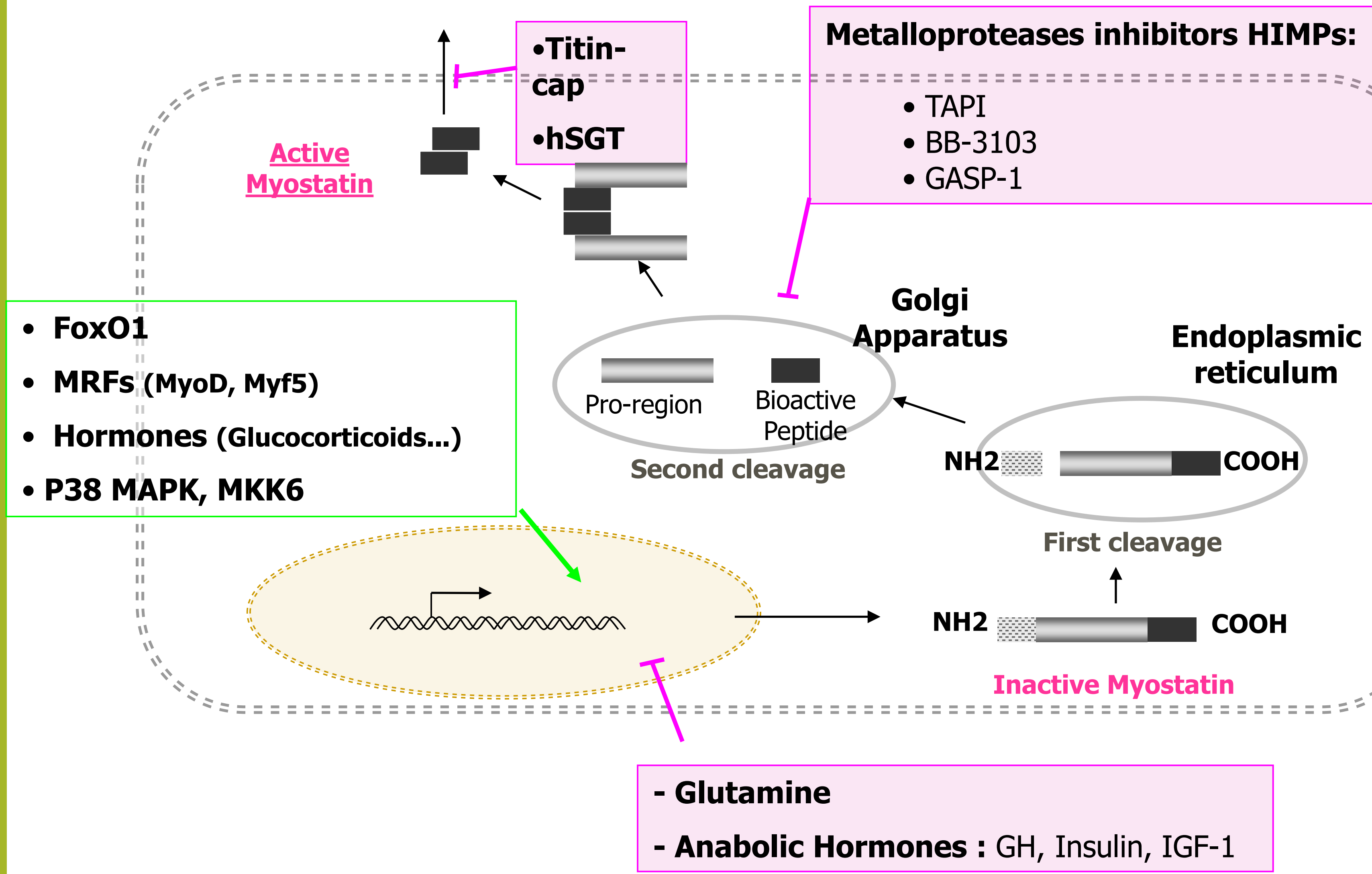
Expression *in vitro*



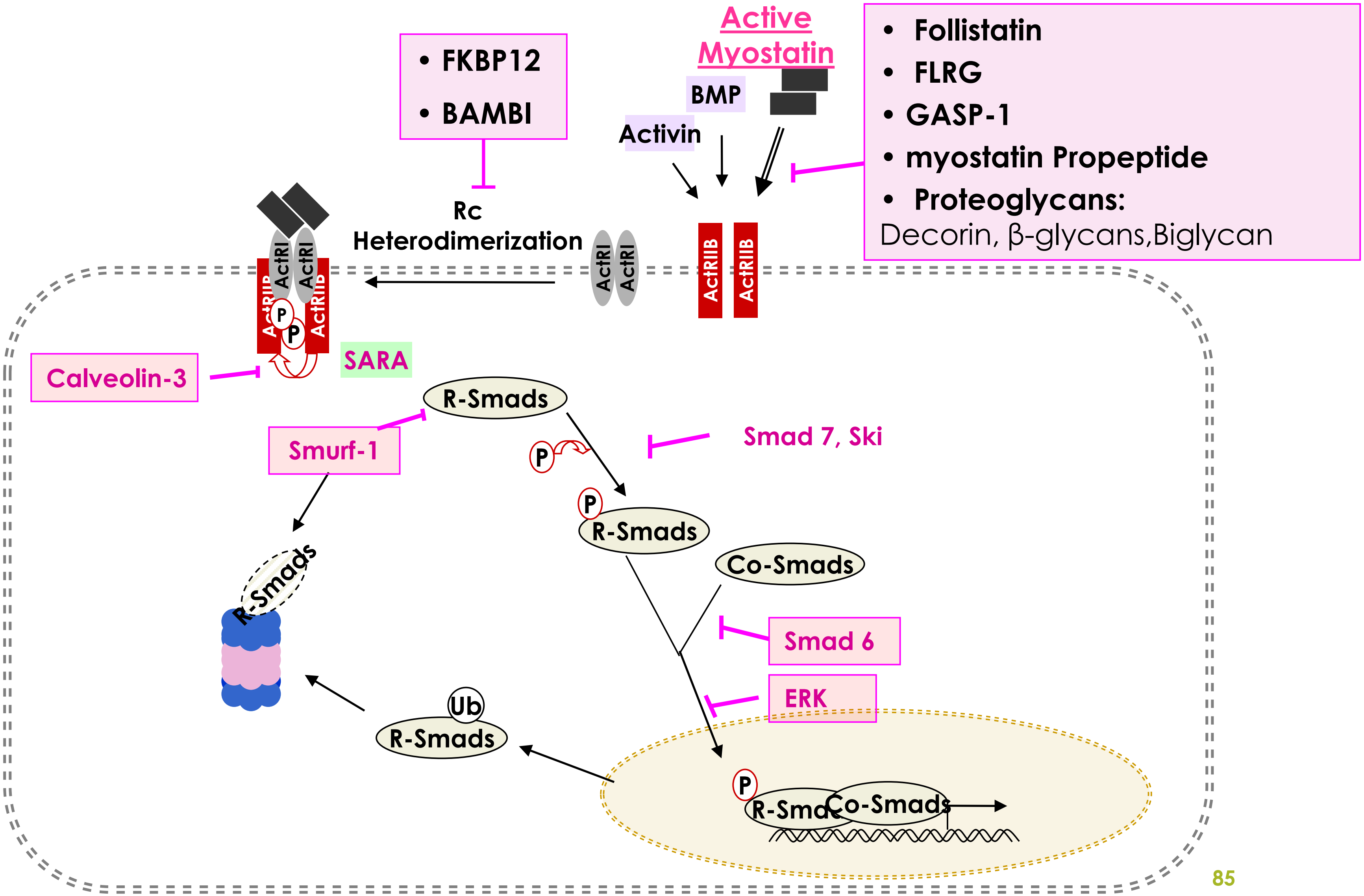


Transient *myostatin* expression in the early differentiation stages

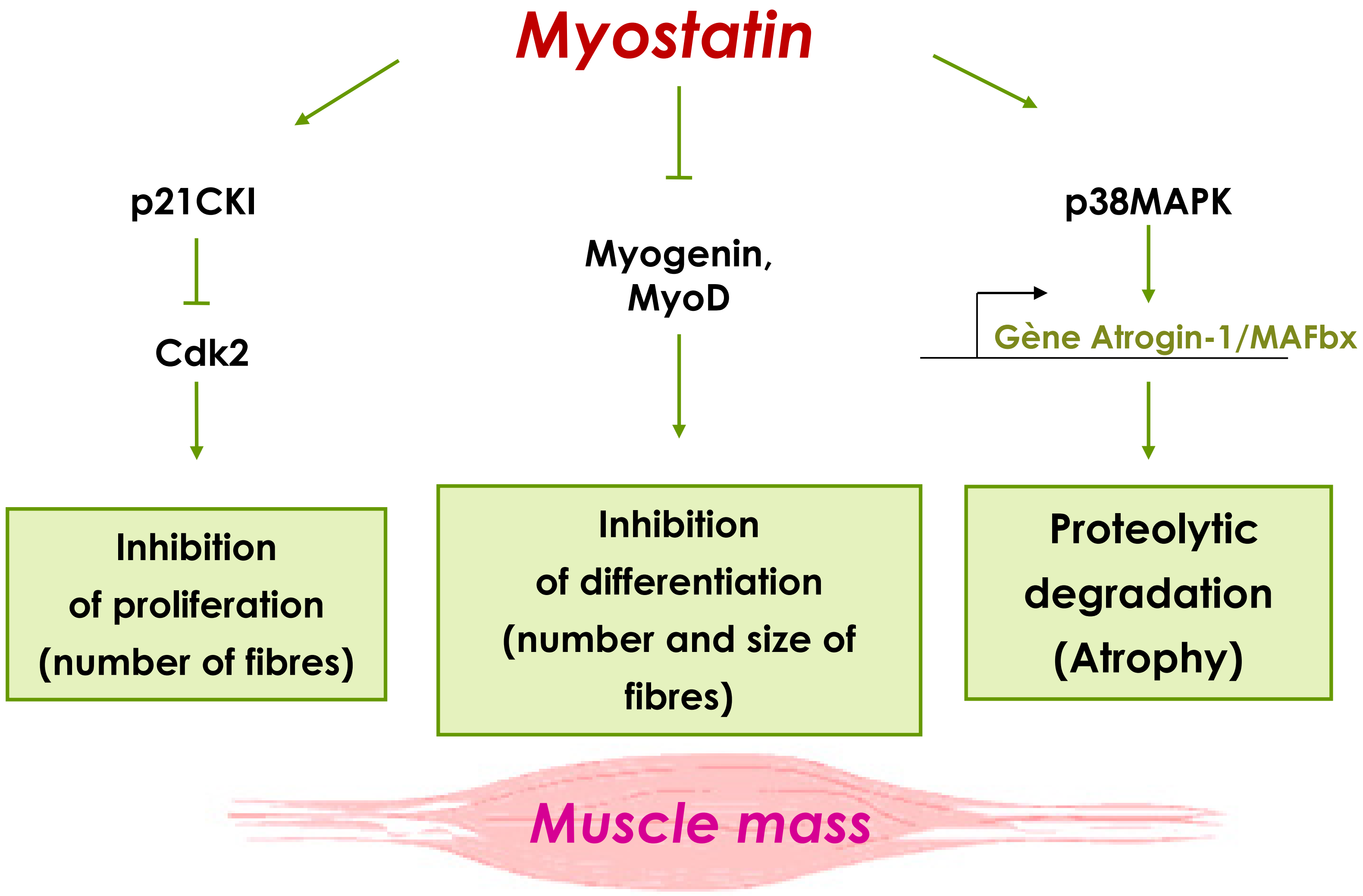
Regulation of MSTN expression



