Proteomics

What's Wrong with My Child

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Questions

- **What is dystrophy？**
- **How do you know if you have dystrophy or not？**
- **How does dystrophin work？**
- **Can we prevent dystrophy？**
- **The future of dystrophy.**

What make Dystrophy?

Dystrophy

- 進行性肌肉萎縮症 ➔非神經性
- 肌肉細胞本身隨著時間及年齡漸進性地損傷與萎縮
- 與基因有關➔sex-linkage

Type of Dystrophy

Becker muscular dystrophy (BMD)貝克型MD
Duchenne muscular dystrophy (DMD) 杜顯型MD

Congenital muscular dystrophy (CMD) 先天性進行性肌肉萎縮症
Amyotrohic Lateral Sclerosis (ALS) 肌萎縮性側索硬化症
Hereditary Polyneuropathy 遺傳周邊神經疾病
Limb Girdle muscular dystrophy 肢帶型MD
The Myotonias 肌強直症
Myotonic dystrophy 萎縮性肌強直
Myotonic Muscular Dystrophy 肌強直性進行型肌肉萎縮症
Spinal Muscle Atrophy 脊髓性肌肉萎縮症

DMD or BMD

- 最常見的2種
- 病程進行較快速
- Occur in boy➔2~3 years-old
- 從日常生活中可以觀察到,並影響日常生活尤甚
- 小腿肌會因纖維化及脂肪化 ➔假性肥大
- 簡易檢測法➔頸部肌肉(最早發病的肌群)
- 關節壓迫性變形
- 呼吸肌群或心臟肌肉 ➔dead

How do you know if you have Dystrophy or Not?

一、家族史

最常見的**Dystrophy**種類 ：

Dunchenne muscular dystrophy (DMD)及
Becker muscular dystrophy (BMD)

皆為性聯隱性基因遺傳

二、行為與表徵

- 經常性跌倒
- 走路左右搖擺
- 蹲下、蹲坐、持物無力
- 小腿肌因纖維化及脂肪化呈現假性肥大
- 手心壓住額頭，測前縮頭動作

三、抽血檢測

- 測量creatine(肌酸肝)在血中的濃度值：

  患者在肌肉未完全萎縮之前，其血液中的creatine濃度值即非常的高，是常人的十至百倍。

- 病童的母親或姊妹帶缺陷基因者，濃度也較常人高。

四、肌肉切片染色及西方點墨法

- 一般組織化學染色：

  觀察肌肉進行性萎縮變化

- 組織免疫染色(immunohistochemistry)：
- 西方點墨法：

  Dystrophin antibody detection

PCR

- DMD有55%是基因缺陷引起

  BMD有70%是基因缺陷引起
- 目前常用的九對primers已可診斷出92-97%的基因缺陷情形。
- 胎兒的羊膜穿刺配合PCR篩檢

How does dystrophin working?

Finder

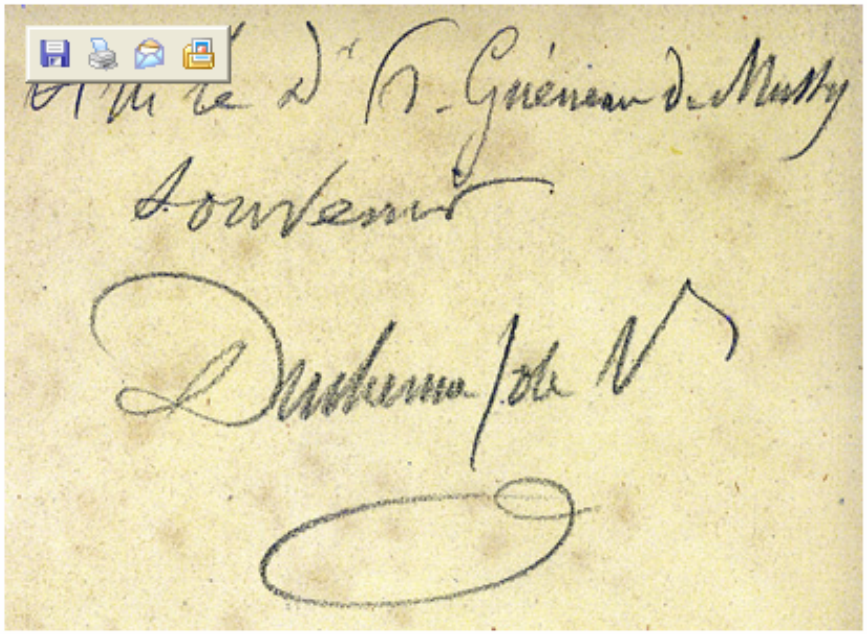
Dystrophinopathies: Clinical

- Dystrophin; Chromosome Xp21; Recessive

Becker
Cardiomyopathy
Cramps
Duchenne
Female carrier
Mental retardation
Microdeletion
Outliers

From NLM

Guillaume-Benjamin Duchenne de Boulogne



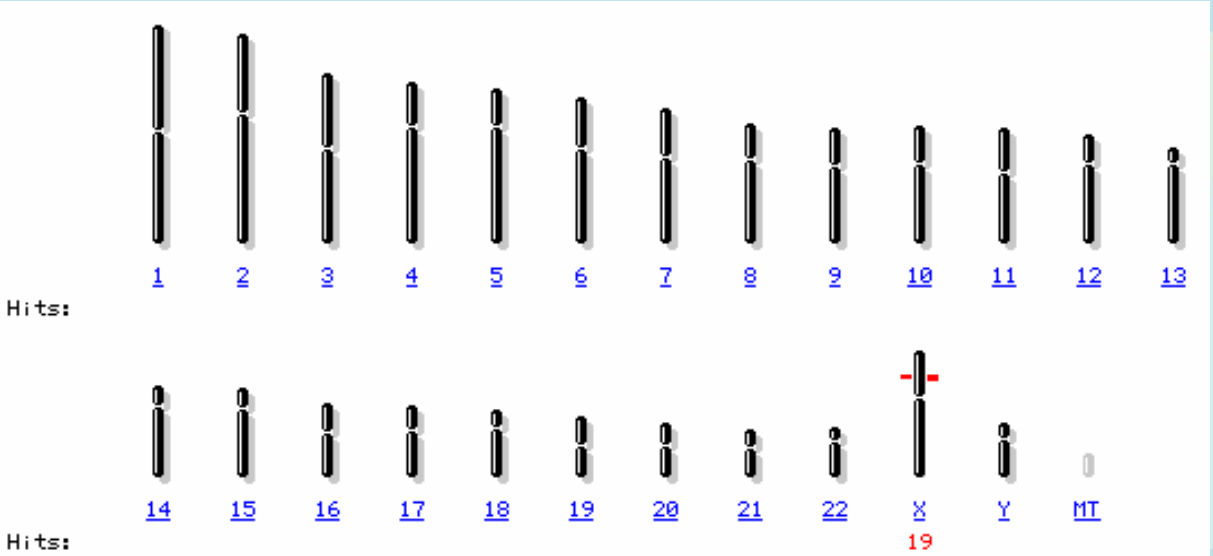
Autographed copy of: De la Paralysie Musculaire Pseudo-hypertrophique...
1868

www.neuro.wustl.edu/neuromuscular/musdist/dmd.html

History

- 1985 年，成功地在性染色體(X-chromosome)上定位出其基因位置，同時發現致病原因是此段基因有部份缺損所致
- 1986　1987，年成功地將這段基因所控制合成的蛋白質完全分析出來
- 1986 年，整個肌縮蛋白的控制基因完全被分離出來，主要位於 Xp2l 處，基因相當大，近 20 mKD

