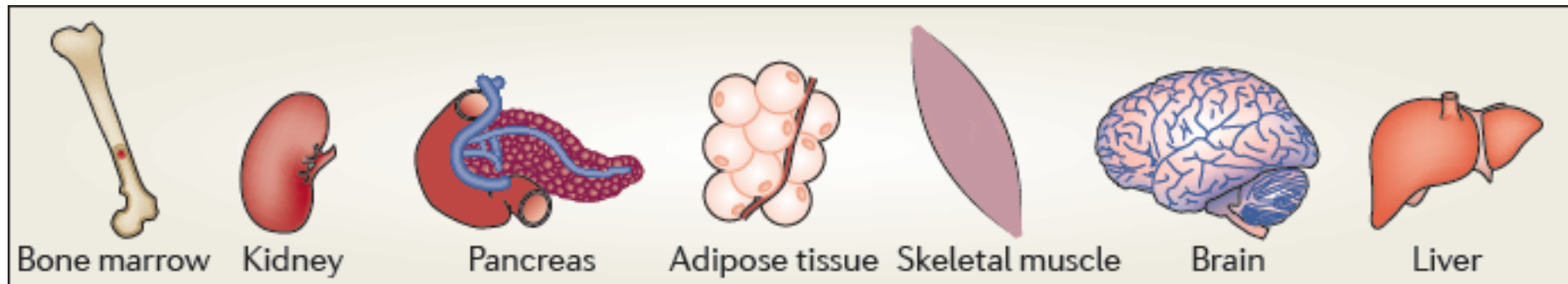
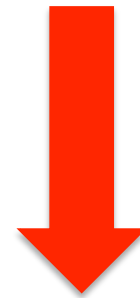


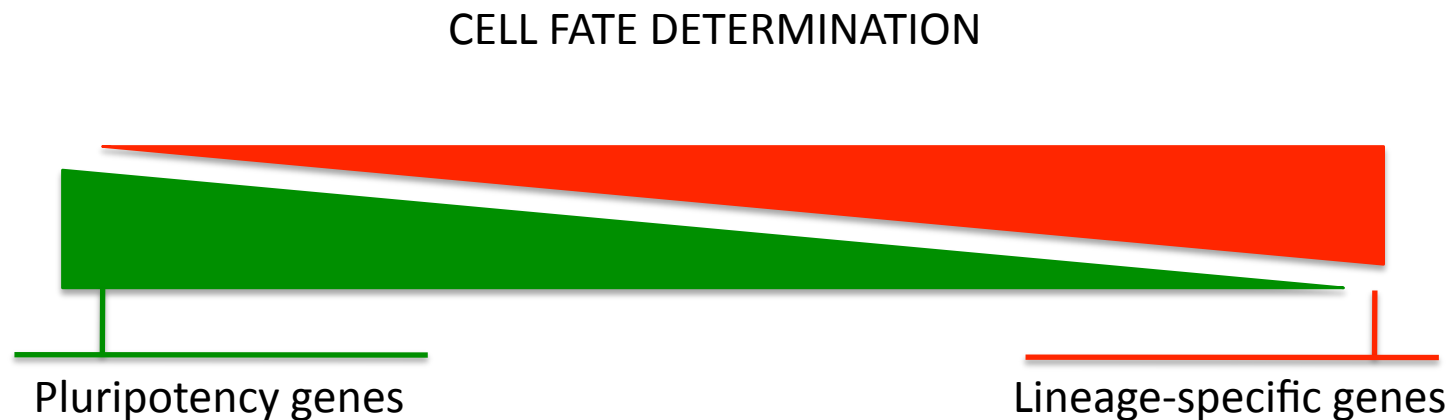
Riprogrammazione Cellulare & Medicina rigenerativa

Regenerative Medicine: We define "regenerative" the branch of medicine that aims to repair organs or tissues damaged by disease, trauma or "simple" aging. The goal is to restore the functioning of these organs, or at least improve it.

The perfect functioning of multicellular organism is pursuit by a progressive specialization of tissue functions, and this is guarantee by a huge amount of genes potentially expressed in a single cell.

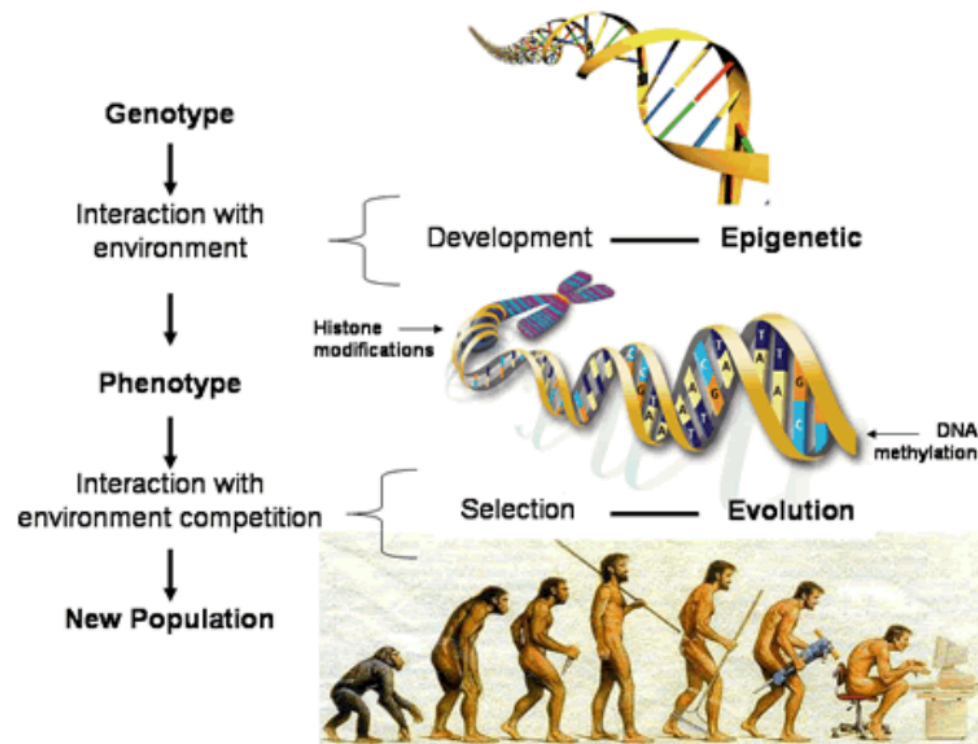


- ✓ In human genome, a subset of circa 30,000 genes is active in different tissues and organs, depending on their regulation by different sets or combinations of transcription factors to generate a more complex and orchestrated **cell specialization**. During development, **cell pluripotency** allows cells to differentiate into virtually **all cell types**. The progressive restriction of pluripotency is reflected by the selective activation of specific subsets of genes, which impart a defined cell lineage

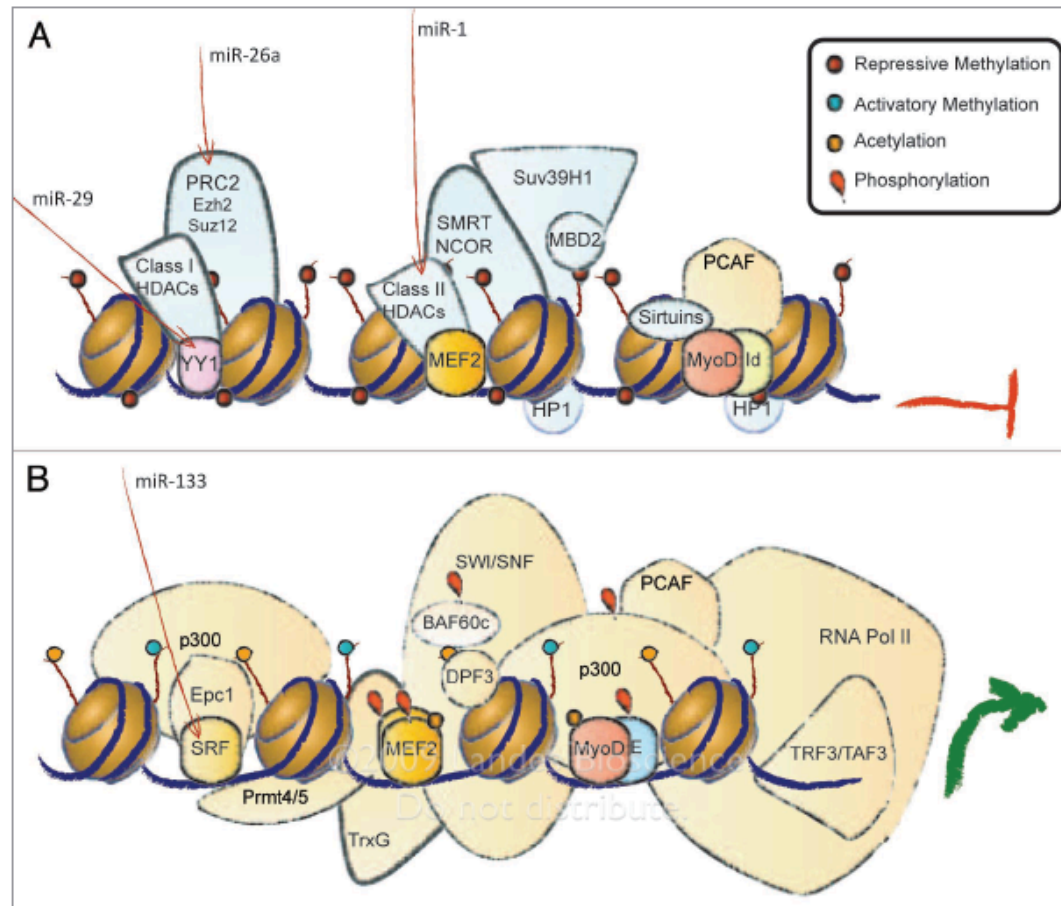


- ✓ Somatic cells, embryonic stem cells, germ cells, are characterized by a crescent ability to **reprogram** gene expression and give rise to different lineage. And the capacity to manipulate cell destiny have profound implications in basic biology, drug development and regenerative medicine.

Cell fate determination is regulated not only by genetic mechanisms, but also by epigenetic modifications through signaling molecules, which together with environmental cues induce appropriate transcriptional and epigenetic responses, essential for determining cell destiny.



Proliferating
satellite cells



Transcription
repressed

Differentiating
satellite cells

Transcription
activated

Transcriptional and epigenetic control of muscle-specific gene expression.

(A) Muscle specific gene promoters are repressed through a combination of mechanisms

MyoD is repressed by the association with transcriptional repressors.

DNA is methylated and chromatin is hypo-acetylated.

(B) Once extracellular cues are transmitted inside the nucleus through signaling cascades, transcriptionally active muscle regulatory regions display:

phosphorylated/acetylated MyoD/E protein heterodimers

Remodeling complex recruited and transcription initiated by Pol II.