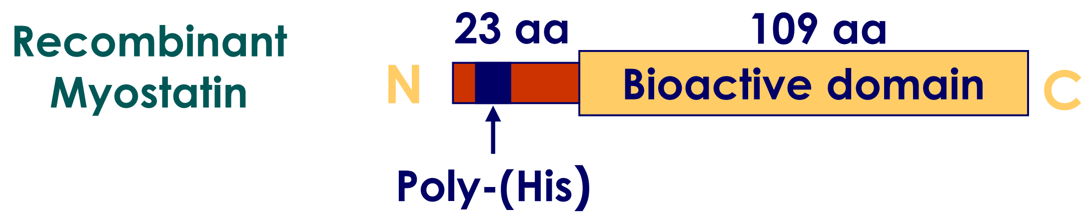
Regulation of MSTN activity

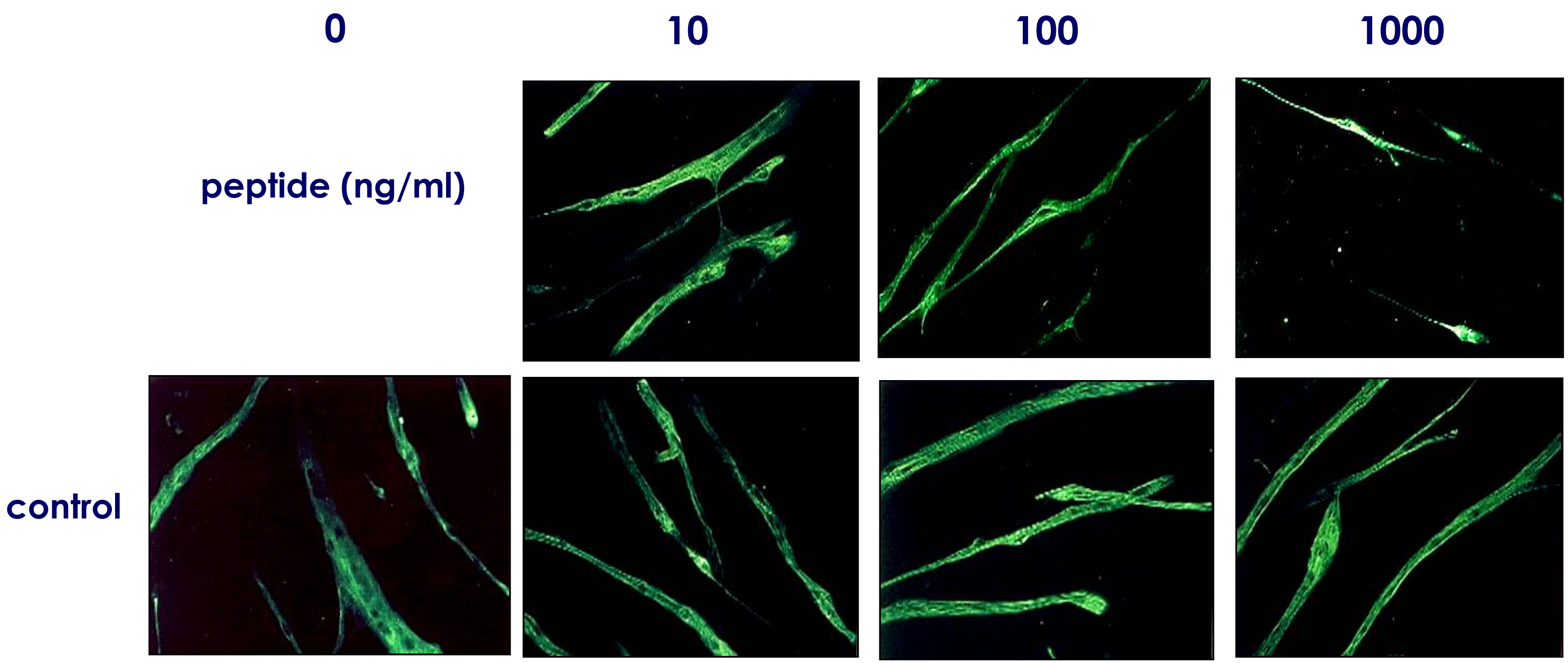


Myostatin target pathways

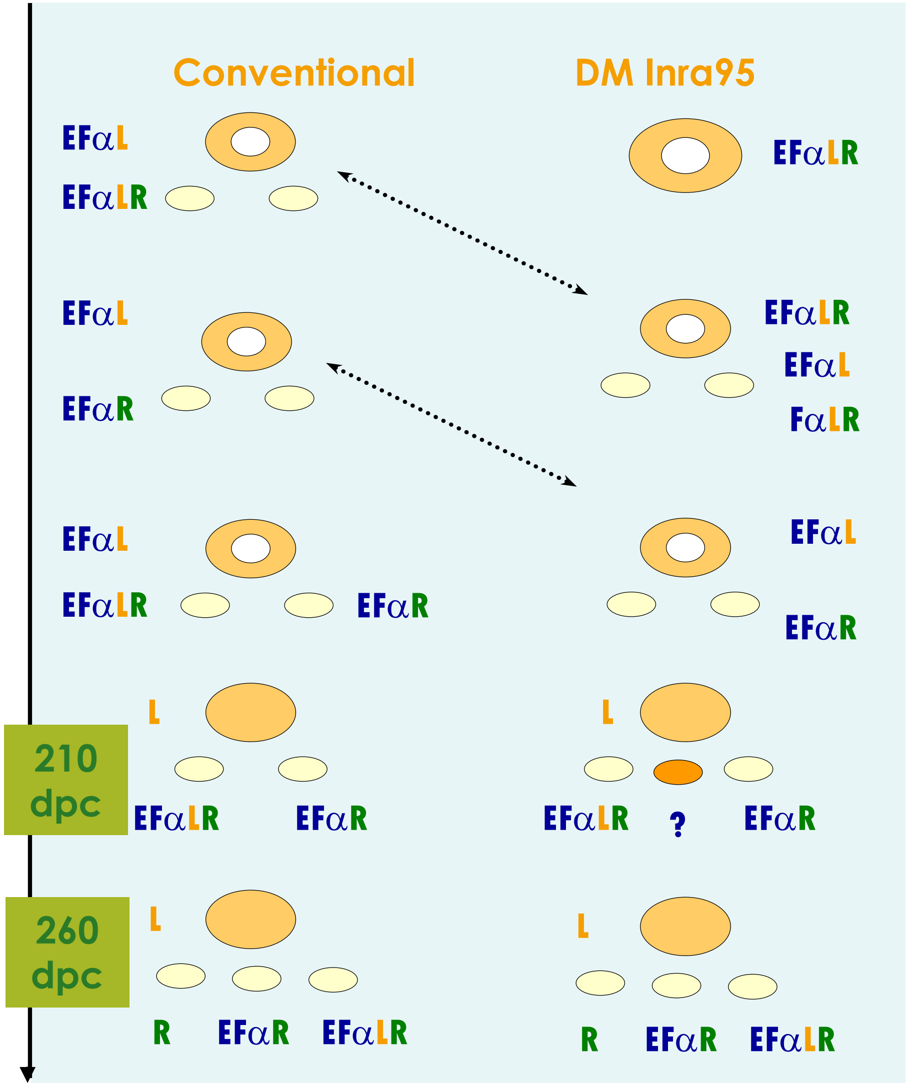


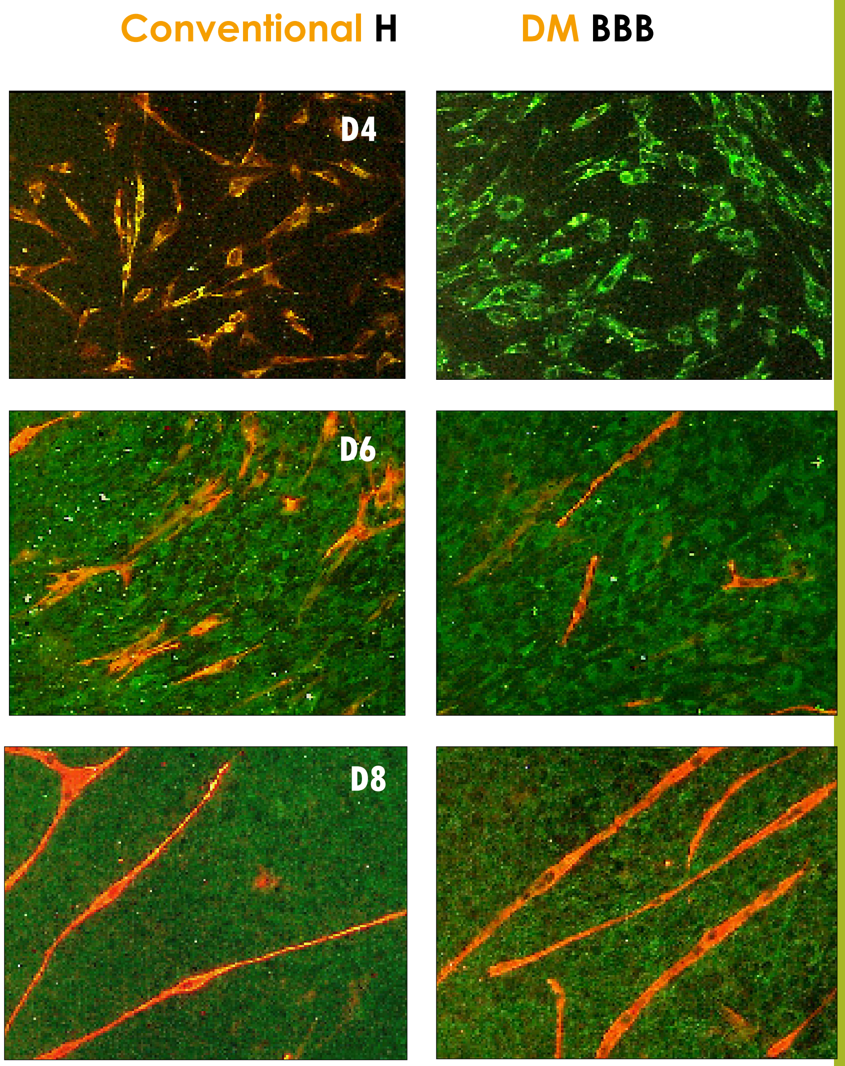
