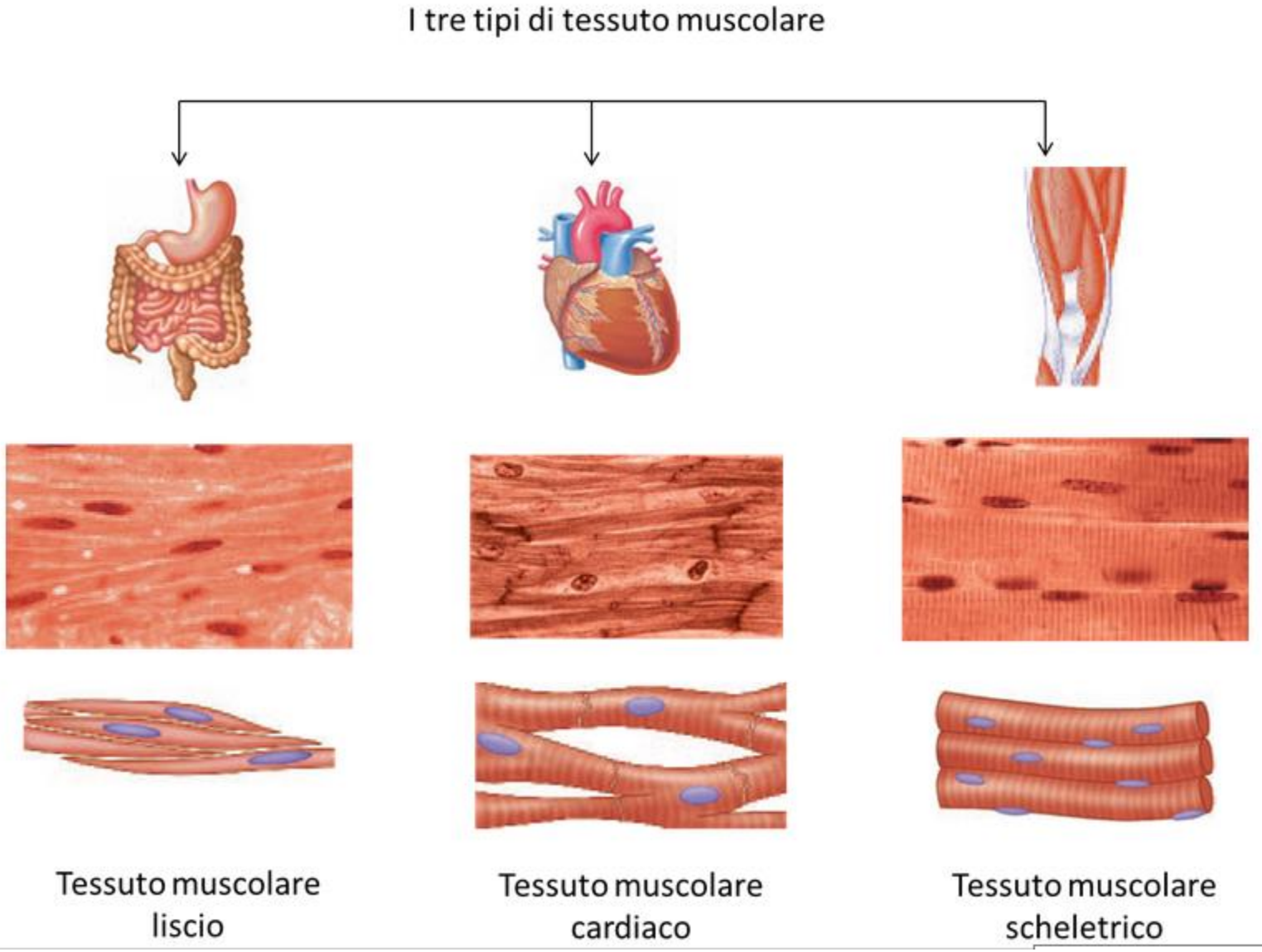
Le cellule satelliti e la rigenerazione muscolare

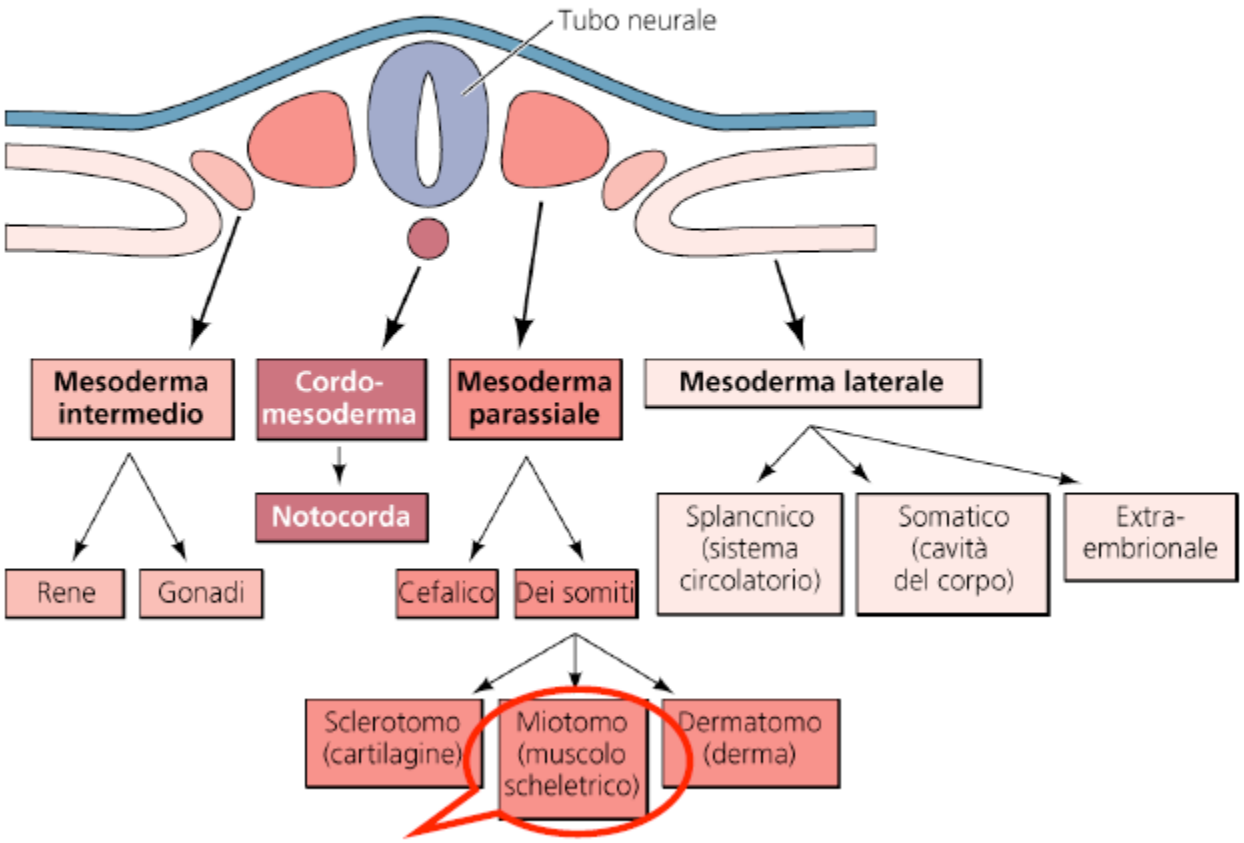
"Dipartimento di Neuroscienze Imaging e Scienze Cliniche"

IL MUSCOLO

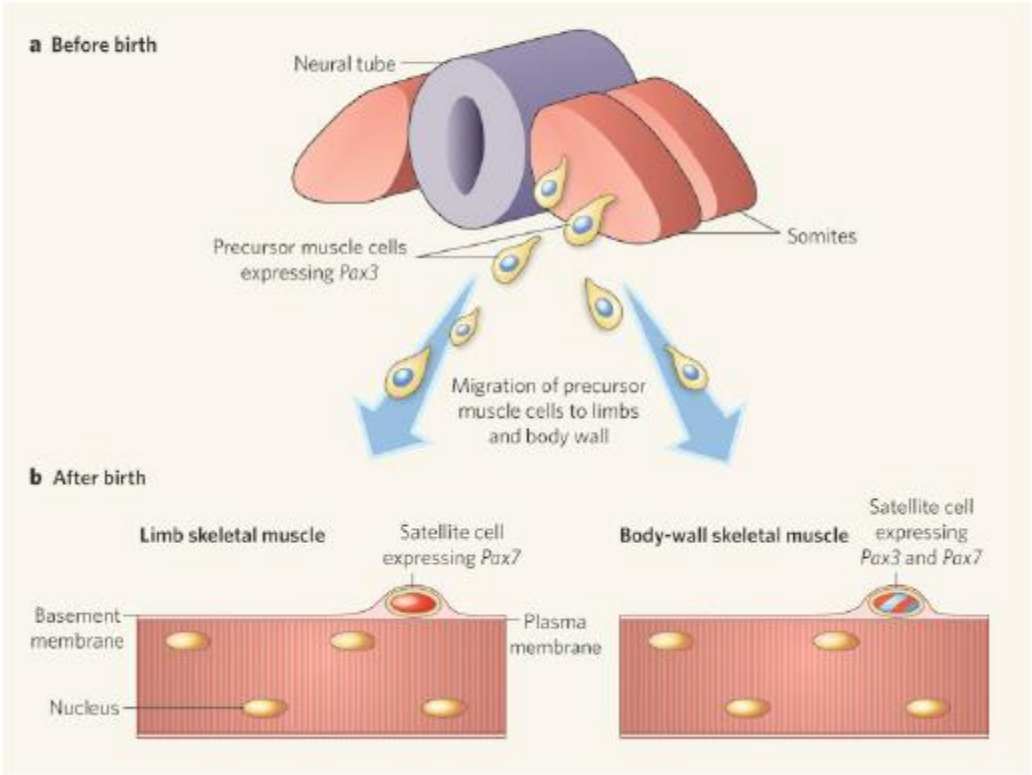
TESSUTO MUSCOLARE



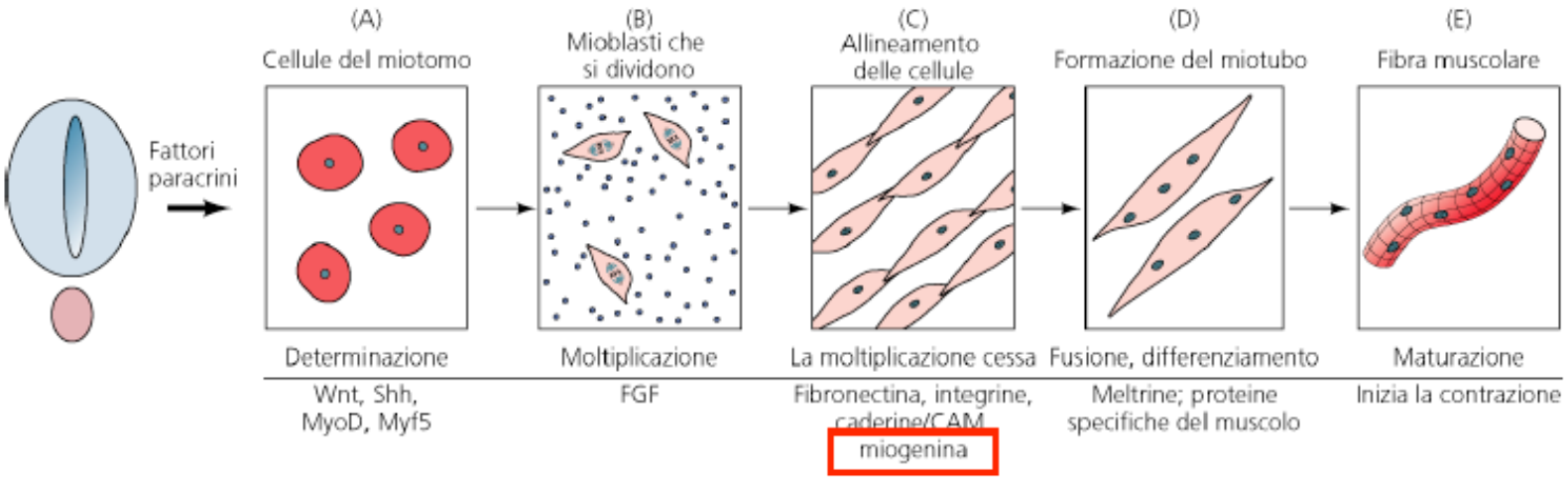
ORIGINE EMBRIONALE DELLE CELLULE MUSCOLARI