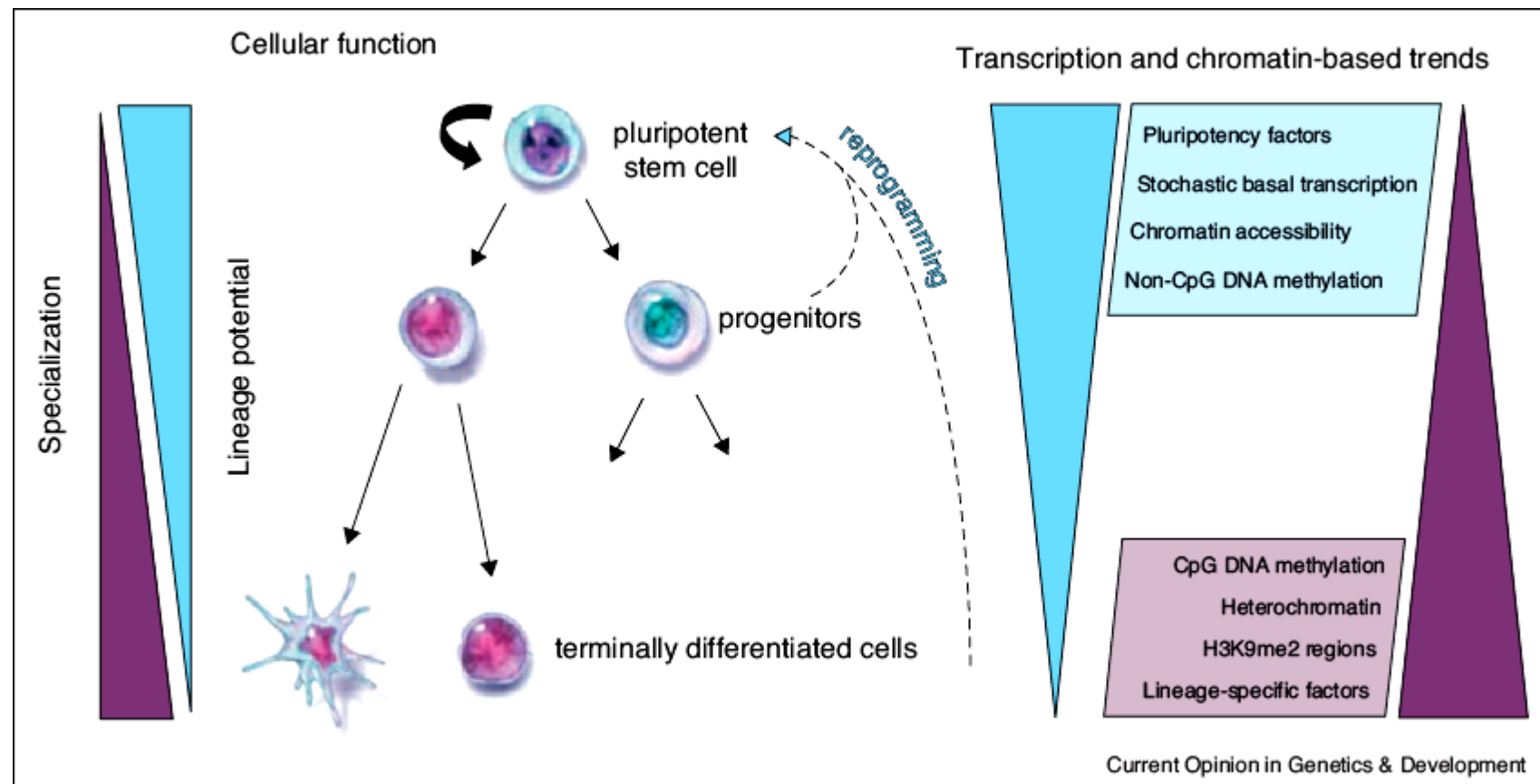
Chromatin is hyperacetylated

Early stages of development:

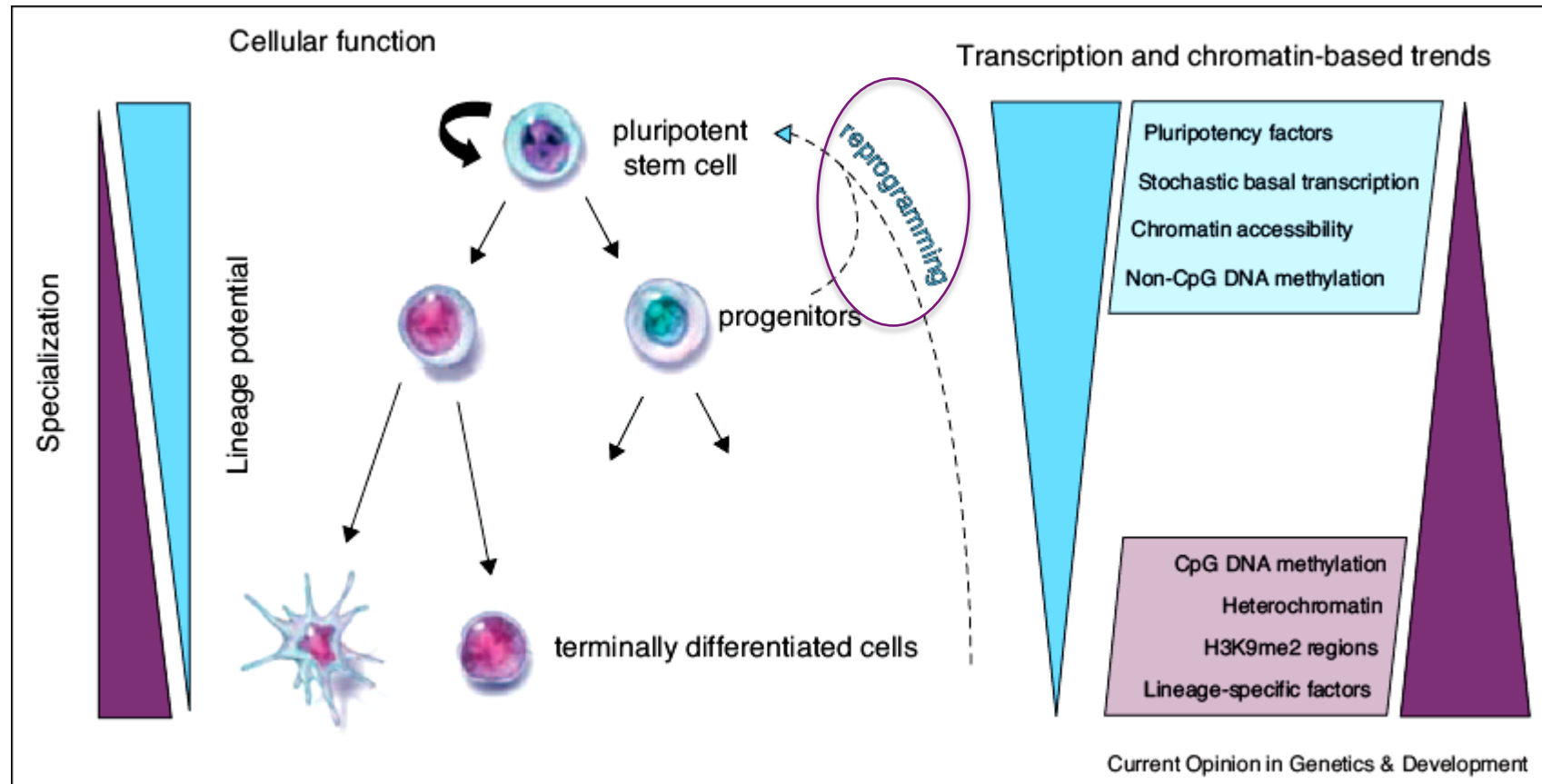
Pluripotency-associated genes are expressed.

Genes that are required later in development are transiently held in a repressed state by Polycomb complex group (PcG) and other chromatin modifying enzymes, which catalyze repressive histone modifications.

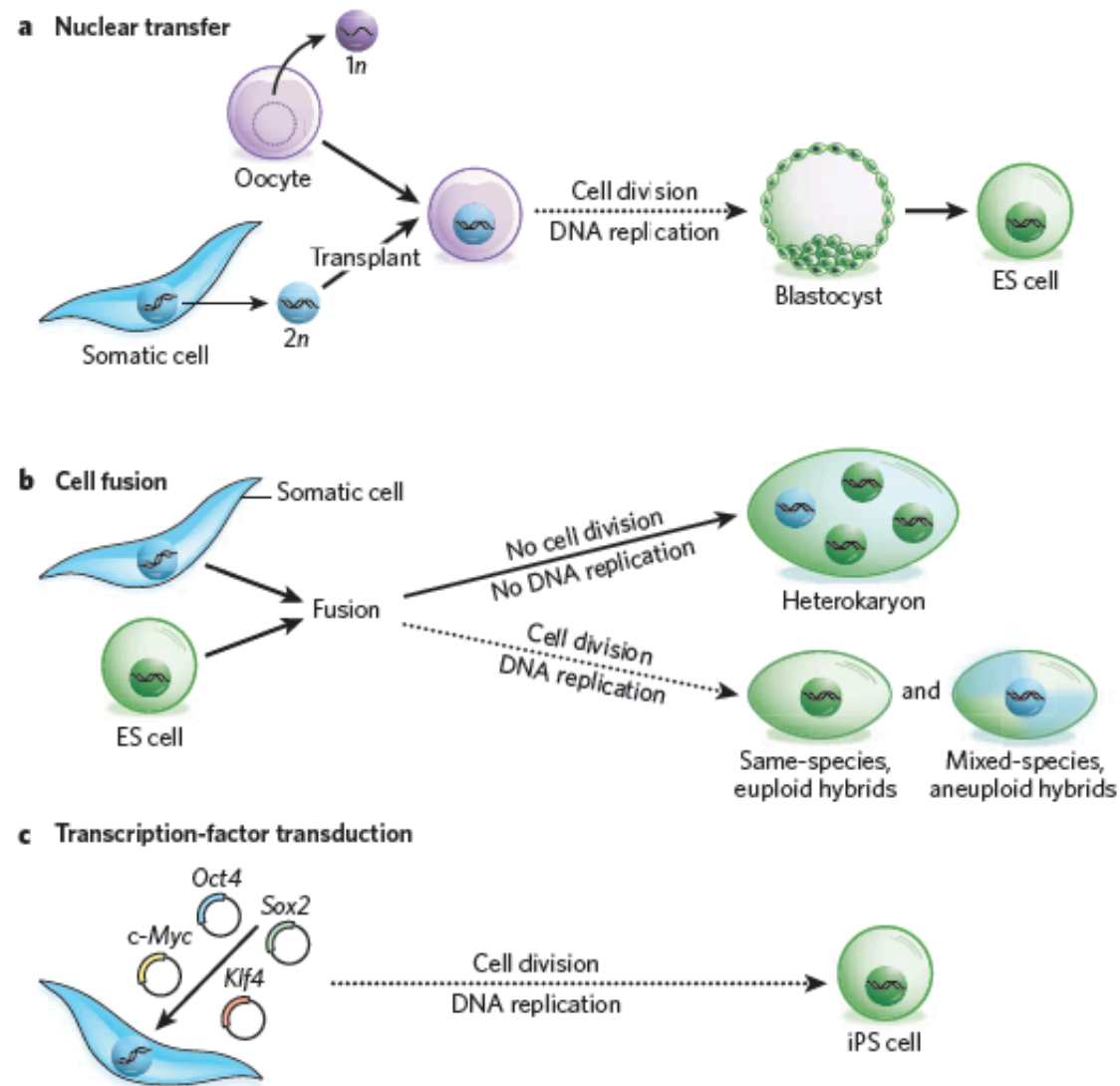
Differentiation: genes that are crucial for pluripotency are silenced by transcription repressors, histone modifications, as well as by DNA methylation (i.e methylation of histone H3K27). Lineage-specific genes are activated (epigenetic modifications, transcription factors).



Reprogramming refers to erasure and remodeling of epigenetic marks, such as DNA methylation, during mammalian development.