Inhibition of differentiation



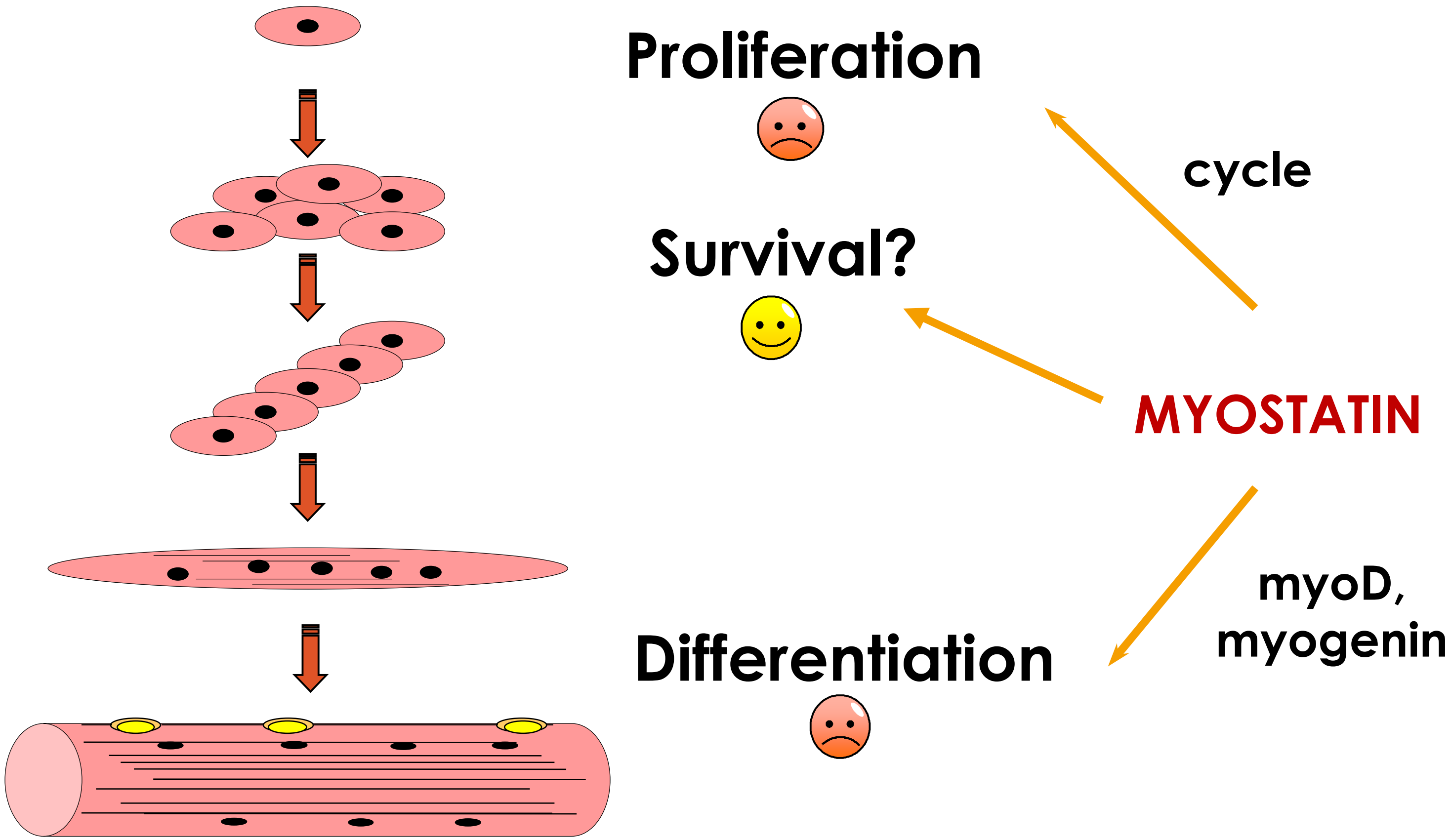


Delay in myogenesis in DM cattle





Molecular action



Regulation of the number of fibres

Take-home message

A member of the TGF-β superfamily

Expression:
Muscle + + +
