MVIMG7470 (Molecular Virology, Immunology and Medical Genetics)
Neuromuscular Biology and Disease, Spring Semester 2014

Duchenne Muscular Dystrophy

Course Instructors: Denis Guttridge, Ph.D. and Jill Rafael-Fortney, Ph.D.

"Dystrophin-glycoprotein complex discovery and associations"
October 30, 2014

Rachelle H. Crosbie-Watson, Ph.D.
Professor and Education Liaison
UCLA Center for Duchenne Muscular Dystrophy
Dept. Integrative Biology & Physiology
Dept. Neurology

CDMD Scientific & Clinical Expertise

- M. Carrie Miceli, Ph.D.
- Stan Nelson, M.D.
- Melissa Spencer, Ph.D.
- April Pyle, Ph.D.
- Ron Victor, M.D.
- Gail Thomas, Ph.D.
- Perry Shieh, M.D., Ph.D.
- Nancy Halnon, M.D.
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- Linda Baum, M.D., Ph.D.
- Eileen Fowler, Ph.D., P.T.
- Marissa Briones, Ph.D.
- Laurie Shaker-Irwin, Ph.D.
- Andrew D. Waton, M.D., Ph.D.

First Time Students Encounter Physicians, Physical Therapies, Pathologists!

Bruin Allies for Duchenne (B.A.D.) Promote DMD Awareness and Education at UCLA

Shelley Tibbs[1], Neigena Mobaligh[1], Aram Namavar[1], Ryan Rosenberry[2], Amanda Popish[2], Courtney Young[2], Rufi Chen[2], Sarah Shidban[2], and Rachelle Crosbie-Watson, PhD[1,3]

[1]Department of Microbiology, Immunology, and Molecular Genetics, [2]Department of Integrative Biology & Physiology, [3]Department of Neurology, University of California Los Angeles, CA 90095

ABSTRACT

The Bruin Allies for Duchenne is an organization that was founded in 2013 by a group of undergraduate students inspired by a four unit course "Molecular Mechanisms and Therapies of Muscular Dystrophy" (PS156) taught by Dr. Rachelle Crosbie-Watson and the CDMD. The class is focused on using disease mechanisms as a pedagogical tool to develop higher order knowledge of basic scientific concepts since students are tasked with integrating concepts from genetics, molecular and cell biology, physiology, immunology, and stem cell biology in order to create molecular 'solutions' to the 'problem' of Duchenne muscular dystrophy (DMD). DMD is a genetic, progressive muscle wasting disease that affects young boys, about 1 in 3,500. The purpose of our organization is to broaden public awareness at UCLA and to the general public about DMD. We seek to support families affected by DMD by providing practical and informational resources. In support of this mission, we have partnered with several groups including the Center for Duchenne Muscular Dystrophy, the Office of Residential Life, the University Committee on Disability, and Coalition Duchenne to host a free screening of the documentary film "Dusty's Trail: Summit of Borneo" as part of UCLA Disability History and Awareness Week. The screening was following by a Discussion Panel including the film's producer, crew, and scientists. The long-term goal of the B.A.D. Associates is to fund research of promising therapies for the treatment of DMD by working through partner organizations.

INTRODUCTION

The Bruin Allies for Duchenne, a UCLA student organization, was united and inspired by a unique upper division physiological sciences course, PS 156, taught by renowned professor and research scientist, Dr. Rachelle Crosbie-Watson. The class is focused on using disease mechanisms as a pedagogical tool to develop higher order knowledge of basic scientific concepts, by focusing on Duchenne muscular dystrophy as the disease model.

Over the ten week course, several guest speakers from the UCLA CDMD lectured on their respective fields of expertise, including genetics, immunology, histology, stem cell research, cardiology, kinematics and other molecular mechanism underlying the disease pathology. The course also delved into the topics of possible therapies, current clinical trials, patient care and management as well as family dynamics among the Duchenne community.

Like the majority of the population, most of us had no previous knowledge of this progressive, muscle wasting disease. Not only did we explore the mechanisms underlying this disease, but we were simultaneously exposed to the personal aspects of DMD through poems from Matthew Stepanian and two documentaries, Darius Goes West and Dusty's Trail: Summit of Borneo.

After just a few weeks, several students united with the common goal of bringing awareness to DMD. With the help of Dr. Crosbie-Watson, the Bruin Allies for Duchenne became an official registered student organization at UCLA.

EVENTS

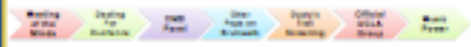


Figure 1. Fellow B.A.D. students Michelle Tibbs and Amanda Popish alongside Dr. Spencer, Dr. Moser and Dr. Nelson at the fundraiser, "Dealing for Duchenne", hosted by Cure Duchenne and the UCLA CDMD to support CDMD activities. Attendance for the B.A.D. students was generously provided by the CDMD.

Figure 2. Fellow UCLA alumni, Josh Bishkin, at DMD PS156 class Discussion Panel alongside scientists specializing in the field, as well as mothers of affected boys who all generously gave their time to attend our class event to promote DMD education.

Figure 3. Screening of the documentary film *Dusty's Trail: Summit to Borneo* during UCLA History and Disability Week, hosted by the Bruin Allies for Duchenne and co-programmed with UCLA CDMD, along with the UCLA Office of Residential Life and the UCLA Committee on Disability (Chair, Dr. Eileen Fowler). The event was well attended by undergraduates at UCLA.

LONG-TERM IMPACT

B.A.D. aspires to:

- Expand our public awareness campaigns to further promote understanding and education of DMD
- Partner the UCLA Center for Duchenne Muscular Dystrophy to establish a volunteer program for the clinical visits of boys with Duchenne.
- Participate in local charity events to raise awareness and funding for the treatment and further peer-evaluated research of DMD
- Provide support and funding to families with affected boys
- Set up an informational website that:
  - Presents basic information on DMD
  - Includes a forum for discussion with families living with DMD, including a section dedicated to an ongoing discussion of significant, current research
- Brand our organization through merchandise, including T-shirts, socks, pens, etc.

Figure 4. The Bruin Allies for Duchenne pens were designed to promote our organization funded by the CDMD1. We are currently designing socks and a B.A.D. logo.

- Continue to partner with sister schools, including the UCI Building Allies for Duchenne student organization
- Extend awareness and outreach programs beyond the UCLA community
- Fund clinical research of significant importance
- Participate in laboratory research (many of us have been inspired to pursue DMD research in CDMD labs.

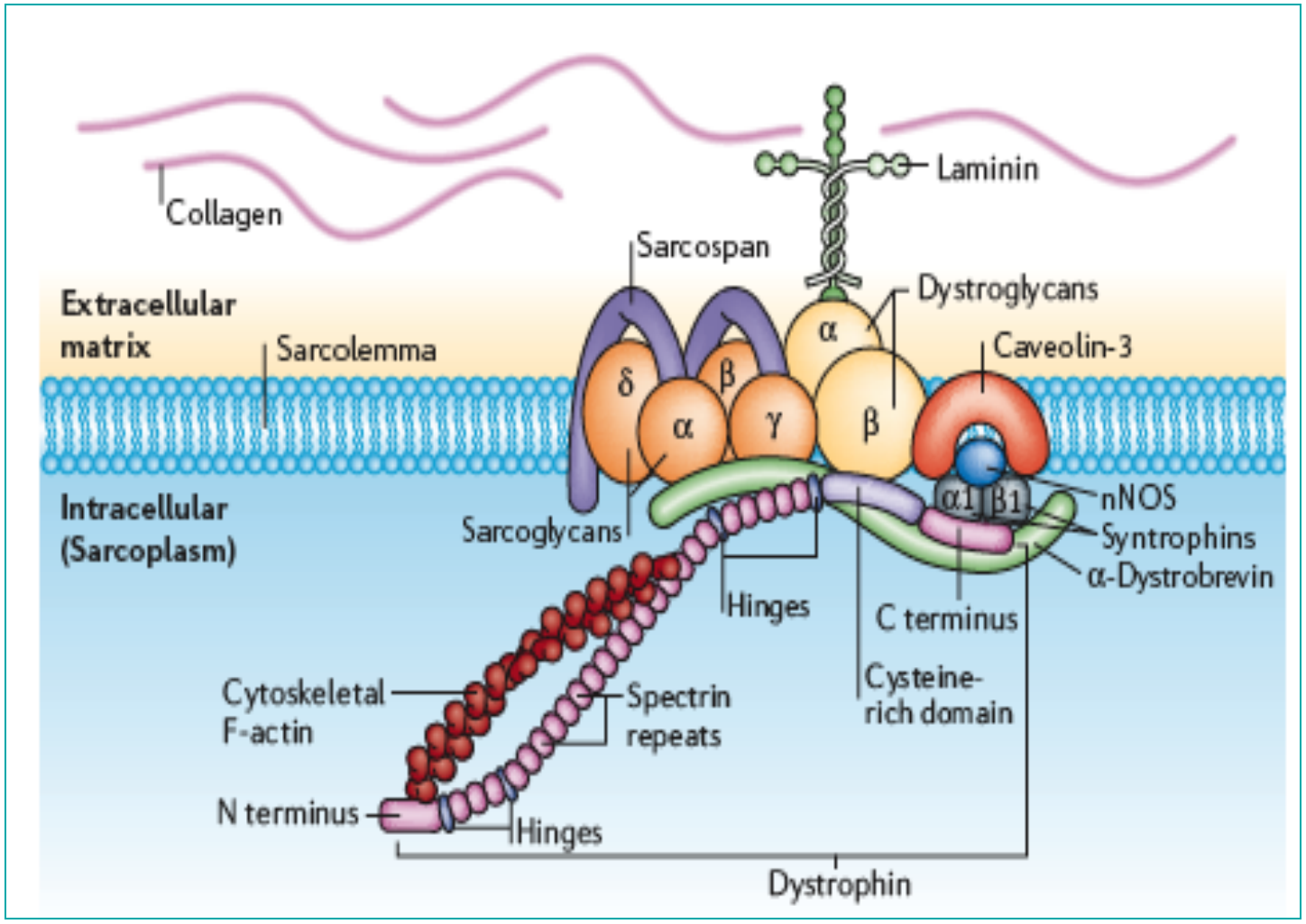
Figure 5. Article "UCLA Professor Seeks to Break Research Stereotypes" that appeared in the Daily Bruin on Oct. 4, 2013. The story featured the PS156 course, which garnered the 2013 Distinguished Teaching Award.

ACKNOWLEDGEMENTS

The Bruin Allies for Duchenne would like to acknowledge the UCLA Office of Residential Life, UCLA Center for Duchenne Muscular Dystrophy, UCLA Office for Students with Disabilities, Coalition Duchenne, and the University Committee on Disability.

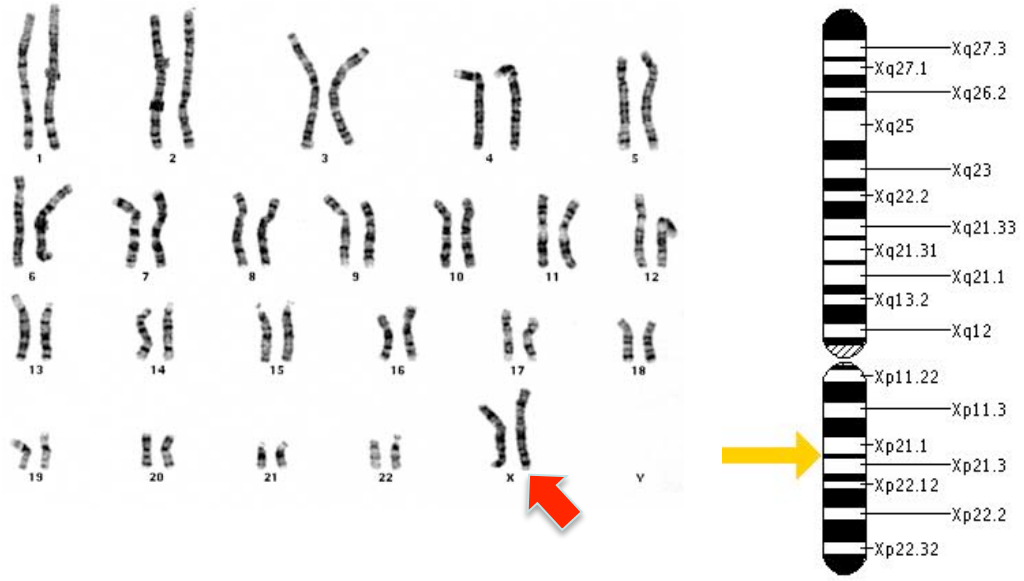
Further, we would especially like to thank Dr. Rachelle Crosbie-Watson, Dr. Eileen Fowler, Ms. Cath Jayasuriya, Ms. Amy Martin, Dr. Melissa Spencer, Dr. M. Carrie Miceli for their continued support of the Bruin Allies for Duchenne.