Different strategies to **reprogram somatic cells** into pluripotent stem cells

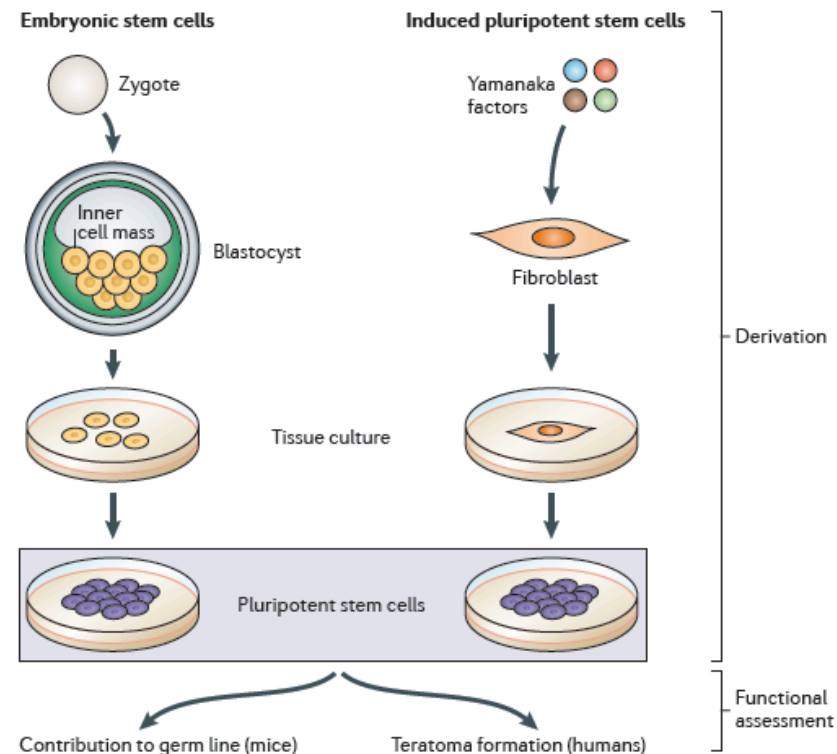


Pluripotent stem cells

- ✓ **Pluripotent stem cells** (PSCs) are defined by their ability to **differentiate** into the hundreds of somatic cell types of an adult animal, and by their ability to continually **self-renew**. PSCs called embryonic stem cells (ESCs) were first isolated in 1981 by culturing the inner cell mass of pre-implantation mouse blastocysts
- ✓ PSC are **inexhaustible**, scalable and **physiologically native** material for experimentation, which is in contrast with other types of patient-derived cell lines that involve oncogenic transformation to ensue long term immortal and scalable culture.
- ✓ The capacity of PSCs to self renew facilitates cell-based genetic or drug screens, which would otherwise be difficult in primary cell lines or mouse models.

Induced pluripotent stem cells (iPSC)

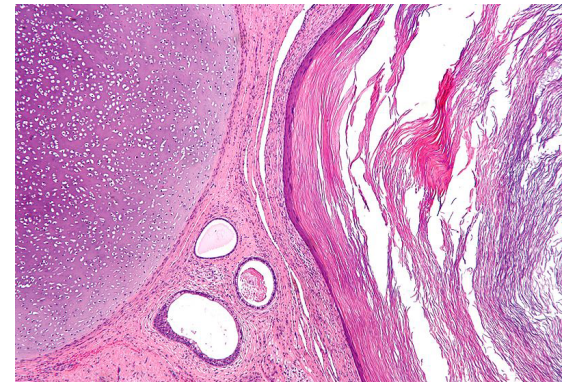
- ✓ In 2006, Yamanaka and colleagues discovered that introducing four transcription factors that are typically expressed in ESCs (the so-called '**Yamanaka factors**': OCT4, SOX2, Krüppel-like factor 4 (KLF4) and MYC) converted mouse skin fibroblasts to PSCs, a powerful example of the general process termed '**reprogramming**' (see the figure, right panel).
- ✓ Reprogramming by overexpressing Oct3/4, Sox2, Klf4, and c-Myc factors occurs as a gradual process whereby **lineage-specific genes** are silenced as **embryonic markers** become activated (i.e. Nanog, Fgf4, Cripto, Dax1).



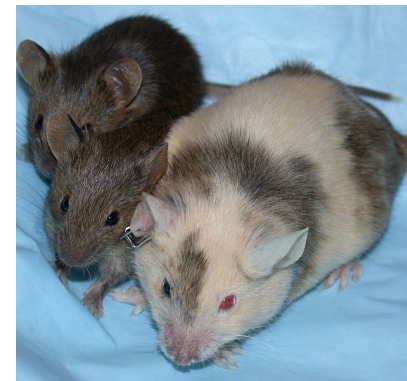
Induced pluripotent stem properties:

1. Expression of stem cells genes and proteins
2. Chromatin methylation patterns
3. Teratoma formation
4. Viable chimera formation
5. Self renewal
6. Capability to differentiate in several lineages

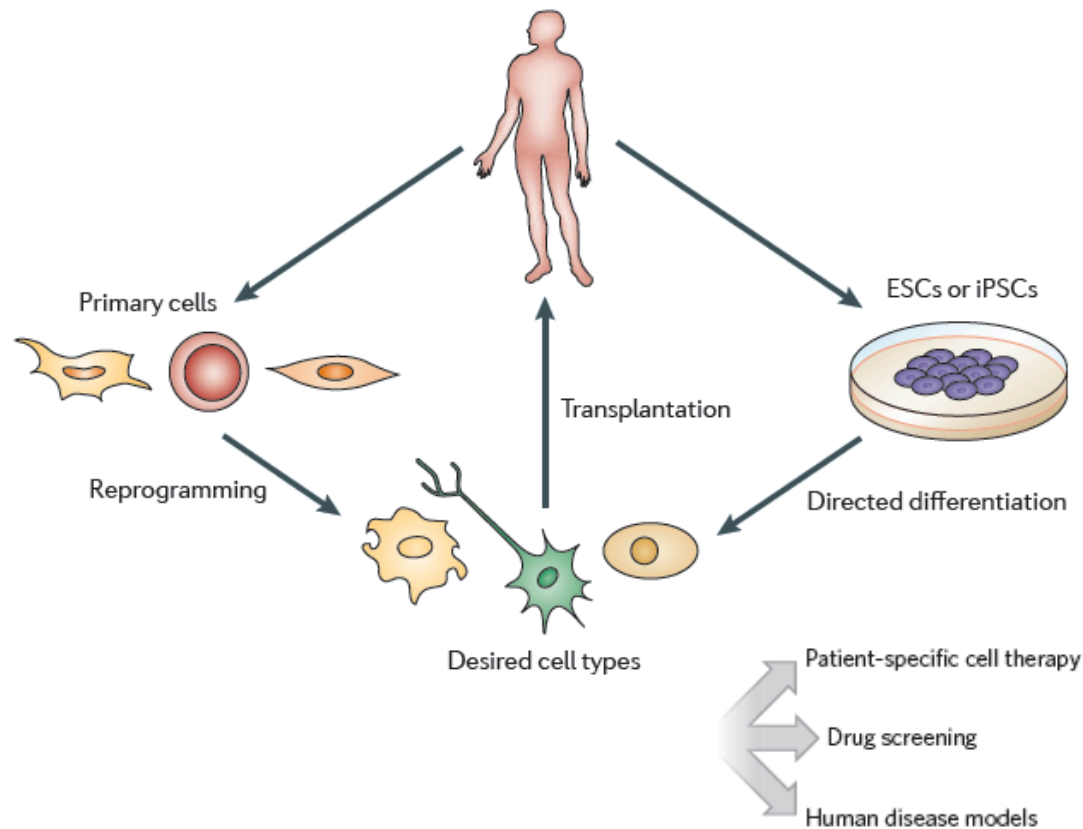
A **teratoma**: encapsulated tumor with tissue organ components resembling normal derivatives of **ALL THREE GERM LAYERS**: mesoderm, endoderm and ectoderm



A **chimera** is a single organism that is composed of two or more different populations of **genetically distinct cells**



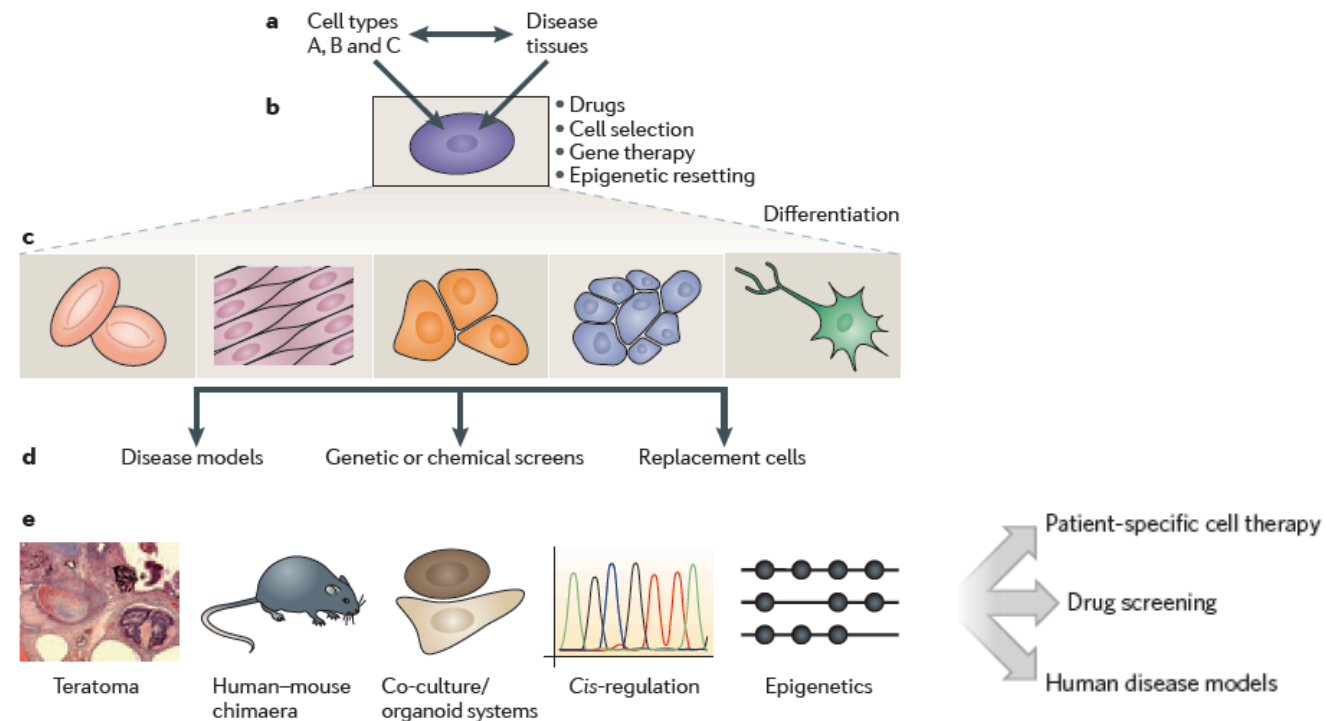
Central strategies of regenerative medicine



Two main strategies for generating patient-specific cells of a desired type.

1. Pluripotent cells to be used for regenerative medicine can be either patient-derived (iPSCs) or non-patient-derived (either embryonic stem cells (ESCs) or iPSCs. Pluripotent cells can be differentiated in vitro to a desired cell state (directed differentiation, right).
2. Primary cells derived from a patient can be used to generate a desired cell type directly (reprogramming, left).

Cells can then be studied in vitro or used for transplantation into patients



Application of iPS.

