

The Stories of Jerry's Boys:

From Gene Discovery to Novel
Drug, Cell and Gene Therapies

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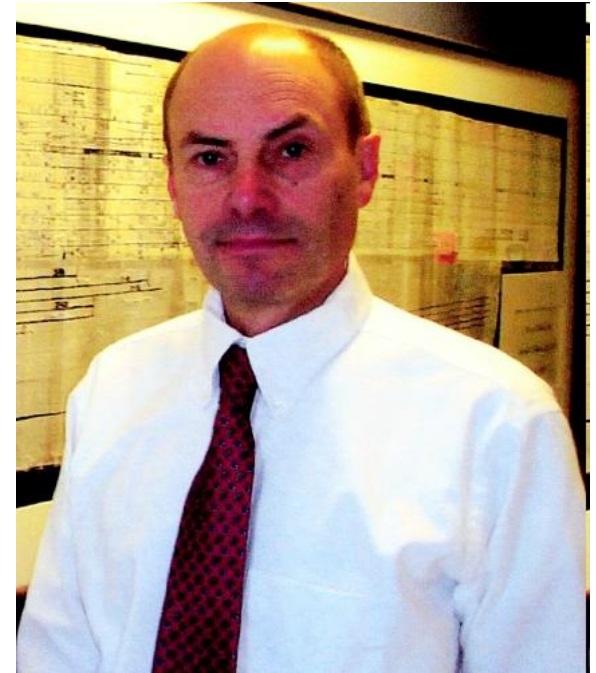
Duchenne and Becker Muscular Dystrophies (DBMD)

- 1: 3,500 boys
- Skeletal muscle disease
 - Wheel chair 12 years
 - Resp/vent assist
- Cardiomyopathy
- Average survival: 20s



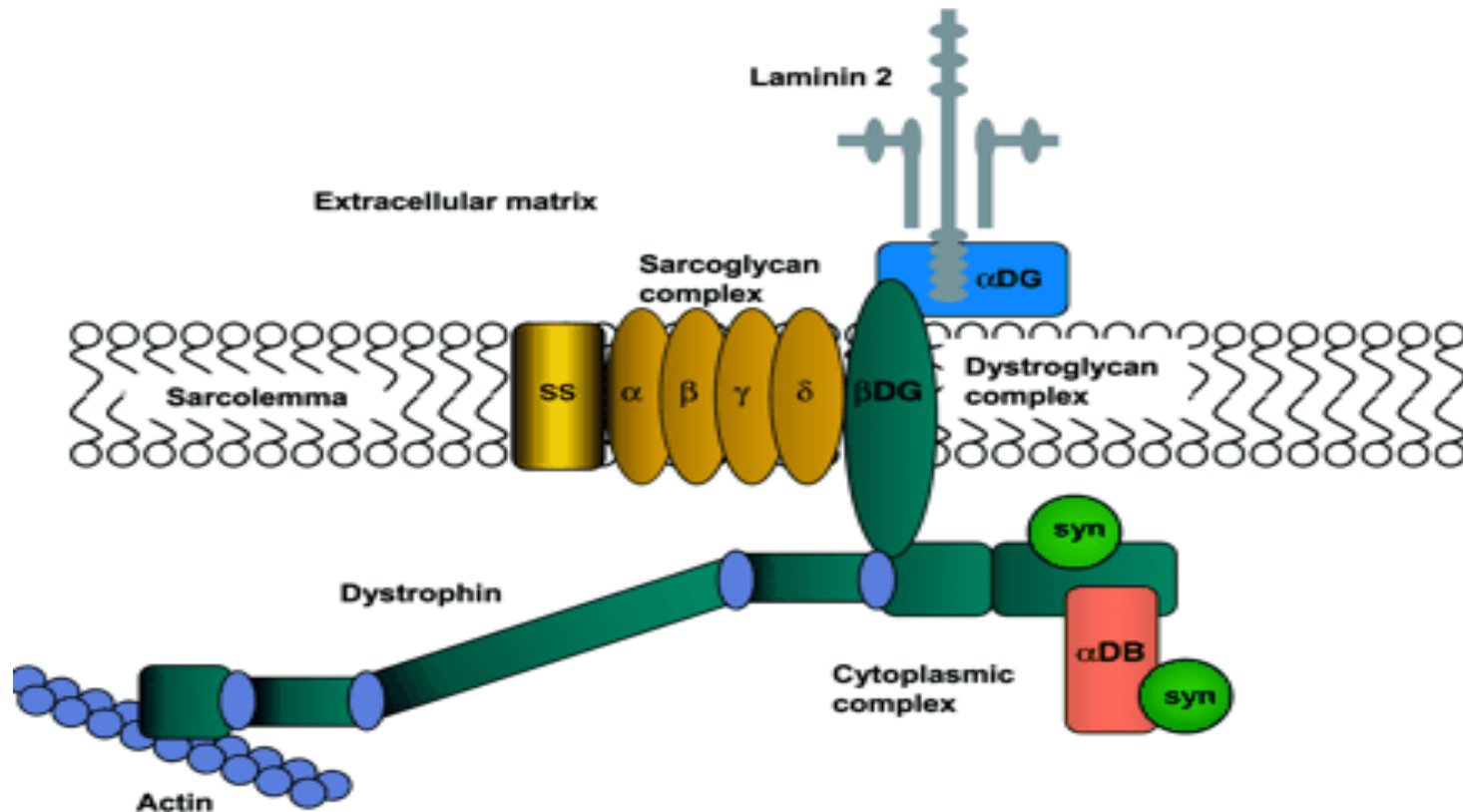
DMD: The gene and protein

Kunkel et al cloned the gene in the Xp21 that encodes *Dystrophin*, the mutation of which causes DMD



Kunkel LM et al Nature 1986, Nature 1986, Nature 1987, Nature 1986, Cell 1986, NEJM 1987 (Nebulin?) and Cell 1987 (Dystrophin)