Location



Search results for query "DMD": 19 hits

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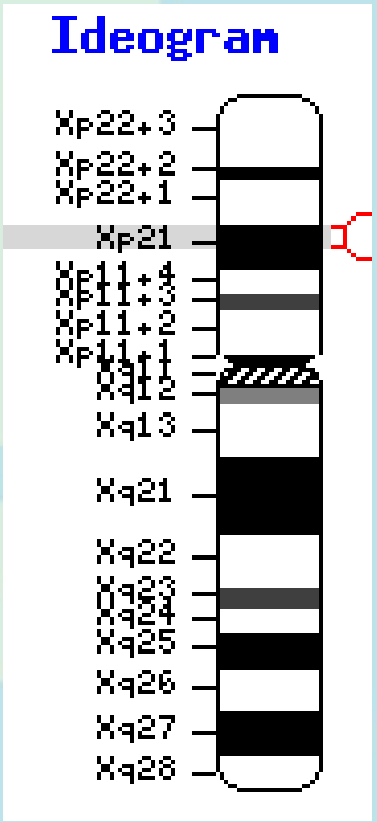
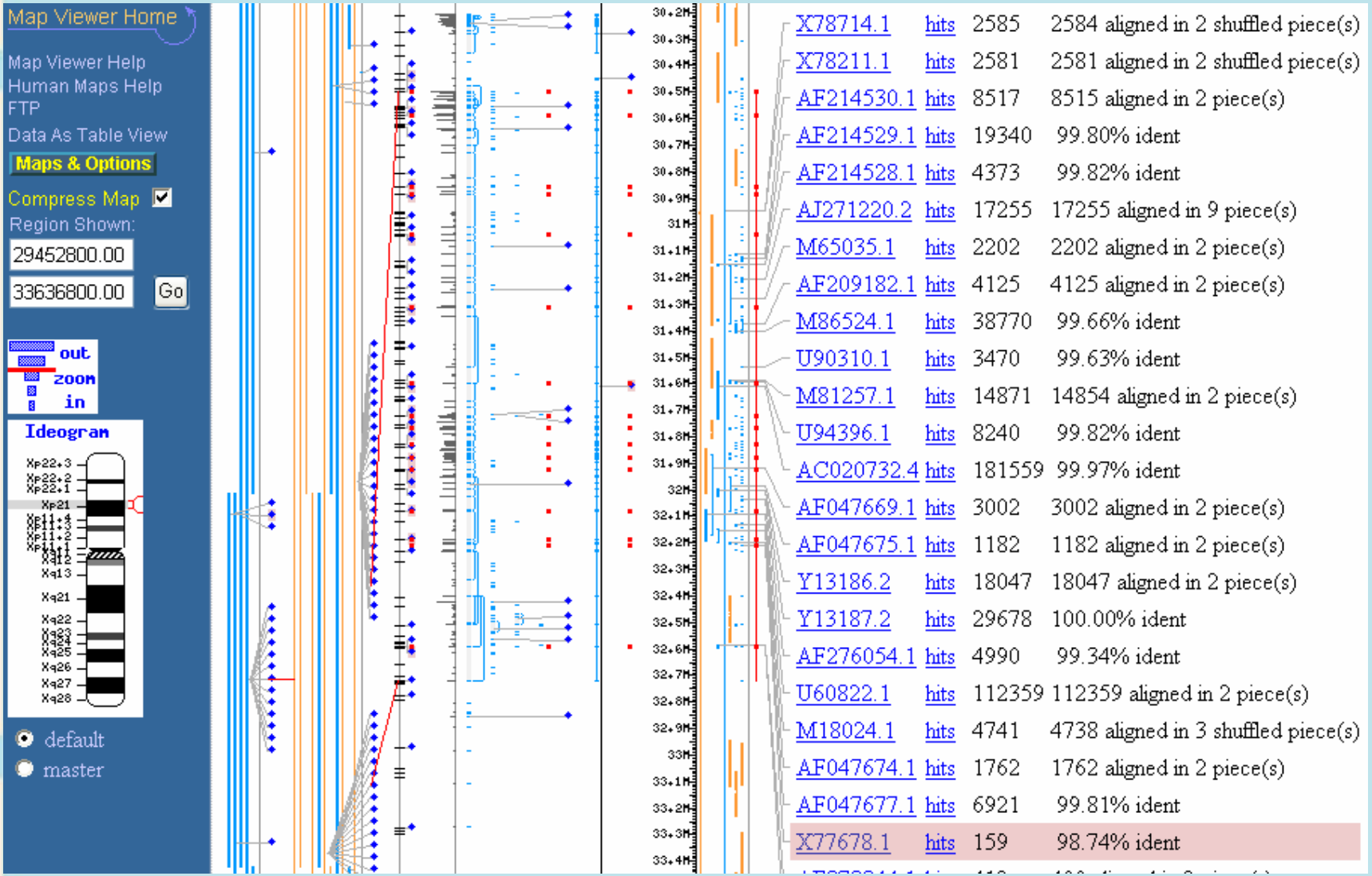
X	DMD	DXS503	STS	STS
X	DMD (13 hits)	DMD	STS	STS
X	DMD	GDB:187368	STS	STS
X	(DMD)	X77678.1	Component	gbDNA
X	DMD	300377	MIM	Morbid

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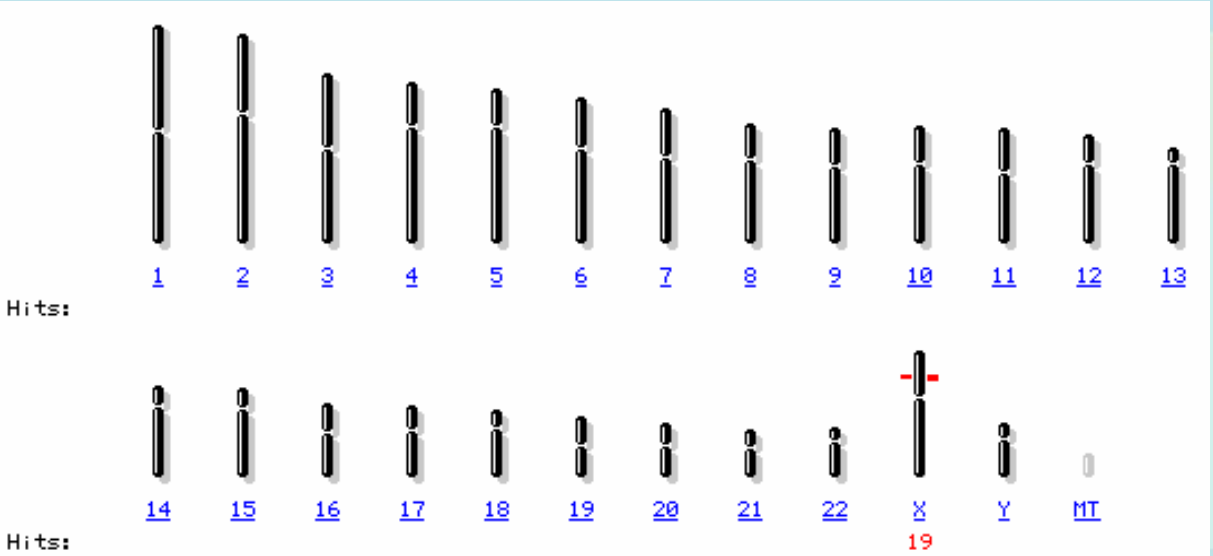
X78211.1 hits 2581 2581 aligned in 2 shuffled piece(s)
AF214530.1 hits 8517 8515 aligned in 2 piece(s)
AF214529.1 hits 19340 99.80% ident
AF214528.1 hits 4373 99.82% ident
AJ271220.2 hits 17255 17255 aligned in 9 piece(s)
M65035.1 hits 2202 2202 aligned in 2 piece(s)
AF209182.1 hits 4125 4125 aligned in 2 piece(s)
M86524.1 hits 38770 99.66% ident
U90310.1 hits 3470 99.63% ident
M81257.1 hits 14871 14854 aligned in 2 piece(s)
U94396.1 hits 8240 99.82% ident
AC020732.4 hits 181559 99.97% ident
AF047669.1 hits 3002 3002 aligned in 2 piece(s)
AF047675.1 hits 1182 1182 aligned in 2 piece(s)
Y13186.2 hits 18047 18047 aligned in 2 piece(s)
Y13187.2 hits 29678 100.00% ident
AF276054.1 hits 4990 99.34% ident
U60822.1 hits 112359 112359 aligned in 2 piece(s)
M18024.1 hits 4741 4738 aligned in 3 shuffled piece(s)
AF047674.1 hits 1762 1762 aligned in 2 piece(s)
AF047677.1 hits 6921 99.81% ident
X77678.1 hits 159 98.74% ident

Location



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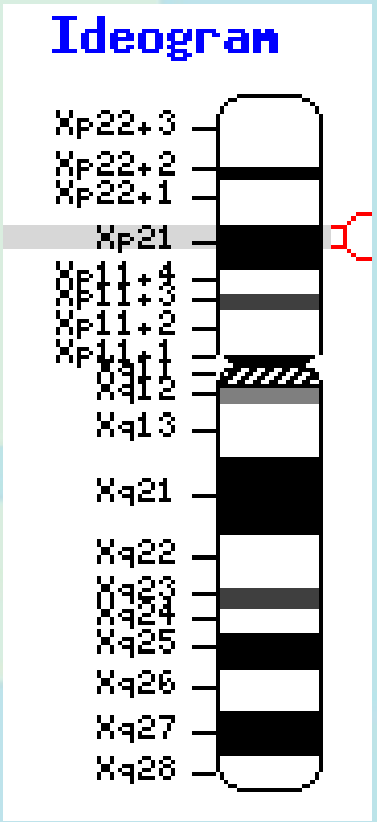