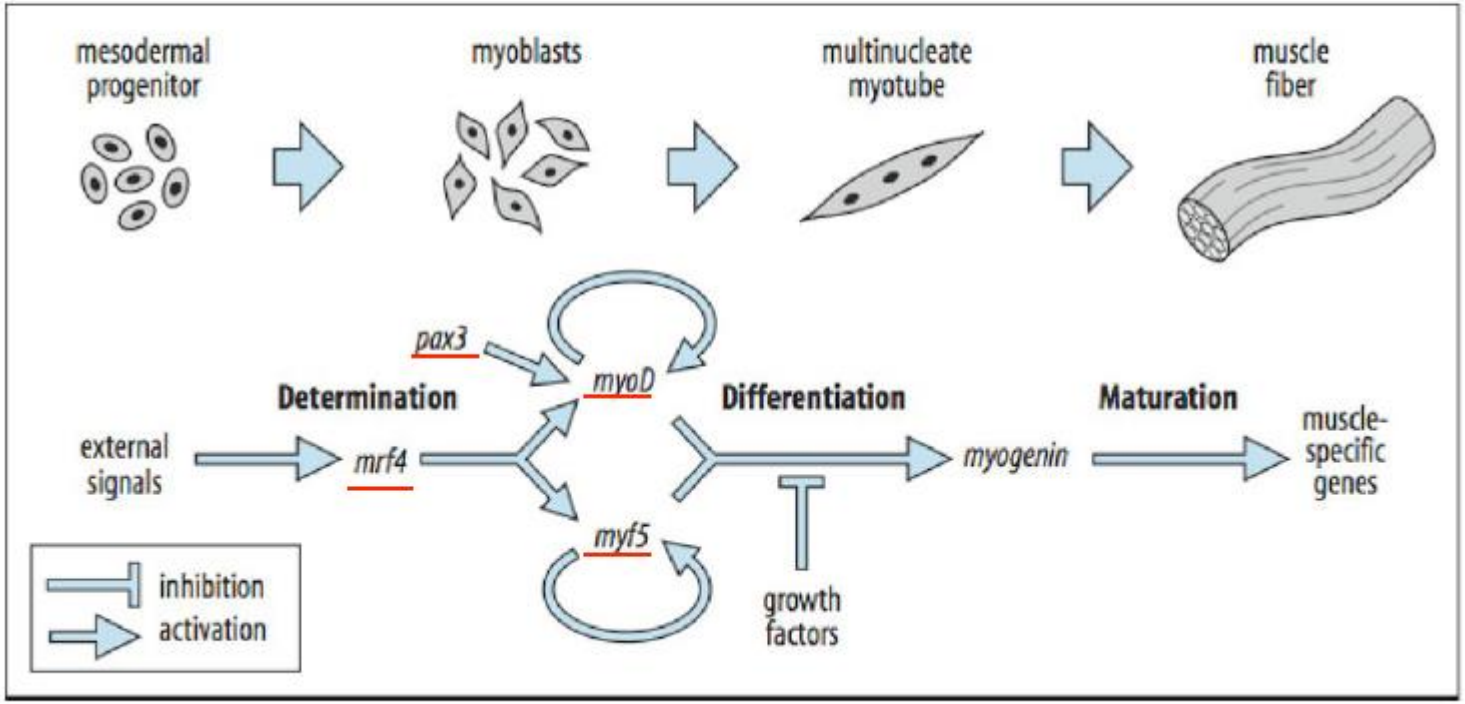
MIGRAZIONE DI MIOBLASTI



MIOGENESI DEL MUSCOLO SCHELETRICO



DIFFERENZIAMENTO DEL MUSCOLO SCHELETRICO

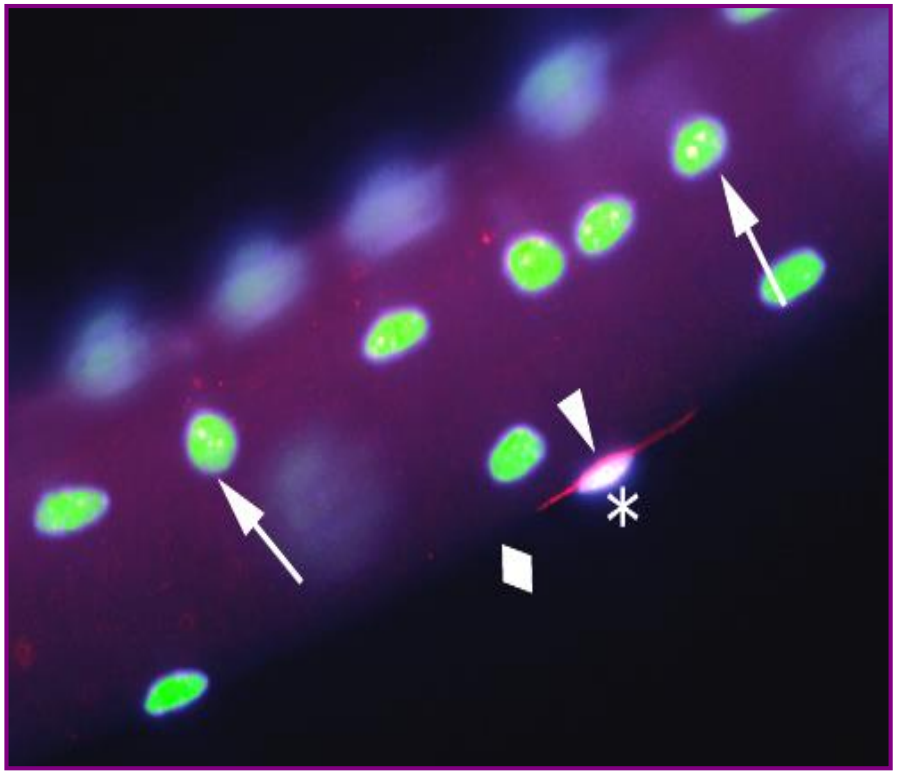


MODULAZIONE DEL MUSCOLO SCHELETRICO

Le cellule muscolari possono modificare le loro proprietà cambiando le isoforme proteiche in esse contenute

- Una volta formatasi, la cellula muscolare può persistere per tutta la vita di un individuo, accrescendosi e modulando le proprie caratteristiche in base alle necessità funzionali.

- Esistono copie multiple dei geni codificanti per le proteine muscolari e molti RNA di tali geni subiscono splicing alternativo, per cui, durante la maturazione cellulare, possono essere prodotte numerose varianti proteiche.



L'immagine mostra la posizione di una cellula satellite in rapporto ai mionuclei (freccia) su una fibra muscolare isolata. La cellula satellite è stata messa in evidenza per mezzo di immunocolorazione con l'anticorpo specifico della M-caderina (testa di freccia e asterisco). La membrana plasmatica è indicata con il rombo.

Cosa succede ad un muscolo danneggiato?

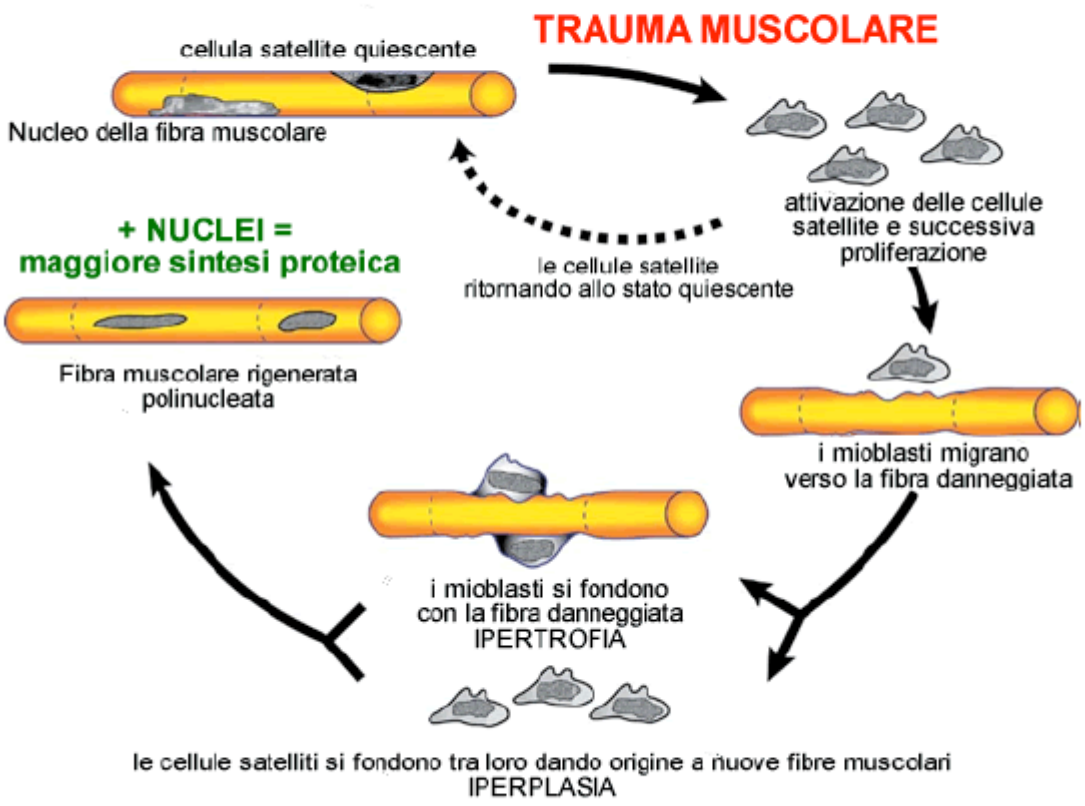
La guarigione coinvolge sempre proliferazione di fibroblasti e sintesi di nuova matrice di tessuto connettivo

MUSCOLO CARDIACO - può rigenerare durante l'infanzia, ma non nell'individuo adulto. È rimpiazzato da tessuto connettivo che forma cicatrici.

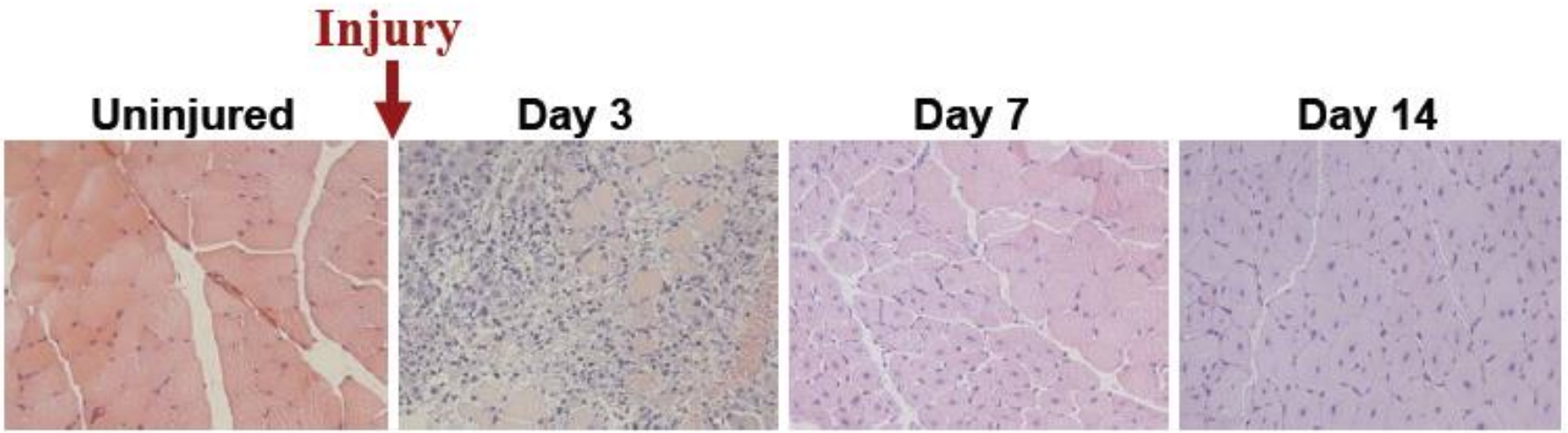
MUSCOLO LISCIO - comincia la proliferazione ed il differenziamento di cellule muscolari lisce primitive in nuove fibre muscolari. Lo stesso processo avviene durante la gravidanza in cui nuovo tessuto muscolare viene aggiunto per aumentare l'utero.

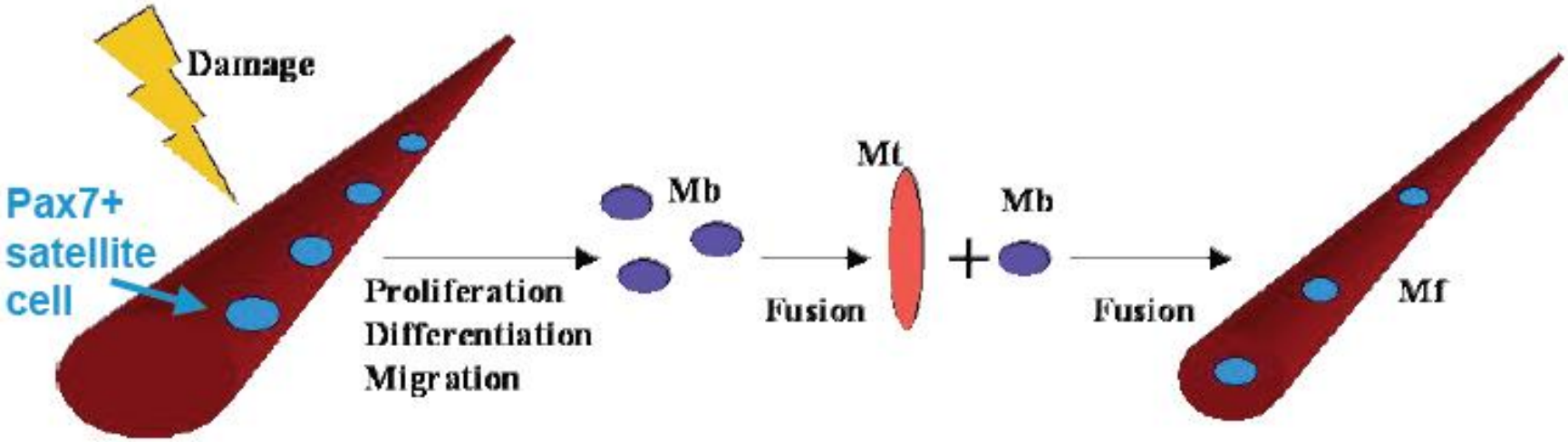
MUSCOLO SCHELETRICO - le cellule satelliti normalmente quiescenti, presenti sotto l'endomisio, cominciano a dividersi e differenziarsi in mioblasti che alla fine fondono per formare nuove fibre muscolari.

RIGENERAZIONE IN SEGUITO A DANNO



Processo riparativo del muscolo scheletrico





RIGENERAZIONE DEL MUSCOLO SCHELETRICO

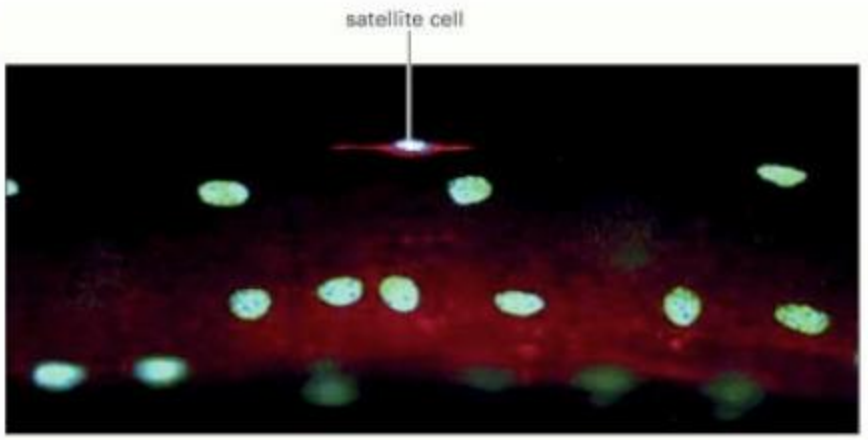
Alcuni mioblasti persistono nell'adulto come cellule staminali quiescenti

- sono cellule appiattite in stretto contatto con la cellula muscolare, mantenute in uno stato di quiescenza, ma disponibili per l'autorinnovamento;

- Si attivano, iniziando a proliferare e poi a fondersi, in risposta a fattori di crescita (es. FGF) o in seguito ad un danno muscolare.

RIGENERAZIONE DEL MUSCOLO SCHELETRICO

Alcuni mioblasti persistono nell'adulto come cellule staminali quiescenti



Rosso: M-caderina

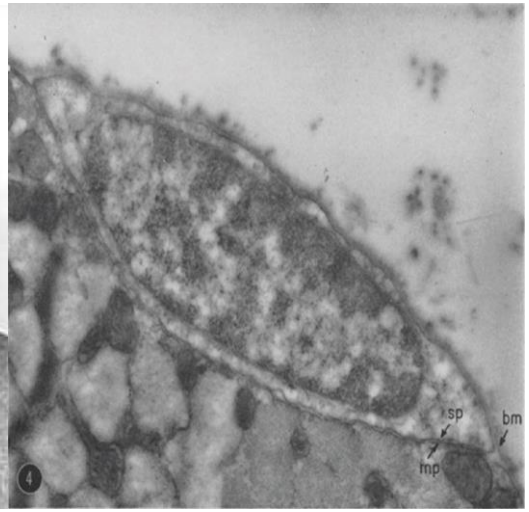
Blu-verde: nuclei

...from the archive

In 1961, two independent studies by Alexander Mauro and Bernard Katz provided the first electron microscopic descriptions of satellite cells in frog and rat muscles.

Alexander Mauro

In: "SATELLITE CELL OF SKELETAL MUSCLE FIBERS", A. Mauro, 1961

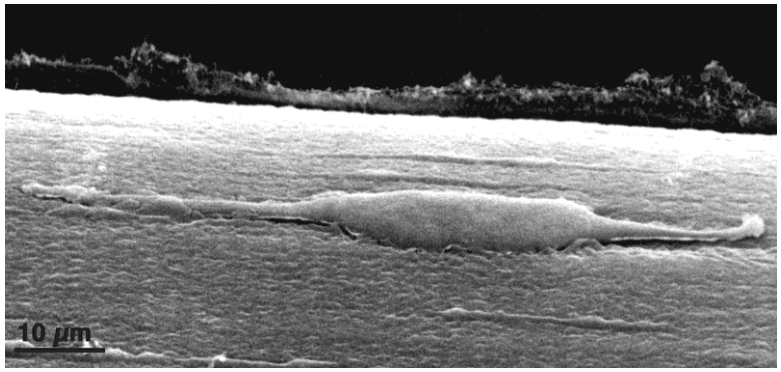


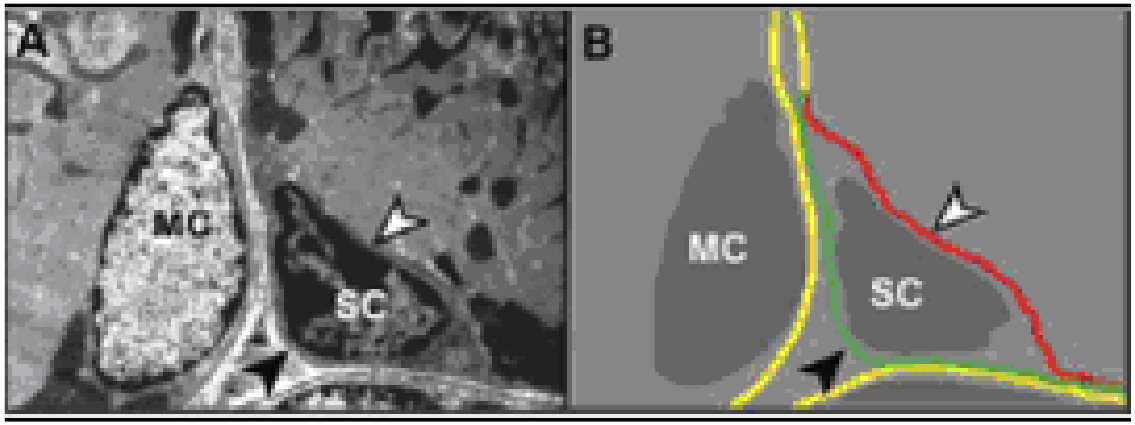
Bernard Katz

Adult skeletal muscle exhibits a remarkable capacity for regeneration following damage, an ability that resides in a population of muscle precursor cells, which have been called satellite cells. In normal muscle these are quiescent mononucleated cells that are sequestered between the basement membrane and the sarcolemma of the mature muscle fibers (Mauro, 1961).