Mamary gland
Adipose tissue

↑ adipose depots
Orientation towards adipogenesis
↑ metabolic effect

Myostatin

Negative regulator of muscle growth

anti-osteogenic factor

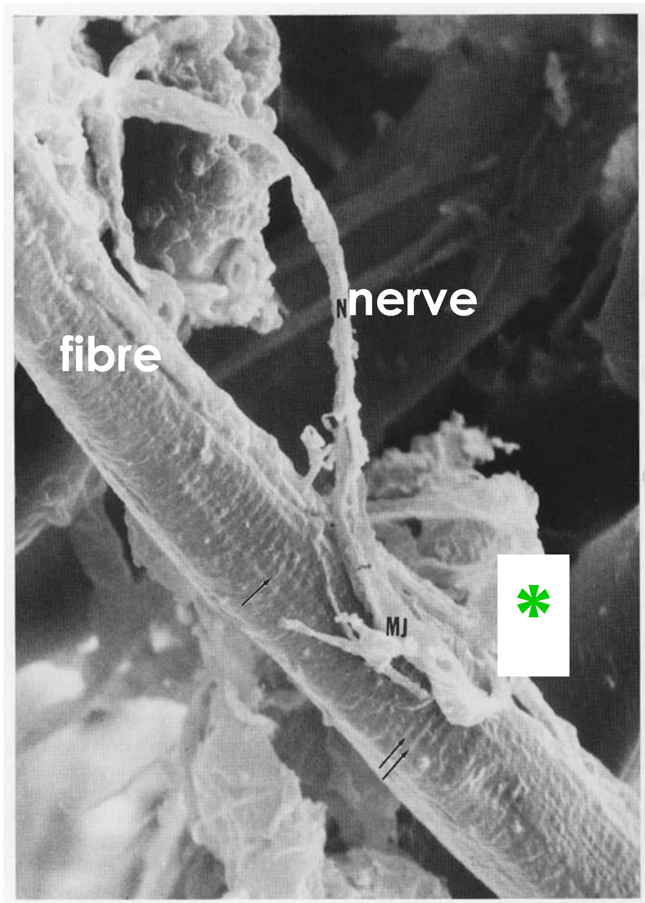
antiproliferative (number of fibres)