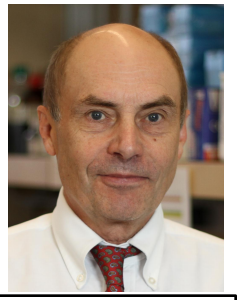
The Dystrophin-Glycoprotein Complex at the Cell Membrane



Genetic Discovery of DMD

- Approximately 1:3,500 male births
- Gene identified in 1986
- Co-discovered by Kunkel and Worton
- Disorder caused by mutations in dystrophin gene





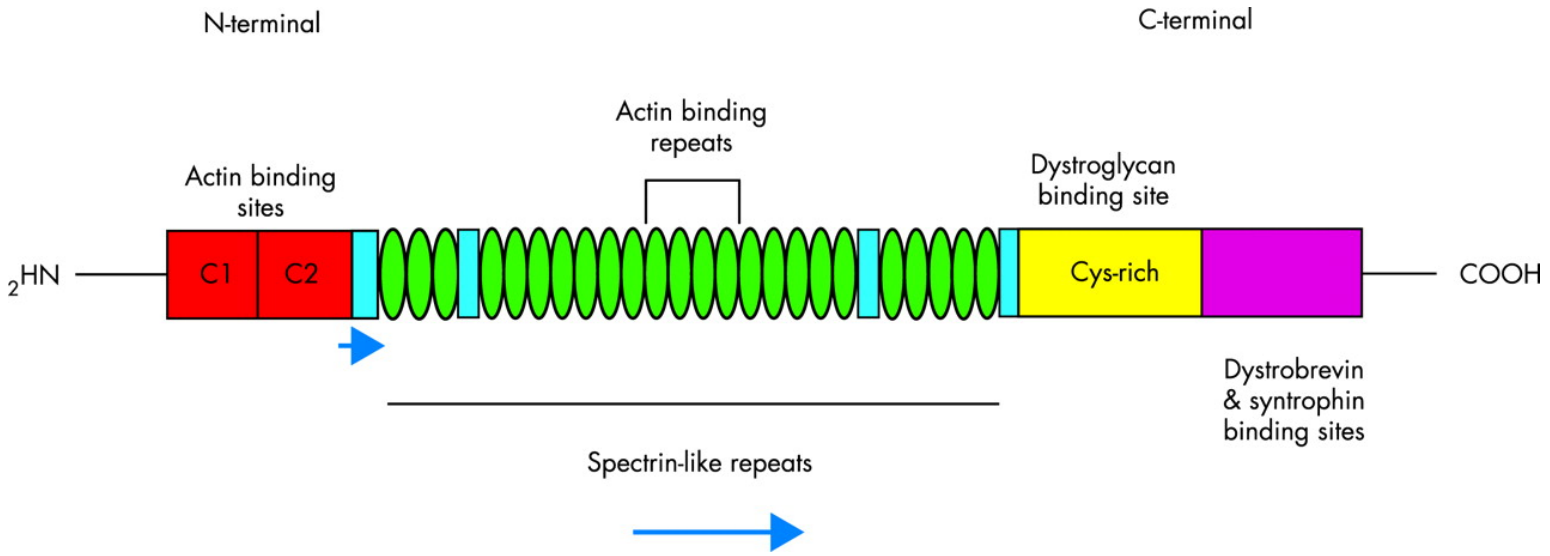
Lou Kunkel, Ph.D.
Harvard University

Ron Worton, Ph.D.
University of Ottawa

DMD Gene Localized to Xp21

Dystrophin Protein

Large (427 kDa) protein
Comprises a small % of total muscle protein (only 0.002%)
Sub-sarcolemma localization in striated and smooth muscles & neuronal tissue
Associated in a complex of transmembrane and peripheral proteins called the "dystrophin-glycoprotein complex" or DGC
Binds to actin and beta-dystroglycan



Key to Protein Domains within Dystrophin:
red: actin binding domain
yellow: beta-dystroglycan binding domain
green: spectrin-like repeats
blue: hinge region
yellow: Cys-rich repeats
purple: C-terminus/dystrobrevin/syntrophin binding sites

Cohen N , Muntoni F Heart 2004;90:835-841

Muscle & Nerve
Volume 34, Issue 2, pages 135-144, 12 JUN 2006 DOI: 10.1002/mus.20586

Louis Kunkel, Ph.D.
Harvard University

dystrophin gene
DMD mutation
dystrophin antibodies

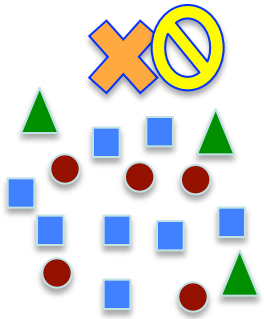
Purification of voltage-gated Ca^{++} channels

Kevin P. Campbell, Ph.D.
University of Iowa

MDA® Muscular Dystrophy Association

Tucson, AZ

Principles of Protein Purification:
1. Apply proteins to column

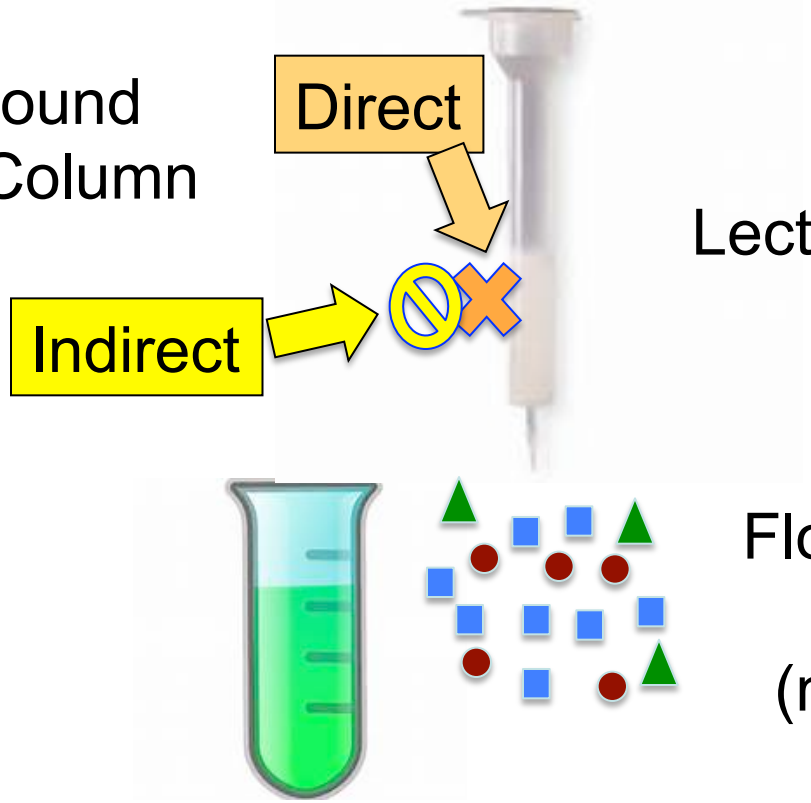


Total Proteins

Lectin Sepharose Column

Principles of Protein Purification
2. Collect the flow-through or void

Proteins Bound
To Lectin Column



Lectin Sepharose
Column

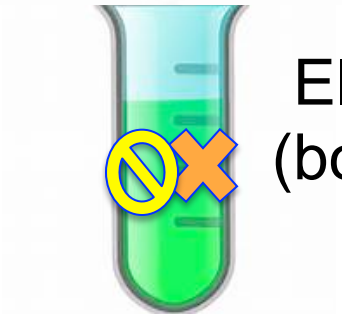
Flow-Through
or Void
(not bound)

Principles of Protein Purification
2. Collect the eluate

WGA=wheat germ agglutinin=lectin that binds carbohydrates

Elution buffer

Lectin Sepharose Column



Eluate (bound)

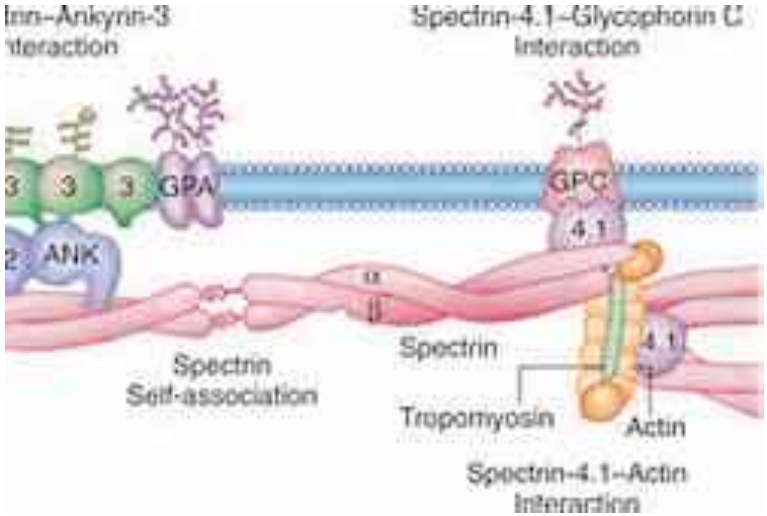
Association of dystrophin and an integral membrane glycoprotein

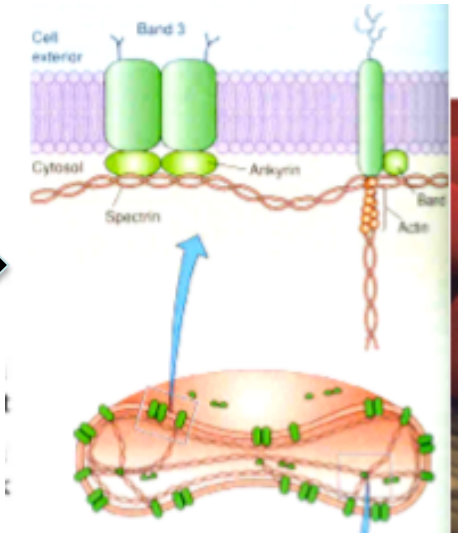
Kevin P. Campbell & Steven D. Kahl

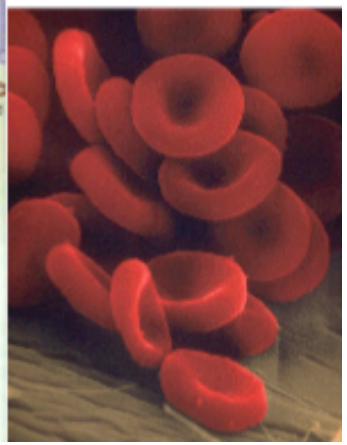
Department of Physiology and Biophysics, The University of Iowa College of Medicine, Iowa City, Iowa 52242, USA

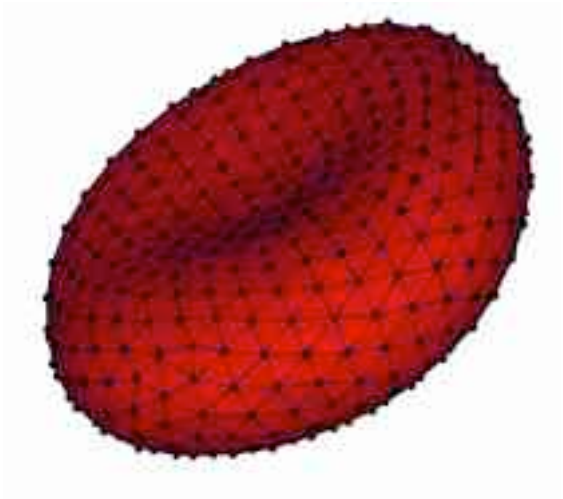
DUCHENNE muscular dystrophy (DMD) is caused by a defective gene found on the X-chromosome[1]. Dystrophin is encoded by the DMD gene and represents about 0.002% of total muscle protein[2]. Immunochemical studies have shown that dystrophin is localized to the sarcolemma in normal muscle but is absent in muscle from DMD patients[3–7]. Many features of the predicted primary structure of dystrophin are shared with membrane cytoskeletal proteins[8], but the precise function of dystrophin in muscle is unknown. Here we report the first isolation of dystrophin from digitonin-solubilized skeletal muscle membranes using wheat germ agglutinin (WGA)-Sepharose. We find that dystrophin is not a glycoprotein but binds to WGA-Sepharose because of its tight association with a WGA-binding glycoprotein. The association of dystrophin with this glycoprotein is disrupted by agents that dissociate cytoskeletal proteins from membranes. We conclude that dystrophin is linked to an integral membrane glycoprotein in the sarcolemma. Our results indicate that the function of dystrophin could be to link this glycoprotein to the underlying cytoskeleton and thus help either to preserve membrane stability or to keep the glycoprotein non-uniformly distributed in the sarcolemma.

Dystrophin Similar to Spectrin



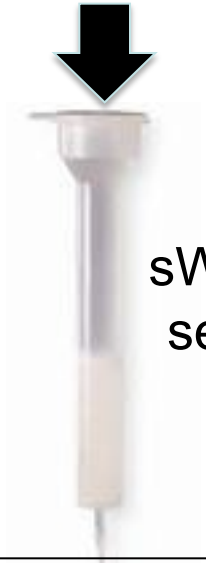






Purification of Calcium Channels from Skeletal Muscle

Rabbit skeletal muscle membranes



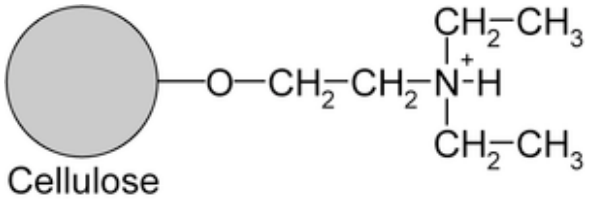
sWGA lectin sepharose column

NAG eluate

DEAE anion exchange column

Step-wise NaCl elution

25mM to 500mM NaCl fractions are collected



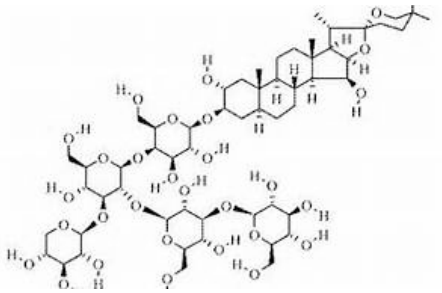
DEAE= Diethylamino ethyl-cellulose
Anion exchanger

What Happens to Dystrophin During Purification (First Part)?

Rabbit skeletal muscle membranes

Solubilize in 1% digitonin buffer with 0.5 M NaCl

WGA sepharose column



digitonin

Void or flow-through (unbound material)

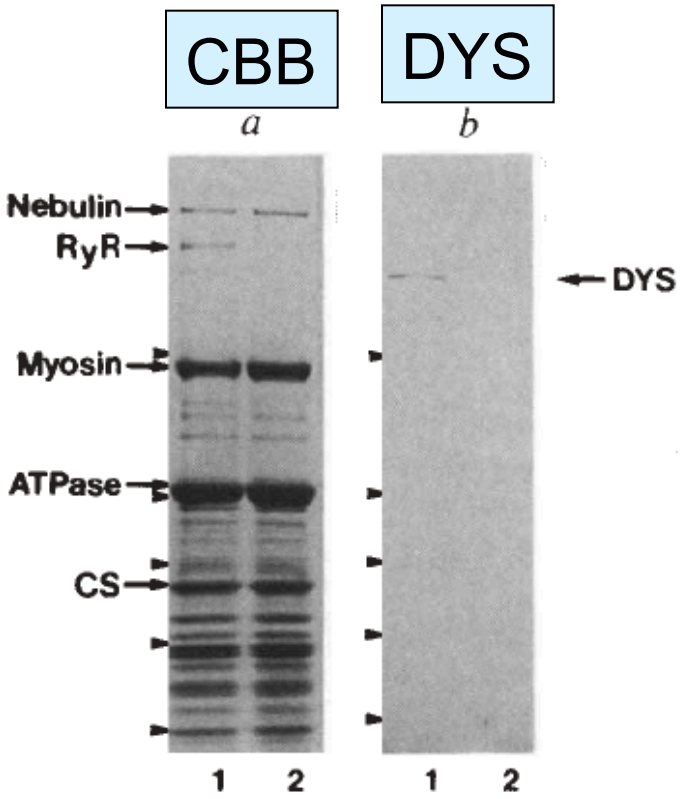
Wash away non-specific proteins

Elute with N-acetyl-D-glucosamine

Fractions eluted from column (bound material)

Succinylated wheat germ agglutinin=lectin that binds glycoproteins

First Results: Dystrophin Retained on sWGA Column



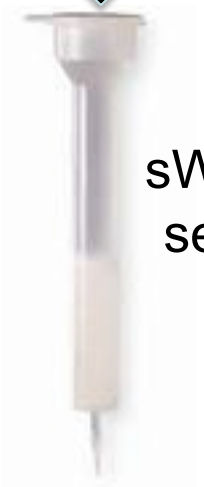
Lane 1: solubilized membranes
Lane 2: sWGA column flow-through (void; unbound material)

Panel A: Coomassie brilliant blue CBB) stained protein gel (to reveal all proteins)
Panel B: Sheep polyclonal anti-dystrophin antibodies (to reveal only dystrophin)

Conclusion: dystrophin itself is a glycoprotein (directly binding to sWGA) or dystrophin is associated with a glycoprotein.