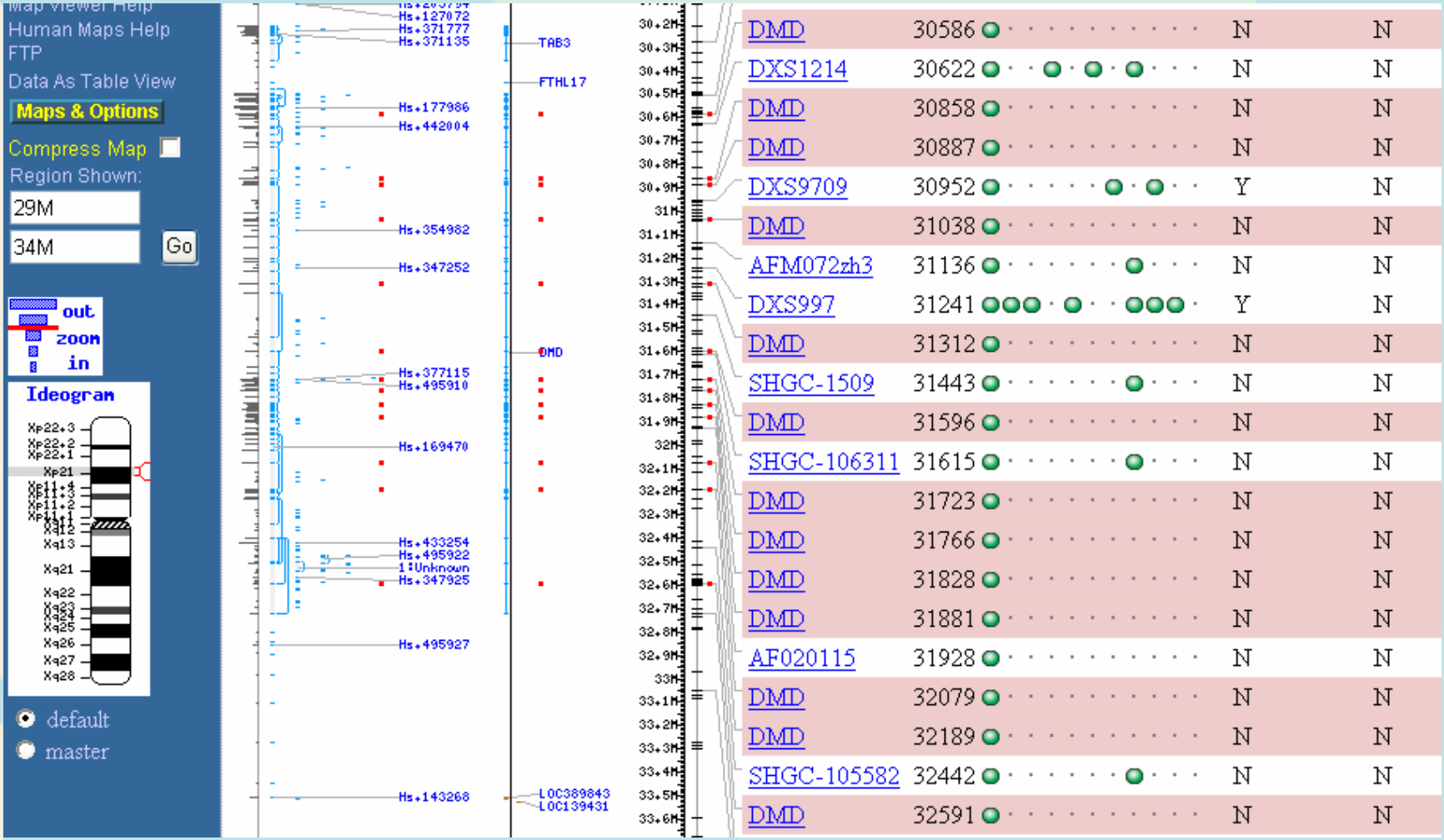
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Maps & Options
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DMD 31038 N N
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DXS997 31241 Y N
DMD 31312 N N
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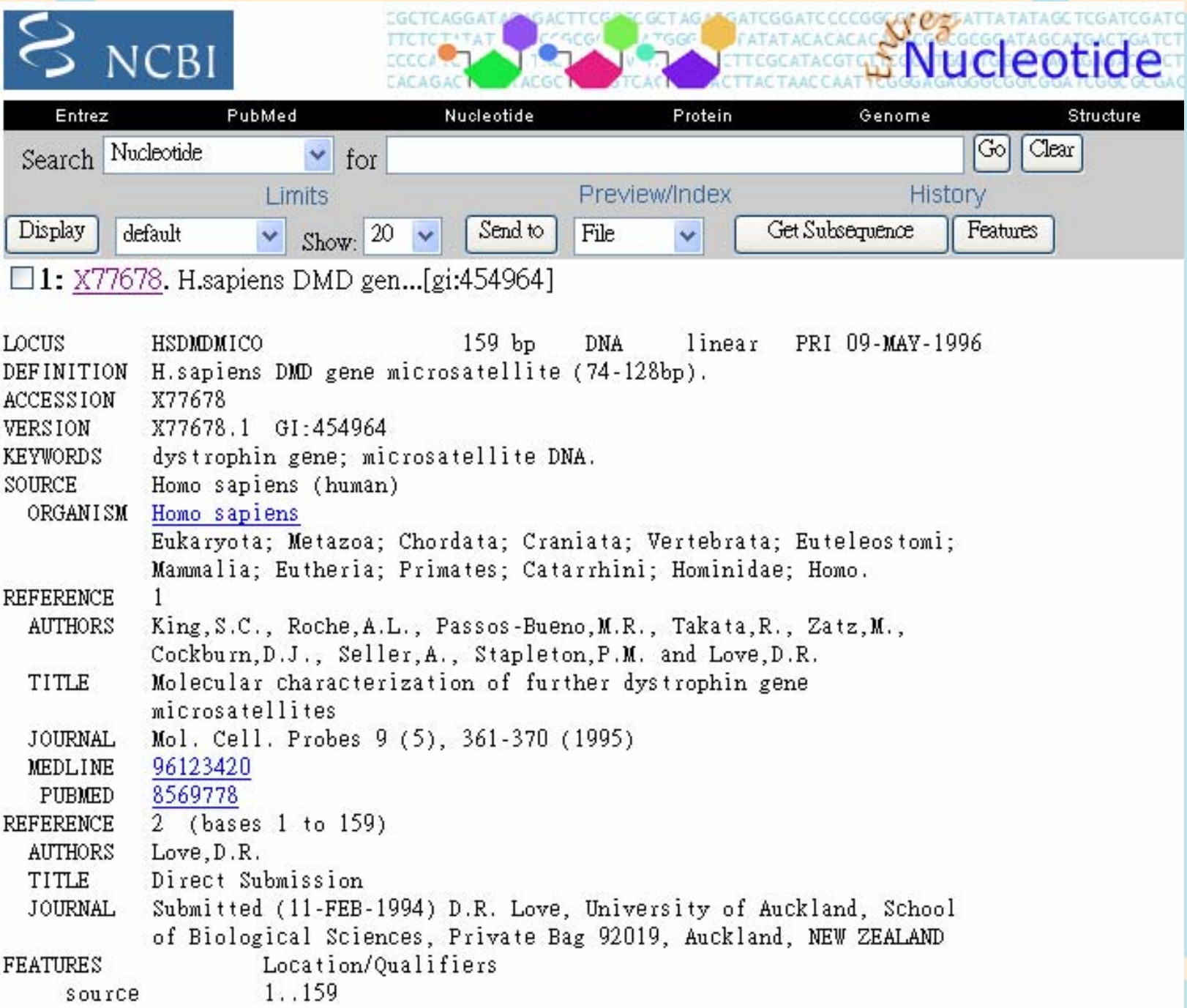
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ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1
AUTHORS King,S.C., Roche,A.L., Passos-Bueno,M.R., Takata,R., Zatz,M.,
Cockburn,D.J., Seller,A., Stapleton,P.M. and Love,D.R.
TITLE Molecular characterization of further dystrophin gene
microsatellites
JOURNAL Mol. Cell. Probes 9 (5), 361-370 (1995)
MEDLINE 96123420
PUBMED 8569778
REFERENCE 2 (bases 1 to 159)
AUTHORS Love,D.R.
TITLE Direct Submission
JOURNAL Submitted (11-FEB-1994) D.R. Love, University of Auckland, School
of Biological Sciences, Private Bag 92019, Auckland, NEW ZEALAND
FEATURES Location/Qualifiers
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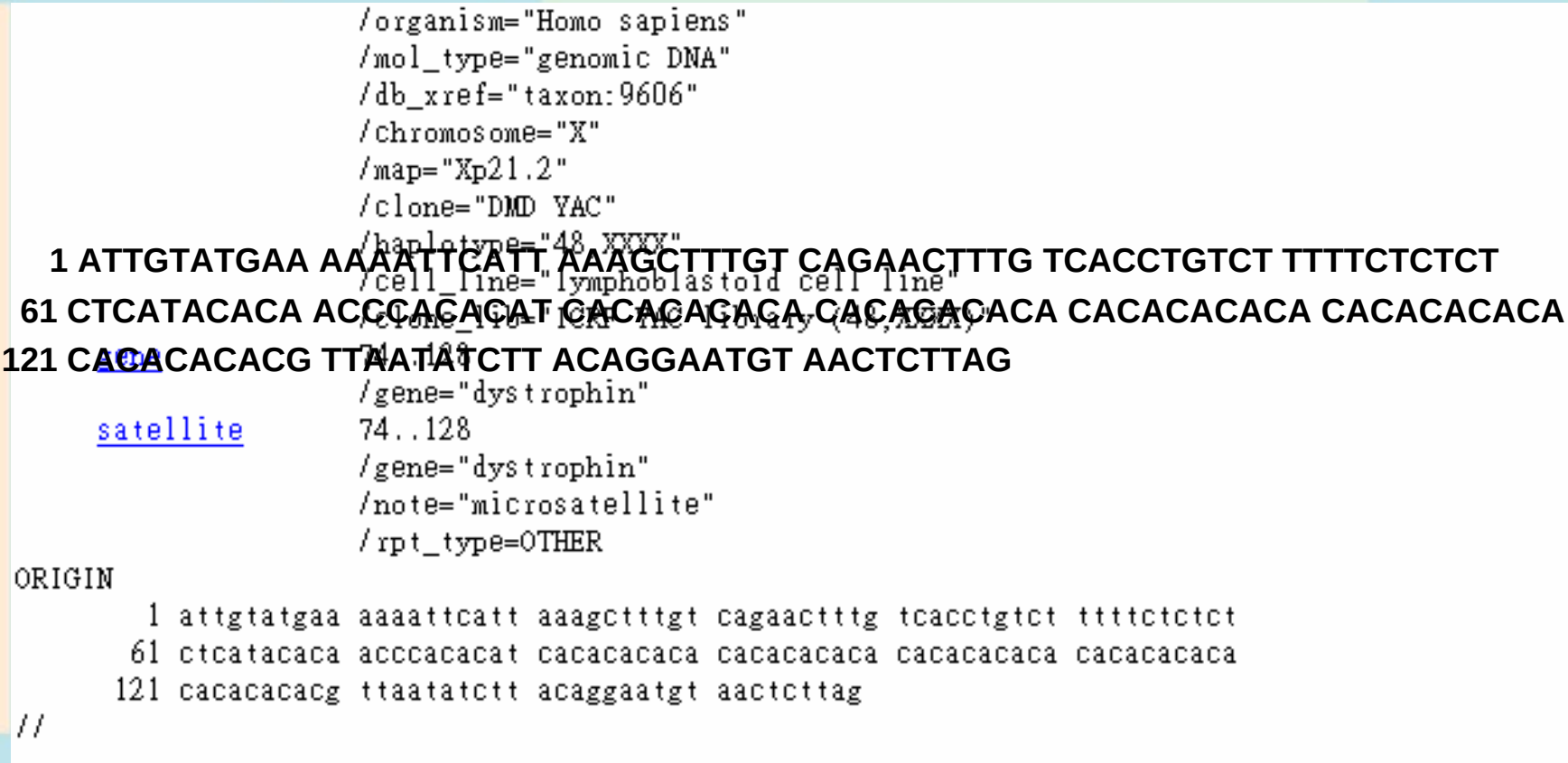

