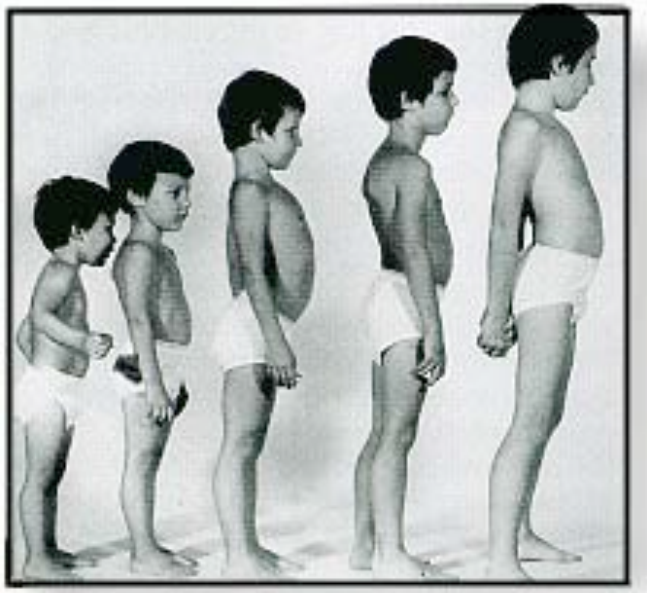
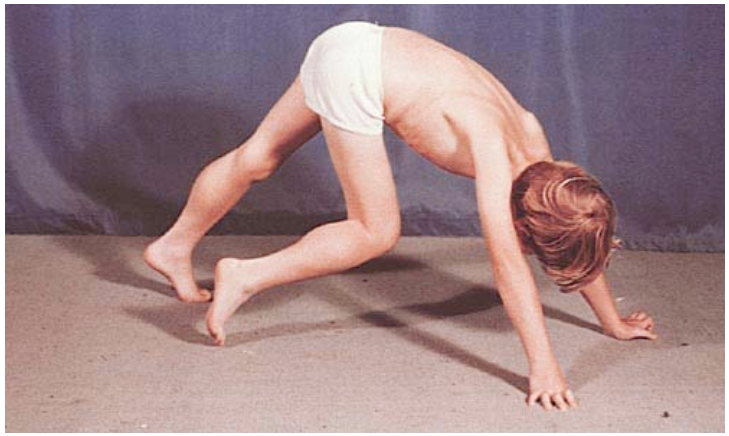
Muscular Dystrophy

November 3, 2008

Duchenne muscular dystrophy

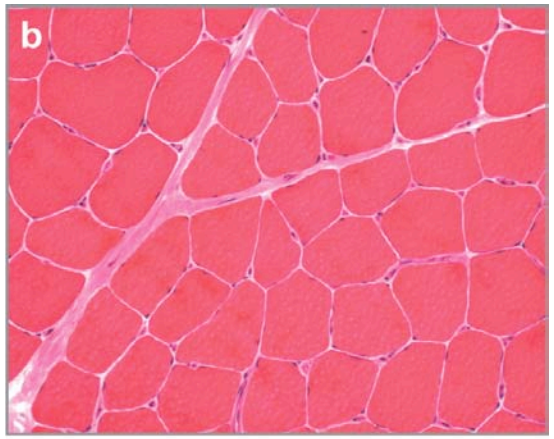




- Common (1/3500 boys)
- X-linked inheritance
- Female carriers may exhibit some symptoms
- Progressive skeletal muscle wasting beginning at around age 3
- Characteristic changes in posture; lordosis, pseudohypertrophy of calves
- “Gower’s maneuver” (climbing up from a sitting position)
- Usually confined to a wheelchair by age 10-12
- Progressive difficulty breathing due to wasting of diaphragm muscle
- Death usually before age 30

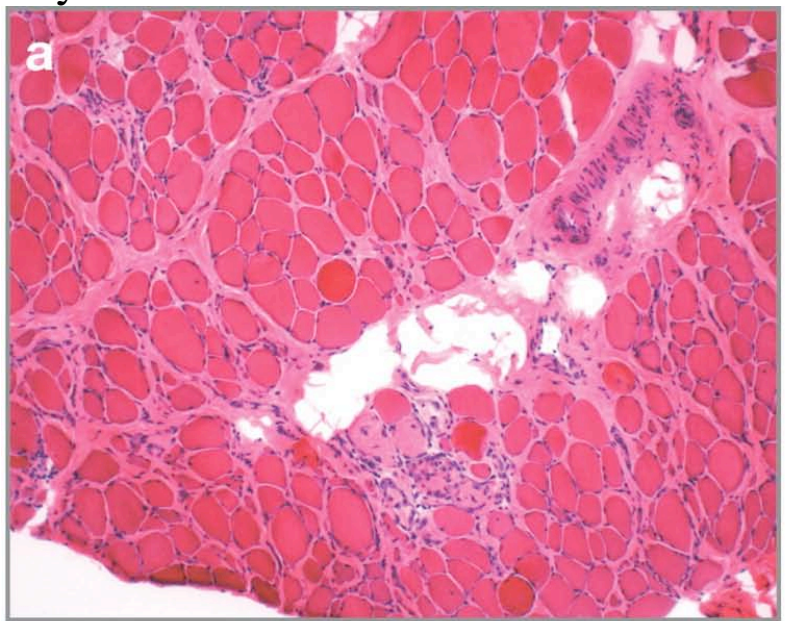
Pathology

normal

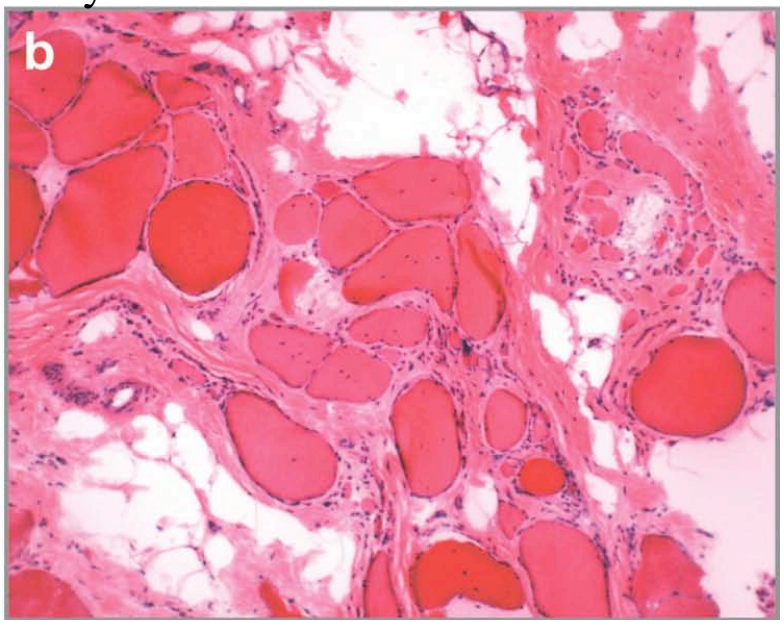


Definitive diagnosis by muscle biopsy
Muscle fibers variable in size
Necrotic fibers with significant inflammation
High serum levels of creatine kinase (also often true in asymptomatic female carriers)

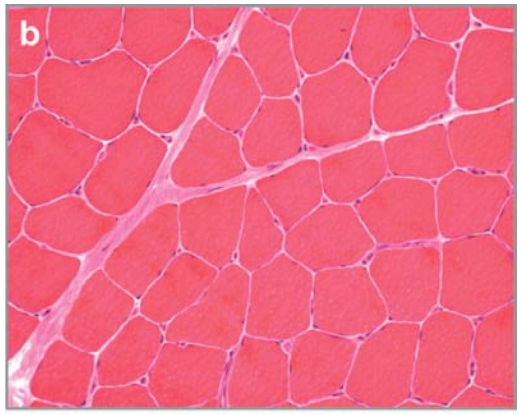
7 yo DMD



9 yo DMD



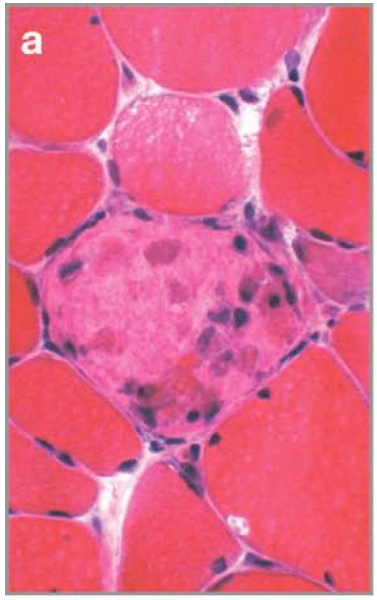
normal



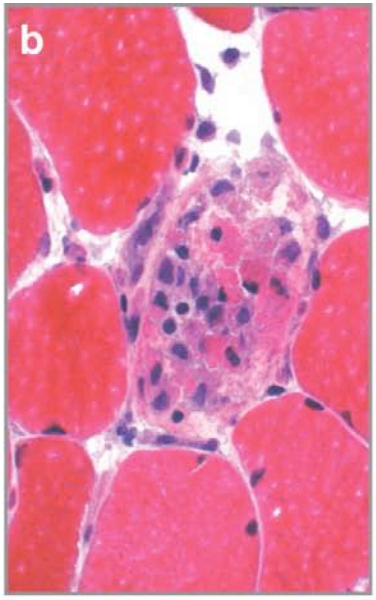
Pathology

Evidence of regenerating muscle fibers; satellite cells proliferate to form new myoblasts, fusion into new muscle fibers with central nuclei