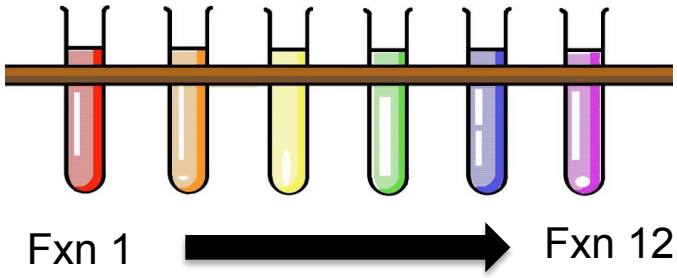
Purification Scheme (First Part)

Rabbit skeletal muscle membranes



sWGA lectin sepharose column

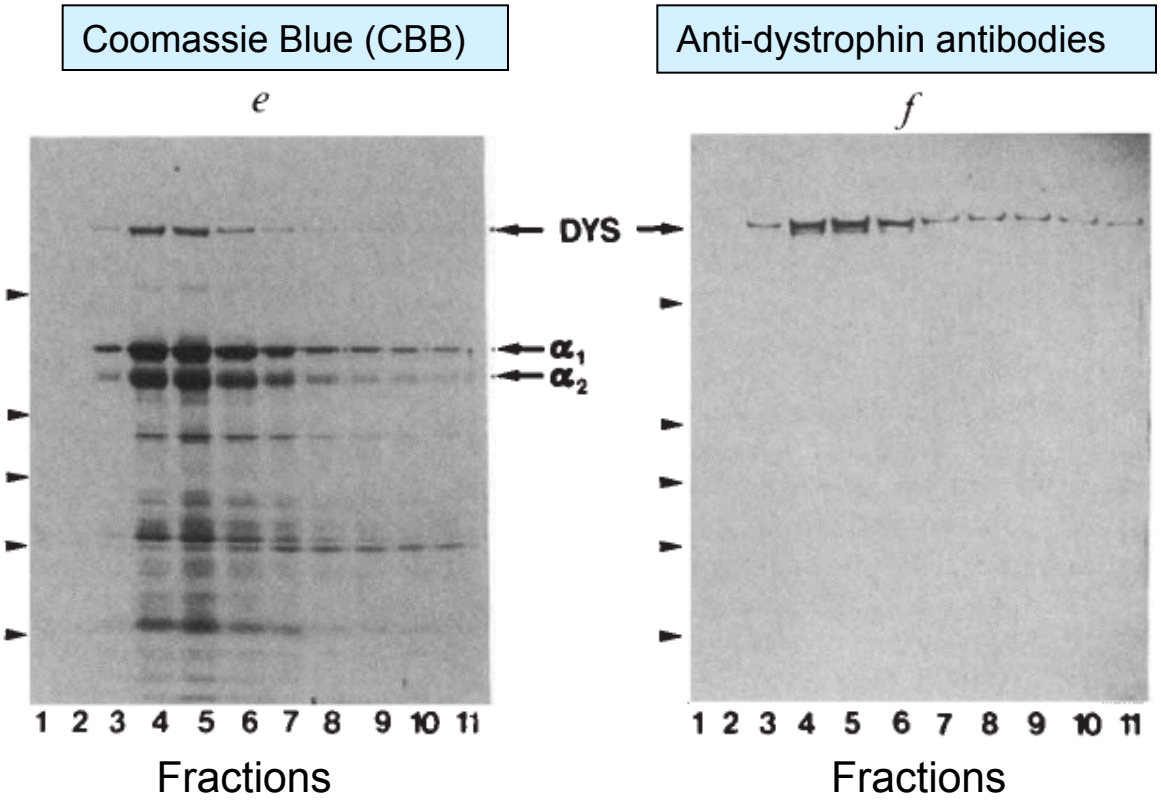
Elute with N-acetyl-D-glucosamine (NAG) and Collect Separate Fractions



Fxn 1 → Fxn 12

Collect fractions 1 → 12

Second Results: Dystrophin Co-Fractionates with DHPR



Panel E: Coomassie brilliant blue CBB) stained protein gel (to reveal all proteins)
Panel F: Sheep polyclonal anti-dystrophin antibodies (to reveal only dystrophin)
α1 and α2 are DHPR subunits

What Happens to Dystrophin During Purification (Second Part)?

Fractions that contain DHPR + DYS

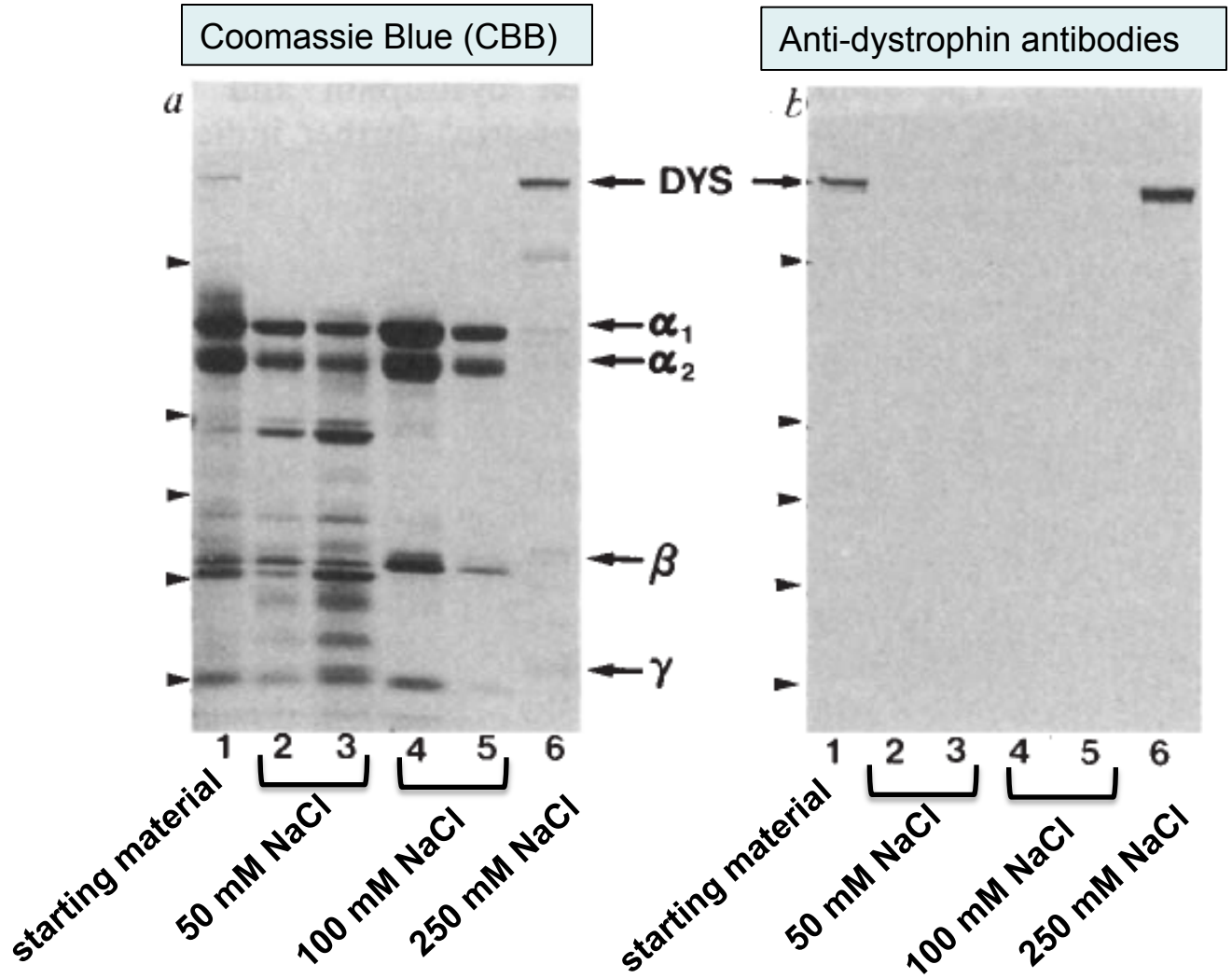
DEAE anion exchange column

Step-wise NaCl elution

50mM NaCl Fraction → 500mM NaCl Fraction

50mM to 500mM NaCl fractions are collected

Third Results: Dystrophin Can Be Biochemically Separated From DHPR



Conclusion: Dystrophin is not associated with DHPR

Deficiency of a glycoprotein component of the dystrophin complex in dystrophic muscle

James M. Ervasti, Kay Ohlendieck, Steven D. Kahl, Mitchell G. Gaver & Kevin P. Campbell*

Howard Hughes Medical Institute and Department of Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, Iowa 52242, USA

Dystrophin, the protein encoded by the Duchenne muscular dystrophy (DMD) gene, exists in a large oligomeric complex. We show here that four glycoproteins are integral components of the dystrophin complex and that the concentration of one of these is greatly reduced in DMD patients. Thus, the absence of dystrophin may lead to the loss of a dystrophin-associated glycoprotein, and the reduction in this glycoprotein may be one of the first stages of the molecular pathogenesis of muscular dystrophy.

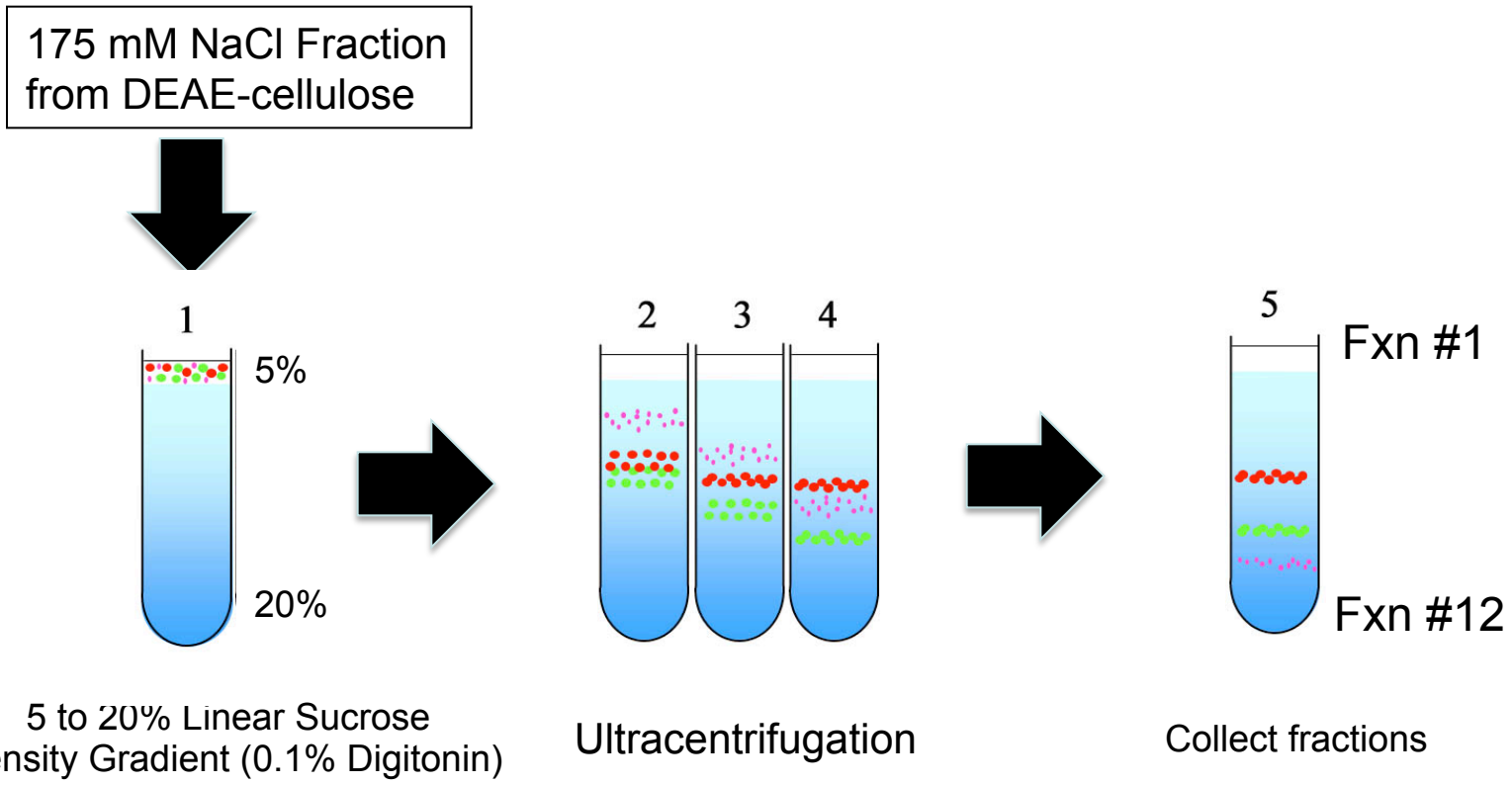
DUCHENNE muscular dystrophy is caused by a defective gene located on the X chromosome. Dystrophin, the high-molecular weight protein product of the DMD gene[1], is localized to the sarcolemmal membrane of normal skeletal muscle[2–5] but is absent from the skeletal muscle of people with DMD[1,2,6], *xmd* dogs[7] and *mdx* mice[1,5] (the last two being possible animal models for DMD). The amino-acid sequence of dystrophin suggests that it is a membrane cytoskeletal protein[8,9] involved in the anchoring of sarcolemmal proteins to the underlying cytoskeleton. But the exact function of dystrophin and its precise role in the resulting necrosis of dystrophic muscle fibres has not been determined. In studies of other genetic diseases involving proteins of the cytoskeleton[10,11], the absence of one component is sometimes accompanied by the loss of another cytoskeletal protein. Therefore, to understand the molecular pathogenesis of DMD, we sought to identify the proteins associated with or bound to dystrophin and to characterize the status of these

dystrophin using sucrose density-gradient centrifugation in the presence of digitonin. We have identified four glycoproteins of apparent relative molecular masses (M_r) 156,000 (156K), 50K, 43K and 35K as integral components of the dystrophin complex. The 156K and 50K glycoproteins are sarcolemmal glycoproteins, as shown by indirect immunofluorescence. Immunoaffinity beads raised against dystrophin and the 50K glycoprotein selectively adsorb the dystrophin–glycoprotein complex. Furthermore, there is a marked reduction of the 156K glycoprotein in muscle from *mdx* mice and DMD patients. These results imply that in dystrophic muscle, the absence of dystrophin may lead to the loss of a dystrophin-associated glycoprotein. This could be the first step in the molecular pathogenesis of muscular dystrophy.