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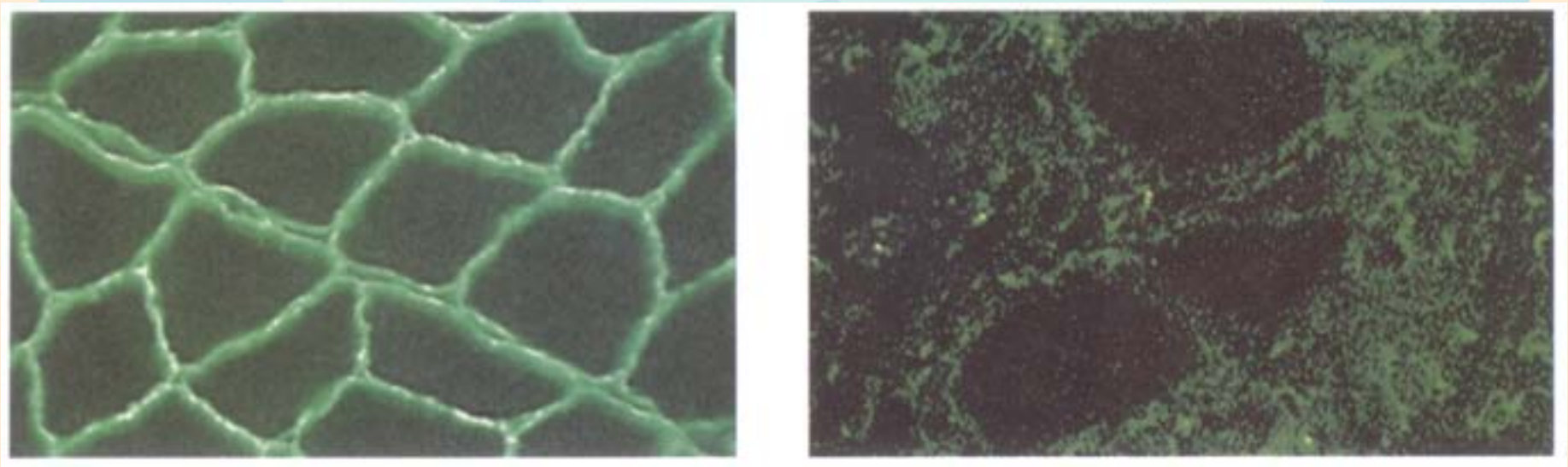
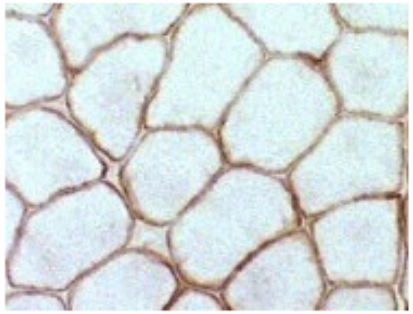
121 CACACACACG TTAATATCTT ACAGGAATGT AACTCTTAG

Fluorescent Immunoperoxidase Staining

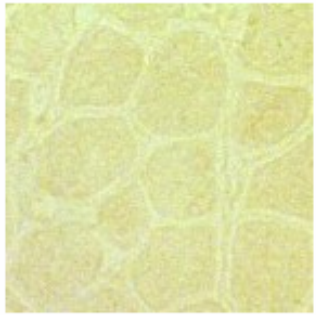


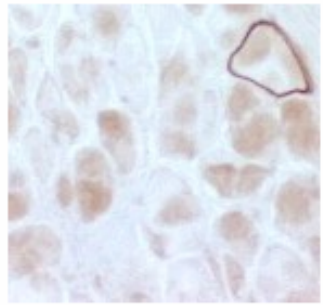
***Genetics* Sixth Edition p.19**

Duchenne Muscular Dystrophy: Dystrophin staining

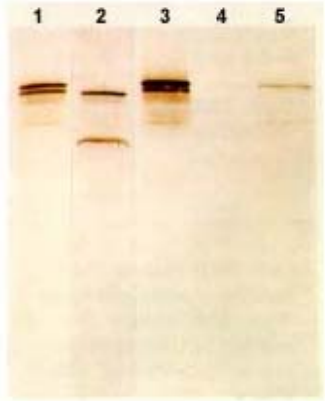


Normal dystrophin staining around the rim of muscle fibers.





Absent dystrophin: Duchenne muscular dystrophy
Left: No staining around the rim of muscle fibers.
Right: No staining of most muscle fibers.
One "revertant" fiber with dystrophin staining.