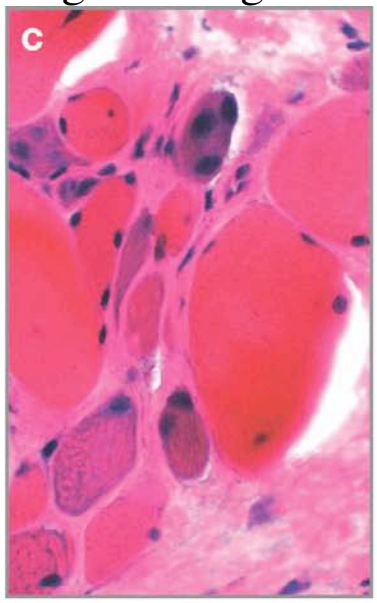
necrotic



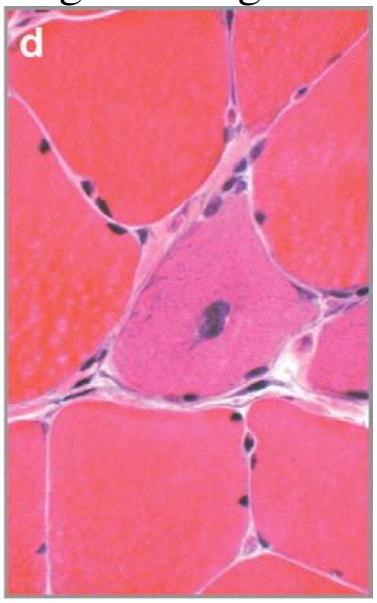
necrotic



regenerating

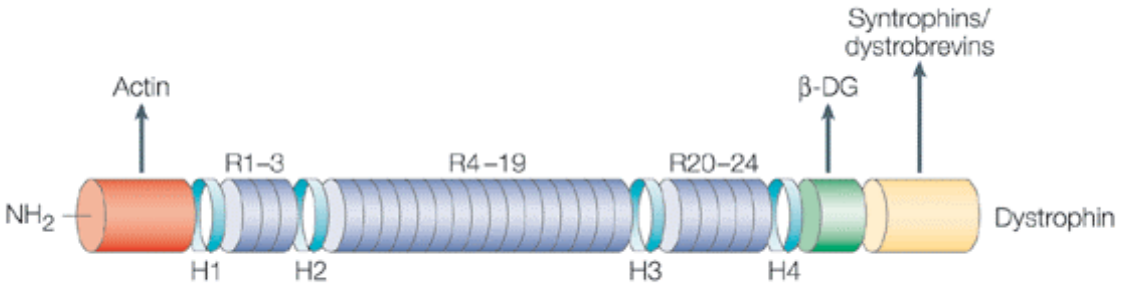


regenerating



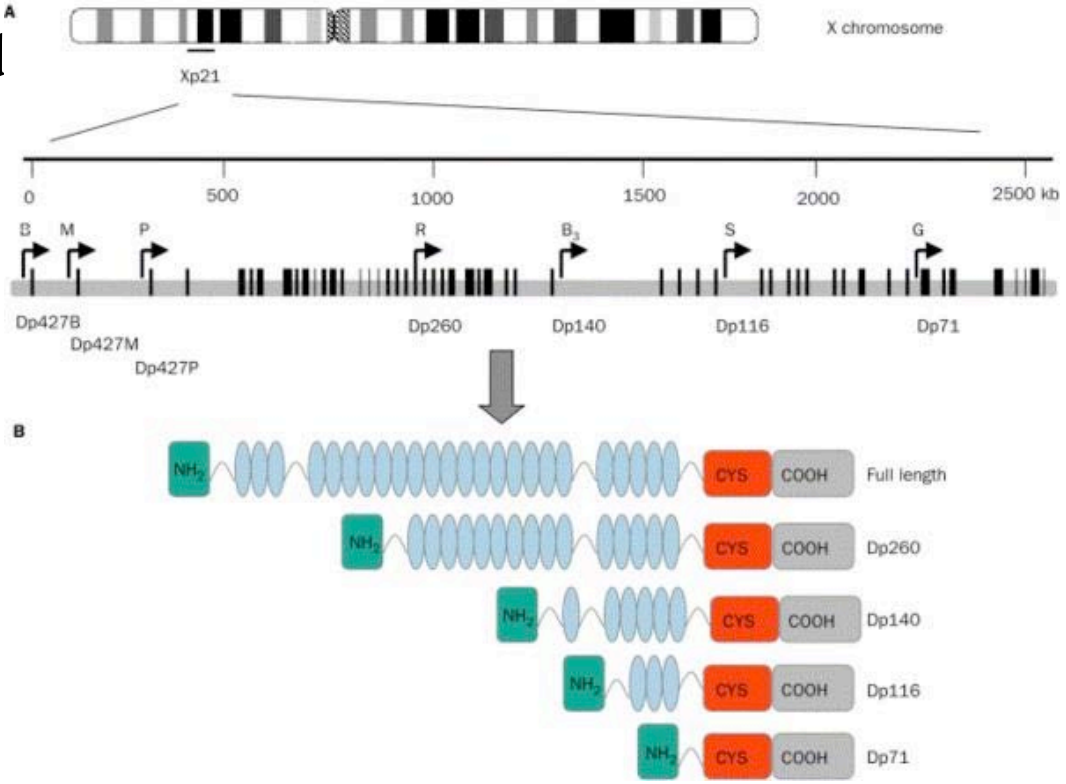
McNally EM, Pytel P. 2007.
Annu. Rev. Pathol. Mech. Dis. 2:87–109

Dystrophin



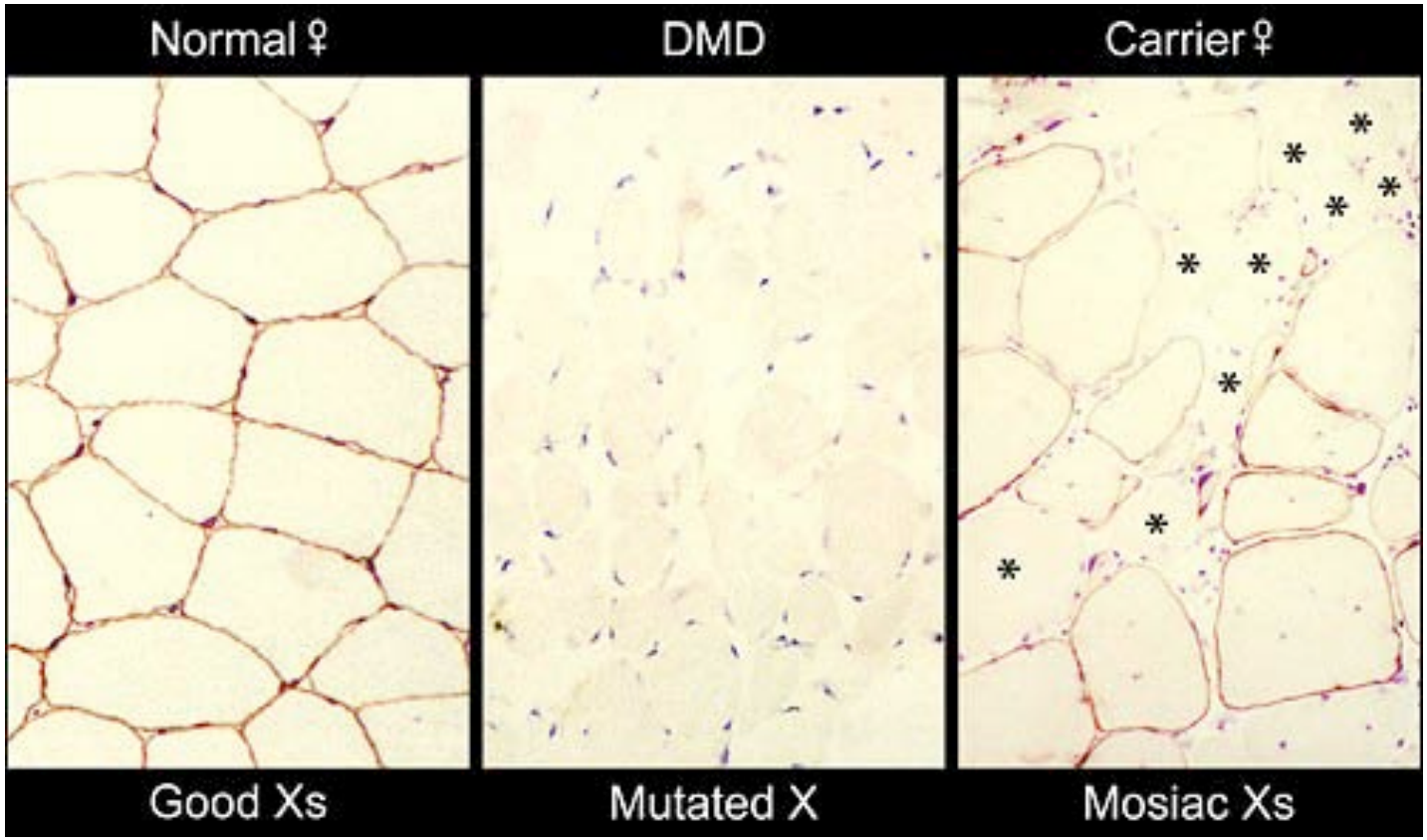
Enormous gene/protein: >3500 amino acids, ~170 nm long
16 hours required for transcription; 79 exons spread over 2.6 Mbp
Most mutations in large spectrin-like coiled coil domain