Cellule Satelliti

Le CS, sono cellule mononucleate localizzate tra la membrana plasmatica e la lamina basale che circonda la fibra muscolare.



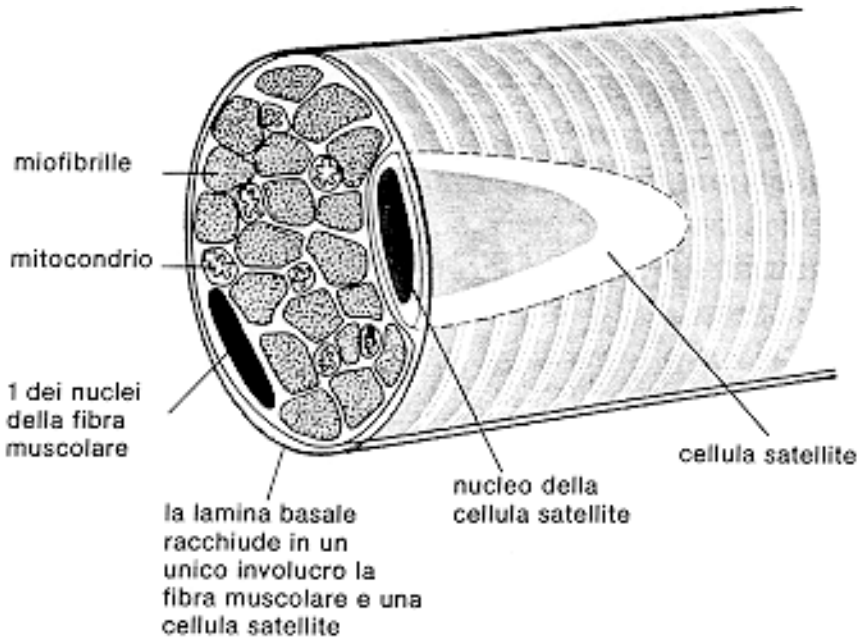


"Muscle stem cells in development, regeneration, and disease" *Shi and Garry*, 2006.

A) Immagini di miscroscopia elettronica che mostra il nucleo di una CS e un mionucleo (MC).

B) Schematizzazione dell'immagine in A che enfatizza come la cellula satellite risieda tra la lamina basale (linea verde) e il sarcolemma (linea rossa).

Cellule Satelliti



- Their location is between the basal lamina and plasma membrane of myofibre

- These cells are responsible for pre- and postnatal muscle growth

- Satellite cells are undifferentiated quiescent mononucleated cells present in muscle

- They are capable of both proliferating and differentiating in order to repair skeletal muscle fibres following injury

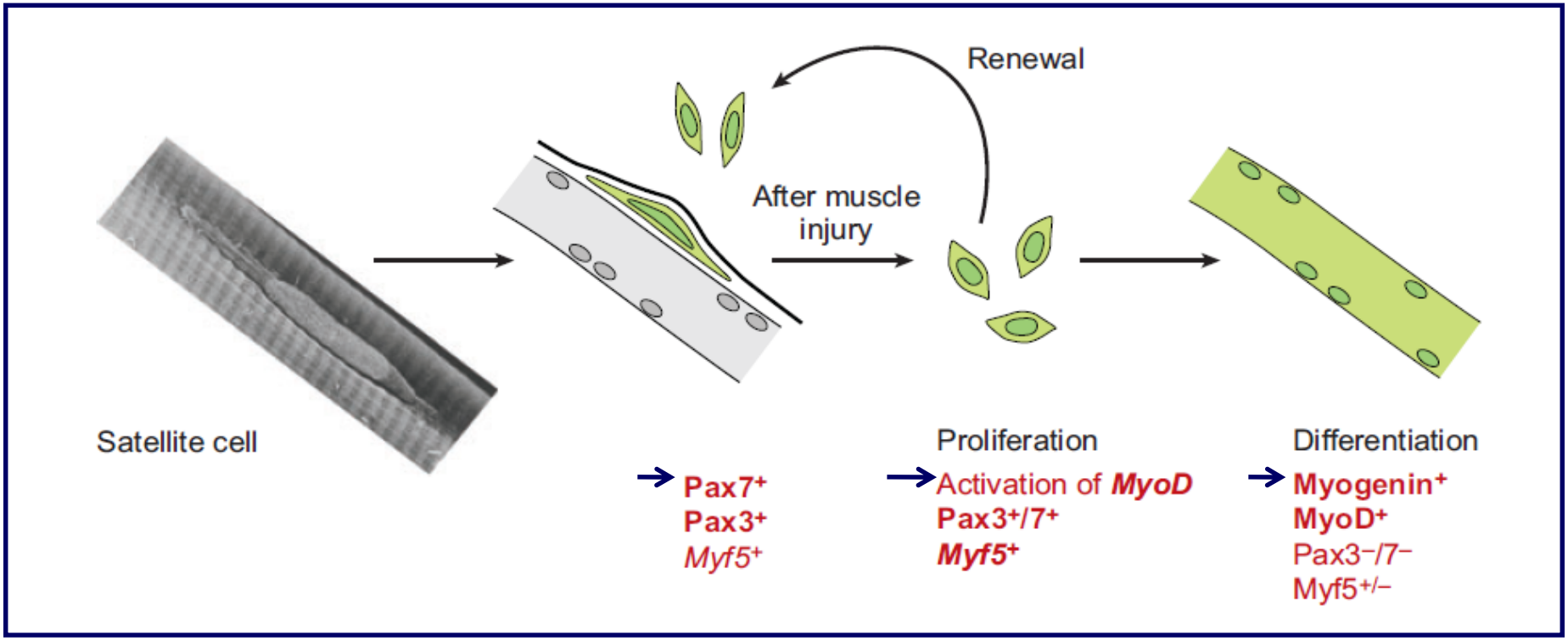
Cellule Satelliti

- L'età è un fattore determinante: alla nascita le CS rappresentano il 20-30% dei nuclei sub-laminari associati alle fibre muscolari dei vertebrati, mentre con la crescita e la maturità questo numero decresce al 5%.

- Sono presenti in tutti i muscoli scheletrici, ma in maniera ineguale tra i diversi muscoli e tra i tipi di fibra: fibre lente hanno un maggior numero di CS rispetto alle fibre veloci.

- Aumentata densità di CS in prossimità delle giunzioni neuromuscolari, dei mionuclei e dei capillari.

CELLULAR AND MOLECULAR REGULATION OF MUSCLE REGENERATION

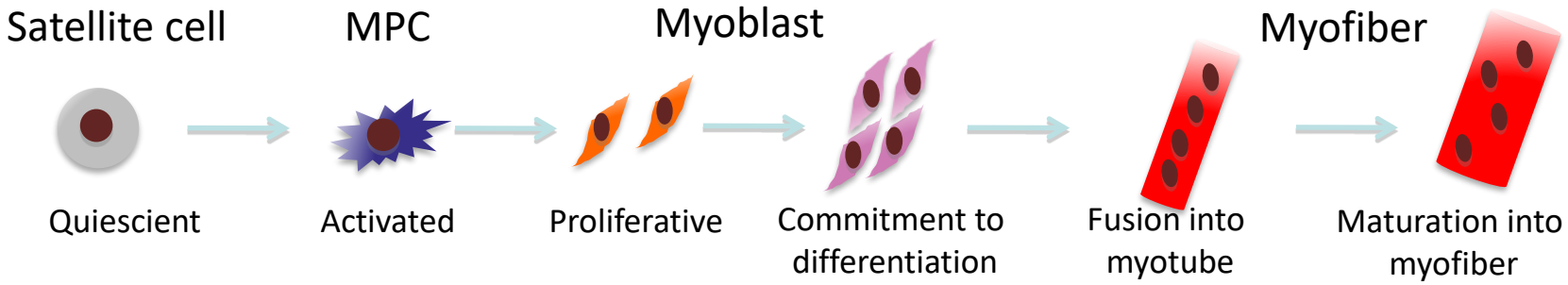


Bold red print indicates the primary factors present at each stage

Buckingham and Relaix Annu. Rev. Cell Dev. Biol. 2007

Molecular Marker	Expressed in...
Foxk1 (previously known as **MNF**)	Quiescent, activated and growing satellite cells
Pax3	Quiescent
Pax7	Quiescent, activated and growing satellite cells
c-Met	Quiescent, activated and growing satellite cells
M-Cadherin	Quiescent, activated and growing satellite cells
NCAM (Neural Cell Adhesion Molecule-1)	Quiescent, activated and growing satellite cells; synaptic junction in adult myofibres
VCAM-1 (Vascular Cell Adhesion Molecule-1)	Quiescent, activated and growing satellite cells
Desmin	Activated and growing satellite cells
Myf5	Activated and growing satellite cells
MyoD	Activated and growing satellite cells
BrdU	Growing satellite cells
PCNA	Growing satellite cells
[^{3}H]timidin	Growing satellite cells

Miogenesi



Pax7
(Pax3)

Pax7
Myf5
MyoD
Pax3

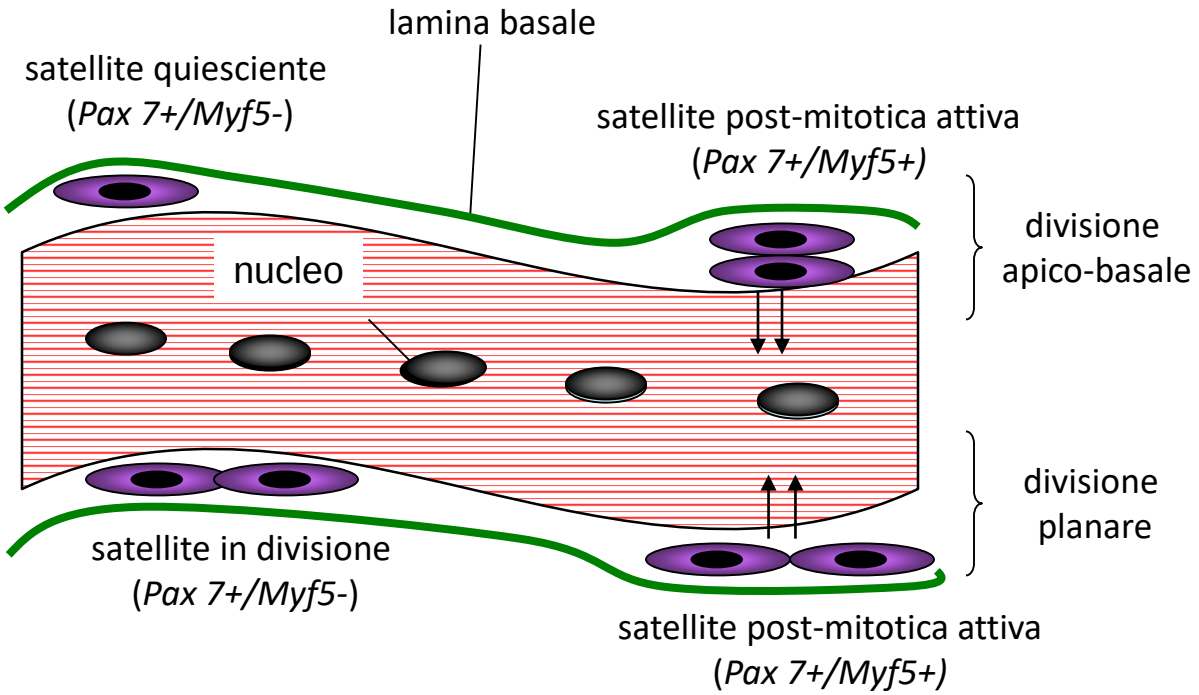
MyoD
Myogenin

Myogenin
MRF4

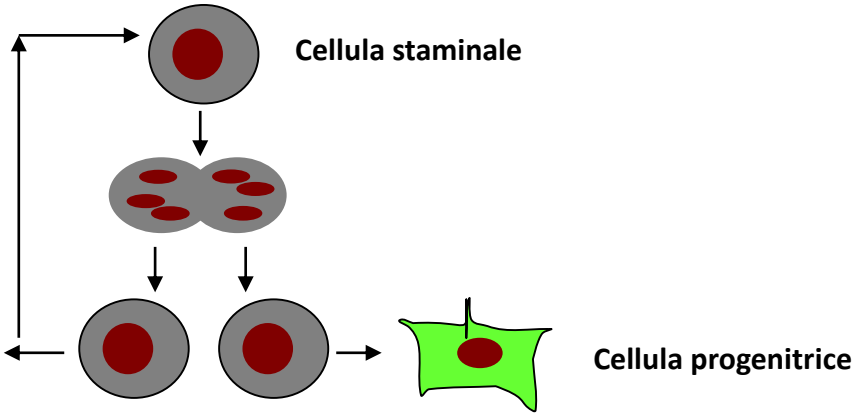
Myogenic Regulatory Factors (MRFs)

Myogenesis control is a complex process, involving several gene regulatory networks, regulated by genes coding the MyoD family of myogenic regulatory factors (MRFs)

Miogenesi adulta



Divisione asimmetrica



Fattori di Crescita

Le CS nello stato di quiescenza vengono attivate da fattori di crescita (fattori di crescita insulino-simili : *insulin-like growth factor* (IGF-I), mIGF-I, *Hepatocyte growth factor* o fattore di crescita degli epatociti (HGF)) che mantengono un equilibrio tra crescita e differenziamento, necessario per il recupero della normale architettura muscolare. È anche influenzata da ormoni quali *growth hormone* (GH), Testosterone, estrogeni.