DMD: The gene and protein

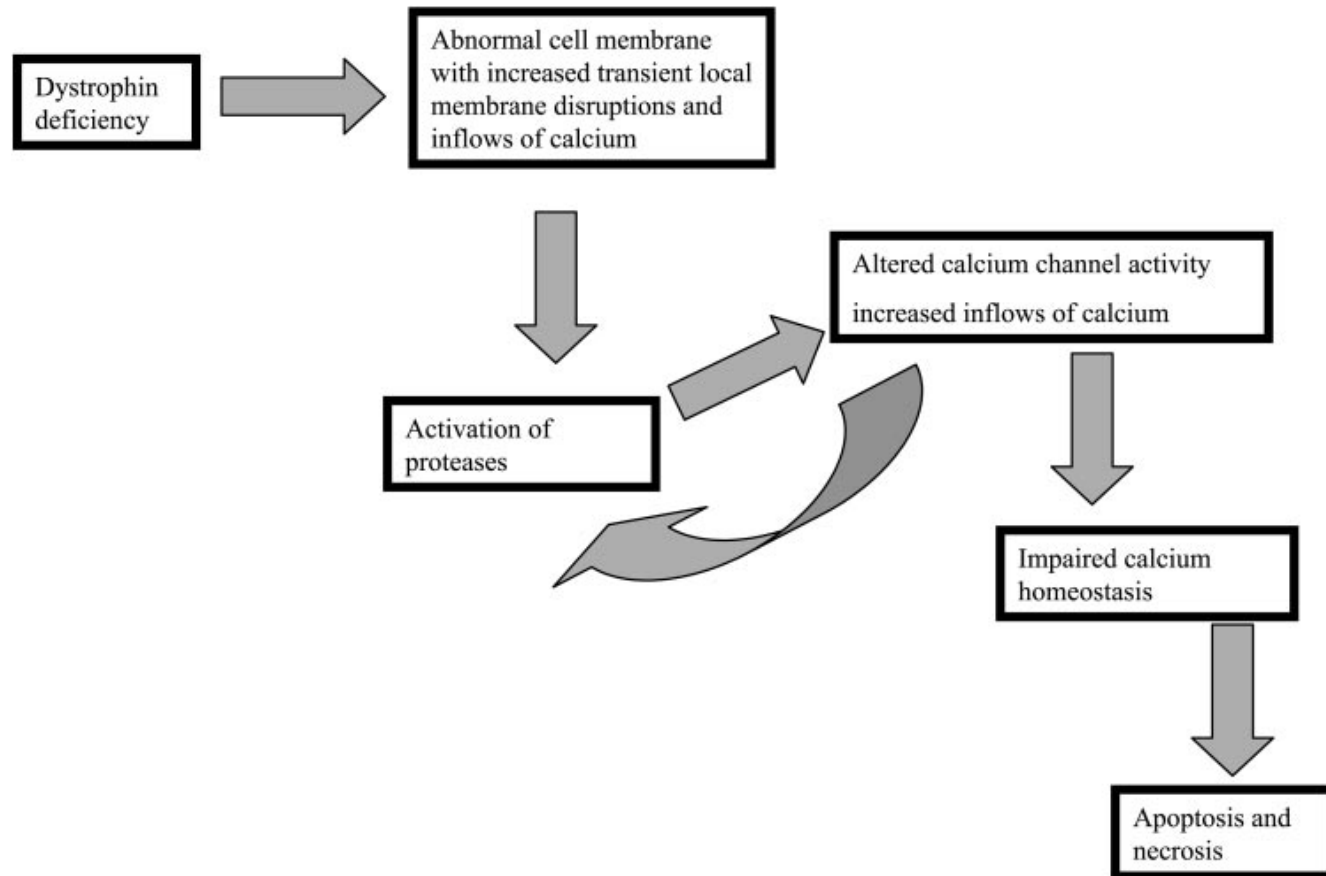


Campbell KP et al and Ozawa E et al Nature 1989;
Cell 1991, Nature 1992, Nat Genet 1995, 1995,
1996, Science 1996

MUSCULAR DYSTROPHIES

- Duchenne Muscular Dystrophy (DMD) Becker Muscular Dystrophy (BMD)
- Emery-Dreifuss Muscular Dystrophy (EDMD): emerin, lamin A or lamin C
- Limb-Girdle Muscular Dystrophy (LGMD):
- Facioscapulohumeral Muscular Dystrophy (FSH or FSHD) (Also known as Landouzy-Dejerine)
- Myotonic Dystrophy (MMD) (Also known as DM or Steinert Disease)
- Oculopharyngeal Muscular Dystrophy (OPMD)
- Distal Muscular Dystrophy (DD) (Miyoshi)
- Congenital Muscular Dystrophy (CMD)

DMD: Pathogenesis



Blake DJ et al Physiol Rev 82:291-329, 2002.

Therapies: Supportive

- Skeletal muscle
 - Steroid
 - Rehabilitation, braces, wheel chair
 - Respiratory/Ventilator support
- Cardiomyopathy
 - ACEI, BB
 - IV Inotropic support

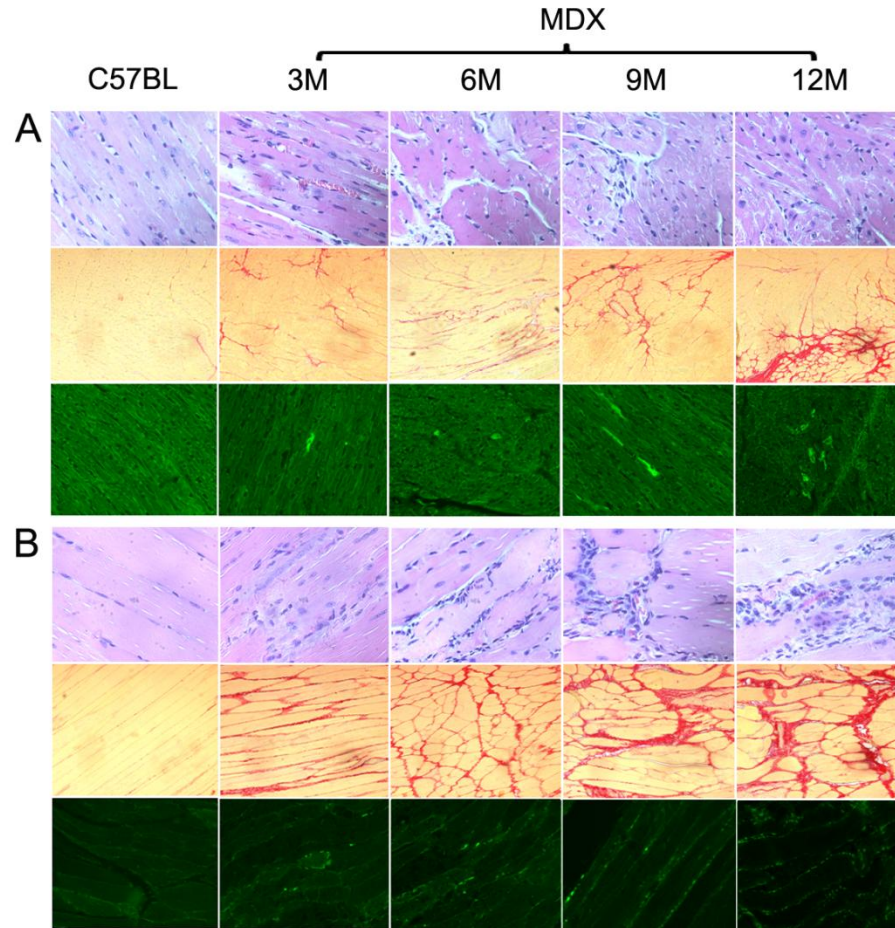
Therapies: Potential Curative

- Potential candidates
 - Small molecule/Drug
 - Gene therapy
 - Cell therapy
- Challenges
 - Rare disease
 - Animal model
 - Local/general disease, skeletal, cardiac, & smooth muscle
 - Skeletal vs cardiac muscle