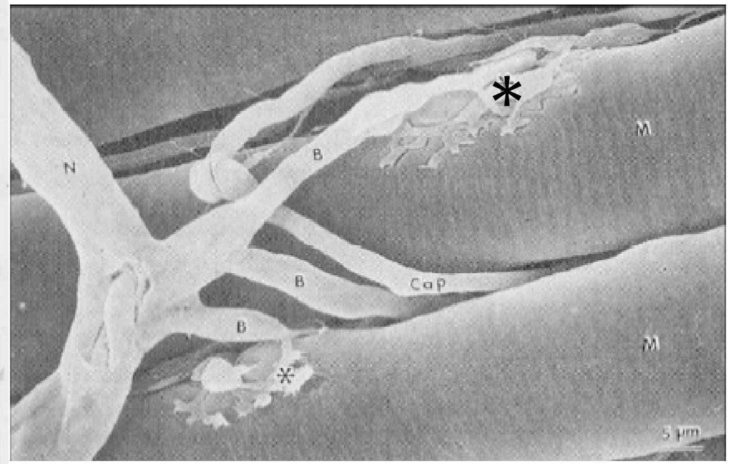
(DM, constitutive ko in mice)

Control of muscle mass (satellite cells, balance hypertrophy/atrophy)

(Ab, conditional ko in mice)

A ROLE FOR INNERVATION





* Neuromuscular Junction

Early synaptogenesis (10th week in humans)

Innervation in the fetus

Innervation

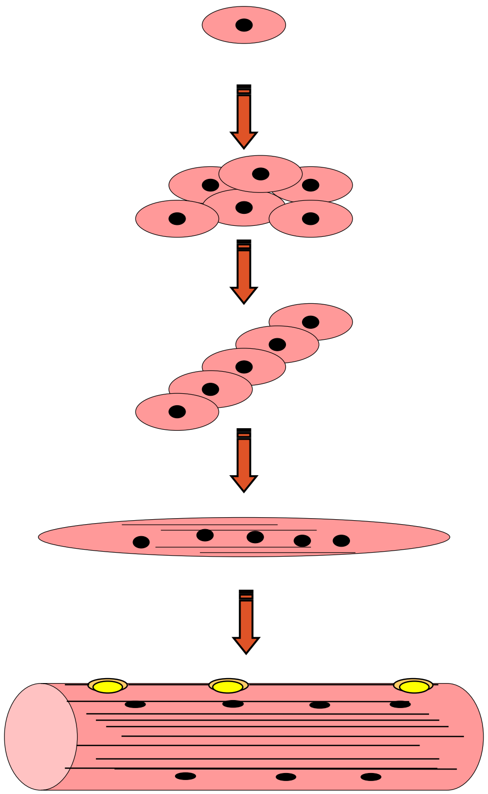
Poly-innervation and thereafter mono innervation

Differentiation of secondary fibres

Acquisition fast fibres properties

HORMONAL REGULATION

T3



proliferation

withdrawal from cycle

differentiation

T3 enhances myogenesis and muscle-gene expression (MyHC, metabolic enzymes)

4-GROWTH

During post-natal life

Postnatal growth

Bovine Muscle

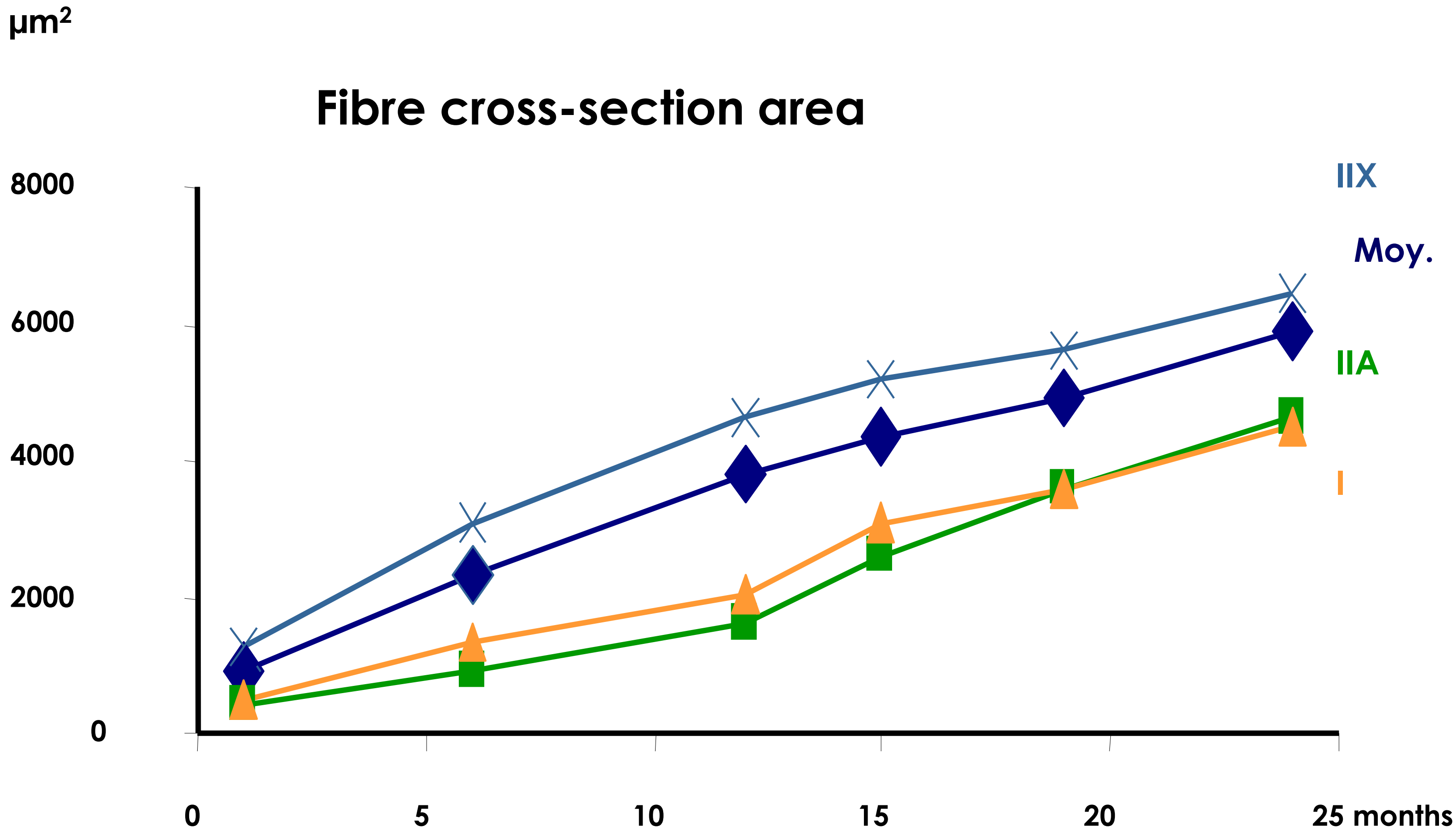
↗ mass between birth and adult stage (x 30)

↗ length of fibres (bone growth)