Tissue-specific promoters and alternatively spliced forms:



Full-length - muscle
DP260 - retinal
DP140 - brain
DP116 - Schwann cells
DP71 - general

Dystrophin distribution in normal and dystrophic muscle



Note female carriers often have some affected fibers due to mosaic X inactivation; elevated serum creatine kinase, sometimes muscle weakness

Muscular dystrophies and related degenerative diseases

Duchenne muscular dystrophy
- All skeletal muscles are affected, usually with cardiac involvement
- Cuased by null or severe hypomorphic mutations in the gene encoding dystrophin, often frame-shift or nonsense mutations or large deletions
- About 2/3 of cases inherited from carrier mother, 1/3 new mutations

Becker muscular dystrophy
- Less severe, later onset, about 1/20,000 boys
- Usually in-frame deletions or point mutations in dystrophin

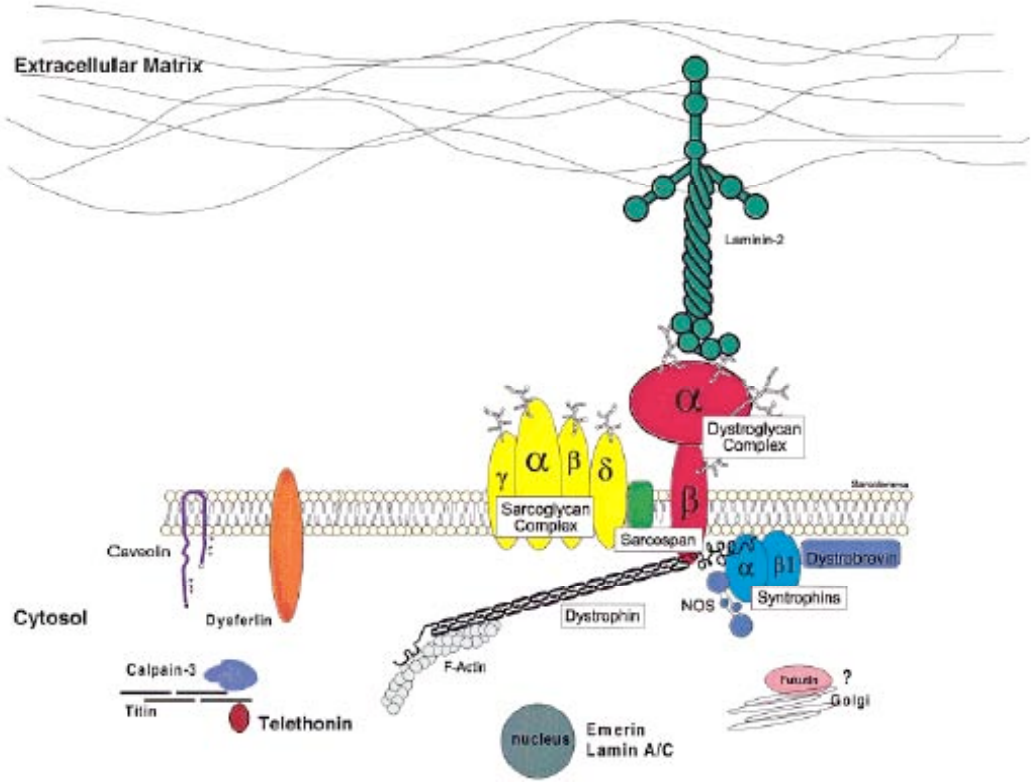
Emery-Dreifuss muscular dystrophy
- Wasting of shoulders and upper arms first, often with cardiomyopathy
- Two genetically distinct forms, both nuclear proteins (lamin A/C, emerin)

Limb girdle muscular dystrophies
- Pelvic and shoulder girdles affected first
- Genetically heterogeneous group of diseases, several sarcoglycans

Congenital muscular dystrophy
- Genetically heterogeneous group, symptoms present at birth
- ~50% due to deficiency in laminin $\alpha 2$

About 40 neuromuscular diseases tracked by mdausa.org include genetic and autoimmune causes

Molecules deficient in various muscular dystrophies



Identified by positional cloning in affected families

Mechanical attachment of skeletal muscle contractile apparatus to extracellular matrix

Possible roles in cell signaling via NOS

Treatment modalities

- Options for degenerative diseases are usually poor
- Supportive therapy includes vitamins, corticosteroids (prednisone), breathing exercises
- Approaches in development:
  - Conventional gene therapy with various vectors
    - Minigenes
  - Alternative gene therapies
    - Antisense suppression
    - Utrophin upregulation
    - *Exon skipping
  - Therapies to increase muscle mass
    - Myostatin blockade
    - Deacetylase inhibition
  - Cell-based therapies
    - Myoblast transplant
    - *Stem cell transplants - bone marrow, ES cells, satellite cells, others?

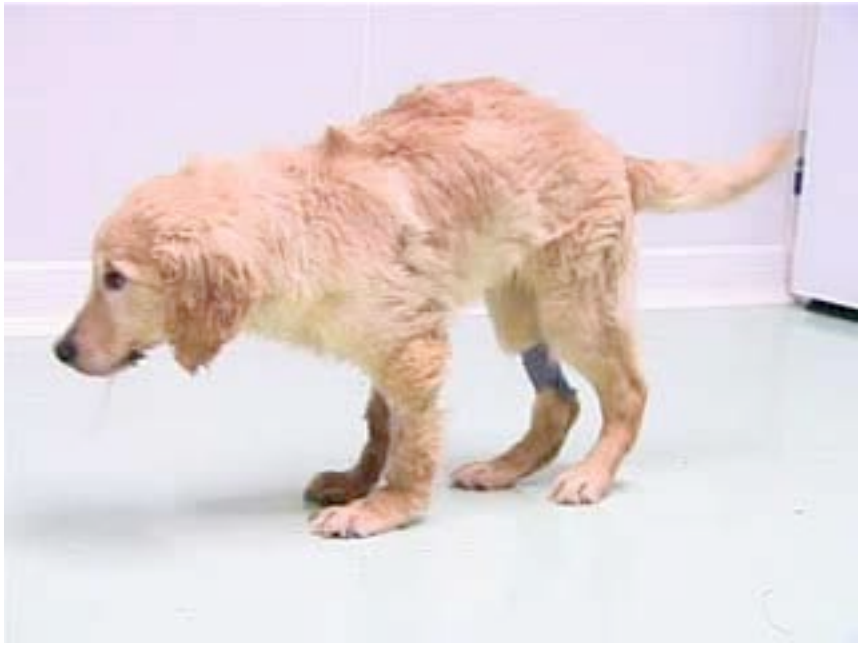
Animal models for DMD

mdx mouse (nonsense mutation in exon 23)

- Pathology does not mimic human disease
- Fiber degeneration, compensation by regeneration
- Myopathy grows less severe later in life
- Normal lifespan

CXMD golden retriever dogs

- Spontaneous mutation, pathology first described in 1988
- Exon skipping, frame shift due to splice site mutation
- Severe muscle degeneration



Conventional gene therapy for DMD

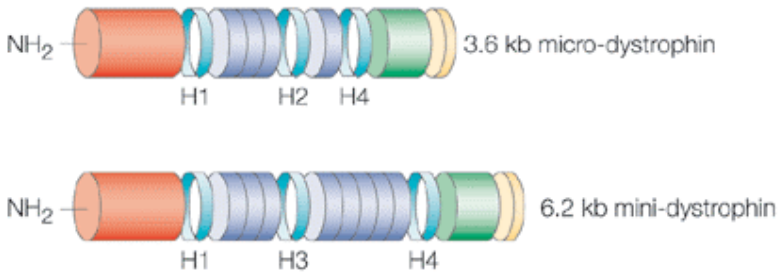
Delivery of full-length gene with adenovirus, herpes simplex virus, or plasmid vectors

High transduction levels are required; work OK in regenerating mouse muscle but persistence is poor

Inflammatory response is a particular problem since degenerating muscle is already inflamed

Viral vectors not yet used in human patients

Minigenes mimic Becker, can be carried by simpler and more efficient vectors. Alleviate degeneration in *mdx* mice.