Dystrophin–glycoprotein complex

This complex was isolated following digitonin-solubilization of rabbit skeletal muscle membranes using WGA–Sepharose and DEAE–cellulose[12] and further purified by sucrose density gradient centrifugation in the presence of 0.1% digitonin. It is evident from the Coomassie blue-stained gel of sequential gradient fractions (Fig. 1*a*) that the dystrophin–glycoprotein complex was separated from the voltage-sensitive sodium channel and the dihydropyridine receptor (Fig. 1). The size of the dystrophin complex was ~18S in comparison with β-galactosidase (15.9S), thyroglobulin (19.2S) and dihydropyridine receptor (20S) standards. Densitometer scanning of the peak dystrophin-containing fractions (10 and 11, Fig. 1*a*) revealed several proteins that co-purified with dystrophin: a broad, diffusely staining component with an apparent M_r of 156K, an 88K protein, a triplet of proteins centred at 59K, a 50K protein, a doublet at 43K and proteins of 35K and 25K.

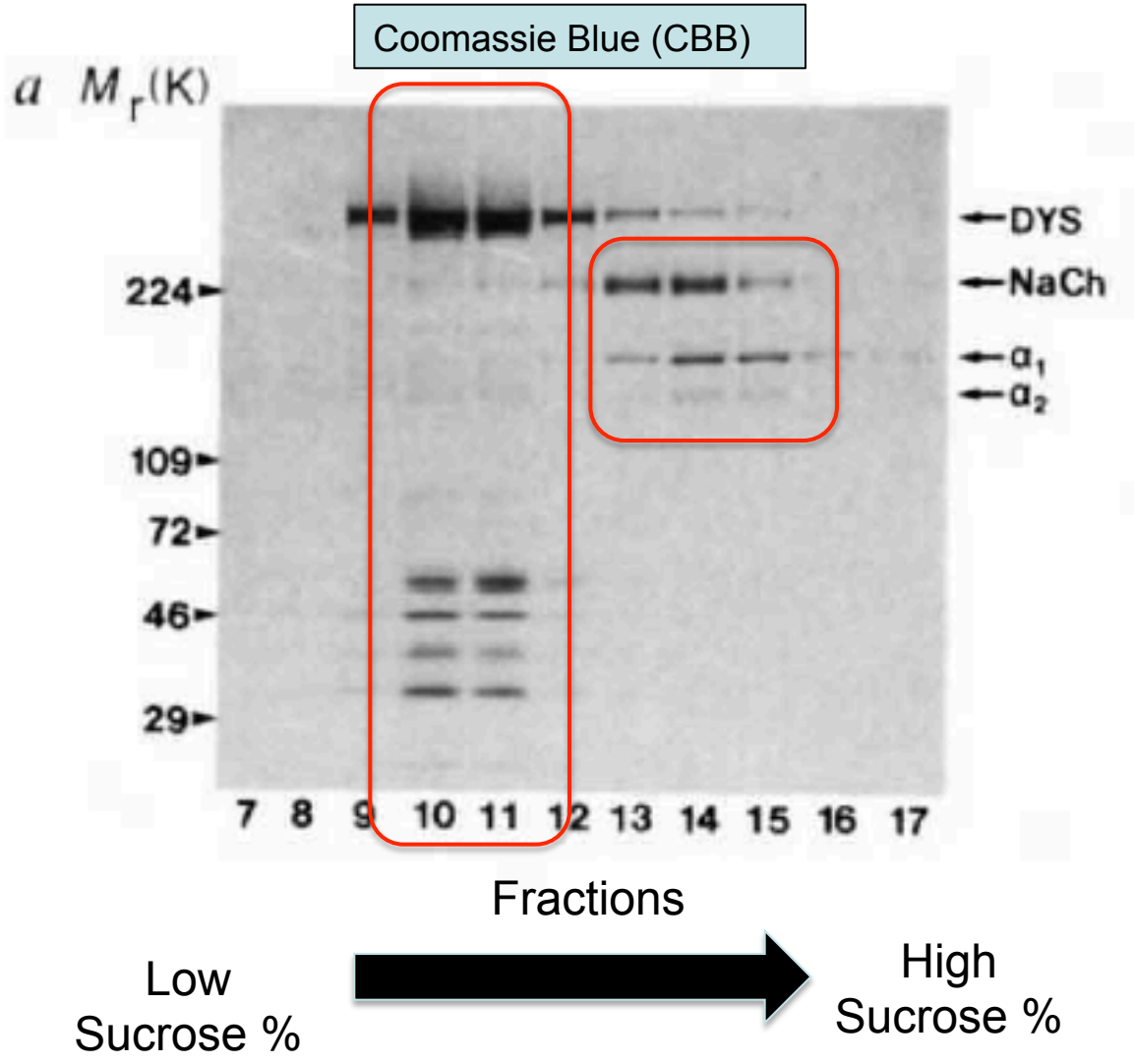
Are There Other Proteins that Associate With Dystrophin During Purification?



Proteins Separate During Ultracentrifugation:

1. Size
2. Frictional coefficient (shape)
3. Complexes

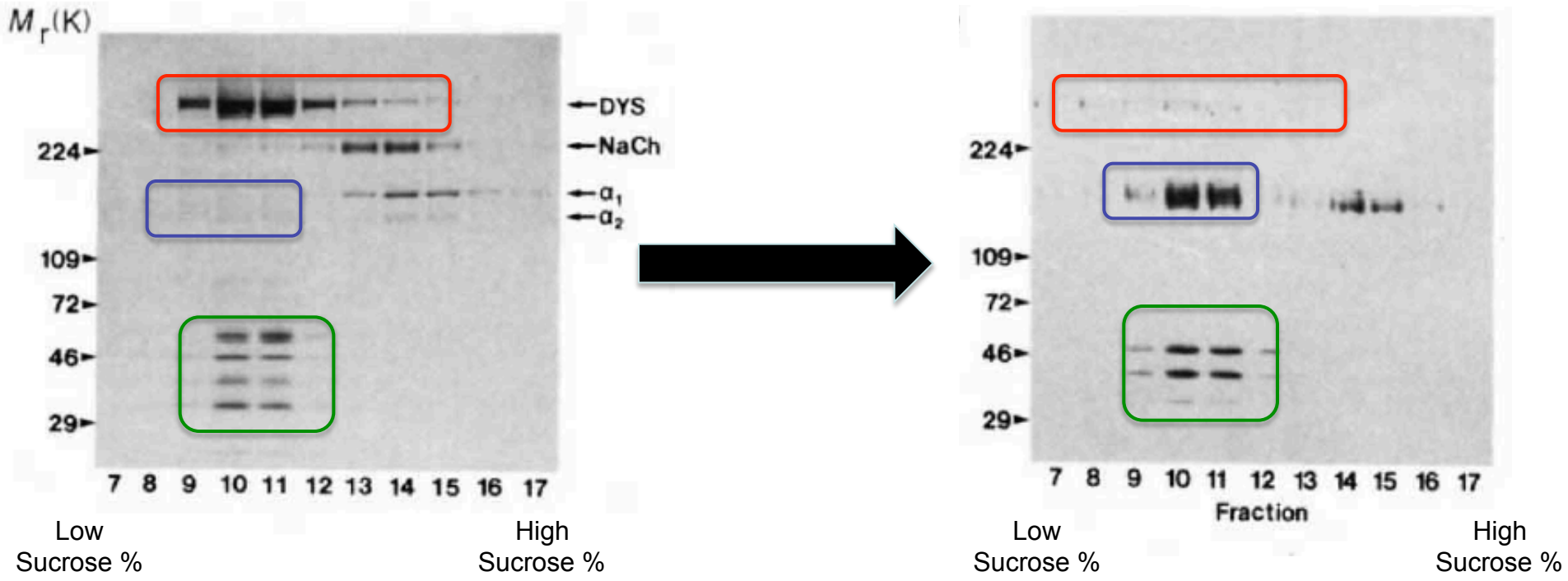
1. Dystrophin Separates from DHPR and Voltage-Sensitive Sodium Channel
2. Dystrophin is Associated with Other Proteins! Is This a Complex?



Is Dystrophin Protein a Glycoprotein?
Does Dystrophin Associate with Glycoprotein?

Coomassie Blue (CBB)

sWGA-peroxidase

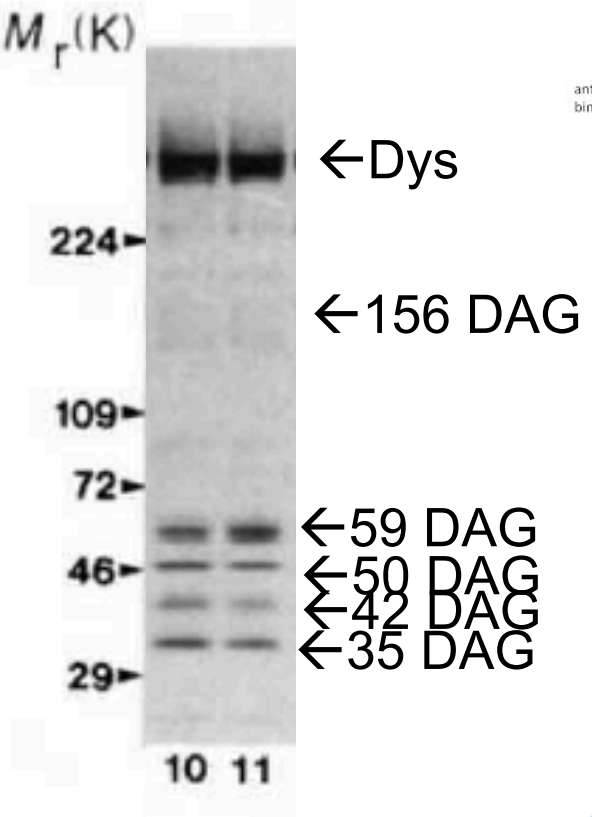


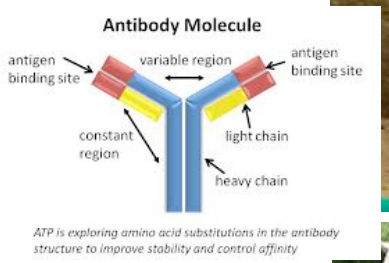
1. Transfer to Nitrocellulose
2. Probe with sWGA-peroxidase to detect carbohydrates

First Demonstration of the Dystrophin-Glycoprotein Complex

Development of Molecular Tools: Antibodies

Purified DGC





20

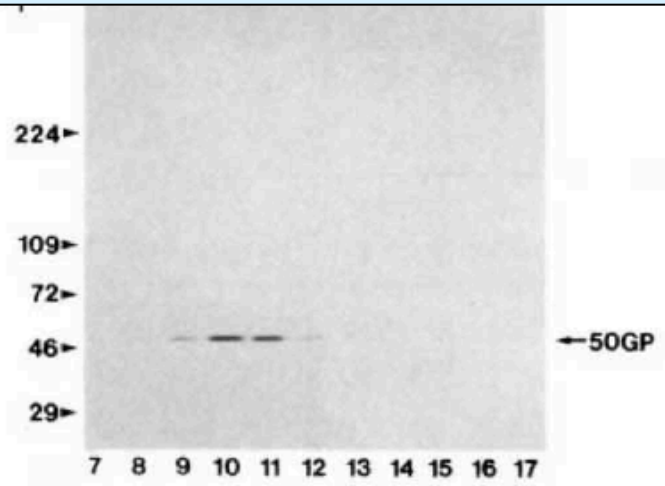
Individual protein bands and entire DGC complex used as immunogens

Monoclonal Antibodies

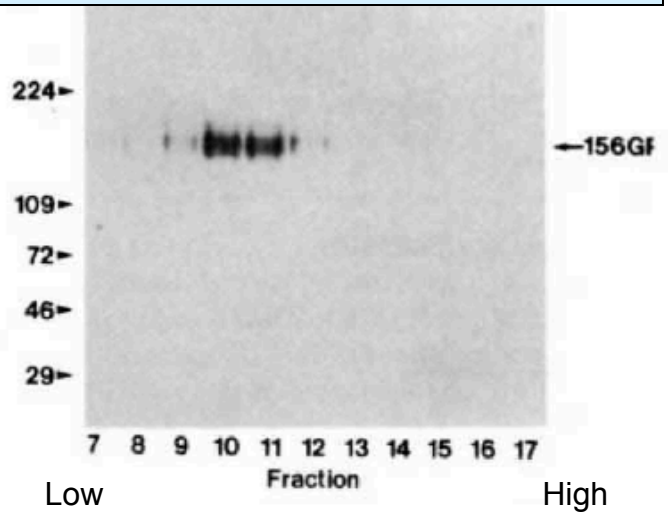
Louise V.B. Anderson, Ph.D.
Newcastle University

Antibodies now available:
Developmental Studies Hybridoma Bank
Vector Labs
Millipore (Fisher)

IVD3 monoclonal antibody
(50kDa DAG=ADHALIN=α-SARGOCLYAN)



VIA41 monoclonal antibody
(156kDa DAG= α-DYSTROGLYCAN)



Cell, Vol. 66, 1121–1131, September 20, 1991,

Membrane Organization of the Dystrophin–Glycoprotein Complex

James M. Ervasti and Kevin P. Campbell
Howard Hughes Medical Institute
and Department of Physiology and Biophysics
University of Iowa College of Medicine
Iowa City, Iowa 52242

Summary

The stoichiometry, cellular location, glycosylation, and hydrophobic properties of the components in the dystrophin–glycoprotein complex were examined. The 156, 59, 50, 43, and 35 kd dystrophin-associated proteins each possess unique antigenic determinants, enrich quantitatively with dystrophin, and were localized to the skeletal muscle sarcolemma. The 156, 50, 43, and 35 kd dystrophin-associated proteins contained Asn-linked oligosaccharides. The 156 kd dystrophin-associated glycoprotein contained terminally sialylated Ser/Thr-linked oligosaccharides. Dystrophin, the 156 kd, and the 59 kd dystrophin-associated proteins were found to be peripheral membrane proteins, while the 50 kd, 43 kd, and 35 kd dystrophin-associated glycoproteins and the 25 kd dystrophin-associated protein were confirmed as integral membrane proteins. These results demonstrate that dystrophin and its 59 kd associated protein are cytoskeletal elements that are tightly linked to a 156 kd extracellular glycoprotein by way of a complex of transmembrane proteins.