Therapies: Potential Curative

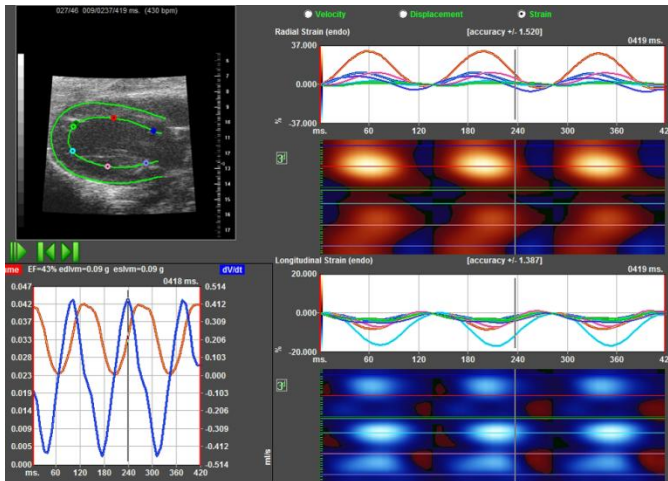
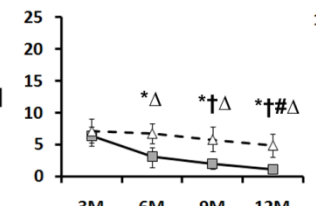
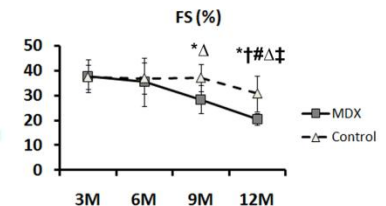
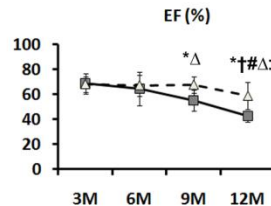
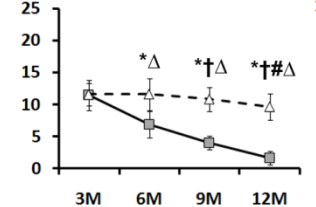
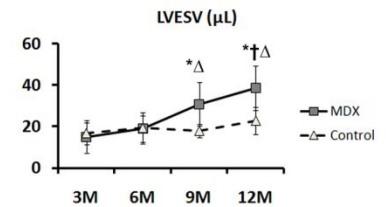
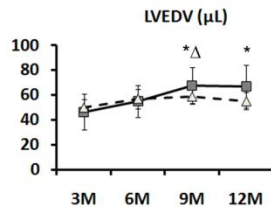
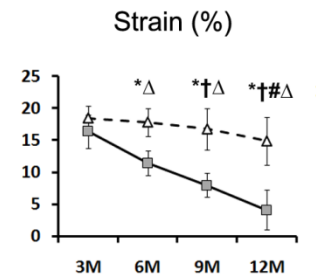
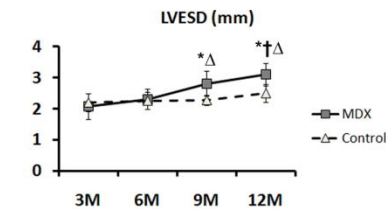
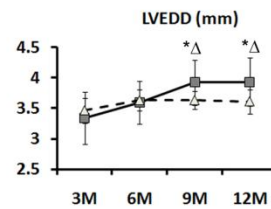
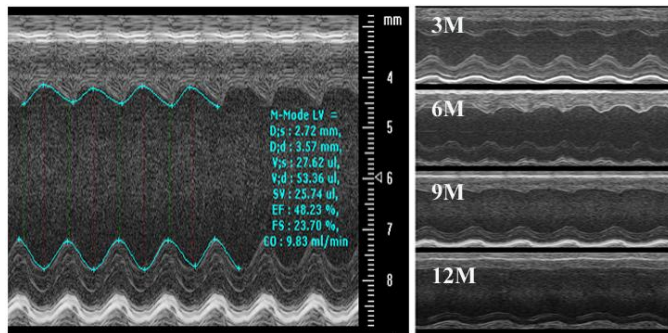
- Animal models
 - *mdx* mouse: point mutation in *Dystrophin* gene
 - Sicinski P, Science. 1989 Jun 30; 244(4912): 1578-80.

Therapies: Potential Curative



Zheng M...Ge S. Circulation. 2010;122:A14483

Therapies: Potential Curative



Zheng M...Ge S. Circulation. 2010;122:A14483

Drug Therapy:

Aminoglycoside antibiotics/ PTC 124

- Aminoglycoside antibiotics/ PTC 124 restore dystrophin function to skeletal muscles of mdx mice in vitro and in vivo
- Some of dystrophin mutation are premature stop codons; aminoglycoside treatment can suppress stop codons and selectively induce ribosomal readthrough of premature but not normal termination codons
- Barton-Davis ER...Sweeney HL. J Clin Invest. 1999 Aug;104(4):375-81
- Welch EM... Sweeney HL *Nature* **447**, 87-91 (3 May 2007)

Drug Therapy: myostatin blockade

Functional improvement of dystrophic muscle by myostatin blockade

Myostatin (GDF8)/TGF- β superfamily is a negative regulator of skeletal muscle growth

Mutations in the myostatin (GDF8)/TGF- β superfamily gene show a marked increase in body weight and muscle mass

Intraperitoneal injections of blocking antibodies for three months resulted in an increase in body weight, muscle mass, muscle size and absolute muscle strength in *mdx* mouse muscle along with a significant decrease in muscle degeneration and concentrations of serum creatine kinase

Bogdanovich S Nature **420**, 418-421 (28 November 2002)

Drug Therapy:

TGF-beta blockade or inhibition

Angiotensin II type 1 receptor blockade attenuates TGF-beta-induced failure of muscle regeneration in multiple myopathic states.

- Increased TGF-beta activity leads to failed muscle regeneration in fibrillin-1-deficient mice**
- Systemic antagonism of TGF-beta through administration of TGF-beta-neutralizing antibody or the angiotensin II type 1 receptor blocker losartan normalizes muscle architecture, repair and function in vivo.**
- Moreover, TGF-beta-induced failure of muscle regeneration and a similar therapeutic response in a dystrophin-deficient mouse model of Duchenne muscular dystrophy.**