- Essentially any source of cells (A, B and C designate distinct lineages), normal or diseased, can be converted into induced pluripotent stem cells (iPSCs).
- With iPSCs or embryonic stem cells (ESCs) in hand, chemical or genetic modifications, cell passaging, or epigenetic modulation can be performed in order to study disease, drive differentiation or select for clones with particular phenotypes.
- Differentiated cell types can be derived and used as disease models
- Unlimited material for cell-based chemical or genetic screens or, potentially, therapeutic tissue sources.
- Disease modelling options include, but are not limited to, teratoma formation assays, human–mouse chimaeras, co-culture/organoid systems, allele-specific expression (ASE) and RNA-seq, and epigenetic assays.

iPSC and regenerative medicine

STEM CELLS[®]

TRANSLATIONAL AND CLINICAL RESEARCH

Efficient Generation of Functional Dopaminergic Neurons from Human Induced Pluripotent Stem Cells Under Defined Conditions

ANDRZEJ SWISTOWSKI,^a JUN PENG,^a QIUYUE LIU,^a PRASHANT MALI,^{b,c} MAHENDRA S. RAO,^{a,d}
LINZHAO CHENG,^{b,e} XIANMIN ZENG^a

Leading Edge
Minireview

Cell

The Human Brain in a Dish: The Promise of iPSC-Derived Neurons

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DOI 10.1016/j.cell.2011.05.034

Stem Cell Res. 2007 Oct;1(1):9-24.

Human embryonic stem cell-derived cardiomyocytes survive and mature in the mouse heart and transiently improve function after myocardial infarction.

van Laake LW, Passier R, Monshouwer-Kloots J, Verkleij AJ, Lips DJ, Freund C, den Ouden K, Ward-van Oostwaard D, Korving J, Tertoolen LG, van Echteld CJ, Doevendans PA, Mummery CL.

nature
cell biology

Conversion of mouse fibroblasts into cardiomyocytes using a direct reprogramming strategy

Jem A. Efe¹, Simon Hilcove¹, Janghwan Kim¹, Hongyan Zhou¹, Kunfu Ouyang², Gang Wang², Ju Chen²
and Sheng Ding^{1,3}

Skeletal muscle regeneration

Skeletal muscle differentiation

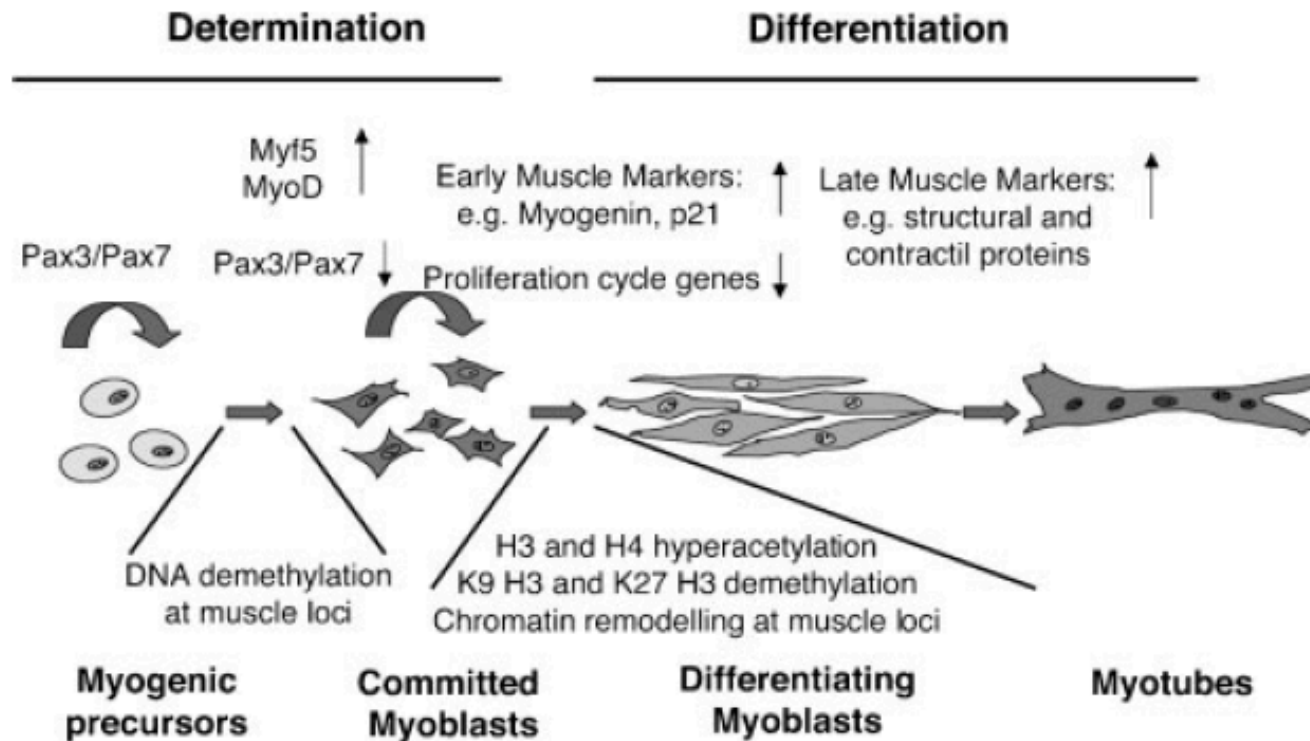
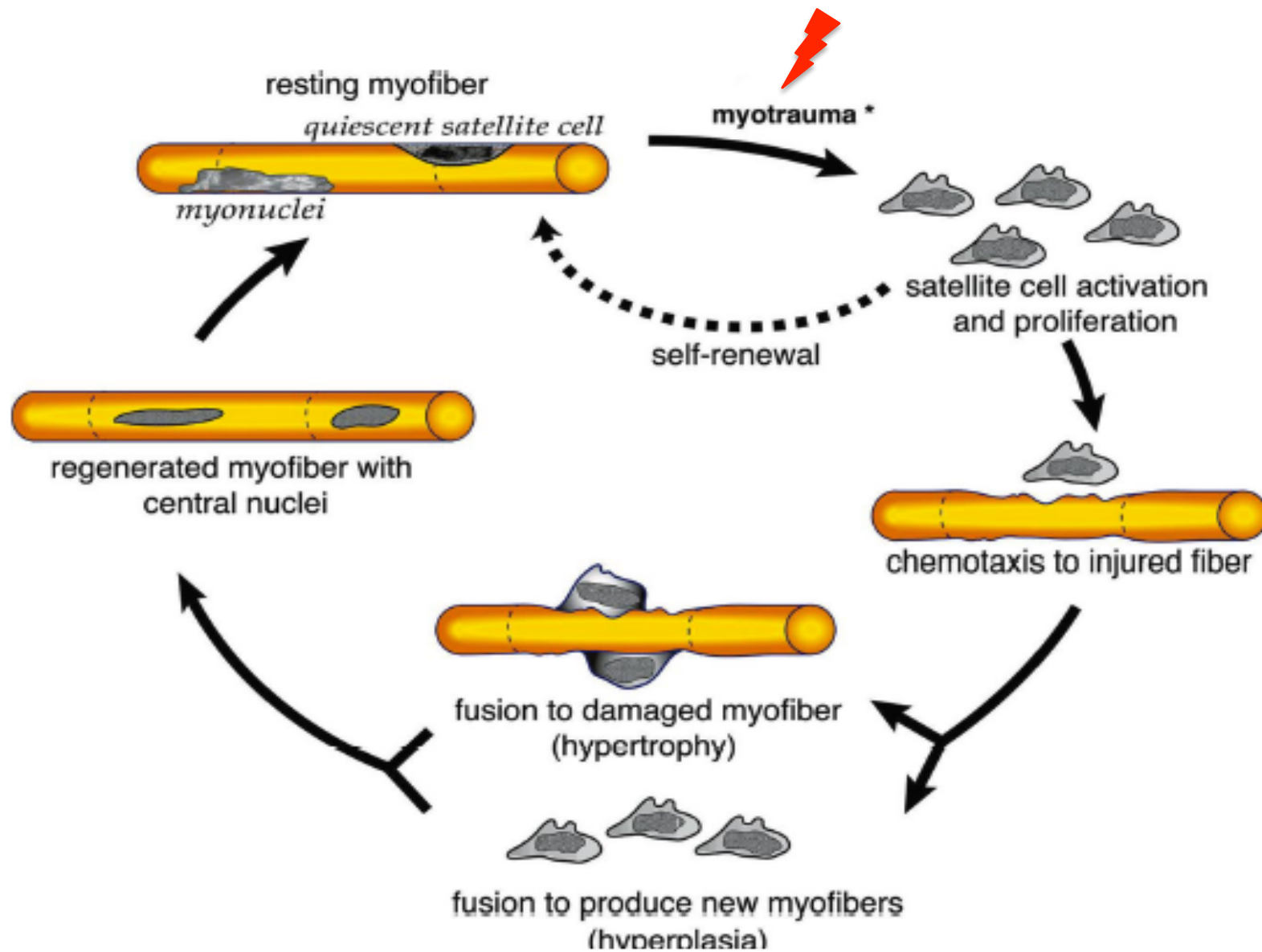


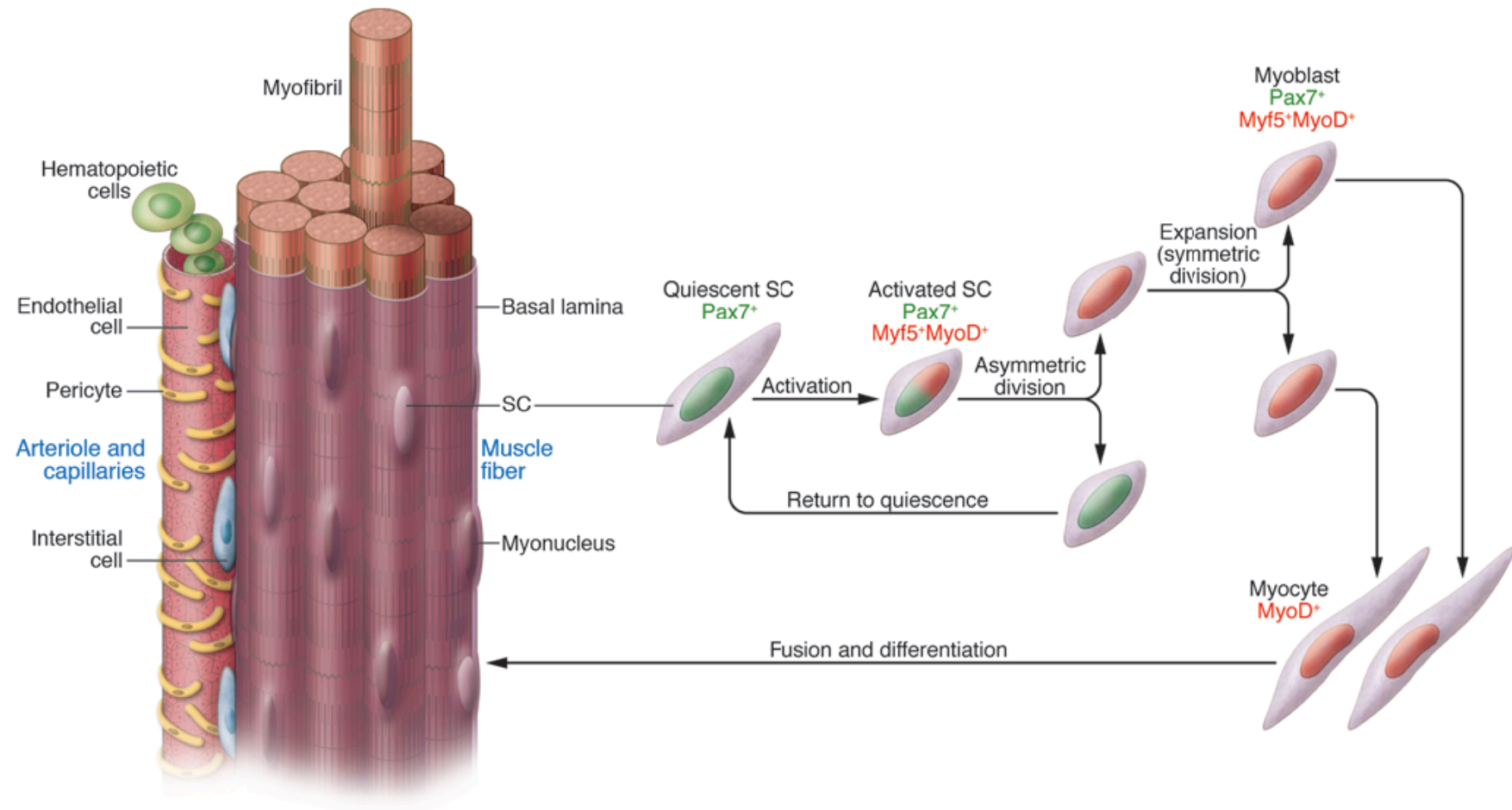
Fig. 1. A schematic representation of the sequential stages that underlie skeletal myogenesis. Determination of the myogenic lineage is conferred by the expression of the early myogenic bHLH proteins, MyoD and Myf5, likely due to DNA de-methylation at their regulatory regions. These muscle regulatory factors (MRFs) initiate myogenesis by activating transcription of muscle genes at previously silent loci, via the combined histone acetylation and demethylation of particular lysines, and the local chromatin remodeling. These events are promoted by the recruitment of chromatin-modifying enzymatic complexes by sequence-specific myogenic activators (e.g. myogenic bHLH and MEF2 proteins.)