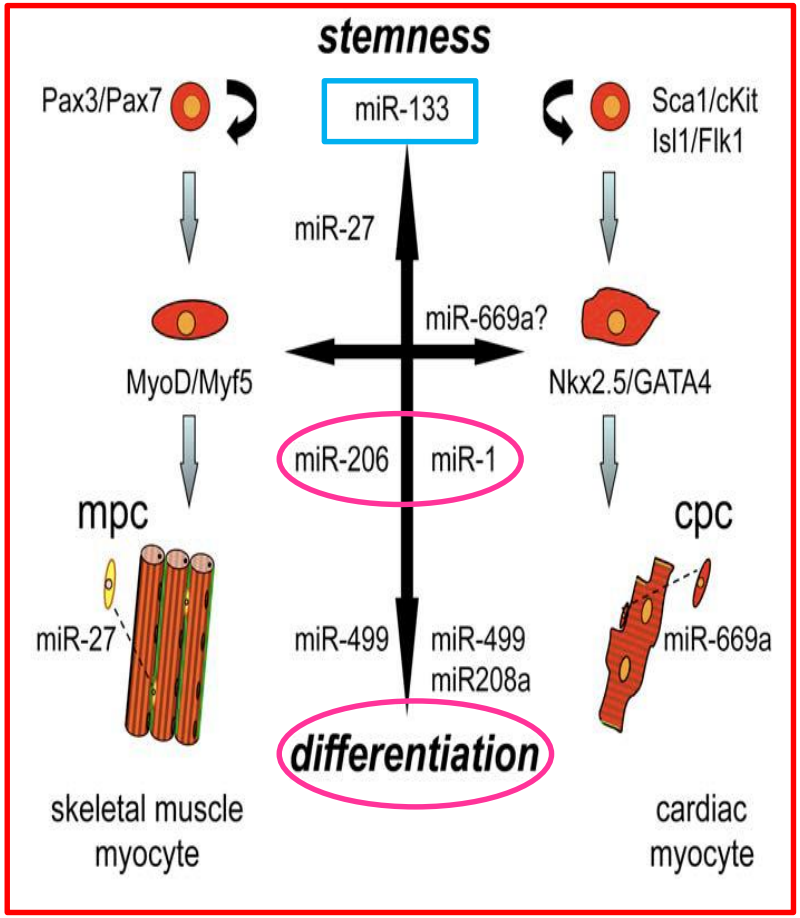
microRNAs

1718 *Current Pharmaceutical Design*, 2012, *18*, 1718-1729

Role of miRNAs in Muscle Stem Cell Biology: Proliferation, Differentiation and Death

Stefania Crippa[2], Marco Cassano[2] and Maurilio Sampaolesi[1,2,*]

MiRNAs are short non-coding RNAs (~22nucleotides), involved in the regulation of gene expression post-transcriptionally

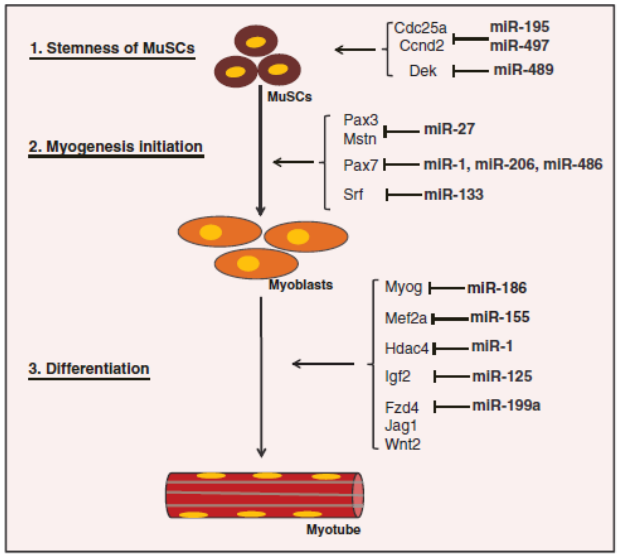


The first miRNA was discovered in **1993** from genetic studies of worm development

The Art of MicroRNA Research

Eva van Rooij

Abstract: **Originally identified as moderate biological modifiers, microRNAs have recently emerged as powerful regulators of diverse cellular processes with especially important roles in disease and tissue remodeling. The rapid pace of studies on microRNA regulation and function necessitates the development of suitable techniques for measuring and modulating microRNAs in different model systems. This review summarizes experimental strategies for microRNA research and highlights the strengths and weaknesses of different approaches. The development of more specific and sensitive assays will further illuminate the biology behind microRNAs and will advance opportunities to safely pursue them as therapeutic modalities. (*Circ Res*. 2011;108:219-234.)**



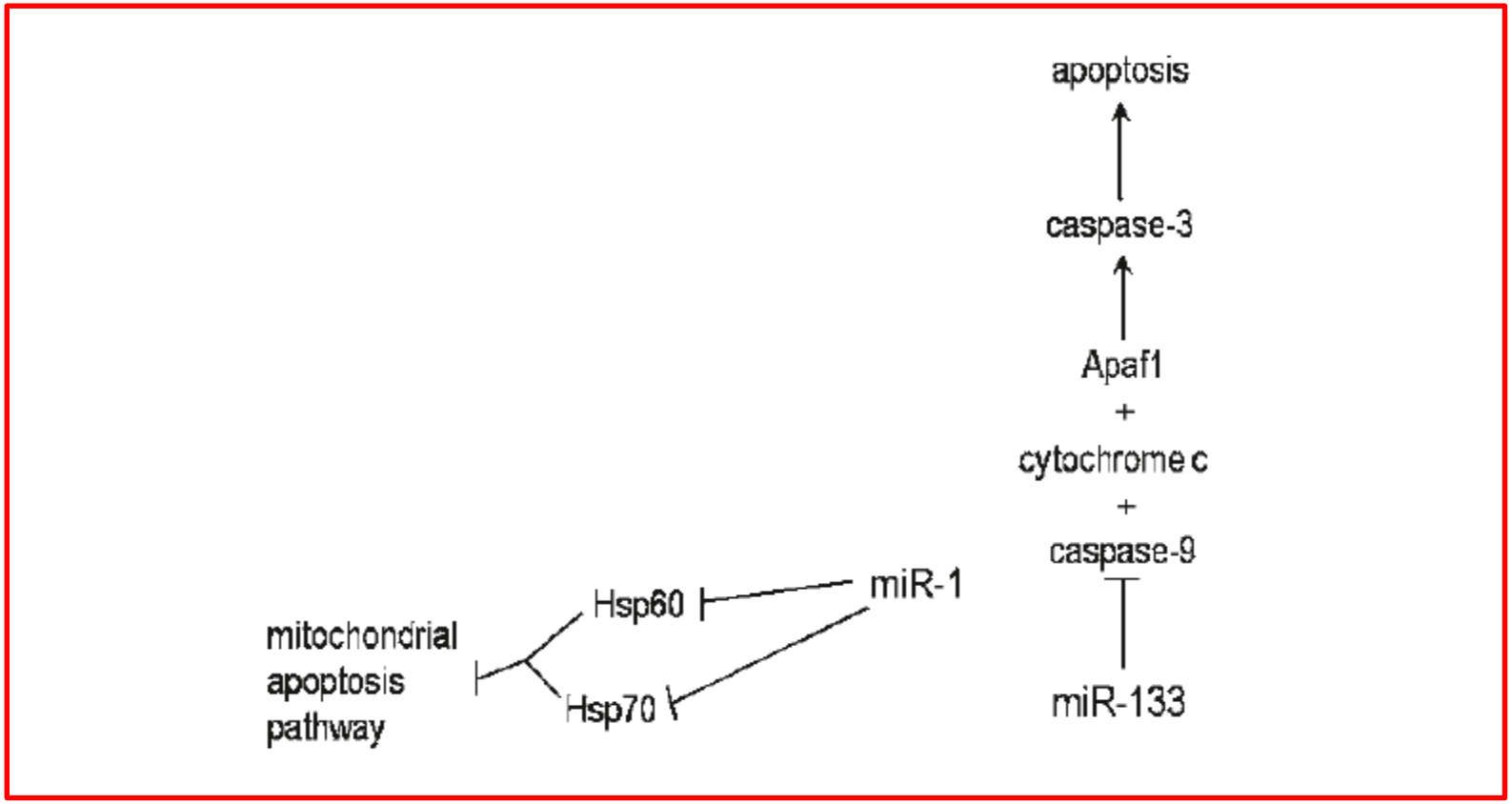
Regulation of Skeletal Muscle by microRNAs

Gabriela Placoná Diniz[*1,2] *and Da-Zhi Wang*[2,3]

ABSTRACT

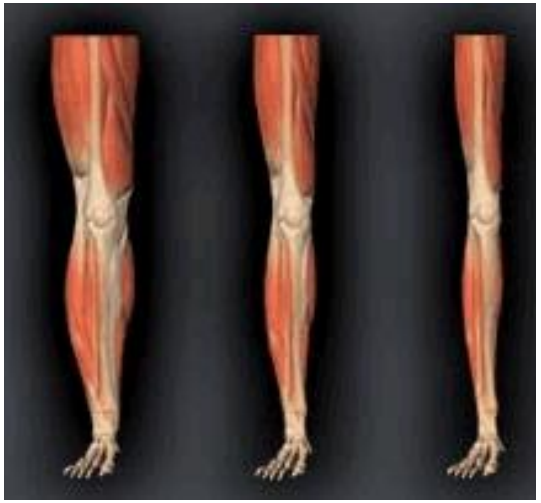
MicroRNAs (miRNAs) are a class of small noncoding RNAs highly conserved across species. miRNAs regulate gene expression posttranscriptionally by base pairing to complementary sequences mainly in the 3′-untranslated region of their target mRNAs to induce mRNA cleavage and translational repression. Thousands of miRNAs have been identified in human and their function has been linked to the regulation of both physiological and pathological processes. The skeletal muscle is the largest human organ responsible for locomotion, posture, and body metabolism. Several conditions such as aging, immobilization, exercise, and diet are associated with alterations in skeletal muscle structure and function. The genetic and molecular pathways that regulate muscle development, function, and regeneration as well as muscular disease have been well established in past decades. In recent years, numerous studies have underlined the importance of miRNAs in the control of skeletal muscle development and function, through its effects on several biological pathways critical for skeletal muscle homeostasis. Furthermore, it has become clear that alteration of the expression of many miRNAs or genetic mutations of miRNA genes is associated with changes on myogenesis and on progression of several skeletal muscle diseases. The present review provides an overview of the current studies and recent progress in elucidating the complex role exerted by miRNAs on skeletal muscle physiology and pathology. *Compr Physiol* 6:1279-1294, 2016.

miRNA, APOPTOSIS AND SARCOPENIA



Adult Stem Cells
Donald G. Phinney editor

Sarcopenia



↓ Massa muscolare

↓ Funzionalità muscolare

↓ Forza muscolare

↓

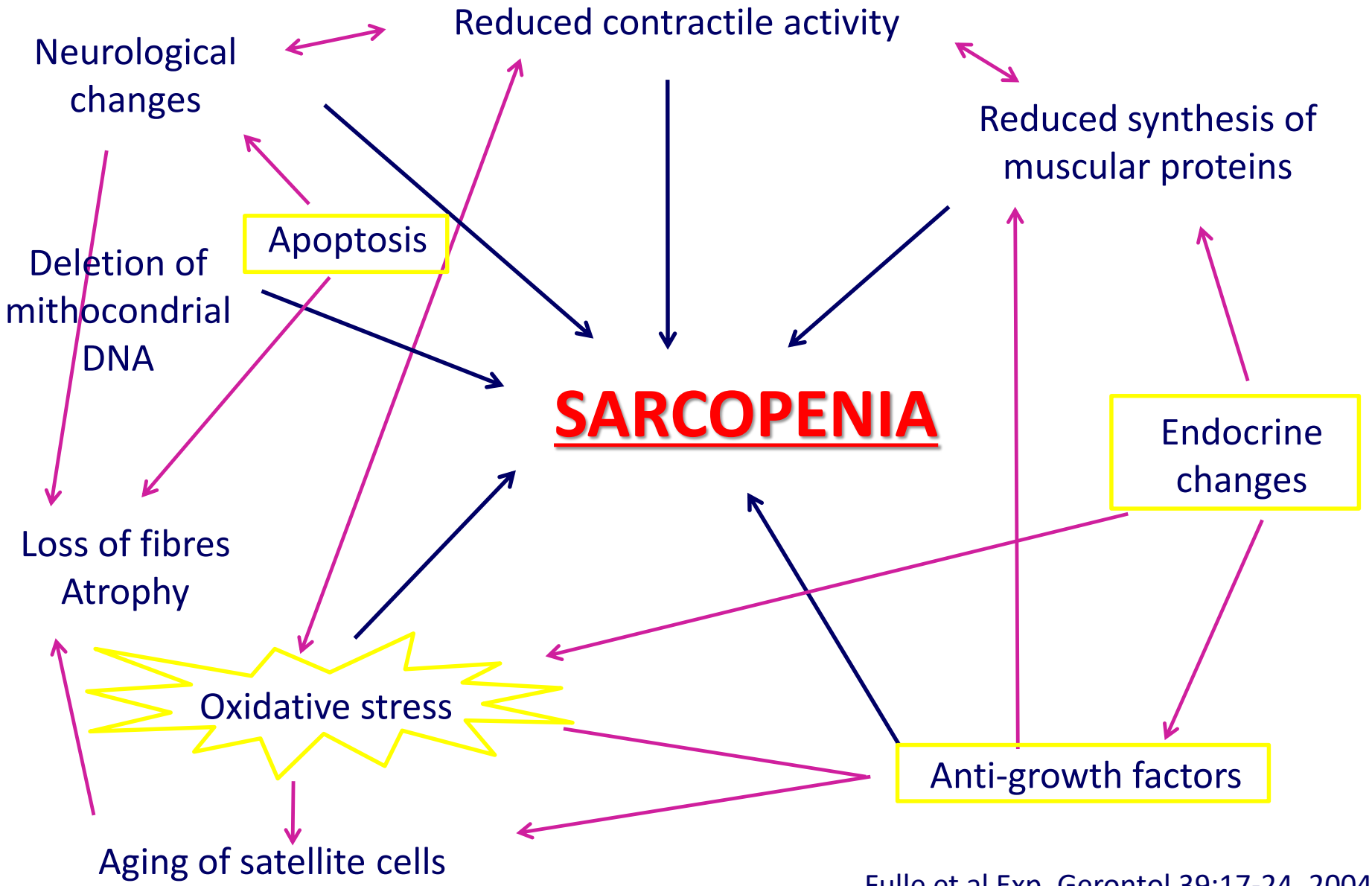
In funzione dell' <u>età</u>

Questa condizione è caratterizzata da uno stato metabolico in cui il muscolo presenta una riduzione della capacità sia di produrre che di sfruttare energia.
La sarcopenia è stata considerata come una conseguenza di alcuni processi multifattoriali tra cui:

- *<u>fattori intrinseci</u>*, quali la diminuzione del numero degli α-motoneuroni, la produzione di GH e la diminuzione dei livelli degli steroidi sessuali;
- *<u>fattori estrinseci</u>* o ambientali come lo scarso esercizio fisico, l'immobilizzazione di un arto, l'alimentazione;

Nell'uomo, passando dai 20 agli 80 anni di età, la massa muscolare decresce circa del 40% con degli effetti negativi sulla mobilità, forza, velocità metabolica e funzione respiratoria

AGING EFFECT ON SKELETAL MUSCLE



Fulle et al Exp. Gerontol 39:17-24, 2004
Pietrangelo et al Exp. Gerontol 44:523-531, 2009