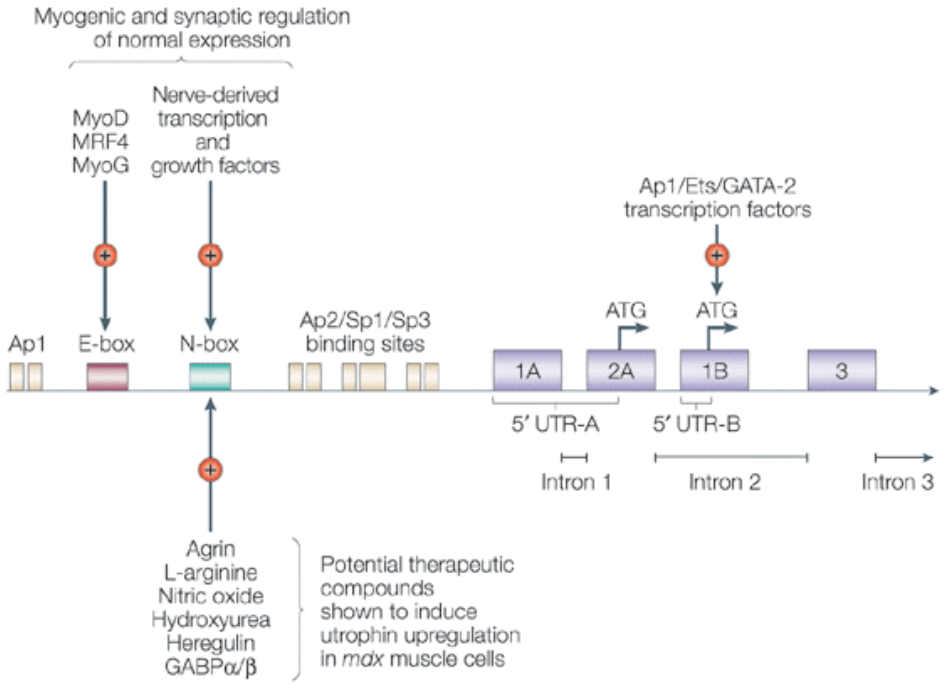


Utrophin as a substitute?

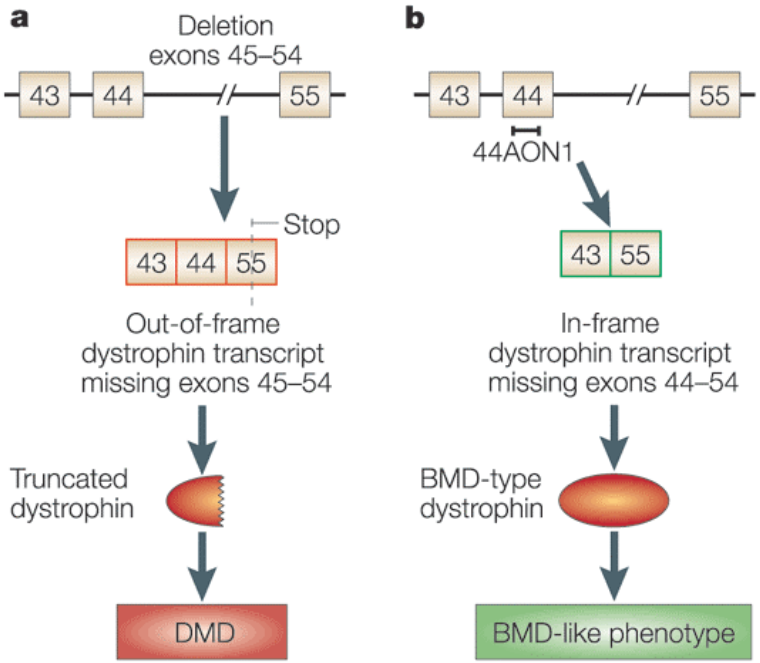
Ubiquitously expressed dystrophin paralog

Found in mature skeletal muscle fibers at neuromuscular junctions; associates with acetycholine receptors

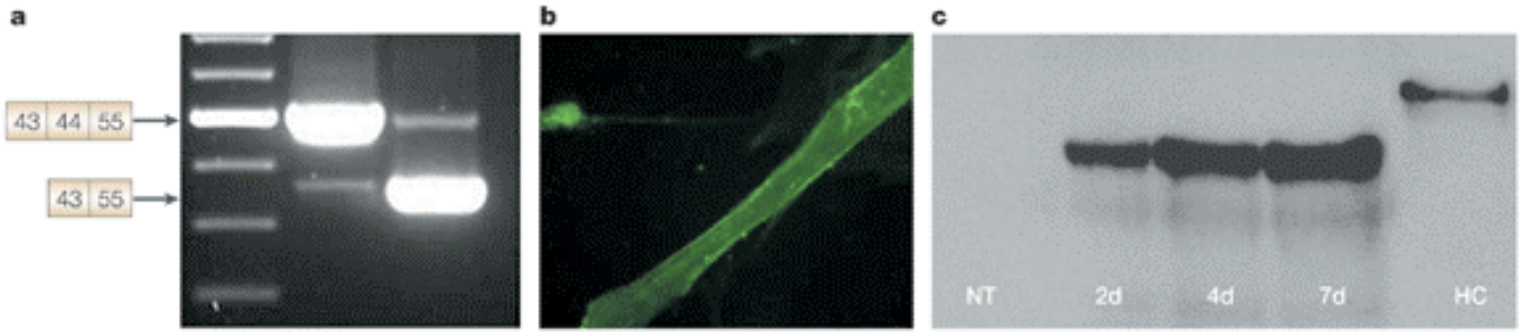
Expression upregulated in *mdx* mice and protein relocates to a dystrophin-like pattern; double knock-out mice have pathology more similar to human DMD