Skeletal muscle regeneration

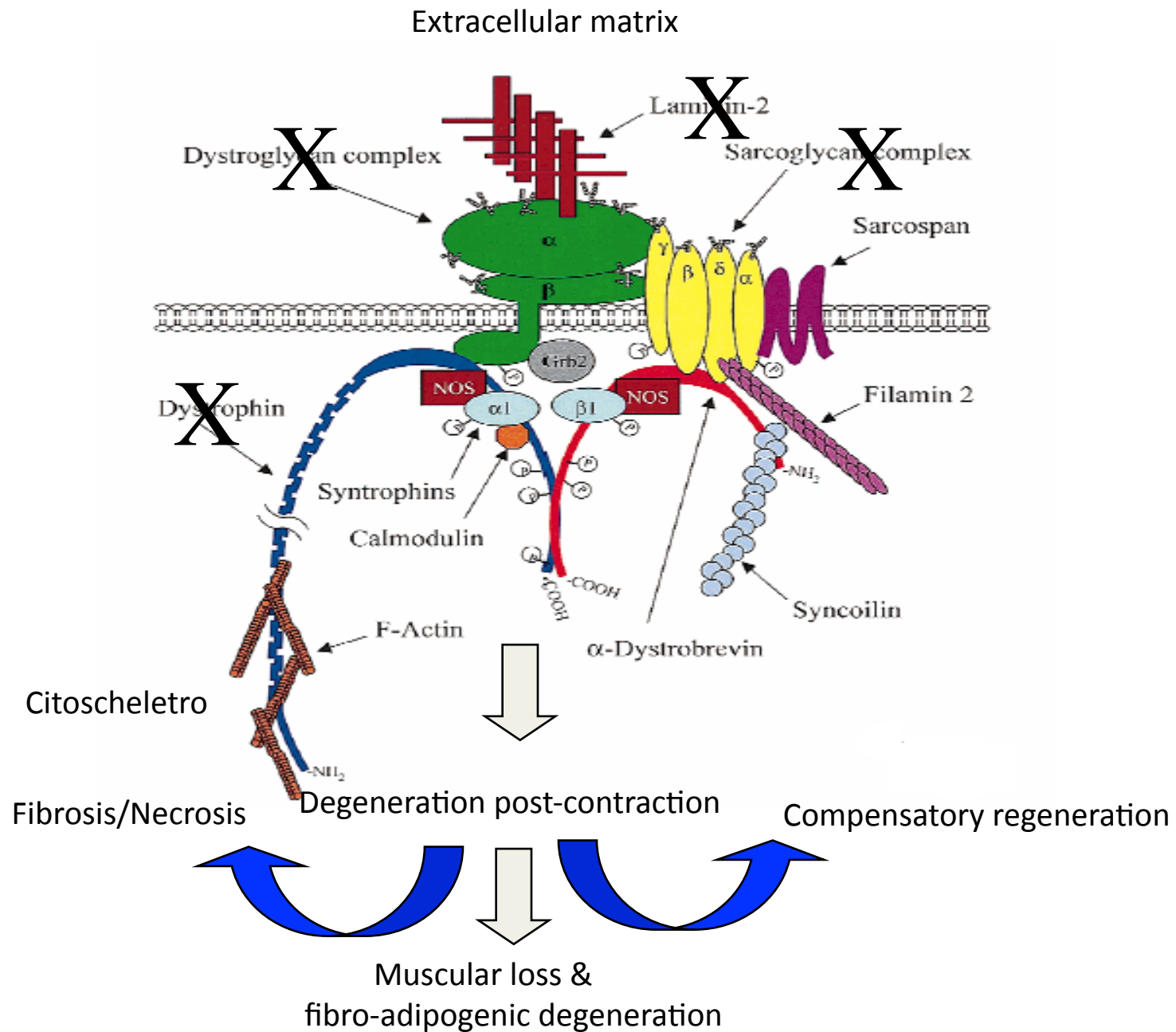
Skeletal muscle has the ability to regenerate new muscle fibers after it has been damaged by injury



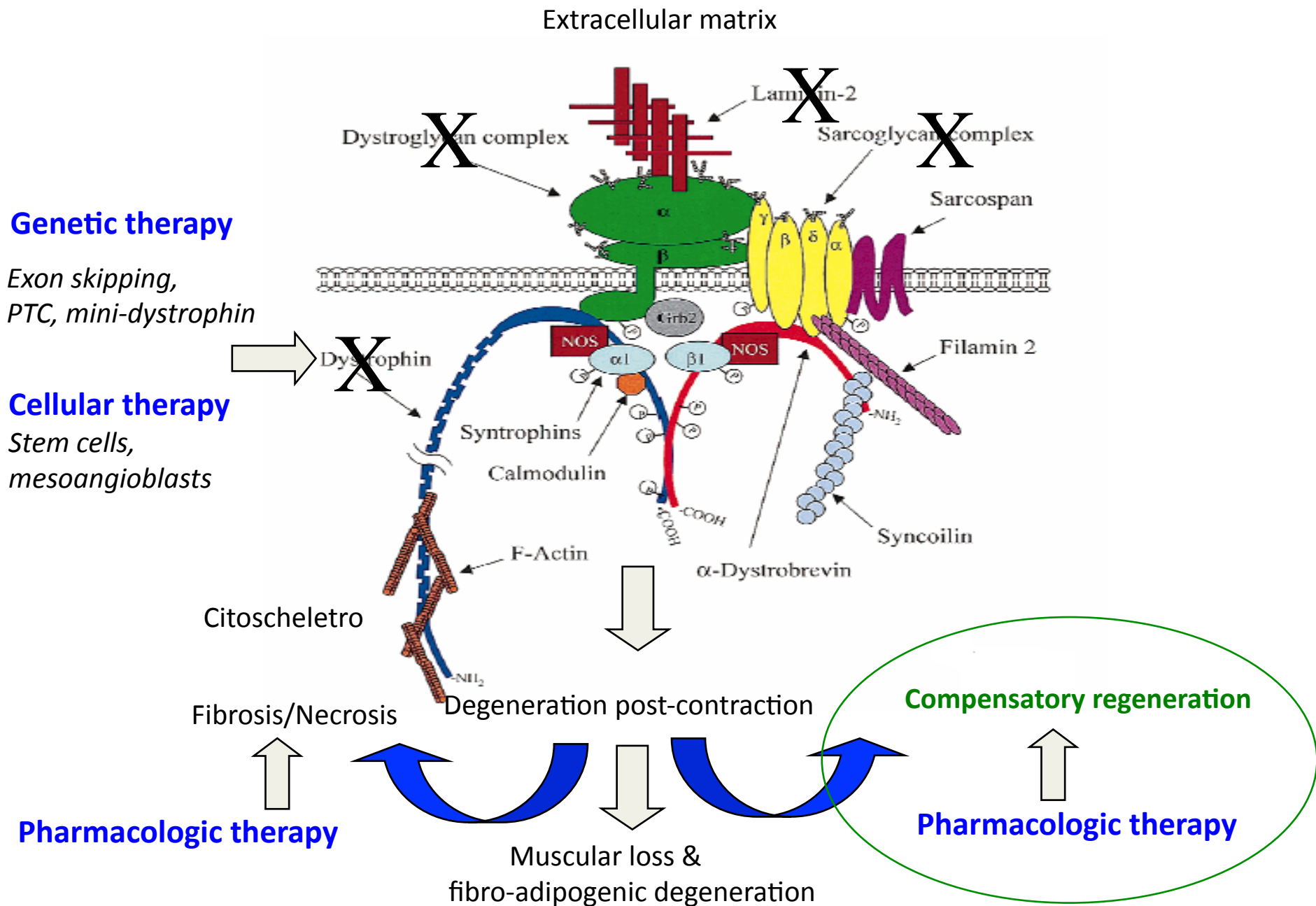
Asymmetric cell division



Pathogenesis of muscular dystrophies



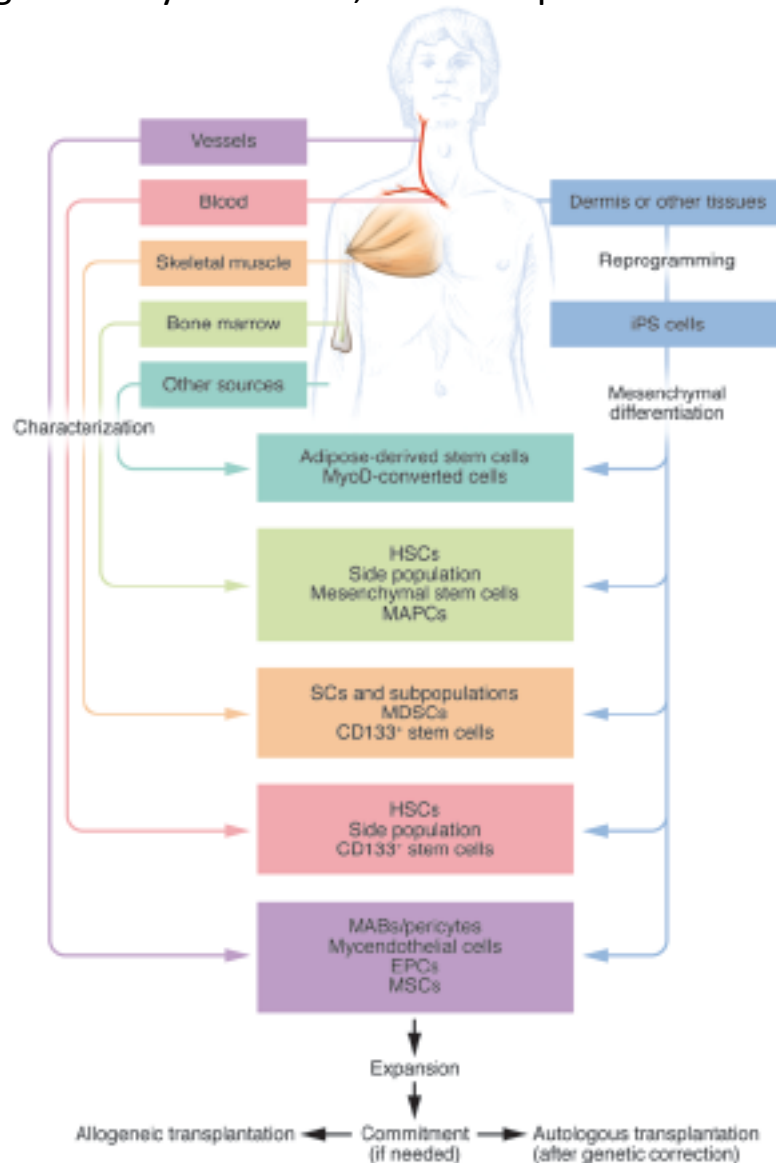
Pathogenesis of muscular dystrophies and therapeutic opportunities



Cellular therapy

Derivation of different stem cells for possible use in skeletal muscle regeneration.