Cohn RD...Dietz HC. Nat Med. 2007 Feb;13(2):204-10

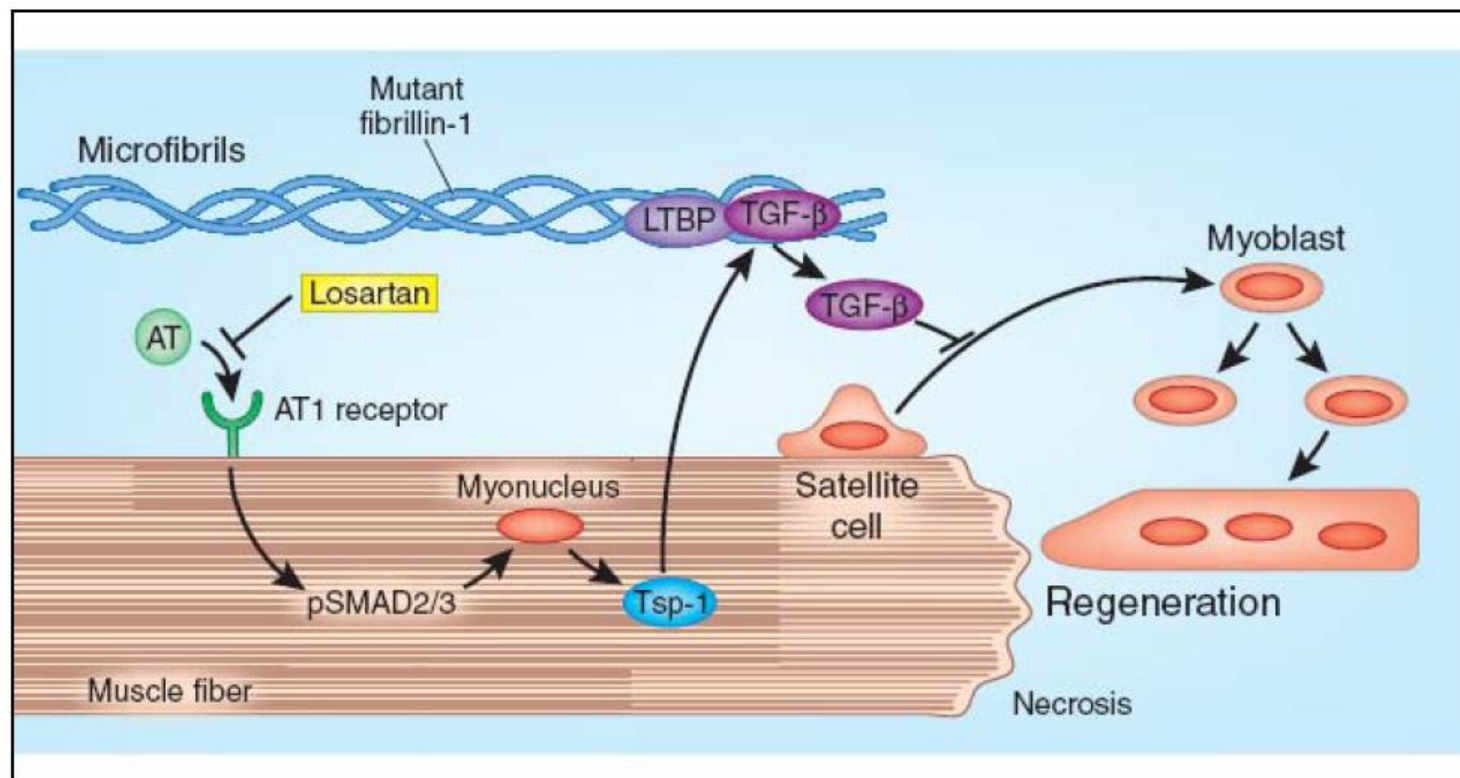


Figure 1. Schematic of the AT1/TGF- β pathways and mechanisms of AT1 blockade by losartan for muscle repair and regeneration in DBMD.

Myoblast transfer

- IV

- Gussoni E et al Nature. 1992 Apr 2;356(6368):435-8.
- Mendell JR, et al N Engl J Med. 1995 Sep 28;333(13):832-8.
- Gussoni E, et al. Nature. 1999 Sep 23;401(6751):390-4.

- IA

- Cell therapy of alpha-sarcoglycan null dystrophic mice through intra-arterial delivery of mesoangioblasts.

Sampaolesi M, et al. Science. 2003 Jul 25;301(5632):487-92

Drug Therapy: Sildenafil

- Defective nitric oxide (NO)/cGMP pathway in early DCM
- *mdx* mice overexpressing, guanylyl cyclase (GC) maintained their contractile performance vs age-matched *mdx*
- Phosphodiesterase inhibitor sildenafil reduced cardiomyocyte damage induced by isoproterenol

Khairallah M et al Proc Natl Acad Sci U S A. 2008 May 13; 105(19): 7028–7033

Gene therapy: Dystrophin

- Overexpression of dystrophin in transgenic mdx mice eliminates dystrophic symptoms without toxicity