Alternative genetic approach: exon skipping

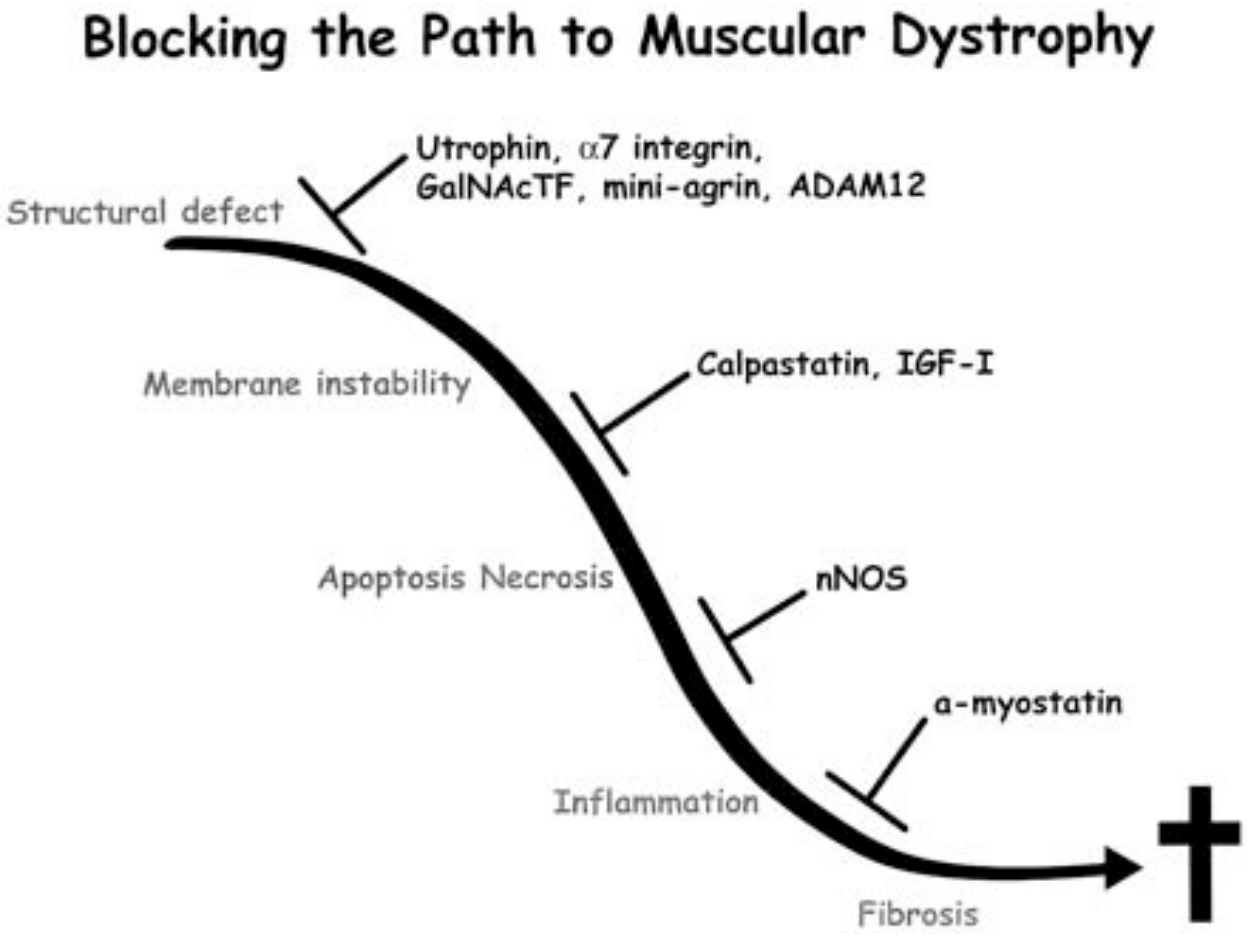


- Related to mechanism of "reversion" in DMD**
- Antisense oligonucleotides block splice site recognition
- Temporary effect in *mdx* mice and cultured human myotubes



Aartsma-Rus et al., 2003, Hum. Mol. Genet., 12:907

A different approach: Target "booster genes" that affect disease progression



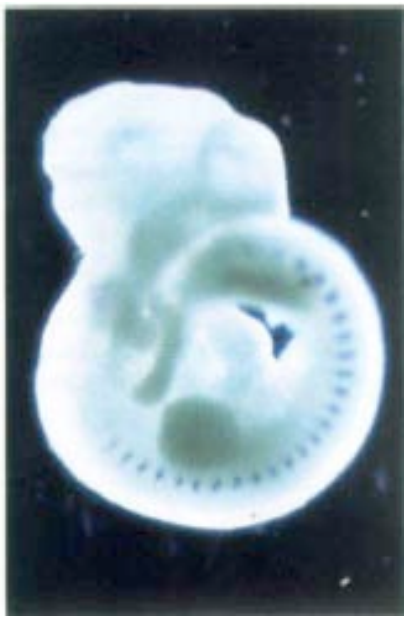
Myostatin as a candidate to increase muscle mass and regeneration in DMD

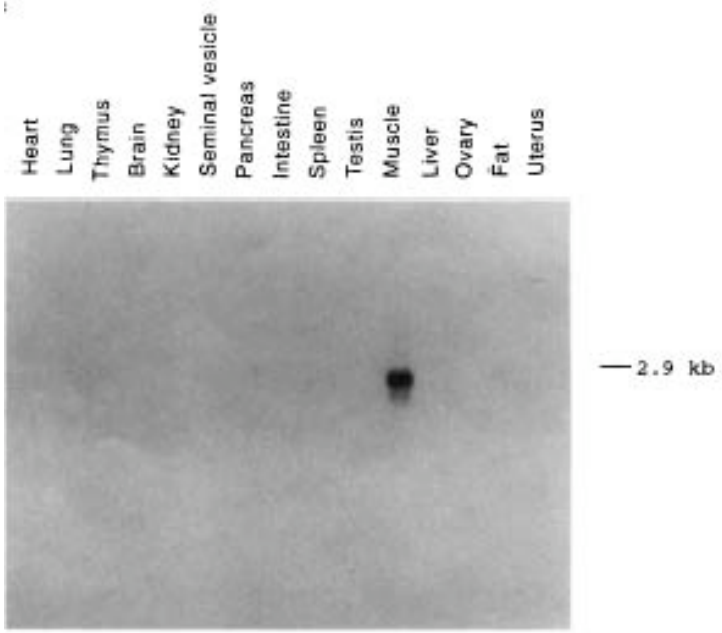
Note regeneration appears to compensate early in disease progression

Candidate for regulation: MYOSTATIN

TGF-β family member, cloned by degenerate PCR

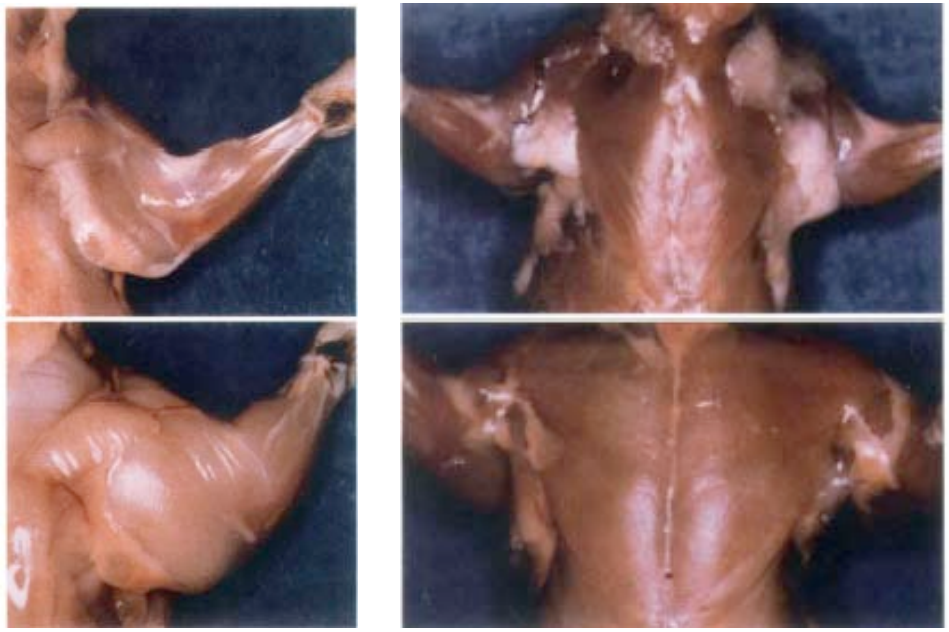
Expressed exclusively in skeletal muscle at all stages of development





McPherron et al., 1997, Nature 387: 83

Myostatin knockout supermice



30% larger than littermates
"Abnormal body shape with pronounced shoulders and hips"
Skeletal muscle mass increases 2-3X
Hyperplasia and hypertrophy (note different dominant negative alleles can separate these two effects)

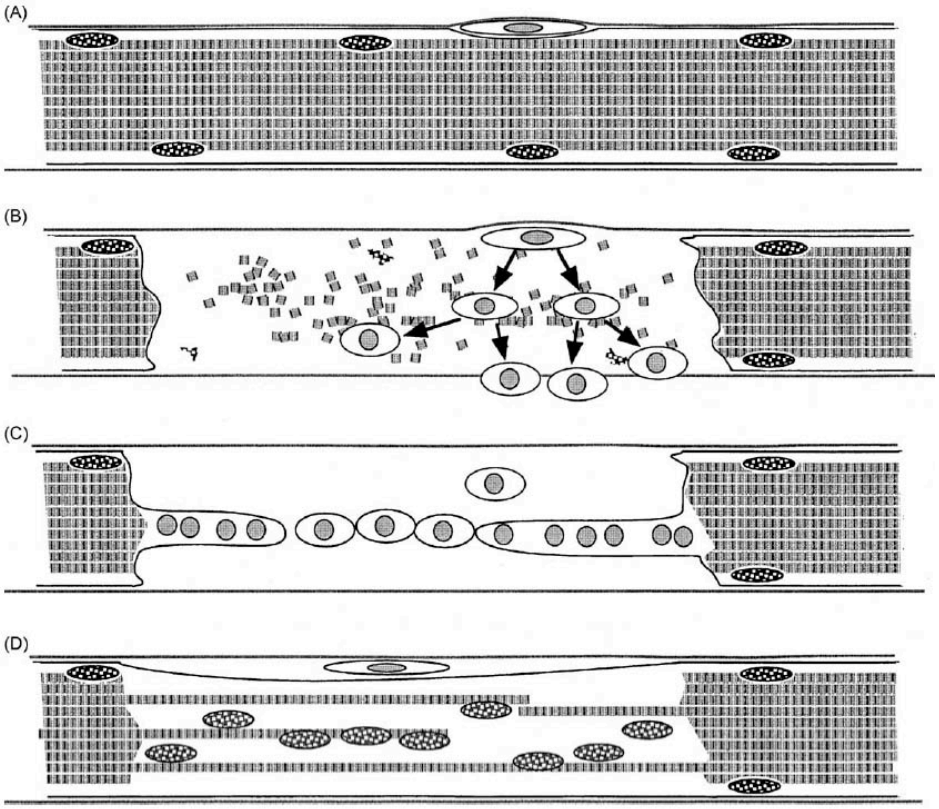
McPherron et al., 1997, Nature 387: 83

Also mysotatin defects in double-muscled cattle

McPherron and Lee, 1997
PNAS 94: 12457

And in humans!