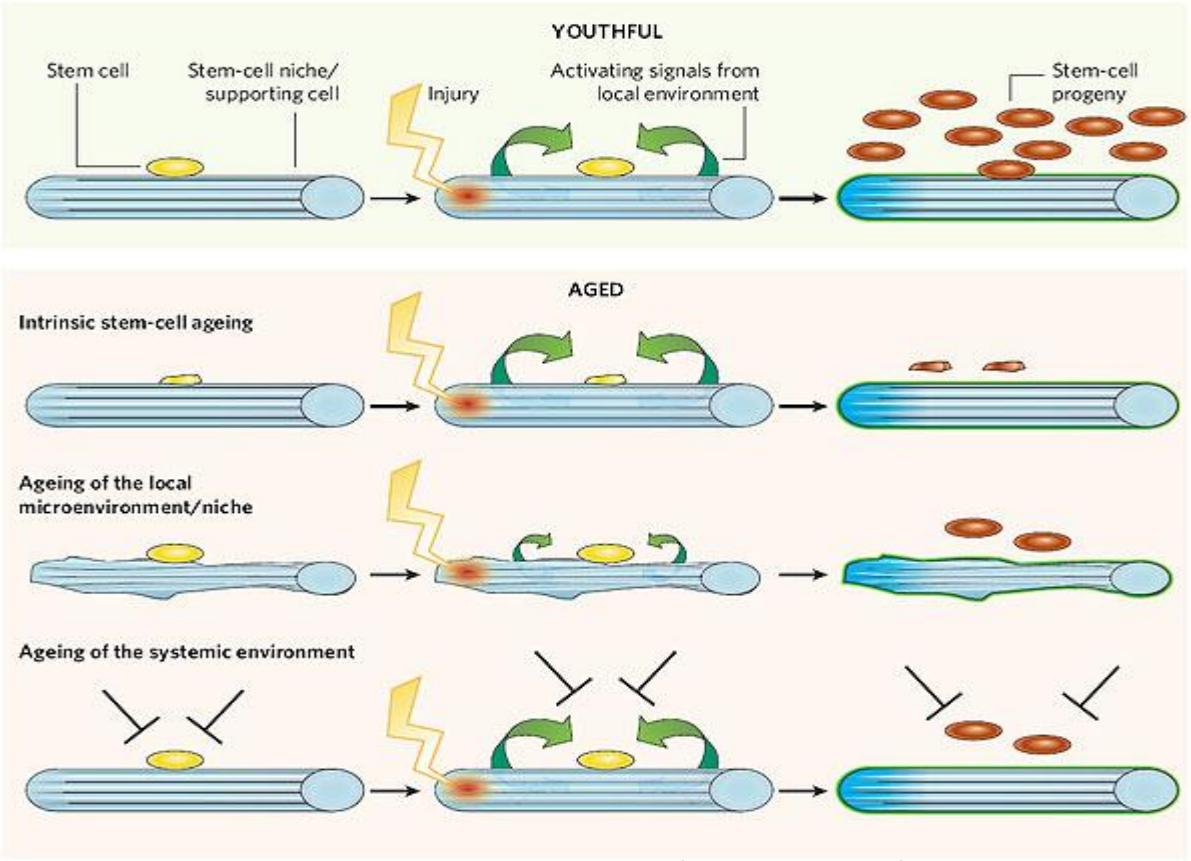
AGE-DEPENDENT CHANGES IN HUMAN SATELLITE CELLS

SARCOPENIA

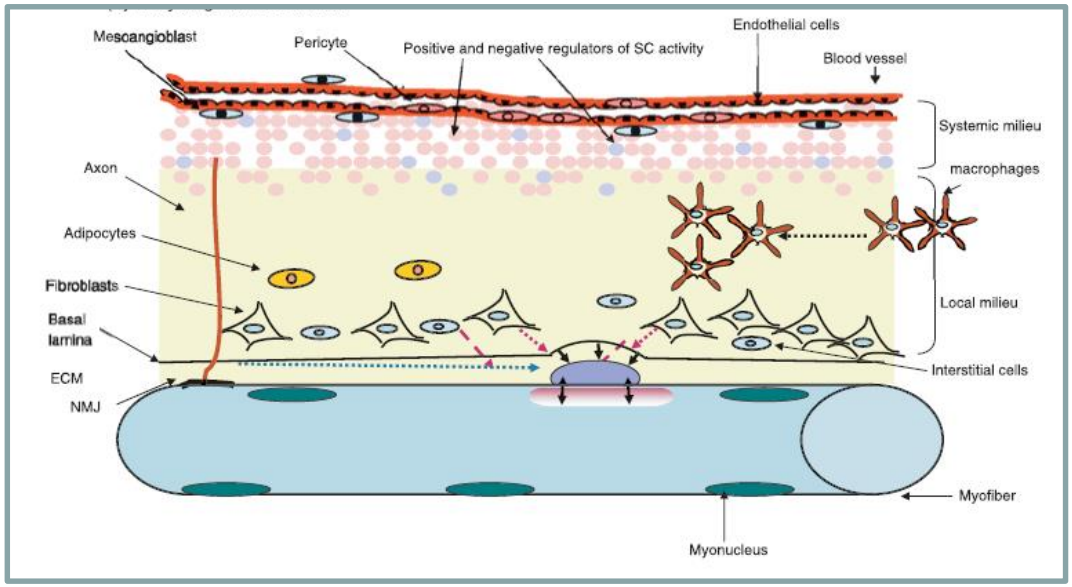
- Intrinsic factors involving changes at molecular and cellular levels
- Extrinsic or environmental factors such as nutrition and exercise

The are many changes at cellular level that contribute to sarcopenia:

In the satellite-cell niche or in the systemic milieu could all result in a diminished functionality of satellite cells in an aged organism, manifested as a decreased propensity to generate sufficient functional progeny for effective regeneration.

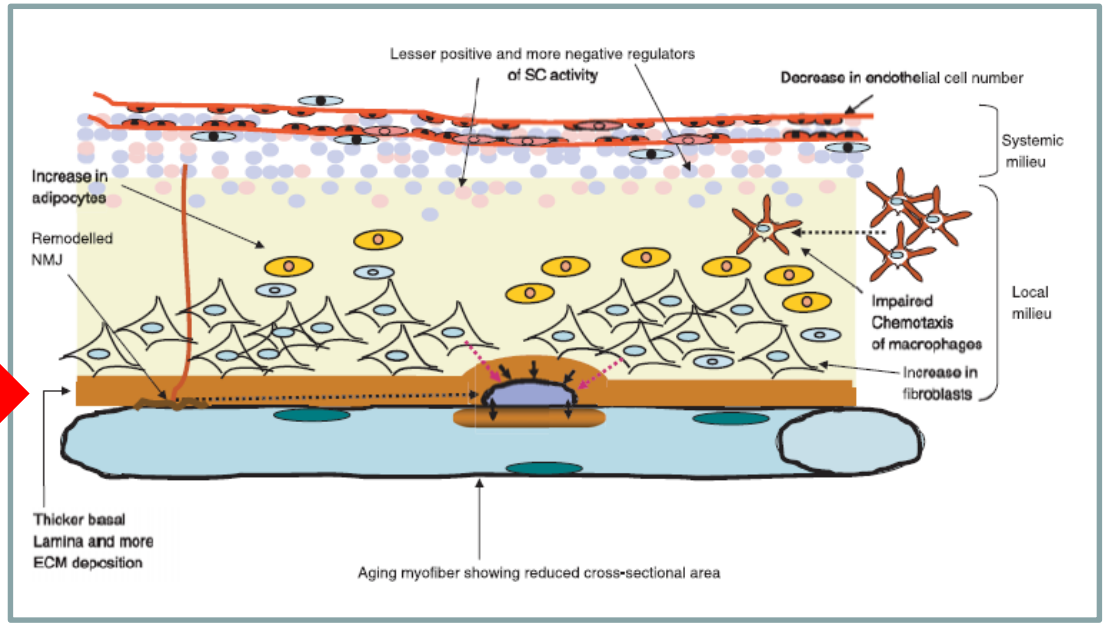


Thomas A. Rando, Nature, 2006



Young satellite cell niche

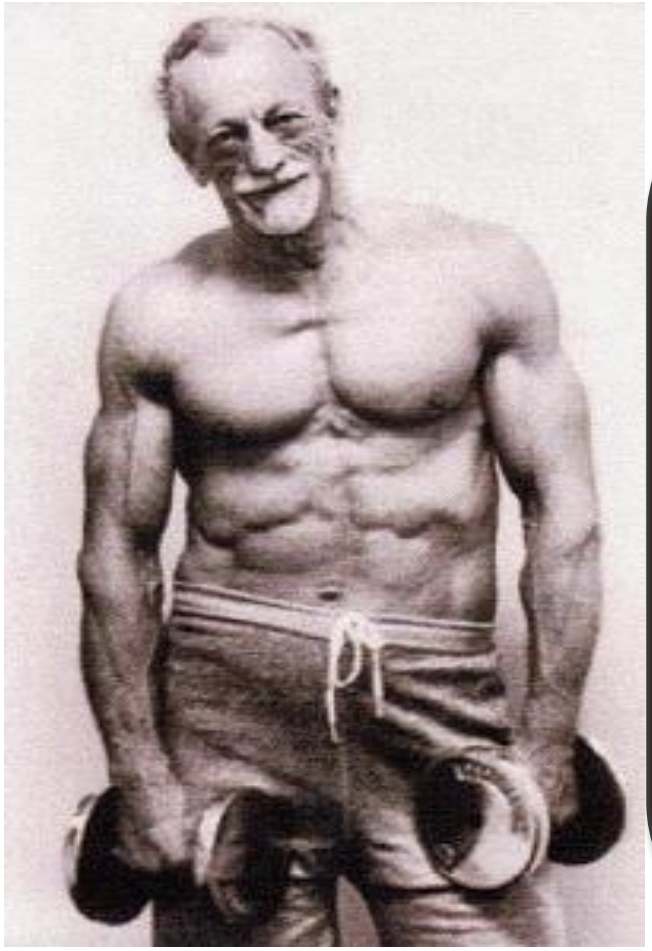
Old satellite cell niche



Gopinath and Rando Aging Cell, 2008

✓Could specific and intensity-graduated training/therapy slows down or contrasts the Sarcopenia progression?

✓And which is the better countermeasures?



AIM of the study

- **to identify the training protocol able to increase the strength of inferior limbs of elderly subjects**

- **to analyse the cellular and molecular mechanisms that increase the strength of inferior limbs of elderly subjects during each training.**

At the beginning and at the end (and at the follow up) of training/stimulation period:

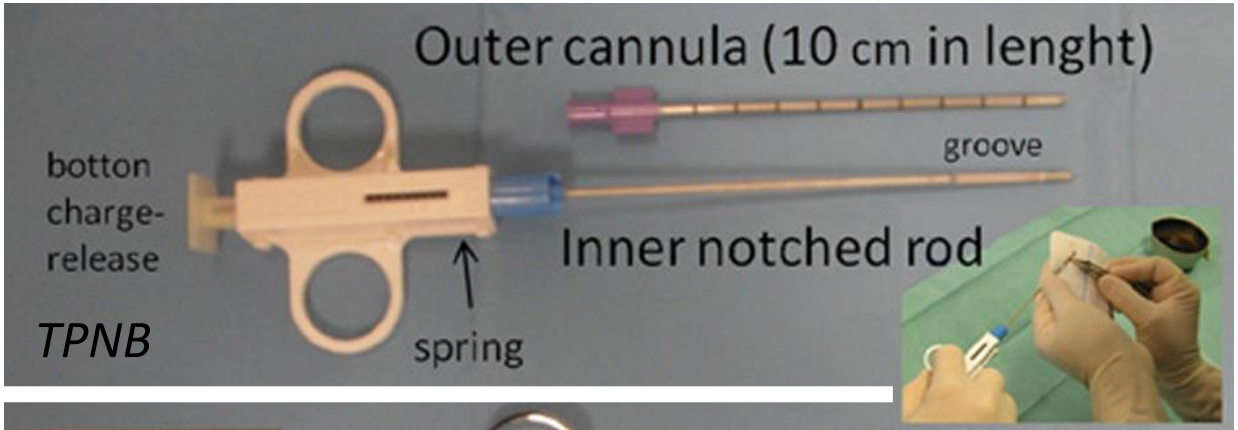
a. Antropometric measurements

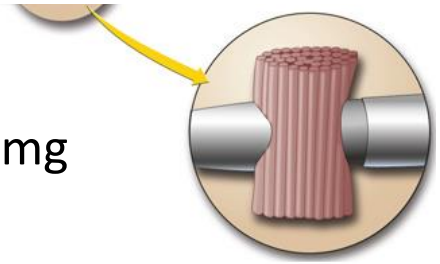
b. Functional evaluation of lower limbs

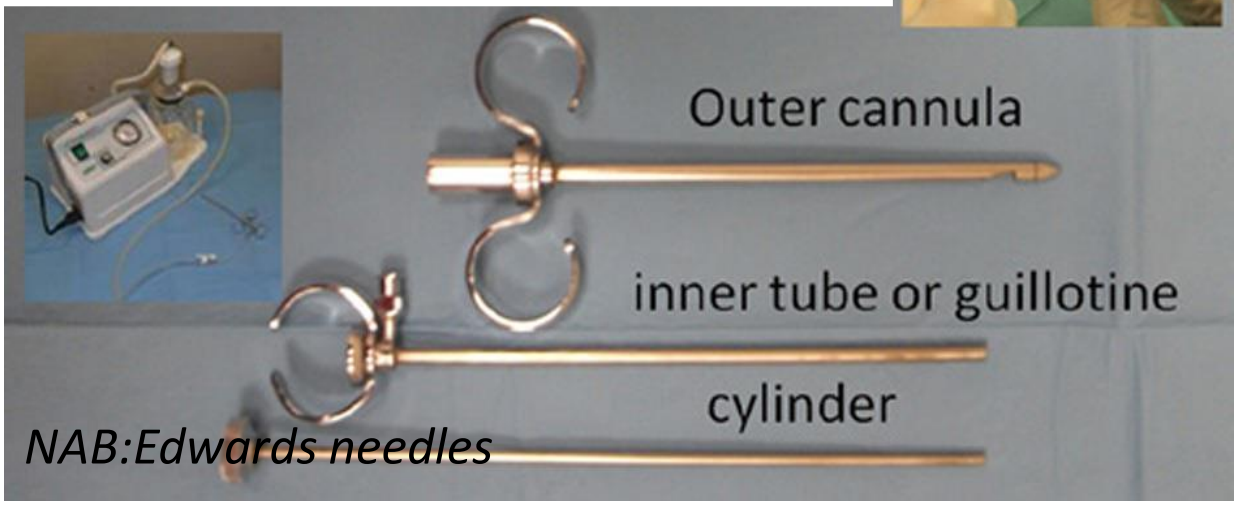
c. Needle biopsies to collect fibers, satellite cells, proteins and RNA

TINY PERCUTANEOUS NEEDLE BIOPSY (TPNB): A NEW APPROACH

Pietrangelo T. et al., *International Journal Molecular Medicine 27: 361-367, 2011*
Tiny percutaneous needle biopsy: An efficient method for studying cellular and molecular aspects of skeletal muscle in humans





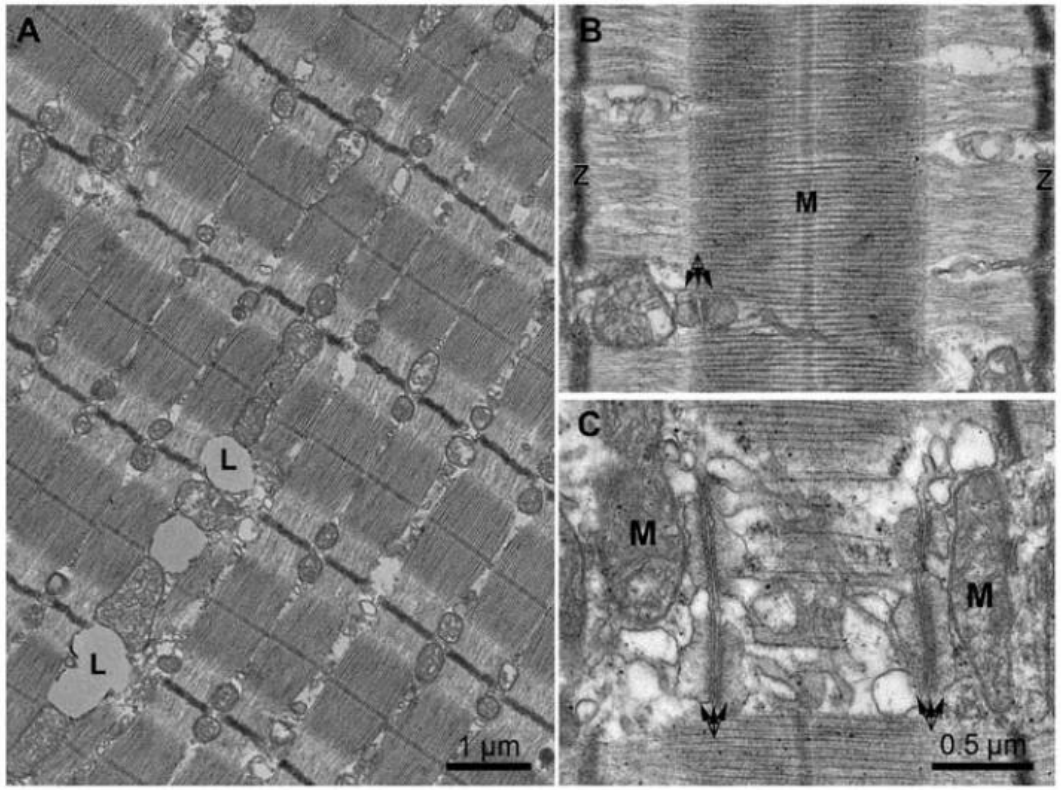


Each sample is a cylinder approximately 0.9-1.1 mm in diameter, with a cross-sectional area of approximately 0.6 mm^2 and a length of about 8-10 mm

INTERNATIONAL JOURNAL OF MOLECULAR MEDICINE 32: 965-970, 2013

A method for the ultrastructural preservation of tiny percutaneous needle biopsy material from skeletal muscle

TIZIANA PIETRANGELO[1], STEFANO PERNI[2], GUGLIELMO DI TANO[1], GIORGIO FANÒ-ILLIC[1] and CLARA FRANZINI-ARMSTRONG[2]



Ultrastructure of a sample from a human vastus lateralis (VL) muscle obtained by TPNB discarded from the needle into a 'K acetate' solution. (A) Low magnification image of the regular striation pattern of the fibers, no sign of local contractures and lipid droplets (L). (B) Details of sarcomeres as well aligned bands, a straight Z line and a prominent M line (M). (C) Architecture of the membrane components with the appropriate filament orientation at the edges of the A band (triple arrows indicate a triad) and the mitochondria (M) appropriately positioned. (A and B) Donor 66 years old; (C) donor 32 years old.