Cox GA...Chamberlain JS Nature. 1993 Aug
19;364(6439):725-9

Gene therapy: Dystrophin

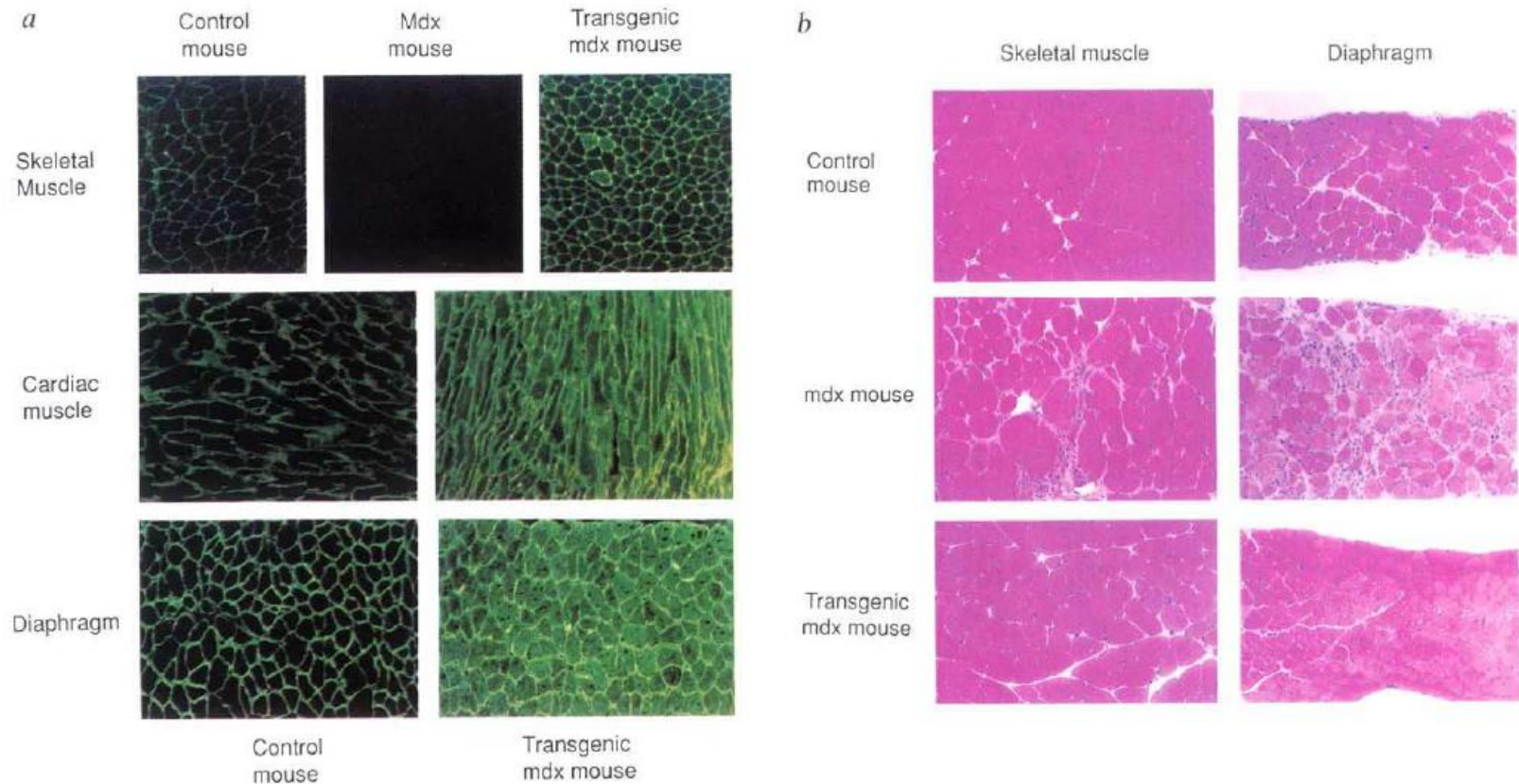


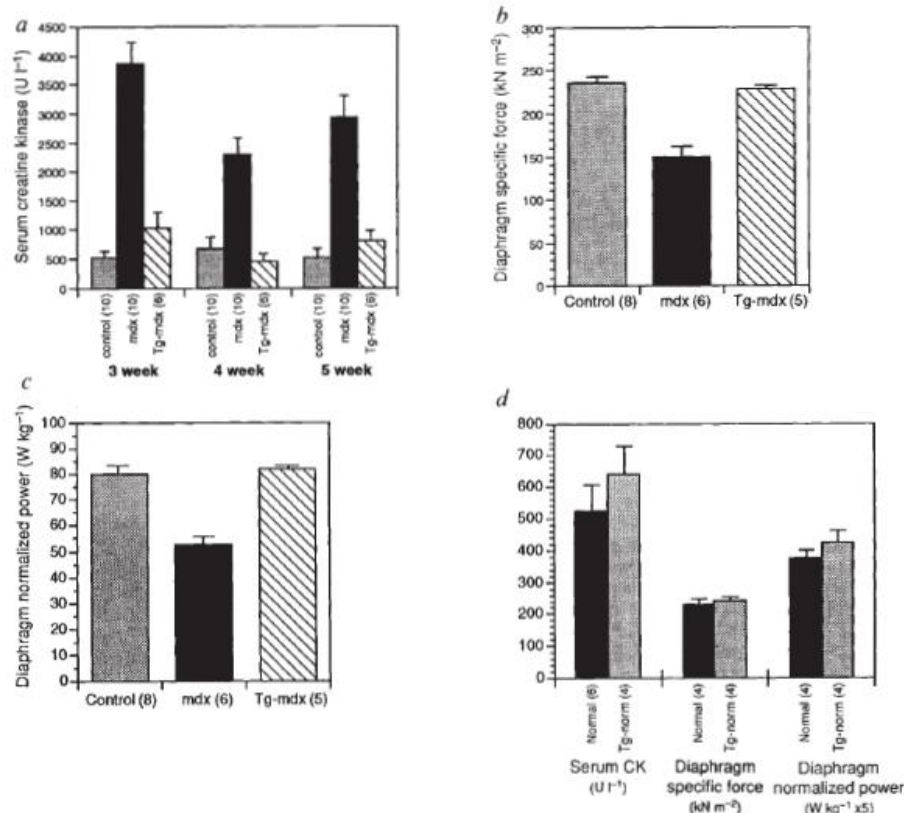
FIG. 2 Immunostaining and histological analysis of age-matched (6-month) C57BL/10 control, *mdx* and transgenic *mdx* muscle. *a*, Dystrophin immunostaining is localized to the sarcolemma of control quadriceps, cardiac and diaphragm muscles and is absent from *mdx* muscle, except for rare revertant dystrophin-positive fibres. (Magnifications: ►

Gene therapy: Dystrophin

FIG. 4 Dystrophin expression in skeletal and cardiac muscles of transgenic *mdx* mice results in control levels of serum creatine kinase (CK). Serum samples were assayed at 3, 4 and 5 weeks of age in F_3 normal heterozygous (+/*mdx*) female, and hemizygous *mdx* and transgenic *mdx* (Tg-*mdx*) male littermates (26 animals in 3 F_3 litters). The serum CK levels of heterozygous (+/*mdx*) mice are not significantly different from wild type, enabling these littermates to be used as normal controls¹⁷. The number of mice (*n*) in each group is shown in parentheses. *b* and *c*, Specific forces and normalized powers developed by diaphragm muscles from a sample of the same littermates as in *a* at 3 months of age. *d*, Comparison of serum CK levels, diaphragm specific force and diaphragm normalized power in phenotypically normal F_3 heterozygous (+/*mdx*) female littermates with (Tg-norm) or without (normal) the dystrophin transgene. Serum CK values were based on an average value of each animal measured at 3, 4 and 5 weeks of age.

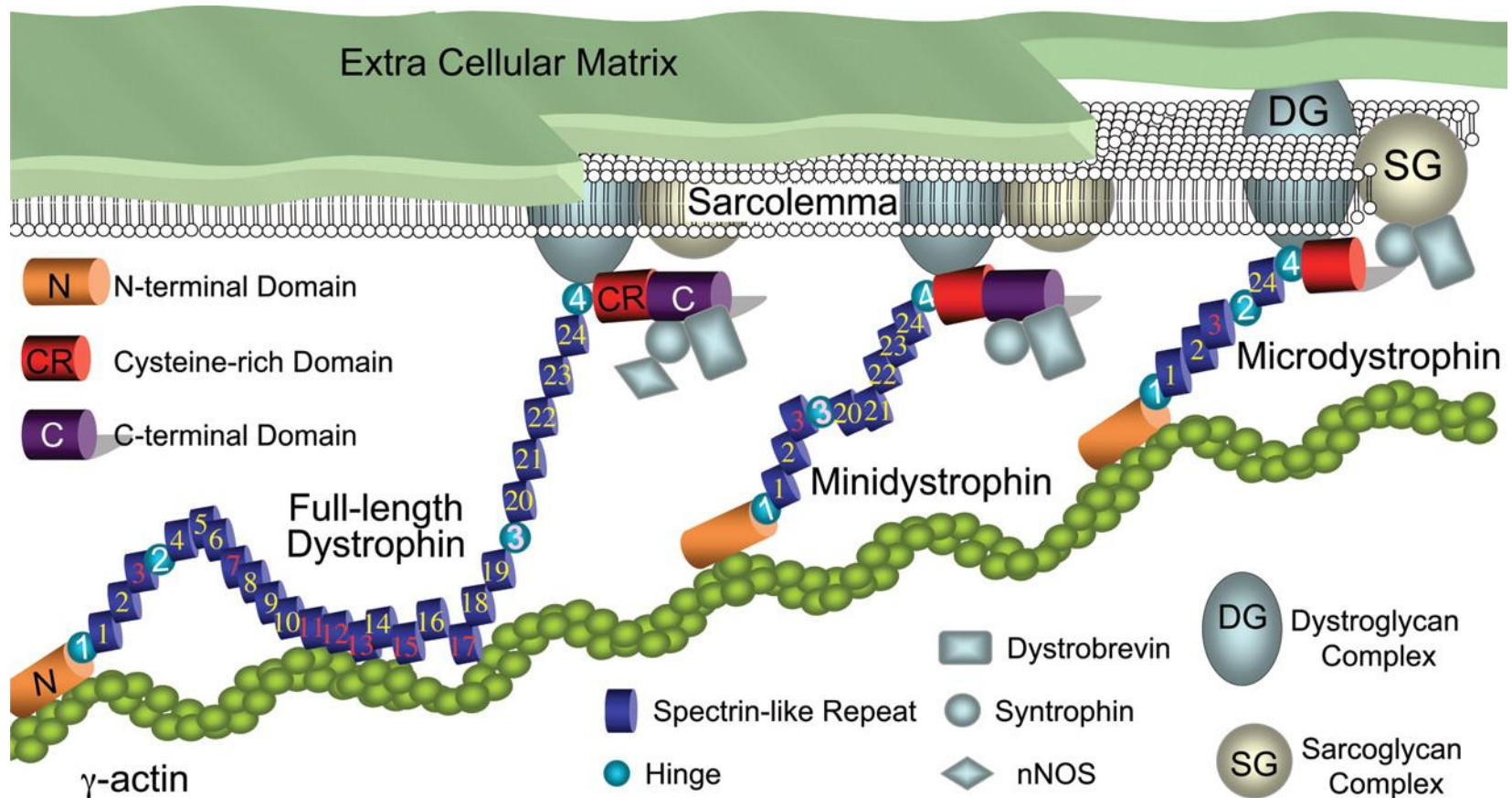
METHODS. Serum samples from mice were obtained from the retro-orbital sinus using heparinized capillary tubes. Serum CK measurements were determined using a coupled enzyme spectrophotometric assay (Sigma). Diaphragm strips 1 to 2 mm wide, including an adjacent section of a single rib and part of the