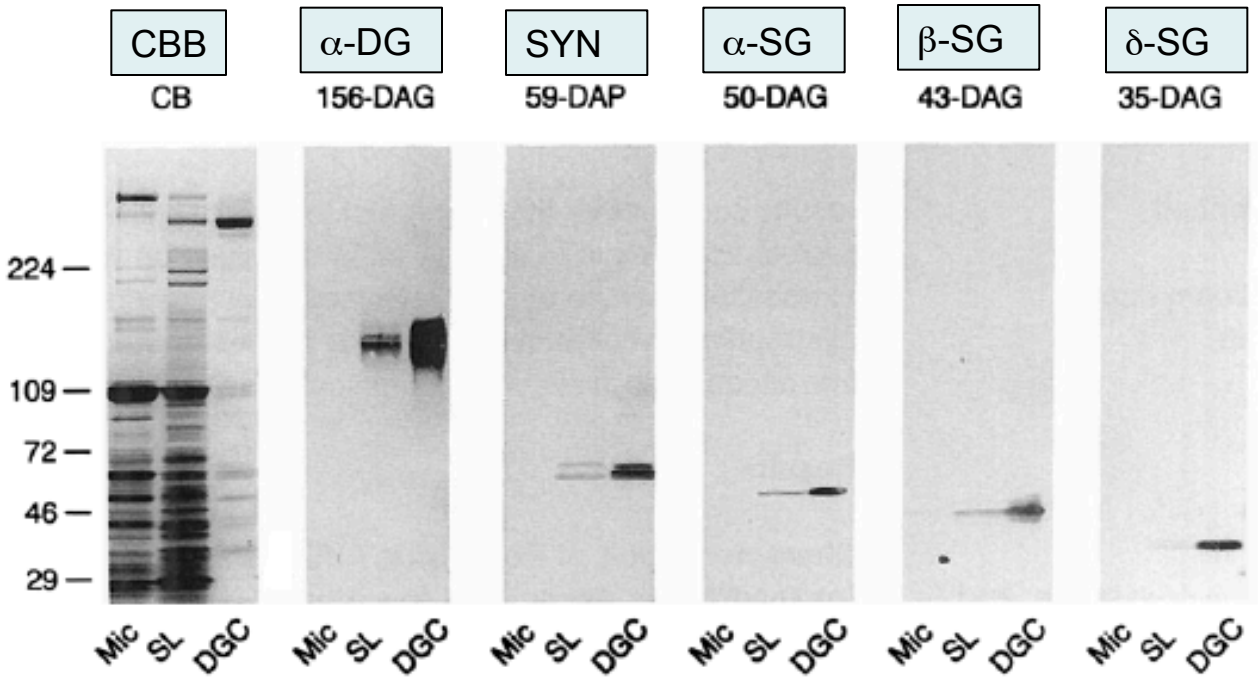
results suggest that the function of the dystrophin–glycoprotein complex is to link the actin cytoskeleton with an extracellular component of skeletal muscle. A model of the dystrophin–glycoprotein complex is proposed that takes into account the available biochemical and structural data.

Results

Characterization of Polyclonal Antibodies Specific for Dystrophin-Associated Proteins

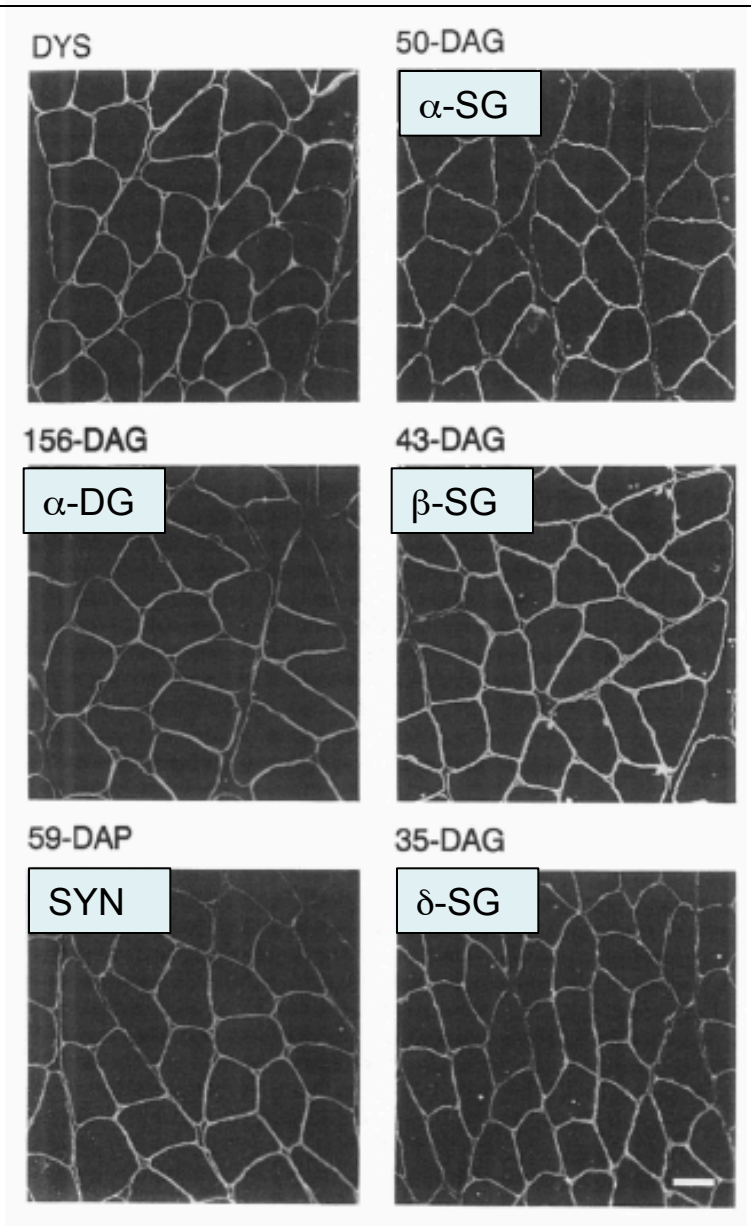
We have previously reported the preparation and characterization of monoclonal antibodies (MAbs) against dystrophin and the 156 kd and 50 kd dystrophin-associated glycoproteins (Ervasti et al., 1990; Jorgensen et al., 1990; Ohlendieck et al., 1991). However, MAb VIA4$_1$ bound very poorly to the native 156 kd dystrophin-associated glycoprotein, while MAb IVD3$_1$ stained the reduced form of the 50 kd dystrophin-associated glycoprotein very weakly on immunoblots (Ervasti et al., 1990; Ohlendieck et al., 1991). In addition, the induction of high-titered ascites from these hybridomas has yet to be successful. These limitations, coupled with the need for specific probes to the 59 kd, 43 kd, and 35 kd dystrophin-associated proteins, compelled us to prepare polyclonal antisera specific for each component of the dystrophin–glycoprotein complex. Antisera from guinea pigs immunized with purified dystrophin–glycoprotein complex (Ervasti et al., 1991) showed immunoreactivity to all components of the complex, with the exception of the 50 kd dystrophin-associated glycoprotein (not shown). Immobilon-P transfer strips containing individual

DAG/DAG Enrich Together: Further Support for the Dystrophin-Glycoprotein Complex



Mic: skeletal muscle microsomes
SL: purified sarcolemma membranes
DGC: purified Dystrophin-Glycoprotein Complex

Protein Components of Dystrophin-Glycoprotein Complex Localize to Sarcolemma



Wild-type mouse cryosections

One Method to Identify Glycoproteins within the DGC

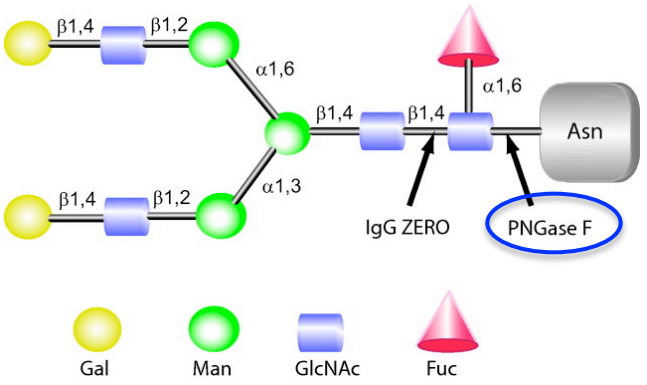
PNGaseF

Purified DGC

37°C, 1hr

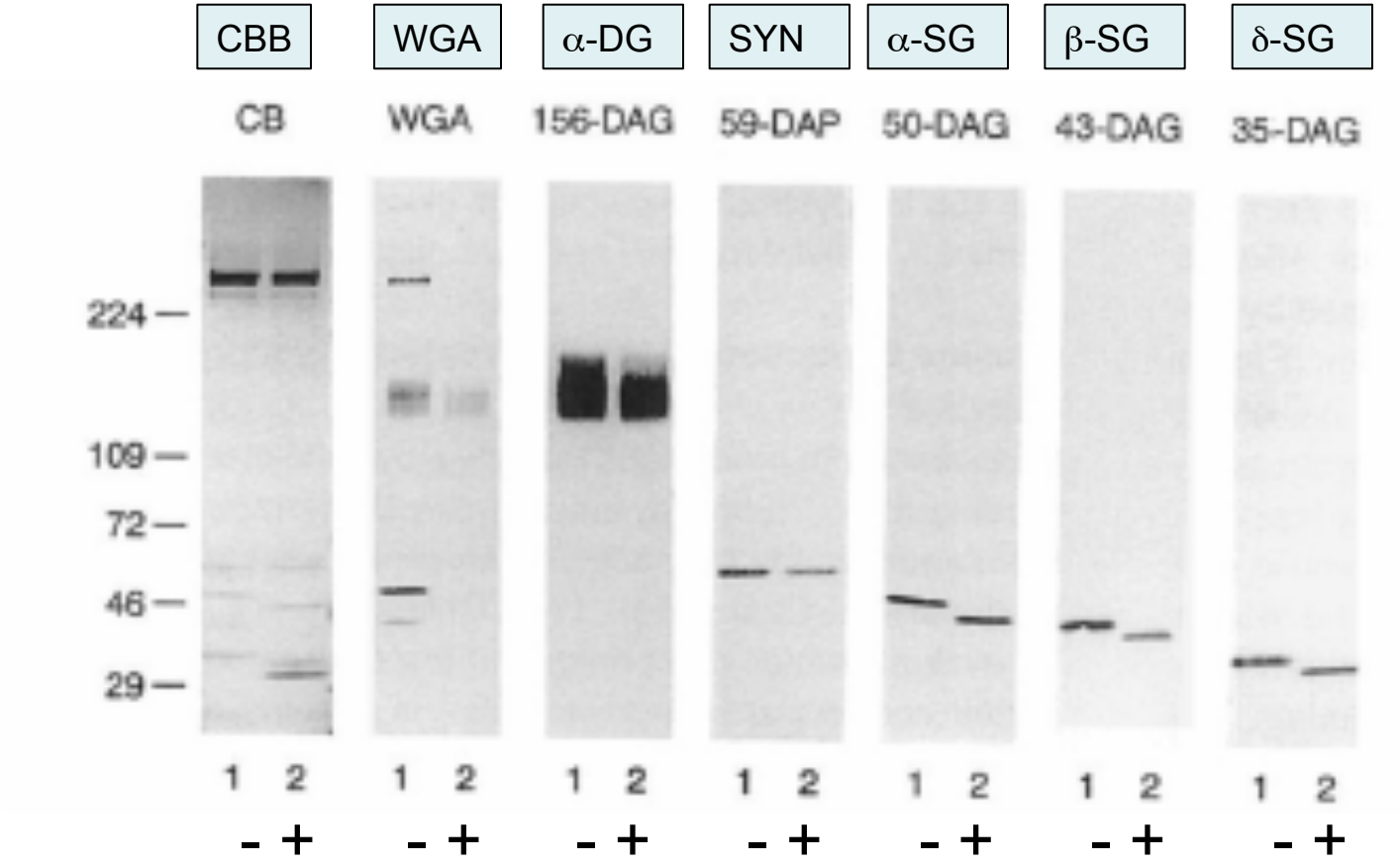
Treated DGC

SDS-PAGE Immunoblot



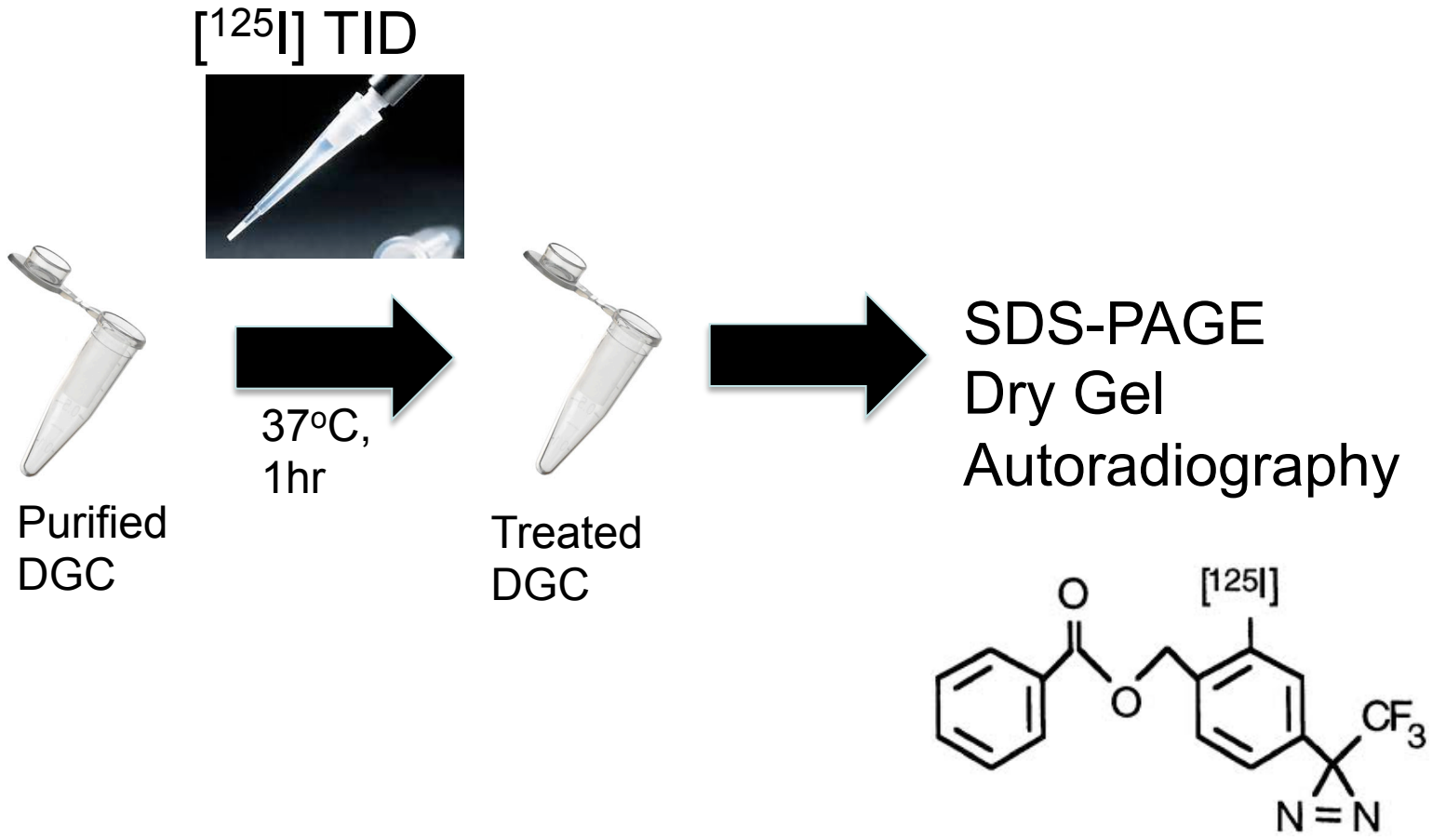
PNGaseF is an enzyme that cleaves N-linked glycans (GlcNAc)

Which Proteins are Glycoproteins?