Source of different myogenic stem cells. Once obtained, the stem cells can be characterized, expanded, genetically corrected, and transplanted



Many different types of mesoderm stem/progenitor cells have been shown to exhibit myogenic potential, usually after drug treatment, genetic modification, or coculture with satellite cells or myoblasts:

Mesenchymal stem cells (MSCs)

Multipotent adult progenitor cell (MAPCs)

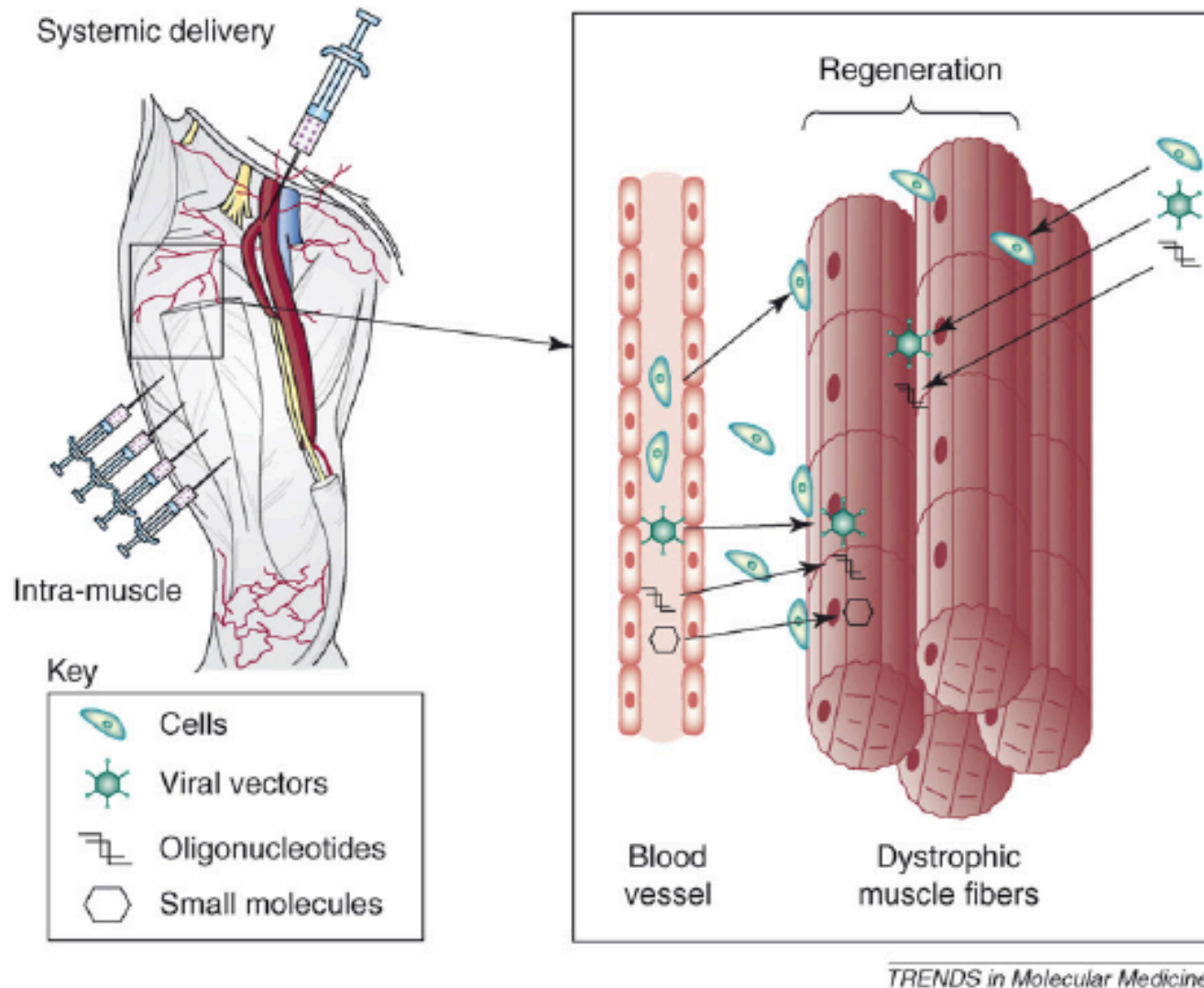
Muscle-derived stem cells (MDSCs)

Mesoangioblasts vessel-associated progenitors (MABs)

Endothelial progenitor cells (EPCs)

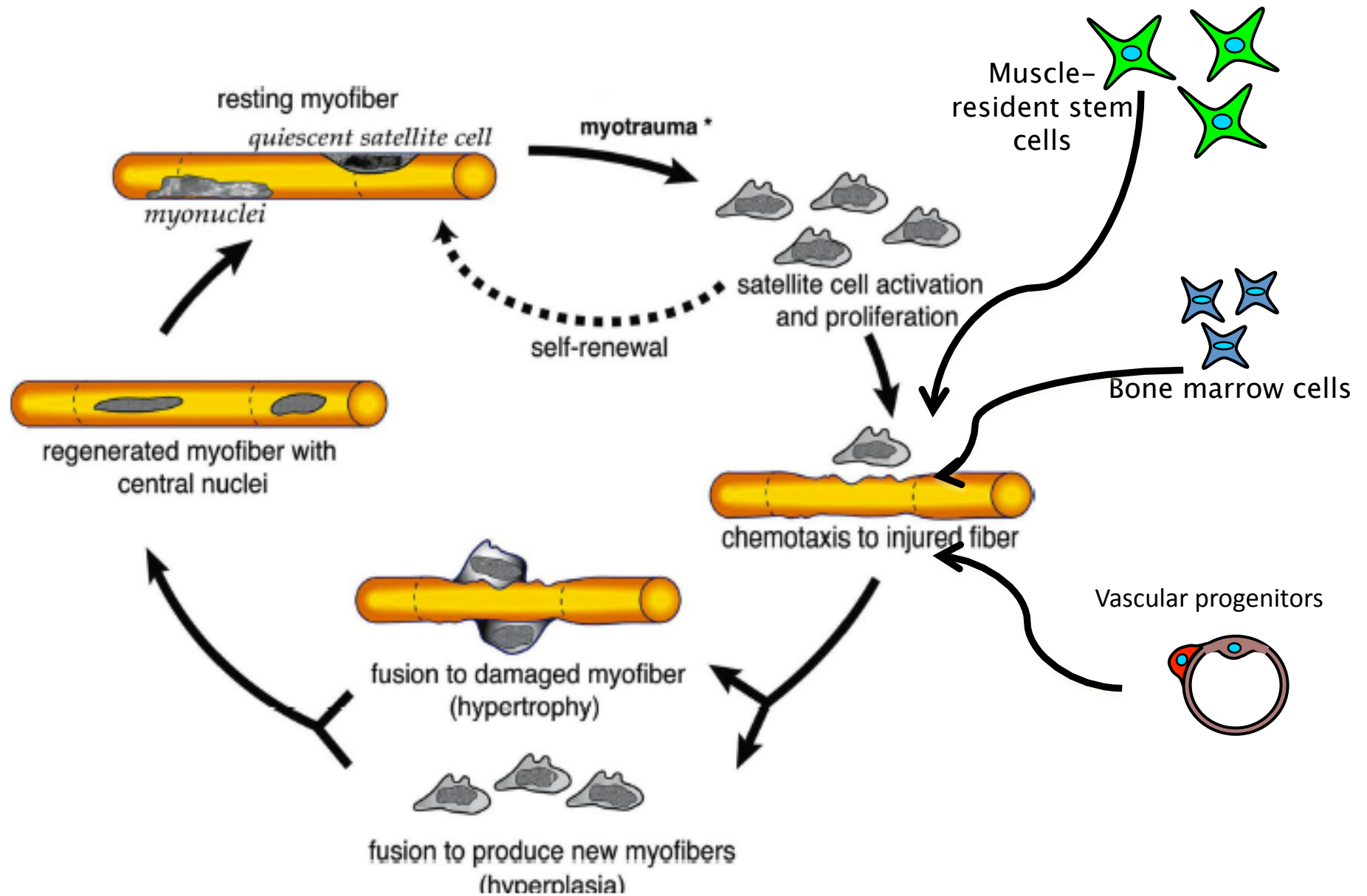
Adipose-derived stem cells (MADS)

Delivery strategies

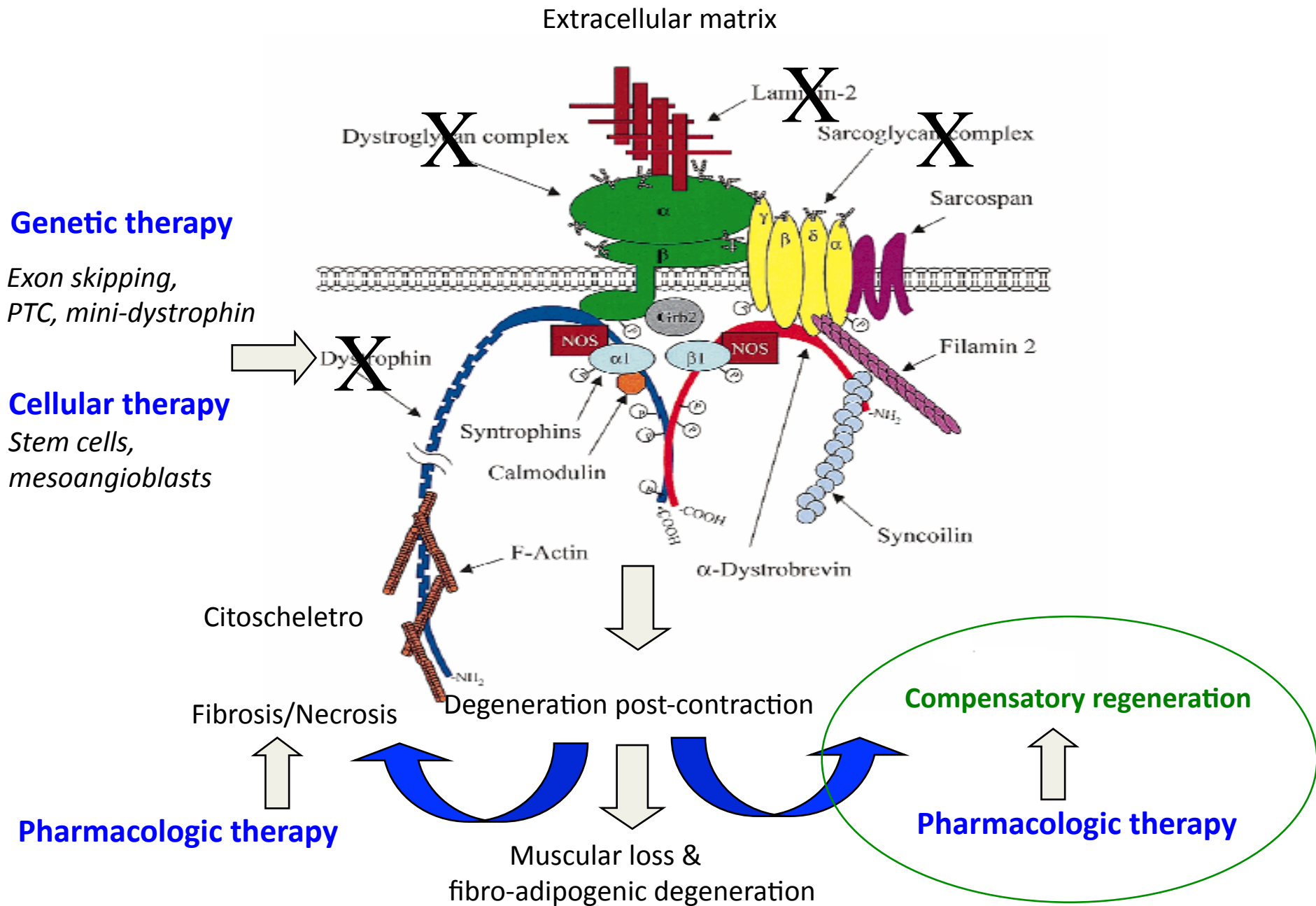


Intramuscular injection will require multiple injections for delivery to only a small volume of a given muscle. Systemic delivery would overcome this limitation, but crossing the vessel wall and the muscle basal lamina could be a problem. Small molecules will usually be administered orally and thus will reach the muscle through the circulation.