central tendon, were cut from the central region of the lateral costal hemidiaphragm and immersed in an oxygenated bath containing mammalian Ringer solution (pH 7.4) at 25 °C. Muscles were adjusted to the optimum lengths (L_0) for the development of isometric force. Force was determined during maximum isometric tetanic contractions. Power output was determined by isovelocity shortenings from 100% L_0 to 90% L_0 during maximum muscle activation. Initiation of the isovelocity shortening ramp and stimulation of the muscle occurred simultaneously.



Stimulation was terminated at the end of the shortening ramp. Power output during a single contraction was calculated as the product of average force and velocity of shortening. The velocity of shortening and the frequency of stimulation were adjusted to elicit maximum power output^{26,27}. After measurements of power, the central tendon and rib bone were trimmed and the muscle was blotted and weighed immediately. Specific force (kN m⁻²) values were normalized to mean cross-sectional area. Power (W) was normalized by muscle mass (W kg⁻¹).

Schematic outline of full-length dystrophin, minidystrophin and microdystrophin and their interaction with other cellular proteins



Gene therapy: microdystrophin

- Systemic delivery of genes to striated muscles using adeno-associated viral vectors.
- Single intravenous administration of recombinant adeno-associated virus pseudotype 6 vectors + vascular endothelium growth factor/vascular permeability factor
- Expression of a functional micro-dystrophin in the skeletal muscles of dystrophin-deficient mdx mice

Gregorevic P...Chamberlain JS. Nat Med. 2004 Aug;10(8):828-34.

Gene therapy: Utrophin

- Utrophin expression is dramatically increased in patients with Duchenne's muscular dystrophy
- Expression of full-length utrophin in 3 lines with different amount of utrophin (Fio, Fer and Fre) prevents muscular dystrophy in mdx mice

Tinsley J...Davies K et al. Nat Med. 1998;Dec;4(12):1441-4

Gene therapy: Utrophin

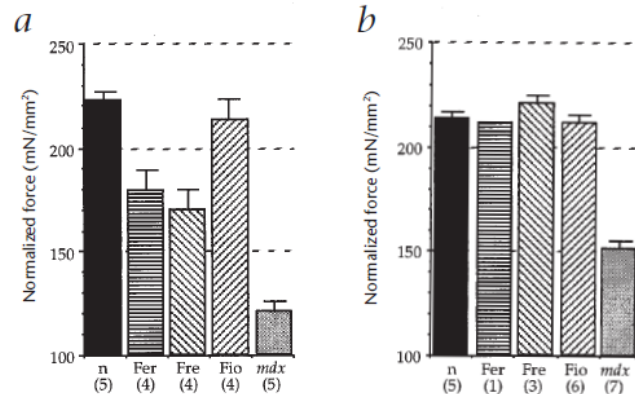
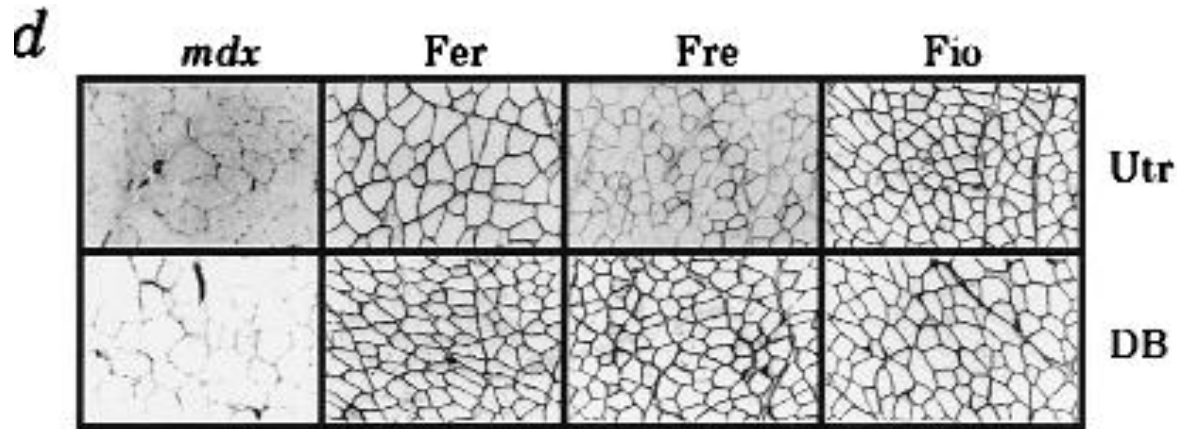