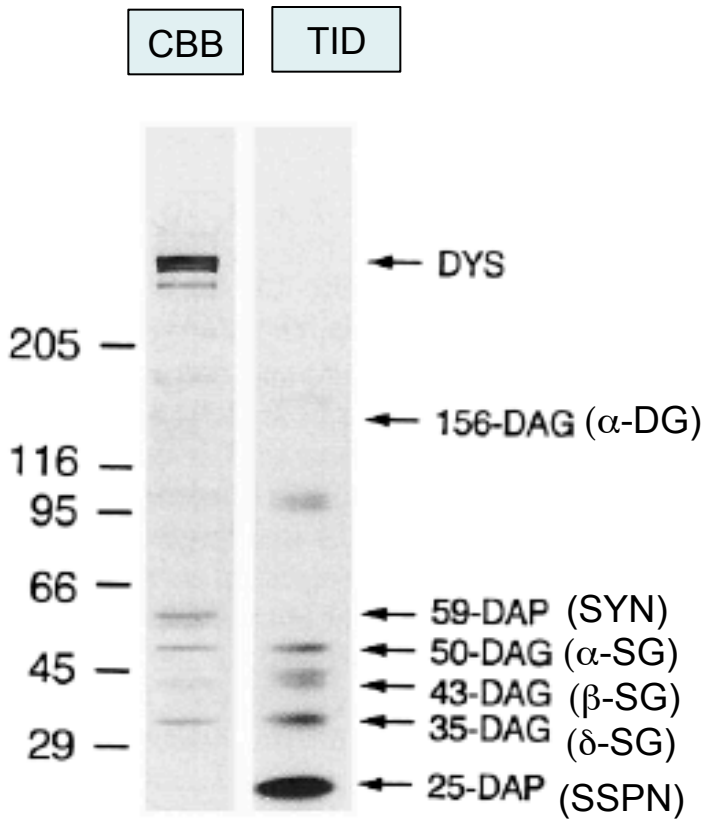
Lane 1. No treatment (control)
Lane 2. PNGase F treatment

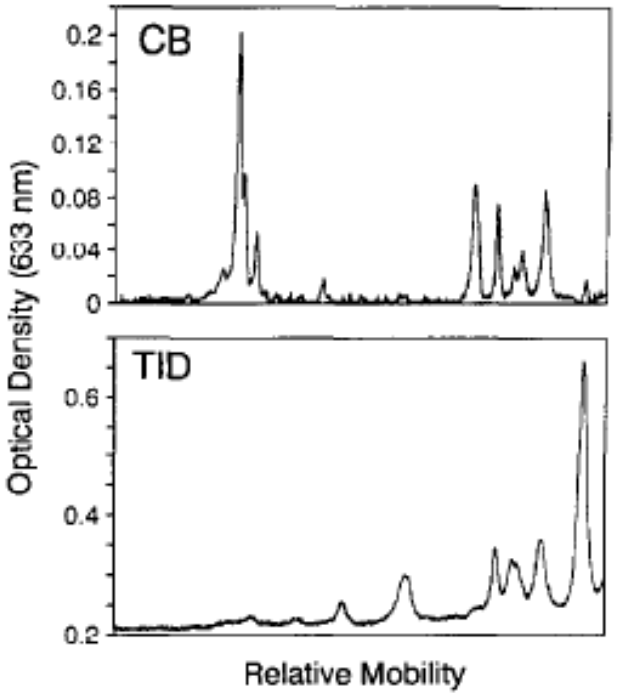
One Method to Identify Transmembrane Proteins within the DGC



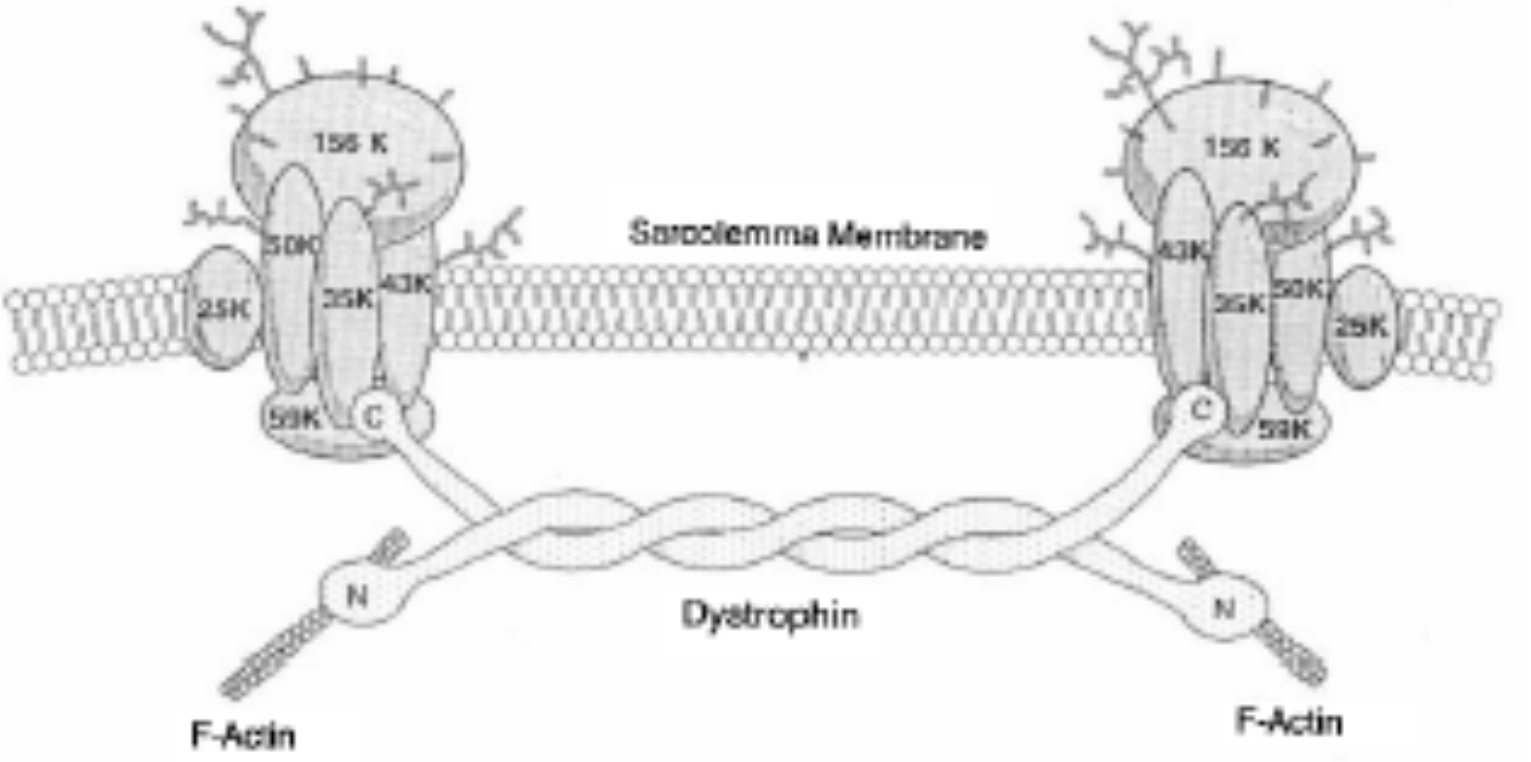
TID: 3-(trifluoro methyl)-3-(m-[^{125}I] iodophenyl diazirine)

Which Proteins are Transmembrane Proteins?





First Model of Dystrophin-Glycoprotein Complex (DGC)



J. Biochem. **108**, 748-752 (1990)

Glycoprotein Complex Anchoring Dystrophin to Sarcolemma[1]

Mikiharu Yoshida[2] and Eijiro Ozawa

National Institute of Neuroscience, NCNP, Kodaira, Tokyo 187

Received for publication, May 7, 1990

We found six groups of proteins, A0-A5, besides dystrophin itself in a dystrophin preparation obtained by the reported method [Campbell, K.P. & Kahl, S.D. (1989) *Nature* 338, 259-262] with some modifications. Their molecular weights were 94, 62, 52, 43, 36, and 24 kDa, respectively. Their molar ratios to dystrophin were 0.14, 2.2, 0.88, 0.90, 1.7, and 0.34, respectively. Each of A1, A3, and A4 was split into several bands. But each group of bands except A3 seemed to behave like the same kind of protein. The doublet of A3 was subdivided into A3a and A3b in the decreasing order of molecular weight. All the A-proteins except A2 were cross-linked with dystrophin molecule by a cross-linker, bis(sulfosuccinimidyl)suberate, suggesting them to be dystrophin-associated proteins. When dystrophin preparation was treated with KI, which is known to break membrane cytoskeletal interactions, as described by Campbell and Kahl, A2, A3, and A4 were absorbed by wheat germ lectin (WGL) Sepharose, but the dystrophin molecule and A1 were not absorbed. On the other hand, A2 and A3b reacted with biotinyl WGL but A3a and A4 did not in blotting analysis. This apparent discrepancy can be explained if we postulate that A3a and/or A4 would associate with A2 and/or A3b. On the basis of these results including stoichiometric considerations, we are of the opinion that the complex of A2·A4 among various possible ones is the most important to anchor dystrophin to sarcolemma. In this A2·A4 complex, A4 but not A2 is directly associated with dystrophin.

Ozawa Identifies DGC: The Race Is On!

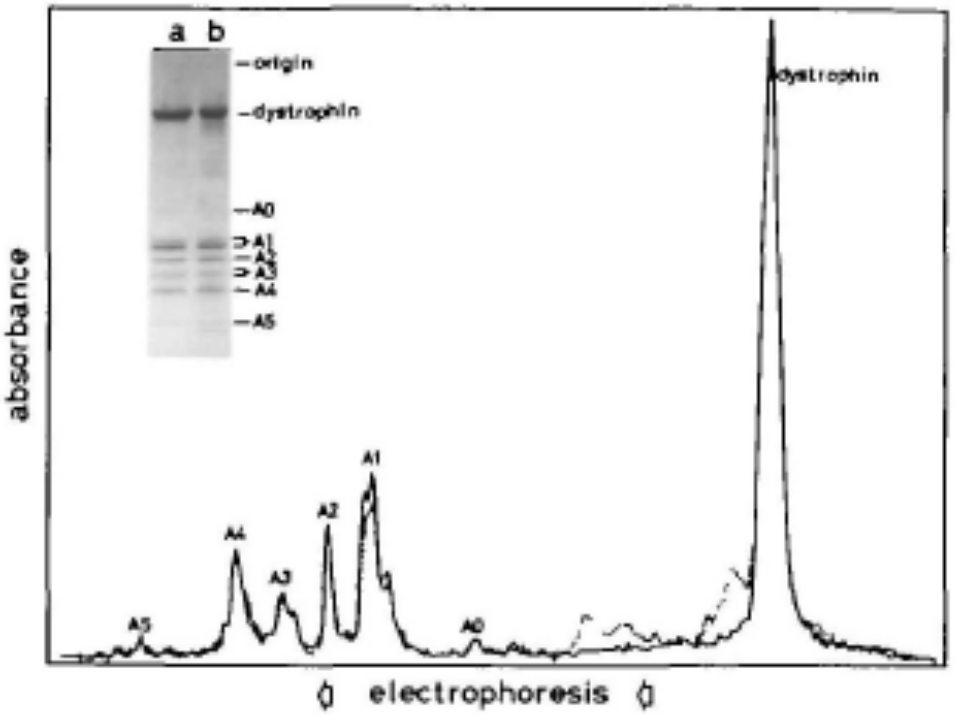


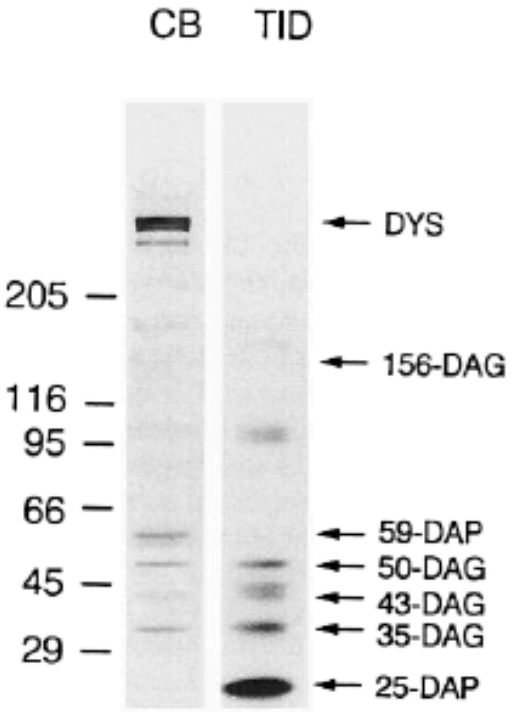
Fig. 3. Densitograms of SDS-PAGE patterns of dystrophin preparations. Dystrophin preparations were electrophoresed by the method of Laemmli (*12*) and stained with Coomassie Brilliant Blue G250. After being destained, gels were scanned with a densitometer. The densitograms were normalized for the dystrophin peak. Solid line, Superose-purified dystrophin; dotted line, monoQ-purified dystrophin. Inset: Direct view of gel patterns. a, Superose-purified dystrophin; b, monoQ-purified dystrophin. A0-A5, dystrophin-associated proteins (see text).