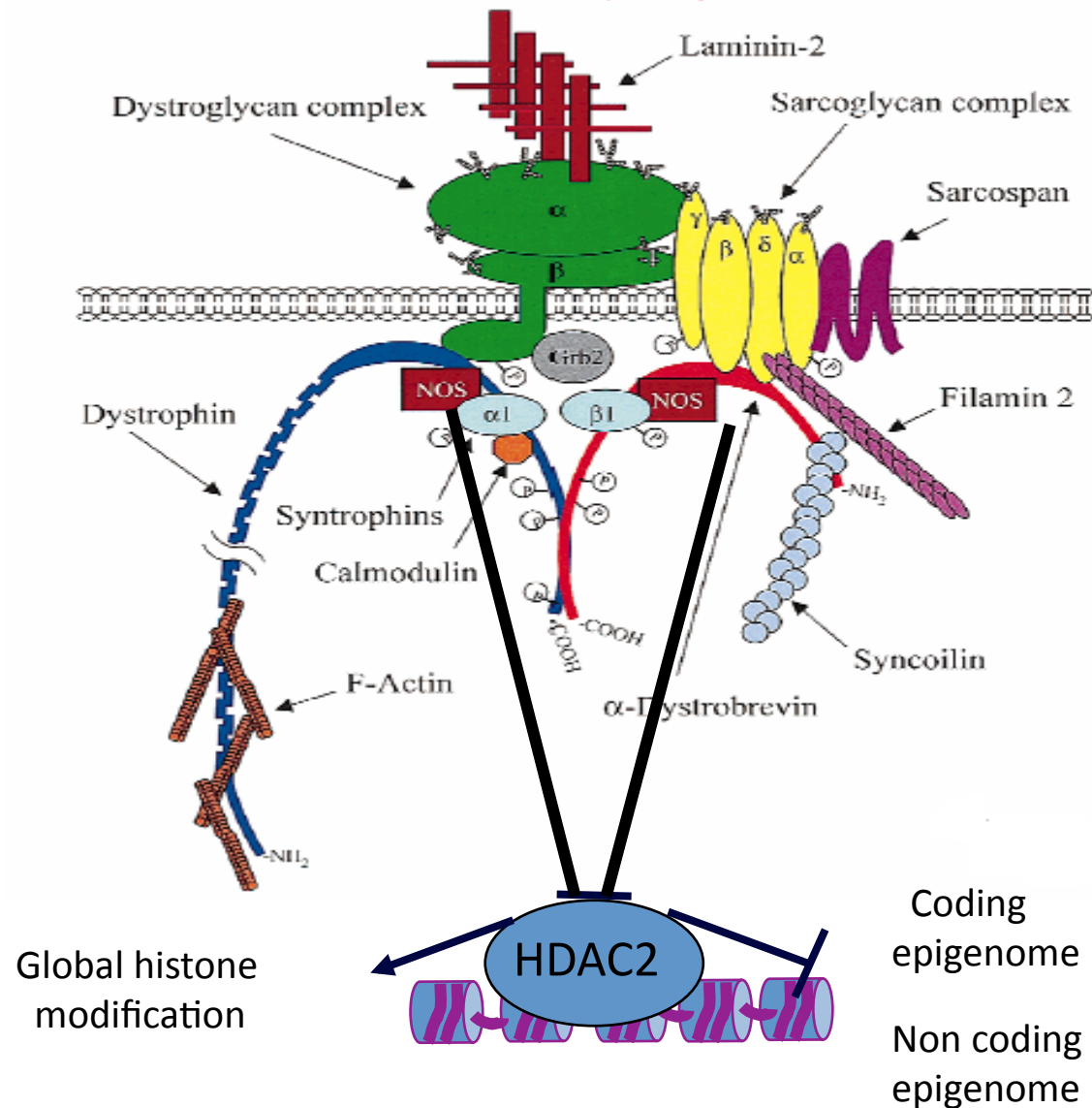
Different progenitors cells participate to skeletal muscle regeneration



Pathogenesis of muscular dystrophies and therapeutic opportunities



Epigenetic control of muscular dystrophy pathogenesis: HDAC as a LINK between genetic defect and epigenetic modulation of disease progression

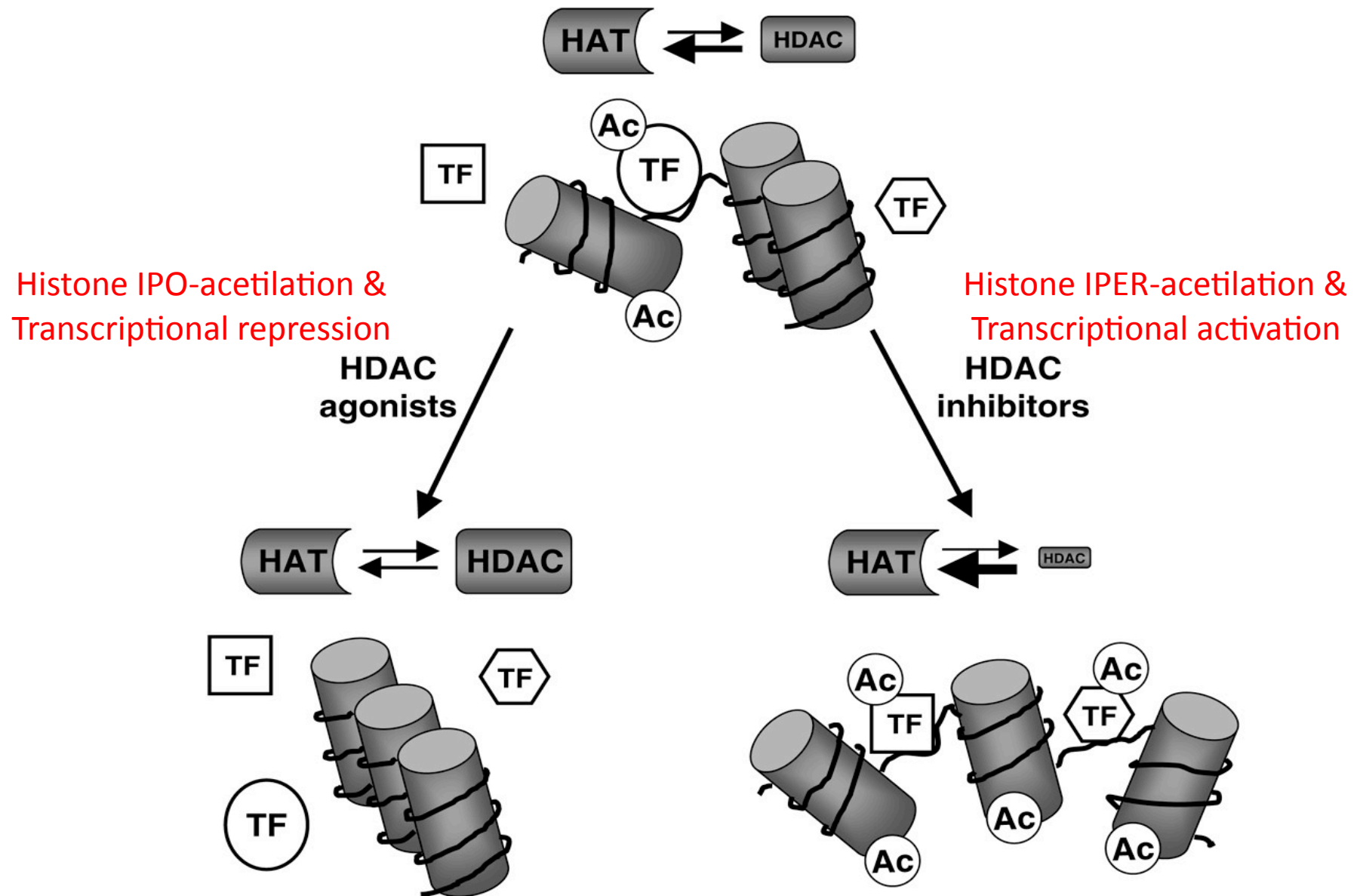


Genetic defect

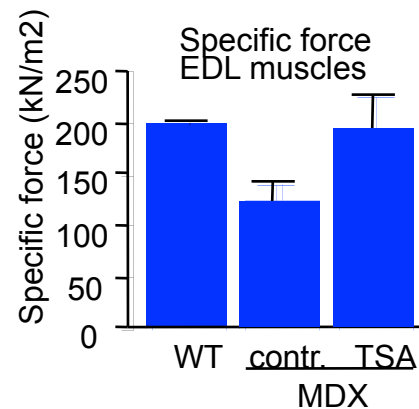
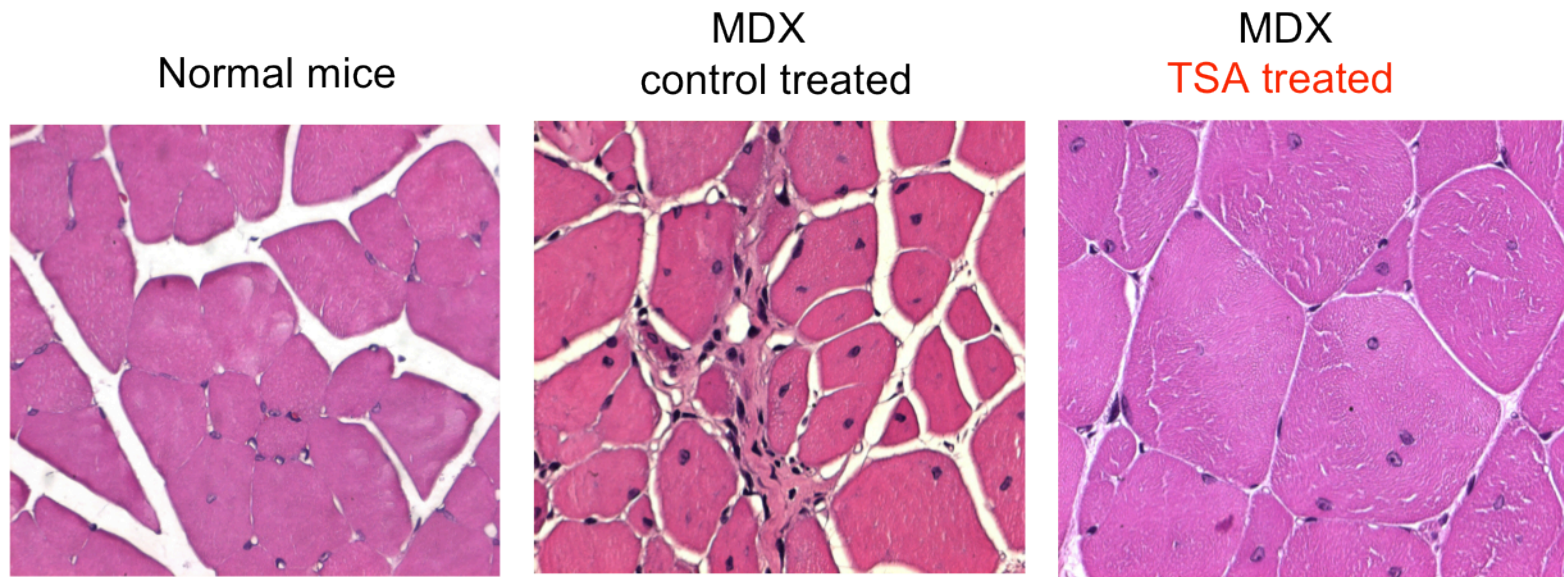
Epigenetic events

- ✓ Enlarging **fiber size** in dystrophic muscles produces **beneficial effects** in MDX mice.
- ✓ Three structurally unrelated **deacetylase inhibitors** share the ability to promote myoblast **fusion** into hyper-nucleated myotubes with an increased size.