NEEDLE BIOPSY

SATELLITE CELLS FIBERS

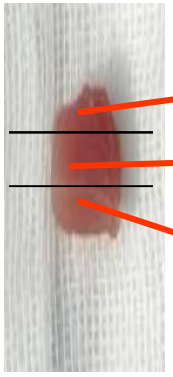
FUNCTIONAL STUDY OF SKELETAL MUSCLE

RNA AND PROTEIN

MOLECULAR ANALYSIS

pre-training

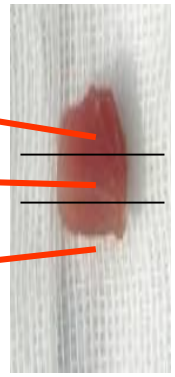
post-training



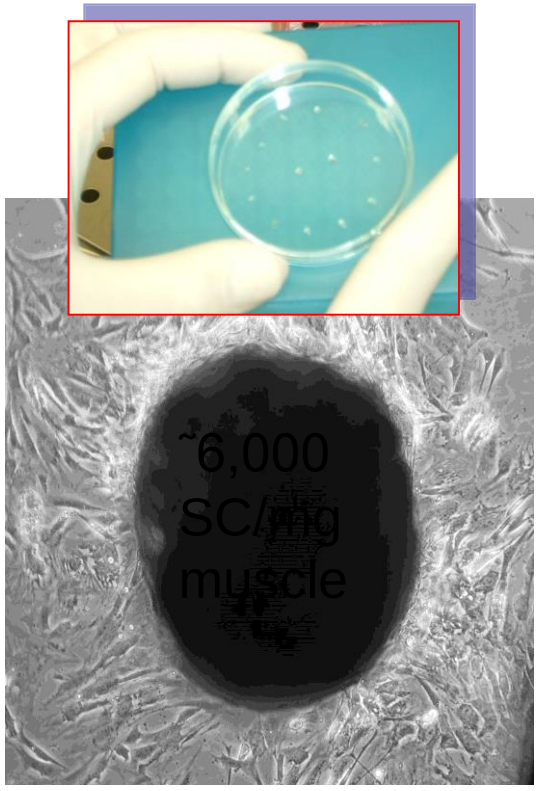
Myogenesis

Analysis on single fiber

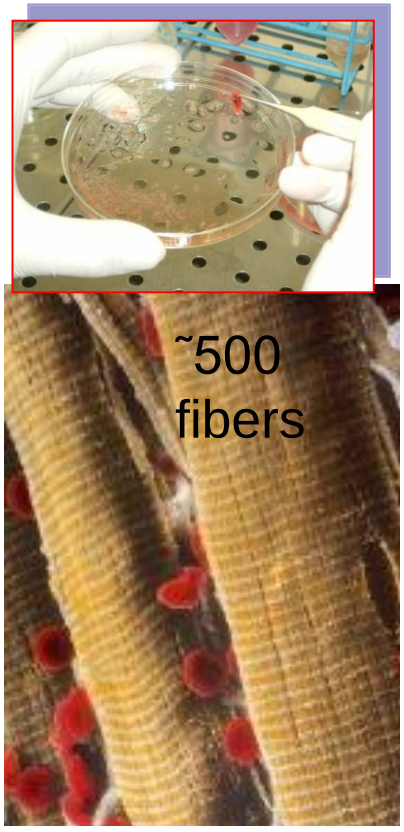
Transcriptional profile



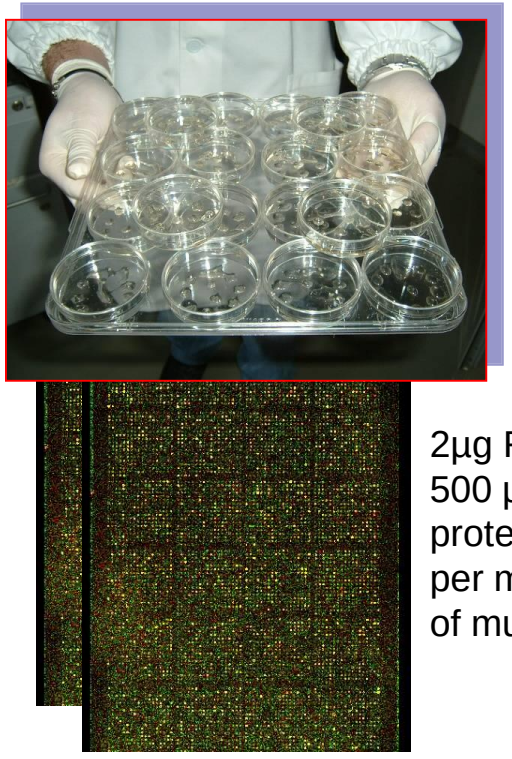
myogenesis



dissociated fibers

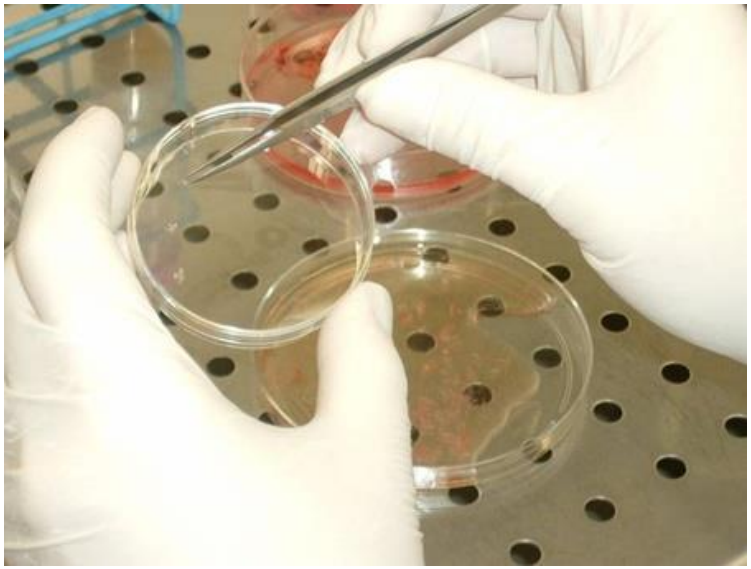


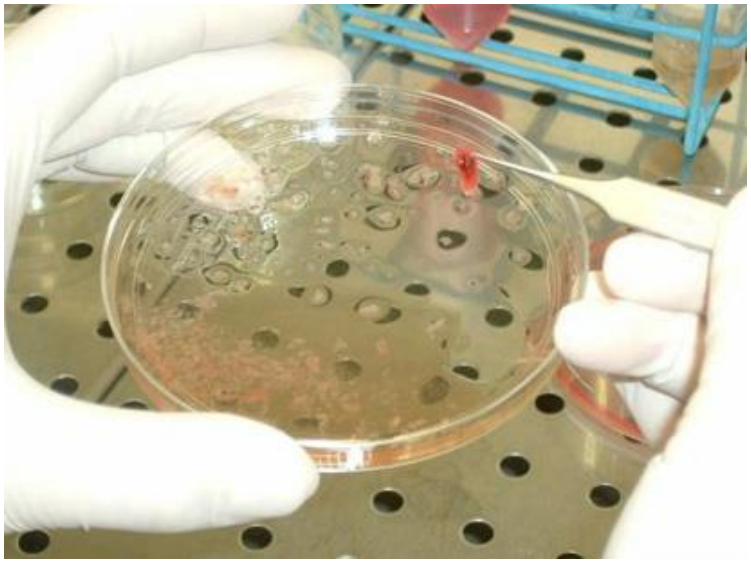
transcriptoma/ proteoma



2µg RNA, 500 µg proteins per mg of muscle

MUSCLE EXPLANT CULTURES





- Biopsies derived from *Vastus Lateralis* muscle were obtained from healthy subjects underwent voluntary biopsy
- Muscle biopsies were processed according to the procedure of Fulle et al.,(2005)

Muscle explant cultures

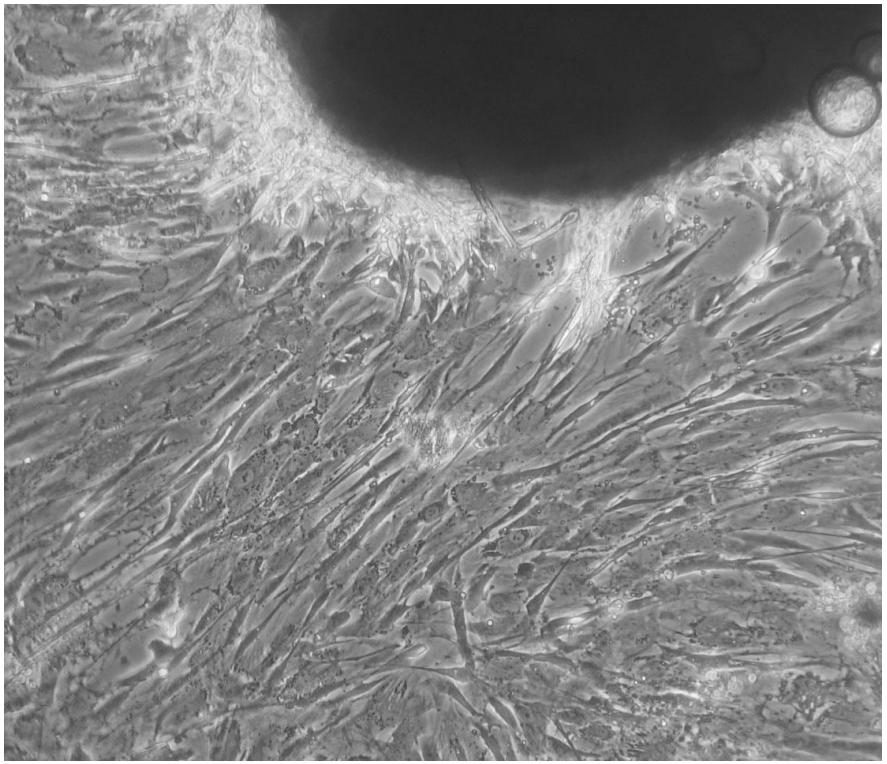
Biopsies from *Vastus Lateralis* (VL) or *Glutaeus Medius* (GM) muscles were obtained from healthy patients that undewent elective orthopedic surgery (after informed consent), and that were non-trained individuals (kindly provided by Dott. Luigi D'Amelio).

Samples were from males and females of different ages divided in 3 groups:

- Adult youngs (from 28 to 48 years old)
- Old individuals (from 69 to 87 years old)
- A five days-old infant (CHQ5B)

Myoblasts from the infant were obtained from an autopsy of quadriceps muscle kindly provided by the Butler-Browne group of Faculté de Médicine Pitié-Salpêtière de Paris.

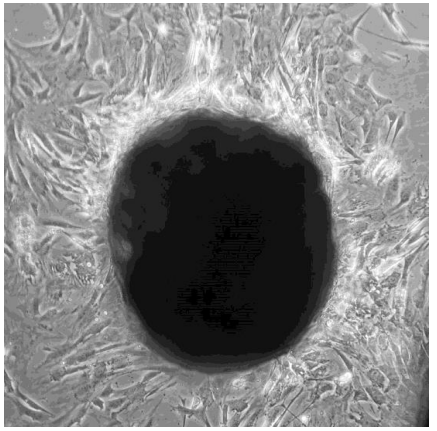
Biopsies (0.1-0.4g of muscle) were immediately minced to get explants that were cultured as described by Decary *(Decary et al., 1996)*.
The first mononucleated cells migrated out of the explants seven to thirteen days after the beginning of the culture independently by donor age.



Explant obtained from a 87 years old healty woman's *Vastus Lateralis* muscle.

MUSCLE EXPLANT CULTURES

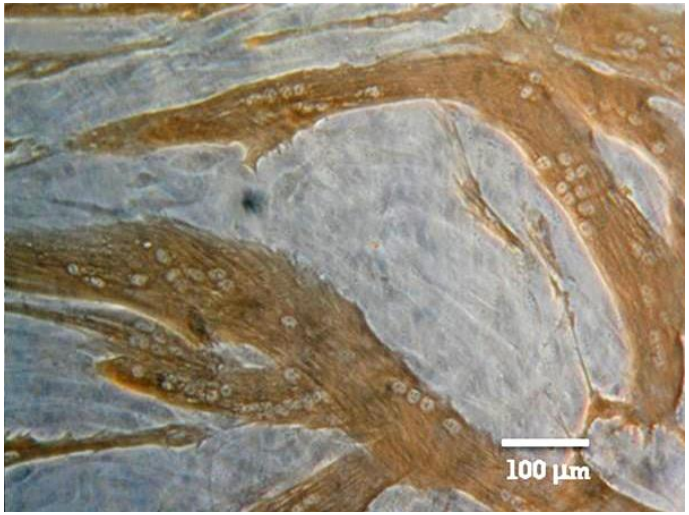
The first mononucleated cells migrated out of the explants within 7-13 days from the beginning of culture



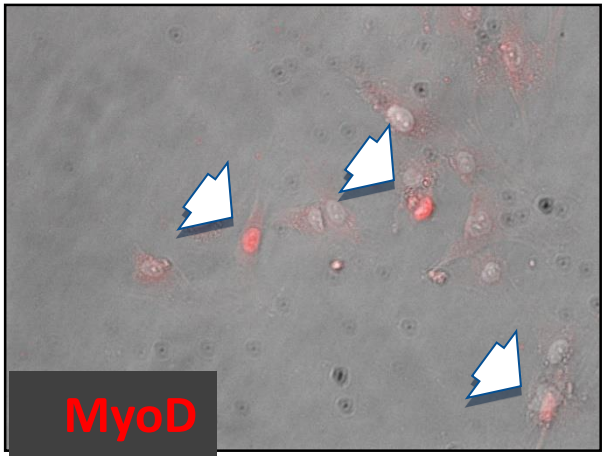
CHARACTERIZATION OF CELL POPULATIONS

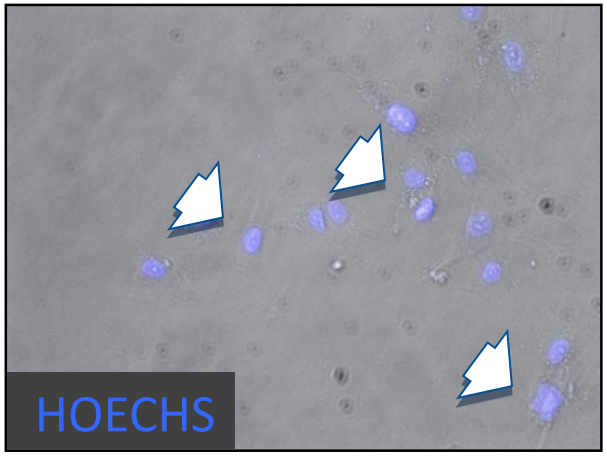
Desmin (myoblasts)