TABLE I. **Molecular weights and molar ratios to dystrophin of the dystrophin-associated proteins in dystrophin preparations.**

Dystrophin-associated protein	Molecular weight (kDa)	Molar ratios to dystrophin[a]	
		Superose-purified dystrophin	MonoQ-purified dystrophin
A0	94	0.14±0.09	0.07±0.04
A1	62	2.23±0.10	1.94±0.22
A2	52	0.88±0.23	0.82±0.15
A3	43	0.90±0.12	0.83±0.16
A4	36	1.73±0.07	1.83±0.10
A5	24	0.34±0.23	0.34±0.12

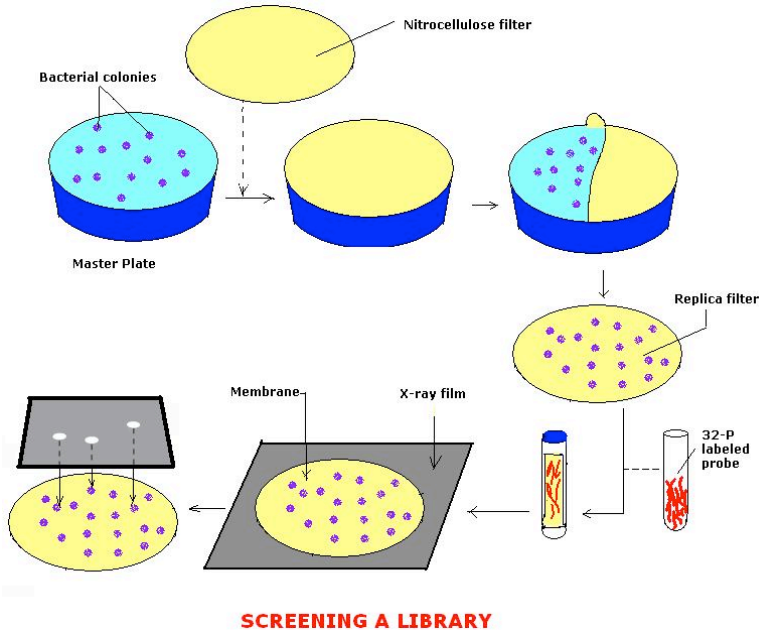
[a]These values are means of different preparations ±SD ($n=3$). The value of 427 kDa was used as the molecular weight of dystrophin (*1*).

Using Molecular Tools (Antibodies, Oligos) to Screen DNA Libraries (Old School)



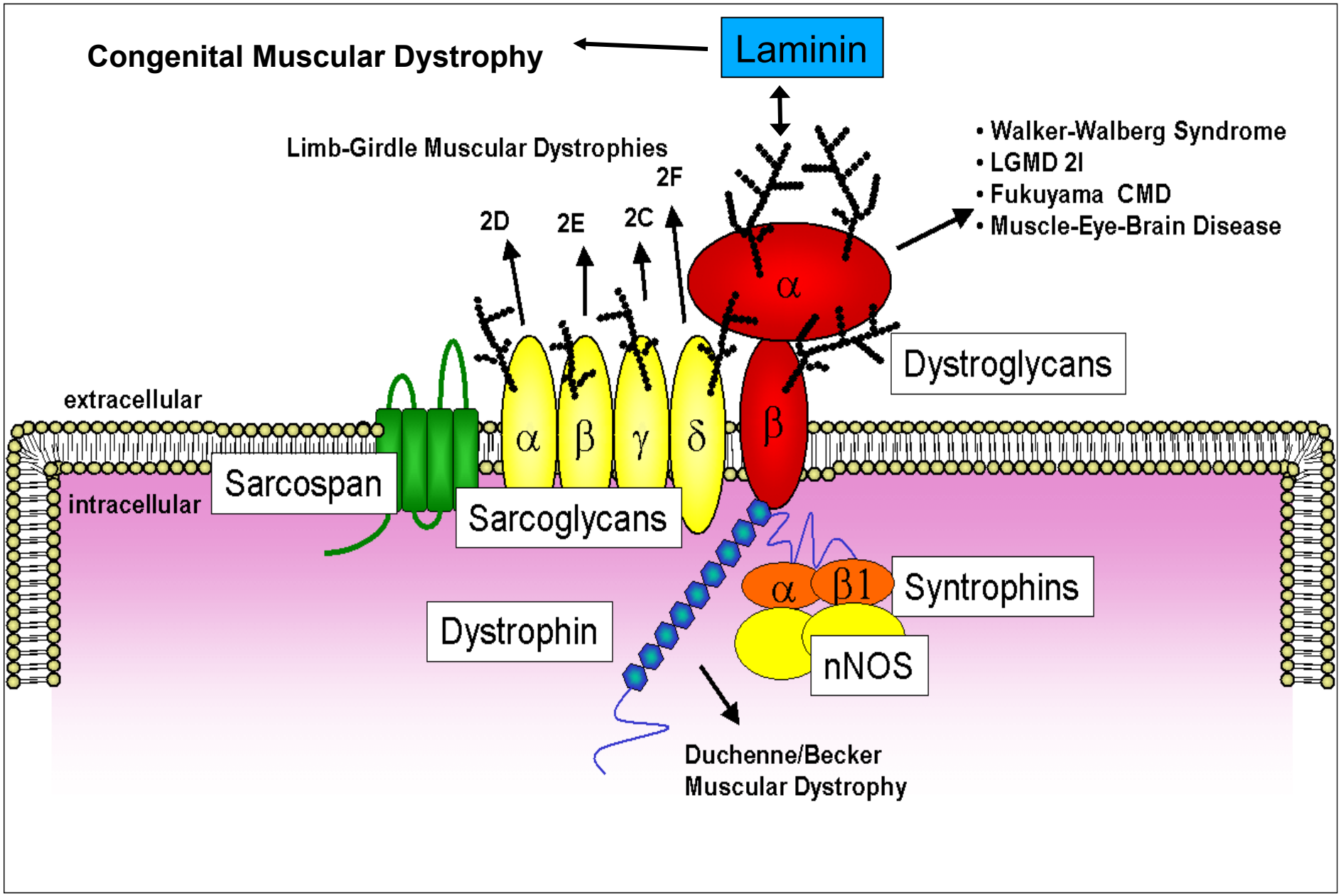
Antibodies

Peptides



Screen GenBank

Muscular Dystrophies Associated with Dystrophin-Glycoprotein Complex



THANK YOU!! QUESTIONS?
RCROSBIE@PHYSCI.UCLA.EDU

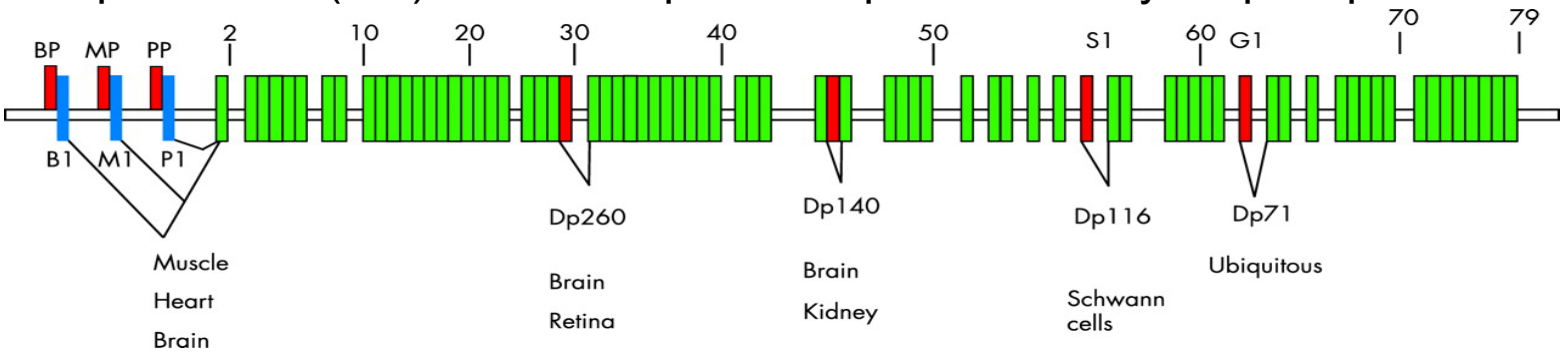
MVIMG7470 (Molecular Virology, Immunology and Medical Genetics)
Neuromuscular Biology and Disease, Spring Semester 2014
Duchenne Muscular Dystrophy
Course Instructors: Denis Guttridge, Ph.D. and Jill Rafael-Fortney, Ph.D.

"Dystrophin-glycoprotein complex discovery and associations"

Rachelle H. Crosbie-Watson, Ph.D.
Professor and Education Liaison
UCLA Center for Duchenne Muscular Dystrophy
Dept. Integrative Biology & Physiology
Dept. Neurology

Dystrophin Gene

- Dystrophin cDNA (exons) is 14.0 kilobases
- Dystrophin is composed of 79 primary exons (green)
- Dystrophin protein is 427kDa; 3500 amino acids
- Transcription of mRNA takes 16 hours
- 7 different promoters (red) → tissue specific expression of dystrophin protein isoforms



Cohen N , Muntoni F Heart 2004;90:835-841

Enrichment of the DAG proteins with Dystrophin: Evidence for a protein complex

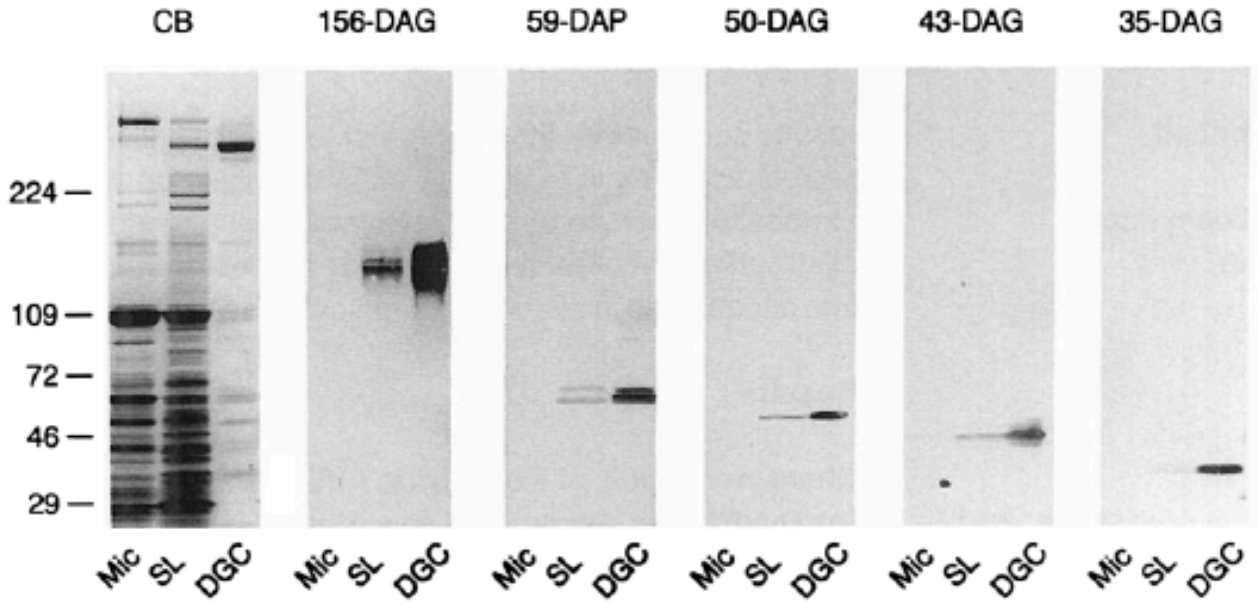


Figure 1. Coenrichment of Dystrophin-Associated Proteins with Dystrophin

Fifty micrograms of KCl-washed skeletal muscle microsomes (Mic), 50 µg of pure skeletal muscle sarcolemma (SL), and 12 µg of dystrophin–glycoprotein complex (DGC) were electrophoretically separated on 3%–12% SDS–polyacrylamide gels and either stained with Coomassie blue (CB) or transferred to nitrocellulose as described in the Experimental Procedures. Nitrocellulose transfers were stained with affinity-purified guinea pig polyclonal antibodies to the 156 kd (156-DAG), 59 kd (59-DAP), 50 kd (50-DAG), 43 kd (43-DAG), or 35 kd (35-DAG) dystrophin-associated proteins. The molecular weight standards ($\times 10^{-3}$) are indicated on the left.

Mic: skeletal muscle microsomes
SL: purified sarcolemma membranes
DGC: purified DGC