Structural and functional chromatin regulation by histone acetylation



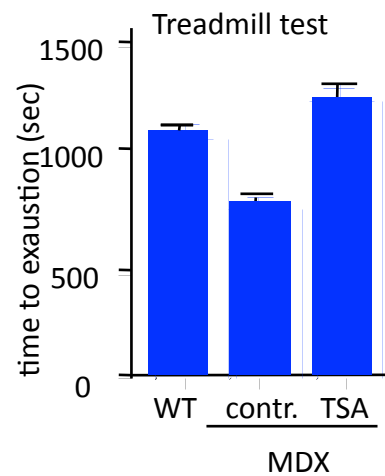
Beneficial effects HDAC inhibitors in MDX mice (mouse model of DMD)



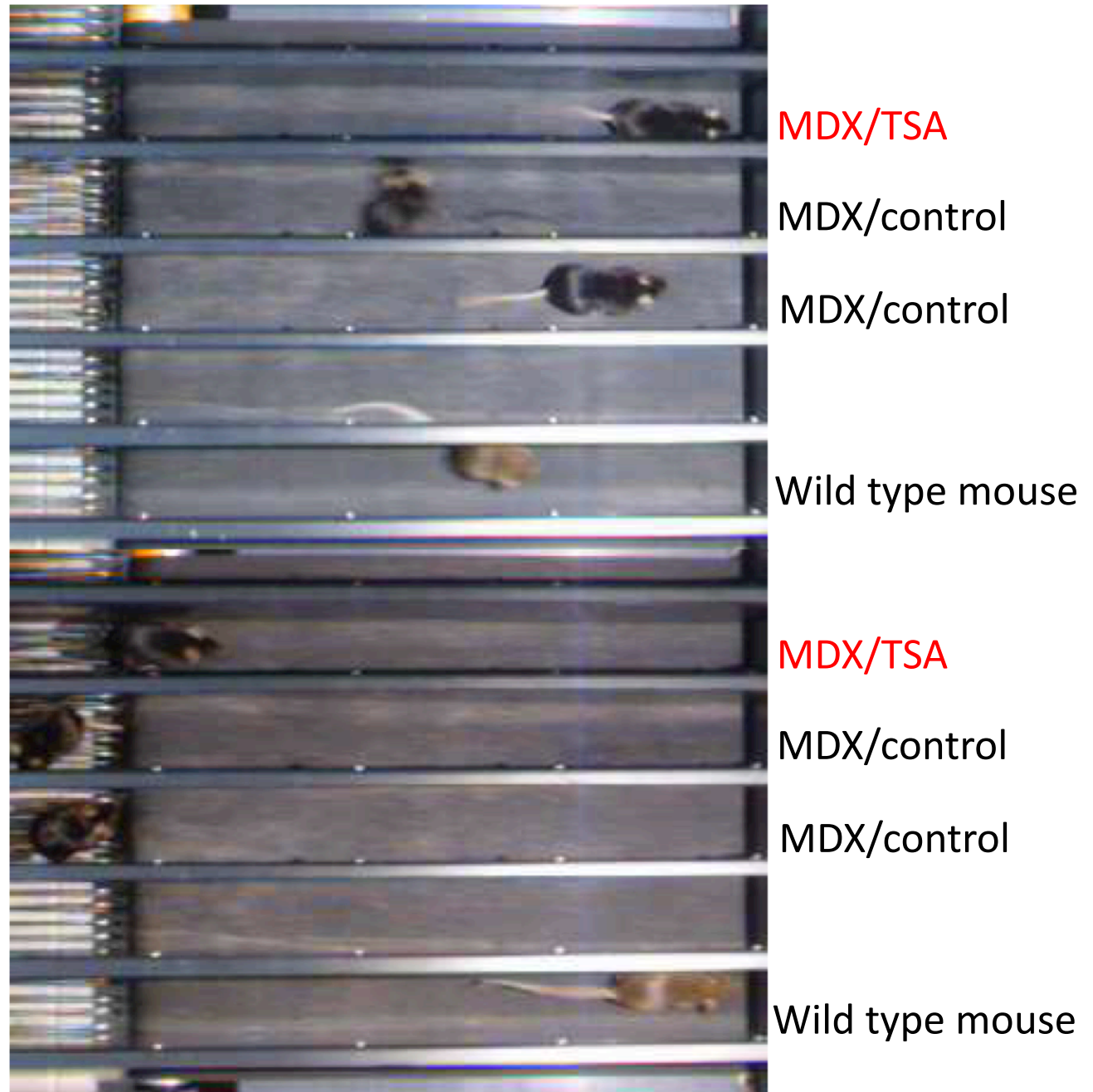
45 day treatment

Therapeutic effect of HDAC inhibitors on Mdx-mice

Beginning of test



End of test



Clinical trials

1. Intramuscular injection of satellite cells (Cell therapy)
2. Autologous transplantation of muscle-derived CD133+ cells (Cell therapy)
3. Mesoangioblast transplantation (Cell therapy)
4. Exon skipping (Gene therapy)
5. Skipping premature termination (PTC124) (Gene therapy)
6. Pharmacological therapy

Givinostat (ITF-2357): inibitore delle HDAC selezionato per clinical trial in pazienti affetti da distrofia muscolare

Known pharmacological profile in children and interactions with steroids

ITF-2357

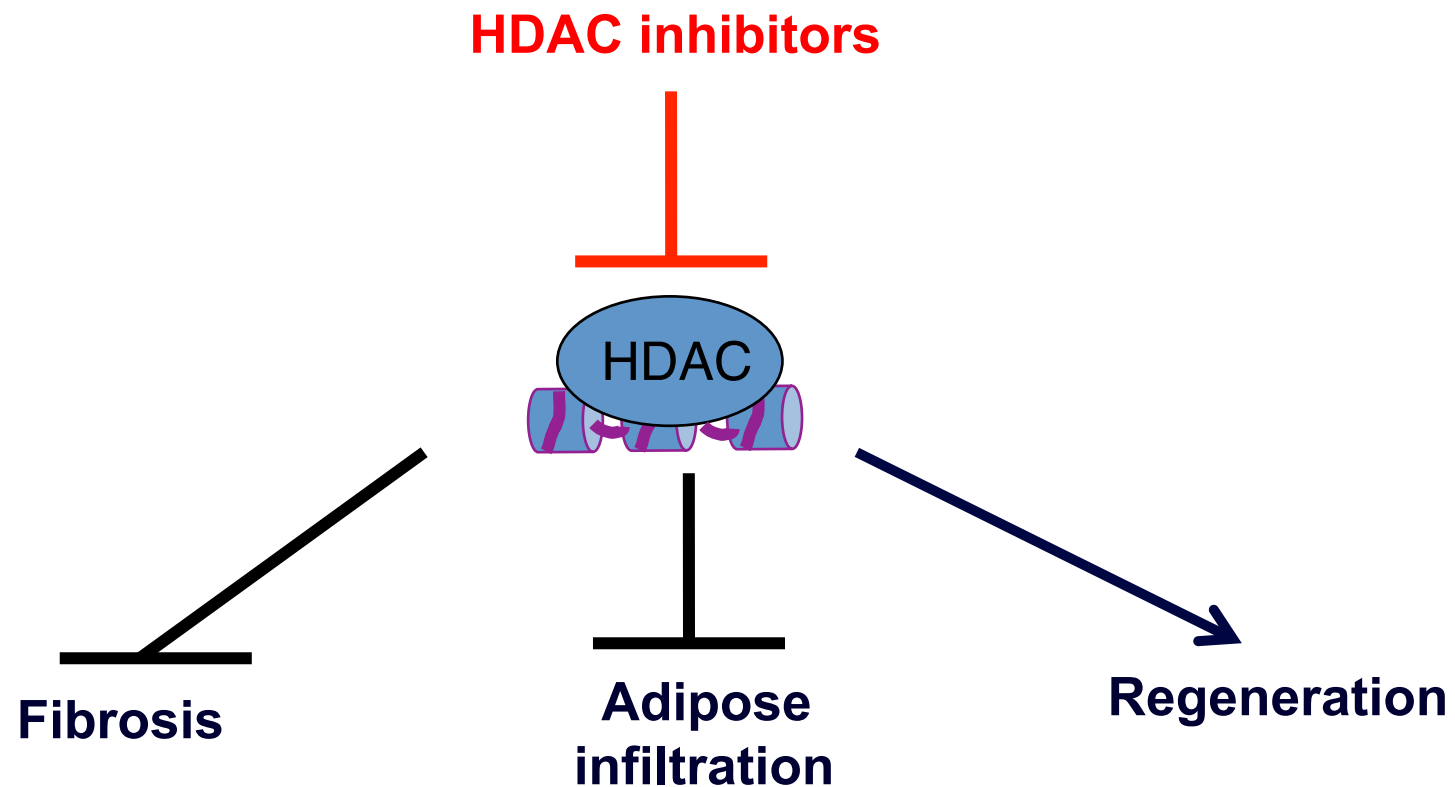
Company	Italfarmaco SpA
Highest Dev Status	Phase 2 Clinical
Indications	Hodgkins disease Acute myelogenous leukemia Multiple myeloma Inflammatory disease Pain Myeloproliferative disorder Juvenile rheumatoid arthritis
Actions	Anticancer Apoptosis stimulator Histone deacetylase inhibitor Analgesic Anti-inflammatory Bradykinin receptor modulator P2X7 purinoceptor agonist Unspecified cytokine receptor antagonist
Technologies	Oral formulation Capsule formulation

In May 2007, a phase I/II trial was initiated in Hodgkin's lymphoma [814683]. In August 2006, a phase II trial in systemic onset juvenile idiopathic arthritis (SOJIA) began [862622], while in September 2006, phase II trials began in autoinflammatory syndromes (AIS) [777056]. By December 2007, a phase II trial had been initiated in MM [858406]. In December 2007, a phase II trial in myeloproliferative disorders was initiated [971115].

Preclinical studies: 2009-2011.

Clinical trial: June 2012

HDAC inhibitors treatment blocks the progression of muscular dystrophy by acting on key events in the pathogenesis



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