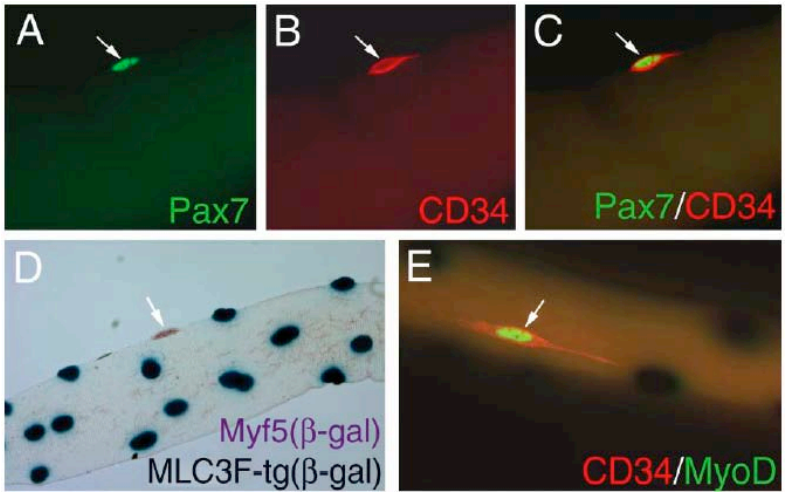
Myostatin regulates satellite cell proliferation and differentiation



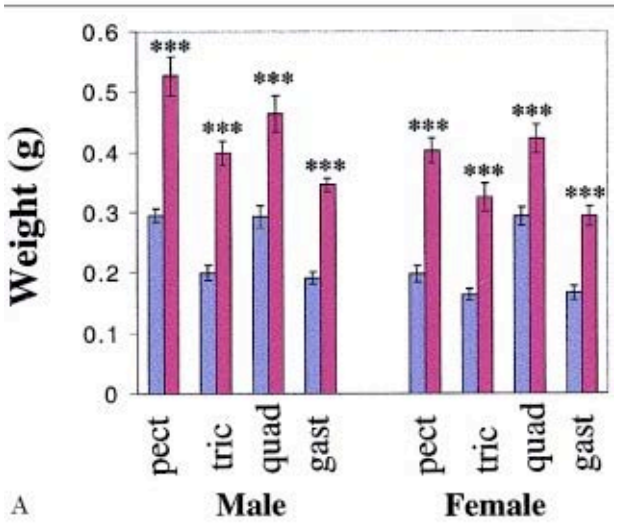
Muscle fiber regeneration
by satellite cell activation

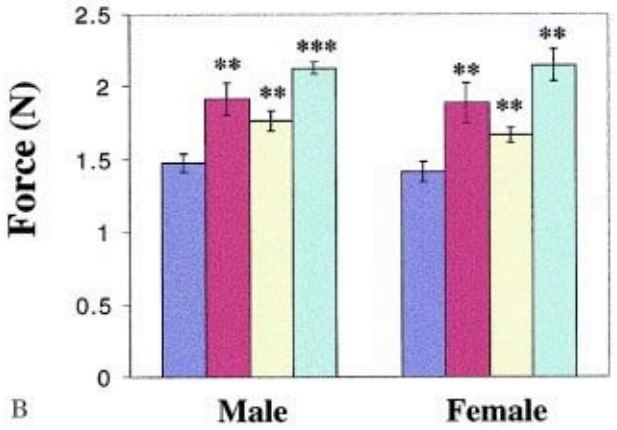
Secreted by mature muscle fibers

In satellite cells, represses cyclin-dependent kinase Cdk2 and upregulates expression of CKI p21



Myostatin KO improves muscle mass and grip strength in *mdx* mice





Blue - mdx
Red - mdx, Mstn-/-
Yellow - wild type
Green - Mstn -/-

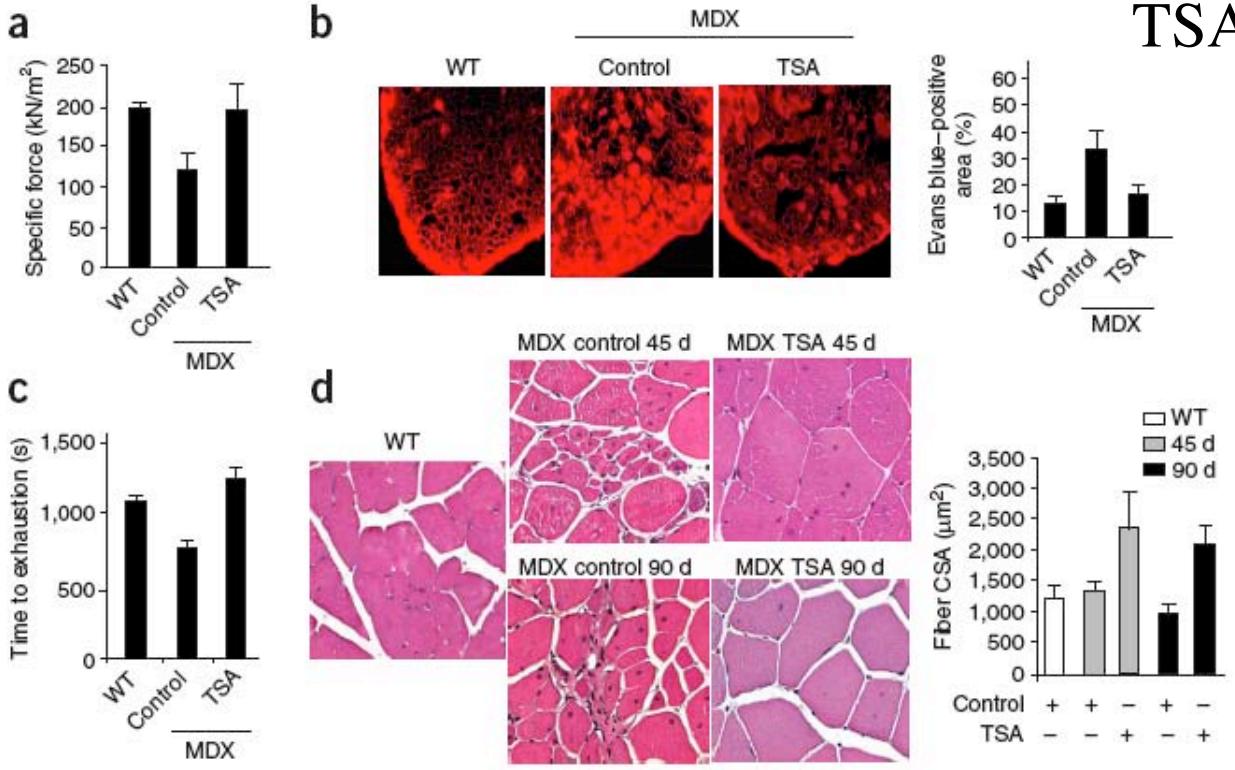
Blockade with antibody has a similar beneficial effect

Wagner et al., 2002, Annal. Neurol. 52:832

Deacetylase inhibitors: indirect regulation of myostatin

Various general deacetylase inhibitors upregulate expression of the myostatin inhibitor follistatin

TSA = trichostatin A

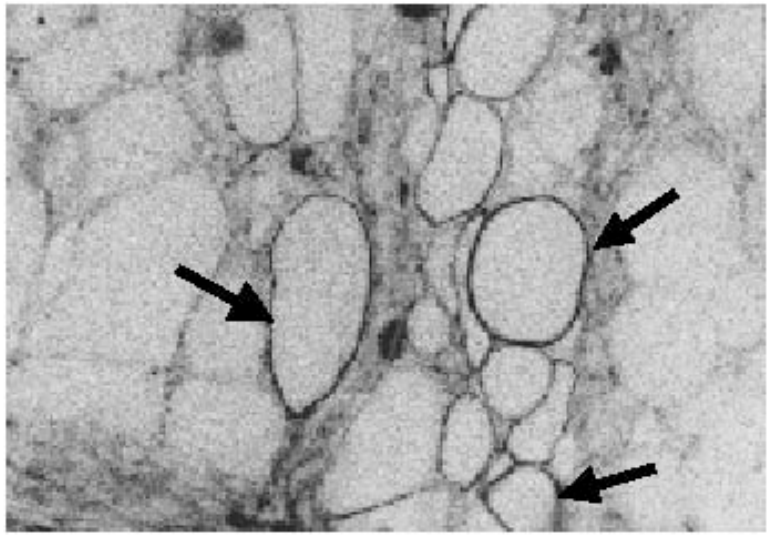


Minetti et al., 2006, *Nature Medicine*, **12**: 1147

Cell-based therapies (transplantation)