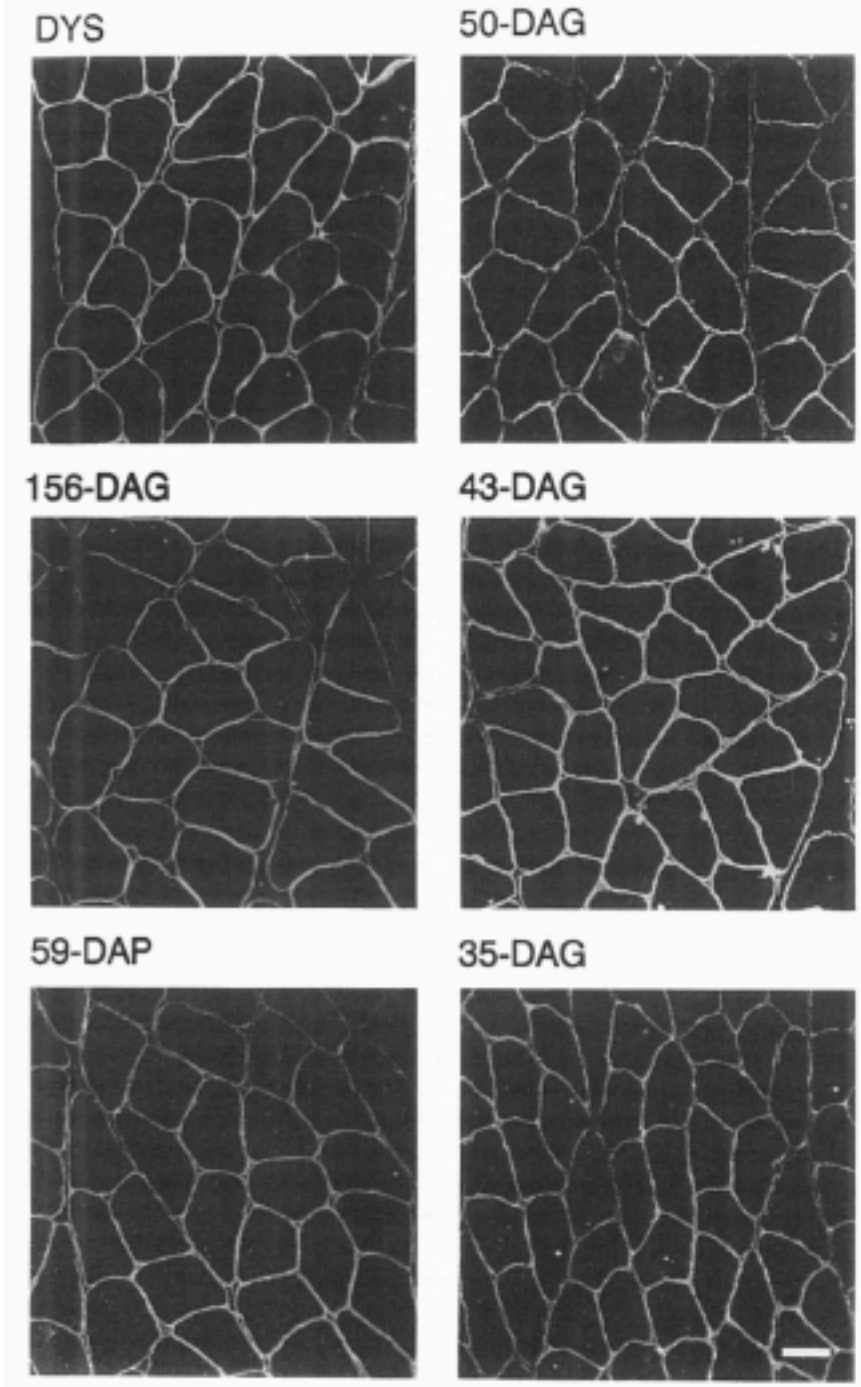
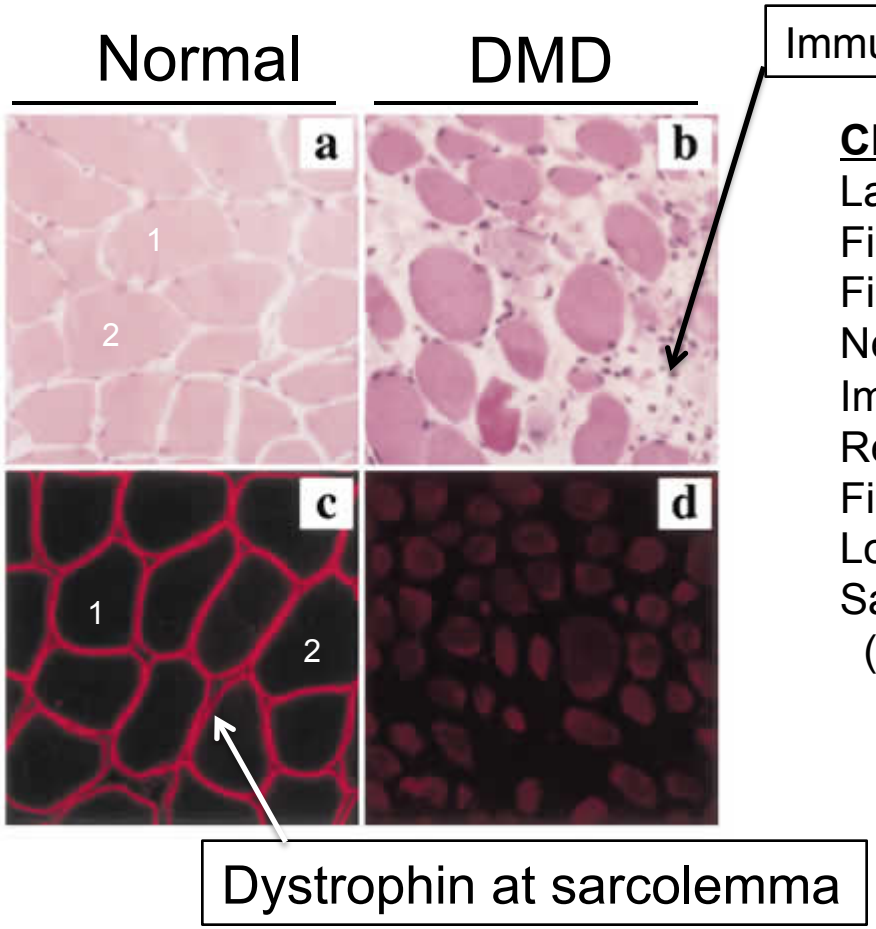


Figure 2. Immunolocalization of Dystrophin-Associated Proteins in Skeletal Muscle

Transverse cryostat sections of rabbit skeletal muscle were labeled by indirect immunofluorescence as described in the Experimental Procedures. Sections were stained with MAb VIA4$_1$ against dystrophin (DYS) or affinity-purified guinea pig polyclonal antibodies specific for the 156 kd (156-DAG), 59 kd (59-DAP), 50 kd (50-DAG), 43 kd (43-DAG), or 35 kd (35-DAG) dystrophin-associated proteins. Bar = 40 μm.

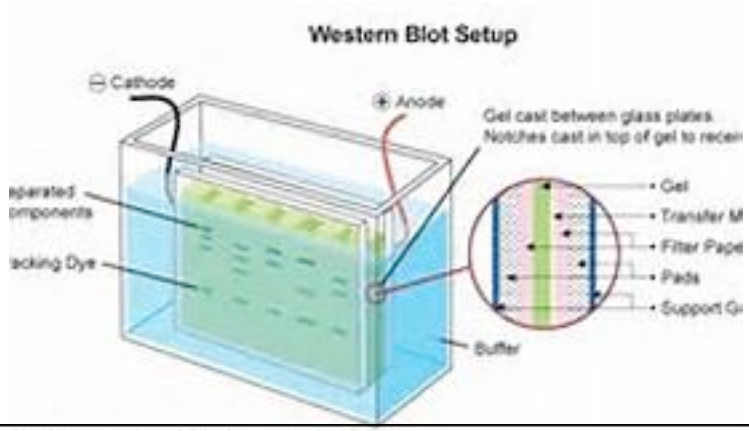
Dystrophin Protein in Normal and DMD Muscle

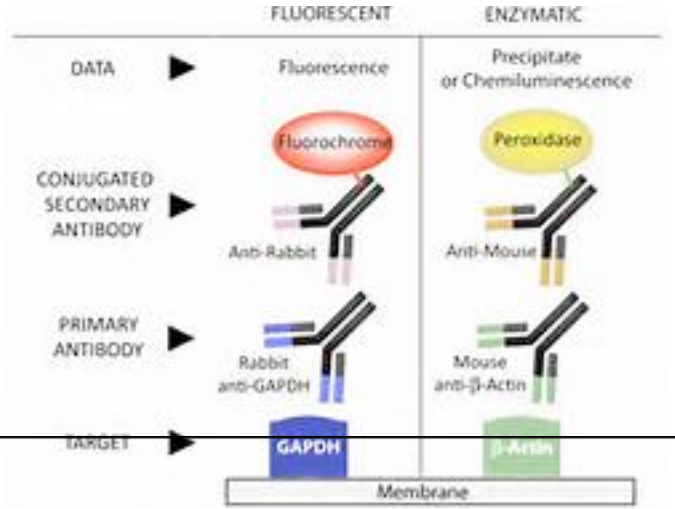


Characteristics of DMD Muscle

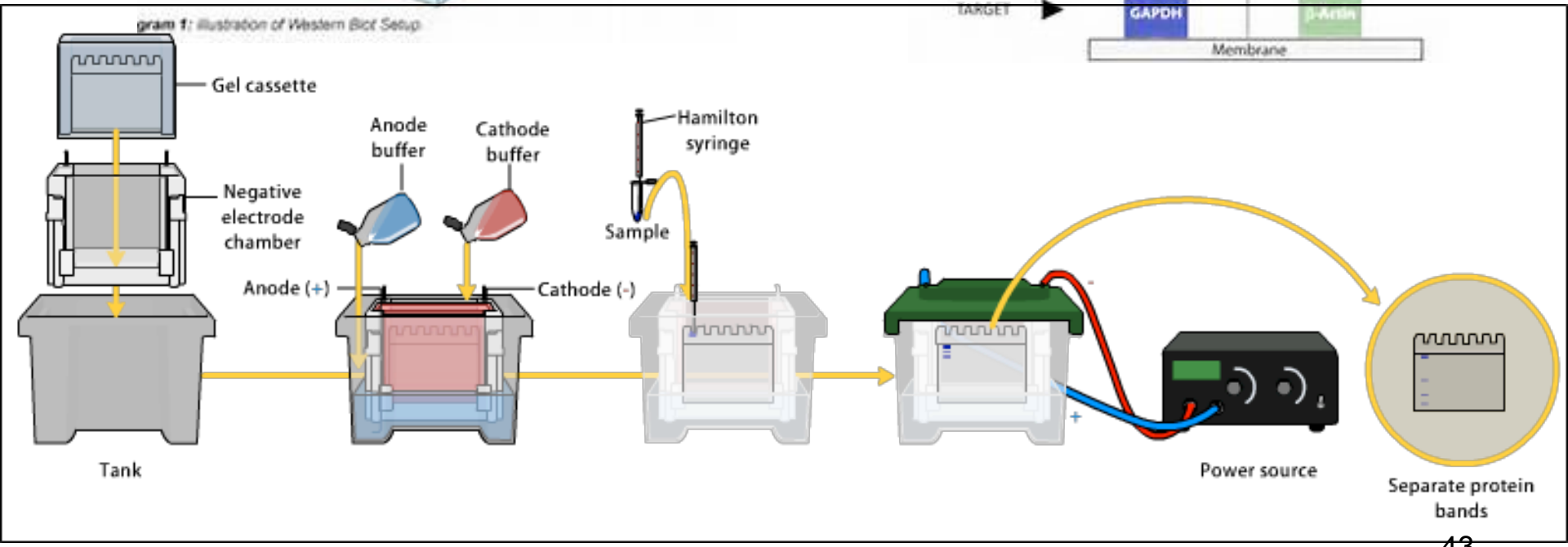
Large variation in fiber size
Fiber regeneration (central nucleation)*
Fiber hypertrophy
Necrotic fibers
Immune cell infiltration
Replacement of muscle by fat
Fibrosis (excess connective tissue)
Loss of dystrophin at sarcolemma
Sarcolemma damage
(indicated by elevated serum levels of muscle creatine kinase)

**When muscle fibers undergo regeneration, the nucleus is in the center of the muscle fiber on a transverse cross-section. This is called central nucleation. In normal muscle, the nucleus is near the sarcolemma.*

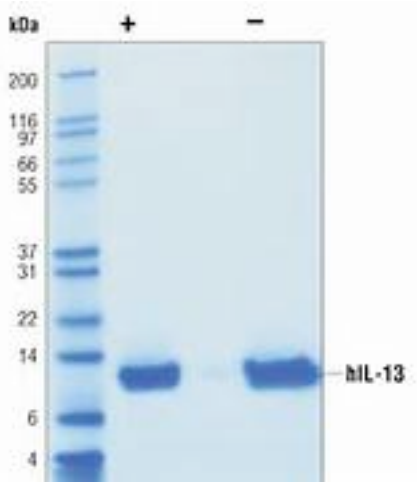


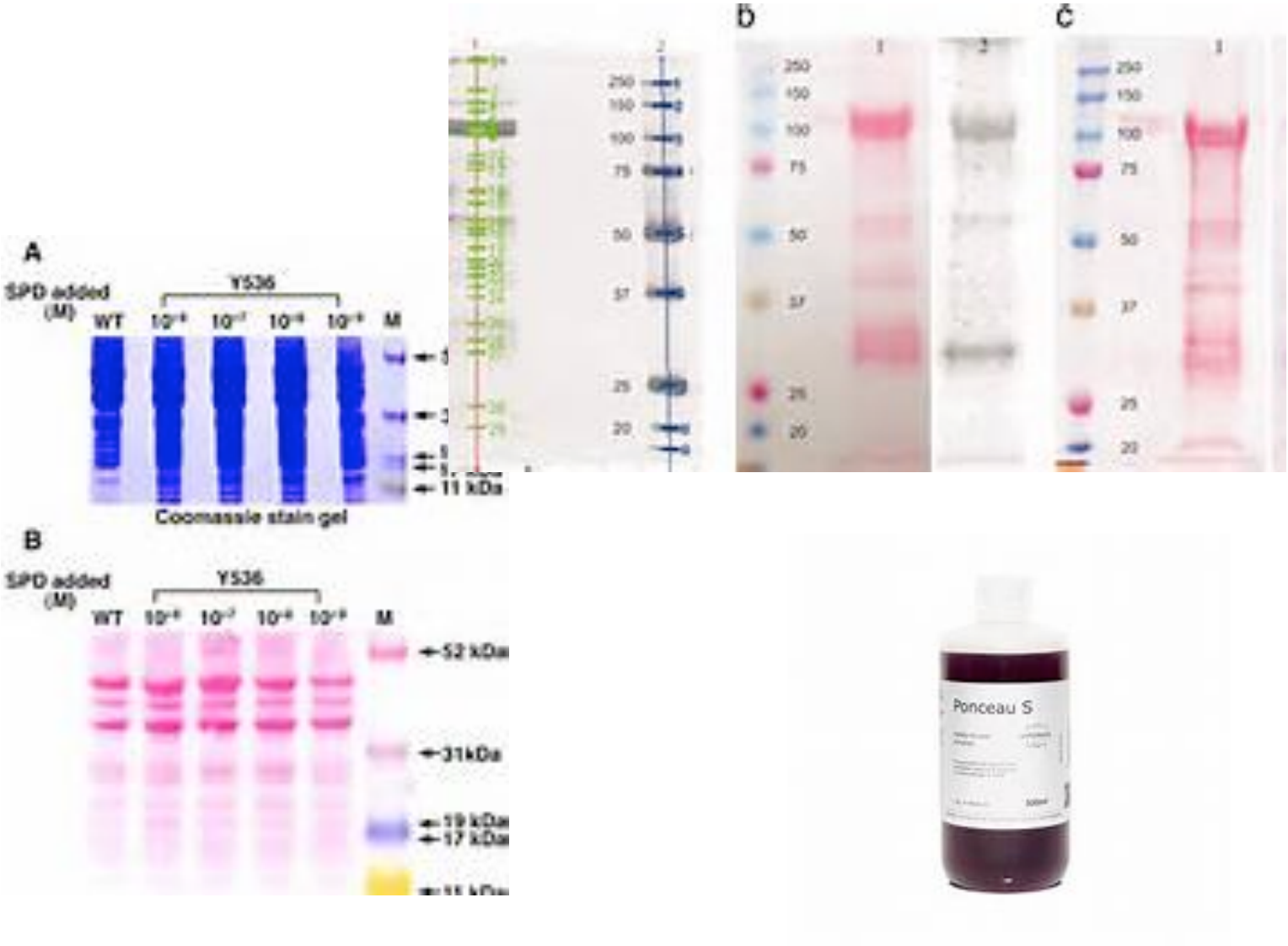


gram 1: Illustration of Western Blot Setup




COOMASSIE BLUE
MEDTECH Forensics


kDa
+
−
200
116
97
66
55
37
31
22
14
6
4
hIL-13


A
SPD added (M)
WT
Y536
M
11 kDa
Coomassie stain gel
B
SPD added (M)
WT
Y536
M
52 kDa
31kDa
19 kDa
17 kDa
b
c
Ponceau S

Purification of the Dystrophin-Glycoprotein Complex (DGC)