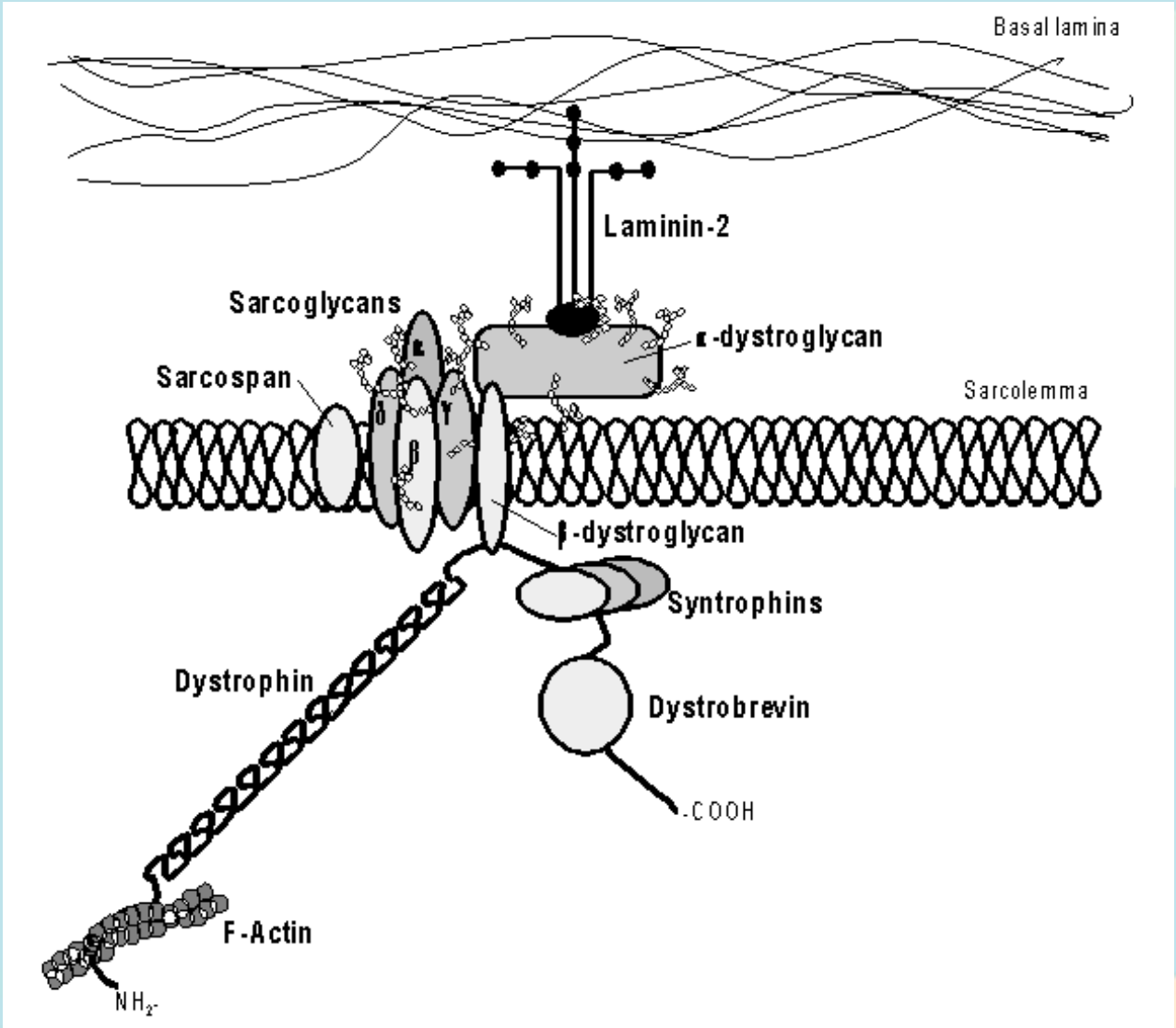


from Novocastra

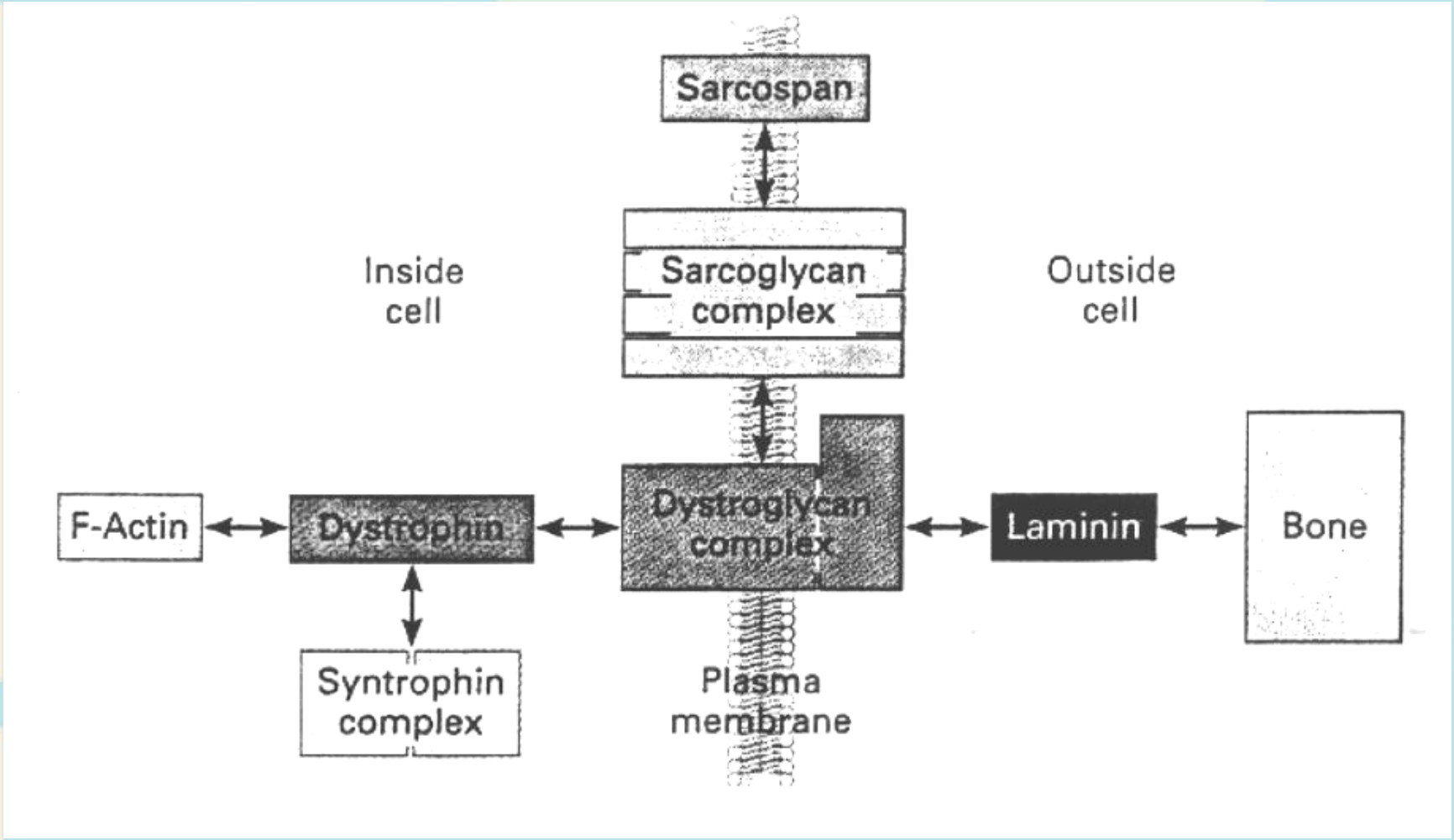
Western blot of dystrophin from dystrophinopathies.
Lane 1: Becker dystrophy; Dystrophin has reduced abundance but normal size.
Lane 2: Becker dystrophy; Dystrophin has reduced size and abundance.
Lane 3: Normal; Dystrophin has normal size and amount.
Lane 4: Duchenne dystrophy; Almost no protein is present.
Lane 5: Duchenne outlier; Dystrophin has severely reduced abundance.

www.neuro.wustl.edu/neuromuscular/pathol/dmdpath.htm

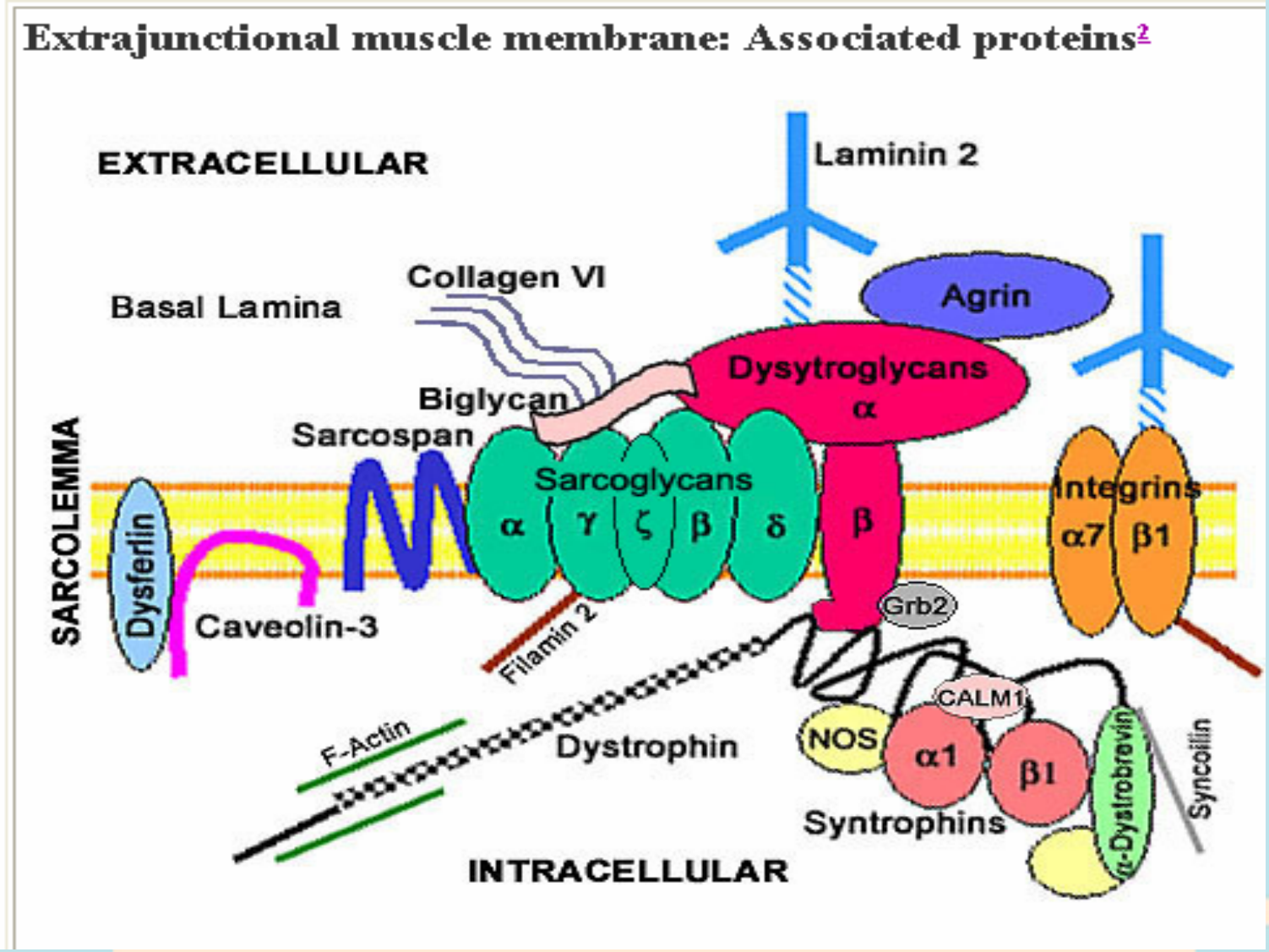
Dystrophin and associated protein interaction