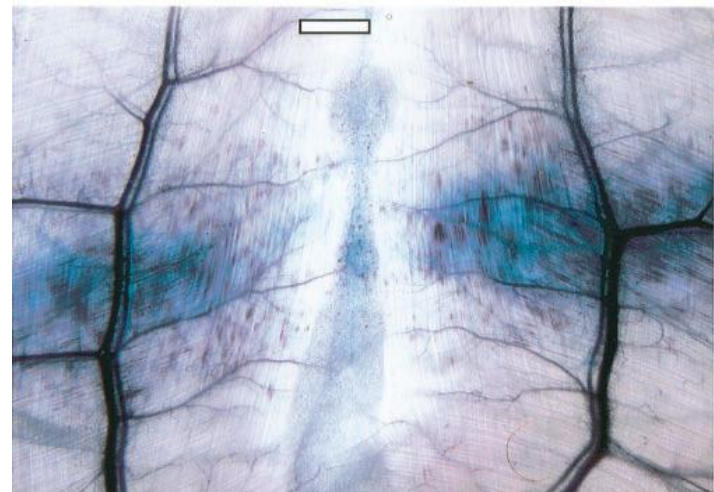
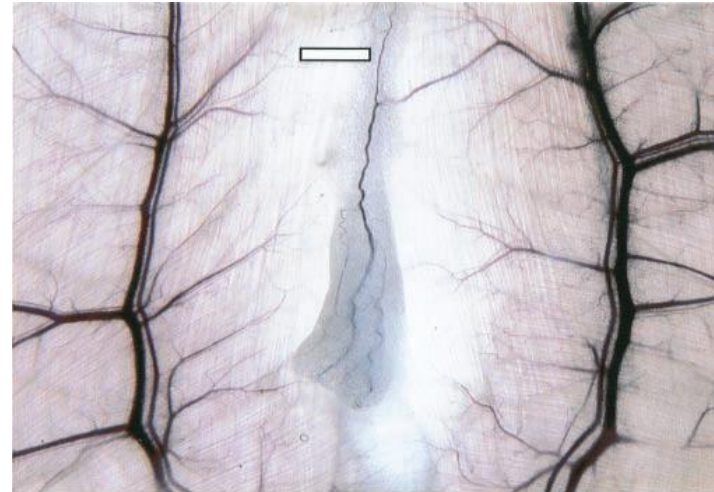
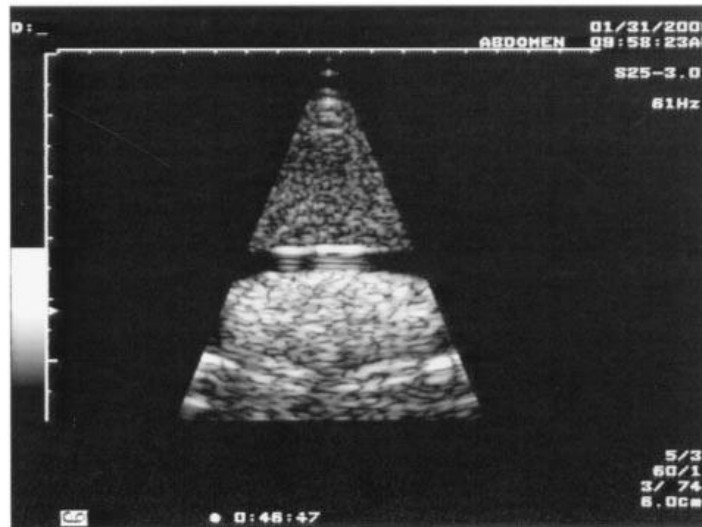
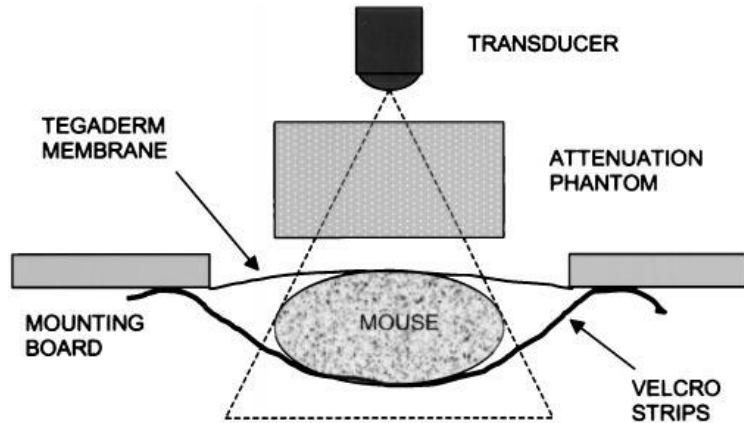
Fig. 2 Mechanics of isolated muscle. **a** and **b**, Evaluation of normalized force (millinewton (mN)/mm²) in diaphragm (**a**) and EDL (**b**). **a**, For the diaphragm, Fer and Fre results were lower than those of normal C57 (n) and Fio mice. **b**, For the EDL, there was no statistically significant difference between the transgenic lines and C57 (n). **c**, % Force Drop measures the sensitivity of the EDL to eccentric contractions. **d**, % Procion Orange is a

Gene therapy: Exon Skipping

- Antisense-induced exon skipping and synthesis of dystrophin in the mdx mouse
- Intramuscular delivery of antisense oligoribonucleotide:liposome complexes
2'-O-methyl antisense oligoribonucleotides have been used to modify processing of the dystrophin pre-mRNA to block motifs involved in normal dystrophin pre-mRNA splicing, we induced excision of exon 23, and the mdx nonsense mutation, without disrupting the reading frame
- mdx myoblasts and in vivo
- Immunohistochemical staining demonstrated the synthesis and correct subsarcolemmal localization of dystrophin and gamma-sarcoglycan in the mdx mouse

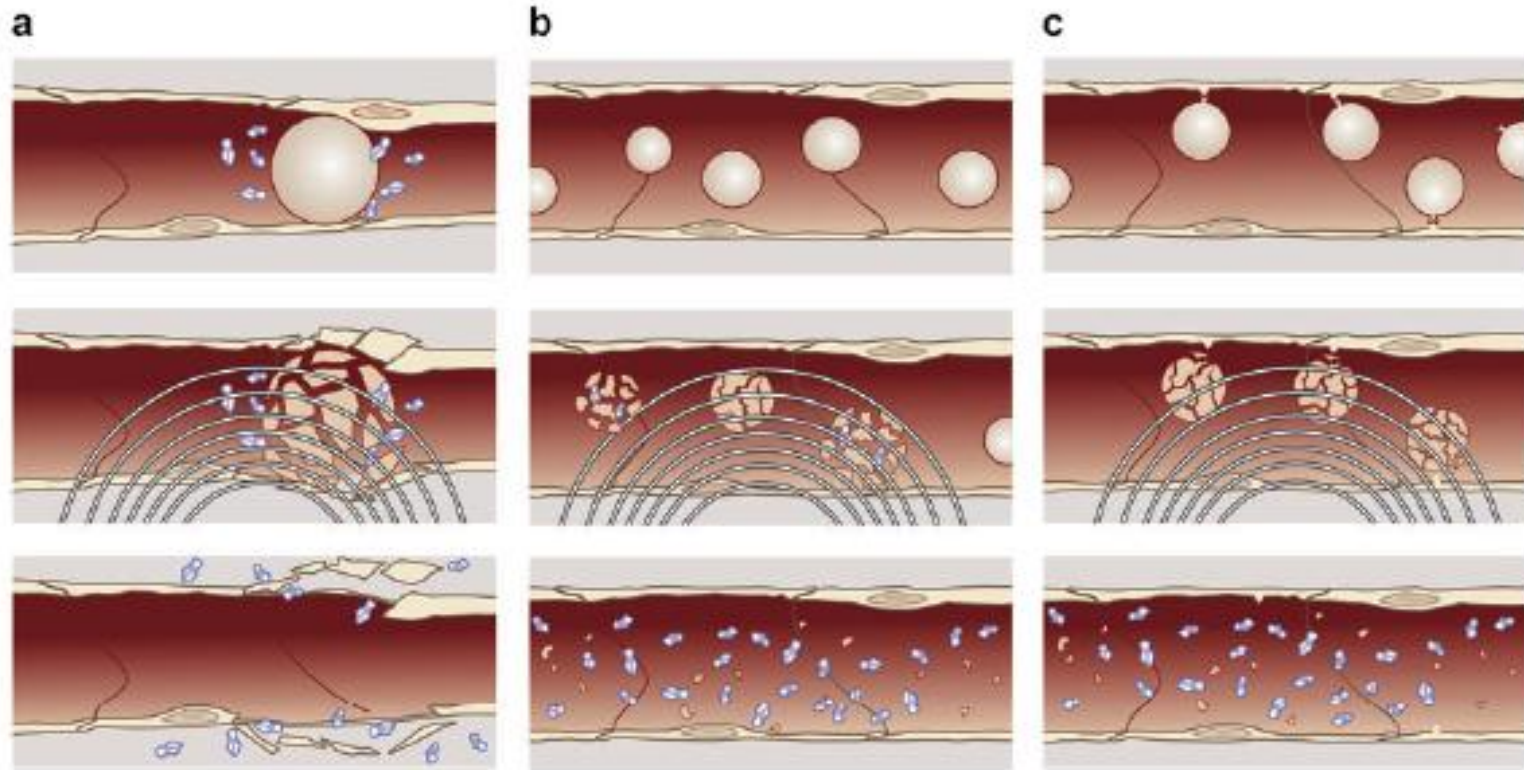
Mann CJ...Wilton SD et al Proc Natl Acad Sci U S A. 2001;98(1):42-7.