↗ fibre section (x 3,6)

↗ DNA content (x 5)
Protein / DNA ratio (x 500)

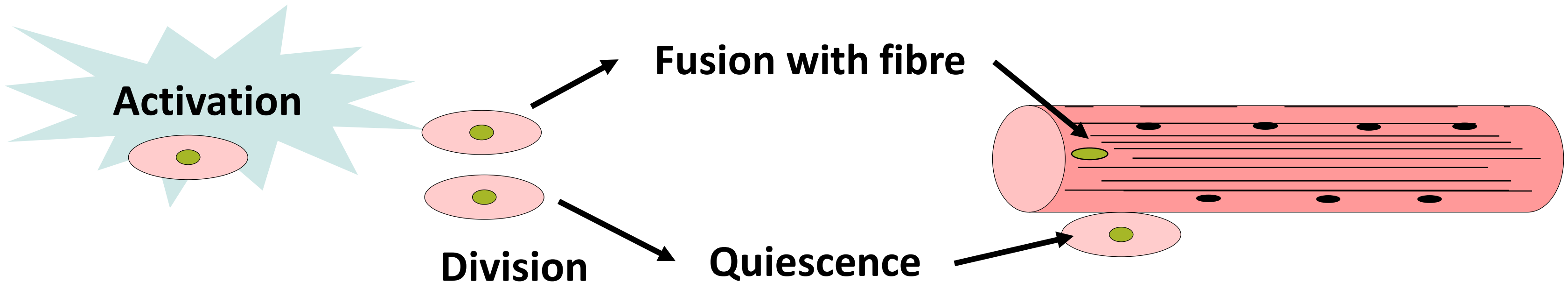
Hypertrophic growth



Crucial role for satellite cells

- A larger number of nuclei / fiber is required to maintain the "DNA unit “ during growth.
- In fibres, nuclei are post-mitotic.

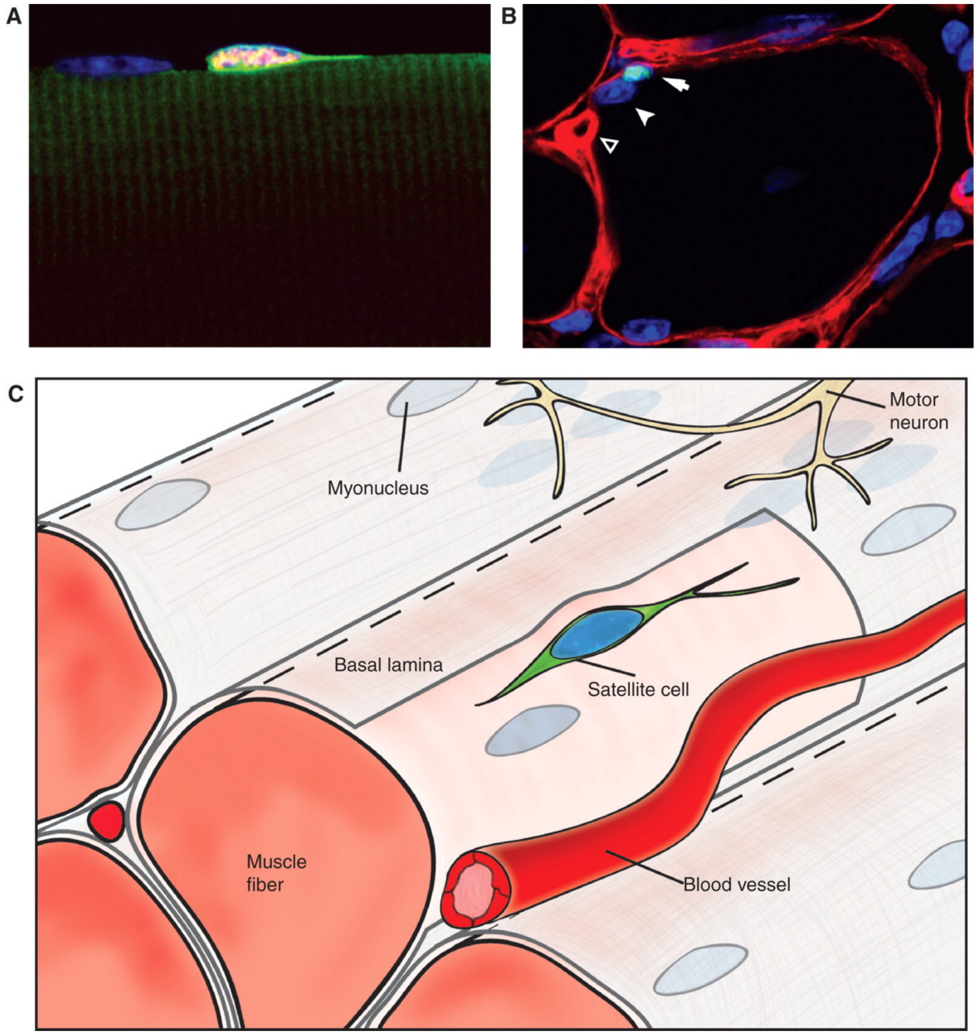
Satellite cells= source of new nuclei



the number of nuclei in the fibres increases,
the number of satellite cells is maintained

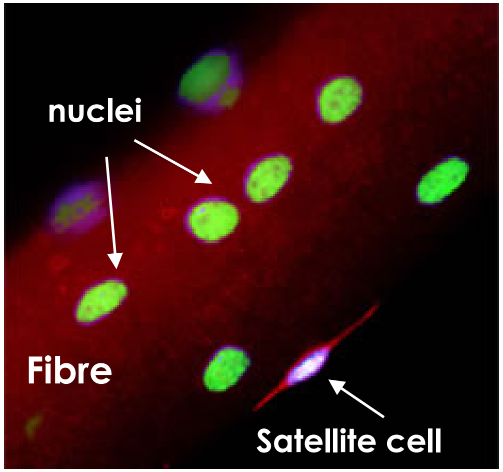
« DNA unit »: volume of cytoplasm controlled by a nucleus

Skeletal muscle and the satellite cell niche



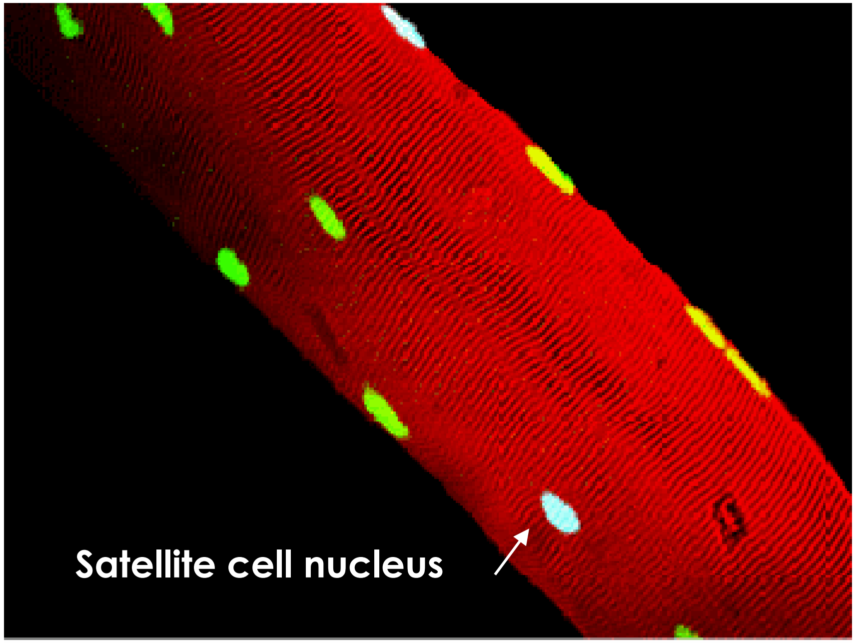
C. Florian Bentzinger et al. Cold Spring Harb Perspect Biol 2012;4:a008342