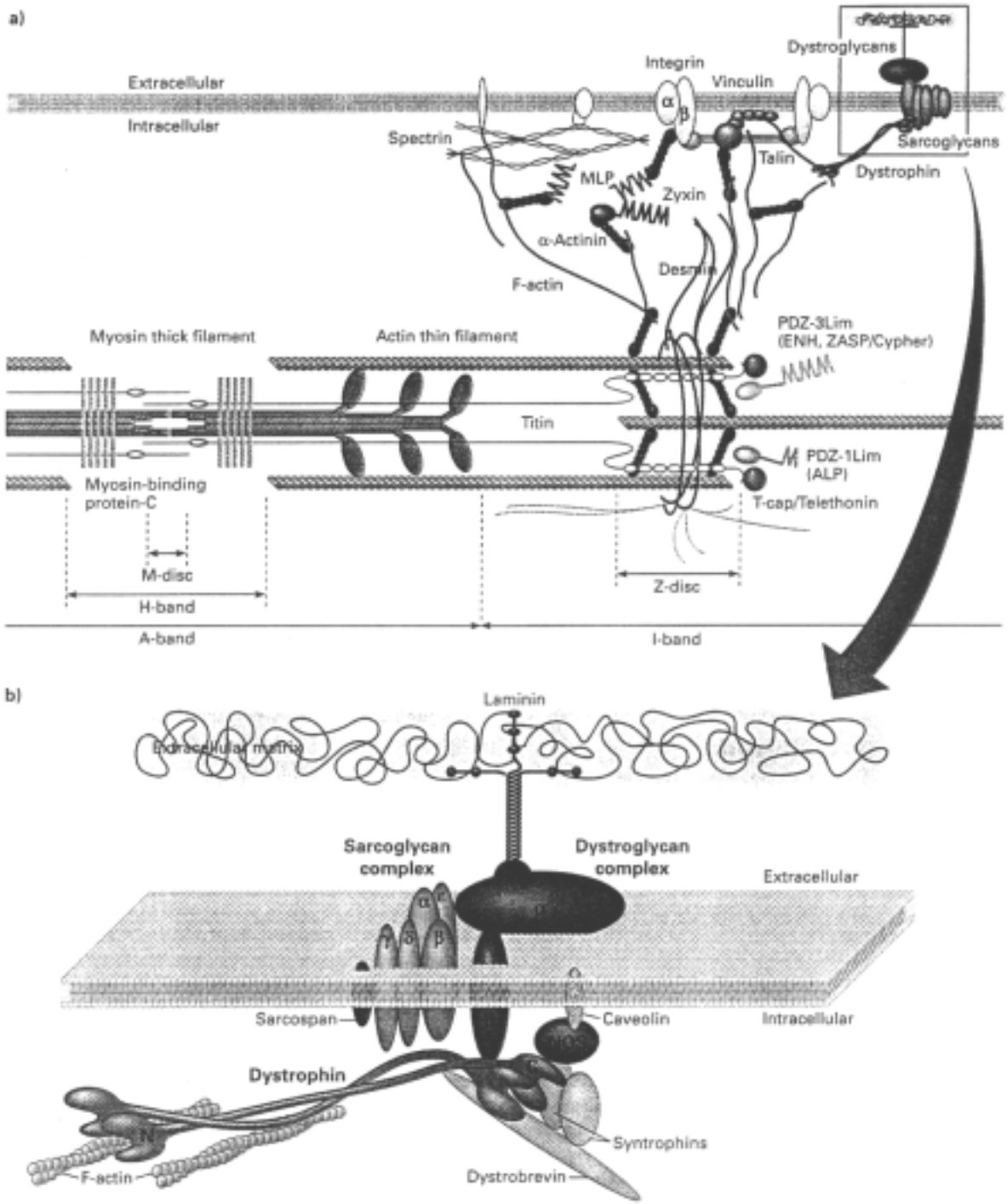


Dystrophin and associated protein interaction



Dystrophin and associated protein interaction




a)
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Intracellular
Integrin
Vinculin
Dystroglycans
Sarcoglycans
Dystrophin
Talin
Spectrin
MLP
Zyxin
α-Actinin
Desmin
F-actin
Myosin thick filament
Actin thin filament
PDZ-3Lim
(ENH, ZASP/Cypher)
Titin
PDZ-1Lim
(ALP)
T-cap/Telethonin
Myosin-binding
protein-C
M-disc
H-band
A-band
Z-disc
I-band
b)
Laminin
Extracellular matrix
Sarcoglycan
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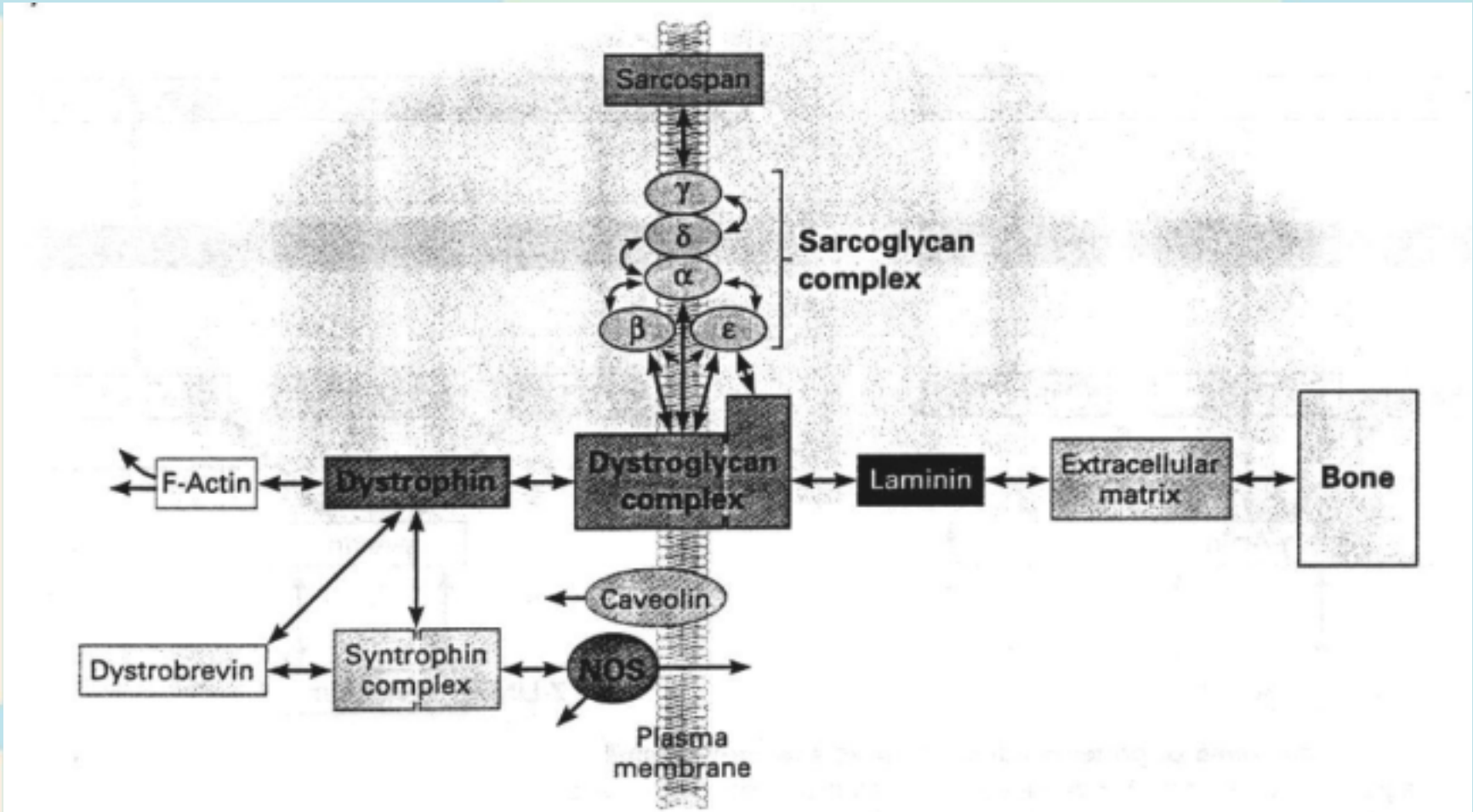
Dystrophin
Syntrophins
Dystrobrevin
F-actin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Sarcoglycan complex
F-Actin
Dystrophin
Dystroglycan complex
Laminin
Extracellular matrix
Bone
Caveolin
Dystrobrevin
Syntrophin complex
NOS
Plasma membrane

Dystrophin v.s. Utrophin

NCBI **Genes and disease** Map

New! Genes and Disease is now fully searchable on the Bookshelf. *Click here.*

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y

NEW **Genome View**

DMD
on the X chromosome
Databases

PubMed
the literature
LocusLink
collection of gene-related information
OMIM
catalog of human genes and disorders

Information

Muscular Dystrophy Association
for Research and Care news
Parent Project

DUCHENNE MUSCULAR DYSTROPHY (DMD) is one of a group of muscular dystrophies characterized by the enlargement of muscles. DMD is one of the most prevalent types of muscular dystrophy and is characterized by rapid progression of muscle degeneration which occurs early in life. All are X-linked and affect mainly males - an estimated 1 in 3,500 boys worldwide.