AAV or others: Ultrasound/microbubble bio-effect

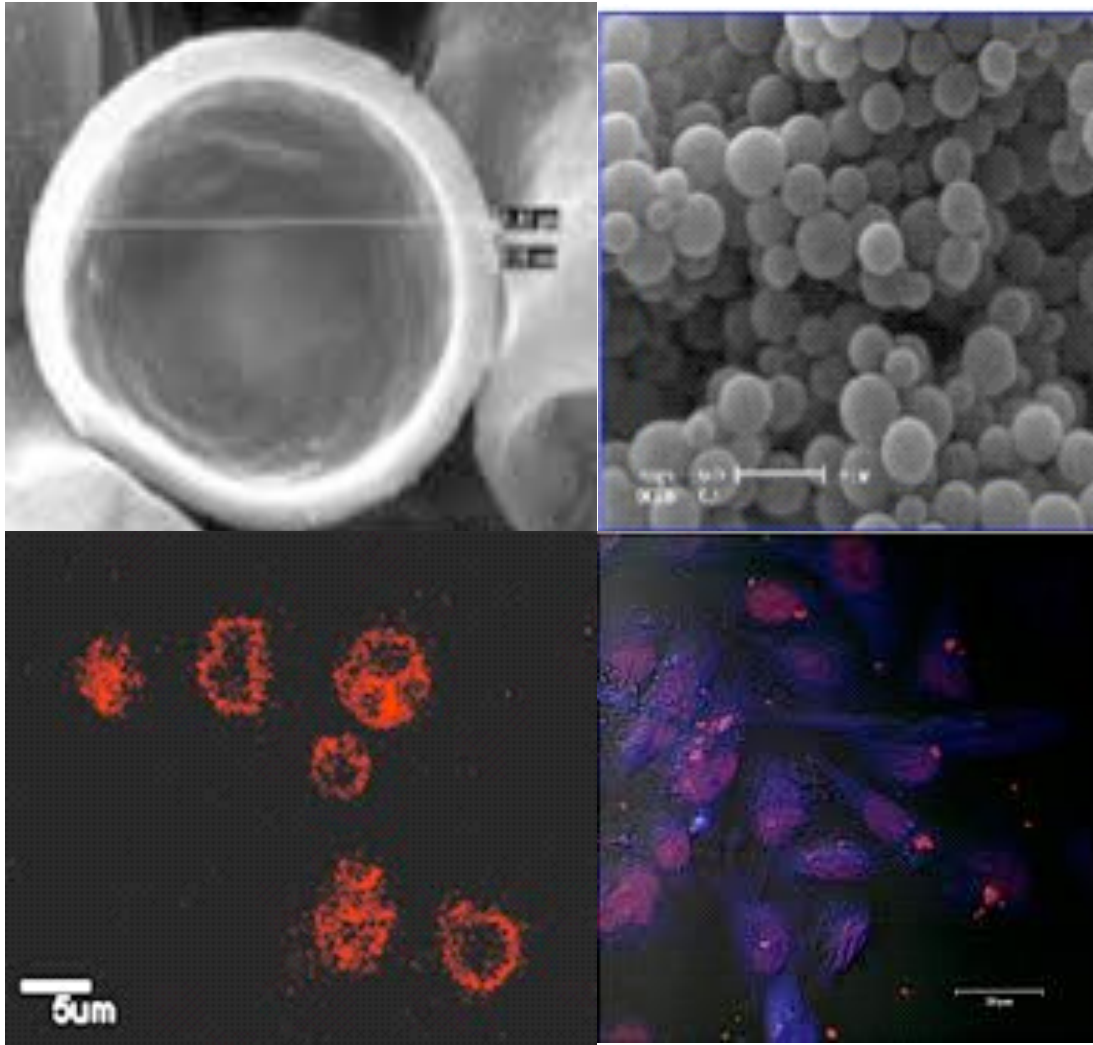


Miller DL et al PNAS 2000;97(18):10179–10184

Ultrasound targeted micro-bubbles destruction (UTMD) and therapy



Ultrasound targeted micro-bubbles destruction (UTMD) and therapy



Cell therapy

- **Conversion of mdx myofibres from dystrophin-negative to -positive by injection of normal myoblasts**
- Myoblast can fuse with host muscle fibres of a different genotype and express the donor genes
- Injected normal muscle precursor cells can fuse with pre-existing or regenerating mdx muscle fibres to render many of these fibres dystrophin-positive and so to partially or wholly rescue them from their biochemical defect

Patridge TA...Kunkel LM Nature. 1989 Jan 12;337(6203):176-9

What have we learned in the last 2 to 3 decades?

- Cloning of dystrophin gene
- DPC (dystrophin protein complex)
- Pathophysiology

Promising Therapies

- Small molecules/drug
 - Gentamycin and PTC 124
 - Myostatin blockade
 - Losartan
 - Sildenafil
- Gene therapy
 - Dystrophin: Full-length, micro-dystrophin
 - Utrophin
 - Exon Skipping
 - AAV or other systems (e.g. UTMD)
- Cell therapy
 - Myoblast?
 - IPC?
 - IV or IA?

Promising Therapies

- PTC124
 - Phase 2
 - Stopped for efficacy
- ACE-031 (Activin Receptor Type IIB (ActRIIB) and myostatin blockade, Acceleron Pharma, Inc.)
 - Phase 2
 - On going
- Gene delivery using viral vector to single skeletal muscle
 - Phase 1 Trial
 - *Arch Neurol.* 2007;64(9):1236-1241
- Antisense oligonucleotides for exon skipping in Duchenne muscular dystrophy with Morpholino oligo
 - Phase 2 in Britain and Belgium

Conclusion and Speculation

...Disease gene discovery is but an obligate first step in the process of making animal models, interrogating pathogenesis, and deriving unanticipated disease mechanisms and rational treatment strategies...

Habashi...and Dietz, Science, 2006