Satellite cells

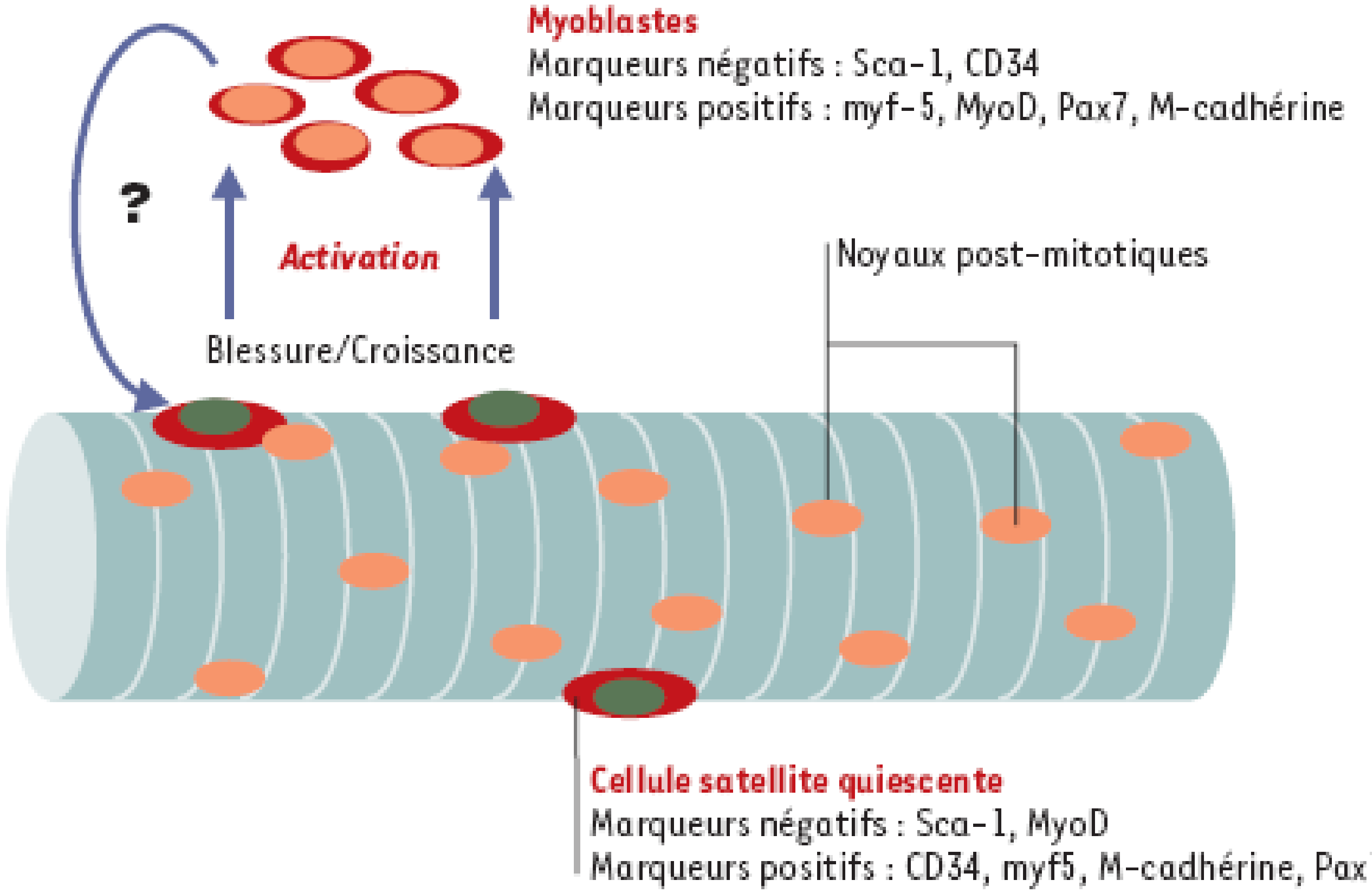


- Adult muscle cells
- located at the periphery of the fibre
- mononucleated
- 4 à 15 % of nuclei

- Quiescent
- involved in growth and regeneration of muscle



Properties

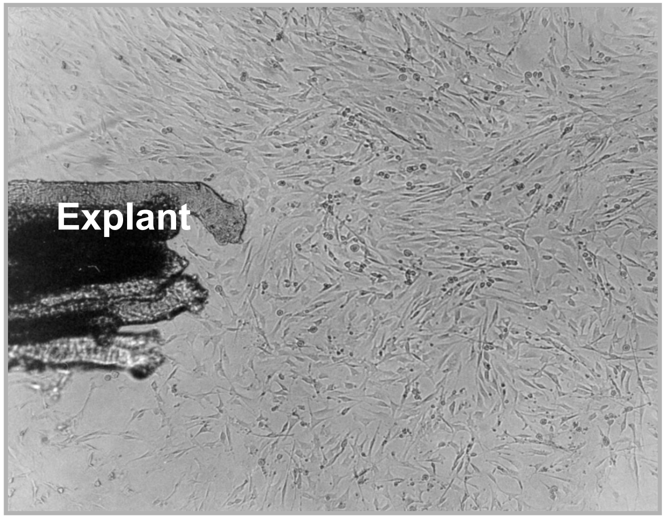


D'après Vincent Mouly, John Beauchamp. **Qu'est-ce qu'une cellule musculaire satellite?** ***M/S n° 6-7, vol. 19, juin-juillet 2003, p696***

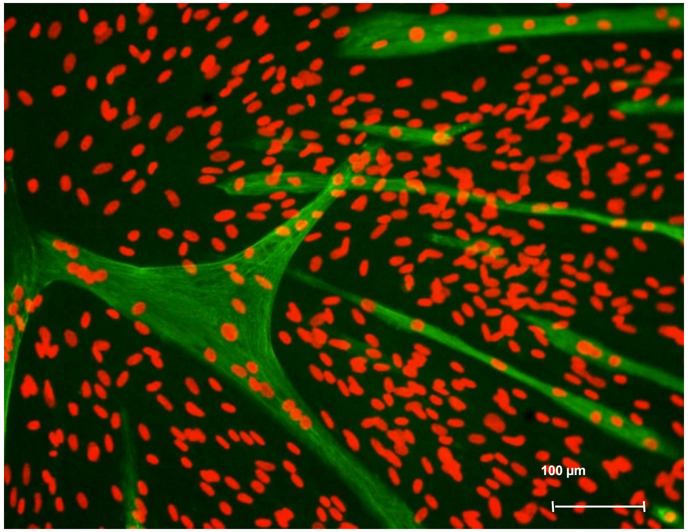
Cell cultures

- Grinding and enzymatic digestion (fetal myoblasts)
- Primo-explantation (satellite cells)