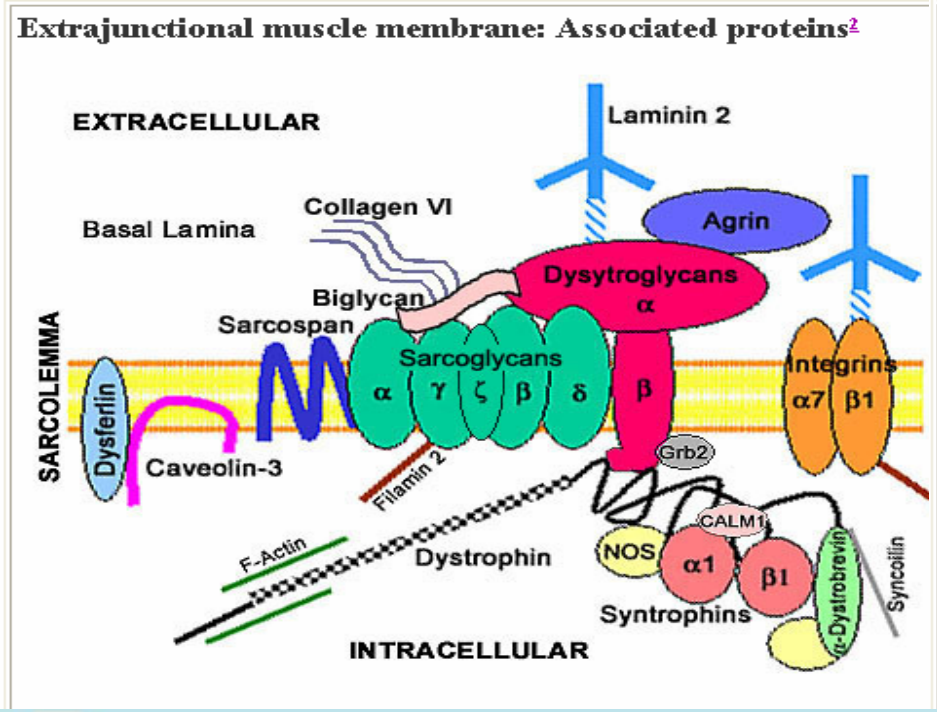
Dystrophin and utrophin are a similar size and have comparable modular architecture. This similarity means that utrophin can sometimes substitute for dystrophin, so providing a potential route for therapy for muscular dystrophy sufferers.

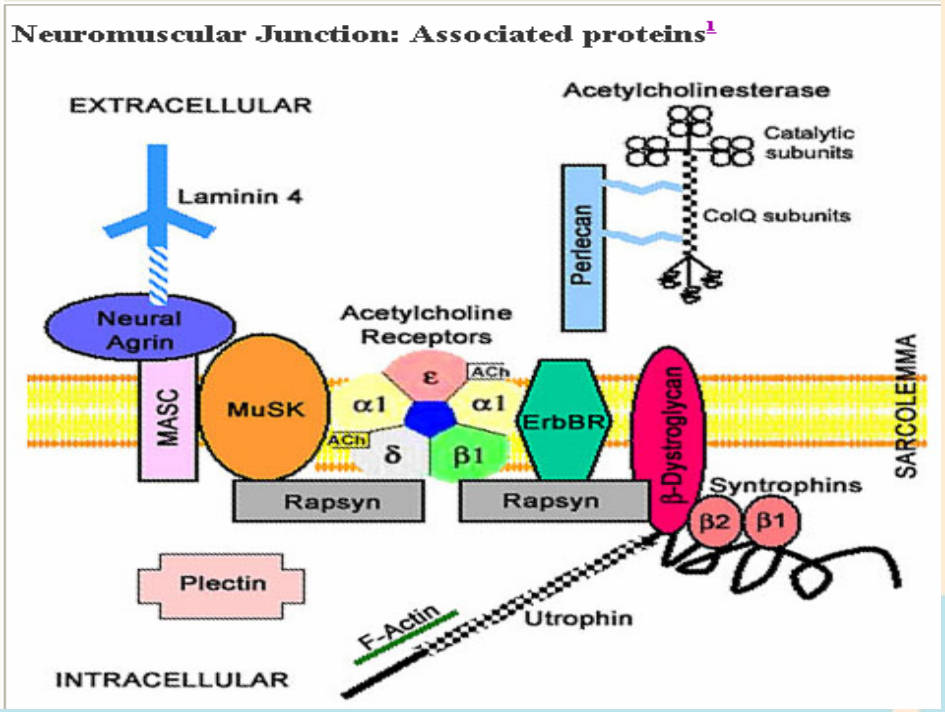
The gene for DMD, found on the X chromosome, encodes a large protein - dystrophin. Dystrophin is required inside muscle cells for structural support: it is thought to strengthen muscle cells by anchoring elements of the internal cytoskeleton to the surface membrane. Without it, the cell membrane becomes permeable, so that extracellular components enter the cell, increasing the internal pressure until the muscle cell 'explodes' and dies. The subsequent immune response can add to the damage.

A mouse model for DMD exists, and is proving useful for furthering our understanding on both the normal function of dystrophin and the pathology of the disease. In particular, initial experiments that increase the production of utrophin, a