Myoblasts can be readily cultured from donor biopsy samples

Human trials; repeated injections into biceps muscle over 6 months gave some fibers (1-10%) expressing donor-derived dystrophin but no clinical improvement



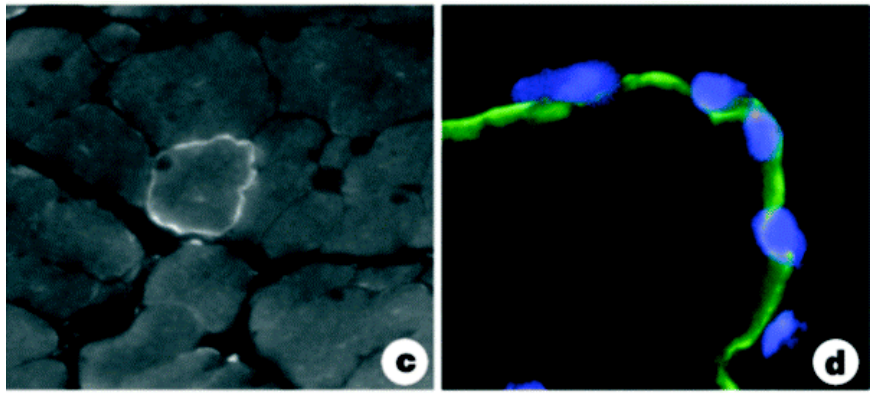
Mendell et al., 1995, NEJM 333: 832

Stem cells?

Satellite cells are rare and difficult to isolate, possibly heterogeneous

Bone marrow stem cells????

- Bone marrow contains multipotent mesenchymal progenitor cells as well as hematopoeitic stem cells
- *mdx* mice have dystrophin-positive fibers with donor nuclei after BMT



Red dot = Y chromosome

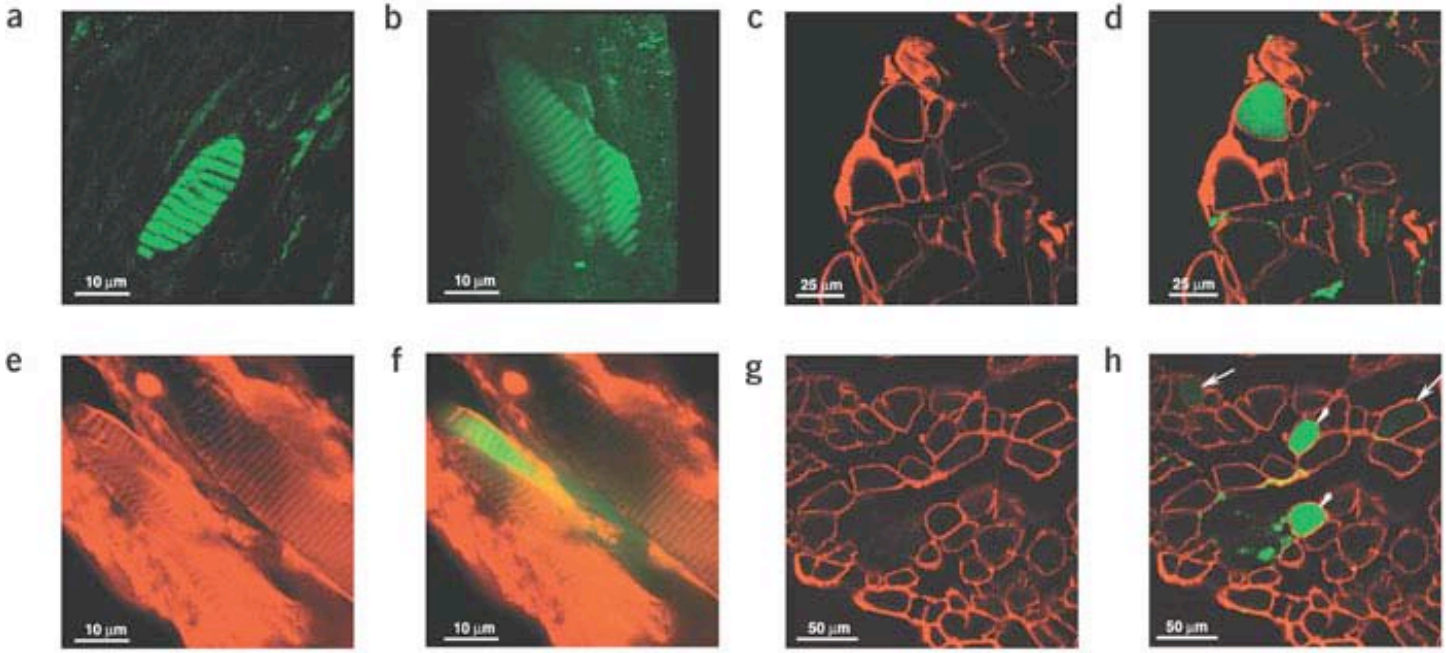
(male donor, female recipient)

Gussoni et al., 1999, Nature 401: 390

DMD patient who had received BMT for X-linked immunodeficiency had donor nuclei persisting in muscle after 13 years, but no expression of donor-derived dystrophin (and there are always a few revertant fibers…)

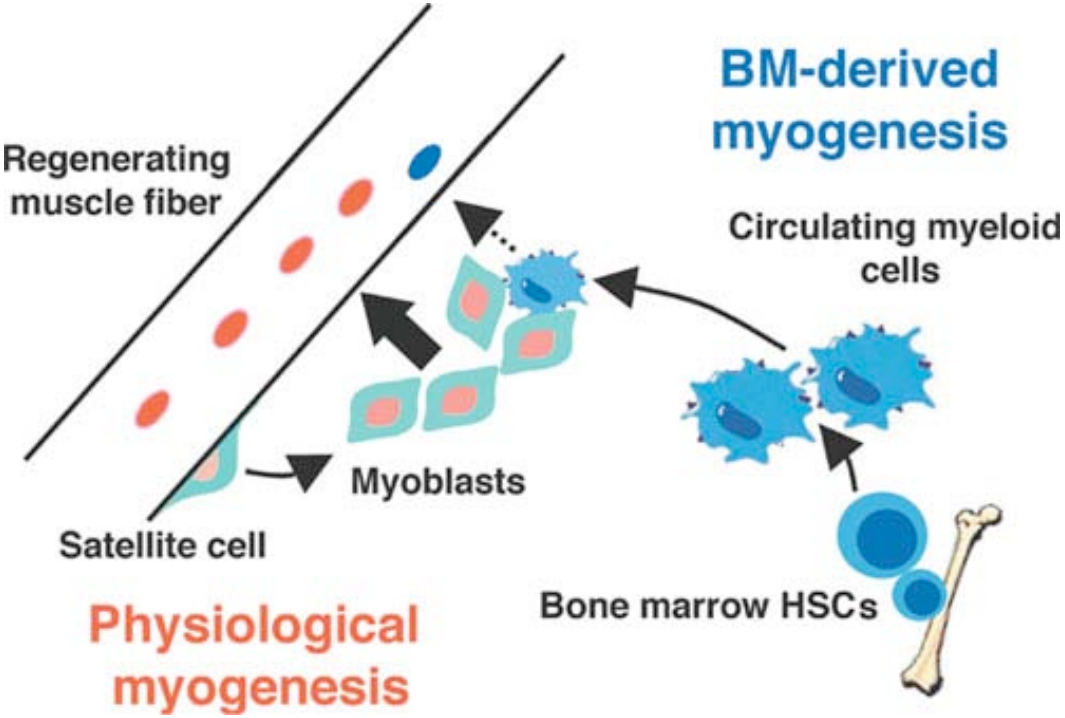
(Gussoni et al., 2002, J. Clin Inv. 110: 807)

Animals reconstituted with a single HSC have some GFP-positive myofibrils: plasticity of adult stem cells?