Satellite cells

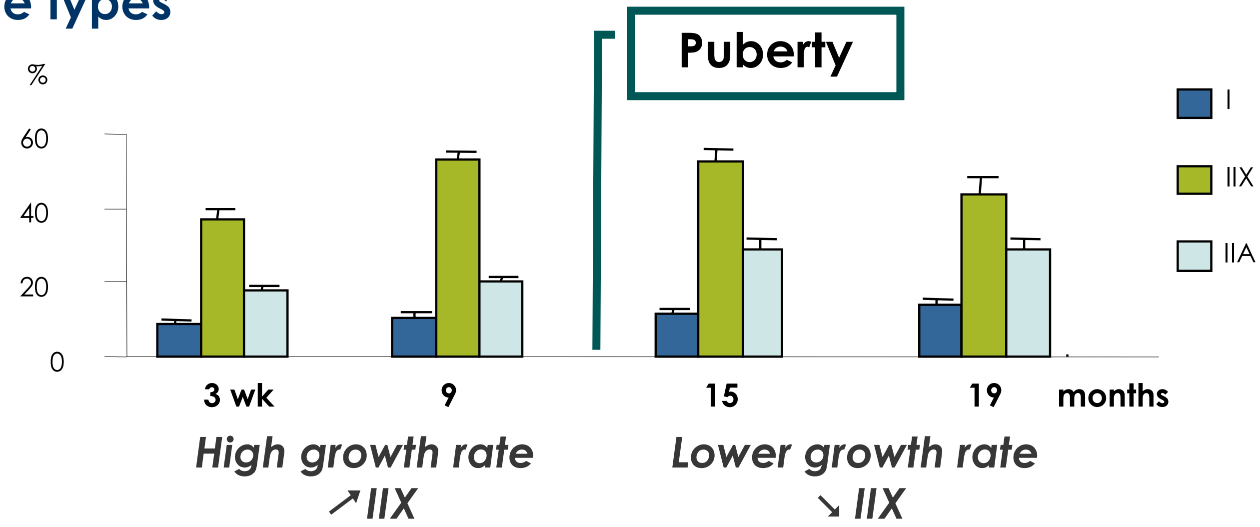


Myoblasts (260 dpc)



Postnatal growth and plasticity

Fibre types



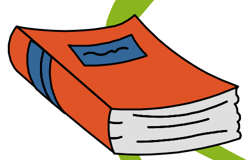
I $\rightleftarrows$ IIA $\rightleftarrows$ IIX

After puberty:

- ↘ fast glycolytic fibres
- more red oxidative muscles

Regulation

Postnatal growth



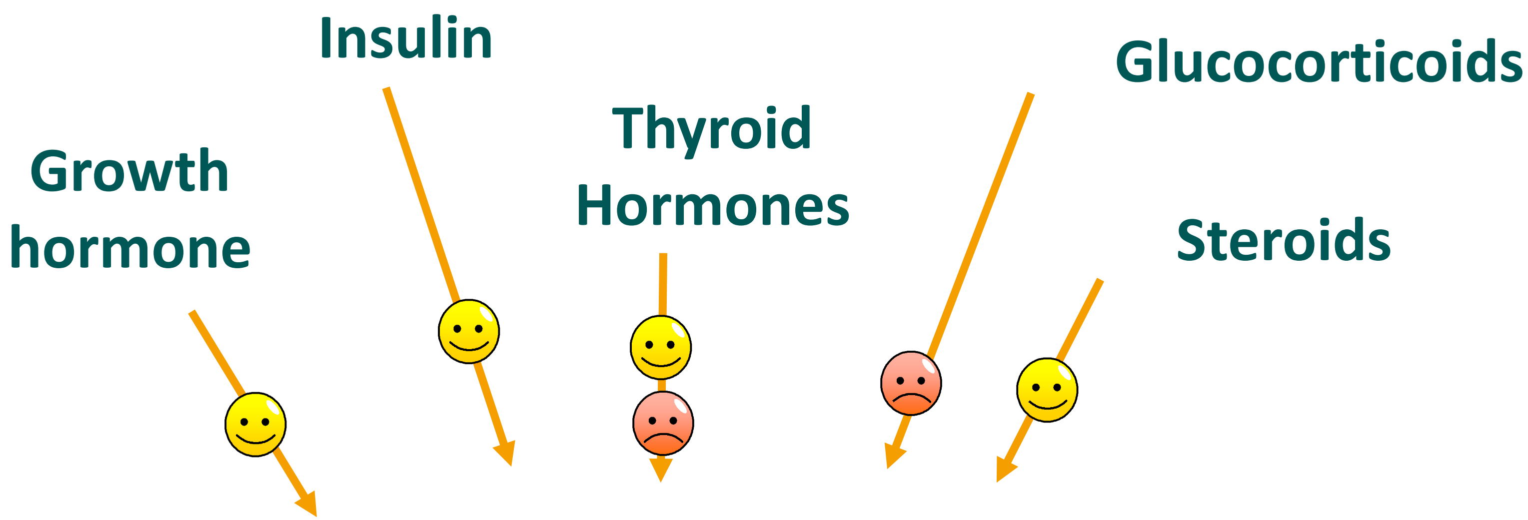
Hormones

Growth factors

IGFs, myostatin

Activity, Innervation

Hormones



HYPERTROPHY :

Anabolic processus

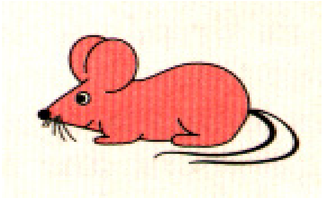
Protein synthesis

Satellite cells

IGFs

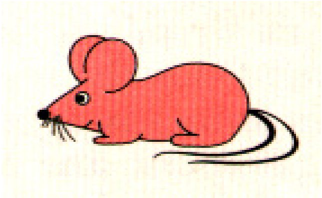
Enhance the size of muscle fibres

- IGF-I (K.O)



Hypotrophy
Hypoplasia

+IGF-I (transgenic)



Muscle Hypertrophy (x 2)

Targets:

- protein synthesis,
- proliferation et differentiation of satellite cells

IGF-I

Mice selected on their high levels of IGF-I at 12 weeks of life have an offspring with more developed muscle mass and less fat deposition than normal mice.

IGF-I

Its expression is regulated in muscle postnatally

- in response to hormones
  - ☺ GH, Insulin, steroids, thyroid hormones
  - ☹ corticosteroids

- ↗ exercice
- ↘ malnutrition

Myostatin

Inhibition of myostatin maturation

Blocking antibodies

Conditional K.O

MUSCLE HYPERTROPHY

Satellite cells

The muscle mass is maintained

Thank you for your attention!