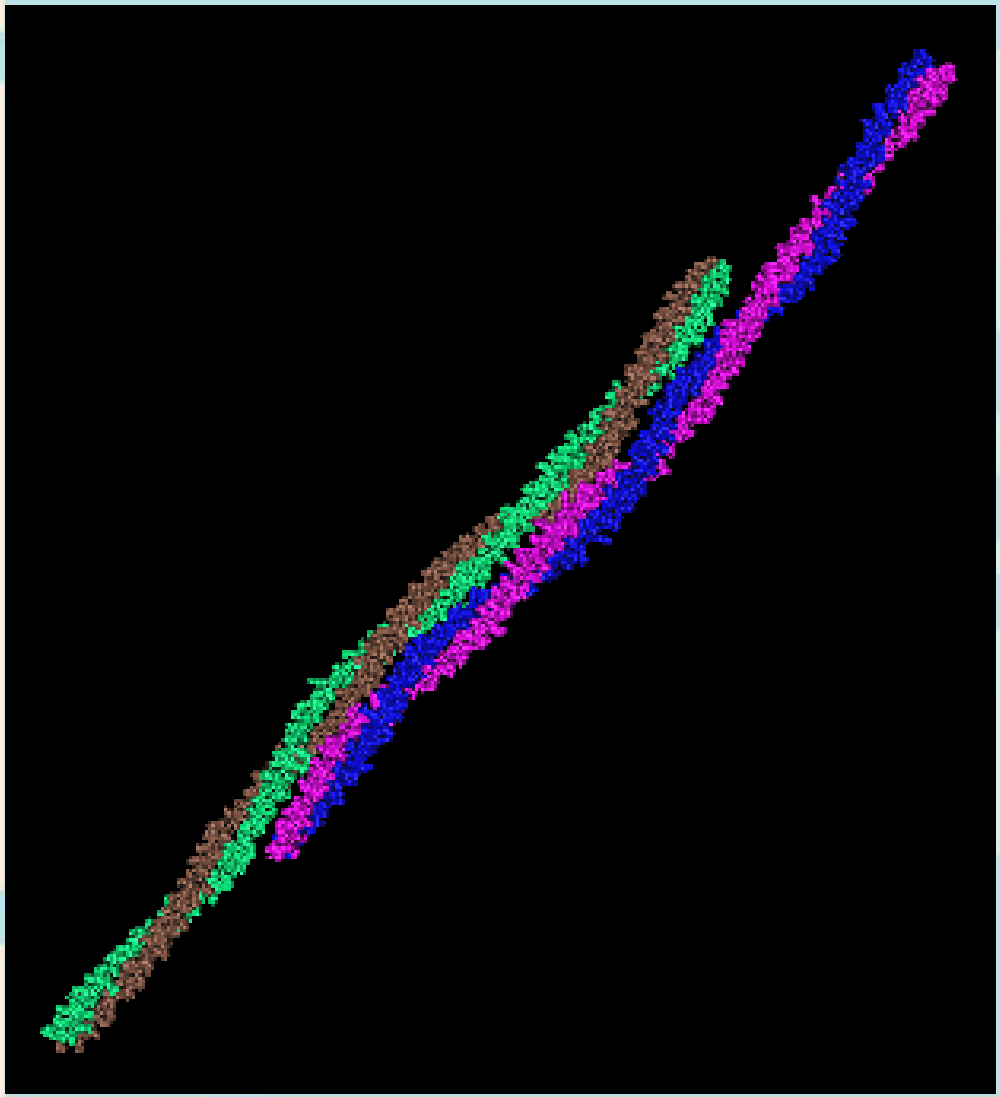
www.ncbi.nlm.nih.gov/disease/DMD.html

Dystrophin v.s. Utrophin

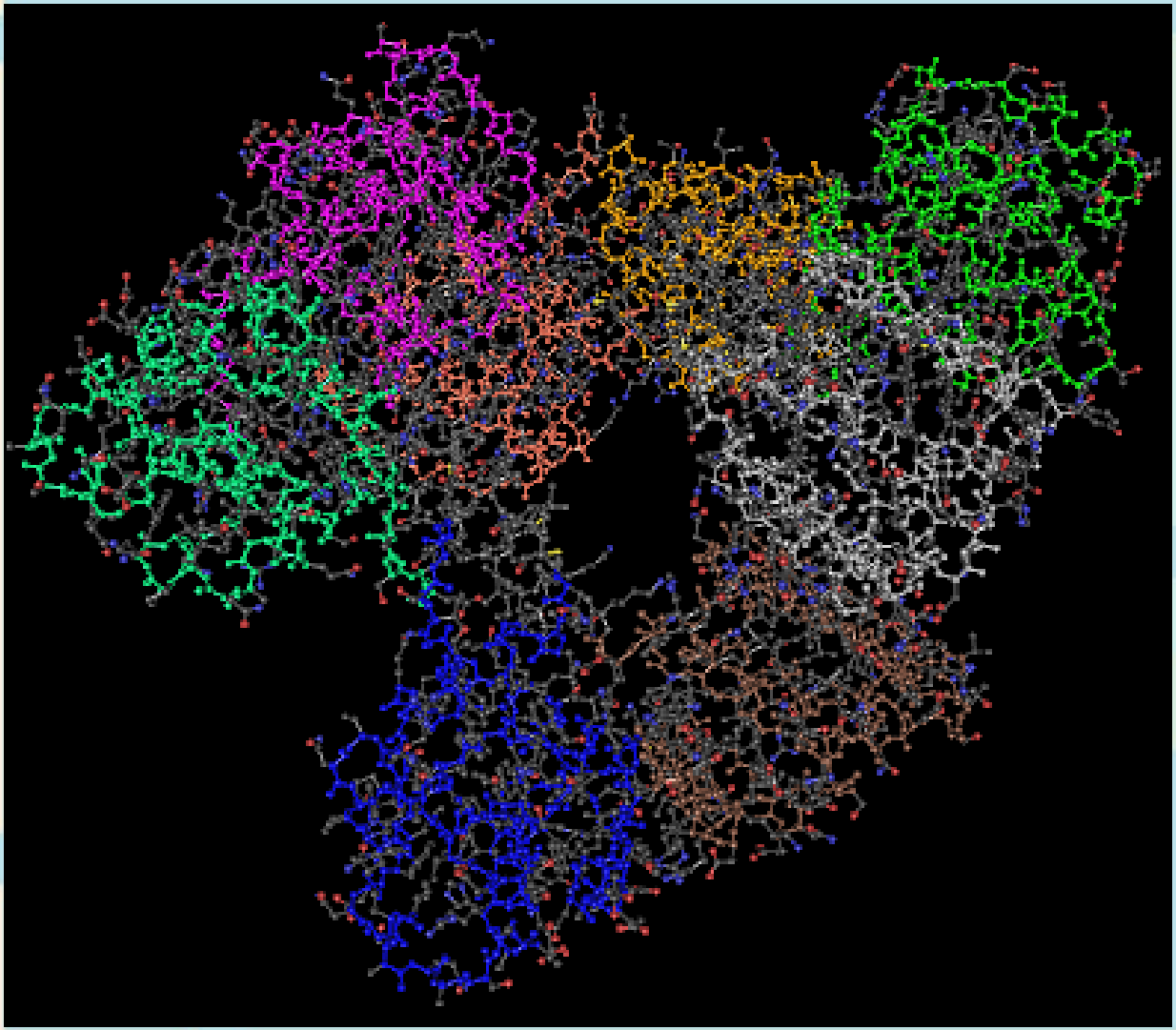




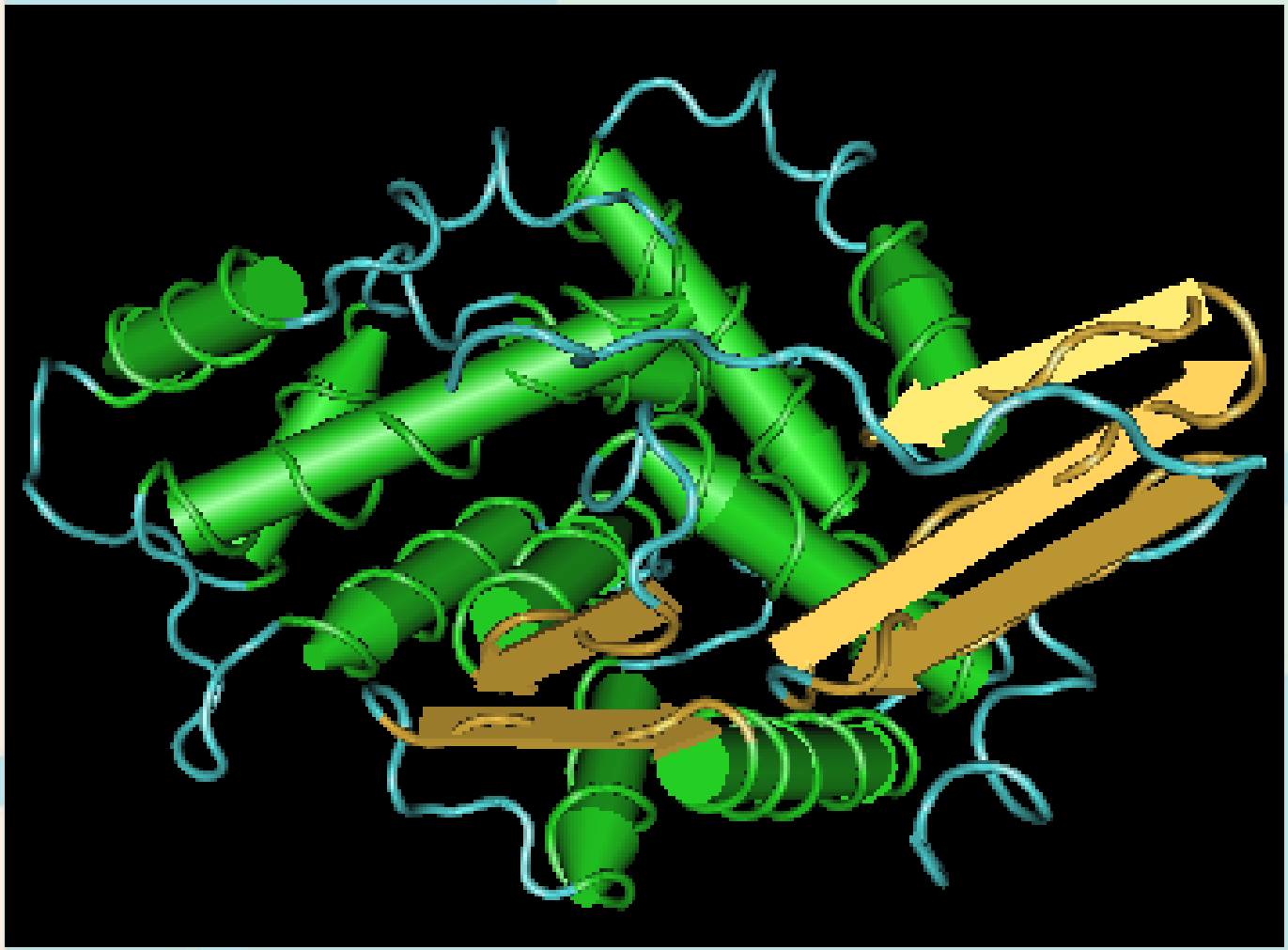
NCBI-MMDB 1C1G



NCBI-MMDB 1DXX



NCBI-MMDB 1EG4



Can we prevent Dystrophy?

Two Point

- 找出帶因者 **(carrier)**
- 帶因婦女產前診斷

找出帶因者

- 家族史分析--適用於大家庭

- Quantify the creatine phosphokinase(CPK)

  CPK, an enzyme in muscle cells.
  The efficiency of this method isn’t great.

- Fluorescent stain the dystrophin protein
- Analyze dystrophin DNA marker
- Examine the gene directly.

  There may be a fragment loss or mutation in this gene. However , the gene is too large to find out the defect.

帶因婦女產前診斷

- **Fetal sex determination**

  絨毛檢查(10週)或羊膜穿刺(15週)

- **DNA marker analysis**

  Compare the DNA marker between mother and fetal

The future of Dystrophy

The future of Dystrophy

- 基因治療：

  將不好的基因，以正常基因取代

- 細胞治療法：

  將帶有正常基因的肌肉細胞注射至功能最大的近端肌肉群中，使正常肌細胞繼續分裂增生，甚至與病態肌細胞融合

The future of Dystrophy

- 復健及支架療法：

  適當的支架輔助及預防變形治療，將有助於病童生活得較有品質，而這也是國內外對這方面治療的主要方針

- 心理的建設：

The End

Thank For Your Attention