Corbel et al., 2003, Nature Medicine, 9:1528

BUT: this is due to fusion of a myeloid cell with a regenerating muscle fiber and not due to trans-differentiation



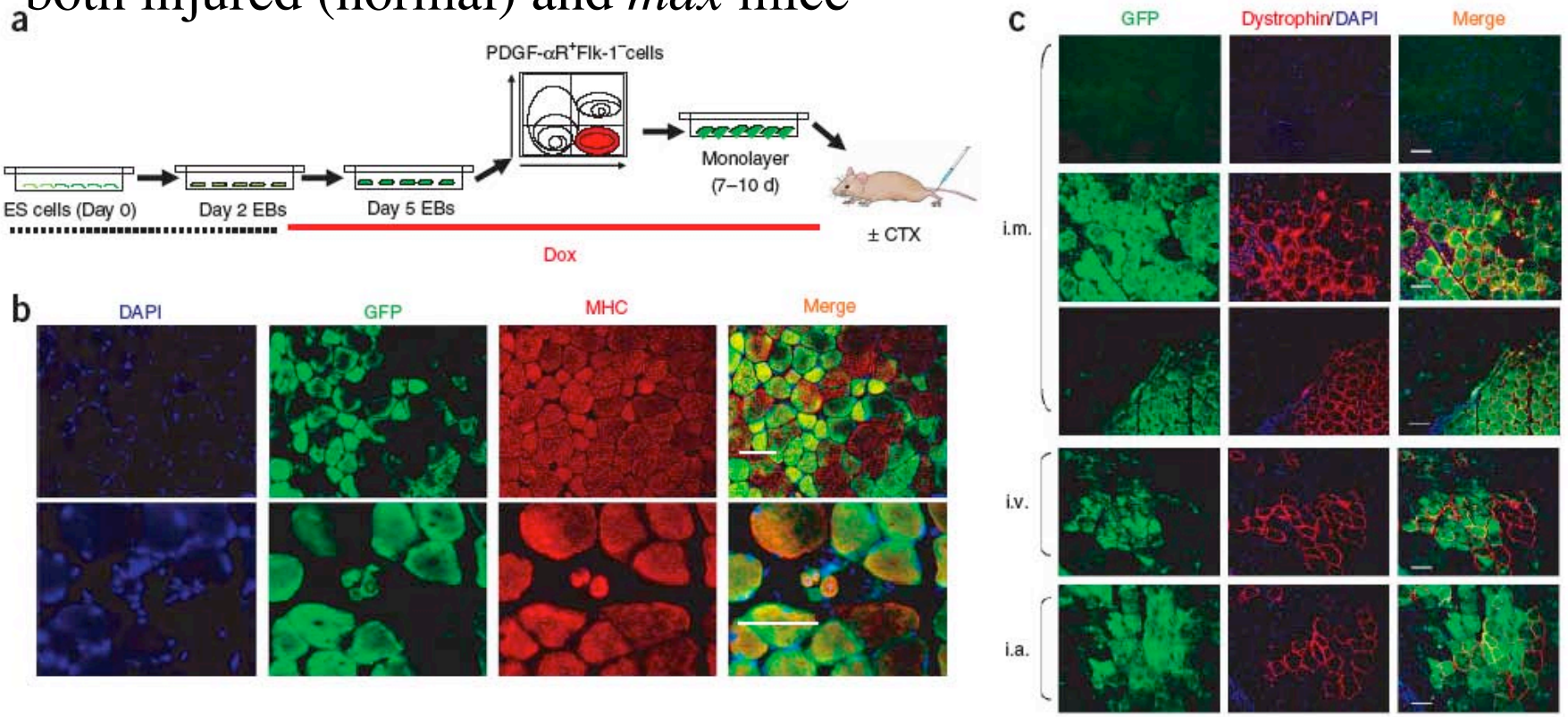
Note similar adhesion and signaling pathways in myoblast fusion and in monocyte fusion to form osteoclasts, also macrophage fusion

Camargo et al., 2003, Nature Medicine, 9:1520

ES cells?

ES cells induced to express Pax3 show increased differentiation into mesodermal lineages

Sorted PDGFαR^{+}, Flk-1^{-} cells can engraft in skeletal muscle of both injured (normal) and *mdx* mice



Darabi et al., 2008, *Nature Medicine* **14**: 134

Alternative stem cell for muscle: mesangioblasts

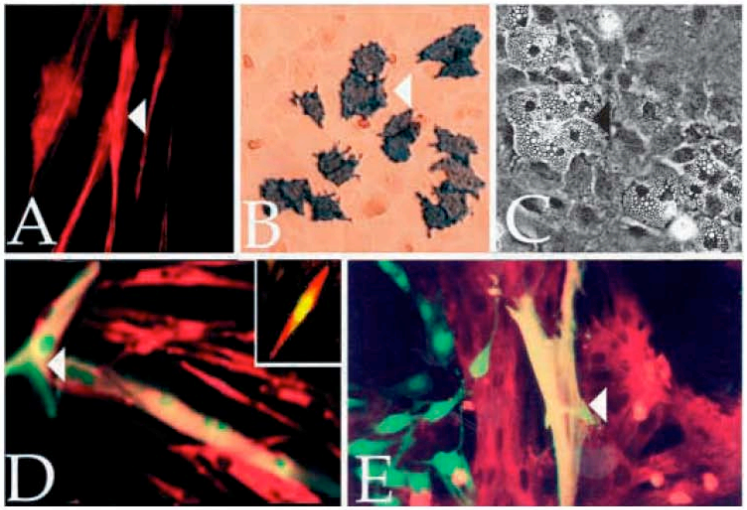
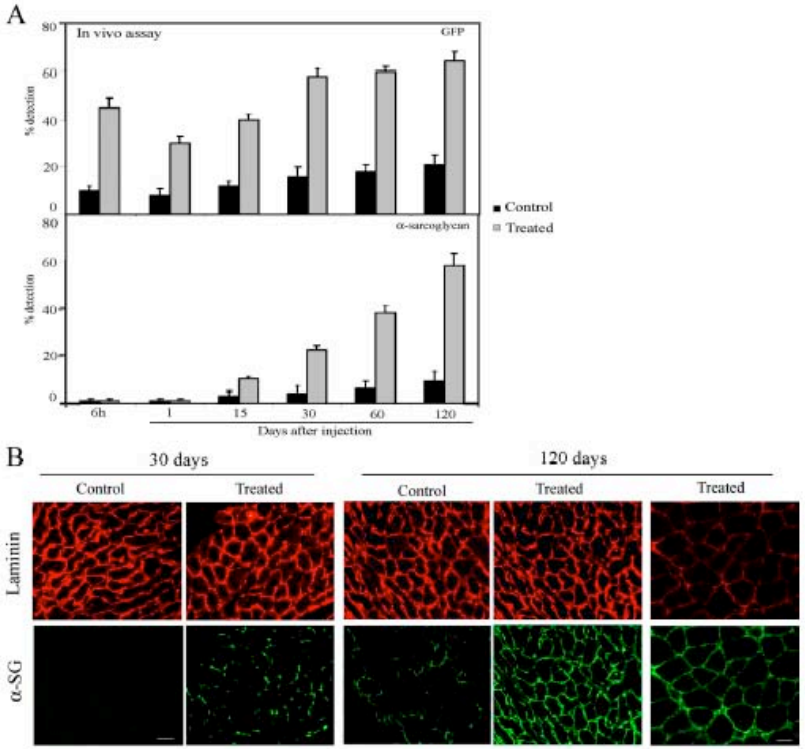


Fig. 6. In vitro differentiation of the aorta derived cell line A4 into different cell types. (A) Smooth muscle cells (SMA positive, arrowheads). (B) Osteoblasts (ALP positive, arrowheads) are detected after treatment with 1 ng/ml BMP2; (C) Adipocytes are detected after treatment with 10 ng/ml of dexamethasone. (D) Skeletal myotubes are detected after co-culture of GFP-labeled A4 cells with C2C12 myoblasts. GFP-positive cells appear green, myocytes and myotubes expressing myosin heavy chains appear red, and cells expressing both appear yellow in the merged image (arrowhead). A mononucleated, differerentiated, GFP-positive myocyte is shown in the inset in D. (E) Cardiocytes are detected after co-culture of GFP-labeled A4 cell with rat neonatal cardiocytes. GFP-positive cells appear green, myocardiocytes expressing cardiac specific troponin 1 appear red and cells expressing both appear yellow in the merged image (arrowhead).

Multipotent stem cells originally isolated from mouse aorta

Treatment with cytokines enhances cell motility and engraftment



Cossu group, 2002 *Development* **129**: 2773; 2006 *J Cell Biol* **174**: 231

Papers for Wednesday

van Deutekom JC, Janson AA, Ginjaar IB, Frankhuizen WS, Aartsma-Rus A, Bremmer-Bout M, den Dunnen JT, Koop K, van der Kooi AJ, Goemans NM, de Kimpe SJ, Ekhart PF, Venneker EH, Platenburg GJ, Verschuuren JJ, van Ommen GJ. "Local dystrophin restoration with antisense oligonucleotide PRO051." *N Engl J Med.* 2007 Dec 27;**357**(26):2677-86.

Sampaolesi M, Blot S, D'Antona G, Granger N, Tonlorenzi R, Innocenzi A, Mognol P, Thibaud JL, Galvez BG, Barthelemy I, Perani L, Mantero S, Guttinger M, Pansarasa O, Rinaldi C, Cusella De Angelis MG, Torrente Y, Bordignon C, Bottinelli R, Cossu G. "Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs." *Nature.* 2006 Nov 30;**444**(7119):574-9.

VOTE!