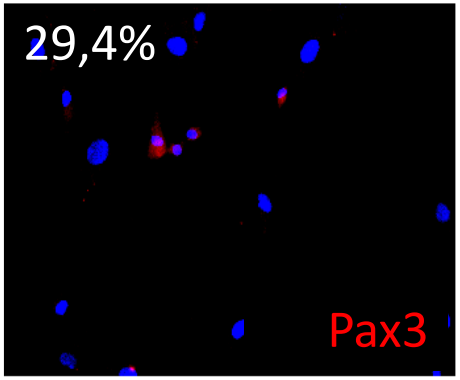
Myosin heavy chains (myotubes)

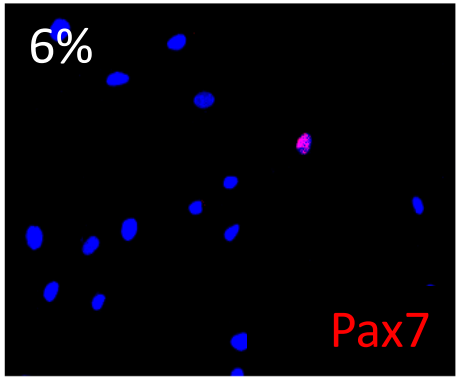


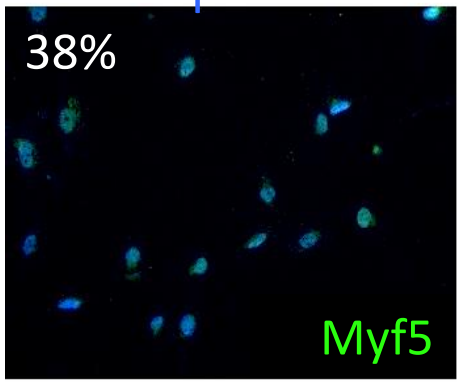
MYOGENIC REGULATORY FACTORS

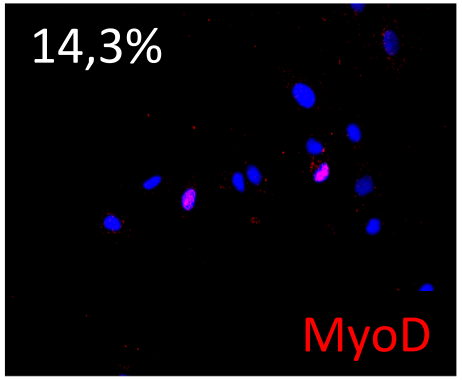


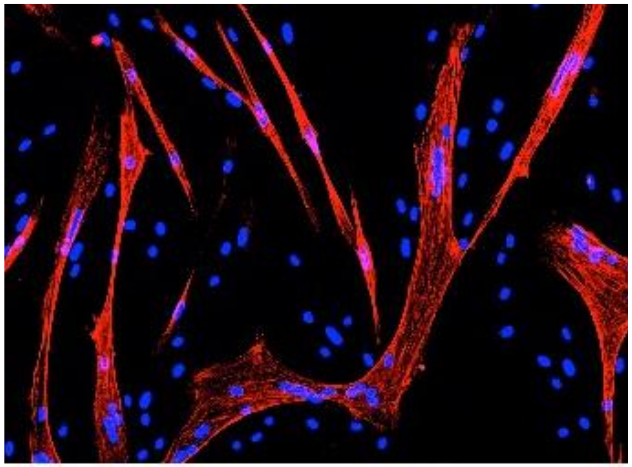






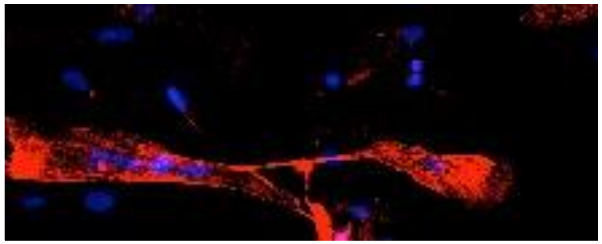




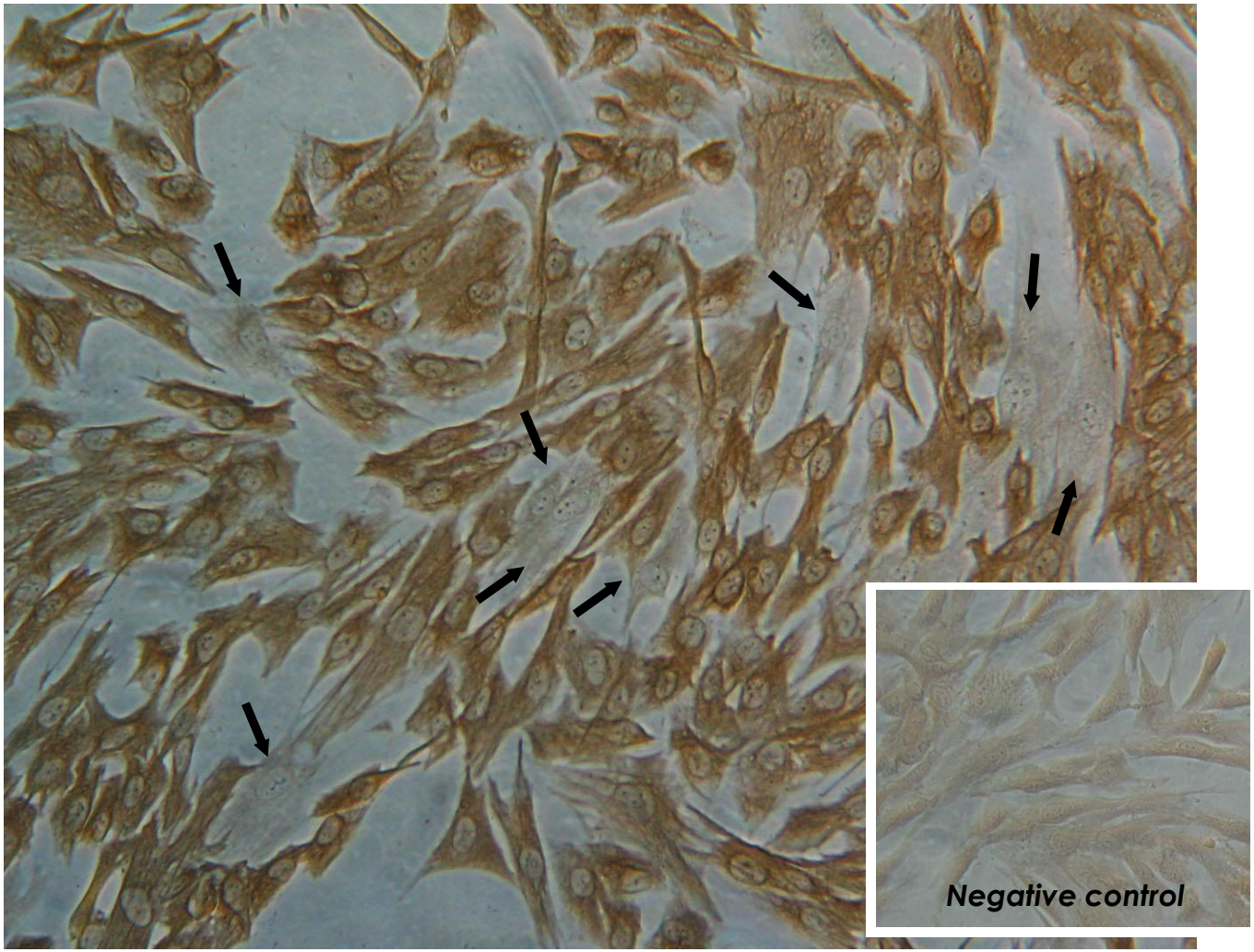


FI=51%

MyHC



Myogenic Purity



CHQ5B sample
Example of Desmin-positive (brown) and Desmin-negative (pale grey) cells.
Revealation by biotin-streptavidin complex method.

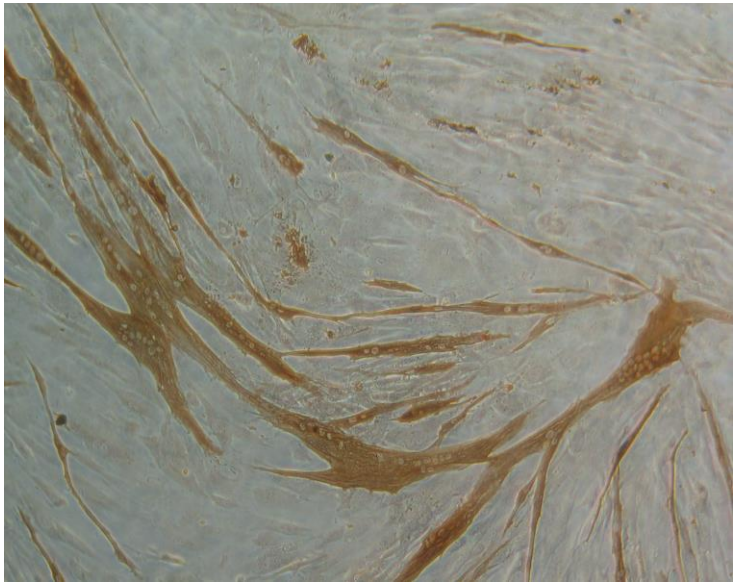
The myogenic purity of each satellite cell culture was estimated using Desmin antibody D33 (Dako, diluition 1:50) as marker *(Behr et al.1994; Kaufman, Foster, 1988;)*.
Desmin is a cytoskeletal intermediate filament protein early expressed in myogenic cell populations.

Fusion Index

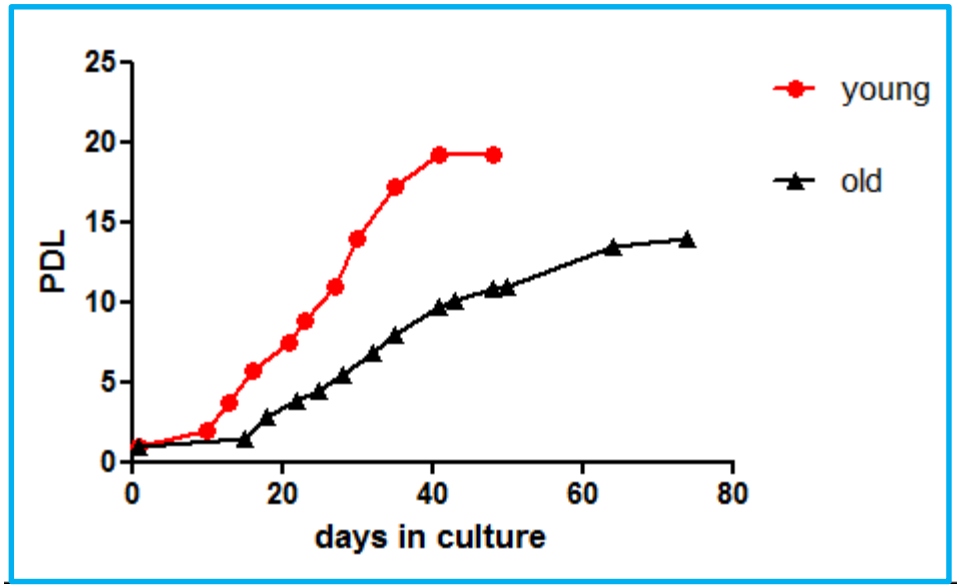
Sample	Miogenic purity	Fusion Index%
CHQ5B	92.3%	85
VL28M	49%	40
VL34M	15%	
VL29M	40%	73
GM48M	40%	42
GM58M	13.6%	
GM69M	60%	45
GM71M	15%	17
VL73F	53%	40
GM76M	37.3%	48
VL81F	76%	22
VL87F	60%	20

The efficiency of differentiation was determined by counting the number of nuclei in differentiated myotubes as percentage of the nuclei total number. The fusion index was calcutated in 7 days differentiated myotubes. The table shows a decrease in the value during ageing; whereas the number of myogenic cells does not change during ageing, the capacity of these cells to fuse with each other seems to decrease

A 7 days differentitated myotube; cells from 48 years old man; example of MF20-positive (brown) and MF20-negative (pale grey) cells. Revealation by biotin-streptavidin complex method.



Life Span

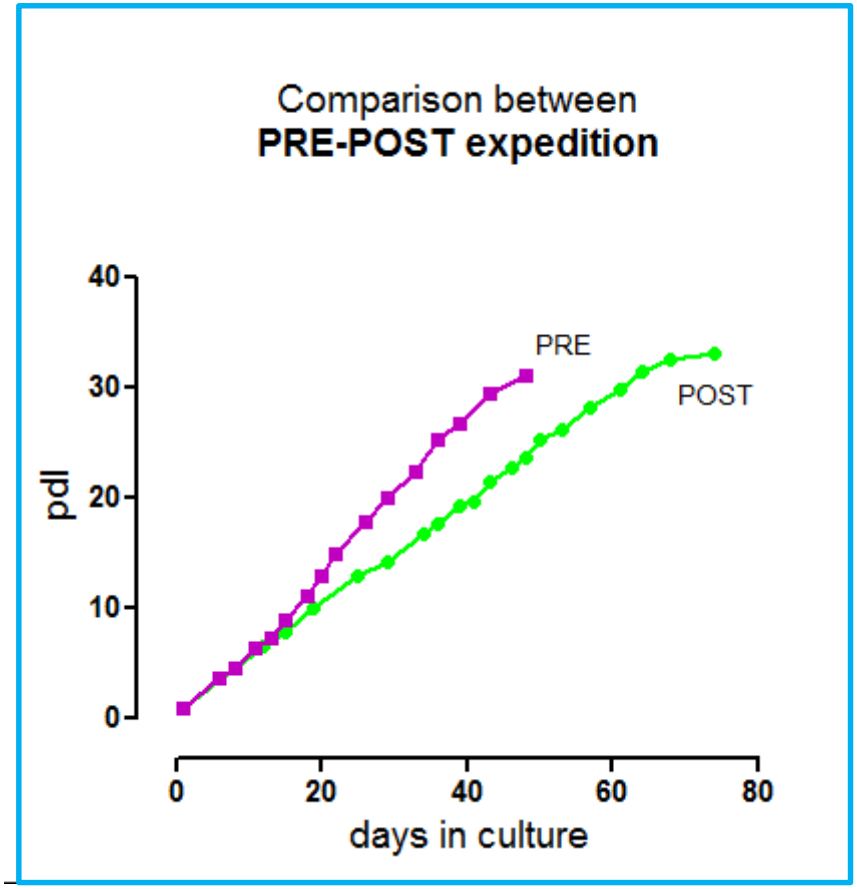


Proliferative life span of human satellite cells: cell populations isolated from young and old donors. At each passage the Population Doubling Level was calculated by counting the number of cells.

CASE REPORT

Climber exposed
to
high altitude

Life Span

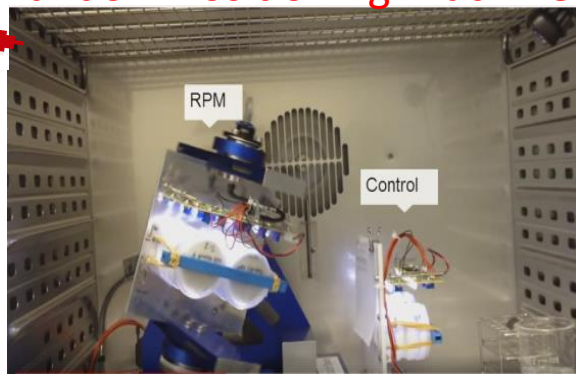

Comparison between
PRE-POST expedition
pdl
40
30
20
10
0
PRE
POST
0
20
40
60
80
days in culture

Tiny percutaneous needle biopsy

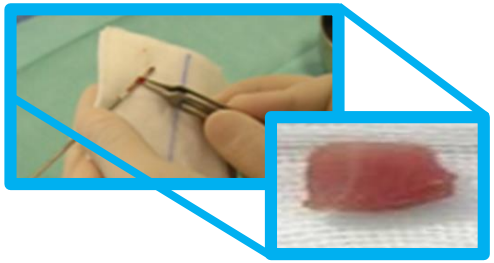
Int J Mol Med. 2011 Mar;27(3):361-7. doi: 10.3892/ijmm.2010.582

Simulazione a terra degli effetti subiti nello spazio

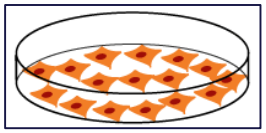
Random Positioning Machine



Biopsie muscolari da *Vastus Lateralis* di soggetti sani



Cellule Satelliti del muscolo isolate



Con / senza Random Positioning

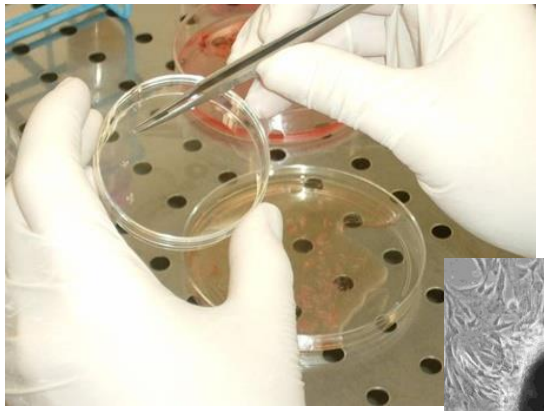
CELLULE SATELLITI **FIBRE**

STUDIO FUNZIONALE DEL MUSCOLO SCHELETRICO

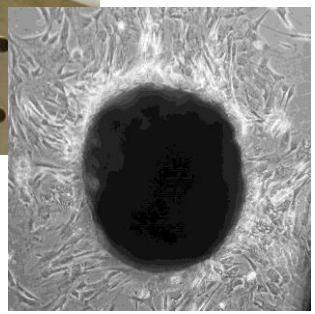
RNA e PROTEINE

ANALISI MOLECOLARE

CULTURE DI CELLULE SATELLITI dalla BIOPSIA MUSCOLARE

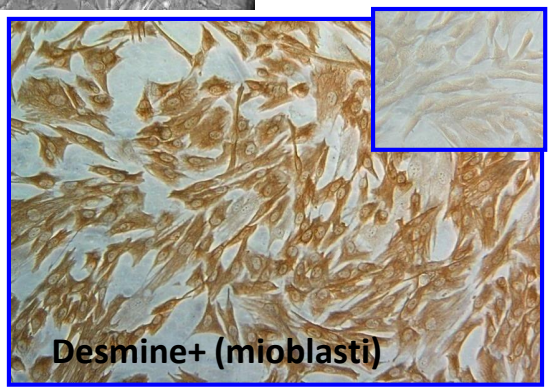


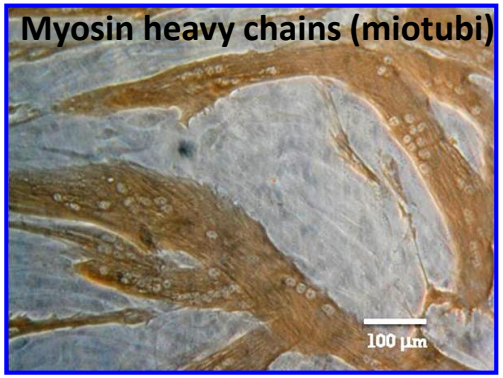
Espianto



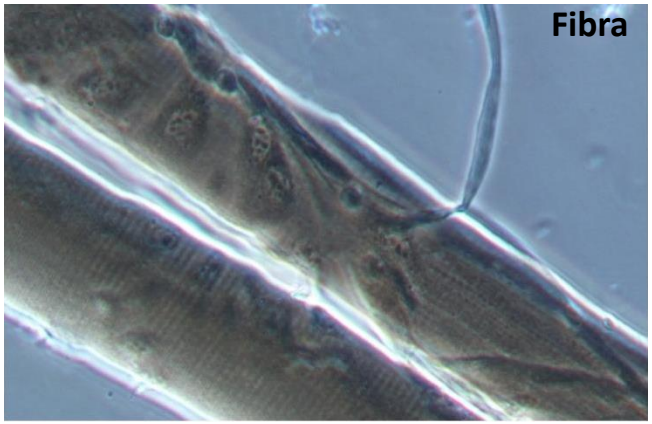
A TERRA
(condizioni normali)

Simulazione dell'effetto IN VOLO
(Random Positioning Machine)



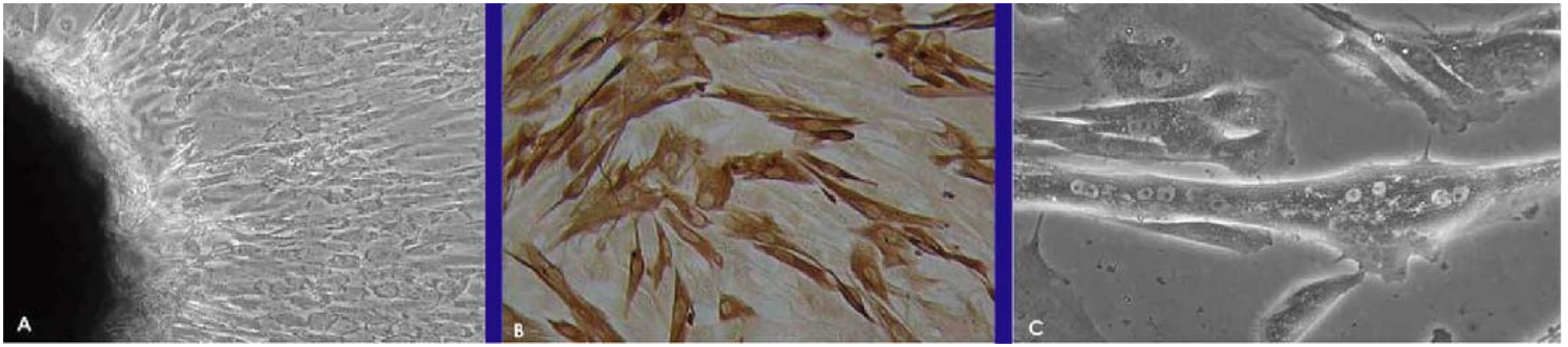


Caratterizzazione della popolazione cellulare e successiva analisi molecolare



Riassumendo……..

Human Satellite Cells



Satellite cells migrating from an explant

desmin positive satellite cells

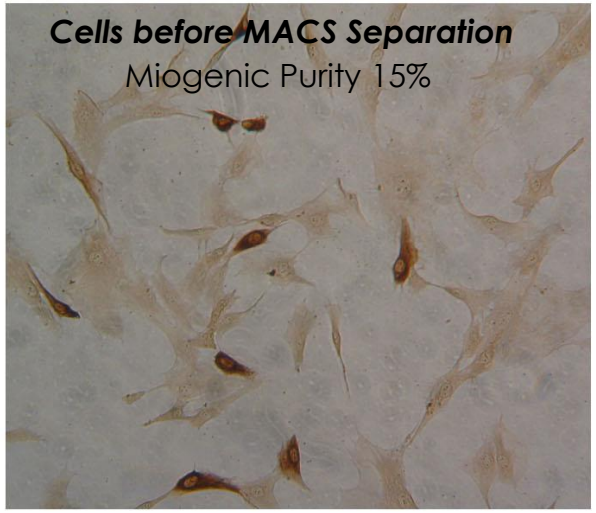
myotubes

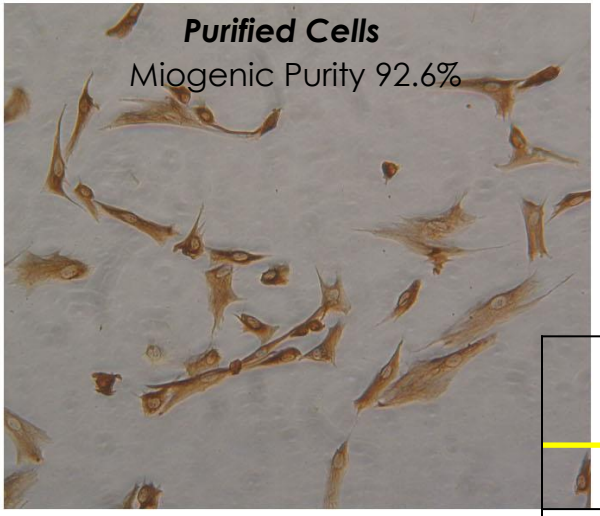
Biopsies from *Vastus Lateralis* muscle

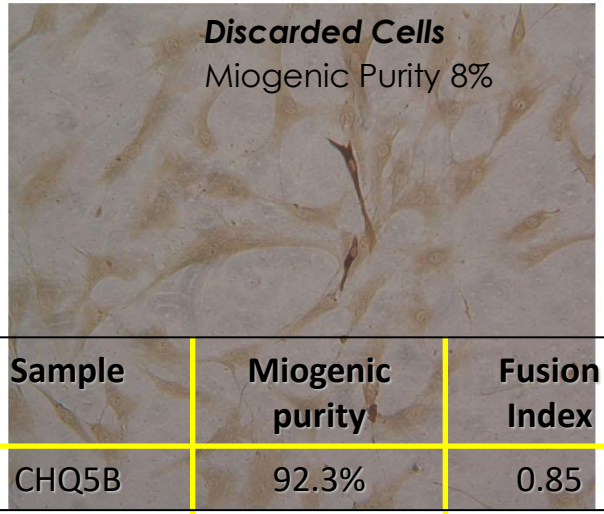
These cells are responsible for pre- and postnatal muscle growth

They are capable to differentiate to repair skeletal muscle fibres following injury

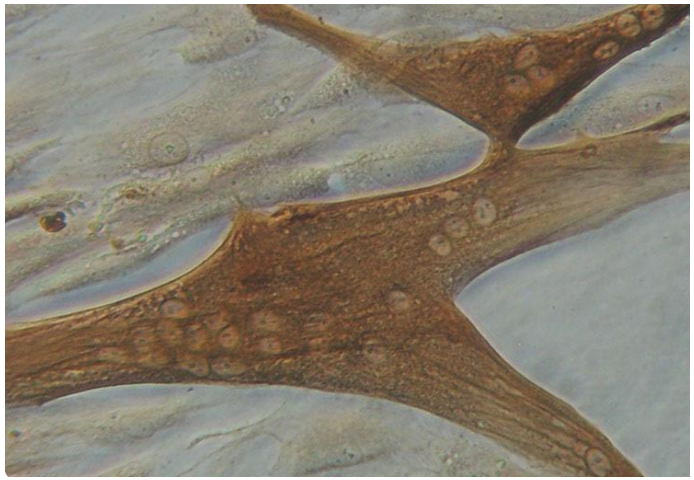
MACS separation tecnique







Cells from a GM - 71 years old man

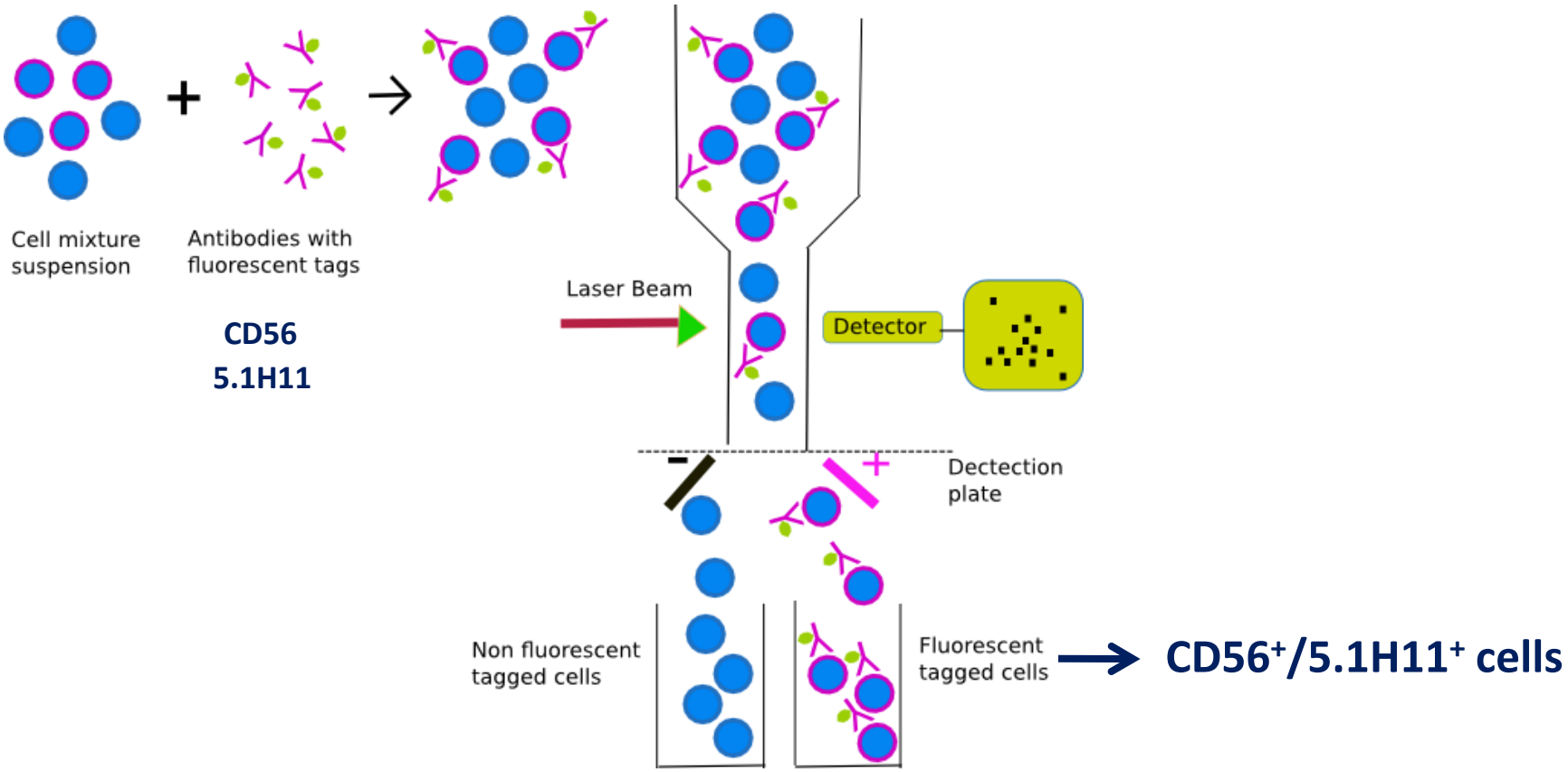


A 7 days differentiated myotube;
staining with MF-20

Sample	Miogenic purity	Fusion Index
CHQ5B	92.3%	0.85
VL28M	49%	0.40
Discarded cells	1.3%	
VL29M	40%	0.73
GM48M	40%	0.42
Discarded cells	8.7%	
GM69M	60%	0.45
Discarded cells	8%	
VL73F	53%	0.40
GM76M	37.3%	0.48
VL81F	76%	0.22
VL87F	60%	0.20

Fluorescence-activated cell sorting (FACS) analysis for myogenic markers

To enrich our cell cultures in myogenic precursors, specific primary antibodies for human myoblasts, **CD56** and **5.1H11** were used.




VI
ASPETTIAMO
IN
LABORATORIO
-INTERNATO di LABORATORIO
-TESI SPERIMENTALE
-CORSO DI FISIOLOGIA MOLECOLARE per i crediti a scelta